

Hepatocurative Effects of Methanolic Root Extract of Sodom Apple (*C. PROCERA*) on CCL₄ Induced Hepatotoxicity Rats

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Abstract: The effects of methanolic root extracts of *C. procera* and livolin on liver function indices of CCL₄ induced hepatotoxicity rats was evaluated. Forty (40) albino rats were grouped into four (I, II, III and IV) of 10 rats each, 120mg/kg CCL₄ was administered to rats in group II, III, and IV intramuscularly followed by oral administration of 10mg/kg livolin and methanolic root extract of *C. procera* to group III and IV respectively. Group I and II serves as positive and negative control. 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 group at 10 days interval for 20 days. The hepatic biochemical markers alanine aminotransferase (ALT), Aspartate amino transferase (AST), alkaline phosphatase (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 lower all the liver function markers and increase the concentration of urea and albumin. This show the hepatocurative effect of the extract against CCL₄ induced rats. These effects may be due to the active components present in the root similar to those found in livolin.

Keywords: *Calotropis procera*, hepatotoxicity, livolin, carbon tetrachloride

1. Introduction

Calotropis procera belongs to the family Asclepiaceae (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, giant-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) Ait [1].

Chemicals in the root includes; Benzoylisolineolone, benzoylineolone, isolineolone and lineolone. Chemicals found in the bark of the tree include; Alpha-amyrin, asclepiad, β -amyrin, α -sitosterol and taraxasterol. Chemicals that are found on the latex exudate of the tree include; γ -lactuceryl, γ -lactuceryl-acetate, γ -lactuceryl-isovalerate, calactin, calotoxin, calotropin, caoutchouc, histamine, proceroside, syriogenin, trypsin, uscharidin, uscharin, uzarigenin and voruscharin. Chemicals found in the seed include; Calotropine, coroglaucogenin, corotoxigenin, fat, frugoside, laurane, linoleic acid, melissyl-alcohol, oleic acid, palmitic acid, protein, stigmasterol and water. Chemicals found in the leaf include; arabinose, calotropagenin, D-glucosamine, glucose, mudarine and rhamnase. Chemicals found in the plant include; giganteol, gigantol, isogiganteol, pseudocalotropagenin, taraxaserol-benzoate, and 3-O-acetyl-calotropin [1].

The following activities have been reported for *Calotropis gigantea*; prevention of insulin resistance hepatoprotective [2] antidiarrhoea, antipyretic and analgesic [3]

antiinflammatory [4] analgesic activity in Eddy's hot plate, and acetic acid-induced writhings and wound healing activity [5]. The milky juice of *Calotropis gigantea* has been reported as a violent purgative and gastrointestinal irritant and used for inducing abortion (Srivastava *et al.*, 2007). The alcohol extract of the flower of *Calotropis gigantea* reported analgesic activity in chemical and thermal models in mice. The crude latex extract exhibited strong proteolytic activity, hydrolyses casein, human fibrinogen and crude fibrin clot in a dose-dependent manner [6]. The aim of this study therefore, is to assess the hepatocurative effects of methanolic extract of *Calotropis procera* in CCL₄ induced hepatotoxicity rats.

2. Material and Methods

2.1 Plant Materials

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 of *Calotropis procera* 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 methanol (BDH) for 2 weeks. The mixture was then filtered using siever, the residue was thrown away while the extract was allowed to dry up at room temperature. The resultant powdered materials were used to prepare the required concentration by dissolving 1g of each powder in 100ml distilled water to make a concentration of 20mg/100ml.

2.2 Experimental Animals

Forty (40) albino rats were obtained from the animal house of physiology Department, faculty of medicine, 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 CCl₄, 10mg/kg livolin and methanolic extract of *C. procera* roots respectively; while group I serve as a control.

2.3 Biochemical Assay

Aspartate amino transferase (AST) and Alanine amino transferase (ALT) was determined by the method of [7] and the method is based on transamination reaction. Alkaline phosphatase (ALP) was assayed by [8]. Serum urea by Diacetylmonoxime method of Nessler's as describe by [9], serum bilirubin by [10], and Serum Creatinine by [11]. Serum Bicarbonate (HCO₃⁻) was determined by [12], Serum Chloride ion (Cl⁻) by [13], Sodium and Potassium was determined by [14].

2.4 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. Result and Discussion

Table 1 and 2 shows the 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 methanolic Extract of *C. procera* root extract and livolin 10 and 20 days respectively, while serum levels of kidney function indices of CCl₄ hepatotoxic rats treated with the extract for 10 and 20 days are represented in table 3 and 4 respectively.

In this investigation, CCl₄ induced toxicity in group II rats by evidently raising the liver function indices, serum activities of AST, ALT, ALP, Total and Direct Bilirubin as compared with positive control (group I). The serum level of the enzymes is increased due to cellular leakage [15]. In CCl₄ induced toxicity, CCl₃[•] is produced as a free radical. It binds to lipoprotein leading to peroxidation of lipid of endoplasmic reticulum. The fact that ALT is raised at both exposure indicates that CCl₄ have induced toxicity in accordance with [16] who reported that rats treated with high dose of CCl₄ 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. The toxicity increases with the increase of day of exposure [15].

Daily oral administration of 10 and 20 doses of 10mg/kg methanolic root extract of *Calotropis procera* root (MRECP) when compared with normal control (Grp I) and toxicity group (Grp II) produced significant increase in ALT and significant decrease in ALB indicate significant liver damage at 10 days exposure. The activities of all the liver function indices normalize after 20 days exposure. The increased ALT values in this group and decreased ALB indicates that MRECP have not cure the induced liver damage at this dose because ALT is the principal enzyme of the liver and hypoalbuminaemia is associated with impaired albumin synthesis in the liver or liver disease [17]. The result of histopathology of MECPR plate 5 after 10 days shows severe liver damage with mild perilobular toxic necrosis. MECPR produced almost similar curative effect with livolin forte (polyunsaturated phosphatidylcholine); hence the values are comparable and very potent against chemically induced liver disease. Although the possible mechanism for the antihepatotoxic properties of MECPR have not been reported yet. It is assumed that the effect of MECPR on liver protection is related to glutathione-mediated detoxification as well as free radical suppressing activity [18]. On the other hand, the kidney parameters show statistically significant decrease in urea, creatinine, chloride and sodium and statistically insignificant increase in bicarbonate and potassium when compared with normal control group Grp I. After 20 days however, the urea concentration remain low an indication of toxicity in the liver, as increased protein utilization probably induced by the extract and/or impairment in the kidney excretory function. As was reported by other workers that hepatic toxicity induced by CCl₄ also causes disorders in kidneys, lungs, testis as well as in blood by generating free radicals [19]; [20]. Findings by [21], [22] and [23] suggested that exposure to this solvent (CCl₄) causes acute and chronic renal injuries. In addition, report on various documented case studies established that CCl₄ produces renal diseases in human [24]. However, the pathogenesis of Carbon tetrachloride (CCl₄) - induced renal dysfunction is not completely known. It may be due to the functional state of liver, or renal injury may develop independently to hepatic events [25].

The kidney condition is improved after 20 days exposure, due to insignificant change in creatinine, chloride, potassium and sodium. Sodium is usually measured with other factors to evaluate problems associated with kidneys, adrenal glands, muscles and nerves. Although other factors can cause body's sodium to go up, these include eating a lot of salt, when you have not been drinking enough fluids, when you have recently eaten licorice, when you have been hyperventilating and when you have kidney disease. Factors such as vomiting or diarrhea, burn, less water, when adrenal glands are not working well, heart or liver disease and vigorous exercise can cause lower body's sodium level [26].

Table 1: Serum activities of ALT, AST and ALP and concentration of ALB, T.BIL and D.BIL for group of CCl₄ induced hepatotoxicity rats orally administered with methanolic extract of *C. procera* and livolin for 10 days.

| Groups | 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 ±6.44 | 4.26 ±0.24 | 1.37 ±0.17 | 4.0 ± 0.3 |
| II (toxicity) | 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(livolin) | 35 ±2.5 | 44.6 ±6.77 | 99.8 ±2.17 | 2.9 ±0.122 ^a | 1.39 ± 0.25 | 2.1 ± 0.2 |
| IV(methanol) | 39 ±1.00 ^b | 51 ±9.62 | 110 10.0 | 3.34 ±0.51 ^a | 1.28 ±0.2 | 6.1 ± 1.4 |

Values in the same column with (^a) and (^b) are significance at P< 0.001 and P< 0.01 and respectively compared to control group in the same column. n =5

Table 2: Serum activities of ALT, AST and ALP, and concentrations of ALB, T. BIL and D.BIL for groups of CCl₄ induced hepatotoxicity rats orally administered with solvents 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 | 28.8±5.933 | 37.6±2.510 | 86.6±3.362 | 3.8 ±0.78 | 1.1 ± 0.07 | 0.8±0.08 |

Values in the same column with (^a) and (^b) are significance at P< 0.001 and P< 0.01 and respectively compared to control group in the same column. n =5

Table 3: Concentration of urea, creatinine, bicarbonate, chloride, potassium and sodium for group of CCl₄ induced hepatotoxicity rats orally administered with some solvent extracts of *C. procera* root and livolin for 10 days

| GROUP | UREA (mg/dl) | CREAT (mg/dl) | HCO ₃ ⁻ (mmol/l) | Cl ⁻ (mmol/l) | K ⁺ (mEq/l) | Na ⁺ (mmol/l) |
|---------------|------------------------|-------------------------|--|--------------------------|-------------------------|--------------------------|
| I(control) | 0.77 ± 0.1 | 24.1 ± 4.9 | 31.2 ± 0.9 | 276 ± 43 | 5.1 ± 1.1 | 315.6± 41.2 |
| II(toxicity) | 0.58 ± 0.04 | 14.3 ± 3.4 | 66.9 ± 5.1 ^a | 209 ± 37.9 ^b | 10.1 ± 0.7 ^a | 278.0± 41.8 |
| III (livolin) | 0.9 ± 0.3 ^a | 30.4 ± 0.2 | 33.4 ± 0.9 | 173 ± 3.6 ^a | 5.8 ± 0.2 | 200.7 ± 3.4 ^a |
| IV(methanol) | 0.7 ± 0.1 ^c | 33.4 ± 2.5 ^c | 43 ± 6.2 | 177.8 ± 6.5 ^a | 4.7 ± 1.8 | 214.7 ± 6.7 ^a |

Values in the same column with (^a), (^b) and (^c) are significance at P< 0.001, P< 0.01 and P< 0.05 respectively compared to control group in the same column. n =5

Results are expressed as mean ± standard deviation.

Table 4: Concentration of urea, creatinine, bicarbonate, chloride, potassium and sodium for group of CCl₄ induced hepatotoxicity rats orally administered with extracts of *C. procera* root and livolin for 20 days

| GROUP | UREA (mg/dl) | CREAT (mg/dl) | HCO ₃ ⁻ (mmol/l) | Cl ⁻ (mmol/l) | K ⁺ (mEq/L) | Na ⁺ (mmol/l) |
|--------------|--------------------------|--------------------------|--|--------------------------|--------------------------|--------------------------|
| I(control) | 1.7 ±0.08 | 17.97 ± 1.8 | 18.9 ± 1.3 | 144.2 ± 13.7 | 2.40 ± 0.57 | 164.3 ± 12.7 |
| II(toxicity) | 1.86 ± 0.2 | 25.6± 1.99 ^a | 30.3 ±0.68 ^a | 162.2 ± 6.7 | 5.30 ± 0.60 ^c | 188.8±6.93 |
| III(livolin) | 0.87 ± 0.2 ^a | 32.6 ± 3.14 ^a | 29.1 ± 1.08 ^a | 106.7 ± 3.2 | 4.40 ± 0.58 | 132.6 ± 5.01 |
| IV(methanol) | 1.33 ± 0.12 ^c | 24.0 ± 1.8 | 31.6 ± 1.3 ^a | 90.2 ± 5.8 | 3.00 ± 1.13 | 116.7 ± 7.8 |

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. Results are express as mean ± standard deviation

4. Conclusion and Recommendations

The *Calotropis procera* extract from methanol possesses some active components similar to those found in Livolin forte, evidence from the fact that it almost lowers the liver biochemical indices as seen in livolin group. It's recommended that other organic solvents should be used to extract the sample, in addition other drugs other than livolin should also be compared. Finally more research should be carried out to characterize the active component responsible for hepatocurative effects in *C. procera*.

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