



## HEPATOPROTECTIVE ACTIVITY OF *CAJANUS CAJAN* AGAINST CARBON TETRACHLORIDE INDUCED LIVER DAMAGE

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### ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of hydroalcoholic extract of the aerial part of *Cajanus cajan* against carbon tetrachloride (CCl<sub>4</sub>) induced liver damage in wistar rats. The extract of *C. cajan* (100, 200 and 400 mg/kg) was administered orally to the animals with hepatotoxicity induced by CCl<sub>4</sub>. Liv. 52 (100mg/kg) was given as reference standard. The extract was effective in protecting the liver against the injury as there was significant reduction in serum enzyme aspartate aminotransferase (AST) and alanine aminotransferase (ALT) and increase in total protein. It was concluded from the study that *C. cajan* possesses hepatoprotective activity against CCl<sub>4</sub> induced hepatotoxicity in rats.

**Keywords:** Hepatoprotective, *Cajanus cajan*, CCl<sub>4</sub>, Liv 52, AST and ALT

### INTRODUCTION

Liver, the key organ of metabolism and excretion, is constantly endowed with the task of detoxification of xenobiotics, environmental pollutants and chemotherapeutic agents. Carbon tetrachloride is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of CCl<sub>4</sub> are largely due to its active metabolite, trichloromethyl radical. These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to formation of lipid peroxidase, which in turn gives products like malondialdehyde (MDA) that cause damage to the membrane<sup>1,2</sup>.

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a perennial member of the family leguminosae. It possess several medicinal properties like anthelmintic<sup>3</sup>, antioxidant<sup>4</sup>, protection against alcohol induced liver damage<sup>5</sup> etc. Based on the above medicinal properties, the present study has been undertaken to investigate the hepatoprotective activity of hydroalcoholic extract of the aerial part of *C. cajan* against CCl<sub>4</sub> induced hepatic damage in rats.

### MATERIALS AND METHODS

#### Plant material

The authenticated plant was collected from Natural Remedies Pvt. Ltd., Bangalore (sample invoice No. D119) and confirmed at Botany Department, Dr. H. S. Gour University, Sagar (M.P).

#### Chemicals and drugs

The following drugs and chemicals were used: Ethanol (RANKEM), AST & ALT estimation kits (Merck), Carbon tetrachloride (RANKEM), total protein estimation kit (Commercial reagents kits from Span Diagnostics) and liquid paraffin (CDH). All chemicals used were of analytical grade

#### Extract preparation

Dried and powdered plant material was extracted with 70% ethanol using soxhlet apparatus. The extract was concentrated and dried at 68°C and kept at 4°C for further studies.

#### Phytochemical test

Phytochemicals screening were performed to detect the presence or absences of various compounds such as tannins, flavonoids, alkaloids etc. as per standard methods<sup>6</sup>.

#### Experimental model

Adult albino rats (Wistar Strain) of either sex weighing between 150 – 200 g body weight were selected for the experimental study. The

animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. They had free access to a commercial pellet diet and water *ad libitum*. The room temperature was maintained at 25±2°C.

#### Experiment

A total of 36 animals were equally divided into 6 groups (n=6 in each group). The Treatment period was for 6 days. Group I served as control and received vehicle (Normal saline) 10 ml/kg p.o. Group-II received CCl<sub>4</sub> (2ml/kg) diluted with liquid paraffin (1:1) given orally on third and sixth day, Group III received CCl<sub>4</sub> and standard drug Liv 52 (100mg/kg p.o.). Similarly, Group IV, V and VI received CCl<sub>4</sub> and *C. cajan* extract 100, 200 and 400 mg/kg p.o. respectively, once daily simultaneously for 6 days. Food was withdrawn 12hrs before CCl<sub>4</sub> administration on the sixth day to enhance the acute liver damage in all the groups except group I animals. Rats were sacrificed on seventh day, 24 h after administration of the last dose. Blood samples were collected by abdominal aorta method and blood was collected in standard sampling tubes and serum was separated within 8 hours at room temperature for use of assay marker enzymes and estimation of total protein. The study was approved by animal ethic committee (1030/9/07/CPCSEA).

#### Enzyme assays

The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were assayed in serum using standard kits from Merck using colorimetric method<sup>7,8,9,10</sup>. The results were expressed as units/litre (U/L).

#### Protein estimation

The level of total protein was estimated in serum of experimental animals by biuret method<sup>11</sup>. Standard kit was obtained from Span diagnostics.

#### Statistical analysis

The significance of difference among the groups was assed using one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test between the data of control and treated groups. The values are expressed in mean± SEM, p< 0.05 were considered significant.

### RESULTS

The effect of hydroalcoholic aerial part extract of *C. cajan* on CCl<sub>4</sub> induced liver damage in rats with reference to the changes in the level of AST, ALT and total protein is shown in Table 1. After assessment of the biochemical parameters from blood collected

from each animal from all the groups, CCl<sub>4</sub> treated animals showed significant increase in the levels of AST and ALT while decrease in the level of total protein as compared to the normal control group. Whereas blood samples analysis from the animals treated with hydroalcoholic extract of the aerial part of *C. cajan* at the dose of 400mg/kg.b.w. showed significant decrease in the levels of serum

marker enzymes and significant increase in the total protein to the near normal value which are comparable to the values observed for standard drug (Liv 52), indicating the recovery of hepatic cells against the damage. The hepatoprotective potential of the plant showed concentration dependent activity.

**Table 1: Effect of *C. cajan* extract on CCl<sub>4</sub> induced hepatotoxicity**

Groups	Treatment	AST (U/ml)	ALT (U/ml)	Total protein
I	Normal Control	54.91 ± 10.30	31.65 ± 4.30	4.89 ± 0.21
II	Toxic (CCl <sub>4</sub> ) Control	187.22 ± 18.50	90.66 ± 8.60	1.57 ± 0.61
III	CCl <sub>4</sub> + Standard drug (Liv 52)	65.31 ± 12.30	42.31 ± 7.50	5.51 ± 0.81
IV	CCl <sub>4</sub> + CC (100mg/kg)	126.76 ± 11.2	68.28 ± 3.46	3.16 ± 0.51
V	CCl <sub>4</sub> + CC (200mg/kg)	101.21 ± 9.66	54.83 ± 4.53	3.78 ± 0.04
VI	CCl <sub>4</sub> + CC (400mg/kg)	72.52 ± 10.12	43.74 ± 6.74	4.34 ± 0.18

Values are expressed as mean ± SD, Six animals in each group, statistical analysis by one way ANOVA, CC= *C. cajan*

## DISCUSSION

One of the most commonly used chemical agents for liver damage in hepatoprotective study is CCl<sub>4</sub><sup>12</sup>. The active radical of this compound is CCl<sub>3</sub> which bind to the macromolecules and induce peroxidative degradation of membrane lipids of Endoplasmic reticulum. This results in the formation of lipid peroxides whose product malondialdehyde (MDA) causes severe membrane damage<sup>13,14</sup>. The extent of hepatic damage is assessed by the elevated levels of serum marker enzyme AST and ALT which is significantly lowered by the extract administration of *C. cajan* in the tested groups showing its hepatoprotective potential. The total protein estimation is useful in hepatoprotective study as its decreased level indicates severe non viral liver cell damage<sup>15</sup>. After CCl<sub>4</sub> administration, the total protein level was lowered which was significantly elevated on treatment with *C. cajan* extract, indicating its protective role against liver cell damage.

The hepatoprotective potential of a drug depends upon its ability in reducing the harmful effects caused by a hepatotoxin<sup>16</sup>. The medicinal property of a plant is due the presence of its chemical constituents. In hepatoprotective study, these phytoconstituents play a vital role in inducing microsomal enzymes thereby accelerating the excretion of CCl<sub>4</sub>, or inhibiting the lipid peroxidation induced by CCl<sub>4</sub><sup>17</sup>. Phytoconstituents such as alkaloids and flavonoid have been found effective in the hepatoprotection against CCl<sub>4</sub> induced liver damage<sup>18,19</sup>. The phytochemical analysis of the hydroalcoholic extract of *C. cajan* showed the presence of such phytochemicals (alkaloid and flavonoid) which may be responsible for the hepatoprotective efficiency of the plant against CCl<sub>4</sub> induced liver damage.

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