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DETECTING THE ROLE OF QUINOA AGAINST THE TOXICITY OF HYDROGEN PEROXIDE, WHICH CAUSES DAMAGE TO TESTICULAR TISSUE, AND SOME PHYSIOLOGICAL PARAMETERS IN MALE RATS

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Abstract: The current study aims to reveal the role of Quinoa seeds against the toxicity of hydrogen peroxide, which causes damage to testicular tissue and some physiological parameters in male rats. In this study 32 adult male albino rats, (wt 180-260 gm with age 3-5 month) obtained from Veterinary college/ Kirkuk University. the rats were randomly divided into four groups, control (n=8), and experimental (n=24) groups. The control group just received 4ml distilled water daily. However, the experimental groups divided into three groups each included 8 male albino rat. The findings showed that the concentration of testosterone (TE), follicle stimulating hormone (FSH) and luteinizing hormone (LH) in second group show significant ($P<0.05$) reduced compared with control rats. While, the concentration of TE, FSH and LH in third and fourth groups show non-significant changes ($P<0.05$) compared with control rats. For oxidative status, that the concentration of malonedialdehyied (MDA), glutathione (GSH) and catalase in second group show significant ($P<0.05$) elevated compared with control rats. While, the concentration of MDA, GSH and catalase in third and fourth groups show non-significant changes ($P<0.05$) compared with control rats. For histological study, the cross section taken from the second group demonstrates a decrease in the number of spermatogonia and spermatocytes as well as the absence of spermatid, the sections that were obtained from the third and fourth groups display spermatogonia, spermatocytes, and spermatids in a semi-normal structure. Therefore, it is concluded from the current study that Quinoa seeds extract has antioxidant activity and enhances and improves the effectiveness of the male reproductive system.

Keywords: Quinoa; Hydrogen Peroxide; Oxidative Stress; MDA; Catalase.

Introduction

Quinoa is a highly nutritious plant that is a member of the Amaranthaceae family. Chenopodium quinoa Wild is its scientific name (fig. 1). It is extremely suited to the various soil types and climate zones, having been domesticated in the Andean highlands [1-3]. Quinoa comes in

250 types and is sold globally. Its classification is determined by the morphology of the plant or by the color of the plant and fruits [4-5]. It is approved for planting throughout Europe, North America, Asia, and Africa. In 1993, the European Community authorized the initiative "Quinoa: a multipurpose crop" [4]. Since the protein levels in quinoa are higher than those in cereals like wheat, rice, and maize and comparable to those in milk, it is regarded as one of the greatest sources of protein from vegetables. Because quinoa is so adaptable to satisfying human needs in space, the National Aeronautics and Space Administration (NASA) has also used it [6–8]. The main portions of the plant that are edible are the seeds of quinoa, which are abundant in lysine, methionine, and carbs (77.6%), protein (12.9%), fats (6.5%), and other amino acids [9]. Compared to cereals like oats, wheat, corn, and rice, it has a higher carbohydrate content [10]. Furthermore, compared to wheat and rice, its seeds contain higher amounts of iron, calcium, potassium, copper, magnesium, and Manganese [11]. Antioxidants including phenolics, ascorbic acid, and carotenoids abound in quinoa seeds [12]. The main source of antioxidant capacity, which is crucial for preventing inflammation and cancer, is these antioxidant chemicals [13]. Quinoa is becoming more and more popular due to its numerous health benefits as well as the fact that it can be used to make gluten-free recipes for those with celiac disease [14]. The greatest prolamine amount found (<2.56 mg/kg) is far less than the threshold needed (<20 mg/kg) for gluten-free food [15]. A number of enzyme systems produce hydrogen peroxide (H2O2), a non-radiative reactive oxygen species, in vivo. Superoxide anion radical (O2) also produces it intraclullarly. H2O2 is a poor oxidizing and reducing agent when used in vivo [16]. Despite being moderately reactive, H2O2 is primarily hazardous when it undergoes the fenton or Halser-Weiss reaction, which converts it into extremely toxic hydroxyl radicals (OH) [17]. Lipid peroxidation (LPO) and the consequent oxidative stress caused by reactive oxygen species (ROS), such as H2O2, are strongly suggested to have a role in the etiology of several illnesses [18–19]. Therefore, the current study aims to reveal the role of Quinoa seeds against the toxicity of hydrogen peroxide, which causes damage to testicular tissue and some physiological parameters in male rats.

Figure (1): the aerial parts of quinoa and its seeds

Methods

Animal model

In order to make that everything is normal and there are no infections, 32 mature male albino rats (weighing between 180 and 260 gm and ages three to five months) were procured from Kirkuk University and the Veterinary College. They were fed a regular pellet diet for two weeks.

Plant extract

The aqueous extract was made using the procedure described in [20], which involved mixing

250 ml of distilled water with 25 g of quinoa powder (1:10), and then using a vibrator to stir the mixture for a while. After 30 minutes at a speed of 150 m/min, let the mixture soak in the refrigerator for 24 hours. Next, strain it through multiple layers of gauze to remove any insoluble plant material. Next, strain it once more using Whatmann No. 1 filter papers. Finally, remove the filtrate and use a device to dry it. For the purpose of creating the concentrations used in the study, the extract is dried under refrigeration (lyophilization) (ALPH 1-2 LD pluo), and the dry powder is stored in an airtight, sterile container in the refrigerator at 4°C until needed [21], the procedure was then repeated. Using the same procedures in succession until a suitable volume of extract is obtained.

Animal groups

The guidelines for laboratory animal care were followed in the treatment of every animal. The rats were then split into four groups at random: eight control groups and twenty-four experimental groups. Every day, the control group was given 4 milliliters of distilled water. Nonetheless, the experimental groups were split into three groups, each consisting of eight male albino rats. The second group (SG) was given 1% hydrogen peroxide in their drinking water for thirty days, the third group (TG) was given 150 mg/kg/day of quinoa, and the fourth group (FG) was given 150 mg/kg/day of quinoa orally along with 50 mg of hydrogen peroxide dissolved in their drinking water for thirty days.

Measurements

Sexual hormones

The concentrations of sexual hormones (testosterone, FSH and LH) were measured using the ELIZA technique and according to the test kit manufacturer's description.

Oxidative status

Using a spectrophotometer, MDA was determined using the colorimetric reaction with thiobarbituric acid (TBA) [22]. The GSH content was calculated by adding 0.5 milliliters of 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB) to 2.3 milliliters of buffer and 0.2 milliliters of the sample. A spectrophotometer was used to evaluate the combination [23]. Catalase was tested using the Biovision-USA kit method.

Histological study

A 4mm punch was used to take testis samples, and 2% xylocaine was administered as an anesthetic. The biopsies underwent standard processing, were embedded in paraffin slices, fixed in 10% formalin, and stained with hematoxylin and eosin before being viewed under a microscope. [24].

Statistical analysis

Minitab was used as a statistical tool for data analysis. Using a one-way analysis of variance (ANOVA), a statistical difference between the means of the experimental groups was examined.

Results and Discussion

Sexual hormones

The concentration of TE (2.04 ± 0.59), FSH (3.17 ± 0.56) and LH (1.32 ± 0.22) in second group show significant ($P<0.05$) reduced compared with control rats (5.72 ± 1.31; 6.47 ± 1.48 and 4.47 ± 1.52 respectively). The concentration of TE (6.03 ± 2.35; 5.19 ± 1.52 respectively), FSH (6.81 ± 1.39; 5.84 ± 1.61 respectively) and LH (4.35 ± 1.14; 4.09 ± 1.29 respectively) in third and fourth groups show non-significant changes ($P<0.05$) compared with control rats as shown in table (1).

Table (1): The concentration of sexual hormones in serum

Parameters / Groups	TE (ng/ml)	FSH (ng/ml)	LH (ng/ml)
CG	5.72 ± 1.31 a	6.47 ± 1.48 a	4.47 ± 1.52 a
SG	2.04 ± 0.59 b	3.17 ± 0.56 b	1.32 ± 0.22 b
TG	6.03 ± 2.35 a	6.81 ± 1.39 a	4.35 ± 1.14 a
FG	5.19 ± 1.52 a	5.84 ± 1.61 a	4.09 ± 1.29 a

When compared to other groups, the testosterone, FSH, and LH concentrations were significantly lower in the rats in the ST group (which received 1% H2O2) than in the other groups. Other investigations produced similar findings [25–26]. The pituitary gland may be impacted by hydrogen peroxide poisoning, which could reduce gonadotropin production, limit steroid manufacture by Leydig cells, and ultimately lower testosterone concentration [27]. Testicular histological alterations in the H2O2 treated group indicated a decrease in testosterone levels, according to the current study. Using quinoa extract, it was discovered in the current study that this extract effectively reduces the harmful effects of hydrogen peroxide, which lowers sexual hormones. The quinoa plant extract's abundance in phenolic compounds, carotenoids, phytosterols, phytoecdysteroids, saponins, betalains, squalene, and phagopyritols accounts for its capacity to affect this treatment [28]. Quinoa belongs to the primary phenolic category, which is made up of flavonoids and phenolic acids. These seeds contain 23 phenolic chemicals, either in free or conjugated forms, according to recent studies. Furthermore, phenolic acids, which include ferulic acid, vanillic acid, and their derivatives, as well as syringaldehyde, quercetin, and kaempferol, are the primary substances found [29–30]. Rats that fed varying amounts of quinoa seed powder in the Wahba et al. study [31] had significantly higher serum concentrations of luteinizing hormone, follicle-stimulating hormone, and testosterone than the positive control group. Additionally, rats' lipid profiles and antioxidant markers increased when quinoa seed powder was administered at various doses. According to the study's findings, quinoa seed powder has strong antioxidant and anti-inflammatory properties. It is also a rich source of minerals and vitamins.

Oxidative status

The levels of MDA (2.38 ± 0.27), GSH (0.129 ± 0.017) and enzyme catalase (0.56 ± 0.17) in second group show high significant changes ($P < 0.05$) compared with control rats (1.42 ± 0.17; 0.281 ± 0.025 and 1.27 ± 0.08 respectively). The levels of MDA (1.15 ± 0.21; 1.53 ± 0.12 respectively), GSH (0.307 ± 0.021; 0.274 ± 0.022 respectively) and catalase (1.35 ± 0.03; 1.13 ± 0.07 respectively) in third and fourth groups show non-significant changes ($P < 0.05$) compared with control rats as shown in table (2).

Table (2): The levels of MDA, GSH and CAT in testis

Parameters / Groups	MDA (mmol/l)	GSH (mol/l)	Catalase (mmol/l)
CG	1.42 ± 0.17 b	0.281 ± 0.025 a	1.27 ± 0.08 a
SG	2.38 ± 0.27 a	0.129 ± 0.017 b	0.56 ± 0.17 b
TG	1.15 ± 0.21 b	0.307 ± 0.021 a	1.35 ± 0.03 a
FG	1.53 ± 0.12 b	0.274 ± 0.022 a	1.13 ± 0.07 a

Similar letters mean there are no significant differences, different letters mean there are significant differences

In addition, there was a noteworthy difference in GSH and MDA following H2O2 in comparison to the control. Considerable reduction in GSH, catalase, and increase in MDA concentration ($p<0.05$) were noted in comparison to the control group's results. Numerous writers have established the oxidative effect of H2O2 in a variety of illness conditions. Superoxide anion and other oxidants, such as H2O2, will grow tenfold under heavy exposure to ROS, including H2O2. This will raise the demand on the body's antioxidant defense system, including GSH, and eventually cause antioxidant depletion [32]. Moreover, an enhanced rise in MDA levels and a decrease in the GSH content of different tissues and blood could accompany the hypercholesterolemia brought on by H2O2 exposure in the current experiment [33–34]. Using quinoa extract in the current investigation revealed that the extract has an antioxidant role because it raised GSH and catalase levels and decreased MDA levels. According to Pasko et al. [35], rats under oxidative stress showed decreased levels of plasma malondialdehyde, antioxidant enzyme activity, and lipid peroxidation when fed a quinoa-based diet. Quinoa was found to be able to lower oxidative stress in the testis, kidney, heart, pancreas, plasma, and lungs in the same study.

Histological study

The sections obtained with the control group, as depicted in figure (2), demonstrate normal spermatogonia, spermatocyte, and spermatid structure. As seen in figure (3), the cross section taken from the second group demonstrates a decrease in the number of spermatogonia and spermatocytes as well as the absence of spermatid. As seen in figure (4-5), the sections that were generated from the third and fourth groups display spermatogonia, spermatocytes, and spermatids in a semi-normal structure.

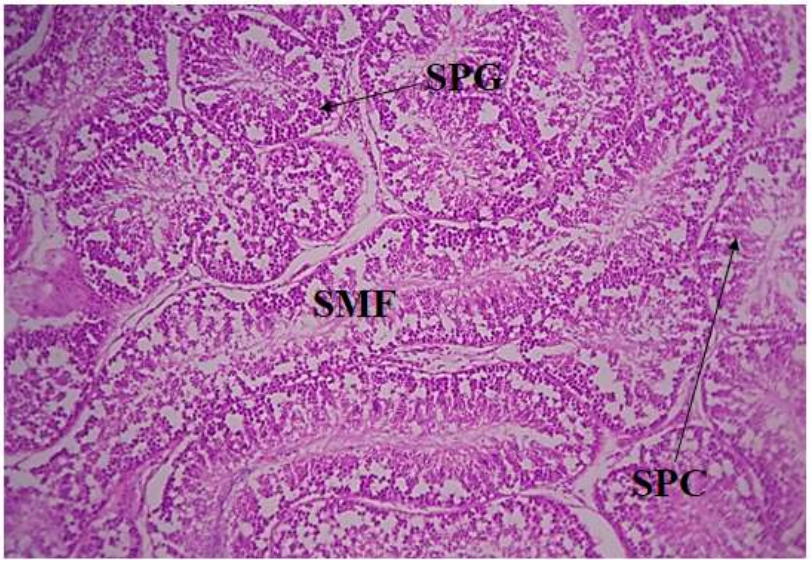

Figure (2): testis of control rat showed normal seminiferous tubules (SMF), normal spermatogonia (SPG), spermatocytes (SPC). H&E 100X

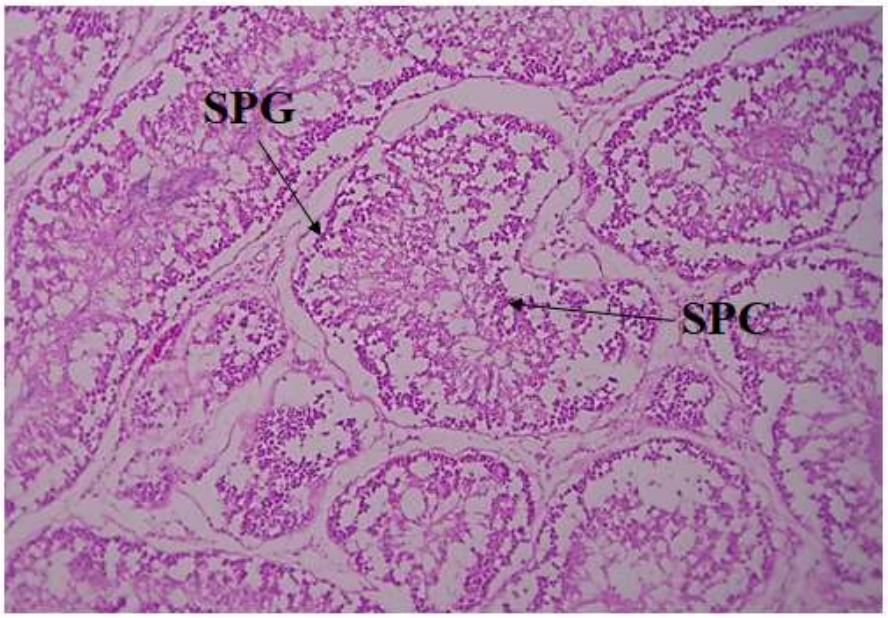

Figure (3): testis of rat in H_2O_2 group showed ruptured of seminiferous tubules wall, decreased number of spermatogonia (SPG) and spermatocytes (SPC). H&E 100X

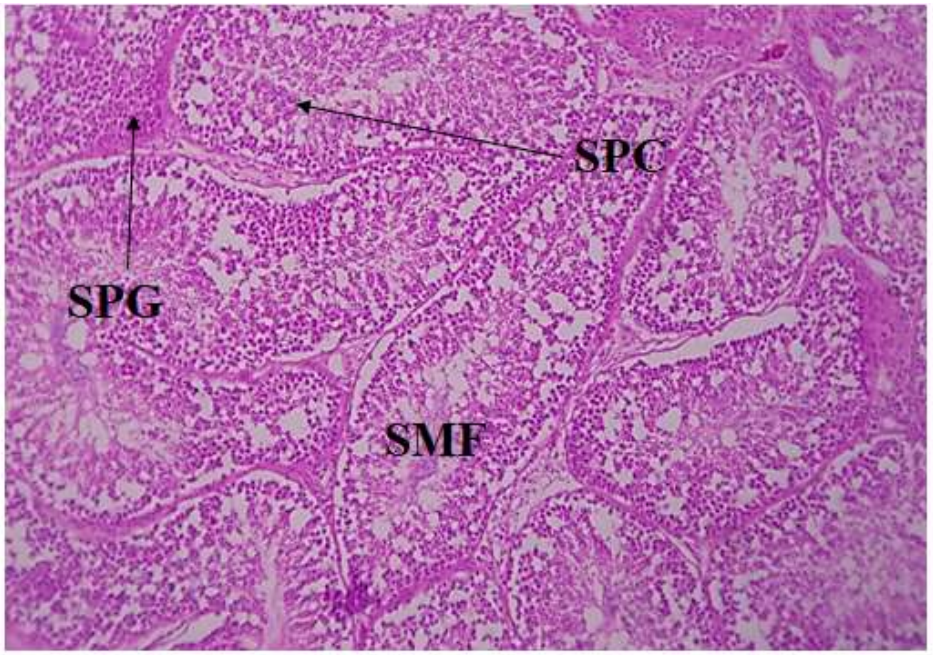

Figure (4): testis of rat in extract group showed normal seminiferous tubules (SMF), normal spermatogonia (SPG), spermatocytes (SPC). H&E 100X.

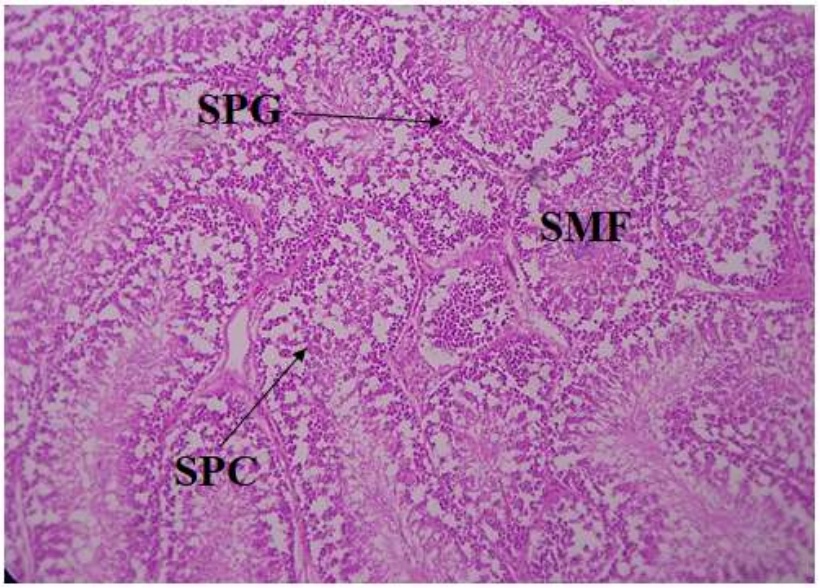

Figure (5): testis of rat in treated group showed semi-normal seminiferous tubules (SMF), normal spermatogonia (SPG), spermatocytes (SPC). H&E

The findings indicated that the second group, which received hydrogen peroxide treatment, had a significant drop in the number of leydig cells, spermatogonia, primary spermatocytes, and spermatids. Reduced numbers of spermatogonia, primary spermatocytes, and spermatids may be indicators of the effects of free radicals and oxidative stress on the testicular tissue. These effects are felt by the plasma membrane, DNA, and macromolecules, and they have a detrimental effect on the division process, which produces new cells during mitosis and meiosis, While Ashok et al. [37] demonstrated that oxidation stress leads to damage to the plasma membrane of sperm cells, causing destruction and a decrease in their count, Halliwell and Gutteridge [36] demonstrated that high concentrations of free radicals (ROS) are harmful to all parts of the living body and physiological functions, causing damage in macromolecules (lipid, protein, carbohydrate, DNA, and RNA). Testis tissue improved when quinoa extract was used in therapy, possibly due to the presence of secondary metabolite groups in quinoa such as phenolics, betaines, glycine betaine, and triterpenoids (saponins, phytosterols, and phytoecdysteroids) [38].

Conclusion

According to the current study, it is concluded from the current study that Quinoa seeds extract has antioxidant activity and enhances and improves the effectiveness of the male reproductive system.

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