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Effect of Ethanol Leaf Extract of *jatropha Curcas* on Some Liver and Kidney Function Biomarkers of Normal Rabbits

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Abstract

This study investigated effects of ethanol leaf extract of *Jatropha curcas* on liver and kidney function biomarkers of male New Zealand rabbits. A total of 20 rabbits were divided into four groups of 5 animals each. Graded concentrations of ethanol extract of the leaf administered thus: 50 mg, 150 mg, 250 mg and 0 mg/kg bodyweight of animals in groups I, II, III and IV (control) respectively for 10 days by oral intubation. Serum liver enzymes and renal function were assessed using standard procedures. Alkaline phosphatase (ALP) activity of all treated rabbits significantly increased ($p<0.05$) in a dose-dependent manner. There was also a significant elevation in activity of aspartate aminotransferase (AST) of rabbits treated with 250 mg/kgbwt (31.0 ± 5.48 U/l) compared to control (10.00 ± 2.44 U/l). However, no significant difference was observed in alanine aminotransferase (ALT) activity. Conjugated bilirubin concentration increased significantly ($p<0.05$) in a non-dose dependent manner. Serum urea concentration of the rabbits treated with 250 mg/kg bodyweight of leaf extract (11.90 ± 0.24 mmol/l) was significantly ($p<0.05$) higher than that of the control (8.01 ± 0.89 mmol/l), while serum creatinine concentration increased significantly ($p<0.05$) in groups treated with 150 and 250 mg/kg body weight. Serum potassium concentration of rabbits showed a significant increase ($p<0.05$) at 150 mg/kg and 250 mg/kg bodyweight, while serum sodium significantly increased ($p<0.05$) in a non-dose dependent manner. These results suggest that ethanol extract of *J.curcas* has some hepatobiliary toxic effect, as well as renal dysfunction

Keywords: Hepatic function, Renal function, *Jatropha curcas*, Rabbits, Phytochemicals.

1. Introduction

It is a common practice in Africa to employ plant parts either as extract, infusion, decoction, tinctures or capsules for the cure or management of various types of ailments such as diabetes mellitus, gastro-intestinal disorder, liver and kidney diseases amongst others (Boadu and Asase 2017). Medicinal plants have an ever increasing role in the development of new drugs and in ensuring an effective healthcare system of many nations including Nigeria. According to Newman, Cragg, and Snader, (2000), at least 119 chemical substances originating from plants can be considered as important drugs for the treatment of various ailments across many nations. Synthetic analogues of these chemical substances of

plant origin are sometimes prepared to increase the drug efficacy and reduce the side effects of the parent chemicals (Azwanida, 2015).

Jatropha curcas, a drought resistant shrub that belongs to the genus Euphorbiaceae, is widely employed in the treatment of various medical conditions including fever, joint rheumatism, jaundice, guinea worm, sores, and various other organ diseases (Villegas, Fernadz, Maldonado, Torres, Zavaleta, Vaisberg & Hammond, 1997). Various scientific works have authenticated the numerous medicinal properties of *Jatropha curcas.* Sarkiyayi, Simon, & Zailani, (2016) reported the antiplasmodial and hepatoprotective effect of aqueous stem bark extract of *Jatropha curcas* on *Plasmodium beighei* infected mice. The ethanol leaf extract was reported by Ehsaan *et al.,* (2011) to to possess antioxidant and anti-inflammatory effects, while the hepatoprotective effect of the methanol leaf extract on cadmium induced toxicity was demonstrated by Adejumobi, Areola, & Babalola, (2015) using rabbits. Ehsaan *et al.* (2011) reported the anti-cancer effect of the metanolic leaf extract of the plant.

Although several studies have validated the medicinal potential of *Jatropha curcas,* only few have considered its safety at relatively low doses. Poisoning from ingestion of Jatropha seeds is well known in veterinary practice. This study is thus designed to evaluate the safety of relatively low doses of ethanol leaf extract of *Jatropha curcas* on normal rabbits.

2. Materials and Methods

2.1 Collection and Preparation of Plant Material

Fresh leaves of *Jatrophacurcas* were collected from the environment of Federal University of Technology,Owerri, Imo State. The leaves were identified and authenticated by a plant taxonomist, F.N. Mbagwu of Imo State University, Owerri and sample prepared and deposited at the Institution's herbarium (Voucher number: IMSUH 311). The leaves were washed under continuous flow of distilled water for 15 min and allowed to dry under shade for 24 h at the Biochemistry laboratory of Federal University of Technology, Owerri. A 500 g part of the leaves were weighed using a triple beam balance (OHAU 750-50, Burlington NC, USA) and dried in an oven (WTC BINDER, 7200 Tuttlingen, Germany) at 60°C until a constant weight was achieved. The dried leaves were packaged in dark polyethylene bags and kept in cold room (7 ± 3°C) for 24 h. The dried leaves were pulverized using Thomas-Willey milling machine (ASTM D-3182, INDIA), after which the ground sample was stored in air-tight plastic bottles with screw caps pending extraction.

2.2 Extraction of Plant material

100 g of the pulverized dried samples of *Jatropha curcas* was extracted with a soxhlet extractor, using 96 % ethanol (BDH, U.K) as solvent to obtain final volume of 200 mL of leaves extract. The extract was further concentrated and recovered in a rotary evaporator for 12 h at 60°C under reduced pressure. The extract was then dried in a desiccator. The dried extract was wrapped in an aluminum foil and stored in air-tight plastic bottles with screw caps at ≤ 4°C. The percentage yield was calculated to be 19.3 %.

2.3 Study Animals

Twenty male rabbits aged 4-6 months and weighing between 750 to 900 g were purchased from the animal house unit of the Department of Pharmaceutical Sciences, University of Nigeria, Nsukka. The animals were randomly divided into four groups and housed in individual iron cages with wire mesh flower. The animals were randomly assigned to the cages and all housed under similar conditions of management and husbandry including regular feeding and washing of drinking troughs. The animals were acclimatized to laboratory conditions for three weeks prior to the commencement of treatment. Throughout the study, the animals were fed with standard rabbit pellets and drinking water *ad libitum*.

2.4 Phytochemical analysis

Freshly pulverized leaves were chemically tested for the presence of phytochemical constituents using standard procedures as described by Edeoga, Okwu & Mbaebie (2005). The following plant constituents were qualitatively analyzed in the plant material: alkaloids, glycoside, flavonoids, saponins, cardiac glycoside, cyanogenic glycoside, tannins, sterodialaglycone and anthracene glycoside.

2.5 Animal Grouping and treatment

The animals were grouped into four groups of five animals each as follows:

Control: Received normal saline daily, for 2 weeks

Group I: Received 50 mg/kg body weight (bwt) of *Jatropha curcas* ethanol leaf extract for 2weeks.

Group II: Received 150 mg/kg bwt of *Jatropha curcas* ethanol leaf extract for 2weeks.

Group III: Received 250 mg/kg bwt of *Jatropha curcas* ethanol leaf extract for 2weeks.

All groups received their respective extract dosage through oral intubation. The animals were all monitored closely throughout the study period.

2.6 Sample collection and preparation

After 14 days of oral administration of extract, all animals were fasted for 24 h and mildly anaesthetized with dichloromethane. Arterial blood was collected through cardiac puncture and used for biochemical analyses. Blood samples were allowed to clot for 45mins at room temperature before separation of serum by centrifugation.

2.7 Biochemical analyses

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed using the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) activity was measured by the optimized method based on DGKC (German Society of Clinical Chemistry, 1972) and SCE (Scandinavian Society of Clinical Chemistry) recommendations. Serum total and conjugated bilirubin were determined using the method of Jendrassik and Grof, (1938), Urea (Searcy, Reardon, & Foreman, 1967) and Creatinine (Bartels and Bohmer, 1971) concentrations were determined using commercial kit, Randox Laboratories

Limited, (UK). Sodium was determined by the modified method described by Maruna (1958) and Trinder (1951) and potassium by the method described by Terri and Sesin, (1958).

2.8 Statistical Analysis

Data obtained from the study were analysed using one-way analysis of variance (ANOVA) and subjected to Dunnett's post-hoc test. The mean difference is considered statistically significant at $p<0.05$ and values were expressed as mean ± standard deviation.

3. Results

Result of the qualitative phytochemical screening revealed the presence of alkaloids, glycosides, flavonoids, saponins and cyanogenic glycosides as well as nutrients like carbohydrates and proteins in the ethanol leaf extract of *Jatropha curcas (*Table 1).

Table 1: Phytochemical Compositions 0f Ethanol Leaf Extract of *Jatropha Curcas.*

S/N	Phytochemical	Result
1	Alkaloids	+
2	Glycosides	+
3	Flavonoids	+
4	Saponins	+
5	Cardiac glycosides	-
6	Cyanogenic glycosides	+
7	Tannins	-
8	Steroidal aglycone	-
9	anthracene glycoside	-

Oral administration of *Jatropha curcas* to the rabbits at 50mg/kg, 150 mg/kg and 250 mg/kg bwt resulted in a dose-dependent increase in the activity of alkaline phosphatase of the animals (Figure 1) compared to the control group. Serum aspartate aminotransferase (AST) activity of all the treated groups were however not affected except for a significant increase observed in 250 mg/kg bwt treated group (Figure 2). The serum alanine aminotransferase (ALT) activity was also not affected significantly in all the treated groups as compared to the control group (Figure 3).The total bilirubin concentration of all the treated groups showed no significant ($p>0.05$) change when compared to the control (Figure 4), except for the group treated with 150 mg/kg bwt of the plant extract. However, an increase was observed in the serum conjugated bilirubin concentration of all the treated groups (Fig.5).

Additionally, a significant increase was also observed in the urea concentrations of the animals treated with 250 mg/kg body weight of the plant extract (Fig. 6), while the creatinine concentration significantly ($p<0.05$) increased in the groups treated with 150 and 250 mg/kg bwt of the extract (Fig. 7) compared to the control group. Serum concentrations of potassium (Fig. 8) increased in a dose dependent manner whereas sodium ion (Figure 9) concentration

equally increased significantly ($p < 0.05$) but reduced at 250 mg/kg body weight of the extract.

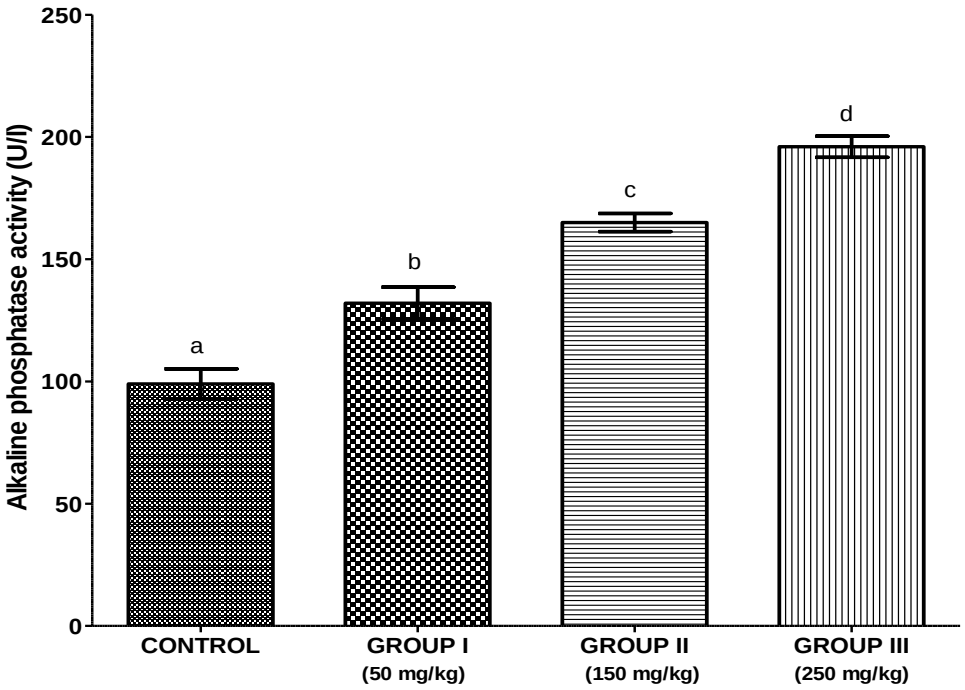

Figure 1: Alkaline phosphatase activity (U/l) of rabbits administered different doses of *Jatrophacurcas* leaf extract.

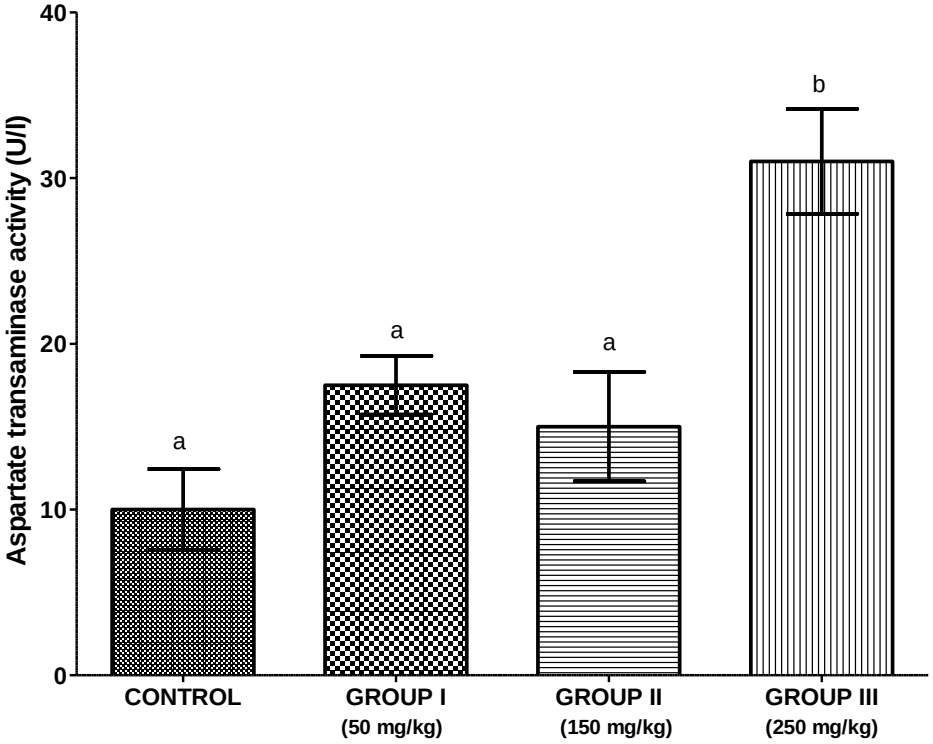

Figure 2: Aspartate transaminase activity (U/l) of rabbits administered different doses of *Jatropha curcas* leaf extract.

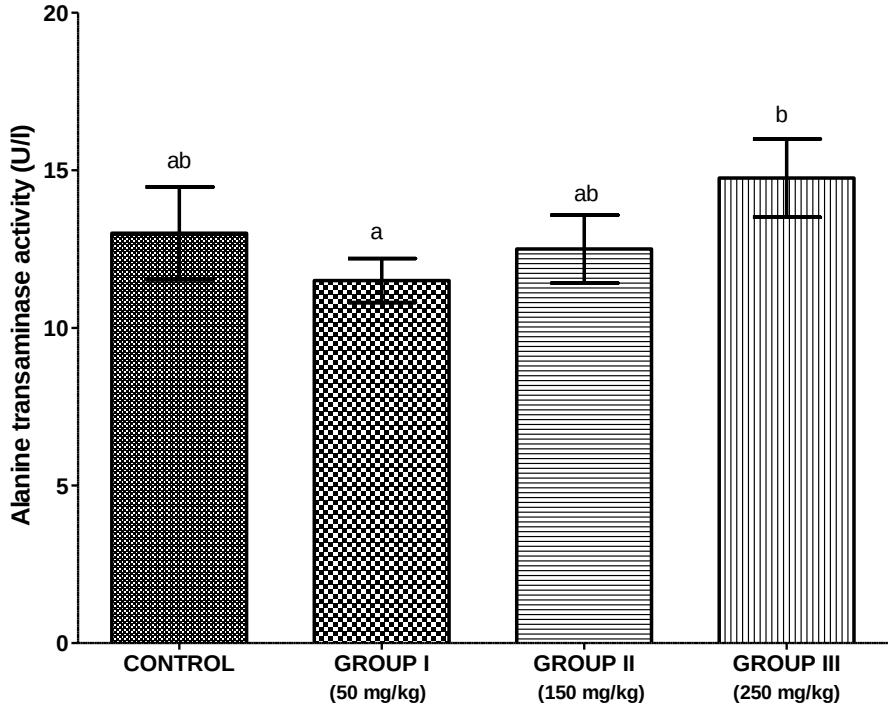

Figure 3: Alanine transaminase activity (U/l) of rabbits administered different doses of *Jatropha curcas* leaf extract.

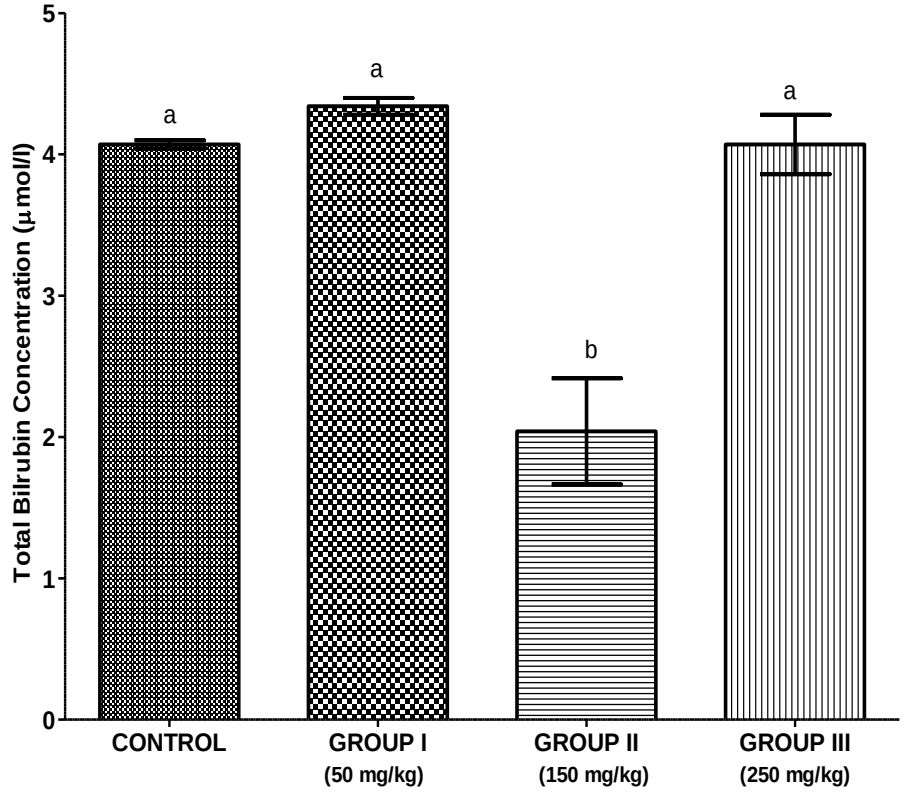

Figure 4: Total bilirubin concentration (µmol/l) of rabbits administered different doses of *Jatrophacurcas* leaf extract.

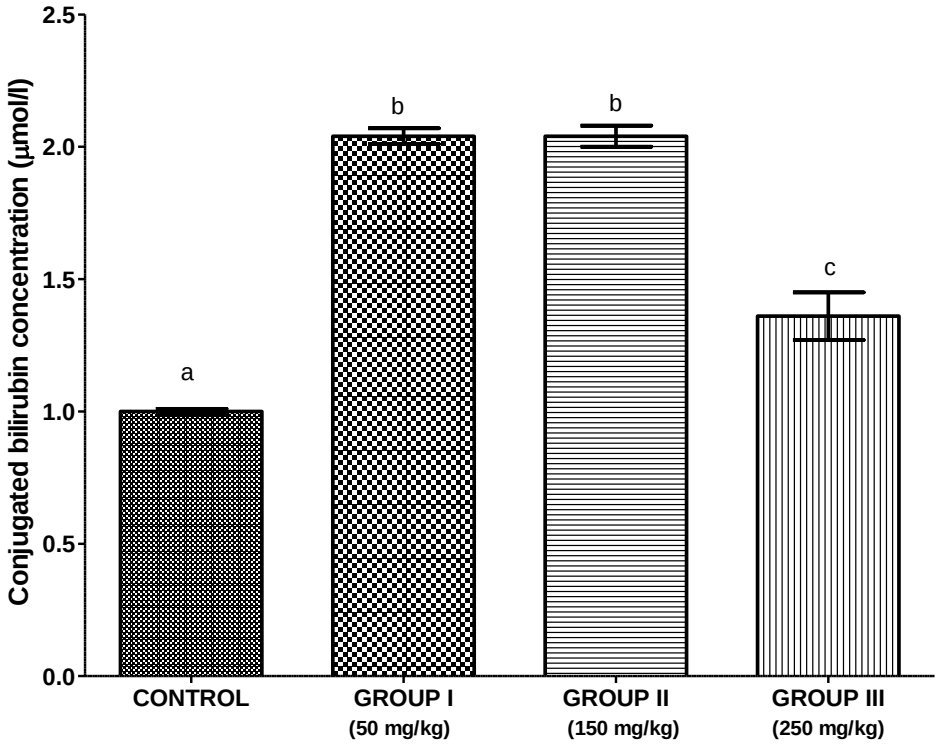

Figure 5: Conjugated bilirubin concentration (µmol/l) of rabbits administered different doses of *Jatrophacurcas* leaf extract.

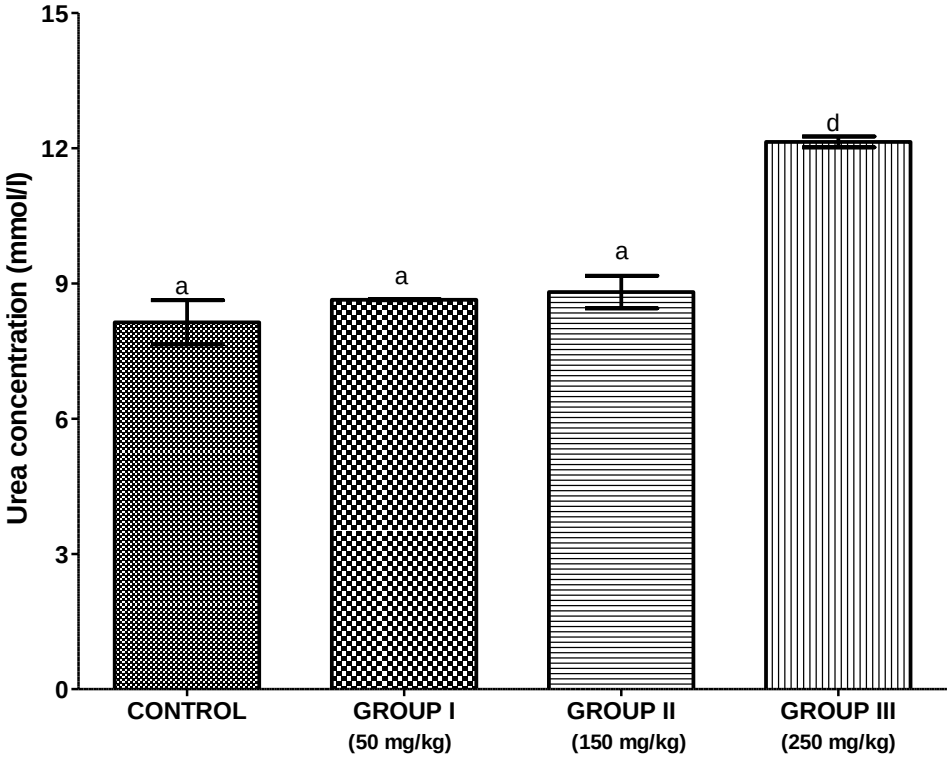

Figure 6: Urea concentration (mmol/l) of rabbits administered different doses of *Jatrophacurcas* leaf extract.

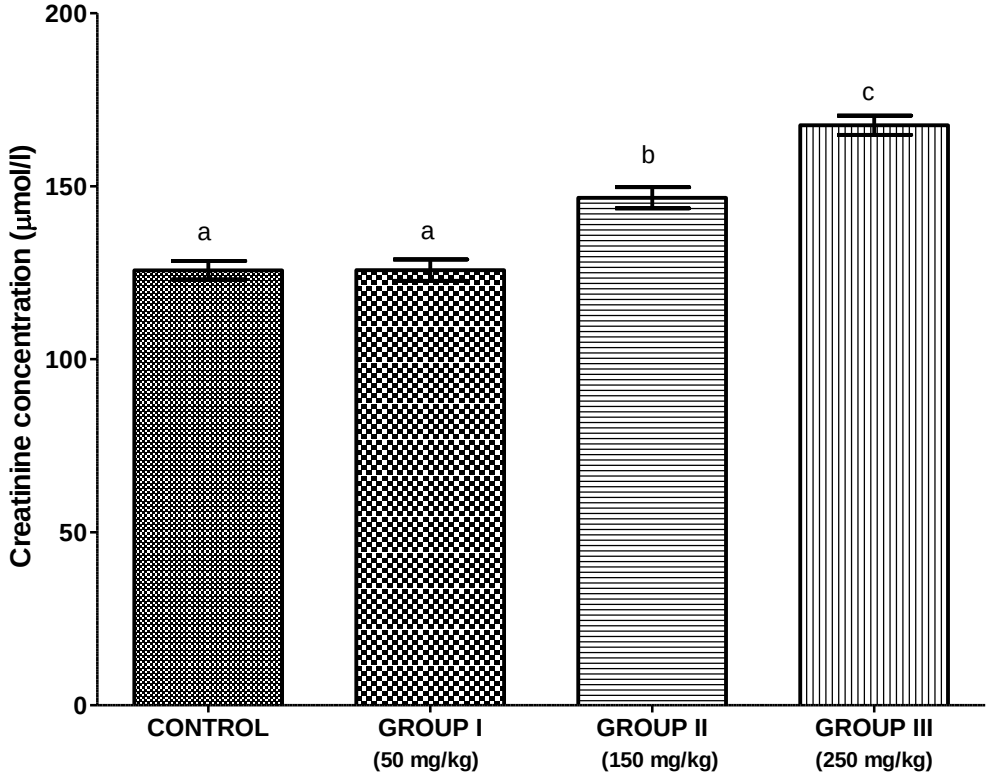

Figure 7: Creatinine concentration (µmol/l) of rabbits administered different doses of *Jatrophacurcas* leaf extract.

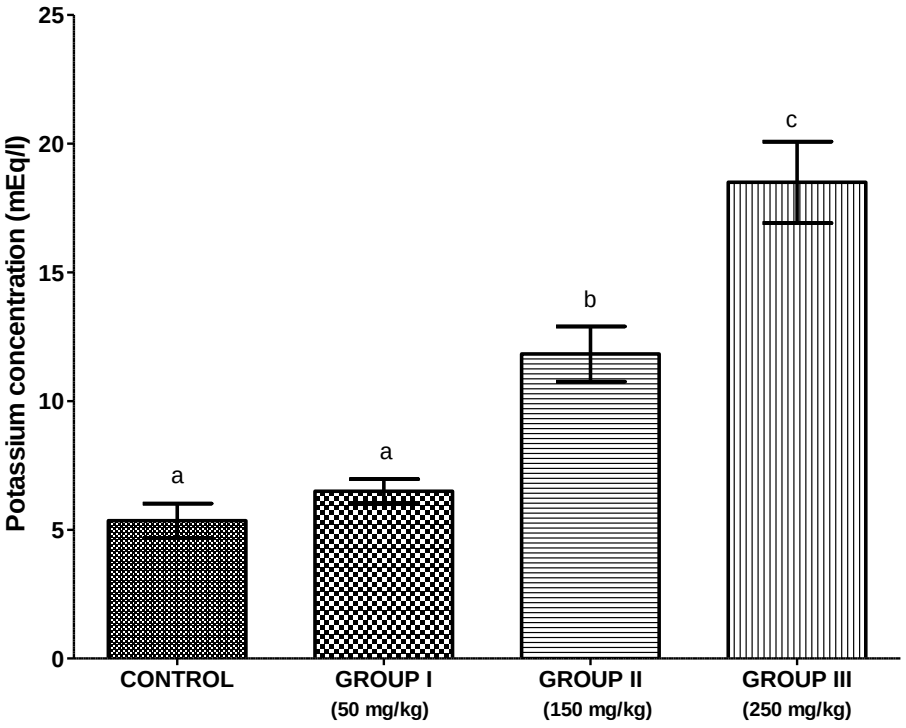

Figure 8: Potassium concentration (mEq/l) of rabbits administered different doses of *Jatrophacurcas* leaf extract.

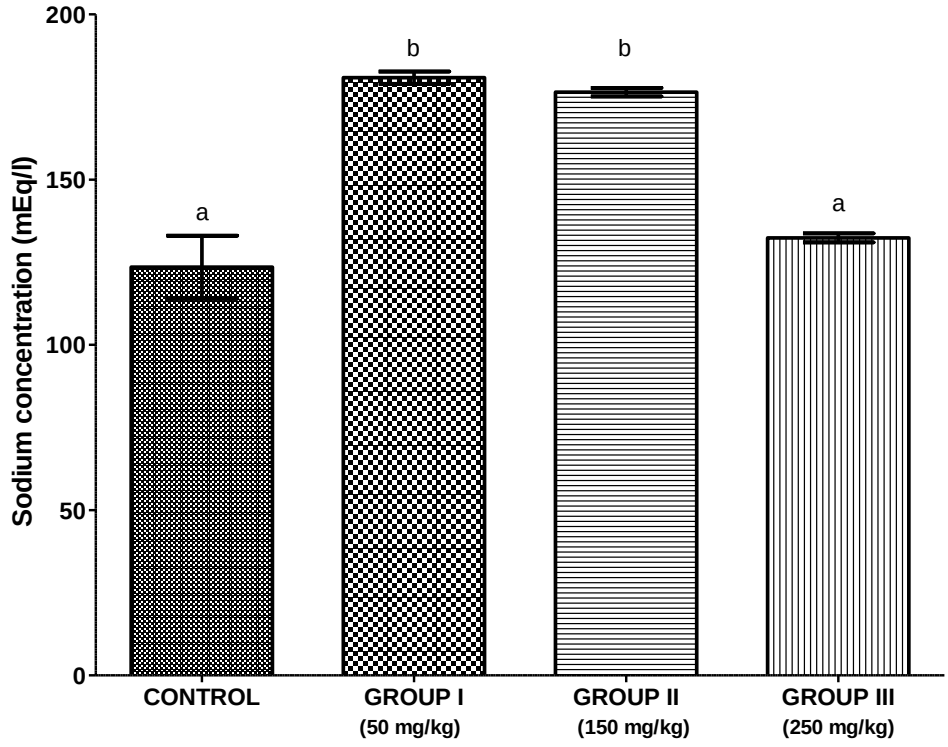

Figure 9: Sodium concentration (mEq/l) of rabbits administered different doses of *Jatrophacurcas* leaf extract.

4. Discussion

Medicinal plants are widely used round the world to cure and manage various ailments. Thus, they form the basic components of our health care system (Ebong, Atangwho, Eyong,& Egbung, 2008). Herbal medicine still remains a major resource for Nigeria and other African countries (WHO, 2013), especially with the advent of healthcare extension programs. These programs are generally based on the assumption that access to and quality of primary healthcare can be improved upon, through transfer of health knowledge and skills to households (Banteyerga, 2011). This changes the thinking, practices and acceptability of herbal medicine by rural dwellers. Healthcare is thus becoming an 'all inclusive affair' as questions concerning safety of drugs are often raised. Knowledge of side effects of medicinal plants have become very imperative in the effective utilization of these agents. *Jatropha curcas* is one of the various medicinal plants that are greatly used in Nigeria for the management of several diseases and this work assessed the safety of ethanol leaf extract of *J.curcas* on normal rabbits.

Treatment of rabbits with 50 mg/kg, 150 mg/kg and 250 mg/kg of *J. curcas* leaf extract resulted in increased activity of the basic liver marker enzymes; ALP (Fig. 1), AST (Fig. 2) and ALT (Fig. 3). Serum activities of ALP, AST and ALT are sensitive indicators of the functionality and integrity of the hepatic system (Meyer and Kulkarni, 2008). The observed increase in the serum activities of these enzymes is an indication of a mild disruption of the normal state of the liver. These observed changes may be attributed to some of the chemical constitutes of *J. curcas.* The phytochemical screen revealed the presence of alkaloids and cyanides amongst other chemicals. Matsuura and Fett-Neto (2015) noted that the toxic

metabolites of alkaloids resulting from actions of microsomal enzymes in the liver, can act locally within the liver cells to cause damage at the chromosomal level. However, the fact that ALT activity did not increase significantly allays fears of overt hepatic dysfunction in the animals.

The study also showed an increase in the concentrations of serum total bilirubin of the rabbits administered the extracts (Fig. 4 and 5). Bilirubin is produced by the hepatocellular system from haem bye-product of degraded erythrocytes. Elevation of bilirubin in the serum is an indication of liver function disruption. Bilirubin is a conventional indicator of liver disease and its elevation in the serum has been associated with hepatocellular damage and hepatobiliary tract obstruction (Emejulu, Alisi, Asiwe, Igwe, Nwogu, & Onwuliri, 2016).

The current study also revealed a significant increase in the serum concentrations of urea and creatinine in animals administered 250 mg/kg of the extract. Increase in serum urea alone may be indicative of excessive protein consumption or a hypercatabolic state, but increase in both serum urea and creatinine concentrations are very sensitive biochemical parameters for the assessment of the status of the kidney and their elevation is suggestive of kidney dysfunction (Awasthy *et al*. 2010). Hence, *J. curcas* leaf extract may negatively affect the kidney when administered at high doses. The serum concentrations of sodium and potassium were also increased significantly in the animals treated with all doses of *Jatropha curcas* ethanol leaf extract (Fig. 8 and 9). This finding is suggestive of a possible mild hyperkalaemic and hypernatremic effects.

Hyperkalemia disrupts normal transmission of electrical signals throughout the nervous system within the body and maintenance of normal heart electrical rhythm. Sodium ion is important for normal muscle and nervous function, and hypernatremia leads to muscle twitching or spasms or even to lethargy. Marked elevations of sodium ion concentration may result in seizure and even coma.

On the other hand, the leaf extract may seem to favour an enhanced renal function by increasing sodium and potassium reabsorption. One could therefore speculate that low concentrations of *J. curcas* leaf extract may be useful in managing hypertension due to its suspected hyperkalaemic effect. This however requires further scientific verification as many other factors would need to be considered.

5. Conclusion

Results obtained from this study showed that oral administration of *Jatropha curcas* leaf extract to rabbits caused significant changes to various serum biomarkers of kidney and liver function. The observed changes were prominent at higher doses of the plant extract, indicating a clear disruption of renal function and a mild disruption of hepatic functions in rabbits. Although the leaf extract has been validated by various studies in rats to contain useful medicinal ingredients which make it a potential agent for the treatment of different ailments, the mild hepatotoxic and nephrotoxic effects of the plant in this study need to be more carefully considered and further investigated.

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