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Haematological Properties of Aqueous Extracts of *Phyllanthus amarus* (Schum and Thonn.) and *Xylopia aethiopica* (Dunal) A. Rich in Albino Rats

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ABSTRACT A study on haematological effects of aqueous extracts from *Phyllanthus amarus* and *Xylopia aethiopica* was investigated in albino rats. The extracts from both plants caused a dose-dependent decrease in erythrocyte sedimentation rate (ESR) with 400mg/kg of *X. aethiopica* causing the least ESR of 2.7 ± 0.6 mm/hr. Significant increases were obtained in red blood cell (RBC) count especially with 100mg/kg of *P. amarus* and *X. aethiopica* that caused 5.6% and 7.8% increases in RBC count respectively ($P < 0.05$). Similar pattern of result was obtained for packed cell volume (PCV). *P. amarus* did not appear to affect haemoglobin concentration, but higher values of HB concentration were obtained for *X. aethiopica*; the difference was, however, not significantly different from the control ($P > 0.05$). Total and differential count studies showed significant increases in the number of circulating leucocytes and neutrophils respectively especially with 100mg/kg of extracts ($P < 0.05$). Assessment of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) gave significantly higher values of ALT for *P. amarus* – treated rats ($P < 0.05$). It was therefore suggested that while both plants can serve as immune boosters and blood tonics, there is need for caution on excessive and prolonged consumption of *P. amarus*.

INTRODUCTION

The use of plants by man for the treatment of various diseases has been in practice and is very popular in many developing countries of the world for over a long period of time (Sofowora 1993; Gill 1990). This practice has gradually gained popularity in some parts of Europe and North America (Leese and William 1994; Odeigah et al. 1999). In Africa especially in the tropical areas, several factors such as poverty and illiteracy still militate against availability and accessibility of Western medical services. The need to have a strong, healthy immune system cannot be overemphasized in our present day society. Most illnesses such as AIDS, and cancer are believed to be immune-related disorders. Herbs used to boost the immune system are generally referred to as immunostimulants. They help to increase the activity of the immune system by mobilizing effector cells which act against all foreign particles, rather than one specific type (Odukoya et al. 2007). Herbs known to have been commonly studied as immunostimulants are *Echinacea* and *Astragalus*.

Information on the use of *P. amarus* and *X. aethiopica* as immune boosting herbs are scanty. However they have been used severally to meet various conditions. *P. amarus* is a supportive herb assisting with circulatory, digestive and skeletal systems (Odukoya et al. 2007). Infusion of its leaves is used for haemorrhoids, venereal diseases, tachycardia and female sterility (Burkill 1994). In addition it has been considered useful in meeting liver disorders. *X. aethiopica* is a tropical West American evergreen tree bearing aromatic seeds usually used as a condiment. The fruit decoction is used to treat bronchitis, asthma and rheumatism (Burkill 1985). They are also used in many herbal preparations to produce xylopic acid, a substance which has been found to have antimicrobial effects (Karioti et al. 2004).

The present study evaluated *in vivo* haematological activity of *P. amarus* and *X. aethiopica* in albino rats. The effects of these medicinal plants on two important liver enzymes: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also assessed. The implication for the use of these plants to treat immune-related and other haematological problems was discussed.

METHODOLOGY

Preparation of Plants Extracts: Whole plant

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of *P. amarus* and seeds of *X. aethiopica* were obtained from a local market in Lagos, Nigeria. They were authenticated at the Forestry Research Institute of Nigeria (FRIN) Ibadan. For the extraction, the plant materials were first washed free of sand, cut into pieces and air-dried before being ground into powder. Seventy-five grams of the powder was extracted with 500ml of distilled water using Soxhlet extraction. The extract was slowly evaporated *in vacuo* to obtain a total yield of 3.59g. Weighed sample of the extract was then used to prepare test solutions of the desired concentration to enable administration of appropriate doses of materials.

Experimental Animals and Administration of Plant Extracts: The animals were 25 healthy male albino rats (160 – 200g) obtained from the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos. They were housed in the Laboratory Animal Care Unit in the Biological Garden of the University of Lagos for a 2-week acclimatization period. Normal rat feed and tap water were provided *ad libitum*. Administration of materials was done using the gavage method. The animals were treated daily for 25 consecutive days.

Experimental Design: The rats were randomly selected and assigned to 5 groups of 5 rats per cage. One group (control) was given ordinary distilled water at 1.0ml/100g body weight (b. wt). The remaining 4 groups were given aqueous extracts of either *P. amarus* or *X. aethiopica* at 100mg/kg and 400mg/kg b.wt. respectively.

Haematological Analysis and Liver Enzyme Assay: Haematological and liver enzyme assays were done at the Biochemistry Department of the Nigerian Institute of Medical Research (NIMR), Lagos. The animals were anaesthetized (ether anesthesia) and blood samples were obtained from the tail into EDTA bottles. The packed cell volume (PCV), haemoglobin (HB) concentration, red blood cell (RBC) count, erythrocyte sedimentation rate (ESR), and white blood cell (WBC) count (total and differential) were determined using the Automated Haematological Analyzer, Sysmex KX-21 (Japan). For liver enzyme assay, the rats were sacrificed using ether fumes and the liver was removed for enzyme assay. Two important liver enzymes were determined: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) using the method of BergMeyer and Bernt (1974).

Data Analysis: The results were analyzed using a statistical software package – SPSS

Version 12. Data were expressed as mean \pm standard error of the mean (mean \pm SEM). Student's t-test was employed for comparison between two sets of data. In those cases where the variables to be compared are three or more, analysis of variance (ANOVA) was done. $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows the results of the RBC count and some other haematological parameters in the experimental animals. *P. amarus* and *X. aethiopica* at 100mg/kg caused increases of 5.6% and 7.8% respectively in RBC count ($P < 0.05$). The effect of the extracts on RBC was not as pronounced with 400mg/kg as with 100mg/kg. Similar pattern of result was obtained for packed cell volume (PCV). *P. amarus* did not appear to affect HB concentration, but higher HB values were observed in *X. aethiopica*-treated rats. It was also observed that extracts from both plants caused a dose-dependent decrease in erythrocyte sedimentation rate (ESR) with 400mg/kg of *X. aethiopica* causing the least ESR of 2.7 ± 0.6 mm/hr as compared to the control ESR of 5.7 ± 0.7 mm/hr ($P < 0.05$).

Table 2 shows the effect of plant extracts on total and differential white blood cell (WBC) counts. Basophils and Eosinophils were produced in insignificant proportion and were therefore not included in the results. It could be seen from Table 2 that both plant extracts caused significant increases in the total and the differential (WBC) counts. The effect was not dose-dependent because 100mg/kg b.wt of both extracts had greater effect than that of 400mg/kg b.wt. Comparing both extracts for their effect on total WBC indicated that *P. amarus* caused greater increase in WBC at both the lower (100mg/kg) and the higher (400mg/kg) doses.

The results of liver enzyme analysis for ALT and AST are shown in Figure 1 (a and b). It could be observed that 100mg/kg of *X. aethiopica* did not cause any increases in the level of both enzymes ($P > 0.05$). *P. amarus*, however, caused significant increases ($P < 0.05$) in the level of ALT with the lower dose of 100mg/kg (37.6 ± 0.7 IU/L) and the higher dose of 400mg/kg (46.0 ± 0.8 IU/L) when compared to the control (16.8 ± 0.6 IU/L). No significant increase in the level of AST was observed in *P. amarus*-treated rats.

Table 1: Effect of the Plant Extracts on Red Blood Cell (RBC) Count, Packed Cell Volume (PCV), Haemoglobin (Hb) Concentration and Erythrocyte Sedimentation Rate (ESR) in Rats.

Group	Treatment	Dose(mg/kg)	RBC($\times 10^6/\text{mm}^3$)	PCV (%)	HB(mg/mm ³)	ESR(mm/hr.)
Control	Dist. water	0	3.60 \pm 0.30	35.7 \pm 4.1	0.12 \pm 0.04	5.7 \pm 0.7
A	<i>P. amarus</i>	100	3.80 \pm 0.34*	37.3 \pm 4.0*	0.12 \pm 0.05	4.7 \pm 0.6
B	<i>X. aethiopica</i>	100	3.88 \pm 4.13*	38.7 \pm 3.9*	0.13 \pm 0.06	4.7 \pm 0.5
C	<i>P. amarus</i>	400	3.81 \pm 3.52*	38.0 \pm 4.1*	0.12 \pm 0.04	3.0 \pm 0.8*
D	<i>X. aethiopica</i>	400	3.81 \pm 3.56*	38.0 \pm 4.2*	0.13 \pm 0.05	2.7 \pm 0.6*

Results are presented as Mean \pm SEM; *-Significant difference from control, P < 0.05 (Student's t-test)

Table 2: Effect of the Plant Extracts on Total and Differential White Blood Cell Count in Rats.

Group	Treatment	Dose(mg/kg)	WBC ($\times 10^3/\text{mm}^3$)	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
Control	Dist. water	0	5.63 \pm 0.6	20.3 \pm 4.5	79.0 \pm 1.0	0.7 \pm 0.2
A	<i>P. amarus</i>	100	9.77 \pm 1.2*	20.3 \pm 4.3	77.0 \pm 0.9	0.7 \pm 0.1
B	<i>X. aethiopica</i>	100	8.23 \pm 0.9*	25.0 \pm 5.0*	74.3 \pm 0.7	0.7 \pm 0.2
C	<i>P. amarus</i>	400	7.15 \pm 0.7*	21.0 \pm 4.0	77.0 \pm 0.9	2.0 \pm 0.3*
D	<i>X. aethiopica</i>	400	6.22 \pm 0.6	21 \pm 4.4	79.0 \pm 0.8	0.0 \pm 0.0

Results are presented as Mean \pm SEM; *-Significant difference from control, P<0.05 (Student's t-test)

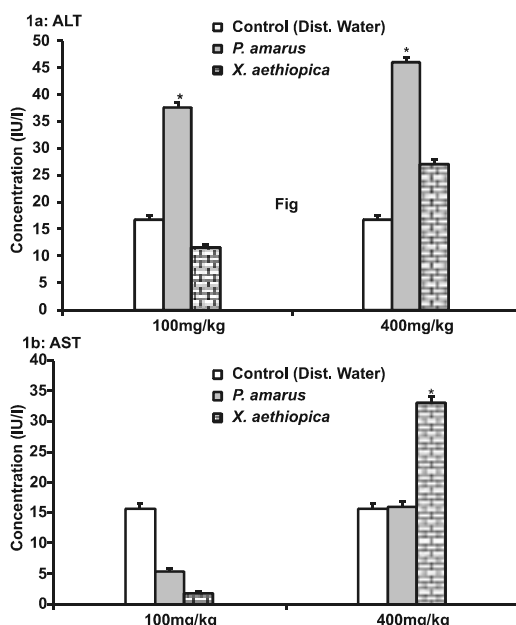


Fig. 1. Effects of Aqueous Extracts of *P. amarus* and *X. aethiopica* on the Level of (1a) Alanine Aminotransferase (ALT) and (2b) Aspartate Amino-transferase (AST) in Rats

(Note: *-significant difference from control; P<0.05)

DISCUSSION

The lower dose of extracts (100mg/kg) used

in this study agrees well with the normal range usually consumed by the general population in Nigeria. The higher dose of 400mg/kg was administered to determine if there are any dose-related haematological and hepatotoxicity effects in the rats. The results showed that the extracts of *P. amarus* and *X. aethiopica* caused increases in WBC count particularly with the dose of 100mg/kg. This finding suggests that the extracts of both plants probably contain agent(s) that stimulate(s) production of leucocytes. The presence of such agents had been reported for *Viscum album* (mistletoe) and other commonly prescribed medicinal plants (Bendich 1993; Al-Mamary 2002; Imoru et al. 2005). The crucial role of WBC in defending the body against infection and tissue damage is well known. Thus the results of this study imply that *P. amarus* and *X. aethiopica* are potent immunostimulants and their use to treat immune-related diseases in herbal medicine can be justified. Such immune boosters are usually recommended to strengthen and harmonize degenerative body systems and assist the immune system to fight invading agents such as bacteria and viruses (Bendich 1993; Al-Mamary 2002).

It is generally believed that as part of the aging process, the immune system is subject to free radical damage, which usually suppresses its activity. Excessive production of free radicals results in oxidative stress and subsequent damage to macromolecules (Meister 1983; Fidelus and Tsan 1986). In a recent study by Odukoya et al. (2007), *P. amarus* and *X. aethiopica* exhibited

antioxidant properties by inhibiting lipid peroxidation and guathanthione oxidation, and this could slow down aging process and improve immune responses. Additional evidence from this study suggests that the immunostimulant properties of these plants may also be mediated through their stimulatory effects on WBC production. In this respect, the observation that 100mg/kg of *X. aethiopica* caused significant increase in circulating neutrophils during differential count is important considering the fact that neutrophils are the main type of WBC that attack and destroy invading bacteria, viruses and other injurious agents.

It was interesting to note that the plants also caused increases in RBC count and the haematocrit (PCV). The resultant decrease in ESR might be attributed to increase in blood viscosity. This is expected because the number of RBC is a major factor contributing to blood viscosity (Marieb 1994). In view of this, the plants can also be of benefit in some anaemic conditions characterized by decrease in erythrocyte number. The results of enzyme analysis for ALT and AST suggest that *X. aethiopica* is non-hepatotoxic at the lower dose of 100mg/kg. *P. amarus*, however, caused significant increase in the level of ALT at both the lower (100mg/kg) and the higher dose (400mg/kg). Burger et al. (2005) have discussed the importance of some liver enzymes in assessing liver damage. Besides the liver, AST is found in many other organs including heart, kidney and brain. Thus a high level of AST does not always indicate that there is a liver problem. ALT, on the other hand, is found primarily in the liver; therefore, a high level of this enzyme observed in *P. amarus*-treated rats in this study probably indicates that prolonged and excessive use of *P. amarus* may be hepatotoxic. This does not agree with the view of Zhou et al. (1997) that the plant is hepatoprotective. More human and animal experimental studies are still needed to clarify this issue.

The haematological principles of *P. amarus* and *X. aethiopica* are not yet known. Previous phytochemical studies have shown the presence of lignans, alkaloids, bioflavonoids and repadusinic acid in *P. amarus*. Iwu et al. (1999) showed that the constituents of *X. aethiopica* include rutin, a volatile aromatic oil, and quercetin. These substances are known to have beneficial haematological and immunological properties: rutin possesses antioxidant properties while

quercetin protects blood vessels especially weak and fragile capillaries against damage (Asekun et al. 2004; Odukoya et al. 2007). In adult vertebrates including man, stem cells in bone marrow give rise to RBC and various types of WBC. It can therefore be inferred that some of the active components in these plants act on the bone marrow to stimulate the production and differentiation of haematopoietic stem cells.

CONCLUSION

The results of this study showed that the two common medicinal plants, *P. amarus* and *X. aethiopica*, have beneficial haematological and immunological properties in albino rats.

RECOMMENDATION

If these results are applicable to man, *P. amarus* and *X. aethiopica* can be used as immune boosters and blood tonics in herbal medicine. There is, however, the need to exercise caution on excessive and prolonged use of these plants especially *P. amarus*.

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