Access this Article online
Quick Response Code:

Website:
jecajournal.com
Doi:
doi.org/10.4314/jeca.v21i2.4

Original Article

Histopathological Assessment of Cardiac Tissue in Arsenic Trioxide-Induced Wistar rats: Effects of Ethanol Leaf Extract of *Irvingia gabonensis*

[1]Olukayode, S.B.; [1]Innih, S.O.; [1]Calmday-Ombo, D.

Abstract

BACKGROUND AND AIM: The toxic effects of drugs and other related substances have attracted concerns from scientists. The passion about the availability of effective and cheap substances against organotoxicity in humans is increasing daily. This work assessed the histopathological changes in the heart of arsenic trioxide-induced Wistar rats and the effects of ethanol leaf extract of *Irvingia gabonensis.*

METHODOLOGY: Forty-two adult Wistar rats weighing between 160-190g were randomly divided into six groups of seven rats each. Group A served as the control, Group B received 10 mg/kg body weight of arsenic trioxide only, Groups C and D received 250 and 500 m/kg body weights of ethanol leaf extract of *Irvingia gabonensis* only respectively, Groups E and F received 250 and 500 mg/kg body weights of ethanol leaf extract of *Irvingia gabonensis* in addition to 10 mg/kg body weight of arsenic trioxide respectively. All administrations were through oral route for 28 days. After sacrifice, cardiac tissues were harvested, fixed in 10% neutral buffered formalin and processed for haematoxylin and eosin staining.

RESULTS AND CONCLUSION: Histological findings show that arsenic trioxide caused vascular distortion, perivascular infiltrates of inflammatory cells and focal myocardial degeneration. Treatment with graded doses of ethanol leaf extract of *Irvingia gabonensis* achieved a remarkable amelioration, with 250 mg/kg body weight having a better ameliorative effect, thus suggesting that ethanol leaf extract of *Irvingia gabonensis* exhibits cardioprotective activity against arsenic trioxide-induced damage and could be a potential cardioprotective substance.

Keywords:
Irvingia gabonensis; arsenic trioxide; vascular distortion; myocardial degeneration.

Submitted: 20th July, 2024
Revised: 24th August, 2024
Accepted: 14th September, 2024
Published: 31st December, 2024

[1]*Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria.*

Address for Correspondence:
Olukayode, S.B.
Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria.
seun.olukayode@bmedsci.uniben.edu

INTRODUCTION

Irvingia gabonensis (bush mango) tree comes originally from Central and West Africa. The fruit of this tree (Oro in Yoruba, Dika in Hausa, Ogbono in Igbo) is usually eaten in these areas and it is known its potential health benefits (Agbor, 1994), because of its richness in minerals, vitamins, fiber and antioxidants. The seeds are high in fat and protein. Ethnomedicinal treatments utilize the bark, kernels, leaves, or roots for a variety of ailments. The bark is mixed with palm oil for treating diarrhea and for reducing the breast-feeding period. The shavings of the stem bark are consumed by mouth to treat hernias, yellow fever, and dysentery, and to reduce the effects of poison in French Equatorial Africa (Ejiofor and Okafor, 1997). The antibiotic properties of the bark help heal scabby skin, and the boiled bark relieves tooth pain. *Irvingia gabonensis* leaf and root extracts have documented inhibitory activity against several bacteria and fungi. Potential mechanisms of action include membrane disruption by terpenoids and inactivation of microbial adhesion, enzymes, and cell envelope transport proteins by ellagic acid-like compounds (Okolo *et al.*, 1995). *Irvingia gabonensis* contains various phytochemicals, including flavonoids, alkaloids, tannins, terpenoids, saponins and polyphenols, which are known to possess antioxidant activity (Mgbemena *et al.,* 2019). These compounds can scavenge and neutralize free radicals, thereby reducing the potential damage caused by oxidative stress. *Irvingia gabonensis* leaf may also help stimulate the body's own antioxidant defenses and has been reported to increase the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) (Sulaimon *et al.,* 2015) These enzymes play a crucial role in neutralizing and detoxifying free radicals (Jeema *et al.,* 2017).

Arsenic trioxide (ATO), a toxic white crystalline compound and a traditional medicine used in China, has been attributed to be effective in acute promyelocytic leukemia (APL) (Lo-Coco *et al*., 2013). However, sudden cardiac death and QT prolongation, from arsenic trioxide-induced

How to cite this article: Olukayode, S.B.; Innih, S.O.; Calmday-Ombo, D. Histopathological Assessment of Cardiac Tissue in Arsenic Trioxide-Induced Wistar rats: Effects of Ethanol Leaf Extract of *Irvingia gabonensis*. J Exp Clin Anat 2024; 21(2): 168-171.
https://dx.doi.org/10.4314/jeca.v21i2.4

cardiotoxicity, limits its clinical usefulness (Wang *et al*., 2022). Reactive oxygen species (ROS) and overload of intracellular calcium have been implicated for the mechanisms of arsenic trioxide-induced cardiotoxicity (Wang *et al*., 2019). There is also increasing evidence that suggest that arsenic trioxide is able to trigger cardiomyocytes primarily by necrosis, apoptosis and autophagy, which have been implicated in cardiac injury, but detailed processes surrounding arsenic trioxide cardiotoxicity remain unlucid (Vineetha *et al*., 2015). Endogenous ingredient metallothionein and some natural compounds like resveratrol, ascorbic acid-tocopherol combination, eugenol, and savianolic acid A have though been demonstrated to possess protective effects against arsenic trioxide-induced cardiotoxicity (Zhao *et al.,* 2008), but at present, there is no effective clinical remedy for arsenic trioxide-induced cardiotoxicity and preventing cardiac cell death is recommended as an effective cardioprotective strategy (Vineetha *et al*., 2015). Caspase-dependent apoptosis is a major type of regulated cell death in cardiomyocytes, in which reactive oxygen species and lipid peroxidation are an important interaction point, and thus reactive oxygen species are expected to be a target to prevent arsenic trioxide-induced cardiotoxicity (Ma *et al.,* 2020). Irvingia gabonensis leaf contains many of the bioactive agents that can possible protect against these reactive oxygen species, (Mgbemena *et al.,* 2019), and since the use of herbal therapy has received widespread attention within the global healthcare system because some pharmaceutical medications are based on a single active ingredient derived from a plant source. The importance of this study is to assess the effects of ethanol leaf extract of *Irvingia gabonensis* on arsenic trioxide-induced damage to the heart in adult Wistar rats.

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant Material

Fresh *Irvingia gabonensis* leaves were harvested in Ekosodin community in Ovie North-East local government area of Edo State. It was identified and authenticated in the Department of Plant Biology and Biotechnology, University of Benin, Benin-City with herbarium number UBH-1153. The leaves were thoroughly washed with tap water, air-dried and then pulverized to powdered form. 120g of the powdered form was soaked in 500ml of 50% ethanol for 72 hours. The crude ethanol extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was freeze-dried, using the method of Kumar (2019) at the Department of Pharmaceutical Chemistry (Natural Product Research Laboratory), Faculty of Pharmacy, University of Benin, Benin-City and refrigerated at -4 Celsius until use.

Experimental Animals

Forty-two (42) Wistar rats weighing 160-190 g were procured from the Animal House, Department of Anatomy, University of Benin. Water and food were provided *ad libitum*. They were exposed to controlled environmental temperature (28 ± 2°C), relative humidity (50 ± 5%) and 12 hour light-dark cycle.

Drugs Preparation

Ten grams (10 g) of arsenic trioxide crystals (500g) was dissolved in 100 ml of distilled water to form arsenic trioxide solution and was administered orally to Wistar rats at a dose of 10 mg/kg body weight. The plant extract (10g) was dissolved in 100 ml of water and was administered orally to Wistar rats at the doses of 250 and 500 mg/kg body weight after LD_{50} (Lorke, 1983) was done.

Animal Groupings:

The animals were randomly divided into six groups of seven animals each.

Group A: (Control) received only 1ml of distilled water daily for a period of 28 days.

Group B: (arsenic trioxide –induced) received 10 mg/kg body weight of arsenic trioxide orally for a period of 28 days.

Group C: received 250 mg/kg body weight ethanol leaf extract of *Irvingia gabonensis* orally for a period of 28 days.

Group D: received 500 mg/kg body weight ethanol leaf extract of *Irvingia gabonensis* orally for a period of 28 days.

Group E: received 250 mg/kg body weight ethanol leaf extract of *Irvingia gabonensis* and 10 mg/kg body weight of arsenic trioxide orally for a period of 28 days.

Group F: received 500 mg/kg body weight ethanol leaf extract of *Irvingia gabonensis* and 10 mg/kg body weight of arsenic trioxide orally for a period of 28 days.

All the animals were sacrificed 24 hours after the day of administration under chloroform anesthesia. Abdomino-thoracic incision was made on the rat to expose the thoracic viscera. Thereafter, the heart was harvested, dissected and immediately fixed in 10% formol saline in a universal bottle for histological analysis.

Histopathological analysis

The formol-saline fixed heart tissues were dehydrated in ethanol (70 to 100 %), cleared in xylene and embedded in paraffin. All tissue sections were examined under a light microscope after staining with hematoxylin and eosin (Drury and Wallington, 1980).

RESULTS

The histopathological findings showed the control group with normal cardiac cytoarchitectures of bundles of cardiomyocytes, interstitial spaces and coronary artery (Figure 1). Rats treated with only arsenic trioxide showed vascular distortion, perivascular infiltrates of inflammatory cells and focal myocardial degeneration (Figure 2). Rats treated with low dose (250 mg/kg) of extract in addition to arsenic trioxide showed again normal cytoarchitecture of bundles of cardiomyocytes, and coronary artery (Figure 3). Rats treated with high dose (500 mg/kg) of

extract in addition to arsenic trioxide showed perivascular infiltrates of inflammatory cells and interstitial oedema (Figure 4).

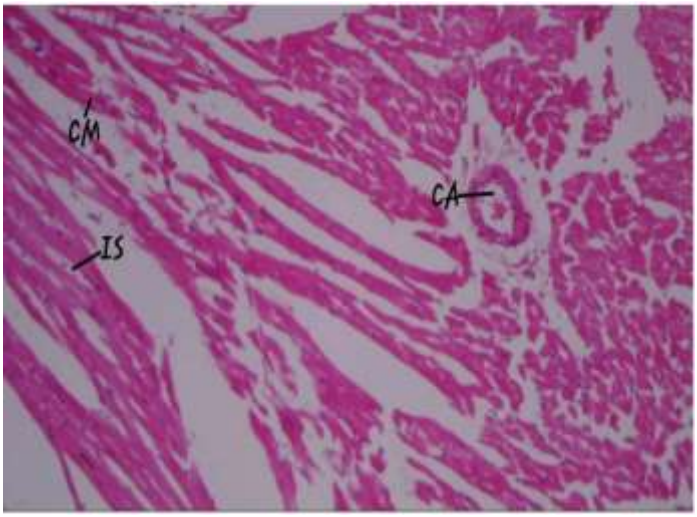

Figure 1. Rat heart. Control. Composed of: bundles of cardiomyocytes (CM), interstitial space (IS), coronary artery (CA): H&E 100x

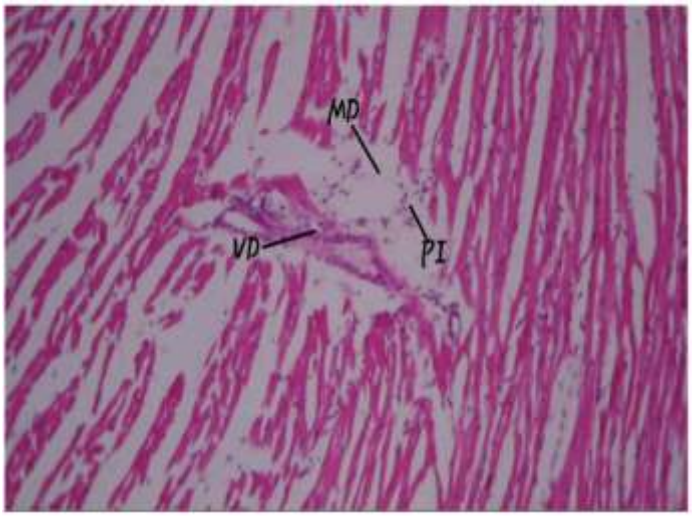

Figure 2. Rat heart given arsenic trioxide only showing: vascular distortion (VD), perivascular infiltrates of inflammatory cells (PI), focal myocardial degeneration (MD): H&E 100x

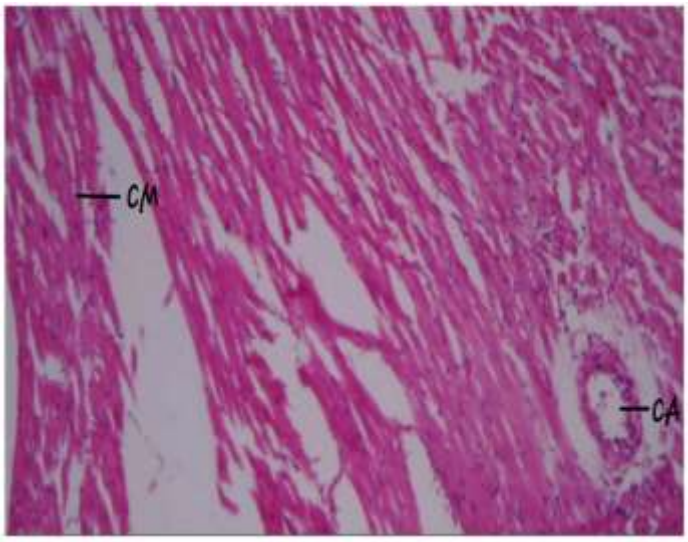

Figure 3. Rat heart given arsenic trioxide + 250 mg Extract showing: bundles of cardiomyocytes (CM), coronary artery (CA), all normal: H&E 100x

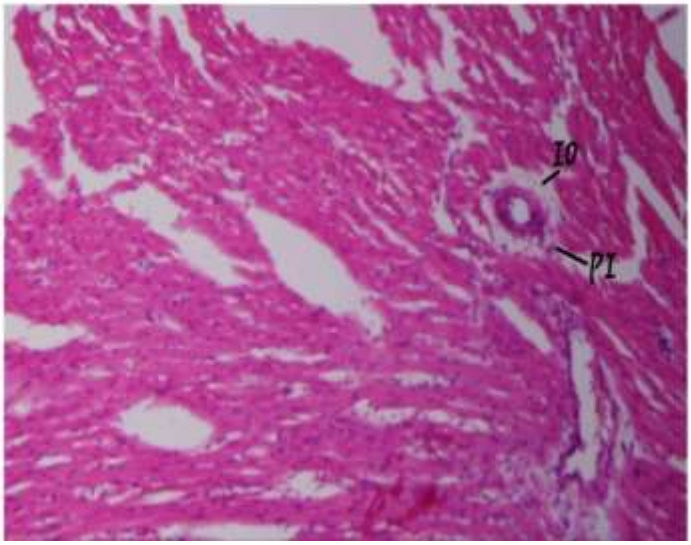

Figure 4. Rat heart given arsenic trioxide + 500mg Extract showing: perivascular infiltrates of inflammatory cells (PI), interstitial oedema (IO): H&E 100x.

DISCUSSION

The toxicities of short and long term arsenic exposure have been documented from case reports, epidemiological studies and animal experiments (Au and Kwong, 2008). Up to 200 human enzymes are inactivated by arsenic. The severity of the toxicity depends on the arsenic compound, and the route, pace and duration of absorption. Oral arsenic is methylated into active metabolites in the liver and clearance from the liver is rapid, but clearance of methylated arsenic metabolites from heart takes a longer duration (Au and Kwong, 2008).

The damage to the cardiac tissue revealed in this study may again be due to overwhelming free radicals or availability of little antioxidant since free radical injury can affect many organs in the body irrespective of their origin and location (Raghu *et al.,* 2009), this could trigger inflammatory response and oxidative stress in the cardiac tissue, as revealed in this study with the histopatholocal features vascular distortion, perivascular infiltrate of inflammatory cells and focal myocardial degenerationsuggestive of myocardial infarction in the group exposed to arsenic trioxide only. This could have damaged and

caused the release of cell contents into the blood. The extent of damage depends on the severity of the membrane damaged. It could also inhibit the activity of various enzymes that are important for cellular function. For example, it probably inhibited the activity of enzymes involved in DNA repair, causing DNA damage to accumulate and disrupted the functioning of enzymes involved in cellular energy production (such as the electron transport chain), leading to cellular dysfunction and cardiac tissue damage (Wang *et al*., 2019) since some evidence suggest that arsenic trioxide is able to trigger cardiomyocytes primarily by necrosis, apoptosis and autophagy, which have been implicated in cardiac injury.

Conclusion: Arsenic trioxide induces cardiac damage, but concurrent treatment with graded doses of ethanol leaf extract of *Irvingia gabonensis* achieved a remarkable measure of amelioration, with the lose dose having a better ameliorative effect, thus providing information that ethanol leaf extract of *Irvingia gabonensis* exhibits cardioprotective potential and could serve as a potential cardioprotective agent as revealed by this study.

REFERENCES

Agbor LON. (1994). Marketing trends and potentials for *Irvingia gabonensis* products in Nigeria. ICRAF-IITA Conference on Irvingia gabonensis; Ibadan, Nigeria.

Au W, Kwong Y. (2008). Arsenic trioxide safety issues and their management. Acta Pharmacologica Sinica; 34(5):296-301.

Doumas BT, Watson WA, Biggs HG. (1971). Albumin standards and the measurement of serum albumin with bromcresol green. Clinical Chimical Acta, 31(1): 87-96.

Drury RA, Wallington EA. (1980). Carleton's Histological Techniques, 5th edition: Oxford University Press, New York 195. Pp. 236.

Ejiofor MA, Okafor JC. (1997). Prospects for commercial exploitation of Nigerian indigenous trees, vegetables, fruits and seeds through food and industrial products formulation. International Tree Crops Journal 9(2):119-129.

Engelhardt G, Blauestein UW. (1970). Ultrasound in the diagnosis of malignant pancreatic tumours. Gut, 11(5), 443-449.

Jemaa HB, Jemia AB, Khlifi S, Ahmed HB, Slama FB, Benzarti A, Elati J, Aouidet A. (2017). Antioxidant activity and a-amylase inhibitory potential of Rosa canina Africa Journal Traditional, Complementary Alternative Medicine.: AJTCAM, 2(16): 9-13.

Jendrassik L, Grof P. (1938). Estimation of total serum bilirubin level by spectrophotometrically in serum and plasma. Biochemistry Zeitschrift, 287(6): 81-89.

Kumar, P. (2019). Lyophilization: An important formulation technique. International Journal of Research Granthaalayah, 7, 11-15.

Lo-Coco F, Avvisati G, Vignetti M. (2013). Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. New England Journal of Medicine; 36(9):111–121.

Lorke D. (1983). A new Approach to Practical Acute Toxicity Testing. Archives of Toxicology, 54, 275-287.

Ma W, Wei S, Zhang B. (2020). Molecular mechanisms of cardiomyocyte death in drug-induced cardiotoxicity. Front Cell Developmental Biology; 8: 434–2020

Ngbemena NM, Ilechukwu I, Okwunodolu FU, Chukwurah JO, Lucky IB. (2019). Chemical composition, proximate and phytochemical analysis of Irvingia gabonensis and Irvingia wombolu peels, seed coat, leaves and seeds. Ovidius University Annals of Chemistry: 30(1): 67.

Okolo CO, Johnson PB, Abdurahman EM, Abdu-Aguye I, Hussaini IM. (2015): Analgesic effects of Irvingia gabonensis stem bark extract. Journal of Ethnopharmacology; 45(2):125-129.

Okolo, HC (1994). Industrial potential of various Irvingia gabonensis products such as oil, Ogbono and juice. ICRAF-IITA Conference on Irvingia gabonensis; Ibadan, Nigeria.

Reitman S, Frankel, S. (1957). Estimation of alanine and aspartate-aminotransferanse activities. American Journal of Clinical Pathology, 28(11): 56-63.

Sulaimon AO, Auta T, Hassan AT. (2015). Evaluation of anti-diabetic activity of Irvingia gabonensis leaf and back in alloxan induced diabetic rats. Biosciences Research Support Foundation: pp 89.

Vineetha VP, Soumya RS, Raghu KG. (2015). Phloretin ameliorates arsenic trioxide induced mitochondrial dysfunction in H9c2 cardiomyoblasts mediated via alterations in membrane permeability and ETC complexes. European Journal of Pharmacology; (75)4:162–172.

Wang L, Liu S, Gao C, Chen H, Li J, Lu J, Yuan Y, Zheng X, He H, Zhang X, Zhang R, Zhang Y, Wu Y, Lin W, Zheng H.(2022). Journal of Human and Experimental Toxicology; (4)1:1-9.

Wang R, Zhang J, Wang S. (2019). The cardiotoxicity induced by arsenic trioxide is alleviated by salvianolic acid A via maintaining calcium homeostasis and inhibiting endoplasmic reticulum stress. Molecules; 24: 543.

Zhao XY, Li GY, Liu Y. (2008). Resveratrol protects against arsenic trioxide-induced cardiotoxicity in vitro and in vivo. British Journal of Pharmacology; 15(4):105–113.