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Hepatoprotective potential of aqueous extract of *Zingiber officinale* leaves using CCl_4 induced model

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ABSTRACT

The hepatoprotective activity of crude aqueous extract of *Zingiber officinale* leaves (ZOL), *Zingiberacae*, was evaluated, using CCl_4 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 CCl_4 induced hepatocellular injury.

Key words: CCl_4, *Zingiber officinale* leaves, extract.

INTRODUCTION

The *Zingiber officinale* (Zingiberaceae) is a slender, perennial rhizhomatous herb; leaves linear, sessile, glabrous; flowers yellowish green in oblong, cylindrical spikes; rhizomes white to yellowish brown, irregularly branched [1,2]. Commonly it is known as Chandrakhya, zanjabil, adraka, sunthi [3]. It is widely cultivated in tropical Asia, Africa [4]. 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 [5]. *Zingiber officinale* rhizomes are used as carminative, stimulant, anti-inflammatory[6], anti-platelet, anti-bacterial, anti-emetic, anti-hyperglycaemic agent, hepatoprotective [7], immuno-stimulant [8], analgesic [9] and in atherosclerosis [10,11]. But studies on leaves are not reported. Therefore it was decided to study the hepatoprotective activitiy 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 grinded. 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[12].

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 6^{th} day. On 6^{th} day CCl_4 was administered intraperitonially. From day 7^{th} to day 15^{th} silymarin was continued.

Group III & IV (Test)

Received extract of ZOL (200 and 400 mg/kg body weight) by oral route from 1^{st} to 6^{th} day. On day 6^{th} CCl_4 in liquid paraffin was administered (1.25 ml/kg) intra-peritoneally. From day 7^{th} to 15^{th} the same extract was continued.

Group V (CCl_4 treated)

Received only vehicle (1ml/kg) by oral route from day 1 to 6. On 6^{th} day CCl_4 in liquid paraffin (1:1) was administered by intra peritoneal route. From day 7^{th} to day 15^{th} no treatment was given.

Serum Analysis

On 5^{th} 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)[13], and total bilirubin[14].

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

S. No.	Groups	Parameters		
		SGOT (u/l)	SGPT (u/l)	Total Bilirubin (mg/dL)
I.	Group I (Control)	35.87± 4.30	27.44± 2.27	0.54± 0.31
II.	Group II (Standard)	42.30± 3.24*	55.81± 1.93*	0.95± 0.46*
III.	Group III (Test) 200mgZOL	100.64± 3.38*	58.38± 1.86*	2.23± 0.87*
IV.	Group III (Test) 400mgZOL	58.76± 2.97*	31.24± 2.11*	1.18± 1.24*
V.	Group I (CCl_4 treated)	129.05± 5.52	82.67± 10.12	3.39± 1.13

The values are Mean± SEM (n=6), *p< 0.05 Vs CCl_4. 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[15]. *P* value <0.05 was considered statistically significant.

RESULTS

Administration of 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 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

CCl_4 is one of the most commonly used hepatotoxin in the experimental study of liver diseases[16]. The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloro methyl radical[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[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 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 *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.

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