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HEPATO-CURATIVE EFFECTS OF CRUDE PHENOL ROOT EXTRACT OF SODDOM OF APPLE (*C. Procera*) ON CCL_4 INDUCED HEPATOTOXICITY IN ALBINO RATS

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ABSTRACTS
The effects of phenol root extracts of C. procera and livolin on liver function indices of CCl_4 induced hepatotoxicity was evaluated on forty (40) albino rats. The animals were grouped into four (I, II, III and IV) of 10 rats each, 120mg/kg of CCl_4 was administered to rats in group II, III, and IV intramuscularly followed by oral administration of 10mg/kg livolin and phenol root extract of C. procera to group III and IV respectively. Analysis of variance (ANOVA) for multiple comparisms test were used to compare the result of the liver and kidney biochemical parameters from the test and control groups at 10 days interval for 20 days. The hepatic biochemical markers Alanine Aminotransferase (ALT), Aspartate Amino Transferases (AST), Alkaline Phosphatases (ALP) of the toxicant group (Gp II) were significantly higher (P<0.001), while group III (treated with livolin) statistically decreased (P<0.05) when compared with control (Gp I), this confirms the toxicity and treatment with livolin respectively. Oral administrations of the extracts at 10 days exposure lower all the liver function markers and increase the concentration of urea and albumin. This is an indication of the hepatocurative effect of the extract against CCl_4 induced rats. However, at 20 days exposure the activities of the liver markers were raised. The Histopathological photomicrograph showed moderate cytolysis and karyolysis.
Keywords: Hepatocurative, Calotropis Procera, hepatotoxicity, cytolysis, Carbon tetrachloride.

1.0 INTRODUCTION

Traditional medicine is the oldest form of health care in the world and is used in the prevention and treatment of physical and mental illnesses. Different societies historically developed various useful healing methods to combat a variety of mild and life-threatening diseases. Traditional Medicine is also variously known as complementary and alternative or ethnic medicine, and it still plays a key role in many countries today (Haidan *et al.*, 2016)

Calotropis procera is a wild growing tropical plant which possesses various medicinal properties. *C. Procera* belongs to the family Asclepiacea (milkweed family) of the Genus Calotropis R. Br. (Calotropis). *Calotropis procera* or Giant milkweed is also known as sodom apple, calotrope, French cotton, small crown flower (English), Tumfafiya (hausa), Epuko (Nupe), Common names; auricular tree, dead sea apple, swallow-wort, apple-of-sodom, gian-milk weed, madar mudar, ruberbush, small crownflower, sodom's milkweed algodón de seda, bomba (Spanish), cotton-france, arbre de soie, and bois canon (French), Latin name: *Calotropis procera* (Ait) (Aliyu, 2006). In Nigeria, traditional medicine, different parts of the plant have been used as purgative, antihelminthic and also in the treatment of diseases, such as leprosy, ulcers, tumors, piles, hepatitis.

2.0 MATERIAL AND METHODS

2.1 Plant Material and Extraction

Root of *C. procera* was collected from Kanya Babba, Babura local government, of Jigawa State. Specimens of the leaves and bark were removed. The root was dugged using hoe and a shovel. The root was allowed to dry under the shade, it was then ground using mortar and pestle. The extract of the plant root was prepared by weighing and soaking of the root powder in phenol (BDH) for 2 weeks.

2.2 Acute toxicity test in albino rats:
Acute toxicity tests of phenol extract of *C. procera* roots were performed separately in male and female rats according to OECD guideline for chemicals tests (OECD, 2001). The limit test at dose level of 13 mg/kg body weight was administered orally (gavage) to six fasted males and females rats per extract. The females were nulliparous and non-pregnant. The animals of different groups were individually observed for 120 min post-treatment and at least once daily for 14 days for mortality and signs of toxicity such as changes in skin and fur, eyes, mucus membranes, convulsion, salivation, diarrhea, lethargy, sleep and coma.

2.3 Experimental animals
Forty (40) albino rats were obtained from the Animal House of Physiology Department, Faculty of Basic Medical Sciences, College of Health Sciences, Bayero University, Kano. The rats were kept in the Departments of Biological Science, Bayero University, Kano for two weeks acclimatization, before they were weighed and separated into different sexes (males and females). The animals were grouped into four groups (I, II, III and IV) of 10 animals each. Group II, III and IV were administered with 120mg/kg CCl_4, 10mg/kg livolin and 13mg/kg phenolic extract of *C. procera* roots respectively; while group I serves as a control.

2.4 Biochemical assay
The liver function indices (AST, ALP, ALT, BIl., ALB) were carried out according to the procedure explained by Clementine and Tar Choon, (2010), while the kidney function test and electrolytes were carried out according to the procedure of Gowder *et al.,* (2010)

2.5 Statistical Analysis
Data were subjected to one-way analysis of variance (ANOVA) and treatment mean were compared to positive and negative control by using Tukey-Kramer Multiple Comparisons Test, a component of GraphPad Instat3 Software (2000) version 3.05 by GraphPad Inc.

3.0 RESULT AND DISCUSSION
Table 1 and 2 showed serum enzyme activities of (ALT, AST, and ALP) and concentrations of albumin (ALB), total bilirubin (T. BIL), and direct bilirubin (D. BIL) for groups of rats orally administered with phenol Extract of *C. procera* root and livolin 10 and 20 days respectively.

The result from this research work indicated that CCl_4 induced toxicity (group II) rats have elevated liver function indices; serum activities of AST, ALT, ALP, total and direct bilirubin as compared to positive control (group I). The increased serum level of the enzymes is due to cellular leakage as shown by Alhassan *et al.,* (2009). In CCl_4 induced toxicity, CCl_3° is produced as a free radical which binds to lipoprotein leading to peroxidation of lipid of endoplasmic reticulum. The fact that ALT is raised at both 10 and 20 exposure indicates that CCl_4 have induced toxicity in accordance with Alhassan *et al.,* 2009 who reported that rats treated with high dose of CCl_4 developed profound hepatic damage and oxidative stress as evidenced by increase in the serum activities of ALT, AST, ALP, total and direct bilirubin that are indicators of cellular leakage and loss of functional integrity of cell membrane in liver.

Daily oral administration of 13mg/kg phenol root extract of C. *procera (PRECP)* produces statistically significant decrease in serum ALB. Hypoalbuminaemia is very common in many diseases including liver disease and kidney diseases. The significant decrease in serum albumin here may be due to liver disease induced by CCl_4 (Alhassan *et al.,* 2009). ALT is considered a more specific and sensitive indicator of hepatocellular injury than AST in rats and dogs (Clementine *et al.,* 2010). The magnitude of ALT increase is usually greater than that of AST when both are increased due to hepatic injury, in part because of the longer half-life of ALT and its higher in liver compared to other tissues and the greater proportion of AST that is bound to mitochondria (Uba *et al.,* 2017). Hepatic dysfunction associated with increased serum ALT activity, with or without increased AST activity, includes hepatocellular necrosis, injury, or regenerative/reparative activity (Clementine *et al.,* 2010). The significant increase in T. bilirubin indicates that too much heamoglobin is being destroyed or may be the liver is not actively treating the haemoglobin it is receiving while the significant increase in D. bil. Indicates that the bile is not being properly excreted which may be as a result in the obstruction in the bile duct or gall bladder (Clementine *et al.,* 2010). Therefore, the increased ALT and T. Bilirubin after 20 days exposure also indicates toxicity either due to long term exposure or the toxic effect of the solvent phenol as it was reported that phenol is neurotoxin as it shuts down the neural transmissions system .It also causes dermatitis, lung edema, can affect the heart and kidney (Gowda *et al.,* 2010).

These findings were further confirmed with Histopathological studies. The Histopathological examination clearly reveals that the hepatic cells and central veins were similar to normal tissue at 10 days in group treated with crude phenol root extract of *C. procera* (13 mg/kg) and treated group (livolin group) in contrast to the group which received CCl_4.

Thus, *C. procera* can be considered as hepatocurative drug as it restores liver damage induced by CCl4 at days. Hence, this extract can be used in poly herbal formulations to provide a synergistic effect with other hepatocurative drugs and thereby preventing the process of initiation and progress of hepatocellular disease (Ibrahim *et al.*, 2016).

TABLE 1: **Serum activities of ALT, AST and ALP, and concentration of ALB, T. BIL and D. BIL for groups of CCl_4 induced hepatotoxicity rats orally administered with phenolic extract of *C. procera* root and livolin for 10 days.**

GROUP	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ALB (mg/dl)	T.BIL (mg/dl)	D.BIL (mg/dl)
I(control)	32 ±4.5	44.6±5.08	92.0± 6.44	4.26 ± 0.24	1.37±0.17	4.0±0.3
II	40 ± 4.1[a]	64.7± 8.6[b]	281 ± 22.5[a]	1.78 ± 0.25[a]	1.8 ± 0.09[b]	8.0 ±0.27[b]
III	35 ± 2.5	44.6±6.77	99.8±2.168	2.9 ± 0.122[a]	1.39 ± 0.25	2.1±0.2
IV	36 ± 1.00	49.2 ± 5.2	110± 6.124	2.9 ± 0.123[a]	1.2 ± 0.08	6.43 ±0.4

Values in the same column with (a) and (b) are significance at P< 0.001 and P< 0.01 respectively when compared with the control.

TABLE 2: **Serum activities of ALT, AST and ALP, and concentrations of ALB, T. BIL and D.BIL for groups of CCl_4 induced hepatotoxicity rats orally administered with phenolic extract of *C. procera* root and livolin for 20 days.**

GROUP	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	ALB (mg/dl)	T.BIL (mg/dl)	D.BIL (mg/dl)
I	23.8±9.58	43.6±3.286	89.4±4.535	3.5± 0.08	0.9± 0.20	0.85±0.1
II	45.6±4.67[a]	59.4±9.43[b]	270±21.335[a]	1.3 ± 0.2[a]	1.43±0.05[b]	2.2± 0.4[a]
III	20.8±5.891	40.6±5.595	95.6±3.130	2.2 ± 0.5	1.118±0.08	1.03±0.2
IV	32±6.819[c]	26±4.183[a]	96±2.449	2.39±0.013	1.5 ±0.17[b]	0.8±0.05

Values in the same column with (a), (b) and (c) are significance at P< 0.001, P< 0.01 and P<0.05 respectively when compared with the control.

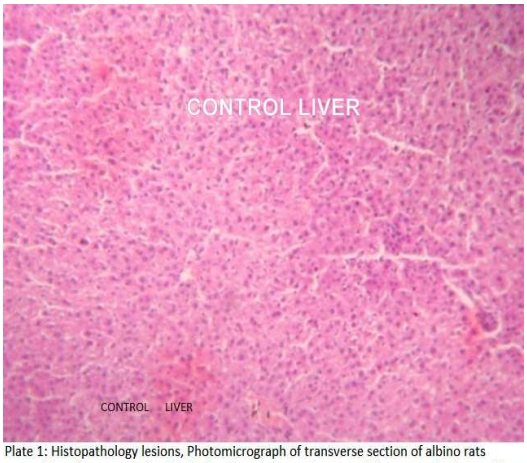

Plate 1: Histopathology lesions, Photomicrograph of transverse section of albino rats control liver from group A with distinct hepatocytes : hepatocellular control veins and the portal triads. H & E stain x 10

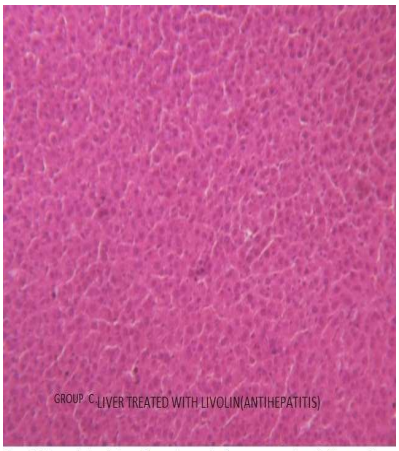

Plate 3: histopathology lesions, Photomicrograph of transverse section of albino rats liver treated with livolin; shows normal liver architecture with the hepatocytes arranged as thin plates separated by fine vascular sinusoid. H & E stain x 10.

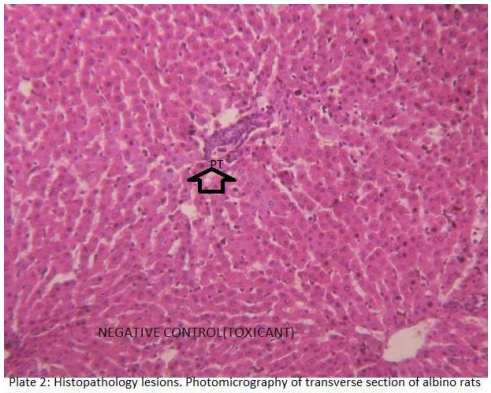

Plate 2: Histopathology lesions. Photomicrography of transverse section of albino rats toxicant of group B. showing severe liver damage with perilobular liver necrosis of the portal area. H & E stain x 10

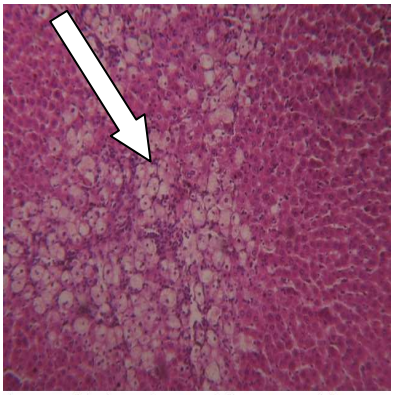

Plate 7: Histopathology lesions ; Photomicrograph of transverse section of albino rats test group G treated with phenolic extract of C. *Procera* root, showing moderate cytolysis and karyolysis. H & E stain x 10

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