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Toxicity Study of Aqueous Leaves Extract of *Jatropha gossypiifolia* from Nigerian in Albino Rats: Serum Biochemistry and Histopathological Evaluation

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Authors' contributions

This work was carried out in collaboration between both authors. Author STM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors STM and IBB managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Confounding factors such as plant species, its location and other environmental associated chemistry were reported to influence the dynamics of phytochemicals from being beneficial health-wise to metabolites that facilitate toxic induced effects. This study investigated the oral sub-acute toxicity profile of the aqueous leaf extracts (ALE) of *Jatropha gossypiifolia* collected from Mubi, Adamawa State, Nigeria. Generalized loss of body weight, weaknesses, dizziness, loss of appetite and restlessness were observed in the acute toxicity study with more severe effects and mortality recorded in the groups exposed to higher doses of 1000 and 2000 mg/kg body weight. In the sub-acute toxicity study, the ALE following the oral administration of 240 mg/kg, 450 mg/kg and 583 mg/kg for 28 days was observed to profoundly alter the normal architecture of the liver and the kidney. The pathological lesions were observed to have disrupted the normal concentration of the serum biomarkers. The ALT concentrations were found to increases to 10.28±1.26 U/L at 250

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mg/kg, 9.38±0.57 U/L at 450 mg/kg and 9.31±0.77 U/L at 583 mg/kg respectively when compared to the control (5.86±0.34 U/L). The concentrations of AST were observed to increase to 49.07±16.2 U/L at 250 mg/kg, 47.10±15.42 U/L at 450 mg/kg and 53.07±10.32 U/L at 583 mg/kg respectively when compared to the control (46.11±9.21 U/L). The activity levels of ALP further shows an increase of 379.50±11.31 U/L at 250 mg/kg, 624.90±22.46 U/L at 450 mg/kg and 662.73±28.62 U/L at 583 mg/kg respectively when compared to the control (349.97±71.70 U/L). It is evident from this study that the ALE of the *J. gossypiifolia species* in addition to its health benefits also contains a cocktail of toxic phytochemicals. Thereby redefined the previous conclusions that the aqueous leaf extract of J. *gossypiifolia* plant is entirely safe. Thus, reinforcing the importance of the uses of indigenous/site-specific experiment, having in mind that some phytochemicals are sensitive to numbers of confounding factors.

Keywords: Jatropha gossypiifolia; acute/sub-acute toxicity; phytochemicals; liver enzymes; rats.

1. INTRODUCTION

Among the vast library of natural products available in nature, plants relative to its spread and varying physiochemistry seems to have the most essential and interesting phytochemicals with great medicinal importance [1]. The phytochemicals relative to its various compositions stand out as a remarkable ingredient in the design of pharmaceutical products [2].

In Nigeria, the search for herbal medicine to remedy the inaccessible pharmaceutical drugs has been a front burner for decades. One among many herbal plants in Nigeria with interesting pharmacological importance is *Jatropha gossypiifolia,* commonly referred to as "bellyache bush" or "black physicnut". The name was coined from Greek words "*jatros,*" which means "doctor" and "*trophe,*" meaning "food," [3]. The phytochemicals derived from the leaves constitutes largely of alkaloids, steroid, phenolic substances and ascorbic acid [4]. Others include moderate amounts of saponins, carbohydrates and trace amount of glycoside and resins [5,6]. These phytochemicals depending on the types of extract and plant parts were reported to play a significant role as antihypertensive, antimicrobial, anti-inflammatory, antioxidant, and antineoplastic agents [3,7].

Herbal or plant-based compounds were assumed to have a wide margin of safety [8]. However, this margin of safety, despite its meaning needs to be factored into the equation for individual species when considering formulating an herbal remedy for human consumption. The crux of the matter is that most medicinal plants consist of yet to be identified constitutes [8] with varying potential to induce toxicity by exerting unforeseen interactions between the herbs and other herbs, especially in concocted form. And to some extend between the herbs and other prescribed drugs [9] which may end up activating different physiological processes downstream.

Different findings were documented regarding the toxicity of *J. gossypiifolia* [5,10]. However, these findings among other factors were observed to differ from each other with respect to species type, plants parts, doses administered, and types of extraction solvents employed [5,10–14]. A cited example shows a single dose of 40 g/kg of raw fresh leaves of *J. gossypiifolia* was able to induce pathological changes in the liver, kidney, lungs and hearts of sheep. According to the study the single dose administered was lethal to the animal [10]. However, Mariz et al. [15], in their own conclusion reported that the administration of medicinal plants in the form of infusions, decoctions, or in dried form could suppress the toxic profiles of the plants. To support Mariz et al. [15] conclusions, the crude ethanol extract of *J. gossypiifolia* leaves were observed to induce relatively low oral acute toxicity in Wistar rats [12,14]. Similarly, oral administration of both the aqueous and ethanol leaves extracts of *J. gossypiifolia* , did not show any sign of toxicity up to 2 g/kg in rats; the investigation was based on acute toxicity study [5]. As interesting as this finding are, the toxicity of the aqueous leaves extract cannot be fully overruled as several confounding factors such as environmental associated chemistry were reported to introduce variations in the chemical compositions in plants [16].

Different parameters were observed to directly influence the degree of toxicity of herbal plants; factors such as soil-water chemistry, breeding conditions, exposure to different forms of pollutions like pesticides, fertilizers and other toxic substances played a role in this direction.

Furthermore, due to the heterogeneous nature of plant-based medicine, the possibility of conflicting end-results between species and within species as regards safety and toxicity related phenomenon remains a sacrosanct task not to ignore [16]. Therefore, before considering *J. gossypiifolia* as important medicinal plants, we find it necessary to carry out an *in vivo* toxicity evaluation (serum biochemistry and histological examination) following both acute and sub-acute toxicity study of the aqueous extracts of the said plants harvested in Mubi, Adamawa state of Nigeria, so as to generate data that might leads to inform decision on the risk/benefit of the plant species for therapeutic purposes. The outcome of the study was envisioned with the sole intention to at least, establish a degree of comfort or more so to rise an alarm for caution to be applied in the use and application of the plant, *J. gossypiifolia* for medicinal purposes.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The leaf of *Jatropha gossypiifolia* used in this study was locally collected from Vimtim village, in Mubi North local government area of Adamawa State, Nigeria. The plant was identified by Mr. Jarafu Ulam Mamza of the department of Botany, Adamawa State University, Mubi and a voucher specimen deposited. The sample was processed following the same methods described by Magili et al. [17]. Briefly, about 100 g of the powdered shade dried leaves sample was dissolved in 100 ml of distilled water and incubated for 24 h on a hot plate. The next day, the filtrate of the sample was collected using cheese cloth, cotton wool and Whatman filter paper respectively. The obtained sample was concentrated on a water bath under consistent heating. The obtained concentrates (extract was stored in fume hood and later used for the study.

2.2 Experimental Animals

The Wistar albino rats used for this study were obtained from the animal house of the National Veterinary Research Institute (NVRI) VOM, Plateau State, Nigeri*a.* The rats were of both sexes, aged between 5-6 months and weighing about 200 g to 250 g. They were housed in clean iron cages and fed with standard animal diet. The rats were used for acute toxicity study to calculate (LD_{50} value), sub-acute toxicity studies and enzyme assay respectively. The harvested organs (liver and kidney) were also examined histologically based on hemotoxyline and eosin (H & E) staining technique. All the experiments using animal models were conducted under the regulations set by the Adamawa state University, Mubi (ADSU) ethics committee's guidelines for the care of laboratory animals (Approval No. A045/2016)

2.3 Acute Toxicity Study

Twelve (12) rats of either sex were divided into four groups of three (3) rats per group which received graded doses (500, 1000 and 2000 mg/kg body weight) of *J. gossypiifolia* aqueous extracts. The animals were divided into a control group and three treatment groups of three rats per group. The control group (Group 1) received orally, normal saline while the three groups were administered orally with the graded doses of the extracts. The animals were observed closely for any gross behavioral effects for twenty four hours (24 h) that is 0 h, 2 h, 6 h 12 h 24^{th} h during which the number of death was recorded for each group [18]. The number of deaths in each group within 24 h was recorded and the LD_{50} values were calculated using the arithmetical method described by Magili et al. [17]. The interval mean of the dead animals in each group of animal was used as well as the difference between doses for the same interval. The product of interval mean and dose difference were obtained. The sum of the product was divided by the number of animal in a group and the resulting quotient was subtracted from the least lethal dose in order to obtain (LD_{50}) value. The data were computed according to the following formula [19].

$$(LD_{50}) = least\ lethal\ dose - \left(\frac{sumof\ (Dose\ Diff\ x\ mean\ death}{N}\right)$$

$$(LD_{50}) = LD - \left(\frac{\sum ab}{N}\right)$$

Where LD is the apparent lethal dose of all the groups, N is the number of animals in each group, a, is the dose difference and b the mean death.

2.4 Sub-acute Toxicity Study

This test expresses the overall side effect observed after repeated administration of the test substance for (2-6 weeks) and enable the determination of the principle behavioral changes

as well as anatomical, physiological, enzyme marker activity and biochemical manifestation of tissue damage provoked by the substance [20,21]. Sixteen (16) rats were selected by randomization and then divided into four groups of four each. The first group served as control while the remaining three groups were given 250,450 and 583 mg/kg body weight of leave extracts of *J. gossypiifolia* single oral dose every day for four weeks. The first day of dosing was taken as D_0 whereas the day of sacrifice was designated as D_{29}.The animals were observed every day for behavioral changes, toxicity symptoms, and signs of poisoning and mortality for a period of 28 days.

2.5 Liver Enzyme Test for Sub-acute Toxicity Study

Blood samples were collected through the tail and centrifuged at 4000 rpm for five minutes using centrifuge, (model 800 electric centrifuges B – Bram Scientific and Instrument Co. England) for serum separation. The serum separated were analyzed to evaluate the activities of liver enzymes, the aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) [22]. The serum were analyzed for activity marker enzymes using spectrophotometer (URINT-810 an automatic Chemistry Analyzer and Reflect on plus Roche - 5069865 and Chemical Kits (Randox Diagnostic kits).The liver and kidney from the control and treated (groups) were removed and fixed in 10% formaldehyde for histopathological examinations [23].

2.6 Histopathological Study of Sub-acute Toxicity

Histopathological investigation of the kidney and liver, were carried out according to the method described by Biswas et al. [23]. After the sacrifice of the rats, the kidney and the liver were harvested from both the treated and control groups for examination of microscopic abnormalities. The organs were preserved in 10% formaldehyde. The tissue was processed with automatic tissue processor (STP 120) by tissue processing method described by Galen and Gambino [24]. Samples were dehydrated in graded (70-100%) alcohol, cleared in xylene I and xylene II. Embedding was done by passing cleared samples in paraffin wax I and wax II. The organ pieces (4 µm thick) were prepared with the help of Microtome (Leica, RM 2145) and the slides were stained with Haematoxylin-Eosin. Microscopic examination was carried out on those organs of both the control and treated groups [25] under microscope (Model Nikon Labophot. 223425 Japan). These slides were photographed through Nikon labophot Advanced Research Microscope, Model 223425 Japan, with Sony Digital 12.1 MEGA PIXELS.

2.7 Statistical Analysis

The obtained results were presented as mean ± SD (standard deviation). All differences are considered significant at 5% level, therefore *P*-values less than 0.05 ($P<0.05$) were considered statistically significant using Analyse-it version 2.3 statistical software for Microsoft Excel.

3. RESULTS

3.1 Acute Toxicity Study (Ld_{50}) of *J. gossypiifolia* Aqueous Leave Extracts

Table 1 shows the effect of the oral administration of the aqueous leave extracts *J. gossypiifolia.* In addition to the clinical signs such as the rubbing of nose and mouth on the floor of the cage, other behavioral changes observed in the experimental groups compared to the control rats includes weaknesses and dizziness, loss of appetite and restlessness. The results shows a mortality recorded at doses of 1000 and 2000 mg /kg. The LD_{50} value was determined as a dose progression factor for the sub-acute toxicity studies.

Table 1. Determination of (LD 50) of aqueous leave extract of *J. gossypiifolia*

Group	Number of rats	Difference of consecutive doses (a)	Number of death rats	Mean death between two consecutive doses (b)	Dose difference x mean death (a *x* b)
Control	3	0	0	0	0
500 mg/kg	3	0	0	0	0
1000 mg/kg	3	500	1	0.5	250
2000 mg/kg	3	1000	1	1	1000

3.2 Sub-acute Toxicity Study of the Aqueous Leave Extracts of *J. gossypiifolia*

The result of the sub-acute toxicity studies of the aqueous extracts of the *J. gossypiifolia* on the liver enzymes activity levels are presented in Figs. 1-3. The result is a fallout of the 28 days of oral administration of the aqueous extracts of the plants to the rats. From the results in Fig. 1, the aqueous leave extract of the plants was observed to induce some disturbance on alkaline phosphatase (ALP) activity levels when compared to the control. A significant (P<0.05) dose dependent increase in the ALP concentration were recorded in all the treatments groups when compared to the control (349.97±71.7 U/L) values.

Similar significant (P<0.05) increase were observed in the alanine transaminase (ALT) activity levels. The aqueous leaves extract of *J. gossypiifolia increase* the concentrations of the ALT from 10.28 ±1.263 U/L in the group treated with 250 mg/kg, while the group treated with 450 mg/kg showed an increase of about 9.38 ±0.567 U/L. Furthermore, 9.31 ±0.768 U/L was recorded in the rats treated with 583 mg/kg. From the results as presented in Fig. 2, the effects were observed not to be dose dependent.

Furthermore, the rats treated with the aqueous leaves extract of *J. gossypiifolia shows* a significant (p<0.05) increase in the concentration level of the serum aspartate aminotransferase (AST). As shown in Fig. 3, an increase of about 49.07 ±16.254 U/L, 47.10 ±15.427 U/L, and 53.07 ±10.321 U/L were recorded in the groups treated with the 250 mg/kg, 450 mg/kg and 583 mg/kg respectively when compared to the control rats (46.17 ±9.214 U/L).

3.3 Histopathological Examination of Sub-acute Toxicity

To further confirm the safety indices of the aqueous leaves extract of *J. gossypiifolia,* an H & E staining was conducted on the liver and kidney tissue sections of the rats. The results is presented in Fig. 4. From the microgram in Fig. 4A, no obvious sign of hepatic degeneration, necrosis, leukocytes infiltration or presence of adipocyte observed in the liver anatomy in all the control groups. However, mild cloudy degeneration of hepatocytes were observed in the microgram of the liver of rats treated with 250 mg/kg of aqueous leaves extract of *J. gossypiifolia* (Fig. 4B). Similar morphological lesion were observed in the rats exposed to 450 mg/kg with more pronounce effect observed in the groups exposed to the 583 mg/kg of the aqueous leaves extract showing an onset of necrotic cells (Fig. 4C and 4D).

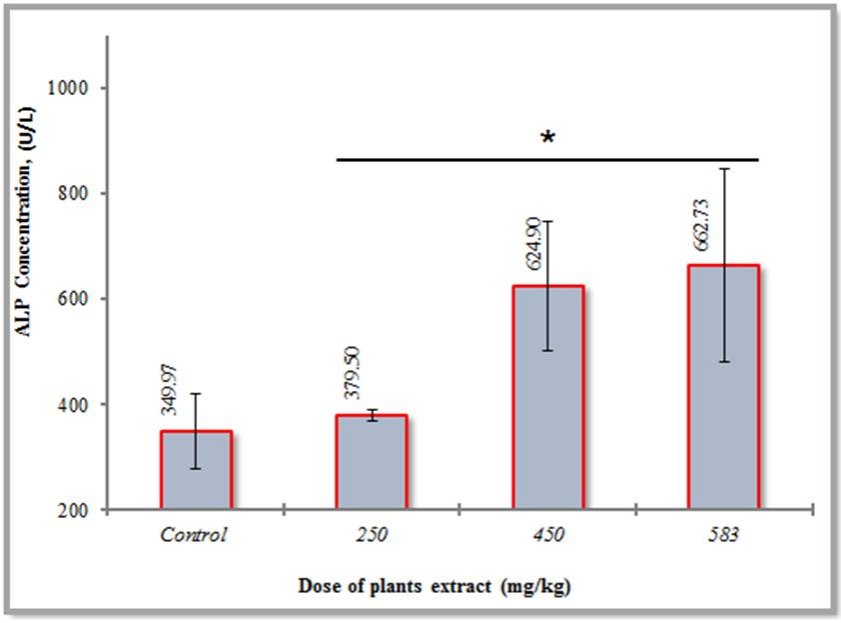

Fig. 1. Effects of the aqueous leaves extract of *J. gossypiifolia* on ALP

*The results are represented as Mean ± SD of three replicate analysis. From the results, * corresponds to significant (p<0.05) values*

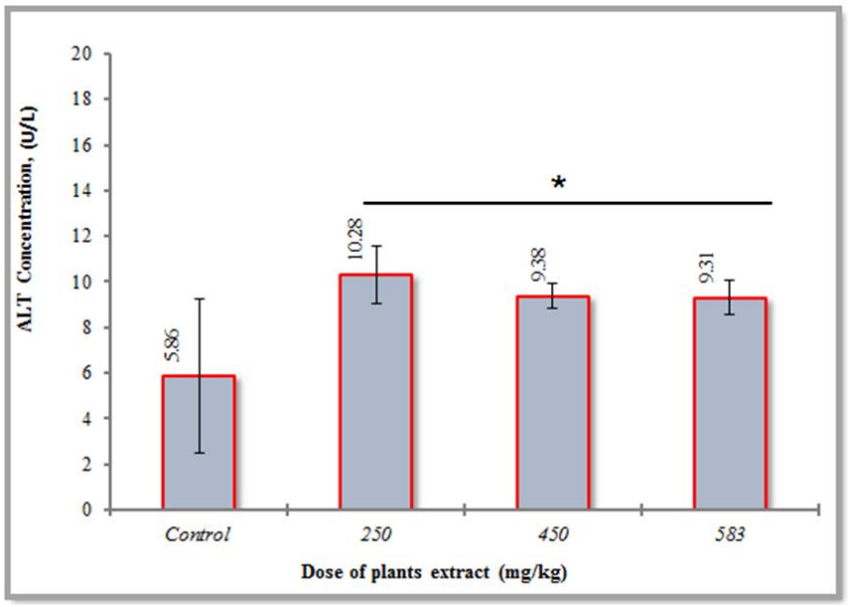

Fig. 2. Effects of the aqueous leaves extract of *J. gossypiifolia* on ALT

*The results are represented as Mean ± SD of three replicate analysis. From the results, * corresponds to significant (p<0.05) values*

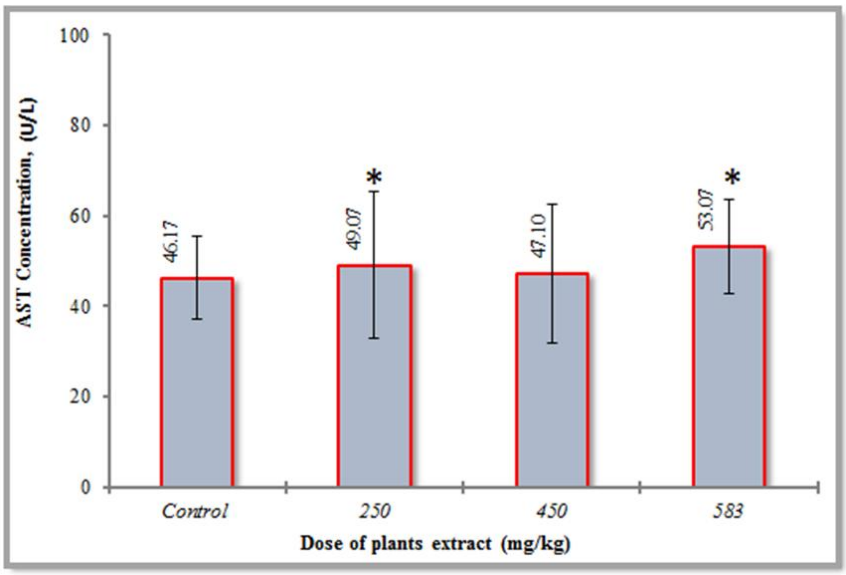

Fig. 3. Effects of the aqueous leaves extract of *J. gossypiifolia* on AST

*The results are represented as Mean ± SD of three replicate analysis. From the results, * corresponds to significant (p<0.05) values*

The H & E tissue section of the kidney of both the treatment and the control groups are presentedf in Fig. 5 (A-D). From the micrograph of the control groups as shown in Fig. 5A, the basic architecture of the kidney such as the glomeruli, nephron cells, distal tubules, and proximal tubules remain intact without any obvious pathological lesion such intra-glomerular congestion or tubular atrophies, tubular degeneration or necrosis recorded. Morphologically, slight congestion, hemorrhages and tubular degeneration were observed in the kidney tissue sections of the rats administered to 250 mg/kg of aqueous leaves extract of

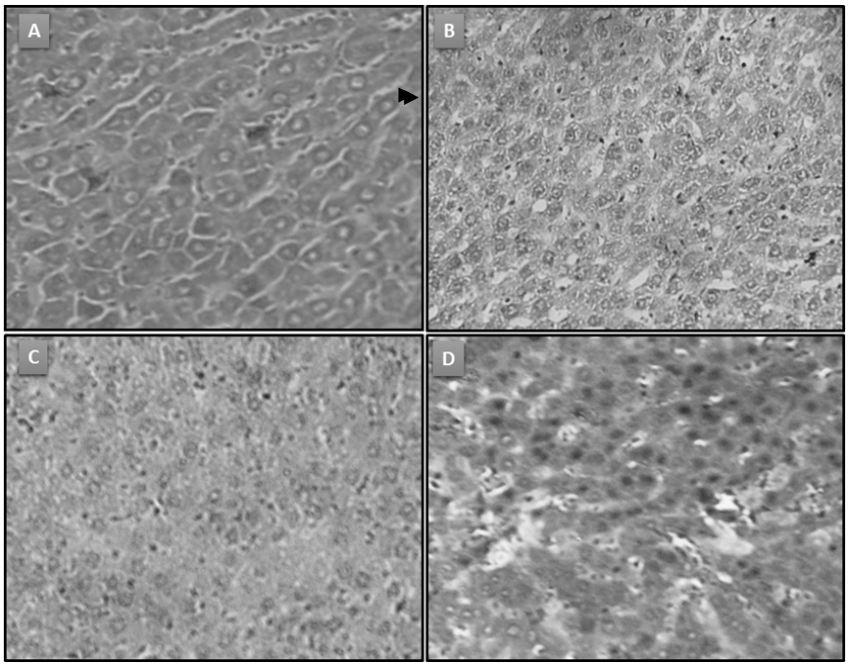

Fig. 4. Micrograph of tissue section of the liver of Albino Rats showing (A) the control; (B) groups treated with 250 mg/kg BW; (C) groups treated with 450 mg/kg BW; (D) groups treated with 583 mg/kg BW. The liver in group B & C shows cloudy degeneration of hepatocytes, while onset of necrosis were observed in group D rats. H and E stain (X 40)

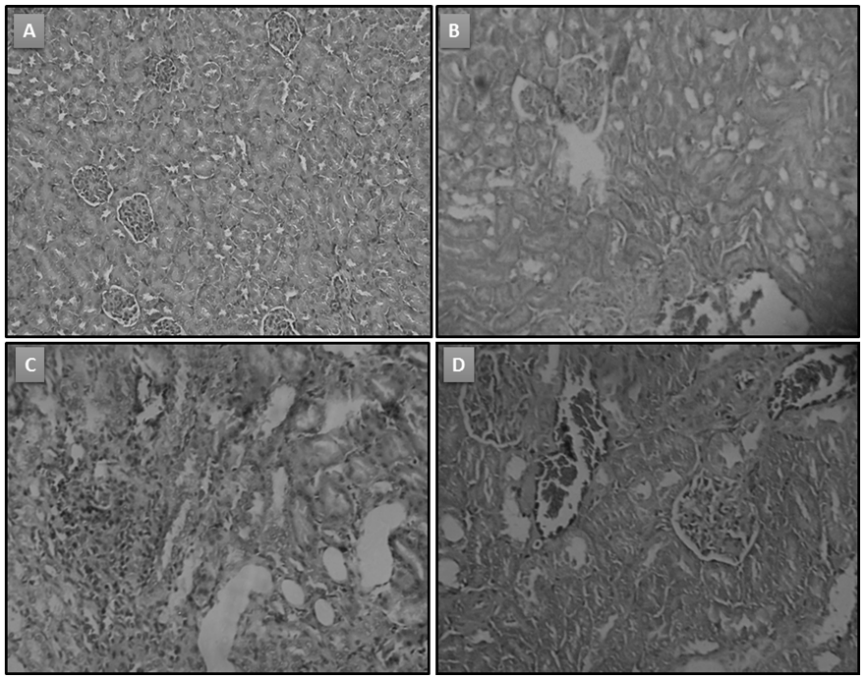

Fig. 5. Micrograph of tissue section of the Kidney of Albino Rats showing (A) the control; (B) groups treated with 250 mg/kg BW; (C) groups treated with 450 mg/kg BW; (D) groups treated with 583 mg/kg BW. The kidney in all the treatment groups shows slight congestion of vessels, hemorrhages and tubular degeneration. H and E stain. (X 40)

J. gossypiifolia (Fig. 5B). Similar morphology were observed in the same organs for the groups exposed to 450 mg/kg and 583 mg/kg of aqueous leaves extract (Fig. 5C & 5D).

4. DISCUSSION

The acute toxicity study conducted following the single dose of 500, 1000, 2000 mg/kg body weight of the aqueous leaves extract administered to the experimental animals has provided additional insight into the toxicity profile of the *J.* gossypiifolia. The investigation conducted was to ascertain the short-time effect of the said medicinal plants and also to serve as a baseline that will be used to establish its lethal dose (LD50) *in vivo* [26]. Following the short-time oral administration, the results thus revealed that the aqueous leaves extract of the *J. gossypiifolia induced* certain degree of toxicity effect in all the experimental groups compared to the control rats. Obvious clinical and behavioral changes observed includes generalized loss of body weight, weaknesses and dizziness, loss of appetite and restlessness with a more severe effects and mortality recorded in the groups exposed to higher doses of 1000 and 2000 mg/kg body weight.

However, the oral acute toxicity effect observed in this study by implication differ with the outcome reported by Nagaharika et al. [5]. The authors carried out an oral acute toxicity study of the aqueous and ethanol leaves extracts of *J. gossypiifolia* [5]. In the study, no significant toxic induced effect recorded in the subject exposed to either the aqueous or ethanol leaves extracts of the plants up to 2 g/kg [5]. While, in another study, the leaves ethanolic extract of this specie were reported to exerts low oral acute toxicity in rats, [12,14]. These results may suggest that the aqueous extract of the leaves, compared to the ethanol extract, may be less toxic possibly due to the differences in the phytochemicals composition which more or less could be directly linked to solvent types used in the extraction [13]. While Nagaharika et al. [5] based on the acute toxicity study conducted declared the aqueous leaves extracts of *J. gossypiifolia* safe and non-toxic. In this present study, a contrary result was observed following the same acute toxicity study of the aqueous leaves extracts of *J. gossypiifolia*. The observed differences recorded in this study as compared to the above cited examples could be ascribed to possible differences in the environmental associated chemistry and climatic factors that may have influence the quality and the quantity of secondary metabolites in the J. *gossypiifolia* specie collected in Mubi, Nigeria [16,27].

As discussed earlier, the concentrations of toxic phytochemicals in plant species varies depending upon confounding factors, which includes environmental stresses on the plant, plant age, individual species susceptibility, plant parts, and seasonal variation [16]. The ability of the plants to transfer and exerts toxic effects may depend on the strength of toxins, quantity consumed, time of exposure, and individual body chemistry. This factors could be the underlining mechanism leading to increased toxic susceptibility observed in this study compared to other findings [27]. These few exceptions call into action to reinforce the importance of the use of indigenous/site-specific experiment that factored into the mix the above mentioned confounding factors, having in mind that some factors influence the physiology and chemical composition of plants despite being of the same species extracted following the same method of preparation.

Several study investigating the phytochemical composition of the aqueous leaves extracts of *J. gossypiifolia reported* the presence of different compositions. Nagaharika et al. [28] and Packialakshmi and Archana [29] reveals largely the presence of alkaloid, steroid, phenolic substances and ascorbic acid. Furthermore, spots corresponding to the presence of alkaloids, terpenes and/or steroids, phenolic compounds, flavonoids, tannins and amines were observed following Thin layer chromatography analysis [6,30]. Though, these phytochemicals were reported to be beneficial health-wise, findings however, reported some rare side toxic effects of some of these phytochemicals. Terpenes, such as diterpenes [31] and alkaloids are reported to be among the toxic compounds in *Jatropha* [3,27]. Even flavonoid specifically apigenin isolated from the leaves of *Jatropha gossypiifolia* [28] and alkaloids were reported to be involved in neurotoxicity or cell signaling disruption [32] affecting neurotransmission and DNA synthesis [32–34]. Thus, the weaknesses, dizziness, loss of appetite and restlessness observed in the treatment groups in this study could be a response to these phytochemicals in the extracts.

The sub-acute toxicity evaluation of the same aqueous leaves extract of *J. gossypiifolia* were investigated *in vivo* with a view to bring into light

the risk-benefit relationship associated with the medicinal use and application of the aqueous leaves extract of *J. gossypiifolia.* Based on the oral sub-acute toxicity study, the aqueous leaves extracts of the *J. gossypiifolia* were observed to profoundly induce an imbalance in the serum enzymes activities. The significantly ($P<0.05$) increased in the serum ALT, AST and ALP in all the treatment groups indicate a disruption in the liver integrity. Biochemistry measurements are often used as indicators to monitor abnormalities within the body physiology. Liver enzymes chemistry are particularly recognized to reflect liver membrane integrity or oxidative stress induction phenomenon, which often initiate transient disturbance in the normal levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) [35,36]. Among the liver enzymes biomarkers, ALT is recognized to be a liver specific enzyme, while ASAT and ALP on the other hand are considered non-specific index because it is distributed not only in the liver but also in the heart, skeletal muscle, kidney, intestine and the brain [35,36].

From the study, the activity levels of ALP and AST were found to be dose dependent in the sense that the severity of damage are likely to increase as the dose administered increases, while an inverse dose dependent effects were observed for ALT, suggesting that there is a likelihood of decreasing effects on ALT, if the dose were increased beyond the 583 mg/kg body weight dose level. The results from this study generally revealed increases in these enzymes suggesting that there are certain level of impact or mild damage to relevant organs, especially liver and kidney. These obvious increases against the control dose implied clinical significance, which is a cause for concern as it would be preferable to have the reducing impact of the administered extract on the blood enzyme parameters that were determined.

Further to the imbalanced exerted on the serum biochemistry, the aqueous leaves extract of the plants were observed to have altered the normal morphology of the liver in all the treatment groups. The micrograph of the liver showed spotty and cloudy degeneration of the hepatocytes as compared to the control group. The histological anomaly observed following the oral administration of the plant extract was observed to be in congruity with the activity levels of the serum biochemistry. The histological lesion on the hepatic cells induced by the plants extract by implication increased the concentration of the serum ALT and AST biomarkers respectively when compared to the control groups. As mentioned earlier, increase in these liver enzymes is a reflection of tissue injury exerted on the liver.

Another histological alteration observed following the oral sub-acute toxicity study in relation to the activity of ALP and the AST is changes in kidney morphology. It was discussed earlier that AST and ALP are considered non-specific indicator for liver injury because it is distributed not only in the liver but also in the heart, skeletal muscle, kidney, intestine and the brain [36]. Thus, the increase in these biomarkers in relation to the liver injury could be attributed also to the observed pathological lesion in the kidney. The pathological picture of the kidney section in the experimental groups was characterized by tubular degeneration, and destruction of the sinusoids. The glomerulus is the primary site of action of several chemicals and it may be injured by any toxic, metabolic and immunologic mechanism [37]. According to Varely [38], toxic irritant substances brought to the kidney by circulatory blood could induces possible pathological lesion in the kidney.

Other study conducted corroborated the presence of toxic metabolite in *J. gossypiifolia.* Studies shows that, the raw leaves of *J. gossypiifolia* induces severe disturbances in the normal metabolic activity of the digestive system, the lung, and heart. Other effect observed includes regressive changes in the hepatic cells and renal tissues [10]. The ethanolic extract based on chronic toxicity study revealed hepatotoxicity and pulmonary damages with various disturbances in the biochemical parameters [13]. Other study also revealed toxic profile of the ethanolic extract of the plant. The study was performed in Wistar rats and the authors observed that the extract was toxic to the kidney and caused increased urea retention in the blood, and induced associated pathological lesion [11].

The phytochemicals identified in the aqueous leaves extract though, beautifully employed as medicinal substrate also contain a cocktail of toxic relationship depending on the confounding factors previously described above. The effects induced by the *J. gossypiifolia* aqueous leaves extract in this study could be related to the physiochemistry of these secondary metabolites. Among the phytochemicals identified in the aqueous leaves extract *J. gossypiifolia,* alkaloid,

terpenes and phenolic compounds (flavonoids and Tannins) are implicated in toxic related phenomenon. This phytochemicals at higher concentrations and physiological conditions can undergo self-transformation from being beneficial health-wise to metabolites that facilitate toxic induced effects [3,5,6,27,29,30]. For example, plant alkaloid toxicity follows different metabolic pathways, but often involves neurotoxicity or cell signalling disruption [34]. Alkaloids may exert toxic effects by inhibiting choline acetyltransferase and DNA synthesis by intercalating with nucleic acid, thereby disrupting the normal neurotically regulating phenomenon [34].

Depending on the body physiology and cell types, the interactions of flavonoids with intracellular signalling pathways usually leads to an unpredictable outcome. Unpredictably results in the formation of free radicals or participate in modulating the stimulation of either pro or anti-oxidant enzymes [39]. Physiologically, copper and iron were observed to catalyze the redox cycle of phenolics compounds, triggering the formation of reactive oxygen species (ROS) and phenoxyl radicals in the process [40,41]. It was further observed that flavonoids with a phenol B ring (*e.g.* apigenin, naringenin) generate ROS and phenoxyl radicals upon oxidation by peroxidase/H_2O_2 [42]. This phenol ring-containing flavonoids or phenolics compound were also reported to caused glutathione oxidation in isolated rat hepatocytes [42]. Other phytochemicals implicated in inducing toxic effects to animal models upon consumption is tanins. Implicated in inducing liver necrosis (Chung 1998) and caused a 4-fold increase in plasma ALT levels after 24 h in CD-1 mice [43]. The toxic mechanism was reported to be partially due to the formation of pro-oxidant intermediates and inhibition of antioxidant enzymes [44].

5. CONCLUSION

Although *J. gossypiifolia* is universally considered a promising plant with great medicinal importance, the study conducted so far further bring into light its toxicity related potentials. Considering that the leaves of *J. gossypiifolia in* its aqueous form is employed empirically as a remedy for various illness in Nigeria. The dynamic toxicity profiles of the aqueous plant extract remain a key factor yet to be explored fully, considering the confounding factors that characterized the individual plant species, its location and other environmental associated chemistry. It is evident from this study that the aqueous leaves extract of the *Jatropha gossypiifolia species* in addition to its health benefits also contain a cocktail of toxic phytochemicals. Based on this study, the oral acute and sub-acute toxicity study conducted redefined the previous conclusions that the aqueous leaves extract of *J. gossypiifolia is* safe. The said aqueous leaves extract was observed to alter the normal architecture of the liver and the kidney. The pathological lesions were observed to disrupt the normal concentration of the serum biomarkers. The activity levels of ALP, ALT and AST significantly increase in response to these pathological lesions.

The outcome of this study further reinforces the importance of the use of indigenous/site-specific experiment, having in mind that some phytochemicals are sensitive to numbers of confounding factors such as soil-water chemistry, breeding conditions, and exposure to different forms of pollutions which invariable induces environmental stresses on the plant. Thus, it will suffice to say that generalizing medicinal plants of the same species safe for human consumption without considering these confounding factors is a risk with an unpredictable outcome.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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