doi.org/10.36721/PJPS.2021.34.6.SUP.2309-2315.1

Therapeutic potential of quinoa seed extract as regenerative and hepatoprotective agent in induced liver injury wistar rat model

Asif Shahzad[1], Muhammad Naeem Faisal[2], Ghulam Hussain[1], Syed Ali Raza Naqvi[3] and Haseeb Anwar[1]***

[1]Department of Physiology, Government College University, Faisalabad, Pakistan
[2]Institute of Physiology and Pharmacology, University of Agriculture, Faisalabad, Pakistan
[3]Department of Chemistry, Government College University, Faisalabad, Pakistan

Abstract: The liver is a fundamental metabolic organ that performs many essential functions including the detoxification of toxic substances present in the body. Exposure to various toxicants leads the liver towards hepatic injury. This study was planned to estimate the hepatoprotective and regenerative efficacy of Quinoa seeds (*Chenopodium quinoa*) extract against carbon tetrachloride (CCl_4) induced liver damage. At a dose of 1ml/kg (153.8mg/kg) body weight carbon tetrachloride (CCl_4) was used intraperitoneally to induce hepatic injury in Wistar rats. Silymarin (30mg/kg body weight, p.o.), an antioxidant was used as a reference standard drug. Subsequently, ethanolic extract of Quinoa seeds (QEE) was administered at 400 and 600mg/kg body weight through oral gavage. Various biochemical and regenerative biomarkers were assessed to evaluate the potential efficacy of QEE in liver tissue regeneration. Results revealed that QEE administration significantly reduced the CCl4-induced raised quantities of alanine transaminase (ALT), aspartate transaminase (AST), and total oxidative stress (TOS) while, significantly improved the level of triiodothyronine (T3), thyroxine (T4), albumin and total protein concentration in QEE treated groups. The expression level of IGF-1, FOXA-2, Stmn-2, SPP-1 was found significantly down-expressed. It is concluded that QEE treatment has the regenerative and hepatoprotective effect.

Keywords: Quinoa seed extract, hepato-protection, regeneration.

INTRODUCTION

Liver is the largest internal metabolic organ that is involved in excretion as well as detoxification of exogenous and endogenous substances. This makes it prone to various damages by chemical agents exposing the organ to various types of diseases including cirrhosis, hepatitis, liver cancer and disorders related to alcohol (Sreelatha *et al*., 2009; Xin *et al*., 2021). These drugs, or environmental pollutants including carbon tetrachloride (CCl_4), thioacetamide, paracetamol generate free radicals or reactive oxygen species (ROS) leading to hepatic injury (Temraz and El-tantawy, 2008; Wei *et al*., 2020). The ROS derived from oxygen metabolism are highly reactive and implicated in tissue damage mediated by lipid peroxidation and covalent binding oxygen (Su *et al*., 2019). They perform an important function in the synthesis of biochemical energy, regulation of intercellular signaling and cell growth (physiological processes) and phagocytosis (immunological). However, when they are synthesized excessively or accumulate inside the living body, they lead to the oxidative stress, a process that could be ascribed to several reasons including a decline in the synthesis or decreased activity of endogenous antioxidant enzymes (super oxide dismutase, catalase and glutathione per oxidase) and/or a decrease in the defense of non-enzymatic entities including as α-tocopherol, β-carotene and vitamin C (Khurelbat *et al*., 2014).

The hepatotoxin, carbon tetrachloride (CCl_4), is frequently employed to induce hepatic injury in experimental animal studies (Xiong *et al*., 2020). The most noteworthy pathological features of CCl4-induced liver toxicity include necrosis, cirrhosis, and fatty liver (Brol *et al*., 2019). The metabolic activation facilitated by cytochrome P450 mediated oxidase resulting in the generation of a extremely toxic radical trichloromethyl that attaches to lipids or proteins inside the endoplasmic reticulum membranes which ultimately leads to lipid peroxidation, oxidative stress and liver damage. Currently, traditional medicinal plants are being frequently used for treating liver-related disorders because synthetic or conventional drugs have restricted usefulness and are toxic posing serious spillover effects (Yeh *et al*., 2012). Many natural products originating from the plants are medicinal in nature having a robust antioxidant potential which may provide protection against liver toxicity triggered by several toxicants (Mitra *et al*., 2000; Umer *et al*., 2010). This antioxidant activity of plants is claimed to be associated to the existence of polyphenols which permit them to scavenge the reactive oxygen species thus leading to antioxidant defense (Madureira *et al*., 2011).

**Corresponding author:* e-mail: drhaseebanwar@gcuf.edu.pk

Quinoa is known as super food because it is a great source of various useful components including different essential amino acids, polyphenols, flavonoids, vitamins, minerals, fiber and antioxidant enzymes. It belongs to *Chenopodiaceae* family and has been grown in South America (Andean region) for many years (Singh and Singh, 2016). Its grains are rich in amino acids such as lysine and methionine that are lacking in cereals (Jancurová *et al*., 2009). A significantly high number of bioactive compounds including flavonoid (quercetin, kampferol and their glycosides), polyphenols (phenolic acids, including ferulic acid, vanillic acid and their derivatives), vitamin E (tocopherols) and carotenoids are present in Quinoa seeds (Laus *et al*., 2017). It has been stated in previous studies that bioactive compounds of the Quinoa are involved in altering the antioxidant status in the living organisms by prevention of oxidative stress (Pasko *et al*., 2010) and also help to lessen the risk of several chronic ailments due to their anti-inflammatory, anticarcinogenic and immunomodulatory activities (Fuentes and Paredes-Gonzalez, 2013).The goal of the present research was to appraise the potential regenerative efficacy of *Quinoa* seeds (*Chenopodium quinoa*) extract against carbon tetrachloride induced liver injury in Wistar albino rats.

MATERIALS AND METHODS

Chemicals

Silymarin, carbon tetrachloride (CCl4), quercetin, ferrozine, ABTS (3-ethylbenzothiazoline-6-sulphonic acid), gallic acid, DPPH (1,1-diphenyl-2-picrylhydrazyl) and assay kits (aspartate transaminase and alanine transaminase, albumin, globulin, total protein, ELISA T3 and T4) were all procured from Sigma-Aldrich (St Louis, MO).

Procurement and identification of quinoa seeds

Quinoa seeds were purchased from the local market of Faisalabad and duly authenticated by a Botanist (Dr. Qasim Ali), Department of Botany Government College University Faisalabad. For future reference, a specimen voucher was assigned and preserved in herbarium vide No. 275-bot-27.

Extraction

Quinoa seeds were extracted (QEE) from ten grams of crushed quinoa seeds mixed with 100mL of 70% (v/v) ethanol. Upon filtration and subsequent evaporation of solution using rotary evaporator, the QEE was stored in refrigerator at 4^0C for further use.

Animals and experimental design

Wistar albino rats (160-180g) were acquired from Department of Physiology, GC University Faisalabad and experiment trial was conducted by ensuing the recommendations of Institutional Bioethics Committee. Rats were allotted into five groups. Hepatic injury was induced in rats by single intra-peritoneal administration of 1ml/kg (153.8mg/kg) bodyweight CCl4. Silymarin (30 mg/kg bodyweight, p.o.), an antioxidant was used as a reference standard drug. The experimental groups were as follows: negative control (NC) group receiving routine rodent diet; positive control (PC) group (CCl_4 treated); standard (STD) group (CCl_4 + silymarin @ 30mg/Kg body weight); treatment 1 (T1) group (CCl_4 +QEE @400mg/Kg body weight) while treatment 2 (T2) group (CCl_4 + QEE @600mg/Kg body weight). All the rats were decapitated at day 7 and 14 of the experiment. Blood samples were collected followed by separation of serum by using standard protocol and preserved at -20°C until the analysis of liver function parameters, oxidative stress markers, serum proteins and metabolic hormones while liver tissue samples were collected in TriZOL reagent (Thermo Fisher Scientific®, USA) and RNA isolation was preformed promptly.

Histopathology of liver tissue

The CCl4 induced liver injury was confirmed by performing histopathology using hematoxylin and eosin (H and E) staining. For this purpose the liver tissues were collected from each decapitated rat, fixed in formalin, embedded in the paraffin and dehydrated with the alcohol. The tissue samples embedded in paraffin were sectioned in the 4-5µm, deparrafinised with xylene, rehydrated and finally stained with the H and E stain. The histology images were observed under light microscope with 10x magnification.

Estimation of liver function enzymes

The serum concentrations of aspartate transaminase (Bioclin® Transaminase AST Kinetic Diagnostic Kit; K048) and alanine transaminase (Bioclin® Transaminase ALT Kinetic Diagnostic Kit; K049) was determined using the commercially available kits.

Determination of oxidative stress markers

Total oxidant status (TOS) and total antioxidant capacity (TAC) were determined in serum samples by using calorimetric method as described by Anwar *et al*. (2021) after minor alterations.

Determination of serum proteins

Serum total protein and albumin levels were estimated by employing Total Protein Monoreagent Diagnostic Kit by Bioclin® (K031) and Albumin Monoreagent Diagnostic Kit by Bioclin® (K040). The concentration of serum globulin was estimated by deducting the level of serum albumin from serum total protein levels.

Determination of metabolic hormones

Serum levels of triiodothyronine (T3) and thyroxine (T4) were estimated by using commercially available ELISA kits.

Gene expression analysis

Relative gene expression analysis was estimated through quantitative real-time PCR (qRT-PCR) by using Maxima SYBR Green/ROX Master Mix (Thermo Fisher Scientific®) on iQ5 Bio-Rad machine with standard protocol and application of the following primers:

IGF-1 (Insulin like growth factor-1): F TGACGGTGAATGAGGTGCAA'3 and R 5' CCGAGCTGGTAAAGGTGAGC'3; FOXA-1 (Forkhead Box A1): F 5' CCTCCCTGGGACTTAACTGT'3 and R 5' GTAGCTGCTCCAGTCGGATG'3; Stmn2 (Stathmin 2): F 5'GATCAACAAGCGTGCTTCCG'3 and R 5'TTCGTGGAGCTTCCGAGATG'3; SPP1 (Secreted Phosphoprotein 1): F 5'GAGACCGTCTGAAACAGCGT'3 and R 5' GCCAAGGATGCTGAGGCTTA'3.

The qRT-PCR data was analyzed by using2*(- ΔΔct) method.

STATISTICAL ANALYSIS

Results were analyzed statistically by using analysis of variance (One-way ANOVA) followed by DMR (Duncan's Multiple Range Test) and final data were presented as mean ± SE. Statistical analysis was performed by using GraphPad Prism version 6 computer software.

RESULTS

Histopathological confirmation of Liver Injury

Hematoxylin and eosin staining was performed to confirm CCl4 induced liver damage. Liver histology of negative control group rats showed regular architecture of hepatocytes. Positive control group showed accumulation of different sizes of lipid droplets accumulation with the change in hepatocyte architecture indicating severe steatosis in the liver cells as compared to negative control group indicating liver injury in CCl4 treated group (Fig 1).

Liver function markers

The outcome of administration of Quinoa seed extract on liver function markers is exhibited in fig. 2. It is revealed that CCl4 considerably ($P<0.05$) raised the serum concentrations of alanine transaminase (ALT) and aspartate transaminase (AST). The administration of quinoa seed extract for a period of 14 days restored the levels of these enzymes towards normal in experimental rats.

Oxidative stress markers

Serum total antioxidant capacity (TAC; mmol/L) & Serum Total Oxidative Stress (TOS; µmol/L)

The effect of administration of Quinoa seed extract on oxidative stress markers is presented in fig. 3. It was noticed that CCl4 considerably raised ($p<0.05$) the serum total oxidative stress by causing a significant ($p<0.05$) reduction in serum total antioxidant capacity. The administration of quinoa seed extract for a period of 14 days exhibited a significant increase ($p<0.05$) and returned to the normal serum TAC level resulting in significant decline in serum TOS in treated groups.

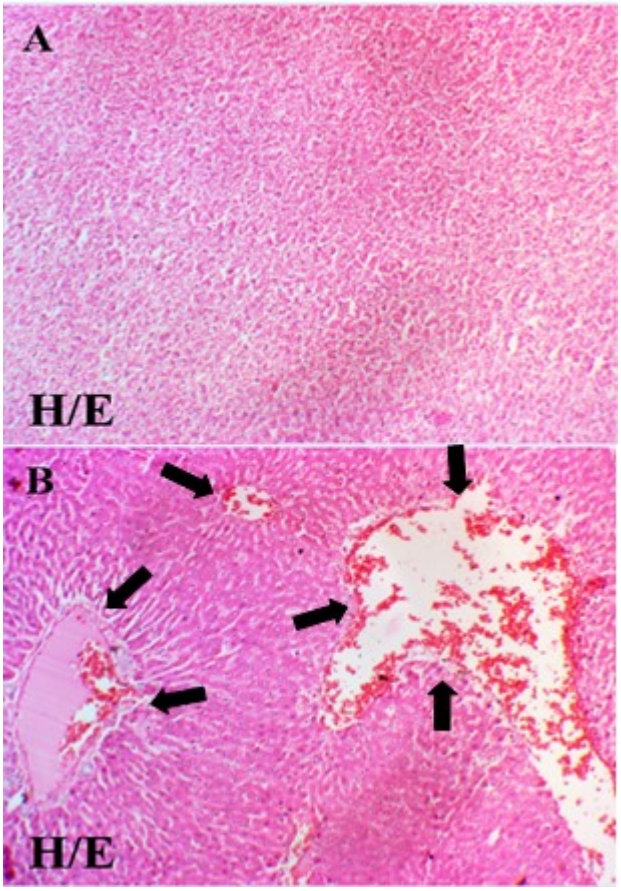

Fig. 1: Photomicrographs showing histopathology of liver tissue (H&E staining; 10X); A: Photomicrograph of rat liver tissue from groups Negative control (NC); B: Positive control (PC) (CCl4 @ 1ml/kg; IP)

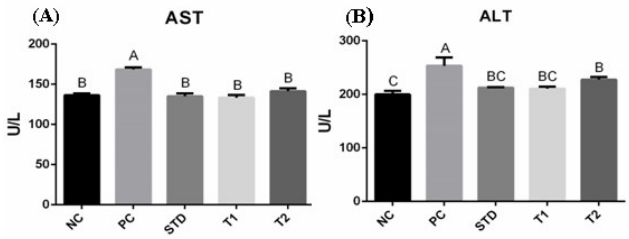

Fig. 2: The outcome of oral administration of Quinoa seed extract (QEE) on liver function markers (A) AST and (B) ALT; mean ± SE in CCl4 induced liver injury rat model. Means with different letters (A-C) indicate significant difference at $p<0.05$. Negative control (NC); Positive control (PC) (CCl4 @ 1ml/kg; IP); STD: CCl4 (1ml/kg; IP) + Silymarin (30 mg/kg, p.o.); T1: CCl4 (1ml/kg; IP) +QEE (400 mg/kg; p.o.); T2: CCl4 (1ml/kg; IP) +QEE (600 mg/kg; p.o.)

Serum proteins

Results of present study after statistical analysis revealed significant differences in albumin, globulin and total protein concentrations in the negative control, positive control and all groups given treatment (fig. 4). The albumin and total protein concentration (mean ± SE) was significantly (P<0.05) decreased in positive control in comparison to negative control group. Silymarin and quinoa seed extract treated groups presented a significant (P<0.05) upsurge and restored normal serum albumin, globulin and total protein levels.

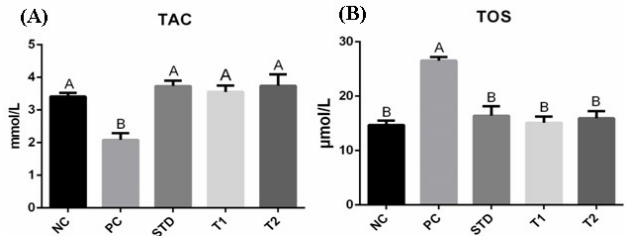

Fig. 3: The effect of oral administration of Quinoa seed extract (QEE) on oxidative stress markers (A) TAC and (B) TOS; mean ± SE in CCl4 induced liver injury rat model. Means with different letters (A-B) indicate significant difference at p<0.05. NC: Negative control (NC); Positive control (PC) (CCl4 @ 1ml/kg; IP); STD: CCl4 (1ml/kg; IP) + Silymarin (30 mg/kg, p.o.); T1: CCl4 (1ml/kg; IP) +QEE (400 mg/kg; p.o.); T2: CCl4 (1ml/kg; IP) +QEE (600 mg/kg; p.o.)

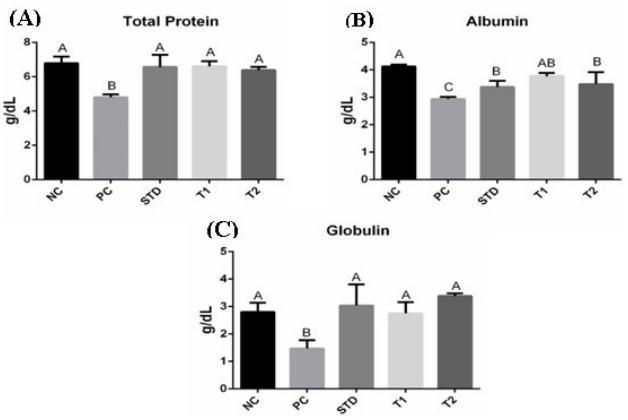

Fig. 4: The effect of oral administration of Quinoa seed extract (QEE) on serum proteins (A) Total protein (B) Albumin and (C) Globulin; mean±SE in CCl4 induced liver injury rat model. Means with different letters (A-C) indicate significant difference at p<0.05. NC: Negative control (NC); Positive control (PC) (CCl4 @ 1ml/kg; IP); STD: CCl4 (1ml/kg; IP) + Silymarin (30 mg/kg, p.o.); T1: CCl4 (1ml/kg; IP) +QEE (400 mg/kg; p.o.); T2: CCl4 (1ml/kg; IP) +QEE (600 mg/kg; p.o.)

Serum thyroid profile

After statistical exploration of triidothyronine (T3) levels, a non-significant effect of different groups was observed (fig. 5). The serum T3 levels were slightly improved in silymarin and quinoa seed extract treated groups in comparison to positive control and negative control groups, however the change is statistically non-significant (P>0.05). However, the mean serum concentration of thyroxine (T4) was considerably (P<0.05) reduced in positive control group in comparison to to negative control and all other treatment groups. Groups treated with silymarin and quinoa seed extract displayed a significant (P<0.05) increase and restored normal serum T_4 level.

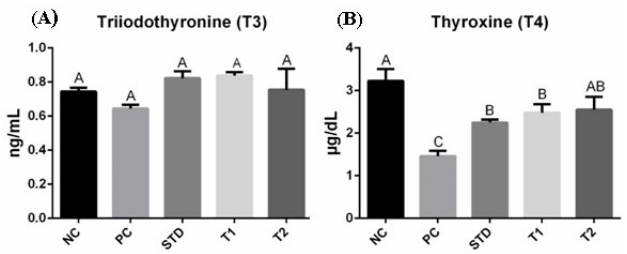

Fig. 5: The effect of oral administration of Quinoa seed extract (QEE) on serum Thyroid (A) Triidothyronine and (B) Thyroxin; mean ± SE in CCl4 induced liver injury rat model. Means with different letters (A-C) indicate significant difference at p<0.05. Negative control (NC); Positive control (PC) (CCl4 @ 1ml/kg; IP); STD: CCl4 (1ml/kg; IP) + Silymarin (30mg/kg, p.o.); T1: CCl4 (1ml/kg; IP) +QEE (400mg/kg; p.o.); T2: CCl4 (1ml/kg; IP) +QEE (600mg/kg; p.o.)

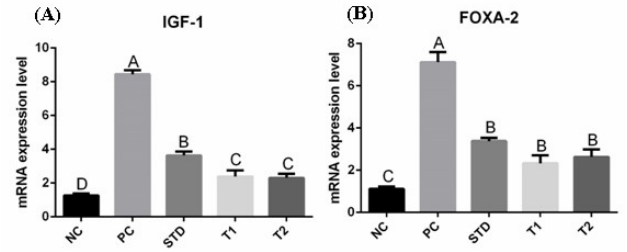

Fig. 6: The effect of oral administration of Quinoa seed extract (QEE) on mRNA expression levels of genes involved in cell regeneration (A) *IGF-1* and (B) *FOXA-2*; mean±SE in CCl4 induced liver injury rat model. Means with different letters (A-C) indicate significant difference at P<0.05. Negative control (NC); Positive control (PC) (CCl4 @ 1ml/kg; IP); STD: CCl4 (1ml/kg; IP) + Silymarin (30mg/kg, p.o.); T1: CCl4 (1ml/kg; IP) +QEE (400 mg/kg; p.o.); T2: CCl4 (1ml/kg; IP) +QEE (600 mg/kg; p.o.)

Growth and cell survival markers

Expression level of regenerative growth markers (*IGF-1 and FOXA-2*) genes are significantly over expressed in positive control (P<0.05) and down expressed among the control, standard, treatment group l and treatment group 2 (fig. 6). In comparison to positive control, the expression level of both genes is down expressed in treatment groups indicating that in treatment group l and treatment group 2 tissue is regenerated with normal parenchyma.

Liver regenerative markers

Expression level of liver regenerative markers *Stmn-2* and *SPP-1* genes are significantly over expressed in positive control (P<0.05) and slightly down expressed among standard, treatment group l and treatment group 2 at day 7 and 14 in comparison to positive control indicating the liver tissue regeneration towards normal parenchyma (fig. 7). Tissue regeneration is ultimately leading towards health and normal liver tissue among all groups except positive control group.

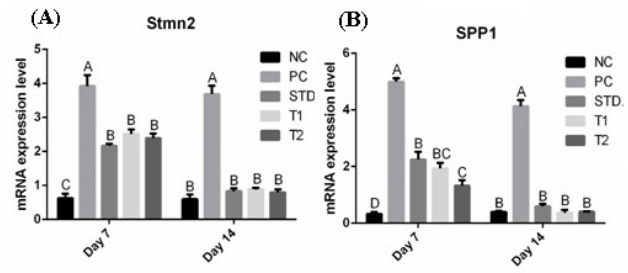

Fig. 7: The effect of oral administration of Quinoa seed extract (QEE) on mRNA expression levels of genes involved in cell regeneration (A) *Stmn2*, (B) *Spp-1*; mean±SE in CCl4 induced liver injury rat model. Means with different letters (A-C) indicate significant difference at p<0.05. NC: Negative control; PC: Positive control (CCl4 @ 1ml/kg; IP); STD: CCl4 (1ml/kg; IP) + Silymarin (30 mg/kg, p.o.); T1: CCl4 (1ml/kg; IP) +QSE (400 mg/kg; p.o.); T2: CCl4 (1ml/kg; IP) +QSE (600 mg/kg; p.o.)

DISCUSSION

The current research was conducted to reveal the defensive and regenerative efficacy of Quinoa seeds extract against CCl4-induced hepatotoxicity in Wistar albino rats after 14 days of treatment. The CCl4 has been commonly used to find new entities having hepatoprotective potential (Jain and Singhai, 2012). In current study, rats treated with CCl4 exhibited significant damage of liver as evidenced by raised level of serum enzyme markers (AST & ALT) and alteration of serum total antioxidant capacity and total oxidative stress by decrease in TAC level with a noteworthy upsurge in TOS level in comparison to negative control group (fig. 1 and 2). It has been reported by previous studies that a rise in AST, ALT, and total oxidative stress level is an imperative feature involved in the pathophysiology of CCl4 -induced hepatic damage (Essawy *et al*., 2012; Jain and Singhai, 2012). The results of current study revealed that the supplementation with Quinoa seed extract considerably reduced CCl4-induced increased levels of hepatic enzymes in serum (AST & ALT) and restored the normal serum TAC and TOS levels.

The results of present research study are supported by previous research studies reporting that the *Chenopodiaceae* family including *Chinopodium murale* and *Chinopodium album* exhibited considerable antioxidant and hepatoprotective efficacy due to the existence of phytochemical compound including phenolic acids and flavonoids in high concentration (Nigam and Paarakh, 2011; Saleem *et al*., 2014). The total protein levels in serum may provide a clue of the type and state of liver injury (Sabiu *et al*., 2014). The hypo/ hyperproteinemia is linked with damage to the liver resulting from a corresponding decline or rise in synthesis of protein. Decrease in the serum levels of albumin, globulin and total proteins was observed in rats after the treatment with CCl4 suggesting hepatic injury. The chief reason of metabolic dysfunction is the oxidative damage of some amino acids in CCl4 induced liver damage (Bandyopadhyay *et al*., 1999). In current study, administration of Quinoa seeds extract ameliorated the oxidative damage and promoted the synthesis of albumin, globulin, and total proteins.

In this study, a significant rise in liver function markers including ALT, AST was observed in CCl4- challenged rats. Induction of acute hepatotoxicity is signified by an elevated serum concentration of liver enzymes which might be associated to breakdown of hepatic tissue allowing the leakage of intracellular enzymes into the bloodstream from the cytoplasm of hepatocytes (Hismiogullari *et al*., 2015). Thus, the increased serum levels of AST and ALT indicate severe liver damage caused by CCL4 exposure. Actually, CCl4 induced liver injury is caused by a reactive intermediate generated as a result of its reductive metabolism leading to lipid peroxidation and consequently hepatotoxicity (Al-Seeni *et al*., 2016; Lu *et al*., 2012).

In this research trial, *in vivo* protective efficacy of Quinoa seeds extracts against thyroid toxicity induced by CCl4 was also explored. Free radicals created by bioactivation of CCl4 cause a decline in the antioxidant enzymes levels and improvement in lipid peroxidation leading to thyroid tissue toxicity (Naz *et al*., 2014). Hypothyroidism was observed by CCl4 treatment in current study as shown by the decreased serum T3 and T4 levels. The T4 and T3 are the two core hormones released by the thyroid gland and are constantly needed for normal growth by living body. Malfunctioning of thyroid gland is depicted by the reduction in level of these hormones (T3 and T4). Treatment of Quinoa seeds extract ameliorated the toxicity caused by CCl4 possibly by improving antioxidant enzyme status, thus resulting in an increased level of T3 and T4 in serum of rats.

Behind the activation of transcription factors, certain elements are directly involved during development of the tissue that include *Nkx2.2, Isl1*, *NeuroD*/BETA2, *Pax6* and *Pax4*. These transcription factors were expressed during the early stages of tissue development, function and during different levels of cellular differentiation

(Catherine *et al*., 2002). Several examples predicted about regulatory circuits involving *Foxa-2* genes in cell survival (Catherine *et al*., 2002). While as, the IGF-1 expression of a muscle-restricted insulin-like growth factor (IGF)-1 (mIGF-1) transgene increased the regenerative process of injured tissue, reducing the inflammatory response and preventing the process of fibrosis (Pelosi *et al*., 2007). IGF-I production in the liver parenchyma cell cycle modulation hence increase the cell replication and regeneration during disease condition (Musaro *et al*., 2004, Sin *et al*., 2002, Yuji *et al*., 2016). The expression levels of Afp mRNA and Spp-1 are associated with bile duct genes as well as a progenitor marker for liver regeneration is upregulated at the site of transacted border of the liver tissue (Viglietto *et al*., 2002). While the protein stathin-1 exerts its effect on microtubules, Stmn-1 usually regulates the microtubules polymerization which is essential for formation of mitotic spindle. Mitotic spindle allows the segregation of chromosomes as well as cell division. Protein statin-1 is highly expressed in dividing cell and expressed markedly due to oxidative stress response (Mass *et al*., 2020).

CONCLUSION

In accordance with the outcomes of current study it is concluded that the Quinoa seed extract plays the hepatoprotective role and shows the regenerative potential against CCl4-induced liver damage in Wistar albino rats. That is evident by the reduction in elevated level of serum liver markers (AST and ALT) and restoration of antioxidant capacity. The regenerative efficacy of Quinoa seed extract is supported by the gene expression analysis of liver regenerative markers including *IGF-1, FOXA-2*, *Stmn-2* and *SPP-1*. Conclusively, our findings suggest that in current experiment Quinoa seed extract is helpful in protecting the hepatic damage and plays regenerative role in the recovery of liver damage in rat model.

REFERENCES

Al-Seeni MN, El Rabey HA, Zamzami MA and Alnefayee AM (2016). The hepatoprotective activity of olive oil and *Nigella sativa* oil against CCl4 induced hepatotoxicity in male rats. *BMC Complement. Altern. Med.,* **16**(1): 1-14.

Anwar H, Hussain G, Rasul A, Ali Shah SM, Naqvi SAR, Bukhari SA, Sohail MU, Faisal MN, Mustafa I, Munir N and Nisar J (2021). Potential role of probiotic species in ameliorating oxidative stress, effect on liver profile and hormones in male albino rat model. *Eur. J. Inflamm.*, **19**(1): 1-9.

Bandyopadhyay U, Das D and Banerjee RK (1999). Reactive oxygen species: Oxidative damage and pathogenesis. *Curr. Sci.*, **77**(5): 658-666.

Brol MJ, Rösch F, Schierwagen R, Magdaleno F, Uschner FE, Manekeller S, Queck A, Schwarzkopf K, Odenthal M, Drebber U and Thiele M (2019). Combination of CCl4 with alcoholic and metabolic injuries mimics human liver fibrosis. *Am. J. Physiol. Gastrointest. Liver Physiol.*, **317**(2): G182-G194

Essawy AE, Abdel-Moneim AM, Khayyat LI and Elzergy AA (2012). *Nigella sativa* seeds protect against hepatotoxicity and dyslipidemia induced by carbon tetrachloride in mice. *J. App. Pharm. Sci.*, **2**(10): 21-25.

Fuentes F and Paredes-Gonzalez XIMENA (2013). Nutraceutical perspectives of quinoa: Biological properties and functional applications: State of the art report on quinoa around the world in 2013, *Chapter 3.5 in Quinoa: Nutritional Aspects of the Rice of the INCAS*, pp.286-299.

Hismiogullari AA, Hismiogullari SE, Karaca O, Sunay FB, Paksoy S, Can M, Kus I, Seyrek K and Yavuz O (2015). The protective effect of curcumin administration on carbon tetrachloride (CCl 4)-induced nephrotoxicity in rats. *Pharmacological Reports*, **67**(3): 410-416.

Jain NK and Singhai AK (2012). Hepatoprotective activity of *Chenopodium album* Linn: *in vitro* and *in vivo* studies. *J Exp. Integr Medi.*, **2**: 331-336.

Jancurová M, Minarovicova L and Dandar A (2009). Quinoa–a rewiev. *Czech J. Food Sci.,* **27**(2): 71-79.

Khurelbat D, Purevkhuu M, Luvsansharav B, Bandi S, Tseveen D, Sanjjav T, Dorjbal E and Miegombo A (2014). The hepatoprotective activity of the herbal preparation salivin against carbon tetrachloride (CCl4) induced hepatotoxicity in rabbits. *Curr. Issues Pharm. Medical Sci.*, **4**(4): 263-266.

Laus MN, Cataldi MP, Robbe C, D'Ambrosio T, Amodio ML, Colelli G, De Santis G, Flagella Z and Pastore D (2017). Antioxidant capacity, phenolic and vitamin C contents of quinoa (*Chenopodium quinoa* Willd.) as affected by sprouting and storage conditions. *Ital. J. Agron.*, **12**(1): 63-68.

Lu B, Xu Y, Xu L, Cong X, Yin L, Li H and Peng J (2012). Mechanism investigation of dioscin against CCl4-induced acute liver damage in mice. *Environ. Toxicol. Pharmacol*., **34**(2): 127-135.

Madureira AR, Amorim M, Gomes AM, Pintado ME and Malcata FX (2011). Protective effect of whey cheese matrix on probiotic strains exposed to simulated gastro-intestinal conditions. *Food Res. Int.*, **44**(1): 465-470.

Mitra SK, Seshadri SJ, Venkataranganna MV, Gopumadhavan S, Udupa UV and Sarma DN (2000). Effect of HD-03-a herbal formulation in galactosamine-induced hepatopathy in rats. *Indian J. Physiol. Pharmacol.*, **44**(1): 82-86.

Naz K, Khan MR, Shah NA, Sattar S, Noureen F and Awan ML (2014). *Pistacia chinensis*: A potent ameliorator of CCl4 induced lung and thyroid toxicity in rat model. *BioMed Res. Int.*, **2014**(1).1-13.

Nigam V and Paarakh PM (2011). Hepatoprotective activity of Chenopodium album Linn. against alcohol induced liver damage. *Int. J. Phytomedicine.*, **3**(4): 511.

Pasko P, Barton H, Zagrodzki P, Izewska A, Krosniak M, Gawlik M, Gawlik M and Gorinstein S (2010). Effect of diet supplemented with quinoa seeds on oxidative status in plasma and selected tissues of high fructose-fed rats. *Plant Foods Hum. Nutr.*, **65**(2): 146-151.

Pelosi L, Giacinti C, Nardis C, Borsellino G, Rizzuto E, Nicoletti C, Wannenes F, Battistini L, Rosenthal N, Molinaro M and Musar A (2007). Local expression of IGF-1 accelerates muscle regeneration by rapidly modulating inflammatory cytokines and chemokines. *FASEB J.*, **21**(7): 1393-1402.

Sabiu S, Wudil AM and Sunmonu TO (2014). Combined administration of Telfaira occidentalis and *Vernonia amygdalina* leaf powders ameliorates garlic-induced hepatotoxicity in Wistar rats. *Pharmacologia*, **5**(5): 191-198.

Saleem M, Ahmed B, Qadir MI, Mahrukh M, Ahmad M and Ahmad B (2014). Hepatoprotective effect of *Chenopodium murale* in mice. *Bangladesh J. Pharmacol.,* **9**(1): 124-128.

Singh KV and Singh R (2016). Quinoa (*Chenopodium quinoa* Willd), functional superfood for today's world: A Review. *World Sci. News*, **58**(1): 84-96.

Sreelatha S, Padma PR and Umadevi M (2009). Protective effects of *Coriandrum sativum* extracts on carbon tetrachloride-induced hepatotoxicity in rats. *Food Chem. Toxicol.*, **47**(4): 702-708.

Su LJ, Zhang JH, Gomez H, Murugan R, Hong X, Xu D, Jiang F and Peng ZY (2019). Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy and ferroptosis. *Oxid. Med. Cell. Longev.*, **2019**(1): 1-13.

Temraz A and El-Tantawy WH, 2008. Characterization of antioxidant activity of extract from *Artemisia vulgaris. Pak. J. Pharm. Sci.*, **21**(4): 321-326.

Umer S, Asres K and Veeresham C (2010). Hepatoprotective activities of two Ethiopian medicinal plants. *Pharm. Biol.*, **48**(4): 461-468.

Viglietto G, Motti ML, Bruni P, Melillo RM, D'alessio A, Califano D, Vinci F, Chiappetta G, Tsichlis P, Bellacosa A and Fusco A (2002). Cytoplasmic relocalization and inhibition of the cyclin-dependent kinase inhibitor p27 Kip1 by PKB/Akt-mediated phosphorylation in breast cancer. *Nat. Med.*, **8**(10): 1136-1144.

Wei X, Wang H, Sun X, Huang X, Xu W, Liang Y and Lin J (2020). 4-hydroxy-2 (3H)- benzoxazolone alleviates acetaminophen-induced hepatic injury by inhibiting NF-κB and activating Nrf2/HO-1 signaling pathways. *Am. J. Transl. Res.*, **12**(5): 2169-2180.

Xin C, Liu S, Qu H and Wang Z (2021). The novel nanocomplexes containing deoxycholic acid-grafted chitosan and oleanolic acid displays the hepatoprotective effect against CCl4-induced liver injury *in vivo*. *Int. J. Biol. Macromol.*, **185**(1): 338-349.

Xiong R, Shan S, Wang X, Zhang X, Yu H, Shi H and Wang X (2020). Aloperine attenuates carbon tetrachloride-induced mouse hepatic injury via Nrf2/HO-1 pathway. *Trop. J. Pharm. Res.*, **19**(5): 983-988.

Yeh YH, Hsieh YL, Lee YT and Hu CC (2012). Protective effects of Geloina eros extract against carbon tetrachloride-induced hepatotoxicity in rats. *Food Res. Int.*, **48**(2): 551-558.

Zhao E. Shen Y, Amir M, Farris AB and Czaja MJ (2020). Stathmin 1 induces murine hepatocyte proliferation and increased liver mass. *Hepatol. Commun.*, **4**(1): 38-49.