

# [A bushy savannah plant biology essay](https://assignbuster.com/a-bushy-savannah-plant-biology-essay/)

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commonly found in fallow farms across northern Nigeria. It is a shrub that growsup to 10 m high. The leaves are alternate, palmate lobed, with stipules. The\*Corresponding author (Email: oaakin@yahoo. com; phone: +2348030824063)ROM. J. BIOCHEM., 49, 1, 3–12 (2012)4 Oluseyi Adeboye Akinloye et al. 2inflorescence consists of bright yellow flowers that are regular or slightly irregularand borne in racemes or panicles. Fruits are elongated 3–5 valve capsulescontaining seeds which are embedded in cotton foam (1). Its local/vernacularnames include " Oja Ikoko"/’Sewutu’ (Yoruba), ‘ Obazi’/’Abanzi’ (Igbo) and" Rawaya"/’Kyamba’ (Hausa). Decoctions of the whole roots have been reported tobe used as remedy for gonorrhoea, jaundice, gastrointestinal diseases, helminthesand bilharzias infestations, as well as for the management of epilepsy (2–5). Togolaet al. (6) have also reported the antimicrobial properties of Cochlospermumtinctorium. Recently, anticonvulsant properties and pharmacological evidence onthe folkloric use of Cochlospermum tinctorium was reported (7–9). To the best ofour knowledge, much of the published data focused on the properties of theCochlospermum tinctorium root bark extract. Thus, the objective of the presentstudy was designed to test the hepatoprotective activity of the methanolic extract ofplant leaf against carbon tetrachloride induced liver damage in rats.

## MATERIALS AND METHODS

PLANT MATERIALS AND PREPARATION OF THE METHANOLIC EXTRACTLeaves of Cochlospermum tinctorium were collected from a local garden inAbeokuta, Nigeria. They were identified and authenticated by Dr. Aworinde D. O.(a plant taxonomist/anatomist) of the Department of Biological Sciences, University of Agriculture, Abeokuta, Nigeria. Some voucher specimen number wassubmitted to the authority for future references. The leaves were washed with water, then allowed to air dry for 5 days anddried in an oven below 50oC, until a constant weight was obtained. The driedleaves were pulverized with a blender into a fluffy mass (fine powder); 200 g ofpowdered leaves were exhaustively extracted with 400 ml of 80% methanol(MeOH) by simple percolation (cold extraction) for five days. The extraction wasrepeated three times, to ensure that the extractable component has been fullyremoved from the plant material. The extracts were pooled together, filtered andconcentrated in vacuum at 40oC, using a rotator evaporator, then about 12. 5 g ofcrude methanol extract was obtained and subsequently referred to asCochlospermum tinctorium methanolic leaf extract (CTMLE). ANIMALSForty white albino rats of either sex, weighing 150–180 g, purchased fromthe animal house of the Department of Veterinary Anatomy, University of Ibadan, Nigeria were used in this study. They were housed in iron cages under hygienicand standard environmental conditions (28±2oC, humidity 60-70%, natural 12 hr3 Hepatoprotective effect of Cochlospermum tinctorium on CCl4-induced toxicity 5light/dark cycle). They were allowed free access to laboratory diet (Ladokun andsons feeds, Nigeria Ltd) and water. They were allowed to acclimatize for twoweeks. The rats were handled with care, according to the Guide for the Care andUse of Laboratory Animals Manual. All experimental protocols were approved bythe Departmental animal ethics committee. CHEMICALS AND REAGENTSThe carbon tetrachloride used by us was manufactured by May and BakerLtd, Dagenham, England. Teco Diagnostic kits (Lakeview Ave. Anaheim, USA)were used for the analysis of biochemical parameters. All the other chemicals wereof analytical grades. PHYTOCHEMICAL SCREENINGThe CTMLE was subjected to various phytochemical tests, in order toidentify the constituent secondary metabolites using standard methods, as describedby Harborne (10), Sofowora (11), and Trease and Evans (12). EXPERIMENTAL DESIGN FOR HEPATOPROTECTIVE ACTIVITYThe rats were randomly divided into four groups of ten each. Thehepatoprotective activity of plant extracts was tested using the CCl4 model. Carbontetrachloride hepatotoxicity was induced in rats according to the method of Rao et al.(13, 14), with slight modifications. Group I (normal control) received only foodand water. Group II (induction control) was given a single intraperitoneal dose of2 mg/kg CCl4. Group III received CCl4 followed by oral administration of CTMLEat the dose of 200 mg/kg b. wt as a fine suspension made by adding sorbitol. GroupIV received CCl4 and then prednisolone (standard anti-inflammatory drug). The experiment was carried out as per the guidelines of committee for thepurpose of control and supervision of experiment on animal care and handling. Theprotocol conforms to the guidelines of the National Institute of Health (NIH). ASSESSMENT OF HEPATOPROTECTIVE ACTIVITYIn the present study, the hepatoprotective activity was estimatedbiochemically and histopathologically. After a week administration/treatment, theanimals were dissected under diethylether anesthesia. Blood from each rat waswithdrawn by cardiac puncture into non-heparinized tubes, allowed to clot for 30minutes at room temperature. Serum was separated by centrifugation at 3000 rpmfor 15 min. The separated sera were used for the estimation of some biochemicalparameters. 6 Oluseyi Adeboye Akinloye et al. 4The level of malondialdehyde (MDA) produced was estimated by the doubleheating method of Draper and Hadely (15). Briefly, 1. 0 ml of 100 g/Ltrichloroacetic (TCA) solution was added to 0. 2 ml serum, placed in a water bathfor 10 min. After cooling in tap water, the mixture was centrifuged at 1000 rpm for10 min and 0. 5 ml of the supernatant added to 0. 5 ml of 6. 7 g/L thiobarbituric acidsolution in a test tube and placed in a boiling water bath for 15 min. The solutionwas then cooled under tap water and its absorbance measured at 532 nm (using aShimadzu UV-VIS 1610 Tokyo Spectrophotometer). The concentration of MDAwas calculated by the absorbance coefficient of MDA-TBA complex 1. 56 x 105 cm-1m-1. Serum alanine aminotransferase (ALT/SGPT) and aspartate aminotransferase(AST/SGOT) were determined according to the method of Reitman and Frankel(16), while cholesterol, bilirubin, urea and glucose levels were measured using theTeco reagent diagnostic kit. Protein concentration was determined by the methodof Lowry et al. (17). For histopathological studies, liver from each animal was removed afterdissection and preserved in Bouin fluid (picric acid+formalin+acetic acid). Then, representative blocks of liver tissue from each lobe were taken and processed forparaffin embedding, using the standard microtechnique (18). Section (5 μm) ofliver stained with hematoxylin and eosin was observed microscopically andphotographed (Olypus, CS21) for histopathological studies.

## STATISTICAL ANALYSIS

The results of biochemical analysis were expressed as mean ± standard errorof mean (Mean ± S. EM). The control and treatment groups were compared byusing one-way analysis of variance (ANOVA). Differences were detected by theTurkey-Kramer multiple comparison test. The level of significance was taken atprobability less than 5%.

## RESULTS

The present study attempted to show the potential hepatoprotective activity ofcrude methanol leaf extract of Cochlospermum tinctorium in carbon tetrachlorideinduced hepatotoxicity. The Cochlospermum tinctorium leaf extract was found tocontain saponins, flavonoids, tannins and alkaloids. The results of CTMLE on some biochemical parameters used as basis for thehepatoprotective index at a dose of 200 mg/kg on rats intoxicated with CCl4 areresumed in Table 1. 5 Hepatoprotective effect of Cochlospermum tinctorium on CCl4-induced toxicity 7Table 1Effects of methanolic extract of Cochlospermum tinctorium on various biochemical parameters in ratswith carbon tetrachloride induced hepatotoxicityValues with different superscript along the same column are significantlydifferent at p <0. 05. The table also shows a comparison of the CTMLE effectsamong the untreated (normal control), carbon tetrachloride treated (inducedcontrol), extract treated group and standard drug treated groups of rats. Data wererepresented as Mean ± Standard Error of Mean (M ± SEM). The results wereanalyzed statistically by one-way analysis of variance (ANOVA), followed byTurkey’s test using SPSS 11. 5 for window Software. P < 0. 05 was regarded asstatistically significant. It was observed that the CCl4 group significantly increased the serum level ofSPGT (5. 75%), SGOT (41. 5%), bilirubin (42. 4%) and cholesterol when comparedto the control group (group 1). However, the plant extract exhibited a significantprotection against CCl4 induced liver injury, as expressed by the reduction in toxinmediated rise in SGPT, SGOT and cholesterol level of rats. There was no statisticalsignificant change in the blood glucose level of rats among all groups. The level ofMDA was significantly higher in CCl4 intoxicated rats by 32. 7%, in comparison tothe normal control group; this is an indication for lipid peroxidation of hepaticcells. Once administered to the CCl4 intoxicated rats, the extract improved the levelof peroxidation by reducing the amount of MDA. The results showed a significantdecrease in MDA levels by 39. 7%, when compared to CCl4-intoxicated rats. Thevalue of MDA was close to that of the control group 1. The results of histopathological studies also provided supportive evidence forbiochemical analysis. For instance, histology of liver section of control animal(group 1) exhibited normal hepatic cells, each with well defined cytoplasm, prominent nucleus and nucleolus with well revealed central vein (Plate 1), whereasthat of the CCl4 intoxicated group animal showed complete loss of hepaticarchitecture with centrilobular hepatic necrosis fatty changes, vacuolization andsinusoid congestion (Plate 2). Treatment with methanol extract of C. tinctoriumshowed a moderate activity of protecting the liver cells against CCl4 injury. Theliver session of CTMLE treated group showed evidence of regeneration. Theseverity of degenerative changes in tubules was lower than in CCl4 untreated group(Plate 3). However, test results in the CCl4 + Predinsolone group were quitecomparable to the control (Plate 4).

## DISCUSSION

The experimental induction of liver damage by CCl4 in this study wasadopted because CCl4 has been known to catabolise free radical-induced lipidperoxidation, damaging the membranes of the liver cells and organelle, causingswelling and necrosis of hepatocytes, and resulting in the release of cytosolicenzymes (ALT, AST AP and GGT) into the circulating blood (19, 20). Also, prednisolone was used as a standard/reference drug or positive control, because itis known to be a hepatic curative agent through its modulatory anti-inflammatoryactions on hepatic disorders irrespective of the cause. The study demonstrates that single dose of CCl4 injection produced elevatedlevels of SGPT, SGOT and cholesterol; an increase in bilirubin and decrease inprotein was also found, which is in good agreement with the results of Etuk et al.(8). Many authors have reported hepatoprotective properties of some medicinalplants, such as Cassia tora (21), Phyllanthus amarus (22), Zizyphus Mauritian(23). However, liver damage with CCl4 is a commonly used model for thescreening of hepatoprotective drugs (24). To the best of our knowledge, thereviewed literature showed that no research has been reported on hepatoprotectiveproperties of leaves. The present biochemical and histopathological analysis of ourplant extract showed a good development in ameliorating carbon tetrachlorideinduceddamaged liver cells. The rise in serum levels of AST, ALT and cholesterolhave been attributed to the damaged structural integrity of the liver, because theyare located in the cytoplasm and are released into circulation after cellulardamages. This is in line with the reports of Sallie et al. (25), and Ashan et al. (26), which show that, when administered to rats, chemicals or drugs often inducehepatotoxicity by metabolic disturbance and activation. For instance, CCl4 is knownto be metabolically activated by the cytochrome P-450 dependent mixed oxidase inthe endoplasmic reticulum to form trichloromethyl radicals (CCl3), whichcombined with cellular lipids and proteins in the presence of oxygen to induce lipidperoxidation (27). Treatment with C. tinctorium methanol leaves extract recoveredthe injured liver to normal after a week administration at a dose of 200 mg/kg bodyweight, which indicates its potential as anti-hepatoprotective agent. The ability of C. tinctorium extract to reduce the level of peroxidation couldbe attributed to the presence of flavonoids, which have been reported to possessanti-oxidant activity which is presumed to be responsible for the inhibitory effecton several enzymes, including those involved in arachidonic acid metabolism (13, 14).

## CONCLUSIONS

From the overall results, it could be inferred that C. tinctorium hashepatoprotective activity and it was assumed that it confers hepatoprotectionprobably as a result of the presence of both enzymic and non-enzymic antioxidants9 Hepatoprotective effect of Cochlospermum tinctorium on CCl4-induced toxicity 11that could bring about free radical suppressing activity. Meanwhile, work is inprogress on other possible protective mechanisms, such that it may lendpharmacological/scientific credence to the ethno-medical claims of the use of thisplant in the management of ailments.