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HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITIES OF AREAL PARTS (EXCEPT FRUITS) OF CICER ARIETINUM AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The Hepatoprotective and antioxidant activity of pet ether, methanol and aqueous extracts of aerial parts (except fruites) of *Cicer arietinum* L against ccl_4 induced hepatotoxicity in rats. The phytochemical investigations of these extracts showed presence of carbohydrates, proteins, phenols and flavanoids. LD_{50} values of all extracts were determined. The extracts did not produce any mortality even at 2000 mg/kg. Hepatotoxicity was induced in wister rats weighing 120-150 gm by oral administration of carbon tetra chloride (CCL_4 1ml/kg/day 20days) in 1ml olive oil per day. The plant extracts were administered to the experimental rats (200 and 400 mg/kg/d p.o for 20days). The Hepatoprotective and antioxidant activity of these extracts was evaluated by liver function biochemical parameters (serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, serum alkaline phosphatase, total bilirubin, lipid peroxidation, superoxide dismutase, catalase, reduced glutathione) and histopathological studies of liver. Pre-treatment of the rats with petroleum ether, methanol and aqueous extract prior to CCL_4 administration caused a significant reduction in the values of SGOT, SGPT, SALP, LPO, total bilirubin and significant increase in SOD, CAT, GSH ($P<0.01$) almost comparable to the silymarin. The hepatoprotective activity was confirmed by histopathological examination of the liver tissue of control and treated animals. Histology of liver sections of the animals treated with the extracts showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration which further evidence the hepatoprotective activity.

Key words: Hepatoprotective activity, Cicer arietinum, L. Carbon tetrachloride (CCl_4), silymarin.

INTRODUCTION

Cicer arietinum, L (Fabaceae) known as chickpea is a well-known plant in the Indian traditional system of medicine and distributed throughout India. The roots are used in treatment of diarrhea, antibacterial and antipyretic activity. [1, 2, 3] The aerial parts and leaves are used as antifungal agents. The seeds are used as anti-inflammatory agents [4]; some important chemical constituents include carbohydrates, tannins, glycosides, proteins, flavanoids and phenols. The seeds of the plant are reported to have in vitro free radical scavenging activity [5], anti inflammatory, anti bacterial and anti fungal activity. The roots of the plant were reported to have antidiarrhoeal activity. Now a days a large number of research work is doing for the efficacy of herbal drugs used in traditional medicine. Modern medicine does not have suitable answers for many conditions such as liver injury, vital organ disorders etc. Liver is the heaviest gland almost completely covered by visceral peritoneum of the body. It plays a major role in metabolism and excretion of the drugs. Around 29 million people in the European union are suffering with chronic liver diseases. Carbon tetrachloride hepatotoxicity is caused by its reactive active metabolite trichloromethane radical (ccl_3^*) bind covalently to the macromolecules and induce lipid peroxidation and

forms lipid peroxides which produce damage to the liver membrane.

MATERIALS AND METHOD

Collection and authentication of plant material: The Plant *Cicer arietinum* was collected during February to March from different region of moinabad, Ranga reddy and authenticated by Dr. Prathibha Devi, Head, Department of Botany, Osmania University, Hyderabad.

Preparation of extracts

Successive solvent extraction: The collected plant was dried under shade, coarsely powdered and processed for successive solvent extraction. The method is based on the extraction of active constituents present in the plant powder using solvents ranging from non polar through polar. The solvents used are petroleum ether, methanol and water.

Method of extraction: using maceration process for 48-72 hrs. The extracts were concentrated with the help of Rotary flash evaporator (Popular India Ltd)

Phytochemical Screening: The extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Animals: All the animal experiments were conducted by the approval of Institutional Animal Ethics Committee **(reg. no- 1349/AC/10/CPCSEA).** During the study period, guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animal Ethics Committee (IAEC) were followed for the maintenance of animals.

Acute toxicity studies: The acute toxicity of the extracts was determined in albino mice, maintained under standard conditions.

Evaluation of anti oxidant and Hepatoprotective activity:

Group - I served as a control and was dosed orally with vehicle i.e. CMC (1ml of 1%w/v) per day, Group - II received an oral dose of CCl_4 (1ml/kg b.w.) in 1ml olive oil per day, Group - III received an oral dose of silymarin (50mg/kg b.w.) along with CCl_4 (1ml/kg b.w.), Group - IV &V received an oral dose of (200, 400mg/kg b.w.) petroleum ether extract per day respectively along with CCl_4 (1ml/kg b.w.), Group - VI & VII received an oral dose of (200, 400mg/kg b.w.) methanol extract per day respectively along with CCl_4 (1ml/kg b.w.), Group - VIII & IX received an oral dose of (200, 400mg/kg b.w) aqueous extract per day respectively along with CCl_4 (1ml/kg b.w.). All groups were administered with their respective drugs for a period of 20 days.

Assessment of hepatoprotective activity:

All animals were killed on day 20 under light ether anesthesia. The blood samples were collected separately into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and biochemical investigations were carried out to assess liver function viz., glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT), serum alkaline phosphatase (sALP), serum bilirubin (total bilirubin)

Anti oxidant enzymes:

The dissected liver tissues were washed with 0.9% saline and homogenate in 5% ice cold phosphate buffer, and then centrifuged at 1000 rpm for 10 min, followed by centrifugation of the supernatant at 1200rpm for 15 min to get the mitochondrial fractions or enzyme extracts viz., superoxide dismutase (SOD),catalase(CAT)[8], reduced glutathione (GSH)[8] ,lipid peroxidation (LP).

Data analysis:

Statistical analysis

Results were subjected to one-way ANOVA. $P<0.05$ was considered significant. The post hoc analysis was carried out by Tukey's multiple comparison tests.

Histopathology:

The liver tissue was dissected out for histological investigation and fixed in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene, and embedded in paraffin. Sections were prepared and then stained with hemotoxylin and eosin (H-E) dye for photo microscopic observation.[7]

RESULTS

Preliminary Phytochemical screening:

All the three extracts obtained from successive solvent extraction were subjected to qualitative chemical evaluation to detect the chemical constituents present in them. Petroleum ether extract revealed the presence of phytosterols. The Methanolic extract shows the presence of carbohydrates, glycosides, saponins, proteins and amino acids, flavanoids, phenolic compounds and tannins. The aqueous extract shows the positive reactions to carbohydrates, glycosides, saponins, amino acids, proteins, phenolic compounds, tannins, and flavanoids. The results are given in table no:1

Acute Toxicity study:
In the present study the three extracts of *Cicer arietinum* L. whole plant except seeds and seed coats were subjected for acute toxicity studies. The animals were fasted overnight prior to the experiment. Fixed dose 500 mg/kg, 1000 mg/kg, 1500 mg/kg, 2000 mg/kg and 3000mg/kg of (OCED Guideline No. 420) method of CPCSEA was adopted for toxicity studies. Neither toxicity substance nor mortality was observed up to a base level of 2000 mg/kg body weight. Hence 1/5th (400 mg/kg) and 1/10th (200 mg/kg) were selected for pharmacological studies.

Biochemical Parameters:
The effect of *Cicer arietinum* on Ccl_4 induced liver damage in rats with reference to biochemical changes in serum is shown in Table-2. The Ccl_4 treated group showed a significant increase in serum SGOT, serum SGPT, serum total bilirubin, indicated the liver injury caused by Ccl_4. Animals treated with high doses of methanol and aqouse extracts shows more significant effect compared with low doses (200mg/kg).The results are given in table no:2

In vivo Antioxidant Studies:
Animals treated with Ccl_4 showed a significant decrease in superoxide dismutase, catalase, GSH, increased MDA levels. The pet.ether group treated with (200mg/kg, p.o, once daily) showed no significance increase in catalase, GSH, SOD and no significant decrease in MDA levels. Whereas group treated with low doses of methanol and aqueous extracts (200mg/kg) showed a significant increase in the catalase, GSH, GST also a significant decrease in MDA. Whereas higher doses of methanol and aqouse extracts (200mg/kg) showed a more significant increase in the catalase, GSH, GST also a more significant decrease in MDA. All these results were compared with a group of animals treated with a positive control group and results are given Table-3.

Histopathological Studies:
The hepatoprotective effect of ***Cicer arietinum* L** was further confirmed by histopathological examination of liver samples from the respective groups. The Histological architecture of CCl4 treated liver sections showed in figure-1.

DISCUSSION

The present study indicated that the high dose of MECA and AECA (400mg/kg) provided significant protection against CCl_4 induced liver damage. The CCl_4 is metabolized by the cytochrome P_{450} system to produce trichloromethyl free radicals, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation leads to altered in functional integrety of hepatic mitochandria leads to hepatic damage. During hepatic damage there is increase in levels of liver enzymes.[9] The phyto constituents present in the extract have the ability to induce microsomal enzymes either by accelerating the excretion of CCl_4 or by inhibition of lipid per oxidation induced by CCl_4 . In the present study, the plant drug was found to be having phyto constituents like tannins, glycosides, proteins, flavanoids and phenols. These phyto constituents were proved of having hepatoprotective activity earlier[10].
The serum SGOT, ALP, SGPT and total bilirubin activities were used as a marker of liver damage. In the present study administration of high dose of MECA (400mg/kg, P.o, once daily) to CCl4 treated rats showed a significant decrease in serum SGOT, ALP, SGPT, TOTAL BILIRUBIN. While the low dose administration of the MECA, AECA extract (200mg/kg, P.o, once daily) did not show any effect on serum SGOT, ALP, SGPT and total bilirubin. The administration of higher dose of AECA showed significant and dose dependent hepatoprotective activity which was compared to the positive control group.

Oxidative stress induced due to the generation of free radicals and decreased antioxidant level in the target cells and tissues has been suggested to play an important role in carcinogenesis.[11] During cell membrane damage, various enzymes leak down to the circulatory fluid and their assessment in serum serves as markers in clinical studies. Scavenging of the free radicals is one of the major antioxidant mechanisms to inhibit lipid peroxidation. Reduced lipid peroxidation was observed by a significant decrease in MDA levels in groups pretreated with standard drug and high dose of extracts simultaneously with a significant elevation in GSH, SOD and CAT activities. In the present study, increase in catalase and reduced GSH activities and decrease in MDA levels were observed in rats treated with silymarin and *Cicer arietinum* indicated that silymarin and *Cicer arietinum* have an antioxidant and hepatoprotective property.

Table 1: Phytochemical investigations of *cicer arietinum* L. extracts.

Test	Pet Ether extract	Methanolic extract	Aqueous extract
Alkaloids			
Dragendorff's Test	-	+	-
Hager's Test	-	+	-
Glycosides			
Borntrager's test	-	+	+
Balget Test	-	+	+
Saponins			
Froth's Test	-	+	+
Phenolics and tannins			
Ferric chloride test	-	-	+
Gelatin test	-	+	+
Proteins & amino acids			
Millons Test	+	+	-
Biuret test	+	+	+
Carbohydrates			
Molisch's test	-	+	+
Benedict's Test		+	+
Flavones and Flavonoids			
Shinoda test	-	+	+

Table 2: Effect of *cicer arietinum* L. extracts on liver parameters in CCl_4 induced hepatotoxic rats.

Group	Treatment	Dose (mg/kg)	SGPT (U/L)	SGOT (U/L)	ALP(KA units)	Total bilrubin (mg/dl)
I	Normal control CMC 1%	1ml	25.11± 0.62	52.81± 0.62	128.26± 8.51	0.37± 0.35
II	Disease Control:CCl_4	1ml	212.50± 1.17^{+++}	245.40± 0.61^{++}	326.32± 8.89^{++}	2.14± 0.21^{+++}
III	Standard (silymarin)	50	52.35±2.41***	48.32±2.49***	162.61± .64***	0.74± .03***
IV	PECA low dose	200	198.63 ± 1.36*	181.71 ± 1.71*	286.68± 7.33*	1.59± 0.31**
V	PECA high dose	400	112.61 ± 2.13**	125.69 ±0.45**	225.38± 5.32**	1.38± 0.03**
VI	MECA low dose	200	124.91 ± 3.15*	112.76 ±1.91*	193.81± 8.62*	1.1± 0.31*
VII	MECA high dose	400	75.33 ±2.61***	90.81±1.83***	158.65± .21***	0.91± 0.02***
VIII	AECA low dose	200	106.61 ±2.96*	92.69 ± 1.35*	196.35± 6.32**	1.02± 0.29*
IX	AECA high dose	400	67.63 ± 1.96***	72.61± 1.65**	171.52± .32***	0.98± 0.06**

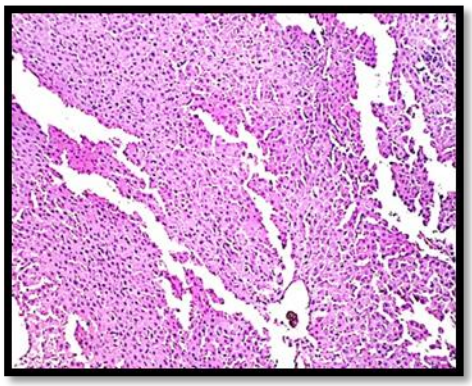
The values were expressed Mean ±SEM

*** - P<0.001, ** - P< 0.01, * - P<0.05 Vs disease control; $^{+++}$ - P<0.001, $^{++}$ - P< 0.01, $^{+}$ - P<0.05 Vs Normal control

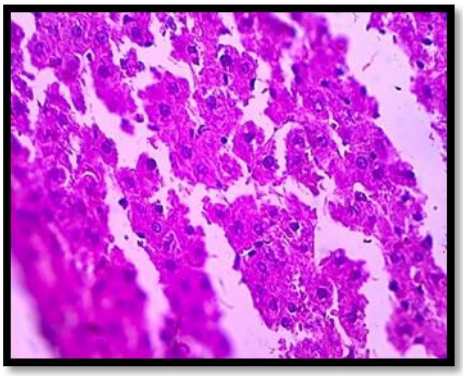
Table 3: Effect of *cicer arietinum* L. extracts on SOD, CAT,GSH and Lipid peroxidation in CCl_4 induced hepatotoxic rats

Group	Treatment	Dose (mg/kg)	SOD (u/mg of protein)	CAT (uM/min/mg of protein)	GSH (µmol/mg of protein)	LPO
I	Normal control	1ml	23.36±2.68	11±0.03	18.5 ± 0.094	0.43±0.02
II	Disease control CCl_4	1ml	12.24±0.11	1.1±0.01	6.2 ±0.7	2.06±0.34
III	Standard (silymarin)	50	20.41±1.62***	9.5±0.1***	16±0.5***	0.37±0.01***
IV	PECA low dose	200	12.23±1.23*	2.5±0.05	7.5±0.6*	0.81±0.49*
V	PECA high dose	400	13.36±2.01*	3.4±0.2	8.2±0.05	0.79±0.28*
VI	MECA low dose	200	16.25±2.43**	3.8±0.05	10.4±0.3	0.43±0.07**
VII	MECA high dose	400	18.17±2.68***	7.6±0.2**	14.01±0.06**	0.40±0.12***
VIII	AECA low dose	200	14.09±1.34**	3.1±0.06	11.08±0.05	0.49±0.08**
IX	AECA high dose	400	17.43±1.94***	7.7±0.2**	14±0.09**	0.42±0.24***

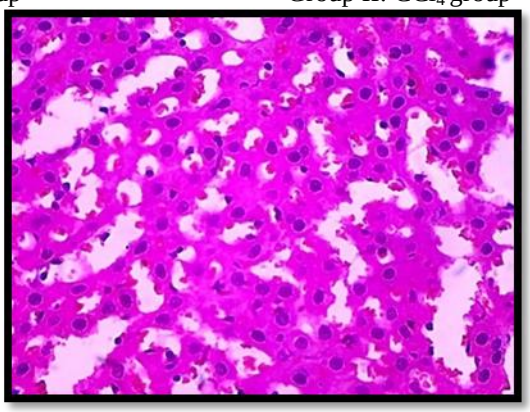
The values were expressed Mean ±SEM; *** - P<0.001, ** - P< 0.01, * - P<0.05 Vs disease control;
$^{+++}$ - P<0.001, $^{++}$ - P< 0.01, $^{+}$ - P<0.05 Vs Normal control



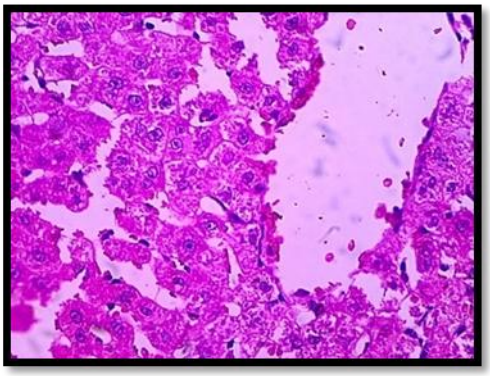
Group I: normal group



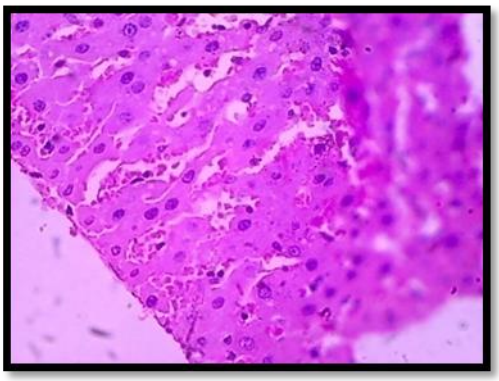
Group II: CCl_4 group



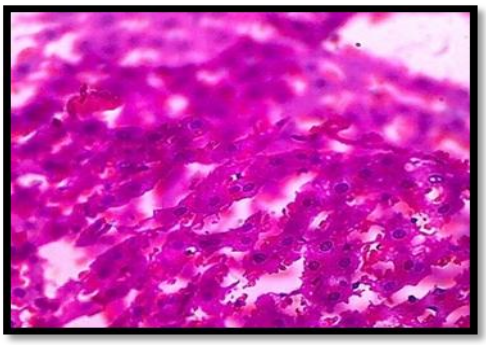
Group III: silymarin group



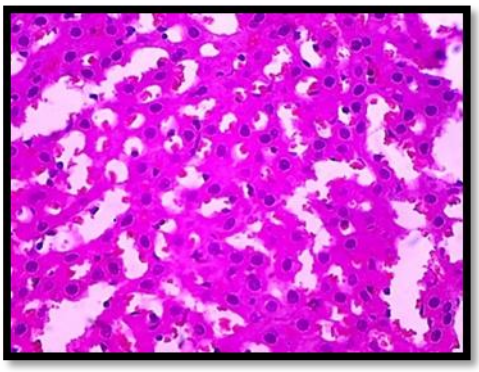
Group IV: PECA low dose group



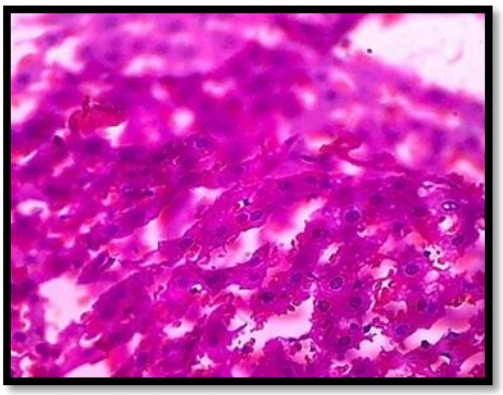
Group II: PECA high group



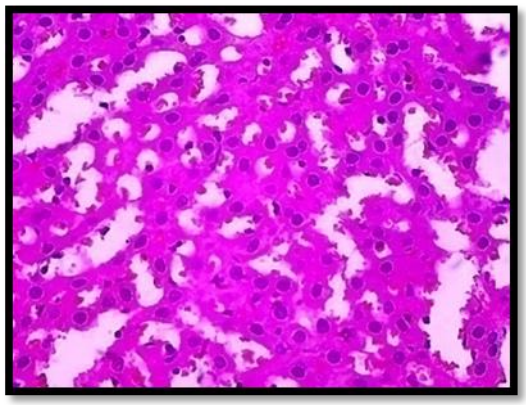
Group VI: MECA low dose group



Group VII: MECA high group



Group VIII: AECA low dose group



Group IX: AECA high group

Figure.No.1 : Histopathological slides of CCl_4 model

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