Original Research Article

OrgantoxicityEffectof Aqueous Extract of *Justiciasecunda*Vahl. Leaf in Wistar Rats

ABSTRACT

Aims:*Justiciasecunda* is a widely distributed plant that is used in the traditional improvement of well-being and treatment of several illnesses and diseases, including anemia. There is limited research on the effect of extracts of the plant on body and organ weights and their effects on organ architecture.

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-IItele, Ogun State, Nigeria, from July 2021 to September 2022.

Methodology: This research therefore investigated the toxicity of an aqueous extract of *J. secunda* leaf on selected organs in male Wistar rats. Mature male rats (15) were divided into three groups (n = 5): Group I (the negative control) received distilled water; Group II and Group III received 200 and 300 mg/Kg doses of the extract, respectively, via oral route, once daily for 14 days. The rats were weighed before being sacrificed; the weights of the kidneys, liver, heart, and spleen were also determined, and histological analysis was carried out on the heart, kidneys, and liver.

Results: There was significant ($p<0.001$) concentration-dependent reduction in body weights with hepatomegaly in the extract-administered rats. The kidneys of Group III rats were

significantly ($p<0.05$) larger than those of the negative control group. Histology revealed severe vascular congestion and edema in the liver of Group III rats and inflammation in the hearts of Group II rats.

Conclusion: This study shows that repeated usage of the aqueous extract of *Justiciasecunda* leaf should be done with caution due to its negative effects on the liver and the heart in rats.

Keywords:*Justiciasecunda*, Hepatomegaly, vascular congestion, weight-reduction, extract inflammatory, toxicity.

INTRODUCTION

A large number of people in different areas of the world use herbal remedies or any of the various components of alternative medicine to treat ailments and disease conditions or to enhance their wellbeing [1, 2]. The use of herbal remedies has become widely accepted for many reasons, including but not limited to the perception that materials from nature are safe, the rising cost and unavailability of orthodox drugs, the large spectrum of side effects arising from the use of orthodox drugs, and the growing resistance of microbes and cancer cells to current clinically used drugs [2]. Adverse drug reactions are a major issue for both orthodox medicine and herbal medicine practitioners [3, 4]. In particular, most herbal products and preparations in rural areas of the world are not standardized, and contamination is rife [5]. Herbal toxicity studies are a big area of research that has received much attention in recent times. Organotoxicity, a test for the toxic effects of bioactive compounds on various organs in living systems, is an aspect of toxicological studies that could be assessed at the cellular level through evaluation of specific

protein activity, metabolite profiling, gene expression via mRNA levels and DNA fragmentation; and also investigated at the tissue level through histological studies.

The traditional use of Justicia species in treatment of various ailments and diseases is well documented in literature. *Justiciasecunda*Vahl is widely distributed in Nigeria and other tropical and pan-tropical regions of the world. 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 [6,7,8]. Phytochemical screening of the leaf extract revealed the presence of saponins, terpenoids, steroids, glycosides, flavonoids, tannins, alkaloids and coumarins [9, 8]. The ethanol extract of the plant leaf was reported to have a negative effect on the heart and kidneys through a significant increase in blood lipid profile, serum electrolytes, creatinine, and blood urea levels with a concomitant increase in rat weight [7]. There is limited information on the effect of the aqueous extract of the leaf on various organs. This study therefore assessed the effect of an aqueous extract of *J. secunda* leaves on body and organ weights and on organ architecture through repeated dose toxicity testing in rats.

Justicia seconda leaves and flowers

MATERIALS AND METHODS

Plant Collection and Authentication

Secunda leaves were collected at Ijebu-Ife, Ogun State, Nigeria, about 3 km away from Hallmark University. The plant sample was authenticated by Mr. D. Esimehhuai of the herbarium, 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. The dried leaves were ground into a partially fine powder using a blender.

Extract Preparation

The powdered leaf sample (40 g) was put into a 250-ml beaker, and 100 ml of distilled water was added. The mixture was stirred and placed in a water bath that was regulated to 80 ℃The mixture was stirred regularly and heated for 3 hours. The mixture was allowed to cool, sieved using a clean sieve cloth, and filtered using Whatman No. 1 paper. The extract was stored in a sealed flask and kept in the refrigerator. Fresh extract was prepared every two days.

Experimental Design

Male Wister rats (15) were kept in clean and dry cages with UV-sterilized dry wood shavings as bedding. The rats were fed the standard pellet chow and were allowed access to food and water *ad libitum*. The rats were kept in a dry room with normal room temperature and a humid condition with a 12 h light and 12 h dark rhythm and allowed to acclimatize for two weeks. The rats were divided into three groups (I, II, and III), each group containing five rats with a weight difference of ± 20 g. The extract was administered orally to the rats once per day for 14 days.

The rats in Group I (the negative control group) were administered water only; Group II and III rats were given a dosage of 200 mg/kg and 300 mg/Kg of body weight of the extract, respectively. The volume of the extract administered to each treatment group was determined mathematically using the formula below. After administration of the extract for 14 days, the rats were weighed, starved overnight, anesthetized, and sacrificed by cervical dislocation. The liver, kidneys, spleen, and heart were harvested and weighed. The organs were preserved in a 10% formalin solution, and histological analyses were carried out on slices of the tissues according to the method previously described by Bancroft, with haematoxylin and eosin staining as previously described by Avwioro..

Data Analysis

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

RESULTS AND DISCUSSION

Effect on Body and Organ Weights

The extract-administered rats showed a dosage-dependent reduction in body weights (Fig. 2). The negative control (Group I), which did not receive the extract, showed an increase in body weight over the same period. The average change in weights in the two treatment groups was

statistically significant, with a p-value < 0.001. The weights of the rats before and after the treatment, including the weights of the various organs harvested from them, are shown in Table 1. Based on final body weights, the kidneys of the Group III rats were statistically bigger ($p<0.05$) than those of the control group. Among the treated groups, the kidneys of Group III rats, those that received 300 mg/Kg dosage, were relatively larger than those of Group II rats that received 200 mg/Kg dosage, but the difference was not statistically significant. The average weight of the livers of Group III rats was more than double the weight of

the negative control group (Group I).There was marginal increase in the weights of the heart and spleen of extract-treated groups above the organs of the negative control group.

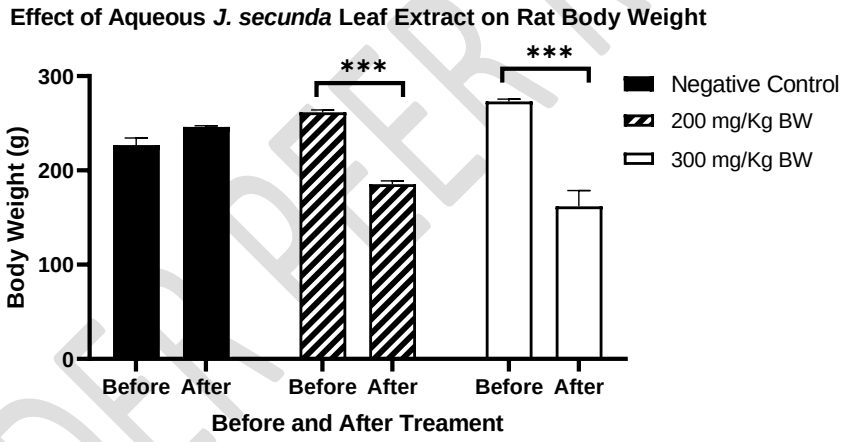

Figure 2: Body weights of rats before and after treatment. ***$p<0.001$ represents significant difference in weight of the extract-treated groups.

Table 1: Comparison of body 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

Group I: negative control; Group II: 200 mg/Kg dosage; Group III: 300 mg/Kg dosage; * represents $p<0.05$ significant difference in weight between the treated group and the negative control.

The organ-body weight ratio is shown in Figure 3. There was no significant difference in ratios obtained for the kidneys, spleen, and heart across the three groups. There was a significant difference in organ-body weight ratios of the liver across the groups. The negative control group (Group I) 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 was statistically significant, $p<0.01$ for Group II and $p<0.001$ for Group III.

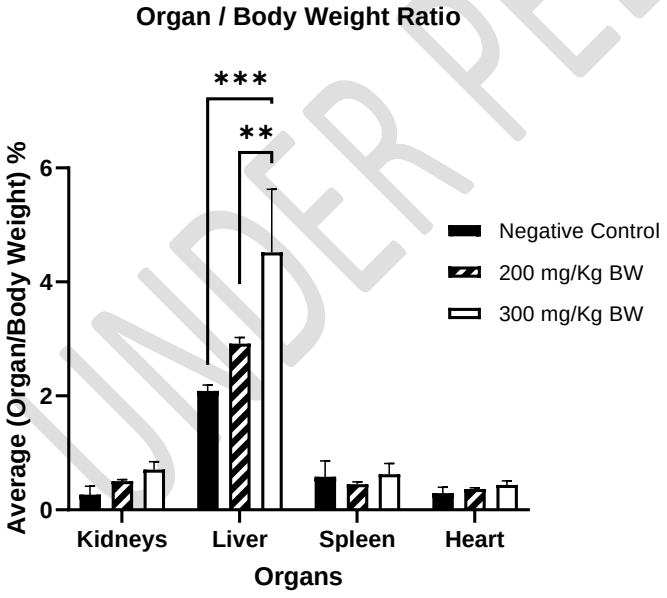

Fig. 3: Organ-body weight ratio of rats treated with aqueous extract of *J. secunda* leaf.

Effects on Organs (Histology)

Sections of kidney tissue from the negative control group and the extract-treated Group II rats show normocellular glomerular tufts disposed on a background containing viable tubules. Vascular congestion is seen, indicated with black arrows. The kidneys of the Group III rats that were treated with the higher dose of the extract did not show signs of vascular congestion or inflammation. The sections of liver tissue in the negative control and treatment groups II show parallel radially arranged plates of hepatocytes with a central vein, a portal vein, 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 edema and congested aggregates of red blood cells are also seen (Severe vascular congestion with edema). The sections of heart muscle of the negative control show interlacing fascicles of cardiac myocytes and myocardial cells, and no abnormalities are seen. In 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 shows interlacing fascicles of cardiac myocytes and myocardial cells with extensive inflammation caused by aggregates of red blood

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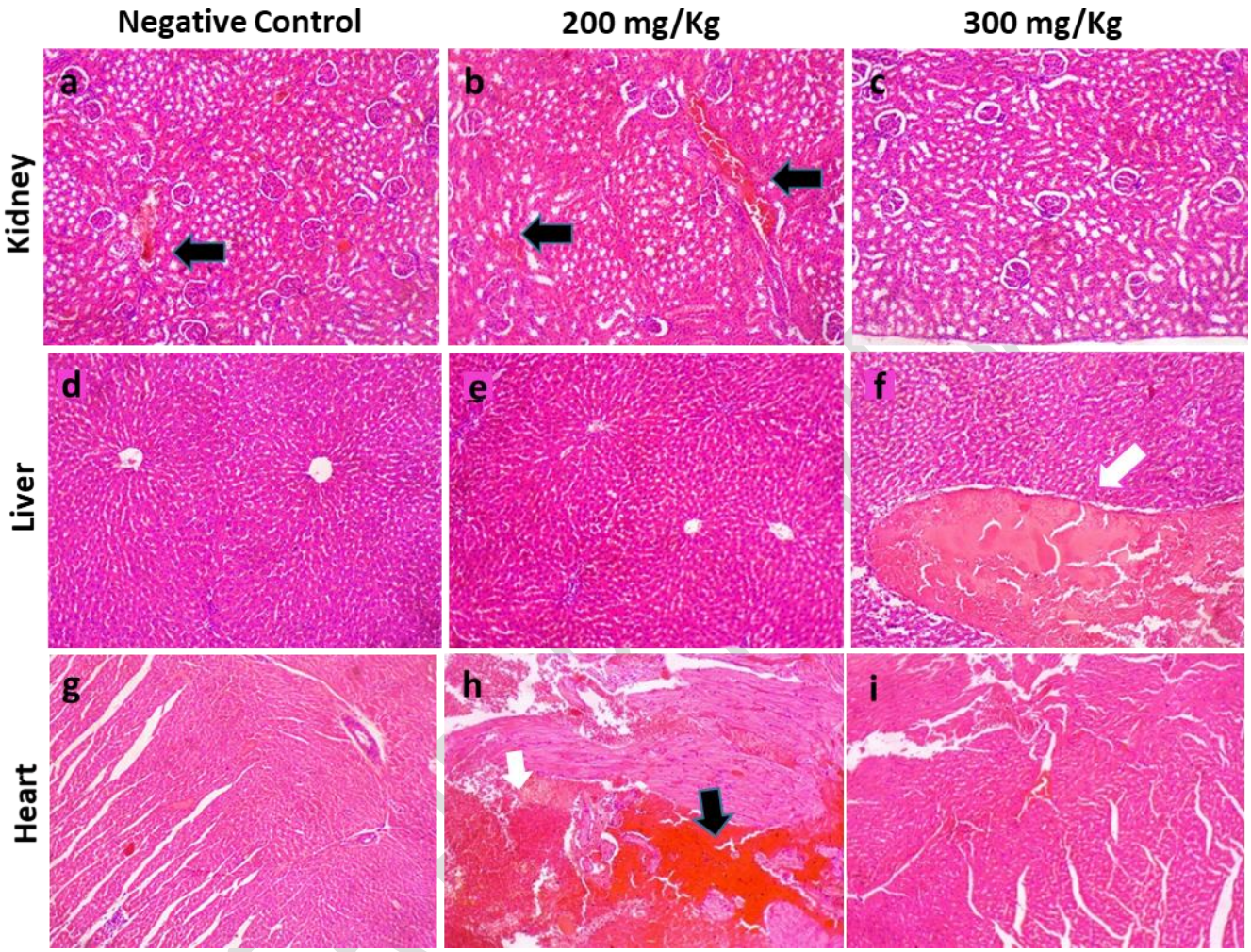

Fig. 4: Photomicrographs of sections of the internal organs of Wistar rats after 2 weeks treatment with *J. secunda* extract. (a), (d), and (g) are sections of the kidney, liver, and heart of the negative control group. (b), (e), and (h) are sections of the kidney, liver, and heart of Group II (200 mg/Kg dose) rats. (c), (f), and (i) are sections of the kidney, liver, and heart of Group III (300 mg/Kg dose) rats. Vascular congestion is indicated with black arrows. Edema and congested aggregates of red blood cells in the liver of the Group III rat are indicated with a white arrow. H&E staining, Magnification: X100.

DISCUSSION

The aqueous extract of *J. secunda* leaf showed weight-reducing potential in a concentration-dependent manner. This finding is in line with the report [10], which showed that the ethanol extract of the plant possesses the potential to significantly reduce blood sugar and induce a state of hypoglycemia. This hypoglycemic potential in *J. secunda* is consistent with findings from other species within the *Justicia genus* [11, 12]. The consistent lowering of blood glucose in the rats by the extract would limit the pathways that utilize glucose for body function, leading to the burning of body fat, leanness of body muscles, and ultimately body weight reduction. The ethanol extract of the leaf was found by [7] to increase blood lipids and body weights in rats; this suggests a difference in bioactivity based on extraction solvent polarity. Ethanol, being a solvent that is less polar than water, would extract more non-polar phytochemicals; the proportion of these non-polar compounds in the ethanol extract may be sufficient to direct the ethanol extract towards increasing body weight as opposed to the weight-reducing effect of the aqueous extract.

The anti-sickling, anti-anemia, and blood-volume-increasing effects of *J. secunda* extract reported in the literature may have additional effects on the size of the liver and kidneys with repeated use of the extract. As seen with the administration of the aqueous extract of *J. secunda* leaf, there was enlargement of the liver (hepatomegaly) in the treated groups in a concentration-dependent manner. Hepatomegaly is a condition that suggests an underlying disease affecting the liver; it could be caused by infections, e.g., viruses (hepatitis), abnormal cell divisions (tumors or hyperplasia), and abnormal accumulation of fats, iron, copper, and proteins in the liver, occlusion of the bile ducts and gallbladder, and the presence of fluid-filled pockets in the liver [13]. The presence of edema in the tissues of the liver suggests that accumulation of fluids and occlusion of

the bile ducts may be part of the underlying factors caused by *J. secunda* leaf extract. Several plant species have been reported to contain phytochemicals that cause liver injuries [14]. The kidneys of the rats treated with 200 mg/Kg of the extract and some of the control group showed signs of inflammation, but in the group treated with a 300 mg/Kg dose, there was no sign of inflammation. This finding points to increased cell proliferation as a reason for the significant increase in kidney size in the 300 mg/Kg treated group.

The extensive inflammation seen in the heart tissues shows the cardiotoxic effect of the extract. Several tropical plant species have been reported to possess cardiotoxic effects when used as herbal remedies [15, 16]. It was reported that an aqueous extract of *J. traquebareinsis* leaf showed cardioprotective effects against isoproterenol-induced myocardial infarction in rats. The dosages (100 and 200 mg/Kg) used by Radhika*et al.* in their study are similar to the ones used in this study (200 and 300 mg/Kg). The inflammatory effect of the extract reported in this study was profound in the rats that received the 200 mg/Kg dosage. Though *J. traquebareinsis* and *J. secunda* are from the same genus, interspecies differences and environmental factors may have contributed to these opposing findings.

CONCLUSION

The increase in the use of herbal remedies to improve wellbeing or treat disease conditions globally should be met with caution due to the possible adverse reactions that the phytochemicals therein could elicit. The dosages, frequency of usage, and duration of use of specific herbal remedies are important factors to be considered in order to prevent adverse reactions, including organotoxicity, which could portend greater danger to the use of herbal remedies. Our findings

show that the aqueous extract of *Justiciasecunda* leaf contains phytochemicals that could be organotoxic when used repeatedly for 14 days.

AUTHORS' CONTRIBUTIONS

IBL is a research project student at BEA. BEA provided resources, planned, and supervised experiments. Analyzed the data and wrote the manuscript. IBL provided resources, performed experiments, and recorded data. AEG provided resources, contributed to the experimental design, and edited the manuscript. JTB provided resources and edited the manuscript, ONM also, contributed to the experimental design, and edited the manuscript and ELO also assisted in providing resources, and data recording. All authors read and approved the manuscript.

Ethical Approval

Animal Ethic committee approval has been taken to carry out this study.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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