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Hepatocurative effect of aqueous leaves extracts of Negro coffee (*Cochlospermum tinctorium)* on carbon tetrachloride induced liver injury in rats

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Abstract: Hepatocurative effect of aqueous leaves extracts of *Cochlospermum tinctorium* against carbon tetrachloride (CCl_4) induced acute hepatic injury was investigated in rats. Forty two rats were used for the study; seventeen used for lethal dose (LD_{50}) determination while the remaining twenty five rats divided into five group each having five rats. Group one served as negative control while group two served as positive control. Group three to five served as test groups. Significantly ($P< 0.05$) higher levels of transaminases, alkaline phosphatase, albumin, bilirubin (total, direct and indirect), were observed in carbon tetrachloride intoxicated rats. However, these parameters were reduced toward normal levels in rat treated with the extract. Histopathological examination revealed regeneration of some damaged liver tissues in group two (positive control), When compared with untreated groups. The lethal dose (LD_{50}) of the leaves extracts of the plant was greater than 5000 mg/kg body weight. This study suggests that Aqueous leaves extract of *Cochlospermum tinctorium* may have curative effect against CCl_4 induced hepatoxicity in rats and appeared to be practically nontoxic.

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INTRODUCTION

Cochlospermum tinctorium is a plant of widespread occurrence in the savannah land throughout the dried parts of the West African region. It's a shrub that grows up to 10m high. The leaves are alternating palmate lobed with stipules. The inflorescence consists of bright yellow flowers that are regular or slightly irregular and borne in raceways or panicles. Fruits are elongated 3-5 valve capsule containing weeds that are embedded in cotton foam[1]. The common vernacular names in Nigerian include Rawaya, Kyamba (Hausa), Obazi Abanzi (Igbo), Sewutu (Yoruba) and Ichachafola (Igala). It's one of the valuable medicinal plants of Africa, which has shown multiple medicinal usages. It's widely used in Senegal and neighboring countries to treat hepatobiliary disorders, particularly icterus[2]. The root decoct ion is used for management of epilepsy[3]. It's a familiar herb in the traditional medicinal preparations in Northern Nigeria, where decoctions of the whole roots are used as remedy for gonorrhea, jaundice and gastrointestinal diseases[4]. *Cochlospermum tinctorium* has been widely used in the treatment of malaria and jaundice or liverish fevers and the research on the roots has so far been focusing on these diseases[5]. The aim of present work was to determine LD_{50}, and evaluate the hepatocurative activity of *Cochlospermum tinctorium* aqueous leaf extract using carbon tetrachloride induced liver damaged rats as experimental model.

MATERIALS AND METHODS

Collection and extraction of plant materials

The leaves of *Cochlospermum tinctorium* were collected from Ologba village, Dekina L. G. A Kogi State in July, 2010. The plant was authenticated at the herbarium of the department of biological sciences, Kogi State University, Anyigba and Voucher specimen (No: 2005) was deposited at the herbarium.

The leaves of C*ochlospermum tinctorium* were shade dried; it was then grinded with mortar and pestle. The dried powdered leaves (5g) was weighed and successively extracted by shaking for two 2h on wrist action shaker after overnight soaking in 40ml of water. The extract was filtered using a blue band filter paper and Buchner funnel and dried on water bath at 45^{o}c. Conc. g/ml=5g/40 ml=0.125 gml^{-1} =125 $mgml^{-1}$.

Experimental animals

Forty two adult (42) male Wister albino rats (120-150g) were purchased and kept in the Department of Biological Sciences, Bayero University, Kano Nigeria; under laboratory condition of 28^{o}C and supplied food (rat chow) and water for the period of the research. Permission from the departmental ethical committee for laboratory use of animals was duly obtained before the animals were put into use.

Induction of liver injury

Carbon tetrachloride (CCl_4) was administered intraperitoneally. This was made by dissolving, 1 ml of (CCl_4) in pure vegetable oil (which was used as a solvent) and the volume was made to 50 ml i.e. 2%

$^{v}/_{v}$ and 120 mg/kg body weight was used to induce hepato toxicity.

Volume of CCl_4 to be administered was determined based on the weight of the rats using the formula below: Volume (ml)=50ml*dosage x weight of rats/100g x 1590mg.

Experimental design

An acute toxicity study of the aqueous leaves extract was carried out by the method described earlier[6]. In the initial phase male albino rats were divided into three groups (Groups I, II, and III) of three mice each. These groups were administered intraperitonealy, doses of 10, 100 and 1000mg/kg body weight of the aqueous extract of the leaves respectively. Animals were observed for 24hours and the number of death recorded, if any. They were also observed for 72h for any sign of delayed toxicity as described[6]. In the second phase, mice were grouped into four groups of one mouse each and administered extract intraperitoneally at doses of 1200, 1400, 1600 and 1800mg/kg. The animals were observed for 24h for any toxicity.in the final phase 2000, 300, 4000 and 5000mg/kg were administered respectively.

For the hepatocurative study, twenty five albino rats were grouped into five containing five rats per group as follows

Group I: Negative control, no extract, no liver toxicity

Group II: positive control, no extract, with liver toxicity

Group III: 50 mg/kg body weight of aqueous leaf extract, after liver toxicity

Group IV: 100 mg/kg body weight of aqueous leaf after liver toxicity

Group V: 150 mg/kg body weight of aqueous leaf after liver toxicity.

Assay for hepatocurative activity

After a period of three weeks (i.e. on the twenty second day) the rats were anaesthetized to minimize pain on the rats; with chloroform and sacrificed. Blood sample was withdrawn by cardiac puncture for determination of biochemical parameters. Aspartate aminotransferase (AST), Alanine amino transferase (ALT), were determined by the method described earlier[7], alkaline phosphatase (ALP) activity was estimated using the Randox kit colorimetric method. Bilirubin was estimated using Tecnoreagent diagnostic kits, and Albumin by the method of Doumas[8]. The tissue sample obtained from the liver was fixed with 10% formalin, stained with haematoxylin and eosin for histopathological analysis.

Statistical analysis

The data are shown as mean±S.E. and statistical significance was evaluated by one way analysis of variance (student t-test).

RESULTS AND DISCUSSION

Acute toxicity studies of oral aqueous leave extract of *Cochlospermum tinctorium* showed zero death up to concentration of 5000 mg/kg body weight, table (1-3). Tables 1 to 3 describe the of Lorke method of acute toxicity Testing (LD_{50}) determination of leave aqueous extract of C. *tentorium*

Many researches have proven the efficacy of CCl4 to induce liver damage by radical induced lipid peroxidation, which compromises membrane integrity of liver cells there by causing swelling and necrosis of the hepatocytes and resulted to the release of cytosolic enzymes such as ALT, AST, ALP and GGT into the circulating blood[9]. This study shows that single dose of CCl_4 injection produced elevated levels of transaminases and cholesterol, this agrees with Etuk *et al*[10].Some authors have reported hepatoprotective property of this plant but no work has ever been done on the hepatocurative to the best of our knowledge. The present study (table 2) shows a dose dependent improvement on the CCl4 damaged liver sequel to intraperitoneal administration of the leaves aqueous extract of *Cocholospermum tinctorium*. The rise in serum levels of AST, ALT and cholesterol have been attributed to the structural integrity of the liver because they are cytoplasmic in nature and released into circulation when liver is damaged[11].

Treatment of the rats with CCl_4 produced visible inflammation of the liver section, distortion of the architecture (necrosis) and fatty changes compared to the control group (figure 1).

Post treatment of the rats in groups 3, 4 and 5 (table 4) with 50,100 and 150mg/kg (body weight) of *Cochlospermum* extract gradually decreased the level of ALT ALP, bilirubin and significant increase ($p<0.05$) in the level of albumin. Similarly histological analysis (figures 3, 4 and5) revealed dose dependent improvement on the architecture and integrity of the hepatocyte, thus a little bit higher concentration of the extract may cure the liver completely. The hepatocurative properties of this plant could be attributed to the presence of tannins in the aqueous extract.

The curative property of this plant extract largely depends on the dosage administered as evidently seen in figures 1, 2 and 3 (i.e. 50mg, 100mg and 150mg) where the regeneration and

decreased in fatty liver is directly proportional to the dosages administered.

Table 1: phase 1 of the LD_{50} Determination

Mg of extract	No. of animal	No. of death
10 g/kg	3	0/3
100 g/kg	3	0/3
1000 g/kg	3	0/3

Table 2: phase 2 of the LD_{50} Determination

Mg of extract	No. of animal	No. of death
1200 mg/kg	1	0/1
1400 mg/kg	1	0/1
1600 mg/kg	1	0/1
1800 mg/kg	1	0/1

Table 3: phase 3 of LD_{50} Determination

Mg of extract	No. of animal	No. of death
2000 mg/kg	1	0/1
3000 mg/kg	1	0/1
4000 mg/kg	1	0/1
5000 mg/kg	1	0/1

Table 4 presents the hepatocurative effect of aqueous leaf extract of *Cochlospermum tinctorium* on carbon tetrachloride induced liver injury in albino rats for a period of three weeks, using the liver function indices. Serum albumin, total bilirubin, directs bilirubin, indirect bilirubin, alkaline phosphatase, alanine transferase and aspartate amino transferase. On the basis of the above results and the recent study on the hepatocurative effects/actions of tannins[12] it could be deduced that the hepatocurative activity of aqueous leaf extract of C. *tinctorium* could be related to the presence of polyphenol compound such as Gallic acid and/ or allergic acid. Caroteniods were detected as the major constituents of the extract which could prevent/cure CCl_4 induced cytotoxity[13]. These carotenoids could also participate in the activity of the aqueous crude extracts.

The acute toxicity studies (tables 1 to 3), recorded zero death, after oral administration of *Cochlospermum tinctorium* aqueous leaf extract up to a dose of 5000 mg/kg body weight. This extract may therefore be considered practically non-toxic according to Hodges and Sterner[14]. According to the theory of Hodges and Sterner any substances which is not lethal at the concentration of 5000mg/kg is considered practically nontoxic.

In summary, this study suggests that administration of *Cochlospermum tinctorium* significantly ameliorates CCl_4 induced hepatotoxicity in rats.

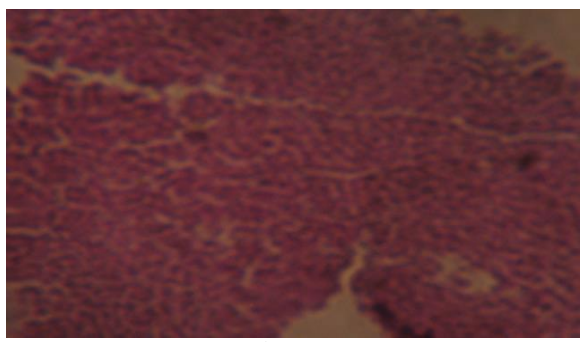

Figure 1(control): Section of the rat liver tissue showing normal hepatocytes X100

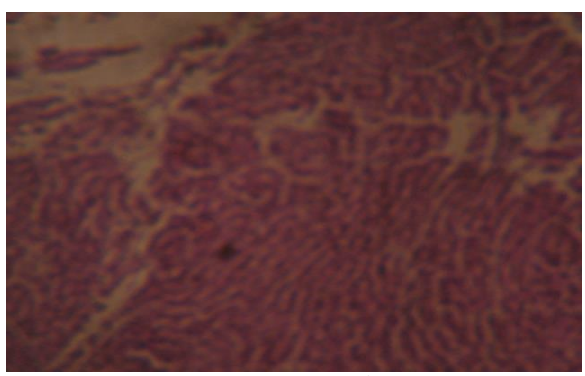

Figure 2 (negative control): section of the rat liver showing inflammations around the portal trials and fatty changes.

Table 4: Effect of *Cochlospermum tinctorium* aqueous extract on Liver function indices after aqueous leave extract administration to rats for three weeks.

Groups (N=5)	Treatment with CRT extract	Serum ALB mg/dl,	Serum TB mg/dl,	Serum DB mg/dl,	Serum IB mg/dl,	Serum ALP (IU/l)	Serum ALT (IU/I)	Serum AST (U/I)
1	Control	5.37±0.28	1.91±0.67	1.05±0.30	0.93±0.71	39.73±3.27	5.28±3.26	10.10±2.87
2	CCl_4	4.70±0.51*[a]	2.95±1.28	1.08±0.96	1.87±1.38	492.94±203.0*[a]	103.96±61.72*[a]	120.56±51.89*[a]
3	50mg/kg+ CCl_4	4.64±0.58*[a]	2.61±1.40	0.87±0.34	1.74±1.25	334.51±181.75*[a]	14.40±7.84	76.75±38.37
4	100mg/kg + CCl_4	4.17±1.00*[a]	2.78±0.69*[a]	0.90±0.31*[a]	1.89±0.97	295.32±138.73*[a]	8.96±8.72	69.61±23.39*[a]
5	150mg/kg + CCl_4	4.61±0.48*[a]	2.00±1.03	0.32±0.19*[a]	1.68±1.04	287.00±254.85*[a]	51.20±40.83*[a]	61.41±18.93

Values are presented as mean±SEM; values in the same column bearing the script* are significantly higher compared to the control at $P<0.05$

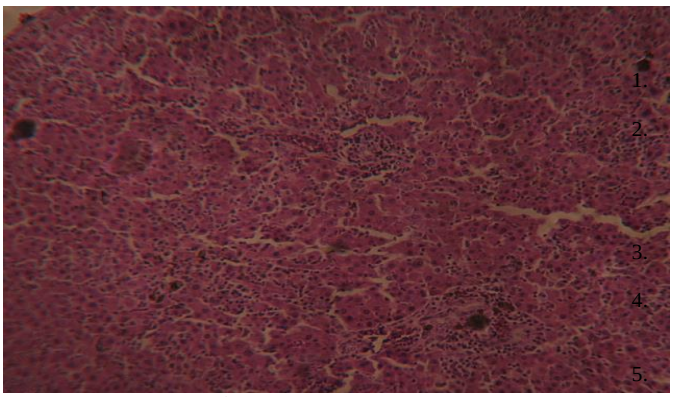

Figure 3: Liver section of (50mg/kg) treated group with decreased inflammation at the portal trials and mild fatty change.

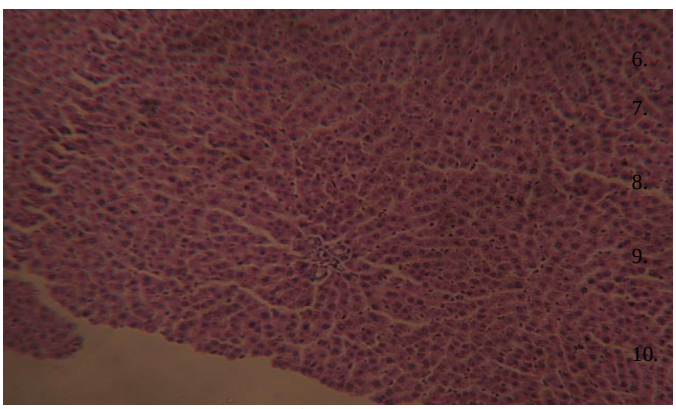

Figure 4: Showing liver section of (100mg/kg) treated group with evidence of regeneration.

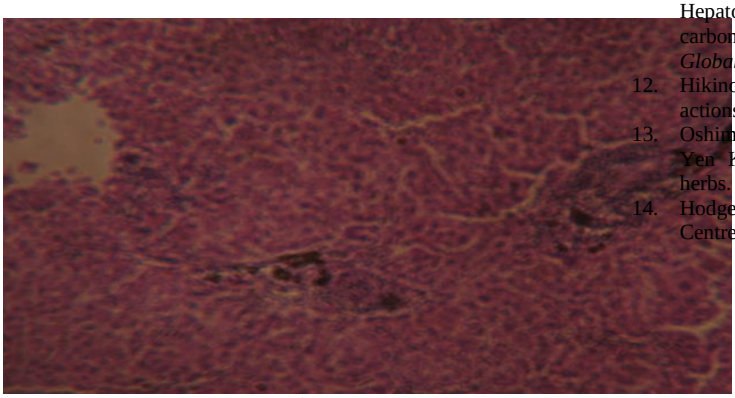

Figure 5: Showing liver section of (150mg/kg) treated group with evidence of regeneration and less area of fatty change.

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