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Hepatoprotective Activity of *Citrullus Lanatus* Seed Oil on CCl_4 Induced Liver Damage in Rats

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Research Article

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Abstract – The present study aimed evaluate the protective effect of *citrullus lanatus* seed oil against CCl4 induced hepatic damage in rat. The hepatoprotective was on carbon tetrachloride induced hepatotoxicity in rats by estimated serum hepatic enzyme levels and hisopathological study of liver tissues. *Citrullus lanatus* seed oil ; CLSO (125mg) and CLSO(250mg) were administered orally for 10 days in rats and compared with standard silymarin (100 mg/kg) orally. The results showed significant decrease in serum ALT, AST and ALP levels treated groups which were increased due to CCl_4 induced liver damage are comparable with standard drug. Histopathological study of liver tissue ravels the hepatoprotective activity of *Citrullus lanatus* seed oil.

Keywords – *Citrullus lanatus* seed oil, CCl_4induced liver damage, serum enzyme levels, Histopathology.

INTRODUCTION

Citrullus lanatus of family Cucurbitaceae is commonly known as water melon and in local name Tarmuz (Hindi), Puchakaya (Telugu). The ripe fruits are edible and largely used for making confectionary. Its nutritive values are also useful to the human health. Fruit is used in cooling, strengthening, aphrodisiac, astringent to the bowels, indigestible, expectorant, diuretic, and stomachic, purifies the blood, allays thirst, cures biliousness, good for sore eyes, scabies and itches and as brain tonic to the brain [1]. It also reported having analgesic and anti-inflammatory activity of roots and leaves [2], antimicrobial activity [3], laxative activity of fruit [4] , anti-oxidant and antiulcerative activity [5].

The liver performs many functions and target organ for toxic drug-induced lesions. The liver transforms and excretes many drugs and toxins. These substances are frequently converted into inactive form by reactions that occur in the hepatocytes. Transformations that occur in the liver that render many drugs water soluble and they readily excreted by the kidneys [6]. The physiological response to injury results such as necrosis, cholestasis, steatosis, inflammation and fibrosis.

Hepatitis is an autoimmune disorder, produce inflammation in the liver, leads to injury or destruction. In most common hepatitis cases (viral hepatitis), specific viruses incite the immune system to fight off infections. Specific immune factors become over-produced that cause injury. Hepatitis caused by drugs, alcohols, chemicals and environmental toxins. CCl_4 is chemical which induce hepatotoxicity through lipid peroxidation by its free radical derivative (CCl_3, CCl_3O_2). Excessive production of the reactive species manifests in tissuethiol depletion, lipid peroxidation, plasma membrane damage etc., culminating into severe hepatic injuryoxic [7].

Carbontetrachloride toxicity begins with the change in endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures. The toxic metabolite CCl_3 radical is produced by microsomal oxidase system binds covalently to the macromolecule and causes peroxidative degradation of lipid membrane of the adipose tissue. [8] Results of this hepatotoxicity increase the serum enzyme levels such as aspartate aminotransferase, alanine aminotransferase and alkaline phosphate.

Present study was conducted to evaluate the protective effect of *citrullus lanatus* seed oil against CCl4 induced hepatic damage in rat.

MATERIAL AND METHODS

Collection and extraction of oil

The seeds of Citrullus lanatus of family Cucurbitaceae were collected from ripe fruits which were obtained from local fruit market, Kodad, Andhra Pradesh. The seeds collected from fruit and dried and extracted with n-hexane to obtain the oil. The percentage yield was 21.59 % w/w.

Physiochemical studies of oil

The oil obtained from water melon seed were tested for qualitative tests for oraganoleptic characters, solubility, specific gravity, refractive index, saponification value, iodine value and chemical tests for oils.

Drugs and Chemicals

Silymarin (Allied FabriChem Private limited, Hyderabad) used as the standard hepatoprotective

drug, Hexane and Carbon tetra chloride (Sd. Fine Chemicals, Mumbai) were obtained from from the institute store and are analytical grade. SGOT, SGPT and ALP enzyme kit (Span diagnostics limited, Surat) were purchased.

Animals

Rats of either sex weighing 150-200 g were used in experiment. Animals were obtained from Anurag Pharmacy College, Kodad. Animals were kept under standard conditions at 23-25^{0}C 12 hr light/dark cycle and given standard pellet diet and water. Before performing the experiment the ethical clearance was obtained from institutional animal ethics committee (IEAC). IAEC No.-177/99/CPCSEA.

Acute oral toxicity studies

Acute oral toxicity study was carried out for n-hexane extracted Citrullus lanatus Seed oil (CLSO) using Acute Toxic Class Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No. 423 in Female Wister rats.

Evaluation of Hepatoprotective activity

For evaluation of hepatoprotective activity of the first day, all animals were randomly divided into five groups of six animals each. Each group of animals were treated with respective vehicles or drugs for 10 days, after 30minutes post dose administration all groups(except group-1 normal) were received CCl_4 at the dose of 1.5ml/kg(1:1 v/v of CCl_4 in olive oil)orally to induced liver damage[10-11].

Group I: Normal(2% Tween80,P.O) for 10days
Group II: Control(2%Tween80, P.O for 10days)with Ccl4 on 10thday
Group III: CLSO (125mg/Kg, P.O for 10days) with Ccl4 on 10thday
Group IV: CLSO (250mg/Kg, P.O for 10days) with Ccl4 on 10thday
Group V: Silymarin (100mg/kg, P.O for 10days) with Ccl4 on 10thday

On 11th day (after 24 hr of CCl_4 administration), the blood samples were collected by retro-orbital puncture from each animal for estimation of hepatic enzyme levels. Blood samples were centrifuged for 15 mins at 3000rpm to separate the serum. Alkaline phosphates (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST) were estimated using standard kits.

Histopathological studies

On the 11th Day, after sacrification of rats by cervical dislocation, liver samples were dissected out and washed immediately with ice-cold saline to remove as much blood as possible. A portion of liver tissue in each group was preserved in 10% formaldehyde solution for histopathological studies.

Haematoxylin and eosin were used for staining and later the microscopic slides of the liver tissue were photographed at magnification 40X.

Statistical Analysis

The statistical analysis was carried by one way ANOVA followed by Dunnet's multiple "t" test. P values < 0.05 (95% confidence limit) was considered statistically significant, using software Graph Pad Prism5.

RESULTS AND DISCUSSION

Preliminary Physicochemical Screening

The CLSO was screened for various Physicochemical test as per the reported methods and found the oil as golden yellow colour, having pungent smell and soluble in organic solvent such as ethanol. It was found of Specific gravity 0.925 at 25^{o}C, refractive index 1.46 at 25^{0}C Saponifiacation value 168.5, iodine value 121.3 and. chemical tests confirms the presence of oil, terpenoids, and phenolic compounds.

Acute oral toxicity studies

The oil was screened for toxicity by oral toxicity studies according to OECD guidelines 423 taking three female wister rats with starting dose of 2000mg/kg body weight. The Citrullus lanatus seed oil was safe up to a dose of 2,000 mg/kg body weight and found in category of class-V, LD_{50} was calculated and LD_{50} was found 2500mg/kg body weight.

Evaluation of Hepatoprotective activity

Hepatoprotective activity of *Citrullus lanatus* Seed oil were evaluated on carbon tetrachloride induced hepatotoxicity in rats by estimated serum hepatic enzyme levels. The results are given in Table-1.

Table-1: Effect of *Citrullus lanatus* Seed oil on carbon tetrachloride induced hepatotoxicity in rats

Treated groups	Hepatic enzyme levels		
	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal	223.2± 5.212	75.67± 3.556	65.33± 5.506
Control(CCl4)	300.3±5.783[a]***	252.0±5.842 [a] ***	111.5± 4.217 [a] ***
CLSO125mg	278.3±5.226 [b]*	221.5±6.495 [b] **	97.00± 5.745 [b ns]
CLSO 250 mg	274.2±5.718 [b] **	215.3±4.394 [b] ***	82.67± 6.761 [b] **
Silymarin 100mg	235.5±4.836 [b] ***	116.7±4.807 [b] ***	75.33± 5.481 [b] ***

Values are in Mean ± S.E.M (n=6); [ns] -Non Significant, *p<0.05, **p<0.01, ***p<0.001
[a] Control compared with Normal, [b] All test groups compared with Control using One way ANOVA followed by Dunnet's "t" test.

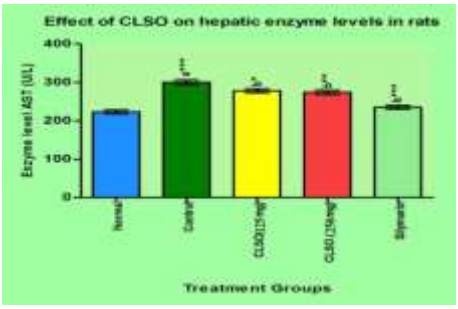

Fig-1: (a)

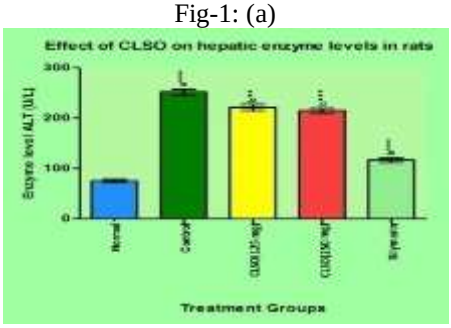

Fig-1: (b)

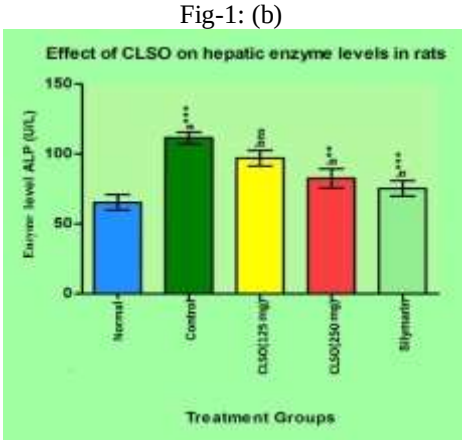

Fig-1: (C)

Fig-1: (a):Effect of *Citrullus lanatus* Seed oil serum on AST enzyme level , (b): Effect of *Citrullus lanatus* Seed oil serum on ALT enzyme level and (c): Effect of *Citrullus lanatus* Seed oil serum on ALP enzyme level on carbon tetrachloride induced hepatotoxicity in rats.

Hepatic cells appear to participate in a variety of enzymatic metabolic activities and administration of carbon tetrachloride (CCl_4) damages hepatic cells and elevate serum level of AST, ALT, ALP and bilirubin significantly. There was significant ($p<0.001$) increase in hepatic enzyme levels were observed in control group and the drug treated animals with CLSO (125mg) and CLSO(250mg) showed reduction in serum enzyme levels and are comparable with standard silymarin.

The results of Histopathological studies of control rat liver treated with carbon tetrachloride exhibited severe necrosis with disappearance of hepatocytes, areas of inflammation and increased sinusoidal spaces. Liver section of the rat treated with 125mg of CLSO and carbon tetrachloride exhibited mild degree of necrosis, normalization of cells and reduced sinusoidal dilation. Liver section of the rat treated with 250mg of CLSO and carbontetrachloride exhibited normalization of cells and reduced sinusoidal dilation along with mild inflammogens. Liver section of the rat treated with Silymarin and CCl_4 exhibited normal hepatocytes. The results are given in Fig-2(a), Fig-2(b), Fig-2(c) and Fig-2(d) respectably.

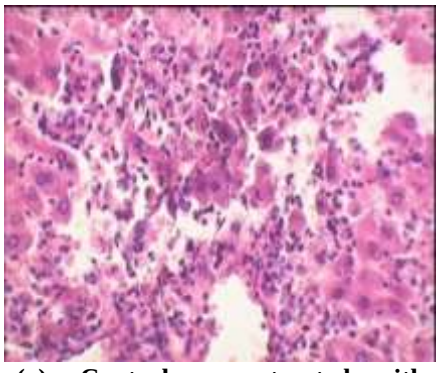

Fig-2 (a): Control group-treated with CCl_4 exhibited severe necrosis with disappearance of hepatocytes, areas of inflammation and increased sinusoidal spaces.

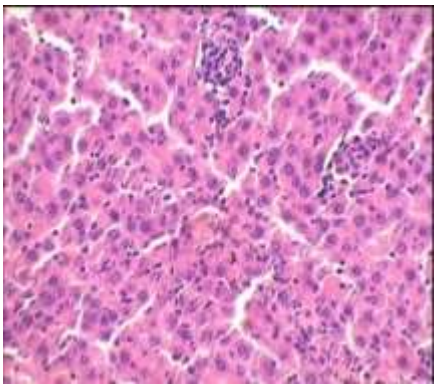

Fig-2 (b): CLSO 125mg/kg- exhibited mild degree of necrosis, normalization of cells and reduced sinusoidal dilation.

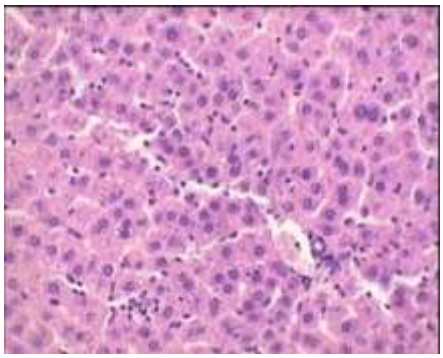

Fig-2 (c): CLSO 250mg/kg- exhibited normalization of cells and reduced sinusoidal dilation along with mild inflammogens.

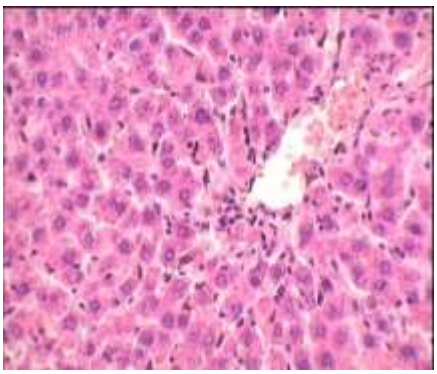

Fig-2 (d): Silymarin 100mg/kg- exhibited normalization of cells and almost exhibited normal hepatocytes.

CONCLUSION

Present study conclude that Citrullus lanatus seed oil posses hepatoprotective activity and presence of phytoconstituents like terpernoids and phenolic compunds in seed oil as reported previously. Further research work is under process for separation of components from *Citrullus lanatus* seed oil to understand the active phytoconstituents.

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