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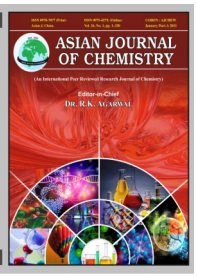

Potential Hepatoprotective Effect of *Solanum xanthocarpum* Against CCl_4 Induced Hepatotoxicity in Albino Rats

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The objective of current study is to assess the hepatoprotective effect of the ethanolic extract of *Solanum xanthocarpum* fruit in rat liver injured with carbon tetrachloride. The damage of the liver was studied in the experimental animals by assessing total protein, total bilirubin, cholesterol, serum glutamate oxaloacetate transaminase (SGOT), triglycerides, lactate dehydrogenase (LDH) and serum glutamate pyruvate transaminase (SGPT), in serum. The effect of co-administration of ethanolic extract of fruits of *Solanum xanthocarpum* (200 mg/Kg) on the above parameters was investigated. Hepatic damage was demonstrated with high levels of all parameters in serum of experimental animals which received carbon tetrachloride. The antioxidant assay of plant extract was proved against hydrogen peroxide and phospho molybdate assay. It is concluded that the fruits of *Solanum xanthocarpum* are rich in antioxidant and acts as a hepatoprotective agent and attenuates the hepatotoxic effect of carbon tetrachloride.

Keywords: Carbon tetrachloride, Hepatic damage, *Solanum xanthocarpum*.

INTRODUCTION

Liver is the most significant, largest organ and first to come across drugs and environmental pollutants, which come into the hepatic portal blood of the human body to regulate important metabolic functions[1]. The vital role of liver is to metabolize foreign compounds. These compounds consumed by human beings through contaminated food and environmental exposure[2]. The drugs used for treating the liver disorders are inadequate and hence it is essential to opt for alternative drugs to substitute for the drugs used at present with doubtful efficacy and safety[3].

India is popular for a plethora of medicinal use of numerous plants (as hepatoprotectants) like *Hibiscus rosasinensis, Azadirachta indica, Andrographis paniculata, , Picrorrhiza kurroa*, *Cassia fistula, Elephantopus scaber* are reported in the literature[4]. *Solanum xanthocarpum* of family *Solanaceae* is a perennial herb widely spread all over India[5]. The fruits of the plant constitute the drugs which are used for numerous medicinal uses like antipyretic, antiinflammatory, anthelmintic, laxative and antiasthmatic activities. It is also effective in treating fever, coughs, asthma, chest pain, enlargement of liver and spleen[6].

Many chemical and pharmacological agents act as hepatotoxin to produce different liver ailments[7]. Reactive free radicals induced by CCl_4 are the initiative for the cell damage by two different mechanisms such as binding to the membrane proteins followed by lipid peroxidation. Administration of CCl_4 to rodents is a commonly used model to investigate the mechanism of hepatic damage[8]. Hence the current study is aimed to assess the protective role of *Solanum xanthocarpum* fruit extract in rat liver injured with carbon tetrachloride.

EXPERIMENTAL

Collection and extraction of plant materials: The fruits of *Solanum xanthocarpum* for the present investigation were collected from the commercial medicinal shop at Kumbakonam. The fruit of *Solanum xanthocarpum* was dried at room temperature (30 ± 2 ºC) and after drying fruit was ground into coarse powder using pulverizer. The extract was prepared by cold percolation method using 500 mg of powder suspended in ethanol in the ratio 1:4 for 48 h. The extract was filtered using muslin cloth and concentrated at (40 ± 5 ºC) refrigerated and used within 2 months[9,10].

in vitro Antioxidant activity

Hydrogen peroxide scavenging activity: Various concentrations of the fruit extract (25-400 µ/mL) in distilled water were mixed with H_2O_2 solution (2 mM/L in phosphate buffer with pH 7.4). The absorbance at 230 nm was calculated

after 10 min against a blank. H_2O_2 scavenging activity of various concentrations was calculated using α-tocopherol[11].

Phosphomolybdate method: 0.1 mL of the extract (100 µg) was added to the reagent (1.0 mL) which contains 4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulfuric acid in a test tube and it was kept for 90 min in a water bath at 95 ºC. After boiling, the tube was cooled and the absorbance was read against the blank at 695 nm. The blank solution was prepared in the same way except adding the extract. The total antioxidant capacity was calculated using α-tocopherol[12].

Experimental animals: Male albino Wistar rats (200-225 g weight) were used for the present study and maintained on 12 h light/dark cycle at approximately 27 ºC in all experiments. The animals were given with pelleted rat chow purchased from Hindustan Lever Ltd., Mumbai and water *ad libitum*. The experiment was performed according to ethical procedures. Animals were sheltered for one week and prior to the experiment they were acclimatized to laboratory temperature and acute toxicity study was carried out.

Hepatic damage induction: Carbon tetrachloride was administered subcutaneously in the abdomen along with olive oil in the ratio of 1:1 to damage the liver of the animal at a dose of 1 mL CCl_4/ kg bw.

Experimental design: Experiments were designed to examine the effect of the ethanolic fruit extract of *Solanum xanthocarpum* on biomarkers indicating hepatic damage caused by carbon tetrachloride. Twenty four rats were separated into four groups with equal size[13].

Group I: Control, received saline (2.0 mL/day) for 15 days.

Group II: Saline of 2.0 mL was received daily for 15 days followed by CCl_4 (1 mL/Kg) on day 15.

Group III: Ethanolic extract of *Solanum xanthocarpum* (200 mg/kg bw) was received daily for 15 days followed by CCl_4 on day 15.

Group IV: Sylimarin (25 mg/kg bw) was received daily for 15 days followed by CCl_4 on day 15.

After 15th day, the animals were sacrificed and various biochemical parameters analyzed. The blood samples of all rats were collected and separated the serum for further analysis[14].

Biochemical estimations: Biochemical estimations of various parameters like total proteins[15], total bilirubin[16], cholesterol, triglycerides[17], SGOT, LDH and SGPT[18] were carried out using serum to assess the acute hepatic damage caused by CCl_4.

Statistical analysis: The outcomes of present investigation were examined by mean ± standard deviation and the significant differences between the groups were assessed by the students *t* test.

RESULTS AND DISCUSSION

Antioxidant activity: Some enzymes are directly inactivated by hydrogen peroxide, an oxidizing agent usually through oxidation of essential thiol (-SH) groups. H_2O_2 will come across the membrane, react with the Cu^+ and Fe^{2+} ions to form hydroxyl radical. These hydroxyl radicals are responsible for the toxic effects to cells and hence it is advantageous to control H_2O_2 which is allowed to accumulate in the cells[19].

The free radicals are scavenged by antioxidants. Table-1 represents the effect of ethanolic extract of *Solanum xanthocarpum* on free radical scavenging. Radical scavenging activity of *Solanum xanthocarpum* increased with increase in concentration. The mixed function oxidase system metabolizes CCl_4 in endoplasmic reticulum of liver. Free trichloromethyl radicals ($CCl_3^{\bullet}$) are formed by the cleavage of the carbon chloride bond. They are extremely unbalanced and then react immediately with membrane proteins and lipids. Moreover, they obstruct a hydrogen atom from the unsaturated fatty acids of membrane lipids or form covalent bonds with unsaturated fatty acids, resulting in the production of chloroform and lipid radicals which react with molecular oxygen, this initiate per oxidative decomposition of phospholipids in the endoplasmic reticulum[20].

TABLE-1
ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *Solanum xanthocarpum* USING HYDROGEN PEROXIDE AND PHOSPHOMOLYBDATE ASSAY

S. No.	Concentration of extract. (µg/mL)	Inhibition (%)	
		H_2O_2	Phosphomolybdate
1	50	41.69	44.04
2	100	53.19	57.93
3	150	66.96	70.71
4	200	95.19	82.80

Biochemical parameters: The level of protein was decreased in group II and it was almost normal in group III when it was compared with control group where as the levels of total bilirubin, cholesterol and triglycerides in serum were increased in group II and almost normal in group III (Table-2). The liver, an important site has its highest rate of protein synthesis. Major protein mass of the organism is severely affected by hepatotoxicity protein waste implies underlying metabolic imbalance and it is expressed by an elevation in the apparent protein degradation rate in the unchanged apparent synthesis rate[21,22].

Hypoalbuminaemia is the most common in the presence of advanced chronic diseases of liver and therefore, decline in the content of total protein can be noticeable as a valuable index of the severity of dysfunction in the damage of chronic liver[23].

Serum marker enzymes: LDH, SGOT and SGPT recorded the higher activity in CCl_4 treated rats when compared with the control (Table-2). In ethanolic extract of *Solanum xanthocarpum* administered group, the levels of the same were retrieving towards the normal level. Assessing the activities of serum marker enzymes, such as SGOT, SGPT, LDH, total proteins, total bilirubin and other biochemical enzymes like cholesterol and triglycerides can make assessment of liver function. In acute inflammatory condition of the liver, the frequency of SGOT elevations will be higher than those of SGOT and will tend to be remaining elevated. The over dose of carbon tetrachloride causes a dramatic increase in the SGPT activity. Serum SGPT level elevated to some extent in nearly all liver disorder[24,25]. SGPT levels are enormous in carbon tetrachloride hepatotoxicity. The increased activity of the marker enzymes in CCl_4 treated rats in the current investigation is mainly due to the damage of the liver caused by the toxin[26].

TABLE-2
ACTIVITY OF ETHANOLIC EXTRACT OF *Solanum xanthocarpum* ON LIPID PROFILE AND LIVER MARKER ENZYMES IN EXPERIMENTAL ANIMALS

S. No.	Parameters	Group I	Group II	Group III	Group IV
1	Serum glutamate oxaloacetate transaminase (U/L)	35.08 ± 0.2	196.9 ± 1.94	56.7 ± 6.3*	49.4 ± 3.6**
2	Serum glutamate pyruvate transaminase (U/L)	97.3 ±1.18	186.7 ± 1.82	49.0 ± 3.82*	47.38 ± 4.3**
3	Lactate dehydrogenase (U/L)	892.2 ± 12.3	1820.42 ± 10.76	974 ± 15.2	696.45 ± 10.03*
4	Protein (g/dl)	4.2± 0.22	2.4 ± 0.02	3.0 ±0.01	4.0 ± 0.16
5	Total bilirubin (mg/dl)	14.0 ± 0.16	17.3 ± 0.18	15.4 ± 0.04	13.0 ± 0.08
6	Cholesterol (mg/dl)	32.92 ± 0.9	98.3 ± 7.9	47.0 ± 5.4*	34.8 ± 2.9**
7	Triglycerides (mg/dl)	90.5 ± 0.064	178.6 ±2.9	102.0 ± 0.86*	96.2 ± 1.2*

Data are expressed as mean ± SD, n = 6. *$p < 0.01$Vs control. **$p < 0.05$ Vs control.

The trend of these enzymes to return close to the normal level in group III rats is a perfect sign of protective role of *Solanum xanthocarpum* fruit extract. Concerning liver diseases, the elevation of serum LDH is observed in hypoxic hepatitis due to shock or heart failure[27]. The elevated level of LDH activity in liver damage is mainly because of the enzyme leakage through damaged hepatocyte membranes, increased LDH production could also be attributable to anaerobic conditions[28]. The serum cholesterol and triglycerides levels were elevated in acute inflammatory condition of liver. The over dose of carbon tetrachloride causes an increase in the serum cholesterol and serum triglycerides level[29].

Conclusion

Overall results conclude that ethanolic extract of *Solanum xanthocarpum* fruit has therapeutic and preventive efficacies in CCl_4 induced hepatotoxicity in rats. Hence, it is possible that the mechanism of hepatoprotection by *Solanum xanthocarpum* may be due to its antioxidant action. Further studies are recommended to elucidate the mechanisms of the hepatoprotective action of this plant and identification of its active agent(s).

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