



EFFECT OF AQUEOUS ROOT EXTRACT OF *COCHLOSPERMUM TINCTORIUM* ON LIVER FUNCTION MARKERS OF ALBINO RATS

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Abstract

The hepatocurative effects of aqueous root extracts of *Cochlospermum tinctorium* against carbon tetrachloride (CCl_4) induced acute hepatic injury were investigated in albino rats. Forty-two albino rats were used for this study; seventeen rats were used for lethal dose (LD_{50}) determination while the remaining twenty-five rats divided into five groups each having five rats. Group I served as negative control while group II served as positive control. Group three III-IV served as test groups. The lethal dose (LD_{50}) of the root extracts of the plant was greater than 5000mg/kg body weight, indicating a relative safety of the extract. Significantly ($P < 0.05$) higher levels of transaminases, alkaline phosphatase, albumin, bilirubin (Total, Direct and Indirect) were observed in carbon tetrachloride intoxicated rats. These parameters were decreased significantly ($P < 0.05$) in rats treated with aqueous root extract of *Cochlospermum tinctorium*, indicating the extract capacity to promote the healing of the damaged hepatocytes. Histopathological examination confirmed the hepatocurative activity of the extract, in which the treated groups showed positive response compared to untreated groups. This finding revealed a possible hepatocurative effect of aqueous root extract of *Cochlospermum tinctorium* against CCl_4 induced hepatotoxicity in rats.



Key words: Hepatocurative, *Cochlospermum tinctorium*, hepatic injury, histopathology, carbon tetrachloride

INTRODUCTION

Phytochemicals are naturally occurring and biologically active plant compounds that have potential health promotion quality, including disease inhibiting capabilities. It has been documented that phytochemical are relatively effective in combating or preventing disease due to their antioxidant and other pharmacological activities (Halliwell and Gutteridge, 1989; Kasaikina *et al.*, 1997; Farombi *et al.*, 1998). Antioxidants protect other molecules from oxidation when they are exposed to free radicals and reactive oxygen species which have been implicated in the etiology of many diseases, including chemically induced liver damage (Koleva, 2000; Farombi *et al.*, 2000; Murtala *et al.*, 2015).

Although, medicinal plants have long been used in traditional medicine for treating different illnesses, but many substances found in them may be harmful to the organism. A large number of plants are known for their teratogenic and abortive properties or for their toxic effects (Mengue *et al.*, 2001). In developing countries, about 80% of the population continue to use medicinal plants and plant products in handling primary medical problems due to their accessibility, availability and affordability of which *Cochlospermum tinctorium* is not an exception (Cherdshewasart *et al.*, 2007; Ganguly *et al.*, 2007).

In Nigeria and other African countries, several roots, leaves, fruits and barks of plants are used for different medicinal purposes, some of which have been discovered in many researches to be rich in secondary metabolites like tannins, alkaloids, flavonoids, phenols, steroids and volatile oils which are responsible for their therapeutic activities (Cowan, 1999; Rabe and Vanstoden, 2000). Continued investigation of the secondary plant metabolites has led to important breakthrough in pharmaceutical and pharmacological areas and has helped tremendously in the development of modern pharmacotherapeutics in Africa and other parts of the world (Nwaogu *et al.*, 2007).

Cochlospermum tinctorium is a plant of widespread occurrence in the savannah land, including the drier parts of the West Africa region. It is a shrub that grows up to 10m high. The plant is commonly known in Nigerian as *Rawaya Kyamba* in Hausa land, *Obazi Abanzi* by Igbo people of south eastern region, *Sewutu* in Yoruba land and *Ichachafolo* in Igala land. *Cochlospermum tinctorium* is one of the valuable medicinal plants of Africa, which has shown multiple medicinal usages. It is widely used in Senegal and neighboring countries to treat hepatobiliary disorders, particularly icterus (Sere *et al.*, 1984). The root decoction is used for management of epilepsy (Burkill, 2000). In Guinea, *cochlospermum tinctorium* is used in the treatment and prevention of diseases due to liver damage (Basilevskaya, 1969; Balde and Diallo, 1981).



Cochlospermum tinctorium is a familiar herb in the traditional medicinal preparations in Northern Nigeria, where decoctions of the whole roots are used as remedy for gonorrhoea, jaundice and gastrointestinal disease (Mann *et al.*, 2003). The roots are used as remedy for malaria, schistosomiasis and are also found to have hepatoprotective effects (Diallo *et al.*, 1992). It has also been widely used in the treatment of malaria and jaundice or liver fevers and the research on the roots has so far been focusing on these diseases (Nergard *et al.*, 2005). As plant material are receiving more attention in sub-Saharan and Savanna regions of Africa for treatment of various ailments, *Cochlospermum tinctorium* has been a potential target for these exploitations by the traditional African healers. It is therefore pertinent to evaluate the safety and acclaimed hepatocurative effect of this pharmacologically valuable plant.

MATERIALS AND METHODS

Plant sample collection

The roots of *Cochlospermum tinctorium* were collected from Ologba village, Dekina Local Government, Kogi State, Nigeria. The plant was authenticated at the Herbarium Unit of the Department of Biological Sciences, Kogi State University, Ayingba and a specimen voucher (No: 205) was issued accordingly.

Preparation of Aqueous Extract

The root sample of *Cochlospermum tinctorium* was shade dried and then ground into powder. The root extract was prepared by weighing 5g of the plant powder into 40ml distilled water.
Conc.(g/cm³) = 5g/40 cm³ = 0.125g/cm³
= 125mg/cm³

Volume of extract to be administered was based on the weight of the rats. The amount given to each rats was obtained using the formulae below;

$$\text{Volume (cm}^3\text{)} = \frac{\text{Dose (mg)} \times \text{Weight of rats (g)}}{\text{Conc of extract (g/cm}^3\text{)} \times 1000\text{g}}$$

Induction of Liver Injury

Carbon tetrachloride was administered intraperitoneally. This was made by dissolving 1cm³ of (CCl₄) in pure vegetable oil (which was used as a solvent) and the volume was made to 50 cm³ i.e. 2% v/v and 120mg/kg body weight was used to induce hepatotoxicity.

Volume of CCl₄ administered was determined based on the weight of the rat using the equation below:

$$\text{Volume (cm}^3\text{)} = \frac{50 \text{ cm}^3 \times \text{dose (mg)} \times \text{weight of rat (g)}}{1000\text{g} \times 1590 \text{ mg}}$$

where:

1590mg is the weight per cm³ of CCl₄.

Dose used was 120 mg/kg.

Study Design



Forty-two (42) experimental albino rats were used for the research work. Seventeen were used for acute toxicity studies (LD₅₀ of aqueous root extract) in three phases (Phase I, Phase II and Phase III), according to Lorke (1983).

The remaining twenty-five (25) albino rats were grouped into five (5) and used for hepatocurative effect studies as follows;

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

Group II, Positive control, no extract, with liver toxicity

Group III, 50mg/kg of aqueous root extract

Group IV, 100mg/kg of aqueous root extract

Group V, 150mg/kg of aqueous root extract

Administration of the extract was done orally and lasted for a period of three weeks using 1ml syringe. The animals were kept under a clean laboratory environment. The rats were fed with growers feed and drinking water. The feeding was two times daily.

Serum Collection

After the period of three weeks (i.e. on the twenty-second day) the rats were sacrificed, their blood collected into a clean and dry centrifuge tube, allowed to coagulate. The coagulated blood was centrifuged using a centrifuging machine at 300 revolutions per minute (rpm) for at least 20 minutes. After centrifugation, the supernatant was collected as the serum and kept in a sample bottle and store in a refrigerator prior to analysis.

Statistical Analysis

The data was expressed as Mean \pm Standard deviation. All the parameters were analyzed by student t-test analysis using GraphPad InStat3 statistical software for window 2006. Values were considered significant at $P < 0.05$.

RESULTS

Table 1: Phase 1: LD₅₀ Determination of aqueous root extract of *Cochlospermum tinctorium*

mg of extract	No. of animal	No. of Death
10mg/kg	3	0/3
100mg/kg	3	0/3
1000mg/kg	3	0/3

Table 2: Phase 2: LD₅₀ Determination of aqueous root extract of *Cochlospermum tinctorium*

mg of extract	No. of animal per extract	No. of Death
1200mg/kg	1	0/1
3000mg/kg	1	0/1
4000mg/kg	1	0/1
5000mg/kg	1	0/1



Table 3: The effects of aqueous root extracts of *Cochlospermum tinctorium* on carbon tetrachloride induced liver injury in albino rat for a period of three weeks.

Groups	Treatment	Serum ALB (g/dl)	Serum TB (mg/dl)	Serum DB (mg/dl)	Serum IB (mg/dl)	Serum ALP (U/l)	Serum ALT (U/l)	Serum AST (U/l)
Group 1	No toxicity	5.37 ±	1.91 ±	1.05 ±	0.93 ±	39.73 ±	5.28 ±	10.10 ±
n = 5	No extract	0.28	0.67	0.30	0.71	13.27	3.26	2.87
Group 2	120mg/kg CCl ₄ +	4.70 ±	2.95 ±	1.08 ±	1.87 ±	492.94 ±	103.96 ±	120.56 ±
n = 5	No extract	0.51 ^a	1.28	0.96	1.38	203.09 ^a	61.72 ^a	51.89 ^a
Group 3	120mg/kg CCl ₄ +	3.83 ±	2.22 ±	0.57 ±	1.66 ±	413.45 ±	41.60 ±	83.00 ±
n = 5	50mg/kg extract	0.57 ^{a,b}	1.45	0.28 ^a	1.59	225.19 ^a	19.90 ^a	15.96 ^{a,b}
Group 4	120mg/kg CCl ₄ +	2.83 ±	2.81 ±	1.51 ±	1.30 ±	371.50 ±	53.15 ±	112.80 ±
n = 5	100mg/kg extract	1.23 ^{a,b}	1.41	1.32	0.75	109.73	28.78 ^a	51.25 ^a
Group 5	120mg/kg CCl ₄ +	3.21 ±	2.20 ±	0.68 ±	1.52 ±	266.54 ±	44.64 ±	66.61 ±
n = 5	150mg/kg extract	0.75 ^{a,b}	0.33	0.64	0.63	111.13 ^a	31.89 ^a	25.22

Values are presented as mean ± standard deviation. Readings bearing 'a' and 'b' indicates significant difference (P<0.05) when compared with Group 1 and 2 respectively.

Key: ALB=Albumin; TB=Total Bilirubin; DB=Direct Bilirubin; IB=Indirect Bilirubin; ALP=Alkaline Phosphatase; ALT=Alanine Aminotransferase; AST=Aspartate Aminotransferase

DISCUSSION

The result of acute toxicity effect of aqueous root extract of *Cochlospermum tinctorium* on albino rats was shown in Tables 1 and 2. The result reveals zero death after oral administration of aqueous root extract up to a dose of 5000mg/kg body weight. The extracts may be considered practically nontoxic according to Hodges and Sterner (2005). This indicated that the extract is safe and could be used for medicinal purposes.

The result of the effect of the extract on liver function markers was shown in table 3 above. In this study, the level of albumin in the negative control was statistically higher (P<0.05) than the test control, though liver damage was suspected. This situation could be attributed to the long half-life of albumin which is about 20 days, making it a poor indicator of acute



liver damage (Vasudevan and Sreekumari, 2005; Murtala *et al.*, 2015). Hypoalbuminaemia is a feature of advanced chronic liver disease, it occurs in acute liver disease only if it is severe and of several weeks' duration. Hypoalbuminaemia in chronic liver disease is often taken as an indication of reduced hepatic albumin synthesis. However, in some cases albumin synthesis may be normal while the distribution or redistribution of albumin in the body is altered.

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have become widely used in the diagnosis of liver disease. The increased hepatic enzymes (AST, ALT and ALP) activity emanated principally from the release of the enzymes from damaged hepatocytes as observed in negative control, in which liver damage was inflicted and treatment was not given (table 3). This was in conformity with previous findings by Nduka (1999) and Murtala *et al.*,(2012) that carbon tetrachloride inflicts acute necrosis upon hepatocytes. Acute liver damage, irrespective of the cause, usually increase the activity in serum more than tenfold but these values may be much higher in acute viral hepatitis and drug-induced hepatitis where there is widespread hepatic necrosis. Carbon tetrachloride is a common model compound for inflicting hepatic injury. Its metabolite, namely trichloromethyl radical is responsible for necrotic capacity of the carbon tetrachloride (Brautbar and Williams, 2009). However, the levels of AST, ALP and ALT in positive and test groups were statistically lower ($P>0.05$) compared the negative control. This suggested a possible healing effect of the extract on chemically induced liver damage. The healing effect could be attributed to the presence of phytochemicals in the root extract that might have antioxidant and other medicinal activities.

Histopathology

The result of histopathological studies shows the liver tissues of rat in group I (fig 1) to be normal, no distortion of the native architecture of the liver cell (i.e. hepatic lobules, central vein, sinusoid, and portal trials were all intact).

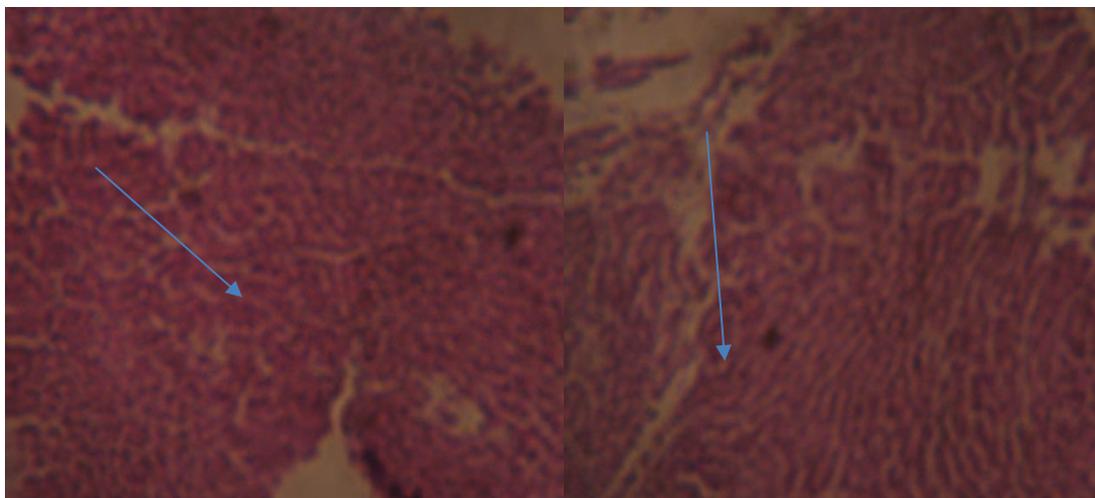


Fig 1: Hepatic tissues of negative control rats (group I); no liver damage, no extract. The arrow indicates normal hepatocytes array.

Fig 2: Hepatic tissues of positive control rats (group I); liver damage, no extract. The arrows indicate CCl_4 induced necrotic areas.

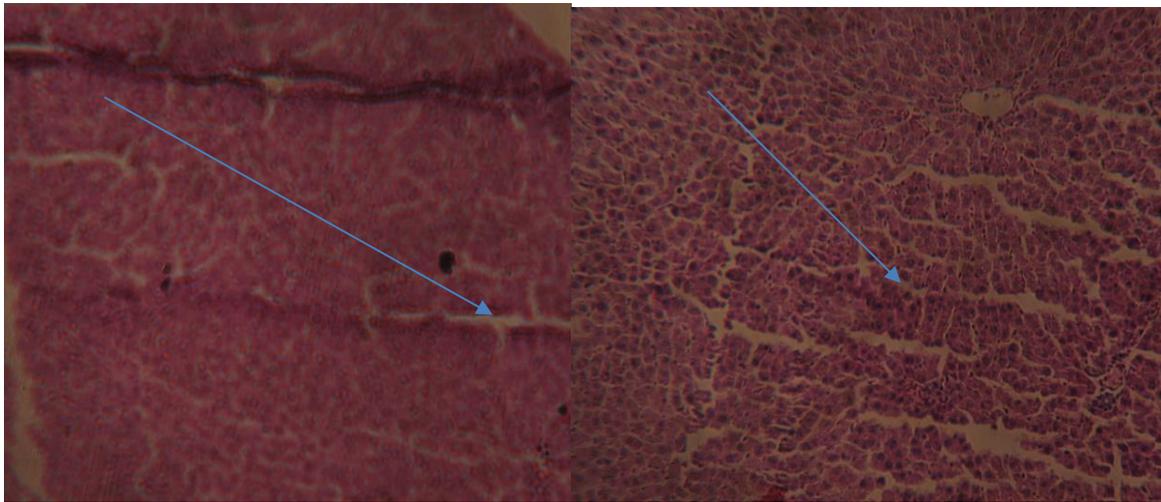


Fig 3: Hepatic tissues of rats treated with 50mg/kg *Cochlospermum tinctorium* root extract for three weeks. The arrows indicate areas responding to the extract treatment

Fig 4: Hepatic tissues of rats treated with 100mg/kg *Cochlospermum tinctorium* root extract for three weeks. The arrows indicate areas responding to the extract treatment

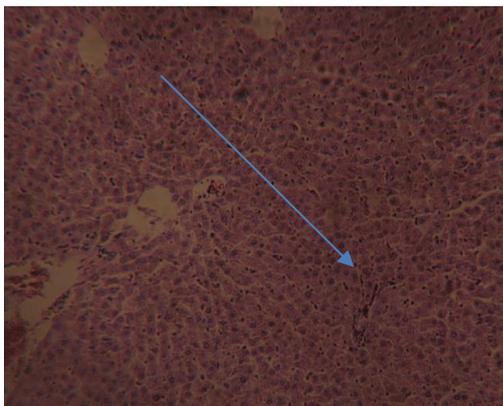


Fig 5: Hepatic tissues of rats treated with 150mg/kg *Cochlospermum tinctorium* root extract for three weeks. The arrows indicate areas responding to the extract treatment

Carbon tetrachloride induced hepatic injury was also confirmed by histopathological examinations of the hepatic tissues of the negative control. In group II (fig 2), the liver tissues appeared inflamed around the portal trials, indicating damage (mixture of lymphocytes, plasmacells and neutrophils present). In group III, IV and V (fig 3,4, and 5), the liver tissues appeared normal, with no significant changes after the treatment, indicating response to the healing capacity of the extract which appeared to be dose dependent.

Conclusion

In conclusion, root extracts of *Cochlospermum tinctorium* may be consider safe for consumption since its LD₅₀ was above 5000mg/kg. Apparent hepatocurative effect was



observed after oral administration of the extracts for three weeks to the CCl₄ induced-liver damaged albino rats.

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