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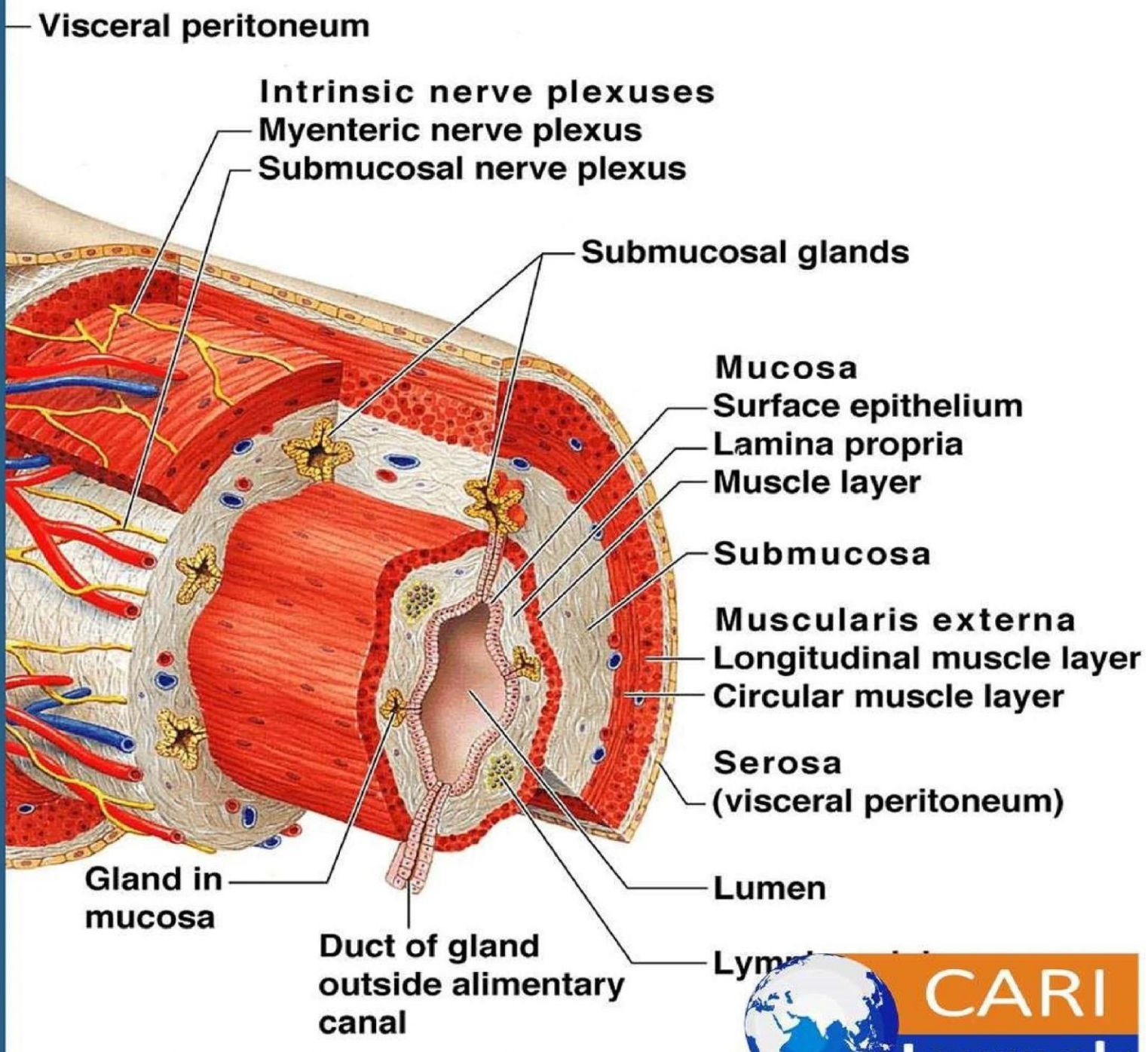

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The Effect of Ethanolic Extract of Justicia Secunda (Bloodroot) on the Liver Function Indices of Albino Wistar Rat

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Abstract

Purpose: Few articles have linked the consumption of some medicinal plants to certain liver diseases. The aim of this study is to investigate the effects of oral administration of ethanolic extract of *Justicia secunda* on the liver of Wistar rats.

Methodology: 30 adult female Wistar rats were grouped into 6, with each group consisting of 5 rats.The 1st group was on a normal diet and distilled water while the other groups received 250mg/bodywt, 300mg/bodywt, 350mg/bodywt, 400mg/body wt, 450mg/ body wt of ethanolic extract *Justicia secunda* for 42 days respectively . Their blood samples were analyzed for total and conjugated bilirubin, total protein, albumin, globulin, gamma-glutamyl transferase, Lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase.

Findings: The liver tissues were also processed for histological examination. There was a statistically significant increase in ALT, AST, and ALP in group 4, and a statistically significant decrease in conjugated bilirubin seen in groups 5 and 6. The Histology revealed that the extract produced moderate but insignificant degeneration of hepatocytes.

Unique contribution to policy, theory, and practice: Long-term administration is likely to contain toxic effects; caution should be taken in consuming this plant.

Keywords: *Justicia secunda*, Liver enzymes, Total protein, Bilirubin

INTRODUCTION

Globally, the role of traditional medicine in the treatment of ailments is crucial to scientists and the general population (Krentz and Bailey, 2005). The World Health Organization (WHO) reports that over 70 percent of the world population resort to herbal medicine for primary healthcare (WHO, 2002). Recent reports from medicinal plants research indicate that extracts from some plants exhibit both hepatotoxicity as evidenced by changes in the profiles of biomarker enzymes (Stickel et al., 2005; Wurochekke et al., 2008; Agbaje et al., 2009; Tiwari and Singh, 2004), and hematotoxicity effects (Ikpi and Nku 2008; Ahumibe and Braide 2009). However, others demonstrated that these have hepatoprotective (Etuk et al., 2009; Akah et al., 2009; Shyamal et al., 2010), and hematopoietic (Chukwuma et al., 2010).

Justicia secunda belongs to the family – Acanthaceae – together with up to 600 other shrubs and herb species. The plants in the family have been reported to treat CNS disorders like mental disorders, headaches, and fever, probably due to their sedative and analgesic properties (Khan et al., 2017, Verdam et al., 2015). Other uses of this plant in traditional medicine are wound healing, and management of anemia and abdominal cramps. Extracts made from only the leaves are the most frequently used, followed by root extracts (Koné et al., 2011).

The liver is the central drug-metabolizing organ and is, therefore, a prime target of drug-related pathologies. Foreign compounds are predominantly bio-transformed in the liver by the action of drug-metabolizing enzymes including microsomal cytochrome P450 enzymes, mixed-function mono-oxygenases, glutathione-S-transferases, sulfotransferases, and UDP-glucuronosyltransferases. Some of these can be induced through variable mechanisms which may lead to large inter-individual variability in susceptibility to drug-related liver damage.

Ingestion of certain herbs have been reported to cause acute and chronic hepatitis, cholestasis, drug-induced autoimmunity, vascular lesions, and even hepatic failure and cirrhosis. Notwithstanding the variously reported medicinal uses of *J. Secunda* in folkloric medicine and authenticated scientific evidence, little or nothing is known about its physiological effects.

This study was based on the hypothesis that the *Justicia secunda* leaves extracts can affect vital organs like the heart, liver, kidney, and blood, and contain important phytochemical constituents and phytonutrients.

MATERIALS AND METHODS

Experimental animals

Thirty (30) Adult Wistar rats were obtained from the animal house of the department of Human Physiology, Faculty of Basic Medical Sciences, College of Medical Sciences, Rivers State University, Rivers state, Nigeria. They were housed in rat cages, in a well-ventilated room with a temperature of 32±2°C.The rats had free access to tap water and rat pellets obtained from Rivers State University Nigeria. The rats were allowed to acclimatize for two weeks before the

experiment. The experiments were conducted according to the approved Institutional animal care guidelines of the Rivers State University, Nigeria. It was an experimental study. This study was carried out from September 2021 to November 2021.

Plant material

Fresh leaves of Justicia secunda of the family Acanthaceae was obtained from a small garden opposite the department of forestry in Rivers State University, Port Harcourt in October 2021. It was identified and authenticated by the department of plant science and biotechnology of Rivers State University. An authentication number of RSU PB 041 was assigned**.**

Preparation of Plant Extraction

The fresh leaves of *Justicia secunda* were washed and air-dried at room temperature. It was completely dried using the hot air oven at 45-50 degrees and blended into fine powder with Binatone blender model FP-850. 50 gm of Justicia secunda was extracted in 500 ml of 99% ethanol using a Soxhlet extractor (Okpara et al., 2022).

Experimental Design

Experimental animals were randomized into six groups and extract was given based on their mean body weight for 6 weeks (42 days)

I. Group A (control): Distilled water

II. Group B: 250mg/body weight (kg)

III. Group C: 300mg/body weight (kg)

IV. Group D: 350mg/body weight (kg)

V. Group E: 400 mg/body weight (kg)

VI. Group F: 450mg/body weight (kg)

Termination/Sacrifice/Organ Collection

Twenty-four (24) hours after the last administration, the rats were sacrificed using the chloroform inhalation method. Each rat was sacrificed and blood samples from each rat were taken via cardiac puncture into lithium heparin sample bottles. The liver was extracted, weighed, and immediately fixed in 5% formaldehyde for histological studies.

Biochemical analysis

The blood samples were centrifuged, plasma aspirated and analyzed for bilirubin, total protein, albumin, globulin, alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase according to standard biochemical methods at the Rivers State University Teaching Hospital, Rivers State Nigeria.

AST/ALT test were performed using Reitman and Frankel Method, ALP test was performed using Kochmar, J.F. and Moss, D.W, Method.,Bilirubin test was performed using Jendrasik and Grof Method, Total Protein test was performed using Biuret Method, Albumin test was performed using Bromoeresol Green Method

Histology

sections measuring approximately 0.2 cm x 0.2 cm were taken from the liver of each rat. They were dehydrated through graded solutions of alcohol ending in two changes of absolute alcohol for 2 hours each. They were cleared in 2 changes of xylene, infiltrated in 2 changes of paraffin

wax for 2 hours each using the automatic tissue processor obtained from Sakura fine tek,Netherlands and embedded in molten paraffin wax. Sections were cut at 4µ with the rotary microtome obtained from Sakura fine tek, Netherlands and stained with haematoxylin and eosin

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 28 software was used for statistical analysis. The Results were expressed by mean ± SEM. Data were subjected to analysis of variance (ANOVA) and Post hoc test. Levels of statistical significance were considered at $P<0.05$.

RESULTS

TABLE 1: EFFECT OF ETHANOLIC EXTRACTS OF *J. SECUNDA* ON THE LIVER OF FEMALE WISTAR RATS

GROUP	AST	ALT	ALP	T.P	ALB	T.B	C.B
1	33.40±2.42	6.30±0.99	33.00±2.51	73.40±1.21	46.00±1.18	6.84±0.39	5.10±0.14
2	34.80±1.36	6.14±0.52	28.58±6.21	77.00±1.95	45.80±1.88	7.20±0.33	4.78±0.38
3	35.80±1.93	7.62±0.56	41.00±1.00	77.80±2.22	48.80±1.28	7.28±0.47	4.96±0.48
4	36.80±1.89*	8.60±0.68*	41.60±1.21*	78.60±1.21	48.60±1.21	7.88±0.34	5.36±0.43
5	32.80±0.93	6.06±00.39	33.20±1.71	73.20±1.71	43.80±1.16	7.38±10.66	3.88±0.26*
6	31.80±0.	6.08±0.	37.00±2.	74.20±1	44.80±1	6.40±0.	3.92±0.

	71	30	16	.60	.56	17	23*

Values are expressed in mean ± SEM, n=5, *p<0.05 statistically significant compared to control

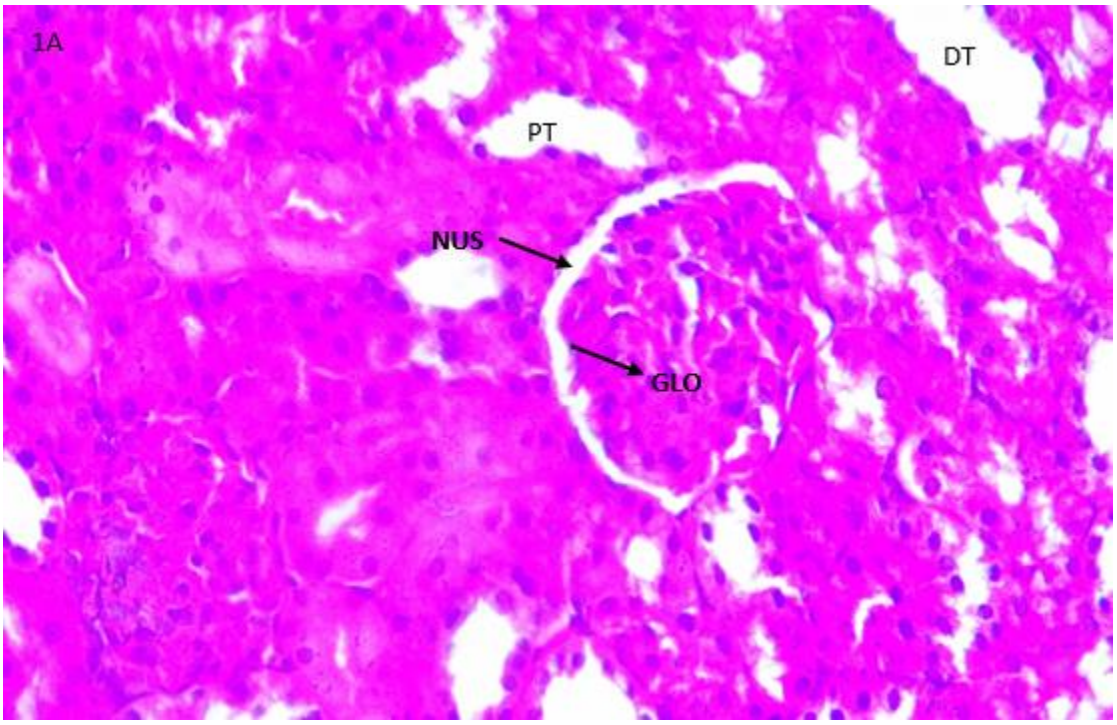

PLATE 1: Photomicrograph section of Kidney tissue from rats given distilled water alone. Section showed normal glomerulus (GLO) with normal urinary space (NUS) and epithelial lining. The proximal (PT) and distal tubules (DT) showed normal cytoarchitecture. H&EX400

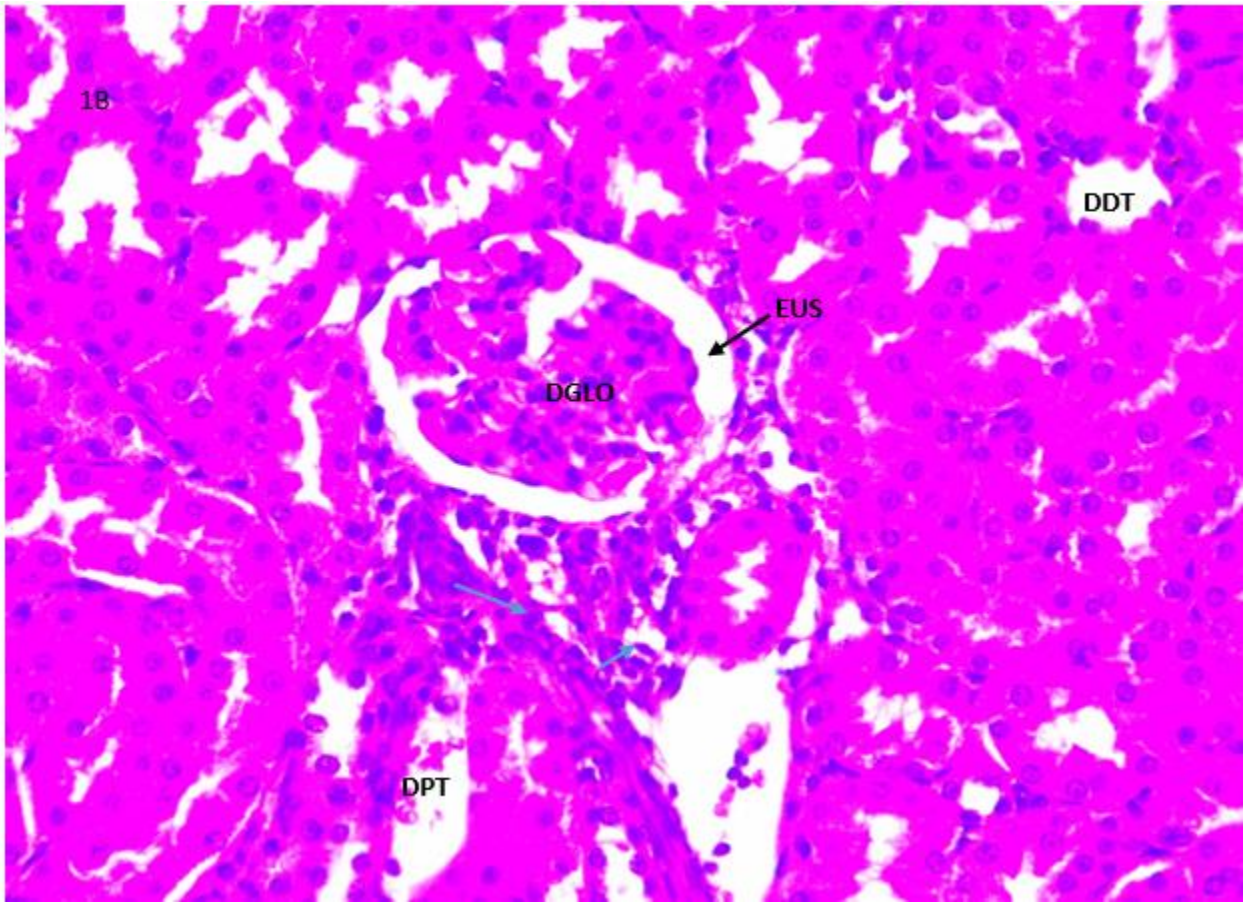

PLATE 2: Photomicrograph section of kidney tissue showing glomerulus with expanded urinary space and infiltration of lymphocytes (bluearrows). Proximal and distal tubules showed cellular tissues damage. H&Ex400

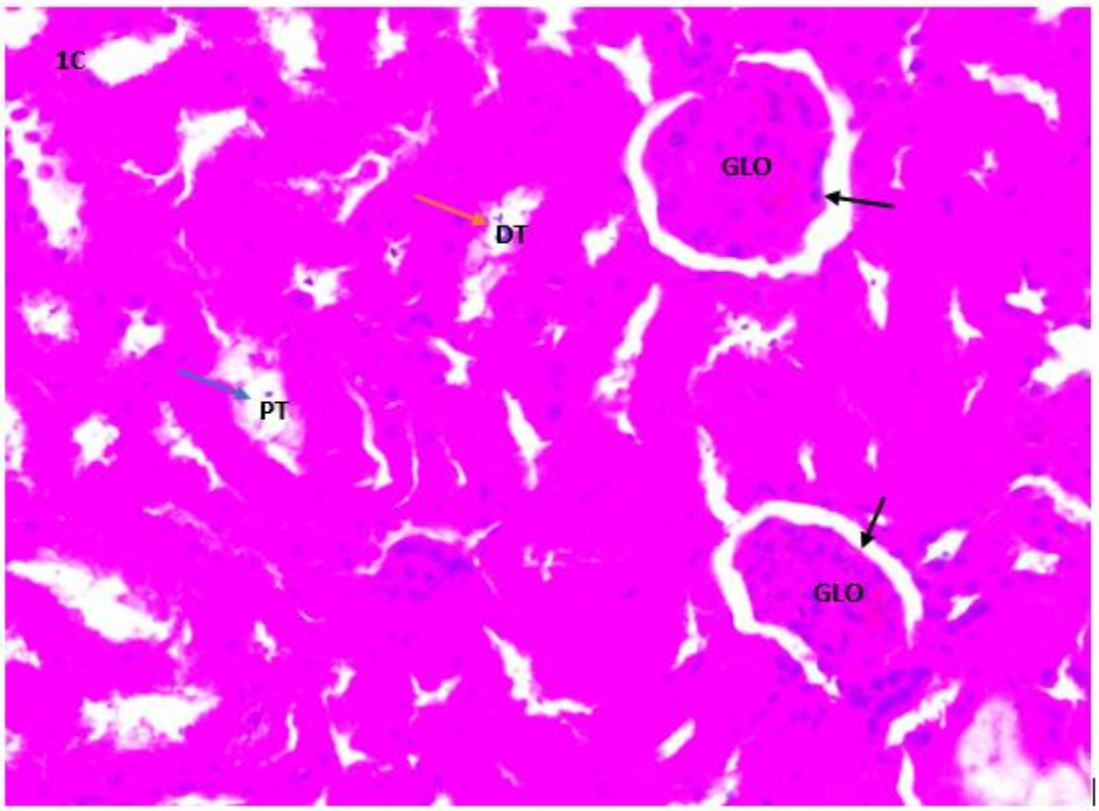

PLATE 3: Photomicrograph section of kidney tissues from group 3. Section showed mild expansion of the urinary space (black arrows) of the glomerulus (GLO) and proximal (Blue arrow) and distal tubules (Red arrow). H&EX400

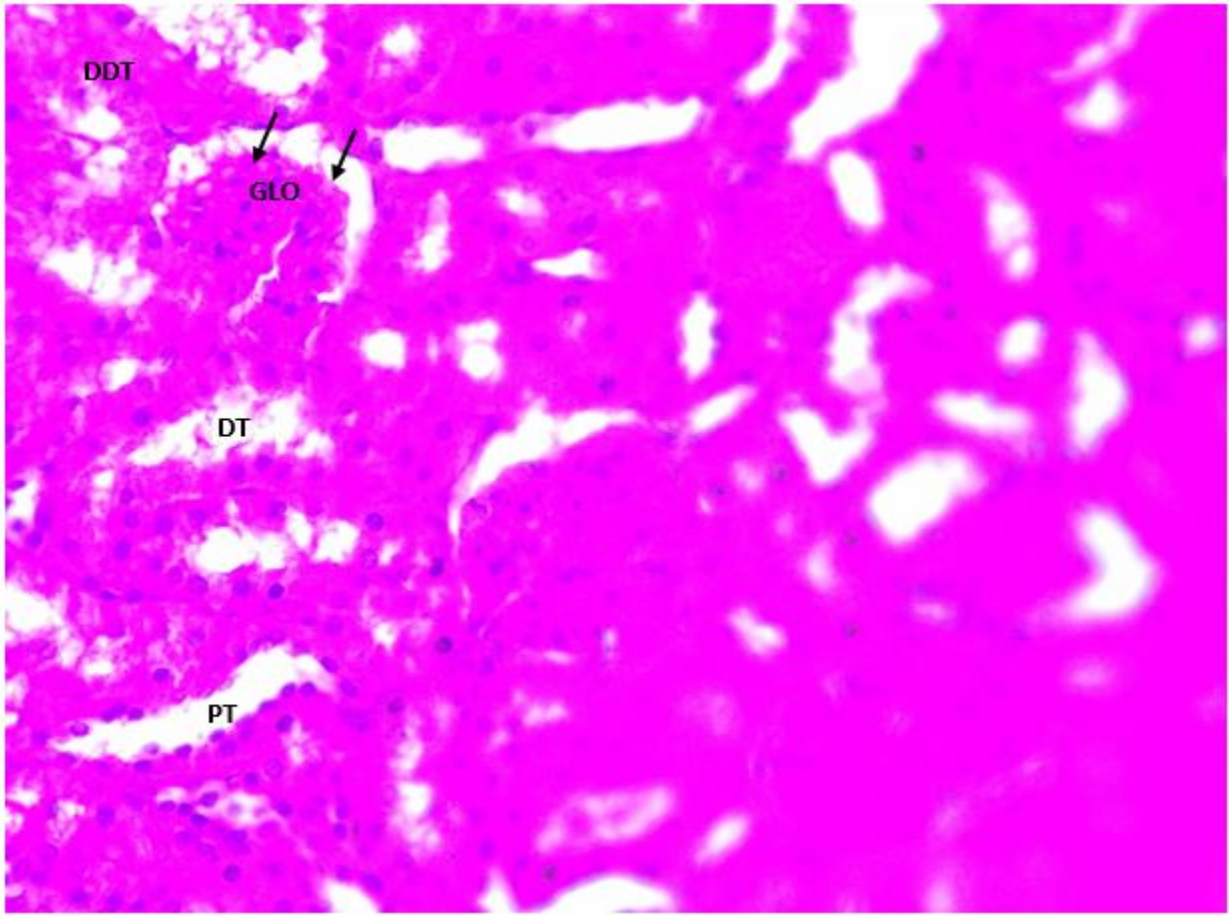

PLATE 4: Photomicrograph section of kidney tissues from rats in group 4. Section showed expansion and distortion (Black arrows) of the glomerulus (GLO). The proximal and distal tubules shows cellular epithelium distortion and degeneration. H&EX400.

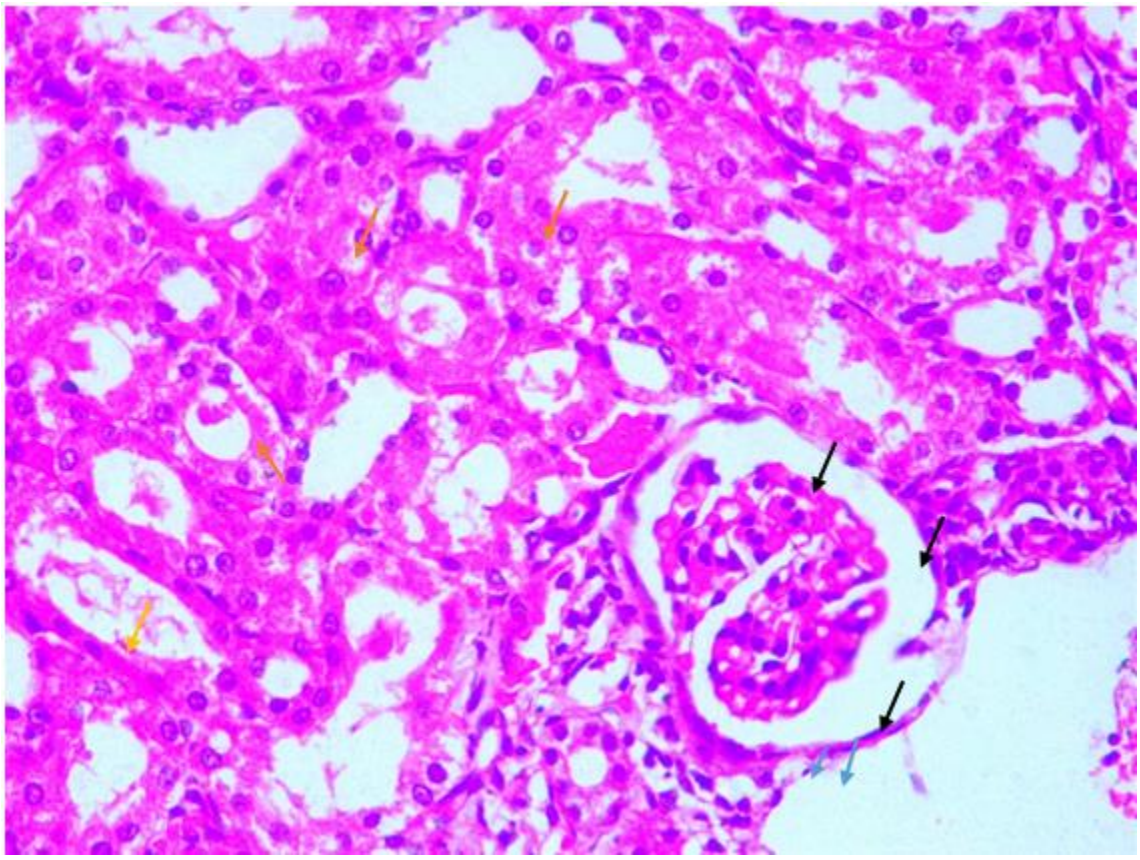

PLATE 5: Photomicrograph section of kidney tissues from rats in group 5. Section showed expansion and distortion of the glomerulus urinary space (Black arrows) with basement membrane fibrosis (Blue arrows).The proximal and distal tubules shows myxoid degeneration (red and yellow arrows). H&EX400.

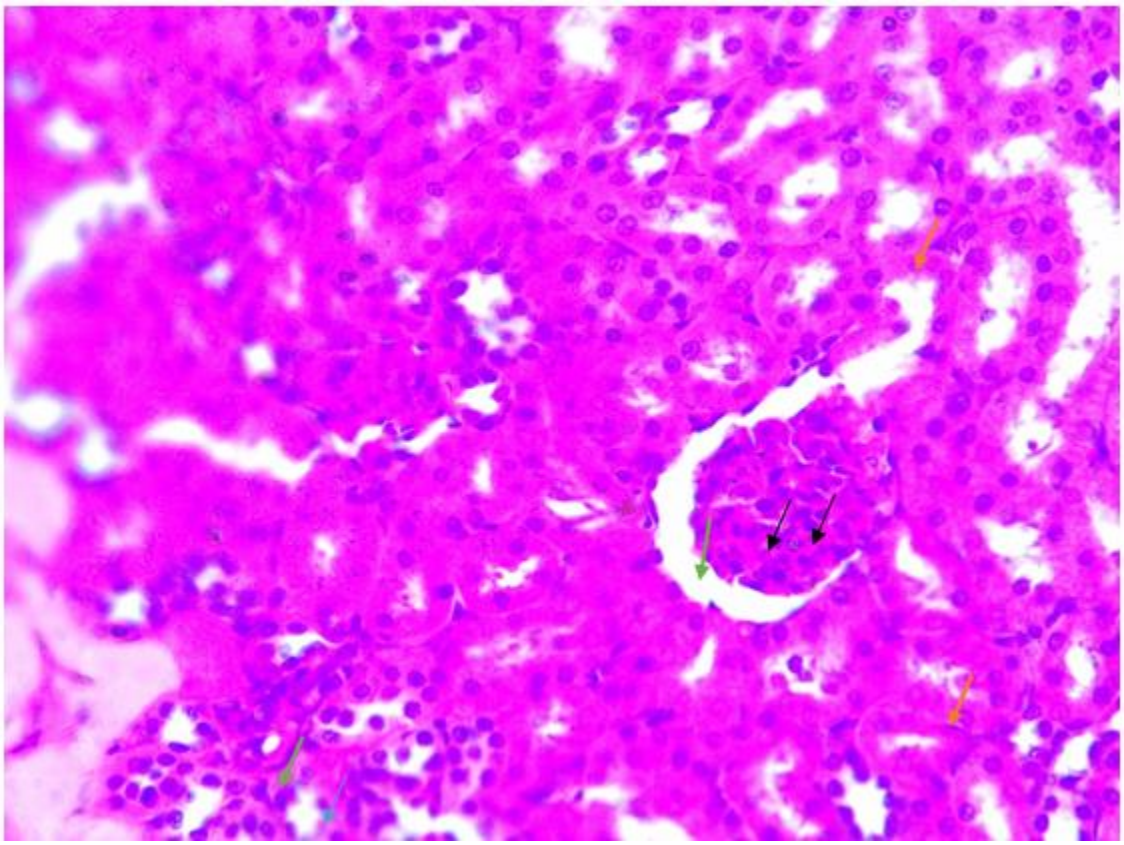

PLATE 6: Photomicrograph section of kidney tissues from rats in group 6. Section showed lymphocytes infiltration (black arrows) into the glomerulus and expansion of the urinary space (green arrow). The proximal and distal tubules show cellular epithelium distortion and degeneration (red arrows). H&EX400.

DISCUSSION

The liver is responsible for most of the metabolic activities that occur in the body, and the use of substances with unknown concentrations is increasing.The major phytonutrients of this plant are carbohydrates, distantly followed by proteins. Notable phyto micronutrients and minerals include zinc, iron, calcium and manganese.it has also been reported that *Justicia secunda* leaf contains cyanide. The implication is that Justicia secunda leaf is not totally safe, potentially due to the cyanide content

The result from the present study showed a statistically significant increase in ALT, AST, and ALP in group 4, and a statistical significant decrease in conjugated bilirubin seen in groups 5 and 6. This implies that the plant extract is likely to cause liver injury, which is in agreement with Bukar *et al.,* (2005). They stated that an increase in serum enzymes such as ALT, ALP, and AST, as well as TP and TB are commonly used as measures of liver injury. The present study is in contrast with

the study reported by Onochie *et al.*, (2020). They reported that extract did not significantly increase the serum/plasma bilirubin levels and liver enzymes of rats.

Results from this study showed that the plant extract had no effect on serum total protein levels and albumin levels. This is because the total protein levels of the treatment group did not increase when compared with the control.

The results obtained for the liver function tests imply that *J. secunda* treatment, under the conditions of this study, exerted a toxic effect on the liver of normal Wistar rats but was not severe and sustained. This is further strengthened by the mild hepatic degeneration observed in some of the histological slides of the liver. This extract is likely to contain some toxic compounds. Long-term administration is likely to contain toxic effects; caution should be taken in consuming this plant. It is possible that a reduction in dose, frequency, and duration of administration may reduce the side effects observed in this study.

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