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HEPATOPROTECTIVE ACTIVITY OF THE AERIAL PARTS OF *CARALLUMA DALZIELII* N. E. BROWN AGAINST CARBON TETRACHLORIDE -INDUCED HEPATOTOXICITY IN RATS

Ugwah-Oguejiofor, C. J.[1]* and Ugwah, O. M.[2]

[1]Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

[2]Department of Pharmacy, Usmanu Danfodiyo University Teaching Hospital, Sokoto

*Corresponding Author e-mail: nenye789@yahoo.com. Tel:+2348036098241

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ABSTRACT

Caralluma dalzielii is a plant whose aerial parts have been used by traditional healers in the North-west Nigeria for the treatment of several ailments including stomach disorders. This research aimed at evaluating the protective effects of the aqueous extract of the aerial parts of *Caralluma dalzielii* against carbon tetrachloride (CCl_4)-induced acute liver injury in Wistar rats. Phytochemical studies were conducted on the extract using standard methods. The extract was administered to the rats in doses of 250, 500 and 750 mg/kg via oral gavage with silymarin as the positive control and distilled water as the negative control for 7 days. After the last pre-treatment dose, oral dose of CCl_4 (1.5 ml/kg) was administered to the study rats. 24 h later, rats were sacrificed and blood samples collected. Aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), total and conjugated bilirubin, total protein, albumin, urea, creatinine and serum electrolytes levels were determined. Histopathological assessment of the liver and kidney tissues were carried out. Phytochemical analysis showed the presence of saponins, flavonoids, alkaloids, sterols, terpenes, glycosides and tannins. The result showed a significant ($p<0.05$) decrease in the AST, ALT, ALP and creatinine serum levels compared with CCl_4- negative control group. Histopathological assessment confirmed that the extract markedly reduced the toxicity of CCl_4 and preserved the architecture of the liver tissues to near normal structure. These results suggest that aqueous extract of aerial parts of *Caralluma dalzielii* may possess hepatoprotective ability.

Keywords: *Caralluma dalzielii*, hepatoprotective, carbon tetrachloride, Silymarin

INTRODUCTION

The liver is one of the vital organs in the body. It plays an important role in the detoxification of various drugs and xenobiotics in the body (Garcea *et al*., 2009; Li *et al*., 2013). Liver injury may arise from intake of certain drugs, excessive alcohol intake, exposure to various toxins and environmental pollutants (Wang *et al*., 2014; Su *et al*., 2017). Any damage to liver can result in irregularities in the general metabolic process causing many disorders ranging from transient elevation in liver or serum enzymes to life-threatening liver cirrhosis and hepatic failure (Bera *et al*., 2012).

Diseases of the liver have become one of the major causes of morbidity and mortality in man and animals all over the globe and hepatotoxicity due to drugs appears to be the most common contributing factor (Bhawna and Kumar, 2009). Two different studies in the Western part of Nigeria have reported prevalence of 4% and 6.5% of patients with chronic liver disease (Nwokediuko and Ijeoma, 2009). At the moment, only few drugs are utilised clinically for the management of liver diseases. In Nigeria for example, only few drugs have been licensed by the Nigerian drug regulatory authority for the treatment of viral hepatitis (Ladep and Taylor-Robinson, 2007). The cost of these drugs is however outside the reach of the minimum wage earner in Nigeria. Therefore, the development of cheaper liver protective agents is of paramount importance in the protection of liver from damage (Shittu *et al*., 2015).

Herbal plants or botanical medicines have been used traditionally by herbalist worldwide for the prevention and treatment of liver disease (Sharma and Kumar, 2010). Liver protective plants have been found to contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotenoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthenes (Bairwa *et al*., 2010). Plant drugs are relatively non-toxic, safe and even free

from serious side effects (Bhawna and Kumar, 2009). *Caralluma dalzielii* is one of such plants that have been utilized traditionally for the treatment of stomach related issues including liver diseases. *Caralluma dalzielii* N.E. Brown belongs to the family *Asclepiadaceae* and is found in abundance in the North-western Nigeria. It is a cactus-like shaped plant with 5-merous flowers which serve to easily identify this species of genus *Caralluma* (Ugwah-Oguejiofor, *et al.*, 2013). In Africa, this species is distributed across the Sahel (Plowes, 2008). The plant is perennial with small, leafless succulents and rarely, with a few fleshy leaves. It can grow up to 1 meter high. Amongst the Hausa tribe of Nigeria, it is known as 'Karan Massallachi'. It is commonly called mosque stalk. It is used traditionally to treat infertility, diabetes, leprosy, rheumatoid arthritis and severe pain on epigastrium (Ugwah-Oguejiofor *et al.*, 2013). The natives chew or sometimes make a decoction of the plant and use it to prevent stomach pain. Other reported ethnomedicinal uses for this plant is for faintness due to fasting, convulsion, emesis, paralysis (Burkill, 1985) and otitis (Inngjerdingen *et al.*, 2004). Previous scientific studies have been carried out on this plant (De Leo *et al.*, 2005; Ugwah-Oguejiofor, *et al.*, 2013). Cytotoxic activities of fractions from this plant have been documented (Oyama *et al.*, 2007). Although the aqueous extract of the aerial parts of *Caralluma dalzielii* is popular in traditional medicine practice in North-west Nigeria as a remedy for various types of pain in the stomach, there has not been any scientific report on its protective effect from liver diseases. The purpose of the study therefore is to evaluate the hepatoprotective activity of the aerial parts of *Caralluma dalzielii* N. E. Brown against carbon tetrachloride-induced hepatotoxicity in Wistar rats.

MATERIALS AND METHODS

Plant collection and identification

The aerial parts of the plant were collected from Sokoto North local government area of Sokoto State in June 2016. It was identified and authenticated by Dr. Halilu Mshelia of the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto. It was stored at the herbarium unit with a voucher number Pcg/UDUS/Asdy/001 issued.

Extraction of plant material

The aerial parts of *C. dalzielii* were air dried under shade for a period of one month and afterwards pulverised with pestle and mortar. Three hundred grams (300 g) of the dried plant powder was macerated in 5 L of distilled water at room temperature for 48 h. It was then filtered and the filtrate evaporated to dryness in a water bath at 70 °C.

Experimental Animals

Male Wistar rats weighing 200 - 250 g obtained from the animal facility centre of Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria were used for the experiment. The animals were acclimatised in our experimental room for 2 weeks before the commencement of the study. Standard commercial feed and water were provided *ad libitum* for the animals. Housing conditions were maintained at 25 °C at 12 h day/ night light cycles. The study was approved by the Animal Research Ethical Committee, Usmanu Danfodiyo University, Sokoto. The care and handling of the animals were according to the established public health guidelines in guide for care and use of laboratory animals (NIH, 1985).

Preliminary phytochemical tests

Phytochemical analysis was carried out using standard procedures (Trease and Evans, 1987; Harborne, 1993).

Hepatoprotective effect against CCl_4-induced hepatotoxicity in rats

Rats were divided into seven groups containing five rats each. Group 1 served as the normal control (distilled water only), group 2 was treated with standard silymarin at an oral dose of 100 mg/kg body weight only, group 3 served as CCl_4 group (negative control, distilled water daily), and group 4 received standard silymarin at an oral dose of 100 mg/kg body weight groups. Groups 5-7 were administered aqueous extract of the aerial parts of *C. dalzielii* at the doses of 250, 500 and 750 mg/kg body weight respectively. All the rat groups were administered oral daily doses for 7 days. On the seventh day, the rats of the groups 3-7 were given a single oral dose of CCl_4 (1:1) in olive oil at 1.5 ml/kg of body weight 30 min after the last dose of extract/distilled water (Mohideen *et*

al., 2003; Mohan *et al*., 2007). After 24 h of CCl_4 administration, rats were anesthetized with chloroform and blood was collected through cardiac puncture into a clean, dry centrifuge tubes. The serum was separated out and used for the determination of aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) (Reitman and Frankel, 1957), alkaline phosphatase (ALP) (McComb and Bowers, 1972), total and conjugated bilirubin, total protein (Gornall *et al*., 1949), albumin (Lowry *et al.,* 1957), urea (Marsh 1965), creatinine (Biod and Sirota, 1948) and serum electrolyte levels estimated using an automated ion selective electrode machine (Audicom electrolyte analyser). The livers and kidneys were immediately removed and fixed in Bouin's solution for histological studies.

Histopathological studies

The fixed tissues were dehydrated in an ascending series of alcohol, cleared in xylene, and embedded in paraffin wax melting at 60 °C. Serial sections (5-μm thick) obtained by cutting the embedded tissue with microtome (Baravalle *et al*., 2006). They were afterwards stained with Mayer's haematoxylin and eosin dyes (H&E) and mounted on a light microscope (x 100) for histopathological examination. Livers were scored for hepatic injury via light microscopy with score 0 = no visible cell damage; score 1 = focal hepatocyte damage on less than 25% of the tissue; score 2 = focal hepatocyte damage on 25-50% of the tissue; score 3 = extensive but focal with hepatocyte lesion; score 4 = global hepatocyte necrosis (Dammag, 2015).

Statistical analysis

The results of the experiment were presented as mean ± standard error of mean (S.E.M). Comparison in all the groups was made using one-way analysis of variance (ANOVA). Student's t-test was employed for comparing the means between groups. Differences were considered significant at $p<0.05$.

RESULTS

Plant extract yield and preliminary phytochemical analysis

The percentage yield was 18.7% w/w. The phytochemical screening gave positive test result for saponins, flavonoids, alkaloids, sterols, terpenes, glycosides and tannins. Phenolic constituents were not found in the tested extract.

Biochemical tests

The rats treated with CCl_4 produced marked elevation in the values of AST, ALT and ALP (Table 1) indicating hepatocellular damage. Serum enzyme pretreated with varying doses of the aqueous extract of the aerial parts of *Caralluma dalzielii*, produced a significant ($p<0.05$) reduction of the serum enzyme levels at all dose levels when compared to the negative control CCl_4 group. However, at the highest dose (750 mg/kg), ALT was not significantly affected when compared to the negative control CCl_4 group (Table 1). Histopathological examination of liver sections of normal and silymarin only control groups showed normal portal triad, liver parenchyma with central vein and radiating column of hepatocytes (Plates 1 and 2). Visible distortion in the liver architecture, hepatocyte necrosis, inflammatory cell infiltration and fatty degeneration with prominent congestion in the central vein (Plate 3) were evident in the photomicrograph of the liver tissues treated with CCl_4 only. The liver sections of the rats of groups 4-7 showed signs of protection as it was evident by mild (Plate 4) or absence of necrosis (Plates 5, 6 and 7) respectively. All groups show varying levels of protection or liver damage. The severity of liver injuries in all the treated groups were scored as presented in table 2.

In the serum electrolyte levels determination, the extract at 250 and 500 mg/kg significantly ($p<0.05$) increased the levels of Na^+ and Cl^- compared to the CCl_4 group. They were however similar to that of the normal control and the silymarin only control groups (Figure 1). Administration of CCl_4 to rats produced an increase in creatinine and reduction in total protein levels (Figure 2). Administration of the extract at 500 and 750 mg/kg to the rats produced creatinine levels that were comparable with the normal and silymarin control groups. They were however significantly ($p<0.05$) lower than the CCl_4 control group. Total protein was significantly increased only at 250 mg/kg of the extract comparable with the normal control group. Urea levels in 250 and 500 mg/kg was significantly ($p<0.05$) higher than that of the CCl_4 control

group (Figure 2). Histology of the kidneys showed regular glomeruli with normal proximal and convoluted tubules in all the treated groups and controls.

Table 1. Effect of pre-treatment with aqueous extract of *Caralluma dalzielii* on CCl_4-induced rat

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	T. B (mg/dl)	D. B (mg/dl)
Normal	207.3 ± 5.2*	117.0 ± 5.6*	729.0 ± 4.7*	0.61 ± 0.06	0.29 ± 0.04
Silymarin	199.8 ±12.1*	89.8 ± 4.8*	662.3 ± 5.7*	0.41 ± 0.01	0.19 ± 0.01
CCl_4	319.8 ± 6.6	471.3 ± 4.2	864.8 ± 0.1	0.62 ± 0.05	0.28 ± 0.05
Silymarin + CCl_4	234.0 ± 10.3*	196.8 ± 21.5*	695.5 ± 0.1*	0.42 ± 0.08	0.18 ± 0.03
250 mg/kg + CCl_4	134.3 ± 4.6*	147.8 ± 19.7*	718.3 ± 0.1*	0.42 ± 0.02	0.23 ± 0.02
500 mg/kg + CCl_4	276.8 ± 12.2*	362.0 ± 11.7*	788.8 ± 0.1*	0.62 ± 0.03	0.27 ± 0.03
750 mg/kg + CCl_4	231.2 ± 14.0*	429.3 ± 40.1	712.0 ± 0.1*	0.71 ± 0.04	0.34± 0.04

Data presented as Mean ± S.E.M. n=5; *p <0.05, significantly different from the CCl_4 group, T.B = total bilirubin, D.B = direct bilirubin.

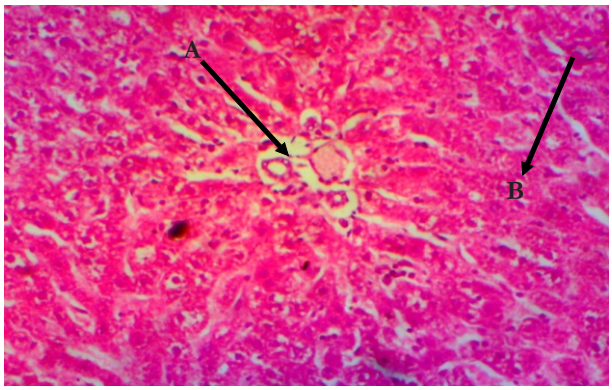


Plate 1: Photomicrograph of liver section of control rats showing normal portal triad, liver parenchyma with central vein (A) and radiating column of hepatocytes (B) (Haematoxylin and eosin x100)

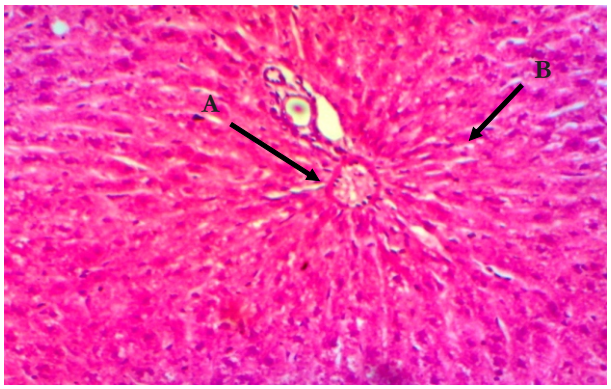


Plate 2: Photomicrograph of liver section of rats treated with silymarin positive control, showing normal portal triad, liver parenchyma with central vein (A) and radiating column of hepatocytes (B) (Haematoxylin and eosin x100)

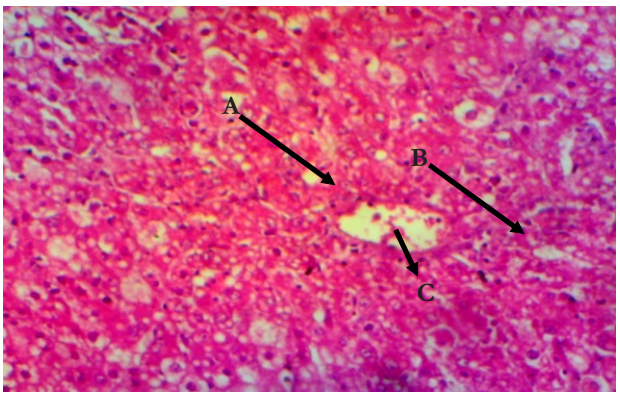


Plate 3: Photomicrograph of liver section of rats treated with carbon tetrachloride negative control, showing visible distortion in the liver architecture (A), hepatocyte necrosis, inflammatory cell infiltration and fatty degeneration (B) with prominent congestion in the central vein (C) (Haematoxylin and eosin x100)

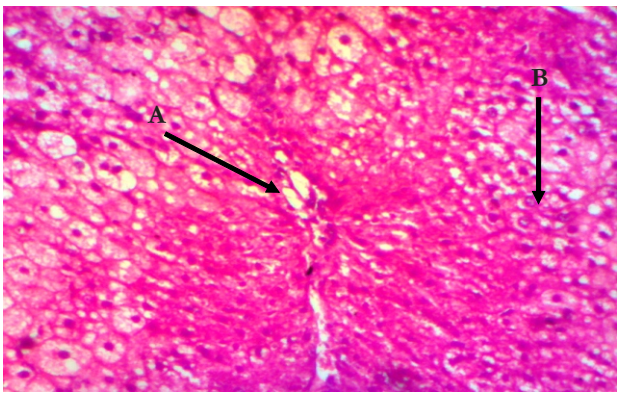


Plate 4: Photomicrograph of liver section of rats treated with carbon tetrachloride (1.25 ml/kg) and silymarin 100 mg/kg, showing mild distortion in the liver architecture and congestion of the central vein (A), mild hepatocyte necrosis, inflammatory cell infiltration and fatty degeneration (B) (Haematoxylin and eosin x100)

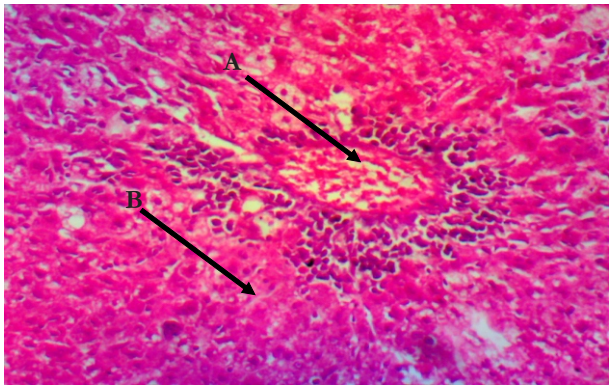


Plate 5: Photomicrograph of liver section of rats treated with carbon tetrachloride (1.25 ml/kg) and *Caralluma dalzielii* extract (250 mg/kg), showing normal portal triad with mild congestion around the central vein (A), fatty degeneration and absence of necrotic tissues (B) (Haematoxylin and eosin x100)

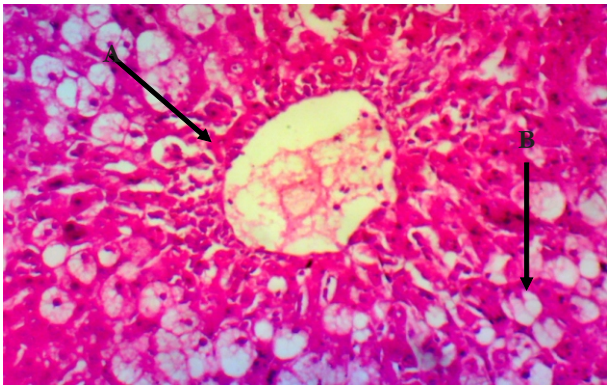


Plate 6: Photomicrograph of liver section of rats treated with carbon tetrachloride (1.25 ml/kg) and *Caralluma dalzielii* extract (500 mg/kg), showing normal portal triad with mild congestion around the central vein (A), prominent fatty degeneration and absence of necrotic tissues (B) (Haematoxylin and eosin x100)

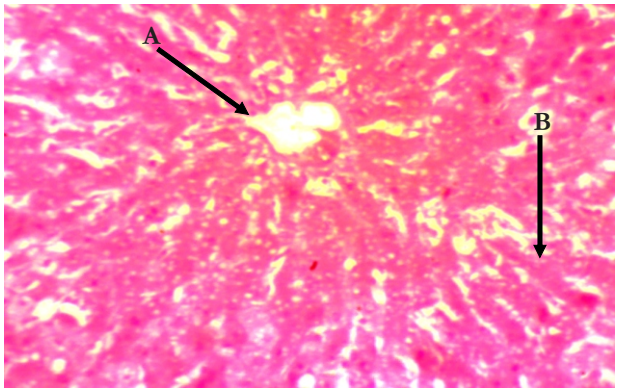
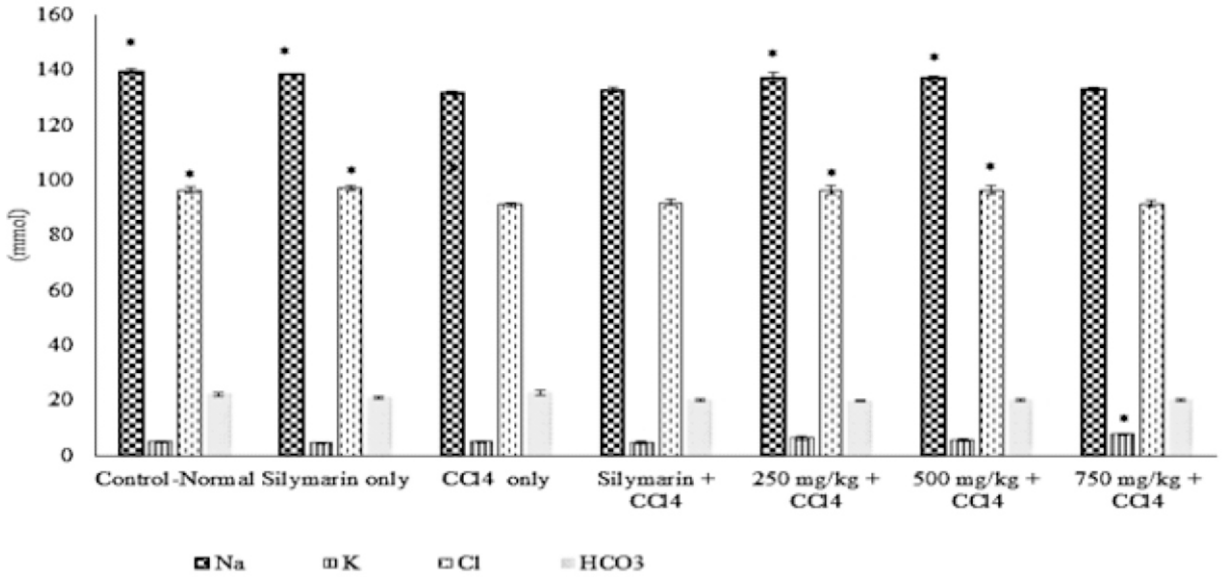


Plate 6: Photomicrograph of liver section of rats treated with carbon tetrachloride (1.25 ml/kg) and *Caralluma dalzielii* extract (750 mg/kg), showing normal portal triad with mild congestion around the central vein (A), absence of fatty degeneration and necrotic tissues (B) (Haematoxylin and eosin x100)

Table 2: Histological injury scores of liver tissues under different doses of AECD in CCl_4-treated rats

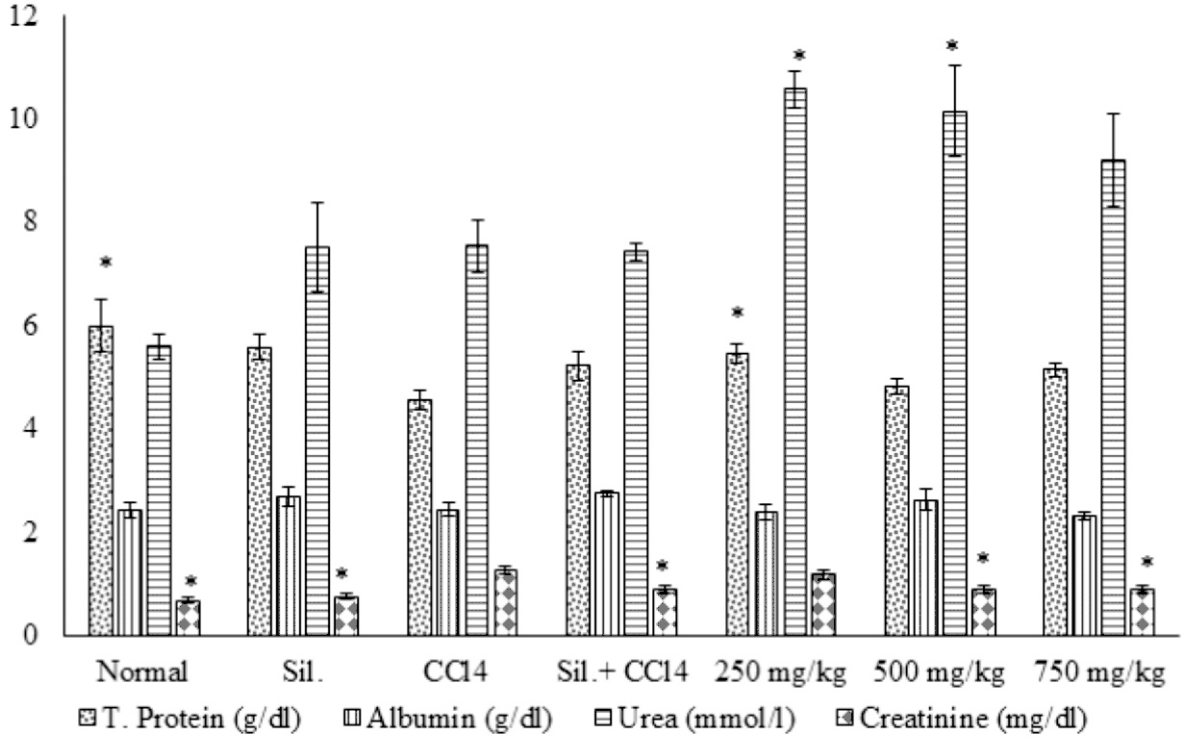
Group	Fatty degeneration	Architecture distortion	Congestion	Necrosis	Inflammation
Normal	0	0	0	0	0
Silymarin	0	0	0	0	0
CCl_4	4	4	3	4	3
Silymarin + CCl_4	2	1	2	1	1
250 mg/kg + CCl_4	1	0	1	0	1
500 mg/kg + CCl_4	2	0	1	0	1
750 mg/kg + CCl_4	0	0	1	0	0

Livers were scored for hepatic injury via light microscopy with score 0 = no visible cell damage; score 1 = focal hepatocyte damage on less than 25 % of the tissue; score 2 = focal hepatocyte damage on 25-50 % of the tissue; score 3 = extensive but focal with hepatocyte lesion; score 4 = global hepatocyte necrosis, AECD = aqueous extract of the aerial parts of *Caralluma dalzielii*



Data presented as Mean ± S.E.M. Na^+= Sodium ion, K^+= Potassium ion, Cl^-= Chloride ion, HCO_3^- = Bicarbonate ion; n= 5 *p <0.05, significantly different from the CCl_4 group

Figure 1: Effect of *Caralluma dalzielii* **on serum electrolyte**



Data presented as Mean ± S.E.M. Sil. =Silymarin, CCl_4= Carbon tetrachloride, T. protein= Total protein; n=5; *p <0.05, significantly different from the CCl_4 group

Figure 2: Effect of *Caralluma dalzielii* **on kidney function parameters**

DISCUSSION

The aqueous extract of the aerial parts of *C. dalzielii* administered prophylactically exhibited significant protection against CCl_4-induced liver toxicity as shown in the result by the reduction in the CCl_4-mediated rise in serum transaminases and ALP. Liver injury induced by CCl_4 is usually employed as a model for testing hepatoprotective drugs (Slater and Greenbaum, 1965). CCl_4-induced hepatotoxicity is believed to involve two phases. In the initial phase, the metabolism of CCl_4 by cytochrome P450 to the trichloromethyl radicals occurs (Kaplowitz, 2000; Bissell *et al.*, 2001). These free radicals bind to lipoprotein and leads to peroxidation of lipids of the endoplasmic reticulum which causes changes in the physical and chemical properties of cellular membranes (Pradeep *et al.*, 2009), thus effecting their fluidity and permeability for ion exchange, resulting in leakage of enzymes in blood and eventually resulting in swelling, cytolysis, and cell death (Basu 2003; Manibusan *et al.*, 2007). The second phase involves the activation of Kupffer cells, which is followed by the production of proinflammatory mediators (Dammag, 2015). CCl_4 effect is generally detected after 24 h of its administration. Hence the withdrawal of the blood for biochemical parameters should be carried out only after 24 h of CCl_4-induction (Sureshkumar and Mishra, 2006).

AST, ALT and ALP are liver enzyme markers that are commonly used to assess liver injury (Kozer *et al.*, 2003). The rise in serum levels of these enzymes has been attributed to the damaged structural integrity of the liver, because they are in the cytoplasmic area and released into circulation after cellular damages (Sallie *et al.*, 1991; Jadeja *et al.*, 2011). The elevated activities of these enzymes are indicative of cellular leakage and loss of the functional integrity of the cell membranes in liver which is always associated with necrosis of the liver cells (Naik and Panda, 2008). In our study, the rise in AST, ALT and ALP levels in the serum observed after oral administration of CCl_4 was significantly reduced by pre-treatment with our extract at all dose levels. This result complied with previous reports that serum levels of transaminases return to normal due to stabilization of plasma membrane as well as repair of hepatic tissue damages caused by CCl_4 (Aniya *et al.*, 2005). Again, reduced total protein content is a useful index of severity of hepatocellular damage (Sureshkumar and Mishra, 2007). Administration of CCl_4 results in the reduction of serum total protein (Bishayee *et al.*, 1995). In our study, our extract at the lowest dose significantly increased the total protein levels caused by the administration of CCl_4. Histopathological study showed extensive fatty degeneration, architecture distortion, congestion, necrosis and inflammation which was induced by oral administration of single dose of CCl_4 (Karakus *et al.*, 2011). However, our extract and silymarin protected the livers. Their histological appearances were similar to that of the control groups. Tissue damage and necrosis were of less extent in these groups than the CCl_4 group.

The kidney plays an important role in maintaining homeostasis of the body by reabsorbing important material and evacuating waste yields. Serum creatinine is commonly used as a measure of kidney function (Rincón *et al.*, 1999). Increase in the level of creatinine in the blood is an indicator of kidney damage (Rahmat *et al.*, 2014). In this study, administration of CCl_4 produced a significant increase in creatinine levels. The increase is indicative of cellular leakage and loss of functional integrity of cell membrane in renal tissue (Al-Duais *et al.*, 2012). Pre-treatment with our extract significantly decreased the serum creatinine level to near normal compared to CCl_4–induced rats. This also suggests that the extract may contain constituents that are responsible for enhancing renal function. Histopathological examination of the kidneys did not produce obvious change in the architecture of both the control and the extract-treated kidneys. This is because the assault was within 24 h period.

Silymarin is used in the treatment of liver diseases (Wong *et al.*, 2012). It acts by enhancing hepatic glutathione and may contribute to the antioxidant defence of the liver (Vargas-Mendoza *et al.*, 2014). It has also been shown that silymarin increases protein synthesis in hepatocytes by stimulating RNA polymerase I activity (Vargas-Mendoza *et al.*, 2014). In the present study, silymarin caused a significant decrease in serum liver enzymes

activity induced by CCl_4 in rats. These results are consistent with previous reports (Mohan *et al.*, 2007). The overall histopathological findings correlate well with the biochemical estimations and suggested that *C. dalzielii* may be effective against CCl_4-induced toxicity in liver.

The preliminary phytochemical studies indicated the presence of saponins, flavonoids, alkaloids, sterols, terpenes, glycosides and tannins in the aqueous extract of the aerial parts of *C. dalzielii*. Since flavonoids have hepatoprotective activity (Svobodová *et al.*, 2007; Vargas-Mendoza *et al.*, 2014), it may be speculated that these constituents of *C. dalzielii* may be responsible for the observed protective effects. However, the role of other constituents cannot be ruled out.

CONCLUSION

In conclusion, the aqueous extract of *C. dalzielii* contains saponins, flavonoids, alkaloids, sterols, terpenes, glycosides and tannins as phytochemical constituents and possess hepatoprotective activity against carbon tetrachloride (CCl_4) -induced liver damage. This activity of the extract may be due to the presence of flavonoids and other phytochemical constituents present in the plant.

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