

HEPATOPROTECTIVE EFFECT OF *CHENOPODIUM QUINOA* SEED AGAINST CCl₄-INDUCED LIVER TOXICITY IN SWISS ALBINO MALE MICE

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ABSTRACT

Objective: To study the effect of *Quinoa* seed on carbon tetrachloride (CCl₄)-induced liver toxicity in Swiss albino male mice.**Methods:** Swiss albino male mice were divided into three groups: Group 1 served as control group; Group 2 served as hepatotoxic group (CCl₄ treated); and Group 3 served as combination of *Quinoa* seed powder (20 mg/kg) + CCl₄-treated group. The effect of these groups on liver tissue was studied after three different periods of 4, 8, and 12 weeks. Liver marker enzyme level of serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, serum alkaline phosphatase and liver antioxidant enzyme level of lipid peroxidation, superoxide dismutase, and reduced glutathione were measured. Further, histopathological analysis of liver was also conducted.**Results:** The results showed that the treatment with *Quinoa* seed powder significantly reduced CCl₄-induced elevated serum level of hepatic enzymes and restored the level of liver antioxidant enzymes toward the normal levels, which was also confirmed by histopathological studies.**Conclusion:** Results of the present study concluded that the *Quinoa* seed (*Chenopodium quinoa*) showed hepatoprotective effect against CCl₄-induced liver damage in Swiss albino male mice.**Keywords:** *Quinoa* seed, Carbon tetrachloride, Liver marker enzymes, Histopathological studies.© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2017.v10i11.20918>

INTRODUCTION

Quinoa is considered as super food since it is a good source of complete protein (it contains all nine essential amino acids), unsaturated fatty acids, minerals, vitamins, fiber, and antioxidants. It is a pseudocereal that has been cultivated in the Andean region of South America for thousands of years and belongs to the Chenopodiaceae family [1-3].

In India, especially in the high-altitude area of the Himalayas and North Indian Plains, *Quinoa*'s ability to produce high-protein grains under ecologically extreme conditions makes it important for the diversification of future agricultural systems. Its grain is rich in amino acids such as lysine and methionine that are deficient in cereals [1,3].

Quinoa seeds contain significant amounts of bioactive compounds, including polyphenols (mainly phenolic acids, including vanillic acid, ferulic acid, and their derivatives, as well as flavonoids, including quercetin, kampferol, and their glycosides) and tocopherols (Vitamin E), tocotrienols and carotenoids [4].

Previous studies [5] observed that bioactive compound of *Quinoa* could change antioxidant status in the organism by preventing oxidative stress and also helps reduce the risk of various chronic diseases risk such as anti-inflammatory, immunomodulatory, and anticarcinogenic [6].

Carbon tetrachloride (CCl₄) has been widely used in animal models to investigate chemical toxin-induced liver damage. The most significant pathological characteristics of CCl₄-induced hepatotoxicity are fatty liver, cirrhosis, and necrosis [7].

The aim of this study was to assess the effect of *Quinoa* seeds against CCl₄-induced liver damage in Swiss albino male mice.

METHODS

Experimental animals

Present experiments were performed using Swiss albino male mice with initial weights of 30-35 g. The mice were kept under standard laboratory conditions, were maintained on natural light and dark cycle and had free access to food and water. All animal procedures were performed in accordance to the Institutional Animal Ethics Committee (Registration No. 1689/PO/a/13/CPCSEA) and in accordance with the recommendations for the proper care and use of laboratory animals.

Experimental chemical and food

CCl₄ chemical was used for inducing liver damage in mice. CCl₄ was administered orally (33 mg/kg body weight in olive oil) for 12 weeks [8]. *Quinoa* seeds were purchased from a local grocery (India). The seeds were washed under running tap water, air-dried, and were then powdered mechanically. The *Quinoa* seed powder was orally administered to mice at a dose of 20 mg/kg body weight. The conversion of experimental doses was based on [9,10].

Experimental design

The animals were divided into three groups of 20 mice each:

- Group 1: Normal vehical control (olive oil) for 12 weeks
- Group 2: CCl₄ (33 mg/kg)-treated group for 12 weeks
- Group 3: Powder of *Quinoa* seed (20 mg/kg) + CCl₄ (33 mg/kg)-treated group for 12 weeks.

All treatments were orally administered continuously using a single dose of body weight. The effects of these groups on animal liver were studied after three different experimental periods of 4, 8, and 12 weeks.

Biochemical analysis

After the completion of 4, 8, and 12 weeks treatment, the mice were killed by cervical dislocation. The blood from heart was immediately

collected into sterilized tubes and was allowed to clot for serum separation. Serum was then separated by centrifugation at 3000 rpm for 10 minutes and was kept for subsequent evaluation of biochemical parameters such as serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), and serum alkaline phosphatase (ALP). The activities of these biochemical parameters were determined using commercially available kits (Accurex Biomedical Pvt. Ltd., and Jeev Diagnostic Pvt. Ltd.). The liver was removed and washed with ice-cold saline solution (0.9%) and stored at -4°C for analysis of liver antioxidant enzyme activity such as lipid peroxidation (LPO), superoxide dismutase (SOD), and glutathione (GSH) that were determined by methods of Ohkawa *et al.* [11], Marklund and Marklund [12] and Moron *et al.* [13], respectively.

Statistical analysis

Data were expressed as mean \pm standard error mean using student's t-test. Statistical significance was considered at $p < 0.05$. $p < 0.01$ were considered as highly significant ($p < 0.01$).

RESULTS

The result of biochemical analysis and liver antioxidant enzyme levels is shown in Figs. 1 and 2.

Histopathological analysis

Subsequent to completion of 4, 8 and 12 weeks treatment, each sample of liver tissue was washed with ice-cold saline solution and fixed in 10%

formalin for histological examination. All specimens were washed in tap water, dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Thin sections of $5\ \mu\text{m}$ were cut and stained with hematoxylin and eosin (H and E). The prepared sections of liver were examined by light microscopy to determine histopathological lesions in liver tissue.

DISCUSSION

The present study was undertaken to demonstrate the protective effect of *Quinoa* seeds against liver toxicity induced by CCl_4 in Swiss albino male mice for 4, 8, and 12 weeks treatment.

CCl_4 is a well-known hepatotoxin and has been widely used to screen new hepatoprotective agents [14]. In the current work, mice that were continuously treated with CCl_4 for 4, 8, and 12 weeks showed significant liver damage as indicated by increased level of serum marker enzyme (SGOT, SGPT, and ALP) and alteration of liver antioxidant enzyme level by decrease in SOD and GSH level with significant increase in LPO level as compared with normal control group (Figs. 1 and 2).

Previous studies showed that rise in SGOT, SGPT, ALP, and LPO level is an important factor in the pathogenesis of CCl_4 -induced liver damage [14,15].

The present results showed that the treatment with *Quinoa* seed powder significantly reduced CCl_4 -induced elevated serum level of hepatic enzymes (SGOT, SGPT, and ALP) and restored the level of liver antioxidant

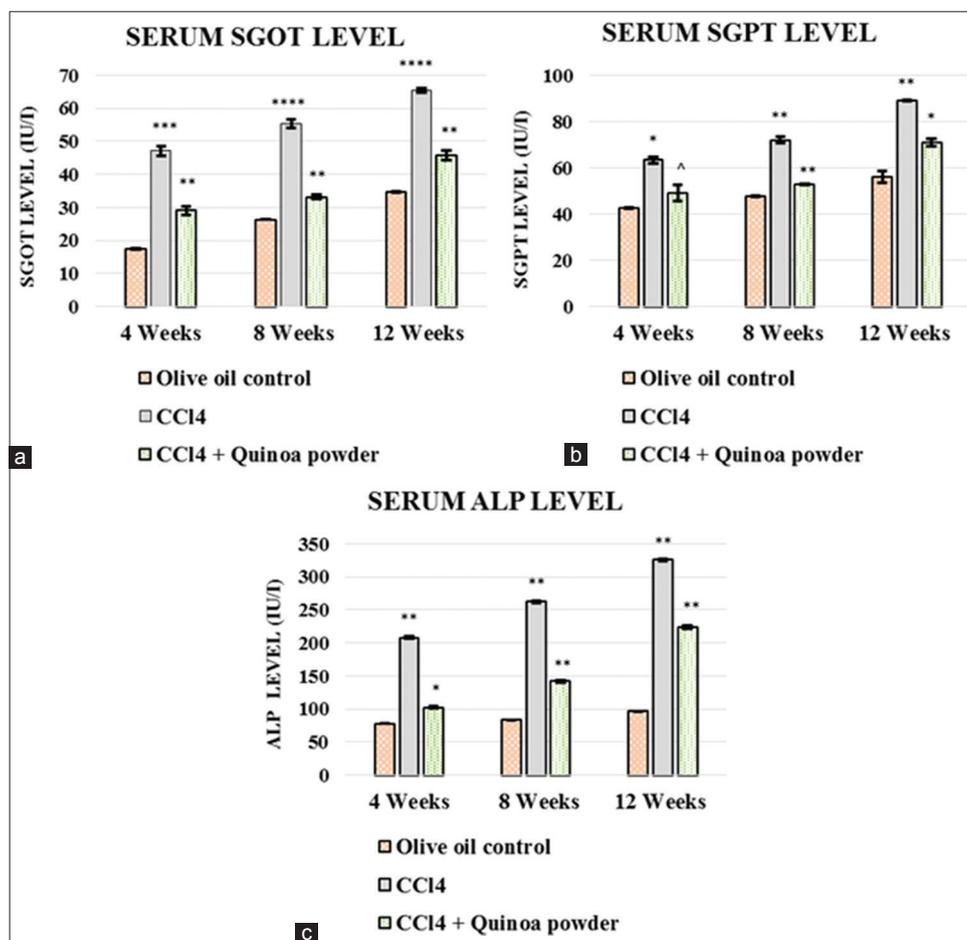


Fig. 1: (a-c) Bar chart depicting serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, and serum alkaline phosphatase levels on different groups of mice after treatment for 4, 8, and 12 weeks. ^ $p > 0.05$ as compared to control (not significant), *significant difference, as compared to control at $0.01 \leq p < 0.05$, **the difference is significant, compared with control at $0.001 \leq p < 0.01$, ***the difference is highly significant, compared with control at $0.0001 \leq p < 0.001$, ****the difference is highly significant, compared with control at $0.00001 \leq p < 0.0001$

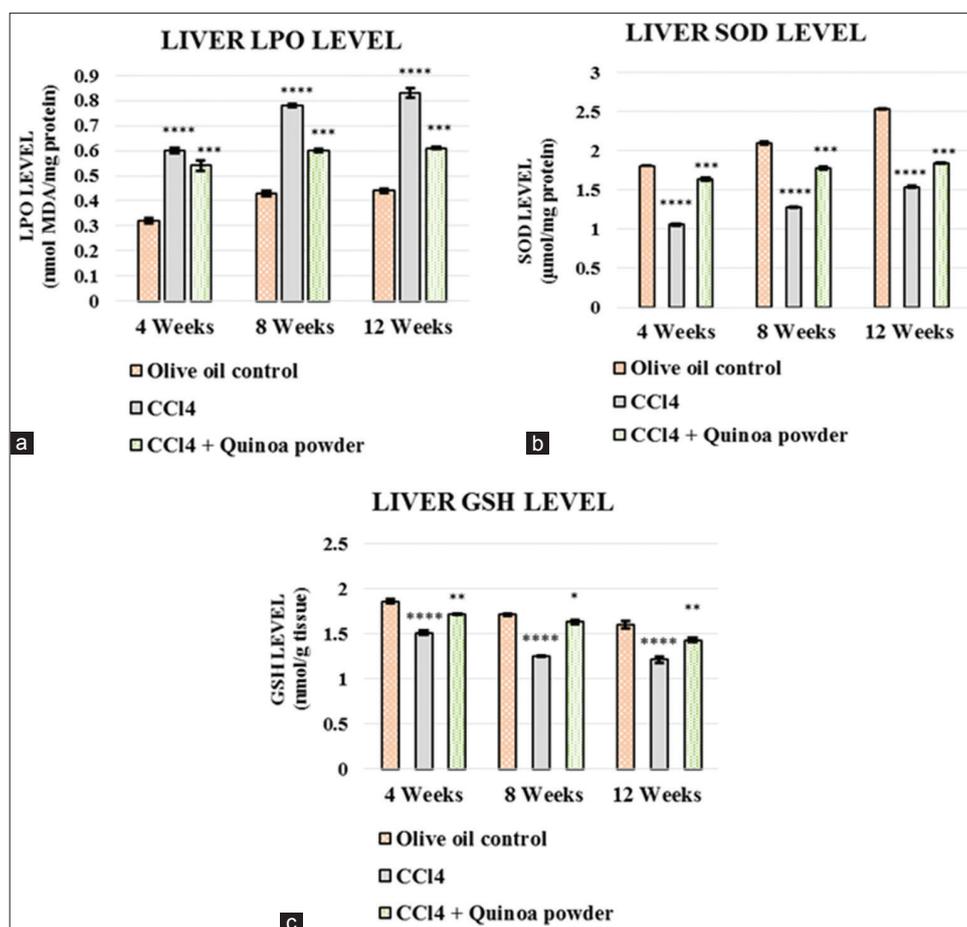


Fig. 2: (a-c) Bar chart depicting liver antioxidant enzyme levels of lipid peroxidation, superoxide dismutase, and glutathione on different groups of mice after treatment for 4, 8, and 12 weeks. Data are shown as mean \pm standard error mean (Figs. 1 and 2). *Significant difference, as compared to control at $0.01 \leq p < 0.05$, **the difference is significant, compared with control at $0.001 \leq p < 0.01$, ***the difference is highly significant, compared with control at $0.0001 \leq p < 0.001$, ****the difference is highly significant, compared with control at $0.00001 \leq p < 0.0001$

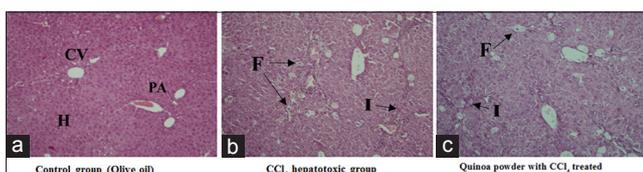


Fig. 3: (a-c) Effect of *Quinoa* powder on CCl_4 -induced hepatotoxicity in mice after 4 weeks treatments. Control group (olive oil) showing normal hepatic architecture with normal central vein, portal vein, and hepatocytes (H); CCl_4 showing fatty degeneration (F) of H with aggregation of inflammatory cells infiltration (I) surrounding the lobule; *Quinoa* powder with CCl_4 treated group showing improvement in the liver tissue reduced fatty degeneration (F) of H and cells infiltration (I) surrounding the lobule. T.S. of the liver section after 4 weeks treatment (H and E $\times 100$)

enzymes toward the normal levels by increase in SOD and GSH level with significant decrease in elevated level of LPO (Figs. 1 and 2).

In the current work, histopathological studies also supported the biochemical analysis and liver antioxidant enzyme activity. Histopathological observations of CCl_4 intoxicated group showed severe fatty degeneration of hepatocytes with aggregation of inflammatory cells infiltration surrounding the lobule and severe steatosis degeneration after 4, 8, and 12 weeks treatment as compared with control group (Figs. 3-5). The histopathological results of CCl_4 were similar to Al-Ghamdi [16], Eidi

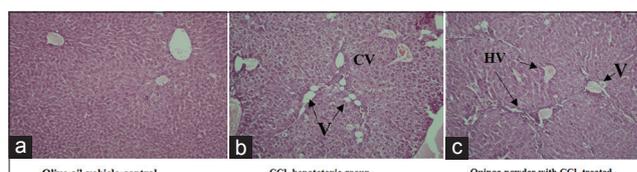


Fig. 4: (a-c) Effect of *Quinoa* powder on CCl_4 -induced hepatotoxicity in mice after 8 weeks treatments. Control group showing normal histological structure such as central vein, portal vein, and hepatocytes; CCl_4 intoxicated group showing vacuolar degeneration (V) with congestion of central vein (CV), *Quinoa* powder with CCl_4 group showing less congestion of hepatic vein (HV) with reduced vacuolar degeneration (V). T.S. of the liver section after 8 weeks treatment (H and E $\times 100$)

et al., [17], Soujanya et al., [18], Cordeiro and Kaliwal [19], Althnaian et al., [20], Sumalatha et al., [21], and Salama et al., [22].

From the histopathological examination of the present work it could be observed that *Quinoa* seed powder coadministered with CCl_4 -treated group showed improvement in the liver tissue to reduced fatty degeneration of hepatocytes, reduced cells infiltration surrounding the lobule and absence of liver steatosis (Figs. 3-5).

The present study results are in agreement with previous studies that reported the Chenopodiaceae family such as *Chenopodium album* and *Chenopodium murale* showed significant hepatoprotective and

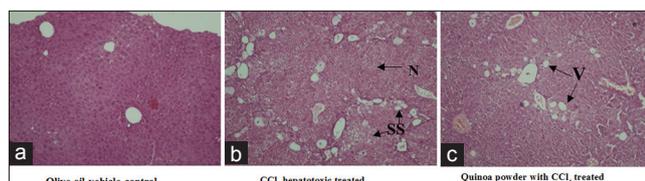


Fig. 5: (a-c) Effect of Quinoa powder on CCl₄-induced hepatotoxicity in mice after 12 weeks treatments. Control mice showing intact basic liver structures such as central vein, hepatocytes; CCl₄ hepatotoxic group showing severe steatosis degeneration (SS) with hepatocyte necrosis (N); Quinoa powder with CCl₄ group showing significant liver protection as evident by the presence of few vacuolar degenerations (V) and absence of liver steatosis. T.S. of the liver section after 12 weeks treatment (H and E ×100)

antioxidant activity due to the presence of high concentration of phytochemical compound such as flavonoids and phenolic acids [23,24].

Nigam and Paarakh observed that alcoholic and aqueous extracts of the aerial parts of *C. album* at the doses of 200 and 400 mg/kg for hepatoprotective activity against alcohol-induced hepatotoxicity using biochemical markers including serum transaminases, alkaline phosphatase, and ALP and by histopathological analysis to the liver tissue further confirmed the reversal of damage induced by hepatotoxin [23].

Similarly, Jain and Singhai worked on hepatoprotective activity of *C. album* Linn.: *In vitro* and *in vivo* studies. They observed that ethanol extract of *C. album* was found to be rich in phenolic and flavonoids and showed significant free radical scavenging activity against diphenylpicryl hydrazyl and superoxide ion radicals. In the *in vivo* studies, ethanol extract of *C. album* at a dose of 100, 200, and 400 mg/kg b.w. showed a significant increase in the levels of GSH, SOD, and CAT along with marked reduction in LPO when compared with CCl₄-induced severe depletion in hepatic GSH, SOD, and CAT with a high level of LPO in rats indicating antioxidant effects [14].

Saleem *et al.* noted that aqueous methanolic extract of *C. murale* (200 and 500 mg/kg) produced significant ($p < 0.001$) decrease in paracetamol induced increased levels of liver enzymes (alanine transaminase, aspartate transaminase, and alkaline phosphatase) and total bilirubin. They concluded that the hepatoprotective properties of a chenopodium plant may be due to the presence of high amount of flavonoids such as quercetin, kampferol, and gallic acid [24].

Pasko *et al.* reported that *Quinoa* seeds can act as a protective agent against fructose-induced changes in rats by reducing LPO and by enhancing the antioxidant capacity of blood (plasma) and heart, kidney, testis, lung, and pancreas. They concluded that the antioxidative system of plasma and selected tissue is more effective when these seeds are present in the diet. These seeds are able to reduce the oxidative stress, which may help alleviate the free radicals generation during pathological state [5].

Thus, the present study observed that *Quinoa* seed powder showed significant antioxidant and hepatoprotective effects that supported the use of *Chenopodium quinoa* as hepatoprotective agent.

CONCLUSION

Results of the present study concluded that the *Quinoa* seed (*C. quinoa*) showed hepatoprotective effects against CCl₄-induced liver damage in Swiss albino male mice as indicated by reduced elevated level of serum liver marker enzymes and restoration of liver antioxidant enzymes toward the normal levels. The hepatoprotective effect of *Quinoa* seed was also supported by the histopathological analysis.

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