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RESEARCH ARTICLE

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC SEED EXTRACT *Cicer arietinum* AGAINST CCL_4 INDUCED HEPATOTOXICITY ALBINO RATS

***S. DIVYA TEJA.BANDA[1], K.SANTHOSHI[1], V.RAVI KUMAR[2]**

*1, 2. Department of Pharmacology, Sri Sai Aditya Institute of Pharmaceutical Sciences & Research, Surampalem, East Godavari Dist. Andhra Pradesh, India.

1. Albino research and Training Institute, Bachupally, Hyderabad, India.

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ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of ethanolic extract of *Cicer arietinum* seeds against CCl_4 induced albino rats. The ethanolic extract of *Cicer arietinum* was administered orally to the animals with hepatotoxicity induced by CCl_4 (0.5ml/kg i.p. with Arachis oil). Silymarin (100 mg/kg) was used in the present study as standard drug for one group. The two doses of Ethanolic seed extract of *Cicer arietinum* (250kg/kg and 500mg/kg p.o) were used in this evaluation. The plant extract was effective in protecting the liver against the injury induced by CCl_4 in rats. This was evident from significant reduction in serum glutamic-pyruvic transaminase (SGPT), serum glutamic oxalo acetic transaminase (SGOT), alkaline phosphatase (ALP) and bilirubin. It was concluded from the result that the hepatoprotective activity of ethanolic extract of *Cicer arietinum* seeds against CCl_4 induced hepatotoxicity in rats.

Key words: *Cicer arietinum,* CCl_4, hepatoprotetive, hepatotoxicity, Silymarin.

INTRODUCTION:

It is useful to divide the mechanism of CCl4 into the following sequence.a) Initial events b) Secondary evoked mechanism c) End stage pathological consequences.

The initial event involves carbon-halogen bond cleavage, probably by a one-electron reduction of CCl_4, by a particular ferrous cytochrome P-450, to form chloride anion and trichloromethyl radical (CCl_3). In next stage, CCl_4-carbon is covalently bound to microsomal lipids and proteins. This placed CCl_4 into a general class of xenobiotics, the toxicity of which appears to depend on their metabolism and subsequent covalent bindings to cellular macromolecules. Protein synthesis could not take place as the specific binding site is already occupied by cytochrome P-450 induced free radicals[1].The per-oxidative decomposition of lipids of the endoplasmic reticulum (ER) initiated by CCl_4 metabolism. Lipid peroxidation generates a wide variety of more or less toxic products, not organic radicals, which presumably could migrate from membrane sites near cytochrome P-450 to the other parts of the cell.

Cicer arietinum is an erect or spreading much branched annual herb, covered all over with glandular hair, extensively cultivated. Whole gram contains saccharose, glucose, fructose, polysaccharides including starch, g-galactan, laevulose and p-galactoarban, beta in, choline, adenine, inositol, phytin, saponin and citric and oxalic acids. The fresh whole germ of sprouting gram contains biochanin A, biochanin B and biochanin C. In the present study we have evaluated the hepatoprotective activity of *Cicer arietinum* against the hepatotoxicity induced rats.

MATERIALS AND METHODS:

Plant material and preparation of extract[2]:

The seeds of *Cicer arietinum* (organic variety) were collected from the market, Miyapur, Hyderabad. The seeds of *Cicer arietinum* were pulverized into fine powder along with the husk and used for extraction. The powdered dried seeds were loaded into the soxhlet extractor and subjected to extraction with 99% ethanol for 18 hrs. at 72^0 C. After extraction the solvent was distilled off and the extract was concentrated to dryness at room temperature.

Drugs and chemicals:

Ethano l(merck specialities private ltd, Mumbai), Paracetmol (granules india ltd, gandi misemma, hyderabad),Silymarin-silybon-70 (hetero pharmacy, Kukatpally, hyderabad).Diagnostics kits- cogent clinical chemistry division of span diagnostics ltd, Gujarat.Gum acacia, Carbon tetrachloride, arachis oil, Formalin- S D

*Corresponding author: S. Divya Teja.Banda | Email: tej.pharma01@gmail.com

fine chem. Limited, Mumbai. Distilled water- central drug house (p) ltd, New Delhi.

Experimental animals:

Albino rats of either sex of weighed between 150-250 gms were taken from the Animal House of Albino research training Institute, Bachupally, HYD-90. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30-70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pellet diet from NIN, Hyd. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC and CPCSEA.

Experimental:

Acute toxicity studies:

Acute oral toxicity studies[3] of the extracts were carried out as per the OECD guidelines, draft guidelines 423 adopted and received from Committee for the Purpose of Supervision and Control of Experiments on Animals (CPCSEA), Ministry of social justice and empowerment, Government of India.

Each of the seed extracts of *Cicer arietinum*, up to a higher dose of 2gm/kg were administered orally to normal rats. During the first four hours after the administration the animals were observed for gross behavioral changes. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia, body weight and mortality were observed up to 48 days. No mortality was observed with oral administration of the extract even at the highest dose of 2gm/kg p.o. The results were mentioned in table no.1.

Hepatoprotective activity:

Wistar albino Rats (180-250g) were used. All the animals were divided into the five groups each group consisting of 6 rats and they received the treatment as follows.

Group I :Vehicle Control received distilled water (10ml/kg p.o.)

Group II: Animals Received CCl_4 (0.5ml/kg i.p. with Arachis oil)

Group III: Animals received Silymarin (100mg/kg p.o.).

Group IV: Animals received Ethanolic extract of *Cicer arietinum* (250mg/kg p.o).

Group V: Animals received Ethanolic extract of *Cicer arietinum* (500mg/kg p.o).

Ethanolic extract of *Cicer arietinum* and vehicles (in distilled water) were administered orally for 7 days. Hepatotoxicity was induced in group IInd, IIIrd, IVth, and Vth, by an injection of CCl_4 (0.5 ml/kg, 1:1 with Arachis oil i.p.) on one day before the dosing of test and standard drugs[4]. On the 8th day blood sample from all Groups of rats were obtained by puncturing retro-orbital plexus. Serum was separated by centrifugation at 3000 rpm at room temperature for 20 min and subjected to biochemical estimations viz. SGPT, SGOT, ALP, Total Bilirubin. The results were mentioned in table no.3The livers of all animals were removed and weiged.Then processed for Histopathological investigations after 24 hrs of biochemical estimation [5].

Statistical analysis:

All the values were expressed as mean ± SEM (standard error mean) for six rats. Statistical analysis was carried out by using PRISM software package (version 5.0). Statistical significance of differences between the control and experimental groups was assessed by One-way ANOVA followed by Dunnett's Multiple Comparison Test. The value of probability, $P \leq 0.05$, $P<0.01$, $P<0.001$ were considered statistically significant.

RESULTS:

The results of body weight and liver weight are shown in Table 2. The results of Hepatoprotective activity of ethanolic extract of *Cicer arietinum* on CCl_4 treated rats are shown in Table3. The hepatic enzymes SGPT, SGOT, ALP, Total Bilirubin in serum were significantly ($P<0.001$) increased in CCL_4 treated animals when compared to normal group. Silymarin (100 mg/kg) treated animals also showed significant decrease in AST[6] ($P<0.01$), ALT[6], ALP[7]and bilirubin ($P<0.001$) levels when compared to CCl_4 treated rats.

DICUSSION:

In recent years, many studies have been undertaken with traditional medicines; in an attempt to develop new drugs for hepatitis[8] .CCl_4 is one of the most important and commonly used hepatotoxic agents in the experimental procedures[9] and it causes liver damage similar to the acute viral hepatitis[10] finally resulting in cell necrosis and consequent cell death[11].The leakage of large quantities of enzymes into the blood stream is often associated with massive necrosis of the liver [12] .The Hepatoprotective potential of ethanolic extract of *Cicer arietinum* evaluated in CCl_4 induced Hepatotoxicity. In this model ethanolic extract of *Cicer arietinum* was administered for 7 days orally 24hrs after single administration of the Hepatotoxicant. The potency and efficacy of ethanolic extract of *Cicer arietinum* was evaluated by measuring plasma biochemical parameters and histopathology of

liver tissue. In CCl_4 induced Hepatotoxicity ethanolic extract of *Cicer arietinum* at a dose of 500mg/kg showed significant reduction in the elevated plasma SGPT,SGOT,ALP,Total Bilirubin confirms the Hepatoprotective effect of ethanolic extract of *Cicer arietinum* at 500mg/kg and also the Histopathological findings showed mild cloud swelling with intact nucleus and nucleolus and reversal of centrilobular necrosis , although ethanolic extract of *Cicer arietinum* 250mg/kg showed reduction in the levels of SGPT ,SGOT , ALP, Total Bilirubin but it was lesser than the 500mg/kg.

Table 1: Effect of ethanolic extract of *Cicer arietinum* in acute toxicity for 48 hrs.

DOSE	NO OF RATS/NO.OF MORATLITY			
	6hr	12hr	24hr	48hr
250mg/kg	6/0	6/0	6/0	6/0
500mg/kg	6/0	6/0	6/0	6/0
750mg/kg	6/0	6/0	6/0	6/0
1000mg/kg	6/0	6/0	6/0	6/0
2000mg/kg	6/0	6/0	6/0	6/0

Table 2: Effect of ethanolic extract of *Cicer arietinum* on body weight and liver weight of rats against CCl_4 induced toxicity.

Group	Treatment	Body weight (g)	Liver weight (g)
Group I	Normal	225.5±11.5	6.73±0.61
Group II	Control (CCl_4 0.5ml/kg i.p. with Arachis oil)	201.0±12.1	11.02±1.21
Group III	Standard Silymarin (100mg/kg p.o.).)$_+$ CCl_4	238.5±13.5***	7.15±1.2***
Group IV	Test-1 (250mg/kg)$_+$ CCl_4	211.7±13.1*	9.2±0.1*
Group V	Test-2 (500mg/kg)$_+$ CCl_4	225.3±9.41**	8.4±0.2**

Values are expressed as Mean ± SEM, (n=6),*P<0.05, ** P<0.01, *** P<0.001

Table 3 Hepatoprotective activity of Ethanolic Extract of *Cicer arietinum* against Carbon Tetrachloride (CCl_4) induced Hepatotoxicity after 7 days.

TREATMENT	SGPT(IU/L)	SGOT(IU/L)	ALP(IU/L)	TOTAL BILIRUBIN(mg/Dl)
I. Normal	64.6±5.16	40.20±4.20	150.3±5.8	0.47±.03
II.Control (CCl_4 0.5ml/kg i.p. with Arachis oil)	241.3±7.8	185.6±10.1	326.1±8.9	1.96±.03
III.Standard Silymarin (100mg/kg p.o.). $_+$ CCl_4	82.6±4.5***	55.5±5.6**	183.33±8.07***	0.67±.031***
IV.Test-1 (250mg/kg)$_+$ CCl_4	122.1±5.8***	107.1±8.5***	230.33±7.4***	0.81±.04**
V. Test-2 (500mg/kg)$_+$ CCl_4	96.0±5.6***	80.5±10.3***	211.6±7.9***	0.71±.05***

Values are expressed as Mean ± SEM, (n=6),*P<0.05, ** P<0.01, *** P<0.001

HISTOPATHOLOGICAL CHANGES OF LIVER IN CCL_4 MODEL

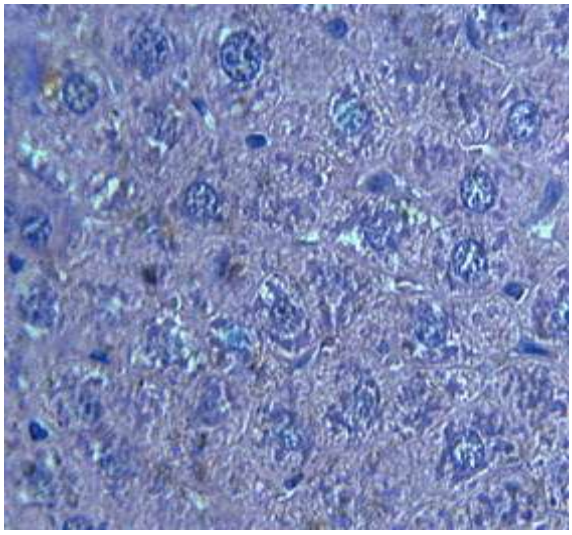

Fig.1 Normal control liver

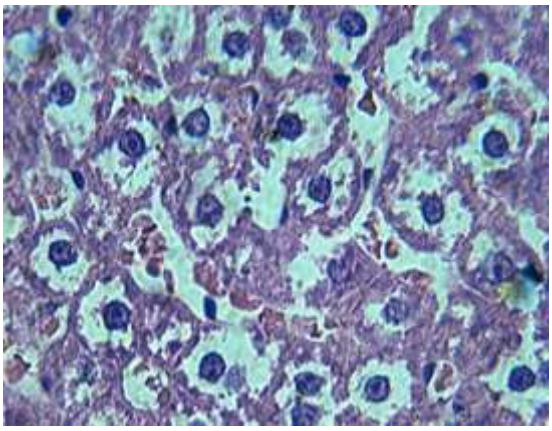

Fig.2 CCl_4 treated rat

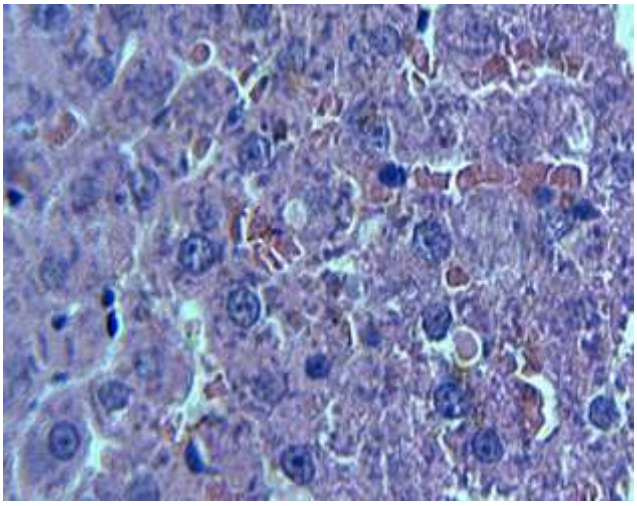

Fig.3 SILYMARIN treated rat

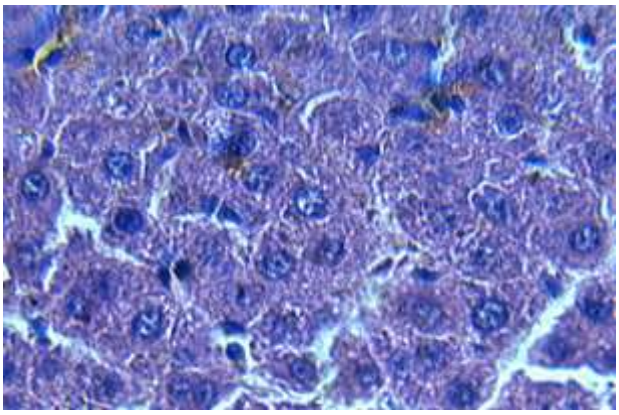

Fig.4 EECA 250mg/kg treated rat

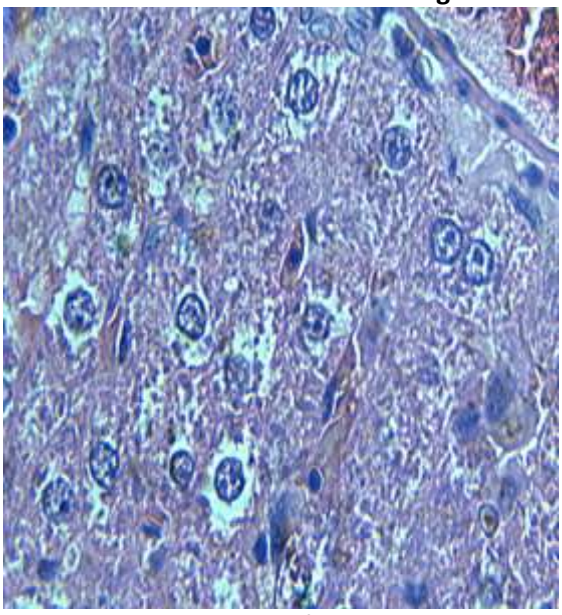

Fig.5 EECA 500mg/kg treated rat

1. Normal control liver has showed normal structure and architecture of liver.
2. CCl_4 treated rat has showed an enlarged hepatocyte with vacuoles. Kupffer cells are compressed with centrilobular necrosis in parenchyma and collection of lymphocytes.
3. SILYMARIN has treated rat showed intact architecture of liver with few vacuoles in cytoplasm .central veins exhibited normal architecture.
4. EECA 250mg/kg treated rat has showed normal central vein with few vacuoles in cytoplasm. Kupffer cells compressed with areas of necrosis.
5. EECA 500mg/kg treated rat has showed normal architecture of liver with reversal of liver centrilobular necrosis with intact nucleus .moderate vacuoles present in cytoplasm.

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