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Evaluation of the toxicity potential of acute and sub-acute exposure to the aqueous root extract of *Aristolochia ringens* Vahl. (Aristolochiaceae)

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ABSTRACT

Ethnopharmacological relevance: Aristolochia ringens Vahl. (Aristolochiaceae) is used traditionally in Nigeria for managing a number of ailments including gastrointestinal disturbances, rheumatoid arthritis, pile, insomnia, oedema, and snake bite venom. Some studies in our laboratory have demonstrated a scientific justification for some of such uses. This study aims at investigating the toxicological actions of the aqueous root extract of *Aristolochia ringens* (AR).

Materials and methods: Brine shrimp lethality assay was carried out using 10, 100 and 1000 µg/ml of the extract. Oral and intraperitoneal acute toxicity tests were carried out using mice. The effect of sub-acute (30 days) repeated oral exposure to the extract at 10, 50 and 250 mg/kg in rats was also evaluated via weekly assessments of body weights and general observations as well as end of exposure haematological, biochemical and histological examinations of blood and tissue samples of treated rats. Phytochemical analyses to determine the presence of aristolochic acid I in the extract was also carried out using high performance liquid chromatography (HPLC).

Results: The aqueous root extract of *A. ringens* showed potential for biological activity and cytotoxicity with an LC_{50} of 175 µg/ml in brine shrimps. AR was found to be relatively safe on acute oral exposure with LD_{50} estimated to be greater than 10 g/kg, while its LD_{50} on intraperitoneal administration was 407.38 mg/kg. Upon 30 days sub-chronic exposure, AR induced significant weight loss in female rats, enlargement of male rats' stomach, oxidative stress in male and female rats' kidney and liver tissues and disruption of leukocytes level in female rats. It also showed evidence of kidney and liver injuries inducible by oxidative damage and the potential to cause male sterility. HPLC revealed the presence of 0.003 mg/1 g of aristolochic acid in AR.

Conclusion: These results show that AR contains detectible aristolochic acid I and has potential to induce toxic responses. Caution must therefore be exercised in its medicinal application especially when required for a prolonged use.

1. Introduction

Aristolochia species have long been known for their relatively wide spread use in traditional medicine. The Aristolochiaceae family of plants consists of hundreds of species mostly distributed along tropical, subtropical, and mediterranean regions of the world (Neinhuis et al., 2005; Wanke et al., 2007). These plants have been cultivated as ornamentals (Wu et al., 2002) and used as abortifacients, emmenagogues (Che et al., 1984), analgesics, anticancers, anti-inflammatory agents, sedatives, muscle relaxants, antihistaminergics, antiallergics, antihelminthics, antimicrobials and antimalarials (Kubmarawa et al., 2007). They have also been used in the management of stomach ache, abdominal pain, rheumatism (Yu et al., 2007), wounds, skin diseases as well as different types of poisonous bites and stings (Wu et al., 2004). According to a report by the International Agency for Research on Cancer, *Aristolochia species* have also been used as diuretics and in the treatment of oedema (International Agency for Research on Cancer (IARC), 2002). In countries such as Nigeria for instance, several species

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of this genus including *A. albida, A. indica* and *A. ringens* are used in the management of various ailments. Users claim that they are highly efficacious when used singly or in combination with other medicinal plants. The antidiarrhoeal activity of a polyherbal preparation, which includes 2 species of this genus (*A. albida* and *A. ringens*) have been demonstrated in our laboratory (Adeyemi et al., 2003).

Aristolochia ringens, also known as *Howardia ringens,* is a perennial bushy glabrous climber, native to tropical America, and introduced to most West African countries as a garden ornamental. It has become naturalized in roadside bushes in Sierra Leone, and in many places in Nigeria (Burkill, 1985). The root of *Aristolochia ringens* is largely used in Nigeria for the management of ailments such as gastrointestinal disturbance, asthma, diabetes and oedema (Odugbemi, 2008). According to personal communications with users, a tiny bite of *A. ringens* root sufficiently alleviates gastrointestinal disturbance. Other accounts reveal the use of these species to manage conditions such as haemorrhoids, fibroids and diabetes. It is also used for the management of haemorrhoids, snake bite, rheumatoid arthritis, fibroids, gastrointestinal disturbances, diabetes (Olabanji et al., 2008) and asthma (Sonibare and Gbile, 2008). Its root is used in relieving body swelling, joint pains, ulcers and its leaves are used for treating general body rashes (Odugbemi, 2008). A teaspoon of the root extract is taken 3 times daily for 3 days for deworming in Nigeria (Idu et al., 2010). Senegalese use its root as an antidote for snake venom (Neuwinger, 2000). It is used in South America for the treatment of ulcer, colic, fever and snake bite (Van Wyk and Wink, 2004). Small portions of *A. ringens* root packed into small rolls are commonly seen among the wares of traditional medicine sellers in Nigeria. Some research findings in our laboratory confirmed the antidiarrhoeal (Adeyemi et al., 2012) and anti-inflammatory (Aigbe et al., 2014) actions of AR. Another study involving our collaboration with another laboratory revealed the anticancer (Akindele et al., 2015) activity of the plant extract.

The vast usefulness of this plant and other members of this genus is however limited by the reported progressive nephropathy and urothelial cancer they induce in humans (Martinez et al., 2002; Meinl et al., 2006). As a result of this, distribution of herbal medicines containing *Aristolochia* extracts has been prohibited in many countries (Neinhuis et al., 2005). The toxic effects have been attributed to aristolochic acids content of this plant genus (Balachandran et al., 2005). However, their continued use in several countries including Algeria, Morocco and Nigeria has been reported by Yamani et al. (2015) and Benarba et al. (2014). One indication of its potentially toxic effect was shown by its antifeedant activity against *Sitophilus zeamais*, the maize weevil. In a study by Arannilewa et al. (2006), the petroleum ether extract of *A. ringens* (1%) showed insecticidal activity, causing 100% mortality of *S. zeamais* by the third and fourth day following application.

To the best of our knowledge, there is yet to be any documented report on the toxic effect of the plant among its users in Nigeria, who claim that it is highly efficacious, hence its continued usage. The common belief that medicinal plants are safer than orthodox drugs also contributes to the increased and sometimes indiscriminate use of medicinal plants. This preclinical study aims at investigating the aqueous root extract of *A. ringens* (AR), to determine its toxicity profile on brine shrimps, mice and rats using acute and sub-acute toxicity test models.

2. Materials and methods

2.1. Plant material

The root of *Aristolochia ringens* Vahl. (Aristolochiaceae) were collected from a local market in Mushin, Lagos, Nigeria. It was identified and authenticated by Mr. T.K. Odewo of the Department of Botany and Microbiology, University of Lagos, Nigeria where a herbarium specimen was deposited with voucher number LUH 4061.

2.2. Preparation of plant extract

Air-dried root (100 g) was macerated in 1000 ml of distilled water in a beaker placed in a refrigerator at 4 °C for 5 days. It was then filtered and the filtrate dried in an oven (Gallenhamp®, England) at 40 °C. The percentage yield of the aqueous root extract of *A. ringens* (AR) obtained was 5.9% (w/w). The extract was stored in a refrigerator at 4 °C and dissolved in distilled water for each experiment.

2.3. High performance liquid chromatographic (HPLC) analysis

This was done using a slight modification of the method described by the British Pharmacopeia Commission (2007). Briefly, the extract was prepared by solid phase extraction and washing with appropriate solvents. Ten (10) µl of the resulting sample thus obtained and different concentrations of the reference standard were injected into stainless steel column (25 cm × 4.6 mm) packed with base-deactivated octadecylsilyl silica gel (Genesis C18) maintained at 30 °C. The reference standard used was aristolochic acid I (Sigma Aldrich, St. Louis, USA). The mobile phase was a mixture of orthophosphoric acid (45 ml) and acetonitrile (55 ml) at 1 ml/min flow rate. An ultraviolet detector set at 225 nm was connected for aristolochic acid I detection and a run time of 5 min was allowed. The concentration of aristolochic acid I in the test samples were determined from the calibration curve obtained with the different concentrations of the reference standard.

2.4. Experimental animals

Artemisia salina (brine shrimps) were kindly supplied by Dr A.A. Sowemimo of the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Nigeria. The shrimps were allowed a 24 h period to hatch in sea water to produce nauplii, which were used in the study. Adult albino mice (average weight of 22 g) or rats (average weight of 160 g) of either sex were obtained from the Animal Housing Centre of the University of Ibadan, Nigeria. The animals were allowed to acclimatize, kept under standard environmental conditions and had access to feed (Livestock Feeds, Lagos, Nigeria) and water *ad libitum*. The experimental procedures were carried out in accordance with the United States National Institute of Health's, (2011) guideline for laboratory animal care and use.

2.5. Brine shrimp lethality test

Artemia salina (brine shrimp) lethality test was carried out using a modification of the procedure described by McLaughlin (1991) as described by Sowemimo et al. (2007). Concentrations 10, 100 and 1000 µg/ml of the extract was prepared in test tubes (three test tubes per concentration) respectively. Three test tubes containing 5 ml of brine water served as control. Using a micropipette, ten shrimp nauplii were added into each of the test tubes. Twenty four (24) hours following the addition of nauplii into each test tube, the number of surviving shrimps were counted and recorded. The LC_{50} of the AR was determined using probit analysis as described by Currell (2015).

2.6. Acute toxicity tests

Mice of either sex were fasted for 12 h prior to the tests. For the oral acute toxicity test, a group of mice were given up to 10 g/kg of AR orally. For the intraperitoneal acute toxicity test, mice were randomly allotted to 4 groups of 5 animals each. Mice in 3 of these groups received AR intraperitoneally at 200, 400 and 600 mg/kg respectively, while mice in the 4th group received the vehicle, distilled water at 10 ml/kg intraperitoneally. The mice were then monitored for symptoms of toxicity and mortality for the first 2 h and for an additional 22 h post treatment. Surviving mice were further observed for 2 weeks for signs of delayed toxicity. The median lethal dose (LD_{50}) was estimated

using Log-dose probit analysis (Miller and Tainter, 1944; Omotoso et al., 2017).

2.7. Sub-acute toxicity test

The procedure was carried out in accordance with the Organization for Economic Cooperation and Development (OECD) Test Guidelines (Organization for Economic Cooperation and Development (OECD), 2007) with slight modifications. Male and female albino rats were randomly divided into four groups of 10 animals per sex. For 30 days, group 1 received distilled water (5 ml/kg daily) orally and groups 2–4 received AR (10, 50 and 250 mg/kg respectively, daily) orally. These doses represent one-fifth of the pharmacologically active dose, the pharmacologically active dose, and five times the pharmacologically active dose (determined from previous studies) respectively (Omotoso et al., 2017). Body weights were determined weekly and the animals were observed for signs of abnormalities throughout the study. At the expiration of 30 days, some of the rats in each group were fasted overnight and blood samples collected by retro-orbital puncture for haematological and biochemical analyses. These animals were then sacrificed by cervical dislocation and various organs excised for morphological and histological evaluations. The testes of male rats were also collected for semen analysis. The other rats in each group were then observed continuously without further treatment for another 14 days before they were sacrificed and their blood and tissue samples collected for analyses.

2.8. Organ weight and morphological assessment of AR treated rats' organs

The organs of treated rats were subjected to macroscopic and microscopic evaluations. The weight and external features of liver, kidneys, brain, heart, lungs, pancreas, spleen, testes or ovaries were determined and assessed for each animal. Organ weights were expressed as relative weights (g/100 g of their body weight).

2.9. Histological analysis of kidney and liver of AR treated rats

Following weight determination, liver and kidney of male and female rats were immediately fixed in 10% buffered formo-saline, processed and embedded in paraffin. The organs were sectioned at 5 μm and stained using haematoxylin and eosin for general tissue-organ architecture examination. Sections were viewed under light microscope. Photomicrographs were obtained using a camera attached to a computer.

2.10. Analysis of haematological, biochemical and electrolyte parameters of AR treated rats

Haematological parameters of whole blood samples were analyzed using automated haematology analyzer. Parameters evaluated include haematocrit, red blood cell (RBC) count, haemoglobin, platelet count, total white blood cell (WBC) count, mean cell haemoglobin concentration (MCHC), mean red cell volume (MCV), mean cell haemoglobin (MCH), neutrophils and lymphocytes. Serum samples were analyzed for biochemical parameters including renal function parameters (including creatinine and urea) as well as liver function parameters (including liver enzymes, albumin, bilirubin, total protein, cholesterol and triglycerides). The concentration of liver enzymes such as alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) were also analyzed using standard protocols involving the screen master automated spectrophotometer and corresponding reagent kits (Yakubu et al., 2006). The concentration of some serum electrolytes was also determined using methods described by Yakubu et al. (2006). Sodium and potassium concentration were measured using flame photometry; chloride and bicarbonate concentration using titrimetric method, and calcium concentration by cresol phthalein complexone method.

2.11. Analysis of oxidative stress biomarkers for liver and kidney of AR treated rats

The levels of antioxidant enzymes; superoxide dismutase (SOD), using autooxidation of epinephrine method (Sun and Zigma, 1978) and catalase (CAT) using Goth's colorimetric method (Goth, 1991) were assessed. The concentration of antioxidant, reduced glutathione (GSH) was also determined following the method described by Sedlak and Lindsay (1968). The extent of lipid peroxidation was also determined via malondialdehyde assay.

2.12. Semen analysis

Male rats' testes with ipsilateral epididymis of sacrificed male rats were removed after incision on the scrotum. Subsequently, semen was expelled out of the epididymis into a beaker placed in water bath at 36 °C. Sperm motility, count and morphology were determined by appropriate microscopic methods as described by Ogli et al. (2009).

2.13. Statistical analyses

Results obtained were expressed as mean ± SEM. Experimental data obtained were analyzed using one way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, two way ANOVA followed by Bonferroni's post hoc test or Student's *t*-test as appropriate. These analyses were carried out using Graph Pad Prism 5 statistical software. Results were considered significant at $p < 0.05$.

3. Results

3.1. HPLC result for aristolochic acid I detection

HPLC analysis revealed the presence of aristolochic acid I in AR (Fig. 1). Standard curve analysis of various concentrations of AR revealed that AR contained up 0.003 mg of the acid in 1 g of the extract.

3.2. Effect of AR in brine shrimp lethality test

In the brine shrimp toxicity test, mortality was observed at all AR concentrations tested. AR at 1000 μg/ml produced 95% mortality in exposed brine shrimps. The median lethal concentration (LC_{50}) of AR in this test was found to be 172.5 μg/ml as shown in Fig. 2.

3.3. Effect of AR in oral and intraperitoneal acute toxicity tests in mice

The extract (10 g/kg) produced no visible morbidity or mortality at 24 h after single oral exposure, and for up to 2 weeks of observation of the treated mice. However upon intraperitoneal administration, AR (200–600 mg/kg) produced dose-dependent severity in abnormal gait, decreased responsiveness to the environment, writhing and pilo-erection. Probit log-dose analysis gave its median lethal dose (LD_{50}) as 407.38 mg/kg for the intraperitoneal route (Fig. 3).

3.4. Effect of sub-acute exposure to AR on body weight of treated rats

At 10 mg/kg, AR significantly ($p < 0.01$) increased the body weight of male rats by the 3rd and 4th week of exposure (Fig. 4). Among female rats on the other hand, AR dose-dependently and significantly ($p < 0.05$) reduced body weight by weeks 3 (at 50 and 250 mg/kg) and 4 (at 10, 50 and 250 mg/kg) as shown in Fig. 5.

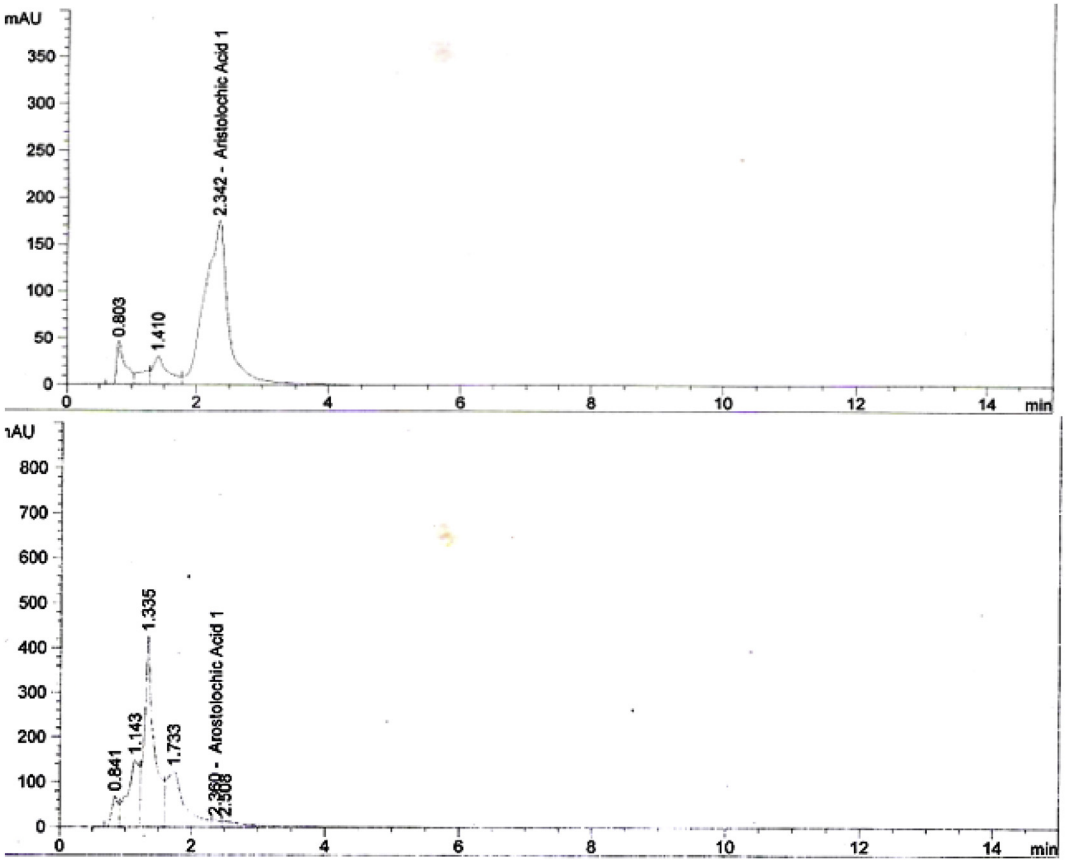

Fig. 1. High performance liquid chromatogram of standard aristolochic acid I (A) and AR (B) at 225 nm.

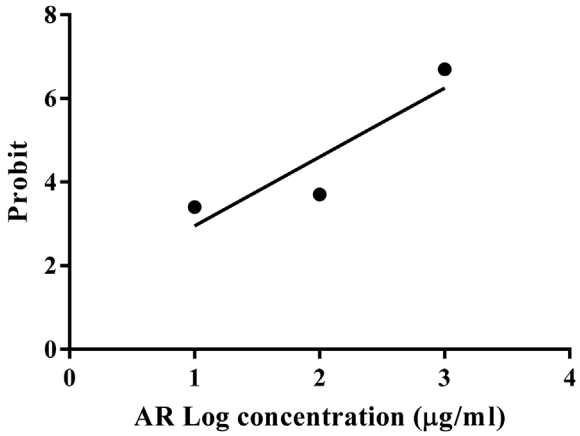

Fig. 2. Graph of probit mortality of brine shrimps exposed to AR versus log concentration of AR. LC_{50} = 172.5 µg/ml. AR-aqueous root extract of *A. ringens*.

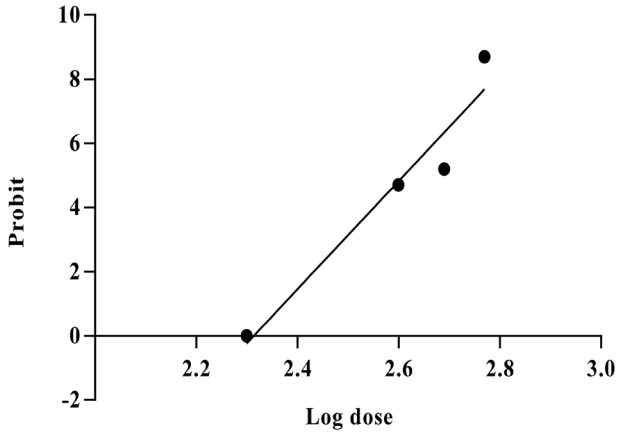

Fig. 3. Graph of probit mortality of mice exposed to AR vs. log dose (mg/kg) for intra-peritoneal acute toxicity test in mice. LD_{50} = 407.38 mg/kg. AR-aqueous root extract of *A. ringens*.

3.5. Effect of sub-acute exposure to AR on weight and histology of selected organs

Tables 1 and 2 show the effect of AR on the weight of some organs of male and female rats respectively. No significant change in the organ weights was observed except for the stomach of male rats, which were significantly ($p < 0/05$) larger at 250 mg/kg of AR. Histological examinations revealed normal liver histology, while indicating histopathology of the male rats' kidney at all the doses tested (Figs. 6 and 7).

3.6. Effect of sub-acute exposure to AR on haematological parameters

In male rats, AR apparently reduced haematocrit at 250 mg/kg, reversal of which was noted in the reversibility phase of the study (Table 3). On the other hand, among female rats, significant ($p < 0.05$) reduction of WBC and significant ($p < 0.01$) increase in differential lymphocytes level was observed at 10 mg/kg of AR at 250 mg/kg were noted in the reversibility study (Table 4).

3.7. Effect of sub-acute exposure to AR on biochemical parameters

At the end of 30 days oral exposure to AR no significant change was observed in the biochemical parameters of male rats. In the reversibility test however, AR significantly decreased urea at 250 mg/kg (Table 5). Among the female rats, after the 30 days treatment, AR significantly

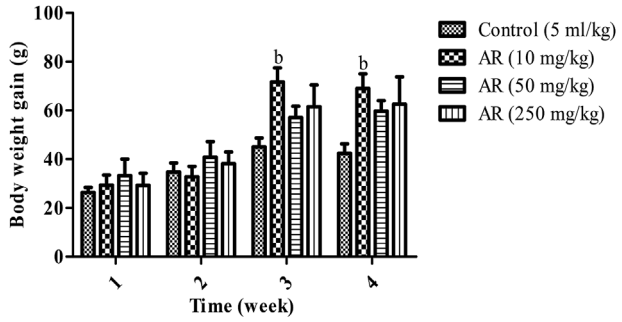

Fig. 4. Effect of AR on weekly body weight of male rats during the 30 days exposure to AR. Bars represent mean ± SEM. (n = 10) $^{b}p < 0.01$ vs. control. (One way ANOVA followed by Tukey's multiple comparison test). AR-aqueous root extract of *A. ringens*.

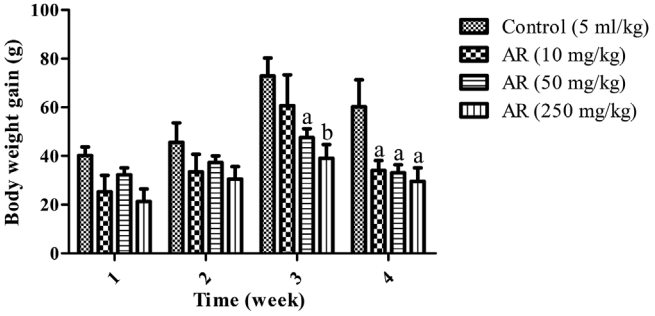

Fig. 5. Effect of AR on weekly body weight of female rats during the 30 days exposure to AR. Bars represent mean ± SEM. (n = 10) $^{a}p < 0.05$, $^{b}p < 0.01$ vs. control. (One way ANOVA followed by Tukey's multiple comparison test). AR-aqueous root extract of *A. ringens*.

($p < 0.05$) reduced serum creatinine at 250 mg/kg and apparently reduced serum albumin in the reversibility study (Table 6).

3.8. Effect of sub-acute exposure to AR on serum electrolytes

There was no significant alteration in serum sodium, potassium and bicarbonate concentrations of male rats by the end of the 30 days treatment and after the reversibility period (Table 7). Similarly in the female rats, no significant alteration was observed in the serum electrolytes assayed in the 30 days treatment or reversibility test (Table 8).

3.9. Effect of sub-acute exposure to AR on liver and kidney oxidative stress biomarkers

By the end of 30 days exposure, no significant alteration in the level of reduced glutathione (GSH), superoxide dismutase (SOD) or catalase was observed in the liver and kidney homogenates of AR treated male rats compared to control. In the reversibility study, dose-dependent increase in GSH and SOD significant at 250 mg/kg was observed in the liver of male rats. In the kidney of these rats, AR dose-dependently decreased SOD levels compared to control. Liver MDA significantly ($p < 0.001$) and dose-dependently increased after 30 days of AR exposure, an effect that reversed following 14 days treatment cessation. Kidney MDA on the other hand, was unaffected following 30 days of AR exposure but significantly ($p < 0.05$ and $p < 0.01$)) increased in the reversibility study (Table 9). Following 30 days of exposure to AR, liver and kidney GSH, SOD and catalase of female rats were not significantly altered, relative to control. However, in the reversibility test, kidney SOD was significantly ($p < 0.001$) reduced by AR (250 mg/kg) as it was in the male rats; while kidney and liver GSH and catalase were unaltered. While kidney MDA remained unaltered in both phases of the study among female rats, liver MDA significantly ($p < 0.001$) increased at 30 days post treatment but this was reversed following 14 days treatment cessation (Table 10).

3.10. Effect of sub-acute exposure to AR on male semen analysis

No significant alteration in sperm morphology or motility was recorded in the 30 days exposure period and in the reversibility test period. However, their sperm count apparently reduced after 30 days of repeated oral exposure to AR and after 14 days of exposure cessation in a somewhat biphasic manner (Table 11).

4. Discussion

Brine shrimp lethality test used to determine the ability of a test compound to produce mortality in laboratory-cultured brine shrimp is a convenient method for monitoring biological activities of natural products (Baravalia et al., 2012). In this study, the mortality of brine shrimps increased with increasing concentration of AR, the LC_{50} of which was found to be 172.5 µg/ml. Compounds with LC_{50} less than 1000 µg/ml are considered bioactive in the toxicity evaluation of plant extracts (Meyer et al., 1982). Such compounds may also be considered to be potentially cytotoxic.

In the acute toxicity tests, no morbidity or mortality was observed in mice treated orally with AR up to 10 g/kg, indicating that the LD_{50} must be greater than 10 g/kg, demonstrating that the aqueous extract of *A. ringens* is safe on oral acute exposure in mice. According to Clarke and Clarke (1977) a substance that failed to cause lethality at 10 g/kg can be considered relatively non-toxic. However, intraperitoneal AR exposure produced dose-dependent severity in ataxia, signifying distorted central nervous system activity. Similarly, in the sub-acute exposure study, a few rats were noted to manifest abnormal head and body backward movements in unusual manners. These findings corroborate the potential of AR to induce central nervous system effects as also demonstrated in a previous study (Aigbe et al., 2014). Writhing, which

Table 1
Effect of sub-acute exposure to AR on organ weights of male rats.

Organ	Organ weight (g per 100 g body weight)			
	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)
Liver	2.75 ± 0.72	2.78 ± 0.20	3.40 ± 0.44	2.74 ± 1.13
Kidneys	0.71 ± 0.08	0.64 ± 0.05	0.71 ± 0.07	0.61 ± 0.20
Brain	0.84 ± 0.07	0.79 ± 0.14	0.97 ± 0.07	0.91 ± 0.10
Heart	0.36 ± 0.02	0.35 ± 0.06	0.40 ± 0.05	0.30 ± 0.12
Lungs	0.99 ± 0.37	0.79 ± 0.29	0.95 ± 0.21	0.72 ± 0.22
Pancreas	0.47 ± 0.19	0.30 ± 0.06	0.41 ± 0.04	0.36 ± 0.19
Spleen	0.50 ± 0.08	0.40 ± 0.11	0.51 ± 0.11	0.33 ± 0.12
Stomach	1.20 ± 0.28	1.42 ± 0.17	1.51 ± 0.29	2.27 ± 0.77^{b}
Testes	1.23 ± 0.04	1.10 ± 0.14	1.31 ± 0.15	0.99 ± 0.16

Values are mean ± S.E.M. (n = 5). $^{b}p < 0.01$ vs control (One way ANOVA followed by Tukey's multiple comparison test). AR-aqueous root extract of *A. ringens*.

Table 2
Effect of sub-acute exposure to AR on organ weights of female rats.

Organ	Organ weight (g per 100 g body weight)			
	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)
Liver	3.44 ± 0.06	3.42 ± 0.34	3.51 ± 0.18	3.69 ± 0.32
Kidney	0.73 ± 0.06	0.80 ± 0.08	0.73 ± 0.06	0.78 ± 0.06
Brain	1.08 ± 0.11	1.10 ± 0.01	1.04 ± 0.05	1.15 ± 0.04
Heart	0.42 ± 0.05	0.47 ± 0.03	0.38 ± 0.05	0.43 ± 0.04
Lung	0.80 ± 0.12	0.99 ± 0.04	0.90 ± 0.13	1.10 ± 0.23
Pancreas	0.33 ± 0.05	0.44 ± 0.09	0.39 ± 0.07	0.42 ± 0.02
Spleen	0.42 ± 0.04	0.47 ± 0.06	0.37 ± 0.07	0.48 ± 0.11
Stomach	1.23 ± 0.18	1.05 ± 0.26	1.34 ± 0.21	1.03 ± 0.27
Ovaries (g)	0.09 ± 0.01	0.10 ± 0.02	0.11 ± 0.01	0.10 ± 0.01

Values are mean ± S.E.M. (n = 5). AR-aqueous root extract of *A. ringens*.

could have been due to the intraperitoneal route of exposure, was also observed in the intraperitoneal acute toxicity test. An LD_{50} of 407.38 mg/kg was obtained for this test. This parenteral route of drug administration usually allows for greater bioavailability of drugs due to increased absorption surface area; hence an increased potential for toxic response and/or lethality at relatively lower doses. According to Looms and Hayes (1996), compounds with LD_{50} of 50–500 mg/kg are considered moderately toxic. Intraperitoneally administered AR can therefore be said to be moderately toxic to laboratory mice.

The extract showed various effects upon prolonged (30 days) exposure in laboratory rats. Other than a significant increase in weight gain at 10 mg/kg, no significant change in the extent of body weight gain was observed in male rats. The relevance of this observation is unclear since although it was sustained for weeks 3 and 4, it was not dose-dependent and it failed to be reproduced in similar studies in our laboratory. On the other hand, significant reduction in weight of female rats was observed by the 3rd and 4th week of exposure in a dose-dependent and apparently time-dependent manner. However the severity of the weight reduction at 250 mg/kg appeared to slightly decrease by the 4th week ($p < 0.05$) compared to the 3rd week ($p < 0.01$) of AR exposures. A more prolonged exposure study may reveal interesting and more insightful outcomes. According to Teo et al. (2002), reduction in weight as observed here could be indicative of an adverse reaction to administered drug. The difference in the effect of AR on body weight of male and female rats may be accounted for by hormonal difference that significantly influence the biological differences between them.

No significant alteration was observed in the weights of vital organs of male and female rats in the study, except for the stomach of male rats. The dose-dependent increase in stomach weight which was significant at 250 mg/kg was also observed in a similar study in our laboratory; giving credence to the stomach enlarging effect of AR. Indeed tumour-like growths were observed in some of the excised stomach tissue as was reported for aristolochic acid by Mengs (1983) and another species, *A. mashuriensis,* by Wang et al. (2018).

Aristolochia species are reported to contain aristolochic acids, which have been implicated as nephrotoxic agents; adverse effects due to aristolochic acid in AR is therefore possible. The histopathological finding of chronic interstitial inflammation and vascular congestion of the male rat kidney tissue as well as the delayed onset significant increase in malondyaldehyde level of male rat kidney homogenates indicate nephrotoxic action of AR possibly mediated via induction of oxidative stress. The corresponding significant decrease in kidney SOD of male rats in the reversibility study further supports this position. Similarly female kidney SOD level also decreased significantly at 250 mg/kg in the reversibility study. Although there was no corresponding MDA increase in female rat kidney, oxidative stress induced renal damage also seems apparent. Renal function parameters such as creatinine and urea however failed to support renal dysfunction potential of AR as alterations of these parameters were not significant. This could be due to the relatively short period of AR exposure among other possible explanations.

On examination of the effect of AR on male rat liver, organ weight, liver function biochemical assays and histological evaluations revealed no significant effect following 30 days exposure to the aqueous root extract of *A. ringens*. However, liver oxidative stress biomarkers assessment reveal oxidative stress with significant increase in MDA level at 250 mg/kg, which in the reversibility study appeared to have been reversed by the upsurge in the level of reduced gluthatione and SOD. Similar liver MDA increase observed for female rats was also reversed following 2 weeks of treatment cessation howbeit without corresponding increase in the levels of the antioxidant enzyme levels.

It is not clear how much of the actions of AR in this study is due to aristolochic acid, considering the facts that the acid is poorly soluble in water and there are other active ingredients in the plant (Wu et al., 2004). Our study revealed the presence of up to 0.003% of aristolochic acid I in AR, this relatively low concentration and the relatively short exposure period could among other reasons account for the nature of observations made in this study. An attempt was also made to examine possible influence of the extract on cytochrome P450 enzymes, CYPs 1A1 and 1A2, being enzymes responsible for metabolizing aristolochic acid (Dračínská et al., 2016) in a preliminary gene expression study. Our findings thus far are yet inconclusive and further studies are ongoing since we believe that such study will contribute to better understanding of the properties and activities of this commonly used plant extract.

The extract showed no significant alteration in the haematological

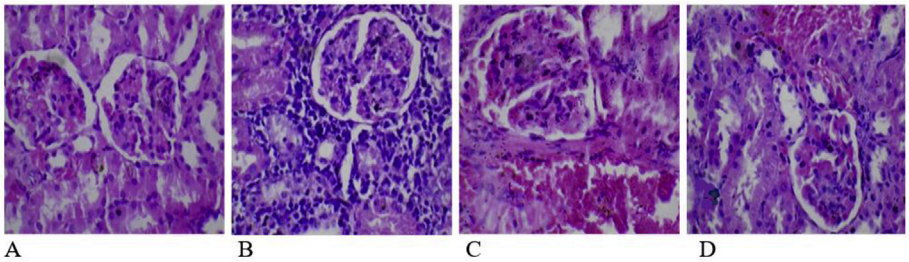

Fig. 6. Photomicrographs of kidney samples of male rats (A) Normal histology of a control rat; (B) Chronic interstitial inflammation and vessel congestion in kidney of rat given AR (10 mg/kg); (C) Vascular congestion in kidney of rat given AR (50 mg/kg) and (D) Vascular congestion in kidney of AR treated rat at AR (250 mg/kg). AR-aqueous root extract of *A. ringens*.

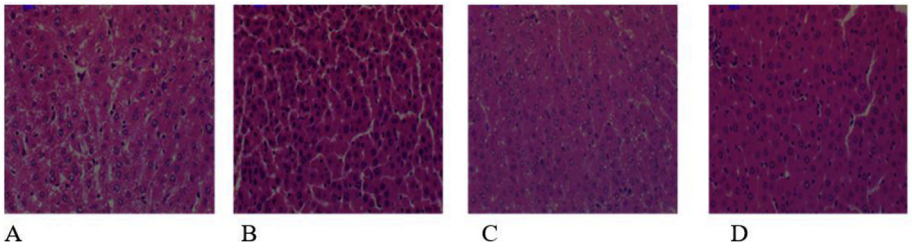

Fig. 7. Photomicrographs showing normal histology for the liver samples of male rats treated with vehicle (A), AR at 10 mg/kg (B), AR at 50 mg/kg (C) and AR at 250 mg/kg (D). AR-aqueous root extract of *A. ringens*.

Table 3
The effect of AR on haematology of male rats.

Haematologic parameter	After 30 days treatment period				After reversibility period			
	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)
RBC (10^{12}/l)	7.72 ± 0.33	7.87 ± 0.38	7.73 ± 0.23	6.80 ± 0.20	8.03 ± 0.44	8.63 ± 0.31	7.91 ± 0.22	8.02 ± 0.23
Haemoglobin (g/dl)	13.40 ± 0.31	14.35 ± 0.50	13.41 ± 0.23	11.97 ± 0.46	15.43 ± 0.49	15.38 ± 0.47	14.48 ± 0.35	15.43 ± 0.34
Haematocrit (%)	46.23 ± 1.49	47.18 ± 2.47	42.84 ± 0.79	36.20 ± 1.51	48.85 ± 2.00	47.00 ± 1.26	43.94 ± 0.95	46.95 ± 0.39
MCH (pg)	18.40 ± 0.33	18.83 ± 0.35	18.46 ± 0.27	18.50 ± 0.83	18.00 ± 0.17	17.90 ± 0.39	18.46 ± 0.41	18.55 ± 0.20
MCHC (g/dl)	30.78 ± 0.25	31.48 ± 0.43	32.00 ± 0.64	31.35 ± 0.48	31.85 ± 0.24	32.30 ± 0.40	32.54 ± 0.47	31.65 ± 0.40
MCV (fl)	60.03 ± 0.85	60.05 ± 1.90	58.04 ± 1.06	58.65 ± 2.14	57.88 ± 0.52	54.15 ± 0.63	57.20 ± 1.08	56.70 ± 0.31
Platelet (10^9/l)	736.0 ± 68.8	748.8 ± 63.6	712.0 ± 26.1	689.3 ± 103.5	701.8 ± 51.3	637.5 ± 27.5	708.0 ± 106.3	742.0 ± 52.4
WBC (10^9/l)	7.43 ± 0.37	9.15 ± 1.02	9.13 ± 0.57	7.61 ± 0.42	12.10 ± 1.28	11.53 ± 0.30	10.34 ± 1.31	9.45 ± 0.84
Neutrophil (%)	28.85 ± 1.12	30.38 ± 4.45	29.14 ± 2.58	38.00 ± 4.57	25.48 ± 8.23	29.80 ± 2.73	25.35 ± 2.44	27.20 ± 3.52
Lymphocyte (%)	61.25 ± 1.81	60.73 ± 1.27	62.60 ± 1.95	50.50 ± 3.28	66.35 ± 8.45	63.83 ± 1.76	64.06 ± 3.73	62.90 ± 3.03

Values are mean ± S.E.M. (n = 4–5) Packed cell volume (PCV), red blood cell (RBC) count, total white blood cell (WBC) count, mean cell haemoglobin concentration (MCHC), mean red cell volume (MCV), and mean cell haemoglobin (MCH). AR-aqueous root extract of *A. ringens*.

parameters tested among male rats. On the other hand, although AR produced no significant alterations in the WBC of female rats following 30 days exposure, after 2 weeks of AR treatment cessation, female rats previously exposed at 250 mg/kg had significant WBC reduction. According to Dinauer and Coates (2008) WBC reduction is indicative of immune system suppression or bone marrow deficiency or failure. This finding therefore suggests possible delayed onset immune system depressant action of AR at the supratherapeutic dose. The relative lymphocytes of female rats treated with 10 mg/kg of AR was significantly ($p < 0.05$) elevated (81.90 ± 0.91%) 2 weeks after cessation of AR exposure. The fact that this effect was not dose-dependent leaves doubts as to whether this lymphocytotic effect can be attributed to AR exposure alone.

Regarding the sperm analysis, no significant effect on sperm motility, morphology and sperm count was observed following 30 days exposure and 14 days treatment cessation. A biphasic trend in reduction of sperm count was however apparent in both the 30 days exposure and reversibility study. Pakrashi and Pakrasi (1977) reported the antispermatogenic potential of *A. albida*, another species of the same genus of plants. This further calls for the need to exercise caution in the use of the plant extract.

5. Conclusion

The findings in this study show that the aqueous root extract of *A. ringens* can be said to be relatively safe on acute oral exposure, but moderately toxic on acute intraperitoneal exposure in mice. However its adverse effects of weight loss in females rats, oxidative stress in male and female rats' kidney and liver and antispermatogenic potential on repeated exposure (up to 30 days) clearly calls for serious caution, especially bearing in mind what is previously known about one of its content, aristolochic acid.

Table 4
The effect of AR on haematology of female rats.

Haematologic parameter	After 30 days treatment period				After reversibility period			
	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)
RBC (10^{12}/l)	7.19 ± 0.19	7.18 ± 0.22	7.02 ± 0.25	7.13 ± 0.38	7.44 ± 0.41	7.75 ± 0.09	8.39 ± 0.35	7.43 ± 0.15
Haemoglobin (g/dl)	13.53 ± 0.14	13.53 ± 0.57	12.44 ± 0.31	12.28 ± 0.95	14.00 ± 0.11	44.28 ± 0.32	14.60 ± 0.57	13.65 ± 0.35
Haematocrit (%)	39.90 ± 8.81	45.95 ± 1.72	36.14 ± 5.94	35.93 ± 8.16	44.33 ± 0.43	40.85 ± 0.73	44.98 ± 1.49	41.35 ± 0.61
MCH (pg)	18.80 ± 0.39	19.48 ± 0.51	17.76 ± 0.23	17.80 ± 0.36	18.43 ± 0.34	17.48 ± 0.71	17.90 ± 0.25	17.80 ± 0.17
MCHC (g/dl)	28.35 ± 0.63	29.35 ± 1.47	28.78 ± 0.40	29.35 ± 0.26	32.13 ± 0.48	32.33 ± 0.26	32.70 ± 0.14	32.18 ± 0.49
MCV (fl)	68.70 ± 2.20	66.45 ± 1.47	62.98 ± 1.52	60.88 ± 1.40	57.83 ± 1.45	52.10 ± 0.44	54.40 ± 0.74	53.65 ± 0.70
Platelet (10^9/l)	632.0 ± 44.1	654.0 ± 88.0	763.2 ± 47.8	681.3 ± 60.4	617.3 ± 20.8	676.0 ± 71.2	553.8 ± 65.7	577.3 ± 46.2
WBC (10^9/l)	7.78 ± 1.24	5.98 ± 0.92	6.02 ± 0.66	9.45 ± 1.33	11.08 ± 1.03	7.93 ± 1.15	9.84 ± 1.01	4.45 ± 0.22$^{\alpha}$
Neutrophil (%)	27.53 ± 5.85	33.48 ± 4.56	34.86 ± 2.28	29.78 ± 3.35	30.78 ± 1.02	12.25 ± 0.62	26.26 ± 1.03	30.97 ± 14.72
Lymphocyte (%)	64.08 ± 5.55	58.48 ± 4.43	54.92 ± 2.53	59.83 ± 5.46	59.08 ± 4.02	81.90 ± 0.91$^{\beta}$	66.04 ± 2.22	51.55 ± 9.64

Values are mean ± S.E.M. (n = 4–5) $^{\alpha}p < 0.05$, $^{\beta}p < 0.01$ vs control in reversibility study (One way ANOVA followed by Tukey's multiple comparison test). Packed cell volume (PCV), red blood cell (RBC) count, total white blood cell (WBC) count, mean cell haemoglobin concentration (MCHC), mean red cell volume (MCV), and mean cell haemoglobin (MCH). AR-aqueous root extract of *A. ringens*.

Table 5
Effect of AR on biochemical parameters of male rats.

Biochemical parameter	After 30 days treatment period				After reversibility period			
	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)
AST (IU/L)	212.10 ± 18.40	215.80 ± 21.20	190.30 ± 14.90	177.20 ± 37.10	93.10 ± 32.60	124.40 ± 4.20	143.90 ± 7.70	97.30 ± 11.60
ALT (IU/L)	49.03 ± 03.10	52.25 ± 03.82	56.10 ± 03.31	55.33 ± 05.63	67.43 ± 16.90	72.45 ± 02.80	98.87 ± 08.54	63.80 ± 10.76
ALP (IU/L)	146.40 ± 21.00	108.60 ± 5.00	146.30 ± 13.50	157.40 ± 12.50	146.10 ± 30.90	287.60 ± 34.40	241.40 ± 34.00	190.90 ± 42.90
Urea (mg/dL)	6.33 ± 0.43	8.20 ± 0.73	8.82 ± 2.03	9.30 ± 1.10	6.25 ± 0.56	7.35 ± 0.50	6.73 ± 0.31	3.88 ± 0.97$^{\alpha}$
Creatinine (μmol/L)	46.89 ± 2.65	58.24 ± 5.45	56.89 ± 1.28	64.80 ± 7.13	34.96 ± 11.76	54.80 ± 10.86	45.08 ± 3.41	32.40 ± 13.83
Bilirubin (μmol/L)	3.87 ± 0.12	3.85 ± 0.03	4.03 ± 0.06	3.70 ± 0.091	1.10 ± 0.58	1.75 ± 0.15	1.30 ± 0.16	0.68 ± 0.11
Albumin (g/L)	37.30 ± 1.08	41.85 ± 1.39	38.64 ± 1.71	35.63 ± 1.79	31.95 ± 6.01	39.60 ± 0.42	36.73 ± 0.90	32.00 ± 7.99
Total protein (g/L)	72.13 ± 2.67	74.88 ± 3.65	74.52 ± 1.36	71.93 ± 3.27	57.88 ± 9.66	73.60 ± 1.25	79.20 ± 4.21	55.45 ± 14.99
Cholesterol (mmol/L)	1.93 ± 0.14	1.86 ± 0.10	2.09 ± 0.21	2.25 ± 0.12	1.95 ± 0.35	2.32 ± 0.08	1.61 ± 0.24	1.25 ± 0.25
TG (mmol/L)	2.38 ± 0.29	1.97 ± 0.19	2.14 ± 0.21	2.31 ± 0.15	1.48 ± 0.51	0.99 ± 0.01	0.82 ± 0.11	0.80 ± 0,29

Values are mean ± S.E.M. (n = 4–5) $^{\alpha}p < 0.05$ vs control in reversibility study (One way ANOVA followed by Tukey's multiple comparison test). AR-aqueous root extract of *A. ringens*, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), triglycerides (TG).

Table 6
Effect of AR on biochemical parameters of female rats.

Haematologic parameter	After 30 days treatment period				After reversibility period			
	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR0 (250 mg/kg)
AST (IU/L)	194.90 ± 18.06	178.00 ± 22.82	207.20 ± 27.74	135.90 ± 29.99	129.90 ± 9.05	149.50 ± 11.09	113.40 ± 16.86	125.20 ± 3.73
ALT (IU/L)	44.07 ± 05.81	41.45 ± 03.15	53.74 ± 06.59	44.10 ± 13.43	88.70 ± 11.61	65.20 ± 2.35	63.45 ± 2.13	71.63 ± 0.38
ALP (IU/L)	166.80 ± 26.12	140.00 ± 5.27	136.50 ± 10.80	114.90 ± 6.63	230.70 ± 2.70	229.70 ± 27.80	182.50 ± 3.90	203.10 ± 3.29
Urea (mg/dL)	8.87 ± 0.54	8.98 ± 1.18	8.54 ± 0.62	8.45 ± 0.43	5.60 ± 0.11	3.45 ± 0.12	4.20 ± 0.56	4.73 ± 0.17
Creatinine (μmol/L)	71.04 ± 4.12	66.98 ± 2.10	68.95 ± 3.85	50.24 ± 2.85^{a}	44.13 ± 0.84	30.27 ± 10.01	39.71 ± 5.21	49.17 ± 0.69
Bilirubin (μmol/L)	3.78 ± 0.30	3.93 ± 0.06	3.86 ± 0.11	3.68 ± 0.26	1.35 ± 0.10	2.43 ± 1.24	2.07 ± 0.18	1.88 ± 0.30
Albumin (g/L)	41.30 ± 1.15	38.20 ± 2.99	41.04 ± 1.01	37.23 ± 1.77	45.40 ± 0.82	30.15 ± 3.69	31.07 ± 1.97	40.10 ± 3.27
Total protein (g/L)	59.35 ± 14.66	75.15 ± 3.48	77.58 ± 3.24	60.33 ± 12.02	76.40 ± 0.59	66.00 ± 10.24	75.07 ± 7.47	79.93 ± 0.46
Cholesterol (mmol/L)	1.80 ± 0.53	1.81 ± 0.16	1.87 ± 0.11	1.53 ± 0.37	1.44 ± 0.05	1.33 ± 0.39	1.72 ± 0.31	1.51 ± 0.01
TG (mmol/L)	0.92 ± 0.25	0.70 ± 0.05	0.86 ± 0.11	0.68 ± 0.19	0.70 ± 0.02	0.75 ± 0.24	0.92 ± 0.19	1.09 ± 0.09

Values are mean ± S.E.M. (n = 4–5) $^{a}p < 0.05$ vs control for the 30 days treatment study (One way ANOVA followed by Tukey's multiple comparison test). AR-aqueous root extract of *A. ringens,* aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), triglycerides (TG).

Table 7
Effect of AR on serum electrolytes of male rats.

Serum electrolyte	After 30 days treatment period				After reversibility period			
	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)
Sodium (mmol/L)	103.50 ± 2.18	109.50 ± 3.50	105.00 ± 2.20	102.80 ± 2.50	131.50 ± 16.50	102.00 ± 12.00	101.00 ± 3.50	120.50 ± 1.50
Potassium (mmol/L)	8.60 ± 1.05	8.45 ± 1.45	12.38 ± 2.28	11.10 ± 1.20	23.10 ± 1.00	30.50 ± 6.50	30.70 ± 1.19	30.50 ± 11.50
Chloride (mmol/L)	110.00 ± 3.58	107.80 ± 5.83	122.40 ± 4.76	138.30 ± 11.01	91.00 ± 5.00	77.50 ± 4.50	79.33 ± 0.33	86.50 ± 1.50
Bicarbonate (mmol/L)	20.00 ± 1.35	19.00 ± 1.35	21.20 ± 3.97	20.75 ± 2.50	12.50 ± 0.50	15.00 ± 2.00	13.67 ± 0.88	12.50 ± 2.50

Values are mean ± S.E.M. (n = 4–5) AR-aqueous root extract of *A. ringens*.

Table 8
Effect of AR on serum electrolytes of female rats.

Serum electrolyte	After 30 days treatment period				After reversibility period			
	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)
Sodium (mmol/L)	96.50 ± 2.40	105.50 ± 4.03	99.40 ± 1.33	84.25 ± 12.24	117.00 ± 1.00	102.50 ± 17.50	162.30 ± 13.12	123.00 ± 4.00
Potassium (mmol/L)	14.45 ± 1.68	10.68 ± 1.99	7.12 ± 1.99	7.23 ± 2.12	19.10 ± 6.10	28.25 ± 5.05	39.13 ± 14.68	20.65 ± 3.65
Chloride (mmol/L)	127.80 ± 15.12	127.00 ± 12.70	133.20 ± 5.68	120.0 ± 4.88	88.00 ± 2.00	90.50 ± 5.50	96.00 ± 4.72	91.00 ± 2.00
Bicarbonate (mmol/L)	16.00 ± 1.08	18.25 ± 2.18	16.60 ± 0.93	19.25 ± 2.02	10.00 ± 3.00	9.00 ± 1.00	10.67 ± 1.33	10.00 ± 3.00

Values are mean ± S.E.M. (n = 4–5). AR-aqueous root extract of *A. ringens*.

Authors contributions

Flora R. Aigbe: Author was involved with conceptualization, experimental design, execution of research protocol, data analysis, data interpretation and manuscript development. **Oluwatoyin M. Sofidiya:** Co-author was involved with co-supervision of research project and manuscript review. **Ayorinde B. James:** Co-author was involved with execution of aspects of research project and data analysis. **Abimbola A. Sowemimo:** Co-author was involved with supervision of aspects of the research project **Olanrewaju K. Akindere:** Co-author was involved with research project execution and data interpretation. **Miriam O. Aliu:** Co-author was involved with aspects of research project execution **Alimat A. Dosunmu:** involved with execution of aspects of research project **Micah C. Chijioke:** Involved with execution of research project.

Table 9
Effect of AR on liver and kidney oxidative stress biomarkers of male rats.

Oxidative stress biomarker	After 30 days treatment period				After reversibility period			
	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)
Liver glutathione (U/mg)	2.39 ± 0.19	2.43 ± 0.17	2.41 ± 0.18	2.05 ± 0.12	1.28 ± 0.18	1.00 ± 0.14	1.37 ± 0.01	1.80 ± 0.05$^{\alpha}$
Liver superoxide dismutase (U/mg)	4.85 ± 0.21	4.44 ± 0.36	4.03 ± 0.26	4.04 ± 0.08	2.63 ± 0.36	1.93 ± 0.24	2.83 ± 0.07	4.06 ± 0.07$^{\beta}$
Liver catalase (U/mg)	19.46 ± 1.00	19.64 ± 1.97	18.71 ± 1.34	18.29 ± 0.30	10.96 ± 1.71	8.76 ± 1.23	11.18 ± 0.66	15.32 ± 0.53
Liver malondialdehyde (U/mg)	0.09 ± 0.01	0.13 ± 0.02^{c}	0.27 ± 0.02^{c}	0.32 ± 0.01^{c}	0.10 ± 0.03	0.16 ± 0.02	0.11 ± 0.05	0.21 ± 0.02
Kidney glutathione (U/mg)	1.92 ± 0.16	2.02 ± 0.20	2.09 ± 0.42	1.49 ± 0.26	2.09 ± 0.20	2.04 ± 0.04	2.30 ± 0.08	2.00 ± 0.07
Kidney superoxide dismutase (U/mg)	3.83 ± 0.16	4.51 ± 0.34	4.70 ± 0.89	2.66 ± 023	4.65 ± 0.15	4.29 ± 0.44	3.69 ± 0.09	3.01 ± 0.20$^{\beta}$
Kidney catalase (U/mg)	17.42 ± 0.86	19.27 ± 1.61	20.10 ± 3.76	12.92 ± 1.67	17.96 ± 0.57	16.43 ± 0.79	17.02 ± 0.73	16.55 ± 0.79
Kidney malondyaldehyde (U/mg)	0.14 ± 0.03	0.20 ± 0.01	0.17 ± 0.03	0.09 ± 0.03	0.08 ± 0.01	0.16 ± 0.03$^{\alpha}$	0.15 ± 0.01$^{\alpha}$	0.19 ± 0.02$^{\beta}$

Values are mean ± S.E.M. (n = 4–5). $^{c}p < 0.001$ vs control in 30 days exposure study; $^{\alpha}p < 0.05$, $^{\beta}p < 0.01$ vs control in reversibility study (One way ANOVA followed by Tukey's multiple comparison test). AR-aqueous root extract of *A. ringens*.

Table 10
Effect of AR on liver and kidney oxidative stress indices in female rats.

Oxidative stress biomarker	After 30 days treatment period				After reversibility period			
	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)
Liver glutathione (U/mg)	2.17 ± 0.20	2.13 ± 0.05	2.14 ± 0.12	1.91 ± 0.06	0.67 ± 0.34	1.20 ± 0.09	1.19 ± 0.04	1.30 ± 2.35
Liver superoxide dismutase (U/mg)	4.01 ± 0.12	3.73 ± 0.14	3.63 ± 0.27	3.33 ± 0.07	2.03 ± 0.20	2.17 ± 0.15	2.36 ± 0.14	2.44 ± 0.05
Liver catalase (U/mg)	17.16 ± 0.96	16.25 ± 0.58	16.65 ± 0.86	15.78 ± 0.26	7.45 ± 0.75	9.76 ± 0.88	9.47 ± 0.39	9.94 ± 0.45
Liver Malondyaldehyde (U/mg)	0.14 ± 0.01	0.18 ± 0.00	0.24 ± 0.02^{c}	0.24 ± 0.01^{c}	0.12 ± 0.01	0.15 ± 0.07	0.13 ± 0.04	0.20 ± 0.01
Kidney glutathione (U/mg)	1.50 ± 0.17	1.49 ± 0.17	2.01 ± 0.13	1.89 ± 0.05	2.13 ± 0.13	2.05 ± 0.19	2.41 ± 0.06	2.08 ± 0.07
Kidney superoxide dismutase (U/mg)	2.88 ± 0.30	2.94 ± 0.44	4.06 ± 0.18	3.94 ± 0.23	3.88 ± 0.25	3.26 ± 0.23	3.72 ± 0.13	2.38 ± 0.11$^{\gamma}$
Kidney catalase (U/mg)	11.04 ± 1.34	14.94 ± 2.30	9.52 ± 1.47	6.10 ± 1.30	16.25 ± 1.14	14.47 ± 1.15	16.73 ± 0.31	15.87 ± 0.71
Kidney malondialdehyde (U/mg)	0.08 ± 0.01	0.12 ± 0.02	0.15 ± 0.03	0.13 ± 0.04	0.21 ± 0.02	0.20 ± 0.04	0.20 ± 0.03	0.23 ± 0.01

Values are mean ± S.E.M. (n = 4–5) $^{c}p < 0.001$ vs control 30 days exposure study; $^{\gamma}p < 0.001$ vs control in reversibility study (One way ANOVA followed by Tukey's multiple comparison test). AR-aqueous root extract of *A. ringens*.

Table 11
Effect of AR on semen parameters.

Parameter	After 30 days exposure				After reversibility period			
	Control (5 ml/kg)	AR (10 mg/kg)	AR (50 mg/kg)	AR (250 mg/kg)	Control (5 ml/kg)	AR (10 mg/kg)	AR50 (50 mg/kg)	AR (250 mg/kg)
Sperm count (million/ml)	55.31 ± 6.82	36.13 ± 7.47	45.25 ± 1.91	34.69 ± 4.46	61.25 ± 1.25	41.88 ± 3.13	48.75 ± 1.25	46.25 ± 2.50
Sperm morphology (%)	12.50 ± 1.11	12.25 ± 2.42	17.60 ± 1.08	17.25 ± 1.93	11.00 ± 1.00	13.00 ± 3.00	17.00 ± 1.00	19.00 ± 1.00
Sperm motility (%)	40.75 ± 5.30	36.00 ± 6.40	41.60 ± 1.89	31.75 ± 4.03	35.50 ± 10.50	39.00 ± 1.00	44.00 ± 1.00	43.50 ± 1.50

Values are mean ± S.E.M. (n = 4–5) $^{a}p < 0.05$ vs control (One way ANOVA followed by Tukey's multiple comparison test). AR-aqueous root extract of *A. ringens*.

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