Original Research Article

TOXICITY EFFECTS OF *JUSTICIA SECUNDA* PLANTS EXTRACTS GROWN IN NIGERIA ON MALE WISTAR RATS

ABSTRACT

Aims: *Justicia secunda*is a plant that belongs to the Acanthaceae family, commonly called bloodroot. *J. secunda* is used in the treatment of several ailments and diseases, including anemia, in folk medicine. There is limited research on the effect of the plant extracts on the body, organ weights, and its architecture. This study was designed to investigate the possible toxic effect of aqueous extracts of *Justicia secunda* leaves on male Wistar rats.

Study Design: This was a laboratory-based study.

Place and Duration of Study: The study was conducted at the Chemistry and Biochemistry Resources Research Laboratory of the Department of Chemistry and Biochemistry of Hallmark University, Ijebu - Itele, Ogun State, Nigeria, from July 2021 to September 2022.

Methodology: This research therefore investigated the toxicity of an aqueous leaf extract from *J. secunda* on male Wistar rats. Three groups (n = 5) of inbred Wister rats were used. The negative control (Group I) received only water; Group II received 200 mg/kg BW; and Group II received 300 mg/kg BW daily for 14 days. The animals were sacrificed by cervical dislocation, and the kidneys, hearts, livers, and spleens were harvested. They were weighed, and histological analysis was carried out on the tissues.

Results: The results showed that there was a significant reduction in body weight and liver enlargement, and histological analysis showed severe vascular congestion and edoema in the

group that received a higher concentration of the extract. There were inflammatory signs in the treatment groups. The kidneys showed only mild vascular congestion.

Conclusion: This study showed that continued use of herbal preparations from *J. secunda* could predispose people to hepatic and cardiac toxicity.

Keywords: Key words: *Justicia secunda,* extract, inflammatory, toxicity, distilled water.

1. INTRODUCTION

Human beings solely depend on nature for some necessities of life, some of which include food, shelter, clothing, fertilizers, and medicine. The use of plants and their extracts or derivatives as medicine can be traced to the beginning of human civilization [1]. In ancient times, our forefathers were compelled to use these plants to ease their suffering caused by wounds, injuries, and terminal illnesses, including physical discomfort. An example is the use of bitter-leaf extract (*Vernonia amygdalina*) to cure stomachache, dysentery, and diarrhea [2]. Phytochemical screening of *Veernonia amygdalina* showed the existence of saponins, glycosides, and tannins, which are well known as bioactive purgative principles. As a result of the benefits that accompany the use of such plants, a large number of the world's population today makes use of these plants in healthcare [3]. Plants with therapeutic attributes or those found with beneficial pharmacological effects on the body are termed medicinal plants or medicinal herbs [1][4]. Medicinal plants which naturally synthesize and produce some secondary metabolites like resins, terpenes, sterols, flavonoids, alkaloids, and volatile oils, which are responsible for the biological characteristics of plant species worldwide [1] [3]. The therapeutic and pharmacological properties of these plants make them essential raw materials for manufacturing various

traditional and modern drugs. In most African countries like Nigeria, which is blessed with herbal plants, plant extracts are useful raw materials in health treatment. An example is the use of ginger extract for weight loss when consumed as tea [4]. The possession of high nutritional value as seen in plant products like ginger, garlic, and walnuts is well known. In America, about one-third of its population depends on herbs for health purposes, though a challenge could be a lack of accurate information about the protection, dosage and efficiency of the herbal remedies [5]. Thus, medicinal plants and herbs play an important role in worldwide culture, food flavoring, and conservation, as well as preventing disease epidemics. Herbs have been contemplated to be safe correspondingly to their records of being employ in the treatment of diseases, according to knowledge piled up over several centuries, while thousands of people die yearly from drugs [6]. The aqueous decoction is an antimalarial medicine in Ghana, which has been observe to have no adverse reaction on the body [7]. Likewise, the aqueous extract of the leaves do not result in cytotoxicity in normal human cells [8]. Despite the medicinal importance of most plants, some toxic effects have been observed. Many plants utilized in traditional medicine or employed as food have demonstrated some toxicity e.g. mutagenic and carcinogenic effects [9]. Plants like *Lantana camara, used as antimalarial*, have been proclaimed to be hepatotoxic in some animals' species, which may be of concern regarding its chronic use in human [10]. *Momordica charantia* is a familiar anti-diabetic and anti-malaria plant, but employed as an abortifacient [11] [12]. Extracts of *Pteroloblum stellatum*, a medicinal plant used in the treatment of tuberculosis, diarrhea, epilepsy, and neuralgia in many African countries, found to elicit DNA-damaging activity on HepG2 cells [13]. Extracts of *Brosimum guadichaudii* and *Caesalpinia ferrea* showed significant concentration-dependent DNA damage in freshwater fish (*Astyanax sp.*); these plants routinely used for the treatment of various diseases, including skin blemishes

and vitiligo in Brazil [14]. Toxicity is therefore the study of the oriental effects of chemicals on living organisms including their symptoms, mechanisms, and treatments. Additionally, it may be referred to the extent in which an exposed tissue is damaged by a chemical substance and its effect(s) on a whole organism or a sub-structural component of an organism, such as the cell cytotoxicity organ [15]. Based on classes, toxicity studies may be acute, subacute, subchronic, or chronic depending on the amount and period of administration of the agents [15]. Acute toxicity investigates the lethal effects produced by a single large-dose exposure to a toxicant lasting no longer than 24 hours. *Justicia secunda* is a plant that is widely distributed in Africa and particularly used traditionally in treating anaemia in Ogun State, south-west Nigeria, where it has locally called "*ewe eje*," perhaps due to the blood-red colour of its tea. There is a wide usage of the plant in this area, and there are reports of other uses of the plant across Africa. The herbal remedies from the plant as well as extracts prepared with various remedies have been reported in the literature to elicit positive responses [16, 17]. The effect of aqueous extracts of the leaves on body weight and organs has not been elucidated. Therefore, there is a need to investigate the toxicity of the plant extract with respect to this identified knowledge gap. *Justicia secunda* is used traditionally in the treatment of various diseases, including anemia. Globally, there is a growing use of herbs in the treatment of various diseases due to the failures of some orthodox medicine, the availability of herbs, and the increasing cost of orthodox medicine. Continuous usage of herbal remedies from *J. secunda* leaves with the aim of boosting blood production may portend some major health issues, including the effect on major body organs, which have very few reports in the literature. The toxic effects on these organs and the entire body have not been completely elucidated. Due to its anti-sickling potential, it is used in the treatment of anemia and tumors and is also known for its anti-inflammatory potential. This research was designed to

examine the possible toxic effects of aqueous extracts of *Justicia secunda* leaves on male Wistar rats in order to determine the effect of daily usage of the extract on body weight and the histological effects of the extract on various organs of the body. *Justicia secunda*, a vascular plant that belongs to the Acanthaceae family, is commonly called bloodroot (kingdom: plantae, class: magnoliopsida, division: tracheophyta) [18]. The plant is widely distributed in Nigeria and other tropical and pan-tropical regions of the world. The images of the plant are shown in Figure 1. The plant has been reported to be used in folk medicine for the treatment or management of various ailments and diseases, including anemia (Sickle Cell Disease), wound healing, fever, headache, epilepsy, hypertension, diabetes, measles, menstrual pains, whooping cough, and gastroenteritis [16] [19][17].The tea made from its extracts can be used for afterbirth treatment, diabetes, and other terminal illnesses. [20][21]. Phytochemical screening of the leaf extract shown the presence of saponins, terpenoids, steroids, glycosides, flavonoids, tannins, alkaloids, and coumarins [22] [17]. Proximate analysis of the plant showed that carbohydrates have the highest proportion, followed by total ash, crude protein, moisture, crude fats, and crude fiber in decreasing order [22]. Pharmacological evidence in support of the use of *Justicia secunda* extracts in the management of sickle cell disease and anemia has been reported in literature. The water extract of the leaf significantly increased red blood cell count and hemoglobin content of blood in rats [17]. The anti-sickling, anti-inflammatory, antibacterial and antihypertensive properties of extracts of *Justicia secunda* have been reported in various studies [23] [24] [25][16][26] [27]. In the assessment of the anti-inflammatory activity of *J. secunda* leaf extract using in vitro and in vivo inflammation models, it was found that varying concentrations of the extract subdued heat-induced BSA denaturation and also caused stability in the erythrocyte membrane [28]. The ethanol leaf extract of the plant was reported to have a negative

effect on the heart and the kidney, as shown by its significant increase of the blood lipid profile, serum electrolytes, creatinine, and blood urea levels with a concomitant increase in rat weight; the LD_{50} was also found to be 3800 mg/kg of body weight [29]. The ethanol leaf extract was reported to possess high antioxidant potential, which it showed by increasing the levels of hepatic superoxide dismutase, catalase, and reduced glutathione in rats, as well as providing protection against acetaminophen-induced liver damage by limiting lipid peroxidation [25]. Blood glucose-lowering studies on the plant showed that there was a decrease in glucose levels released by the liver [30].

Fig. 1: Images of *J. secunda* parts. (A), *J.secunda* leaves (B) Apical images of *J.secunda* (Imagesfrom google.com)

The level of studies on the toxicity of *J. secunda* extracts is quite low; there is a need to research the various forms and dimensions of the toxicity of the plant. This will direct the usage and help in the standardization of herbal remedies. The following research gaps have been identified: (1) the identification of the components of *J. secunda* leaf extract that are responsible for the hematinic activity; (2) the effect of the leaf extract on fertility, studied through the modulatory

effects on fertility hormones, sperm count, and sperm morphology; (3) the genotoxic effect, if any, on circulating blood corpuscles, bone marrow, and sperm cells; and (4) the effect of the extracts on organs and tissue architecture as well as the differential organ body weight ratio. This study, therefore, will investigate the effects of the aqueous extract of the leaves on body weight, tissue architecture, and the differential organ-body weight ratio.

2. MATERIALS AND METHODS

2.1 Plant collection and processing

Samples were collected in Ijebu-Itele, Ogun State, Nigeria, about 2 kilometers from Hallmark University. The plant sample was authenticated by Mr. D. Esimehhuai of the Department of Botany, University of Ibadan The plant was cleaned by separating the sand particles from the plant itself and air-dried at room temperature for 5 weeks to avoid loss of nutrients under sunny conditions. The dried leaves were ground into a partially fine powder using a blender.

2.2 Extract Preparation

100ml of distilled water added 40 gram of powdered leaf of sample in 250-ml beaker. The mixture was stirred and placed in a regulated water bath to heat to boil, the mixture was then stirred regularly and allowed to be heated for 3 hours, it was allowed to cool using a clean white cloth as a sieve, the mixture was sieved into a beaker, and a red, blood-like extract was observed. To determine the volume of the extract and to remove any leaf material still present in it, the extract was re-filtered using a filter funnel and filter paper and placed in a measuring cylinder. The volume of the extract was recorded. The extract was stored in a sealed flask and kept in the refrigerator before use. Fresh extract was prepared every two days.

2.3 Determination of Concentration of Extract

The concentration of the extract was then determined using the gravimetric method. Three clean watch glasses were weighed empty, and their masses were recorded (Wx). 1 ml of the extract was measure using the micropipette into each watch glass and allowed to dry completely on a hot plate that was set at 90 ℃. The weight of each glass and extract was determined and recorded as Wx. The actual weight of the extract was determined by Wx_2–Wx_1. The average weight of the extract in the three replicates was determined and recorded. The percentage yield of the extraction process was determined using the mathematical equation below:

$$\% \text{ yield} = \frac{\text{Mass of extract in 1 ml } \left(\frac{\text{g}}{\text{ml}}\right) X\ Volume\ of\ extract}{\text{Mass of leaf material}}\ X\ 100\%$$

2.4 Experimental Animal

A total number of fifteen male Wister rats were obtained from a local breeder at Imota, Lagos state. The wistar male rats was divided into three groups (I, II, and III), each group containing five rats. The rats were weighed on a weighing balance which was the basis for their grouping. The rats were kept in clean and dry cages with UV-sterilized dry wood shavings as beddings. Different parts of the rats were marked to differentiate one rat from another. The average weight of each group was determined. The rats were fed the standard pellet chow and were allowed access to food and water *and libitum*. The rats were preserve in a dry room with normal room temperature and humid condition with 12 hours light and 12 hours dark rhythm, it was allow acclimatizing for two weeks.

2.5 Administration of Extracts

Each rat was weighed before treatment commenced. The extract was administered to the rats orally using a 2 ml syringe that was mounted on a cannula daily for 2 weeks. The rats in Group I were labelled as the negative control and were administered the vehicle (water only), Group II rats were given a dosage of 200 mg per Kg body weight, while Group II rats were administered 300 mg per Kg body weight of *J. secunda* extract. The volume of the extract to be administered to each treatment group was determined mathematically using the formula below:

$$\text{Volume (ml)} = \frac{\text{Animal weight (g) X Dosage } (\frac{mg}{Kg})}{\text{Concentration of extract (mg/ml) X 1000 g}}$$

2.6 Animal Sacrifice

After administration of the extract for fourteen days, the rats were starved overnight before the sacrifice. Each rat was put in a desiccator containing cotton wool with diethyl ether which made them. On a flat board, the rats were sacrificed. Some organs (liver, kidney, spleen, heart) were harvested and their weights were recorded. The organs of each rat were preserved in universal bottle containing the 10% formalin solution.

2.7 Histological Analyses

The liver, kidneys and the heart harvested from the rats were processed for histological analyses. Portions of each organ were placed in a well labelled embedding tissue cassette before being processed using the automatic tissue processor (Microm STP 125. Thermo-fisher - USA). The duration of the tissue processing was 17 h which included 1 h of fixation in 10% neutral buffered formalin solution, 8 h of tissue dehydration in six different graded alcohol solutions, starting from 70% alcohol to absolute alcohol; 2 ½ h of clearing to replace the alcohols from the tissues in two changes of xylene, and finally, 4 h of infiltration /impregnation in 3 changes of molten paraffin wax which was maintained at 60^{o}C.

The slide preparations were done based on the method previously described by [24]. Using the rotatory microtome (Microm HM 325 Thermo-fisher - USA), each of the block was trimmed to expose their surfaces and sections were cut at 4microns, which were gently placed on well labeled slides. With the aid of a curved floating forceps, the sections were floated out on a hot water bath already maintained at 45^{o}C. The labeled slides were then used to pick the sections that were free of creases, while ensuring that each of the sections adhered to the center of the slide. The slides were subsequently dried on a hot air oven already maintained at 60^{o}C to ensure proper attachment.

Haematoxylin and Eosin (H&E) staining method was used to stain the sections and it was done manually based on the steps previously described by [31].All histologic slides were reviewed using a microscope (Leica MD-500, Leica, Microsystems. Germany) with comments made on findings.

2.8 Data Analysis

Experimental values are presented as the Mean ± SEM (standard error of the mean). One-way Analysis of Variance (ANOVA) followed by post-hoc test (Tukey test) for multiple comparisons was used to determine statistical significance of differences between means, using the Graph Pad Prism Software (Version 8.0.1) (Graph Pad Software Inc., CA, USA). A p-value <0.05 was considered statistically significant. Histological analyses are presented as photomicrographs with comments on findings.

3. RESULTS AND DISCUSSION

3.1 Effect of Aqueous *J. secunda* Extract on Body Weight

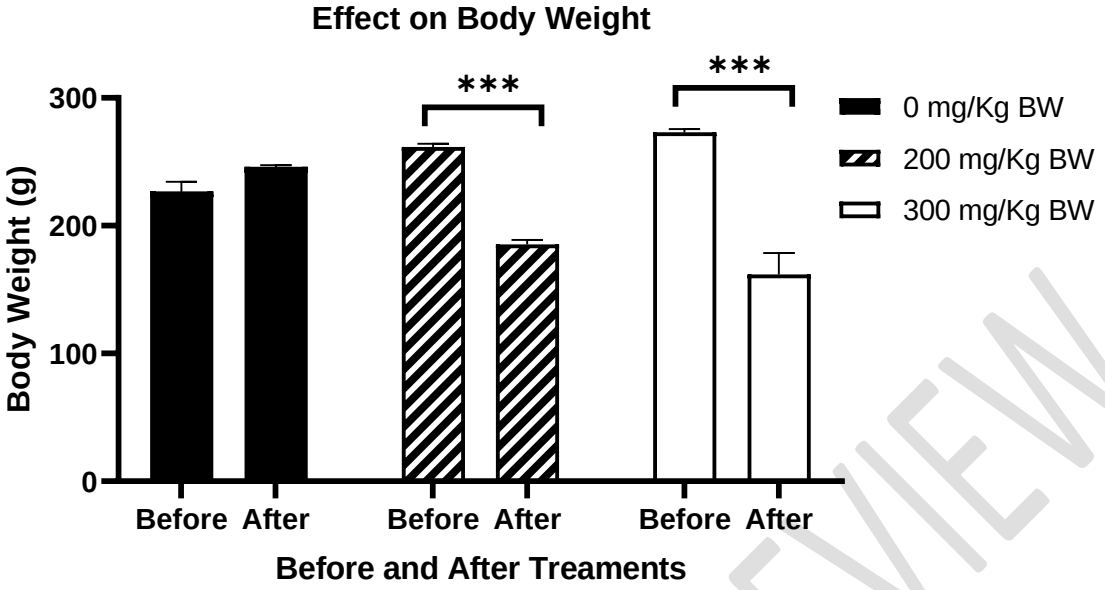

Fig. 2: The effect of Aqueous *J. secunda* extract on the body weights of Wister rats after two weeks.

Daily orally administration of the extract. *** $p<0.001$ represents significant deference between the weights of the rats in each group before and after and after treatments.

There was dosage dependent-dependent reduction in body weights of the rats that were administered the extract (Fig. 2). The negative control (Group I) which did not receive the extract showed increase in body weight over the same period. The average change in weights in the two treatment group was statistically significant with p-value < 0.001. The weights of the rats before the treatment and after the treatment including the weights of the various organs harvested from them are shown in Table 1. Relatively, the spleen of the group that received 300 mg/Kg BW dosage was large than those of the group that received 200 mg/Kg BW dosage, the relative weights of the spleens were close but Group II which received the 200 mg/Kg BW dosage had reduced spleen weight. Relatively, there was no significant difference in the weight of the hearts of animals in the three groups after treatment.

Table 1: Body Weights and Organ Weights.

	Average Body Weight (g)		Average Organ Weight (g)			
	Before	After	Liver	Kidneys	Spleen	Heart
Group I	227 ± 7.6	246 ± 1.3	2.09 ± 0.10	0.27 ± 0.14	0.58 ± 0.28	0.30 ± 0.10
Group II	262 ± 2.7	185 ± 3.5	2.92 ± 0.10	0.50 ± 0.02	0.45 ± 0.04	0.36 ± 0.02
Group III	273 ± 2.5	162 ± 16.7	4.52 ± 0.96	0.71 ± 0.12	0.63 ± 0.16	0.44 ± 0.07

The organ-body weight ratio is show in Figure 3. There was no significant difference in weights of the kidneys, spleen and the heart across the three groups. There was significant difference in organ-body weight ratios of the liver across the groups. The negative control group (Group I – 0 mg/Kg BW) has the lowest organ-body weight ratio and the treatment groups had larger liver weights which showed a concentration-dependent response to the treatment. The difference between the liver-body weight ratio of the treatment groups and that of the negative control were statistically significant, $p<0.01$ for Group II and $p<0.001$ for Group III.

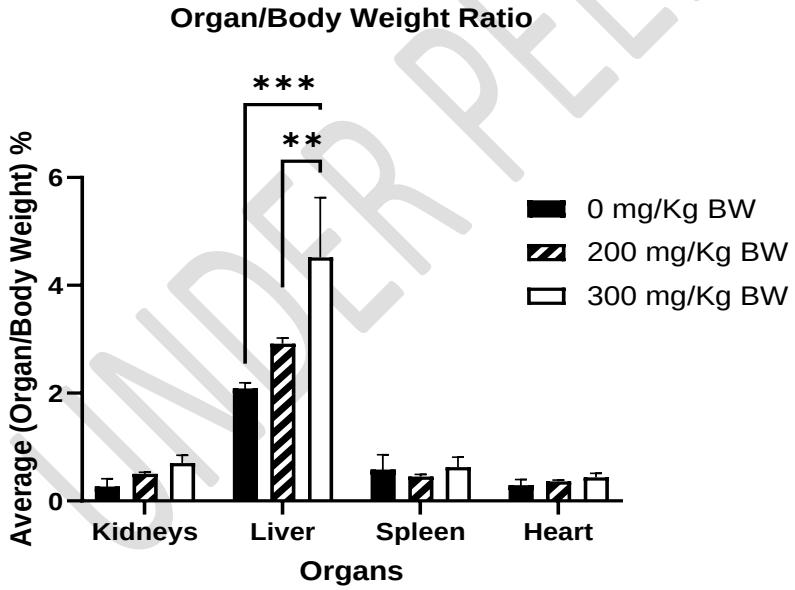

Fig. 3: Organ-Body Weight Ratio of Wister rats treated with aqueous extract of *J. secunda* leaves.

Histological Effects of Aqueous Extracts of *J. secunda*

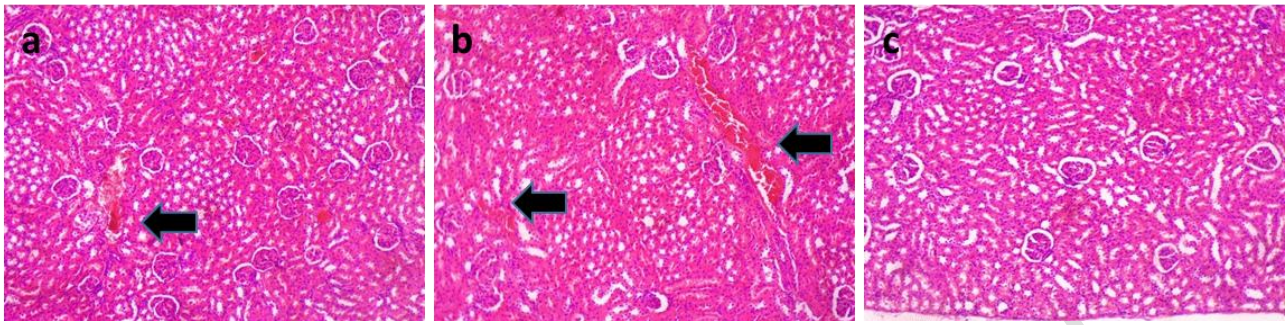

Fig. 4: Photomicrographs of sections of the kidneys of Wister rats after 2 weeks treatment with *J. secunda* extract. (a) Negative control (0 mg/Kg BW) (b) Group II (200 mg/Kg BW) (c) Group III (300 mg/Kg BW).

Sections of kidney tissue show normocellular glomerular tufts disposed on a background containing viable tubules. Vascular congestion is seen, indicated with black arrows. H & E staining. Magnification: X100

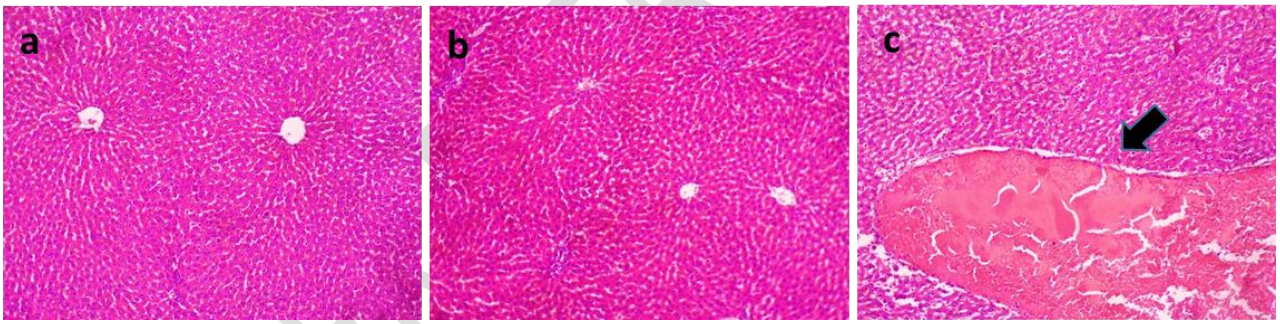

Fig. 5: Photomicrographs of sections of the liver of Wister rats after 2 weeks treatment with *J. secunda* extract. (a) Negative control (0 mg/Kg BW) (b) Group II (200 mg/Kg BW) (c) Group III (300 mg/Kg BW).

Sections of liver tissue in the negative control and treatment Group I shows parallel radially arranged plates of hepatocytes with the central vein (CV), portal vein (PV), and the basophilic portion with the nucleus and the acidophilic cytoplasm of the acinar cells. No abnormality was seen in these tissues. The liver tissue of treatment group III shows parallel radially arranged plates of hepatocytes, with the portal space and periportal zone filled with a smooth to slightly

floccular pink fluid material common with edoema, and congested aggregates of red blood cells are also seen (severe vascular congestion with edoema). H&E staining; magnification: X100

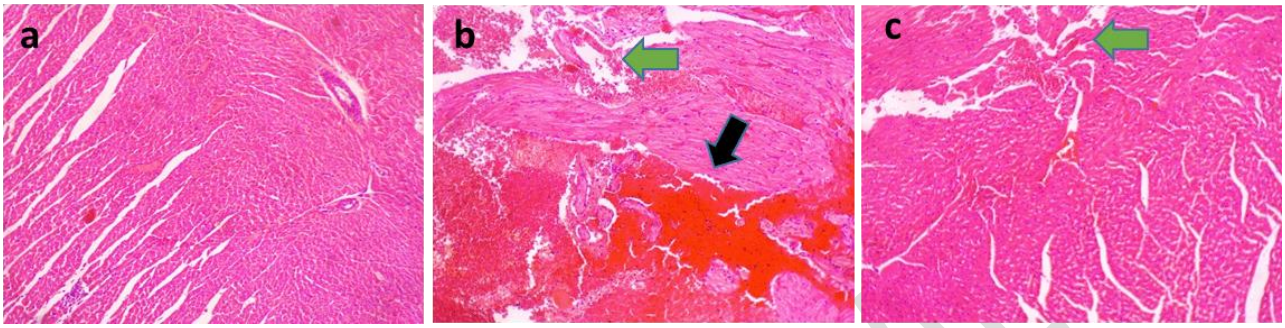

Fig. 6: Photomicrographs of sections of the heart of Wister rats after 2 weeks treatment with *J. secunda* extract. (a) Negative control (0 mg/Kg BW) (b) Group II (200 mg/Kg BW) (c) Group III (300 mg/Kg BW).

Histologic sections of heart muscle of the negative control show interlacing fascicles of cardiac myocytes / myocardial cells and no o abnormalities are seen. In the treatment Group II, areas of the myocardium and vesicles show several aggregates of red blood cells and smooth to slightly floccular pink fluid material common with edema. The heart muscle of Group III show interlacing fascicles of cardiac myocytes/ myocardial cells with extensive inflammation caused by aggregates of red blood cell.

The liver is an organ of the body useful in producing bile to breakdown food into energy, cleaning toxins from the blood, controlling fat storage and cholesterol. For some individuals, enlarged liver can result from excess intake of toxins especially alcohol, sometimes medications. Chronic exposures to toxins increase the risk of liver enlargement also called hepatomegaly. For some others, liver enlargement may be due to growth of cancer cells, excess fats in the liver, abnormal accumulation of fats in the liver at pregnancy and sometimes it may be genetic or inherited disorders.

Symptoms of liver enlargement may be fatigue, yellowing of the eyes or skin, painful abdomen, nausea and vomiting [32]. This dysfunction treated by liver biopsy, which involves removing a

liver tissue sample for testing to detect the cause of the enlargement [33], This is often caused by obesity, alcohol abuse, heart failure, liver cancer, hepatitis and sickle cell anaemia. Complications of liver enlargement may be cirrhosis, liver cancer and liver failure [17]. The reduction in body weight of the rat is in support of the previous Hypoglycaemiant study by [26] on the ethanol extract of *Justicia secunda.* From the above observation, the plant might contain a compound which has an effect on the hormones of the male rats. The histological effects on the kidney indicate that the plant showed no significant effect on the kidney of the rats. This result is supported by the body weight of the rats.

4. CONCLUSION

The herbal shrub *Justicia secunda* is an antianeamic plant that is useful in antianeamia and the treatment of wounds, injuries, and illnesses like fever, headache, hypertension, diabetes, measles, and menstrual pain. For the test for toxicity in male Wistar rats, the blood-like extract of the shrub was administered in various proportions to the treatment groups II and III, and observations were made. There was a significant reduction in body weight with a corresponding significant reduction in liver enlargement in the male Wistar rats. The reduction in body weight of the male Wistar rats supports the previous hypoglycemic research carried out by mea. On the ethanol extracts of *Justicia secunda*, which implies that the extracts from *Justicia secunda* could be useful in weight loss. Also, from the research carried out, the rats' bodies showed severe vascular congestion, leading to inflammation and edoema. Group III, which received the highest dose of the extracts as observed from the results of the toxicity test on the body and the organs of the rats, it is deduced that the *Justicia secunda* plant extracts, despite their herbal benefit, must be controlled while consumed. This implies that the higher the dosage consumed by humans, the greater the bodies and the organs damage. Hence, there is a possibility of excessive consumption

of *Justicia secunda* extracts to reduce body weight by decreasing body fat but increase the weight of the organs of the body. Also, the excess intake of *Justicia secunda* may increase the risk of pressure as a result of overload on the body organs, which can lead to high blood pressure, heart failure, and other vascular injuries. This research therefore shows that continuous consumption of *Justicia secunda* causes hepatic (liver) and cardiac (heart) toxicity in male Wistar rats.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are included within the article

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