HEPATOPROTECTIVE EFFECTS OF METHANOLIC LEAF EXTRACTS OF CASSAVA (*MANIHOT ESCULENTA* CRANTZ) ON CCL_4 INDUCED RATS

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Carbon tetrachloride (CCl_4) as a hepatotoxin has been used to induce liver fibrosis in animals. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloromethyl radical. The liver plays a central role in the metabolism of drug and xenobiotics, protein synthesis and in maintaining biological equilibrium in organisms. Therefore, this study was designed to evaluate the hepatoprotective effects of methanolic leaf extracts of cassava (*Manihot esculenta* Crantz) on CCl_4 induced rats. Methanol extract of dried cassava leaves was administered to albino rats for 7 days. The hepatoprotective effects of the methanol extract against the toxic effect of the CCl_4 was evaluated by assaying for some biochemical parameters such as AST, ALT, Albumin, total Protein and Bilirubin levels in the experimental animals. The results indicated that there was no significant difference between the group of rats treated with 200 mg/kg ascorbic acid and that of positive control (Rats induced with CCl_4 without any treatment) for AST level, while a significant difference ($p<0.05$) was observed in the ALT level. The methanol extract of cassava leaf pre-treatment significantly reduced CCL_4-induced elevation in serum levels of AST, ALT, Total Bilirubin but significantly ($p<0.05$) elevated serum contents of Total Protein and Albumin. Thus, the results showed that administration of methanol extract of cassava leave has a hepatoprotective effect on the liver. Due to the increased attention given to natural herbs worldwide, these encouraging results may have future clinical importance.

Key words: Carbon tetrachloride, Hepatotoxins, liver, *Manihot esculenta*.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub of the Euphorbiaceae family and a staple food for over a billion people in Africa, Latin America and Asia (Chetty et al., 2013; De Souza et al., 2017). In Africa, cassava is the second most important calorific source with its tuberous roots often providing over a quarter of the daily calorie consumption (De Souza et al., 2017), and even more for the rural poor. In many countries of sub-Saharan Africa, cassava leaves also provide an important source of protein, vitamins, and micronutrients for humans and livestock (Lukuyu et al., 2014; Latif and Muller, 2015). Cassava is an important staple crop in sub-Saharan Africa, due to its high productivity even on nutrient poor soils (Obata et al., 2020). Cassava is characterized by a high content of cyanogenic glucosides. Linamarin is the major cyanogenic glucoside in cassava, with significant amounts accumulating in all organs including leaves, stems, and roots (Picmanova et al., 2015). The amino acid profile of cassava leaves is higher than that of soybean protein and also contains moderate levels of phytochemicals that are important as natural antioxidant components of plant food products (Christopher and Sherry, 2008). Natural antioxidants, particularly in fruits and vegetables, have gained increasing interest among consumers as antidotes to aging and associated chronic diseases (Wenying et al., 2003). Flavonoids are phenolic substances isolated from a wide range of vascular plants, and more than 8150 different types have been reported (Andersen and Jordheim, 2006). They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding

repellents, and for light screening (Pieatta, 2000). They also bind and/or inactivate metal ions to prevent generation of Reactive Oxygen Species (ROS) and serve as chain-breaking entities which scavenge and destroy ROS (Benzie, 2003). Passos et al. (2009) argued that Cassava Leaves (CL) deserves more attention as a source of protein and nutrients for human nutrition and should get as much attention as the tubers, since it contains high amounts of fiber, vitamins B1, B2, C, crude protein (17.7-38.1% DM: dry matter basis), essential amino acids, and carotenoids. CL possesses compounds that have pharmacological properties, including anti–inflammatory, anti-oxidative, and renal protection (Udino et al., 2010). These pharmacological actions are believed to be due to phenolic compounds in CL such as tannins, anthocyanidins, cyanidin, and delphinidin (Udino et al., 2010).

Liver and its function

The liver is the largest solid organ, gland and one of the most vital organs that functions as a centre for metabolism of nutrients and excretion of waste metabolites (Ozougwa, 2014). The cells that constitute the adult liver originate from the ventral foregut definitive endoderm during embryogenesis (Watt et al., 2007). The liver weighs approximately 1500g and accounts for approximately 2.5% of adult body weight (Moore and Dalley, 2006). The surface of the liver is smooth and dome shaped, where it is related to the concavity of the inferior surface of the diaphragm. The liver lies mainly in the right upper quadrant of the abdomen where it is hidden and protected by the thoracic cage and diaphragm. The normal liver lies deep to the ribs 7-11 on the right side and crosses the midline towards the left nipple (Moore and Dalley, 2006). The Liver function majorly in controlling the flow and safety of substances absorbed from the digestive system before distribution of these substances to the systemic circulatory system (Allen, 2002). A total loss of liver function could lead to death within minutes, demonstrating the importance of the liver (Ozougwu, 2014). Therefore, the aim of this study is to determine the hepatoprotective effects of methanolic extract of *M. esculenta Crantz* leaves on the liver in vivo.

Objectives of the study

The Objectives of the study includes carrying out extract of the leaves of *Manihot esculenta Crantz* using methanol solvent; to administer the plant extract on rats; and to determine the possible hepatoprotective effects of the plant extracts on liver of the rats induced with CCl_4.

MATERIALS AND METHODS

Plant collection and identification

Cassava leaves were collected from a farmland in Abraka, Ethiope East Local Government, Delta State and thereafter identified at the Department of Botany, Faculty of Science, Delta State University, Abraka.

Preparation of plant material

The leaves of *M. esculenta* were air-dried for two weeks and pulverized to fine powder, using sterile blender to avoid contamination and 150 g of the powdered leaves was weighed and transferred into a beaker which was extracted using 600 ml of methanol for 24 h and was filtered through double layer of cheesecloth and then Whatmann No 1 Filter Paper. Then it was concentrated using rotary evaporator under reduced pressure and temperature and was thereafter concentrated to dryness in a water-bath at 50°C and was stored in airtight containers at 4°C temperature until used.

Experimental animals

Twenty five Albino rats *(Rattus norvegicus domestica)* of wistar strain were procured from Department of Anatomy, Delta State University, Abraka, Nigeria. The animals were allowed 2 weeks of acclimatization before commencement of the experiment. They were fed on standard laboratory diet (Top Feed Nig. Ltd) and water *ad libitum* throughout the duration of the experiment.

Experimental design

A total number of 25 Albino rats of wistar strain were divided into 5 groups with 5 rats in each group.

Group A: Rats treated with 200 mg/kg ascorbic acid daily for 7 days.

Group B: Untreated rats induced with CCl_4 (positive control).

Group C: Rats treated with 200 mg/kg methanol

extract of cassava leaf daily for 7 days.
Group D: Rats treated with 70 mg/kg methanol extract of cassava leaf daily for 7 days.
Group E (Control): Normal untreated rats.
Treatment of the rats was done orally for seven days and on the fifth day. Animals in groups A, B, C, D, and E were challenged with 1.5 ml/kg of 20% CCl_4 in olive oil and on the seventh day, the rats were euthanized in an airtight glass chamber saturated with chloroform and after opening up the rats surgically, blood samples were collected for the analyses.

Preparation of samples
The blood samples collected in plain container were centrifuged at 3000 g for 15 min to obtain serum used for the biochemical assays.

Biochemical assays
The following liver enzymes were studied to investigate the hepatoprotective effect of the extract on the experimental animals used for the study. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by the colorimetric method of Reitman and Frankel (1957), using commercial assay kit from Randox Laboratories Ltd, Co. Antrim, United Kingdom. Alkaline phosphatase (ALP) was estimated by the colorimetric method of Rec (1972), using assay kits from Randox Laboratories Ltd. Serum protein and serum albumin were estimated by Biuret method and Bromocresol Green (BCG) binding method respectively using a commercial assay kit from Randox Laboratories Ltd. Serum globulin level was calculated as the difference between total protein and albumin, while albumin globulin (A/G) ratio was obtained from the division of the values of albumin and globulin. Total and conjugated bilirubin was determined using commercial kits from Randox Laboratories Ltd and colorimetric method as described by Jendrassik and Grof (1938).

Statistical analysis
The results are presented as mean ± SD and were analyzed using ANOVA followed by Turkey Kramer's multiple comparison test on Graphpad Instant Software version, version 6.0 (Graph Pad Software, San Diego, CA, USA), and values of $p<0.05$ were considered significant.

RESULTS

Effect of methanol extract of cassava leaves on the AST activities in CCL4 induced rats
The results for the effects of methanol extracts on the AST activities in the liver of CCL_4 induced albino rats are shown in Figure 1. No statistical difference was seen when Group A (standard) was compared with the positive control group (group B) and all other groups. However, statistical differences ($p<0.05$) were observed when Groups A, C, D, and E were compared with the positive control group B. Thus, higher activity was seen in the positive control group in comparison to all other groups.

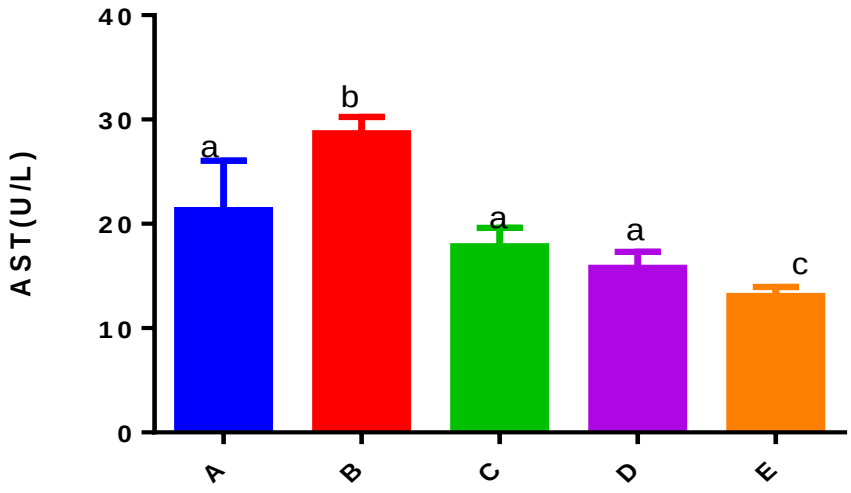

Figure 1. Effect of methanol extract of cassava leaves on the AST activities in CCL4 induced rats.
*Bars with different letters differ significantly ($p<0.05$).

Effect of methanol extract of cassava leaves on the ALT activities in CCL4 induced rats

As shown in Figure 2 for ALT activities, significant differences were seen between Group A (Standard) and Group B, C, D and E. While there was no significant difference observed between Group B (Positive control) and Group D.

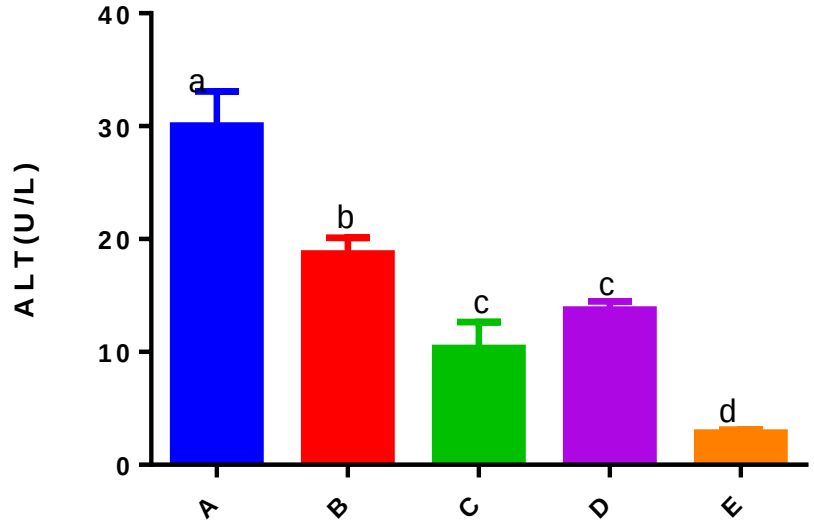

Figure 2. Effect of methanol extract of cassava leaves on the ALT activities in CCL4 induced rats
*Bars with different letters differ significantly ($p<0.05$)

Effect of methanol extract of cassava leaves on the total protein levels in CCL4 induced rats

As shown in Figure 3 for total protein concentration, no significant difference was observed between Group A and B as well as Group D and E. Nevertheless, higher concentration was recorded in Group D and E.

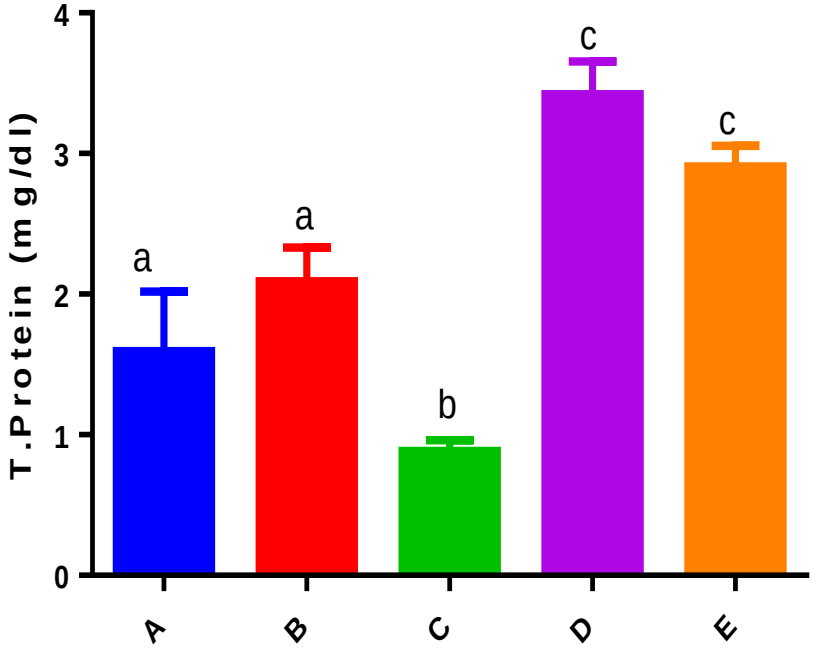

Figure 3. Effect of methanol extract of cassava leaves on the total protein levels in CCL4 induced rats.
*Bars with different letters differ significantly ($p<0.05$)

Effect of methanol extract of cassava leaves on the albumin Levels in CCL4 induced rats

As shown in Figure 4 for Albumin concentration, there is significant difference between Group A (Standard) and Group B, C, D, and E. It implies Albumin level is reduced when the toxin CCL4 was administered to the rats and the cassava extracts had no effect on the Albumin level.

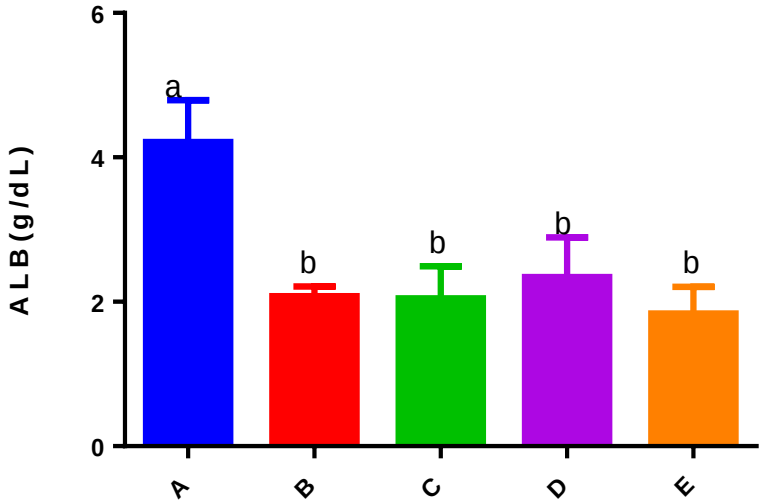

Figure 4. Effect of methanol extract of cassava leaves on the albumin levels in CCL4 induced rats
*Bars with different letters differ significantly ($p<0.05$).

Effect of methanol extract of cassava leaves on the total bilirubin levels in CCL4 induced rats

As shown in Figure 5 for Total Bilirubin Concentration, no significant difference was observed between group A and group B, D and E. However, significant difference was observed between group C and all other groups. The concentration of Total Bilirubin was highest in group C.

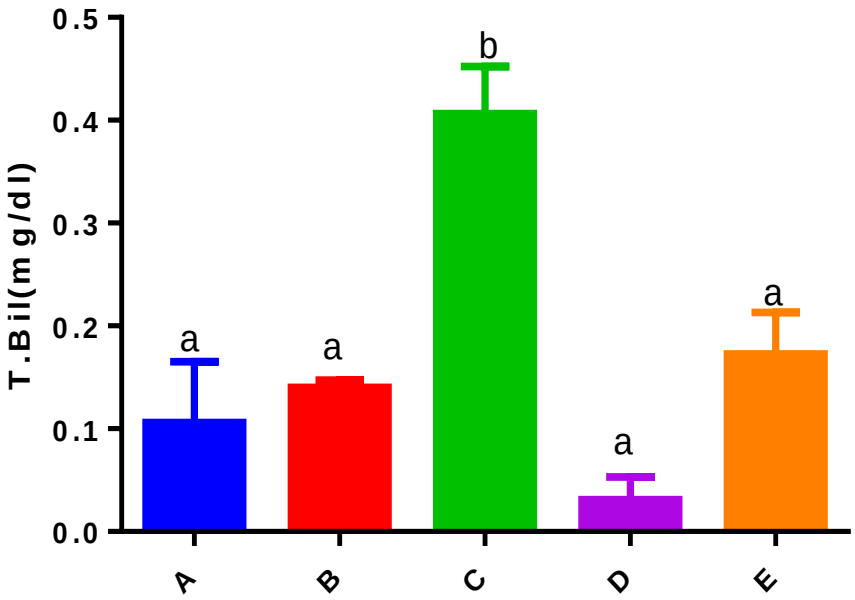

Figure 5. Effect of methanol extract of cassava leaves on the total bilirubin levels in CCL4 induced rats.
*Bars with different letters differ significantly ($p<0.05$).

Effect of methanol extract of cassava leaves on the direct bilirubin levels in CCL4 induced rats

As shown in Figure 6 for direct bilirubin concentration, no significant differences were observed between Group A, B, D and E. While significant difference was observed between group C and other groups. Higher concentration was also recorded in group C compared to all other groups.

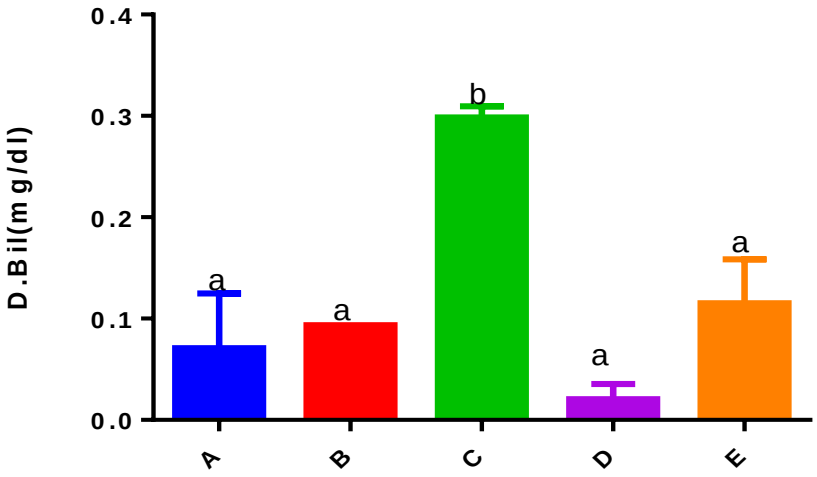

Figure 6. Effect of methanol extract of cassava leaves on the direct bilirubin levels in CCL4 induced rats. *Bars with different letters differ significantly ($p<0.05$).

Effect of methanol extract of cassava leaves on the indirect bilirubin levels in CCL4 induced rats

As shown in Figure 7 for indirect bilirubin concentration, no significant difference was observed between Group A, B, D and E. However, significant differences occurred between Group C and other groups. Meanwhile, Group C showed higher concentration of indirect bilirubin.

Where: Group A=Rats treated with 200 mg/kg ascorbic acid daily for 7 days; Group B= Untreated rats induced with CCL4 (positive control); Group C= Rats treated with 200 mg/kg methanol extract of cassava leaves daily for seven days; Group D= Rats treated with 70 mg/kg methanol extract of cassava leaves daily for 7 days; Group E= (Control): Normal untreated rats.

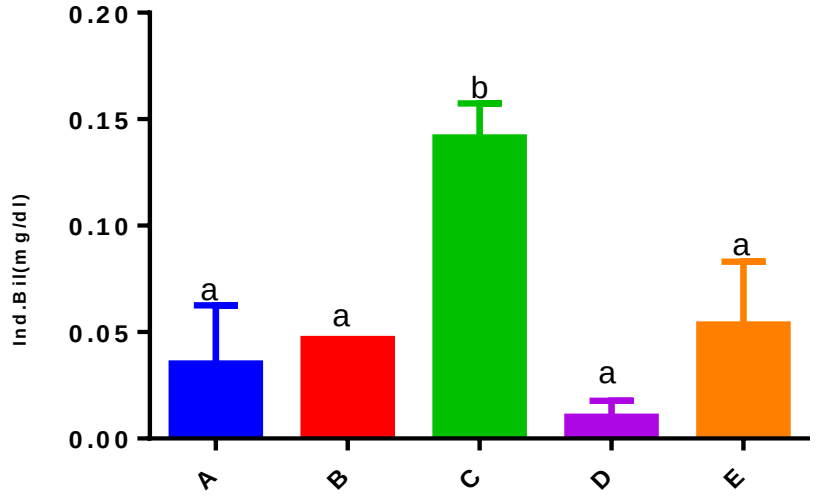

Figure 7. Effect of methanol extract of cassava leaves on the indirect bilirubin levels in CCL4 induced rats *Bars with different letters differ significantly ($p<0.05$).

DISCUSSION

Carbon tetrachloride (CCL_4) is a hepatotoxin that has been used to induce liver fibrosis in animals. One of the major consequences of liver injury caused by CCL_4 is lipid peroxidation, which is mediated by the production of free radicals derived from CCL_4 and when repeatedly administered in low dose, induces liver cirrhosis (Muriel and Escobar, 2003). The results for AST and ALT obtained from this study agree with those reported by Melo et al. (2008), who observed an increase in the ALT activity. Frazini et al. (2008) and Teixeira et al. (2009) reported that ALT is found primarily in the liver and hence considered a more sensitive indicator than AST; since it exist in all body tissue, especially the heart, liver, skeletal muscle, kidneys, brain, pancreas, leukocytes and erythrocytes. Methanol extract of cassava leaves pre-treatment significantly reduced CCl_4 induced elevation in serum levels of AST, and ALT. The positive effect and impact of the extract on health generally is that it acts against CCL4-induced liver fibrosis, through its antioxidant property. Albumin is the most abundant protein in the blood plasma constituting about 50 to 65% (Ribeiro et al., 2006). The findings from this study showed significant difference between Group A (standard) and group B (positive control). Significant differences were also observed between Group A and all other groups. The standard (Group A) showed a higher albumin level than the control (Group B) and all other groups, indicating that CCL_4 decreased albumin levels in the serum of animals. The decrease in albumin production appear to be related to hepatic fat accumulation because, according to Nicoluzzi et al. (2000), this buildup causes a reduction in the synthesis ability of the liver and the consequent reduction in albumin concentration. The methanol extract pre-treatment however elevated serum content of albumin in the CCL4 induced rats. The significant difference between the positive control (Group B) and the control (Group E) showed that CCL_4 administration led to a decrease in serum level of total protein. However, treatment with methanol extract of cassava leaves caused an increase in serum protein concentration. Therefore, there was no significant difference between the normal untreated rats (Group E) with high protein concentration and the Group (D). Total Protein reduction may arise in incidences of liver injury due to the increase in capillary permeability and decrease in the liver ability to synthesize mainly albumin (Adhal and Manning, 2008). There was a significant difference between group C and every other group in terms of total, direct and indirect bilirubin concentration. Similarly, there was significant reduction ($p<0.05$) in the bilirubin levels when the CCL4 intoxicated rats were treated with the methanol extract of cassava leaves (El Rabey et al., 2019).

Conclusion

The administration of methanolic leaf extract of *M. esculenta* showed potent hepatoprotective effect against CCl4 induced hepatotoxicity in the experimental rats. This is because the leaf extract was able to reduce significantly all the elevated biochemical parameters caused by the hepatotoxic effect of the toxicant, carbon tetrachloride. Due to the increased attention given to natural herbs worldwide, these encouraging results may have future clinical importance.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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