**Original Article**

# Effect of Administration of Root Ethanolic Extract of *Aristolochia Ringens* on the Liver Functional Indices of Male Wistar Rats

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*Received: 28.05.2016* *Accepted: 11.07.2016*

## ABSTRACT

**Background:** The alcoholic decoction of root ethanolic extract of *Aristolochia ringens* is taken orally to treat various ailments in South-west Nigeria without prior knowledge of its potential toxic effect. Therefore, this study aimed at assessing the toxicity potentials of root ethanolic extract of *A. ringens* on functional indices and histology of the liver.

**Methods:** Twenty male rats were randomized into four groups of five animals each. Group A (control) received 0.5 ml of distilled water, group B, C and D received 75, 150 and 300 mg/kg b. wt. of the extract respectively. The administration was done orally and lasted for fourteen days.

**Results:** The extract significantly reduced the activities of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) as well as reduction in the level of serum albumin and direct bilirubin ($P$<0.05) while the level of total bilirubin increased. The activities of these enzymes i.e. ALP, ALT and AST increased in the serum at all the doses investigated.

**Conclusion:** Ethanolic extract from *A. ringens* root may not be completely safe when administered repeatedly.

**Key words:** *Aristolochia ringens*, Functional indices, Hepatocytes, Toxicity.

**IJT 2017 (1): 55-58**

## INTRODUCTION

The use of medicinal plants by traditional medicine practitioners (TMPs) in the management of several disorders dates back to time immemorial and is increasing in recent times [1]. A wide range of bioactive found in plants are used as phyto-pharmaceuticals. These secondary metabolites evolved in medicinal plants probably as biochemical defense system against predatory attacks, microbial or viral infections [2]. Several plants possessed medicinal agents for the treatment of various diseases and one of such plant is *Aristolochia ringens*.

*A. ringens* is an ornamental plant belonging to the family Aristolochiaceae. The plant originated from America but now grows in a number of African Countries. It has a long stems, leave of about 17 cm and the flower is about 18 cm. In Nigeria, the local names include "Ako-igun" (Yoruba; South west Nigeria) and "Dumandutsee" (Hausa; North Nigeria). The root of this plant has been reported in South west Nigeria for the treatment of various ailments ranging from asthma and diarrheal [3, 4], cancer [5] as well as diabetes [6].

Increasing the use of medicinal plants and their phytoconstituents, as well as the scarcity of scientific studies on their safety, has raised concerns on toxicity [7, 8]. Therefore, there is the need to assess the potential toxic effects of these plants not only in the normal experimental animals but also in the diseased state

## MATERIALS AND METHODS

### *Experimental Animals*

A total of twenty male albino rats (*Rattus norvegicus*) weighing 135±5.97 g were purchased from the animal holding units of the Department of Biosciences and Biotechnology (Biochemistry Unit), College of Pure and Applied Sciences,

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Kwara State University Malete, Nigeria. They were acclimatized for a week to standard housing condition. Water and rat pellets were provided *ad-libitum.*

### *Plant Materials, Chemicals and Reagents*

*A. ringens* roots were collected from Idumota, Lagos State, South-West Nigeria. It was authenticated at the herbarium unit of the Botany Department, University of Lagos, where a voucher specimen (LUH 6234) was deposited. Diagnostic kits for ALT, AST, ALP, GGT, bilirubin and albumin were obtained from Randox Laboratory Limited, United Kingdom. Other chemicals and reagents used were of analytical / research grades.

### *Preparation of the Extracts*

The root of the plant was air dried to constant weight at room temperature and thereafter grinded into fine powder. The powdered root of *A. ringens* (200 g) was soaked with 2000 ml of (70%) ethanol for 24 h with intermittent shaking, filtered and evaporated using vacuum-assisted rotary evaporator at 40 $^{o}$C to give a yield of 24.25 g (12.17 % w/w). The dried extract was kept in air- tight container at 4 $^{o}$C and labelled REAR.

### *Phytochemical Analyses*

The phytochemical screening and the GCMS analysis of root ethanolic extract of *A. ringens* was determined and reported by Sulyman et al. [6].

### *Animal Grouping and Extract Administration*

Twenty rats were completely randomized into four group of five rats each (A-D) as follow:

- NC (Normal Control) received 0.5 ml of distilled water.
- NT1 (Normal rats treated with extract) received 75 mg/kg b.wt. of REAR.
- NT2 (Normal rats treated with extract) received 150 mg/kg b.wt. of REAR.
- NT3 (Normal rats treated with extract) received 300 mg/kg b.wt. of REAR.

The administration was done orally using a metal oropharyngeal cannula and lasted for fourteen (14) days.

The research adhered strictly and conformed to the Principles of Laboratory Animal Care (NIH Publication, No. 85-23).

### *Preparation of Serum and Tissue Homogenates*

The procedure described by Yakubu et al. [9] was used in the preparation of serum. The rats were thereafter quickly dissected, liver excised and transferred into ice-cold 0.25 M sucrose solution and used as a medium for homogenization. (1:5 wt/vol). The homogenates were kept frozen overnight to ensure maximum release of the enzymes located in the cells of the tissues before being used for the various biochemical assays.

### *Determination of Biochemical Parameters*

The procedures described in the assay kits from Randox Laboratory for albumin, bilirubin, ALP, GGT, ALT, and AST were determined in the serum and liver according to the method described.

### *Statistical Analysis*

The results were expressed as mean ± Standard error of mean of five replicates. Data obtained were subjected to one-way analysis of variance and means found to be significantly different at $P < 0.05$ were separated by Duncan Multiple Range Test.

## RESULTS

The extraction from root ethanolic extract of *A. ringens* using 70% ethanol yielded 24.25 g of REAR extract per 200 g of dried plant sample (12.17 % w/w). The activities of ALP, ALT, AST and GGT in rat liver significantly reduced throughout the period of administration at all doses investigated, whereas the concentration of protein increased significantly at all dose investigated. In addition, the levels of serum albumin and direct bilirubin significantly reduced in a dose dependent manner while there was an increase in the level of serum total bilirubin in all the doses investigated (Table 1). The serum total protein concentration increased throughout the experimental period, but the activities of serum ALP, ALT and AST increased throughout the period of administration (Table 2).

**Table 1.** Effect of Root Ethanolic Extract of *Aristolochia ringens* on Liver Function Parameters of Wister Rats.

| Treatment | Control | 75 | 150 | 300 |
|---|---|---|---|---|
| T.Bilirubin (µmol/L) | 1.13±0.05[a] | 2.50±0.07[b] | 4.12±0.01[c] | 5.86±0.02[d] |
| D.Bilirubin (µmol/L) | 0.62±0.03[d] | 0.98±0.02[c] | 0.38±0.07[b] | 0.14±0.07[a] |
| Albumin (g/L) | 11.20±0.70[d] | 10.20±0.03[c] | 8.25±0.75[b] | 6.75±0.20[a] |
| ALP (U/L) | 1471.19±0.09[d] | 1130.22±0.64[c] | 1034.66±0.67[b] | 527.60±0.20[a] |
| ALT (U/L) | 79.30±0.25[d] | 72.68±0.90[c] | 63.51±0.66[b] | 48.63±0.13[a] |
| AST (U/L) | 144.02±0.55[d] | 125.81±0.42[c] | 92.00±0.50[b] | 31.36±0.44[a] |
| GGT (U/L) | 704.52±0.77[d] | 938.56±0.76[c] | 629.81±0.42[b] | 665.25±0.56[a] |
| T. Protein (g/L) | 1.14±0.06[b] | 1.55±0.02[b] | 1.05±0.76[a] | 1.99±0.23[a] |

n=5±SEM, Values with superscripts a, b, c and d are considered significantly different ($p<0.05$)

**Table 2:** Effect of Root Ethanolic Extract of *Aristolochia ringens* on the Activity of Some Serum Enzymes of Wister Rats.

| Treatment | Control | 75 | 150 | 300 |
|---|---|---|---|---|
| ALP (U/L) | 35.15±0.10[a] | 46.80±0.09[b] | 57.87±0.17[c] | 67.58±0.05[d] |
| ALT (U/L) | 23.21±0.90[a] | 35.19±0.60[b] | 56.45±0.50[c] | 68.27±0.24[d] |
| AST (U/L) | 23.13±0.50[a] | 34.49±0.60[b] | 45.40±0.25[c] | 46.01±0.01[d] |
| T. Protein (g/L) | 1.08±0.07b | 0.60±0.01[a] | 0.85±0.23[a,b] | 1.01±0.04[c] |

n=5±SEM, Values with superscripts a, b, c and d are considered significantly different ($p<0.05$)

## DISCUSSION

The use of herbal medicine to treat various ailments is increasing in developing countries [10]. More than half of the world's population depends either directly or indirectly on the traditional ways of treating ailments, which involve the use of plant decoction [11, 12]. The use of herbal remedies by the traditional medicine practitioners (TPMs) has raised many concerns because of the inadequate scientific information on the toxicity and adverse effects of these plants [8]. These plants contain some secondary metabolites that may cause adverse effects. The present study shows that root ethanolic extract of *A. ringens* may adversely affect the hepatic functions of male rats.

The measurement of the activities of various enzymes in the tissues and body fluids plays a significant role in disease investigation, diagnosis and tissue cellular damage [13]. Changes in the levels of normal range of enzymes localized in specific cells indicate functional toxicity of such cells and these alterations occurs prior to obvious cellular architectural degeneration that are observed on histological examination [15] and is required in certain amounts for proper functioning of organs. The reduction in the activity of the liver ALP observed in this study may be due to damage to the plasma membrane leading to loss of this enzyme into the extracellular fluid [16]. This was reflected with a mild increase in the serum ALP. Other enzymes such as the transaminases occupy a central position in the metabolism of amino acids. The decrease in the activities of the ALT and AST observed in the liver of extract treated rats may be a result of tissue membrane damage causing cytosolic leakage of the enzymes into the extracellular fluid and this was reflected in the activities of the serum ALT and AST where the activities increased. GGT (Gamma Glutamyl Transferase) is the most sensitive enzymatic indicator of hepatobiliary disease [17]. It is a membrane – localized enzyme that plays a major role in glutathione metabolism and resorption of amino acids from the glomerular filterate and from the intestinal lumen [18]. Reduction in the activities of GGT in the liver further lends credence to selective toxicity and indications of cellular toxicity of the extract on the tissues.

The concentrations of total proteins, bilirubin and albumin in the serum of rats could indicate the state of the liver [19]. The increase in total bilirubin levels observed in this study might be an indication of impairment in the functional capacity of the liver [20] and could be a consequence of severe defects in bilirubin transport, which may cause haemolysis and thus lead to jaundice [18]. The decrease in serum albumin might be attributed to liver dysfunction resulting from damage, infection and or loss of albumin from the body [21]. Alterations in the

liver histology in this study further lend credence to the selective toxicity of the extract. This, however, could explain the alterations in the functional parameters of the liver investigated.

## CONCLUSION

The root ethanolic extract of *A. ringens* despite its efficacy in the treatment of various ailments may have deleterious effect when administered repetitively.

## ACKNOWLEDGEMENTS

The authors are very thankful to the laboratory technologists of Biochemistry Laboratory of Department of Biosciences and Biotechnology, Kwara State University for providing some of the reagents used during this study.

The authors declared that no conflicts of interest with respect to this research, authorship, and/or publication of this article.

## REFERENCES

1. Da Costa Lopes L, Albano F, Laranja GAT, Alves LM, e Silva LFM, de Souza GP, et al. Toxicological evaluation by in vitro and in vivo assays of an aqueous extract prepared from Echinodorus macrophyllus leaves. Toxicol Lett 2000;116(3):189-98.
2. Balick MJ, Cox PA. Plants, people, and culture: the science of ethnobotany: Scientific American Library; 1996.
3. Adeyemi O, Aigbe F, Badru O. The antidiarrhoeal activity of the aqueous root extract of Aristolochia ringens (Vahl.) Aristolochiaceae. Nig Qt J Hosp Med 2012;22(1):29-33.
4. Sonibare M, Gbile Z. Ethnobotanical survey of anti-asthmatic plants in South Western Nigeria. Afr J Tradit Complementary Altern Med 2008;5(4):340-5.
5. Akindele AJ, Wani Z, Mahajan G, Sharma S, Aigbe FR, Satti N, et al. Anticancer activity of Aristolochia ringens Vahl.(Aristolochiaceae). J Tradit Complemen Med 2015;5(1):35-41.
6. Sulyman AO, Akolade JO, Sabiu S, Aladodo RA, Muritala HF. Antidiabetic potentials of ethanolic extract of Aristolochia ringens (Vahl.) roots. J Ethnopharmacol 2016;182:122-8.
7. Suffness M, Douros J. Current status of the NCI plant and animal product program. J Nat Prod 1982;45(1):1-14.
8. Saad B, Azaizeh H, Abu-Hijleh G, Said O. Safety of traditional Arab herbal medicine. Evid-Based Complemen Altern Med 2006;3(4):433-9.
9. Yakubu M, Akanji M, Oladiji A. Aphrodisiac potentials of the aqueous extract of Fadogia agrestis (Schweinf. Ex Hiern) stem in male albino rats. Asian J Androl 2005;7(4):399-404.
10. Obici S, Otobone FJ, da Silva Sela VR, Ishida K, da Silva JC, Nakamura CV, et al. Preliminary toxicity study of dichloromethane extract of Kielmeyera coriacea stems in mice and rats. J Ethnopharmacol 2008;115(1):131-9.
11. Gram H. Summary of WHO guidelines for the assessment of herbal medicines. Switzerland: World Health Organization. 1993:13-4.
12. Saggu S, Divekar H, Gupta V, Sawhney R, Banerjee P, Kumar R. Adaptogenic and safety evaluation of seabuckthorn (Hippophae rhamnoides) leaf extract: a dose dependent study. Food Chem Toxicol 2007;45(4):609-17.
13. Malomo S. Toxicological implications of ceftriaxone administration in rats. Proteins 2000;5:34-8.
14. Akanji MA. A comparative biochemical study of the interaction of some trypanocides with rat tissue cellular system. [Phd Thesis] University of Ife, Ile Ife Nigeria,1986.
15. Wright PJ, Plummer DT. The use of urinary enzyme measurements to detect renal damage caused by nephrotoxic compounds. Biochem Pharmacol 1974;23(1):65-73.
16. Malbica J, Hart L. Effect of adenosine triphosphate and some anti-inflammatory agents on a purified lysosomal fraction having high acid phosphatase and labile β-glucuronidase activity. Biochem Pharmacol 1971;20(8):2017-26.
17. Mayne PD. Clinical Chemistry in Diagnosis and Treatment. 6th Ed. Arnold international students,1998.p.196-205.
18. Kaplan LA, Pesce AJ. Clinical chemistry: Theory, Analysis and correlation. 3rd Ed. A Harcourt Health Science Company, Mosby, London ,1996.P. 140-52.
19. Ganong WF. Review of medical physiology. 20th Ed. London: Lange Medical Books/McGraw-Hill Medical Publishing Division, 2001. p.414–7.
20. Moudgil KD, Narang BS. The liver and the biliary system. In: Talwar GP, Srivastava LM, Moudgil KD (eds.) Textbook of biochemistry and human biology. 2nd ed. New Delhi: Prentice-Hall of India Pvt Ltd, 1989. p.271–3.
21. Naganna B. Plasma proteins. In: Tawlar GP, Srivastava LM, Moudgils KD (eds.) Textbook of biochemistry and human biology. 2nd ed. New Delhi: Prentice- Hall of India Pvt Ltd, 1989. p.172–81.