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The Effects of *Eleaphorbia drupifera* Latex on the Liver Function Parameters using Albino Wistar Rat Model

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Abstract: This study was aimed at evaluating the effect of eleophorbia drupifera latex on the histology and liver functions using albino wistar rat model. The area selected for the study was University of Calabar botanical garden, Cross River State, where the latex was tap from eleophorbia drupifera's plant. Twenty-one male albino wistar rats, weighed between 110-130 grams were used for this study. The animals were divided into three groups of seven animals each per group and kept in well-ventilated clean cages for twenty-eight days period. At the end of the experiment, liver function parameters (AST, ALT, ALP, Total Protein, Albumin, Globulin, Bilirubin- both conjugated and unconjugated) were determined using Enzyme Linked Immunosorbent Assay (ELISA) kits while standard method were used in histological evaluation of the organs. The effect of the latex on the liver function of wistar rats after 28 days dose- dependent pattern administration indicated hepatocellular damage with the significant increase in the activities of liver enzymes such as ALT, AST, ALP while the serum bilirubin (serum total bilirubin, conjugated bilirubin and unconjugated bilirubin) was significantly high ($P<0.05$). It is concluded that the latex contains substances that are deleterious to the liver. While the plant may contain active medicinal principles and rich mineral content, caution should be exercised when utilizing the plant for therapeutic purposes.

Key points: Eleophorbia drupifera, Liver enzymes, Histology and active medical principles.

INTRODUCTION

The use of plants of bioactive compound for the management and treatment of various diseases is believed to be the oldest existing means that humanity has employed in an attempt to cope with illnesses. More than eighty percent of the population in the developing nations are dependent upon the traditional or folk systems of medicines mainly because they are easily accessible, widely affordable and cultural familiarity.

Eleophorbia drupifera is the medicinal plant that belongs to family of *Euphorbiaceae,* with wider application in traditional medicine owing to its pharmacological properties like anti-hypertensive, anti-bacterial, anti-tumor etc. (Schiff, 1970). The latex is commonly used as pain relieve antidote, it can be applied topically against ring worm, insect stings, scorpion stings and snake bites. It is also applied to the gums for the treatment of toothache and extraction of worm from guinea worm sore. Similarly, the root bark sap of the plant is use in the dressing of skin rashes wounds (Schiff, 1970).

The latex of *E. drupifera* possesses skin irritant effect, and it is reported to promote inflammatory reactions (Kinghorn and Evans, 1975; Abo, 1994). In addition, the extracts of the latex of *E. drupifera* inhibited replication and cytopathicity of HIV strains, even after delayed treatment as reported by Ayisia and Nyadedoz, 2003.

Statement of the problem

Excess of publications existed on the physiological effects of medicinal plants, and these effects are attributable to the presence of bio-active principles in the plant. Phytochemicals exert a wide range of physiological actions that could be beneficial or harmful to the body depending on the interaction of several factors. The adverse effects exhibited by the plant latex could be due to accumulation of toxic substances and phytochemicals in the host plant which when consumed on long or short term by humans or animals can result in cytotoxicity. Knowledge of plant phytochemical constituents and toxic components is useful for understanding of the group of metabolites either secondary or primary are responsible for an observed physiological as well as biochemical action that are either adverse or beneficial to human. Ingestion and utilization of *E. drupifera* latex for medicinal purposes is very common in most communities in Nigeria and other West African Countries due to its easiest accessibility, cultural affinity and cheapest means of obtaining the plant part. It has been reported extensively that consistent consumption of *E. drupifera* latex for disease treatment result in severe toxic effects such as loss of appetite, drowsiness, loss of hair, swelling of jaw and intestine (Akpiri *et al*., 2013). It has also been reported that the latex promotes inflammatory reactions (Abo, 1994). The increasing incidence of complications associated with the use of *E. drupifera* resulting from generation of free radicals and other toxic metabolites as reported by Abo (1994) calls for serious concern and further evaluation of the toxic components and possible effects of this plant on the liver function parameters becomes imperative.

Justification of the study

Knowledge of the physicochemical properties of plant latex or its extract is useful in ethnobotanical studies as imprecise knowledge on this can be detrimental on the use of plants in ethnomedicine as some are relatively toxic. There is a dearth of report on the determination of the effect of latex of *E. Drupifera* on the liver function inspite of the numerous reported uses, hence the need for this study. Examination of the effects of *E. Drupifera* latex on the liver function parameters of wistar rat model will provide knowledge of the possible health risk that may be posed by continuous use of this plant's latex for therapeutic purposes by man.

Methodology

Study Area

The area selected for the study was botanical garden, University of Calabar and Faculty of Basic Medical Science Animals house, University of Calabar, Cross River, Nigeria.

Collection and Analysis of Samples

Fresh latex of *Eleophorbia drupifera* was obtained from the botanical garden, University of Calabar, Calabar. This plant was authenticated by a botanist in the herbarium unit, Department of Botany, University of Calabar, Calabar.

Extraction and preparation of Latex Solution of *Eleophorbia drupifera*

A clean sample bottle was washed with detergent and distilled water, and was sterilized in an autoclave at 40^0C with 16mmHg for four hours to void any contamin ants. The latex was tapped from the *E. drupifera* tree using tissle. The liquid latex was collected into the treated bottle and corked to prevent possible microbial contamination. Four-point eight percent (4.8%) solution of diluted latex was prepared by dissolving 0.5ml of the raw latex in 10mls of distilled water. The prepared latex was preserved in the refrigerator at the temperature of 4^oC until require for administration.

Experimental Animals

A total of 21 male Wistar rats weighing between 110-130 grams were purchased from the animal house of the Department of Physiology, Faculty of Basic medical Science, University of Calabar, Calabar, Cross River State, Nigeria. The animals were allowed to acclimatised for a period of seven

days in the Department of Biochemistry's animal house, Faculty of Basic medical Science, University of Calabar, Calabar, Cross River State, Nigeria. Thereafter, they were reweighed and were housed in conventional standard wooden cages with top mesh wire covers under standard laboratory conditions (relative humidity of 45%, room temperature of 25^0C) and were exposed to twelve hours light and dark cycle with adequate ventilation. They were fed with normal rat chow or pellet and water *ad libitum* for one week, before administration of the latex for 28 days.

Animal grouping and administration

The study comprised of twenty-one mature male animals divided into three groups of seven animals per group:

Group A (control): Animals in this group served as control and were fed normal rat chow or pellet and water *ad libitum*.

Group B (Low dose): Animals in this group served as a low dose group and were orally administered with 0.5ml of 4.8% prepared solution of the latex of *E. drupifera* once daily while they were fed normal rat chow or pellet and water *ad libitum*.

Group C (High dose): Animals in this group served as high dose group and were orally administered with 0.7mls of 4.8% prepared solution of the latex of *E. drupifera* while they were fed with normal rat chow or pellet and water *ad libitum*.

Table1: Experimental design.

Group	Number of Animals	Regimen + water *ad libitum*	Duration
A	7	Normal Pellet	28 days
B	7	Low Dose (0.5ml/kg/bw) of diluted latex of *E. drupifera*	28 days
C	7	High Dose (0.7ml/kg/bw) of diluted latex of *E. drupifera*	28days

Blood Collection

After 28 days period of administration and feeding, the rats were subjected to an overnight fast before sacrifice. In accordance with the standard guidelines (European Treaty Series, 2005), they were anaesthetized with chloromethane. Thereafter, their thorax were opened and whole blood collected via cardiac puncture with sterile syringes and needles into a well-labelled plain sample bottles for assay of biochemical indices. The samples (blood) were allowed to stand for one hour to clot at room temperature and spinning with MSE model (England) table-centrifuged at 2000rpm for 10mins to obtain the serum and later stored in a refrigerator until it is needed for biochemical assay. While the organ (liver) exercised from the animals was fixed into sterile universal bottles with 10% buffered formalin for histopathological assessment.

Biochemical Evaluation

All biochemical investigations were carried out using standard methods with the help of Randox Kits.

Histopathological Assessment

The histology of the liver in the control and test groups were carried out by the principle of Drury and wallington. Tissue fixed in 10% buffered formaldeyde were dehydrated via series of graded concentrations (50%, 70% and 90%) of ethanol. The specimens were cleared in xylene, and mounted using a drop of Canada balsam on the specimens and covered with the glass cover slip for microscopic study. High powered photographs of the sections of the liver were taken in bright field at X40 and X100.

Statistical Analysis

Data obtained from the experiment was expressed as Mean ± SEM and analysis done using the Analysis of Variance' ANOVA'. f-ratio and Statistical Package for Sectional Scientists (SPSS version 2.10). Values at $P < 0.05$ were considered significant in comparison with appropriate control.

RESULTS

Effects of the latex of *Eleophorbia drupifera* on serum liver enzymes

The result of the effects of the latex of *Eleophorbia drupifera* on serum liver enzymes are presented in table2.

AST: Aspartate Aminotransferase

The serum concentration of AST of animals in the test groups: low dose (178.57 ±1.19µmol/l) and high dose (198.57± 1.83 µmol/l) respectively were significantly greater ($P < 0.05$) than the control group (159.43 ± 1.59µmol/l).

ALT: Alanine Aminotransferase

The serum concentration of ALT of animals in the test groups: low dose (80.86±1.06µmol/l) and high dose (131.29±2.20µmol/l) respectively showed significant increase ($P < 0.05$) as compared to control (68.29 ±1.42µmol/l).

ALP: Alkaline Phosphatise

The serum concentration of ALP of animals in the test groups: low dose (274.00 ±1.62µmol/l) and high dose (316.00 ±3.02µmol/l) respectively were significantly greater ($P < 0.05$) than control group (201.00 ±1.44µmol/l).

Comparatively, low and high dose groups showed significant increase in the activities of the three enzymes in the dose- dependent pattern as compared to the enzymes in the control groups

Effects of the latex of *Eleophorbia drupifera* on serum bilirubin

The result of the effects of the latex of *Eleophorbia drupifera* on serum bilirubin is presented in table3.

Serum Total Bilirubin:

The serum concentration of total bilirubin of animals in the groups: low dose (16.53± 0.28µmol/l) and high dose (22.43± 0.57µmol/l) were significantly higher ($P<0.05$) compared to control (12.87 ± 0.38µmol/l).

Conjugated Bilirubin:

The serum concentration of Conjugated bilirubin of animals in the groups: low dose (11.19± 0.86µmol/l) and high dose (15.37± 0.45µmol/l) showed significant increases ($P < 0.05$) compared to control group (6.57± 0.25µmol/l).

Unconjugated Bilirubin

The serum concentration of Unconjugated bilirubin of animals in the groups showed insignificant changes ($P>0.001$) with reduction in the group administered low dose (5.34± 0.81µmol/l) and increase in the high dose group (7.06± 0.43µmol/l) all compared to control (6.30± 0.25µmol/l).

Percentage Conjugation of Bilirubin:

The serum concentration of percentage conjugated bilirubin of animals in the groups increased significantly ($P < 0.05$) in groups administered low dose (67.59± 4.88 percent) and high dose (68.61± 1.59 percent) of the latex relative to that of control (51.06± 1.24 percent).

From the above results, the serum conjugated bilirubin, unconjugated bilirubin and total bilirubin levels were greater in the high latex dose group than the low latex dose and therefore indicate that serum bilirubin levels increased in direct proportion to the dose of the latex administered.

Effects of the latex of *Eleophorbia drupifera* on serum proteins

Results of the effects of the latex of Eleophorbia drupifera on serum proteins

Concentration is presented in table 4.

Total Protein:

The serum concentration of total protein of animals in the groups administered low dose (62.86±0.98g/l) and high dose (55.86±0.51g/l) were significantly lowered (P<0.001) compared to control (68.29 ± 0.63g/l).

Albumin:

The serum concentration of albumin of animals showed significant decrease (P<0.001) in group administered high dose (32.29± 0.48g/l) and non-significant decrease (P>0.001) in the low dose group (37.14± 0.48g/l) all compared to control group (40.29± 2.84g/l).

Globulin:

The serum concentration of globulin of animals showed significant reduction in groups administered low dose (24.86 ± 0.55g/l) and high dose (24.71± 0.80g/l) relative to control (31.14 ± 0.37g/l).

Table 2: Effects of the latex of Eleophorbia drupifera on serum liver enzymes. Values are expressed as Mean±Standard Error of Mean (SEM), n=7. * Significant at P< 0.05 compared with the control group.

Group	AST (µmol/l)	ALT (µmol/l)	ALP (µmol/l)
Control	159.43 ± 1.59	68.29 ±1.42	201.00 ±1.44
Low Dose	178.57 ±1.19	80.86±1.06	274.00 ±1.62
High Dose	198.57± 1.83	131.29±2.20	316.00 ±3.02

Table 3: Effects of the latex of Eleophorbia drupifera on serum bilirubin. Values are expressed as Mean±Standard Error of Mean (SEM), n=7. * Significant at P< 0.05 compared with the control group.

Group	Total Bilirubin (µmol/l)	Conjugated Bilirubin (µmol/l)	Unconjugated Bilirubin (µmol/l)	Percentage Conjugation of Bilirubin (µmol/l)
Control	12.87 ± 0.38	6.57± 0.25	6.30± 0.25	51.06± 1.24
Low Dose	16.53± 0.28	11.19± 0.86	5.34± 0.81	67.59± 4.88
High Dose	22.43± 0.57	15.37± 0.45	7.06± 0.43	68.61± 1.59

Table 4: Effects of the latex of Eleophorbia drupifera on serum protein. Values are expressed as Mean±Standard Error of Mean (SEM), n=7. * Non-significant at P< 0.001 compared with the control group

Group	Total Protein (g/l))	Albumin (g/l))	Globulin (g/l))
Control	68.29 ± 0.63	40.29± 2.84	31.14 ± 0.37
Low Dose	62.86±0.98	37.14± 0.48	24.86 ± 0.55
High Dose	55.86±0.51	32.29± 0.48	24.71± 0.80

Effects of the latex of *Eleophorbia drupifera* on histology of the liver

Photomicrographs of the control group (Plates 1) shows normal histology of liver with distinct central vein, sinusoids, hepatocytes and the portal triad.

Photomicrograph of the low dose group (Plates 2) also show normal histological appearance of the central vein, sinusoids and hepatocytes as well as the portal triad consisting of the hepatic artery, portal vein and bile duct.

Photomicrograph of the high dose group (Plates 3) show congested portal vein, with inflammatory filtrate around the hepatic artery and distorted hepatocytes.

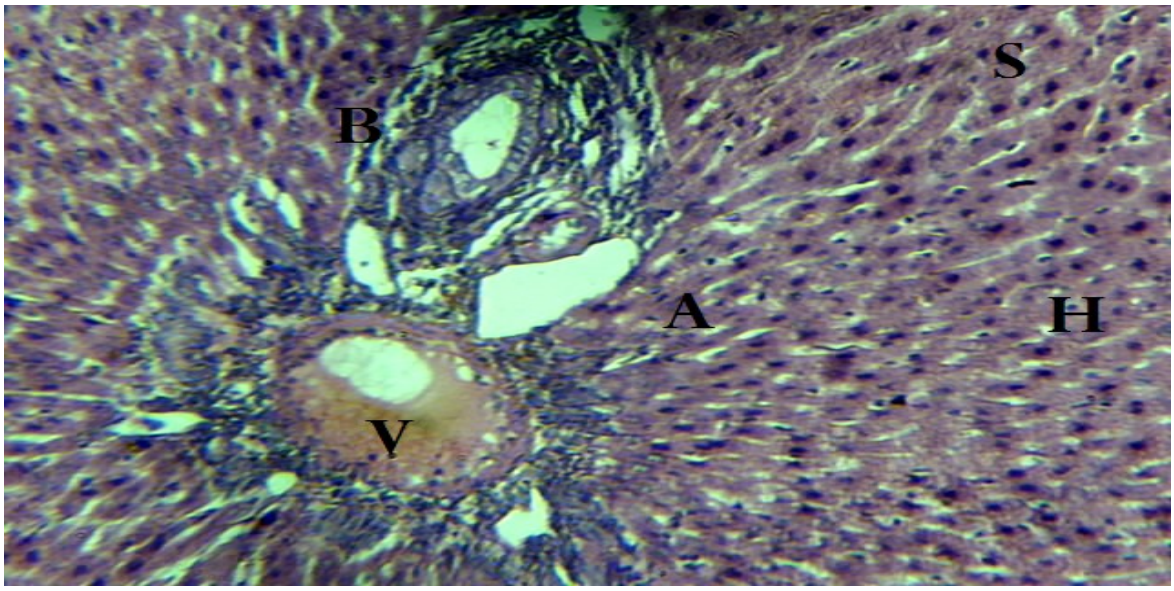

Plate 1: Photomicrograph showing liver sections of rats in the control group (Group 1). Magnification X100 (H&E Stain). Photomicrograph shows normal histology of liver. The portal triad consisting of hepatic artery (A), bile duct (B) and portal vein (V) are prominent. The central vein (CV), sinusoids (S) and hepatocytes (H) also show normal liver cytoarchitecture.

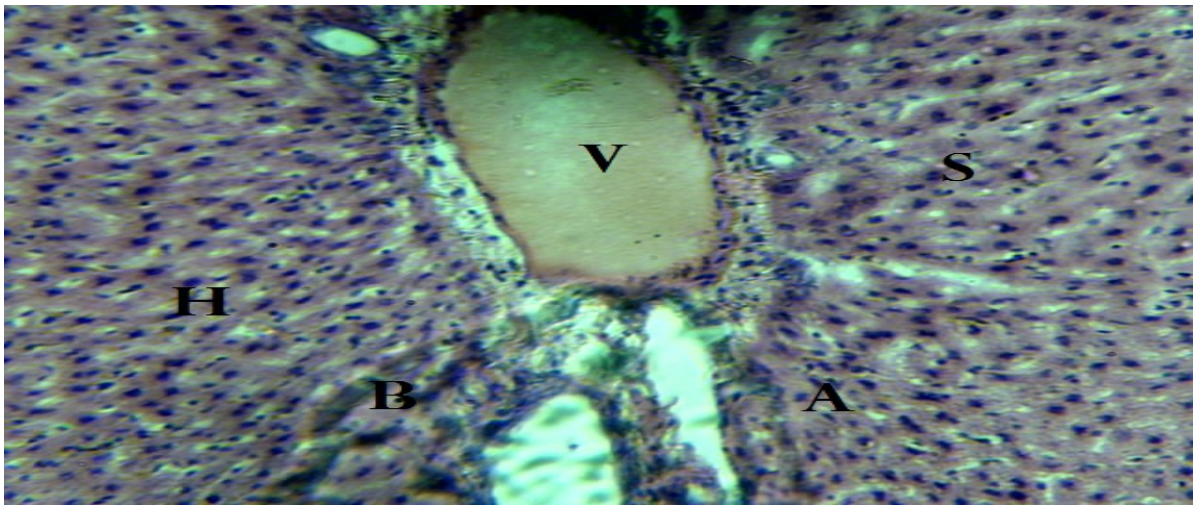

Plate 2: Photomicrograph showing liver sections of rats in the low dose group (Group 2). Magnification X100 (H&E Stain). Photomicrograph shows normal histology of the liver. Seen are the portal vein (V), hepatic artery (A) and bile duct. The hepatocytes (H) and sinusoids(S) are well arranged

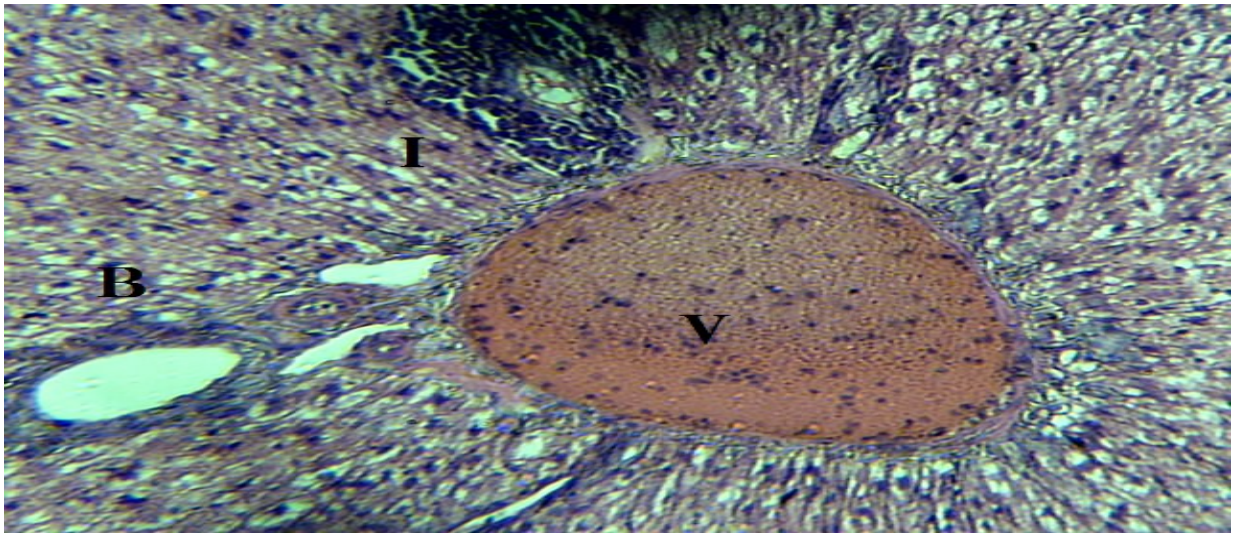

Plate 3: Photomicrograph showing liver sections of rats in the high dose group (Group 3). Magnification X100 (H&E Stain). Photomicrograph shows congested portal vein (V), with inflammatory filtrate around the hepatic artery (A) and hypertrophy of the hepatocyes.

DISCUSSION

The current study was embarked upon to examine the effect of *Eleophorbia drupifera* latex on Serum liver enzymes, serum proteins and bilirubin concentrations which are useful indicators of health status of the liver. Measurement of hepatic serum enzymes such as ALT, AST and ALP serve as a means for assessment of liver health status and estimation of these enzymes is a more reliable test for detecting liver abnormalities Burtis, *et al.,* 2012 and it is employed as markers of hepatocellular damage Burtis, *et al.,* 2012. Damage to hepatic cells result in the leakage of these cellular enzymes into the blood circulation, culminating in an abnormal rise in the activities of these marker enzymes in the blood. As the site of synthesis of albumin and most serum proteins, the levels of albumin with its normal range of **3.5 – 5.0 g/dL (**Tietz, 2014**)** and total protein with its normal range of 6.0 to 8.3 g/dl (McPherson & Pincus, 2017) reduce significantly in severe hepatic diseases. Although, the change in concentration of albumin is usually non-specific to the liver, albumin is often reduced in liver cirrhosis with a compensatory rise in β –globulin and γ– globulin levels (Mayne, 1994). Hepato-cellular injury is often accompanied by cellular swelling that can constrict bile canaliculi and thereby result in cholestasis (obstruction of bile flow). Cholestasis is often accompanied by abnormal concentrations of bilirubin in blood. Cholestasis may be as a result of intrahepatic or extrahepatic causes. Intrahepatic cholestasis may be due to hepatocyte damage. Extrahepatic cholestasis is often due to biliary stones, or inflammation of the biliary tract. This obstruction leads to regurgitation of substances normally excreted in the bile into the blood circulation and subsequently result in accumulation of substances such as conjugated and unconjugated bilirubin, bile salt and cholesterol in the blood. Liver damage in the case of intrahepatic cholestasis leads to alteration in uptake, conjugation and secretion of bilirubin, hence, a rise in unconjugated bilirubin levels higher than conjugated form. In cases of extrahepatic obstruction, conjugated form tends to be higher (Nsirim, 1997; Bolarin 2010). In this study, administration of low dose and the high dose of the latex of *Euphorbia drupifera* caused significant elevations of all the three hepatic serum enzyme markers (AST, ALT and ALP) in a dose dependent pattern (Table 2). Serum total bilirubin and conjugated bilirubin concentration of groups administered low dose and high dose were significantly higher compared to control (Table 3). Levels of unconjugated bilirubin showed non- significant changes (Table 3). Serum total bilirubin, conjugated bilirubin and unconjugated bilirubin were higher in the high latex dose group than the low latex dose indicating a dose-dependent alteration in serum bilirubin levels. Serum total protein

concentration, albumin and globulin levels of groups administered low dose and high dose were significantly lowered compared to control (Table 4). It is evident in the light of the above findings that administration of the latex of *Euphorbia drupifera* to the rats resulted in hepatocellular injury in a dose dependent manner. It can be inferred that *Euphorbia drupifera* contains substances that are capable of causing injury to body tissues, Okon *et al.,* (2024). The low polyphenol content of the latex could not offer protection from the deleterious effect of these substances which according to phytochemical screening by Okon *et al.,* (2024) might be an alkaloid or saponin compounds of the latex. It is known that alkaloids (though they exhibit pharmacological activities) are toxic substances produced by plant and often serve as feed deterrents to herbivores. It has been documented that latex of *Euphorbia* genus is poisonous to grazing animals. According to Isaac *et al.,* (2010) acute toxicity of *E. heliscopia (*specie of the same genus) in various organs of rats has been reported. According to the author, "the main phytochemical compounds in the latex of *Euphorbia drupifera* are of di- or tri-terpene esters (e.g., resiniferatoxin), 1-inositol, catechuic and pyrrogalic tannins and xanthoramnine (an alkaloid). The latex acts as a wound healer as well as adeterrent for herbivores. The terpene ester constituent determines how caustic and irritating to the skin. In contact with mucous membranes (mouth, eyes, nose), the latex can induce extremely painful inflammation. In the course of experiment, the animals were discovered to react with irritating effect to the letax (which confirm the present of resiniferatoxin or terpene ester) much stronger than capsaicin, the "hot" substance found chillies". Adedapo *et al., (*2005) have reported that *Euphorbia hirta* contains substances that have potential adverse effects on serum biochemistry of rats and advised that *E. hirta*must be used with caution as a medicinal plant.

Beside these hazardous effects, the saponin and polyphenol content of *eleophorbia drupifera* justifies its traditional use for medicinal purposes such as treatment of hypertension and diabetes. Saponins have been shown to possess hypocholesterolemic activities while polyphenols are known for their strong antioxidant activities making this plant useful in the management of cardiovascular health related conditions. However, the alkaloid content of the plant especially the latex calls for caution in the use of the plant for therapeutic purpose because of potential cytotoxic effects associated with the plant. Besides, as stated by Roseline *et al.,* (2013), several terpene esters are also known to have carcinogenic effects. The negative effect of the latex on serum parameters of liver health is further supported by the photomicrographs of the liver obtained for the test groups (plates 1-3). The photomicrograph showed hepatocellular inflammation in the group administered high dose of the latex (plates 3) while the low dose and control groups had normal histology (plates 1-2). This histological examination supports the assertion that the cytotoxic effect of the latex in dose-dependent and may as well depend on the duration of exposure as the liver did not show any remarkable changes in weight at the time of examination.

It is interesting to also note that in the course of the experiment, the test animals became less active, weak, allergic to the latex as evident in coughing; mild nasal bleeding as well as depressed appetite to food were observed. These observations are apparently related to the reported and observed cellular toxicity reported by Konya *et al.,* (2013) on the latex of the *euphobia*genus on animals. Roseline *et al.,* (2013) reported behavioural changes such as continuous itching immediately after feeding with leaf extract, reduced activity, loss of appetite, drowsiness, swollen jaws and subsequent death. A similar observation was noted in this study. Initial concentrated latex resulted in instant death of mice and rats. Rat's latter administered diluted latex survived throughout the experimental period but gradual decline in health was noticed especially in the high dose group and eventual death.

Summary and conclusion

In conclusion, the effect of the *Eleaphorbia drupifera* latex on livers of rats were carried out in this study. Results of the investigation show that administration of the latex of *Eleophorbia drupifera* resulted in hepatocellular injury in a dose-dependent pattern.

Hence, Eleophorbia drupifera latex contains substances that causes injury to the liver. The low polyphenol content of the latex could not offer protection from deleterious effect of these

substances which according to phytochemical screening might be an alkaloid or saponin compounds. While the plant may contain active medicinal principles and rich mineral content, caution should be exercised when utilizing the plant for therapeutic purpose.

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