Advanced Journal of Microbiology Research Vol. 2017
Available online at http://internationalscholarsjournals.org/journal/ajmr

Full Length Research Paper

Regenerative action of *Cochlospermum tinctorium* aqueous root extract on experimentally induced hepatic damage in rats

Etuk E. U[1*], Francis U. U[2], Garba I[3]

[1&3]Department of Pharmacology, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.
[2]Department of Pharmacognosy and natural medicine, University of Uyo, Nigeria.

Accepted 15 May, 2017

The hepatoprotective effect of aqueous root extract of *Cochlospermum tinctorium* on carbon tetrachloride (CCl_4) on induced hepatic damage in rats was reported. The present study examined the curative action of the plant extract on experimentally induced hepatic damage in rats. Wistar rats were divided into normal control, induction control, extract and prednisolone treated groups. Hepatotoxicity was induced in rats by intraperitoneal administration of CCl_4 (30% in olive oil) for 5 days. Treatment group received 200 mg/kg of extract post hepatotoxicity induction orally for 7 days. The animals were sacrificed on the 8th day, blood and hepatic tissue collected for liver function test and histopathological analysis respectively. Administration of carbon tetrachloride induced hepatic damage in the rats was evidenced by a significant increase ($P < 0.05$) in the blood clotting time, serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP) and bilirubin as compared to the control. There was also a significant reduction in the serum total protein, serum albumin and reduced glutathione levels. Treatment with the extract reversed the values of all the biochemical parameters to near normal values in control. The histopathological reports collaborate with the biochemical analysis results. Oral administration of aqueous root extract of *Cochlospermum tinctorium* for 7 days has significantly reversed hepatic damage produced by CCl_4 in wistar rats.

Key words: *Cochlospermum tinctorium*, carbon tetrachloride, hepatotoxicity, wistar rats.

INTRODUCTION

Cochlospermaeae is a well known plant family in herbal medicine. The species *Cochlospermum tinctorium* A. Rich (CTR), the plant of interest in this study is widely distributed in the savannah area of west and central Africa. An inventory on African hepatoprotective remedies puts *C. tinctorium* in third position based on the number of countries in which its use is cited (Abondo et al., 1990). A recent study has shown that the plant has heaptoprotective effect against carbon tetrachloride induced toxicity in rats. But apart from the preventive actions, drugs are also needed for the treatment of existing pathological conditions. The aetiological factors of hepatic diseases are multiple and developing a single agent capable of preventing hepatic diseases at all times appears elusive. Alternatively, finding a potent drug that can regenerate hepatic functions irrespective of the initial cause of the damage appears more feasible.

Approximately two million people die annually from hepatic related disorders in the world (Roger et al., 2001). There are no reliable curative drugs for the treatment of hepatic diseases in modern medicine. But a number of medicinal plants have been recommended for the treatment of liver disorders (Sanmugapriya and Venkataraman, 2006). The efficacies of most of these medicinal plants are not yet validated. Thus investigation of medicinal plants with potential hepatic regenerative activity becomes very important. The present study examined the ability of *C. tinctorium* root extract to reverse hepatic damage produced by administration of carbon tetrachloride and restore nor-mal hepatic functions in rats.

* Corresponding author. E-mail: etuk2005@yahoo.co.uk. Tel: +2348054693770, fax.23460231514.

Table 1. Effect of post-treatment with CTR extract on blood clotting time and serum enzymes in CCl_4 induced hepatotoxicity in rats.

Group	Treatment	Clotting time (s)	AST (uL^{-1})	ALT (uL^{-1})	ALP (uL^{-1})
A	CONTROL	275.0 ± 6.9	39.4 ± 1.1	54.6 ± 0.8	126.2 ± 1.0
B	CCl_4	620.6 ± 2.1	109.0 ± 1.5	121.8 ± 1.1	300.8 ± 1.1
C	CTR (200 mg/kg) + CCl_4	290.3 ± 5.4	50.0 ± 0.7	80.1 ± 0.5	130.8 ± 1.4
D	Pred (2 mg/kg) + CCl_4	382.5 ± 6.0	68.8 ± 0.9	62.5 ± 0.9	206.2 ± 1.7
One-way	F	865.97	789.31	1236.4	3789.0
ANOVA	df	23	23	23	23
	P	0.001	0.001	0.001	0.001

Values are mean ± SEM; n = 6 rats in each group. Comparison was between control vs treatment groups; and then CCl_4-treated Vs CTR + CCl_4-treated groups. $P < 0.05$; all values are significant.

MATERIALS AND METHODS

This study was conducted in the Department of Pharmacology, College of Health Sciences, Usmanu Danfodiyo University, Sokoto (UDUS), Nigeria between the months of March and September, 2007.

Experimental animals

Wistar rats weighing 280 – 300 g of either sex were obtained and kept in the animal facility of Department of Pharmacology, UDUS, for about two weeks before the commencement of the study. They were kept in a well ventilated room with free access to rat feeds and tap water. Animals were randomly assigned to the treatment groups (n = 5). Permission from the departmental ethical committee for laboratory use of animals was duly obtained before the animals were put into use.

Preparation of plant extract

The dried powdered root (200 g) of *C. tinctorium* was extracted with distilled water using a Soxhlet apparatus. The filtrate was concentrated to dryness in an oven at 45 $^{\circ}$C and the yield calculated. The extract was stored in a close container and preserved at - 17 $^{\circ}$C until required for use in the study. On the day of the experiment, fresh extract was reconstituted in distilled water at required concentration and put into use.

Treatment of hepatotoxic rats with extract

Carbon tetrachloride (CCl_4) hepatotoxicity was induced in rats according to the method of Rao et al. (2006) with little modifications. Animals were divided into six groups (n = 5). Group A (normal control) were treated with daily dose of olive oil (1 ml/kg body weight p.o.) for 5 days. Group B (induction control) were dosed intraperitoneally daily with CCl_4 (30% in olive oil) for 5 days.

Group C received prednisolone (2 mg/kg b.w., p.o.) a standard anti-inflammatory drug for 7 days after hepatotoxicity induction with CCl_4 as in group B.

The animals in group D were treated orally with 200 mg/kg (body weight) of the extract for 7 days after hepatotoxicity induction with CCl_4 as in group B. The dose of the test extract was selected based on the basis of an earlier work. On the 8th day, clotting time for each rat was determined by the method of Lee et al. (1996). Thereafter, the animals were anaesthetized with chloroform and sacrificed. Blood sample was withdrawn by cardiac puncture, centrifuged and serum separated and preserved for biochemical analysis. The liver samples were collected and preserved in 10% formalin for histopathological analysis.

In the biochemical analysis, the method of Reitman and Frankel (1957) was used in determining aspartate amino transferases (AST) and alanine amino transferase (ALT) activity in the serum. Alkaline phosphatase (AP) level was estimated by Randox kit Colorimetric method. The serum total bilirubin was obtained by using Jendrassik and Graf method (1997) while Doumas method (1997) was used to estimate the serum total protein and albumin. The tissue sample obtained from the liver of each rat was divided into two portions. The first portion was perfused with cold 0.86% KCL, homogenized and centrifuged to obtain post mitochondrial super-natant for estimation of reduced liver glutathione (Slack and Lindsay, 1996). The remaining portion was fixed with 10% formalin; and stained with haematoxylin and eosin before the slides were examined under a microscope.

Statistical analysis

The results of biochemical analysis were expressed as mean plus standard error of mean (MEAN ± S.E.M) . The control and treatment groups were compared by using one way analysis of variance (ANOVA). Further differences were detected by Turkey-Kramer multiple comparison test. The level of significance was taken at probability less than 5%.

RESULTS

The results shown in Table 1 revealed that, the transaminases (ALT and AST), alkaline phosphatase (AP) enzymes and serum total bilirubin were significantly increased ($P < 0.05$) in CCl_4 – treated rats (group B) when compared to the control (group A). Administration of the extract returned the enzymes and bilirubin levels in the intoxicated rats near to the normal values in control group. Also a significant decrease in serum total protein, serum albumin and reduced liver glutathione levels were observed in the CCl_4– treated rats when compared to control group (Table 2). The treatment with 200 mg/kg body weight of the extract raised the values of the affected parameters in the hepatotoxic rats to near the normal values in the control rats. The effect of the extract in restoring the biochemical values in the treated animals

Table 2. Effect of post-treatment with CTR extract on serum total protein, albumin, and reduced liver glutathione in CCl_4 induced hepatotoxicity in rats.

Group	Treatment	STP (100g/ml)	SA (mg/dl)	STB (g/dl)	GSH (mM/g tissue)
A	CONTROL	6.9 ± 0.9	2.9 ± 0.8	1.7 ± 0.1	6.1 ± 0.1
B	CCl_4	6.3 ± 0.3	2.6 ± 0.8	4.8 ± 0.0	1.5 ± 0.0
C	CTR(20 mg/kg) + CCl_4	8.1 ± 0.7	3.1 ± 0.6	2.2 ± 0.1	4.1 ± 0.1
D	Pred(2 mg/kg) + CCl_4	9.0 ± 0.6	4.2 ± 0.6	1.8 ± 0.3	3.9 ± 0.4
One-way	F	3.343	0.9733	78.152	78.851
ANOVA	df	23	23	23	23
	P	0.4249	0.4249	0.001*	0.001*

Values are mean ± SEM; n = 6 rats in each group. Comparison was between control vs treatment groups, and then CCl_4- treated versus CTR + CCl4-treated groups. With P < 0.05; values marked* are significant; g = gram; ml = millilitre; dl = decillitre ; mM = millimole.

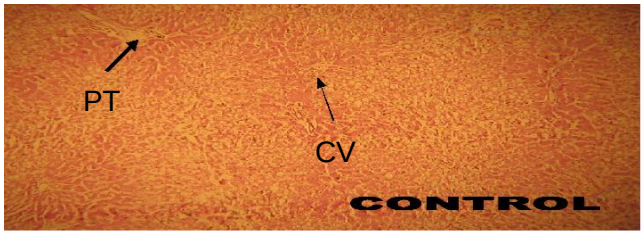

Plate 1. Cross section of rat liver showing normal architecture (× 50). CV: central vein, PT: portal tract.

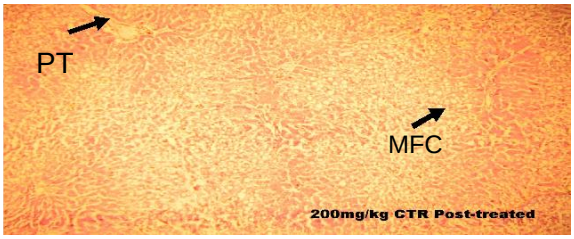

Plate 3. 200 mg/kg CTR post treated liver section showing mild fatty change (MFC).

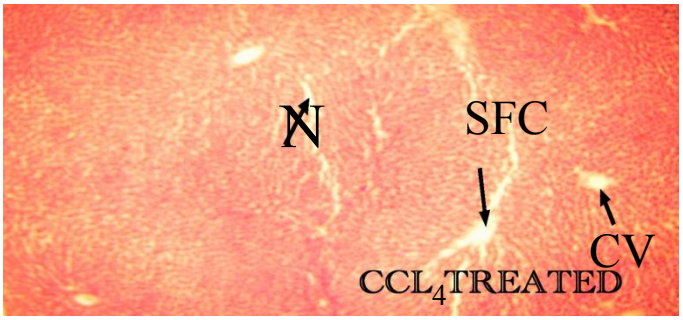

Plate 2. CCl_4 treated liver section showing severe fatty change (SFC) and necrosis (N) (× 50).

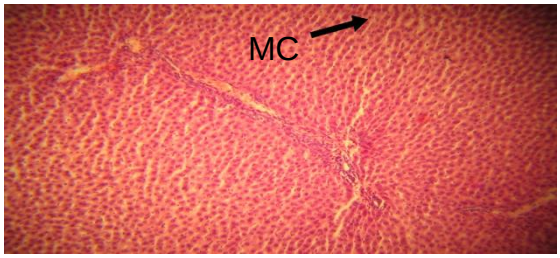

Plate 4. 2 mg/kg PRD post treated liver section showing moderate congestion (MC).

to near normal levels was more significant compared to the prednisolone treatment. Treatment with the extract also reduced the blood clotting time in the rats from 620.6± 2.1 s in the CCl_4 treated rats to 290.3 ± 5.4 s.

The histopathological findings collaborated with the biochemical results. Plate 1 displayed normal hepatic architecture in the rats. Administration of CCl_4 produced severe distortion of the hepatic architecture in the rats (Plate 2). A severe vacuolar fatty change and moderate number of lymphocytes infiltrations were observed. Treatment with the extract reversed the fatty change, restored the normal architecture and reduced lymphocytes infiltration (Plate 3). There was moderate congestion retained in the prednisolone treated group (Plate 4).

DISCUSSION

In this study, the experimental induction of liver damage was achieved by CCl_4 administration in rats. This was evidenced by the elevation in the liver transaminases activity, bilirubin and the histopathological lesions observed. Bilirubin, albumin, transaminases, phosphorlipids and cholesterol assay are sensitive tests to substantiate the functional integrity of the liver and severity of necrosis (Edmundstone et al., 1985). Abdel-Hamid (2006) used a similar method to induce fatty liver in male rats. Chemicals and drugs such as CCl_4 catabolized radicals induced lipid peroxidation, damaged the membranes of liver cells and organelles, caused the swelling and necrosis of hepatocytes and resulted to the release of cytosolic enzymes such as AST, ALT, AP and

Gamma – Glutamate transpeptidase into the circulating blood (Venukumar et al., 2002). The present result showed that CCl_4 administration significantly increased serum transaminase activity, alkaline phosphatase and bilirubin levels while the protein synthesis in the liver was concomitantly inhibited. These observations are in accordance with what was previously reported (Venukumar et al., 2002).

Treatment with *C. tinctorium* aqueous root extract significantly reversed the changes and recovered the five parameters near to normal control values. The effect of this extract might be as a result of the presence of some phytochemical compounds with inherent antioxidant properties capable of inhibiting the free radicals scavenging activity or its ability to regenerate the depressed endogenous antioxidant substances such as reduced liver glutathione as seen in this result. It was reported that, antioxidant activity or inhibition of the generation of free radicals is important in the protection against CCl_4 induced liver lesion (Behattacharyya et al., 2003). It is well recognized that, free radicals are critically involved in various pathological conditions such as arthritis, inflamemation and liver diseases (Quambo et al., 1998).

Prednisolone was used as a standard drug in this study not because it is a known hepatic curative agent but because it is one of the drugs reported to have modulatory actions on hepatic disorders irrespective of the cause. Steroids have marked anti-inflammatory and immunosuppressive effects which makes them useful for disease conditions such as lupus erythematosus, arthritis, ulcerative colitis, hepatitis and nehphritic syndrome (Graig and Stitzel, 1986) . The ability of this extract to reduce clotting time in the rats to less than half the period in the CCl_4 treated rats is very significant. Uncontrolled hemorrhage is a major complication of hepatic diseases. The reduction in clotting time by the extract may be associated with the increase in serum protein levels as recorded in this study.

Overall, the seven days oral treatment with aqueous root extract of *C. tinctorium* has restored normal hepatic functions in CCl_4 intoxicated rats and this makes the plant extract a potential curative agent for liver disorders.

REFERENCES

Abdel – Hamid NM (2006). Diphenyl dimethyl bicarboxylate as an effective treatment for chemical-induced fatty liver in rats. Afr. J. Biomed. Res. 9: 77-81.

Abondo A, Mbenkum F, Thomas D (1990). Ethnobotany and the medicinal plants of the Korup rainforest project area, Cameroon. Mshigeni KE (ed) Proceedings of International Conference on Traditional Medicinal Plants. Arusha, Tanzania, Feb 18 – 23. Dares Salam Uni. Press. ISBN – 9976 60 2294. pp 112-124.

Bhattacharyya D, Mukherjee R, Pandit S, Das N, Sur TK (2003). Prevention of carbon tetrachloride induced hepatotoxicity in rats by Himoliv, a polyherbal formulation. Indian J. Pharmacol. 35:133-135.

Doumas BT, Watson W, Homer G (1997). Albumin standards and the measurement of serum albumin with bromcresol green. Clinica Chimica Acta. 258(1): 21-30.

Edmonson HA. Peters RL (1985). Anderson's pathology. 8th edition Kissane K M.(ed.), C.V. Mosby, St. Louis Press, USA. pp 1096 - 1212.

Graig J, Stittzel D (1986). Delta Viral hepatitis Histopathology and Course. Pathol. Ann. J. 27(1): 12-17.

Jendanssik L, Goffan P (1997). Bilirubin, Colorimetric method. Biochem. Z. 297: 81-93.

Lee J, Son KH, Chang HW, Do JC, Yang KY, Kang SS, Kim HP (1996). Haematological profile following chemical induced hepatotoxicity in rats. Arch. Pharm Res. 16: 25 -31.

Rao GMR, Chandana P, Palpu Annie S (2006). Hepatoprotective effects of rubiadin, a major constituent of Rubia cordifolia (Linn) J. Ethnopharmacol. 103(3): 484-490.

Reitman S, Frankel S (1957). Hepatic disorders. Am. J. Clin. Pathol. 28(1):53-56.

Sanmugapriya E, Venkataraman S (2006). Studies on the hepatoprotective and antioxidant actions of *Strychnos potatorum* Linn seeds on CCl_4 - induced hepatic acute injury in experimental rats. J. Ethnopharmacol. 105: 154-160.

Slack J, Lindsay RH . Estimation of blood proteins bound sulphydril groups in tissue with Eliman's reagent. Anal. Biochem. 1968. 25: 192-197.

Roger D. Pamplona R (2001). Liver toxicity Encyclopedia of Medicinal plants.. (1): 392 – 395.

Venukumar MR, Latha MS (2002). Hepatoprotective effect of the methanolic extract of *Curculigo orchioides* in $CC1_4$ – treated male rats. Int. J. Pharmacol. 34: 29 – 75.