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Research Article

STUDIES ON HEPATOPROTECTIVE PROPERTIES OF DIFFERENT EXTRACTS OF CANSCORA DECUSSATA (SCHULT) AGAINST CARBON TETRACHLORIDE INDUCE HEPATOTOXICITY

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ABSTRACT

Liver is a vital organ play major role in metabolism and excretion of xenobiotics from the body. Liver injury or liver dysfunction is a major health problem. The present study was carried out to evaluate the hepatoprotective role of whole plant extracts of *Canscora decussata*. Hepatoprotective activities of aqueous and methanolic extracts of *C. decussata* were examined against carbon tetrachloride induced liver damage in rabbits using silymarin as control. Enzyme activities of Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Alkaline Phosphatase (ALP) and bilirubin were analyzed. Methanolic extract exhibited significant hepatoprotective activity. Aqueous extract exhibited moderate activity over carbon tetrachloride treated animals. Results confirms the traditional-ethno medicinal use of *C. decussata* as a potential source of hepatoprotective agent.

Keywords: Bilirubin, *Canscora decussata*, Hepatoprotective, Serum biochemical parameters

INTRODUCTION

The search for compounds with novel properties to deal with the situation of the disease is still ongoing. The role of traditional medicines in solving health problems is valuable globally. This is even more impressive considering the fact that about 80% of people living in less developed countries rely exclusively on traditional medicines for their health care needs. The WHO listed 21000 plants used for medicinal purposes in all parts of the world[1]. Herbal medicines are low-cost and free of adverse effects [2]. Since ancient times different plants and herbs are used to treat various ailments. It is clear from history that the pharmaceutical preparations are based on plant material. Although there is an abundance of availability of synthetic drugs for the treatment of many diseases, herbal remedies have been used long to treat patients who suffer from liver disease. During the past decade, plants have regained importance as remedies and famous because of the fact that it is relatively safe, non-toxic and cost-effective too [3]. Therefore, many folk remedies from plant origin are tested for its potential hepatoprotective liver damage in experimental animal model. Carbon tetrachloride (CCl_4) induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts [4]. The *Canscora decussata* (Family: Gentianaceae) one of such plant locally known as Sankha-hulee, has been used in the traditional medicine for the treatment of various diseases including insanity, epilepsy nervous debility etc, and found to contain triterpines, alkaloids, flavonoids and xanthones [5]. Recently, it has been reported that it also has modulatory effects on hematological parameters in alloxan-induced diabetic rabbits [6]. Previously, It has also been reported that magostin-3,6-di-O-glucoside and mangiferin, a C-glucoside from *C. decussata* roots provides a definite protection against experimentally induced carbon tetrachloride liver injury in albino rats [7], the present study was proposed to evaluate hepatoprotective activity of aqueous and methanolic extracts of *C. decussata* whole plant in CCl_4 induced hepatotoxic rabbits.

MATERIAL AND METHOD

Plant Material

Whole plant of *C. decussata* (Schult) was collected from urban areas of Lahore (Pakistan) during the month of August 2010 and got identified and authenticated by taxonomist of University of Sargodha, Sargodha. A voucher specimen (No. UOS/CD/333) was deposited in Faculty of Pharmacy University of Sargodha, Sargodha.

Preparation of powdered plant extracts

The whole plant were washed with water to remove adhering dirt and then completely dried under shade. They were powdered with a Chinese herbal grinder and the powdered material was stored in well closed cellophane bags at 4 ºC in a refrigerator. The powdered material was used for preparation of methanolic and aqueous extract by cold maceration, the percentage yield was 19.5% and 13.33 %respectively. The methanolic extract was dried with rotary evaporator while aqueous by lyophilizer.

Carbon tetrachloride induced hepatotoxicity

Group I (control) animals were administered with distilled water (1 ml/Kg) once daily for seven days. Group II (CCl_4) received distilled water (1 ml/Kg) for seven days and CCl_4, liquid paraffin (1:1, 0.5 ml/Kg) after 24 h of last dose of vehicle. Group III served as positive control and received standard drug silymarin (100 mg/kg) orally once daily for seven days. Test group animals (group IV-VI) were administered orally doses of 150, 300 and 500 mg/kg of methanolic extract respectively, while groups VII-IX were received orally doses of 150, 300 and 500 mg/kg of aqueous extract respectively OD for seven days. The groups III-IX were administered simultaneously CCl4: liquid paraffin (1:1, 0.5 ml/kg) after 24 h of administration of silymarin and extracts on 7th day. Blood was collected, allowed to clot and serum was separated by centrifugation at 2500 rpm at 37°C for 15 min and analyzed for various biochemical parameters [8,9].

Enzyme Assay

The enzyme assay was determined for serum ALT, ALP, AST and bilirubin using commercially available kits (Randox).

Statistical analysis

Data obtained from this work were analyzed statistically using Students't-test and ANOVA (One- or Two-way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means will be considered significant at 5% level of significance ($P < 0.05$).

RESULTS

Pretreatment Hepatoprotective Effects

CCl_4-treated group had ALT, AST, ALP and bilirubin level which was significantly higher than control (Table 1). The methanolic extract, aqueous extract and silymarin pretreatment inhibited the rise of serum ALT, AST, ALP and bilirubin levels effectively as their values were significantly decrease as compare to CCl_4 treated group. The lower doses of aqueous extract did not significantly affect the CCl_4-induced rise of serum ALT enzyme and bilirubin whereas the higher doses show moderate effect while methanolic extract show good control in rise of serum enzymes level (Table 1).

Table 1: Pretreatment hepatoprotective effects of methanolic extract, aqueous extract, and Silymarin on carbon tetrachloride-induced rise of ALT, AST, ALP and bilirubin (total and direct)

Group	Treatment	ALT [IU/L]	AST [IU/L]	ALP [IU/L]	DB (g/dl)	TB (g/dl)
1.	Normal Control	61.05 ± 1.85	57.55 ± 2.49	138.31 ± 4.57	0.91 ± 0.03	0.18 ±0.07
2.	CCl4 Control	116.70 ± 4.04	216.04 ± 5.74	446.39 ± 6.58	1.47 ± 0.05	1.23±0.21
3.	Silymarin + CCl4	56.26 ± 3.62**	89.49 ± 3.09**	139.94 ± 2.36**	0.49 ± 0.03**	0.36±0.07**
4.	ME (150 mg/kg) + CCl4	106.79 ± 4.94*	121.15 ± 4.61**	159.86 ± 2.59**	1.46 ± 0.11	0.89±0.17*
5.	ME (300 mg/kg) + CCl4	93.42 ± 1.58 *	90.79 ± 2.13**	192.41 ± 2.51**	1.08 ± 0.07*	0.63±0.11*
6.	ME (500 mg/kg) + CCl4	65.11 ± 4.04**	72.37 ± 2.56**	144.35 ± 4.33**	0.4 ± 0.02**	0.38±0.09**
7.	AE (150 mg/kg) + CCl4	113.09 ± 5.94^{NS}	201.45 ± 6.71*	391.61 ± 3.59*	1.46 ± 0.11^{NS}	1.19±0.08^{NS}
8.	AE(300 mg/kg) + CCl4	100.21 ± 3.58 *	192.69 ± 4.23*	349.61 ± 6.56**	1.38 ± 0.07*	0.96±0.15*
9.	AE(500 mg/kg) + CCl4	93.51 ± 6.04**	170.37 ± 4.51**	293.35 ± 3.83**	1.04 ± 0.02*	0.84±0.07**

Where, DB= Direct bilirubin and TB= Total bilirubin, ME= methanolic extract, AE= aqueous extract.

Values are mean ±SEM, n=6. Symbols represent statistical significance.

*P< 0.05, **P<0.01 as compared to CCl_4 - intoxicated group; NS - not significant.

DISCUSSION

CCl_4 is one of the most commonly used hepatotoxins in the experimental study of liver diseases. CCl_4 causes altered permeability of membrane resulting in leakage of hepatic marker enzymes (AST and ALT) from cells into the circulation. Hence, elevation in levels of these enzymes in plasma acts as a reliable marker for assessing hepatotoxicity [10]. AST predominantly found in mitochondria of hepatocytes. ALT is more specific to liver, and thus is a better parameter for detecting liver injury. Serum ALP and bilirubin is also associated with liver cell damage. The ALT, AST and ALP activity and serum bilirubin level are largely used as most common biochemical markers to evaluate liver injury [11,12]. Administration of hepatotoxic agent caused a significant elevation of enzymes level such as AST, ALT, ALP and bilirubin level has been attributed to the damage structural integrity of liver indicating development of hepatotoxicity [13]. The administrations of plant extracts have prevented the increased serum marker enzymes AST, ALT, ALP level and bilirubin level. This is in agreement with the commonly accepted view that serum levels of AST, ALT and ALP return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes [14]. The hepatotoxic effects of CCl_4 are largely due to generation of free radicals [15]. Drugs having antioxidant activity are effective in treating CCl4 induced hepatotoxicity. *Canscora decussata* may have antioxidant activity due to presence of triterpines, alkaloids flavonoids, and xanthones [5] because number of scientific reports indicated that certain flavonoids, triterpenoids and steroids have protective effect on liver due to its antioxidant properties [16]. The methanolic extract exhibits the excellent hepatoprotective properties as indicated by maximum prevention of increased serum biochemical parameters on CCl_4 induced toxicity than aqueous extract. All the studied plants extract contain antioxidants and hepatoprotective activity through regulatory action on cellular permeability, stability and suppressing oxidative stress. Phytochemically, *Canscora decussata* contains triterpines, flavonoids, sesquiterpene and sesquiterpene, xanthones, alkaloids might play role in hepatoprotective activity [17].

In the view of above study, it has becomes evident that the methanolic extract of *Canscora decussata (*Schult) exhibit hepatoprotective activity may be due to the presence of some active compound(s) in the methanol solvent more .However, it requires further studies to isolate the active constituent (s) from the extract and to find out its exact mechanism of action.

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