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Research Article

Assessment of hepatoprotective activity of *Caralluma cicatricose* against CCl_4-induced liver damage in rabbits

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ABSTRACT

Liver disease has become one of the serious health problems as it is exposed to many kinds of xenobiotics and therapeutic agents. Certain medicinal plants had been used to cure some liver diseases. The present study aimed to evaluate the hepatoprotective effect of methanolic extract of *Caralluma cicatricose* (MECC) in rabbits with acute liver injury induced by carbon tetrachloride at a single dose of 1.25 ml/kg b.w. as a mixture with olive oil. MECC was administered in doses of (0.5, 1 and 1.5 g/kg b.w.) *via* oral gavage by intragastric tube for 8 days. The SGOT, SGPT, ALP, total protein, creatinine, blood urea nitrogen and blood glucose level were measured and histopathology of liver was performed. The results of the rabbits treated with MECC were compared with that of Liv-52. *C. cicatricosa* showed a significant dose-dependent reduction ($P< 0.05$) in the hepatic enzymes levels, blood urea nitrogen, blood glucose level and improvement of serum protein. The histopathological studies in the liver of rabbits also supported that *C. cicatricosa* extract markedly reduced the toxicity of CCl_4 and preserved the histoarchitecture of the liver tissue to near-normal structure. These results suggested that *C. cicatricose* may be acting as a natural hepatoprotective agent that prevents hepatic injury induced by $CC1_4$ and this may be due to its active phytoconstituents such as flavonoids and glycosides which present in the plant.

Keywords: Carbon tetrachloride, *Caralluma cicatricosa*, Hepatoprotective, Rabbits.

INTRODUCTION:

The liver is one of the important organs in the body. It plays a pivotal role in regulating various physiological processes. It helps in the maintenance, performance and regulating homeostasis of the body. It is involved in almost all the biochemical pathways to growth, fighting against disease, nutrient supply, energy provision and reproduction. In addition, it aids metabolism of carbohydrate, protein and fat detoxification, secretion of bile and storage of vitamins [1]. Unfortunately, the liver is often abused by environment, toxins, alcohol and over-the-counter drug use (xenobiotics) which can damage the liver and eventually lead to hepatitis, cirrhosis and liver diseases[2]. About 25,000 deaths found every year due to liver disorders[3]. Therefore, treatment of liver diseases is extremely important. Medicinal plants have been used for the treatment of several human diseases over the centuries and have been very important in the health care delivery of every nation [4].

The genus *Caralluma* comprises about 200 species distributed throughout Africa and Asia. The majority of these species are indigenous to the Indian sub-continent and Arabian Peninsula[5]. The etymology of the word "*Caralluma*" is from the Arabian, "qarh al-luhum", meaning wound in the flesh or abscess, probably from the floral odor in some taxa[6]. Some species of *Caralluma* are edible and form part of the folk medicine system of many countries[7]. These are commonly used in traditional medicine as remedies to relive wide variety of illness and health problems [8]. *Caralluma* species have shown anti-inflammatory, anti-nociceptive[9,10], antidiabetics[11,12], hepatoprotective[13], gastric mucosa protecting [14], antimalarial[15], anti-oxidant[16], anti-trypanosomal[15,17], appetite suppressant[18] and cytotoxic[19,20,21,22] activities.

Caralluma cicatricosa is belonging to the family of Asclepiadaceae, which is also known as the milkweed family because many of its members contain milky latex [23].Caralluma cicatricosa is a succulent and angular plant which growing wild in western and Arabian Peninsula. In Yemen it's found in Taiz, Maaber, Milhan, Manakha and mountainalArays[24]. In traditional Yemeni folk medicine

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C. cicatricosa is used in case of diabetes and in peptic ulcer and its juice as drops for ear inflammation. There are no phytochemical and pharmacological work has been done on C. cicatricosa. In view of traditional uses as well as the diverse biological activities so far reported for different Caralluma species, the C. cicatricose was chosen.

CCl4 is a well-known hepato-and nephrotoxicant [25,26,27,28] and proves highly useful as an experimental model for the study of certain hepatotoxic effects[29,30]. The changes associated with CCl4-induced liver damage are similar to that of acute viral hepatitis[31].

MATERIALS AND METHODS

Drugs and Chemicals

Drugs and reagents were used include: Carbon Tetrachloride from (BDH Chemicals Ltd, Poole, England), standard Kits for assay of Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP) activities in addition to, total protein, urea and creatinine levels were purchased from (SPINREACT, Spain). Liv-52 (Himalaya Drugs, India) from local supplier. All other reagents and solvents were of highest purity and analytical grade.

Plant Collection and identification

The Fresh plant was collected from various locations around Ibb city in April and May 2014 and authenticated by the taxonomist Prof. Abdulnaser Al Gifri from the department of biology, faculty of education, Aden University, Yemen.

Plant extraction

The plant roots were removed and stem (bark) of plant were washed in floating water, cut into smaller pieces and dried under shade for 14 consecutive days. The dried stems of plant were grounded with a mechanical mill (SM MARSHAL-SUPERY DRY GRINDER-MODEL NO:SM-830) and passed through sieves (No-60), to obtain fine mass. The powdered material (256.8 g) was cold macerated in 70% v/v Methanol in conical flask (V1000 ml) for three days (72 hours) with frequent stirring. Then the extract was filtered using cotton and concentrated under vacuum at 40^{0}C using a rotary evaporator (Buchi-type) and kept in closed container at approximately -20 ^{0}C.

Plant extracts yield and phytochemical analysis

The yield percentage of the extract was calculated using the following equation[32].

$$\text{The Yield (\%)} = \frac{\text{Weight of the extract(g)}}{\text{Weight of the ground plant material (g)}} \text{x100}$$

The 70% methanolic bark extract was subjected to a preliminary phytochemical screening for the presence of tannins, alkaloids, flavonoids, glycosides (reducing sugars), anthraquinones, sterols and saponins by simple qualitative methods[33,34].

Experimental animals

Adult healthy rabbits of weights (900g-1200g) and aged 8 month±1 week were obtained from a local supplier, city of IBB (IBB, Yemen). Animals were housed in metal cages (80×80×50 cm) with soft wood shavings as bedding and fed with clean water and food throughout the period of the experiment. The animals were acclimatized for one week before starting the experiment. The study protocol was approved by the University Ethics committee before any experiment can be conducted.

Acute toxicity study

Acute toxicity study was conducted by 5 female rabbits according to the Organization for Economic Cooperation and Development (OECD) revised up-and-down procedure for acute toxicity testing (OECD guideline 425, 2001)[35]. Non-pregnant female rabbits weighing 900-1000g were divided and housed singly in metal cages for one week before the start of experiment. Rabbits were fasted overnight from food, but not water, prior to dosing and weighed before the extract was administered orally. A dose of 5000 mg/kg was dissolved in distilled water at an administration volume of 10 ml/kg body weight based on the individual animal body weights obtained on the day dosing and given to the first rabbit by oral gavages, and this rabbit was observed for mortality and clinical signs for the first hour, then hourly for 3h and finally periodically until 48h. If the animal survived, then four additional animals were given the same 5000 mg/kg dose sequentially at 48-h intervals.

All of the experimental animals were maintained under close observation for 14 days, and the number of rabbits that died within the study period was noted. The LD50 was predicted to be above 5000 mg/kg if three or more rabbits survived.

Experimental design

Forty two male rabbits were grouped into seven groups each contain six animals. Group (I) served as the normal control and received only 0.5% CMC (carboxymethyl-cellulose) suspension by a gastric gavage throughout the experimental period. Group (II) treated by methanolic extract of *C. cicatricose* (MECC) 1000 mg/kg b.w. Group (III) served as the toxin control and received a 0.5% CMC suspension by a gastric gavage. Group (IV), (V), and (VI) received MECC 500 mg/kg b.w., 1000 mg/kg b.w. and

1500 mg/kg b.w. respectively while animals in group (VII) received liv-52 (5ml/kg b.w.)[39] for seven consecutive days. On the eighth day, animals groups III, IV, V, VI & VII were given single oral dose of CCl_4 (1.25 ml/kg) [36,37,38] diluted in olive oil at rate of 1:1(v:v) Sixty minutes after the last administration of the vehicle, extract and liv-52[40].

Blood Collection

Twenty four hours later[41,42], overnight fasting rabbits were deprived of food but allowed for free access of drinking water. Animals were anaesthetized with ether and blood was collected by direct cardiac puncture into tubes without anticoagulant and allowed for 30 minutes to clot at room temperature. After the clotting of the blood, it was centrifuged at 4000 rpm for ten minutes (HettichMikro 220R centrifuge, DJB lab care, UK) at 25°C for separation of serum. The supernatant was stored in the freezer at -21^{o}C until analysis [43].

Biochemical analysis

Serum levels of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total proteins, creatinine, urea and glucose were determined kinetically using commercially available kits.

Histopathological Examination

At the end of the experimental period (8 days), rabbits were anesthetized and sacrificed; liver was removed and kept in 10% formaldehyde solution and send to University of Science and Technology hospital laboratory were tissue processed and stained with hematoxyline and eosin for histological examination[44,45]**.**

Statistical analysis

Statistical analysis of the results was done using the computer software program "SPSS v.21". Comparison between different groups was carried out using the one-way analysis of variance (ANOVA) followed by least significant difference test (LSD test). Difference was considered significant when P is less than 0.05.

RESULTS

Plant extracts yield and preliminary phytochemical analysis

The percentage yield of the 70% methanolic crude extract which was obtained by cold extraction was (21.18% w/w).The results of the phytochemical screening revealed the presence of steroids, flavonoids, tannins, glycosides, saponins, carbohydrates and triterpenes in the 70% of MECC steam. Alkaloids and anthraquinones were not found in the tested extract.

Acute toxicity

The single oral administration of MECC in rabbits was non-toxic up to a dose of (5000 mg/kg) as no mortality was detected at the end of experiment (14 days).

Biochemical tests

The results showed that CCl_4 produced a significant increase in AST, ALT & ALP levels and a significant reduction in the total protein level, indicating hepatocellular damage. On other hand, administration of MECC at the doses of 500mg, 1000mg and 1500 mg/kg b.w. produced a highly significant fall in the AST, ALT & ALP levels and a significant rise in the total protein levels in a dose dependent manner. Similar results were also obtained with liv-52+CCL_4 which considered as a standard group. There was no significant alteration in control rabbits treated solely with MECC. (Table 1)

The administration of CCL_4 alone induced a significant increase in blood urea nitrogen (BUN), creatinine levels compared to control group. on other side, orally pretreatment of animals with MECC+CCL_4 and Liv-52+CCL_4 reduced the level of blood urea nitrogen significantly in these groups with respect to rabbits intoxicated with CCL_4.However,there is no significant decrease in creatinine level in animals pretreatment with MECC+CCL_4 and MECC. The result showed significant increase in blood sugar level in animal models treated with CCL_4 compared with control (Table2). Similarly, there were significant reductions in blood sugar values of animals pre-treated with MECC+CCL_4 and Liv-52+CCL_4 compared with CCl_4 treated group. Additionally, the level of glucose in the animals that pretreated with MECC, MECC+CCL_4 and Liv-52 was significantly decreased compared to normal group.

Histopathology findings

Liver morphological changes were scored, described and summarized in control, MECC treated, CCl4 treated, CCl_4+MECC treated & Liv-52 treated groups (Table 3). Liver sections of control group and rabbits pretreated with MECC showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces, and central vein. However, CCl_4 application constituted histopathological changes in the liver of rabbits. These changes were characterized by dilation of central vein with noticeable hemorrhage. Moreover, hepatocyte necrosis, inflammatory cells infiltration, fatty degeneration, and hydropic degeneration were found in rabbits 24 hours after CCl_4 treatment (Figure 1). However, liver sections of the rabbits received MECCandLiv-52 prior to CCl_4 intoxication showed less histopathological changes in a dose dependent manner when compared to CCl_4 group.

Table1: The effect of methanolic extract of *Caralluma cicatricose* associated with CCl_4-induced hepatic damage on liver function tests in rabbits.

Group	ASTU/L	ALTU/L	ALPU/L	TP g/dL
Normal control (5%CMC5mL/kg p.o.)	80.250±1.956	71.100±6.049	130.250±6.503	5.450±0.085
MECC (1g/kg p.o.)	75.250±0.540	57.350±4.148	118.500±5.529	5.250±0.140
CCl_4 (1.25ml/kg p.o.)	395.667±31.110*	438.817 ±43.331*	441.333±32.368*	4.083±0.429*
CCl_4+MECC (0.5g/kg p.o.)	284.267±54.930*#	341.500 ±21.603*#	225.767±18.003*#	5.150±0.232#
CCl_4+MECC (1g/kg p.o.)	264.500±37.825*#	290.800 ±41.201*#	223.800±29.091*#	5.250±0.096#
CCl_4+MECC (1.5g/kg p.o.)	225.667±17.091*#	235.267 ±39.457*#	181.000±24.324*#	5.317±0.168#
CCl_4+liv52 (5ml/kg p.o.)	190.350 ±12.766*#	175.880 ±24.279*#	205.800±26.164*#	5.483±0.140#

Data expressed in mean±SEM, n=6.*: P<0.05, significant difference from the normal control group; #: P<0.05, significant difference from the CCL_4 group

Table 2: The effect of methanolic extract of *Caralluma cicatricose* associated with CCl_4 toxicity on kidney function tests and Fasting blood sugar in rabbits.

Group	Blood urea nitrogen(mg/dL)	Creatinine (mg/dL)	Fasting blood sugar (mg/dL)
Normal control (5% CMC 5mL/kg p.o.)	55.450±3.593	0.958±0.013	137.750±5.522
MECC (1g/kg p.o.)	58.583±4.669	1.023±0.019	90.250±9.975*
CCl_4(1.25ml/kg p.o.)	75.667±7.805*	1.417±0.185*	153.000±15.438*
CCl_4+MECC(0.5g/kgp.o.)	49.000±1.414#	1.110±0.066	89.833±1.537*#
CCl_4+MECC(1g/kg p.o.)	50.667±3.018#	1.260±0.117	88.167±1.447*#
CCl_4+MECC(1.5g/kgp.o.)	53.167±2.960#	1.400±0.328*	83.333±3.383*#
CCl_4+liv52 (5ml/kg p.o.)	47.167±0.703#	0.948±0.057#	86.517±2.696*#

Data expressed in mean±SEM, n=6.*: P<0.05, significant difference from the normal control group;#: P<0.05, significant difference from the CCL_4group

Table 3: Histological injury scores of liver under different doses of MECC in rabbits treated with CCl_4.

Group	Injury Score [a]				
	Fatty degeneration	Architecture distortion	congestion	Necrosis	Inflammation
Control	0	0	0	0	0
MECC (1 g/kg b.w.)	0	0	0	0	0
CCL_4 (1.25ml/kg)	4	3	3	3	3
MECC (0.5 g/kg + CCl_4)	3	3	2	2	2
MECC (1 g/kg + CCl_4)	2	2	2	2	1
MECC (1.5 g/kg + CCl_4)	1	1	2	3	3
Liv-52 (5mL/kg + CCl_4)	1	1	2	2	2

[a] Livers were scored for hepatic injury via light microscopy with score 0 = no visible cell damage; score1 = 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 lesions; score 4 = global hepatocyte necrosis.

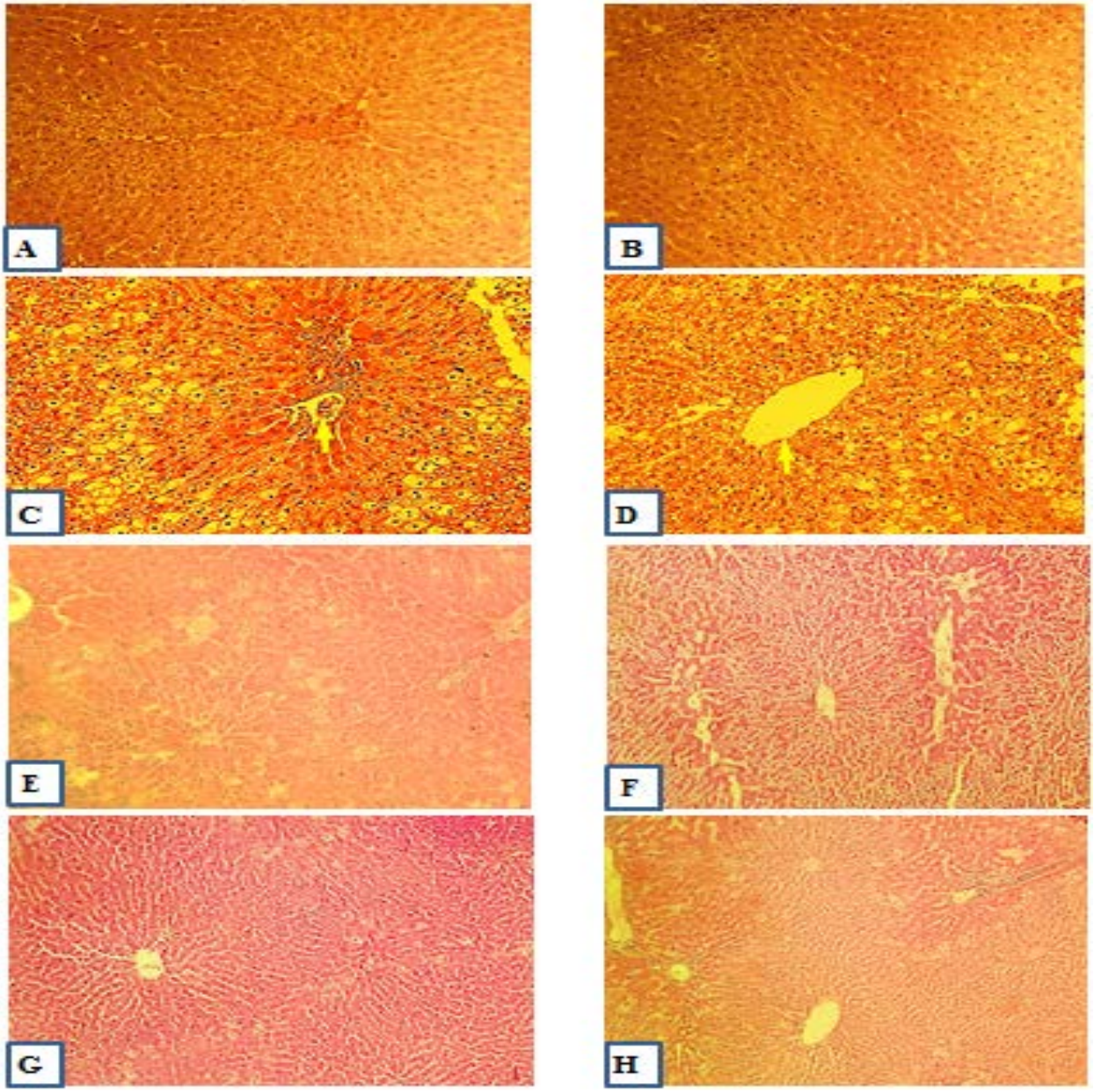

Figure 11: Photographs of liver sections of CCl_4 (1.25 ml/kg) toxicity in rabbits (hematoxylin and eosin stained,10x). **(A)** Normal control, **(B)** MECC 1 gram/kg,**(C&D)** Toxin control (CCl_4), **(E)** MECC0.5 gram/kg +CCl_4, **(F)**MECC 1 gram/kg + CCl4, **(G)** MECC 1.5gram/kg and**(H)** Liv-52 5ml/kg + CCl_4.

Discussion

Phytochemical screening of *C. cicatricosa* has revealed the presence of steroids, flavonoids, tannins, glycosides, saponins, carbohydrates and triterpenes. Phytoconstituents like flavonoids, triterpenoids, saponins and alkaloids are known to possess hepatoprotective activity[46,47]. *C. cicatricosa* possesses a significant protective effect against hepatotoxicity induced by CCl_4 which may be attributed to the individual or combined action of phytoconstituents present in it. The liver-protective herbal drugs have been shown to contain a variety of chemical constituents like phenols, coumarins, ligands, essential oils, monoterpenes, carotenoids, glycosides, organic acids, lipids, xanthene etc.[48,1]. Thus it is possible that other secondary metabolites of the plant as observed in the present study may be responsible for the hepatoprotective activity of the plant.

In the current study, acute toxicity test was done to establish if any adverse affects of the administration of MECC on some observable parameters. Changes in body

weight have been used as an indicator of adverse effects of drugs and chemicals[49,50]. No significant changes were observed in the general behavior, body weight, suggesting that at single oral doses administered, MECC had no effect on the normal growth of rabbits. From the results of acute oral toxicity study of the methanolic extract, it can be concluded that LD50 of the drug is greater than 5000 mg/kg bodyweight, which means that even a dose of 5000 mg/kg bodyweight of methanolic extract of stem *of C. cicatricosa* is safe for administration.

CCl_4-induced hepatotoxicity is believed to involve two phases. The initial phase involves the metabolism of CCl_4 by cytochrome P450 to the trichloromethyl radicals ($CCl3^{\bullet}$ and/or $CCl3OO^{\bullet}$)[51,52], a free radical that binds to lipoprotein and leads to peroxidation of lipids of the endoplasmic reticulum which causes changes in the physical and chemical properties of cellular membranes[53,54], thus effecting their fluidity and severity of liver injury[63]. 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 hepatonecrosis[64,65]. In this study, significant increase in AST, ALT and ALP levels in the serum was observed after oral administration of CCl_4, indicated that CCl_4 intoxication compromised the integrity of the hepatic cell membranes [66,67,68]. Pretreatment with MECC+CCL_4 and Liv52+CCL_4 significantly decreased levels of AST, ALT and ALP towards the normal value. These observations are in agreement with the commonly accepted view 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[69].

Reduction in total protein content can be deemed as a useful index of severity of hepatocellular damage[70]. In the present study, CCl_4 intoxication reduced the serum total protein level which is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of cytochrome P-450 enzymes leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver[71].Previous studies have showed that administration of CCl_4 to a diversity of animal types results in a rapid reduction in protein synthesis in the liver [66,72,73]. The pretreatment of MECC+CCL_4 restored the total protein level that suggests the stabilization of endoplasmic reticulum leading to protein synthesis[59].

The kidney assistances in keeping homeostasis of the body by reabsorbing important material and evacuating waste yields. Creatinine is a commonly used as measure of kidney function[74]. The increase in the level of creatinine in the blood considered an indicator to the kidney damage[75]. In this experiment, oral administration of CCl_4 resulted in a significant increase in creatinine and blood urea nitrogen concentrations. The observed increase is indicative of cellular leakage and loss of functional integrity of cell membrane in renal tissue[76,77].In the current study, the pretreatment with MECC+CCl_4 significantly decreased serum urea levels in a dose dependently manner, compared to CCl_4–induced rabbits by enhancing the renal function that is generally impaired in CCl_4–induced rabbits. On other hand, elevated serum level of creatinine in the CCl_4 group in this study was found to reduce insignificantly sequel to administration of MECC in a dose-dependent manner. But the dosage of 1500mg/kg b.w. increased serum creatinine level significantly compared to normal model. This suggests that the high dose of this plant extract may precipitate kidney damage.

permeability for ion exchange, resulting in leakage of enzymes in blood and finally results in swelling, cytolysis, and cell death[55,56,57]. The second phase of CCl_4-induced hepatotoxicity involves the activation of Kupffer cells, which is accompanied by the production of proinflammatory mediators[58]. The effect of CCl_4 is generally observed after 24 h of its administration and then values slowly normalized. Hence the withdrawal of the blood for biochemical parameters should be carried out only after 24 h of CCl_4 intoxication[59,42].

The liver marker enzymes (AST, ALT and ALP) are largely used as most common biochemical markers to evaluate liver injury[60,61]because these enzymes are placed in cytoplasmic area of the cell and are released into circulation in case of cellular damage[62]. Thus, the activities of these enzymes in serum can reflect the

In the present study, serum glucose level was higher in CCL_4 treated rabbits. The elevation of glucose level could be recognized to destruction of hepatocytes brought by CCl_4 intoxication[78,79]or decreasing of glycogen contents due to degradation of glycogen to glucose in hepatocytes after treatment with CCl_4 which leads to increase in glucose level in the blood[80]. The hypoglycemic effect of *C. cicatricosa* observed in the present work was consistent with the observation on other Caralluma extract[11]. The possible mechanism may be by potentiation of insulin effect either by increasing the pancreatic secretion of insulin from β-cells

of islets of Langerhans or by increasing the peripheral glucose uptake[81].

Liv-52 is widely used in treatment of liver diseases of varying origins. It enhances tocopherol levels, which inhibits lipid peroxidation and scavenges free radicals[82]. In the present study, Liv-52 caused a significant decrease in serum enzymes activity induced by CCl_4 in rabbits. These results were in agreement with previous investigation[39].

On the contrary, pre-treatment with MECC alone did not show any significant elevation in the amino transferases activities and kidney biochemical parameters when compared to control. This finding is anther pointer to the safety of this extract for consumption at this concentration.

Histopathological study showed extensive fatty degeneration, architecture distortion, congestion, necrosis and inflammation which successfully induced by oral administration of single dose of CCl_4[83,84]. The histological appearance of MECC and liv-52 groups was partly similar to that of the control group, and tissue damage and necrosis were of less extent in these groups than the CCl_4 group in a dose-dependent manner. As well as, no abnormalities or no changes observed in liver sections of rabbits pretreated by MECC and this indicated safety of the plant on liver organ. The overall histopathological findings are well correlating with the biochemical estimations and suggested that *C. cicatricosa* may be effective against CCl_4-induced changes in liver.

CONCLUSIONS

This study has shown that the methanolic extract of *Caralluma cicatricosa*is is safe in experimental rabbits up to a dose of 5000 mg/kg and has hepatoprotective activity against carbon tetrachloride-induced liver damage. This activity of the extract may be due to the presence of flavonoids and other phytochemical constituents present in the plant.

FUTURE WORK

Recommended future work is to look at the effect of *C. cicatricosa* on diabetic animals as well as to isolate the chemical compound(s) responsible for hepatoprotection. Moreover, sub-acute and chronic evaluation of the extract should be carried out in evaluating the safety profile of *C. cicatricosa*.

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CONFLICT OF INTERESTS

There is no conflict of interests regarding the publication of this paper.

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