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ORIGINAL ARTICLE **OPEN ACCESS**

Hepatoprotective activity of *Pedilanthus tithymaloides* in Carbon tetrachloride treated rats

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ABSTRACT

The present investigation was aimed to evaluate the hepatoprotective effects of ethanolic extract of Pedilanthus tithymaloides (PT) leaves on carbon tetrachloride (CCl_4) induced liver toxicity. PT extract was administered orally in the dose of 200 mg/kg and 400 mg/kg body weight for 7 days and liver toxicity was induced by administration of CCl_4(1ml/kg) subcutaneously on alternative day for a week. Silymarin (25 mg/kg body weight) was used as a standard drug. Serum biochemical markers were determined along with antioxidants enzymes in liver homogenates. The PTtreated groups show significantly decrease in biochemical markers (SGOT, SGPT, ALP and total bilirubin) in dose dependent manner which were increased significantly in toxic control group (CCl_4treated). Whereas total proteins level was increased significantly. Pre-treated groups with PTalso produced significant reversal in levels of liver homogenates (LPO, SOD, CAT, GSH, GST and GPx). The present study shows that ethanolic extract of PT possesses hepatoprotective action against CCl_4induced hepatotoxicity.

Keywords:*Pedilanthus tithymaloides, carbon tetrachloride, hepatoprotective, subcutaneously.*

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INTRODUCTION

The liver also plays crucial role in altering of one chemical form to another and its clearance from body. When definite therapeutic agents are administered in overdoses and sometime while administered inside the therapeutic limits may also damage organs. Additional chemicals like those utilized in laboratories, industries, herbal chemicals and natural drugs can leads to liver harm are known as hepatotoxins [1]**.** Exposure to hepatotoxins includes accidental exposure to phosphorous [2], carbon tetrachloride [3], toxic or over doses of drugs such as paracetamol, isoniazid, augmentin, trimethoprim, sulfamethoxazole, phenytoin etc. [4] or ferrous sulphate [5] causes liver injury. The present treatment for liver diseases with Western medicine are often limited in efficacy carry the risk of adverse effects and are often too costly, especially for the developing world [6,7]. Therefore, treating liver diseases with plant derived compounds which are accessible and do not require laborious pharmaceutical synthesis seems highly attractive. Furthermore, in spite of the advances in conventional medicine in the last decades, professionals and the lay public of developed countries pay increasing attention to phytomedicine[8].

MATERIAL AND METHODS

Plant material

The fresh leaves of *Pedilanthus tithymaloides* were collectedlocally and authenticated by Dr.Y. S. Sarangdevot, Professor,B.N. College of Pharmacy, Udaipur Rajasthan. The air-dried leaves of *Pedilanthus tithymaloides* were ground into course powder. About500g of powder wastransferred in glass jar containing 600ml of ethanol, kept at room temperature for 7 days with shaking 3-4 times in a day. The extract was filtered through Whatman No. 1 filter paper. The extract was concentrated by rotary evaporator (RE-2 Aditya Scientific, India) and allow to air dry for complete evaporation of solvent. The dried ethanolic extract was transferred to air tight container and kept in desiccators for further uses [9,10].

Animals

The Albino Wistar rats (male and female) of weighing between 170 to 200g were used for the experiments. They were housed in the cages under the standard laboratory condition at a temperature of 23±2°C, humidity 60-70% and 12 hr light/dark cycles.The animals were feed with standard pellet diet and water was given *ad libitum*. The animals were maintained as per the CPCSEA regulations and the

study protocol was approved by the Institutional Animal Ethical Committee at Bhupal Nobles' College of Pharmacy, Udaipur.

Study design and experimental protocol

Animals were randomly divided into 5 groups of 6 rats each. Animal of Group I served as a control group, received subcutaneously administration of liquid paraffin at a dose of 1 ml/kg on alternate days, for a week. The animals of Group IIwere served as a toxic control and received CCl_4 subcutaneously, in a suspension ofliquid paraffin (1:2 v/v) at a dose of 1 ml/kg onalternate day for a week. Group III animals served as a standard group were given silymarin orally daily at a dose of 25 mg/kg and CCl_4 in a suspensionof liquid paraffin (1:2 v/v) subcutaneously at a dose of 1 ml/kg body weighton alternate day for a week. Group IV and Group Vwere received extract orally daily at a dose of 200 and 400 mg/kg respectively and CCl_4 in a suspensionof liquid paraffin (1:2 v/v) subcutaneously at a dose of 1 ml/kg body weighton alternate day for a week [11,12].

Liver weight

After sacrificing the animals and processing of isolation, livers were placed on filter paper to remove excess moisture and then were finally weighed on electronic weighing balance[13].

Estimation of biochemical parameters

The biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT),serum glutamate pyruvate transaminase (SGPT),alkaline phosphatase (ALP), total proteinand total bilirubin were estimated by using procedure described in assay kit (Lab Care Diagnostics India, Pvt. Ltd).The oxidative stress parameters like lipid peroxidation (LPO), catalase (CAT) superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx) and Glutathione S-transferase (GPx) were alsoestimated in liver homogenates [14,15,16].

Statistical analysis

The data were expressed as mean ± S.E.M. Statistical differences at $P < 0.05$ between the groups were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test using Graphpad Prism software.

RESULTS AND DISCUSSION

Effect of *Pedilanthus tithymaloides*on liver weight

The liver weightwas increase significantly in group II carbon tetrachloride treated animals as compared to the untreated normal control groupI. The weight of liver decreases significantly in group III (CCl_4 silymarin 25mg/kg) and groupV (CCL_4+ PT 400mg/kg) when compared to Group-II (CCL_4 treated)as given in Table1.

Effect of *Pedilanthus tithymaloides*onserum biochemical markers

The levels of biochemical markers, viz. SGOT, SGPT, ALP and total bilirubin were significantly increased in group II CCl_4 treated animals, as compared to the control untreated group I animals. Whereas the level of total proteins was significantly decrease in CCl_4 treated group II. The groups III and V, treated with silymarin 25 mg/kg and *Pedilanthus tithymaloides* dose of 400 mg/kg respectively followed by CCl_4, showed significant decrease in the level of serum biochemical marker, as compared with the carbon tetrachloride treated group II as given in Table 2. The level of total proteins was also significantly increased in both groups III and V. Whereas, group IV treated with *Pedilanthus tithymaloides* dose of 200 mg/kg showedsignificant decrease in the levels of SGPT, SGOT and ALP and did not show any significant changes in the levels of total bilirubin and total proteins.

Table 1: Effect of *Pedilanthus tithymaloides*on liver weight

Group/Treatment	Liver weight (gm/100 body weight)
Control I with LP	3.17 ± 0.14
Group II LP + CCl_4	4.91 ± 0.70[a]
Group III LP + CCl_4 +silymarin (25 mg/kg)	3.49 ± 0.26[b]
Group IV LP + CCl_4 + PT(200 mg/kg)	4.25 ± 0.32[NS]
Group V LP + CCl_4 + PT(400 mg/kg)	4.02 ± 0.60[d]

All values represent mean ± SEM (n = 6)

P Value: a p<0.001 significantly different groups when comparing with control.

b P < 0.001, c P < 0.01, d P < 0.05 significantly different groups when comparing with Group-II (CCl_4 treated).

NS indicates non-significant groups when comparing with Group-II (CCl_4 treated).

Table 2: Effect of *Pedilanthus tithymaloides* on serum biochemical markers

Group/Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Total Protein (mg/dl)
Control I with LP	69.36 ± 7.77	80.52 ± 10.78	51.60 ± 3.62	0.66 ± 0.09	7.01 ±0.26
Group II LP + CCl_4	168.69±19.38[a]	223.12 ± 24.55[a]	117.55 ± 12.33[a]	1.37 ± 0.24[a]	4.83 ± 0.95[a]
Group III LP + CCl_4 +Silymarin (25 mg/kg)	106.55±8.19[b]	117.81 ± 7.48$_b$	62.50 ± 5.64[b]	0.87 ± 0.09[c]	6.73 ± 0.35[c]
Group IV LP + CCl_4 + PT (200 mg/kg)	143.55 ±13.46[d]	193.06 ± 13.54[d]	98.60 ± 8.39[d]	1.25 ± 0.16[NS]	5.84 ± 1.02[NS]
Group V LP + CCl_4 + PT (400 mg/kg)	134.75 ± 11.78[c]	177.09 ± 14.36[c]	95.30 ± 10.53[d]	1.03 ± 0.11[d]	6.20 ± 0.54[d]

All values represent mean ± SEM (n = 6)
P Value: a p<0.001 significantly different groups when comparing with control.
bP <0.001, cP <0.01, d P <0.05 significantly different groups when comparing with Group-II (CCl_4 treated).
NS indicates non-significant groups when comparing with Group-II (CCl_4 treated).

Effect of *Pedilanthus tithymaloides* on liver homogenates

The changes in the level of biochemicals parameters of liver homogenates in different groups of animals are given in Table 3. The levels of liver antioxidants enzymes (SOD, CAT, GSH, GPx and GST) reduced significantly in Group-II (CCl_4 treated) as compared with untreated group I. Whereas the level of LPO increased significantly in Group II (CCl_4 treated). The groups III and IV, treated with silymarin 25mg/kg and *Pedilanthus tithymaloides* dose of 400 mg/kg followed by CCl_4 showed significantly increase in the level of these antioxidants enzymes, as compared with the CCl_4 treatedgroup II. Whereas, significantly decrease in level of LPO. The group IV treated with *Pedilanthus tithymaloides* dose of 200 mg/kg showedsignificant increase in the levels of CAT and GST as compared with the CCl_4 treated group II.

Table 3: Effect of *Pedilanthus tithymaloides* on liver homogenates

Group/Treatment	LPO (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (U/mg protein)	GPX (U/mg protein)	GST (nmol/mg protein)
Control I with LP	0.49 ± 0.08	92.86 ± 6.72	30.67 ± 2.78	3.69 ± 0.29	4.26 ± 0.28	1.28 ± 0.12
Group II LP + CCl_4	1.75 ± 0.42[a]	54.25 ± 9.10[a]	13.32 ± 4.04[a]	2.19 ± 0.61[a]	2.30 ± 0.55[a]	0.47 ± 0.29[a]
Group III LP + CCl_4 +Silymarin (25 mg/kg)	0.78 ± 0.17[b]	86.28 ± 6.28[b]	23.25 ± 2.47[c]	3.51 ± 0.18[c]	3.95 ± 0.36[b]	1.32 ± 0.14[b]
Group IV LP + CCl_4 + PT(200 mg/kg)	1.45 ± 0.19[NS]	67.01 ± 6.37[NS]	20.31 ± 2.48[d]	2.62 ± 0.32[NS]	3.13 ±0.63[NS]	0.82 ± 0.10[d]
Group V LP + CCl_4 + PT(400 mg/kg)	1.31 ± 0.13[d]	72.55 ± 8.11[d]	22.13 ± 3.11[c]	2.65 ± 0.46[NS]	3.29 ± 0.48[d]	0.93 ± 0.13[c]

All values represent mean ± SEM (n = 6)
P Value: a p<0.001 significantly different groups when comparing with control.
b P < 0.001, c P < 0.01, d P < 0.05 significantly different groups when comparing with Group-II (CCl_4 treated).
NS indicates non-significant groups when comparing with Group-II (CCl_4 treated).

Carbon tetrachloride (CCl_4) is widely used as a solvent for dissolving non-polar compounds such as fats and oils. The acute toxicity of CCl_4 is well established from many animal studies. Most hepatotoxic effects of CCl_4, was attributed to its metabolism by cytochrome P450, especially by P450 2E1, to yield toxic trichloromethyl radicals ($CCl_3^{\bullet}$) and peroxide radical ($^{\bullet}OOCCL_3$) which are highly reactive. These radicals bind to the macromolecule and induce peroxidative degradation of membrane phospholipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This in turn leads to the formation of lipid peroxide which ultimately produces toxic aldehyde inducing liver damage[17].Serum biochemical markers are the commonly usedmarkers in the assessment of liver damage because these are cytoplasmic in location and are released in to the circulation after cellular damage [18,19].

Whereas, lipid peroxidation (LPO) is a destructive process in liver injury after administration with CCl_4. Increase in LPO leading to liver damage and failure of antioxidant defense mechanisms to prevent formation of free radicals [20]. SOD and CAT constitute a team of mutually supportive antioxidant enzymes which provide defense against reactive oxygen species. SOD provide the protection from oxidative stress by dismutation of highly reactive and potentially toxic superoxide radical to H_2O [21]. Glutathione is an important naturally occurring antioxidant and it prevents the hydrogen of methylene

moiety of unsaturated fatty acids to be abstracted. The binding of acetaldehyde, a metabolite of ethanol with glutathione may contribute to reduction of levels of glutathione [22]. The metabolites of CCl_4, directly reduce the levels of glutathione. GPx is very important enzyme which provide protect against chemically induced oxidative destruction process of lipid and proteins [23]. The GST is multifunctional enzyme which involved in the metabolism of a broad variety of xenobiotics and endogenous compounds and provides protection against toxic compound by metabolised them [24].

In the present investigation, treatment withtwo different dosages of the ethanolic extract of *Pedilanthus tithymaloides* (200 and 400 mg/kg p. o.) significantly reversed these elevated levels of biochemical markers, viz. - SGOT, SGPT, ALP and total bilirubin, whereas significantly increases in total proteins level. The results obtained were comparable withthose of the silymarin 25 mg/kg treated group.In our present study, 400mg/kg p. o. dose of *Pedilanthus tithymaloides* was more effective, as compared to 200 mg/kg dose. It is thus concluded that the ethanolic extract of *Pedilanthus tithymaloides*at an oraldose 400mg/kg/day, is effective against the hepatotoxicity induced by CCl_4.

CONCLUSION

The results of this present study have demonstrated that ethanolic extract of *Pedilanthus tithymaloides* leaves can be effective treatment against hepatic injury. Further studies are needed to establish the therapeutic potential, safety, mechanism and active compounds of the drug involved in the treatment of hepatotoxicity.

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