PROTECTIVE EFFECT OF NERIUM INDICUM ON CCl₄ INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT
Methanolic flowers extract of Nerium indicum was evaluated for hepatoprotective in rats. The plant extract (500 and 1000 mg/kg, p.o.) showed a remarkable hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in liver tissues. Carbon tetrachloride induced a significant rise in Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT) and alkaline phosphatase (ALP). Treatment of rats with different doses of plant extract (500 and 1000 mg/kg) significantly (P<0.001) altered serum marker enzymes levels to against carbon tetrachloride treated rats. The activity of the extract at dose of 300 mg/kg was comparable to the standard drug, silymarin (100 mg/kg, p.o.). Histopathological changes of liver sample were compared with respective control. Results indicate the hepatoprotective properties of Nerium indicum against carbon tetrachloride induced hepatotoxicity in rats.

KEY WORDS: - Nerium indicum, Hepatoprotective activity, carbon tetrachloride.

INTRODUCTION
Nerium Indicum (N. oleander Linn; N. Odorum soland) is a Well Known ornamental plant with leathery evergreen leaves and handsome clusters of red or pink or white flower. The plant originates from the Mediterranean region and is indigenous to indo-Pakistan subcontinent.¹-² The plant contains cardiac glycosides in its leaves, stems and flowers and is known as one of poisonous plants; poisoning and fatal cases for domestic animals and humans due to ingestion of this plant were reported.³ Nerium oleander's or Nerium Indicum leaves contain two principles: nerin and oleandrin, glucosides with properties similar to digitalin the main toxin of oleander is oleandrin.⁴ The Plant has Certain Toxic Properties due to the presence of digtoxin like steroidal Glycosides. The plant also used for the treatment of mange in rabbits.⁵ The bark contains toxic glycosides: rosaginin and nerlin, volatile oil, fixed oil. Leaves and flowers are considered cardiotonic diuretic and emetic. Whole plant believed to have anticancer properties.⁶ The extract of Nerium indicum are still used in homeopathy. In human beings, toxicity manifests as nausea, vomiting, colic, decreased appetite, dizziness, drowsiness, bradycardia and irregular heart beats, pupillary dilation, and sometimes unconsciousness attributed to digitalis poisoning.⁷
MATERIAL AND METHODS

**Plant material:** - The fresh flowers of *Nerium indicum* Mill were collected from Sagar, Madhya Pradesh, India. The sample was identified by Prof. Madhuri Modak, plant taxonomist, Department of Botany, M.V.M. College, Bhopal, Madhya Pradesh, and the voucher specimen was deposited at Department of Botany, M.V.M. College, Bhopal.

**Preparation of extract:** - The fresh organs of the plant were dried and grounded. A sample of the grounded flowers was accurately weighed. The powdered plant material (100 g) was repeatedly extracted in a 500 mL round bottomed flask with 300 mL solvents of increasing polarity starting with petroleum ether and methanol. The reflux time for each solvent was 40 cycles. The extracts were cooled at room temperature, filtered, and evaporated to dryness under reduced pressure in a rotary evaporator. [8]

**Experimental animals:** - Wistar albino rats (180 to 200 g) and Wistar mice (24-30 g) of either sex procured from Institute of Animal Health and Vety. and Biologicals, Rasalpura, Mhow, 453 446 (Madhya Pradesh, India) (Reg. No. 5007/SAS/2006-07) were used for this study. They are maintained under standard conditions (temperature 22 ±2°C, relative humidity 60±5% and 12 h light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellet diet and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol. All the procedures were performed in accordance with Institutional Animal ethics committee constituted as per the direction of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), (Reg. No. - 1252/AC/09/CPCSEA) under ministry of animal welfare division. Government of India, New Delhi, India.

**Acute toxicity study:** - The acute toxicity for methanolic extracts of *Nerium indicum* was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline No. 423) method of CPCSEA was adopted for toxicity studies.

**Hepatoprotective activity:** - The animals were divided into four groups of six Wistar albino rats each. The animals were fasted for 24 h prior to carbon tetrachloride treatment. Group I was maintained as normal control received normal saline 5 ml/kg orally. All the animals of group II to IV received carbon tetrachloride at dose of 1 ml/kg, subcutaneously for two successive days (2nd and 3rd day). Group I animals were maintained as carbon tetrachloride control without any drug treatment. Group II animals were treated with Silymarin (100 mg/kg, orally) which served as standard group. Group III and IV animals were treated with 500 and 1000 mg/kg methanolic extract respectively by oral route. The vehicle or drug treatment was carried out orally from 1st day to 5th day with concurrent administration of carbon tetrachloride on 2nd and 3rd day. During the period of drug treatment the rats were maintained under normal diet and water *ad libitum*. The animals of all the groups were sacrificed by light ether anesthesia on 6th day. The blood sample of each animal was collected separately by carotid artery into sterilized dry centrifuge tubes and allowed to coagulate for 30 min. Serum was separated by centrifugation 3000rpm for 15 min. The serum was used...
to estimate serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and serum alkaline phosphatase (ALP). Livers were removed and preserved in 10% formalin solution for histopathological studies. [9-14]

Statistical analysis: - The values are represented as mean ± S.E.M, and statistical significance between treated and control groups was analyzed using of One way ANOVA, Followed by Dunnett’s test where P<0.001 was considered statistically significant.

RESULTS & DISCUSSION: - Effect of methanolic flower extract of *Nerium indicum* on CCl4 induced liver damage in rats with reference to biochemical changes in serum is shown in Table 1. At the end of the 5th day treatment, blood sample of CCl4 treated control animals showed significant increase in the level of SGPT, SGOT and ALP. Pretreatment with *Nerium indicum* flower extract at 500 and 1000 mg/kg showed marked decreased of SGPT, SGOT and ALP as compared to the CCl4 treated group. The maximum protection was shown by methanolic extract at the dose of 1000 mg/kg body weight. Protection against CCl4 induced hepatic damage at 500 mg/kg dose of extract was negligible in all these biochemical markers. The group of rats treated with methanolic extract of *Nerium indicum* at a dose of 500 and 1000 mg/kg prevented carbon tetrachloride induced reduction in ascorbic acid significantly (P< 0.001).The standard reference drug Silymarin prevented CCl4 induced reduction in ascorbic acid significantly. Histopathological examination of liver sections of control group showed disarrangement of normal hepatic cells with centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration were observed in CCl4 intoxicated animals (Fig.1. A). The liver sections of the rats treated with methanolic extract of *N. indicum* and silymarin followed by CCl4 intoxication showed a sign of protection as it was evident the absence of necrosis and vacuoles (Fig.1. B, C, and D).

CONCLUSION

The present study was conducted to evaluate the hepatoprotective activity of methanolic extract of aerial parts of *Nerium indicum* in CCl4-induced hepatotoxicity in wistar rats. The methanolic flowers extract of *Nerium indicum* have shown very significant hepatoprotection against CCl4-induced hepatotoxicity in wistar rats by reducing serum total bilirubin, SGPT ,SGOT and ALP levels. Histopathological studies also confirmed the hepatoprotective nature of the extract.

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REFERENCES

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1. **Table 1** Effects of methanolic flowers extract of *Nerium indicum* on certain serum biochemical parameters in CCl4 induced hepatotoxicity in rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Biochemical’s parameters</th>
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<tbody>
<tr>
<td></td>
<td>SGPT (IU/L)</td>
</tr>
<tr>
<td>Control (CCl4 1 ml/kg)</td>
<td>38.16 ± 0.440</td>
</tr>
<tr>
<td>Silymarin + CCl4 (100 mg/kg)</td>
<td>45.39 ± 0.402***</td>
</tr>
<tr>
<td><em>N. indicum</em> + CCl4 (500 mg/kg)</td>
<td>63.48 ± 0.624***</td>
</tr>
<tr>
<td><em>N. indicum</em> + CCl4 (1000 mg/kg)</td>
<td>46.36 ± 0.635***</td>
</tr>
</tbody>
</table>

2. Values are express as mean ± SEM of 6 observations, statistical comparisons as follows:

significant at  ***p < 0.001 compared to control group.

**Fig. 1: Microphotographs of liver section taken from rats**

A  B  C  D
Microphotographs (10 x 45) of liver section taken from rats. **A**, CCl4 control group (1 ml/kg); **B**, Silymarin (100mg/kg) + CCl4 **C**, methanolic extract (500mg/kg) +CCl4; **D**, methanolic extract (1000mg/kg) +CCl4