ABSTRACT

Increased blood pressure has been associated with exposure to petroleum products by inhalation or administration of contaminated water. The mechanism of action has however not been elucidated. This study was undertaken to investigate the effect of petroleum products on cardiovascular functions using three different routes of administration and the possible involvement of oxidative stress in the mechanism of action. Sprague Dawley rats were divided into four main groups; control, diesel (automotive gas oil), kerosene (dual purpose kerosene) and petrol (premium motor spirit). Each of the groups except control was subdivided into three; ingestion, inhalation and water contaminated groups; with 10 rats in each group and sub-group. The control was not exposed to any treatment. The diesel, kerosene and petrol sub-groups, were exposed to their corresponding contaminants via ingestion, inhalation and water contamination respectively. Each administration and exposure lasted eight weeks. The results showed that blood pressure and heart rate were significantly increased ($p<0.05$) in the treated groups when compared with control. However, there was insignificant reduced blood pressure in petrol ingestion group. The significant increase in the blood pressure and heart rate persisted one week after stoppage of exposure in all the groups; suggesting that exposure to petroleum products could cause hypertension; the effect could be as a result of the ability of petroleum products to cause sensitization of the vascular smooth muscle to catecholamines, elicited by impaired endothelium-dependent and -independent vasomotor function. Baroreflex response was significantly ($p<0.05$) increased in diesel and kerosene groups compared with control, however the increase in the petrol was only significant for a while and returned to the control level by 25 seconds. This suggests that with exposure to petrol the baroreceptors still reset the blood pressure to a new higher than normal value as though normal. The activities of baroreceptors have been altered by diesel and kerosene such that they could no longer reset the arterial blood pressure. Exposure to

diesel was most deleterious in all except in the inhalation group. There was reduced body weight gain ($p<0.05$) in all exposed rats, which was most severe in the diesel group, when compared with control as well as kerosene and petrol groups. Exposure to petroleum products dissolved fat and lipid in the body causing degeneration of fat store. The reduced weight gain might also be associated with decreased food intake seen in the study especially the diesel group. Anaemia was implicated as there were significant ($p<0.05$) reductions in RBC, PCV, and Hb. Platelets and lymphocyte reduced significantly in diesel and petrol groups but not significant in kerosene groups. A significant increase in WBC was recorded in diesel, kerosene and petrol groups. Liver damage as a result of increased reactive oxygen species was more severe in the diesel ingestion group as Alanine amino transferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase (ALP) increased significantly $p<0.05$) in all the groups. The results also showed significant increase in lipid peroxidation, as concentration of MDA increased significantly ($p<0.01$) in all the treated groups. Catalase (CAT), Glutathione reductase (GSH) and superoxide dismutase (SOD) activities were ($p<0.05$) reduced significantly in serum and tissue homogenate at varying proportions in the different groups. In the urine samples there was significant reduction in creatinine and urea value compared with control, while creatinine and urea in serum increased significantly suggesting renal function impairment. **In conclusion,** exposure to petroleum products resulted in increased blood pressure and heart rate; the baroreceptors were impaired by diesel and kerosene; caused anaemia, reduced platelets and lymphocytes and caused renal dysfunction. The severity of the effects was most severe in the diesel groups. This study suggests that afore-mentioned observations are partly caused by oxidative stress by altering the levels of CAT, GSH, SOD and MDA.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND TO THE STUDY

The primary cardiac function is to impart energy to blood in order to generate and sustain an arterial blood pressure necessary to provide adequate perfusion of organs. Cardiovascular dysfunctions such as hypertension, stroke, and coronary heart disease are associated with many underlining disease conditions and environmental factors. Hypertension is the most prevalent of all cardiovascular events (Kaplan, 2006; Julia et al., 2012). Petroleum products are frequently used in our environment and are ubiquitous environmental toxicant; which affect many physiological parameters and cause stress in man and animals (Ovuru and Ekweozor, 2004). Interest on the adverse effects of petroleum hydrocarbons has grown in recent years, and focus has been on the deleterious effects of these products on various systems in the body. Exposure could be by ingestion of food crop from polluted soil and contaminated sea foods, inhalation of vapour in the environment, (from vehicle exhaust, as petrol attendant or refinery worker) or drinking contaminated water. Contamination with petroleum products is increasing both in rural and urban areas. Acute exposure also occurs following spillage, blowouts, pipeline-vandalization and tanker accidents. There is increased use of petroleum products for power generation in domestic and industrial environment (Anigbogu and Ojo, 2009). Diesel, gasoline and kerosene are among the commonly used fractionated products of crude petroleum. These fractions contain aliphatic, aromatic and a variety of other branched saturated and unsaturated hydrocarbons at variable concentrations. The constituents of the vapours from these fractions, to a greater extent, depend on the composition of their liquid forms and carbon content, which varies with the brand and storage period (EHC 20, 1982; Henderson et al., 1993; Kato et al. 1996; Anderson et al., 1995).

Petroleum products are integral part of our modern lives. It is almost impossible to avoid exposure to hydrocarbon from petroleum products, whether it is from gasoline fumes at pumps, spilled crankcase oil on asphalt solvent used at home or work or pesticide applications that use petroleum product as carrier. Petroleum products are used in the domestic environment and are still frequently stored in the homes or garage in unmarked container or beverage bottles. They may also have attractive aromas and be brightly coloured. It is not surprising therefore that most cases of exposure in the homes involve accidental ingestion by young children/adults. A larger proportion of childhood poisoning occurs in the pre-school group. Children under 6 years accounted for more than 50% of all exposures reported to poisons centre in the USA in 1996, of which 2.4% involved hydrocarbons (U.S. EPA 2009). Contamination with petroleum product is not only limited to the marine environment. Pollution at drill sites and oil spills on land used for agricultural purposes, as well as petroleum or diesel waste; pose serious risks exposure to occupational public, terrestrial wildlife, mammals and livestock raised on these lands (Igwebuike et al., 2007). Some components of petroleum have the potential to bioaccumulate within susceptible aquatic organisms and thus passed by trophic transfer to other levels in the food chain (Gardner et al., 1991).

The adverse effects of bioaccumulation in exposure to petroleum products are manifold and vary depending on the concentration of the substances and the length of time that one is exposed. Breathing petroleum vapour can cause nervous system defects such as headache, nausea, dizziness and respiratory irritation (Steffe et al., 1996).

Very high exposure to petroleum products can cause coma and death (Levy and Pappano, 2007). Liquid petroleum products which come in contact with the skin can cause irritation and some are absorbed through the skin. Chronic exposure to petroleum products may affect the nervous system, blood and kidneys (Levy and Pappano, 2007). Gasoline is known to contain small amounts of benzene, a known human carcinogen

(Periago and Prado, 2005).

The toxicity of petroleum product is related to its hydrophobicity (Freedman, 1995) because lipid solubility is an important factor in the passage of petroleum components through the plasma membrane of cell as well as the degree of membrane damage (Igwebuike et al., 2007). The most common sources of petroleum contamination can either be from stationary petroleum storage systems or when released to the environment. Contamination can occur from a storage system through;

- Leaks in pipes and joints
- Leaks from corroded tanks
- Over fills and spills while filling tanks

When petroleum products are released into the environment, the petroleum and the products can contaminate the soil, groundwater, surface water and air. Explosive vapours from discharged petroleum products can accumulate in confined space such as an abandoned tank, a subsurface cable vault, and sewer or beneath buildings.

1.2 Major Petroleum Products

1.2.1 Diesel

Diesel, called automotive gas oil (AGO) is produced by fractional distillation of petroleum 220-250^{o}C (392° F-662° F), typically contains 15-25 carbon atoms. Chemical formula for common diesel fuel is $C_{15}H_{23}$. There are alternatives that are not derived from petroleum, such as biodiesel, biomass to liquid (BTL) or gas to liquid (GTL) diesel, are increasingly being developed and adopted. To distinguish these types, petroleum-derived diesel is increasingly called petrol diesel**.** In the UK, diesel fuel for on-road use is commonly abbreviated **DERV**, standing for Diesel Engined Road Vehicle, which carries a tax premium over equivalent fuel for non-road use. Diesel has a higher density than gasoline and is simpler

to refine from crude oil. It is most commonly used in transportation in a diesel engine. The generated heat (J/kg) obtained by the burning of diesel in air is 13762J/kg. Petroleum-derived diesel is composed of about 75% saturated hydrocarbons and 25% aromatic hydrocarbons. The hazard experience with petro diesel is that when it spilled on a road, will stay there until washed away by sufficiently heavy rain, whereas gasoline will quickly evaporate. A **diesel engine** is an internal combustion engine, developed by Rudolf Diesel in 1893 (ATSDR, 1995). **Synthetic diesel** can be produced from any carbonaceous material, including biomass, biogas, natural gas, coal and many others. The raw material is gasified into synthesised gas, which after purification is converted by the Fischer-Tropsch process to a synthetic diesel. **Fatty-acid methyl ester** (FAME), perhaps more widely known as biodiesel, is obtained from vegetable oil or animal fats (biolipids) (Demirbas, 2008); which have been trans-esterified with methanol. It can be produced from many types of oils, the most common being rapeseed oil (rapeseed methyl ester, RME) in Europe and soybean oil (soy methyl ester, SME) in the USA. (Torgov et al., 1994), Diesel displaced coal and fuel oil for steam-powered vehicles in the latter half of the 20th century, and is now used almost exclusively for the combustion engines of self-powered rail vehicles (locomotives and railcars).

1.2.2 Kerosene

Kerosene called dual purpose kerosene (DPO) because it is most commonly used as jet fuel and as heating fuel. It is a thin clear liquid formed from a complex mixture of hydrocarbon ($C_{14}H_{30}$) with density of 0.78 - 0.81/cm^3. Kerosene was first described by al-Razi (Rhazes) as a distillation of petroleum in 9th-century Baghdad. It is obtained from fractional distillation of crude oil between 150 and 270^{o}C resulting in a mixture of carbon chain that typically contain between 12 and 15 carbon atom per molecule (Ofusori et al., 2009). In the United Kingdom, kerosene is also known as paraffin. The word kerosene comes from the Greek

word “keros” meaning wax. The lethal dose (LD_{50}) of kerosene for 70 kg adult is 100 ml (Patel et al., 2004).

In the early 21st century, kerosene was used to power New York City transit buses. Now, kerosene is used as fuel in portable stoves, kerosene space heaters, and in liquid pesticides. It is the major domestic cooking fuel in many Nigerian homes. It is called dual purpose kerosene because of its dual-use as a fuel that is both "Motor vehicle and non-road, locomotive or marine. A short, one-off exposure to kerosene is unlikely to result in any long-term effects. However, a severe form of lung injury called pneumonitis may occur if liquid kerosene is aspirated directly into the lungs, for example, whilst manually siphoning a tank or from inhaling vomit after swallowing kerosene toxicity is principally due to pulmonary complications if it is inhaled while being ingested (aspiration). Although kerosene is not poisonous, medical advice should be obtained immediately when swallowed as there is a risk of short term lung damage if vomiting occurs. Frequent skin exposure may lead to skin damage (dermatitis); there is a threshold level of exposure to kerosene above which adverse health effects evolves. Breathing large quantities of kerosene vapour or drinking kerosene based liquids may cause non-specific signs such as dizziness, headache, and vomiting (Oyekale et al., 2012; Mahdi, 1988).

1.2.2.1 Kerosene Toxicological overview

Health Effects of Chronic / Repeated Exposure

The most common health effect associated with chronic / repeated kerosene exposure is Dermatitis (Ritchie et al., 2003) which may be associated with insufficient or inappropriate use of personal protective equipment (PPE) in occupational environments. Lung effects (such as dyspnoea) have been reported, but tend to be associated with “high level” exposures (Ritchie et al., 2003). It is conceivable that similar lung and skin effects may be observed in some individuals following a single, acute exposure

Dermal / ocular exposure

Dermatitis was observed in mice topically exposed to kerosene (applied in muslin cloth) for 15 to 60 minutes each day for one week which resolved within three weeks (Upreti, et al., 1989) Pathological changes (hyperplasia and visual scores of irritation) were also observed in mice exposed twice a week for two weeks to deodorised kerosene, but the lesion severity did not correlate with tumour-promoting activity when compared against four other petroleum products (Walborget al., 1998) When applied three times a week for up to six weeks, repeated cycles of necrosis and regeneration were observed that were deemed sufficient to represent an epigenetic mechanism for tumourigenesis.

Pulmonary pathology (inflammatory cell infiltrates and morphological changes to tracheal epithelia) and cardiovascular changes (resembling atherosclerosis) have been observed in guinea pigs following exposure to high concentrations (up to 34 g m-3) of kerosene aerosol for 15 minutes per day over a three week period(Noa et al., 1987, 1985,1984). Continuous (3 month) exposure of rats and mice to up to 1 g m-3 aviation fuel (JP-8) vapour resulted in male rat-specific pathology (nephropathy) that was not thought to be of relevance to humans (Mattieet al., 1991). In a study carried out by Mann et al., (1977), absorption of kerosene was investigated in primates, the result showed that primates absorbed kerosene from the gastrointestinal tract, but the volumes are very small and do not cause gross neurologic signs.

Lee and colleagues (2000), in an experiment found out that cleaning with kerosene resulted in mean carcinogen-DNA adduct levels in the lung which were significantly higher than even the positive controls, regardless of cleaning time. Garciá Mesa and his colleagues in another experiment discovered that the subchronic exposure to vapors of kerosene or its combustion fumes, induced an increase in the activity of lysosomal enzymes in lungs which can be an explanation of the inflammatory response induced in lungs by this agent.

1.2.3 Petrol

Petrol, called premium motor spirit (PMS) gasoline, or petroleum-derived liquid mixture was primarily used as fuel in internal combustion engines. It is obtained from fractional distillation of crude oil between $20^{o}C$ - $60^{o}C$, contains 5-11 carbon atom. Easily vapourized, highly flammable and easily ignited. It is also used as a solvent, mainly known for its ability to dilute paint. Exposure to petrol vapour in confined or poorly ventilated areas may cause rapid onset of unconsciousness (Pino et al., 2004). The generated heat (J/kg) obtained by the burning of gasoline in air is 12,200 J/kg. The energy Density depends on the grade and source.

1.2.3.1 Petrol Toxicological Overview

Petrol is a complex mixture of aliphatic and aromatic hydrocarbons derived from blending fractions of crude oil with brand-specific additives. The actual composition of petrol will vary according to the source of crude oil, the manufacturing process and between batches. As with other hydrocarbon solvents, petrol has anaesthetic (narcotic) properties (Table 2). Petrol also contains a number of potentially neurotoxic chemicals including n-hexane, benzene, butadiene, toluene, ethylbenzene, xylene and trimethyl pentane (Ritchie et al., 2001). The approximate concentration of each constituent in liquid petrol and vapour are given in the table below.

Table 1: Average concentration of potentially neurotoxic constituents of liquid petrol and vapour.

	Concentration %w/w)	
Chemical	Liquid	Vapour
Benzene	2.5 (0.2 – 4.7)	1.77 (0 – 5.4)
1,3-Butadiene	<0.1	0.65 (0 – 4.6)
Ethylbenzene	2.6 (1.0 – 5.4)	0.009 (0 – 0.1)
n-Hexane	2.5 (0.8 – 5)	1.37 (0 – 6.5)
Toluene	11.4 (2.7 – 21.0)	1.63 (0 – 7.1)
Xylene	10.6 (5.8 – 15.8)	0.48 (0 – 2.1)

Cairney et al., 2002; Cecil et al., 1997)

Numbers in brackets refer to range of values. Vapour values expressed as percentage of total hydrocarbons recovered from air samples obtained during the manual filling of cars (conditions and duration not reported)

General toxicity

Dysfunction of the central nervous systemis the predominant pathological condition associated with chronic exposure to high levels and such effects arising from frequent, recreational exposure (‘sniffing’ or ‘huffing’) have been extensively documented (Edminster and Bayer, 1985; Cairney, 2002; Flanagan, and Ives, 1994; Cairney et al., 2004,2005). There is currently insufficient evidence to unequivocally link chronic (occupational) exposure to petrol with other pathological conditions (Kovarik, 2005). This may be because petrochemical workers are potentially exposed to a wide range of chemicals in addition to other confounding factors (IARC,1989). Historically, lead has been identified as the principal component of petrol responsible for neurotoxicity (Kovarik, 2005),and studies have demonstrated a link between lead body burden and neurological deficits as a result of petrol

abuse (‘sniffing’ or ‘huffing’) (Seshia, 1978). However, it should be noted that the volatility of tetraethyl lead (TEL) is relatively low (0.4 mm Hg at 25ºC) and so prolonged dermal exposure associated with the practice of petrol sniffing is likely to be the predominant route of entry for TEL rather than inhalation (Toxicology update, 1989). Since 2000, petrol has only been commercially available in ‘unleaded’ form within the UK and most of Europe. This policy limits the concentration of lead in marketable petrol to less than 0.005 g L-1as defined at Annex I of the 1998 EU Directive (EU, 1998). Whilst there is a known association between chronic petrol exposure and renal cancer in male rats (Halder et al., 1985; Olson et al., 1987; Short et al., 1987), there is currently no evidence to link petrol exposure and renal cancer in humans (Trump et al., 1984; IARC, 1989). It is generally accepted that the susceptibility of male rats is mediated via a specific protein (α-2-microglobin) which is absent in other mammals (Olson et al., 1990, 1987).

1.3 Octane Rating

Octane rating or octane number is a standard measure of the performance of a motor or aviation fuel. The higher the octane number, the more compression the fuel can withstand before detonating. In broad terms, fuels with a higher octane rating are used in high-compression engines that generally have higher performance. In contrast, fuels with low octane numbers (but high cetane numbers) are ideal for diesel engines. Use of gasoline with low octane numbers may lead to the problem of engine knocking (Werner et al., 2007).

1.3.1 Measurement methods

Research Octane Number (RON) is the most common type of octane rating worldwide. RON is determined by running the fuel in a test engine with a variable compression ratio under controlled conditions, and comparing the results with those for mixtures of iso-octane and n-heptane. Motor Octane Number (MON) or the aviation lean octane rating, is a better measure

of how the fuel behaves when under load, as it is determined at 900 rpm engine speed, instead of the 600 rpm for RON (Werner et al., 2007). MON testing uses a similar test engine to that used in RON testing, but with a preheated fuel mixture, higher engine speed, and variable ignition timing to further stress the fuel's knock resistance. Depending on the composition of the fuel, the MON of a modern gasoline will be about 8 to 10 points lower than the RON, however there is no direct link between RON and MON. Normally, fuel specifications require both a minimum RON and a minimum MON

Anti-Knock Index (AKI): In most countries, including Australia and all of those in Europe, the "headline" octane rating shown on the pump is the RON, but in Canada, the United States and some other countries, like Brazil, the headline number is the average of the RON and the MON, called the **Anti-Knock Index** (**AKI**, and often written on pumps as **(R+M)/2**). It may also sometimes be called the **Pump Octane Number** (**PON**), because of the 8 to 10 point difference noted above, the octane rating shown in Canada and the United States is 4 to 5 points lower than the rating shown elsewhere in the world for the same fuel.

Observed Road Octane Number (**RdON**), is derived from testing gasolines in real world multi-cylinder engines, normally at wide open throttle. It was developed in the 1920s and is still reliable today. The original testing was done in cars on the road but as technology developed the testing was moved to chassis dynamometers with environmental controls to improve consistency. The selection of octane ratings available at the pump can vary greatly from region to region, for example in Saudi Arabia: Two types of fuel are available at all gas stations in Saudi Arabia. "Premium 91" (RON 91) where the pumps are coloured green, and "Super Premium 95" (RON 95) where the pumps are coloured red.
In United Kingdom: 'regular' petrol has an octane rating of 95 RON, with 97 RON fuel being widely available as the Super Unleaded. Tesco and Shell both offer 99 RON fuel. In United States: octane rating is displayed in AKI. In the Rocky Mountain (high elevation) states, 85

AKI (90 RON) is the minimum octane, and 91 AKI (95 RON) is the maximum octane available in fuel (Werner et al., 2007).

1.4 Inhalant abuse

Inhalants are broad range of drugs whose volatile vapours are taken in via the nose and trachea. They are taken in by volatalization. While some drugs are used for medical purposes, as in the case of nitrous oxide (a dental anxiolytic), this article focuses on inhalant abuse as recreational drugs that are used for their intoxicating effect. Inhaling volatile substances because of their intoxicating effect is called huffing. Most inhalant drugs that are used non-medically are ingredients of household or industrial chemical products that are not intended to be concentrated or inhaled. Examples include: Hydrocarbons: gasoline, kerosene, butane, Toluene, xylene. Others include: glue, photocopier fluid, aerosol, paint thinner, cleaning and lighter fluids. The recreational use of inhaling hydrocarbons and other volatile solvents for the purposes of creating a euphoric state is becoming increasingly common. Methods used for this abuse, including "sniffing" (directly inhaling vapours), "huffing" (placing a hydrocarbon-saturated rag over the mouth and nose and then inhaling), or "bagging" (inhaling via a plastic bag filled with hydrocarbon vapours) (Barnes, 1985). Petrol sniffing is a major problem in Aboriginal communities across four Australian states. It destroys health and families. It has claimed over 100 Indigenous lives from 1981 to 2003 across Australia. The practice was first observed in 1951, and is believed to have been introduced by US servicemen stationed in the nation's top end during World War II (MacLean, 2007).

Figure 1: Young boys sniffing petrol for recreational purpose
(National Institute on Drug Abuse (NIDA), 1985, 2012)

The national Institute on Drug abuse (NIDA) is an arm of National Institute of Health, U.S. Department of Health and Human services. NIDA has a mission of leading the nation in bringing the power of Science to bear on drug abuse and addiction. There is a Prevention Research Branch (PRB) aimed at giving support to a programme of research on prevention of initiation of drug use, progression to abuse and dependence, and transmission of drug related HIV infections (Robertson et al., 2012).

1.5 Effect of sniffing

Inhalant users inhale vapours or aerosol propellant gases using plastic bags held over the mouth or by breathing from an open container of solvent such as gasoline or paint thinner. Once these solvents or gases are inhaled, the extensive capillary surface of the lungs rapidly absorb the solvent or gas and blood levels peak rapidly. The intoxication effects occur so quickly that the effects can resemble the intensity of effects produced by intravenous injection of other psychoactive drugs (Joseph, 2005).

The effects can vary widely depending on dose and what type of gas or solvent. In short term, many users experience headache, nausea and vomiting, slurred speech, loss of motor

coordination and wheezing. Inhalants are a large class of drugs and therefore exhibit a variety of mechanism of action. Anaesthetic gases used for surgery, such as nitrous oxide or enflurane act as NMDA receptor antagonist, open channel blockers that bind to the inside of the calcium channels on the outer surface of the neuron, and provide high levels of NMDA receptor blockers for a short period of time. The normal NMDA antagonist such as ketamine, binds to a regulatory site on NMDA sensitive calcium transporter complex and provide slightly lower levels of NMDA blockade, but for a longer and more predictable duration. One of the major risks is hypoxia which can occur due to inhaling fumes from a plastic bag or from using proper equipment but not adding oxygen or room air. The hypoxic effect of inhalants can cause damage to many organ systems (particularly the brain, which has very low tolerance for oxygen deprivation. Solvents have many potential risks in common, including pneumonia, cardiac failure or arrest and aspiration of vomitus (Weimann, 2003). Brain damage is typically seen with chronic long term use as opposed to short-term exposure. Toxicity may result from pharmacological properties of the drug. Excess NMDA antagonism can completely block calcium influx into neurons and provoke cell death through apoptosis, although this is more likely to be a long term result of chronic solvent abuse than a consequence of short term use (Koblin, 1990; Yu et al., 2002; Filley et al., 2004).

In Nigeria, crude oil is predominantly found in the riverine areas and over the years the local population have used crude oil for various ailment such as gastrointestinal disorders, burns, foot-rot, leg ulcer, etc. (Orisakwe et al., 2009). However, diesel, kerosene and petrol are used by people all over the country. Petrol sniffing was also found being practiced in Ilorin and Abuja by young sports men (personal communication).

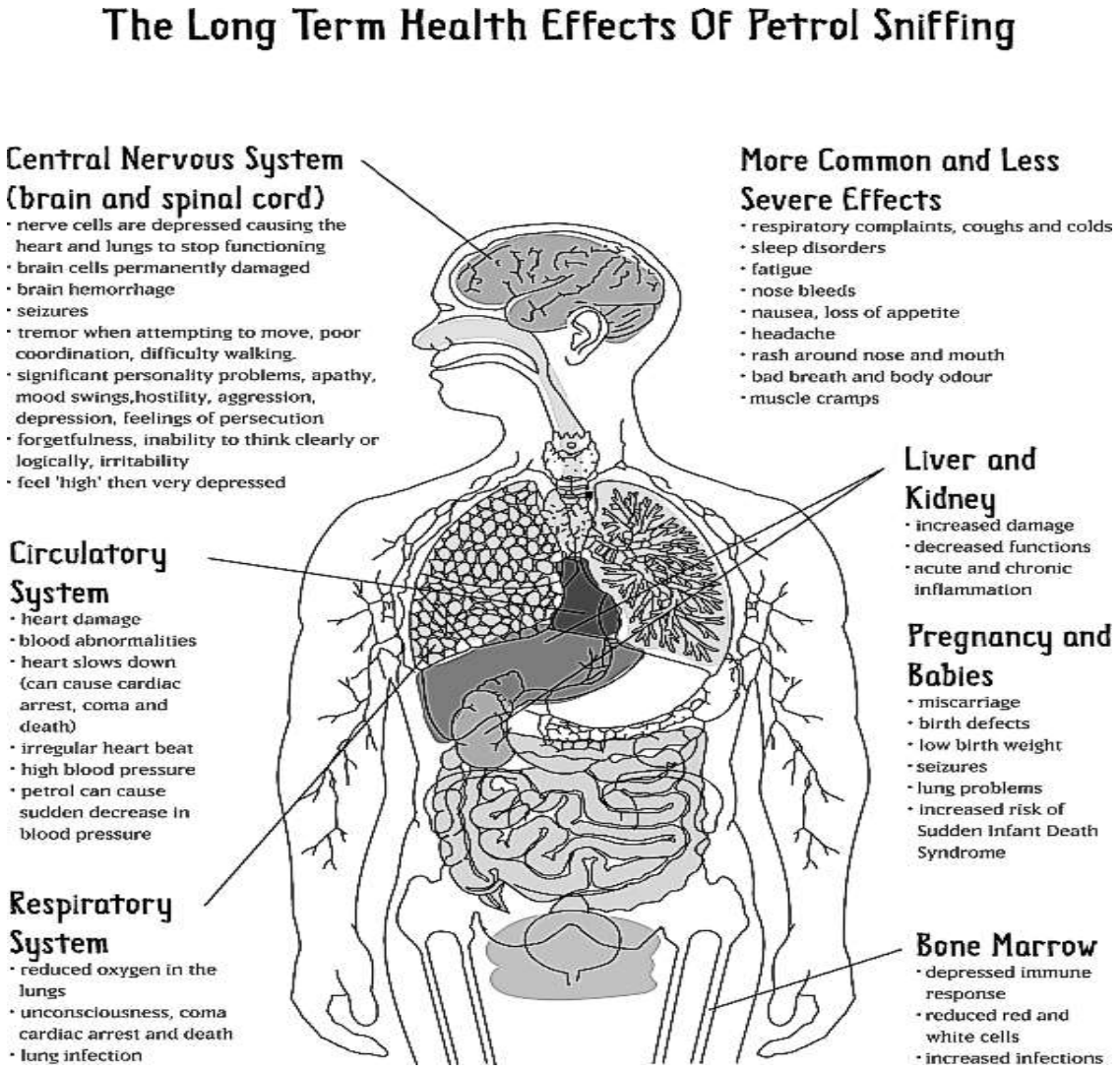


Figure 2: Effect of petrol sniffing on body system (National Institute on Drug Abuse (NIDA), 1985).

The principal adverse effect arising from ingestion of petrol and kerosene is chemical pneumonitis secondary to aspiration of vomitus. Ingestion of petrol and kerosene or acute exposure to vapour may lead to general signs of intoxication such as mild central nervous system (CNS) symptoms (dizziness, headache, nausea) and vomiting. Skin exposure to kerosene may result in dermatitis through the extraction of endogenous skin lipids. While kerosene is not considered a direct-acting dermal carcinogen, chronic skin exposure may result in tumourigenesis (National Institute on Drug Abuse, 1985).

1.5.1 Absorption, Distribution, Metabolism, and Excretion

Very limited data indicate that petrol and kerosene are poorly absorbed from the gastrointestinal tract and is distributed to various tissues, although accumulation is low. Another study in humans suggests that respiratory toxicity may occur as a result of aspiration from vomiting and gastrointestinal absorption (Mattorano et al., 2004). However, aspiration is the primary concern following ingestion. There is also some suggestion from case studies that renal toxicity may occur in humans following exposure to diesel fuel vapour. Renal toxicity may occur following dermal contact with diesel fuel. No data were located regarding the metabolism or excretion of fuel oils following any of the three routes of exposure. Acute, intermediate, and chronic data are needed to assess the relative rates and extent of absorption, distribution, and excretion of fuel oils with respect to all three routes of exposure, as well as with respect to time or dose. Also, data are needed to determine whether dermal absorption of diesel fuel vapour can occur to induce renal toxicity. (Barrientos, 1977; Reidenberg et al., 1964).

1.6 Diesel Particulate Matter (Dpm)

Figure 3: Diesel exhaust from a diesel engine (National Institute on Drug Abuse (NIDA),

1985)

Diesel fuel and the products of its combustion represent one of the toxins most commonly encountered by people living in both urban and rural areas of the world. As nations become more heavily populated, there will be increasing reliance on diesel fuel to power mass transportation and commercial vehicles, as well as heavy machinery involved in construction, farming, and mining (Irina et al 2008).

Diesel exhaust (known as clag when emitted by diesel locomotives or diesel engine emissions in scientific papers) is the exhaust gas of a diesel engine. Diesel exhaust has been found to contain many toxic air contaminants. Diesel engine emits a complex mixture of air pollutants, composed of gaseous and solid material. The visible emission in diesel exhaust is known as particulate matter (DPM) which includes carbon particle or "soot". Diesel exhaust also contains a variety of harmful gases and over 40 other known cancer-causing substances. It is a carcinogen which causes lung cancer and associated with bladder cancer.

Diesel exhaust pollution accounts for over one quarter of the total hazardous pollution in the air and a disproportionately high share of the load of sickness and death caused by pollution (Attfield et al. 2012). Exposure to diesel exhaust and diesel particulate matter (DPM) is a known occupational hazard to truckers, railroad workers and miners using diesel powered equipment in underground mines. Adverse health effects have also been observed in the general population at ambient atmospheric particle concentrations. In March 2012, U.S. government scientist showed that underground miners exposed to high level of diesel fumes have threefold increased risk for contracting lung cancer compared with those exposed to low levels. Average life expectancy was reduced by about 1.5 years, comparing the cities with highest and lowest high DPM levels. This translates to a loss of about 14 years of life for people who died from disease associated with DPM exposure (U.S. EPA, 2009). Exposure have been linked with acute short term symptoms such as headaches, dizziness, light headedness, nausea coughing, difficult or laboured breathing, tightness of chest and irritation

of the eyes, nose and throat. Long term exposure can lead to chronic, more serious health problems such as cardiovascular disease, cardiopulmonary disease and lung cancer. Ambient traffic related air pollution was associated with decreased cognitive function in older men (Coble and Stewart, 2010; IARC/WHO, 2012).

Several recent research publications have added to concerns regarding adverse health effect from exposure to diesel exhaust. First, a study of rail road worker employed between 1959 and 1996 found that lung cancer mortality was elevated in jobs associated with work on trains powered by diesel locomotives, suggesting that diesel exhaust contributed to lung cancer mortality in this study group. However, lung cancer mortality did not increase with increasing years of work in these jobs (Garshick et al., 2003, 2004a &b).

A study investigated transient exposures to diesel exhaust and their effects on cardiovascular function, linking traffic- related pollution to cardiovascular effects such as acute myocardial infarction (heart attacks) (Mills et al. 2005).

Thirty healthy men were exposed to diluted diesel mechanist in exposure chambers. They found that inhalation of diesel exhaust at the levels found in urban environments impaired two important aspects of vascular function in humans i.e. the regulation of vascular tone and endogenous fibrinolysis. This finding provides a potential mechanism that links air pollution to heart diseases including heart attacks (Mills et al. 2005).

Another research conducted on the emissions from heavy duty diesel engines have shown marked disastrous effects of petroleum products on human being in one set of studies, toxic pollutant emissions were measured from an in use 1998 model year diesel transit bus equipped with either an oxidation muffler or a catalysed particulate filter found similar result as above (DPM). In addition to the health effects outlined above, it is estimated that exposure to diesel DPM have carcinogenic effects. For example IARC (1989) concluded that diesel engine exhaust is a probable human carcinogen, and based on these IARC findings, the state of California under the safe drinking water and toxic enforcement of 1986 (proposition 65)

identified diesel exhaust as a chemical known to cause cancer. Diesel vehicles constitute only about 5% of road vehicles; however, they could contribute from 10% to 75% of visibility degradation in urban areas. Apart from the carcinogenic effects of diesel at its visibility reduction ability, it is also a potent global warming agent. Diesel engines emit soot, or black carbon particles which then become airborne. Diesel is responsible for more than half of black carbon emissions in the United States. DPM also affects the cardiovascular system and the pulmonary system. DPM is also a contributing factor to hospital admissions and emergency room visits for cardiopulmonary cases such as asthma (Schwartz and Zanobetti 2003; Zanobetti et al, 2003). By age 18, children exposed to higher level of DPM 2.5, and elemental carbon (all products of fossil fuel) combustion, especially diesel) are five times more likely to have underdeveloped lungs compared to teenagers living in communities with lower pollutants levels and will likely never recover (Gauderman et al. 2004).

Evidently, diesel poses an enormous threat on our health. Chief amongst these are the acute lower respiratory infections (ALRI) in childhood, particularly pneumonia. Exposure to diesel is also associated with chronic bronchitis and chronic obstructive pulmonary disease. All these adverse effects and the predisposing factors have remained a major concern to physiologist over the years (Gauderman et al., 2004; Kenneth et al., 2006).

DPM deposits in the lung and components can be absorbed in the body, the general, particles 10μm or less in diameter can be inhaled into the lungs (U.S. EPA, 2004). Not all inhaled particles deposit in the lung, and many are exhaled. Particles about 0.5μm in diameter are minimally deposited in the airways, with higher deposition rates for particles can dissolve in the fluid lining the airways, and then be absorbed into the body. Insoluble particles are cleared by more complex mechanisms.

Diesel PM contains toxic chemicals including compounds that are known to cause damage to genetic material (DNA) and are considered to cause cancer. For example, one

class of compound typically present on diesel PM is polycyclic aromatic hydrocarbons, or PAH. Some PAHs have been classified as probable human carcinogens by the U.S. EPA and by the international agency for research on cancer (IARC, 1989), a World Health Organization group. These compounds have also been shown to damage DNA and also be absorbed into the bloodstream after diesel PM exposure, and are therefore considered to be available to damage cells in tissues such as the lung (U.S. EPA, 2002). Benzene, the first toxic air contaminant listed by the state, and a known human cancer causing agent for leukemia, has been reported not only in the gaseous phase of diesel exhaust, but also is present on diesel PM itself (U.S. EPA, 2002). Other cancer causing compound such as formaldehyde, acetaldehyde, acrolein, and 1, 3 butadiene are present in diesel exhaust (IARC, 1989; USEPA, 2002) primarily in the gas phase. Diesel exhaust is also considered to pose a reparatory hazard to humans based on extensive studies. A few epidemiologic studies have investigated the responses of human subjects specifically exposed to diesel PM and the diesel PM content of the outdoor pollution mix. However, the extensive animal toxicology, literature on the health impacts of diesel exhaust PM leads to the conclusion that diesel exhaust PM is at least as toxic as the general ambient PM mixture. ARB has made quantitative estimates of the public health impacts of diesel exhaust PM based on this equivalency assumption. We estimate that current state-wide levels of diesel PM contribute to 3, 500 deaths (range: 1,000 to 6, 400) annually (CARB, 2008). Additional health impacts can result from exposure to secondary diesel PM that is formed in the atmosphere from oxides of nitrogen, emitted from diesel engine exhaust.

Specific studies that link motor vehicle-related PM exposure to premature death include:

- Elderly people living near major roads had almost twice the risk of dying from cardiopulmonary causes.
- PM from motor vehicles was linked to increased mortality.

- Fine PM (PM2.5) from mobile sources accounted for three the mortality as did PM 2.5 from coal combustion sources.

Diesel PM is also a contributing factor to hospital admissions and emergency room visits for cardiopulmonary diseases (Pope, 1989; Schwartz et al. 2003; Sheppard, 2003; Zanobetti and Schwartz, 2003). We estimate thousands of hospital admissions for cardiopulmonary causes, emergency room visits, asthma attacks, and millions of lost work days each year in California due to PM (CARB, 2008). At least 10% of these impacts (see below) are related to diesel PM. In addition, preliminary evidence suggests that diesel PM exposure may facilitate development of new allergies (Diaz- Sanchez et al., 1999; Kleinman et al. 2005). By age 18, children exposed to higher levels of PM 2.5, Nox, acid vapour, and elemental carbon (all products of fossil fuel combustion, especially diesel) are five times more likely (7.9% versus 1.6%) to have underdeveloped lungs (80% of normal, equivalent to 40-years olds) compared to teenagers living in communities with lower pollutant levels, and will likely never recover (Gauderman et al. 2004).

In addition, several "intervention" studies report significant reduction in the number of adverse health impacts following either removal or reduction of a PM emission source. For example, the Southern California Children's Health Study reported improved lung function growth rates for young children who relocated from a high PM area to a lower PM area (Avol et al., 2001).

The Niger- Delta area of Nigeria is rich in petroleum resources, and the exploitation of these resources has resulted in the discharge of petroleum hydrocarbon into the fresh water environment. This exposes the fauna and flora of the associated ecosystem to the toxic effects of crude petroleum (Wogu and Okaka, 2011).

Petroleum based chemicals can cause serious health problems overtime. Research showed that this can occur from exposure in the workplace, home and also from many consumer

products. Due to the time delay in exposure and the onset of symptoms, as well as complexity of processes involved, these illness are often overloaded, diagnosed wrongly or denied (by physicians representing the chemical or insurance industry).

1.7 Populations that are Unusually Susceptible

In California diesel particulate matter (PM) contributes to an estimated 3,500 premature deaths each year as well as thousands of hospital admissions, asthma attacks and other respiratory symptoms, and lost workdays. Many diesel emission sources such as heavily traveled roadsports, and rail yards are concentrated near densely populated areas, which leads to higher exposures and greater health consequences for our children [(California Air Resource Board (CARB, 2008)].

A susceptible population will exhibit a different or enhanced response to fuel oils than will most persons exposed to the same level of fuel oils in the environment. Reasons include genetic make-up, developmental stage, health and nutritional status, and chemical exposure history. These parameters result in decreased function of the detoxification and excretory processes (mainly hepatic and renal) or the pre-existing compromised function of target organs. For these reasons we expect the elderly with declining organ function and the youngest of the population with immature and developing organs will generally be more vulnerable to toxic substances than healthy adults.

No information was located regarding the toxicity of fuel oils in susceptible populations. The human data, in general, were based upon case studies that reported ingestion of kerosene by children. Although children were not shown to be particularly susceptible to kerosene in these studies, it was obvious that children are more likely to be exposed to kerosene accidentally than adults. In particular, children that are 5 years old or younger often mistakenly drink kerosene because it was accessible to them. In one animal study, it was found that younger rats are more susceptible to kerosene toxicity than are older rats. A single

oral dose of 400 mg/kg kerosene killed 27% of the adult rats, 66% of the 5-week -old rats and 100% of the 10-day-old rats (Deichmann et al., 1944). It is not known whether kerosene would also be more toxic in younger humans as compared to older humans.

1.8 Statement of Problem

Interest of scientists in the adverse effects of petroleum products has grown in recent years, and researchers have focused on the deleterious effects of these products on various systems in the body. Inhalant abuse, the deliberate inhalation of hydrocarbons as a form of recreational drug use, has become a significant health issue affecting children and adolescents (Steffe et al., 1996). Their low cost, ready availability and ease of use contribute to this problem. Inhalation is most commonly achieved by sniffing, huffing or bagging (McHugh, 1987). Epidemiologic data state that, inhalants are the second most widely used class of illicit drugs. Petrol sniffing is a major problem in Aboriginal communities across four Australian states. It destroys health and families. In Nigeria exposure to Petroleum products is on the increase as a consequence of leakage which may result from vandalization, erosion, construction, urbanisation etc. Occupational exposure as well as prolonged scarcity (Akintonwa et al., 2005; Wardley-Smith, 1983; Jackson et al., 1989).

Sudden death from inhalation of petroleum distillates is well recognized in misusers of volatile substances (Steffe et al., 1996; Chalmers, 1991). Over the years, there have been various concerns about both acute exposure as a result of the daily use of petroleum products and the chronic exposure by drinking contaminated water especially from petroleum refineries. People are not taking cognisance of the daily and continuous exposure to petrol and diesel fumes from generators in almost all households and working places in Nigeria as a result of continuous need for power generation.

Evidently, researchers have shown that petroleum products cause tachycardia, dysrhythmias and hypertension and damage to the brain. However, the mechanism of action on human body has not been ascertained. The quest for a lasting solution to the toxic effect of petroleum products through the knowledge of its actions on the body is the notating factor for this research work. Although some work has been done on effect of petroleum product on various body systems, the effect on cardiovascular functions is still poorly understood (Joseph, 2005).

1.9 Aim of the Study

The general aim is to investigate the effect of petroleum products on cardiovascular functions using three different routes of administration and the possible involvement of oxidative stress in the mechanism of action. This is to produce useful animal model to mimic cardiovascular events in patients following exposure to petroleum products.

1.10 Objectives

1. To measure blood pressure and heart rate, after exposure to petroleum product via three different routes of administration
2. To evaluate the baroreflex responses using method of bilateral carotid occlusion
3. To analyze the haematological indices, liver enzymes and biochemical properties in serum and urine
4. To evaluate the antioxidant activities and lipid peroxidation in serum and homogenate of vital organs (heart, liver, brain, kidney and lungs)
5. To assess the direct effects of petroleum products on vital organs through histological analysis of the vital organs (kidney, heart, brain, liver and lung) at the end of exposure to diesel, kerosene and petrol.

1.11 Scope of The Study

This study was carried out using the rat model, randomly selected from the Animal House of Ladoke Akintola University of Technology. The experimental procedure took place at the Animal House, College of Medicine University of Lagos. At the end of the experiment the Grass polygraph (model 7D and pressure transducer model 7PIF was used to measure the blood pressure and heart rate. Other analyses carried out included: haematological, electrolyte, biochemical and antioxidant studies as well as histology of some visceral organs were carried at various corresponding laboratory within University of Lagos.

1.12 Significance of The Study

This research work will enlighten the populace on how to handle the petroleum products so as to reduce the mortality rate caused by petroleum products due to human daily exposure to these products without taking cognisance of the effect. Imbalance in the production of reactive oxygen species and ability to correct or remove the intermediate from the system.

1.13 List of Abbreviations and Acronyms

DFG: Diesel food ingestion group -rats in this group were given 1000g feed mixed with 15 ml diesel.

DIH: Diesel inhalation group- rats in this group exposed to inhalation of diesel in the fume chamber for 5 minutes as the case may be

DWC: Diesel water contaminated group-rats in this group were given water mixed with diesel at the rate of 2.5ml/kg body weight. Mixing was done by shaking at ratio 1:1

KFG: Kerosene food ingestion group -rats in this group were given 1000g feed mixed with 15ml kerosene.

KIH: Kerosene inhalation group- rats in this group exposed to inhalation of kerosene in the fume chamber for 5 minutes as the case may be

KWC: Kerosene water contaminated group-rats in this group were given water mixed with kerosene at the rate of 2.5ml/kg body weight. Mixing was done by shaking at ratio 1:1

PFG: Petrol food ingestion group -rats in this group were given 1000g feed mixed with 15ml petrol.

PIH: Petrol inhalation group- rats in this group exposed to inhalation of petrol in the fume chamber for 5 minutes as the case may be

PWC: Petrol water contaminated group-rats in this group were given water mixed with petrol at the rate of 2.5ml/kg body weight. Mixing was done by shaking at ratio 1:1

SBP: (Systolic blood pressure) is the maximum pressure exerted by circulating blood upon the walls of blood vessels

DBP: (Diastolic blood pressure) is the minimum pressure exerted by circulating blood upon the walls of blood vessels. Increased levels of both the systolic and diastolic pressure are indicators of concern for cardiovascular problems.

MAP: (Mean arterial blood pressure), it can be approximately determined from measurements of the systolic pressure P_{syst} and the diastolic pressure P_{dias} while there is a normal resting heart rate, (Klabunde, (2007). $MAP = P_{dias} + 1/3 (P_{sys} - P_{dias})$

RBC: (Red blood count) is the number of red blood cells per volume of blood, and is reported in either millions in a microliter or millions in cubic millimeter. It is a blood test that tells how many red blood cells (RBCs) an individual has.

WBC: White blood count or leukocytes count, is the number of white blood cells per volume of blood, and is reported in either thousands in a microliter or millions in cubic millimeter. A high white blood cell count could mean that there is inflammation somewhere in the body.

Hb: Haemoglobin, abbreviated as Hb or Hgb) is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates. Haemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body (i.e. the tissues) where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism, and collects the resultant carbon dioxide to bring it back to the respiratory organs to be dispensed from the organism. In mammals, the protein makes up about 97% of the red blood cells' dry content, and around 35% of the total content

PCV: (packed cell volume) is the volume percentage (%) of red blood cells in blood. It is normally about 45% for men, 40% for women and 37.6-50.6% for rats.

PLT: (Platelets, or **thrombocytes)** are small, irregularly shaped clear cell fragments (i.e. cells that do not have a nucleus, and 2–3 μm in diameter. The principal function of platelets is to prevent bleeding.

CAT: **Catalases** is a common enzyme found in nearly all living organisms exposed to oxygen. It is produced by aerobic organisms ranging from bacteria to man. Catalase

is haem-containing proteins that catalyse the conversion of hydrogen peroxide (H_2O_2) to water and molecular oxygen, thereby protecting cells from the toxic effects of hydrogen peroxide

SOD: Superoxide dismutase is an enzyme that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen. It is found in both the dermis and the epidermis, and is key to the production of healthy fibroblasts (skin-building cells).

GSH: **Glutathione** is a tripeptide of glutamic acid, cysteine, and glycine, existing in reduced (GSH) and oxidized (GSSG) forms and functioning in various redox reactions: in the destruction of peroxides and free radicals, as a cofactor for enzymes, and in the detoxification of harmful compounds.

MDA: **Malondialdehyde** is the organic compound with the formula $CH_2(CHO)_2$. It is one of the most frequently used indicators of lipid peroxidation. It is generated from reactive oxygen specie (ROS), and as such is assayed in vivo as a bio-marker of oxidative stress.

ROS: **Reactive oxygen species** are chemically reactive molecules that contains oxygen.

1.14 Operational Definition of Terms

The **blood–brain barrier** (**BBB**) is a separation of circulating blood from the brain extracellular fluid (BECF) in the central nervous system (CNS). It occurs along all capillaries and consists of tight junctions around the capillaries that do not exist in normal circulation

Diesel particulate matter is part of a complex mixture that makes up diesel exhaust. Diesel exhaust is commonly found throughout the environment and is estimated by EPA's National Scale Assessment to contribute to the human health risk. Diesel exhaust is composed of two phases, either gas or particle and both phases contribute to the risk. The gas phase is composed of many of the urban hazardous air pollutants, such as acetaldehyde, acrolein, benzene, 1,3-butadiene, formaldehyde and polycyclic aromatic hydrocarbons. The particle phase also has many different types of particles that can be classified by size or composition. The size of diesel particulates that are of greatest health concern are those that are in the categories of fine, and ultrafine particles.

Anaemia: is a decrease in number of red blood cells (RBCs) or less than the normal quantity of haemoglobin in the blood. However, it can include decreased oxygen-binding ability of each haemoglobin molecule due to deformity or lack in numerical development as in some other types of haemoglobin deficiency.

CHAPTER TWO

2.0 LITERATURE REVIEW

Cardiovascular disease remains the leading cause of death in the world. It was estimated that 61,800,000 Americans have cardiovascular disease, which can include high blood pressure, coronary heart disease (heart attack and chest pain), stroke, birth defects of the heart and blood vessels, and congestive heart failure. Cardiovascular diseases kill more people than the next seven causes combined–including cancer. Cardiovascular disease is a class of diseases that involve the heart or blood vessels (arteries, capillaries and veins) (Maton, 1993). It refers to any disease that affects the cardiovascular system, principally cardiac disease, vascular diseases of the brain and kidney, and peripheral arterial disease (Bridget, 2010). The causes of cardiovascular disease are diverse but atherosclerosis and/or hypertension are the most common. Additionally, with aging come a number of physiological and morphological changes that alter cardiovascular function and lead to subsequently increased risk of cardiovascular disease, even in healthy asymptomatic individuals (Dantas et al., 2012).

Cardiovascular diseases remain the biggest cause of deaths worldwide, though over the last two decades, cardiovascular mortality rates have declined in many high-income countries. At the same time, cardiovascular deaths and disease have increased at a fast rate in low- and middle-income countries. Although cardiovascular disease usually affects older adults, the antecedents of cardiovascular disease, notably atherosclerosis, begin in early life, making primary prevention efforts necessary from childhood (McGill et al., 2008). There is therefore increased emphasis on preventing atherosclerosis by modifying risk factors, such as healthy eating, exercise, and avoidance of smoking (Mendis et al., 2011).

2.1 HUMAN CARDIOVASCULAR SYSTEM

The circulatory system is an organ system that passes nutrients (such as amino acids, electrolytes and lymph), gases, hormones, blood cells, etc. to and from cells in the body to help fight diseases, stabilize body temperature and pH, and to maintain homeostasis.

This system may be seen strictly as a blood distribution network, but some consider the circulatory system as composed of the cardiovascular system, which distributes blood, and the lymphatic system, which returns excess filtered blood plasma from the interstitial fluid (between cells) as lymph. While humans, as well as other vertebrates, have a closed cardiovascular system (meaning that the blood never leaves the network of arteries, veins and capillaries), some invertebrate groups have an open cardiovascular system. The most primitive animal phyla lack circulatory systems. The lymphatic system, on the other hand, is an open system providing an accessory route for excess interstitial fluid to get returned to the blood (Dwivedi and Dwivedi, 2007).

2.1.1 Pulmonary circulation

The pulmonary circulatory system is the portion of the cardiovascular system in which oxygen-depleted blood is pumped away from the heart, via the pulmonary artery, to the lungs and returned, oxygenated, to the heart via the pulmonary vein.

Oxygen deprived blood from the vena cava, enters the right atrium of the heart and flows through the tricuspid valve (right atrioventricular valve) into the right ventricle, from which it is then pumped through the pulmonary semilunar valve into the pulmonary artery to the lungs. Gas exchange occurs in the lungs, whereby CO_2 is released from the blood, and

oxygen is absorbed. The pulmonary vein returns the now oxygen-rich blood to the heart (West, 2008).

2.1.2 Systemic circulation

Systemic circulation is the circulation of the blood to all parts of the body except the lungs. Systemic circulation is the portion of the cardiovascular system which transports oxygenated blood away from the heart through the Aorta from the left ventricle where the blood has been previously deposited from pulmonary circulation, to the rest of the body, and returns oxygen-depleted blood back to the heart. Systemic circulation is, distance-wise, much longer than pulmonary circulation, transporting blood to every part of the body (West, 2008).

2.1.3 Coronary circulation

The coronary circulatory system provides a blood supply to the muscles of the heart. As it carries oxygenated blood to muscles, it is by definition a part of the systemic circulatory system. It arises from the aorta and drains through the coronary sinus into the right atrium. Back flow of blood through its opening during atrial systole is prevented by the Thebesian valve. The smallest cardiac veins drain directly into chambers of the heart (West, 2008).

2.1.4 The Heart

The heart pumps oxygenated blood to the body and deoxygenated blood to the lungs. In the human heart there is one atrium and one ventricle for each circulation, and with both a systemic and a pulmonary circulation there are four chambers in total: left atrium, left ventricle, right atrium and right ventricle. The right atrium is the upper chamber of the right side of the heart (Fig.3). The blood that is returned to the right atrium is deoxygenated (poor in oxygen) and passed into the right ventricle to be pumped through the pulmonary artery to

the lungs for re-oxygenation and removal of carbon dioxide. The left atrium receives newly oxygenated blood from the lungs as well as the pulmonary vein which is passed into the strong left ventricle to be pumped through the aorta to the different organs of the body (West, 2008).

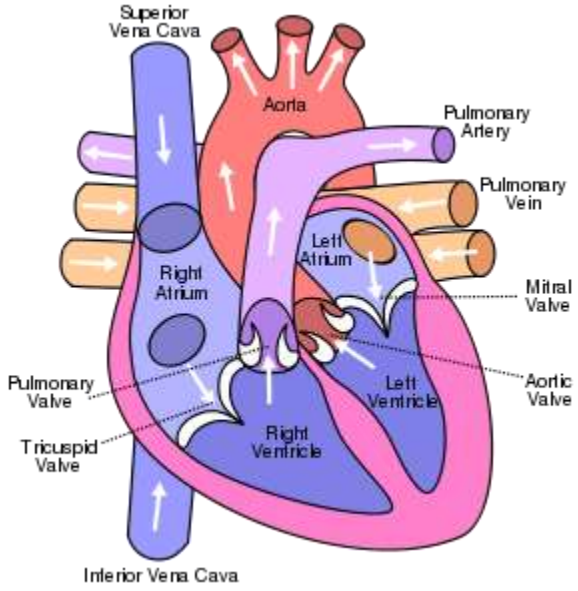


Figure 4: Longitudinal section of the heart showing the atrium, ventricles and the major vessels

2.2 CARDIOVASCULAR FITNESS

This is the ability of the heart and lungs to supply oxygen-rich blood to the working muscle tissues and the ability of the muscles to use oxygen to produce energy for movement. This type of fitness is a health-related component of physical fitness that is brought about by sustained physical activity. A person's ability to deliver oxygen to the working muscles is affected by many physiological parameters, including heart rate, stroke volume, cardiac output, and maximal oxygen consumption.

Understanding the relationship between cardiorespiratory endurance training and other categories of conditioning requires a review of changes that occur with increased aerobic, or

anaerobic capacity. As aerobic/anaerobic capacity increases, general metabolism rises, muscle metabolism is enhanced, haemoglobin rises, buffers in the bloodstream increase, venous return is improved stroke volume is improved, and the blood bed becomes more able to adapt readily to varying demands. Each of these results of cardiovascular fitness/cardiorespiratory conditioning will have a direct positive effect on muscular endurance, and an indirect effect on strength and flexibility (Moran, 2010).

2.3 CARDIAC OUTPUT (Q)

Cardiac output is the volume of blood pumped by the heart per minute (ml/min). Cardiac output is a function of heart rate and stroke volume. The heart rate is simply the number of heart beats per minute. The **stroke volume** is the volume of blood, in milliliters (ml), pumped out of the heart with each beat. Increasing either heart rate or stroke volume increases cardiac output. Cardiac Output in ml/min = heart rate (beats/min) X stroke volume (ml/beat) An average person has a resting heart rate of 70 beats/minute and a resting stroke volume of 70 ml/beat. The cardiac output for this person at rest is: Cardiac Output = 70 (beats/min) X 70 (ml/beat) = 4900 ml/minute (Guyton et al., 2006).

2.3.1 Clinical uses of cardiac output

Q is regulated principally by the demand for oxygen by the cells of the body. If the cells are working hard, with a high metabolic oxygen demand then the Q is raised to increase the supply of oxygen to the cells, while at rest when the cellular demand is low, Q is said to be at baseline. Q is regulated not only by the heart as it pumps, but also by the function of the vessels of the body as they actively relax and contract thereby increasing and decreasing the resistance to flow. When Q increases in a healthy but untrained individual, most of the increase can be attributed to an increase in heart rate (HR). Change of posture, increased

sympathetic nervous system activity, and decreased parasympathetic nervous system activity can also increase cardiac output (Levy et al., 1997; Rowell and Loring, 1993; Braunwald, 1997).

A parameter related to stroke volume (SV) is Ejection Fraction (EF). EF is the fraction of blood ejected by the Left Ventricle (LV) during the contraction or ejection phase of the cardiac cycle or Systole. Prior to the start of Systole, the LV is filled with blood to the capacity known as End Diastolic Volume (EDV) during the filling phase or diastole. During Systole, the LV contracts and ejects blood until it reaches its minimum capacity known as End Systolic Volume (ESV), it does not empty completely. Clearly the EF is dependent on the ventricular EDV which may vary with ventricular disease associated with ventricular dilatation. Even with LV dilatation and impaired contraction the Q may remain constant due to an increase in EDV.

Stroke Volume (SV) = EDV – ESV

Ejection Fraction (EF) = (SV / EDV) × 100%

Cardiac Output (Q) = SV × HR

Cardiac Index (CI) = Q / Body Surface Area (BSA) = SV × HR/BSA

HR is Heart Rate, expressed as BPM (Beats per Minute)

BSA is Body Surface Area in square metres.

Diseases of the cardiovascular system are often associated with changes in Q, particularly the pandemic diseases of hypertension and heart failure. Cardiovascular disease can be associated with increased Q as occurs during infection and sepsis, or decreased Q, as in cardiomyopathy and heart failure. The ability to accurately measure Q is important in clinical medicine as it

provides for improved diagnosis of abnormalities, and can be used to guide appropriate management. Q measurement, if it were accurate and non-invasive, would be adopted as part of every clinical examination from general observations to the intensive care ward, and would be as common as simple blood pressure measurements are now.

2.3.2 Measuring cardiac output

There are a number of clinical methods for measurement of Q ranging from direct intra-cardiac catheterisation to non-invasive measurement of the arterial pulse. Each method has unique strengths and weaknesses and relative comparison is limited by the absence of a widely accepted "gold standard" measurement. Q can also be affected significantly by the phase of respiration; intra-thoracic pressure changes influence diastolic filling and therefore cardiac output. This is especially important during mechanical ventilation where Q can vary by up to 50% across a single respiratory cycle. Q should therefore be measured at evenly spaced points over a single cycle or averaged over several cycles. Invasive methods are well accepted, but there is increasing evidence that these methods are neither accurate nor effective in guiding therapy, so there is an increasing focus on development of non-invasive methods (Binanay et al., 2005; Pasche et al., 2005).

2.3.3 The Fick principle

The Fick principle was first described by Adolf Eugen Fick in 1870 and assumes that the rate at which oxygen is consumed is a function of the rate of blood flow and the rate of oxygen picked up by the red blood cells. The Fick principle involves calculating the oxygen consumed over a given period of time from measurement of the oxygen concentration of the venous blood and the arterial blood. Q can be calculated from these measurements:

- V_{O2} consumption per minute using a spirometer (with the subject re-breathing air) and a CO_2 absorber
- The oxygen content of blood taken from the pulmonary artery (representing mixed venous blood)
- The oxygen content of blood from a cannula in a peripheral artery (representing arterial blood)

From these values, we know that: $V_{O2} = (Q \times C_A) - (Q \times C_V)$

Where C_A = Oxygen content of arterial blood

- C_V = Oxygen content of venous blood. This allows us to say

$Q = (VO_2/[C_A - C_V])*100$

and therefore calculate Q. While considered to be the most accurate method for Q measurement, Fick is invasive, requires time for the sample analysis, and accurate oxygen consumption samples are difficult to acquire. There have also been modifications to the Fick method where respiratory oxygen content is measured as part of a closed system and the consumed Oxygen calculated using an assumed oxygen consumption index which is then used to calculate Q. Other modifications use inert gas as tracers and measure the change in inspired and expired gas concentrations to calculate Q (Innacor, Innovision A/S, Denmark).

Additionally, the calculation of the arterial and venous oxygen content of the blood is a straightforward process. Almost all oxygen in the blood is bound to haemoglobin molecules in the red blood cells. Measuring the content of haemoglobin in the blood and the percentage of saturation of haemoglobin (the oxygen saturation of the blood) is a simple process and is readily available to physicians. Using the fact that each gram of haemoglobin can carry 1.34

ml of O_2, the oxygen content of the blood (either arterial or venous) can be estimated by the following formula:

Oxygen content of blood= (haemoglobin)(g/dl) ×1.34 (ml O_2/g of haemoglobin) × saturation of blood (percent)+ 0.0032 × partial pressure of oxygen(torr)

2.3.4 Dilution methods

This method was initially described using an indicator dye and assumes that the rate at which the indicator is diluted reflects the *Q*. The method measures the concentration of a dye at different points in the circulation, usually from an intravenous injection and then at a downstream sampling site, usually in a systemic artery. More specifically, the Q is equal to the quantity of indicator dye injected divided by the area under the dilution curve measured downstream (Hall, 2005).

$$Cardiac\ output = \frac{Quantity\ of\ Indicator}{\int_0^\infty Concentration\ of\ Indicator \cdot dt}$$

The trapezoid rule is often used as an approximation of this integral.

2.3.5 Ultrasound dilution method

Ultrasound Dilution method was firstly introduced in 1995 by Krivitski, and it was used extensively to measure flow and volumes with extracorporeal circuits condition CO status™ technology is based on ultrasound indicator dilution. Blood ultrasound velocity (1560–1585 m/s) is a function of total blood protein concentration (sums of proteins in plasma and in red blood red cells), temperature etc. Injection of body temperature normal saline (ultrasound velocity of saline is 1533m/sec) into a unique AV loop decreases blood ultrasound velocity, and produce dilution curves. CO status establishes an extracorporeal circulation through its

unique AV loop with two pre-existing arterial and central venous lines in ICU patients. When the saline indicator is injected into the A-V loop, it is detected by the venous clamp-on sensor on the AV loop before it enters the patient's right heart atrium. After the indicator traverses the heart and lung, the concentration curve in the arterial line is recorded and displayed on the CO status HCM101 Monitor. Cardiac output is calculated from the area of the concentration curve by the classic Stewart-Hamilton equation. It is a non-invasive procedure only by connection the AV loop and two lines of a patient. There lacks general methods to measure cardiac output in paediatric ICU patients, CO status has been demonstrated to be a safe and reproducible too (Krivitski et al., 2008).

2.3.6 Cardiac output and respiration

Q is affected by the phase of respiration with intra-thoracic pressure changes influencing diastolic heart filling and therefore Q. Breathing in reduces intra-thoracic pressure, filling the heart and increasing Q, while breathing out increases intra-thoracic pressure, reduces heart filing and Q. This respiratory response is called stroke volume variation and can be used as an indicator of cardiovascular health and disease. These respiratory changes are important, particularly during mechanical ventilation, and Q should therefore be measured at a defined phase of the respiratory cycle, usually end-expiration.

2.3.7 Combined cardiac output

Combined cardiac output (CCO) is the sum of the outputs of the right and left side of the heart. It is useful in foetal circulation, where the cardiac output from both sides of the heart partly work in parallel by the foramen ovale and ductus arteriosus, both directly supplying the systemic circulation.

2.4 Cardiovascular Functions

The functions that are performed by this organ system can be categorized into three main domains: transport, regulation and protection. The heart and blood vessels carry out these functions in coordination with the other systems for the proper functioning of the body. **Transportation**: The components of the cardiovascular system work collectively so as to transport oxygen from the lungs to the various cells of the body. Oxygen and nutrients that are assimilated in the blood after the process of digestion are circulated via blood to various parts of the body. The removal of carbon dioxide which is produced by the cells and transportation of hormones from various endocrine glands are also performed by this organ system. This function of transport is mainly carried out by blood and the network of blood vessels.

Protection: White blood cells that are present in the blood protect the body from infection and diseases. Proteins and antibodies required for destroying viruses, bacteria and disease-causing germs are also provided by blood to the various parts of the body. Another important function is to protect the body from excessive blood loss through the process of blood clotting during an injury.

Regulation: Another important function is the regulation of the concentration of hydrogen ions (pH) in the body, and the regulation of the body temperature and body heat. It also regulates the salt and water content of the cells in the body (McGee, 2009).

Vascular tone refers to the degree of constriction experienced by a blood vessel relative to its maximally dilated state. All arterial and venous vessels under basal conditions exhibit some degree of smooth muscle contraction that determines the diameter, and hence tone, of the vessel. Basal vascular tone differs among organs. Those organs having a large vasodilatory capacity (e.g., myocardium, skeletal muscle, skin, splanchnic circulation) have

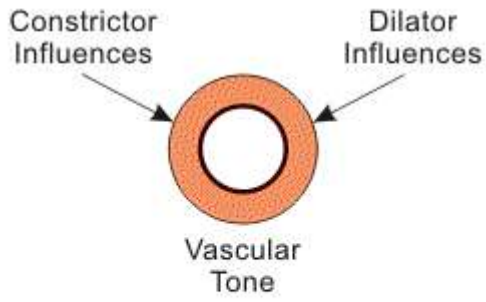
high vascular tone, whereas organs having relatively low vasodilatory capacity (e.g., cerebral and renal circulations) have low vascular tone.

Vascular tone is determined by many different competing vasoconstrictor and vasodilator influences acting on the blood vessel. These influences can be separated into extrinsic factors that originate from outside of the organ or tissue in which the blood vessel is located, and intrinsic factors that originate from the vessel itself or the surrounding tissue. The primary function of extrinsic factors is to regulate arterial blood pressure by altering systemic vascular resistance, whereas intrinsic mechanisms are important for local blood flow regulation within an organ. Vascular tone at any given time is determined by the balance of competing vasoconstrictor and vasodilator influences. In general, extrinsic factors (neurohumoral) such as sympathetic nerves and circulating angiotensin II increase vascular tone (i.e., cause vasoconstriction); however, some circulating factors (e.g., atrial natriuretic peptide) decrease vascular tone.

Intrinsic factors include:

- Myogenic mechanisms (originating from vascular smooth muscle), which increase tone.
- Endothelial factors such as nitric oxide and endothelin can either decrease or increase tone, respectively.
- Local hormones/chemical substances (e.g., arachidonic acid metabolites, histamine and bradykinin can either increase or decrease tone.
- Metabolic by-products or hypoxia, which generally decrease tone.

The mechanisms by which the above influences either constrict or relax blood vessels involve a variety of signal transduction mechanisms that ultimately influence the interaction between actin and myosin in the smooth muscle (Klabunde, 2007).



Vasoconstrictor and vasodilator influences acting on arteries and veins determine their state of vascular tone, which is the balance between constrictor and dilator influences.

Figure 5: Blood vessel showing vasoconstriction and vasodilator influences

2.4.1 Heart rate

Heart rate is the number of heartbeats per unit of time - typically expressed as beats per minute (bpm) - which can vary as the body's need for oxygen changes, such as during exercise or sleep. The measurement of heart rate is used by medical professionals to assist in the diagnosis and tracking of medical conditions. It is also used by individuals, such as athletes, who are interested in monitoring their heart rate to gain maximum efficiency from their training. The R wave to R wave interval (RR interval) is the inverse of the heart rate. Heart rate is measured by finding the pulse of the body. This pulse rate can be measured at any point on the body where an artery's pulsation is transmitted to the surface - often as it is compressed against an underlying structure like bone - by applying pressure on it with the index and middle finger. The thumb should not be used for measuring another person's heart rate, as its strong pulse may interfere with discriminating the site of pulsation (Gellish, 2007). A more precise method of determining pulse involves the use of an electrocardiograph, or ECG (also abbreviated EKG). Continuous electrocardiograph monitoring of the heart is routinely done in many clinical settings, especially in critical care medicine. Commercial heart rate monitors are also available, consisting of a chest strap with electrodes. The signal is

transmitted to a wrist receiver for display. Heart rate monitors allow accurate measurements to be taken continuously and can be used during exercise when manual measurement would be difficult or impossible (such as when the hands are being used); (Robergs and Landwehr 2002; Farazdaghi and Wohlfart, 2001).

2.4.2 Recovery heart rate

This is the heart rate measured at a fixed (or reference) period after ceasing activity; typically measured over a 1 minute period. For death, it has been hypothesized that a delayed fall in the heart rate after exercise might be an important prognostic marker. Less than 30 bpm reduction at one minute after stopping hard exercise was a predictor of heart attack. More than 50 bpm reduction showed reduced risk of heart attack. Training regimes sometimes use recovery heart rate as a guide of progress and to spot problems such as overheating or dehydration. After even short periods of hard exercise it can take a long time (about 30 minutes) for the heart rate to drop to rested level (Christopher, 1999).

2.4.3 Heart rate reserve

Heart rate reserve (HRR) is a term used to describe the difference between a person's measured or predicted maximum heart rate and resting heart rate. Some methods of measurement of exercise intensity measure percentage of heart rate reserve. Additionally, as a person increases their cardiovascular fitness, their HR_{rest} will drop, thus the heart rate reserve will increase. Percentage of HRR is equivalent to percentage of VO_2 reserve.

$$HRR = HR_{max} - HR_{rest}$$

2.4.4 Resting heart rate

Resting heart rate (HR_{rest}) is a person's heart rate when they are at rest: awake but lying down, and not having immediately exerted them. Typical healthy resting heart rate in adults is 60–80 bpm, with rates below 60 bpm referred to as bradycardia and rates above 100 bpm referred to as tachycardia. Note however that conditioned athletes often have resting heart rates below 60 bpm. Tour de France cyclist Lance Armstrong has a resting HR around 32 bpm, and it is not unusual for people doing regular exercise to get below 50 bpm. Other cyclists like Miguel Indurain and Alberto Contador have reported resting heart rates in the mid-20s below 32 bpm (Christopher, 1999).

2.4.5 Heart rate abnormalities

Tachycardia is a resting heart rate more than 100 beats per minute. This number can vary as smaller people and children have faster heart rates than average adults.

Bradycardia is defined as a heart rate less than 60 beats per minute although it is seldom symptomatic until below 50 bpm when a human is at total rest. Trained athletes tend to have slow resting heart rates, and resting bradycardia in athletes should not be considered abnormal if the individual has no symptoms associated with it. Again, this number can vary as children and small adults tend to have faster heart rates than average adults. Miguel Indurain, a Spanish cyclist and five times Tour de France winner, had a resting heart rate of 28 beats per minute, one of the lowest ever recorded in a healthy human (Wohlfart and Farazdaghi, 2003).

Arrhythmias are abnormalities of the heart rate and rhythm (sometimes felt as palpitations). They can be divided into two broad categories: fast and slow heart rates. Some cause few or

minimal symptoms. Others produce more serious symptoms of light headedness, dizziness and fainting (Wohlfart and Farazdaghi, 2003).

2.4.6 Heart rate as a risk factor

A number of investigations indicate that faster resting heart rate has emerged as a new risk factor for mortality in homeothermic mammals, particularly cardiovascular mortality in human beings. Faster heart rate may accompany increased production of inflammation molecules and increased production of reactive oxygen species in cardiovascular system, in addition to increased mechanical stress to the heart. Generally, the number of heart beat over life time is considered as an allotment. Those who have faster heart rate are using their heart beat allotment faster, therefore likely having a reduced lifespan. An Australian-led international study of patients with cardiovascular disease has shown that heart beat rate is a key indicator for the risk of heart attack. The study, published in The Lancet in 2008 studied 11,000 people, across 33 countries, who were being treated for heart problems. Those patients whose heart rate was above 70 beats per minute had significantly higher incidence of heart attacks, hospital admissions and the need for surgery. University of Sydney professor of cardiology Ben Freedman from Sydney's Concord hospital, said "If you have a high heart rate there was an increase in heart attack, there was about a 46 percent increase in hospitalizations for non-fatal or fatal heart attack (Danny, 2008). Standard textbooks of physiology and medicine mention that heart rate (HR) is readily calculated from the ECG as follows: HR = 1,500/RR interval in millimeters, HR = 60/RR interval in seconds, or HR = 300/number of large squares between successive R waves. In each case, the authors are actually referring to instantaneous HR, which is the number of times the heart would beat if successive RR intervals were constant (Wohlfart, 2003, Christopher, 1999, Danny, 2009)

2.4.7 Control of Heart Rate

Heart rate is normally determined by the pacemaker activity of the sinoatrial node (SA node) located in the posterior wall of the right atrium. The SA node exhibits automaticity that is determined by spontaneous changes in Ca^{++}, Na^{+}, and K^{+} conductances. This intrinsic automaticity, if left unmodified by neurohumoral factors, exhibits a spontaneous firing rate of 100-115 beats/min. This intrinsic firing rate decreases with age.

Heart rate is decreased below the intrinsic rate primarily by activation of the vagus nerve innervating the SA node. Normally, at rest, there is significant vagal tone on the SA node so that the resting heart rate is between 60 and 80 beats/min. This vagal influence can be demonstrated by administration of atropine, a muscarinic receptor antagonist, which leads to a 20-40 beats/min increase in heart rate depending upon the initial level of vagal tone. For heart rate to increase above the intrinsic rate, there is both a withdrawal of vagal tone and an activation of sympathetic nerves innervating the SA node. This reciprocal change in sympathetic and parasympathetic activity permits heart rate to increase during exercise, for example. Heart rate is also modified by circulating catecholamines acting via β_1-adrenoceptors located on SA nodal cells. Heart rate is also modified by changes in circulating thyroxin (thyrotoxicosis causes tachycardia) and by changes in body core temperature (hyperthermia increases heart rate). SA nodal dysfunction can lead to sinus bradycardia, sinus tachycardia, or sick-sinus syndrome. The maximal heart rate that can be achieved in an individual is estimated by Maximal Heart Rate = 220 beats/min − age in years Therefore a 20-year-old person will have a maximal heart rate of about 200 beats/min, and this will decrease to about 170 beats/min when the person is 50 years of age. This maximal heart rate is genetically determined and cannot be modified by exercise training or by external factors. Stimulation of the sympathetic nerves releases hormone nor-epinephrine at the sympathetic

nerve endings. The precise mechanism by which this hormone acts on cardiac muscle fibre is somewhat nuclear, but the belief is that it increases the permeability of the fibre membrane to sodium and calcium ions. In the sinus node, an increase of sodium-calcium permeability causes a more positive resting membrane potential and also causes increase rate of upward shift of the diastolic membrane potential towards the threshold level for self-excitation, thus accelerating self-excitation and therefore increasing heart rate (Ferrier and Howlett 2004; Klabunde, 2007; Kleber and Rudy 2004).

2.5 Blood Pressure

Blood pressure (BP) is the pressure exerted by circulating blood upon the walls of blood vessels, and is one of the principal vital signs. During each heartbeat, BP varies between a maximum (systolic) and a minimum (diastolic) pressure (Pickering et al., 2005). The mean BP, due to pumping by the heart and resistance to flow in blood vessels, decreases as the circulating blood moves away from the heart through arteries. Blood pressure drops most rapidly along the small arteries and arterioles, and continues to decrease as the blood moves through the capillaries and back to the heart through veins (Klabunde, 2007). Gravity, valves in veins, and pumping from contraction of skeletal muscles, are some other influences on BP at various places in the body. The term blood pressure usually refers to the pressure measured at a person's upper arm. It is measured on the inside of an elbow at the brachial artery, which is the upper arm's major blood vessel that carries blood away from the heart. A person's BP is usually expressed in terms of the systolic pressure and diastolic pressure (mmHg), for example 120/80 (Klabunde, 2007).

2.5.1 Measurement of blood pressure

Arterial pressure is most commonly measured via a sphygmomanometer, which historically used the height of a column of mercury to reflect the circulating pressure (Booth, 2009). BP values are reported in millimetres of mercury (mmHg), though aneroid and electronic devices do not use mercury. For each heartbeat, BP varies between systolic and diastolic pressures. Systolic pressure is peak pressure in the arteries, which occurs near the end of the cardiac cycle when the ventricles are contracting. Diastolic pressure is minimum pressure in the arteries, which occurs near the beginning of the cardiac cycle when the ventricles are filled with blood. An example of normal measured values for a resting, healthy adult human is 120 mmHg systolic and 80 mmHg diastolic (written as 120/80 mmHg, and spoken (in the US) as "one-twenty over eighty") **(**Pesola et al., 2001).

Systolic and diastolic arterial BPs are not static but undergo natural variations from one heartbeat to another and throughout the day (in a circadian rhythm). They also change in response to stress, nutritional factors, drugs, disease, exercise, and momentarily from standing up. Sometimes the variations are large. Hypertension refers to arterial pressure being abnormally high, as opposed to hypotension, when it is abnormally low. Along with body temperature, respiratory rate, and pulse rate, BP is one of the four main vital signs routinely monitored by medical professionals and healthcare providers. Arterial pressures are usually measured non-invasively, without penetrating skin or artery. Measuring pressure invasively, by penetrating the arterial wall to take the measurement, is much less common and usually restricted to a hospital setting and research (Deakin and Low, 2000).

2.5.2 Non-invasive measurement

The non-invasive auscultatory and oscillometric measurements methods are simpler and quicker than invasive measurements, they require less expertise, have virtually no complications, less unpleasant and less painful for the patient. However, non-invasive methods may yield somewhat lower accuracy and small systematic differences in numerical results. Non-invasive measurement methods are more commonly used for routine examinations and monitoring.

2.5.3 Palpation method

A minimum systolic value can be roughly estimated by palpation, most often used in emergency situations. Historically, students have been taught that palpation of a radial pulse indicates a minimum BP of 80 mmHg, a femoral pulse indicates at least 70 mmHg, and a carotid pulse indicates a minimum of 60 mmHg. However, at least one study indicated that this method often overestimates patients' systolic blood pressure. A more accurate value of systolic BP can be obtained with a sphygmomanometer and palpating the radial pulse. The diastolic blood pressure cannot be estimated by this method. The American Heart Assoc. recommends that palpation is used to get an estimate before using the auscultatory method (Pickering et al., 2005; Laurent, 2003).

2.5.4 Auscultatory method

Auscultatory method: aneroid sphygmomanometer with stethoscope

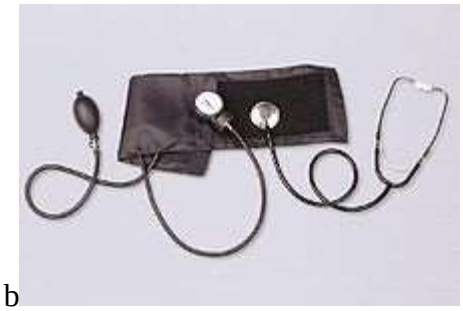
a  b 

Figure 6: a. Mercury sphygmomanometer

b. aneroid sphygmomanometer with stethoscope

The auscultatory method (from the Latin word for *listening*) uses a stethoscope and a sphygmomanometer. This comprises an inflatable (Riva-Rocci) cuff placed around the upper arm at roughly the same vertical height as the heart, attached to a mercury or aneroid manometer (Fig. 4). The mercury manometer, considered the gold standard, measures the height of a column of mercury, giving an absolute result without need for calibration, and consequently not subject to the errors and drift of calibration which affect other methods. The use of mercury manometers is often required in clinical trials and for the clinical measurement of hypertension in high risk patients, such as pregnant women. A cuff of appropriate size is fitted smoothly and snugly, and then inflated manually by repeatedly squeezing a rubber bulb until the artery is completely occluded. Listening with the stethoscope to the brachial artery at the elbow, the examiner slowly releases the pressure in the cuff. When blood just starts to flow in the artery, the turbulent flow creates a "whooshing"

or pounding (first Korotkoff sound). The pressure at which this sound is first heard is the systolic BP. The cuff pressure is further released until no sound can be heard (fifth Korotkoff sound), at the diastolic arterial pressure. The auscultatory method is the predominant method of clinical measurement (Pickering et al., 2005).

2.5.5 Oscillometric method

The oscillometric method was first demonstrated in 1876 and involves the observation of oscillations in the sphygmomanometer cuff pressure which are caused by the oscillations of blood flow, i.e. the pulse. The electronic version of this method is sometimes used in long-term measurements and general practice. It uses a sphygmomanometer cuff like the auscultatory method, but with an electronic pressure sensor (transducer) to observe cuff pressure oscillations, electronics to automatically interpret them, and automatic inflation and deflation of the cuff. The pressure sensor should be calibrated periodically to maintain accuracy. Oscillometric measurement requires less skill than the auscultatory technique, and may be suitable for use by untrained staff and for automated patient home monitoring. The cuff is inflated to a pressure initially in excess of the systolic arterial pressure, and then reduces to below diastolic pressure over a period of about 30 seconds. When blood flow is nil (cuff pressure exceeding systolic pressure) or unimpeded (cuff pressure below diastolic pressure), cuff pressure will be essentially constant. It is essential that the cuff size is correct: undersized cuffs may yield too high a pressure, whereas oversized cuffs yield too low a pressure. When blood flow is present, but restricted, the cuff pressure, which is monitored by the pressure sensor, will vary periodically in synchrony with the cyclic expansion and contraction of the brachial artery, i.e., it will oscillate. The values of systolic and diastolic pressure are computed, not actually measured from the raw data, using an algorithm; the computed results are displayed. Oscillometric monitors may produce inaccurate readings in patients with heart and circulation problems, which include arterial sclerosis, arrhythmia,

preeclampsia, pulsus alternans, and pulsus paradoxus. In practice the different methods do not give identical results; an algorithm and experimentally obtained coefficients are used to adjust the oscillometric results to give readings which match the auscultatory results as well as possible. Some equipment uses computer-aided analysis of the instantaneous arterial pressure waveform to determine the systolic, mean, and diastolic points. Since many oscillometric devices have not been validated, caution must be given as most are not suitable in clinical and acute care settings (Pickering et al., 2005; Laurent, 2003).

2.5.6 White-coat hypertension

For some patients, BP measurements taken in a doctor's office may not correctly characterize their typical value. In up to 25% of patients, the office measurement is higher than their typical BP. This type of error is called white-coat hypertension (WCH) and can result from anxiety related to an examination by a health care professional. The misdiagnosis of hypertension for these patients can result in needless and possibly harmful medication. WCH can be reduced (but not eliminated) with automated BP measurements over 15 to 20 minutes in a quiet part of the office or clinic (Pickering et al., 2005; Jhalani et al., 2005).

2.5.7 Invasive measurement

Arterial blood pressure (BP) is most accurately measured invasively through an arterial line. Invasive arterial pressure measurement with intravascular cannulae involves direct measurement of arterial pressure by placing a cannula needle in an artery (usually radial, femoral, dorsalis pedis or brachial). The cannula must be connected to a sterile, fluid-filled system, which is connected to an electronic pressure transducer. The advantage of this system is that pressure is constantly monitored beat-by-beat, and a waveform (a graph of pressure

against time) can be displayed. This invasive technique is regularly employed in human and Veterinary intensive care medicine, anaesthesiology, and for research purpose (Booth, 1977.

2.5.8 Classification of blood pressure

The following classification of blood pressure applies to adults aged 18 and older. It is based on the average of seated BP readings that were properly measured during 2 or more office visits

Table 2: Classification of blood pressure for adults

Category	**systolic, mmHg**	**diastolic, mmHg**
Hypotension	**< 90**	**< 60**
Normal	**90 – 120**	**and 60 – 80**
Pre-hypertension	**121 – 139**	**or 81 – 89**
Stage 1 Hypertension	**140 – 159**	**or 90 – 99**
Stage 2 Hypertension	**≥ 160**	**or ≥ 100**

(Chobanian et al., 2003; American Heart Association, [2011, 2005]).

2.5.9 Mean arterial pressure (MAP)

The mean arterial pressure (MAP) is the average over a cardiac cycle and is determined by the cardiac output (CO), systemic vascular resistance (SVR), and central venous pressure (CVP), (Klabunde, 2007).

$$MAP = (CO. SVR) + CVP$$

MAP can be approximately determined from measurements of the systolic pressure P_{syst}

and the diastolic pressure P_{dias} while there is a normal resting heart rate, MAP= P_{dias} +1/3 ($P_{sys} – P_{dias}$) (Klabunde, (2007).

2.5.10 Pulse pressure

The up and down fluctuation of the arterial pressure results from the pulsatile nature of the cardiac output, i.e. the heartbeat. The pulse pressure is determined by the interaction of the stroke volume of the heart, compliance (ability to expand) of the aorta, and the resistance to flow in the arterial tree. By expanding under pressure, the aorta absorbs some of the force of the blood surge from the heart during a heartbeat. In this way the pulse pressure is reduced from what it would be if the aorta wasn't compliant. The loss of arterial compliance that occurs with aging explains the elevated pulse pressures found in elderly patients. The pulse pressure can be simply calculated from the difference of the measured systolic and diastolic pressures (Klabunde, 2007).

$$P_{pulse} = P_{syst} - P_{diast}$$

2.5.11 Disorders of Blood pressure

Disregulation disorders of blood pressure control include high blood pressure, blood pressure that is too low, and blood pressure that shows excessive or maladaptive fluctuation.

High blood pressure can be an indicator of other problems and may have long-term adverse effects. Sometimes it can be an acute problem, for example hypertensive emergency. All levels of arterial pressure put mechanical stress on the arterial walls. Higher pressures increase heart workload and progression of unhealthy tissue growth

(atheroma) that develops within the walls of arteries. The higher the pressure, the more stress that is present and the more atheroma tend to progress and the heart muscle tends to thicken, enlarge and become weaker over time. Persistent hypertension is one of the risk factors for strokes, heart attacks, heart failure and arterial aneurysms, and is the leading cause of chronic renal failure. Even moderate elevation of arterial pressure leads to shortened life expectancy. At severely high pressures, mean arterial pressures 50% or more above average, a person can expect to live no more than a few years unless appropriately treated. In the past, most attention was paid to diastolic pressure; but nowadays it is recognised that both high systolic pressure and high pulse pressure (the numerical difference between systolic and diastolic pressures) are also risk factors. In some cases, it appears that a decrease in excessive diastolic pressure can actually increase risk, due probably to the increased difference between systolic and diastolic pressures (see the article on pulse pressure). However, in a study of people with heart valve regurgitation, there was a decreased severity of aortic and mitral regurgitation when a person's diastolic blood pressure decreased, whereas when the diastolic blood pressure increased, there was an increased severity.

Blood pressure that is too low is known as hypotension. Hypotension is a medical concern only if it causes signs or symptoms, such as dizziness, fainting, or in extreme cases, shock. When arterial pressure and blood flow decrease beyond a certain point, the perfusion of the brain becomes critically decreased (i.e., the blood supply is not sufficient), causing light-headedness, dizziness, weakness or fainting. Sometimes the arterial pressure drops significantly when a patient stands up from sitting. This is known as **orthostatic hypotension** (postural hypotension); gravity reduces the rate of blood return from the veins below the heart back to the heart, thus reducing stroke volume and cardiac output. When people are healthy, the veins below their heart quickly constrict and the heart rate increases

to minimize and compensate for the gravity effect. This is carried out involuntarily by the autonomic nervous system. The system usually requires a few seconds to fully adjust and if the compensations are too slow or inadequate, the individual will suffer reduced blood flow to the brain, dizziness and potential blackout. Increases in G-loading, such as routinely experienced by aerobatic or combat pilots 'pulling Gs', greatly increases this effect. Repositioning the body perpendicular to gravity largely eliminates the problem. Other causes of low arterial pressure include:

- Sepsis
- Haemorrhage - blood loss
- Toxins including toxic doses of BP medicine
- Hormonal abnormalities, such as Addison's disease

Shock is a complex condition which leads to critically decreased perfusion. The usual mechanisms are loss of blood volume, pooling of blood within the veins reducing adequate return to the heart and/or low effective heart pumping. Low arterial pressure, especially low pulse pressure, is a sign of shock and contributes to and reflects decreased perfusion. If there is a significant difference in the pressure from one arm to the other, that may indicate a narrowing (for example, due to aortic coarctation, aortic dissection, thrombosis or embolism) of an artery (Mancia et al., 2007).

2.6 The Baroreceptor

Baroreceptors are sensors located in the blood vessels of several mammals. They are a type of mechanoreceptor that detects the pressure of blood flowing through them, and can send messages to the central nervous system to increase or decrease total peripheral resistance and

cardiac output. Baroreceptors act immediately as part of a negative feedback system called the baroreflex. As soon as there is a change from the usual blood pressure mean arterial blood pressure, returning the pressure to a normal level. They are an example of a short-term blood pressure regulation mechanism. Baroreceptors detect the amount of stretch of the blood vessel walls, and send the signal to the nervous system in response to this stretch. The nucleus tractus solitarius in the medulla oblongata recognizes changes in the firing rate of action potentials from the baroreceptors, and influences cardiac output and systemic vascular resistance through changes in the autonomic nervous system (Stanfield, 2008).

Baroreceptors can be divided into two categories: high-pressure arterial baroreceptors and low-pressure baroreceptors (also known as cardiopulmonary (Levy and Pappano, 2007) or volume receptors (Stanfield, 2008).

2.6.1 High-pressure (Arterial) baroreceptors

Arterial baroreceptors are present in the aortic arch and the carotid sinuses of the left and right internal carotid arteries. The baroreceptors found within the aortic arch enable the monitoring of the blood being delivered to all the blood vessels via the systemic circuit, and the baroreceptors within the carotid sinuses monitor the blood pressure of the blood being delivered to the brain. Arterial baroreceptors are stimulated by pressure changes in the arteries. The baroreceptors can identify the changes in the blood pressure, which can increase or decrease the heart rate. They are sprayed nerve endings that lie in the tunica adventitia of the artery, not drug-binding molecules as the term receptor may suggest. A change in the mean arterial pressure induces depolarization of these sensory endings, which results in action potentials. These action potentials are conducted to the central nervous system by axons and have a direct effect on the cardiovascular system through autonomic neurons (Stanfield, 2008). Hormone secretions that target the heart and blood vessels are affected by

the stimulaton of baroreceptors. If blood pressure falls, such as in hypovolaemic shock, baroreceptor firing rate decreases. Signals from the carotid baroreceptors are sent via the glossopharyngeal nerve (cranial nerve IX). Signals from the aortic baroreceptors travel through the vagus nerve (cranial nerve X) **(**Bray et al., 1999). If the arterial pressure is severely lowered, the baroreflex is activated (Guyton and Hall, 2006). Baroreceptors respond very quickly to maintain a stable blood pressure, but they respond only to short-term changes. Over a period of 1–2 days, they will reset to a new value (Guyton and Hall, 2006). Thus, in people with essential hypertension the baroreceptors behave as if the elevated blood pressure is normal and aim to maintain this high blood pressure. The receptors then become less sensitive to change (Levy and Pappano, 2007).

2.6.2 Low-pressure baroreceptors

The low-pressure baroreceptors, are found in large systemic veins, in pulmonary vessels, and in the walls of the right atrium and ventricles of the heart (Stanfield , 2008). The low-pressure baroreceptors are involved with the regulation of blood volume. The blood volume determines the mean pressure throughout the system, in particular in the venous side where most of the blood is held. The low-pressure baroreceptors have both circulatory and renal effects; they produce changes in hormone secretion, resulting in profound effects on the retention of salt and water; they also influence intake of salt and water. The renal effects allow the receptors to change the mean pressure in the system in the long term. Denervating these receptors 'fools' the body into thinking that it has too low blood volume and initiates mechanisms that retain fluid and so push up the blood pressure to a higher level than it would otherwise have.

2.6.3 Baroreceptor dysfunction

Baroreceptors are integral to the body's function: Pressure changes in the blood vessels would not be detected as quickly as in the presence of baroreceptors. When baroreceptors are not working, blood pressure continues to increase, but, within an hour, the blood pressure returns to normal as other blood pressure regulatory systems take over (Guyton and Hall, 2006).

2.7 BAROREFLEX

The baroreflex or baroreceptor reflex is one of the body's homeostatic mechanisms for maintaining blood pressure. It provides a negative feedback loop in which an elevated blood pressure reflexively causes heart rate and thus blood pressure to decrease; similarly, decreased blood pressure depresses the baroreflex, causing heart rate and thus blood pressure to rise. The system relies on specialized neurons, known as baroreceptors, in the aortic arch, carotid sinuses, and elsewhere to monitor changes in blood pressure and relay them to the brainstem. Subsequent changes in blood pressure are mediated by the autonomic nervous system. Atrial natriuretic peptide forms a parallel negative feedback loop in an endocrinological contrast to the renin-angiotensin system.

2.7.1 Anatomy of the reflex

Baroreceptors include those in the auricles of the heart and vena cavae, but the most sensitive baroreceptors are in the carotid sinus and aortic arch. The carotid sinus baroreceptors are innervated by the glossopharyngeal nerve (CN IX); the aortic arch baroreceptors are innervated by the vagus nerve (CN X). Baroreceptor activity travels along these nerves, which contact the nucleus of the tractus solitarus (NTS) in the brainstem. The NTS sends

excitatory fibers (glutamatergic) to the caudal ventro-lateral medulla (CVLM), activating the CVLM. The activated CVLM then sends inhibitory fibers (GABAergic) to the rostral ventrolateral medulla (RVLM), thus inhibiting the RVLM. The RVLM is the primary regulator of the sympathetic nervous system, sending excitatory fibers (glutamatergic) to the sympathetic preganglionic neurons located in the inter-medio-lateral nucleus of the spinal cord. Hence, when the baroreceptors are activated (by an increased blood pressure), the NTS activates the CVLM, which in turn inhibits the RVLM, thus inhibiting the sympathetic branch of the autonomic nervous system leading to a decrease in blood pressure. Likewise, low blood pressure causes an increase in sympathetic tone via "disinhibition" (less inhibition, hence activation) of the RVLM. The NTS also sends excitatory fibres to the Nucleus ambiguus (vagal nuclei) that regulate the parasympathetic nervous system, aiding in the decrease in sympathetic activity during conditions of elevated blood pressure (Sleight, 1995).

2.7.2 Baroreceptor activation

The baroreceptors are stretch-sensitive mechanoreceptors. When blood pressure rises, the carotid and aortic sinuses are distended, resulting in stretch and therefore activation of the baroreceptors. Active baroreceptors fire action potentials ("spikes") more frequently than inactive baroreceptors. The greater the stretch, the more rapidly baroreceptors fire action potentials. These action potentials are relayed to the nucleus of the tractus solitarius (NTS), which uses frequency as a measure of blood pressure. As discussed previously, increased activation of the NTS inhibits the vasomotor center and stimulates the vagal nuclei. The end result of baroreceptor activation is inhibition of the sympathetic nervous system and activation of the parasympathetic nervous system. The sympathetic and parasympathetic branches of the autonomic nervous system have opposing effects on blood pressure. Sympathetic activation leads to an elevation of total peripheral resistance and cardiac output

via increased contractility of the heart, heart rate, and arterial vasoconstriction, which tends to increase blood pressure. Conversely, parasympathetic activation leads to a decreased cardiac output via decrease in heart rate, resulting in a tendency to decrease blood pressure. By coupling sympathetic inhibition and parasympathetic activation, the baroreflex maximizes blood pressure reduction. Sympathetic inhibition leads to a drop in peripheral resistance, while parasympathetic activation leads to a depressed heart rate (reflex bradycardia) and contractility. The combined effects will dramatically decrease blood pressure. Similarly, sympathetic activation with parasympathetic inhibition allows the baroreflex to elevate blood pressure (Boron et al., 2005).

2.7.3 Effect of baroreceptor on Heart Rate

Baroreceptor stimulation usually produces inhibition of efferent cardiac sympathetic fibres and actvation of efferent cardiac vagal fibres. However, baroreflex tachycardia response to hypotension was very small in magnitude. The chronotropic response to bilateral carotid occlusion is a mild tachycardia as reported by Iturriaga et al., (1988). The baroreflex may be responsible for a part of the low-frequency component of heart rate variability, the so called Mayer waves, at 0.1 Hz (Bisognano, 2006; Sleight, 1995).

2.7.4 Baroreflex activation therapy for treatment of resistant hypertension

Published feasibility studies have shown that a pacemaker-like device designed to electrically activate the baroreflex, also known as baroreflex activation therapy, significantly lowers blood pressure in patients with treatment resistant hypertension. One study published on a group of 16 patients reported an average systolic blood pressure reduction of 34 mmHg after three months of treatment and 35 mmHg after 24 months. A drop in systolic blood

pressure of at least 20 mmHg was achieved in 12 of 16 (75%) patients at 2 years and 5 of 16 (31%) achieved a systolic BP of less than 140 mmHg at 2 years, (Teba et al 2012). Results published on a separate group of 10 patients from another feasibility trial reported an average systolic blood pressure reduction of 24 mmHg after three months of treatment. Baroreflex activation therapy devices are not currently available outside of clinical research studies (Bisognano, 2006; Scheffer et al., 2008, 2010).

2.8 The Antioxidants

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. **(S**ies, 1997 and Bjelakovic et al., 2007).

2.8.1 History of the antioxidant

The term antioxidant originally was used to refer specifically to a chemical that prevented the consumption of oxygen. In the late 19th and early 20th century, extensive study was devoted to the uses of antioxidants in important industrial processes, such as the prevention of metal corrosion, the vulcanization of rubber, and the polymerization of fuels in the fouling of internal combustion engines (Matill, 1947). Early research on the role of antioxidants in biology focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity (German, 1999). Antioxidant activity could be measured simply by placing the fat in a closed container with oxygen and measuring the rate of oxygen consumption.

However, it was the identification of vitamins A, C, and E as antioxidants that revolutionized the field and led to the realization of the importance of antioxidants in the biochemistry of living organisms (Jacob, 1996; Knight, 1998). The possible mechanisms of action of antioxidants were first explored when it was recognized that a substance with anti-oxidative activity is likely to be one that is itself readily oxidized (Moreau and Dufraisse, 1922). Research into how vitamin E prevents the process of lipid peroxidation led to the identification of antioxidants as reducing agents that prevent oxidative reactions, often by scavenging reactive oxygen species before they can damage cells **(**Wolf, 2005). In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they can damage vital components of the cell (Sies, 1997; Davies, 1995). However, since reactive oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level **(**Rhee, 2006).

The reactive oxygen species produced in cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and free radicals such as the hydroxyl radical (OH) and the superoxide anion (O_2^-) (Valko et al., 2007). The hydroxyl radical is particularly unstable and will react rapidly and non-specifically with most biological molecules. This species is produced from hydrogen peroxide in metal-catalyzed redox reactions such as the Fenton reaction (Stohs, 1995).

These oxidants can damage cells by starting chemical chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins (Sies, 1997). Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms, **(**Nakabeppu et al., 2006 and Valko, 2004); while damage to proteins causes enzyme inhibition, denaturation and protein degradation (Stadtman, 1992).

2.8.2 Metabolites

Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation (Sies, 1997). These compounds may be synthesized in the body or obtained from the diet. The different antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed. Some antioxidants are only found in a few organisms and these compounds can be important in pathogens and can be virulence factors. The relative importance and interactions between these different antioxidants is a very complex question, with the various metabolites and enzyme systems having synergistic and interdependent effects on one another (Chaudière and Ferrari, 1999; Sies, 1993). The action of one antioxidant may therefore depend on the proper function of other members of the antioxidant system. The amount of protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts **(**Vertuani et al., 2004). Some compounds contribute to antioxidant defence by chelating transition metals and preventing them from catalysing the production of free radicals in the cell. Particularly important is the ability to sequester iron, which is the function of iron-binding proteins such as transferrin and ferritin. Selenium and zinc are commonly referred to as antioxidant nutrients, but these chemical elements have no antioxidant action themselves and are instead required for the activity of some antioxidant enzymes, as is discussed below.

2.8.3 Catalase

Catalases are enzymes that catalyse the conversion of hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor (Chelikani et al., 2004; Zámocký and Koller, 1999). This protein is localized to peroxisomes in most eukaryotic cells. **(**Río et al., 1992). Catalase is an unusual enzyme since, although hydrogen peroxide is its only substrate, it follows a ping-pong mechanism. Here, its cofactor is oxidised by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate (Hiner et al., 2002). Despite its apparent importance in hydrogen peroxide removal, humans with genetic deficiency of catalase — "acatalasemia" — or mice genetically engineered to lack catalase completely, suffer few ill effects (Mueller et al., 1997).

2.8.4 Glutathione

Glutathione is a cysteine-containing peptide found in most forms of aerobic life (Meister and Anderson, 1983). It is not required in the diet and is instead synthesized in cells from its constituent amino acids (Meister, 1988). Glutathione has antioxidant properties since the thiol group in its cysteine moiety is a reducing agent and can be reversibly oxidized and reduced. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins, as well as reacting directly with oxidants. Due to its high concentration and its central role in maintaining the cell's redox state, glutathione is one of the most important cellular antioxidants). In some organisms glutathione is replaced by other thiols, such as by mycothiol in the Actinomycetes, or by trypanothione in the Kinetoplastids (Fahey, 2001; Fairlamb and Cerami, 1992). The glutathione system includes glutathione, glutathione reductase, glutathione peroxidases and glutathione *S*-transferases. This system is found in animals, plants and microorganisms

(Meister and Anderson, 1983). Glutathione peroxidase is an enzyme containing four selenium-cofactors that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides. There are at least four different glutathione peroxidase isozymes in animals. Glutathione peroxidase 1 is the most abundant and is a very efficient scavenger of hydrogen peroxide, while glutathione peroxidase 4 is most active with lipid hydroperoxides. Surprisingly, glutathione peroxidase 1 is dispensable, as mice lacking this enzyme have normal lifespans; but they are hypersensitive to induced oxidative stress. In addition, the glutathione *S*-transferases show high activity with lipid peroxides These enzymes are at particularly high levels in the liver and also serve in detoxification metabolism

2.8.5 Superoxide dismutase

Superoxide dismutase (SODs) is a class of closely related enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide (Zelko et al. 2002; Bannister et al., 1987). SOD enzymes are present in almost all aerobic cells and in extracellular fluids (Johnson and Giulivi, 2005). Superoxide dismutase enzymes contain metal ion cofactors that, depending on the isozyme, can be copper, zinc, manganese or iron. In humans, the copper/zinc SOD is present in the cytosol, while manganese SOD is present in the mitochondrion **(**Bannister et al., 1987). There also exists a third form of SOD in extracellular fluids, which contains copper and zinc in its active sites (Nozik-Grayck et al., 2005). The mitochondrial isozyme seems to be the most biologically important of these three, since mice lacking this enzyme die soon after birth (Melov et al., 1998). In contrast, the mice lacking copper/zinc SOD (Sod1) are viable but have numerous pathologies and a reduced lifespan (see article on superoxide), while mice without the extracellular SOD have minimal defects (sensitive to hyperoxia); **(**Reaume et al., 1996). In plants, SOD isozymes are present

in the cytosol and mitochondria, with an iron SOD found in chloroplasts that is absent from vertebrates and yeast (Van et al., 1997).

2.8.6 Oxidative stress in disease

Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease **(**Christen, 2000; Nunomura et al., 2006). Parkinson's disease, the pathologies caused by diabetes, rheumatoid arthritis, and neuro-degeneration in motor neuron diseases **(**Cookson and Shaw, 1999). In many of these cases, it is unclear if oxidants trigger the disease, or if they are produced as a secondary consequence of the disease and from general tissue damage (Valko et al., 2007). One case in which this link is particularly well-understood is the role of oxidative stress in cardiovascular disease. Here, low density lipoprotein (LDL) oxidation appears to trigger the process of atherogenesis, which results in atherosclerosis, and finally cardiovascular disease (Van-Gaal et al., 2006). A low calorie diet extends median and maximum lifespan in many animals. This effect may involve a reduction in oxidative stress, while there is some evidence to support the role of oxidative stress in aging in model organisms such as Drosophila melanogaster and Caenorhabditis elegans, (Larsen, 1993; Helfand, 2003). The evidence in mammals is less clear (Sohal et al., 2002; Rattan, 2006). Indeed, a 2009 review of experiments in mice concluded that almost all manipulations of antioxidant systems had no effect on aging (Pérez et al., 2009). Diets high in fruit and vegetables, which are high in antioxidants, promote health and reduce the effects of ageing; however antioxidant vitamin supplementation has no detectable effect on the aging process, so the effects of fruit and vegetables may be unrelated to their antioxidant contents (Thomas, 2004; Ward, 1998). One reason for this might be the fact that consuming antioxidant molecules such as polyphenols and vitamin E will produce changes in other parts

of metabolism, so it may be these other effects that are the real reason these compounds are important in human nutrition. (Azzi, 2007; Aggarwal, 2006).

2.8.7 Effects of antioxidants on health

The brain is uniquely vulnerable to oxidative injury, due to its high metabolic rate and elevated levels of polyunsaturated lipids, the target of lipid peroxidation **(**Reiter, 1995). Consequently, antioxidants are commonly used as medications to treat various forms of brain injury. Here, superoxide dismutase mimetics (Warner et al., 2004); sodium thiopental and propofol are used to treat reperfusion injury and traumatic brain injury, **(**Wilson and Gelb, 2002); while the experimental drug NXY-059 **(**Lees et al., 2006; Yamaguchi et al., 1998); are being applied in the treatment of stroke. These compounds appear to prevent oxidative stress in neurons and prevent apoptosis and neurological damage. Antioxidants are also being investigated as possible treatments for neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Matteo and Esposito, 2003; Rao and Balachandran, 2002); and as a way to prevent noise-induced hearing loss. (Kopke et al., 2007).

2.8.8 Disease prevention

People who eat fruits and vegetables have a lower risk of heart disease and some neurological diseases (Stanner et al., 2004), and there is evidence that some types of vegetables, and fruits in general, protect against some cancers. Since fruits and vegetables happen to be good sources of antioxidants, this suggested that antioxidants might prevent some types of diseases. This idea has been tested in clinical trials and does not seem to be true, as antioxidant supplements have no clear effect on the risk of chronic diseases such as

cancer and heart disease (Stanner et al., 2004; Shenkin**,** 2006). This suggests that these health benefits come from other substances in fruits and vegetables (possibly flavonoids), or come from a complex mix of substances (Cherubini et al., 2005; Lotito and Frei, 2006).

While several trials have investigated supplements with high doses of antioxidants, the "Supplémentation en Vitamines et Mineraux Antioxydants" (SU.VI.MAX) study tested the effect of supplementation with doses comparable to those in a healthy diet Over 12,500 French men and women took either low-dose antioxidants (120 mg of ascorbic acid, 30 mg of vitamin E, 6 mg of beta carotene, 100 μg of selenium, and 20 mg of zinc) or placebo pills for an average of 7.5 years. The investigators found there was no statistically significant effect of the antioxidants on overall survival, cancer, or heart disease. In a post-hoc analysis they found a 31% reduction in the risk of cancer in men, but not women **(**Hercberg et al., 2004).

2.8.9 Measurement and levels in food

Measurement of antioxidants is not a straightforward process, as this is a diverse group of compounds with different reactivities to different reactive oxygen species. In food science, the oxygen radical absorbance capacity (ORAC) has become the current industry standard for assessing antioxidant strength of whole foods, juices and food additives (Cao et al., 1993; Ou et al., 2001). Other measurement tests include the Folin-Ciocalteu reagent, and the Trolox equivalent antioxidant capacity assay (Prior et al., 2005).

Antioxidants are found in varying amounts in foods such as vegetables, fruits, grain cereals, eggs, meat, legumes and nuts. Some antioxidants such as lycopene and ascorbic acid can be destroyed by long-term storage or prolonged cooking (Xianquan et al., 2005) Rodriguez-Amaya, 2003). Other antioxidant compounds are more stable, such as the polyphenolic antioxidants in foods such as whole-wheat cereals and tea **(**Baublis et al., 2000; Rietveld and

Wiseman, 2003). The effects of cooking and food processing are complex, as these processes can also increase the bioavailability of antioxidants, such as some carotenoids in vegetables (Maiani et al., 2008). In general, processed foods contain fewer antioxidants than fresh and uncooked foods, since the preparation processes may expose the food to oxygen (Henry, 2002).

Other antioxidants are not vitamins and are instead made in the body. For example, ubiquinol (coenzyme Q) is poorly absorbed from the gut and is made in humans through the mevalonate pathway (Turunen et al., 2004). Another example is glutathione, which is made from amino acids. As any glutathione in the gut is broken down to free cysteine, glycine and glutamic acid before being absorbed, even large oral doses have little effect on the concentration of glutathione in the body (Witschi et al., 1992; Flagg et al., 1994). Although large amounts of sulfur-containing amino acids such as acetylcysteine can increase glutathione, (Dodd et al., 2008); no evidence exists that eating high levels of these glutathione precursors is beneficial for healthy adults (Van-de-Poll et al., 2006). Supplying more of these precursors may be useful as part of the treatment of some diseases, such as acute respiratory distress syndrome, protein-energy malnutrition, or preventing the liver damage produced by paracetamol overdose (Dodd et al., 2008; Wu et al., 2004).

2.9 The Creatinine

Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). In chemical terms, creatinine is a spontaneously formed cyclic derivative of creatine. Creatinine is chiefly filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion). There is little-to-no tubular reabsorption of creatinine. If the filtering of the kidney is

deficient, blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR). The GFR is clinically important because it is a measurement of renal function. However, in cases of severe renal dysfunction, the creatinine clearance rate will be "overestimated" because the active secretion of creatinine will account for a larger fraction of the total creatinine cleared Ketoacids, cimetidine and trimethoprim reduce creatinine tubular secretion and therefore increase the accuracy of the GFR estimate, particularly in severe renal dysfunction. (In the absence of secretion, creatinine behaves like inulin.)

A more complete estimation of renal function can be made when interpreting the blood (plasma) concentration of creatinine along with that of urea. BUN-to-creatinine ratio (the ratio of blood urea nitrogen to creatinine) can indicate other problems besides those intrinsic to the kidney; for example, a urea level raised out of proportion to the creatinine may indicate a pre-renal problem such as volume depletion. Men tend to have higher levels of creatinine because they, in general, have more skeletal muscle mass than women. Vegetarians have been shown to have lower creatinine levels (Burke, 2003).

2.9.1 Diagnostic use of creatinine

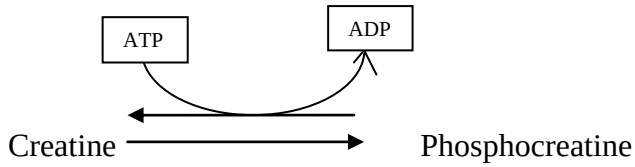
Measuring serum creatinine is a simple test and it is the most commonly used indicator of renal function. A rise in blood creatinine level is observed only with marked damage to functioning nephrons. Therefore, this test is not suitable for detecting early-stage kidney disease. A better estimation of kidney function is given by the creatinine clearance (CrCl) test. Creatinine clearance can be accurately calculated using serum creatinine concentration and some or all of the following variables: sex, age, weight, and race, as suggested by the American Diabetes Association without a 24-hour urine collection (Gross et al., 2005). Some laboratories will calculate the CrCl if written on the pathology request form, and the

necessary age, sex, and weight are included in the patient information. A recent Japanese study suggests that a lower-serum creatinine level is associated with an increased risk for the development of type 2 diabetes in Japanese men (Harita et al., 2008). Creatinine concentration is also checked during standard urine drug tests. High creatinine levels indicate a pure test, whereas low amounts of creatinine in the urine indicate a manipulated test, either through the addition of water in the sample or by drinking excessive amounts of water. In the United States, creatinine is typically reported in mg/dL, whereas, in Canada and a few European countries, μmol/litre may be used. 1 mg/dL of creatinine is 88.4 μmol/L. The typical human reference ranges for serum creatinine are 0.5 to 1.0 mg/dL (about 45-90 μmol/L) for women and 0.7 to 1.2 mg/dL (60-110 μmol/L) for men. While a baseline serum creatinine of 2.0 mg/dL (150 μmol/L) may indicate normal kidney function in a male body builder, a serum creatinine of 1.2 mg/dL (110 μmol/L) can indicate significant renal disease in an elderly female. More important than absolute creatinine level is the trend of serum creatinine levels over time. Creatinine levels may increase when ACE inhibitors (ACEI) or angiotensin-II receptor blockers (ARBs) are taken. Using both ACEI & ARB concomitantly will increase creatinine levels to a greater degree than either of the two drugs would individually. An increase of <30% is to be expected with ACEI or ARB use.

2.9.2 The creatine kinase

Creatine is a nitrogenous organic acid that occurs naturally in vertebrates and helps to supply energy to all cells in the body, primarily muscle. Creatine kinase (CK), also known as phosphokinase is an enzyme expressed by various tissues and cell types. CK catalyses the conversion of creatine and consumes Adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine phosphate (ADP). This CK enzyme reaction is reversible such that ATP can be generated from PCr and ADP (Bong et al., 2008). There are

three isoenzymes: CK-MM, CKBB and CKMB. The gene for the subunits are located on different chromosomes: B on 14q32 and Mon 19q13. In addition to the three cytosolic CK isoforms, there are two mitochondrial creatine kinase isoenzymes, the ubiquitous and sarcometric (Schlattner et al., 2006). In normal conditions, there is very little creatine kinase (CK) circulating in the blood of the average, healthy human being. Elevation of CK is indicative of damage to muscle and heart. It is therefore indicative of injury such as myocardial infarction, myositis, myocarditis or any cardiac or muscular disease.



Clinically, CK is often determined routinely in a medical laboratory. It is also determined specifically in patients with chest pain or if acute renal failure is suspected. Normal values are usually between 60 and 174 IU/L. Elevation of CK is an indication of damage to muscle. It is therefore indicative of injury, rhabdomyolysis (severe muscle breakdown), myocardial infarction (heart attack), myositis and myocarditis. There is an inverse relationship in the serum levels of T3 and CK in thyroid disease. In hypothyroid patients, with decrease in serum T3 there is a significant increase in CK. Therefore, the estimation of serum CK is considered valuable in screening for hypothyroid patients (Hekimsoy and Oktem, 2005). Lowered CK can be an indication of alcoholic liver disease and rheumatoid arthritis (Schlattner et al., 2006; Wallimann et al., 1994; Hekimsoy and Oktem 2005). Isoenzyme determination has been used extensively as an indication for myocardial damage in heart attacks. Troponin measurement has largely replaced this in many hospitals, although some centers still rely on CK-MB.

2.10 The Urea

Urea or carbamide is an organic compound with the chemical formula $(NH_2)_2CO$. The molecule has two amine ($-NH_2$) residues joined by a carbonyl (-CO-) functional group. Urea serves an important role in the metabolism of nitrogen-containing compounds by animals and is the main nitrogen-containing substance in the urine of mammals. Being solid, colourless, odorless (although the ammonia which it gives off in the presence of water, including water vapor in the air, has a strong odor), neither acidic nor alkaline, highly soluble in water, and relatively non-toxic, urea is widely used in fertilizers as a convenient source of nitrogen. Urea is also an important raw material for the chemical industry. The synthesis of this organic compound by Friedrich Wöhler in 1828 from an inorganic precursor was an important milestone in the development of chemistry. The terms urea and carbamide are also used for a class of chemical compounds sharing the same functional group RR'N-CO-NRR', namely a carbonyl group attached to two organic amine residues. Example includes carbamide peroxide, allantoin, and hydantoin. Ureas are closely related to biurets and related in structure to amides, carbamates, diimides, carbodiimides, and thiocarbamides (Greenan et al., 1995). Urea was first discovered in urine in 1773 by the French chemist Hilaire Rouelle. In 1828, the German chemist Friedrich Wöhler obtained urea by treating silver cyanate with ammonium chloride in a failed attempt to prepare ammonium cyanate (Williams, 2001).

$$AgNCO + NH_4Cl \rightarrow (NH_2)_2CO + AgCl$$

This was the first time an organic compound was artificially synthesized from inorganic starting materials, without the involvement of living organisms. The results of this experiment implicitly discredited vitalism: the theory that the chemicals of living organisms are fundamentally different from inanimate matter. This insight was important for the development of organic chemistry. His discovery prompted Wöhler to write triumphantly to

Berzelius: "I must tell you that I can make urea without the use of kidneys, either man or dog. Ammonium cyanate is urea." For this discovery, Wöhler is considered by many the father of organic chemistry. Urea is synthesized in the body of many organisms as part of the urea cycle, either from the oxidation of amino acids or from ammonia. In this cycle, amino groups donated by ammonia and L-aspartate are converted to urea, while L-ornithine, citrulline, L-argininosuccinate, and L-arginine act as intermediates. Urea production occurs in the liver and is regulated by N-acetylglutamate. Urea is found dissolved in blood (in the reference range of 2.5 to 7.5 mmol/liter) and is excreted by the kidney as a component of urine. In addition, a small amount of urea is excreted (along with sodium chloride and water) in sweat. Amino acids from ingested food which are not used for the synthesis of proteins and other biological substances are oxidized by the body, yielding urea and carbon dioxide, as an alternative source of energy (Sakami and Harrington 1963). The oxidation pathway starts with the removal of the amino group by a transaminase, the amino group is then fed into the urea cycle. Ammonia (NH_3) is another common by-product of the metabolism of nitrogenous compounds. Ammonia molecules are smaller, more volatile and more mobile than urea's. If allowed to accumulate, ammonia would raise the pH in cells to toxic levels. Therefore many organisms convert ammonia to urea, even though this synthesis has a net energy cost. Being practically neutral and highly soluble in water, urea is a safe vehicle for the body to transport and excrete excess nitrogen. In water, the amine groups undergo slow displacement by water molecules, producing ammonia and carbonate anion. For this reason, old, stale urine has a stronger odour than fresh urine (Greeman et al., 1995). The handling of urea by the kidneys is a vital part of human metabolism. Besides its role as carrier of waste nitrogen, urea also plays a role in the countercurrent exchange system of the nephrons, that allows for reabsorption of water and critical ions from the excreted urine. Urea is reabsorbed in the inner medullary collecting ducts of the nephrons (Walter, 2007); thus raising the osmolarity in the medullary interstitium surrounding the thin ascending limb of the loop of Henle, which in turn causes

water to be reabsorbed. By action of the urea transporter 2, some of this reabsorbed urea will eventually flow back into the thin ascending limb of the tubule, through the collecting ducts, and into the excreted urine. This mechanism, which is controlled by the antidiuretic hormone, allows the body to create hyperosmotic urine, that has a higher concentration of dissolved substances than the blood plasma. This mechanism is important to prevent the loss of water, to maintain blood pressure, and to maintain a suitable concentration of sodium ions in the blood plasma. In aquatic organisms the most common form of nitrogen waste is ammonia, while land-dwelling organisms convert the toxic ammonia to either urea or uric acid. Urea is found in the urine of mammals and amphibians, as well as some fish. Birds and saurian reptiles have a different form of nitrogen metabolism, that requires less water and leads to nitrogen being excreted in the form of uric acid. It is noteworthy that tadpoles excrete ammonia but shift to urea production during metamorphosis. Despite the generalization above, the urea pathway has been documented not only in mammals and amphibians but in many other organisms as well, including birds, invertebrates, insects, plants, yeast, fungi, and even microorganisms **(**Kurzer and Sanderson, 1956).

2.10.1 Medical uses of urea

Urea is used in topical dermatological products to promote rehydration of the skin. If covered by an occlusive dressing, 40% urea preparations may also be used for nonsurgical debridement of nails. This drug is also used as an earwax removal aid. Certain types of instant cold packs (or ice packs) contain water and separated urea crystals. Rupturing the internal water bag starts an endothermic reaction and allows the pack to be used to reduce swelling. Like saline, urea injection is used to perform abortions. Urea is the main component of an alternative medicinal treatment referred to as urine therapy. The blood urea nitrogen (BUN) test is a measure of the amount of nitrogen in the blood that comes from urea. It is

used as a marker of renal function. Urea labelled with carbon-14 or carbon-13 is used in the urea breath test, which is used to detect the presence of the bacteria *Helicobacter pylori* (*H. pylori*) in the stomach and duodenum of humans, associated with peptic ulcers. The test detects the characteristic enzyme urease, produced by *H. pylori*, by a reaction that produces ammonia from urea. This increases the pH (reduces acidity) of the stomach environment around the bacteria. Similar bacteria species to *H. pylori* can be identified by the same test in animals such as apes, dogs, and cats (including big cats) (Sakami and Harrington, 1963).

2.10.2 Urea analysis

Urea is readily quantified by a number of different methods, such as the diacetyl monoxime colorimetric method, and the Berthelot reaction (after initial conversion of urea to ammonia via urease). These methods are amenable to high throughput instrumentation, such as automated flow injection analyzers (Baumgartner et al., 2005); and 96-well micro-plate spectrophotometers (Greenan et al., 1995).

2.11 The Liver Enzymes

2.11.1 Aspartate amino-transferase (AST) formerly called serum glutamic oxaloacetic transaminase (SGOT). AST is normally found in red blood cells, liver, heart, muscle tissue, pancreas, and kidneys. Low levels of AST are normally found in the blood. When body tissue or an organ such as the heart or liver is diseased or damaged, additional AST is released into the bloodstream. The amount of AST in the blood is directly related to the extent of the tissue damage. After severe damage, AST levels rise in 6 to 10 hours and remain high for about 4 days. The AST test may be done at the same time as a test for alanine aminotransferase, or ALT. The ratio of AST to ALT sometimes can help determine whether the liver or another organ has been damaged. Both ALT and AST levels can test for liver damage. An aspartate

aminotransferase (AST) test is done to: Check for liver damage and identify liver disease, especially hepatitis and cirrhosis.

2.11.2 Alanine amino-transferase (ALT) is an enzyme found primarily in the liver and kidney. It was originally referred to as serum glutamic pyruvic transaminase (SGPT). Normally, a low level of ALT exists in the serum. ALT is increased with liver damage and is used to screen for and/or monitor liver disease/injury. Alanine aminotransferase (ALT) is usually measured concurrently with AST as part of a liver function panel to determine the source of organ damage. It catalyzes the transfer of an amino group from alanine to α-ketoglutarate, the products of this reversible transamination reaction being pyruvate and glutamate.

glutamate + pyruvate ⇌ α-ketoglutarate + alanine (Wang et al. 2012).

2.11.3 Alkaline phosphatase (**ALP**, **ALKP**) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins, and alkaloids. The process of removing the phosphate group is called *dephosphorylation*. As the name suggests, alkaline phosphatases are most effective in an alkaline environment. It is sometimes used synonymously as **basic phosphatise.** Alkaline phosphase is found in all tissues of the body, but most of it is derived from the liver, bile duct, kidney, bone, and the placenta (Kim and Wyckoff, 1991).

2.12 History of Petroleum

One of the first wells that produced oil which was drilled near Marietta, Ohio, in 1814 (Hildreth 1833 P. 64). The well was actually drilled for salt water; the oil was a useless by product which often spoiled the well. The Ohio well was almost 500 feet deep and produced about a barrel or 50L of oil per week, which worth about 50-75 cents / gallon.

In 1818, another salt well produced oil in south-eastern Kentucky. It was known as the 'Beatty well", named after the owner of the land on which it was drilled (Shepherd, 1988). Since then, various discoveries have been made which have contributed immensely to the economy of the world.

2.12.1 History of the Nigerian petroleum industry

Crude oil was discovered in commercial quantity in Nigeria in 1956 at Oloibiri in the Niger Delta. The discovery was made by Shell – BP, at the time the sole concessionaire. Nigeria joined the ranks of oil producer in 1958 when its first oil field came on stream producing 5, 100 barrels per day (bpd).

2.12.2 Petroleum

Petroleum or crude oil is a naturally occurring, toxic, flammable liquid consisting of a complex mixture of hydrocarbons of various molecular weights, and other organic compounds, that are found in geologic formations beneath the Earth's surface. Petroleum is recovered mostly through oil drilling. It is refined and separated, most easily by boiling point, into a large number of consumer products, from gasoline and kerosene to asphalt and chemical reagents used to make plastics and pharmaceuticals (Kvenvolden, 2006). Petroleum is a mixture of a very large number of different hydrocarbons; the most commonly found molecules are alkanes (linear or branched), cycloalkanes, aromatic hydrocarbons, or more complicated chemicals like asphaltenes. Each petroleum variety has a unique mix of molecules, which define its physical and chemical properties, like color and viscosity (British Petroleum, 1975). The alkanes, also known as paraffins, are saturated hydrocarbons with straight or branched chains which contain only carbon and hydrogen and have the general formula C_nH_{2n+2}. They generally have from 5 to 40 carbon atoms per molecule, although

trace amounts of shorter or longer molecules may be present in the mixture. The alkanes from pentane (C_5H_{12}) to octane (C_8H_{18}) are refined into gasoline (petrol), the ones from nonane (C_9H_{20}) to hexadecane ($C_{16}H_{34}$) into diesel fuel and kerosene (primary component of many types of jet fuel), and the ones from hexadecane upwards into fuel oil and lubricating oil. At the heavier end of the range, paraffin wax is an alkane with approximately 25 carbon atoms, while asphalt has 35 and up, although these are usually cracked by modern refineries into more valuable products. The shortest molecules, those with four or fewer carbon atoms, are in a gaseous state at room temperature. They are the petroleum gases. (Ebbing, 2005)

2.13 The Hydrocarbons

Hydrocarbons are heterogeneous group of organic substance that are primarily composed of carbon and hydrogen molecules. They are quite abundant in modern society. Some of the most commonly used hydrocarbons include gasoline, lubricating oil, motor oil, mineral spirits, lighter fluid / naphtha, lamp oil and kerosene. Other common sources of hydrocarbons include dry cleaning solutions, paint, spot remover, rubber cement and solvents. In addition, many volatile substances that contain hydrocarbons (e.g. glue propellants) are commonly abused for their euphoric effect (Micheal et al., 2009).

2.13.1 Classification of hydrocarbons

Hydrocarbon can be classified as being aliphatic, in which the carbon moieties are arranged in a linear or branched chain or aromatic in which the carbon moieties are arranged in a ring. (Michael et al., 2009).

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of chemicals that occur naturally in coal, crude oil and gasoline. PAHs also are present in products made from fossil fuels, such as coal-tar pitch, creosote and asphalt. When coal is converted to natural gas, PAHs can

be released. PAHs are found throughout the environment in the air, water and soil, and can remain in the environment for months or years. Levels of PAHs in urban air may be 10 times greater than those found in rural areas. Polycyclic aromatic hydrocarbons (PAHs) are considered human carcinogens and are encountered in a wide range of occupational settings such incomplete combustion, pyrolysis of fossil fuels, coal and coke production, and iron and steel foundries, as well as in the ambient environment in air pollution and cigarette smoke. PAHs exposure has been found to provoke an imbalance in cardiac autonomic control, as well as stimulating sympathetic nerve activity.

Benzo (a) pyrene is a classic PAH and carcinogen, found in the environment. It has been found to have the ability to reach the brain tissue by crossing the blood-brain barrier or directly passing through olfactory nerve and accumulate in cerebellum (Mei Zhang et al. 2008). Hydrocarbons can be derived from either petroleum or wood. Petroleum distillates include petrol, diesel, kerosene, gasoline and naphta, whereas wood derived hydrocarbons include truponte and pine oil (Micheal et al., 2009). Hydrocarbons are obtained by destructive distillation or carbonization of coal (heating coal in the absence of air) at 1000-1300^{0}C to produce coal gas and coal tar (Olajire and Oderinde, 1996).

2.13.2 Categories of exposure to hydrocarbons

Hydrocarbon exposure can be divided into the following broad categories;

i. Non-intentional non-occupational exposure
ii. Recreational exposure
iii. Occupational exposure
iv. Intentional exposure

i. **Non-intentional non-occupational exposure;** Accidental ingestion is the most frequent type and commonly involved young children tasting hydrocarbon. Typically, children do not drink large quantities as hydrocarbons generally taste

bad. Adults and older children. Occasionally consume hydrocarbon if liquid is placed in an unlabelled can or bottle resulting in accidental ingestion.

ii. **Recreational exposure:** Inhaling hydrocarbon or other volatile solvents for the purpose of producing a transient state of euphoria is now common. This is common in junior high and high school aged children.

iii. **Occupational exposure:** this type of exposure is most often industrial a worker has either a dermal exposure to the liquid or an inhalational exposure to the vapours.

iv. **Intentional exposure:** this type of exposure usually involves consuming a large amount of the hydrocarbon as an oral ingestion during a suicide attempt.

2.14 Effects of Hydrocarbons on Body Systems

Research on health disorders resulting from petroleum based chemicals used in consumer products and job environments has become something to reckon with. Petroleum based chemicals are being found to cause significant nutritional effects for the nervous system and immune system after a prolonged exposure. Illnesses identified in a chemical research include;

i. Adult and child cancer

ii. Numerous neurological disorder

iii. Immune system weakening

iv. Autoimmune disorders

v. Asthma and allergies

vi. Infertility and abortion/miscarriage

vii. Learning disability and mental retardation (Richard et al., 2009).

2.14.1 Effect of hydrocarbons on the cardiovascular system

Exposure to hydrocarbons can result in cardio-toxicity. Cardiac manifestation of hydrocarbons effects are thought to be responsible for numerous reports of sudden death with abuse by inhalation. It has been reported that patients exposed to hydrocarbons may complain of dispel or syncope. In addition, because of sensitization of the myocardium to catecholamine, a relatively young and previously healthy patient can present with a full cardiac arrest after being suddenly startled or following strenuous athletic events. A common scenario for the cardiac arrest patient was a teenager who was bagging alone in a dark room and got startled when his parent opened the door. This was possibly as a result of hypoxia and ventricular tachycardia following a large catecholamine exposure to the myocardium. This syndrome is more common following exposure to halogenated hydrocarbons as well as aromatic hydrocarbon. Most importantly, the mechanism is believed to be sensitisation of the myocardium to endogenous catecholamines which can predispose the patient to dysarhythmias (tachydysrhythmias) and hypotension which can result in syncope or sudden death (Wax and Uhler, 2006).

2.14.2 Effects of hydrocarbons on the pulmonary system

Pulmonary complications, especially aspiration of hydrocarbon, are the most frequently reported adverse effects of hydrocarbon exposure while most aliphatic hydrocarbons have little gastrointestinal absorption, aspiration frequently occurs, either initially or in a semi delayed fashion as the patient coughs or vomits, thereby resulting in pulmonary effects, once aspirated, the hydrocarbons can create a severe pneumonias.

Hydrocarbon pneumonitis results from a direct toxic effect by the hydrocarbon on the lung parenchyma. The type II pneumocytes are most affected, resulting in decreased surfactant production. This decrease in surfactant, results in alveolar collapse, ventilation

perfusion mismatch and hypoxemia. Hemorrhagic alveoli can subsequently occur, which peaks 3 days after ingestion. The end result of hydrocarbon aspiration is interstitial inflammation, intra- alveolar hemorrhage and edema, hyperemia, bronchial necrosis and vascular necrosis, rare pulmonary complications include the development of pneumothorax, pneumatocele fistula.

2.14.3 Effect of hydrocarbons on the nervous system

The central nervous system (CNS) toxicity occasionally observed following hydrocarbon exposure appears to be indirect and secondary to the pulmonary involvement. Experiments using paraffin in balloons suggest that the primate brain is resistant to the direct effect of kerosene; and that the most potent cause of CNS damage is hypoxia or simply asphyxiation secondary to pneumonitis. Many of the hydrocarbons that affect CNS directly are able to make their way across the blood brain barrier because certain hydrocarbons are highly lipophilic. In addition for individuals who are huffing or bagging the act of rebreathing can result in hypercarbia, which can contribute to decreased level of arousal. Prolonged abuse of hydrocarbons can result in white matter degeneration (leukoencephalopathy) and atrophy. In addition, prolonged exposure to certain hydrocarbon (e.g. n-hexane, methyl-n-butyl ketone (MnBk) can result in peripheral neuropathy, blurred vision, sensory impairment, muscle atrophy and parkinsonism (Rhodes et al., 2005).

2.14.4 Effects of hydrocarbons on the liver

The chlorinated hydrocarbons in particular carbon tetrachloride, are hepatotoxic. Usually, the hepatotoxixcity results after the hydrocarbon undergoes phase 1 metabolism, thereby inducing free radical formation. These free radicals subsequently bond with hepatic macromolecules and ultimately cause lipid peroxidation; the metabolites create a covalent

bond with the hepatic macromolecules, thereby initiating lipid peroxidation (Hochommovit 1975). The common histopathologic pattern is centrilobular (zone 111) necrosis. Liver function tests results can be abnormal within 24 hours after ingestion of petroleum product and clinically apparent jaundice can occur within 48 – 96 hours. Methylene chloride, a hydrocarbon commonly found in paint remover, is metabolized via the P450 mixed function oxidase system in the liver to carbon monoxide. Unlike others, co exposure with methylene chlorine, formation can continue for a prolonged period of time (Anderson and Meeker, 1996).

2.14.5 Effects of hydrocarbons on the kidney

Long term (chronic) exposure to toluene, an aromatic hydrocarbon can result in a distal renal tubular acidosis and present with an anion gap acidosis. A patient may have chronic exposure either via an occupational environment or by repeated recreational inhalation. Glomerulo-nephritis and chronic tubulointerstitial nephritis has been associated with long term hydrocarbon exposure (Brouette and Anton, 2001).

2.14.6 Effect of Hydrocarbon on haemopoietic system

Prolonged exposure to certain hydrocarbon can lead to an increased risk of plastic anaemia, multiple myeloma, and acute myelogenous leukemia. In addition, haemolysis has been reported following the acute ingestion of various types of hydrocarbons possibly due to damage to red blood cell membrane and induction of lipolysis by lipid solubilisation. Petrol also contains a small amount of benzene (<1%), which is a known human carcinogen (Brouette and Anton, 2001).

2.14.7 Effects of hydrocarbons on the gastrointestinal (GIT) system

Many of the hydrocarbons create a burning sensation because they are irritating to the gastrointestinal mucosa. Vomiting has been reported in up to one third of all hydrocarbon exposure.

2.15 More Information on Exposure of kerosene to The Environment

Kerosene is found in the environment as a result of site or transport vehicle. Exposure to kerosene is common in modern society. In Nigeria, kerosene is the major household fuel, derived from petroleum distillates. It is one of the most dangerous hydrocarbons. Exposure to kerosene can occur through accidental ingestion (Nwafor, 1999). Dermal contact or oral ingestion in a suicide attempt.

2.15.1 Toxic effects of kerosene

The toxic effect of kerosene is related to its physical properties, which include high volatility, low viscosity and low surface tension. Highly volatile compound with low viscosity and low surface tension are likely to be inhaled or aspirated into the respiratory system. Kerosene is poorly absorbed after ingestion and absorption is rapid after inhalation or pulmonary aspiration (Nwafor, 1999). Acute kerosene intoxication in a near drawing event often results in severe respiratory and cardiac failure with a high fatality rate (Seger et al., 1999.

2.15.2 Kerosene poisoning in a child

Kerosene poisoning is common in modern society especially in the peadiatric age group and in community where kerosene is a major household fuel. The exact incidence of kerosene poisoning in Nigeria is not always reported. Generally, cases are under reported as some patients are asymptomatic. Kerosene, a petroleum distillate hydrocarbon is a central nervous system depressant, a gastrointestinal irritant, respiratory irritant but it is poorly absorbed after

ingestion, however absorption following inhalation or pulmonary aspiration is rapid. This can lead to vomiting

There is a case of 2 year old girl who was brought to the children emergency room of UNTH Enugu with attired consciousness and noisy breathing following kerosene ingestion.

The baby presented with the following signs

- Coughing and choking with a noisy breathing
- Vomiting (Emesis), an episode of seizure, Pink mucous membrane, respiratory distress, Well hydrated and febrile with a temperature of 38.6^0C, Comatose and in a state of unconsciousness (Nwafor, 1999)

2.15.3 Effects of kerosene on the pulmonary system

A case was reported of four patients who were drowned in a river contaminated by kerosene. The results of the clinical examination done on the patients were;

The four patients developed acute respiratory failure, cardio-myopathy was present in three patients and a persistent hypokalemia in two patients the onsets of the symptoms was delayed, which led to the underestimation of the severity of their illness. Two of the four patients died. The diagnosis of hydrocarbon intoxication was based on broncho-aveolar large results, neutrophilic alveolitic with the presence of lipid laden macrophages, and evidence of lipoid pneumonia from the autopsy performed on the patience (Seger et al., 1999).

2.15.4 Complications of kerosene poisoning

Aspiration pneumatic is the most common complication of hydrocarbon ingestion, followed by central nervous system and cardiovascular compilations. Though some cases may be asymptomatic, presently, acute respiratory distress is usually noticed as a result of pneumonitis and bronchospasm. These can also be intra alveolar haemorrhage.

Respiratory symptoms generally begin in the first few hours after exposure and usually resolve in 2-8 days. Complications include: Hypoxia, Bacterial pneumonia and emphysema, seizures and death.

Bacterial pneumonia is a result of secondary bacterial infection common organism implicated include staphylococcus pyrogenes, staphylococcus pneumonic haemophilis influenca and gram negative bacilli (Goldstein et al., 1999). Kerosene is not considered to be a cancer – causing substance (carcinogen) but repeated exposure of animals to kerosene has caused skin cancer (Chilcott, 2006).

2.16 Death from Petrol Inhalation after an Armoured Vehicle Rollover

An incident was reported of armoured vehicle that rolled over after the days exercise The 37 year old army major driving the vehicle lost control and this resulted in the vehicle toppling over into a ditch during which the patient sustained an injury to his head.

During the accident the upturned vehicle had spilt its load of petroleum fuel into the ditch in which the vehicle came to lie. This had the effect of allowing petroleum vapours to accumulate in the air being breathed by the casualty. The patient however remained trapped within the turret opening by his leg. He was conscious from time of the accident until he got arrested about five minutes later. During this time he was reportedly fully orientated and able to maintain a lucid conversation with the uninjured parties. He later died and post-mortem examination was conducted shortly after death and the following findings noted (Ahn et al., 2006; Daoji and Dag, 2004). The following were the findings:

The skull contained a 3 cm linear base of skull fracture ruining transversely in the middle cranial fossa on the left. The brain was generally swollen, no haemorrhages or contusions were seen and appearances were consistent with diffuse cerebral oedema. The bronchi contained heavily blood-stained fluid, there were bilateral pleural effusions

consisting of 500 ml of straw coloured fluid. The lung parenchyma was markedly congested and oedematous. The ventricles, atria valves, and endocardium appeared normal, the coronary arteries and aorta were free from atherosclerosis. The stomach contain 50 ml of slightly blood stained fluid, but otherwise normal the liver had a mottled appearance with areas of pallor on cut section consistent with fatty change and areas of congestion. The remainder of the gastrointestinal system was normal. The urogenital, lympho-reticular, endocrine and musculoskeletal systems were normal. Histological examination revealed normal myocardium, congested kidneys, with granular debris in Bowman's space consistent with shock, congested lungs with haemorrhage and oedema, brain tissue revealed no evidence of established ischemia or axonal retraction balls. Routine toxicological analysis revealed no evidence of any abnormalities.

The cause of death was recorded as:

1a cerebral oedema

1b Cardiac arrhythmia

1c Inhalation of petrol (gasoline) fumes.

Sudden death from inhalation of petroleum distillates is well recognized in misusers of volatile substances Fatal accidental exposure is usually associated with ingestion of the fuel during siphoning and fatal exposure during appropriate use is rare. There are several recognized mechanisms by which death from petroleum exposure may occur. Arrhythmias secondary to sensitization of myocardium to catecholamines, vasovagal events, CNS respiratory depression, hypoxia and hypercapnia all have been suggested and some are dependent on the mechanism of administration (Steffe et al., 1996; Chalmer, 1991).

The use of defibrillation in the above mentioned patient's case was delayed because of his remote location and lack of appropriate equipment, earlier defibrillation may have improved outcome, however the use of defibrillation in this environment brings the danger of explosion and probably would not have been feasible from a safety point of view.

The proposed mechanism of death in this case is myocardial sensitization to endogenous catecholamines and this is supported by the absence of any other significantly life threatening injury and the absence of any evidence to support the other mechanisms that have been proposed above. Vasovagal events are associated with the application of cold liquid or spray to the oropharynx (Steffe et al., 1996). CNS respiratory depression would be evidient from the history with a gradually reducing level of consciousness and there is no reason that the patient should have become hypoxic or hypercapnic from the history. The authors are unaware of any specific toxicological or biochemical markers, which would support the conclusion that acute inhalation of petrol, caused the death.

Conclusively, toxicity from hydrocarbon exposure can be thought of as different syndromes, depending on which organ system is predominately involved. Organ systems that can be affected by hydrocarbons include the pulmonary, neurologic, cardiac, gastro intestinal, hepatic, renal, dermatologic and hematologic systems, the pulmonary system is the most commonly involved system.

2.17 Benzene

2.17.1 Major Uses and Sources of Emissions

Benzene is a natural constituent of crude oil, and is most basic petrochemicals. It is an aromatic hydrocarbon and the second [n]- annulene ([6]-annulene, a cyclic hydrocarbonwith continuous pi bond. Benzene is colourless and highly flammable liquid with sweet smell.. It is an important component of gasoline because it has a high octane number.

in the course of natural processes and human activities that involve the combustion of organic matter such as wood, coal and petroleum products. The main industrial use of benzene is as a starting material for the synthesis of other chemicals. Most benzene feedstock is imported, but some is manufactured at an Australian steelworks as a by-product of coal coking. Large

quantities of benzene are produced during the refining of petroleum and retained as a component of petrol. Petrol vehicle emissions are the predominant source of benzene in the environment. (NICNAS, 2001) In the past, benzene has been widely used as a multipurpose organic solvent, however, this use has been actively discouraged. As gasoline (petrol) additive, benzene increases the octane rating and reduces knoking. As a consequence, gasoline often containedseveral percent benzene before 1950s, when tetraethyl lead replaced it as the mostwidely used antiknock additive. With the global phaseout of leaded gasoline benzene has made a comeback in some nations

2.17.2 Effects of Chronic Human Exposure

Benzene increases the risk of cancer and other illness. It is a notorious cause of bone marrow failure (Smith, 2010). Benzene has been clinically linked with aplastic anaemia acute leukemia and bone marrow abnormalities. Specifically associated with acute myeloid leukemia, aplastic anaemia, lymphoblastic leukemia and chronic myeloid leukemia. The critical human health effects from long term exposure to benzene are bone marrow depression and leukaemia, specifically acute non-lymphocytic leukaemia Benzene is classified as a human carcinogen. It is considered to be a genotoxic carcinogen for which no threshold has been established (NICNAS 2001, US EPA 2000, WHO, 2000).

2.17.3 Modes of Action

Several reviews of benzene metabolism and the proposed mechanisms of toxicity have been published (Ross, 1996; Snyder, 2000; Snyder et al., 1993; Snyder and Hedli, 1996; Yardley-Jones et al., 1991). Exposure to benzene can result in haematotoxicity, immunotoxicity and carcinogenicity in humans and animals. Haematotoxicity resulting from chronic benzene exposure can present as anaemia, aplastic anaemia, leukopenia, lymphocytopenia,

thrombocytopenia, or pancytopenia (Aksoy, 1989). While the liver is the initial site for the biotransformation of benzene, hepatotoxicity is not a consequence of benzene exposure. Subsequently, these metabolites become localised within the bone marrow (Rickert et al., 1979) where they undergo activation by peroxidase enzymes, which are present in bone marrow. While individual benzene metabolites appear not to induce bone marrow toxicity, the combination of phenol and hydroquinone has been shown to induce the same effects on bone marrow as benzene (Eastmond et al., 1987). This effect appears to be due to the ability of phenol to act as a co-oxidant in the activation of metabolites.

2.18 The Animals used in This Study

The Sprague Dawley rat is an outbred multipurpose breed of albino rat used extensively in medical research (Mordes et al., 2007). Its main advantage is its calmness and ease of handling. This breed of rat was first produced by the Sprague Dawley farms (later to become the Sprague Dawley Animal Company) in Madison, Wisconsin. These rats were first bred in 1925. The breeding facilities were purchased first by Gibco and then by Harlan (now Harlan Sprague Dawley) in January 1980. The average litter size of the Sprague Dawley rat is 10.5 rats. The adult body weight is 250–300 g for females, and 450–520 g for males. The typical life span is 2.5–3.5 years. These rats typically have increased tail to body length ratio compared with Wister rats (Krinke et al., 2000).

This work is therefore aimed at studying the effect of diesel, kerosene and petrol, on cardiovascular functions and possible role of oxidative stress as part of the mechanism of action.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 The Petroleum Products

The petroleum products used were bought from Total Petrol Station Mushin Lagos; and kept sealed in plastic container, throughout the study period except when required for use. The petroleum products were identified and analysed by staff of chemistry department of the University of Lagos, Akoka, Lagos, Nigeria; using gas chromatography mass spectrometer (GCMS). Distilled water was used in administering the petroleum products to the rats to avoid any form of impurity.

3.2 The Animals used

Adult male Sprague Dawley rats randomly selected from the same source with weight range of (140-200g) were used. The age ranges from 12-15 weeks as at the onset of the acclimatisation. They were obtained from the Animal House of Ladoke Akintola University of Technology, Ogbomoso. They were housed in plastic cages with steel perforated cover and maintained under standard conditions. The feed used was pelleted mash made by Livestock feeds, Nigeria LTD, Mushin, Lagos. The was always crushed to powder after weighing every day. Feed and water were provided *ad- libitum*. The rats were identified at the Department of Cell Biology and Genetics University of Lagos.

A total of four hundred and fifty rats were used for the whole experiment. Fifty rats were used for preliminary studies using various previous researchers methods that would be adopted. The project work was done in three phases. The first phase was designed to study blood pressure, heart rate, baroreflex activities and histology. The second phase was to determine all biomedical, antioxidant and enzyme activities; while the third phase was for further study of blood pressure, and observation of post- exposure recovery. One hundred and

fifty gram of the livestock feed was given to the ten rat in all the groups using the formula of Perret-Gentil, (2004). Each rat was designed to have 15g/day.

At each of the phases, the rats were divided into four main groups (control, diesel, kerosene and petrol). Each of the groups except control was then subdivided into three (ingestion, inhalation and water contaminated groups) with **ten** rats in each sub-group. The animals were acclimatized for two weeks, before the commencement of the study. The control was not exposed to any treatment. The diesel, kerosene and petrol sub-groups, were exposed to their corresponding contaminants via food ingestion, inhalation and water contamination respectively. Each administration and exposure lasted eight weeks. These three routes of administration were so chosen because they are the common routes through which humans are exposed

3.3 Grouping of the Animals

3.3.1 Control Group - Control rats were not exposed to any petroleum product

3.3.2 Diesel group:

DFG- (Diesel food ingestion group): Rats fed with1.5 % diesel-contaminated feed (Hayes et al., 2010).

DIH- (Diesel Inhalation group): Rats made to inhale diesel vapour for five minutes as done by Uboh et al., (2005).

DWC- (Diesel water contaminated group) Rats in this group had 2.5ml/kg body weight of water contaminated with diesel orally (Anigbogu andOjo, 2009).

3.3.3 Kerosene group

KFG- (Kerosene food ingestion group) Rats fed with1.5% kerosene contaminated feed

KIH- (Kerosene inhalation group) Rats made to inhale kerosene vapour for five minutes

KWC- (Kerosene water contamination group) Rats in this group had 2.5ml/kg body weight of water contaminated with kerosene orally.

3.3.4 Petrol group

PFG- (Petrol food ingestion group): Rats fed with1.5% petrol contaminated feed (Hayes et al., 2010).

PIH- (Petrol inhalation group): Rats were made to inhale petrol vapour for five minutes

PWC- (Petrol water contaminated group): Rats in this group had 2.5ml/kg body weight of water contaminated with petrol orally.

There were 10 rats in each group, all of them were given feed and water *ad-libitum*. At the end of the of exposure period, all the rats were subjected to experimentation

3.4 Procedure for Exposing the Rats

3.4.1 The control group: Animals in this group were given normal feed and water *ad-libitum*. They were not exposed to any petroleum product, but were handled in the same manner as test groups

3.4.2 DFG group

Rats in this group were given feed mixed with diesel at rate of 15ml of diesel per 1000g of feed daily, for eight weeks (Hayes et al., 2010). The feed was made available *ad-libitum*. The left-over was measured in order to know the consumption rate. Left over measure whenever there was.

3.4.3 DIH Group

A modified nose inhalation exposure method described by Uboh et al. (2005) was used. The cages housing the test groups were placed in respective exposure chambers with calibrated beakers of 1000cm^3 containing 500cm^3 of diesel. The diesel was allowed to evaporate freely within the exposure chambers at ambient humidity and temperature. All animals were exposed to vapours 5 minutes daily generated from direct evaporation of the diesel. At the end exposure each day, the animals were transferred to free section of the animal house. The initial and final volumes of diesel were recorded before and after daily exposure. The daily differences in volume were used to estimate relative concentration of vapours used in this exposure method. The reactions of the rats were noted in each case. The rats were given normal feed and water *ad-libitum* thereafter.

3.4.4 DWC group

Water mixed with diesel at ratio 1:1 was administered to rats at the rate of 2.5ml/kg body weight daily for eight weeks (Anigbogu and Ojo, 2009). The mixing was done by shaking. The contaminated water was given orally using the oral cannula to deposit it in the esophagus. The rats were given normal feed and water *ad-libitum*.

The Kerosene (KFG, KIH, and KWC) and petrol (PFG, PIH, and PWC) groups were treated the same way as described above for the diesel group. Left over was measured in all cases when there was any, for the diesel, kerosene and petrol groups.

3.4.5 Limitation

In this study, dermal contact as route of administration could not be used because earlier attempt resulted in exposing the rats to skin infection. The rats have fur and thick hair as a barrier for skin study, shaving the hair on the skin exposes them to infection and other environmental hazards that could later the results

Procedures involving animals and their care were performed in accordance with the

guidelines of the Institution Animal Ethics Committee and NIH guidelines for the care and use of animals.

3.5 Studies on effect of Petroleum Products on Blood Pressure, Heart Rate and Baroreflex Responses

3.5.1 Anaesthesia

Rats were anaesthesized with 1% chloralose and 25% urethane mixture (BDH chemicals limited, Poole, England) given intra-peritoneally at a standard dose of 5ml/ kg body weight. ` The anaesthetic agent was stored in the refrigerator at 4^{o}C until use. At the time of use, it was always warmed by placing it in boiling water for about 5 min. before administering to rats.

The anaesthesia was maintained with additional 0.5ml of the urethane/chloralose mixture intravenously through the femoral vein at 30 minutes intervals to maintain a state of light surgical anaesthesia throughout the period of the experiment.

The degree of anaesthesia was determined by checking for the absence of the corneal reflex, lack of response to painful stimuli such as pinching the sole of the lower limb, lack of pinkish coloration of the tongue and diminished or absence of muscular tone.

After ascertaining the induction of anaesthesia, the animal was placed supine on the dissecting board and fastened to it. All animals were dissected under the same condition. The surgical equipments were sterilized; they were washed/dried and heated in the oven after each experiment.

3.5.2 Cannulation of the Trachea

The trachea was exposed by blunt dissection and cannulated in all experiment to improve alveolar ventilation. All tissue and fascia around were neatly teased out and loop of ligature placed on it. An incision was then made in between two tracheal rings. A polyethylene cannula with an internal diameter similar to that of the trachea was inserted. The cannula was

firmly tied in place with a strong ligature. This allowed the animal to breath freely from the atmosphere.

3.5.3 Cannulation of the femoral artery

The femoral vessels were located in femoral triangle in the inguinal region together with the femoral nerve. An incision was made on the skin over this region and by careful blunt dissection, the vessels were exposed. The f**emoral artery** was cannulated for recording of systemic arterial blood pressure. The cannula was place centrally facing the direction of the heart. The **femoral vein** was cannulated centrally and was used for maintenance doses of anaesthesia.

3.5.4 Balancing of Polygraph

The blood pressure was measured by the pressure transducer using the Grass Polygraph model 7D (Grass instruments Limited, Quincy, Mass, USA). To balance the driver amplifier all control knobs were first turned to zero position and the input knob turned to “Bridge 2k” and the “½ amp High freq” switch turned to position “15” The polarity knob was set to “cal” position and a suitable baseline chosen using the “baseline position” switch. This sets the pen to a position corresponding to zero input from the preamplifier. The pre amplifier was balanced after the driver amplifier has been balanced. The driver amplifier was set on calibration and the baseline at a convenient position on the recording paper. The polygraph was balanced and the pressure transducer calibrated. The pen-writer adjusted to a standard deflection of 2cm representing 100mV. The sensitivity and balance voltage on the low level dc pre-amplifier was then set to maximum sensitivity (0.01mV/cm) and the balance voltage increased until the pen-writer returned to baseline. The polygraph speed was set up at 5mm/sec.

3.5.5 Measurement of blood pressure

The femoral artery was canulated, connected to the polygraph and pressure transducer for recording of blood pressure. The transducer was calibrated prior to use using mercury manometer. The phasic blood pressure was recorded on the tracing paper. Heart rate was calculated from the blood pressure tracing and Mean arterial blood pressure (MAP) calculated using the formula: MAP = systolic b.p. + 1/3 pulse pressure (Manzour et al., 2004; Klabunde, 2007).

3.5.6 Measurement of baroreflex response

The common carotid arteries lie deep along the side of the trachea. By careful dissection, the right and left carotid arteries were lifted and separated from the vago- sympathetic nerve trunk. A proximal ligature tied around it to occlude blood flow. A bulldog clip was then placed distally as possible on the artery while a second ligature was placed loosely around it proximal to the bulldog clip. The carotid artery was occluded using the bulldog clip for 30 seconds and the blood pressure was also recorded continuously. From the tracing, the heart rate and MAP was determined every 5 seconds. Baroreflex response was then calculated continuously for 30 seconds from ratio of the change in the Heart rate (ΔHR) and change in MAP (MAP) every 5 seconds from 0 seconds to 30 seconds.

3.6 Measurement of Body and Organ Weight

Body weight of each rat in all the groups was taken every week for eight weeks. An initial weight was taken before the experiment began. This served as baseline. The mean weight gain/lose was found and percentage growth/weight gain, calculated from the overall mean weight gain/lose at the end of the eight weeks.

Afterwards, rats were sacrificed by cervical dislocation; each animal was fastened to the dissection board and midline incision made to excise the heart, kidney, brain, lungs and liver.

The organs were placed in the petri-dish containing normal saline they were flushed with the normal saline in order to dislodge the blood from its chambers; mopped with cotton wool, and weighed. The wet weight of the organs was estimated and the ratio of the organ weight (wet weight) to that of the body gave organ weight index. The procedure carried out according to Bailey et al., (2004) and Wimmer et al., (1985).

3.7 Procedure for Blood Collection

At the end of the eight weeks of each exposure, blood samples were collected from 5 rats from each group into plain blood sample bottles and ethylene-diamino-tetra-acetic acid (EDTA) contained sample bottles. The blood was collected by inserting capillary tube into the periorbital sinus and allowed to drop gently into the plain and EDTA contained sample bottles (Abatan et al. 2008). The blood in plain sample bottles were spun at 3,000 rpm for 15 minutes. The serum was then removed using the Pasteur's pipette into pendov tube and stored in the refrigerator at -20^{o}C until time of analysis. The blood collected into the EDTA bottles were gently rocked to avoid its clotting, kept at room temperature, for 30 minutes before carrying out the analysis. The Electrical haemo-analyser (Sysmetex KX-21N) was used to determine the following: Red Blood cell count (RBC), Haemoglobin (Hb) concentration, Packed cell volume (PCV), Platelets and White Blood count (WBC) were produced. Serum from blood collected into the plain bottles was analyzed for:

i. Liver enzymes activities: Alanine Amino Transferase (ALT), Aspartate amino transferase (AST), Alkaline phosphatase (ALP)
ii. Biochemical properties: total protein, albumin, creatinine and urea.
iii. Antioxidants activities: Catalase (CAT), Glutathione reductase (GSH), Superoxide dismutase (SOD), as well as concentration of Malondialdehyde (MDA).

3.8 Preparation of the Homogenate

At the end of the exposure, rats were sacrifice by cervical dislocation and the brain, heart, kidney, liver and lung were removed for antioxidant analysis. Each organ was removed rapidly, and cut into pieces, the tissues was homogenized in KCl (10 mM) phosphate buffer (1.15%) with ethylene-di amine tetra acetic acid (EDTA; pH 7.4) and centrifuged at 12,000 rpm for 30 minutes. The supernatant (post.mitochondrial fraction) was used to assay for the antioxidant enzymes- catalase, glutathione-reductase and superoxide dismutase according to the method of Alisi et al., (2011).

3.9 Studies on effect of Petroleum Products on Liver Enzyme and Biochemical Properties in Serum

The control and treated rats were sacrificed by cervical dislocation, the liver was excised out for further biochemical estimation. The estimation of aspartate transaminase (AST) and alanine transaminase (ALT) in liver were done by the method of Reitman and Frankel, (1957) reused by Paliwal et al., (2009). The data generated was analysed statistically according to Fischer and Yates 1950. Alkaline phosphatase (ALP) was estimated by flourometric method for the estimation and detection of serum alkaline phosphatase developed by Johnson, (1969); and used by Guilbault et al., (1971) using naphthol AS-MX phosphate as substrate.

- Creatinine level in serum was determined using the Dash and Sawhney 2002
- Urea was determined by the colorimetric method developed by Boale and Craft (1961) modified and used by Clark et al., (2007); by HPLC with fluorescence detection after automated derivation with xanthydrol

3.10 Studies on Effects of Petroleum Products on Biochemical Properties in Urine

Daily urinary collection of the rats was carried out using the metabolic cages. Each day a rat from each group was removed from the main cage and placed in a metabolic cage. It was left for 24 hours in the metabolic cage. The urine was collected through the funnel under the cage into a container in which few drops of toluene have been added to preserve the urine. At the end of the 24 hours the urine volume was measured and some removed to be stored in the freezer for determination of biochemical properties. Creatinine urea was determined as done for the serum sample above. Total protein and albumin was measured using the method of Savory et al., (1976).

3.11 Studies on Effects of Petroleum Products on Antioxidant Activities

3.11.1 Determination of catalase enzyme activity

Catalase activity was assayed by the method of Aebi (1974). A 0.1ml portion of serum was added to cuvette containing 1.9mL of 50mM phosphate buffer (pH 7.0). Reaction was started by addition of 1.0mL of freshly prepared 30mM H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically at 240nm using the equation for a first-order reaction. The result was expressed as units /mg protein where one unit is the amount of enzyme that hydrolysis 1 µmol of H_2O_2 per minutes and per milligramof protein at 30^oC and pH 8.0

Procedure

To 0.3ml (300) of extract sample, 1.8ml of 30ml M $H_2O_2$was added

Phosphate buffer was used as a blank and their absorbance readings were taken at 240nm, at 60s intervals for 5mins.

3.11.2 Determination of reduced Glutathione (GSH) activities

Reduced glutathione (GSH) was estimated by its reaction with dithio-bis-2-nitrobenzoic acid

(DTNB) that gives a yellow coloured complex with absorption maximum at 412 nm

Glutathione reductase activity was estimated by the method of the reaction mixture consists of 2.75mL of sodium phosphate buffer (0.1 M; pH 7.4), 0.1mL reduced glutathione (l mM), 0.1mL supernatant in a total volume of 3.0 mL. The changes in the absorbance was recorded at 340 nm and enzymes activity was calculated as nanomoles of 1-chloro-2,4-dinitro benzene (CDNB) conjugate formed/min/mg protein using a molar extinction coefficient of 9.6×103M−1 cm−1 (Raja et al., 2007; Habig et al., (1974).

Reagents

10mM DTNB

20nM EDTA

0.2 M Tris buffer

Procedure

100µl sample was added to 1ml of 0.2µTris- EDTA buffer pH 8.2

0.9ml of 20mM EDTA, pH 1.7 was added

20ml of 10mM DTNB was added and the sample was allowed to incubate at room temperature for 30minutes. The mixture was centrifuged and absorbance of the supernatant read against distilled water at 112nm

Reagent blank = 100ml distilled water + 1ml Tris- EDTA buffer +0.9 EDTA+20ml DTNB

Calculation

GSH = OD/∑×V/v

∑ = Extinction co efficient (1.3× 10-1 M-1 cm-1)

V = volume of sample in reaction mixture.

Where OD = absorbance

V = Total volume of reaction

3.11.3 Determination of Malondialdehyde concentration

Lipid peroxidation in the supernatant fractions was determined spectrophotometrically by assessing the concentration of thiobarbituric acid reactive substances (TBARS) according to the method of Ohkawa et al. (1979) as described by Liu et al. (1990). The results were expressed in malondiadehyde (MDA) formed relative to an extinction coefficient of 1.56 x 106 mol/cm. Lipid peroxidation (LPO) level was estimated by measuring the pink coloured chromophore formed by the reaction of thiobarbituric acid with malondialdehyde (MDA) to give a red specie absorbing at 535nm

Reagents

Stock TCA-HCL reagent

15%w.v tri-chloroacetic acid

0.375%w/v thiobarbituric acid

0.25N hydrochloric acid

This solution may be mildly heated to assist the dissolution of the thiobarbituric acid

Procedure

Combine 1.0ml of sample with 2.0 ml of TCA-HCL and mix thoroughly. The solution is heated for 15min. in a boiling water bath. After cooling, the flocculent precipitate is removed by centrifugation at 1000g for 10min. the absorbence of the sample is determined at 535nm against a blank that contains all the reagent minus the sample The Malondialdehyde concentration of he sample can be calculated using an extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$

Calculation

MDA (nmol/ml) = OD/$\sum$ ×V/v

OD = Absorbance(0ptica density) of salt

$\sum$ = Molecular extinction coefficient

V = Total volum of reacting sample

V = volume of the sample.

3.11.4 Determination of superoxide dismutase (SOD) Activities

Superoxide dismutase (SOD) activity was assayed as formerly described by Fridovich (1989). The ability of the superoxide dismutase to inhibit the autoxidation of adrenaline was the basis of the SOD assay. This is determined by the increase in absorbance at 320nm. The reaction was carried out in 0.05m sodiumcarbonate buffer pH 10.2 and was initiated by the addition of 3×10^{-1} epinephrine in 0.005N HCl

Reagents

3× 10-1 M epinephrine

0.05M sodium carbonate

0.005N hydrochloric acid

Procedure

Sample—0.02ml

Buffer—3.00ml

Epinephrine—0.03ml

Blank reagent—3.00ml buffer +0.02ml water +0.03ml epinephrine

Absorbance measured at 320nm for 3-5mins.

Calculation

$A_{320}/\sum \times V/v$ A_{320} = change in absorbance at 320nm/min.

$\sum$ = Molar extinction coefficient (4020M cm-1)

V = total volume of reacting sample v= volume of the sample

3.12 Studies on Effects of Petroleum Products on Histological Analysis of Some Visceral Organs

The heart, brain, liver, lungs and kidneys collected, were later fixed in buffered formalin solution for 24 hours and removed from the solution.

They were dehydrated through ascending grade of absolute alcohol (10%, 20%, ... 80%, 90%); when dehydration was completed, the tissue were cleared in xylene, infiltrated and embedded in paraffin wax.

Thin section of about 5micron was cut on rotary microtome. Five sections from each organ were mounted per slide and stained with heamatoxyline and Eosin (H and E) (Carleton's histological technique, 4^{th} edition)

3.13 Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) of a complete randomized design model, while significant difference were separated using Duncan multiple range test. All analysis is done using Graph Pad Prism 5. P value of less than 0.05 was taken as statistically significant. All results were presented as Mean ±SEM. Tables, histogram and line graph were used for graphical presentation.

CHAPTER FOUR

4.0 RESULTS

4.1 Effects of Petroleum Products on Blood Pressure (mmHg)

The Systolic blood pressure (SBP) in all diesel groups (DFG: 203±1.23mmHg, DIH: 139.1±0.4 mmHg, DWC: 144.8±1.36 mmHg) increased significantly ($p<0.05$) when compared with control (115.9±1.27 mmHg) (figure7). The effect was highest in DFG and lowest in the DIH.

The diastolic blood pressure (DBP) in all diesel groups (DFG: 173±1.23mmHg, DIH: 112.8±0.49 mmHg, DWC: 103±1.23 mmHg) increased significantly ($p<0.05$) when compared with control (81.8±1.96 mmHg), when compared with other groups the effect was highest in the DFG and lowest in the DWC subgroup.

The Mean arterial blood pressure (MAP) increased significantly ($p<0.05$) in all diesel groups (DFG: 183±0.97 mmHg, DIH: 121.5±0.36 mmHg, DWC: 116.9±1.13 mmHg) compared with control (93.13±1.6). It was highest in the DFG and lowest in the DWC subgroup.

The systolic blood pressure (SBP) increased significantly in all kerosene groups (KFG: 148.4±1.17 mmHg, KIH: 160.0±0.84 mmHg, KWC: 181.2±0.73 mmHg) compared with control (115.9±1.27 mmHg), the value was highest in the KWC) and lowest in KFG (figure 8). The diastolic blood pressure (DBP) and MAP in all kerosene groups (KFG: 87.2±0.37 mmHg, KIH: 105.2±1.02 mmHg, KWC: 155.4±1.03 mmHg) increased significantly ($p<0.05$) when compared with control, the value was highest in KWC and lowest in KFG (figure 8).

The SBP (PFG-104.4±0.74 mmHg, PIH-196.2±1.74 mmHg, PWC-135.6±1.29 mmHg) DBP (PFG-73.6±3.54 mmHg, PIH-135.8±0.73 mmHg, 98.2±1.11 mmHg) and MAP (83.87±2.56, 155.9±0.73 mmHg, 110.7±1.14 mmHg) increased significantly ($p<0.05$) compared with control (115.9±1.27 mmHg, 81.8±1.96 mmHg, 93.13±1.6 mmHg). The increase was highest in the PIH. However there was insignificant reduction in the petrol food ingestion group (PFG) when compared with control (figure 9). Exposure to diesel appeared to have stimulated the highest increase 203.0±1.23 mmHg) in blood pressure compared with petrol (196.2±1.74 mmHg) and kerosene (181.2±0.84 mmHg).

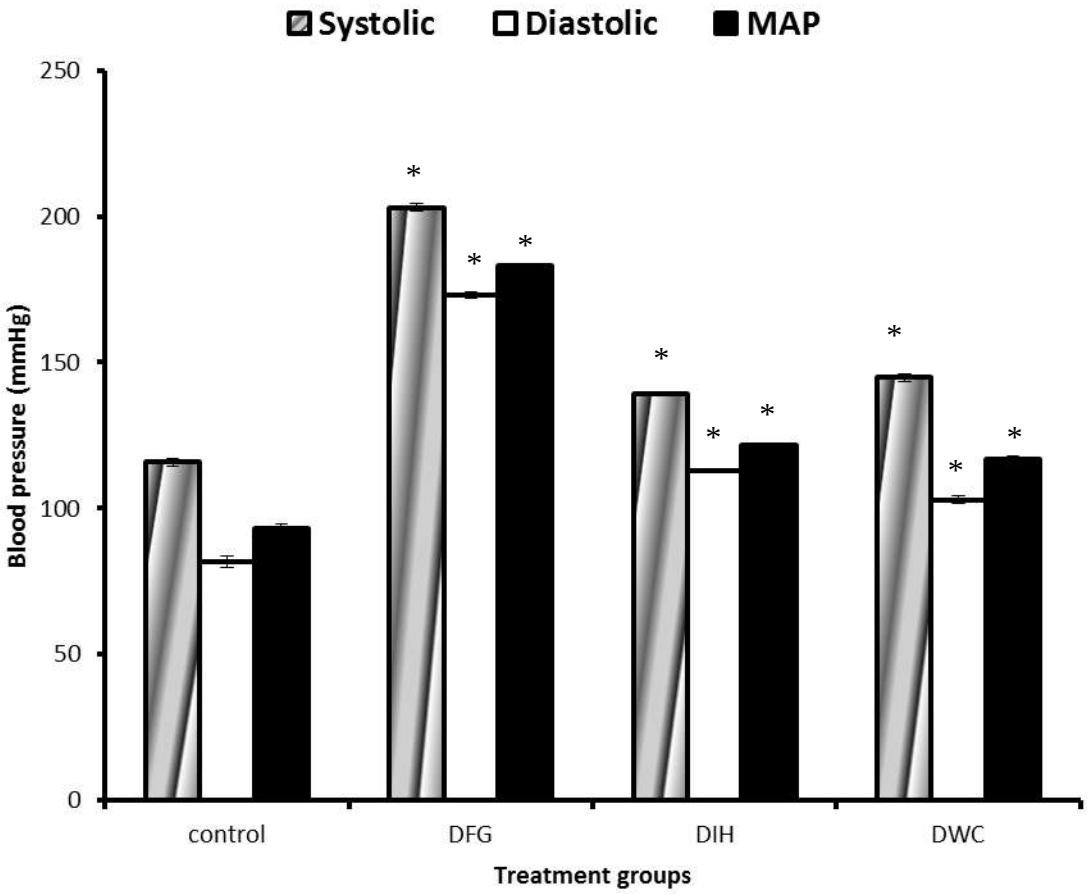


Figure 7: Effects of diesel on blood pressure of adult male rats

DFG- Diesel food ingestion group,

DIH- Diesel inhalation group and

DWC- Diesel water contaminated group;

Result presented as Mean ± SEM,

*= $p<0.05$; *=significantly increased

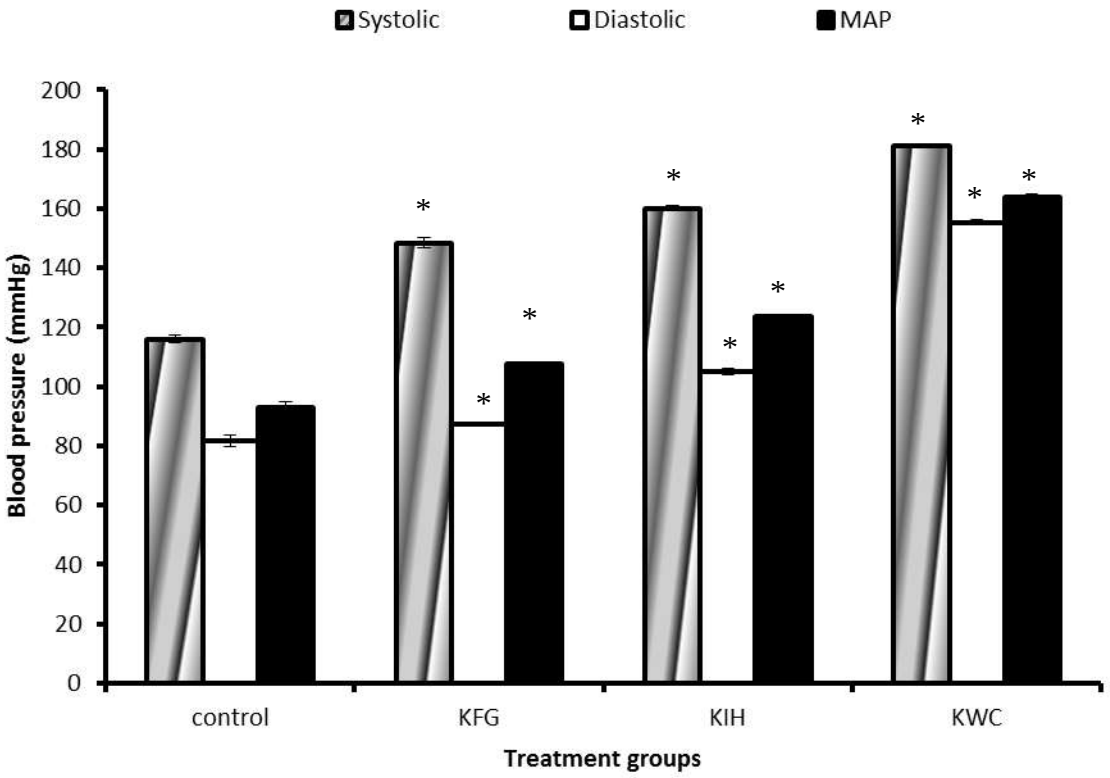


Figure 8: Effects of kerosene on blood pressure of adult male rats

KFG- kerosene food ingestion group,

KIH- kerosene inhalation group

KWC- kerosene water contaminated group;

Result presented as Mean ± SEM,

*= $p<0.05$; *=significantly increased

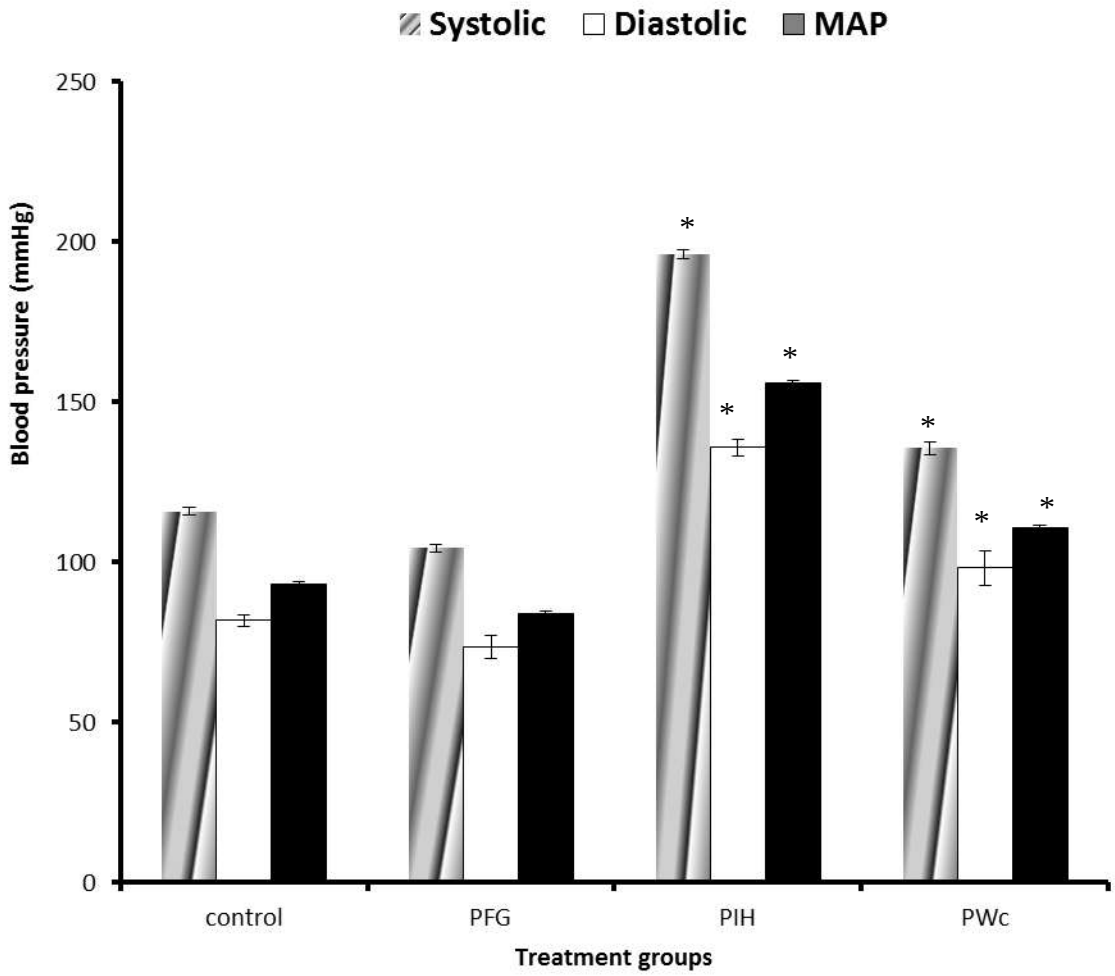


Figure 9: Effects of petrol on blood pressure of adult male rats

PFG- petrol food ingestion group,

PIH- petrol inhalation group

PWC- petrol water contaminated group.

Result presented as Mean ± SEM

*= $p<0.05$; *=significantly increased

4.2 Effects of Petroleum Products on Heart Rate

The results in figure 10 showed that there was significant increase ($p<0.05$) in the heart rate (HR) of all diesel groups (DFG: 502.4± b/min, DIH: 442.4± b/min, DWC: 466.6± b/min) when compared with control (415.4± b/min). However, the increase was most prominent in DFG group and least in DIH group when compared within groups.

Figure 11 result showed that there was significant increase ($p<0.05$) in the heart rate of (KIH: 444.4± b/min and KWC: 486.8±) group, when compared with control. However, the increase in the (KFG: 386.8± b/min,) group was insignificant.

Figure 12 result showed that there was significant increase ($p<0.05$) in the heart rate (HR) of (PFG: 463.2± b/min and PIH 451.2±: b/min) when compared with control. There was most no significant increase in the PWC group.

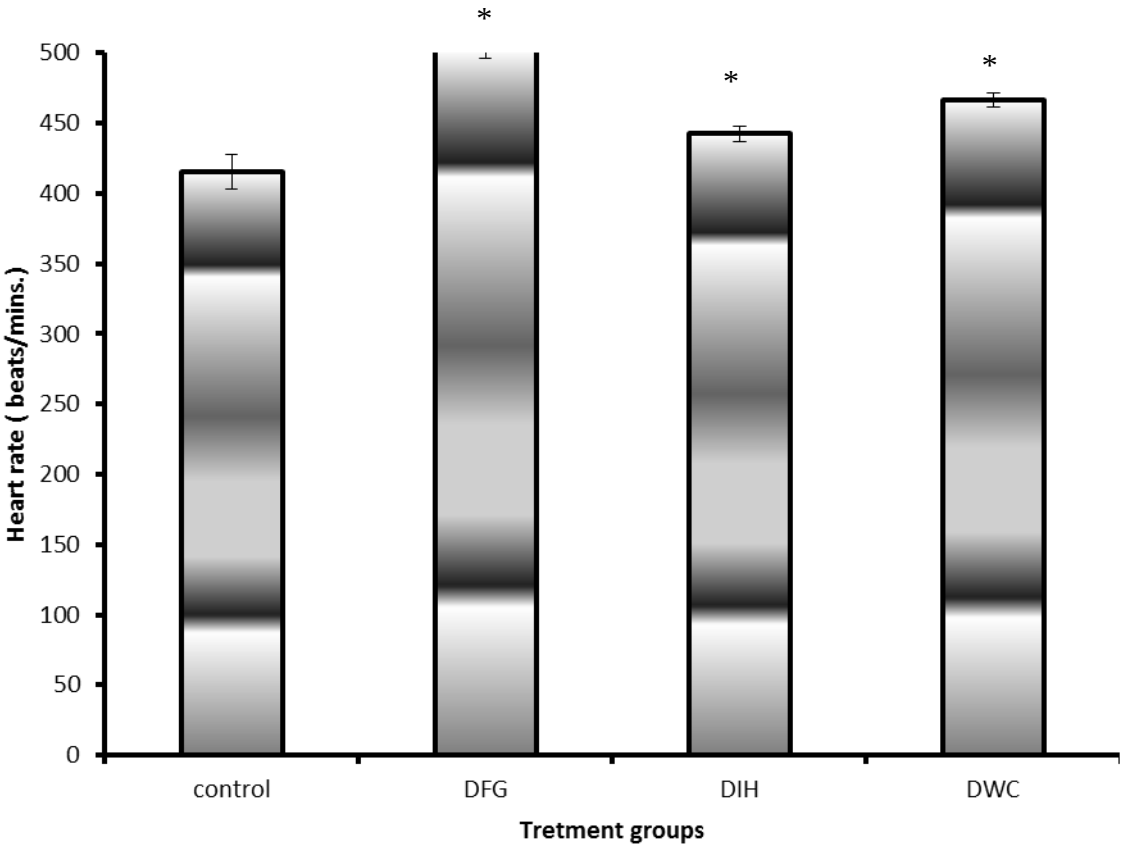


Figure 10: Effects of diesel on Heart Rate of adult male rats

DFG- diesel food ingestion group,

DIH- diesel inhalation group,

DWC- diesel water contaminated group.

Result presented as Mean ± SEM,

*= $p<0.05$; *=significantly increased

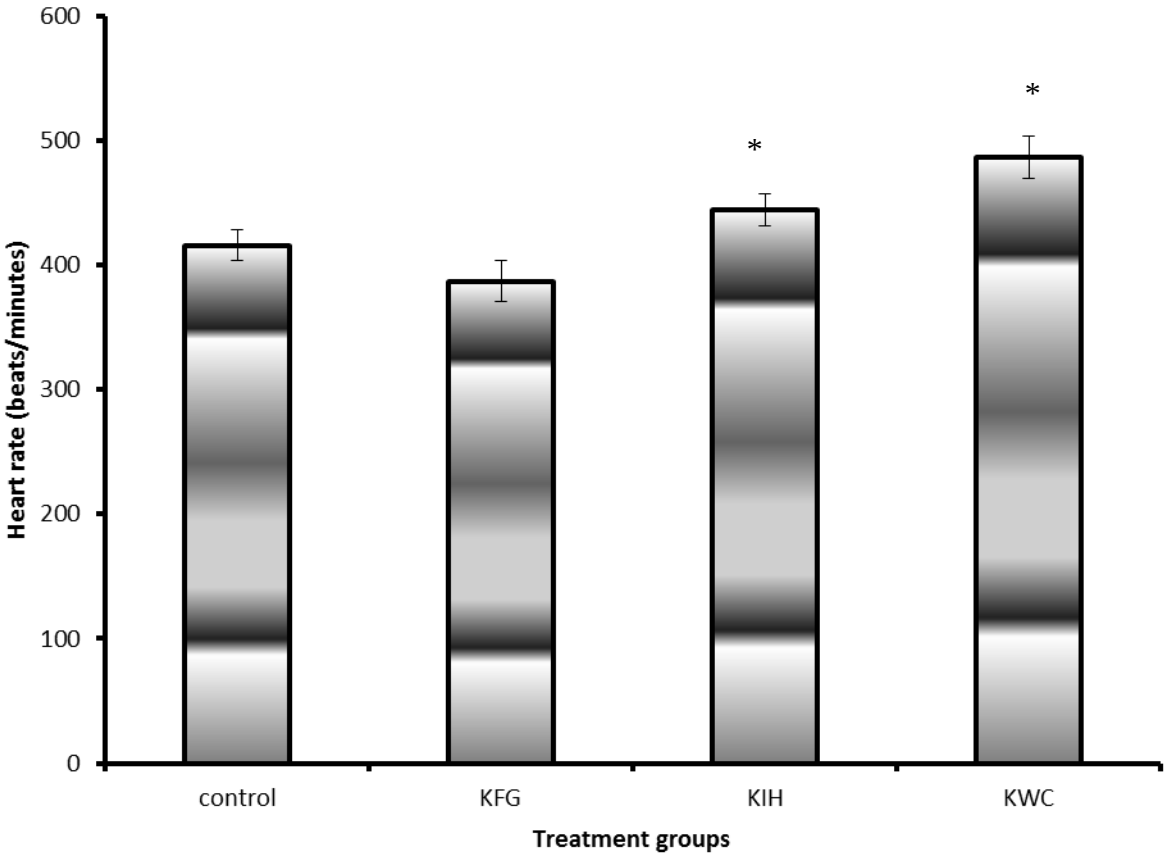


Figure 11: Effects of kerosene on Heart Rate of adult male rats

KFG- kerosene food ingestion group,

KIH- kerosene inhalation group,

KWC- kerosene water contaminated group.

Result presented as Mean ± SEM

*= $p<0.05$; *=significantly increased.

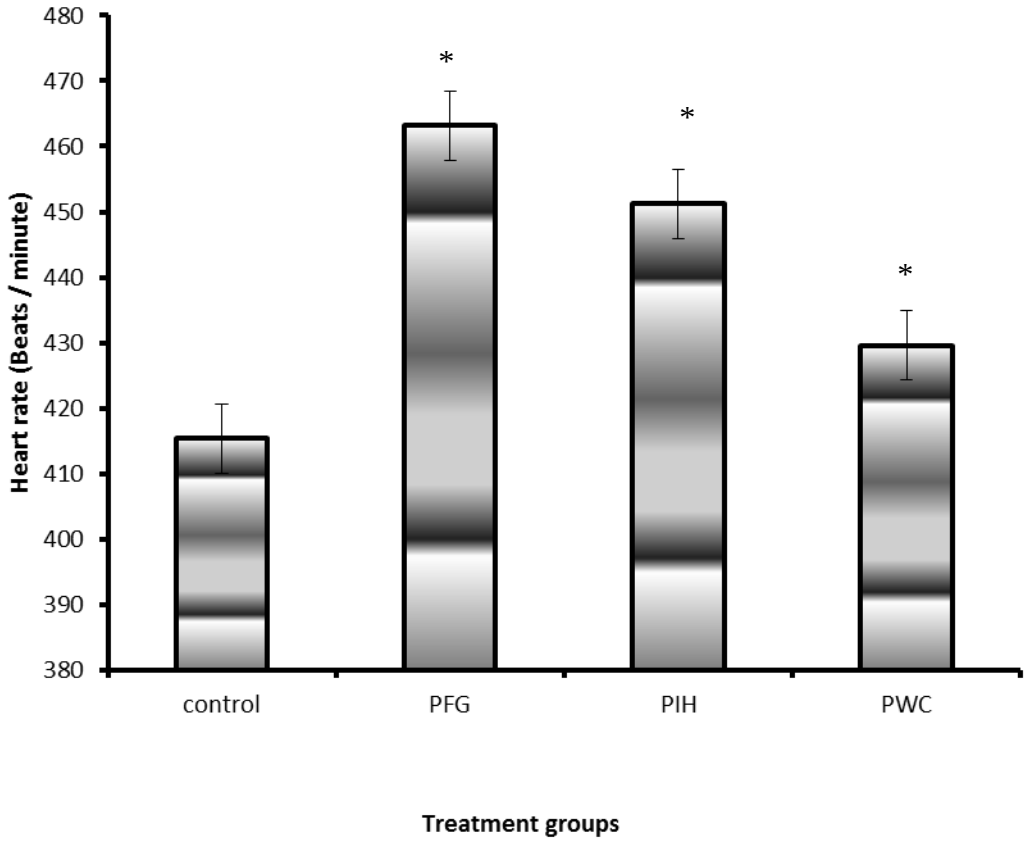


Figure 12: Effects of petrol Heart Rate of adult male rats

PFG- petrol food ingestion group,

PIH- petrol inhalation group,

PWC- petrol water contaminated group.

Result presented as Mean ± SEM,

*= $p<0.05$; *=significantly increased

4.3 Effects of Petroleum Products on Baroreflex Responses

Figure 13 showed the result of baroreflex response in diesel exposed rats. From the results, the DIH group did not show any response for 5 seconds, this is followed by a significant increase ($p<0.05$) that was progressive till the end of the manipulation at 30 seconds; the peak rate of change was between 6 & 7 beats/min./mmHg. The DWC group did not show any significant response till 10 second. After 10 seconds there was progressive and significant increase till 20 seconds (up to about 4 beats/min./mmHg).

Figure 14 showed the result of baroreflex response in kerosene exposed rats. From the results, The KFG group and the KWC group did not show any significant increase in response compared with control group. The KIH group on the other hand showed a significant increase up to 5 seconds (as high as 15 beats/min/mmHg before the progressive drop) and reduced progressively till 30 seconds, but still with significantly higher response than the control group.

Figure 15 showed the results of baroreflex response in petrol exposed rats. From the results, there was no significant increase in the response of PFG group and PWC group compared with control. The PIH group showed significant increase ($p<0.05$) in response at 5 seconds (2 beats/min./mmHG). This is followed by a progressive fall up to 25 seconds; after which it intersect with the response of the control group and fall below it.

Baroreflex response was induced by bilateral carotid occlussion continuously for 30 seconds and determined by change in heart rate and MAP ($\Delta HR/\Delta MAP$).

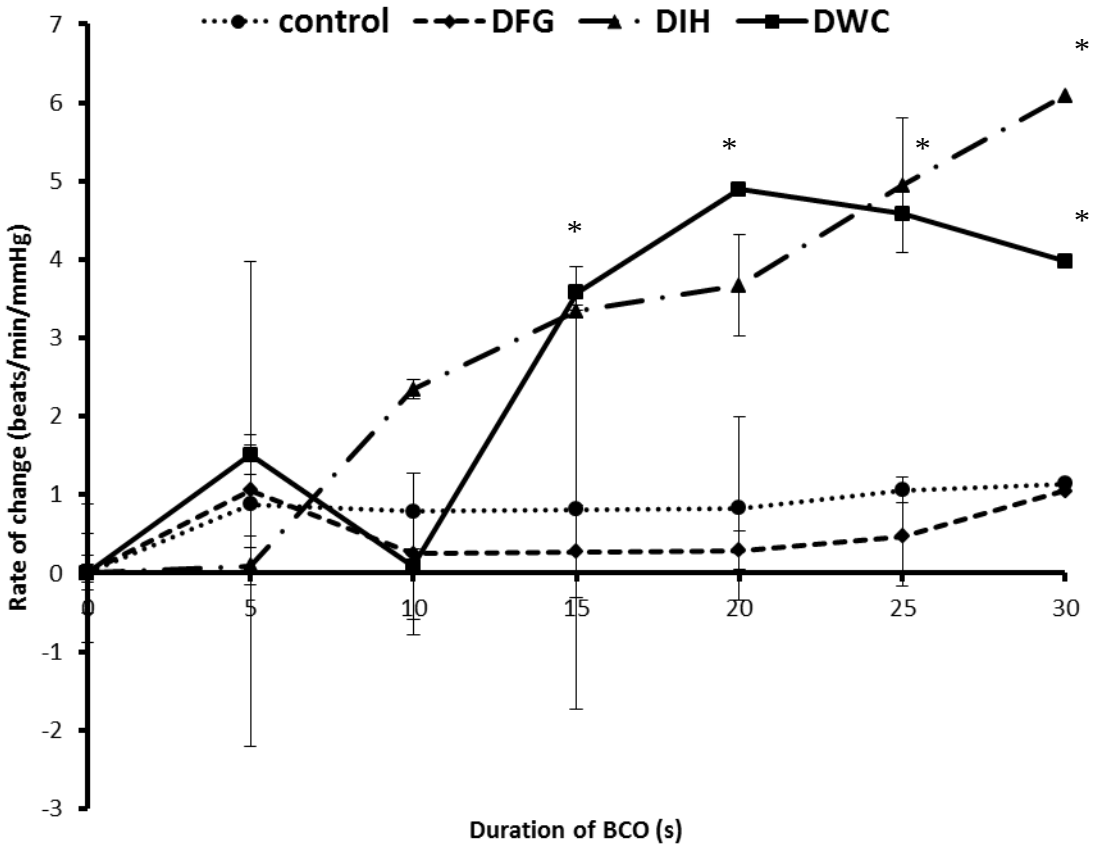


Figure 13: Effect of exposure to diesel on baroreflex responses for 30 secs in adult male rats

DFG- diesel food ingestion group,

DIH- diesel inhalation group

DWC- diesel water contaminated group.

ΔHR/ΔMAP = baroreflex sensitivity

*= $p<0.05$; *=significantly increased

Result presented as Mean ± SEM,

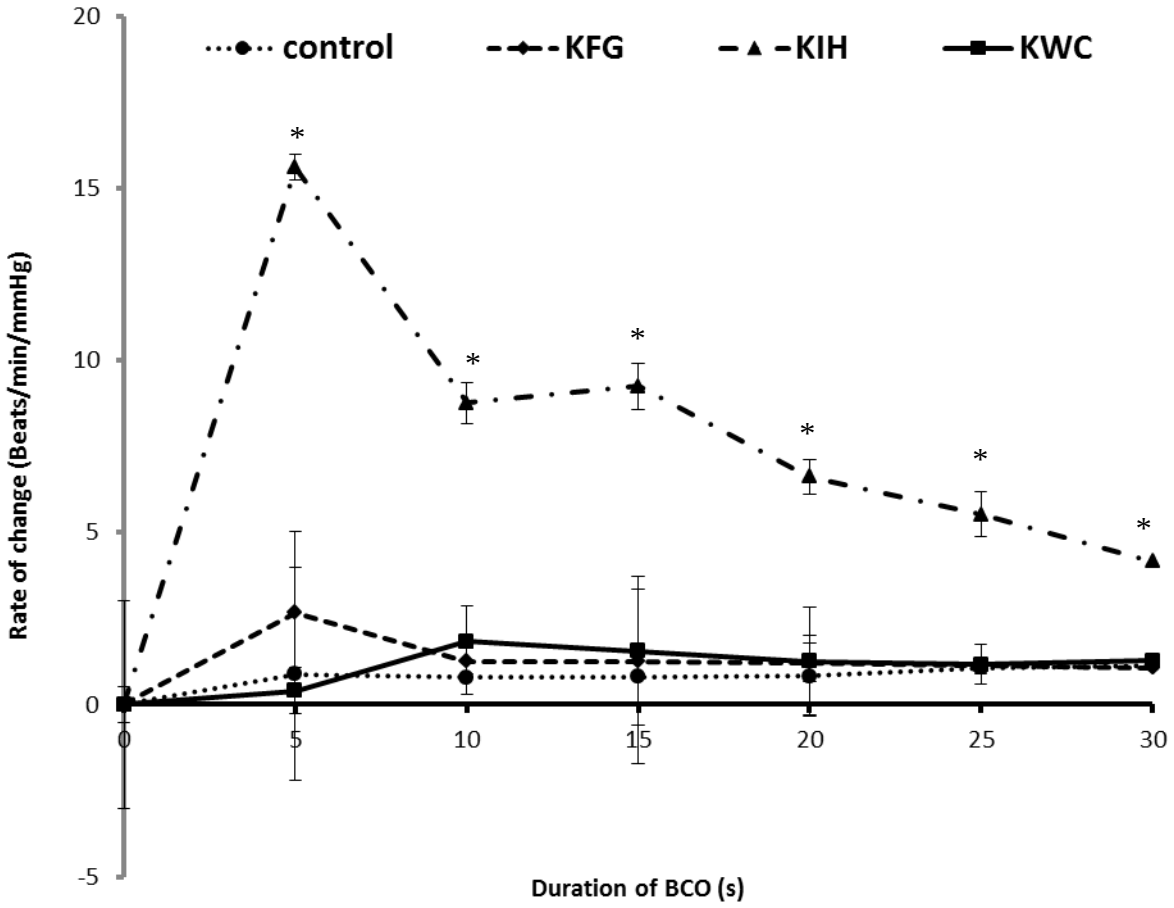


Figure 14: Effect of exposure to kerosene on baroreflex responses for 30 secs in adult male rats

KFG- kerosene food ingestion group,

KIH- kerosene inhalation group

KWC- kerosene water contaminated group.

ΔHR/ΔMAP = baroreflex sensitivity

*= $p<0.05$; *=significantly increased

Result presented as Mean ± SEM,

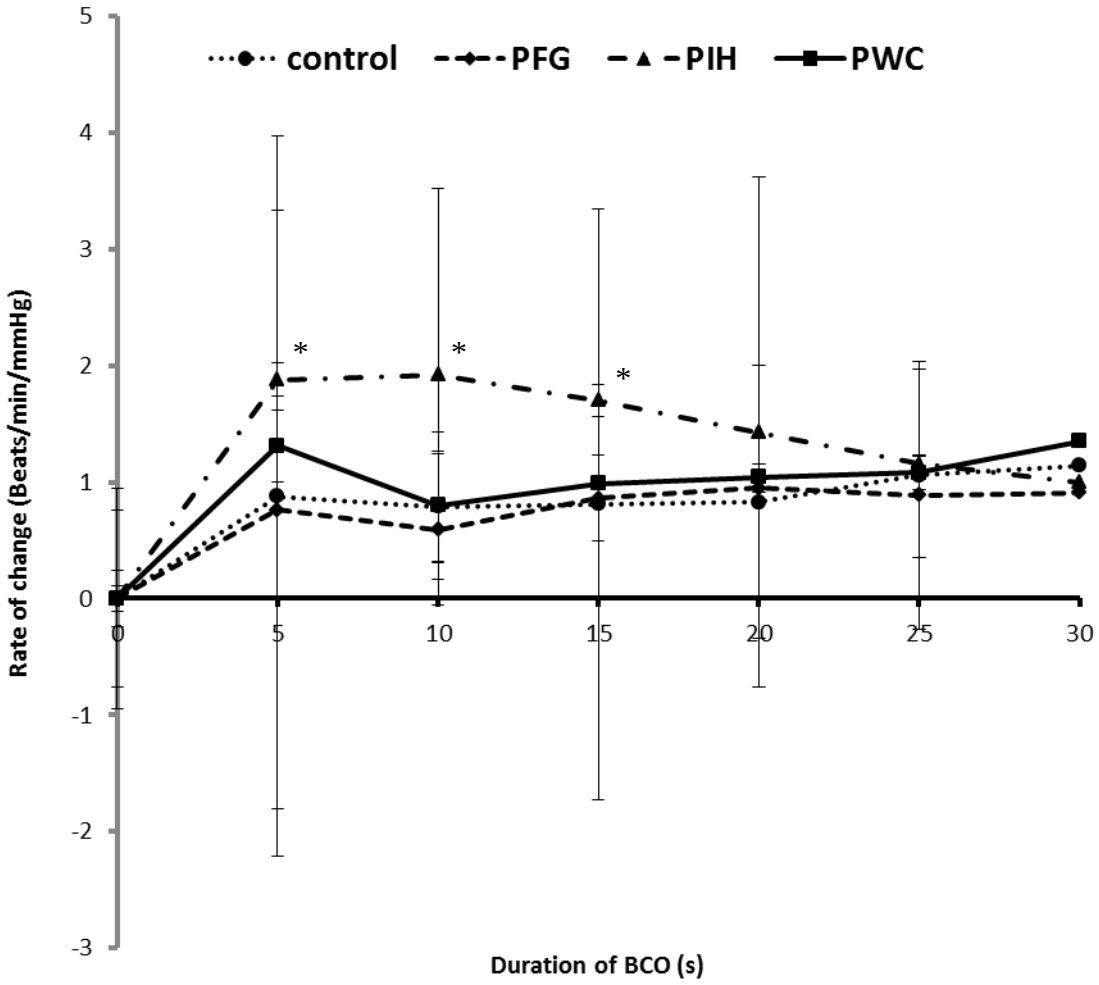


Figure 15: Effect of exposure to petrol on baroreflex responses for 30 secs in adult male rats

PFG- petrol food ingestion group,

PIH- petrol inhalation group

PWC- petrol water contaminated group. The

ΔHR/ΔMAP= baroreflex sensitivity

*= $p<0.05$; *=significantly increased

Result presented as Mean ± SEM,

4.4 Blood Pressure and Heart Rate after one Week of Stopping exposure to Petroleum Products

Figure 16 showed that the increase in blood pressure measured in all the rat groups persisted one week after removal of source of exposure when compared with control. This result was generated from the inhalation sub-groups

From the results, there is persistent significant increase ($p<0.05$) in SBP of diesel: 191.01±5.34 mmHg, kerosene: 155.2±4.21mmHg, and petrol: 135.9± 3.87mmHg) compared with control: 114.7±3.23mmHg

Significant increase was also observed in DBP of Diesel: 130.8± 3.78 mmHg, kerosene: 102.4±2.15 mmHg and petrol: 112.2±2.18 mmHg compared with control: 80.2±1.02mmHg

The significant increase ($p<0.05$) in DBP and MAP was most prominent in diesel group and lowest in the kerosene group compared with control.

From the result, the increase in HR persisted (diesel: 441.2±5.23 b/min, kerosene: 435.8±5.61 b/min, petrol: 431.6±4.89) compared with control: 404.6±1.78mmHg; one week after withdrawal of source of exposure as shown in figure 17. There significant increase ($p<0.05$) was most prominent in the diesel group and lowest in the petrol group.

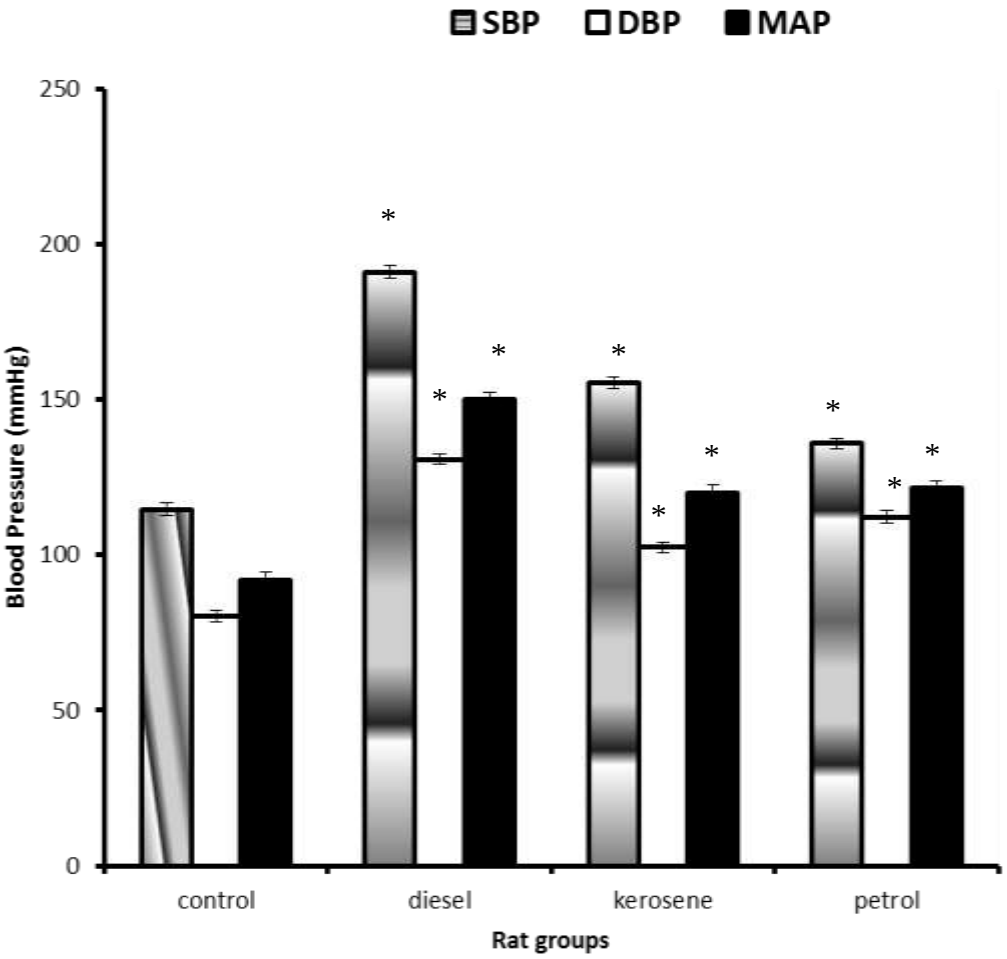


Figure 16: Blood pressure persistence after stoppage of exposure to petroleum products

This result was from the inhalation subgroup of diesel, kerosene and petrol

*= $p<0.05$; *=significantly increased

Result presented as Mean ± SEM,

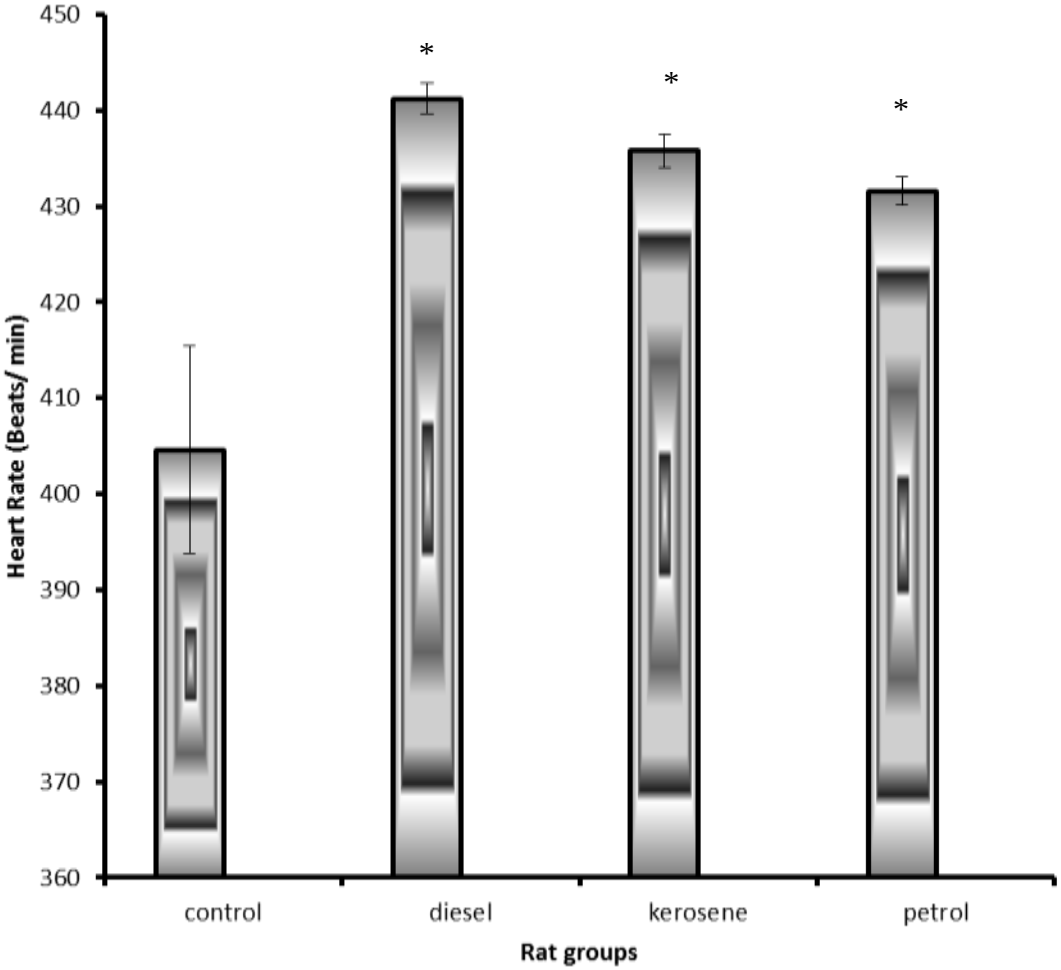


Figure 17: Heart rate persistence after stoppage of exposure to petroleum products

This result was from the inhalation subgroup of diesel, kerosene and petrol

*= $p<0.05$; *=significantly increased

Result presented as Mean ± SEM,

4.5 Effect Diesel, Kerosene and Petrol on Body Weight Changes in Adult Male Rats

It was noted that the rats were not rushing to eat the feed with diesel and kerosene, but they eventually ate. Left-over of 25-30 g was recorded at the unset but by the fourth week, rats adapted and the left-over reduced to 5-10g in the diesel but none in petrol and kerosene.

The body weight changed in all diesel groups of rats during the study period are shown in figure 18. There was progressive loss in weight of diesel food ingestion (DFG) group. Weight changes in DIH and DWC reduced more gradually compared with control. However the reduction in weight changes, became significant at 6-8 weeks ($p<0.05$). There was significant weight loss in DFG, DIH and DWC groups compared with control (Table 1).

The body weight changes in all kerosene groups are shown in figure 19. There was gradual loss in weight of the KFG group which became significant ($p<0.05$) after the second week. The weight loss continued till the end of the exposure period (8^{th} week). The KIH and KWC group had minimal decrease in weight till the end of 2^{nd} week when it became significant.

The body weight changes in the petrol groups are shown in figure 20. There was significant reduction in weight changes in the PFG, PIH and PWC from 3^{rd} to 4^{th} week of exposure. The percentage weight loss in all petrol groups was significant compared with control (<0.05).The selection of rats into the various groups was random, but because it's a toxicity test consideration was given to keeping biger rats in the test groups to be able to carry out the various analysis.

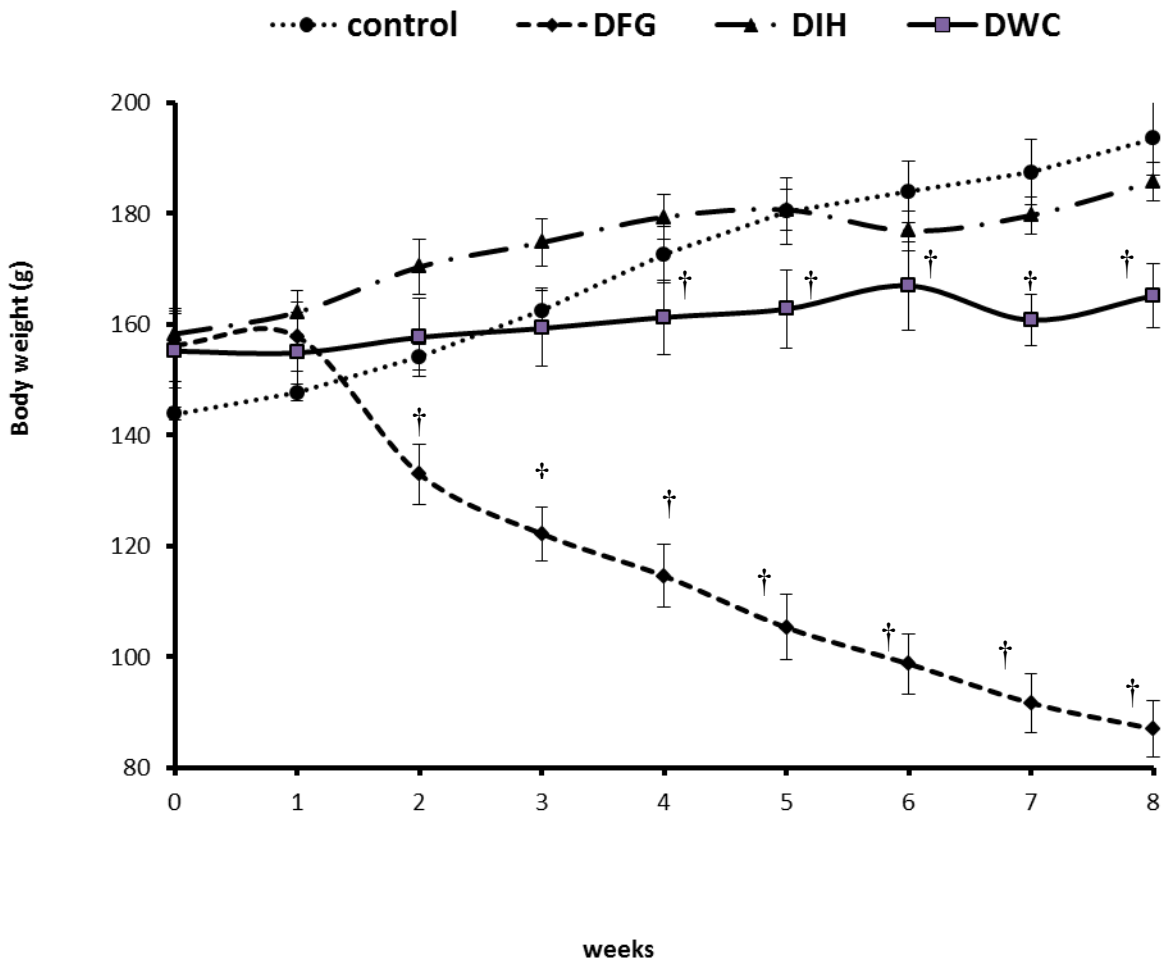


Figure 18: Effects of diesel on weekly mean body weight in adult male rats.

DFG- Diesel food ingestion group,

DIH- Diesel inhalation group

DWC- Diesel water contaminated group.

Result presented as Mean ± SEM,

† = P<0.05, †= significantly reduced

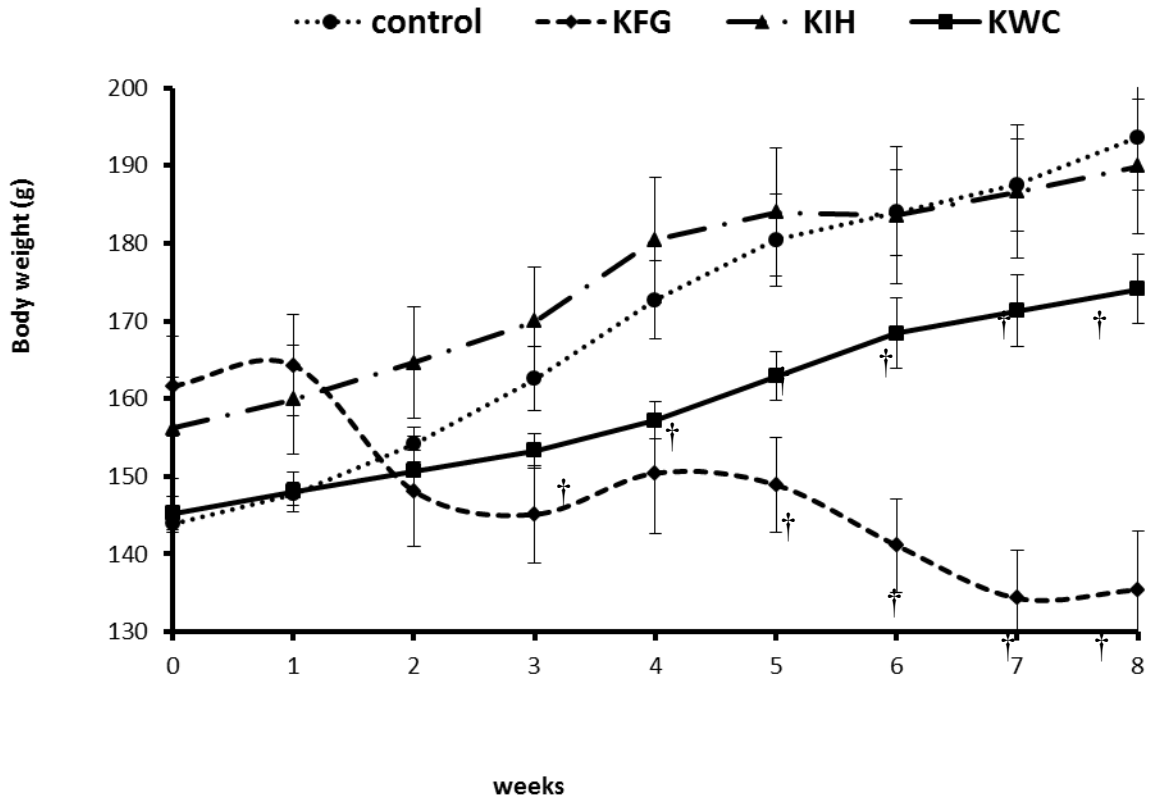


Figure19: Effects of kerosene on weekly mean body weight in adult male rats.

KFG- kerosene food ingestion group,

KIH- kerosene inhalation group

KWC- kerosene water contaminated group.

Result presented as Mean ± SEM,

† = P<0.05, †= significantly reduced

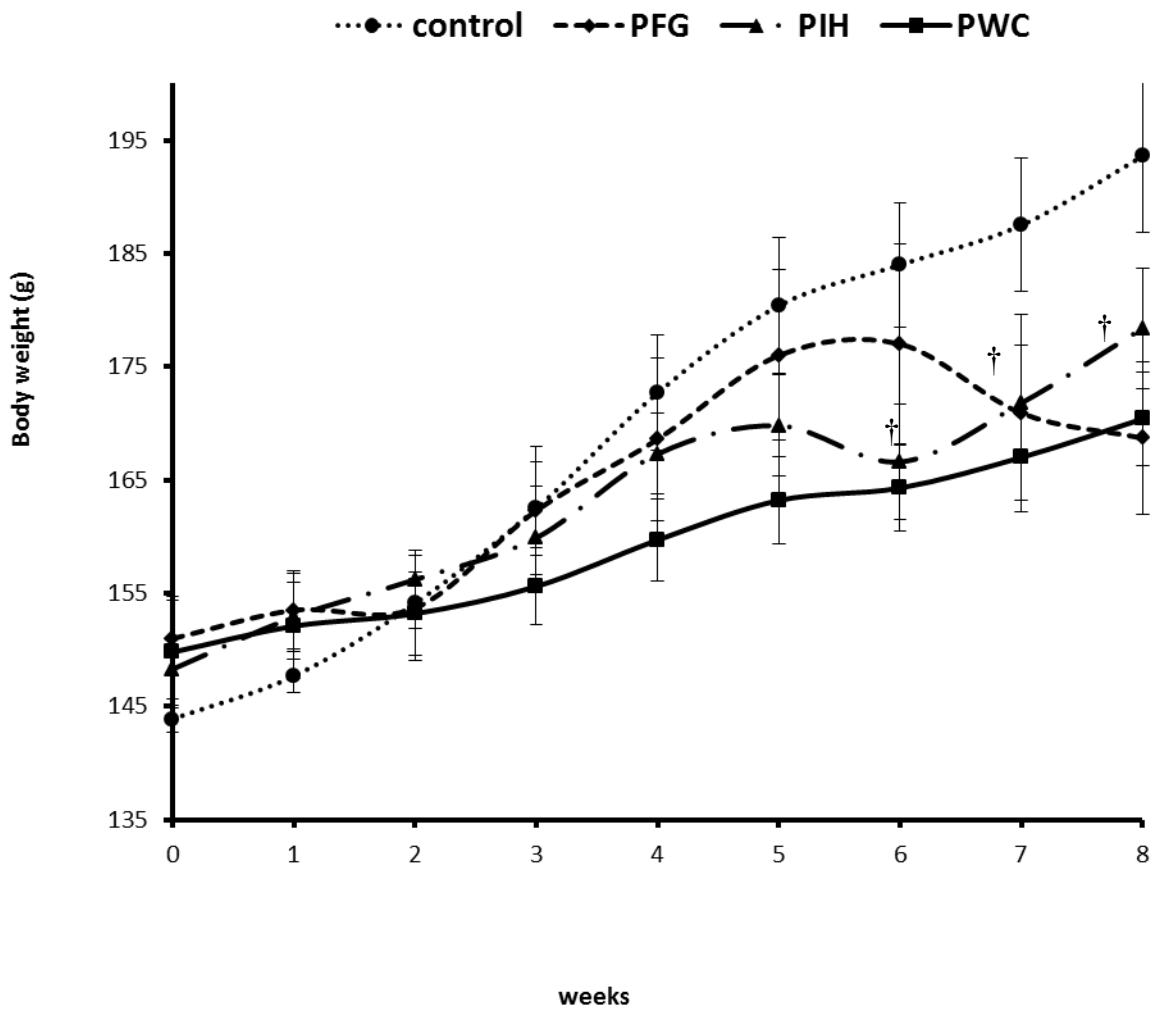


Figure 20: Effects of petrol on weekly mean body weight in adult male rats.

PFG- Petrol food ingestion group,

PIH- Petrol inhalation group

PWC- Petrol water contaminated group

Result presented as Mean ± SEM,

† = P<0.05, †= significantly reduced

Table 3: Effect of diesel on mean body weight Changes over eight weeks of exposure in adult male rats

Group	Initial weight (g)	Final weight(g)	Weight change	% weight gain $=\frac{weightchange}{initialweight}\times 100$
Control	146.0±1.95	193.60±6.72	47.6±4.77	32.6±2.45
DFG	154.9±7.46	85.5±5.44	-69.1±2.02	- 44.6±0.27††
DIH	162.2±3.95	188.45±4.3	26.3±0.35	16.2±0.08†
DWC	147.0±9.69	160.6±6.4	13.6±3.2	9.25±0.33†

DFG- Diesel food ingestion group,

DIH- Diesel inhalation group

DWC- Diesel water contaminated group.

Result presented as Mean ± SEM,

† =P<0.05, †= significantly reduced

††= p<0.01,

Table 4: Effect of kerosene on mean body weight Changes over eight weeks of exposure in adult male rats

Group	Initial weight (g)	Final weight(g)	Weight change	% weight gain $=\frac{weightchange}{initialweight}\times 100$
Control	146.0±1.95	193.60±6.72	47.6±4.77	32.6±2.45
KFG	164.3±6.47	135.4±7.49	-28.9±2.02	-17.59±0.31††
KIH	158.4±7.5	189.9±8.67	31.5±1.17	19.89±0.16†
KWC	145.2±3.41	173.9±4.56	28.7±1.15	19.76±0.33

KFG- kerosene food ingestion group,

KIH- kerosene inhalation group

KWC- kerosene water contaminated group.

Result presented as Mean ± SEM,

† =P<0.05, †= significantly reduced

†† = p<0.01

Table 5: Effect of petrol on mean body weight Changes over eight weeks of exposure in adult male rats

Group	Initial weight (g)	Final weight(g)	Weight change	% weight gain $=\frac{weightchange}{initialweight}\times 100$
Control	146.0±1.95	193.60±6.72	47.6±4.77	32.6±2.45
PFG	152.7±3.87	168.7±6.73	16.0±2.86	10.48±0.73†
PIH	152.0±7.5	178.4±5.31	26.4±2.19	17.36±0.29†
PWC	148.4±5.63	168.7±4.72	20.3±0.91	13.68±0.16†

PFG- Petrol food ingestion group,

PIH- Petrol inhalation group

PWC- Petrol water contaminated group.

Result presented as Mean ± SEM,

† =P<0.05, †= significantly reduced

4.6 Studies on Effects of Petroleum Products on Haematological indices of rats exposed to diesel, kerosene and petrol

Note: RBC was measured in ($5\times10^6/mm^3$)

Table 5 shows the haematological indices of diesel groups

RBC (DFG: 4.32±0.08, DIH: 4.22±2.6, and DWG: 3.91±0.18) reduced significantly in all the sub-groups of diesel compared with control (5.97±0.14).

PCV (DFG: 35.04±0, DIH: 36.9±1.87, DWC: 35.88±0.29) reduced compared with control group (40.76±2.09), however the reduction was insignificant.

Hb (DFG: 8.84±0.12 g/dl, DIH: 7.99±0.41 g/dl, DWC: 11.34±0.19 g/dl) reduced significantly in DFG and DIH, compared with control group (13.02±0.19 g/dl)

Platelets (DFG: 197.2±3.89 g/dl, DIH: 190.6±4.45 g/dl, DWC: 277.2±3.83g/dl) reduced significantly when compared with control group (421.6±23.40 g/dl).

Lymph reduced significantly (DFG: 31.22±1.24 %, DIH: 39.2±1.2 %, DWC: 37.00±0.6), compared with control group (48.8±1.02%). It also reduced significantly in KFG, KIH as well as PFG and PIH compared with control group. There was significant increase in white blood count (WBC) in DFG: [(12.88±0.36 ($5\times10^4/mm^3$)] and DWC: [12.48±0.19 ($5\times10^4/mm^3$)] while Neutrophils count increased significantly in all the three groups compared with control group.

Table 6 shows haematological indices of kerosene groups. RBC reduced significantly only in KWC group. PCV and Hb reduced significantly in KFG and KIH groups while Platelets and Lymph reduced significantly in KWC groups. WBC increased significantly in KFG and KWC groups compared with control group.

Table 7 shows haematological indices of petrol groups. RBC reduced significantly in all the three sub-groups, PCV and Hb reduced significantly in PFG and PIH compared with control. Platelets and Lymph reduced in all the exposed groups compared with control. WBC increased significantly in PIH group compared with control group.

Table 6: Effects of diesel on Haematological indices of adult male rats

	Control	DFG	DIH	DWC
RBC($5\times10^6/mm^3$)	5.97±0.14	4.32±0.08†	4.22±2.6†	3.91±0.18†
PCV (%)	40.76±2.09	35.04±0.84†	36.9±1.87†	35.88±0.29†
Hb (g/dl)	13.02±0.19	8.84±0.12†	7.99±0.41†	11.34±0.19
PLT (g/dl)	421.6±23.40	197.2±3.89†	190.6±4.45†	277.2±3.83†
WBC($5\times10^4/mm^3$)	9.88±0.24	12.88±0.36*	10.5±0.4	12.48±0.19*
Lymph (%)	48.8±1.02	31.22±1.24†	39.2±1.2†	37.00±0.6†
Neutr (%)	45.64±0.49	59.08±0.28*	52.68±0.69*	55.98±0.29*

DFG- Diesel food ingestion group,

DIH- Diesel inhalation group

DWC- Diesel water contaminated group.

Result presented as Mean ± SEM,

† =P<0.05, † = significantly reduced

*=p<0.05, * = significantly increased

Table 7: **Effects of kerosene on Haematological indices of adult male rats**

	Control	**KFG**	**KIH**	**KWC**
RBC ($5\times10^6/mm^3$)	5.97±0.14	5.4±0.12	5.35±0.19	4.83±0.36†
PCV (%)	40.76±2.09	38.58±0.4†	37.62±0.67†	39.00±0.13
Hb (g/dl)	13.02±0.19	11.60±0.4†	10.67±0.33†	14.26±0.13
PLT (g/dl)	421.6±23.40	388.2±13.63	319.8±27.62†	339.2±2.87†
WBC($5\times10^4/mm^3$)	9.88±0.24	12.26±0.26*	9.3±0.22	11.20±0.11*
Lymph (%)	48.8±1.02	29.40±0.55†	43.3±0.75	33.68±0.96†
Neutr (%)	45.64±0.49	56.48±0.24*	53.38±0.75*	51.82±0.25*

KFG- kerosene food ingestion group,

KIH- kerosene inhalation group

KWC- kerosene water contaminated group.

Result presented as Mean ± SEM,

† =P<0.05, †= significantly reduced

*=p<0.05, * = significantly increased

Table 8: **Effects of petrol on Haematological indices of adult male rats**

	Control	**PFG**	**PIH**	**PWC**
RBC ($5\times10^6/mm^3$)	5.97±0.14	4.49±0.15†	4.86±.22†	4.84±0.11†
PCV (%)	40.76±2.09	35.95±0.73†	36.96±1.99†	38.74±0.28
Hb (g/dl)	13.02±0.19	9.1±0.13†	9.42±0.34†	13.38±0.18
PLT (g/dl)	421.6±23.40	290.6±14.34†	229.6±17.62†	361.0±6.2†
WBC($5\times10^4/mm^3$)	9.88±0.24	10.41±0.17	12.60±0.29*	10.9±0.18
Lymph (%)	48.8±1.02	34.10±0.24†	46.10±1.4	39.94±0.23†
Neutr (%)	45.64±0.49	51.34±0.15	56.74±0.42*	52.52±0.36*

PFG- Petrol food ingestion group,

PIH- Petrol inhalation group

PWC- Petrol water contaminated group

Result presented as Mean ± SEM,

† =P<0.05, †= significantly reduced

*=p<0.05 * = significantly increased

4.7 Result Showing Effects of Petroleum Products on Liver Enzyme Activities in Serum of Adult Male Rats

Aspartate Amino-transferase (AST) In (table 8) AST was found to be significantly increased in diesel, kerosene and petrol groups (DFG-215.9±2.2 (IU/L), DIH- 182.40±6.75 (IU/L), DWC- 234.6±9.64 (IU/L) compared with control (62.4±10.83 (IU/L). Highest value was recorded in kerosene (KFG) followed by diesel (DWC).

Alanine Amino-transferase (ALT), Ther was significant increase in DFG: 83.56±4.99, DIH: 87.18±0.70, DWC: 95.71±2.74 compared with Control: 75.76±3.32. Significant increase was also observed in KFG: 115.5±9.80, KIH: 77.6±12.48 with highest value in KFG.

The AST/ALT ratio showed significant increase in kerosene group and PIH group.

Alkaline phosphatase (ALP) increased significantly, in DFG: 340.4±10.00, DIH: 347.7±25.17, compared with control (312.6±8.97). There was also significant increase in KIH: 384.1±32.92 PFG: 356.7±37.89, with highest value in the kerosene inhalation group. Kerosene appeared to have caused more damage on the liver and kidney, from the result of liver function test.

Table 9: Effects of diesel on serum liver enzyme activities in adult male rats

	Control	DFG	DIH	DWC
AST (IU/L)	162.4±10.83	215.9±2.2*	182.40±6.75*	234.6±9.64*
ALT (IU/L)	75.76±3.32	83.56±4.99*	87.18±0.70*	95.71±2.74*
ALP (IU/L)	312.6±8.97	340.4±10.00*	347.7±25.17*	332.8±9.8*
AST/ALT	2.14±3.26	22.58±0.44	2.09±9.64	2.45±3.51

DFG- Diesel food ingestion group,

DIH- Diesel inhalation group

DWC- Diesel water contaminated group.

Result presented as Mean ± SEM,

†= P<0.05†= significantly reduced,

*= p<0.05 * = significantly increased

Table 10: Effects of kerosene on serum liver enzyme activities in adult male rats

	Control	KFG	KIH	KWC
AST(IU/L)	162.4±10.83	273.1±30.12*	219.3±12.78*	215.5±5.09*
ALT(IU/L)	75.76±3.32	115.5±9.80*	77.6±12.48*	74.42±3.60
ALP(IU/L)	312.6±8.97	330.5±6.28	384.1±32.92*	336.3±4.42
AST/ALT	2.14±3.26	2.36±3.07	2.83±	2.90±3.55

KFG- kerosene food ingestion group,

KIH- kerosene inhalation group

KWC- kerosene water contaminated group.

Result presented as Mean ± SEM,

†= P<0.05, †= significantly reduced,

*= p<0.05, * = significantly increased

Table 11: Effects of petrol on serum liver enzyme activities of adult male rats

	Control	PFG	PIH	PWC
AST(IU/L)	162.4±10.83	221.7±37.72*	204.7±11.01*	216.3±27.06*
ALT(IU/L)	75.76±3.32	89.95±4.20*	78.78±0.28*	107.6±3.75*
ALP(IU/L)	312.6±8.97	356.7±37.89*	331.9±7.61*	325.3±6.36
AST/ALT	2.14±3.26	2.46±8.98	2.60±39.32	2.01±7.22

PFG- Petrol food ingestion group,

PIH- Petrol inhalation group

PWC- Petrol water contaminated group.

Result presented as Mean ± SEM,

†= P<0.05, †= significantly reduced,

= p<0.05 = significantly increased

4.8 Effects of Petroleum Products on Serum Biochemical Properties of Adult Male Rats

From the result in tables 11, 12 &13 there was significant increase ($p<0.05$) in serum creatinine level in diesel DFG: 64.10±3.6, DIH: 66.85±1.48 DWC: 69.15±1.76

Kerosene: KFG: 100.70±3.3 KIH: 70.63±1.8, KWC: 76.20±1.13 and petrol PFG: 82.10±5.40 PIH: 65.01±2.02, PWC: 77.07±3.89 groups compared with control (44.83±0.96).

Urea from the result in tables 11, 12 &13, the serum urea level increased significantly ($p<0.05$) in all diesel DFG: 9.83±0.39, DIH: 8.60±0.11, DWC: 8.96±0.38 groups, compared with control (7.92±0.13). Significant increase was also found in KFG: 11.93±1.23, KIH: 9.38±0.10 and PFG: 8.92±0.60 groups. However it reduced significantly in PIH: 5.00±0.56.

Total protein (TP) and serum albumin (SA) From tables 11, 12 and 13, there was significant increase in serum TP and SA level in DFG: 83.23±7.5, KWC and PIH: 72±2.53groups compared with control. However, there was significant decrease in TP and SA level in KFG and KWC groups compared with control.

Table 12: Effects of diesel on serum biochemical properties of adult male rats

	Control	DFG	DIH	DWC
CREA(µmol/L)	44.83±0.96	64.10±3.6*	66.85±1.48*	69.15±1.76*
Urea(mmol/L)	7.92±0.13	9.83±0.39*	8.60±0.11*	8.96±0.38*
ALB(g/L)	42.5±0.19	61.28±6.94*	36.98±0.87†	42.19±0.91
TP (IU/L)	72.46±0.73	83.23±7.5*	64.58±0.74†	64.56±1.44†

DFG- Diesel food ingestion group,

DIH- Diesel inhalation group

DWC- Diesel water contaminated group.

Result presented as Mean ± SEM,

†= P<0.05, †= significantly reduced,

= p<0.05 = significantly increased

Table 13: Effects of kerosene on serum biochemical properties of adult male rats

	Control	KFG	KIH	KWC
CREA(µmol/L)	44.83±0.96	100.70±3.3*	70.63±1.81*	76.20±1.13*
Urea(mmol/L)	7.92±0.13	11.93±1.23*	9.38±0.10*	7.87±0.55
ALB(g/L)	42.5±0.19	31.66±2.45†	42.76±0.75	36.5±0.9†
TP (IU/L)	72.46±0.73	67.30±2.46†	68.64±0.29†	78.00±0.54

KFG- kerosene food ingestion group,

KIH- kerosene inhalation group

KWC- kerosene water contaminated group.

Result presented as Mean ± SEM,

†= P<0.05, †= significantly reduced,

= p<0.05 = significantly increased

Table 14: Effects of petrol on serum biochemical properties in of adult male rats

	Control	PFG	PIH	PWC
CREA (µmol/L)	44.83±0.96	82.10±5.40*	65.01±2.02*	77.07±3.89*
Urea (mmol/L)	7.92±0.13	8.92±0.60*	5.00±0.56†	7.66±1.19
ALB (g/L)	42.5±0.19	44.6±0.98	72±2.53*	40.04±1.80
TP (IU/L)	72.46±0.73	73.26±1.23	73.09±2.37	67.64±5.99†

PFG- Petrol food ingestion group,

PIH- Petrol inhalation group

PWC- Petrol water contaminated group.

Result presented as Mean ± SEM,

†= P<0.05, †= significantly reduced,

=p<0.05,=significantlyincreased

4.9 Effects of Petroleum Products on Biochemical Properties in Urine of Adult Male Rats

Urea in urine of diesel treated rats DFG: 290.9±2.12, DIH: 271.6±0.15, DWC: 151.8±25.67 reduced significantly compared with control (411.9±23.31). Significant reduction was also observed in the kerosene group KFG: 337.4±21.48, KIH: 337±21.48 and KWC: 298.00±29.87 compared with control: 411.9±23.31; as well as in the petrol PFG: 301.8±15.69, PIH: 255.5±1.03and PWC: 174.5±4.16; when compared with control.

Creatinine in urine of treated rats reduced significantly DFG: 651.1±16.19, DIH: 730.0±3.30 and DWC: 349.9±12.12 compared with control: 995.0±12.99; KFG: 852.2±6.98, KIH: 783.2±7.54, KWC: 730.0±23.30,: as well as PFG: 747.2±11.71, PIH: 661.8±13.34, PWC: 794.5±6.87 when compared with control.

There was significant increase in concentration of albumin in DFG: 2.12±0.04, DIH: 2.00±0.15 , DWC: 2.14±0.07 , KIH: 1.92±0.32, and PIH: 3.42±0.54.

Total protein also increased significantly in DIH: 23.3±0.43, KWC: 23.22±0.72 and PIH: 22.38±0.87. This increase as well as the fluctuation infers that with exposure to petroleum products the body was unable to absorb protein and the kidney and liver were damaged.

Table 15: **Effects of diesel on biochemical properties in urine of adult male rats**

	Control	**DFG**	**DIH**	**DWC**
CREA(mmol/L)	995.0±12.99	651.1±16.19†	730.0±3.30†	349.9±12.12†
Urea(mmol/L)	411.9±23.31	290.9±2.12†	271.6±0.15†	151.8±25.67†
ALB (g/L)	1.53±0.25	2.12±0.04*	2.00±0.15*	2.14±0.07*
TProt (g/L)	12.08±1.89	12.58±0.06	23.3±0.43*	12.40±0.24

DFG- Diesel food ingestion group,

DIH- Diesel inhalation group

DWC- Diesel water contaminated group.

Result presented as Mean ± SEM,

†= P<0.05, †= significantly reduced

*= p<0.05, * = significantly increased

Table 16: Effects of kerosene on biochemical properties in urine of adult male rats

	Control	KFG	KIH	KWC
CREA(mmol/L)	995.0±12.99	852.2±6.98†	783.2±7.54†	730.0±23.30†
Urea(mmol/L)	411.9±23.31	337.4±21.48†	317±12.64†	298.00±29.87†
ALB(g/L)	1.53±0.25	1.80±0.19	1.92±0.32*	1.64±0.06
TProt(g/L)	12.08±1.89	12.14±0.92	18.24±1.84	23.22±0.72*

KFG- kerosene food ingestion group,

KIH- kerosene inhalation group

KWC- kerosene water contaminated group.

Result presented as Mean ± SEM,

†= P<0.05, †= significantly reduced,

*= p<0.05, * = significantly increased

Table 17: Effects of petrol on biochemical properties in urine of adult male rats

	Control	PFG	PIH	PWC
CREA(mmol/L)	995.0±12.99	747.2±11.71†	661.8±30.34†	794.5±6.87†
Urea (mmol/L)	411.9±23.31	301.8±15.69†	255.5±1.03†	174.5±4.16†
ALB (g/L)	1.53±0.25	1.88±0.17	3.42±0.54*	1.97±0.15
TProt (g/L)	12.08±1.89	12.54±0.16	22.38±0.87*	12.54±0.18

PFG- Petrol food ingestion group,

PIH- Petrol inhalation group

PWC- Petrol water contaminated group.

Result presented as Mean ± SEM,

†= P<0.05, †= significantly reduced,

*= p<0.05, * = significantly increased

4.10 Effects of Petroleum products on Creatine Kinase

Creatine kinase (CKMB) was found to increase significantly in rats expose to inhalation of diesel: 277.4±5.32, kerosene: 175.6±4.05 and petrol: 162.8±4.25; compared with control: 107±2.75.

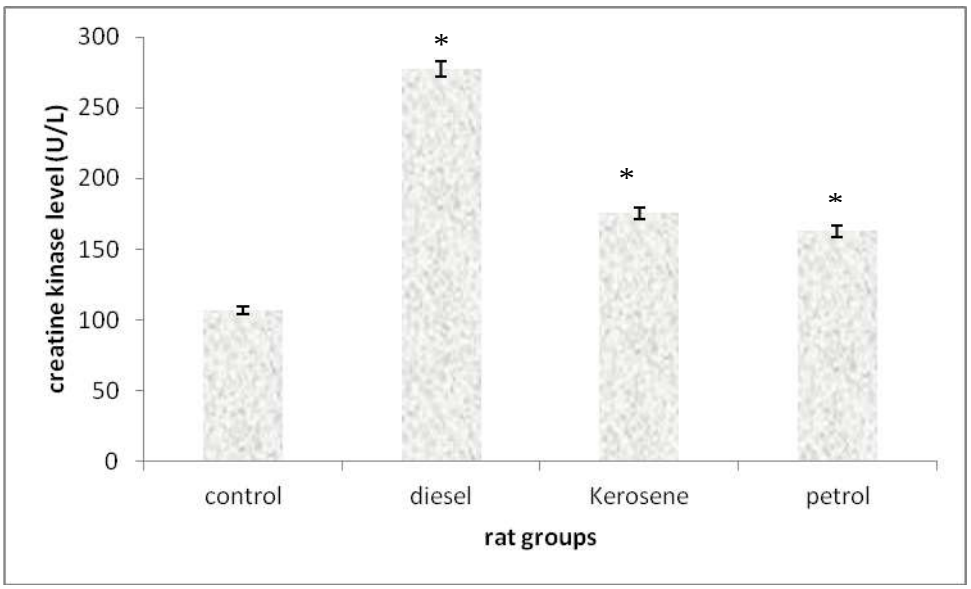


Figure 21: Effect of inhalation of diesel, kerosene and petrol on creatine kinase of Sprague Dawley rats.

*= P<0.05 *= significant difference

4.11 Effects of Petroleum Products on Serum Antioxidant Activities and Lipid Peroxidation in Adult Male Rats

The result showed significant reduction in the level of **Catalase** in DFG: 162.7±1.82, DWC: 157.2±3.33 groups compared with control: 443.6±29.82. Significant reduction was also observed in KIH: 162.7±1.82 and KWC: 326.4±4.07 as well as PWC: 158.0±1.70

Glutathione reduced significantly in DFG and DIH groups; KWC: 29.95±5.39as well as PIH: 25.84±2.75 and PWC: 38.40±0.76

Superoxide dismutasereduced significantly in DIH: 31.19±3.85, DWC: 19.66±0.48, KIH: 22.69±3.71, KWC: 27.56±5.09 as well as PIH: 18.96±0.01. From this result, the effects of antioxidant enzymes activities were suppressed after exposing the rats to petroleum products.

From result tables 17, 18 and 19 there is significant increase in MDA in all the diesel kerosene and petrol sub-groups.

Table 18: Effects of diesel on antioxidant activities and lipid peroxidation in serum of adult male rats

	Control	DFG	DIH	DWC
CAT(µmol/L/min)	448.6±10.16	322.1±17.80†	425.7±9.7	157.2±3.33†
GSH (µmol/L/mg prot.)	46.86±1.52	42.06±4.64†	27.90±4.25†	43.32±1.01†
MDA (µmol/L/mg prot.)	0.90±0.24	4.67±0.12**	8.50±0.17**	2.28±0.08**
SOD(µmol/L/min)	38.14±0.47	33.49±0.34	31.19±3.85†	19.66±0.48†

DFG- Diesel food ingestion group,

DIH- Diesel inhalation group

DWC- Diesel water contaminated group.

Result presented as Mean ± SEM,

†= P<0.05, †= significantly reduced,

*= p<0.05, * = significantly increased

**= p<0.01

Table 19: Effects of kerosene on antioxidant activities and lipid peroxidation in serum of adult male rats

	Control	KFG	KIH	KWC
CAT(µmol/L/min)	448.6±10.16	443.6±29.82	162.7±1.82†	326.4±4.07†
GSH (µmol/L/mg prot.)	46.86±1.52	52.73±4.06	29.95±5.39†	43.48±0.12†
MDA (µmol/L/mg prot.)	0.90±0.24	6.71±0.48**	12.88±1.22**	4.42±0.01**
SOD(µmol/L/min)	38.14±0.47	37.82±0.09	22.69±3.71†	27.56±5.09†

KFG- kerosene food ingestion group,

KIH- kerosene inhalation group

KWC- kerosene water contaminated group.

Result presented as Mean ± SEM,

†= P<0.05, †= significantly reduced,

*= p<0.05, * = significantly increased

**= p<0.01

Table 20: Effects of petrol on antioxidant activities and lipid peroxidation in serum of adult male rats

	Control	PFG	PIH	PWC
CAT(µmol/L/min)	448.6±10.16	403.9±16.2†	158.0±1.70†	543.2±3.73
GSH (µmol/L/mg prot.)	46.86±1.52	46.76±0.61	25.84±2.75†	38.40±0.76†
MDA (µmol/L/mg prot.)	0.90±0.24	4.80±0.69**	15.57±0.97**	7.33±0.10**
SOD(µmol/L/min)	38.14±0.47	37.95±0.40†	18.96±0.01†	34.49±0.09†

PFG- Petrol food ingestion group,

PIH- Petrol inhalation group

PWC- Petrol water contaminated group.

Result presented as Mean ± SEM,

†= P<0.05, †= significantly reduced,

*= p<0.05, * = significantly increased

**= p<0.01

4.12 Effects of Diesel on Antioxidant Activities and Lipid Peroxidation in Visceral Organs

A demonstration of an *in vitro* oxidative stress of five different tissues: brain, heart, kidney, liver and lung; investigated following exposure to diesel by food ingestion, inhalation and drinking in contaminated water are shown in figures 22, 23, 24 and 25. Natural antioxidant defense systems were disturbed as seen in the figures.

Catalase is significantly reduced in the heart, kidney and liver compared with control

GSH is significantly reduced in brain, heart, liver and lung of all the subgroups

SOD is significantly reduced in the brain, heart and liver of ingestion and water contamination groups.

The MDA concentration in diesel sub-groups showed that there was significant increase in the MDA level of the homogenate of all the sub-groups. The significant increase shown in the homogenate of the organs is an indication of severe lipid peroxidation.

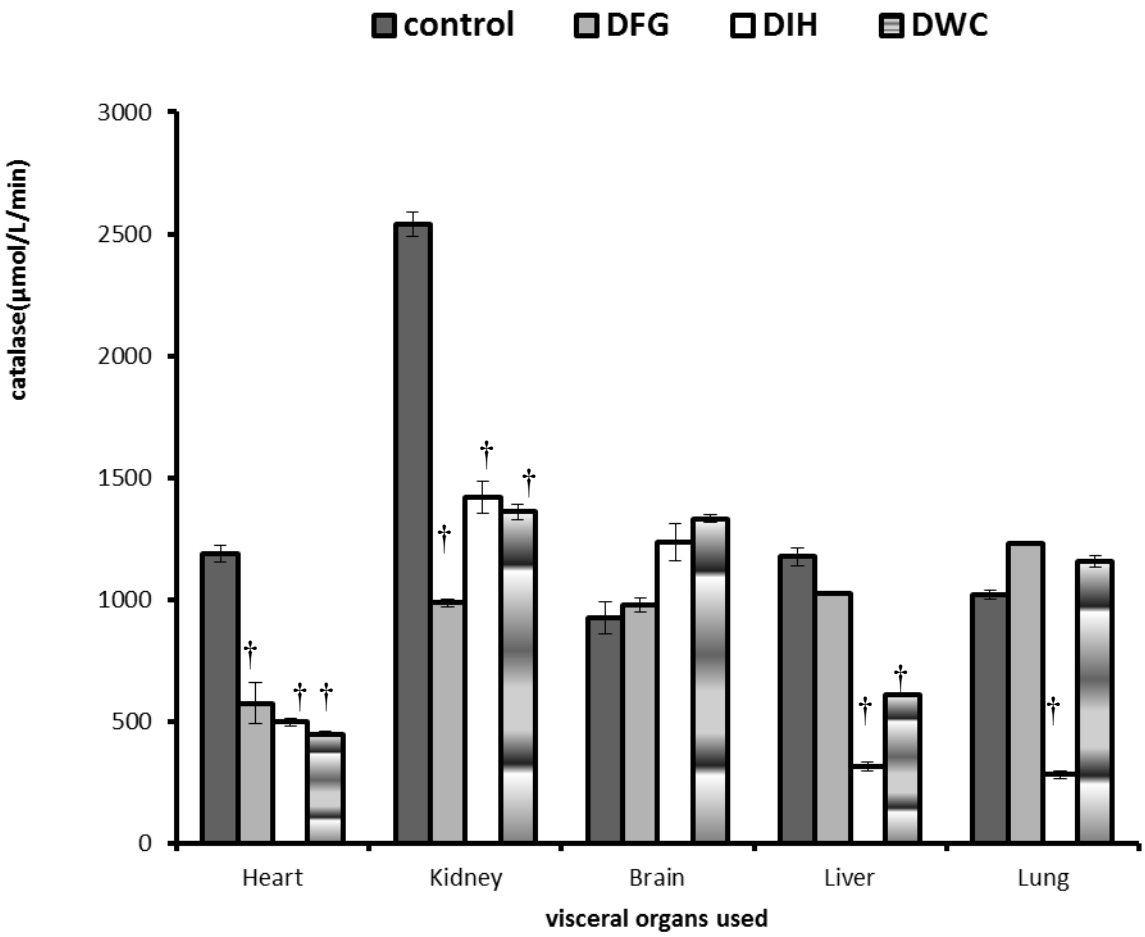


Figure 22: Effects of diesel on catalase of the homogenate of visceral organs in adult male rats

Control

Catalase

Glutathione reductase

Super oxide dismutase

†= $p<0.05$; †= significant reduction

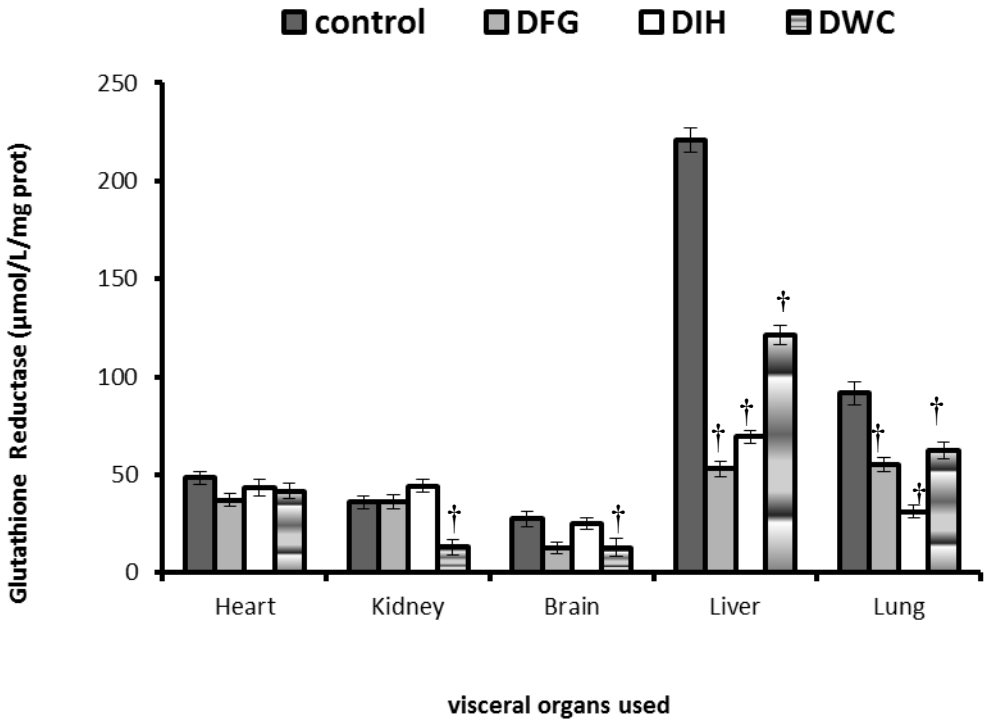


Figure 23: Effects of diesel on Glutathione reductase of the homogenate of visceral organs in adult male rats

Control

Catalase

Glutathion reductase

Super oxide dismutase

†= $p<0.05$; †= significant reduction.

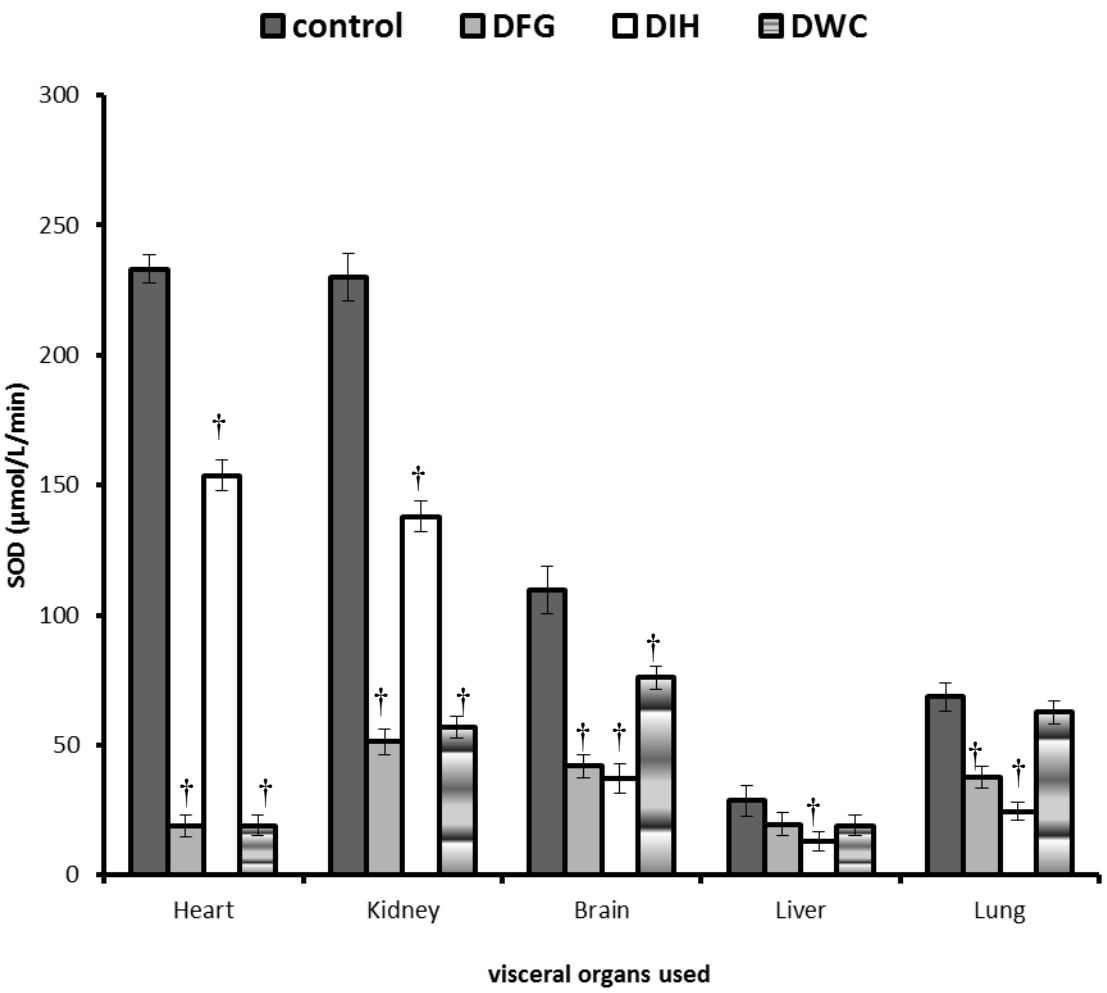


Figure 24: Effects of diesel on catalase of the homogenate of visceral organs in adult male rats

Control

Catalase

Glutathion reductase

Super oxide dismutase

†= $p<0.05$; †= significant reduction

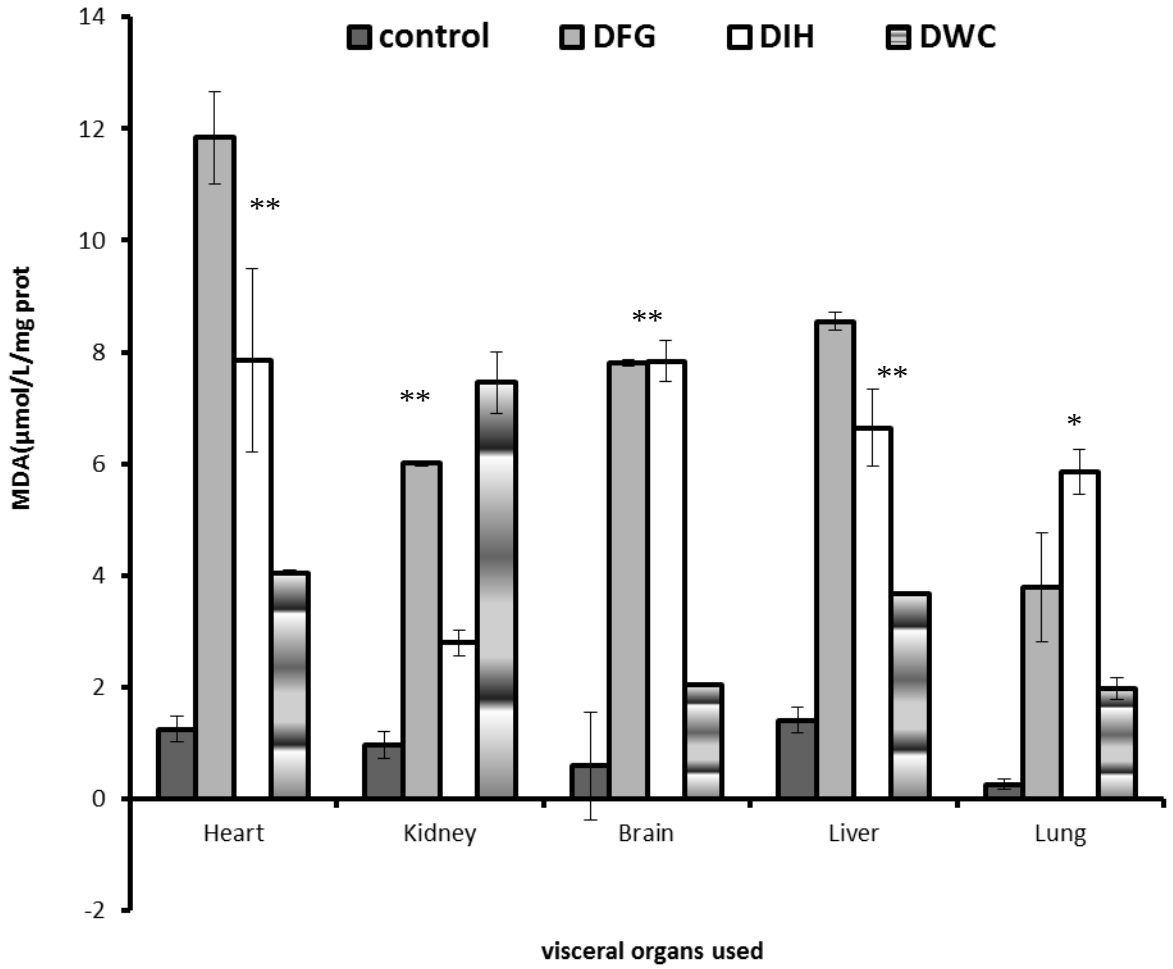


Figure 25: **Effects of diesel on catalase of the homogenate of visceral organs in adult male rats**

Control

Catalase

Glutathion reductase

Super oxide dismutase

**= p<0.01 *= significant increase

*= p<0.05

4.13 Effects of Kerosene on Antioxidant Activities and Lipid Peroxidation in Visceral Organs

Increased production of reactive oxygen species seems to have caused important biochemical modification in the events of exposure to kerosene. The effects have disturbed natural antioxidant defense systems, changing antioxidant enzyme activities in various tissues: brain, heart, kidney, liver and lungs as shown in figures 26, 27, 28 and 29.

From figure 26, catalase was significantly decreased in the heart, kidney, brain and liver more obvious in the KFG and KWC groups

From figure 27, GSH was significantly reduced in kidney, liver and lung especially in the KFG group.

From figure 28, SOD reduced significantly in heart, kidney, brain, liver and lung especially the KFG and KIH groups.

From figure 29, MDA significantly increased in all the tissue and all groups especially n KIH and KWC groups.

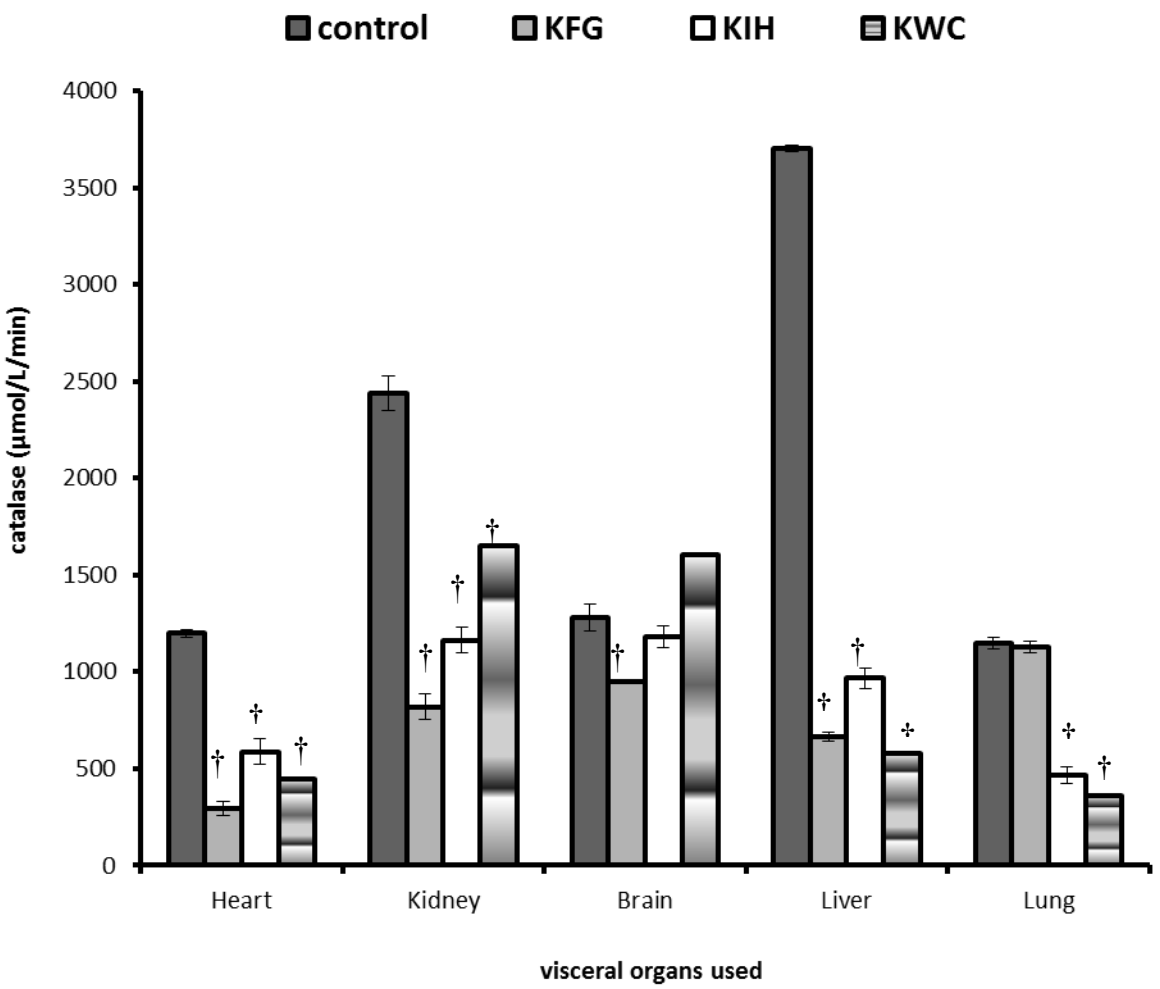


Figure 26: Effects of kerosene on catalase of the homogenate of visceral organs in adult male rats

Control

Catalase

Glutathion reductase

Super oxide dismutase

†= $p<0.05$; †= significant reduction

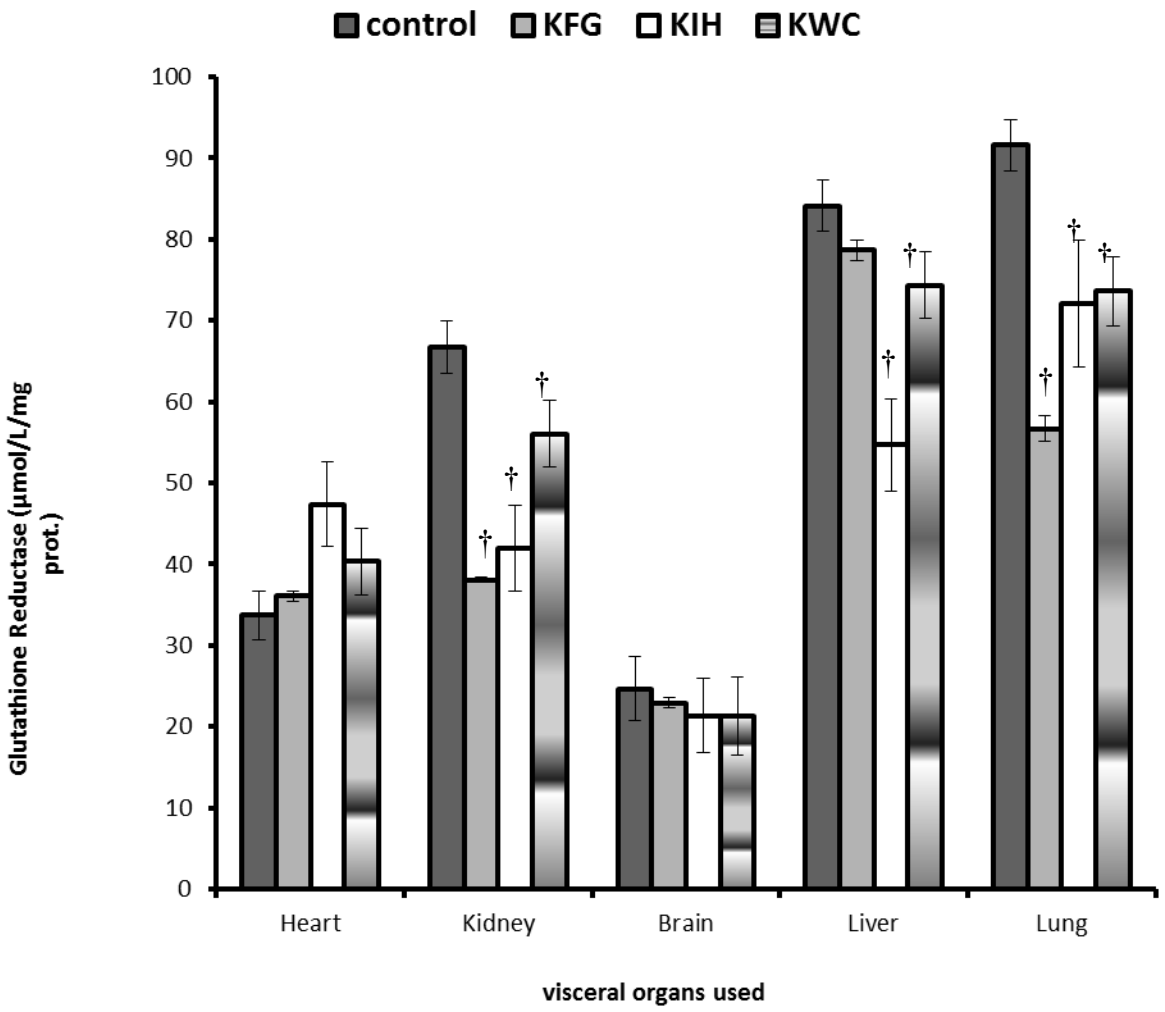


Figure 27: Effects of kerosene on Glutathione reductase (GSH) of the homogenate of visceral organs in adult male rats

Control

Catalase

Glutathion reductase

Super oxide dismutase

†= $p<0.05$; †= significant reduction

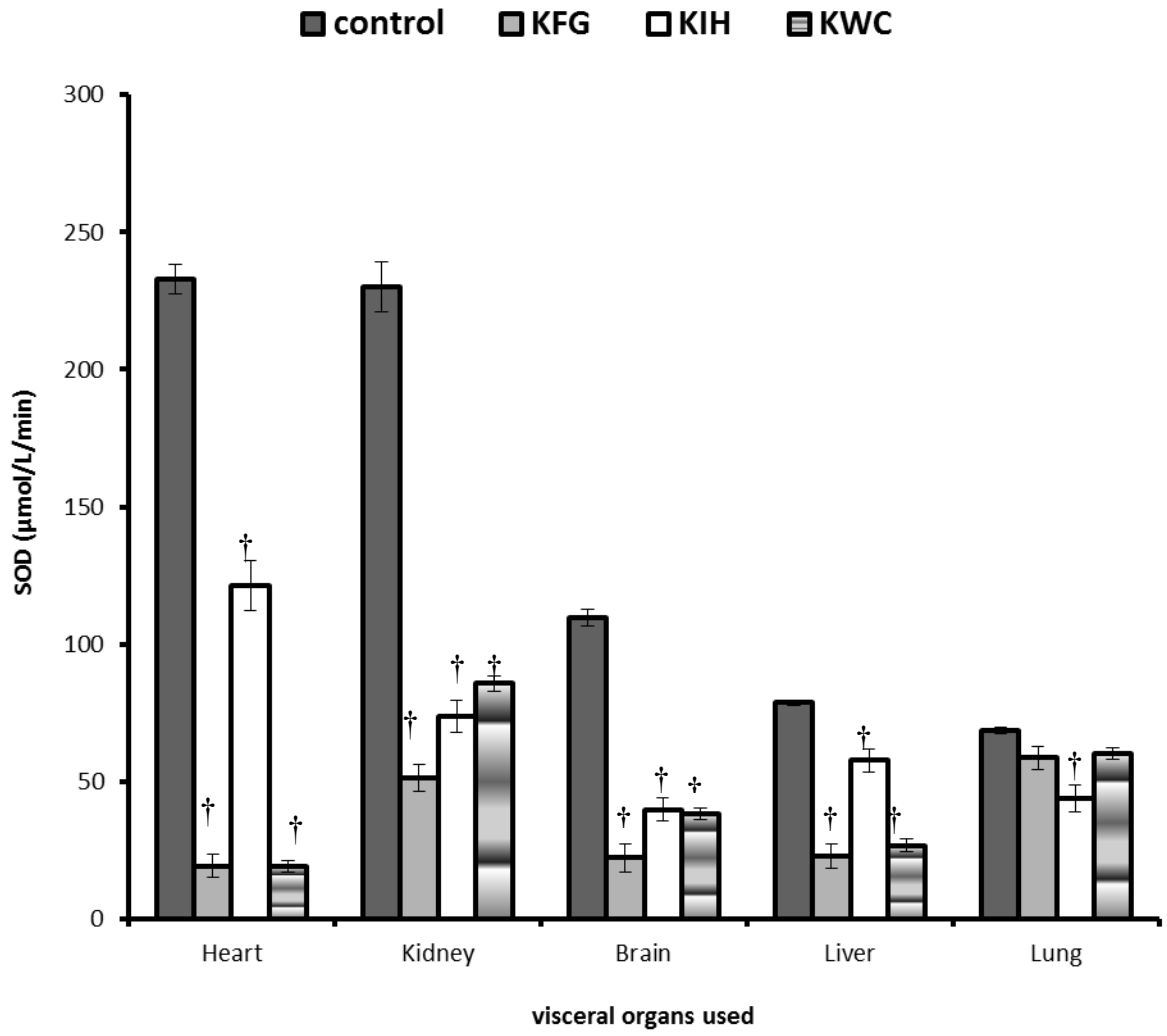


Figure 28: Effects of kerosene on Superoxide dismutase (SOD) of the homogenate of visceral organs in adult male rats

Control

Catalase

Glutathion reductase

Super oxide dismutase

†= $p<0.05$; †= significant reduction

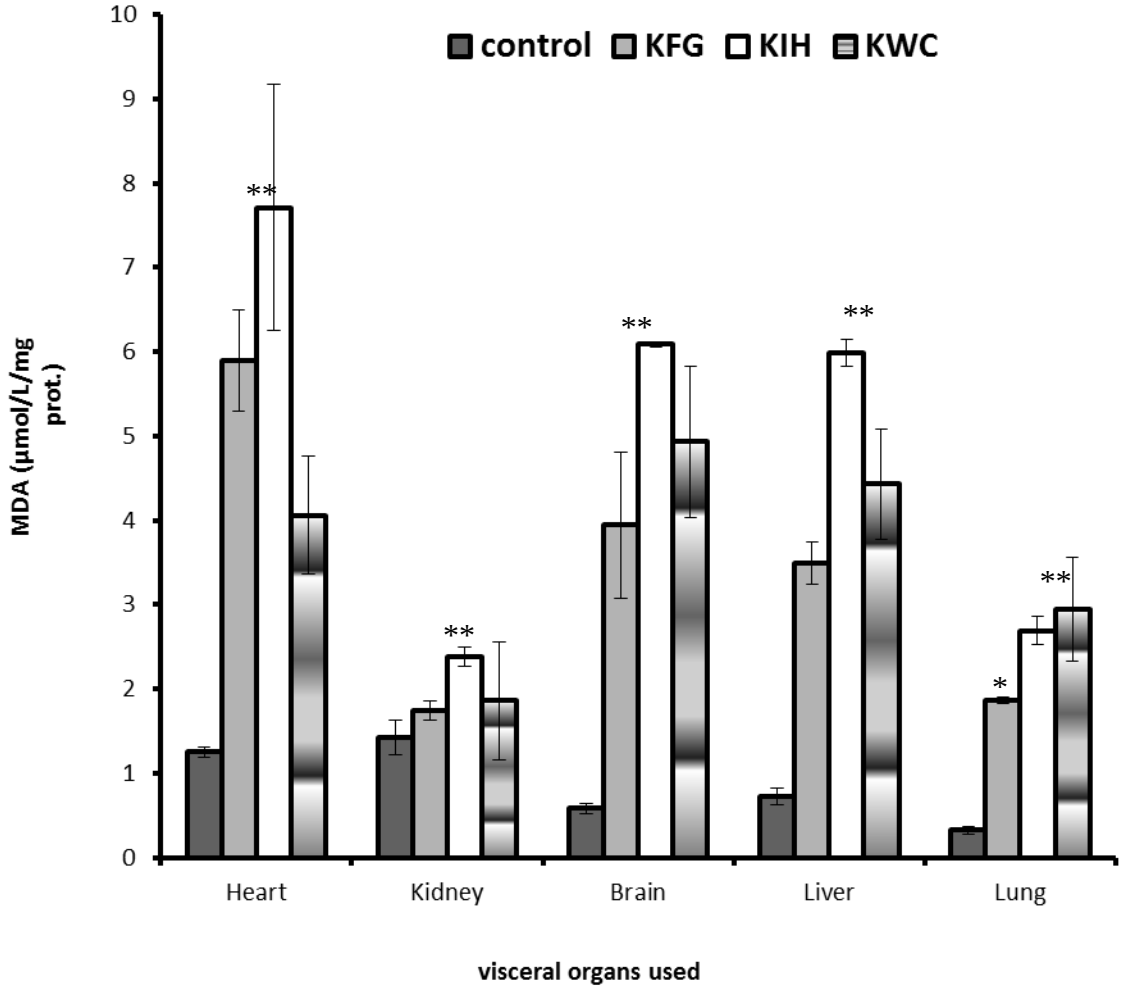


Figure 29: Effects of kerosene on Malondihaldehyde (MDA) of the homogenate of visceral organs in adult male rats

Control

Catalase

Glutathion reductase

Super oxide dismutase

**= p<0.01; *= significant increase

*=p<0.05

4.14 Effects of Petrol on Antioxidant Activities and Lipid Peroxidation in Visceral Organs

Figures 30, 31, 32, 33 demonstrate oxidative stress and lipid peroxidation in the brain, heart, kidney, liver and lungs of the exposed rat groups.

From figure 30, there is significant reduction in catalase heart, kidney, liver and lung homogenate especially the KFG and KWC groups.

Figure 31, The Glutathione reduced significantly in kidney, liver and lung. However, there is an increase in the heart

Figure 32, The SOD reduced significantly in ingestion and water contamination group of heart, kidney and brain homogenate.

Figure 33, shows the result of MDA concentration in the homogenate of control group and petrol sub-groups. The result shows that there is significant difference in the MDA concentration of the homogenate of all the groups especially the PFG and PIH groups. The significant increase shown in the homogenate of the organs is an indication of severe lipid peroxidation.

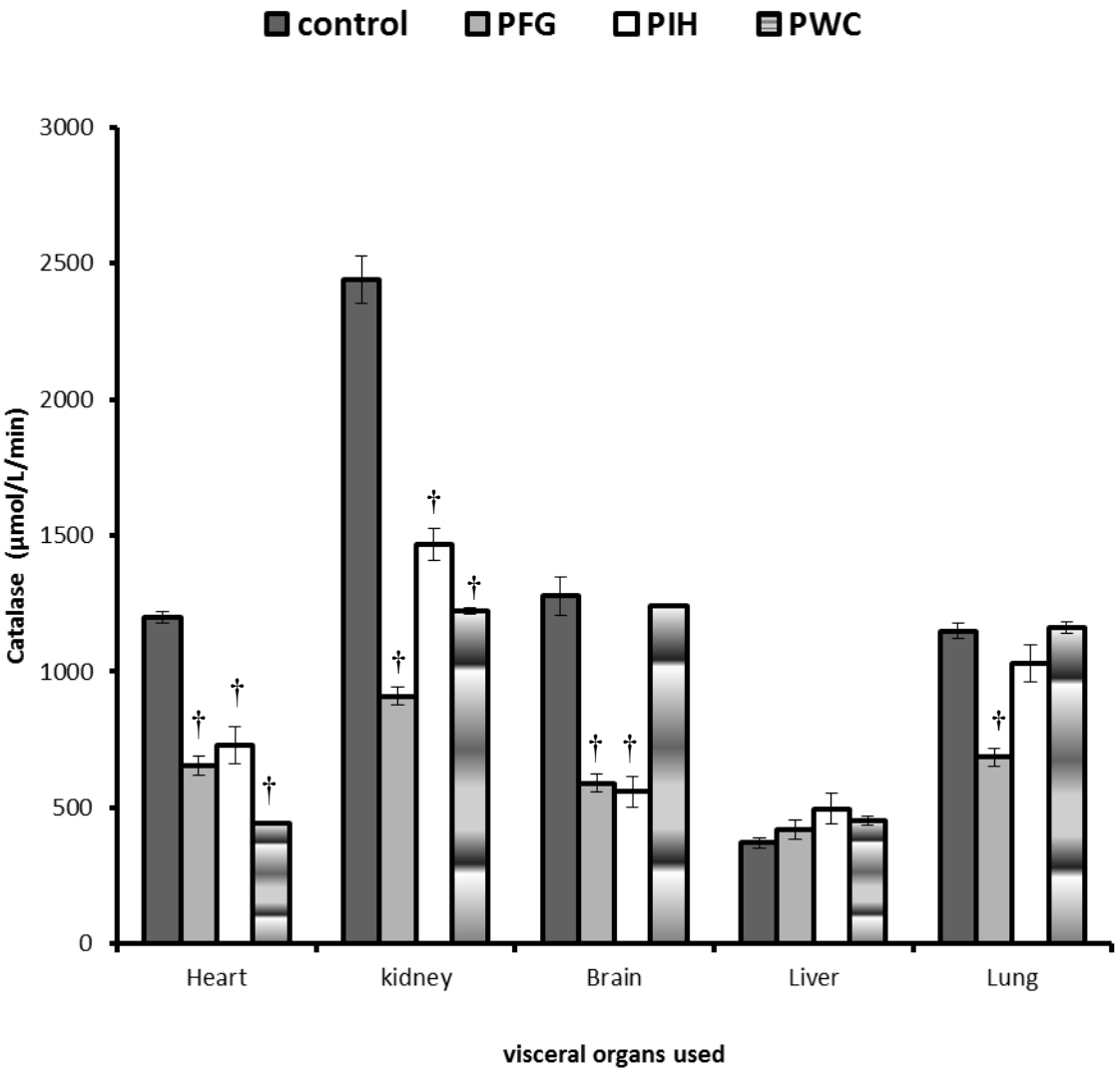


Figure 30: Effects of petrol on Catalase of the homogenate of visceral organs in adult male rats

Control

Catalase

Glutathion reductase

Super oxide dismutase

†= $p<0.05$; †= significant reduction

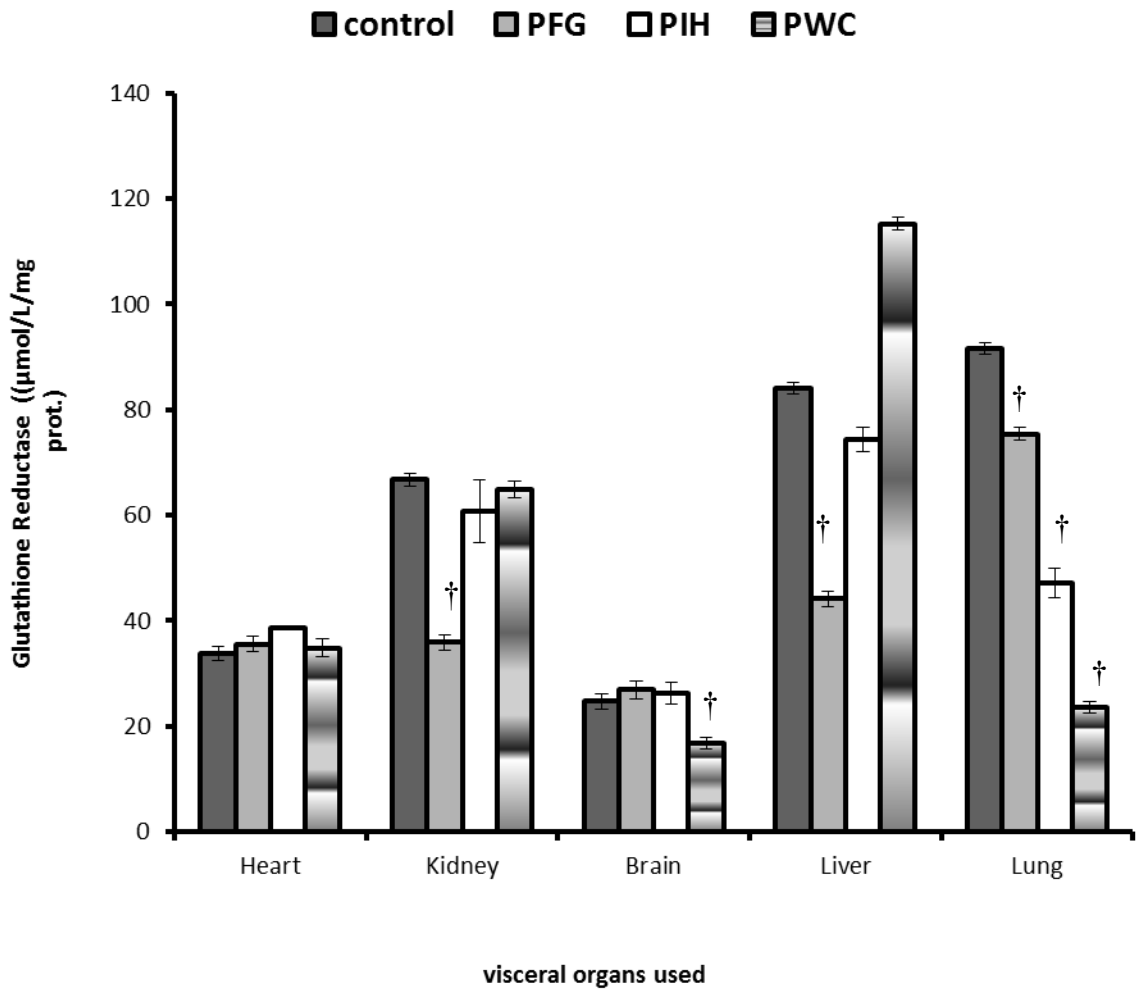


Figure 31: Effects of petrol on Glutathione reductase (GSH) of the homogenate of visceral organs in adult male rats

Control

Catalase

Glutathion reductase

Super oxide dismutase

†= $p<0.05$; †= significant reduction

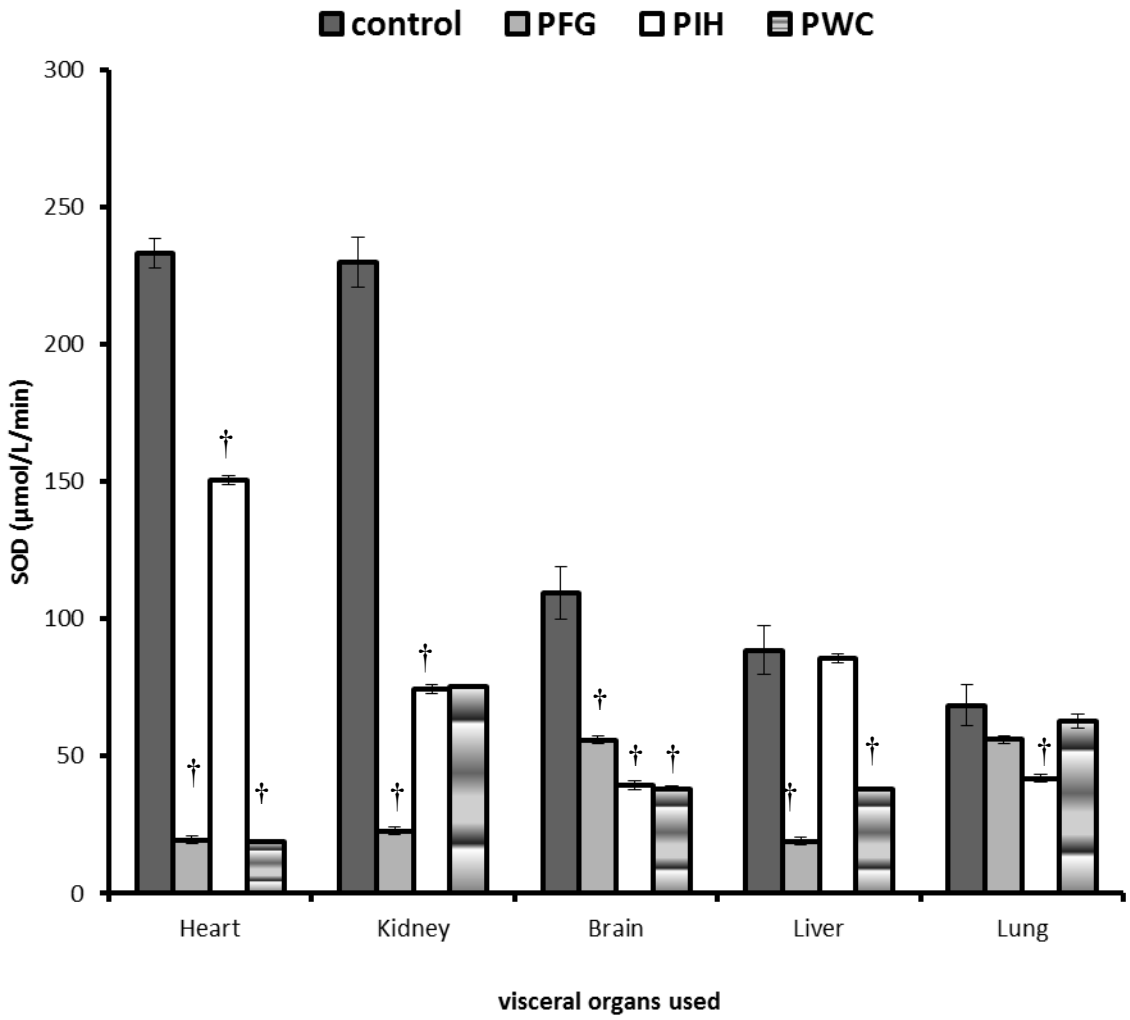


Figure 32: Effects of petrol on Superoxide dismutase (SOD) of the homogenate of visceral organs in adult male

Control

Catalase

Glutathion reductase

Super oxide dismutase

†= $p<0.05$; †= significant reduction

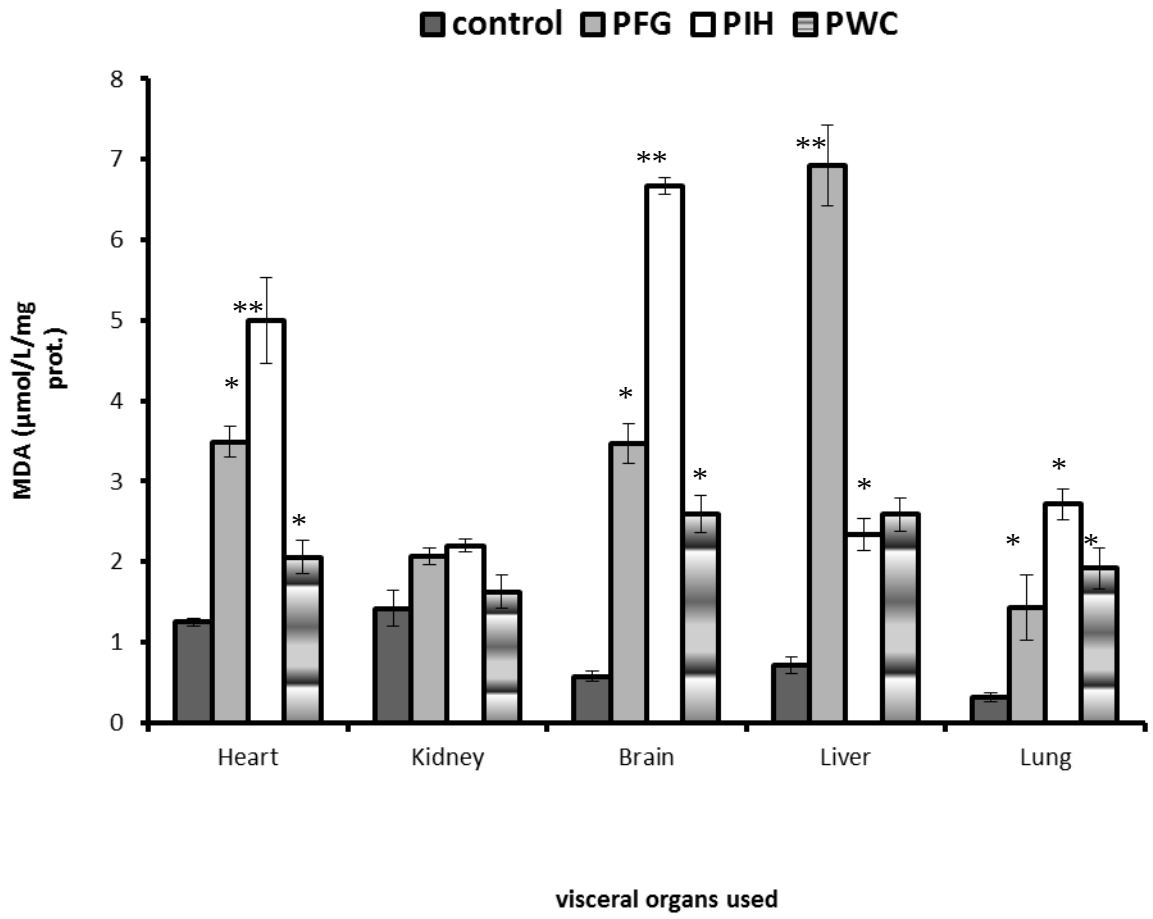


Figure 33: Effects of petrol on Malondialdehyde (MDA) of the homogenate of visceral organs in adult male rats

Control

Catalase

Glutathion reductase

Super oxide dismutase

**= p<0.01; *= significant increase

*=p=0<0.05

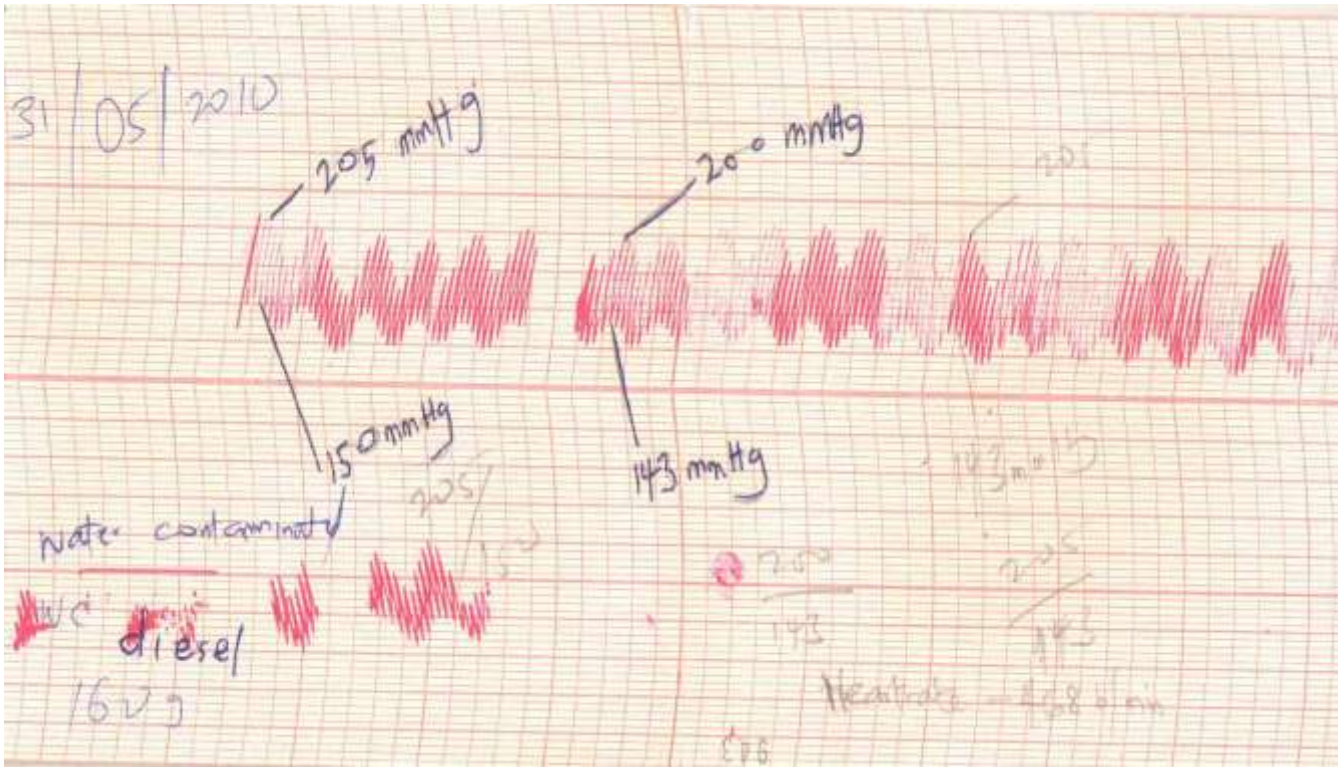


Figure 34. Tracing of blood pressure measurement in diesel water contaminated group of adult male rats

SBP= 205mmHg

DBP= 150mmHg

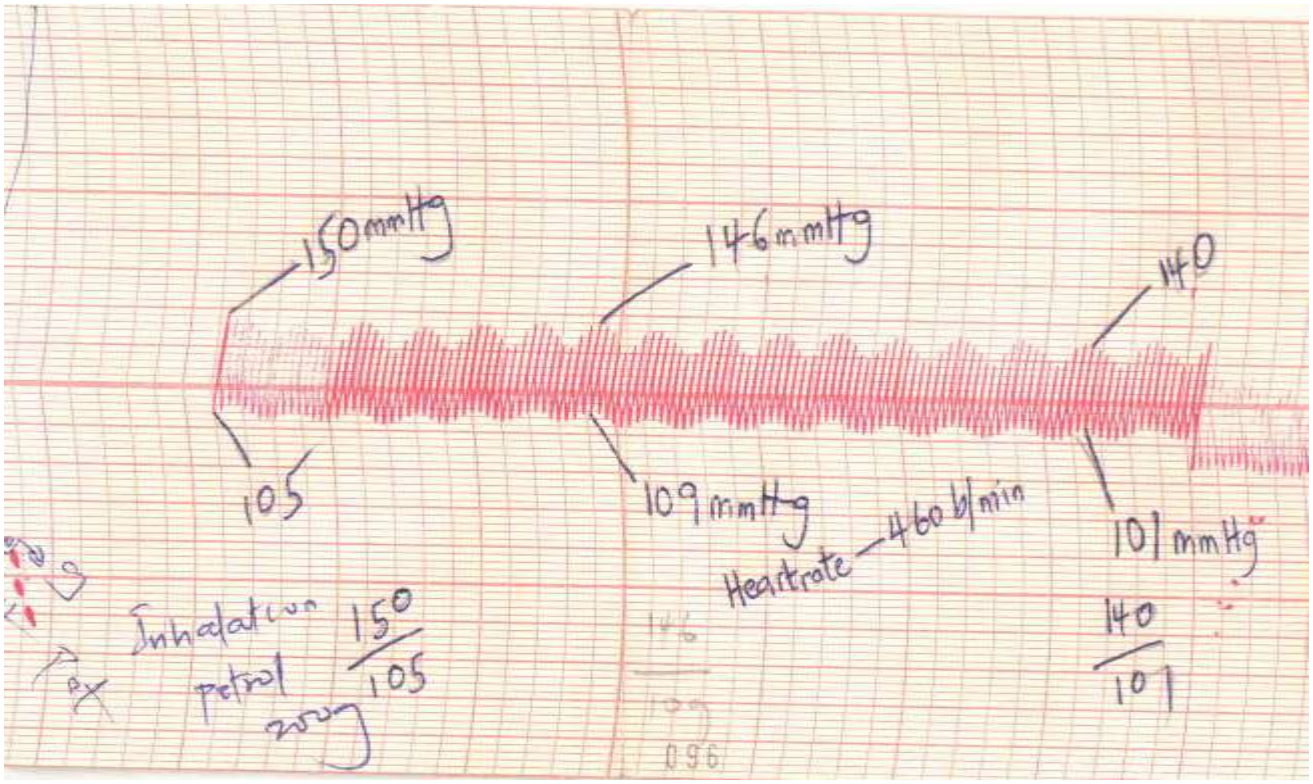


Figure 35. Tracing of blood pressure measurement in petrol inhalation group of adult male rats

SBP= 150 mmHg

DBP= 105 mmHg

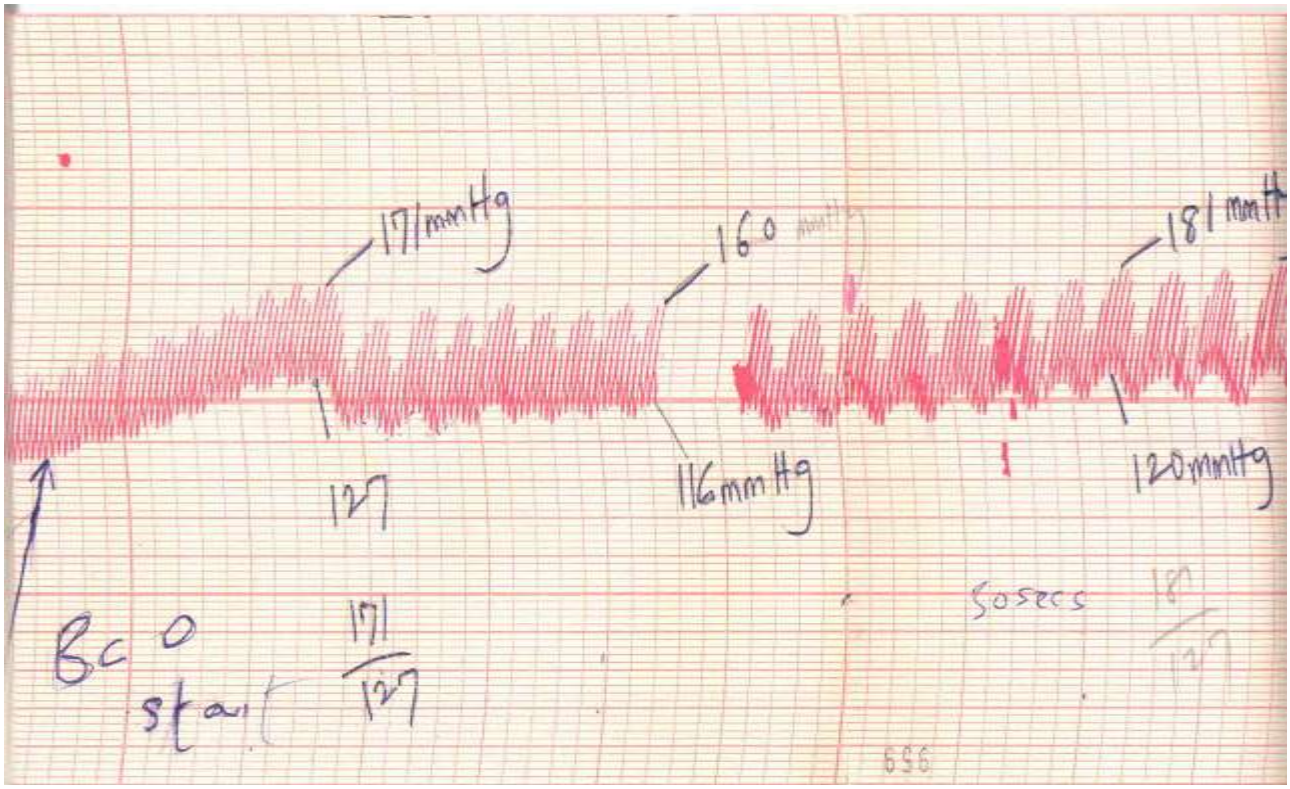


Figure 36: Tracing of bilateral carotid occlusion measurement in petrol inhalation group of adult male rats

SBP= 171 mmHg

DBP= 127 mmHg

Figure 37. Picture of Polygraph model 7D used for measuring blood pressure

4.15 Effects of Petroleum Products on Histological Analysis of the Brain, Heart, Kidney, Liver and Lungs

The direct effect of exposure to petroleum products on tissue was checked by histological analysis of brain, heart, kidney, liver and lung.

Plates 1-4 showed the results of histological analysis of **brain** of rats in control, diesel, kerosene and petrol exposed groups. Results showed there was ranging degrees of cellular degeneration which was most severe in the diesel exposed rats.

Plates 5-8 showed the result of histological analysis of **heart** of rats in control, diesel, kerosene and petrol exposed groups. Results showed that there was ranging degree of cellular degeneration and loss of myocytes. The degeneration was most severe in the diesel group

Plate 9-12 showed the result of histological analysis of **kidney** of rats in control, diesel, kerosene and petrol groups. Results showed there was terminal hydropic degenerative changes (E) in the diesel group, terminal degenerative changes in the tubules, glomeruli and the interstitium in kerosene group and showing area of degeneration in medulla, glomeruli and the tubules in the petrol group

Plate 13-18 showed the results of histological analysis of **liver** of rats in control, diesel, kerosene and petrol groups. Results showed that there was widespread hepatocellular necrosis predominantly centrilobula. It is peri-portal in origin

Plate 19-20 showed the result of histological analysis of **lung** of rats in control, diesel, kerosene and petrol groups. Results showed widespread degeneration of the alveoli and parenchyma in (diesel and kerosene group) as well as haemorrhage and edema in petrol group. The degeneration was most severe in the diesel group

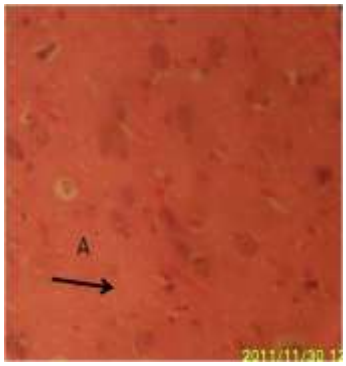


Plate 1: photomicrograph of the brain in control rats showing normal cells (H&E stain**).**

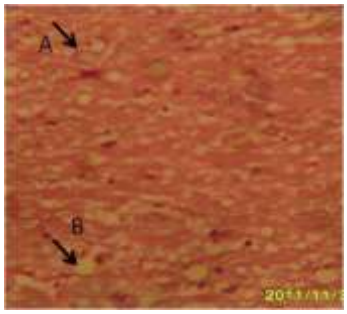


Plate 2: photomicrograph of the brain tissue in rats exposed to diesel (DIH) showing ranging degree of cellular necrosis (A&B) (H&E stain**).**

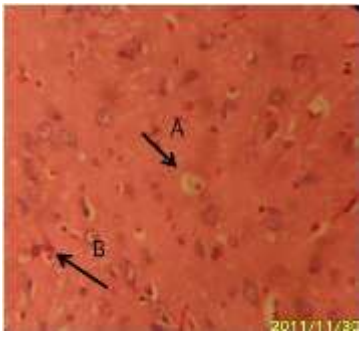


Plate 3: photomicrograph of the brain in rats exposed to kerosene (KIH) showing haemorrhage(B) and cellular degeneration(A) (H&E stain**)**

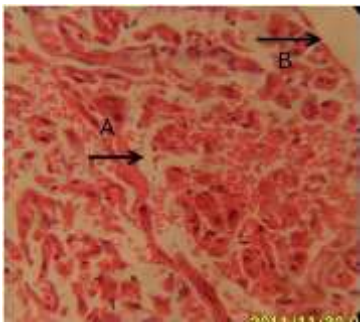


Plate 4: Photomicrograph of the brain in rats exposed to petrol (PIH) showing apical (B) and cellular degeneration (A) (H&E stain**)**

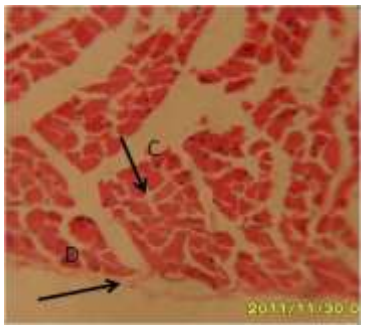


Plate 5: Photomicrograph of cardiac tissue in control rats showing normal myocytes(C) and normal apex(D) (H&E stain)

Plate 6: Photomicrograph of cardiac tissue in rats exposed diesel (DIH) showing ranging degree of cellular degeneration (C) that appeared to have originated from the apex(D)

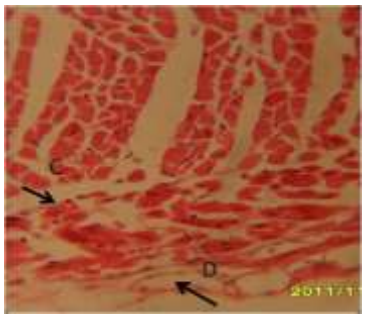


Plate 7: Photomicrograph of cardiac tissue in rats exposed to kerosene (KIH) showing focal loss of myocyte/ degeneration (H&E stain).

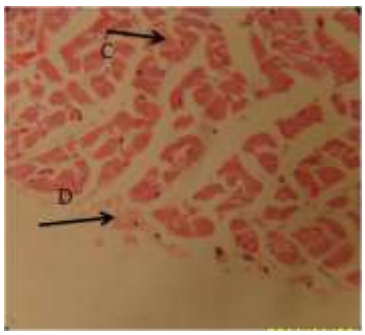


Plate 8: Photomicrograph of cardiac tissue in rats exposed to petrol (PIH) showing area of mild degeneration(C) and some degree of apical degeneration(D) (H&E stain).

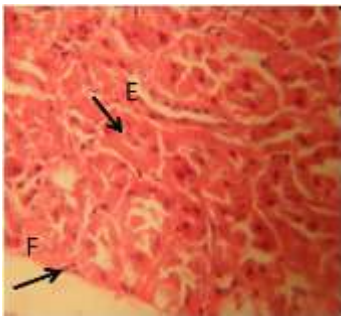


Plate 9: photomicrograph of kidney in control rats showing normal kidney cell **(**H&E stain**)**

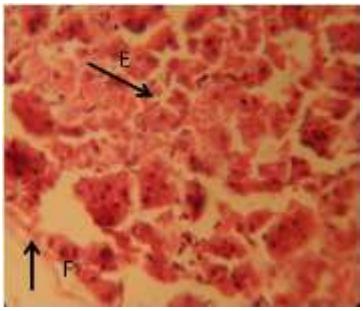


Plate 10: photomicrograph of kidney in rats exposed to diesel (DIH) showing terminal hydropic degenerative changes (E) (H&E stain**)**

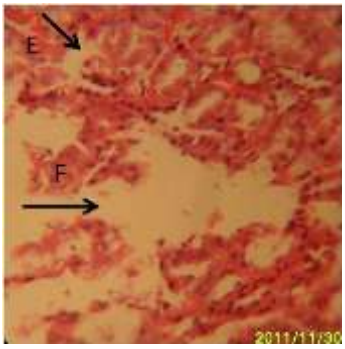


Plate11: photomicrograph of kidney in rats exposed kerosene (KIH) showing terminal degenerative changes in the tubules, glomerulus and the interstitium; (H&E stain**).**

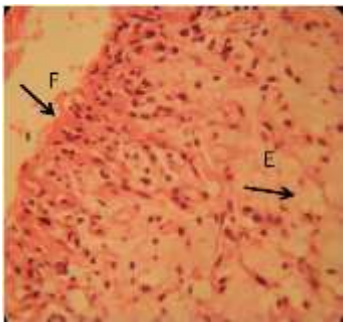


Plate 12: Photomicrograph of the kidney in rats exposed to petrol (KIH) showing area of degeneration in medulla, glomeruli and the tubules **(**H&E stain**)**

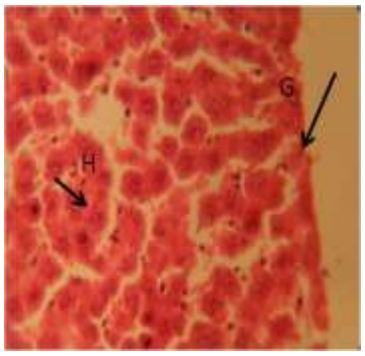


Plate 13: Photomicrograph of liver showing normal Hepatic cells (H&E stain)

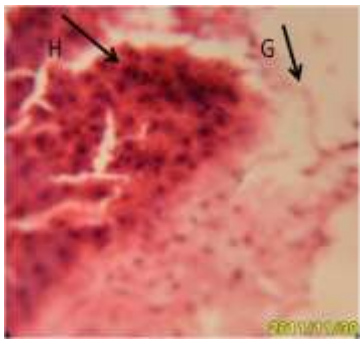


Plate 14: Photomicrograph of liver in rats exposed to diesel (DIH) showing widespread hepatocellular necrosis (G) predominantly peri- portal (H) **(**H&E stain**)**

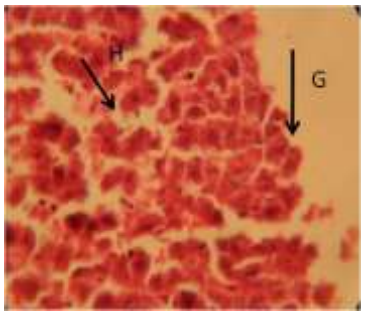


Plate15: Photomicrograph of liver in rats exposed to kerosene (KIH) showing widespread hepatocellular necrosis (H) predominantly peri-portal (G) **(**H&E stain**)**

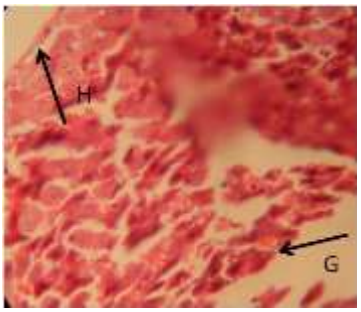


Plate 16: Photomicrograph of the liver in rats exposed to petrol (PIH) showing hepatocellular hemorrhage and degeneration (G), originating from the capsule (H) **(**H&E stain**)**

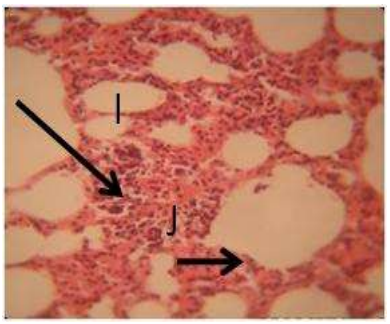


Plate 17: photomicrograph of lung in control rats showing normal parenchyma and alveoli (H&E stain**)**

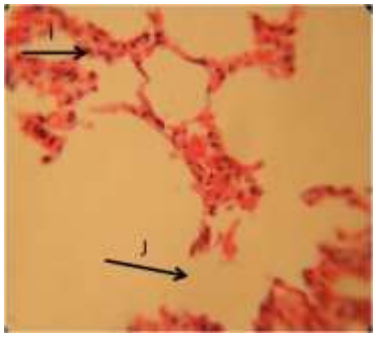


Plate 18: photomicrograph of lung in rats exposed to diesel (DIH) showing widespread degeneration of parenchyma and alveoli wall **(**H&E stain**)**

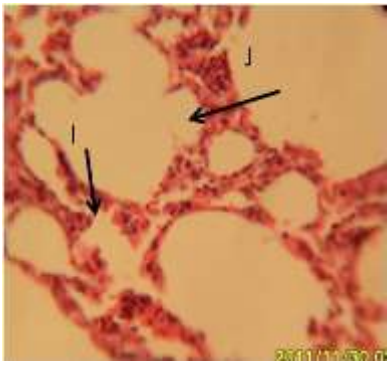


Plate 19: photomicrograph of lung in rats exposed to kerosene (KIH) showing widespread degeneration (I) of parenchyma and alveoli wall (J) **(**H&E stain**)**

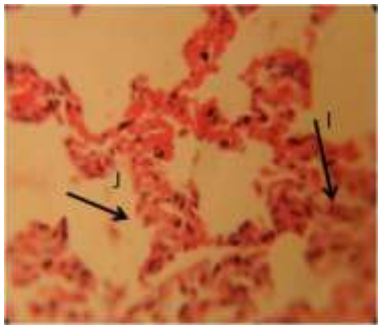


Plate20: photomicrograph of lung in rats exposed to petrol (PIH) showing widespread degeneration (I) of parenchyma and alveoli with edema of the alveoli (J) (H&E stain**)**

CHAPTER FIVE

5.0 DISCUSSION

5.1 Discussion of methods

Rat model was used for this study because it might be difficult getting human volunteer. Previous workers have used rats for similar studies (Uboh et al., 2005).

Selection of rats used in this study considered the toxic nature of the study so that we prepared for toxic effects that may affect weight or other body parameters, selection of the rats was therefore done in such a way that larger rats were in the treated groups so that when invasive blood pressure was to be measure there will be no difficulty inserting cannula into the blood vessels. The age range chosen 12-15 weeks, plus the eight weeks of the test would have made all the rats to be full adult knowing fully well that age is a major factor that affect blood pressure

This study was carried out using three main routes of contact with petroleum products (direct ingestion of feed mixed with petroleum products, inhalation using the modified nose inhalation adopted by Uboh et al., (2005), and water contaminated with petroleum product using the method of Anigbogu and Ojo, (2009). This work also considered three refined petroleum products- diesel, kerosene and petrol. The carbon content was highest in diesel (15-25carbon atoms) followed by kerosene (12-15 carbon atoms) and lastly by petrol (5-11 carbon atoms). These two factors contributed to the result we got and their effects on the cardiovascular functions.

Urithane (25%) and cloralose 1% (BDH chemicals limited, Poole, England) was used as the anaesthesia before measuring blood pressure. This is because mixture of urethane and chloralose has been found to be compartible and will not stimulate any change in blood pressure in anaesthetic state. It was given intra-peritoneally at a standard dose of 5ml/ kg body weight

Invasive means of measuring the blood pressure was adopted because it is very stable and more accurate than the non invasive means. The grass polygraph model 7D (Grass instruments Limited, Quincy, Mass, USA) with the polygraph speed set up at 5mm/sec after appropriate caliberation. Because the blood pressure measurement was invasive and terminal, we could not measure the time when the rise in blood pressure commenced and how it progressed till eighth weeks of the exposure to petroleum products.

Biochemical analysis for creatinine, urea, albumin and total protein and creatine kinase were included in the study to assess the health status of the renal system. Knowing fully well that defect with the renal system would also have an effect on cardiovascular function. Histology and Creatine kinase assessment was brought in to the study to affirm the physical status of the organs relating the effect of petroleum product on the organs that resulted in the overall cardiovascular dysfunction.

The rats in the feeding group actually took their feed although at different rate. Petrol is very volatile, the odour in the perol feed disappears faster than kerosene and diesel. This actually made the feeding rate to vary but they eventually ate the feed.

5.2 DISCUSSION OF RESUTS

5.2.1 Blood pressure

The findings in this study showed that exposure to petroleum products resulted in significant increase in systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP) and heart rate (HR) in all diesel and kerosene treated groups compared with control. In the diesel group, diesel food ingestion (DFG) induced the highest blood pressure compared with diesel inhalation (DIH) and Diesel water contaminated groups (DWG) and with the kerosene and petrol groups. In the kerosene group, the KWG induced the highest blood pressure compared with the others in the group. The increase which was highest in the diesel appears to have some relationship with the hydrocarbon component of the compound. diesel contains the highest number of carbon.

In the petrol group, there was significant increase in blood pressure and heart rate of PIH and PWC compared with control while there was an insignificant reduction in the PFG. The volatility of petrol made it to have little or no effect on the blood pressure in the PFG group. One week after stoppage of exposure, the blood pressure persisted in the same manner. This persistence seen in the blood pressure and HR suggests that exposure to petroleum products could cause hypertension.

In the study, there was significant increase in creatine kinase in all the sub groups. In normal conditions, there is very little creatine kinase (CK) circulating in the blood of the average, healthy human being. Elevation of CK is indicative of damage to muscle and heart. It is therefore indicative of injury such as myocardial infarction, myositis, myocarditis or any cardiac or muscular disease (Schlattner et al., 2006). The result of histology of the heart also showed ranging degree of degeneration from the apical region including loose of myocyte. All the above mentioned depict lose of some of the physiological properties of the heart that are responsible for carrying out the cardiovascular functions. The cells of the conducting systems of the heart is suspected to have involved in degeneration, affecting rhythmicity of

the heart, this can also be attributed to sudden death as a result of myocardial infarction and heart failure.

5.2.2 Effects of Petroleum Products on Baroreflex Response

The significant increase seen baroreflex responses in diesel and kerosene (inhalation and water contaminated groups) and not in the petrol treated rats suggest that the sensors in the diesel and kerosene groups have been suppressed. Baroreceptors are sensors located in the blood vessels and act as part of the baroreflex negative feedback mechanism in the short-term regulation of arterial pressure (Levy and Pappano, 2007). These mechanoreceptors reset in the maintenance of a normal arterial pressure (Ganong, 2007). This suggests that with exposure to petrol, the baroreceptors are still capable of re-setting the arterial blood pressure to a new higher than normal value as though normal. Petrol contains fewer hydrocarbon compared with either kerosene or diesel, thereby producing pressorlytic effect. On the other hand, the baroreceptors have been seriously altered by diesel and kerosene in diesel- and kerosene-exposed animals; such that they could not reset or adjust the arterial blood pressure as before. The increased carbon content is suspected to be contributing factor

This persistence is a marker of the degree of damage done by the petroleum products generally and on individual consideration. Diesel and kerosene were seen above to have elicited higher baroreflex response; similarly the persistence in blood pressure and heart rate was highest in diesel group followed by kerosene group and least in petrol.

It is noteworthy that petroleum products are risk factors of cardiovascular dysfunctions (Berkowitz and Booth, 1978; Anderson et al., 2003; El-Menyar, 2009) which are major challenges among refinery workers, inhalant abusers, and the general public at large since they form an essential component of life, thus accounting for clinical complications leading to increased mortality (LoVecchio and Fulton 2001; Ritchie et al., 2003).

5.2.3 Effect of Petroleum Product on Body Weight changes

The progressive loss in weight seen in the diesel food ingestion group (DFG) suggest that the food might not have been absorbed and metabolised. The weight changes observed in other diesel groups dropped after the 5^{th} week and balanced again after 7^{th} week. This suggests inconsistency in body growth and development on exposure to diesel. There is an irregularly progressive fall in body weight changes in the kerosene food ingestion group (KFG). The KIH and KWC group experience reduced percentage weight gain compared with control. In the petrol group there was progressive growth with reduced percentage rate compare with control. The impairment seen is most prominent in the diesel group. These significant reductions seen in body weight noted particularly from the fifth week in kerosene and petrol subgroup; could be linked with the hydrocarbon component of the compounds. This finding agrees with previous studies (Hanson and sharp, 1978; McHugh, 1987) that showed that petroleum solvents dissolve fat and lipids in the body with resultant degeneration of fat store in the body. Hydrocarbons caused degeneration of the liver and small intestine with consequent poor absorption of nutrients (Davoodi and Claireaux, 2007). It also agreed with some previous finding that petrol caused irritation of the intestinal wall.

During cellular metabolism significant increase in the level of **free radical** species in various tissues (Lam et al., 1994; Bondy et al., 1995) is generated. The generated free radicals and reactive metabolites can interact and disrupt the cell membranes of the affected tissues thereby causing the tissue enzymes and other metabolites to leak out and increase the plasma concentrations as observed in this study. This may interact with absorption conversion of the feed consumed into useful nutrients required by the body, thus accounting for the reduced body weight gain when compared to the control (Patrick-Iwuanyanwu et al., 2011) .

A significant decrease has been found in weight of mice following exposure to diesel fuel

(Schultz et al., 1981). This also conforms to our histological findings in liver which shows general degeneration originating from the capsule.

5.2.4 Effect of Petroleum Products on Haematological Indices

The haematological results of the control rats were in consonance with the standard value of previous researchers (Johnson, 1996).

Alteration in some haematological parameters and indices were recorded in adult male rats exposed to diesel, kerosene and petrol via ingestion in food, inhalation and drinking contaminated water. The mean RBC values showed significant reduction in all the diesel and petrol sub groups. This shows that diesel induces more damage to the haemopoietic system than kerosene. The reduction in kerosene was only significant in water contaminated group (KWC). This result is in agreement with previous work done by Eyong et al., (2004), Ovuru and Ekweozor (2004). These researchers observed similar heamatotoxic effects in rats and rabbit following ingestion of crude oil and refined petroleum product.

The PCV was significantly lower in all diesel and kerosene subgroups as well as petrol food and inhalation group. The observed decreased PCV is believed to be as a result of decreased RBC which is consistent with the findings of Eyong et al., 2004, Itan and Udofia 2011.

Another probable reason for the observed decrease in RBC counts may be due to perturbations of crude oil hydrocarbons on growth or differentiation inducers involved in erythropoiesis such as erythroid colony forming unit, according to Keller and Synder, (1986)

Haemoglobin concentration showed significant reduction in all the subgroups of diesel, kerosene food ingestion and inhalation, as well as petrol food ingestion and inhalation. This result is consistent with the work of Suzanne, (2003) who reported that chronic ingestion of

petroleum oil by birds caused oxidative chemical damage to haemoglobin resulting in reduced haemoglobin level in birds.

The reduction in RBC, PCV and Hb suggest anaemia and reduced tissue perfusion which could also expose the body tissue to increased oxidative stress. Anaemia, chronic inflammation and poor nutritional status promote development of oxidative stress and reduced antioxidant status (Azinge et al., 2001). The toxic component in petroleum product changed blood chemistry and interfered with blood cell production hence the reduction in the indices according to report by Ovuru and Ekweozor, (2004).

White blood cells function primarily in body defense against foreign bodies and this is often achieved through leucocytosis and antibody production (Marieb, 1995; Robbin and Angel, 1976). There was a significant increase in the white blood cells (WBC) in ingestion group. This result is not consistent with the work of Okoro et al., (2006); Ovuru and Ekweozor, (2004); Dede and Kagbo, (2002); they reported a decrease in WBC after exposure to crude petroleum. The platelets (PLT) and lymphocyte count were reduced significantly in diesel in the inhalation group, in the diesel and petrol of ingestion group.

The decrease in platelets and lymphocyte observed in this study may result in impairement of the immunity and so the body will be liable invation by foreign organism

5.2.5 Effects of Petroleum Products on Liver Enzymes

The obtained data from this study revealed that there was significant increase in the aminotransferases (AST and ALT) and alkaline phosphatase (ALP). This finding is in agreement with the Uboh et al., (2005). Report showing that 4 hours/day of kerosene and petrol fume inhalation in a period of 2 weeks significantly increased the concentration of ALP and aminotransferase in an experimental rat group in comparison to the control group.

In their study, Uboh et al. have proposed that increased activity of aminotransferase, especially of ALT, in the experimental group is caused by liver cell injury due to increased permeability of hepatocyte cell membranes or focal necrosis of hepatic cells. They documented that the increase in concentration of AST in the experimental group may be due to abnormal dynamic properties of cellular membranes following exposure to inhaled fumes These marker enzymes are cytoplasmic in origin and are released into the circulation after cellular damage (Lin et al., 2000). The level of serum ALT activity has been reported to be increased as a result of liver injury in patients developing severe hepatotoxicity (Beckett et al., 1989). In all the studied parameters, ALT is specific to the liver, whereas AST is also found in the cardiac muscle, the kidneys and the brain. Alkaline phosphatase (ALP) comprises a group of enzymes present in a variety of tissues including those of the liver and kidney. An increase of up to three times in AP levels is relatively non-specific. Redlich and colleagues, (1988) reported that occupational liver diseases may occur more frequently in workers exposed to occupational fumes than in the general population, and stated that improvement in conditions with liver enzyme abnormalities were seen after modification of the workplace. The increased concentration of ALP in the experimental group reported in this study is in accord with the results of Jadhav et al., (2007) and Marques et al., (2006).

5.2.6 Effect of Petroleum Products on Serum Biochemical Properties

There was significant increase observed in creatinine and urea concentration in the serum of all the exposed groups, as well as the significant reduction in creatinine and urea values in the urine when compared with control were indication of renal impairment. The factors that regulate cardiovascular functions (rennin-angiotensin mechanism) in the renal system will be affected. Secretion of rennin by the juxtaglomerular apparatus of the

kidney is known to play a significant role in the regulation of blood pressure. Renal impairment will there affect the secretion of rennin. Previous researchers have found long term (chronic) exposure to hydrocarbons resulted in distal tubular acidosis and present with an anion gap acidosis; as well as Glumerulo-nephritis and chronic tubule-interstitial nephritis (Brouette and Anton, 2001).

The significant increase seen in total protein and albumin values in urine of treated rats compared with control indicate renal pathology and confirm the observation of Bartimaeus and Jacobs, 2003; Yaqoob et al., 1993). Hypertension appears to be an important indicator for increased serum creatinine and progressive decline in renal function (Ishida et al., 2001). This infers that there was damage to liver and kidney as well as inability to absorb enough protein because of the effect of the petroleum product on the body. The severity of the effects is directly related to degree of damage.

5.2.7 Effect of Petroleum Products on Antioxidant Activities and Lipid Peroxidation in Serum and Homogenate

In this study, diesel was found to induce significant reduction in the activities of catalase (CAT), Glutathione reductase (GSH) and superoxide dismutase (SOD) in all the groups. The significant reduction seen in the activities of catalase, SOD and GSH, showed that the antioxidants have been used up by the body. This reduction seen is as a result of depletion of antioxidant activities due to oxidative stress. According to Aberare et al., (2011), in their work on lipid peroxidation in rats exposed to premium motor spirit, once there is liver damage the disturbance of cell membrane integrity is likely to cause some membrane lipids to be released into circulation; while on the other hand, it causes the tissue to compromise its effectiveness in regulating lipid metabolism. Robertson et a., (2001), found out in their work that one of the main hypotheses of mechanism of hepatocyte injury from petrol fume

metabolism is associated with oxidative stress and lipid peroxidation resulting from the imbalance between pro-oxidant and antioxidant chemical species. These oxidative processes produce free electrons, H_2O_2, and reactive oxygen species (ROS) while depleting the potent antioxidants, glutathione and vitamin E in accordance with the report of McCullough et al., (2002). Significant increase in malondihaldehyde (MDA) concentration in all the groups in the serum and tissue showed a high degree of lipid peroxidation.

Petroleum hydrocarbons and other related carbon-containing compounds are converted into free radicals or activated metabolites during their oxidation in the cells, especially mammalian liver and kidney cells. It is these activated metabolites that react with some cellular components such as membrane lipids to produce lipid peroxidation products (Onwurah, 1999).

5.2.8 Effects of Petroleum Products on Histology of Some Vital Organs

In this study there is ranging degree of cellular necrosis, as well as haemorrhage seen in the **brain** of the diesel and kerosene groups. This finding can be used to explain the derangement seen in baroreflex response to diesel and kerosene

There was ranging degree of necrosis, loss of myocytes, haemorrhage and some degree of apical degeneration in the heart. The presence of this lesion can be used to explain the dysfunction cardiovascular activities seen in this study. Damage is most severe in the diesel group

The terminal hydrophic degeneration in the tubules of glomeruli and the interstitium seen in the kidney confirms the renal toxicity suspected by abnormal concentration of creatinine urea, total protein and albumin in serum and urine of treated rats.

The widespread hepatocellular degeneration that originated from the capsule seen in the liver of this study confirms the lipid peroxidation and increase in serum liver enzyme activities in all exposed rats.

The lungs showed widespread necrosis of the alveoli and parenchyma in the diesel and kerosene group; haemorrhage and edema in the petrol group.

It has been reported that metabolism of aliphatic and aromatic hydrocarbons, the major constituents of petroleum and petroleum-derivatives, generate significant increase in the level of **free radical** species in various tissues (Lam et al., 1994; Bondy et al., 1995). The generated reactive intermediates can interact and disrupt the cell membranes of the affected tissues thereby causing the tissue enzymes and other metabolites to leak out and increase the plasma concentrations as observed in this study.

This concurs with significantly reduced urine creatinine and urea as well as increased total protein when compared with control group, Tubular dysfunction was also prominent with petroleum oil exposure as seen in our result (Yaqoob et al., 1993).

The moderate tissue degeneration and hemorrhage seen in the brain of the rats exposed to petrol, diesel and kerosene suggests brain tissue and vascular damage, in this study. In addition, the brain tissue and vascular damage is attributable to increase in lipid peroxidation and decrease in antioxidant enzymes in the brain tissue following exposure to the petroleum products. The high mean arterial blood pressure, heart rate and baroreflex activities reported is attributable to degeneration to brain tissue and vessel. This supports the alteration in cardiovascular centre at the medulla that produced increase in MAP, HR and baroreflex activities in this study.

5.3 Summary of Findings

Objectives	Findings
1. Blood pressure and heart rate, following exposure to petroleum products	The Blood pressure and Heart rate increased significantly compared with control in all groups, except in petrol ingestion group. The elevation was highest in the petrol inhalation group. The increase persisted one week after stoppage of exposure, suggesting that the exposure cause hypertension
2. The baroreflex responses using the method of bilateral carotid occlusion, following exposure to diesel, kerosene and petrol	There was significant increase in the baroreflex responses in the diesel and kerosene group suspected to be as a result of impairment in the activities of the baroreceptors. On the other hand inhalation of petrol induced insignificant increase baroreflex response which returned to normal value after twenty five seconds. This suggests that the reflexes have been altered in the diesel and kerosene exposed groups such that they could not reset the blood pressure. On the other hand with petrol exposure, the baroreceptors are still capable of reseting the arterial blood pressure to a new higher than normal value as though normal; this was suspected to be due to pressor effect as a result of reduced number of carbon in the petrol compared with diesel and kerosene
3. Haematological indices, liver enzymes and biochemical properties in serum and urine	There was significant reduction in RBC, Hb, and PCV suggesting anaemia. There was significant increase in WBC observed except in the kerosene group. Significant reduction in platelets and lymphocyte count which points towards reduced body immunity. The activities of AST, ALT and ALP were significantly elevated in all the rat groups used in the study. The total protein and albumin

	were reduced in the serum and elevated in the urine when compared with the corresponding control group. Concentrations of serum creatinine and urea were higher when compared with control while urine concentration were lower when compared with corresponding control This confirms renal toxicity that have been reported by previous workers
4. Antioxidant activities and lipid peroxidation in serum and tissue of heart, liver, brain, kidney and lungs	In this study it was found out that CAT, GSH and SOD activities were reduced in serum of almost all the treated groups while the MDA level increased significantly in all the groups, as marker of lipid peroxidation. The result was similar in the tissue homogenate of five visceral organs (brain, heart, kidney, liver and lungs). The degree of damage varies with the petroleum products.
5.Histological analysis of vital organs	Plates 1-20 showed the effect of diesel, kerosene and petrol on the brain, heart, kidney, liver and lungs. The Photomicrograph showed the series of derangement in the tissues.

5.4 Conclusion

Exposure to petroleum products resulted in increased blood pressure and heart rate; baroreflex responses increased in the diesel and kerosene groups as a result of impairment of the baroreceptors. It caused anaemia, reduced platelets and lymphocytes count and renal dysfunction. The effects were most severe in the diesel groups especially food ingestion group compared with other groups. This study suggests that afore-mentioned observations are partly caused by oxidative stress by altering the levels CAT, GSH, SOD and MDA.

5.5 Contributions to Knowledge

1. The study establishes that there was alteration of baroreflex responses by diesel and kerosene while the effect is less severe in the petrol group.
2. Elevated blood pressure has been associated with exposure to petroleum products in previous researches, but this study has established that the elevated blood pressure could persist even after stoppage of exposure.
3. This study establishes that continuous exposure to petroleum products promotes lipid peroxidation and reduced antioxidant activities irrespective of route of exposure. (Previous workers have used only inhalation route).

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APPENDIX 1

Diesel Tanker Explodes At MTN Premises in Abuja Posted: July 27, 2012 - 19:06

APPENDIX 2

Exhaust from a petrol operated car

APPENDIX 3


Exhaust from a diesel operated car

APPENDIX 4


Waste burning in incinerator

APPENDIX 5

Diesel exhaust from an engine in a power station