Hepatoprotective Effect of Aqueous Extract of Solanum macrocarpon Leaves against Carbon tetrachloride-Induced Liver Damage in Rats

Olusola Bolaji Adewale1, Omotade Ibidun Oloyede2, Amos Onasanya1, Israel Idowu Olayide1, Scholastica Onyebuchi Anadozie, Adewale Oluwaseun Fadaka3

1Department of Chemical Sciences, Biochemistry Unit, Afe Babalola University, P.M.B. 5454, Ado-Ekiti, Nigeria.
2Department of Biotechnology, Ekiti State University, P.M.B. 5354, Ado-Ekiti, Nigeria.

ARTICLE INFO

Article history:
Received on: 15/06/2015
Revised on: 30/06/2015
Accepted on: 17/07/2015
Available online: 04/09/2015

Key words:
Carbon tetrachloride;
Chemoprotective;
Hepatoprotective;
Phytochemical; Solanum macrocarpon.

ABSTRACT

Liver damage is a growing concern of today’s modern society. The increasing incidence of exposure to toxic agents has contributed to liver diseases. There is therefore need for hepatoprotective agents. This study was aimed at investigating the protective effect of aqueous extract of the leaves of Solanum macrocarpon against CCl₄-induced liver damage in rats. Six groups of four animals each were used for the investigation. Group 1 served as control, groups 2, 3 and 4 animals were pre-treated with leaf extract of Solanum macrocarpon at 250mg/kg, 500mg/kg and 750mg/kg body weight respectively for 14 days prior to a single intraperitoneal administration of CCl₄. Animals in groups 5 and 6 received only the extract at a dose of 750mg/kg body weight and CCl₄ respectively. All animals were sacrificed 24 h after the administration of CCl₄. The liver functions tests were performed in addition to their histopathological evaluation. Results obtained showed significant adverse changes in the levels of all measured parameters in CCl₄-treated rats. However, pre-treatment with aqueous extract of S. macrocarpon prevented the adverse changes. Our findings suggest that S. macrocarpon protects the liver against CCl₄-induced damage. This could be attributed to the presence of phytochemical compounds in the plant.

INTRODUCTION

Exposure to carbon tetrachloride (CCl₄), a well-known hepatotoxin, is known to induce oxidative stress and causes liver injury by the formation of free radicals. Free radical-induced lipid peroxidation is one of the major causes of cell membrane damage which leads to a number of pathological conditions (Slater, 1984; Oberley, 1988; Halliwell, 1993). Therefore, there is a great need for the development of agents with potent antioxidant effect against the CCl₄ – induced tissue pathology. Studies showed that various plant extracts could have the potential to protect organs against CCl₄ - induced oxidative stress by altering the levels of increased lipid peroxidation as well as enhancing the decreased activities of antioxidant enzymes (Rajesh and Latha, 2004). Solanum macrocarpon is an important medicinal plant with rich source of nutritional and medicinal constituents. It is widely cultivated in West Africa where it serves as an important fruit and leaf vegetable. The leaves are considered very nutritious and are used in preparing soups and stews. The leaves were known to be rich in protein, fat, crude fiber, calcium and zinc (Oboh et al., 2005). Bukenya-Ziraba and Bonsu reported that the leaves have a variety of medicinal uses such as in Sierra Leone for the treatment of throat problems; in Kenya, for the treatment of stomach problems (Bukenya-Ziraba and Bonsu, 2004). The fruit or the leafy part of the plant was reported to be used for the treatment of constipation, ulcers, tooth ache and the leaves as snake bite remedy (Oladiran, 1989). The leafy part of Solanum macrocarpon was effective for treatment of skin disease, infections and sores when applied to the infected areas (Edijala et al., 2005). Generally, the plant has been used as indigenous medicine for the treatment of several ailments such as asthma, allergic rhinitis, nasal catarrh, skin infections, rheumatic disease and swollen joint pains, gastro-esophageal reflux disease, constipation, dyspepsia and also in weight reduction (Dalziel, 1937). The aqueous fruit extract of the plant was reported to exhibit lipid lowering activities as well as renal and hepatoprotective effects in diet-induced hypercholesterolemic rats (Sodipo et al., 2009a,b; Sodipo et al., 2009c, 2011b).
The plant was also shown to improve hematological parameters in hypercholesterolemic and triton-induced hyperlipidemic rats (Sodipo et al., 2011b). The leaves of *S. macrocarpon* have also been revealed to contain high phenolic and flavonoid content, thus possessing a potent antioxidant activity which can offer good protection against oxidative damage to some body tissues (Adewale et al., 2014). The aim of this study was to evaluate the protective potential of aqueous extract of the leaves of *Solanum macrocarpon* against CCl₄-induced liver damage in rats. To the best of our knowledge, this study provides first line of information that aqueous extract of the leaves of *S. macrocarpon* at different doses could be effective in preventing hepatic insufficiency occasioned by CCl₄-intoxication. This is particularly important since traditional consumption of *Solanum macrocarpon* is usually by aqueous medium.

**MATERIALS AND METHODS**

**Plant collection, Identification and Extraction**

The leaves of *Solanum macrocarpon* were obtained from Bisi market in Ado-Ekiti, Ekiti State, and were authenticated at the Department of Plant Science, Ekiti State University, Ado-Ekiti, Nigeria. The voucher specimen of the plant is available and a sample of the plant leaf was deposited at the Departmental Herbarium for future reference. The leaves were air-dried, blended and soaked in distilled water for 24 h. It was then filtered using Whatman filter paper no. 1 and the filtrate was concentrated at 50°C using the rotary evaporator, following which it was dried by means of a freeze dryer. The extract was administered orally using gavage according to the required doses of 250, 500 and 750 mg/kg body weight for 14 days.

**Chemicals**

All chemicals and reagents used were of analytical grade while the water was glass distilled.

**Experimental animals**

Twenty-four female albino rats of Wistar strain weighing between 120 - 180 g purchased from the central animal house of Afe Babalola University, Ado-Ekiti were used for the study. The animals were housed in cages under laboratory conditions in the departmental animal house and were acclimatized for 14 days before starting experiments. They were provided with standard pelleted feed and water *ad libitum*. After acclimatization period, the animals were divided into groups. All animal procedures were in strict accordance with the Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press, Washington, DC, USA, 2011). All animal experiments were approved by the animal care committee of Afe Babalola Research Center, Ado-Ekiti.

**Grouping of animals**

Six groups of four rats each were studied; Group 1 served as normal control; Groups 2, 3 and 4 were pretreated with aqueous extract of *Solanum macrocarpon* at doses of 250mg/kg, 500mg/kg and 750 mg/kg body weight for 14 days respectively prior to CCl₄ administration. Groups 5 and 6 received only 750mg/kg extract and CCl₄ respectively.

Administration of CCl₄ was done after 14 days of *Solanum macrocarpon* treatment and following an overnight fast. Animals were sacrificed 24 hours after CCl₄ administration. The extract was administered orally using gavage according to the required doses.

**Administration of CCl₄**

Where necessary, each animal received 3 ml/kg body weight of a 1:1 preparation of CCl₄ and groundnut oil (in the ratio 1:1). Administration of CCl₄ was by a single intraperitoneal injection. Administration was done after 14 days day treatment with *S. macrocarpon*, and following an overnight fast.

**Preparation of plasma and liver homogenate**

The rats were sacrificed 24 h after CCl₄ administration by cervical dislocation and then dissected. Blood samples were collected from the heart using the heart puncture technique. Blood was collected in heparinized bottles. The blood samples were subsequently centrifuged at 3000 x g for 10 min using a bench centrifuge to obtain plasma. The plasma obtained was separated, and transferred into fresh plain sample bottles and used for the subsequent biochemical analyses. The liver of each animal was excised immediately after sacrifice. They were rinsed in ice-cold 1.15% potassium chloride, blotted with filter paper and weighed. Weighed portions were minced with scissors in 4ml of ice-cold 0.1M phosphate buffer, pH 7.4 and homogenized in a Potter-Elvehjem homogenizer.

The homogenates were later centrifuged using refrigerated centrifuge at 12,000 x g for 15 min at 4°C to obtain clear supernatant, which was used for subsequent biochemical analyses.

**Biochemical analyses**

Biochemical analyses carried out includes measurement of the activities of plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (γ-GT or GGT) and alkaline phosphatase (ALP); plasma total protein and albumin concentrations. The determination of the concentrations of these biochemical parameters was done using commercially available test kits, products of Randox Laboratories (Crumlin, United Kingdom).

**Antioxidant assays**

Lipid peroxidation was assessed by measuring the formation of thiobarbituric (TBA) reactive substances according to the method described by Varshney and Kale, (1990). Catalase (CAT) activity was determined according to the method of Sinha, (1972). Superoxide dismutase (SOD) activity was measured as described by Misra and Fridovich, (1972).
Histopathological assessment

Liver sections from rats of different groups were fixed in 10% neutral formalin solution, dehydrated in graded alcohol, and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with hematoxylin-eosin for light microscopic analyses.

To eliminate bias the slides which were coded, examined and photographed by a qualified histopathologist who had no knowledge of the treatment groups.

Statistical analysis

Statistical analysis was done by one-way analysis of variance (ANOVA) using SPSS 16 for windows software package. Post hoc testing was performed for inter-group comparisons using the Least Significant Difference (LSD) test according to the method described by Zar, (1984). In all instances p values < 0.05 were considered statistically significant.

RESULTS

Administration of CCl₄ produced significant toxic effects on the liver, as shown by the data obtained from the parameters investigated (Figures 1 to 3).

Marker enzymes

Figure 1 showed the effect of aqueous extract of the leaves of Solanum macrocarpon on CCl₄-induced changes in some marker enzymes. When compared with the control, CCl₄ treatment gave rise to significant increase (p < 0.05) in the marker enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP). However, pretreatment of rats with aqueous extract of S. macrocarpon (AESM) at 250 mg/kg, 500 mg/kg and 750 mg/kg body weight produced a significant decrease in the activities of these enzymes when compared with CCl₄ group.

As regards the different pretreatment doses of the extract, there was a significant difference in the activities of ALT, GGT and ALP when compared with 250 mg/kg b.w. dose as shown in figure 1. Treatment with AESM at 750 mg/kg b.w. produced a comparable result with that of the control.

Liver function parameters

Figure 2 showed the effect of aqueous extract of the leaves of Solanum macrocarpon on CCl₄-induced changes in plasma total protein and albumin levels. A significant decrease (p<0.05) in the levels of these parameters was observed following a single intraperitoneal administration of CCl₄ when compared with the control group. Pretreatment of rats with aqueous extract of C. rubens leaves at 250 mg/kg, 500 mg/kg and 750 mg/kg body weight produced a significant increase (p<0.05) in the levels of total protein and albumin when compared with CCl₄ group. There was a significant difference in the level of albumin in the pretreatment group at 250 mg/kg b.w. when compared with both 500 mg/kg and 750 mg/kg b.w. as shown in figure 2.

Antioxidant enzymes and lipid peroxidation

As shown in figure 3, intraperitoneal administration of CCl₄ resulted in marked hepatic injury as revealed by significant decrease (p<0.05) in the activities of antioxidant enzymes (SOD and catalase) and significant increase (p<0.05) in the level of MDA respectively when compared with the control group. Pretreatment with the leaves extract of S. macrocarpon at all the three dose levels (250 mg/kg, 500 mg/kg and 750 mg/kg b.w.) resulted in a significant increase (p<0.05) in the activities of these enzymes and prevented the observed peroxidation when compared with the toxicant group. A significant difference was observed in these parameters during the pretreatment with the lower dose when compared with the higher doses as shown in figure 3. Treatment of rats with 750 mg/kg b.w. extract produced a non significant difference in these parameters as compared with the control group.

![Fig. 1: Effect of aqueous extract of Solanum macrocarpon on CCl₄-induced changes in the plasma in ALT, AST, GGT and ALP. AESM 1, AESM 2 and AESM 3 are the pretreatment doses of aqueous extract of Solanum macrocarpon at 250mg/kg, 500mg/kg and 1000mg/kg body weight respectively. Values are expressed as Mean ± SEM (n=4) *p< 0.05 when compared with Control group; **p<0.05 when compared with CCl₄ group; ***p< 0.05 comparison among the pretreatment groups.](image-url)
Histopathology of the Liver

Histopathological examinations as presented in plate 1 revealed that liver section from normal rats showed mild lymphocytic infiltration and no significant lesions (plate 1 (a)). The liver of rats pretreated with 250mg/kg AESM (aqueous extract of *Solanum macrocarpon*) showed periportal congestion, mild lymphocytic parenchyma infiltration and fragmentation of the hepatocytes with progressive occlusion of the sinusoid (plate 1 (b)). Rats pretreated with 500 mg/kg (plate 1 (c)) and 750 mg/kg (plate 1 (d)) AESM, lymphocytic infiltration with the degeneration of the hepatocytes were observed respectively. However, rats treated with 750 mg/kg dose of the extract showed mild lymphocytic infiltration and no significant lesions (plate 1 (e)). However, evidence of cellular degeneration in the hepatocytes and multiple foci of hepatocellular were seen on sections of rats treated with CCl₄ only (plate 1 (f)).
DISCUSSION

Hepatotoxicity, an injury to the liver is associated with impaired liver function caused by free radicals derived from oxygen on exposure to drugs, chemical and non-infectious agents (Navarro and Senior, 2006). Various report confirmed that CCl₄ induces hepatic injury by producing free radicals (Tirkey et al., 2005; Yang et al., 2008; Lavanya et al., 2009). Also, the onset and progression of CCl₄-induced toxicity is tightly linked to the generation of reactive oxygen species (Rechnagel et al., 1989). Carbon tetrachloride is now known to be metabolized to a highly reactive intermediate, trichloromethyl radical (CCl₃*) (Fadhel and Amran, 2002). Trichloromethyl radical and trichloromethylperoxyl radical derived from CCl₄ are known to be involved in a number of deleterious interactions with biological molecules, which includes proteins, lipids and DNA (Packer et al., 1978). This is believed to be part of the critical chemical process in the aetiology of CCl₄-induced tissue damage. Increase in the levels of the liver marker enzymes (AST, ALT and GGT) following CCl₄ administration may have resulted from the leakage of these enzymes into the circulation, which may be attributed to the damaged structural integrity of the liver, because these enzymes are located in the cytoplasm (Bilgin et al., 2011). Plasma levels of ALT and AST have been shown to be important parameters in the diagnosis of liver damage (Williamson et al., 1996; Renugadevi and Prabu, 2010). Liver cells are particularly rich in the transaminases (ALT and AST), as well as alkaline phosphatase and γ-glutamyltransferase. Increased activities of plasma ALP may be due to its increased synthesis in the presence of increasing biliary pressure. Plasma alkaline phosphatase is known to increase as a result of biliary obstruction as seen in cholestatic disease of the liver. GGT is a membrane-bound enzyme and increased plasma level is an indicator of tissue damage (Vasudha et al., 2006). Carbon tetrachloride negatively interferes with protein metabolism probably by inhibiting the synthesis of proteins such as albumin (Iniahe et al., 2008) which may be attributed to the decreased levels of total protein and albumin observed in this study. The susceptibility of the liver cell to oxidative insult occasioned by CCl₄ can be linked to the failure of the antioxidant mechanism to prevent free radical damage thereby leading to lipid peroxidation and ultimately tissue damage (Morakinyo et al., 2012). Lipid peroxidation is a marker of oxidative stress. Evaluation of hepatic antioxidant status in the present study revealed a significantly elevated MDA levels, and a concomitant decrease in the activities of the antioxidant enzymes (SOD and catalase). Thus CCl₄-mediated damage to the liver was secondary to the establishment of oxidative stress.

The ability of the aqueous extract of S. macrocarpon leaves to significantly prevent all the observed biochemical lesions is worthy of note. We therefore speculate that the action of the plant extract may revolve around the possibility that the extract effectively down-regulate the amount of CCl₄ or its toxic metabolites reaching the liver.

It may also be possible that S. macrocarpon may actually act as a free radical scavenger or quencher, thus preventing the oxidative damage to the cell. Based on the findings from this study, it can be suggested that aqueous extract of the leaves of S. macrocarpon in a dose dependent manner holds great hope in preventing liver damage occasioned by CCl₄ toxicity.

Plate 1: Photomicrograph of rat liver sections (Mag. x 400): (a). Normal control; (b). 250 mg/kg AESM + CCl₄; (c). 500 mg/kg AESM + CCl₄; (d). 750 mg/kg AESM + CCl₄; (e). 750 mg/kg AESM only; (f). CCl₄ only.
CONCLUSION

It is confirmed based on the results of this experiment that free radicals are important in the pathogenesis if CCl₄-induced hepatotoxicity. Therefore, doses up to 750 mg/kg body weight of aqueous extract of the leaves of Solanum macrocarpon have been able to prevent the injuries associated with the adverse effect of CCl₄.

ACKNOWLEDGEMENT

We appreciate Afe Babalola University for the encouragement given in terms of facilities and other support.

REFERENCES


Dalziel JM. The Useful Plants of West Tropical Africa (New York: Longman) 1st edn., 1937; 433-441.


How to cite this article: