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Effect of Oral Administration of Ethanolic Leaf Extract of *Acanthospermum hispidum* DC on Carbon Tetrachloride Induced Acute Liver Injury in Rats

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Abstract: The effects of administering *Acanthospermum hispidum* dc ethanolic leaf extract on patients of hepatitis were studied on acute hepatitis induced by carbon tetrachloride (20% CCl4/olive oil, 1.5 mL kg^{-1}, p.o) in rats. The effects were monitored by estimating the serum transaminases levels and the histopathological changes in the livers of experimental rats. The pretreatment of the animals with *Acanthospermum hispidum* dc leaf extract (0.3-2.0 g kg^{-1} orally) significantly elevated the activities of the serum transaminases as well as the hepathotoxin-induced histopathological changes in the livers of the experimental rats. But in cases of no hepatitis the observed effect of the leaf extract was normal like that of the control.

Key words: *Acanthospermum hispidum* dc, effects, CCl_4, Hepatitis

INTRODUCTION

In recent years following the demonstration of the presence of active principles against many diseases and infections in a variety of plant extracts, there has been a great surge of public interest in the use of herbs and plants. Some scientists have viewed this phenomenon as a modern herbal-renaissance[1]. Though pharmaceutical industries are moving towards synthetics and biotechnology research in search of novel medicine in the 21st century; consumers are expressing a greater awareness of the risks of using synthetic products[2,3]. Therefore, attention is now focused on the various aspects of toxicity of such plants extracts[4] in order to have a good understanding of the safety potentials and level of toxicity (if any).

Acanthospermum hispidum dc is a medicinal plant commonly known as ewe onitan meta in western Nigeria. The leaves are used locally for the treatment of acute tuberculosis, other types of cough, diarrhea, dysentery, typhoid and pneumonia. There is no record of its scientific study on its effects on liver. The aim of the present study was to investigate the effects of *Acanthospermum hispidum* dc leaf extract in an experimental model of acute liver injury in rats. In that hepatitis patients who have also contracted any of the diseases that are curable by the use of the leaf extract will be handled with extreme care in order not to alleviate the hepatitis condition thereby leading to death of the patient. Therefore this study describes the effects of *Acanthospermum hispidum* dc in an animal model of hepatotoxicity.

MATERIALS AND METHODS

Plant material: The plant was collected from an herbal health practitioner in Ogbomoso, Nigeria and taken to biology laboratory of Ladoke Akintola University of Technology, Ogbomoso, Nigeria for identification.

Preparation of plant leaf extract: The leaves were air dried in the laboratory for a period of two weeks. It was then ground into fine powder using a sterilized food blender and stored in a sealed plastic container until when required. The leaves were extracted by adding 500 g of it into one liter of ethanol.

Experimental animals: White albino rats (Sprague dawley) weighing approximately 200 g were obtained from the animal breeding unit of the department of Animal Production and Health, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. The animals were housed in clean cages and fed with rat pellets and water *ad libitum*. The animals were then divided into 6 groups of 10 rats each. They were allowed to acclimatize to their new environment for two weeks before administration of the leaf extract.

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Chemicals and reagents: Carbon tetrachloride (Wako Pure Chemical Industries Ltd). Kits for determining sGPT (B 8122) and sGOT (B 8120) activities.All other chemicals were of reagent grade and were used without further purification.

Acute toxicity test: The rats were divided into 6 groups of 10 animals each. The animals were then treated with graded doses (0.3, 0.5, 1.0, 2.0, 5.0 and 10.0 g kg^{-1}) of the extract by oral administration (p.o) and the median lethal dose (LD 50) was determined as described by Litchfield and Wilcoxon (1949). These animals were continually observed for general symptoms of toxicity and mortality after 72 h.

CCl_4-induced hepatotoxicity in rats: The rats were divided into six groups of 10 rats and CCl4 (1.5 ml/10ml/kg, in olive oil) was administered to animals of the first five groups orally. The sixth group received olive oil alone (10 mL kg^{-1}, p.o) and was used as a vehicle control. The extracts (0.3, 1.0 and 2.0g 10 mL^{-1} kg, were fed to the rats in groups C, D and E respectively three times, each at 6, 24 and 42 h time periods before CCl_4 administration; while the remaining two groups were i.e., one treated with CCl_4 (group B) and the other vehicle (group A) treated only, were given no more treatment. All animals were killed 48h after CCl_4 administration, blood was withdrawn from the carotid artery and the sera were separated for each study.

Assessment of liver function: The collected blood was centrifuged at 3000 rev/min, using a centrifuge (centurion model GP (CD295-30)) at 4°C for 10 mins to separate the sera. The activities of Glutamate-Oxalate-Transaminase (sGOT) and Glutamate-Pyruvate-Transaminase (sGPT) were measured, using the method described by Mohun and Cook. Protein concentration was determined by the Biuret method, Spectronic (Bausch and Lomb, USA) was used for all measurements.

Histopathological observations: After blood draining, liver sections were taken from each lobe of the liver. The tissue was fixed in 10% neutral formalin, dehydrated with different ethanol solutions (50-100%) and embedded in paraffin.it was then cut into 4-5 μm thick sections, stained with haematoxylin-eosin and observed under a photomicroscope.

Statistical analysis: Data were expressed as mean±SEM (n = 6) and statistically assessed by one-way Analysis of Variance (ANOVA) .the difference between the drug treated animal and control groups was evaluated by student's t-test.

RESULTS AND DISCUSSION

The ethanol extract of *Acanthospermum hispidum* dc was found to be safe for further biological studies as no lethality was observed at doses as high as10g kg^{-1} p.o in the rats. Table 1 shows the serum enzyme levels of rats in the *Acanthospermum hispidum* dc treated and other groups. Administration of CCl_4 resulted in a marked increase of serum GOT and GPT, which were significantly different from those of the vehicle control (group B vs A in Fig. 1). Treatment of rats with the *Acanthospermum hispidum* dc ethanolic leaf extract caused an increase of sGOT and sGPT induced by CCl_4-intoxication.

The histopathological changes associated with the unhepatoprotective activity of the *Acanthospermum hispidum* dc extract basically support the results of the serum enzymes. The liver of the CCl_4 intoxicated rats showed deformity of the sinusoids, which is responsible for the hemorrhage seen in the tissue. There was broad infiltration of the lymphocytes and kupffer cells around the central vein and loss of cellular boundaries, massive fatty change and necrosis.

The histopathological pattern of the livers of the rats treated with extracts of *Acanthospermum hispimum* dc showed almost the same pattern with that of the CCl_4-intoxicated ones but the cells were seen to be highly extra hepatic cholestasis. The centre of the cells was filled with concentrated bile pigment, which is responsible for the degeneration of the liver cells. The kupffer cells were not seen at all. The central hepatic vein was heavily hemorrhaged.

CCl_4 is metabolized by the mixed-function oxidase system in the endoplasmic reticulum of the liver. The carbon-chloride bond cleavage results in the formation of trichloromethyl free radicals (*CCl_3), which are highly unstable and immediately react with membrane components[5]. Covalent bonds are formed with unsaturated fatty acids resulting in the production of chloroform and lipid radicals. The lipid radicals then react with molecular oxygen, which initiates the peroxidative

Table 1: Effect of oral administration of *Acanthospermum hispidum* dc on some selected enzymes

Group	sGOT(1u L^{-1})	sGPT (1u L^{-1})
Control (NS+vehicle)	133.5±1.41	36.3±1.6
CCl_4 intoxication (NS+CCl_4 (20% CCl4/olive oil, 1.5 mL kg^{-1}, (p.o) Treatment	395.2±61.6[a]	112.2±13.8[a]
AH (0.3g kg^{-1})+CCl^4	396.5±20.5[b]	114.8±6.5[b]
AH (1.0 g kg^{-1})+CCl^4	399.8±15.6	118.2±5.2[b]
AH (2.0 g kg^{-1})+CCl^4	405.8±25.2[c]	130.5±8.1[b]
AH alone	135.8±2.51	37.9±1.4

Each value represents mean±SEM (n = 6). Student's t-test was performed.[a]$p<0.01$ significantly different from control group.[b]$p<0.01$. [c]$p<0.05$ significantly different from CCl_4-intoxicated group. NS: Normal saline

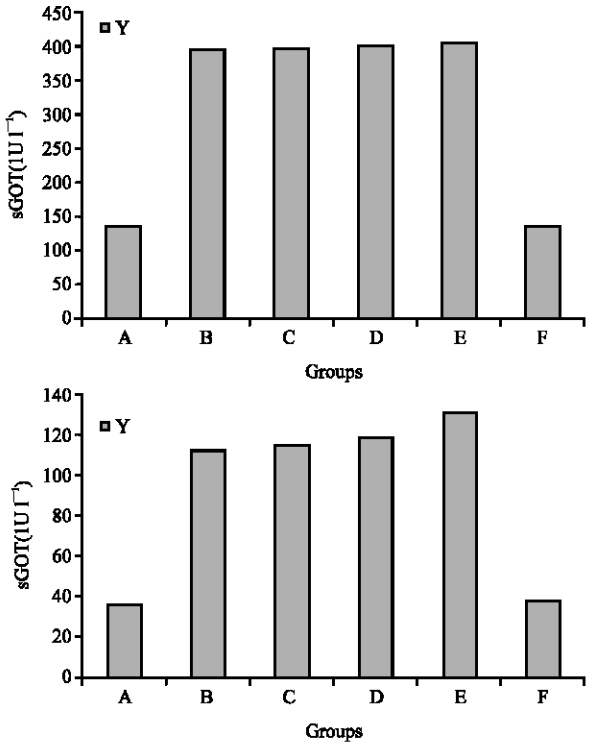

Fig. 1: The prophylactic effects of Acanthospermum hispidum dc leaf exract on sGOT and sGPT in CCl_4-treated rats. A: Vehicle control; B: CCl_4/olive oil (1.5 mL kg^{-1}); C: AH-0.3 g/kg+CCl_4; E: AH-2.0 g.kg+CCl_4; F: AH alone. Values represent the mean±SEM of ten rats. *p<0.005, **p<0.001 (student-test, compared with B, p<0.01 (campared with A). Each value represents mean±SEM (n = 6). Student'st-test was performed. [a]p<0.01 signigicantly different from control group. [b]p<0.01 [c]p<0.05 significantly different from CCl4-intoxicated group. NS: Normal Saline

decomposition of phospholipids in the endoplasmic reticulum. This peroxidation process results in the release of soluble products that may affect other membranes such as cell membrane[6].

The study indicated that treatment with the herbal extract of *Acanthospermum hispidum* dc appeared to enhance the CCl4-induced hepatotoxicity as monitored by the sGOT and sGPT.

The present study has demonstrated that ethanolic extract of *Acathospermum hispidum* dc enhances the damage of an already damaged liver but has no effect on normal liver.

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