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EFFECT OF *CALITROPIS PROCERA* AQUEOUS ROOT EXTRACT AGAINST CCl_4 INDUCED LIVER TOXICITY IN RABBITS

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ABSTRACT
The hepatocurative effect of aqueous root extract of Calitropis Procera on CCl_4 induced hepatotoxicity in rabbits was studied in groups of rabbit and the levels of liver enzymes; aspartate amino transferase (AST), alanine amino transferase (ALT) and alkaline phosphatase (ALP). Serum concentrations of total bilirubin and direct bilirubin were found to be significantly ($p<0.05$) higher in group induced with hepatotoxicity, with significantly ($p<0.05$) lower total protein and urea compared to the control group. These findings confirmed induction of hepatotoxicity. Three different dosages were orally administred (1.0g/kg, 2.5g/kg and 3.6 g/kg) twice daily for 9 days. The C. Procera root extract was found to significantly ($p<0.05$) reduce the serum levels of AST, ALT, and ALP, serum concentration of total bilirubin in rats with hepatotoxicity compared to the the control group and significantly ($p<0.05$) incerased the serum concentration of total protein and urea. These indicates the possible hepatocurative effects of aqueous root extract of C. Procera on CCl_4 induced liver toxicity.
Keywords: C. procera , curative, carbon tetrachloride and liver disease.

INTRODUCTION

Traditional medicine or folk medicine practice (Herbalism) based on the use of plants and plant extracts is an excellent source of therapeutic agents. This has continued to attract attention in the global search for effective methods of using plants (e.g. seeds, stems, leaves, roots and bark etc) for the treatment of many diseases affecting humans (Sofowora, 1993). Study of herbs dates back over 5,000 years to the Sumerians, who described well-established medicinal uses of such plants as laurel, caraway and thyme. The Egyptians of 1000 B.C. are known to have used garlic, opium, castor oil, coriander, mint, indigo and other herbs for medicine (Alan *et al.,* 1995). Phytomedicine has been used in the treatment of sickle cell disease, and a systematic review of the available data indicates that it reduces the number of crisis periods associated with severe pain experienced over a six-month period. Furthermore, no adverse side effects were noted following the use of the phytomedicine (Cordeiro and Oniyangi, 1998). Stem bark extract of *Khaya senegalensis* was shown to have potency against liver diseases and high dose over 200mg/Kg could induce liver damage (Sule *et al.,* 2008). Many plants synthesize secondary metabolites with pharmacuetical properties and at least 12,000 have been isolated – a number estimated to be less than 10% of the total (Sofowora, 1993). *Calotropis procera* belongs to the family *Asclepiadaceae* (milkweed family) of the Genus *Calotropis R. Br*. (*calotropis*). This plant is found in dry savannah and other arid areas, mostly anthropogene occurring around villages (Aliyu, 2006). *Calotropis procera* is a shrub, up to 5.5m high, occasionally branchless to a height of 2.5m and the bark is fibrous, scaly, deeply fissured when old, grey to light brown. All parts of the plant exude a white latex when cut or broken (Aliyu, 2006). The plant *Calotropis procera* has shown to have many ethnomedicinal uses some of which includes; aphrodisiac, laxative, poison antidote, emetic, snake bite, ringworm, guinea worm, scorpion stings, venereal sores, ophthalmic disorders, diuretics, stomach tonic, antidiarhoetic, asthma, leprosy, arthritis, analgesic (Clara, 2001; Parotta, 2001). The plant is a purgative and is used in the treatment of embrocation, headache and catarrh, conjunctivitis, skin diseases and in wound dressing (Agharkar, 1991). The plant has also shown positive effects in the treatments of cold, cough, dyspepsia, elephantiasis, epilepsy, leprosy, malaria, odontectomy, hemiplegia, rheumatism, small pox, syphilis and sore (Duke, 1994). It has long been used for abortive and suicidal purposes (Weber, 2003). Since the plant has been reported to be used as medicine against number of diseases, the work reported in this paper was carried out to investigate the effect of queous root extract of C*alotropis procera* against CCl_4 induced liver damage in rabbits, with the view to investigate the acclaimed liver disease(s) alleviating effect of the plant.

MATERIALS AND METHODS

Collection and Preparation of Root Extract

Root of *Calotropis procera* was collected from Fegi, Sharada quarters, Gwale L.G.A. of Kano state in May 2010. The root was dugged out, cleaned and dried under shade before grinding using mortar and pestle and stored in plastic container. The aqueous root extract was prepared by soaking, filtration after 24hrs, adjusted to 1.0g/cm^3 concentration and stored in refrigerator.

Reagents

Reagents used are commercially prepared reagent kits for Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total Bilirubin (TB), total protein and urea obtained from Randox Laboratories, Antrim, UK, were used to assay the serum enzymes activity and other parameters.

Animals and Design of Experiment

Rabbits (70) weighing between 520-1200g ware used for this experiment. The animals were allowed free access to both food and water while they acclimatized with the environment for one week. The animals were divided into 5 groups (I, II, III, IV and V), groups I and II had five (5) and thirty (30) rabbits respectively and groups III, IV and V had twenty five (25) rabbits each.

Group I - Negative control, they were neither treated with CCl_4 nor *Calotropis procera* aqueous root extract, but were allowed free access to food and water.

Group II - Positve control and were administered with 100mg/Kg body weight CCl_4 subcutaneously as reported by Alhassan *et al* (2009) but no *Calotropis procera* aqueous root extract administred.

Groups III - Administering with 100mg/kg body weight CCl_4 and 1.0 g/Kg body weight *Calotropis procera* aqueous root extract for 9 days.

Groups IV - Administering with 100mg/kg body weight CCl_4 and 2.5 g/Kg body weight *Calotropis procera* aqueous root extract for 9 days.

Groups V - Administering with 100mg/kg body weight CCl_4 and 3.6 g/Kg body weight *Calotropis procera* aqueous root extract for 9 days

After 48hrs, 5 rabbits from groups I and II were sacrificed to confirm the inducement of liver damage by CCl_4 according to Alhassan *et al* (2009). Following oral administration of the aqueous root extract of *Calotropis procera* (1.0mg/kg, 2.5mg/kg and 3.6mg/kg twice a day) five animals were sacrificed from each group after the 3rd, 6th and 9th day, blood sample was collected into clean test tube, allowed to clot and then centrifuged at 5000 revolution per minute for 3 minutes in a centrifuge. The serum obtained was then used to analyze the levels of AST, ALT, ALP, total bilirubin, direct bilirubin, total protein and urea in order to asses the liver function.

Estimation of Biochemical Parameters

Aspartate amino transferase (AST) and Alanine amino transferase (ALT) was determined by the method of Reitman and Frankel (1957) which is based on transamination reaction. Alkaline phosphatase (ALP) was assayed by Rec (1972). Serum urea was determined by Diacetylmonoxime method as reported by Evans (1968), serum bilirubin by the method of Malloy and Evolyn (1937) and assay of serum total protein by Biuret method (Chalwa, 1999).

Statistical Analysis

The data was statistically analysed using GraphPad Instat3 Software (2000) version 3.05 by GraphPad Inc for analysis of variance.

RESULTS AND DISCUSSION

Table 1 compares the serum levels of AST, ALT, ALP, T.bil., D. bil., T. protein and urea for normal and hepatotoxic rabbits 48 hours after subcutaneous injection of 100mg/kg CCl_4 to confirm inducement of hepatotoxicity by the CCl_4. The serum activities of AST, ALT, and ALP of hepatotoxic rabbits were found to be 83.00 ± 15.6IU/L, 50.54 ± 13.80IU/L and 146.00 ± 22.63IU/L respectively. AST, ALT and ALP are non plasma specific enzymes and are mostly found in tissues (kidney, heart etc) , liver inclusive. The enzymes were reported to be higher than normal in serum when there is liver necrosis (Keith and Robert, 2001; Price and Stevens, 2003). These values are significantly higher ($P<0.05$) in group II compared to Group I and it indicates possible induction of liver damage and confirm that CCl_4 can induce liver damage (Obi and Uneh, 2003; Alhassan *et al.,* 2009). Although certain factors such as sex differences, haemolysis of red blood cells, presence of activators and inhibitors and presence of pyridoxine (vitamin B6), may influence the levels of AST in the serum since the concentration of AST in erythrocyte is roughly ten fold than normal serum level (Price and Stevens, 2003). An earlier report by Marsh *et al* (1965) indicated that dietary supplementation of pyridoxine results in increase in AST by 25-50%. The concurrent elevations of serum AST together with ALT and ALP indicates that the above factors may likely not be the cause of the elevated serum enzyme activities, but rather it is more likely to be due to the toxicity induced by CCl_4 (Nduka, 1999). The concentration of serum T. bil. level in positive control (group II) (table 1) increased significantly 1.92 ± 0.26mg% ($P<0.05$) when compared to that of normal control group I. The values of serum total bilirubin and direct bilirubin indicates the presence of hyperbilirubunaemia, this may indicates liver dysfunction. However, hyperbilirubunaemia is not always present in well compensated cirrhosis (Price and Alberti, 1985) and the presence of increased direct bilirubin is probably an indication of acute hepatitis induced by CCl_4. The serum levels of total protein in positive control (group I) was found to be 11.70 ± 0.24mg% which showed a significant decrease ($p<0.05$) compared to normal control (Group II). This decrease in serum total protein level in positive control group is associated with hepatic damage and should be expected as the hepatic synthesis of both albumin and globulin may be impaired due to impairment of hepatic function and the effect may be severe in chronic hepatitis.

The serum level of urea in negative control I, was found to be 38.93 ± 17.4mmol/L. This value show a significant decrease when compared to normal control at ($P<0.05$). This decrease in serum urea level may be associated with severely reduced liver function as reported by Ansley *et al* (1978) that in a severely reduced liver function, a true intolerance of ammonia was seen and thus neurological signs after a heavy protein meal or substantially reduced urea levels may be seen.

The administration of different concentration (1.0g/kg, 2.5g/kg and 4.0g/kg) of aqueous root extract of *C. Procera* to CCl_4 hepatotoxicity rabbits (group III, IV and V) for 3, 6 and 9 days resulted in the significant ($P< 0.05$) reduction in the level of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline Phosphatase (ALP), Total bilirubin and Direct bilirubin in group III, IV and V compared to group II (Table 2, 3 and 4). The significant decrease in these liver function indices in *C. Procera* administered animals especially in CCl_4 hepatotoxicity rabbits treated with 4.0g/kg (Group V) aqueous root extract of *C. procera* for 9 days might be due to decreased leakage of liver enzymes by the hepatocytes. This indicates that, the *C. Procera* extract reduces or reversed the toxic effect of CCl_4 hence; prevent leakage of enzymes into the blood circulation.

The reduction in serum total protein and urea in group II (administered with 100mg/Kg CCl_4/untreated) compared with group III, IV and V (*C. Procera* treated rabbits) was also recorded which was significantly elevated on treatment with *C. Procera* extract, indicating its curative effect against liver cell damage. Clawson (1989) reported that, the decline in protein content may be due to defect in protein biosynthesis as well as disruption and disassociation of polyribosomes from endoplasmic reticulum following administration of CCl_4. According to Rajesh and Latha (2004), the improvements in the level of total protein after treatment with the natural products may be due to the promotion of ribosome assembly on endoplasmic reticulum which facilitates uninterrupted protein biosynthesis.

In conclusion, the ability of *C. Procera* in reducing the ALT, AST, ALP, T. bil. and D. bil. levels was more pronounced at 4.0g/kg compared with 1.0g/kg and 2.5g/kg aqueous root extract of *C. procera* after oral administration for 9 days and the effect may be associated with the phytochemical contents of the plant.

Table 1: Serum AST, ALT and ALP activities (IU/L), levels of T. bil., D. bil and T. protein (mg/dl) and urea (mmol/l) of rabbits 48 hours after treatment with CCl_4.

Groups	AST	ALT	ALP	T.Bil.	D.bil.	T.protein	Urea
I	9.75	9.30	139.05	0.60	0.47	21.50	46.60
n = 5	±	±	±	±	±	±	±
No CCl_4 administered	0.78[a]	3.39[b]	8.56[c]	0.28[d]	0.24[e]	1.97[f]	9.33[g]
II	83.00	50.54	146	1.9	1.33	11.7	38.93
n = 5	±	±	±	±	±	±	±
100mg/Kg CCl_4 administered	15.6[a]	13.8[b]	22.63[c]	0.26[d]	0.09[e]	2.4[f]	17.4[g]

n = number of rabbits

Values are expressed as mean±standard deviation Values in the same column bearing similar super script are significantly different at P< 0.05.

Table 2: Serum AST, ALT and ALP activities (IU/L), levels of T. bil., D. bil and T. protein (mg/dl) and urea (mmol/l) of CCl_4 hepatotoxicity rabbits treated with 1.0g/kg aqueous root extract of *C. procera* after 3, 6 and 9 days.

Groups	AST	ALT	ALP	T.Bil.	D.bil.	T.protein	Urea
I	9.75	9.30	139.05	0.60	0.47	21.50	46.60
no extract no CCl_4 administered	$\pm 0.78^{a,b,c,*}$	$\pm 3.39^{e,f,g,h}$	$\pm 8.56^{i,j,k,l}$	$\pm 0.28^{m,n,o,p}$	$\pm 0.24^{q,r,s,t}$	$\pm 1.97^{u,v,w,x}$	$\pm 9.33^{y,z,\#,\$}$
II	81.08	51.64	148	1.8	1.43	10.9	39.33
100mg/Kg CCl_4 administered	$\pm 16.4^{a}$	$\pm 12.6^{e}$	$\pm 21.48^{i}$	$\pm 0.23^{m}$	$\pm 0.07^{q}$	$\pm 1.9^{u}$	$\pm 15.2^{y}$
III	38.00	44.00	142.00	1.71	1.49	7.20	59.00
6.0g/kg extract administered	$\pm 2.97^{b}$	$\pm 9.90^{f}$	$\pm 4.24^{k}$	$\pm 0.13^{n}$	$\pm 0.06^{r}$	$\pm 0.86^{v}$	$\pm 2.83^{z}$
III	36.20	41.81	139.05	1.42	1.32	7.34	31.70
12.0g/Kg extract administered	$\pm 1.27^{c}$	$\pm 1.85^{g}$	$\pm 4.82^{j}$	± 0.16	$\pm 0.45^{s}$	$\pm 0.79^{w}$	$\pm 1.34^{\#}$
III	29.40	37.08	135.23	1.14	1.32	7.87	28.00
18.0g/Kg extract administered	$\pm 2.26^{*}$	$\pm 2.93^{h}$	$\pm 2.02^{l}$	± 0.20	$\pm 0.30^{t}$	$\pm 0.47^{x}$	$\pm 4.24^{\$}$

Values are expressed as mean±standard deviation Values in the same column bearing similar super script are significantly different at P< 0.05.

Table 3: Serum AST, ALT and ALP activities (IU/L), levels of T. bil., D. bil and T. protein (mg/dl) and urea (mmol/l) of CCl_4 hepatotoxicity rabbits treated with 2.5g/kg aqueous root extract of *C. procera* after 3, 6 and 9 days.

Groups	AST	ALT	ALP	T.Bil.	D.bil.	T.protein	Urea
I	9.750	9.30	139.05	0.60	0.47	21.50	46.60
no extract no CCl_4 administered	$\pm 0.78^{a,b,c,d}$	$\pm 3.39^{e,f,g,h}$	$\pm 8.56^{i,k,l,m}$	$\pm 0.28^{n,op,q}$	$\pm 0.24^{r,s,t,u}$	$\pm 1.97^{v,w,x,y}$	$\pm 9.33^{z,*,\#,\$}$
II	80.05	50.56	139	1.90	0.65	11.0	40.04
100mg/Kg CCl_4 administered	$\pm 13.3^{a}$	$\pm 13.6^{e}$	$\pm 20.39^{i}$	$\pm 0.19^{n}$	$\pm 0.04^{r}$	$\pm 1.72^{v}$	$\pm 13.1^{z}$
IV	36.90	37.60	155.30	1.73	1.72	8.80	50.35
15.0g/kg extract administered	$\pm 7.64^{b}$	$\pm 5.80^{f}$	$\pm 6.93^{k}$	$\pm 0.04^{o}$	$\pm 0.12^{s}$	$\pm 0.71^{w}$	$\pm 0.35^{*}$
IV	29.82	29.80	135.75	1.20	1.40	9.66	43.70
30.0g/Kg extract administered	$\pm 1.52^{c}$	$\pm 1.13^{g}$	$\pm 7.42^{l}$	$\pm 0.28^{p}$	$\pm 0.14^{t}$	$\pm 0.77^{x}$	$\pm 1.98^{\#}$
IV	27.79	29.44	135.47	1.65	1.89	7.58	34.54
45.0g/Kg extract administered	$\pm 1.54^{d}$	$\pm 3.44^{h}$	$\pm 7.20^{m}$	$\pm 0.92^{q}$	$\pm 0.02^{u}$	$\pm 0.81^{y}$	$\pm 8.82^{\$}$

Values are expressed as mean±standard deviation Values in the same column bearing similar super script are significantly different at P< 0.05.

Table 4: Serum AST, ALT and ALP activities (IU/L), levels of T. bil., D. bil and T. protein (mg/dl) and urea (mmol/l) of CCl_4 hepatotoxicity rabbits treated with 4.0g/kg aqueous root extract of *C. procera* after 3, 6 and 9 days.

Groups	AST	ALT	ALP	T.Bil.	D.bil.	T.protein	Urea
I	9.750	9.30	139.05	0.60	0.47	21.50	46.60
no extract no CCl_4 administered	± 0.78 ^{a,b,c,d}	± 3.39 ^{e,f,g,h}	± 8.56 ^{i,j,k,l}	± 0.28 ^{m,n,o,p}	± 0.24 ^{q,r}	± 1.97 ^{u,v,w,x}	± 9.33 ^{s,t,y,z}
II	76.16	430.56	133	1.20	0.58	19.03	38.13
100mg/Kg CCl_4 administered	± 11.2 ^{a}	± 12.4 ^{e}	± 16.25 ^{i}	± 0.10 ^{m}	± 0.03	± 1.64 ^{u}	± 11.20 ^{s}
III							
24.0g/kg extract administered	28.80	30.15	135.50	1.35	1.20	7.60	40.60
	± 2.55 ^{b}	± 1.63 ^{f}	± 6.36 ^{j}	± 0.07 ^{n}	± 0.28 ^{q}	± 0.57 ^{v}	± 0.85 ^{t}
IV	27.51	29.15	128.25	1.15	1.21	7.65	48.78
48.0g/Kg extract administered	± 2.27 ^{c}	± 3.04 ^{g}	± 3.04 ^{k}	± 0.21 ^{o}	± 0.42 ^{r}	± 0.92 ^{w}	± 2.51 ^{y}
V	22.46	28.55	122.92	1.65	0.84	8.17	41.00
72.0g/Kg extract administered	± 2.34 ^{d}	± 2.04 ^{h}	± 3.93 ^{l}	± 0.21 ^{p}	± 0.23	± 0.23 ^{x}	± 15.40 ^{z}

Values are expressed as mean±standard deviation Values in the same column bearing similar super script are significantly different at P< 0.05.

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