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Original Research Article

Hepatoprotective effect of desi and kabuli cultivars of *Cicer arietinum* L (chick peas) against carbon tetrachloride-induced toxicity in rats

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Abstract

Purpose: *To determine the hepatoprotective potential of ethanol extracts of desi and kabuli cultivars of Cicer arietinum L. (chick peas).*
Methods: *Hepatotoxicity was induced in rats using oral administration of carbon tetrachloride (CCl_4). The rats were then orally administered different doses of the ethanol extracts of desi and kabuli cultivars of Cicer arietinum L. for 21 days. Oxidative stress parameters and hepatoprotective profiles were determined in serum samples using standard procedures. The effect of the treatments on liver histology was also determined.*
Results: *Administration of extracts of desi and kabuli cultivars of Cicer arietinum L. to CCl_4 treated rats at a dose of 300 mg/kg resulted in a significant ($p \leq 0.05$) decrease in oxidative stress parameters, whereas catalase activity significantly increased ($p \leq 0.05$); on the other hand, ALT and AST levels were decreased significantly ($p \leq 0.05$), when compared to the control group.*
Conclusion: *High doses of Cicer arietinum L (desi and kabuli cultivars) seem to have hepatoprotective and antioxidant effects on CCl_4-induced toxicity in rats. This finding underscores the therapeutic importance of Cicer arietinum L. as a plant with hepatoprotective properties.*

Keywords: *Cicer arietinum, Phenolics, Hepatotoxicity, Chick peas, Catalase*

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INTRODUCTION

Cicer arietinum L. (*desi* and *Kabuli*) is a leguminous pulse which belongs to the *Fabaceae* family. It has high nutritional profile and it is a good source of minerals and unsaturated fatty acids [1]. *Cicer arietinum* L. also contains bioactive compounds like polyphenols, antioxidants and vitamins which play vital roles in enhancing immunity against cardiovascular and metabolic diseases, as well as cancer [2]. In general, daily intake of legumes has beneficial effect in the management of various chronic heart conditions and metabolic diseases [3]. *Cicer arietinum* L. also contains sugars and proteins. Meat is a good source of

protein [4]. However, meat is more expensive and more scarce than plant-based proteins, and it contributes significantly more to global warming than *Cicer arietinum* L. [5]. Moreover, legumes are used by vegetarians as a meat substitute for protein and energy, so much so that they are referred to as 'the poor man's meat' [5].

Studies have associated *Cicer arietinum* L. with hypocholesterolemia due to its contents of various biological components such as phytic acid and complex proteins [6-8]. Legumes contain various micronutrients such as water-soluble vitamins, fat-soluble vitamins, macro minerals and trace minerals [9,10]. Globally, plant-based diets are now frequently used as relatively cheap sources of valuable nutrients which are normally required by humans [11, 12].

Limited data are available on the nutritional and compositional status, and the bioactive potential of *Cicer arietinum* L. However, not much is known on the biological role of *Cicer arietinum* L. with respect to its hepatoprotective effect. Plant-based antioxidant compounds are safer and more beneficial than synthetic compounds.

The aim of this research was to investigate the hepatoprotective potential of the ethanol extract of *Cicer arietinum* L. in rats.

EXPERIMENTAL

Collection of *Cicer arietinum* L.

Newly-developed high-yield lines of *Cicer arietinum* L. (desi and kabuli) named *Balkasar* and *COOP-6* were chosen during the sowing season from mid-September to mid-November, 2018 at Ayub Research Centerand Punjab, Pakistan. Fully mature seed pod samples of the chick peas were selected and collected. The samples were authenticated Dr. Hameed from Botany Department, University of Agricultural, Faisalabad. A voucher sample (no. Ch-Am-2018) was kept in the departmental herbarium.

Preconditioning and storage of seeds

Seeds from the selected lines of *Cicer arietinum* L. were removed from their dried black pods and washed with water to remove unwanted matter e.g. dust particles. Thereafter, a paper towel was used to remove any residual water from the seeds. Then, the seeds were air-dried until a constant weight was obtained. The dried seeds were milled with an electric miller, and the resultant course powder was preserved in sealed containers.

Extract preparation

Ethanol extracts were prepared according to the method previously described, using orbital shaker with absolute ethanol and 80% aqueous ethanol (80:20 ethanol:water, v/v) [13]. The ethanolic extracts were concentrated in a rotary evaporator (SciLogix Re-100 Pro) and kept at -4 ^{o}C [14].

Animals, diet and *in vivo* studies

Twenty-four albino rats aged 8 - 10 weeks (mean weight = 150 ± 7.5 g) were used in the study. The experimental rats were housed in a room with all standard facilities, and were provided *ad libitum* access to drinking water and normal diet. The rats were acclimatized for 7 days in the animal room with 12-h light/12-h dark cycle at 26 ^{o}C and 40 – 60 % relative humidity. They were weighed weekly, and the daily amount of feed consumed by the animals was determined. The experimental rats were randomly assigned to 6 groups: negative control (NC), positive control (PC), high-dose desi *Cicer arietinum* L. group (G1A), low-dose desi *Cicer arietinum* L. group (G1B), high-dose kabuli *Cicer arietinum* L. group (G2A), and low-dose kabuli *Cicer arietinum* L. group (G2B). Each group had 4 rats in it (n = 4). Table 1 shows the details of treatments and doses used in the various groups. The ethanolic extract of *balkaser* cultivar of desi *Cicer arietinum* L. and *COOP-6* cultivar of *kabuli Cicer arietinum* L. were administered orally (300 and 150mg/kg) for 21 days. The experiment was carried out in accordance with the guidelines outlined by the American Association for Laboratory Animal Science (AALAS) rules for ethical use of laboratory animals in research [15]. The current study was also certified by Government College University Faisalabad, and Ethical Committee for Animal Care (IRB no. 211; study no. 20189: Ref no. GCUF/ERC: 211).

Rats in all the groups were sacrificed on the 22nd day of experiment after regular oral administration of ethanolic extract of *Cicer arietinum* L. Blood was collected in vacuum containers without anticoagulant, and serum samples were obtained by centrifuging the blood samples for 4 min at 4000 g. The serum was analyzed for total antioxidant capacity (TAC) and total oxidative stress (TOS) potential according to the methods described earlier [16]. Hydrogen peroxide (H_2O_2) standard curve was prepared using a concentration range of 0.38-6.26 µmol/L, and was used to measure the TOS of selected serum samples.

Table 1: Grouping and animal treatments

Group	Diet and treatment
Negative control (NC)	Commercial chow normal diet (CMD) for 21 days
Positive control (PC)	Single oral dose of CCl_4 in olive oil (1:1 v/v) on day 14
G1A	CMD for 21 days Single oral dose (CCl_4+Olive oil 1:1 v/v) on day 14 Daily oral dose of desi (*Cicer arietinum* L.) extract at a dose of 300mg/kg bwt for 21 days;
G1B	CMD for 21 days, Single oral dose of CCl_4 in olive oil (1:1 v/v) on day 14 Daily oral dose of desi (*Cicer arietinum* L.) extract (150mg/kg bwt) for 21 days;
G2A	CMD for 21 days, Single oral dose of CCl_4 in olive oil (1:1 v/v) on day 14 Daily oral dose of kabuli (*Cicer arietinum* L.) extract (300mg/kg bwt) for 21 days
G2B	CMD for 21 days, Single oral dose of CCl_4 in olive oil (1:1 v/v) on day 14Daily oral dose of kabuli (*Cicer arietinum* L.) extract (150mg/kg bwt) for 21 days

The lowest measurable concentration was 0.13 µmol H_2O_2 L^{-1}, with less than 3% precision. The linearity of $H_2O_2L^{-1}$was maintained at 200 µmol, and intra-assay CV was kept below 10 %. Trolox standard curve was prepared using a concentration range of 0.1 - 1.5 mmol/L for determination of TAC of the samples, and the results were expressed in mmol of TroloxL^{-1}. The lowest measurable concentration was 0.18 mmol Trolox L^{-1}, with less than 3% precision. The linearity of $H_2O_2L^{-1}$ was maintained at 6 mmol, while intra-assay CV was < 3 %. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were assayed using Randox kits. The ranges of detection for ALT and AST were 7.20 – 1039 U/L and 9.7 – 603 U/L, respectively. Catalase was assayed using the method as previously described [17].

Statistical analysis

All analyses of serum samples were performed in triplicate. Data are presented as mean ± SD. All statistical analyses were carried out with GraphPad Prism 6.0, using one-way (ANOVA). Differences were assumed statistically significant at $p \leq 0.05$.

RESULTS

Yield of extracts

The extract yields from lines of desi and kabuli *Cicer arietinum* L. varied significantly, with values in the range of 4.78±0.23-10.16±0.51g/100g dry weight. Overall, extract yield from kabuli *Cicer arietinum* L. (COOP-6) was significantly higher than that from desi *Cicer arietinum* L. cultivar *balkasar* ($p \leq 0.05$). These results are shown in Figure 1.

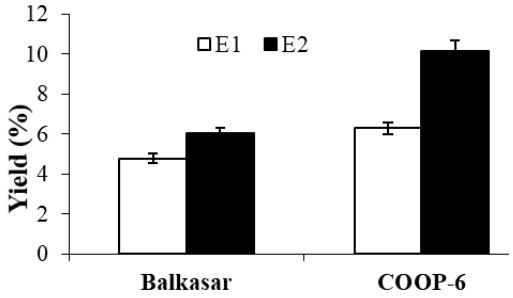

Figure 1: Extract **y**ields from desi and kabuli *Cicer arietinum* L. E1: absolute ethanol extract; E2: 80% ethanol extract

Change in body weights of rats

The mean body weight of rats used in the current study was unchanged during the treatment period Figure 2.

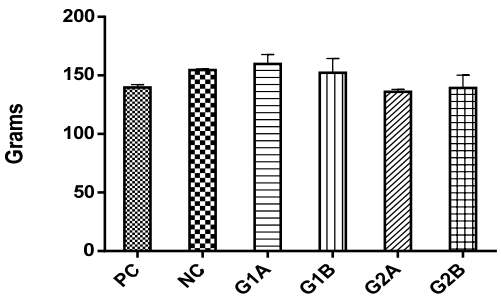

Figure 2: Body weights of rats (g ± SE) in different groups: PC: +ve control treated with CCl_4 only; NC: -ve control (CCl_4 without any treatment; G1A: Desi *Cicer arietinum* L. extract (300mg/kg bwt), G1B: desi *Cicer arietinum* L. extract (150mg/kg bwt), G2A: kabuli *Cicer arietinum* L. extract (300mg/kg bwt), and kabuli *Cicer arietinum* L. extract (150mg/kg bwt)

Total oxidant status

As shown in Figure 3, mean serum TOS was markedly (p≤0.05) reduced in G1A (2.54±0.42), G1B (2.81±0.33), G2A (6.47±0.27), G2B (6.62±0.51 and NC (6.33± 0.29) groups, when compared to PC group (7.70±0.58). Moreover, serum TOS values in G1A and G1B groups were significantly lower than those of G2A and G2B groups (p≤0.05).

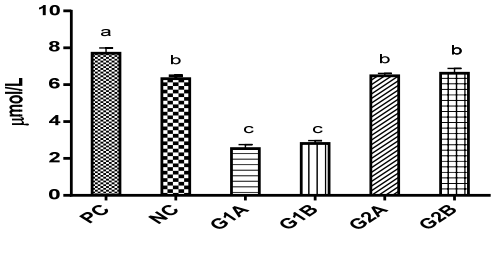

Figure 3: Mean serum TOS levels. Values are expressed as µmol of H_2O_2 $_{equiv}L^{-1}$ ± SEM). PC: positive control group treated with CCl_4 only, NC: negative control group (untreated), G1A: desi *Cicer arietinum* L. extract group (300 mg/kg), G1B: desi *Cicer arietinum* L. extract group (150 mg/kg), G2A: kabuli *Cicer arietinum* L. extract group (30 0 mg/kg), and G2B: kabuli *Cicer arietinum* L. extract (150mg/kg bwt). All treatments were given orally for 21 days

Total antioxidant capacity

Mean serum TAC levels were markedly increased (p ≤ 0.05) in G1B group (2.78 ± 0.08) and G1A group (2.38 ± 0.20) than in PC and G2B groups. Moreover, TAC level was higher (p ≤ 0.05) in G2A, G2B and NC groups than in PC group. These results are presented in Figure 4.

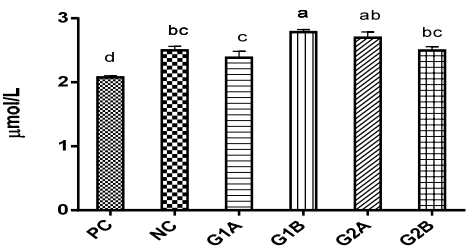

Figure 4: Mean serum TAC ofn the various groups. Values are expressed as mmol of $Trolox_{equiv}L^{-1}$ ± SEM. PC: positive control group treated with CCl_4 only, NC: negative control group (untreated), G1A: desi *Cicer arietinum* L. extract group (300mg/kg), G1B: desi *Cicer arietinum* L. extract group (150mg/kg), G2A: kabuli *Cicer arietinum* L. extract group (300 mg/kg), and G2B: kabuli *Cicer arietinum* L. extract (150 mg/kg). All treatments were given orally for 21 days

Changes in serum ALT

There was a significant (p≤0.05) decrease in mean serum ALT in G1B group (66.63 ± 26.71 U/L), when compared to PC group (177.70 ± 13.78U/L). In addition, ALT was markedly lowered (p ≤ 0.05) in G1A group (86.18 ± 24.67), G2Agroup (103.33 ± 24.19) and G2B group (119 ± 16.14), relative to PC group. These results are shown in Figure 5.

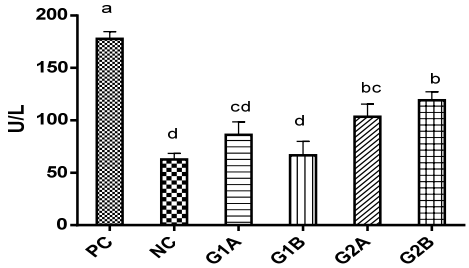

Figure 5: Mean serum ALT (U/L±SEM) levels. PC: positive control group (CCl_4 only), NC: negative control group (untreated), G1A: desi *Cicer arietinum* L. extract group (300 mg/kg), G1B: desi *Cicer arietinum* L. extract group (150 mg/kg), G2A: kabuli *Cicer arietinum* L. extract group (300mg/kg bwt), and G2B: kabuli *Cicer arietinum* L. extract (150 mg/kg). All treatments were given orally for 21 days

Changes in serum AST

As shown in Figure 6, significant (p≤0.05) decreases were seen in serum AST levels in G1B (87.23 ± 9.20U/L), G1A (111.86 ± 13.40U/L) and G2B (116.28 ± 31.97U/L) groups, relative to PC group (165.24±16.43). Serum ALT decreased in G2A group, when compared to PC group, but the decrease was not statistically significant.

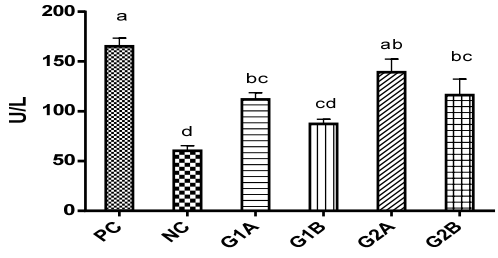

Figure 6: Mean serum AST levels. Values are expressed as (U/L±SEM). PC: positive control (CCl_4 only), NC: negative control group (untreated), G1A: desi *Cicer arietinum* L. extract group (300 mg/kg), G1B: desi *Cicer arietinum* L. extract group (150 mg/kg), G2A: kabuli *Cicer arietinum* L. extract group (300 mg/kg), and G2B: kabuli *Cicer arietinum* L. extract (150 mg/kg). All treatments were given orally for 21 days

Catalase

Significant ($p \leq 0.05$) increases were observed in serum catalase in G1B (3.20 ± 0.23) and G1A (2.93 ± 0.17) groups, when compared to PC group, while serum catalase activity was comparable among the negative control, G2A, G2B and PC groups. These results are shown in Figure 7.

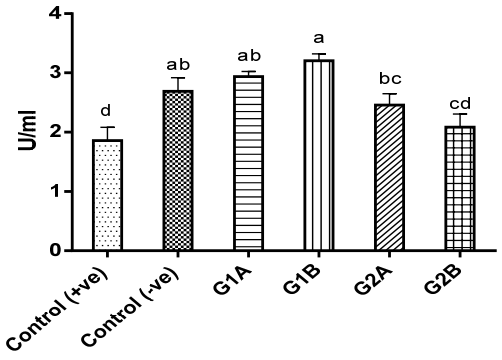

Figure 7: Mean serum catalase activities. Values are expressed as (U/mL ± SEM). PC: positive control group (CCl_4 only), NC: negative control group (untreated), G1A: desi *Cicer arietinum* L. extract group (300 mg/kg), G1B: desi *Cicer arietinum* L. extract group (150 mg/kg), G2A: kabuli *Cicer arietinum* L. extract group (300 mg/kg), and G2B: kabuli *Cicer arietinum* L. extract (150 mg/kg). All treatments were given orally for 21 days

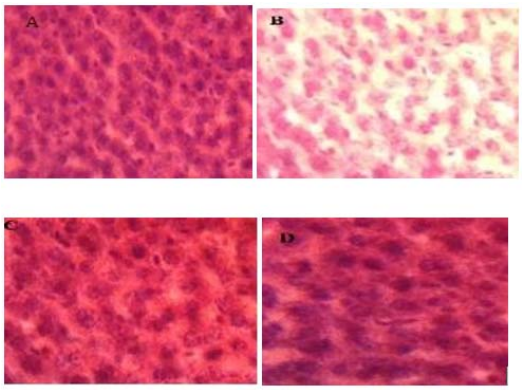

Figure 8: Effect of various treatments on liver histopathology. **A:** normal liver cells; B: CCl_4-induced hepatotoxicity; C: lower dose desi chick pea oral treatment (150 mg/kg); D: higher dose desi chick pea oral treatment (300 mg/kg)

DISCUSSION

Ethanolic extract yield varied significantly among cultivars of desi and kabuli (*Cicer arietinum* L.) in the current study. Overall, percentage extract yield of kabuli *Cicer arietinum* L. was significantly higher than that of desi *Cicer arietinum* L. cultivar. The findings on the percent yield in this study are in close agreement with previously published report [18].

In the current study, the percent yield of *Cicer arietinum* L. was higher in 80% aqueous ethanol than the yield in absolute ethanol. This is due to the lower polarity of absolute ethanol, when compared to 80% aqueous ethanol. Therefore, 80% ethanol, rather than absolute ethanol is usually used for the extraction of bioactive compounds from fruits and plants, mainly due to its good match, in nature and polarity with naturally occurring anti-oxidative compounds [19]. Antioxidants derived from plants are mostly polar. Phenolic and flavonoid compounds are extracted in 80% ethanol. The bioactive potential of these compounds (phenolic and flavonoids) play vital roles in beneficial effects of legumes [20].

In the current study, *Cicer arietinum* L. extract at high dose (300mg/kg bwt) mitigated the cytotoxic effects of CCl_4-derived free radicals. Therefore, TOS level was highly decreased in G1A and G2A groups, when compared with the low extract dose (150mg/kg bwt) groups (G1B and G2B). Decreases observed in TOS levels at the higher dose of *Cicer arietinum* L. were comparable to NC. Higher dose of desi *Cicer arietinum* L. (*balkasar)* and kabuli *Cicer arietinum* L. (*COOP-6*) tended to minimize the cytotoxic effects produced by CCl_4 in rats. This might be due to the higher concentration of phenolic and flavonoids contents and subsequent scavenging activity of desi *Cicer arietinum* L. (*balkasar*) and kabuli *Cicer arietinum* L. (COOP-6), leading to reduction in TOS levels in the fragile microenvironment of CCl_4-induced liver toxicity [21,22]. Total antioxidant capacity (TAC) of desi *Cicer arietinum* L. extract was significantly higher in G1A and G1B groups, when compared to PC, NC, G2A and G2B groups. The antioxidant capacity seen in the current study is comparable with those in a previous report [22]. The current results obtained are comparable to previous results for yellow soybean (38.0 µTroloxeq/g and 94.9 µTroloxeq/g) and 65 µTroloxeq/g for common beans [23-25]. It has been reported that higher levels of phenolic content activate the antioxidant enzymes, resulting in greater antioxidant capacity [26].

Serum ALT and AST levels were higher in PC than in NC, while serum levels of ALT and AST were decreased significantly in G1A and G1B groups, when compared to PC, but the results were not comparable to NC. These results suggest normal and healthy hepatic enzyme

levels, which confirms recovery from CCl_4-induced hepatotoxicity. Serum ALT and AST levels were also reduced in G2A and G2B groups, when compared to G1A and G1B groups.

Serum catalase was also significantly higher in G1A and G1B, relative to PC and other experimental groups. However, catalase level was high in G1A and G1B groups.

CONCLUSION

The ethanol extracts of high-yield cultivars of *Cicer arietinum* L. (desi and kabuli) are rich in phenolic compounds and flavonoids. Desi *Cicer arietinum* L. has higher capacity for reducing oxidative stress and decreasing CCl_4-induced increases in serum ALT and AST levels in rats. Thus, *Cicer arietinum* L. exerts its hepatoprotective effects in CCl4-treated rats through enhancement of antioxidant capacity. However, further investigations should be carried out to determine the hepatoprotective effect of *Cicer arietinum* L. in humans.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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