ORIGINAL ARTICLE

Hepatoprotective Effect by Methanolic Extract of *Dinothrombium tacnitorium* in Albino rats

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ABSTRACT

Products of animal origin in traditional medicines are accountable for their healing importance. Objective: The goal was to appraise hepatoprotective effect by methanolic extract of *Dinothrombium tacnitorium* in Albino rats. Study design: It was a randomised control study. Methodology: Aqueous methanolic extract (70% v/v) of *Dinothrombium tacnitorium* (Dt.Cr) was prepared followed by succeeding evaporation using rotary evaporator. Liver toxicity was done by introduction of CCl_4 (2 ml/kg, p.o) in paraffin oil on 7th day of experiment. Administration of methanolic extract of *Dinothrombium tacnitorium* (300mg/kg body weight/day) orally protected the CCl_4 caused elevation of liver enzymes that include Aspartate aminotransferase, Alanine aminotransferase, Alkaline Phosphatase and total bilirubin. The hepatic enzymes level elevation in the CCl_4 alone treated animals. Administration of methanolic extract to CCl4 challenge protected against the hepatic toxic agent. Conclusion: The findings thus suggested that the methanolic extract of *Dinothrombium tacnitorium* (Dt.Cr) can be used as hepatoprotective agent against CCl_4-induced hepatotoxicity in albino rats. Key words: *Dinothrombium tacnitorium,* Carbon-tetrachloride, hepatic markers and albino rats.

Keywords: dinothrombium tacnitorium, methanolic extract, hepatoprotective effect

INTRODUCTION

Among global health problems are the liver diseases. New drugs are not very efficient for its eradication. Major burden of deaths globally (46%) are due to hepatic complications as reported by World Health Organization (WHO). After cancer and heart diseases, liver diseases are the biggest cause for high mortality rate worldwide[1].

Liver is the major metabolic and excretory organ. It metabolizes carbohydrates, protein. It also does fat detoxification and regulation of body homeostasis. Poor drug habits and alcohol expose it to various liver complaints[2]. Exposure to Alflatoxin and CCl_4 cause conditions such as Jaundice and hepatitis, accounting for highest death rate[3].

Carbon tetrachloride (CCl_4) is commonly employed as hepatotoxin in animal models[4]. Silymarin, the active constituent of *Silybum marianum*, has antioxidant and hepatoprotective properties. It regains hepaic antioxidant status. Its extract is used as a herbal remedy against hepatotoxicity traditionally[5].

In modern world, animals derived drugs play an important role as alternative treatment option globally. Among the 252 essential drugs, 8.7% constitute animal origin chemicals)[6]. In Pakistan, 31 substances derived from animals constitutes 9% all traditional medicines[7].

Dinothrombium tacnitorium has been used in many medical ailments like paralysis, malaria, urogenital disorders as remedy for centuries[8]. Its antibacterial, antifungal and gastroprotective effects have been established years ago[9]. The current project was designed to investigate the hepatoprotective action by methanolic extract of *Dinothrombium tacnitorium* in Albino rats given CCl_4.

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Study design and equipments: It was a randomised control study. Reagents used in current research included Diagnostic kits by Humans, Silymarin, Carbon Tetrachloride, distilled water, Digital electronic balance, Grinder, Vortex Mixer, Incubator, Centrifuge machine, Rotary Evaporator. All the chemicals were of analytical grade.

Table1: Group treatment schedule

Groups	Days	Day 7th
Normal control	Distilled H_20 (4 ml/kg)	Distilled H_20 (4 ml/kg)
Intoxicated	Distilled H_20 (4 ml/kg)	Distilled H_20 (4 ml/kg) +CCl4 (2 ml/kg)
Rx. group 1	Dt.Cr 30 mg/kg	Dt.Cr 30 mg/kg+ CCl4 (2 ml/kg)
Rx. group 2	Dt.Cr 100 mg/kg	Dt.Cr 100 mg/kg+ CCl4(2 ml/kg)
Rx. group 3	Dt.Cr 300 mg/kg	Dt.Cr 300 mg/kg+ CCl4(2 ml/kg)
S.control	Silymarin 25 mg/kg	Silymarin 25 mg/kg + CCl4(ml/kg)

Extract Preparation: One kg of Red Velvet Mites was acquired locally. The species *Dinothrombium tacnitorium* identification was done by the zoology department, IUB. A coarse paste of Red Velvet Mites was waterlogged using 70% v/v aqueous methanol. It was carried for 03 days. Crude extract was extracted from filtrate after filtration by using Rotary Evaporator. Final extract was stored till future use[8].

Animals: In the study, male Wistar albino rats (200-250g) and Swiss albino mice (18-30g) were used. They were upheld at a temperature (25±2°C) and humidity (55-55%) along with 12 hour light and dark cycle. Animals were given standard diet and tap water ad libitum. Acclimatization of subjects was done for seven days before the start of study[10].

Acute toxicity assay: Five groups were made containing 5 mices in each group of both genders. Overnight fasting was done. Normal control group was given normal saline (10ml/kg p.o). Other remaining groups were treated with doses of crude extract (0.3, 1, 3, 5, 10g/kg p.o) respectively, in-order to see toxic effects from zero hour till day 14.

Animal model of hepatotoxicity: Equal division of animals into different groups was done. CCL_4 was given (p.o) to all groups except control group to induce toxicity on 7th day as per schedule. The animals were sacrificed 12 h after the last treatment of CCl_4. Blood was collected to analyze it for liver enzymes and markers by using standard kit methods (10). Treatment schedules (Table 1).

Statistical analysis: ANOVA with Bonferroni test was employed for analysis of data and Mean ± S.E.M was used for expression of results. Significant (*) result values if $p<0.05$.

Acute toxicity studies showed that the extract used in study was practically non toxic to liver at particular given doses since the hepatic markers were in normal range.

Table 2: Serum (AST) & (ALT) Levels in Albino rats after extract treatment

Groups	AST Levels (IU/L)	ALT Levels (IU/L)	P-value
Control	64.61±4.44	30.40 ±1.37	
Intoxicated	258.8±12.22	125.8±4.46	
Dt.Cr. (30 mg/kg) + CCl_4	208.4±16.63	109.3±3.03	<0.01*
Dt.Cr. (100 mg/kg) + CCl_4	167.2±8.6	77.81±3.2	<0.001**
Dt.Cr. (300mg/kg) + CCl_4	85.63±3.51	49.29±1.9	<0.001**
Silymarin(25 mg/kg)+ CCl4	71.36±3.96	35.42±2.8	<0.001**
*Statistically Significant			

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Table 3: Serum (ALP) & (T.B) Levels in Albino rats after extract treatment

Groups	Levels of ALP IU/L	T.B Level (mg/dl)	P-value
Control	129.3 ±3.01	0.48±0.02	
Intoxicated(CCl_4 2 ml/kg)	447.0±6.81	2.95±0.11	
Dt.Cr. (30 mg/kg)+ CCl_4	417.2±13.2	2.41±0.16	<0.01*
Dt.Cr. (100 mg/kg)+ CCl_4	277.9±3.52	1.29±0.04	<0.001**
Dt.Cr. (300mg/kg)+ CCl_4	179.6±6.04	0.61±0.02	<0.001**
Silymarin(25 mg/kg)+ CCl4	140.0±3.81	0.58±0.06	<0.001**

DISCUSSION

Medicines of modern era have limitations for alleviation of hepatic diseases. Drug treatment from herbal or animal origin has attracted patients towards them for many decades. The study was carried out on Red velvet mite methanolic extract, *due to* its traditional uses in medical history[9].

In current project, CCl_4-induced hepatotoxicity was done in male Wistar Albino rats to evaluate methanolic extract of Dt.Cr. for its hepatoprotective property[11]. Our work was in line with previous studies who used same agent for induction. Paradoxically, paracetamol was the inducing agent in other studies[10].

Hepatotoxicity are linked with improper liver functioning because of any reason[12]. Silymarin was used as standard in our study to compare different strengths of *Dinothrombium tacnitorium* extract for its hepatoprotective action. It was used as standard by many researchers so our work was in line with past researchers[5]. The liver function enzymes that included Alkaline phosphatase, Aspartate transaminase, Alanine transaminase and total bilirubin were evaluated.

Acute toxic assessments were carried out in current project in 25 mice. Critical toxic behavioral changes were noted from zero hour till day 14 continuously in our study. In other studies such changes were noticed but from zero hour till day 7 paradoxically[10].

Protocol adopted in current study regarding number of animals and groups was same as done in previous animals studies to see different hepatoprotective effect of *Fumairia indica* plant extract but with some modifications[13].

Different doses of extract was given to treatment groups in current study. Extract of Fabaceae leaves was explored as hepatoprotective agent in one previous work. The extract at a dose of 50,100 and 400 mg/kg body wt. exhibited orally. Paradoxically, the extract at a dose of 30,100 and 300 mg/kg body wt. exhibited orally in current project to treatment groups respectively[10].

Our study had a number of limitations like financial constrains and less resources. No histopathology of lever tissue was done. Only liver function tests were done to evaluate hepatoprotective effect of extract in present study. Current project was a rare study in a sense that it assessed the protection property of *Dinothrombium tacnitorium methanolic* extract, animal origin, against hepatotoxicity induced by CCl_4.

CONCLUSION

The findings suggested that this methanolic extract can be employed as hepatoprotective agent against hepatotoxicity.

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Findings: The findings of *Dinothrombium tacnitorium methanolic* extract with different doses received to animals showed significant decrease in their serum hepatic biomarkers.

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