Hepatoprotective potential of aqueous extract of *Zingiber officinale* leaves using CCl₄ induced model

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ABSTRACT

The hepatoprotective activity of crude aqueous extract of *Zingiber officinale* leaves (ZOL), *Zingiberaceae*, was evaluated, using CCl₄ induced hepatic damage in male *Wistar albino* rats. Various biochemical parameters like serum glutamic pyruvate transaminase (SGPT), serum glutamic oxaloacetate transaminase (SGOT) and total bilirubin were studied. The aqueous ZOL extract afforded significant protection against CCl₄ induced hepatocellular injury.

Key words: CCl₄, *Zingiber officinale* leaves, extract.

INTRODUCTION

The *Zingiber officinale* (*Zingiberaceae*) is a slender, perennial rhizomatous herb; leaves linear, sessile, glabrous; flowers yellowish green in oblong, cylindrical spikes; rhizomes white to yellowish brown, irregularly branched. Commonly it is known as Chandrakhya, zanjabil, adraka, sunthi. It is widely cultivated in tropical Asia, Africa. The rhizomes contain volatile oil, resinous matter, starch and mucilage. Oil of ginger consists of monoterpenes, sesquiterpene hydrocarbons and the sesquiterpene alcohol. The pungency of ginger is due to gingerol. *Zingiber officinale* rhizomes are used as carminative, stimulant, anti-inflammatory, anti-platelet, anti-bacterial, anti-emetic, anti-hyperglycaemic agent, hepatoprotective, immuno-stimulant, analgesic and in atherosclerosis. But studies on leaves are not reported. Therefore it was decided to study the hepatoprotective activity of aqueous leaf extract of *Zingiber officinale*.

MATERIAL AND METHODS

Plant Material and Extraction

The leaves of *Zingiber officinale* [ZO] (*Zingiberaceae*) were collected from Bhopal and was deposited as a voucher specimen [no: VNSIPCG0928] in the herbarium of Department of Pharmacognosy, VNS Institute of Pharmacy, Bhopal. The samples were cut into small pieces and dried in shade for 5 days. The dried leaves were then ground. Sixty grams of the pulverized material was macerated in distilled water for 72 hours with occasional shaking in dark. Macerate was decanted and filtered. The marc was pressed and filtration was done 2-3 times. The macerates were concentrated to give aqueous extract [11.13% w/w].

Phytochemical studies

Freshly prepared extracts were subjected to phytochemical screening tests for the detection of various constituents using conventional protocol.

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Animals
Albino rats of Wistar strain (150-200 g) of either sex were procured from the central animal house of the institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 20°C; relative humidity 60-70%) in a 12 h light-dark cycle. The rats were given a standard laboratory diet and water ad libitum. Food was withdrawn 12 h before and during the experimental hours. All experimental protocols were approved by the institutional animal ethics committee (reg. no.778/03/C CPCSEA).

Procurement of Diagnostic kit and chemicals
Diagnostic kits used for estimation of SGPT, SGOT and total bilirubin were obtained from Star diagnostics. All chemicals used for the experiments were of analytical grade.

Acute toxicity study
No adverse effect or mortality was detected in albino rats up to 3 g/kg, p.o. of ZO leaves during the 24 h observation period.

Drugs and Dosing Schedule

Model
Thirty male albino rats weighing between 120-220g were taken. Animals were divided into 5 groups of 6 rats each.

Group I (Control)
Served as control which received only vehicle, 4% tragacanth solution (1ml/kg/day) by oral route.

Group II (Standard)
Received Silymarin present in solution at a dose of 10mg/kg body weight by oral route up to 6th day. On 6th day CCl4 was administered intraperitonially. From day 7th to day 15th silymarin was continued.

Group III & IV (Test)
Received extract of ZOL (200 and 400 mg/kg body weight) by oral route from 1st to 6th day. On day 6th CCl4 in liquid paraffin was administered (1.25 ml/kg) intra-peritoneally. From day 7th to 15th the same extract was continued.

Group V (CCl4 treated)
Received only vehicle (1ml/kg) by oral route from day 1 to 6. On 6th day CCl4 in liquid paraffin (1:1) was administered by intra peritoneal route. From day 7th to day 15th no treatment was given.

Serum Analysis
On 5th day, after treatment period the animals of all groups were anaesthetized with chloroform and sacrificed. Blood was withdrawn by direct puncturing the heart and serum was separated by centrifugation at 3000 rpm at 30°C for 15 min. and analyzed for various biochemical parameters; Serum transaminases viz. Serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT)12, and total bilirubin14.

Statistical Analysis
Results of biochemical parameters are reported as mean±S.E.M. Statistical significance

Table 1: Level of serum hepatic enzymes after treatment with aqueous extract of Zingiber officinale leaves

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Groups</th>
<th>Parameters</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>SGOT (u/l)</td>
</tr>
<tr>
<td>I.</td>
<td>Group I (Control)</td>
<td>35.87± 4.30</td>
</tr>
<tr>
<td>II.</td>
<td>Group II (Standard)</td>
<td>42.30± 3.24*</td>
</tr>
<tr>
<td>III.</td>
<td>Group III (Test) 200mgZOL</td>
<td>100.64± 3.38*</td>
</tr>
<tr>
<td>IV.</td>
<td>Group III (Test) 400mgZOL</td>
<td>58.76± 2.97*</td>
</tr>
<tr>
<td>V.</td>
<td>Group I (CCl4 treated)</td>
<td>129.05± 5.52</td>
</tr>
</tbody>
</table>

The values are Mean± SEM (n=6), *p< 0.05 Vs CCl4. One way analysis followed by Dunnet’s test SGOT- serum glutamate oxaloacetate transaminase, SGPT- serum glutamate pyruvate transaminase
was determined by one way analysis of variance followed by Dunnet's $t$-test\textsuperscript{15}. $P$ value <0.05 was considered statistically significant.

**RESULTS**

Administration of $\text{CCl}_4$ led to significant hepatocellular damage as evident from increase in serum activities of Serum glutamate oxaloacetate transaminase (SGOT) (129.05 U/ mL), serum glutamate pyruvate transaminase (SGPT) (82.67 U/ ml), and total bilirubin (3.39 mg/dl) concentration as compared to normal control group (35.87 U/ mL, 27.44 U/ml and 0.54 mg/dl, respectively). Treatment of rats with aqueous extract of leaves at a dose of 200 mg/ kg and 300 mg/kg body weight, i.p. exhibited significant reduction ($P$ <0.05) in $\text{CCl}_4$ induced elevation of serum glutamate oxaloacetate transaminase (SGOT) (100.64 U/ml, 58.76 U/ml), serum glutamate pyruvate transaminase (SGPT) (58.38 U/ml, 31.24 U/ml) and total bilirubin (2.23 mg/ dl, 1.18 mg/dl) in a dose dependant manner.

**DISCUSSION**

$\text{CCl}_4$ is one of the most commonly used hepatotoxin in the experimental study of liver diseases\textsuperscript{16}. The hepatotoxic effects of $\text{CCl}_4$ are largely due to its active metabolite, trichloro methyl radical\textsuperscript{17,18}. These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides, which in turn give products like malondialdehyde (MDA) that cause damage to the membrane. This is evidenced by an elevation in the serum marker enzymes. The increase in the levels of serum bilirubin reflected the level of jaundice and increase of transaminases was the clear indication of cellular leakage and loss of functional integrity of cell membrane\textsuperscript{19}. Aqueous extract has significantly reduced these liver enzyme levels and has increased the level of total bilirubin in the serum in a dose dependant manner, which indicates hepatoprotection. Furthermore, results of hepatocellular damage caused by $\text{CCl}_4$ and its recovery by aqueous extract suggest that the drug might be considered a potential source of natural hepatoprotective agents, which could be related to free radical scavenging properties of flavonoids present in the high concentration in the extract of the plant.

Also all the effects of aqueous extract of $\text{ZOL}$ were comparable with those of Silymarin, a proven hepatoprotective drug. Further isolation of active principles responsible for hepatoprotective activity is currently under progress in our lab.

**REFERENCES**

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