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Hepatoprotective effect of *Pterocarpus marsupium* against carbon tetrachloride induced damage in albino rats

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Abstract

Medicinal plants play a key role in human health care. *Pterocarpus marsupium* is one of the plants used in treatment of diabetes mellitus and the present study was aimed to assess hepatoprotective effect of the plant against CCl_4 induced hepatotoxicity. Wistar albino rats were divided into four groups. Group I was normal control group; Group II, the hepatotoxic group was given CCl_4 (2ml/kg body weight intraperitoneally); Groups III received CCl_4+ Plant extract (100mg/kg b.w orally); Group IV received only the plant extract. Liver markers were assayed in serum and liver tissue. Levels of marker enzymes such as alanine transminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) and bilirubin were increased significantly in Group II. These enzymes were significantly decreased in Group III treated with plant extracts. The present investigation suggest that the plant had a good protective effect on CCl_4 induced hepatic injury.

Introduction

The liver holds a unique position in the human body because of its gastrointestinal connections and varied functions. Liver receives large amount of nutrients and noxious compounds entering the body through the digestive tract and portal vein[1]. As a result of its continuous involvement,

it is susceptible to toxic injuries caused by certain agents and hence any damage to hepatic cells will disturb body metabolism[2]. In spite of the tremendous advancement in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help in regeneration of hepatic cells[3]. Nature has bestowed on us a very rich botanical wealth and a large number of diverse type of plants growing in different parts of the country. Plants form a major part of the therapeutic ingredients in almost all systems of medical sciences[4].

Herbal medicines have been used since the dawn of civilization to maintain health and to treat diseases. Medicinal plants commonly included in ayurvedic recipes for liver nutrients have drawn much attention and research investigations induced on several natural plant products used as liver protectives have been well documented[5]. *Pterocarpus marsupium* is one of the drugs being used in the treatment of diabetes mellitus. Hence, the present study aimed to assess the Anti-hepatotoxic effect of the plant against CCl_4 induced hepatotoxicity in rats.

Materials and Method

The plant was obtained from SKM, Sidha Pharmaceutical, Erode. The powder form of the plant was used for analysis. The decoction of the drug was prepared by taking 10gm of drug powder in 100ml of water and boiled for 10 minutes. The filtrate of this solution was used for the study. The hepatoprotective effect was assessed in experimental rats. Male Albino rats of Wister strain weighing 120-150gm were selected. They were housed under standard condition and maintained on a standard diet and divided into 4 groups and treatment protocol as follows.

Group I : Control rats (6no).

Group II : Negative control – Induction of hepatotoxicity by injecting CCl_4 with paraffin oil (1:1), 2ml/kg body weight intraperitoneally on 2nd and 3rd day.

Group III : The drug was administered orally for 5 days;CCl_4 in paraffin oil (1:1,2ml/kg bw) was given intraperitoneally on 2nd and 3rd day.

Group IV : Positive control, the animal were administered the plant decoction orally for 5 days (100mg/kg b.w orally). The animals were sacrificed on 6th day.

All the animal were anesthetized with chloroform and blood was drawn and serum was collected .The liver was removed for histopathology and biochemical studies. AST[6], ALT[6], ALP[7], LDH[8] in serum and liver and bilirubin[9] in serum were analyzed according to the standard protocols.

Results And Discussion

The activity of hepatic marker enzymes AST, ALT, ALP and LDH were assessed in serum and liver homogenate in different groups of rats.

From Table-1 and 2 it was very obvious that there was a significant increase in

AST, ALT, LDH, ALP levels in group II (CCl_4 treated) rats when compared to the normal rats in both the serum and liver. CCl_4 induced hepatotoxicity depends on reductive dehalogenation of CCl_4 catalyzed by Cytochrome P450 in the liver cells endoplasmic reticulum. It has become clear that a cascade of secondary metabolc activities is evoked by the initial events of CCl_4 metabolism and that the secondary mechanisms are responsible for ultimate plasma membrane disruption and death of cell[10,11]. The mechanisms by which toxic metabolites are formed, include formation of electrophiles or free radicals, which can form covalent adducts with cellular macro molecules, inducing proteins, lipids and nucleic acids, leading to disruption of their function[12]. There is evidence that the responsible metabolite is a free radical (CCl_4 and the derived peroxy radical CCl_3OO). These appear to produce peroxidation of the unsaturated lipids of cellular membranes and probably convert other cellular molecules to secondary free radicals that extend the injury[13,14,15,16]. There is reason to believe that native, nonmetabolized CCl_4 also may contribute the leakage of intracellular enzymes, coenzymes and electrolytes from the hepatocytes and entry of calcium and other ions into cytosol[17,18]

In Group III plant extracts seems to offer protection, which was evident from the significant reduction of all the enzymes in serum and liver when compared with group II. Similarly increased level of bilirubin in group II was noted which might be due to destruction of erythrocytes by toxic metabolites leading to over production or failure to excrete bilirubin. Administration of plant extracts in group III decreased the elevation.

The results of the histopathological studies of section of liver of control and experimental rats carried out to test the toxicity of aqueous extract of the plant on it obtained are tabulated in table-3 and shown in figure 1.

Thus it can be concluded that CCl_4 produces fatty changes and was well brought out in these animal sections. But on treatment with the drug the liver showed microvascular steotosis only. Thus the plant extract offered protection against CCl_4 and shows its hepatoprotective effect in Albino rats

Table 1
Levels of the Enzymes and Bilirubin in serum of Control and Experimental groups

Groups	AST[a]	ALT[a]	ALP[b]	LDH[c]	Direct Bilirubin (mg/100 ml)	Total Bilirubin (mg/100ml)
I (control)	10.1 ± 0.922	13.06 ± 0.481	33.19 ± 0.860	34.12 ± 2.17	0.26 ± 0.012	1.50 ± 0.026
II (CCl_4 injected)	35.93 ± 1.79*	35.43 ± 2.351*	56.19 ± 2.49*	57.01 ± 0.364*	0.44 ± 0.023*	2.33 ± 0.076*
III(CCl_4+Plant Extract)	29.06 ± 2.283#	27.8 ± 3.12#	25.02 ± 0.706#	43.27 ± 1.375#	0.32 ± 0.018#	1.78 ± 0.076#
IV (Plant Extract)	11 ± 1.048$	13.04 ± 1.255$	33.28 ± 0.838$	34.17 ± 3.64$	0.25 ± 0.022$	1.56 ± 0.042$

Values are expressed by mean ± SD
a – mg pyruvate liberated /100 ml of serum at 37^0C
b - mg of phenol liberated /100 ml of serum at 37^0C
c - mg pyruvate liberated /100 ml of serum at 37^0C
* - P(<0.05) Significant when group II compared with group I
# - P(<0.05) Significant when group III compared with group II
$ - P(<0.05)Not Significant when group IV compared with group I

Table 2

Levels of the Enzymes in Liver of Control and Experimental groups

Values are mean± SD

a – mg pyruvate liberated /mg protein at 37^0C

b- mg of phenol liberated /mg protein at 37^0C

c - mg pyruvate liberated /mg protein at 37^0C

*- P(<0.05) Significant when Group II compared with group I

#- P(<0.05) Significant when Group III compared with group II

$- P(<0.05)Not Significant when Group IV compared with group I

Table 4. Histopathlogical Observations in Control and Experimental Groups

Groups	Animal group			Histopathological examination
	AST[a]	ALT[a]	ALP[b]	LDH[c]
I (control)	0.244 ± 0.029	0.289 ± 0.016	2.67 ± 0.05	0.374 ± 0.017
II (CCl_4 injected)	0.433 ±0.032*	0.504 ± 0.047*	4.56 ± 0.52*	0.498 ± 0.10*
III (CCl_4 + Plant Extract)	0.310 ± 0.05#	0.407 ± 0.011#	3.02 ± 0.07#	0.415 ± 0.008#
IV (Plant Extract)	0.241 ± 0.02$	0.304 ± 0.028$	2.65 ± 0.08$	0.375 ± 0.019$

Group I – Normal

Group II – CCl_4 injected

Group III – CCl_4 injected + plant Extract

Group IV – Plant extract injected rats

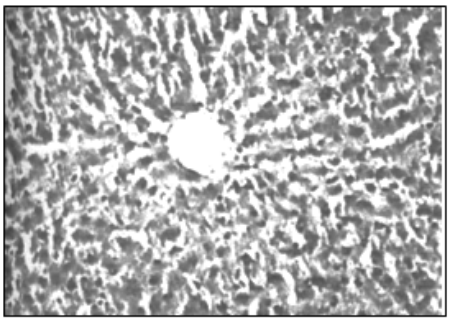
Normal liver architecture

Lobular hepatocellular Necrosis steatosis and fibrosis

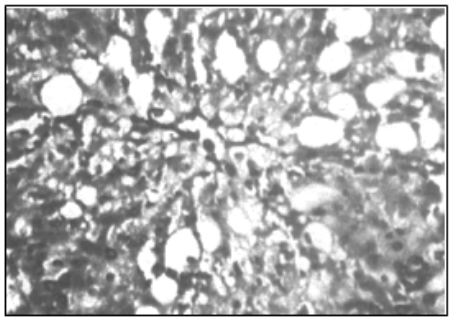
Minimal hepatocellular necrosis

Normal liver architecture

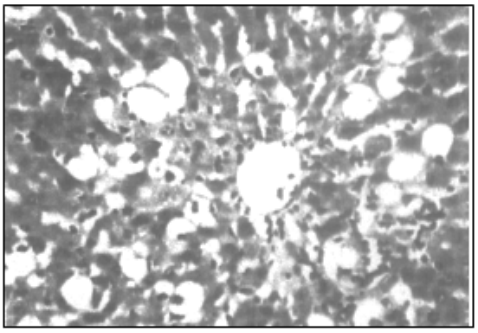
CONTROL GROUPS



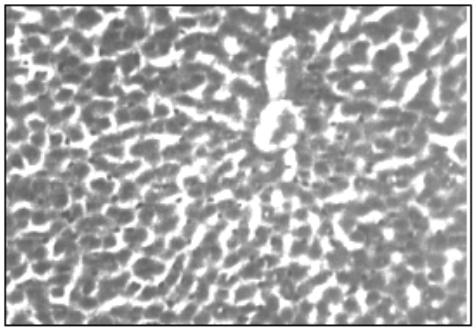
Group I – Normal control
Normal liver architecture



Group II – CCl_4 control
Lobular necrosis, steatosis and balloon degeneration



Group III – CCl_4 + plant extract
Zone I & 2 normal, Minimal isolated hepatocellular necrosis in zone 3



Group IV – Plant extract
Normal liver architecture

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