EVALUATION OF SUBACUTE TOXICITY OF METHANOL EXTRACT OF *JUSTICIA CARNEA* LEAVES IN WISTAR ALBINO RATS

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ABSTRACT

The subacute toxicity of methanol extract of Justicia carnea leaves on rats was evaluated. Thirty six male rats (70 – 100 grams) were randomly divided into four groups replicated thrice with each replicate having three rats. Group A (control group), and groups B, C and D (test groups) were treated with 0, 200, 400 and 800 mg/kg of extract respectively. Liver and kidney function markers, antioxidant activities, lipid profile and haematological indices determined after 28 days of treatment indicated that the liver enzymes markers, protein profile of the extract treated groups showed no significant changes (p>0.05), except the group treated with 800 mg/kg BW of extract which showed significant decrease (p<0.05) in alanine transaminase (ALT) activity. Significant (p<0.05) reduction was observed in malondialdehyde level in group treated with 800 mg/kg extract, whereas superoxide dismutase and catalase activities of the extract treated groups were not significantly (p>0.05) different. No significant changes (p>0.05) was observed in the concentrations of the haematological indices, kidney markers and lipid profile of the test groups when compared with normal control. The histopathology examination of the liver and kidney of the rats in control, 200 and 800 mg/kg extracts group showed normal tissue architecture; while rats administered 400 mg/kg BW of the extract had mild portal inflammation with interface necrosis. The findings of this study suggest that the methanol extract of J. carnea may have antioxidant and hepatoprotective properties and be relatively safe for albino rats following acute consumption.

Keywords: *Justicia carnea*, Subacute toxicity, Serum liver markers, Kidney markers, Antioxidant markers, Lipid profile, Haematological indices

INTRODUCTION

Plants and herbs have been used since olden times to cure various diseases. Approximately 80% of all medicines were derived from herbs as at the middle of the nineteenth century (Arsad *et al*., 2013). They are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Sathisha, 2013). Herbal medicine flourishes today as the primary form of medicine for perhaps as much as 80% of the world's population (WHO, 2002). A specific part of the plant (root, leaves, fruit, flowers and seeds) is

usually used in traditional preparations or as pure active principles formulated into a suitable preparation. Many medicines commonly used today are of herbal origin. About 25% of prescription drugs contain at least one active ingredient derived from plant material (Taylor *et al.,* 2001; Saad and Said, 2011). Plant derived medicines are used in all civilizations and cultures, and hence, plants have always played a key role in health care systems worldwide. In most developing countries, the indigenous modes of herbal treatment are parts of the culture and the dominant method of healing therapy. These remedies, with a considerable extent of effectiveness, are socially accepted and economically viable and mostly, are the only available source (WHO, 2003).

These remedies have natural therapeutic potentials which aid in combatting ailments such as obesity, cardiovascular disorders, arthritis, osteoporosis, diabetes, renal and liver diseases (Kunle *et al*., 2012). There is a perception that herbal remedies, because of their natural derivation, are devoid of adverse or toxic side effects when compared with the synthetic drugs used in conventional medicine (Latha *et al*., 2010). According to the World Health Organization, in spite of the wide use of medicinal plants, their efficacies have rarely been tested (WHO, 2008) and it is therefore necessary to evaluate and standardize various herbal formulations used in the management of a myriad of diseases. Although the use of these plants has shown promising potential with high global demand, there are still concerns about not only their use but also their safety (Obidike and Salawu, 2013).

Justicia carnea Lindl. (Lamiales: Acanthaceae) commonly referred to as the Brazilian plume flower, Brazilian-plume, flamingo flower or jacobinia is a flowering perennial plant native to the Atlantic forest ecoregions of eastern Brazil. The genus *Justicia* was named after a Scottish Gardner, James Justice, in the 18th century (Onyeabo *et al.,* 2017). In Nigeria, the shrubs of *J. carnea* are grown around homesteads and act as fences, which are easy to grow and propagate from stem cuttings by pushing the cut stems 1 to 2 inches into the soil (Mabberley, 1997). It has been reported that *J. carnea* is rich in macronutrients and trace elements such as iron and calcium (Rasheed *et al.,* 2013). Presently, the interest in studying and understanding the constituents of plants with healing properties have increased. It is widely distributed in Nigeria and used in homes as a decorative plant. It is well used in eastern Nigeria as blood tonic made by decoction (Onyeabo *et al.,* 2017). Traditionally, several species of *Justicia* are used in the management of several ailments such as: inflammation, gastrointestinal disorders, diarrhoea, liver diseases, rheumatism and arthritis (Corrêa and Alcântara, 2012; Onyeabo *et al*., 2017). They also possess anti-inflammatory, anti-allergy, anti-tumor, anti-viral and analgesic activities (Radhika *et al.,* 2013) including antioxidant activity (Medapa *et al.,* 2011) and hepatoprotective activity (Ukpabi-Ugo *et al*., 2019). The phytochemical analysis showed that phenols, tannins, alkaloids, anthraquinone, saponins, flavonoids and reducing sugars were present in the leaves of *Justicia carnea* (Makunga *et al*., 2008; Onyeabo *et al.,* 2017). It has been widely use as antimicrobial, antioxidant, hypocholesterolemic and anti-cancerous and this may associated with the bioactive constituents like phenols and flavonoids present in it (Oloruntola *et al.,* 2022).

The reason for increasing interest in herbal medicines is the belief that because these medicines are natural and have been traditionally used, they are safe and harmless (Arsad *et al*., 2013). Nevertheless, their natural origin is not a guarantee of safety, as many studies concerning the risks associated with the use of herbal products and its dosage have been reported (Vaes and Chyka, 2000; Whiting *et al*., 2002). Hence, scientific information regarding the safety of this plant for use as an alternative medicine is very important before it is further developed into a new medicinal herbal therapy. Therefore, the objective of the present study was to determine the subacute toxicity of methanol extract of *J. carnea* leaves in Wistar albino rats.

MATERIALS AND METHODS

Collection and Identification of Plants: Fresh leaves of *J. carnea* were collected from Government College in Umuahia North L.G.A, Abia State, Nigeria in 2019. The plant was identified (ITIS, 2019) and authenticated by a plant taxonomist in the Department of Forestry, Michael Okpara University of Agriculture Umudike, Abia State, Nigeria. Voucher specimen (MOUAU/VPP/2019/04) was deposited in the departmental herbarium for referral purposes.

Preparation of Plant Materials: The freshly collected leaves were washed with clean water and air-dried at room temperature; 1110 g of the dried leaves were pulverized into coarse powder by use of miller and macerated in 4.5 litres of absolute methanol for 72 hours with intermittent stirring to facilitate extraction. The extract was filtered using Whatman No. 1 filter paper. The filtrate was concentrated in a water bath at 60^{o}C to obtain the crude extract.

Animals: A total of forty five (45) healthy male Wistar rats weighing between 70 – 100 grams obtained from the Animal Breeding Unit of the College of Veterinary Medicine, University of Nigeria, Nsukka were used for the study. The animals were housed in well ventilated stainless-steel cages under standard laboratory condition and were given commercial rat feed (Finisher mash, Chikun Feeds, Crown Flour Mill Limited, Lagos State, Nigeria, with crude protein of 19.90% and metabolizable energy of 3209.64 Kcal) and water *ad libitum.* They were allowed to acclimatize for two weeks before commencement of the experiment. The guidelines and the experimental protocols was approved by the ethical committee, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria The experimental rats were maintained using the guide for the care and use of laboratory animals (NRC, 2011). The experiment was laid done in a complete randomized design of four treatments with three replicate each, and each replicate having three rats. Group B, C and D (test groups) were given 200, 400 and 800 mg/kg BW of the extract respectively while group A which served as control group was given 5 ml/kg of water for 28 days. Nine rats were used for acute toxicity study.

Acute Oral Toxicity Test: Oral acute toxicity study of methanol extract of *J. carnea* was carried out using the 'Up-and- Down' method of testing in mice and rats at single doses of 0, 500 and 2000 mg/kg in accordance with the Organization for Economic Development (OECD) guideline No. 425 (OECD, 2008). 3 rats were used for each dose level in the study. An animal was picked at a time, weighed and dosed with the equivalent volume of extract dissolved in distilled water. The extract was administered orally using a gavage. Each animal was observed after dosing for the first 5 minutes for signs of regurgitation and kept in a metallic cage according to the specifications of OECD (2008). The animals were monitored for 14 days to assay for the long-term possible lethal outcome.

Experimental Design: Thirty six rats were randomly assigned to four groups (A – D) of nine animals each and they were treated as follows: group A (normal control) received distilled water (5 ml/kg) only, groups B – D received 200, 400 and 800 mg/kg of methanol extract of *J. carnea* respectively. The initial dose (200 mg/kg) was a 10% reduction of the LD_{50} (2000 mg/kg) that was subsequently double for C (400 mg/kg) and D (800 mg/kg) treatments. The treatments were administered once per day orally via gavage for 28 days. Twenty-four (24) hours after the last treatment on day 28, blood samples were collected through the median canthus into plain sample and EDTA container for haematological indices and biochemical analysis and thereafter, the rats were anaesthetized with chloroform fume in a desiccator, laparotomized and the liver and kidney were excised immediately and fixed in 10% formalin for histopathological examination.

Biochemical Analysis

Assay of liver and kidney function markers: The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline

phosphatase (ALP) activities as well as serum bilirubin, urea, creatinine, albumin and total protein levels were determined using a commercially available Randox reagent kit (Randox Diagnostics, United Kingdom) following the manufacturer's guide foe each assay.

Assay of serum antioxidant activity and lipid peroxidation: The catalase and superoxide dismutase (SOD) activities were determined as described by Aebi (1984) and Beauchamp and Fridovich (1971) respectively. Lipid peroxidation level was estimated by spectrophotometrical determination of the thiobarbituric acid reactive substance content as described by Wallin *et al.* (1993).

Determination of lipid profile: Cholesterol, triacylglycerol and high density lipoprotein concentration were determined by the methods as described by Allain *et al.* (1974), Tietz (1990) and Grove (1979) respectively. Low density lipoprotein and very low density lipoprotein were calculated according of Friedewald formula (Friedewald *et al.,* 1972) and Wilson *et al*. (1981) respectively.

Determination of Haematological Indices: Red and white blood cell counts were determined by the method described by Schalm *et al.* (1975). Haemoglobin concentration, packed cell volume and differential leucocyte count were determined by the method of Brar *et al*. (2000).

Histological Examination: The liver and kidney were excised and transferred to a sterile universal container containing 10% neutral formalin. They were processed and embedded in paraffin wax to provide a hard support for sectioning. Every third section was mounted in glass slide and stained with Haematoxylin and Eosin and photomicrographed.

Statistical Analysis: Data collected were analysed with one way analysis of variance (ANOVA). Significant means were separate with post-hoc least significant difference (LSD) multiple comparisons. Significance was accepted at $p \leq 0.05$. Results were expressed as Mean ± SEM. Data analysis was done using Statistical Package for Social Scientists (SPSS) version 16.0.

RESULTS

Acute Toxicity: The acute toxicity study did not show any toxicity sign and symptom at 500 and 2000 mg/kg. No morbidity signs and mortality were observed in the treated groups at both doses during acute toxicity study. Furthermore, the animals did not display any drug-related changes in behavior, breathing, skin effects, water consumption, impairment in food intake and temperature. Therefore, the extract was deemed to be safe at dose of 2000 mg/kg, and the median lethal dose (LD_{50}) was considered to be > 2000 mg/kg.

The results as shown in Table 1 showed that there was no significant changes ($p>0.05$) in the concentrations of total bilirubin, total protein, albumin, globulin and AST and ALP activities in the group administered with the extract when compared with the normal control. However, the rats administered 800 mg/kg BW of *J. carnea* extract showed a significant decrease ($p<0.05$) in the ALT activity in relative to the control group.

The results as shown in Table 2 showed no significant changes ($p>0.05$) in the activities of the serum antioxidant enzyme (CAT) in the extract treated groups but a marked increase was noticed in SOD activities in 800 mg/kg BW extract group when compared to the control group, also MDA level lowered significantly ($p<0.05$) only in the group treated with 800 mg/kg BW of extract when compared with control group

The results of effect of methanol extract of *J. carnea* leaves on kidney function markers and lipid profile of rats as shown in Table 3 showed that there was no significant changes ($p>0.05$) in the concentrations of kidney function markers (urea and creatinine) and lipid profile parameters (cholesterol, triacylglycerol, high density lipoprotein, low density lipoprotein and very low density lipoprotein) in the extract treated groups comparable with the control group.

Table 1: Effect of methanol extract of *Justicia carnea leaves* on serum liver function markers in albino rats

Parameters	A	B	C	D
	Control group	200 mg/kg of *J. carnea* methanol extract	400 mg/of *J. carnea* methanol extract	800 mg/of *J. carnea* methanol extract
ALT (IU/L)	23.87 ± 1.54[b]	24.60 ± 2.03[c]	23.67 ± 4.02[b]	14.40 ± 1.61[a]
AST (IU/L)	66.13 ± 6.97	66.13 ± 8.78	65.13 ± 8.26	56.40 ± 2.12
ALP (IU/L)	33.68 ± 5.59	35.63 ± 4.97	32.22 ± 2.47	28.26 ± 2.89
Total Protein (g/dl)	5.43 ± 0.95	5.50 ± 0.23	5.57 ± 1.01	5.17 ± 0.34
Albumin (g/dl)	3.41 ± 0.19	3.54 ± 0.09	3.12 ± 0.22	3.47 ± 0.13
Globulin (g/dl)	2.02 ± 0.77	1.95 ± 0.27	2.45 ± 0.96	1.70 ± 0.44
Total bilirubin (mg/dl)	0.66 ± 0.03	0.71 ± 0.01	0.66 ± 0.03	0.73 ± 0.06

Values are expressed as mean ± SEM. Values on the same row with different letter superscripts are significantly different ($p<0.05$). ALT- alanine transaminase, AST - aspartate transaminase, ALP - alkaline phosphatase

Table 2: Effect of methanol extract of *Justicia carnea* leaves on serum antioxidant enzymes activities and lipid peroxidation

Group	Treatment	MDA (Nanomole/g protein)	SOD (µg Protein)	CAT (µg Protein)
A	Control	53.01 ± 11.13[bc]	2.54 ± 0.81[a]	64.53 ± 7.76
B	200 mg/kg	54.93 ± 4.28[c]	2.30 ± 0.06[a]	64.56 ± 4.68
C	400 mg/kg	51.27 ± 9.21[b]	2.55 ± 0.30[a]	66.32 ± 13.66
D	800 mg/kg	47.91 ± 2.75[a]	6.26 ± 3.77[b]	67.20 ± 2.80

Values are expressed as mean ± SEM. Values on the same column with different letter superscripts are significantly different ($p<0.05$). MDA – malondialdehyde, SOD - superoxide dismutase, CAT - catalase

Table 3: Effect of methanol extract of *Justicia carnea* leaves on kidney function markers and lipid profile of albino rats

Parameters	A	B	C	D
	Control group	200 mg/kg of *J. carnea* methanol extract	400 mg/kg of *J. carnea* methanol extract	800 mg/kg of *J. carnea* methanol extract
Urea (mg/dL	21.55 ± 1.26	22.06 ± 4.90	21.69 ± 2.77	20.11 ± 0.82
Creatinine (mg/dL)	0.66 ± 0.02	0.71 ± 0.01	0.66 ± 0.03	0.73 ± 0.06
CHOL (mg/dl)	61.47 ± 5.61	60.55 ± 5.73	49.85 ± 6.32	55.96 ± 5.73
TRIG (mg/dl	61.57 ± 11.87	51.76 ± 6.22	36.86 ± 6.16	42.35 ± 3.59
HDL (mg/dl)	9.79 ± 0.26	8.47 ± 1.61	8.73 ± 1.83	7.94 ± 0.92
VLDL (mg/dl)	12.31 ± 2.37	10.35 ± 1.25	7.37 ± 1.23	8.47 ± 0.72
LDL (mg/dl)	39.37 ± 6.23	41.73 ± 3.57	33.74 ± 4.16	39.56 ± 5.65

No significance difference ($p>0.05$) when compared among the groups. CHOL - cholesterol, TRIG - triacylglycerol, HDL - high density lipoprotein, LDL - low density lipoprotein, VLDL - very low density lipoprotein.

The results of the haematological indices as shown in Table 4 showed that no significant difference ($p>0.05$) was observed in the red blood cell count, packed cell volume, haemoglobin concentration, white blood count and differential leucocyte counts in the group treated with different doses of extract when compared with the control group.

Histopathology: Histopathological examinations were performed on the liver and kidney to assess whether or not organs or tissues were damaged.

Table 4: Effect of methanol extract of *Justicia carnea* leaves on haematological indices in albino rats

Parameters	A	B	C	D
	Control group	200 mg/kg of *J. carnea* methanol extract	400 mg/kg of *J. carnea* methanol extract	800 mg/kg of *J. carnea* methanol extract
HB (g/dl)	19.07 ± 0.84	18.33 ± 0.88	17.80 ± 0.90	18.33 ± 0.66
PCV (%)	48.33 ± 1.67	47.67 ± 1.45	45.00 ± 3.51	47.33 ± 0.88
RBC ($x10^6/mm^3$)	7.89 ± 0.27	7.70 ± 0.21	7.25 ± 0.56	7.70 ± 0.16
WBC ($x10^3/mm^3$)	11.07 ± 0.04	11.33 ± 0.55	11.65 ± 0.26	10.33 ± 0.55
Lymphocyte %	55.33 ± 3.18	53.67 ± 1.45	56.33 ± 0.67	52.33 ± 1.33
Neutrophil %	36.67 ± 2.03	38.33 ± 0.88	37.00 ± 1.53	40.00 ± 1.15
Monocyte %	5.67 ± 0.33	5.33 ± 0.88	4.00 ± 0.58	5.67 ± 0.88
Eosinophil%	2.33 ± 0.88	2.00 ± 0.58	2.67 ± 0.67	2.00 ± 0.58
Basophil %	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

No significance ($p>0.05$) when compared among the groups. HB – haemoglobin, PCV - packed cell volume, RBC - red blood cells; WBC - white blood cells

None of the organs in the rats given daily methanol extract of *J. carnea* doses (200 and 800 mg/kg BW) had any cellular morphological alterations or abnormalities in relative to rats in the control group. Rats administered 400 mg/kg BW of the extract had mild portal inflammation with interface (piecemeal) necrosis but without fibrosis or steatosis (Figure 1). Figure 2 represents that the kidney of the rats administered different doses of the extract that did not show any abnormalities or alteration in treated group comparative to control group.

DISCUSSION

Justicia carnea is an unconventional medicine traditionally used for managing a myriad of diseases (Onyeabo *et al.*, 2017; Akintimehin *et al.*, 2021). The assumption that herbal preparations/remedies are safe and effective has influenced the indiscriminate use of such remedies, most especially among rural communities, where these remedies can be administered for a long period of time without considering the dose or concentration that will bring about toxic side effects (Ben-Arye *et al.*, 2016). The main reason of evaluating the safety of any medicinal plant is to identify the nature and significance of adverse effect, establish the exposure level at which this effect is observed and determine the dose ranges that are safe for subsequent studies (Ibrahim *et al.*, 2016).

Acute toxicity test assesses the adverse effects that occur within a short time after administration of a single dose of a test substance. This testing is performed principally in rodents and is usually done early in the development of a new chemical or product to provide information on its potential toxicity (Loha *et al.*, 2019). For acute toxicity study, methanol extract of *J. carnea* leaves were given to rats at a dose of 0, 500 and 2000 mg/kg BW. No signs of toxicity and mortality were recorded in the groups at administered doses during the study period. It was also observed that there were no changes in water and food consumption in any of the animals given the single dose of *J. carnea* extract. Therefore, the LD_{50} of the extract may be greater than 2000 mg/kg. According to OECD criteria under its Globally Harmonised Classification System (GHS) for chemical substances and mixtures, substances with LD_{50} > 2000 – 5000 mg/kg are categorized as unclassified or category 5 (OECD, 2008) hence methanol extract of *J. carnea* extract may, therefore, be considered relatively safe on acute exposure. The present finding from the acute toxicity study agreed with the study of Onyeabo *et al.* (2017) who reported that there was non-toxic nature of the ethanol extract of *J. carnea* leaves up to 5000 mg/kg. Orjiakor (2016) reported that aqueous extract of *J. carnea* leaves up to 5000 mg/kg showed no sign of toxicity and mortality, which also align with our results.

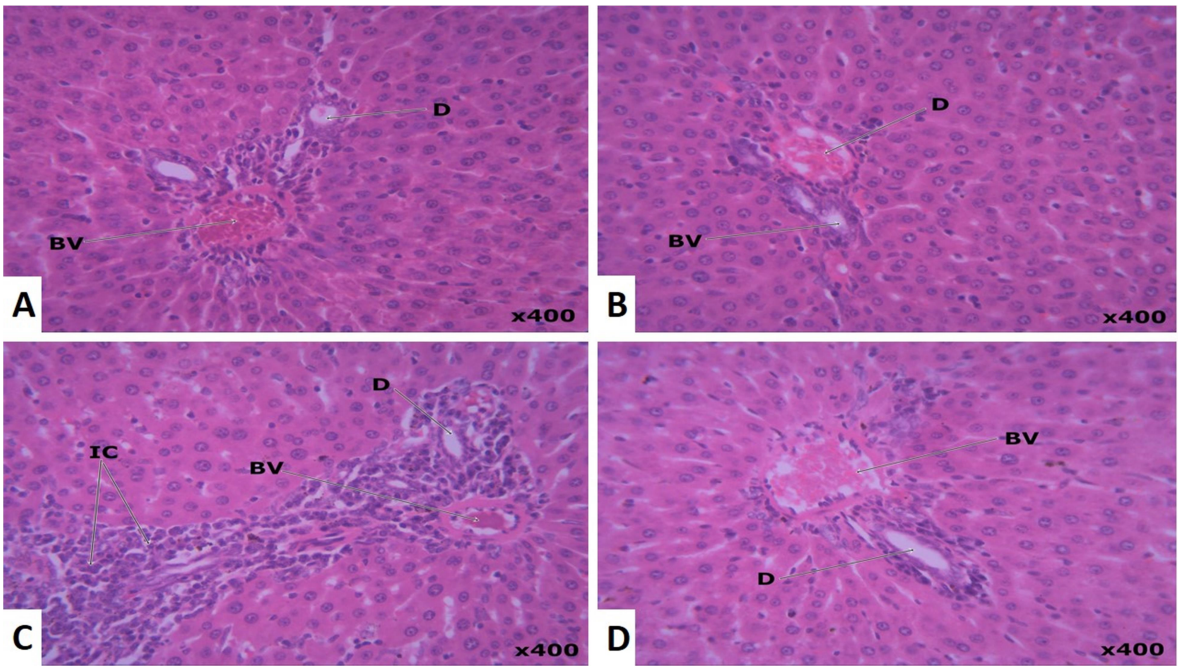

Figure 1: The photomicrograph (H & E, x400) of the liver of rats exposed to methanol extract of *Justicia carnea leaves for 28 days.* *Legend: A = normal control; B = extract, 200 mg/kg; C = extract, 400 mg/kg; D = extract, 800 mg/kg; IC=Inflammatory cells; BV= Blood vessel. D= Hepatic ductile*

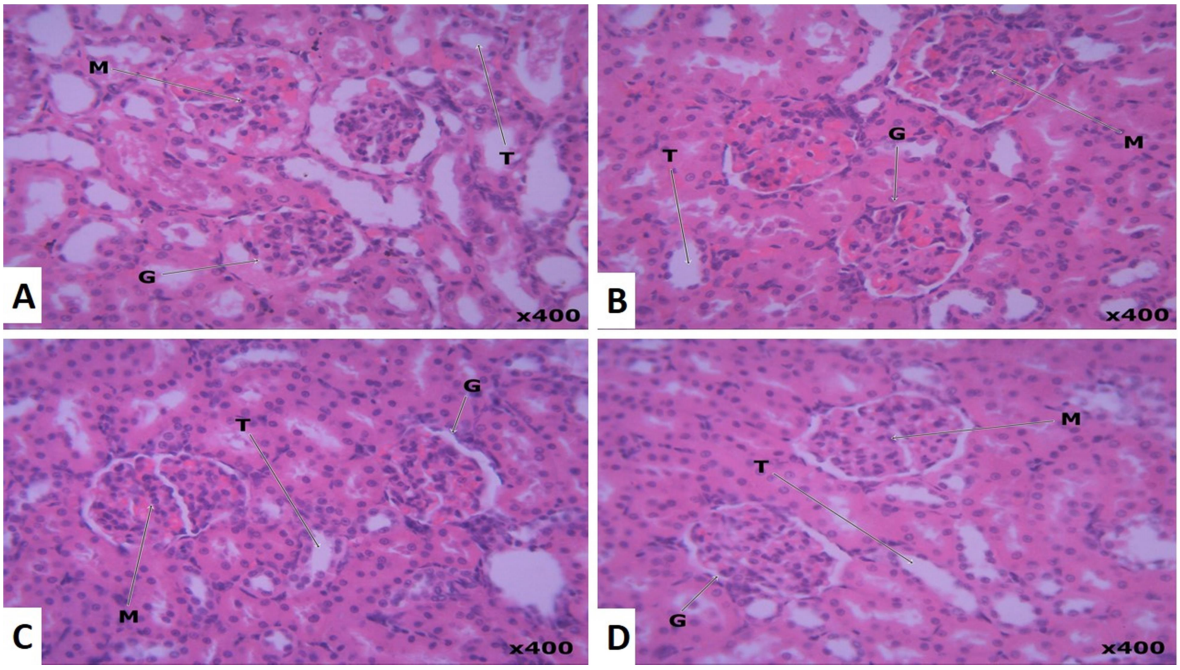

Figure 2: The photomicrograph (H & E, x400) of the kidney of rats exposed to methanol extract of *Justicia carnea leaves for 28 days.* *Legend: A = normal control; B = extract, 200 mg/kg; C = extract, 400 mg/kg; D = extract, 800 mg/kg*

Acute toxicity data are usually of limited clinical application. Therefore, sub-acute toxicity study was carried out. Substances administered in chronic disease conditions may need repeated dosing for toxicological evaluation (sub-acute toxicity study) since daily use may result in accumulation in the body with gradual effects on tissues and organs (Abotsi *et al.,* 2011;

Bariweni *et al*., 2018). Haematological, biochemical and target organ effects are usually not observable in acute toxicity testing so sub-acute toxicity testing is useful in assessing these effects and also important in establishing human safety especially in the development of pharmaceuticals (Ugwah-Oguejiofor *et al*., 2019). In this study, no deaths were recorded after oral administration of 200, 400 or 800 mg/kg of the extract for 28 days. The animals did not present any behavioral changes when subjected to toxicity screening for this experimental period.

The most common parameters used to assess liver function are ALT, AST and ALP activities (El Hilaly *et al.,* 2004). AST is an enzyme found in the cytoplasm and mitochondria in different tissues, including the heart and skeletal muscles, liver, kidneys, pancreas and erythrocytes (Vroon and Israili, 1990). This enzyme is used as a marker of liver injury in certain species of animals, including cattle, goats, sheep, horses and pigs. ALT is a liver-specific enzyme in dogs, cats, rabbits, rats and primates (Dina *et al*., 2011). It is localized primarily in the cytosol of hepatocytes and is considered to be a sensitive marker of hepatocellular damage in these animal species when compared to the levels of AST. It can provide a quantitative assessment of the degree of damage sustained by the liver (AL-Mamary *et al*., 2002). The elevated activities of these enzymes corroborate with tissue damage, especially the hepatobiliary and kidney tissue (Dina *et al*., 2011; Iweala *et al.,* 2019). In this present study, the ALT activity decreased significantly ($p<0.05$) in 800 mg/kg BW extract group when compared with the control group, whereas AST and ALP activity did not show any significant changes in the extract group in relative to control group although a marked decrease was observed in 800 mg/kg BW extract group, this may also be as a result of the antioxidants present in the leaves extract. This result was in line with the previous work of Patrick-Iwuanyanwu and Wegwu (2008), who reported that the decrease in the liver enzymes activities observed may be due to the presence of antioxidants in the plant which protected the hepatocyte membrane and Onyeabo *et al*. (2017) reported the presence of phenol, flavonoids and some antioxidant vitamins like Vitamin A, C and E may have contributed to this effect. From this observation, the methanol extract of *J. carnea* may possess hepatoprotective property at 800 mg/kg BW of extract administered.

Bilirubin passes through the liver and is eventually excreted out of the body. Bilirubin levels higher than the usual level may indicate different types of liver or bile duct problem. Once liver is damaged, it may not be able to excrete bilirubin properly, hence bilirubin elevation (Singh *et al*., 2011). This study recorded no significant difference in the bilirubin concentration, these further buttresses the fact that the extract at all doses administered maintained the functional integrity of the liver cells was in accordance with the findings of Vetriselvan and Subasini (2012).

Albumin and other blood proteins are primarily synthesized in the liver. Albumin serves as a carrier for molecules of low water solubility including hormones, bile salts, unconjugated bilirubin, free fatty acids, calcium and some drugs, this functional role of albumin makes it a reliable marker for diagnosis liver disease (Ruot *et al*., 2000). According to Ruot *et al.* (2000), inflammatory responses are mostly triggered by a decrease in plasma albumin levels. Serum proteins (also blood or plasma proteins) are proteins present in blood that serve many different functions, including transport of lipids, hormones, vitamins and minerals in the circulatory system and the regulation of a cellular activity and functioning of the immune system. When the liver becomes damaged or injured, the hepatocytes may not be able to synthesize the proteins efficiently, hence, leading to the reduction in serum concentration of albumin and total protein. In this study, there was no significant difference in the levels of total protein and albumin concentration thus indicating that there was little or no reduction in hepatocyte number consequently leading to normal synthesis of these proteins (Iweala *et al.,* 2019).

Measurement of plasma urea has been used for many years as an indicator of kidney function. Plasma urea is usually increased in

acute and chronic renal diseases (Tietz, 2000). Urea clearance falls as the kidney fails and as a result, urea tends to accumulate with diseased kidneys that are unable to excrete these substances at normal rate thus leading to raise in blood urea levels (Tietz, 2000; Féres *et al*., 2006). Creatinine is produced endogenously and released in to body fluids at a constant rate and its plasma concentration is maintained predominantly by glomerular filtration. The elevation in urea and/or creatinine is diagnostic of kidney malfunction (Sood *et al*., 2015). The findings of this study suggest that the extract do not produce nephrotoxic effects at the doses administered and for the duration. Histology of the kidney rats did not produce any toxic changes confirming the safety of the extract in the kidney which is also in accordance with the results.

Superoxide dismutase (SOD) is a major enzyme of defence in the antioxidant system against free radicals causing oxidative stress. It catalyses the dismutation reaction of superoxide ion into oxygen and hydrogen peroxide. Catalase (CAT) enzyme decomposes hydrogen peroxide to water and oxygen thereby enhancing acquisition of tolerance to oxidative stress by preventing accummulation of free radicals in the system (Mirunalini *et al.,* 2010). The result from this study showed that there was no significant difference ($p>0.05$) in the activity of SOD and CAT for all test groups compared to the control group which suggests that the extracts did not contribute to the depletion of SOD and CAT and this was in agreement with the findings of Ojha *et al.* (2009).

Malondialdehyde (MDA) is a commonly used biomarker for assessment of lipid peroxidation amongst many secondary product formed during lipid peroxidation. Its elevated levels may reflect the degree of lipid peroxidation injury in the hepatocytes (Messarah *et al.,* 2013; Mistry *et al.,* 2013). Elevated MDA usually indicates a compromise of functional integrity of the cell. Ukpabi-Ugo *et al*. (2019) reported that an increase in serum MDA levels observed in the rats treated with CCl_4 alone when compared with the normal control may be due to increased lipid peroxidation caused by CCl_4 induced in the rats. The findings of this present study showed a significant decrease ($p<0.05$) in the concentration of MDA in the group administered with 800 mg/kg BW of the extract, whereas groups administered with 200 and 400 mg/kg extract showed no significant changes ($p>0.05$) in MDA level. This suggests that the extract may likely possesses free radical scavenging activity (Samy *et al.,* 2007).

Evaluation of lipids such as TC, TG, HDL-C, LDL-C and VLDL can provide vital information on predisposition of the heart to atherosclerosis and lipid metabolism as well as other associated coronary heart diseases (Linton *et al*., 2019; Shabana *et al.,* 2020). Our findings showed that there was no significant difference in the lipid profile parameters in the extract groups when compared with control group. This suggests that this plant may not cause any form of obesity and heart disease at the dose administered for the duration.

Evaluation of haematopoietic indices can be used to assess the deleterious effects of toxic agents (Agbaje *et al*., 2009; Ibrahim *et al*., 2016,) and is of great importance in determining the health status of an individual (Burtis *et al*., 2012) therefore, change in the haematopoietic system can be used for prediction of toxicity in animals (Olson *et al*., 2000). Alteration of concentrations of PCV, RBC, Hb, MCV, MCH and MCHC is important in the diagnosis of anaemia (Njinga *et al.,* 2020). These parameters do not only depict the harmful effects of herbal remedies, but also reveal their blood-relating potential. There were no significant changes on RBC, Hb and PCV in the extract group in relative to control group after sub-acute administration of extract. This suggests that the extract may not cause any abnormalities in the haematological parameters, indicating that this plant extract may not likely cause any abnormalities in humans, including bleeding, anemia or bone marrow suppression. This was in agreement with the report of Arsad *et al*. (2013). The increased release of WBCs is a notable biomarker of stress and also aids in defending the body against some inflammatory conditions, such as bacterial infections, leukemia and haemorrhage. WBC and

differentials such as lymphocytes (the main effectors cells of the immune system) are used as indicators of the system's response to toxic and exogenous substances including plants (Adedapo *et al*., 2004). The result obtained from this study revealed that methanol extract of *J. carnea* leaves did not cause any significant changes in the level of WBC count, or in their subtypes, including neutrophils, lymphocytes, monocytes and eosinophils, at any of the doses relative to the control group indicating that the plant extract is nontoxic, this aligned with the report of Loha *et al*. (2019).

In the histopathological study of the liver, rats treated with doses of 200 and 800mg/kg of the extract showed no significant difference compared to normal control. It showed a well preserved liver architecture, The portal triads are evenly spaced around a central vein and there is mild portal inflammation without interface necrosis , fibrosis, steatosis whereas the rats treated with 400 mg/kg BW of the extract showed severe portal inflammation with interface necrosis but without fibrosis or steatosis, this was almost in accordance with the study of Akintimehin *et al*. (2021) who reported slight abnormalities in the central veins of the rats administered 500 mg/kg BW and above of ethanol extract of *J. carnea*. The sections of the kidneys of treated rats showed normal general structure of the kidney and the normal appearance of glomeruli and tubules. The proximal convoluted tubules, distal convoluted tubules, and macula densa are intact. The result was further supported by the values of biochemical parameters of the blood (such as urea, creatinine, and total protein), which are main indicator of kidney damage. On the contrary, the current findings disagree with the work of Akintimehin *et al*. (2021) thus the difference may be from the solvent used for extraction. However, a well-designed subchronic and chronic toxicity studies should be carried out in order to set the clear picture of the safety of the plant part before developing *J. carnea* leaf based health product

Conclusion: The findings of this study, suggest that the methanol extract of *J. carnea* may have antioxidant and hepatoprotective property and may be relatively safe following sub-acute consumption of the administered doses.

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