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Research article

Studies on the hepatoprotective properties of seed coat extract of *Cicer arietinum* species

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Abstract

The present study was done to evaluate the hepatoprotective properties of the seed coat of *cicer arietinum* or Bengal gram in carbon tetra chloride ($CC1_4$) produced liver toxicity in rats. Depending up on the ethano-medico exploration, *Cicer arietinum* species (Desi and kabuli type) were selected and screened for their hapatoprotective activity. The extractions were done using n-hexane, ethanol and water. Hexane and water extracts were not active. Ethanol extract showed hepatoprotective activity. The ethanol extract showed maximum activity than n-hexane and water extract with a dose of 60 mg/kg body weight. The liver protection effect of this extract was compared with a standard reference herbal drug silymarin (100mg/kg). The efficacy of this extract was comparable with the standard drug. Thus, the present work reveals that the ethanolic extract of *cicer arietinum* seed coat (Desi) type is promising for its hepatoprotective property. Therefore further studies can lead to an invention of a hepatoprotective drug.

Key words: Cicer arietinum, Carbon tetra chloride, Hepatoprotection, ALT- Alanine amino transferase, AST-Aspartate amino transferase, ALP-Alkaline phosphatase.

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1. Introduction

The liver is the most important vital organ in the vertebrates [1]. It is involved in the metabolism and excretion of various xenebiotics from the human body. The risk of liver toxicity is increasing day by day because of the increasing exposure to toxic chemicals. Liver problems are very serious problems. Conventional medicines are not satisfactory for the treatment of liver diseases. Herbs play an important role in managing the liver diseases. Therefore development of liver protective herbal medicines is very important and also its need is very urgent [2].

Scientific validation was not done so far for the medicinal plants in the treatment of liver diseases [4]. In folklore medicines so many medicinal plants are available and they are used to treat certain diseases also, but no scientific evidence was there. One such medicine is the seed coat of *cicer arietinum* or Bengal gram seed coat

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belongs to the family *Fabaceac*e. The seed as a whole is used in Ayurveda [7] which is a traditional system of medicine. The seed coat is also used the treat liver diseases. Traditionally Egyptian used the gram to gain weight. The other uses include treatment against cough, headache, sore throat etc. [9]. Pulmonary, anal and uterine problems can be overcome by using boiled gram [8]. Datura mixed with gram was used as laxatives in edema. Face pack can also be prepared using powered gram seed. Powered seed is used for dandruff also. The taste of leaves is sour and it is used as an astringent to the relieve bowels, to improve appetite and used to treat bronchitis. The seed can also be used as a tonic, and an appetizer in the treatment of anthelmintic [10]. It also cures burning. It is also reported that it can be used in skin diseases, bronchitis, leprosy throat complaints and blood troubles.

Mainly leaves are used as purgatives. It is also useful in cold pains, tonic to hair. It is also good for liver diseases, for complaints of the chest, foul mouth, enlargement of spleen etc. In Yunani system of medicine, it is reported that it can be used for fever, inflammation, skin diseases etc. Dysmenorrheal diseases can be cured by using the decotion of the plant. "Vinegar" the exudation acid can be used as an astringent. It is also used as an antidote for snake venom. In Europe the Bengal gram seeds were used for its diuretic and anthelmintic action. The infusion of gram plant is used to prevent the pain of urinary calculi in some parts of India. Presently the investigation is done to evaluate or to determine the effect of seed coat of Bengal gram or *cicerarietinum an*d different extracts (n- hexane, ethanol and water) were done on carbon tetra chloride induced liver damages in rats [2].

2. Experimental

Chemicals and animals

Carbon tetra chloride (CCl_4) was purchased from Nice chemicals, Ernakulam, Kerala. All the chemicals and solvents used were of analytical grade. This was used to determine the toxicity produced by the liver. The experimental protocol was approved by Animal Ethics committee as per CPCSEA guideline.

Plant materials and preparation of extracts

Bengal gram or *cicer arietinum* were collected from the local market of Thiruvalla in the month of August. The specimens were identified and were deposited in the Herbarium. The seeds were dried in the shade. The seed coats were removed carefully and dried under shade and powdered. It was then extracted using n- hexane, ethanol or water [2]. The hexane and ethanol extracts were then dried using rotary evaporator under a pressure of 40^0C.

Design of experiment

Effect of *cicer arietinum* desi and kabuli type on carbon tetra chloride induced liver toxicity

Water suspension:-

Rats were divided randomly in to four groups of containing six animals:

Group 1- Normal control: Administered with distilled water 2ml/kg for 4 days. On day 3, liquid paraffin 2ml was administered.

Group 2- Carbon tetra chloride control animals: Administered with distilled water 2ml/kg for 4 days. On day 3, Carbon tetra chloride diluted in liquid paraffin 2ml was administered.

Group 3- *Cicer arietinum* seed coat desi type animals: Administered with seed coat

suspension 500mg/kg for 4 days. On day 3, Carbon tetra chloride diluted in liquid paraffin 2ml was administered.
Group 4- *Cicer arietinum* seed coat kabuli type animals: Administered with seed coat suspension 500mg/kg for 4days. On day 3, CCl_4 diluted in liquid paraffin 2ml was administered [2]. This was done after the administration of water suspension of seed coat of *cicer arietinum* (desi and kabuli type). All the rats were sacrificed on the 5th day. This was done using mild anesthesia and the blood samples were collected and used for biochemical evaluation [5].

Effect of *cicer arietinum* desi type on carbon tetra chloride induced liver toxicity

Water suspension:-
Rats were divided randomly in to three groups containing six animals
Group 1- Normal control: Administered with distilled water 2ml/kg for 4 days. On day 3, liquid paraffin 2ml was administered.
Group 2- Carbon tetra chloride control animals: Administered with a single dose of 2ml of carbon tetra chloride on the third day. The third group was administered with a water suspension of *cicer arietinum* seed coat desi type 500mg/kg in 2% acacia gum. This was continued for 4 days. On the third day all the group was given with a single dose of 2ml of carbon tetra chloride. This was done after the administration of water suspension of seed coat of *cicer arietinum.* All the rats were sacrificed on the 5th day. This was done using mild anesthesia and the blood samples were collected and used for biochemical evaluation.

Effect of different extracts of *cicer arientunum* seed coat

This study was done using 12 groups of rats which consisted of 6 rats in a group.
Group 1- Normal control group: They were administered with a single dose of liquid paraffin on the third day.
Group 2- Carbon tetra chloride control group: The animals were administered with a single dose of CCl_4 and liquid paraffin in the ratio1:1 on the day three.
The group 3rd to group 11th animals was administered with different concentrations of different extracts for 4 days.
Group 12 were administered with standard drug silymarin for four days. The group 3rd to group 12 received a single dose of carbon tetra chloride and liquid paraffin (1:1) in day 3, just 60 min after the oral administration of silymarin. All the groups were sacrificed on the 5th day using anesthesia. The blood samples were taken for further analysis.

Evaluation of serum transminases, alkaline phosphates and bilirubin
The activities of serum aspartate transaminase (AST, EC 2.6.1.1), Alanine transaminase (ALT, EC 2.6.1.2), and Alkaline phosphatase (ALP, EC 3.1.2.1), were assayed by Spectrophotometric method followed by standard procedures using commercially available kits (SPAN Diagnostics, India)[3]. The serum bilirubin was evaluated spectrophotometrically using diagnostic kits [4].

Statistical analysis
All the values are expressed in the mean +SD (standard deviation) and the values of different groups were evaluated using one way analysis (ANOVA) techniques.

3. Results

The seed coats of *cicer arietinum species* showed increased in the serum ALT (Alanine transaminase) and AST

(Aspartate transaminase) activities compared with normal control groups (Table 1). Treatment with seed coat species prevented the elevated serum levels. But seed coat of desi type showed remarkable protective property compared to kabuli type. Therefore it was taken for detailed analysis.

Table 1. Effect of *cicer arietinum* species (desi and kabuli type) on carbon tetra chloride induced liver toxicity

GROUPS	ALT(IU/L)	AST(IU/L)
Normal control	2.25± 44.83	4.63± 87.67
CCl4 toxicity	11.77± 234.60	7.76± 255.27
Cicer arietinum Seed coat:- Kabuli type+CCl_4	4.71± 127.56	3.90± 139.29
Cicer arietinum Seed coat: Desi type+CCI_4	3.13± 88.59	2.70± 137.19

Effect of *Cicer arietinum* desi type on Carbon Tetra Chloride Induced Liver Toxicity

The water suspentions effects of *cicer arietinum* (desi type) on the serum markers for the hepatic damage produced by carbon tetra choride in rats were indicated in the (Table 2). In carbon tetra choride treated groups, the levels of AST, ALT, ALP and total bilirubin levels were increased when compared with the normal control groups [6]. The *Cicer arietinum* desi type suspension (500mg/kg) reversed the changes and was comparable with normal groups. The effects of *Cicer arietinum* desi type were found to be very promising.

Effect of different extracts of *Cicer arietinum* on CCl_4 induced liver damage on rats

Table no. 3 shows the efficiency of different extracts of *Cicer arietinum* desi type, on the changes by inducing CCl_4 toxicity in ALT, serum tranaminases, ALP, and bilirubin levels. Different extracts (n hexane, Water ant ethanol) of *Cicer arietinum* exhibited varying levels of liver protection against carbon tetra choride toxicity. The alcoholic extract exhibited maximum protection against carbon tetra choride toxicity. The water and hexane extract were in active. The liver protective activity of alcoholic *Cicer arietinum* extract (60mg/kg) was almost equal to silymarin (100mg/kg). The alcoholic *Cicer arietinum* extract exhibited maximum protection in the dose of 60mg/kg did not produce any increase in the liver protective activity at a higher dose of 120mg/kg.

Table 2. Results of serum markers on the efficacy of *cicer arietinum desi type*

Groups	Serum ALT(IU/L)	Serum AST (IU/L)	Serum ALP (K.A unit)	Bilirubin mg/dl
Normal Control	50.15± 3.52	94.34 ± 7.15	85.43 ± 6.13	0.37 ± 0.05
CCl_4 Control	248.71±2.53	26.56 ± 23.13	221.13±19.51	1.21 ±0.15
C. A. (seed coat) (500mg/kg)+ CCl_4	97.33 ± 7.14	121.11±11.26	106.40 ± 893	0.77 ±0.07

CCl_4-carbon tetra chloride, CA-cicer arietinum suspension values

Table 3. Effect of different extract of *Cicer arietinum* seed coat against CCl_4 induced hepatotoxicity in rat

Serum				
Groups	**ALT (IU/L)**	**AST (IU/L)**	**ALP (K. A. UNIT)**	**Bilirubin (mg/dl)**
Normal Control	37.47 ± 3.52	11.84 ± 7.75	82.44 ± 7.14	0.64 ± 0.05
CCl_4 Control	220.10 ± 17.5	296.43 ± 23.33	198.24 ±16.99	1.36 ± 0.32
CAH + $CCl_4$30mg/kg	219.43± 16.79	279.15± 25.31	184.30±17.46	1.22 ± 0.11
CAH + CCl_4 60mg/kg	204.40± 16.48	244.19±21.41	168.50±15.04	0.87 ± 0.11
CAH + $CCl_4$120mg/kg	216.47± 17.41	259.40± 21.89	174.40 ±15.74	1.11 ± 0.40
CAE + CCl_4 30mg/kg	103.41± 7.72	196.15± 16.31	131.30±11.36	0.75 ± 0.07
CAE +CCl_4 60mg/kg	60.27 ± 5.41	131.40± 12.36	96.55 ± 9.75	0.54 ± 0.07
CAE + CCl_4 120mg/kg	71.27 ± 5.21	146.40± 11.85	108.40± 8.34	0.81 ± 0.07
CAW + CCl_4 30mg/kg	181.43± 14.68	237.15± 18.92	176.41±16.79	1.38 ± 0.15
CAW + CCl_4 60mg/kg	146.30± 14.17	188.50± 15.38	124.25±11.41	0.81 ± 0.08
CAW + CCl_4 120mg/kg	171.37± 15.40	214.40±21.62	134.60±11.26	1.11 ± 0.11
Silymarin +CCl_4 100mg/kg	51.24 ± 5.71	118.61± 7.8	91.62 ± 9.75	0.51 ± 0.07

4. Discussion

The study reports that for the first time the hepatoprotective activity of ethanolic extract of *Cicer arietinum* seed coat against CCl_4 induced toxicity in rats are identified. The results gives validity for the folk lore claim for Bengal gram or *Cicer arietinum* seed coat as a drug for hepato protection. But the dose determination is an important factor for providing maximum liver protection. It can be concluded that the optimum activity was found at a dose of 60mg/kg and further increase in the dose (120mg/kg) did not exhibit any increase in the activity.

Conclusion

The result of this study reveals that the ethanolic extract of *Cicer arietinum* (desi type) seed coat have the capacity to cure hepatic damage which was induced by carbon tetra chloride.

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