Protective Effect of the Red Quinoa Seeds versus Oxidative Stress Induced by Alloxan and Carbon Tetrachloride in Experimental Rats

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ABSTRACT

The Red Quinoa seeds (RQ seeds) shows a higher number of total phenolic and flavonoids content. Therefore, the protective effect of the RQ seeds versus oxidative stress induced by Alloxan and carbon tetrachloride (CCl_4) may treatment in rats. Fifty-six healthy male albino rats were randomly divided into three main groups. The first group of healthy-rats (8 rats) was negative control and fed on commercial diet. The second group (24 rats) received CCl_4 injections subcutaneously (for two weeks); while, the third group (24 rats) were fasted for 24 hours, injected with one dose prepared Alloxan by intraperitoneal injection to induce oxidative Stress. Then both main 2 groups were divided into 3 subgroups (8rats /each) and treated by different concentrations of RQ seeds as for 6 weeks. The rats were weighed weekly and at the end of the experimental, the animals were fasted overnight, anesthetized; sacrificed and weighted the organs excise" liver, kidney and Pancreas". Results showed that stress groups (Alloxan and CCl_4) were significantly increased risk for oxidative stress. However, the results indicated that a diet fortified at 5g and 10g RQ seeds improves weight gain, increased antioxidant enzymes (SOD, CAT and GPx), reduces liver enzymes, kidney function, lipid profiles, serum glucose and reduces the risk of organ functions (liver, kidney and pancreas), related to oxidative stress, when compared to stress groups. More specifically, a diet with 10g RQ seeds reduced the adverse effect of oxidative stress.

Key words: Red quinoa seeds; Oxidative stress; CCl_4; Alloxan.

INTRODUCTION

Quinoa (*Chenopodium quinoa Willd*) has been identified as a potential antioxidant source due to its high concentration of flavonoids, phenolic compounds, and other bioactive compounds. **(Ayyash, *et al*., 2018).** Quinoa is high in protein, contains all essential amino acids, is high in unsaturated fatty acids, and has a low glycemic index (GI). It also contains vitamins, minerals, and other beneficial compounds and is naturally gluten-free. Quinoa is a versatile grain that can be prepared in a variety of ways. **(Tang, *et al*., 2015**). The evidence suggests that phenolic compounds play an important role in health maintenance and disease risk reduction, with no negative side effects. **(Rein, *et al*., 2013),** and may have important roles in inhibiting free radicals, which avoids oxidative stress **(Pasko, *et al*., 2010a).**

Oxidative stress is defined as an imbalance between the occurrence of reactive oxygen species and the body's detoxification or repairing mechanisms. **(Romá-Mateo, *et al*., 2015).** When the normal redox state of cells is disrupted, toxic peroxides and free radicals are produced, which damage cell lipids, proteins, and DNA. Oxidative stress from oxidative metabolism can cause DNA strands to break, resulting in underlying damage. The generation of hydroxyl radicals, superoxide radicals, and hydrogen peroxide results in the indirect base damage of reactive oxygen species (ROS). **(Bhattacharya, 2015).** Because some reactive oxidative species also function as cellular messengers in redox signalling, oxidative stress can disrupt normal cellular signalling. In humans, oxidative stress is thought to be a possible cause of cancer and Alzheimer's disease. **(Romá-Mateo, *et al*., 2015)**, atherosclerosis **(Bonomini, *et al*., 2008)**, and depression **(Jiménez-Fernández, *et al*., 2015).** Toxic chemical exposure can cause hepatocyte damage via metabolic activation of reactive oxygen species (ROS) **(Kohen and Nyska, 2002).** Carbon tetrachloride (CCl_4) is a xenobiotic that is widely used in animal models to study hepatotoxicity by initiating lipid peroxidation. **(Khan, *et al*., 2012).** Through the formation of reactive metabolic trichloromethyl radicals (CCl3) and peroxy trichloromethyl radicals (OOCCl3), bio-activation of the phase I cytochrome P450 system induced by CCl_4 can cause

acute and chronic tissue injuries. Trichloromethyl can react with sulfhydryl groups (glutathione and protein thiols), as well as antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD). Trichloro-methyl-free radicals' overpro-duction enhances the membrane lipid peroxidation, finally leading to liver steatosis, fibrosis, or cirrhosis **(Hefnawy and Ramadan, 2013).** These free radicals can covalently bind to macromolecules such as proteins, lipids, and nucleic acids **(Khan, *et al*., 2012**).

Alloxan (the β-cell toxin) is a model substance in type 1 diabetes. ROS were produced as a result of the use of alloxan. Free radicals are formed during this redox reaction, which results in Alloxan's beta cell toxicity. **(Lenzen, 2008**). There is a significant relationship between diabetes and lipid profile abnormalities, which may induce a high risk of cardiovascular diseases **(Sangwan and Singh, 2018).**

The objective of the present research to study the protective effect of the RQ seeds against oxidative stress induced by Alloxan and CCl_4 treatment in rats.

MATERIALS &METHODS:

Materials:

Red Quinoa (*Chenopodium quinoa Willd*) seeds were obtained from Abu Auf stores for selling agricultural crops and medicinal and aromatic plants in Cairo - Egypt. Al - Gomhoria Chemicals Company in Egypt supplied the carbon tetrachloride and alloxane. The experimental animals (male albino white rats) were obtained from the Giza Agricultural Research Center's animal house. Commercial diet were obtained from Agricultural Research Center in Giza.

Methods:

Preparation of RQ seeds:

Red Quinoa Seeds (*Chenopodium quinoa*) were washed with water at 60ºC (with agitation) during one hour with seeds to water ratio of 1:10 (w/ w). Then, drying was carried out at 60ºC using a convective dryer and then ground according to **Margarerita, *et al.*, (2010).**

Chemical analysis of RQ seeds:

- **Nutritional composition** of RQ Seeds (moisture, protein, total fiber, fat and ash) was determined according to **AOAC (2012)** as follow: Moisture content of samples was determined using oven-dry technique. Protein content was determined using the Kjeldahl method (Gerhardt, Germany) and 5.83 multiplied the nitrogen content of the samples. Fat content was determined using a Soxhlet with petroleum ether as the extraction solvent. Total carbohydrate was calculated by difference. **Saponin** in Red quinoa seeds were determined using method according to **Uematsu, *et al*., (2000)**. **Total phenolic** content was determined using Folin-Ciocalteau regent **(McDonald, *et al*., 2001). Total flavonoids** were determined using aluminium chloride colorimetric method **(El-Olemy, *et al*., 1994). Antioxidant activity measured by DPPH assay:** The scavenging activity of the Red quinoa seeds on DPPH radicals was assayed according to the method of **(Roche, *et al*., 2005).** Thirty microliters of RQ seeds (40, 80 and 100 µg/ml) were mixed with 3 ml of 0.2 mM DPPH in HPLC methanol. Absorbance at 515 nm was determined after adding the extract. Inhibitory concentration at 50% was also calculated (IC_{50} values) and denoted as Trolox equivalent anti-oxidant capacity (TEAC). The DPPH radical scavenging activity of the test substance was calculated by the following equation:

$$\textbf{Antioxidant activity (\%)} = (A_{\text{Control}} - A_{\text{sample}})/A_{\text{control}} \times 100$$

- *Where: $A_{control}$, was the absorbance of the control sample and A_{sample}, the absorbance in the presence of the sample*

Biological experiment:

Fifty-six male Albino rats weighing about 150±20 g were obtained from the Agricultural Research Center, Giza, Egypt. The animal groups were kept in an atmosphere of filtered; pathogen-free air and water and maintained at a temperature between 20-25ºC with a 12 h light/dark cycle and light cycle (8-20 h) and relative humidity of 50%. The animals acclimatized for one week as an adaptation period. The animals were randomly divided into three main groups. The

first group of healthy group (8rats) was fed on commercial diet (protein 21, fat 3.3, Crude fiber 3.3, Dl. Methionine 0.4, Vitamins mixed 1, Minerals mixed 4, and) Carbohydrates 67.1) g %. While the second group (24 rats) injected with CCl_4 in paraffin oil (50% v/v 2ml/kg) twice per week by subcutaneous injection (for two weeks) to induce oxidative Stress **(Jayasekhar, *et al.*, 1997).** The third group (24 rats) were fasted for 24 hours, injected with prepared Alloxan using citrate buffer 0.1M (pH = 4.6) as vehicle, at a dose of 150 mg Alloxan/kg body weight by intraperitoneal injection according to **Szkudelski, (2001)**. At the third day, Alloxan injection was carried out, blood glucose was examined and animals with glucose concentration higher than 200 mg/dl were considered as diabetic rats and have oxidative Stress.

After that, the second and third main groups were divided into 3 subgroups of (eight rats each). The first subgroup fed commercial diet only for 6 weeks as represented stress/ positive control group. Treated subgroups (2 and 3) fed commercial diet supplemented with two doses of RQ seeds (5 and 10%) respectively. Rats were weighed weekly and at the end of the experimental feeding period, the animals were fasted overnight, anesthetized and sacrificed. They were then quickly dissected to excise the liver, kidney and P pancreas and weighted it.

Histopathology Technique:

Tissue samples from the liver, kidney and pancreas were fixed in 10% neutral formalin for 24 hours immediately after dissection, then collected and dehydrated in a concentration of alcohol, cleaned in xyline, and embedded in paraffin wax. Tissues were sectioned at 3 micron intervals and stained with hematoxylin and eosin. (**Banchroft and Stevens, 1996**). The light microscope for detection of any histopathological alteration were examined them.

Biological parameters:

Biological evaluation of the different tested diets was carried by determination of body weight gain (BWG %) and relative weight of organs according to **Chapman, *et al.*, (1959)**.

BWG% = [(Final weight - Initial weight) / (Initial weight)] X 100

Organ relative weight = (Organ weight / Final weight) X 100

Biochemical analysis:

Blood was drawn from the orbital plexus venous with fine capillary tubes, placed in centrifuge tubes without anticoagulant, and allowed to clot. After centrifugation (3000 rpm for 15 minutes), serum samples were analysed using bio-diagnostic kits.**Barham and Trinder, (1972),** described the determination of the Serum uric acid. Serum urea nitrogen was determined according to the method described by **Batton and Crouch, (1977)**. Method of **Tietz, (1986),** qualified to determine serum creatinine. Superoxide dismutase: was determined according to the method described by **Nishikimi, *et al*., (1972)**. Catalase was determined according to the method described by **Aebi, (1984)**. Glutathione peroxidase**:** was determined according to the method described by **Paglia and Valentine, (1967)**. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were determined by calorimetrically method of **Reitman and Frankel, (1957**). Total protein was determined according to the method described by **Gornall, *et al*., (1949)**. Albumin was determined according to the method described by **Doumas, (1971).** Total bilirubin, direct bilirubin and indirect bilirubin were determined according to the method described **Walter and Gerade, (1970)**. Determination of Serum Glucose in the serum was according to the method described by **Trinder, (1969)**. Determination of Serum Cholesterol was determined according to the method described by **Allain, *et al*., (1974)**. Serum Triglycerides (TG) were determined according to the method described by **Fossati and Principe, (1982)**. Evaluation of High-density lipoprotein cholesterol (HDL-c was determined according to the method described by **Burstein, (1970)**. Low-density lipoprotein cholesterol (LDL-c): calculated according to equation described by **Friedwald, *et al*., (1972)**. The concentration of the sample was calculated from the following equation:

LDL-c con. (mg/dl) = Total cholesterol - (TG /5 (vLDL) + HDL-c)

Statistical Analysis:

The collected data were subjected to an analysis of variance. Duncan's multiple range test was used to compare means at a 5% level of significance. The PROC ANOVA

procedure of the Statistical Analysis System **2006 (SAS)** was used for the analysis. **(Snedecor and Cochran, 1979).**

RESULTS & DISCUSSION

Saponin analysis:

Saponins, which are mostly found on the outside of quinoa, are not only anti-nutritional but also responsible for the bitter taste of quinoa. Their contents are determined by a variety of factors such as ecotype, irrigation level and water salinity, processing, and cooking conditions. **(Nowak, *et al*., 2016).** Despite the fact that there is no saponin legislation or maximum residue level set in the EU and UK **(Ojinnaka, 2016)**, was hinge is still the most common method used to remove saponins from quinoa seeds in order to improve their nutritional and sensory quality. **(Suárez-Estrella and Torri, 2018).**

The findings revealed that quinoa seeds contain saponin (0.516 %) and that soaking after washing was beneficial (0.152 %). According to **Koziol, (1992)**, quinoa was classified as "sweet" if it contained less than 0.11 % saponin on a fresh weight basis or "bitter" if it contained more than 0.11 % saponins. .**Gomez-Caravaca, *et al*., (2011**) mentioned that, Saponin content ranged from 5.6 to 7.5 % of total composition of whole quinoa flour.

Chemical analysis of RQ seeds:

Table (1) shows the approximate composition of Red quinoa seeds. Carbohydrates were found to be the most important macronutrient, followed by protein, fat, and ash. The proximate values of (RQ seeds) are comparable to those obtained by **Abd-Allah, *et al*., (2020)**.In addition to their nutritional value, the proteins of RQ seeds have been suggested to exert some beneficial effects and to be a source of bioactive peptides **(Vilcacundo, *et al*., 2018**). **Guzman - Maldonado and Paredes -Lopez, (1998)** reported that the protein content of quinoa is between 11.0 and 15.0% that agree with the present results.

Total phenolic compounds and total flavonoids content:

RQ seeds had a higher content of both total phenolic compounds (TPC) and total flavonoids (TF), as shown in Fig. (1) (9.97mg/g and 7.1 mg/g dry weight (D.W), respectively). These findings are comparable to those obtained by **Abd-Allah, *et al*., (2020)**. As a result, quinoa emerges as a viable option for the development of new

treatments and therapeutic options capable of preventing diseases associated with oxidative stress. Quinoa contains more phenolic compounds than other Andean pseudocereals such as kaiwa (Chenopodium pallidicaule) and kiwicha (Amaranthus caudatus) **(Repo-Carrasco-Valencia, *et al*., 2010)** and compared with traditional cereals such as wheat **(Alvarez-Jubete, *et al*., 2010).** Quinoa seeds are also a good source of flavonoids, with levels 5–10 times higher than in flavonoid-rich berries. (**Repo-carrasco-valencia, *et al*., 2010)**. Furthermore, the antioxidant activity of quinoa is highly correlated with the presence of phenolic compounds. **(Tang, *et al*., 2015).**

The radical scavenging effect was found to increase with increasing ratios of RQ seeds were given in (Table 2). A positive correlation was found between the total ascorbic acid (AC) and TPC agreeing with some reports in the literature, which have attributed the AC to other compounds apart from TPC, such as certain vitamins **(Orsavová, *et al*., 2019)**. The total AC is primarily attributed to the TPC and TF found in quinoa **(Liu, *et al*., 2020)**. Furthermore, the AC of RQ is related to its betalains content. **(Escribano, *et al*., 2017).**

Han, *et al*., 2019 discovered that RQ seeds had higher antioxidant activity than black quinoa. **Tang, *et al*., (2015)** discovered a link between total phenolic components in quinoa and DPPH. According to **Gordillo, *et al*., (2016),** who evaluated antioxidant potential extracted from Quinoa flour and from whole cereals (wheat, barley, millet, rice, and buckwheat), Quinoa presented higher antioxidant potential than the other whole cereals, implying that Quinoa possesses potent free radical-scavenging compounds.

Table (3) shows the final body weights and weight gain of all rat groups. The initial body weights of all rat groups were significantly lower when compared to the healthy group. Whereas the final body weights of all rat groups were not significantly different, with the exception of inducted groups (stress by CCl_4 group and stress by Alloxan group) which were significantly lower ($P \leq 0.05$).On the other hand, treatment groups with two doses of 5, 10% RQ seeds, increased body weight gain significantly. These findings were because the treatments used, which included

varying concentrations of RQ seeds, may improve appetite and increase weight gain. Furthermore, these findings are consistent with **Halaby, *et al*., (2017)**, who discovered that a diet fortified with 30% and 40% Quinoa seeds powder (QSP) can improve body weight gain, feed consumption, and feed efficiency ratio.

The data in table (4) revealed a significant increase ($P \leq 0.05$) in the relative weight of liver in the stress groups (Alloxan or CCl_4) when compared to the healthy group. As seen in the same table, after 6 weeks of treatment, there was a significant decrease ($P \leq 0.05$) in all treatment groups compared to stress groups. This finding is consistent with **Ali, (2019)** and **González, *et al*., (2014)**, who found that quinoa seeds significantly reduced liver weight while increasing heart and kidney weights. According to **Kanki, *et al*., (2003),** the liver is a detoxification organ that breaks down toxic substances and metabolites of the administered substances. The endoplasmic reticulum of the hepatocytes is responsible for this breakdown, and as a result, the hepatic cells are severely damaged**.** At the same trend, a significant increase ($P \leq 0.05$) in relative weight of kidney was observed in the two stress groups compared to the healthy group. When compared to the stress group, all treatment groups experienced a significant decrease ($P \leq 0.05$). The relative weight of the pancreas decreased significantly ($P \leq 0.05$) in both stress groups compared to the healthy group. All treatment groups were highly significant ($P \leq 0.05$) improved in relative weight of pancreas.

The results in (Table 5) revealed a significant ($P \leq 0.05$) reduction in antioxidant markers, SOD, CAT, and GPx in both oxidative stress groups when compared to the healthy group. The antioxidant system's depletion with the CCl_4 group could be attributed to CCl_4-generated cellular ROS production and the subsequent depletion of the antioxidant cellular system. **(Muhammad, *et al*., 2009)**. These findings are consistent with those of **Tirkey, *et al*., (2005)**, who found that CCl_4 causes significant oxidative stress in rat liver. Alloxan is a diabetic-induced cytotoxic agent that causes cell death and apoptosis through the production of reactive oxygen species (ROS), superoxide radicals, and hydrogen peroxide, resulting in hyperglycemia. **(Szkudelski, 2001)**. Alloxan-

induced diabetic animals experience oxidative stress as a result of persistent and chronic hyperglycemia, which depletes the activity of the antioxidative defence system and promotes the generation of de novo free radicals. **(Baynes and Thorpe, 1997).** After 6 weeks of treatment, the oxidative Stress groups fed different concentrations of RQ seeds showed a significant increase ($P \leq 0.05$) in SOD, CAT, and GPx levels when compared to the two oxidative Stress groups, with the exception of SOD in the Stress groups by Alloxan. Quinoa supplementation in the diet of oxidative stress-induced rats reduced plasma malondialdehyde levels and increased antioxidant enzyme activity. **(Pasko, *et al*., 2010).** As bioactive compounds, phenolic and flavonoids found in RQ seeds (see Fig.1) may be effective in preventing CCl_4-induced oxidative stress and liver inflammation. **Li, *et al*., (2018a)** investigated the ability of quinoa ethanolic extract to reduce oxidative stress and preserve antioxidant enzyme activity, as well as its ability to inhibit lipid peroxidation hydroxyl radical. Another possible mechanism is quinoa ethanolic extract's ability to correct cardiac cells' deficient thiol status by increasing GSH synthesis. Furthermore, quinoa ethanolic extract has a strong antioxidant effect, as it may prevent free radical-induced myocardium damage through its free radical scavenging effect, which may be mediated by polyphenols, phytosterols, and flavonoids in quinoa ethanolic extract. **(Konishi, *et al*., 2004).**

Table (6) shows the results of liver enzymes for all groups. When compared to the control group, CCl_4 and Alloxon stress caused a significant ($P<0.05$) increase in serum ALT and AST levels until the end of the experiment. Furthermore, when compared to the control group, CCl_4 and Alloxon stress caused a significant ($P<0.05$)) decrease in total protein, serum ALP, and total bilirubin content of tissues. This increase could be attributed to cellular damage and structural integrity leakage in the liver cells. Similarly, CCl_4 and Alloxon administration induced elevation of direct bilirubin and indirect bilirubin, which are considered indicators of cholestasis and pathological alterations of the biliary flow when compared with the healthy group. The presence of the

highest concentration of direct bilirubin in the serum indicates liver damage caused by CCl_4 and Alloxon. Similarly, other studies found that rats intoxicated with CCl_4 had higher levels of liver marker enzymes and bilirubin. **(Lalitsingh, *et al.*, 2010).** Moreover, CCl_4 has observed the histological changes of the liver, such as structure damage, hepatocellular necrosis, leucocyte infiltration, in stress groups and Alloxon animals (see Fig. 2). These findings are consistent with the findings of **Li, *et al*., (2011b)** during hepatocellular damage; these enzymes are released into the bloodstream from the cytoplasm following the rupture of the hepatic plasma membrane. Furthermore, CCl_4 destroys hepatic cells and blocks bile ducts, resulting in an increase in serum total bilirubin levels. **(Shah and Shah, 2012).** Furthermore, CCl_4 caused a significant decrease in serum albumin levels, the most important protein synthesized in the liver. This is consistent with the findings of **Li, *et al*., (2011 b)**, who considered this to be an indication of hepatocyte damage and loss of functional integrity. Intoxication with CCl_4 causes hypomethylation of cellular components; in the case of RNA, this is thought to inhibit protein synthesis. **(Lalitsingh, *et al*., 2010).**

Supplementation of RQ seeds in the diet of oxidative stress-induced rats when quinoa seeds 10g were given to rats, followed by quinoa seeds 5 g, liver functions were significantly restored when compared to both stress groups. Phenolic and flavonoids, which are bioactive compounds found in RQ seeds (see Fig.1), may have an efficient activity in preventing induced oxidative stress and liver inflammation. The findings agreed with those of **Ali, (2019)** and **Repo-Carrasco-Valencia, (2010)**, who discovered that enzyme activities such as SPGT and SGOT were significantly reduced in rats fed quinoa seeds. These findings are consistent with **Halaby, *et al*., (2017)**, who found that quinoa seeds reduced ALT (30%) and AST (40%) enzymes in rats with induced hypercholesterolemia.. Saxena, et al., (2017), confirmed the effects of quinoa extracts on elevated serum ALT and AST enzymes in rats against CCl4-induced oxidative stress**.** Previous research has also found anthocyanins, betacyanins, and flavonoids in RQ seed (quercetin and rutin).These compounds have been proven to

perform ant-oxidative and anti-inflammatory effects in rats with hepatic damage **(Lin, *et al*., 2019).**

Results presented in table (7) summarize the analysis of the different ratios of RQ seeds on serum urea, uric acid and creatinine in oxidative stress rats by CCl_4 and Alloxon -induced, when compared to the healthy group. Stress groups showed highly significant ($P<0.05$) elevation serum urea, uric acid and creatinine levels until the end of the experiment compared with healthy group. High creatinine levels indicate that a person is suffering from kidney failure, which can be caused by elevated cholesterol levels, as discovered by **Barakat and Mahmoud, (2011)**. Results from the current study closely correspond with those of **Zevallos, *et al*., (2014)** demonstrated that renal damage in hypercholesterolemia might be associated with an increase in serum urea nitrogen levels, are indicating glomerular and tubular kidney dysfunction. RQ seed supplementation in the diet was given to rats subjected to oxidative stress. When rats were given quinoa seeds 10g, followed by quinoa seeds 5 g, their kidney functions were significantly improved when compared to both stress groups. The current study's findings agree with the assumption of dietary fiber improves kidney function level **(Altunkaynak, *et al*., 2008).** Some quinoa vitamins and minerals are important because they act as antioxidants in kidney cell membranes, such as selenium, magnesium, phytosterols, folic acid, and tocopherols, which are thought to have antioxidant anti-carcinogenic properties or work as anti-inflammatory agents. **(Repo-Carrasco- valencia, *et al*., 2003).**

The results of lipid profile for every group are gathered in (Table 8). Stress by CCl_4 and Alloxon causes severe a significant ($P<0.05$) elevation in the serum levels of TC, TG, LDL-C and VLDL-C as compared with healthy group this was associated with significant decrease $p < 0.05$ in HDL-C in these groups levels

at the end of the experiment compared with healthy group. HDL-C promotes catabolism by assisting in the transport of excess cholesterol from peripheral tissue to the liver. **(Makni, *et al.*, 2008).** Furthermore, our findings are consistent with those of **Wang, *et al.*, (2012)**, who found that an increase in HDL-C ratio is one of the most important identifiers for any anti-hypercholesterolemia agent. The link between CCl_4 exposure and high serum lipid levels is complex, and it could be due to either increased lipoprotein synthesis or decreased removal of lipoproteins. Inhibiting hepatic lipoprotein lipase activity may result in decreased removal. **(Zhang, *et al.*, 2012).** Furthermore, it has been demonstrated that CCl_4 alters the activity of lipid metabolism enzymes in the liver. This can limit bile acid biosynthesis, which is the only significant route for cholesterol elimination from the body. **(Sun, *et al.*, 2012).**

RQ seeds supplementation in the diet administered to oxidative stress-induced rats significant ($P<0.05$) decrease in the serum levels of TC, TG, LDL-C and VLDL-C, and significant increase $p < 0.05$ in HDL-C was found in rats groups received quinoa seeds 10g, followed by quinoa seeds 5 g as compared to both stress groups. Dietary fiber from these grains can inhibit absorption of dietary cholesterol **(Takao, *et al.*, 2005)** and can bind to biliary acid, which may increase cholesterol catabolism or fermentation of the fiber in the colon and produce short-chain fatty acids contributing to decrease in cholesterol synthesis in the liver **(Escudero, *et al.*, 2006). Takao, *et al.*, (2005)** discovered that quinoa protein inhibits the expression of HMG-CoA reductase. Squalene is thought to be the cause of these changes because it is an inhibitor of this enzyme and improves triglyceride levels after adding protein fraction isolated from

quinoa seeds. A quinoa protein-enriched fraction was discovered to prevent the increase of total cholesterol levels in mice plasma and liver by inhibiting bile acid re-absorption in the small intestine and controlling cholesterol synthesis and catabolism. (**Aluko and Monu, 2003).**

Other authors speculated that the fibre, saponins, could cause the hypocholesterolemic effect of quinoa or squalene found in these seeds. **(Ryan, *et al*., 2007). Foucault, *et al*., (2011)** demonstrated the ability of quinoa to reduce serum glucose, (TG), and total and low-density lipoproteins (LDL) cholesterol levels in male Wister rats fed a fructose-enriched diet, thereby inhibiting the negative effects of fructose on (HDL). These findings suggest that quinoa can protect against oxidative stress by increasing antioxidant capacity and decreasing lipid peroxidation in animal plasma and tissues. Quinoa's ability to prevent obesity has also been studied. **(Farinazzi-Mach-ado, *et al*., 2012).**

The plasma glucose level results for each group are compiled in (Table 9). The level of glucose in the two stress groups was found to be significantly higher ($P < 0.05$) than in the healthy group. Through the generation of cytotoxic ROS, Alloxan selectively induces pancreatic β-cell death. **(Wang and Wang, 2017)** which contribute to the development and progression of diabetic complications **(Kaneto, *et al*., 2006).**

Significant reduction ($P < 0.05$) in the blood glucose levels was observed in rats groups received quinoa seeds 10g, followed by quinoa seeds 5 g as compared to both the stress groups. RQ seed contains a number of compounds that may be linked to its hypoglycemic effect. For starters, fibre may modulate the postprandial insulin response because it induces satiety, and some epidemiological studies have discovered an inverse relationship between dietary fiber

consumption and the development of type II diabetes. In addition, certain poly-hydroxylated steroids have been attributed to this bioactivity **(Graf, *et al*., 2015).** While the content of TPC and tocopherols was primarily responsible for lowering blood, glucose levels when a quinoa-based cereal bar was consumed on a daily basis **(Paśko, *et al*., 2010).** Furthermore, the protein content of quinoa was related to the low-glycemic index because this macronutrient slows digestion and stomach emptying. **(Shin, *et al*., 2013).**

Histopathological examination: Liver

In the current work, histopathological studies also supported the biochemical analysis and liver antioxidant enzyme activity. Histopathological examination of the liver sections from healthy group (normal rats fed on commercial diet only) showed no histopathological alteration and the normal histological structure of the central vein and surrounding hepatocytes in the parenchyma (Fig. 2). While in the CCL_4 stress group, vascular degeneration was detected in the cytoplasm of the hepatocytes in a diffuse manner, along with pyknosis in the nuclei of some of the other hepatic cells, as well as inflammatory cell infiltration in the portal area. Furthermore, the portal area showed hyperplasia, newly formed bile ducts, and portal vein dilatation (Fig.2). The histopathological results of CCl4 were similar to **(Eidi, *et al*., 2012)** Liver of animals affected by CCL_4 and fed on (5 g RQ seeds) showed apoptosis was detected in some few individual hepatocytes surrounding the central vein. In contrast, no histopathological alteration in liver was observed in the animals affected by CCl_4 and fed on (10 g RQ seeds) (Fig.2).These results agree with the results of table 5.

In the Alloxan stress group (fed a commercial diet), degenerative changes were detected in a diffuse manner all over the hepatocytes in the

parenchyma, along with a few inflammatory cells infiltration in the portal area, compared to the normal control (Fig.3). The livers of animals affected by Alloxan and fed on (5 g RQ seeds) showed few inflammatory cells infiltration in the portal area, whereas no histopathological changes in the livers of animals affected by Alloxan and fed on (10 g RQ seeds) were observed (Fig.3). The current study findings are consistent with those of **Saleem, *et al*., (2014)**, who found that members of the *Chenopodiaceae* family, such as *Chinopodium album and Chinopodium murale*, have significant hepatoprotective and antioxidant activity due to high concentrations of phytochemical compounds such as flavonoids and phenolic acids.

Kidney

Rats fed only commercial diet (healthy group) had no histopathological changes and the glomeruli and tubules at the cortex had normal histological structure (Fig.4). While animals fed, a commercial diet plus CCL_4 injections (stress group) showed degeneration in the tubular lining epithelium as well as necrobiotic changes in a diffuse manner, the glomerular tufts showed atrophy with congestion in the cortical blood vessels, as well as swelling and degeneration in the corticomedullary lining tubular epithelium (Fig.4). Furthermore, nuclear pyknosis was found in some lining tubular epithelium at the cortex of animals affected by CCL_4 and fed on (5 g RQ seeds). There were no histopathological changes in the kidneys of animals exposed to Alloxan and fed (10 g RQ seeds) (Fig.4). When animals were fed a commercial diet and given Alloxan (stress group), the tubular lining epithelium showed diffuse degenerative changes all over the cortical portion, whereas the glomerular tufts had vacuolization in the lining endothelium versus the normal group (Fig. 5). Nuclear pyknosis was found in some lining tubular epithelium at the cortex of animals affected by Alloxan and fed on (5 g RQ seeds).

Furthermore, no histopathological changes were observed in the kidneys of animals exposed to Alloxan and fed (10 g RQ seeds), as reported in (Fig.5). Some quinoa vitamins' bioactive ingredients are important because they act as antioxidants in kidney cell membranes, such as selenium, magnesium, phytosterols, folic acid, and tocopherols, which are thought to have antioxidant anti-carcinogenic properties or work as anti-inflammatory agents. **(Repo-Carrasco, 2003).**

Pancreas

The rats (healthy group) exhibited no histopathological changes, and the normal histological structure of the islands of Langerhans cells as the endocrine portion, as well as the acini and duct system as the exocrine portion, was observed (Fig.6). Furthermore, animals injected with CCL_4 (stress group), animals affected by CCL_4 and fed on (5 g RQ seeds), and animals fed on (10 g RQ seeds) showed no histopathological changes (Fig.6). Meanwhile, in animals injected with Alloxan (stress group), atrophy was observed in islet of Langerhans cells in a diffuse manner throughout the pancreatic lobules. Through the generation of cytotoxic ROS, Alloxan selectively induces pancreatic -cell death. **(Wang and Wang, 2017)** As recorded, there were no histopathological changes in animals exposed to Alloxan and fed (5 g RQ and 10 g RQ seeds) (Fig.7). Quinoa can regenerate antioxidant species, which can then attack free radicals and protect tissues from oxidative damage **(Matsuo, 2005)**.

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Table (1): Proximate composition percentage dry weight basis (D.W.) of RQ seeds

Samples	Moisture %	Protein %	Total fiber%	Fat %	Ash %	Total Carbohydrate %
Red quinoa seeds	12.00	14.24	21.86	9.44	1.58	40.88

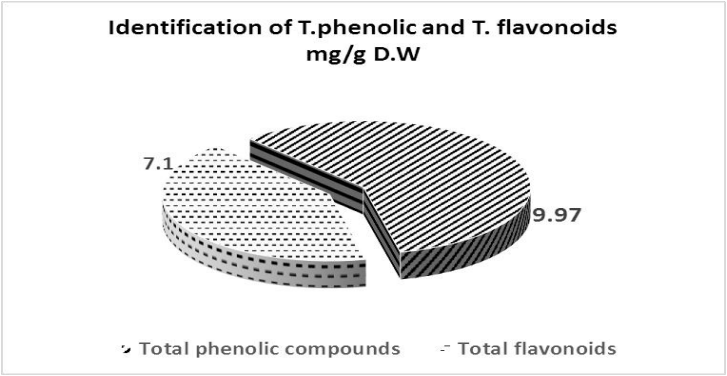

***Fig. (1)**: Identification of Total phenolic compounds and Total flavonoids of RQ seeds*

Table (2): The free radical scavenging assay using different ratios of RQ seeds mg / ml sample and ascorbic acid

Sample	Sample concentration (mg/ml)	DPPH%	IC_{50}
RQ seeds	40	51.581	38.77
	80	53.063	
	100	53.557	
Ascorbic acid	40	95.010	21.05
	80	97.184	
	100	98.419	

Table (3): Mean body weight gain (g) of experimental rats, which inducted oxidative stress by CCl_4 and Alloxon and treated with different concentrations of RQ seeds.

Body weight (g)	Groups					
	Healthy group (G1)	Stress groups by CCl_4 (G2)			Stress groups by Alloxan (G3)		
		stress only	stress + 5g(RQ)	stress + 10g (RQ)	stress only	stress + 5g(RQ)	stress + 10g (RQ)
IBW	170.00^{Abd} ±10.5	175.66^{A} ^{Bb}±9.85	180.8^{Ac} ±7.33	178.33^{Ae} ±6.02	179.83^{Aa} ±14.43	165.16^{Be} ±5.49	176.66^{ABf} ±10.31
FBW	226. 5 ^{Ca} ±17.59	191.67^{D} ^{a} ±10.31	266.5 ^{Ba} ±17.90	277.83^{Aa} ±8.82	201.83Da ±18.35	251.67^{Ba} ±20.08	259.17^{Ba} ±9.5
BWG/ wk %	33.24^{C} ±6.21	9.13^{D} ±1.50	47.28^{B} ±5.57	55.85^{A} ±4.27	12.17^{D} ±3.26	52.18^{A} ±7.59	46.96^{B} ±6.88

** Data are presented as means ± SDM (n=8).*

a, b, c and d: Means with different letter among treatments in the same column are significantly different ($P \leq 0.05$)

A, B, C and D: Means with different letter among treatments in the same row are significantly different ($P \leq 0.05$)

IBW= Initial body weight; FBW= Final body weight; BWG= Body Weight gain; RQ: Red quinoa seeds;

Table (4): Mean relative weight of organs for rats, which inducted oxidative stress by CCl_4 or Alloxon and treated with different concentrations of RQ seeds.

Organs weight (%)	Groups						
	Healthy group (G1)	Stress groups by CCl_4 (G2)			Stress groups by Alloxan (G3)		
		stress only	stress + 5g(RQ)	stress + 10g(RQ)	stress only	stress + 5g(RQ)	stress + 10g(RQ)
Liver	2.61^{CD} ±0.24	4.69^{A} ±0.48	2.57^{CD} ±0.36	2.35^{D} ±0.28	3.99^{B} ±0.60	2.89^{C} ±0.39	2.31^{D} ±0.37
Kidney	0.61^{CB} ±0.04	0.79^{A} ±0.07	0.72^{AB} ±0.09	0.53^{C} ±0.041	0.75^{Ab} ±0.25	0.65^{ABC} ±0.07	0.56^{C} ±0.10
Pancreas	0.20^{A} ±0.02	0.08^{B} ±0.02	0.19^{A} ±0.04	0.20^{A} ±0.03	0.07^{B} ±0.02	0.16^{A} ±0.03	0.21^{A} ±0.6

** Data are presented as means ± SDM (n=8). RQ: Red quinoa seeds*

A, B, C and D: Means with different letter among treatments in the same row are significantly different ($P \leq 0.05$)

Table (5): CAT, SOD and GPx (U/I) levels of experimental rats, which inducted oxidative stress by CCl_4 and Alloxon and treated with different concentrations of RQ seeds.

Parameters (U/L)	Groups						
	Healthy group (G1)	Stress groups by CCl_4 (G2)			Stress groups by Alloxan (G3)		
		stress only	stress + 5g(RQ)	stress + 10g (RQ)	stress only	stress + 5g(RQ)	stress + 10g(RQ)
SOD	21.30^{A} ±0.53	14.64^{B} ±0.11	17.93^{AB} ±0.7	20.17^{A} ±1.12	13.96^{B} ±0.34	14.14^{B} ±0.34	14.58^{B} ±6.7
CAT	3.62^{AB} ±0.10	2.54^{C} ±0.10	3.13^{B} ±0.69	3.49^{AB} ±0.07	2.43^{C} ±0.15	3.38^{AB} ±0.05	3.84^{A} ±0.13
GPx	3.86^{A} ±0.09	1.74^{D} ±0.14	2.90^{C} ±0.08	3.56^{B} ±0.075	1.870^{D} ±0.06	3.67^{AB} ±0.19	3.87^{A} ±0.08

** Data are presented as means ± SDM (n=8).*

A, B, C and D: Means with different letter among treatments in the same row are significantly different ($P \leq 0.05$)

RQ: Red quinoa seeds; SOD: superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase

Table (6): Liver functions of experimental rats, which inducted oxidative stress by CCl_4 and Alloxon and fed on different concentrations of RQ seeds.

Parameters	Groups						
	Healthy group (G1)	Stress groups by CCl_4 (G2)			Stress groups by Alloxan (G3)		
		stress only	stress + 5g(RQ)	stress + 10g (RQ)	stress only	stress + 5g(RQ)	stress + 10g (RQ)
ALT(U/I)	29.00^{E} ±2.61	84.33^{A} ±8.76	37.16^{CD} ±5.64	32.66^{ED} ±3.7	75.16^{B} ±5.95	42.83^{C} ±3.9	37.83^{CD} ±3.19
AST(U/I)	61.83^{F} ±2.32	172.00^{A} ±11.4	92.50^{C} ±5.32	76.50^{EF} ± 4.4	142.00^{B} ±10.4	82.66^{D} ±5.75	72.66^{E} ±5.28
Total Protein (g /dl)	6.60^{AB} ±0.82	3.65^{D} ±0.302	5.63^{C} ±0.28	6.50^{AB} ±0.44	3.83^{D} ±0.39	6.06^{CB} ±0.52	6.71^{A} ±0.45
Albumin (mg /dl)	3.20^{A} ±0.25	2.20^{B} ±0.21	3.11^{A} ±0.12	3.30^{A} ±0.21	2.31^{B} ±0.28	3.10^{A} ±0.18	3.35^{A} ±0.3
Total Bilirubin (mg /dl)	0.26^{B} ±0.05	0.56^{C} ±0.06	0.27^{B} ±0.05	0.21^{A} ±0.04	0.64^{C} ±0.04	0.26^{A} ±0.04	0.17^{A} ±0.05
Direct Bilirubin (mg /dl)	0.11^{AB} ±0.04	0.24^{C} ±0.03	0.16^{AB} ±0.06	0.10^{AB} ±0.05	0.29^{C} ±0.01	0.10^{AB} ±0.04	0.09^{A} ±0.09
Indirect Bilirubin (mg /dl)	0.16^{AB} ±0.04	0.32^{C} ±0.03	0.11^{AB} ±0.08	0.11^{AB} ±0.03	0.35^{C} ±0.05	0.16^{BC} ±0.06	0.08^{A} ±0.08

** Data are presented as means ± SDM (n=8). Data in a row with different superscript letters are statistically different (P ≤ 0.05). AST: aspartate amino transferase; ALT: alanine amino transferase; RQ: Red quinoa seeds;*

Table (7): Kidney function (mg/dl) of experimental rats, which inducted oxidative stress by CCl_4 and Alloxon and fed on different concentrations of RQ seeds.

Parameters	Groups					
	Healthy group (G1)	Stress groups by CCl_4 (G2)			Stress groups by Alloxan (G3)	
		stress only	stress + 5g(RQ)	stress + 10g (RQ)	stress only	stress + 5g (RQ)	stress + 10g (RQ)
Urea (mg/dl)	36.83^{DC} ±3.6	57.83^{A} ±5.34	43.33^{B} ±3.39	36.50^{DC} ±5.75	59.33^{A} ±3.98	41.00^{BC} ±7.56	32.33^{D} ±3.14
Uric Acid (mg/dl)	2.50^{B} ±0.29	3.26^{A} ±0.175	2.23^{BC} ±0.207	2.15^{C} ±0.122	3.26^{A} ±0.327	2.31^{BC} ±0.293	2.20^{BC} ±0.29
Creatinine (mg/dl)	0.43^{C} ±0.05	0.61^{AB} ±0.03	0.40^{CD} ±0.06	0.30^{D} ±0.089	0.68^{A} ±0.16	0.51^{CB} ±0.09	0.30^{D} ±0.09

* Data are presented as means ± SDM (*n=8).* Data in a row with different superscript letters are statistically different ($P \leq 0.05$). RQ**:** Red quinoa seeds**;**

Table (8): Effect of different concentrations of RQ seeds on serum Lipid Profile (mg/dl) in various group of rats, which produced oxidative stress by CCl_4 and Alloxon induction

Parameters	Groups						
	Healthy group (G1)	Stress groups by CCl_4 (G2)			Stress groups by Alloxan (G3)		
		stress only	stress + 5g(RQ)	stress + 10g (RQ)	stress only	stress + 5g(RQ)	stress + 10g (RQ)
TC (mg/dl)	95.95^{EF}± 3.47	162.36^{B} ±4.54	117.83^{C} ±6.8	99.46^{E} ±3.35	220.33^{A} ±8.4	109.66^{D} ±5.7	91.00^{F} ±4.6
LDL-c (mg/dl)	23.88^{E}±5. 26	121.16^{B} ±6.6	57.06^{C}± 4.84	24.93^{E} ±1.69	179.40^{A} ±3.41	40.86^{D} ±1.89	21.76^{E} ±1.27
HDL-c (mg/dl)	62.66^{AB}± 2.94	29.00^{D} ±4.77	51.16^{C}± 4.31	65.66^{A}± 2.34	23.00^{E}± 1.41	59.83^{B} ±1.45	60.50^{B} ±0.99
TG (mg/dl)	45.50^{C}±2 .43	61.00^{B} ±7.38	48.0^{C} ±5.48	44.33^{C} ±3.78	89.66^{A} ±1.45	44.83^{C} ±0.87	43.66^{C} ±1.05
VLDLc (mg/dl)	9.10^{C} ±0.49	12.20^{B} ±1.48	9.60^{C} ±1.1	8.86^{C} ±0.76	17.93^{A} ±0.29	8.96^{C} ±0.17	8.73^{C} ±0.21

Data are presented as means ± SE (n=8). Data in a row with different superscript letters are statistically different (P ≤ 0.05). TC: Serum total cholesterol; TG: Serum triglyceride HDL-C: High-density lipoproteins cholesterol; LDL-C: Serum low-density lipoproteins cholesterol; VLDLc: Serum very low-density lipoproteins cholesterol

Table (9): Effect of different concentrations of RQ seeds on blood glucose concentration (mg/dl) of experimental rats, which inducted oxidative stress by CCl_4 and Alloxon.

Parameter	Groups						
	Healthy group (G1)	Stress groups by CCl_4 (G2)			Stress groups by Alloxan (G3)		
		stress only	stress + 5g(RQ)	stress + 10g(RQ)	stress only	stress + 5g(RQ)	stress + 10g (RQ)
Glucose	97.00^{Db} ±1.7	154.66^{c} ^{d} ±3.4	129.50^{Cf} ±3.3	102.16^{Dg} ±4.2	249.17^{Aa} ±20.8	131.83^{Cf} ±3.1	100.67^{Dg} ±2.7

** Data are presented as means ± SDM (n=8).*

a, b, c and d: Means with different letter among treatments in the same column are significantly different ($P \leq 0.05$)

A, B, C and D: Means with different letter among treatments in the same row are significantly different ($P \leq 0.05$)

RQ: Red quinoa seeds; WK: Week

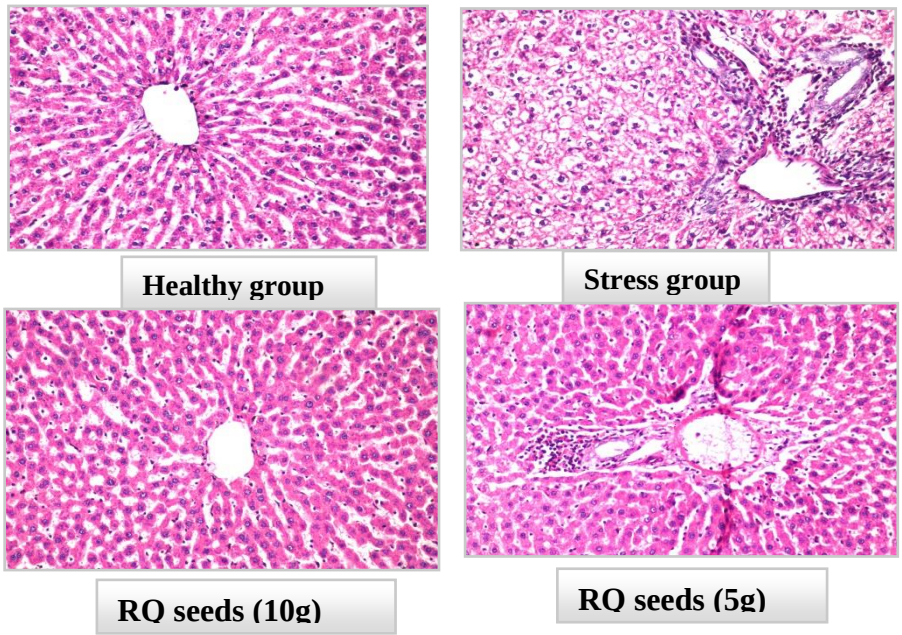

Fig. (2): photomicrographs of hematoxylin – eosin stained liver in CCl_4 induced oxidative stress in liver

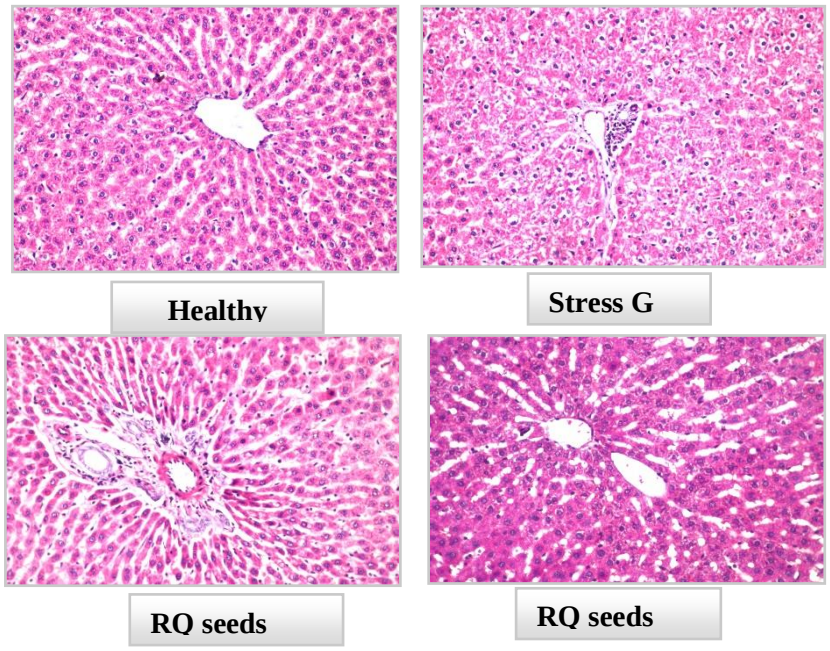

Fig. (3): photomicrographs of hematoxylin – eosin stained liver In Alloxan induced oxidative stress in liver

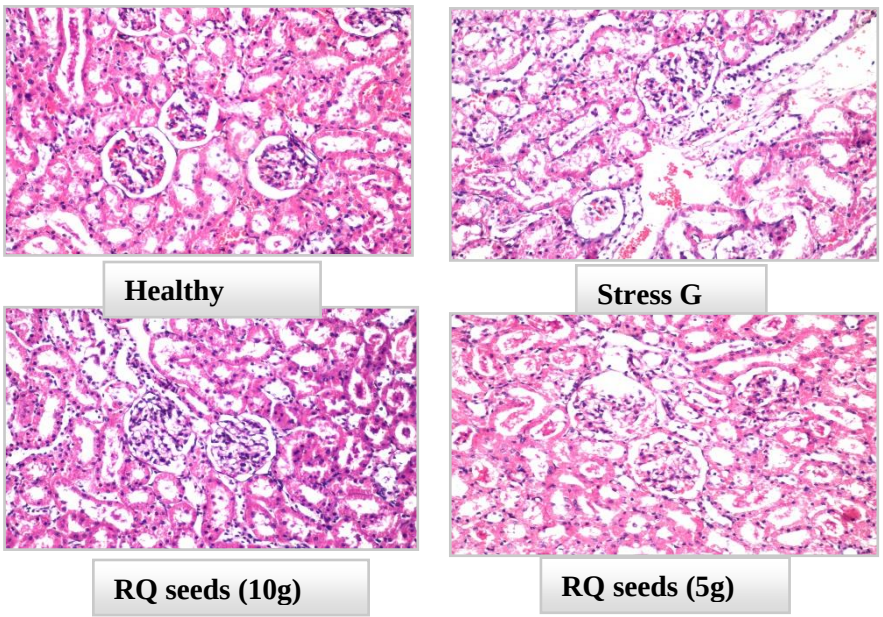

Fig (4): photomicrographs of hematoxylin – eosin stained kidney in CCl_4 induced oxidative stress in Kidney

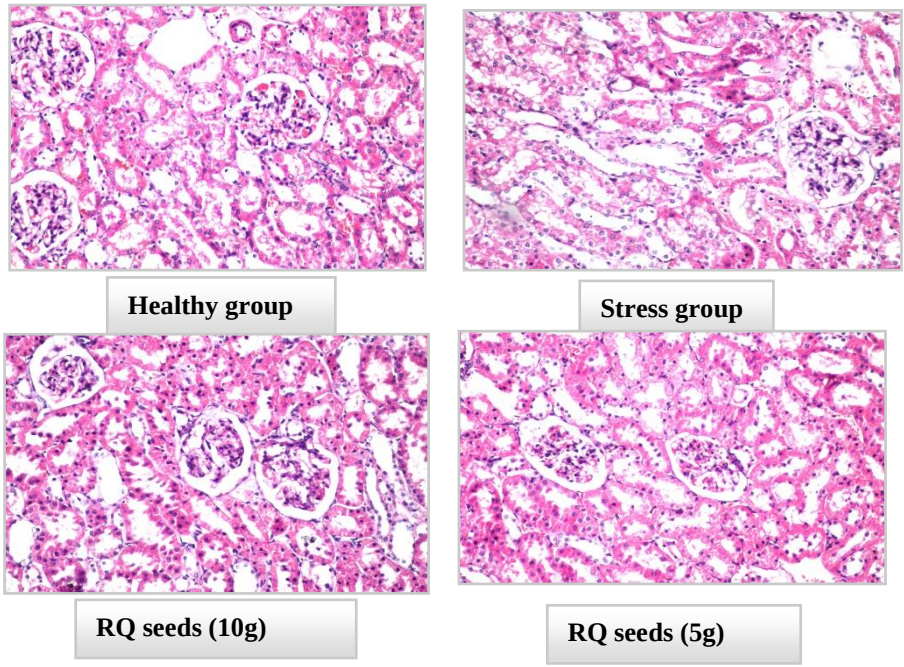

Fig (5): photomicrographs of hematoxylin – eosin stained kidney in Alloxan induced oxidative stress in Kidney

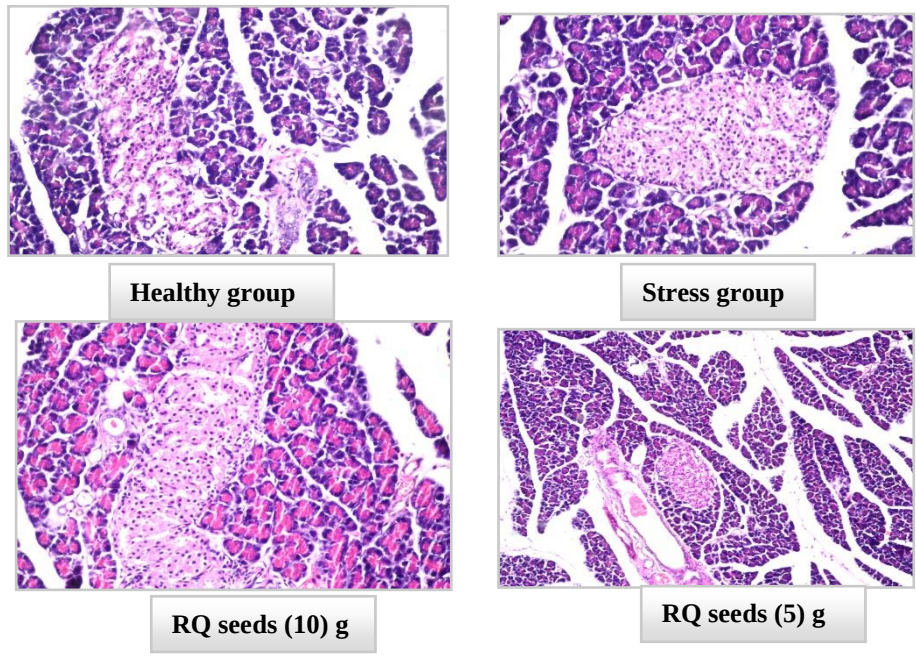

Fig (6): photomicrographs of hematoxylin – eosin stained kidney in CCl_4 induced oxidative stress in pancreas

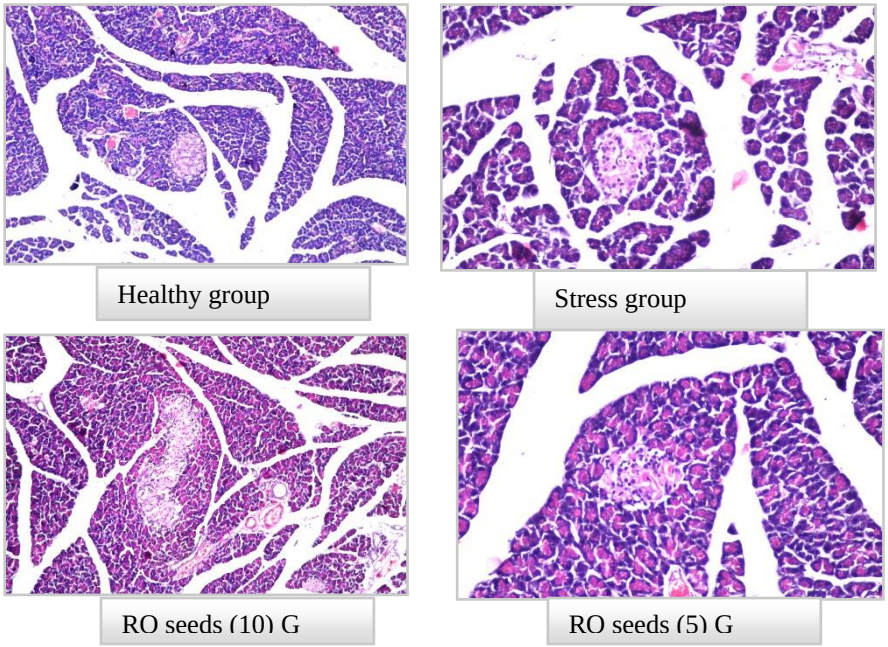

Fig (7): photomicrographs of hematoxylin – eosin stained kidney in Alloxan induced oxidative stress in pancreas

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التأثير الوقائي لبذور الكينوا الحمراء ازاء الإجهاد التأكسدي الناجم عن الألوكسان ورابع كلوريد الكربون في الجرذان التجريبية

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الملخص العربي

تحتوي (بذورالكينوا الحمراء) على المركبات الفينولية والفلافونويد. لذلك وجدت ارتباطات إيجابية بين إجمالي المكونات الفينولية و اختبار القدرة التأكسدية. تم تقسيم ستة وخمسين من ذكور الجرذان البيضاء إلى ثلاث مجموعات رئيسية. تم تغذية المجموعة الأولى من الحيوانات السليمة على نظام غذائي قياسي. بينما تم حقن المجموعة الثانية (٢٤ جرذ) برابع كلوريد الكربون عن طريق الحقن تحت الجلد (لمدة أسبوعين) للحث على الإجهاد التأكسدي ، ثم قسمت الفئران إلى ٣ مجموعات فرعية (ثمانية جرذان لكل منها) وعولجت بتركيز مختلف من بذورالكينوا الحمراء. المجموعة الثالثة (٢٤ جرذ) منعوا من الطعام لمدة ٢٤ ساعة ، ثم تم اجراء حقن ألوكسان عن طريق الحقن داخل الصفاق. في اليوم الثالث ، من حقن الألوكسان وفحص نسبة الجلوكوز في الدم. قسمت الجرذان إلى ٣ مجموعات فرعية (كل منها ثمانية جرذ) وعولجت بتركيزان مختلفان من بذور الكينوا الحمراء لمدة ٦ أسابيع. تم وزن الفئران أسبوعيًا وفي نهاية فترة التغذية التجريبية ، تم صيام الحيوانات طوال الليل وتخديرها وتشريحها. ثم تم استئصال الكبد والكلى والبنكرياس. تم وزن الأعضاء، خلص البحث إلى أن مجموعات الإجهاد التأكسدي التي تم حقنها بالألوكسان ورابع كلوريد الكربون كانت معرضة بشكل ملحوظ لخطر الأكسدة. ومع ذلك ، أشارت النتائج إلى أن اتباع نظام غذائي مدعم بنسبة ١٠ جم من بذور الكينوا الحمراء يقلل من خطرالإجهاد التأكسدي .

الكلمات المفتاحية: *بذور الكينوا الحمراء ؛ مضادات الأكسدة ، رابع كلوريد الكربون؛ ألوكسان.*