



Assessment of Hepatoprotective Activity of Caralluma Tuberculata Stem Against CCl₄-Induced Liver Damage in Rabbits in Sherani Distruct of Balochistan



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Abstract

Liver disease has become one of the serious health problems as it is exposed to many kinds of xenobiotic and therapeutic agents. Acute liver failure (ALF) is a potentially fatal complication of severe hepatic illness resulting from viral hepatitis or drug use. Some species of Caralluma are edible and form part of the folk medicine system of many countries. These are commonly used in traditional medicine as remedies to relive wide variety of illness and health problems. Silimycin is the extract of silybum marianum, or milk thistle, and consists of seven flavonolignans (silibinin, isosilibinin, silychristin, isosilychristin and silydianin) and a flavonoid (taxifolin). Caralluma species have shown anti-inflammatory, anti-nociceptive antidiabetics Hepatoprotective gastric mucosa protecting antimalarial antioxidant, anti-trypanosomal, appetite suppressant and cytotoxic activities. The present study aimed to evaluate the hepatoprotective effect of Methanolic Extract of Caralluma Tuberculata (MCCTE) 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. MCCTE was administered in doses of 500mg/kg b.w.) Via oral gavage by intragastric tube for 15 days. The SGOT, SGPT, ALP, total protein. The results of the rabbits treated with MCCTE were compared with that of standard drug showed a significant reduction ($P < 0.05$) in the hepatic enzymes levels, improvement of serum protein. These results suggested that MCCTE may be acting as a natural hepatoprotective agent that prevents hepatic injury induced by CCl₄ and this may be due to its active phytoconstituents such as flavonoids which present in the plant.

Keywords: Silimycin; Caralluma tuberculata; Acute liver failure; Hepatoprotective Activity; CCl₄

Introduction

In the past decade, there has been a great increase in the use of complimentary treatments such as herbal remedies in the treatment of various diseases [1] Liver is the largest and most complex internal organ in the body, which plays an important role in maintenance of internal environment with the help of its multifarious and several functions [2]. It is connected to intermediary metabolism of proteins, fats, and carbohydrates as well as in the synthesis of number of plasma proteins [3], such as albumin, fibrinogen and in the production of various enzymes, formation and excretion of bile [4] such as paracetamol, carbon tetrachloride, thioacetamide and isoniazid catabolize the radicals, bring on lipid peroxidation, damage the membranes of liver cells and organelles, cause the inflammation and necrosis of hepatocytes and leads to the liberation of cytosolic enzymes into the systemic transmission [5]. The most common disease of the liver is jaundice can be presented as yellow coloration of eye sclera, skin and mucous membrane due to increase amount

of bilirubin in body, having prehepatic, hepatic or post-hepatic causes [6].

Plants and plant products are part of the vegetarian diet and several them exhibit medicinal properties. Silymarin is a standardized mixture of antioxidant flavonolignans (silybin and silibinin) extracted from the medicinal plant Silybum marianum [7] Silymarin is the extract of silybum marianum, or milk thistle, and consists of seven flavonolignans (silibinin, isosilibinin, silychristin, isosilychristin and silydianin) and a flavonoid (taxifolin) [8]. Among these substances, silybin is mainly prevalent and has the most important biological effect. It makes up about 70% of the total composition of silymarin in the form of two diastereo-isomeric compounds: silybin a and silybin b [9].

Silymarin was proved to have a protective effect against experimental hepatotoxicity by regulating the actions of the ultrastructure of liver cells and improving the performance of

hepatic enzymes and bile production [10]. The purpose of this study was to evaluate the Hepatoprotective effect of the stem of *C. Tuberculata* against the CCL_4 induced hepatotoxicity in rabbits (Figure 1). The local people has been using this plant since long for bitter tonic, febrifuge, stomachic and carminative useful in rheumatism internal wounds, diabetes, throat pain, fever, paralysis and joint pain, weight loss, consumed as vegetable and for economical purposes. Furthermore, the other uses such as fresh plant is chewed, ear inflammation, Dysmenorrhea, abdominal pain, constipation and hepatitis B & C Peptic ulcer.



Figure 1: Caralluma Tuberculata Plant in Sherani Distract.

Materials and Methods

Selected plants material

Caralluma Tuberculata are edible and form part of the folk. This plant belonging to the family of Asclepiadaceae, which is also known as the milkweed family because many of its members contain milky latex. Some species of *Caralluma* are edible and form part of the folk medicine system of many countries [11]. It is commonly used in traditional medicine as remedies to relive wide variety of illness and health problems [12].

Samples collection area

The Fresh plant was collected in April and May from various locations around the hill of village Ahmadedergah near to Tahkht-a- Suleiman district Sherani Balochistan, Pakistan (Figure 2). The collected samples authenticated by department of Pharmacognosy faculty of Pharmacy, University of Baluchistan, Quetta under the specimen's voucher.



Figure 2: Sample Collection Area of Sherani District.

Preparation of extract

The fresh plants *Caralluma Tuberculata* were exposed to washing, then desired materials were left for 60 minutes after that the stem were chopped in small pieces and dehydrated at 25°C for 60 days under shade. The dried fragments were milled into fine powder [13]. Macerated in methanol at room temperature, occasional shaking as well as stirring done on alternate day for fifteen days followed filtration [14]. The extracts obtained were concentrated via Heidolph rotary serial number 519-00000-003, made of (Germany) evaporation at (45°C). Semi-solid (CMEs) was obtained, kept at 4°C until screened for further processing [15] (Figure 3).

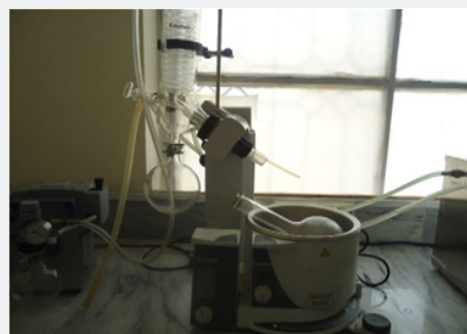


Figure 3: Heidolph rotary used for processing.

Experimental animals

Healthy adult rabbits (1000-1300g) were obtained from the Center for Advanced studies in Vaccinology and Biotechnology (CASVAB), UoB. The animals were kept in a well-ventilated room and the animals were exposed to 12 hrs day and night cycle with a temperature between $20 \pm 3^\circ C$. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed ad libitum, supplied by this institution. All the experiments were performed after obtaining prior approval of the Animal Ethics Committee (AEEC) of university of Balochistan, Quetta (Figure 4).



Figure 4: Experimental Rabbits at CASVAB.

Drugs and reagents

Drugs and Chemicals Drugs and reagents were used include: Carbon Tetrachloride from ((Merck) standard Kits for assay of Aspartate transaminase (AST), Alanine transaminase (ALT),

Alkaline phosphatase (ALP) activities in addition to, total protein, bilirubin, albumin levels were purchased from the local supplier. All other reagents and solvents were of highest purity and analytical grade [16].

Extract dose preparation

The dose was based on the body weight (mg/kg) and calculated through following formula

$$\frac{\text{Animal Weight (kg)} \times \text{dosage (mg / kg)}}{\text{Concentration of drug (mg / ml)}} = \text{Drug Volume in ml or cc}$$

The Methanolic crude extracts (MCE) were dissolved and diluted with distilled water. Controlled animals were treated with an equal volume of saline solution of raw pieces [17].

Experimental design

The animals were distributed into 4 groups 5 rabbits in each group.

Control rats given physiological saline solution 10 mL/kg body wt, p. o for 14 days only.

Rabbits given silymarin (25 mg/kg body wt / p. o for 14 days only.

Rabbits given Caralluma Tuberculata (500 mg/kg body wt / p o) for 14 days only.

Rabbits given CCL₄ + Caralluma Tuberculata (1.25 ml/kg b.w. as a mixture with olive oil + 500 mg/kg body wt / p o) respectively on day 16th.

Rabbits given CCL₄ + silymarin (25 mg/kg body wt / p. o for 14 days only on day 16th

At the end of the experimental period in 24h after last treatment the animals were killed by cervical decapitation. Blood was collected without anticoagulant for the separation of serum.

Biochemical analysis

Blood samples were taken into centrifuge tube with rubber caps labeled and centrifuged at 6000 rpm for 15-20 minutes. Serum biochemical parameter such as Transaminases (AST and ALT), AkP, GGT, Bilirubin and protein levels were estimated according to standard methods. The supernatant was stored in the freezer at -21 0C until analysis [18] (Figure 5).

Time period

Upon carrying out all the animal trials, the experiment was finished subsequently after 15 days the oral dose (30 to 40 min) of the material [19].

Administration route

The oral route was used for plant extract, standard drug and CCL₄(Figure 6).



Figure 5: Lab Balance” used for weighing”.



Figure 6: Oral Feeding.

Statistical analysis

All statistical analysis, including the calculations of mean ± SE, the Student’s t-test, and the simple linear regression analysis, were carried out using SPSS and Graph Pad Prism programs. Significance was set at P< 0.05 level.

Results

Hepatoprotective test

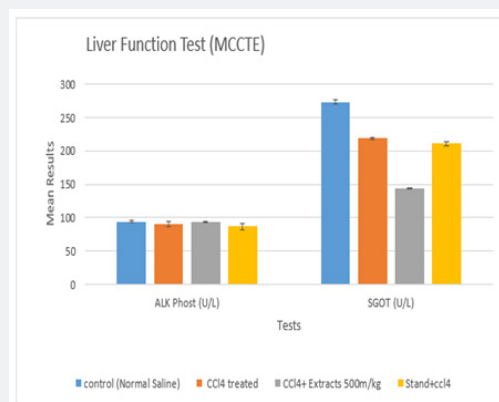


Figure 7: Hepatoprotective activity. Effects of MCCTE LFT Rabbit.

Liver function test: Findings in the current test of TB, DB, ALT, AKP, GGT and SGOT for the controlled group were, 0.76± 0.060, 0.032± 0.008 (mg/dL) 4.2± 0.802, 2.28± 0.10, 72.28±0.107, and 233± 2.818 (U/L), while, the same parameters were calculated in CCL₄ treated group as 0.86± 0.075, 0.064±0.006 (mg/dL), 4.6± 0.680, 90.4± 3.467, 2.28± 0.107 and 278.4± 1.439 (U/L), respectively (Figure 7). Furthermore, the same parameters were

found in the group treated with CCL₄ and MCOLE 500mg, as 5.56± 0.150, 0.02± 0.003 (mg/dL) 5.1± 0.044, 2.12± 0.049, 93.34± 1.193 and 149.8± 1.322, (U/L) [20]. Whereas, for the Standard drug (Silimyarin) and CCL₄ treated group, values were 0.7± 0.0838, 0.028± 0.008, 3.4± 0.680 (mg/dL), 2.18± 0.080, 86.8± 4.544, 2.18± 0.0802 and 210.6± 2.587 (U/L), as shown in the (Table 1) [21].

Table 1: Hepatoprotective activity Effects of MCCTE in LFT rabbit.

Test	TB (mg/d)	DB (mg/dl)	SGPT (U/L)	GGT	AK P (U/L)	SGOT (U/L)
control (Normal Saline)	0.76± 0.060	0.032± 0.008	4.2± 0.802	2.28± 0.107	90.8± 1.500	233± 2.818
CCL ₄ treated	0.86± 0.075	0.064± 0.006	4.6± 0.680	2.44± 0.150	98.4± 3.467	278.4± 1.439
CCL ₄ ± Extracts 500m/kg	5.56± 0.150	0.02± 0.003	5.1± 0.044	2.12± 0.049	93.34± 1.193	149.8± 1.322
Stand ± CCL ₄	0.7± 0.0838	0.028± 0.008	3.4± 0.680	2.18± 0.080	86.8± 4.544	210.6± 2.587

Protein profile (PP): The values for TP, Al, Gb, in the current study were 6.18± 0.097, 3.38± 0.132 and 3.42± 0.165 (g/dL), while, the values of the same parameters in the CCL₄ treated group were, 6.54± 0.116, 3.46± 0.121 and 3.44± 0.136 (g/dL), whereas, the same parameters for the MCCTE 500mg/kg and CCL₄ treated group, 5.72± 0.080, 3.34± 0.068 and 3.42± 0.143 (g/dL), respectively. Furthermore, these parameters for the standard drug ± CCL₄ were calculated as 6.36± 0.133, 3.64± 0.211 and 3.54± 0.163 as shown in the (Table 2).

Table 2: Hepato-protective activity Effects of MCCTE on PP.

Groups	TP (mg/dl)	Al(mg/dl)	Gb (mg/dl)
Controlled)	6.18± 0.097	3.38± 0.132	3.42± 0.165
CCL ₄ treated	6.54± 0.116	3.46± 0.121	3.44± 0.136
CCL ₄ ± Extracts 500m/kg	5.72± 0.080	3.34± 0.068	3.42± 0.143
Stand±ccl ₄	6.36± 0.133	3.64± 0.211	3.54± 0.163

Values are the mean number of activities ± standard errors mean (n=5), (P<0.05) PP= Protein profile, TP = total protein, Al = Albumin, GB= Globulin (Figure 8).

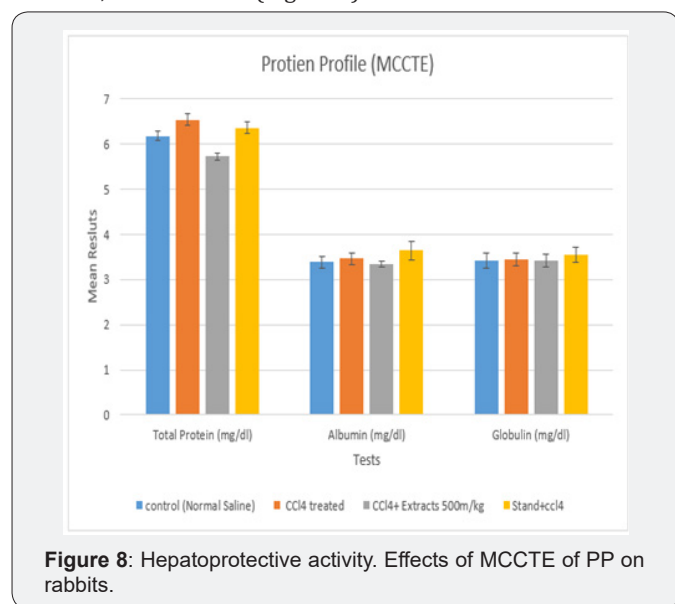


Figure 8: Hepatoprotective activity. Effects of MCCTE of PP on rabbits.

Discussion

Phytochemical screening of Methanolic Crude *C. tuberculata* Extract (MCCTE) has revealed the presence of steroids, flavonoids, tannins, glycosides, saponins, carbohydrates and triterpenes. Phyto-constituents like flavonoids, Triterpenoids, saponins and alkaloids are known to possess Hepatoprotective activity [22]. MCCTE possesses a significant protective effect against hepatotoxicity induced by CCL₄ which may be attributed to the individual or combined action of phytoconstituents present in plant [23]. 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 [24].

The effect of CCL₄ 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 CCL₄ intoxication [25]. The liver marker enzymes (AST, ALT and ALP) are largely used as most common biochemical markers to evaluate liver injury [26] because these enzymes are placed in cytoplasmic area of the cell and are released into circulation in case of cellular damage [27]. Thus, the activities of these enzymes in serum can reflect the severity of liver injury [28]. 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 hepato-necrosis [29].

In the present study CCL₄ ± liquid paraffin administrated animals showed increased level of AST, SGOT, GAMA. GT, TP, Globulins. DB, TB, SGPT (ALT), but a decreased level of AKP and albumin, similarly, the standard drug (Silimyarin) showed defending property against CCL₄ treated group [30]. Where as, close results were obtained by the MCCTE (500mg/kg) treated group against CCL₄ during the present study [31]. Treatment with MCCTE+CCL₄ significantly decreased levels of AST, ALT and ALP towards the normal value. These observations agree 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 CCL₄ [32].

Reduction in total protein content can be deemed as a useful index of severity of hepatocellular damage. In the present study, CCL₄ intoxication reduced the serum total protein level which is attributed to the initial damage produced and localized in the endoplasmic reticulum. This indicated a decrease of cytochrome P-450 enzymes leading to its functional failure with a decline in protein synthesis and buildup of triglycerides leading to fatty liver [33]. Earlier studies exhibited that administration of CCL₄ to various types of animal results in a rapid reduction in protein synthesis in the liver [34]. The treatment of MCCTE+CCL₄ returned the total protein level which proposes the maintenance of endoplasmic reticulum leading to protein synthesis [35].

Conclusion

The present study produced convincing evidence that Methanolic Crude Extract (MCE) of Wild *C. tuberculata* has a Hepatoprotective effect against chemically induced CCL₄.

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