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RESEARCH ARTICLE

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF *DENDROBIUM NORMALE* FLOWERS AGAINST CCL_4 INDUCED LIVER DAMAGE IN RATS

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ABSTRACT

Objective: Objective: In the present work, hepatoprotective activity of methanolic extract of *Dendrobium normale* were tested against carbon tetrachloride (CCl_4) induced hepatotoxic in rats.
Methods: CCL_4 has been used as the screening model for hepatoprotective activity
Results: The results indicated an increase in serum biochemical parameters like serum glutamate oxaloacetate transminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphate (ALP) and total bilirubin (TB) levels are sensitive indices for hepatic damage. The ability of the above mentioned extracts to maintain the biochemical parameters level near to normal values are indication of their hepatoprotective potential.
Conclusion: The present investigation showed hepatoprotective activity against CCL_4 induced liver damage in rats

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INTRODUCTION

Plants drugs are known to play a vital role in the management of liver diseases. There are numerous plants and polyhedral formulations claimed to have hepatoprotective action. However, numerous medicinal prepartions have been advocated a traditional system of medicine, speciallyin ayurvedic, for treating liver disorders. Only a small proportion of hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their efficiency. India is sitting on a gold mine of well recorded and traditionally well-practised knowledge of herbal medicine. This country is perhaps the largest producer of medicinal herbs and is rightly called the botanical garden of the world. India officially recognizes over 3000 plants for their medicinal value. It is generally estimated that over 6000 plants in India are in use in traditional, folk and herbal medicine, representing about 75% of the medicinal needs of the third world countries (Kokate et al., 1996; Vogel, 1991; Dubey et al., 2004).

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Medicinal plants had been in use since 5000 BC oldest known herbal is Pent'sao written by emperor Shen-Nung around 3000 BC. It contains 365 drugs one for each day of the year. Indians worked meticulously to examine and classify the herbs. Charaka made 50 groups of 10 herbs, each of which would suffice an ordinary physician's need. Similarly Sushruta arranged 760 herbs in 7 distinct sets based on to some of their common properties. Charaka says ''There is no substance in the world that has no medicinal value, provided you know how to use it (Rajshekharan, 2002; Handa, 1991).

MATERIALS AND METHODS

Plant material

The plant collected from the Sri Venkateswara University, Tirupathi and indentified, Authenticated by taxonomist.

Preparation of plant extracts

The shade dried powder of the plant was collected and it was treated with methanol then extracted by percolation process.

Animals

Wistar albino rats of either sex weighing between 200-250 g were obtained from Mahaveer Enterprises, Hyderabad. The animals were housed under standard environmental conditions (temperature of 22 ± 1^0 C with an alternating 12 hrs light- dark cycle and relative humidity of 60±5%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Ethical Committee and by animal regulatory body of the government.

Drugs and chemicals

Carbon tetra chloride (CCl_4), Poona Chemical Laboratory, Pune, India, Silymarin-Nature remedies, Bangalore, Karnataka, India, Estimation kits (SGOT, SGPT, SALP, BILIRUBIN) - SPAN Diagnostics, Surat, India. All the other chemicals were obtained from local sources and were of analytical grade.

Experimental Procedure

Albino rats of either sex (200-250 g) were used in the study. The animals were fed with standard diet and water *ad libitum* two weeks before and during the experimental period. Each methanolic extract was tested at 400 mg/kg dose level. The animals were divided in to 5 groups (I-VI), each consisting of 6 animals. Group I received 5% gum acacia suspension and acts as a normal control and Group II received CCl_4 at a dose of 1 ml/kg orally (p.o.) acts as negative control. Groups III-VI were treated with selected drugs (Silymarin and plant extracts) for 5 days before the commencement of experiment and on day 6^{th} of the experiment, blood samples were collected (6^{th} day) at 0 hr in all groups and CCl_4 was administered to all groups except group I (normal control) one hour after the administration of drugs. On 7^{th} day blood samples were collected from all groups by retro orbital puncture and serum was separated by centrifugation and used for the estimation of blood serum biochemical parameters (SGOT, SGPT, SALP and T. BILI.) using autoanalyser according to standard procedures. Finally, the liver sections were carefully dissected out, subjected to histopathology study

Statistical analysis

The mean±SEM values were calculated for each parameter. Percentage reduction in biochemical parameters with the test samples was calculated by considering the difference between the hepatotoxin treated group and the control group as 100% reduction. For the determination of significant inter group difference, each parameter was analyzed separately using one way analysis of variance (ANOVA) followed by Dunnet's test was carried out to assess the hepatoprotective potency of different extracts of the plants.

DISCUSSION

Carbon tetrachloride intoxication in normal rats produced elevated levels of serum biochemical parameters SGOT (72.25±0.22 to 502.26±0.31 IU/L), SGPT (63.34±0.59 to392.21±0.32 IU/L), SALP (134.21±0.20 to 779.41±0.26 IU/L) and T.BILI. (0.43±0.01 to 4.66±0.67mg/dl) significantly, indicating acute hepatocellular damage.

RESULTS AND DISCUSSION

Evaluation of the hepatoprotective activity

Table 1. Protocol for study of hepatoprotective

S. No.	Group	Treatment
1	Group I	Receives vehicle orally, 1 ml/kg (2% gum acacia)
2	Group II	Receives CCl_4orally at a dose of 1 ml/kg in paraffin oil (1:1).
3	Group III	Receives CCl_4 orally at a dose of 1 ml/kg + Silymarin orally at a dose of 50 mg/kg.
7	Group IV	Receives CCl_4 orally at a dose of 1 ml/kg + Methanolic extract of *Dendrobium normale,* orally at a dose of 100 mg/kg.
8	Group V	Receives CCl_4 orally at a dose of 1 ml/kg + Methanolic extract of *Dendrobium normale,* orally at a dose of 200 mg/kg.
9	Group VI	Receives CCl_4 orally at a dose of 1 ml/kg + Methanolic extract of *Dendrobium normale,* orally at a dose of 400 mg/kg.

Table 2. Effect of methanolic extract of *Dendrobium normale* flowers against CCl_4 - induced hepatotoxicity in albino rats

S. No.	Treatment group	Serum biochemical parameters			
		SGOT (IU/L)	SGPT (IU/L)	SALP (IU/L)	T.BILI. (mg/dl)
1	Control (2% gum acacia 1ml/kg p.o.)	72.25±0.22	63.34±0.59	134.21±0.20	0.43±0.01
2	Hepatotoxin - CCl_4 (1ml/kg p.o.)	502.26±0.31***	392.21±0.32***	779.41±0.26***	4.66±0.67***
3	Standard- Silymarin (50 mg/kg p.o.)	129.16±0.33***	110.93±0.31***	225.41±1.21***	1.49±0.21
4	Methanolic extract of *Dendrobium normale* (100mg/kg p.o.)	310.15±0.24***	248.76±0.36***	532.07±0.84***	3.12±0.01**
5	Methanolic extract of *Dendrobium normale* (200mg/kg p.o.)	220.06±0.93***	176.21±0.03***	375.36±0.28***	2.14±0.04
6	Methanolic extract of *Dendrobium normale* (400mg/kg p.o.)	127.06±0.54***	108.42±0.21***	238.23±0.14**	1.65±0.02**

Values are mean ± SEM, n=6, Significance: *P<0.05, **P<0.01, ***P<0.001

Table 3. Percentage reduction of various serum biochemical parameters due to treatment with methanolic extract of *Dendrobium normale* flowers against CCl_4 - induced hepatotoxicity in albino rats

Treatment	SGOT	SGPT	SALP	T.BILI.
Standard- Silymarin (50 mg/kg p.o.)	86.76	85.52	85.86	74.94
Methanolic extract of *Dendrobium normale* (100mg/kg p.o.)	44.67	43.61	38.33	36.40
Methanolic extract of *Dendrobium normale* (200mg/kg p.o.)	65.62	65.67	62.62	59.57
Methanolic extract of *Dendrobium normale* (400mg/kg p.o.)	87.25	86.29	83.87	71.15

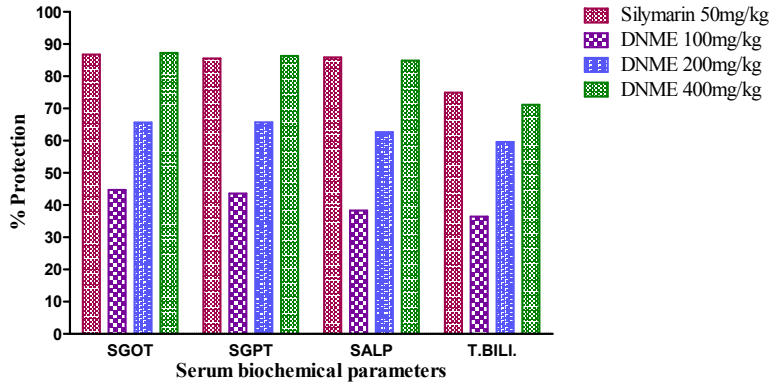
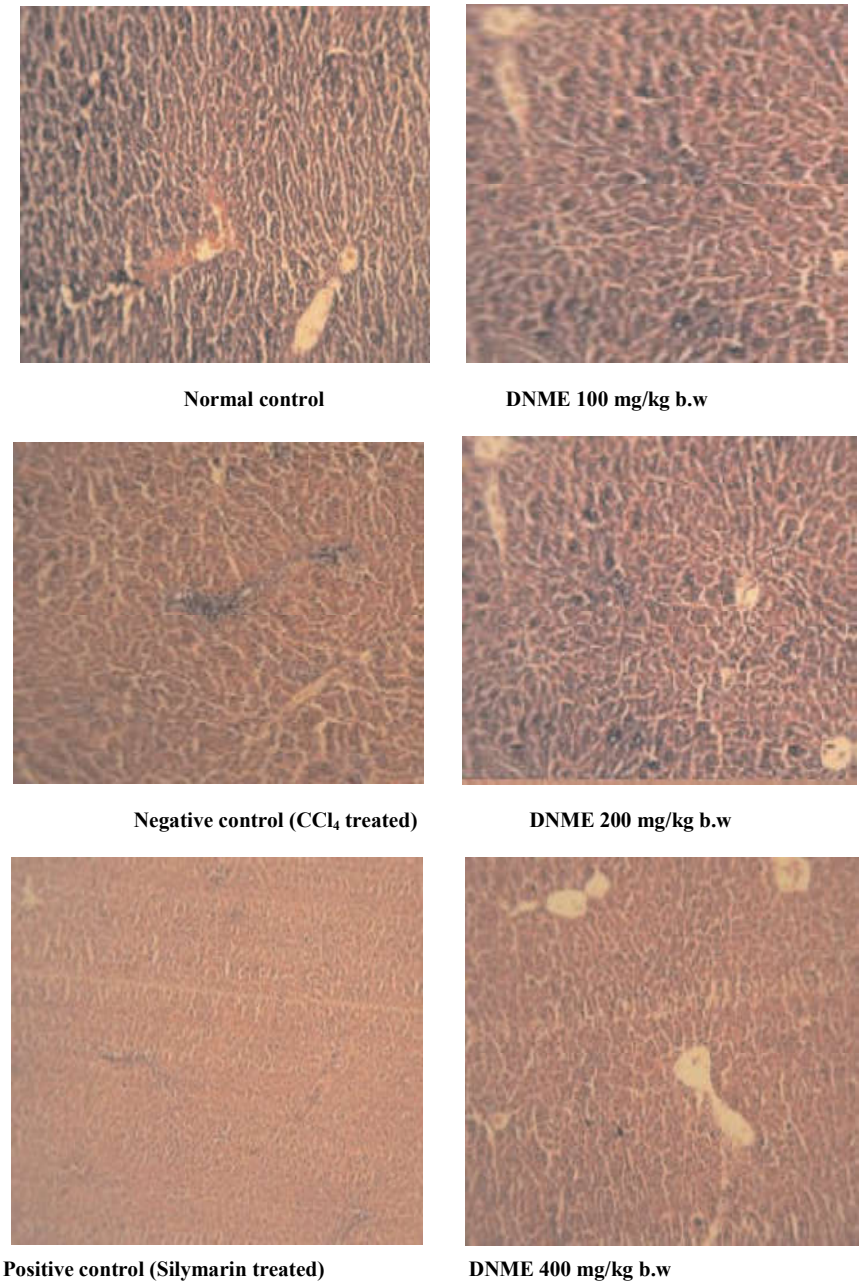


Figure 1. Percentage reduction of various serum biochemical parameters due to treatment with methanolic extract of *Dendrobium normale* flowers against CCl_4 - induced hepatotoxicity in albino rats

Histopathology: Photomicrographs of liver sections



Normal control **DNME 100 mg/kg b.w**

Negative control (CCl_4 treated) **DNME 200 mg/kg b.w**

Positive control (Silymarin treated) **DNME 400 mg/kg b.w**

Figure 2. Effect of methanolic extract of *Dendrobium normale* flowers against CCl_4 - induced hepatotoxicity in albino rats

Normal control: Showing cords of hepatocytes around the central vein; prominent nucleus and nucleolus, **Negative control:** Showing liver with focal hepatocytic damage and inflammatory collection, **DNME 100 mg/kg b.w:** Showing minimal inflammatory collection and damaged hepatocytes, **DNME 200 mg/kg b.w:** Showing minimal inflammatory collection and damaged hepatocytes, **DNME 400 mg/kg b.w:** Liver appearing normal no foci of damage or inflammation collection, **Positive control:** Liver appearing near to normal condition

The percentage reduction of various serum biochemical parameters in case of standard drug Silymarin in CCl_4 intoxicated rats revealed a significant reduction in the levels of SGOT, SGPT, SALP and T.BILI. (86.76, 85.52, 85.86 and 74.94%).When compared to the CCl_4 toxic control group, the groups treated with the methanolic extract of whole plant of *Dendrobium normale* at doses of 100 mg/kg, 200mg/kg and 400mg/kg in CCl_4 intoxicated rats exhibited a significant reduction of SGOT (44.67, 65.62 and 87.25 %), SGPT (43.61, 65.67, and 86.29 %), SALP (38.33, 62.62 and 83.87 %) and T.BILI. (36.40, 59.57 and %) levels (table1,2&figure1) All the doses show significant activity and at the dose of 400mg/kg shown more potent hepatoprotective activity than remaining doses because of effect on percentage reduction in elevated levels of biochemical parameters. The comparative efficacy of the extract tested for their hepatoprotective activity, the relationship between dose and percentage reduction in each case were depicted.

Histopathology

Histopathological examination of the liver sections of the control group showed normal architecture of the liver with distinct hepatic cells, sinusoidal spaces and central vein . The liver section of CCl_4 intoxicated group showed complete disarrangement of normal hepatic cells with intense centrilobular necrosis, vacuolization, fatty changes, sinusoidal haemorrhages and dilatation. The liver sections of Silymarin treated rats showed a normal hepatic architecture with normal hepatocytes, sinusoidal spaces, less vacuole formation, absence of necrosis and less visible changes as compared to control group. Histopathological examination of liver sections of the rats treated with test methanolic extracts of *Dendrobium normale* doses of 100 mg/kg b.w 200 mg/kg b.w and 400 mg/kg b.w showed recovery from CCl_4 induced liver damage as evident from normal hepatocytes and with higher dose of 400mg/kg b.w showed significant attenuation of inflammatory and necrotic changes and cellular architecture of liver was preserved indicating a marked protective activity similar to that observed in Silymarin treated rat liver sections and the effect was found to be dose dependant.

Conclusion

From the above results, we can conclude that, the selected medicinal plant showed significant hepatoprotective activity.

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