Alloxan-Induced Oxidative Stress and Its Impact on the Reproductive System in Male Albino Rats and the Protective Role of Malva Parviflora Seed Extract

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Abstract

The current study aimed at evaluating the impact of the protective role of the aqueous extract of Malva parviflora seeds to reduce oxidative stress induced by the use of Alloxan on the male reproductive system and on a number of biochemical and histological variables and sperm parameters and comparing the protective role of the extract with Insulin Clargine, the study used 35 rats distributed in plastic cages in seven groups, 5 rats for each group, which included the first group, the healthy control, the second group was the control group, which was injected subcutaneously with Alloxan at a concentration of 130 mg/kg, the third group, the healthy control, which was treated with aqueous extract of Malva parviflora seeds at a concentration of 100 mg/kg by tube feeding every 24 hours, the fourth healthy group was treated with Insulin Clargine at a concentration of 5 IU/kg subcutaneously every 48 hours, the fifth group was injected with Alloxan at a concentration of 130 mg/kg subcutaneously, which was treated with extract of Malva parviflora seeds orally at a concentration of 100 mg/kg every 24 hours, the sixth group was injected with Alloxan at a concentration of 130 mg/kg subcutaneously, which was treated with Insulin Clargine at a concentration of 5 IU/kg subcutaneously every 24 hours, and the seventh group was injected with Alloxan at a concentration of 130 mg/kg subcutaneously and treated with Insulin drug Clargine subcutaneously at a concentration of 5 IU/kg every 24 hours with the aqueous extract of Malva parviflora seeds at a concentration of 100 mg/kg every 24 hours. The results showed that the group of rats in the infected control had a significant decrease at the probability level ((p≤0.05) in the concentrations of each of the interstitial cell stimulating hormone (ICSH), the sperm hormone (SSH) stimulating hormone and the testicular saturation hormone Testosterone and a decrease in the concentration of glutathione, superoxide dismutase (SOD) and catalase (CAT) enzyme, a decrease in sperm parameters represented in the number of concentration and motility, and an increase in the number of distorted ones compared to the control group. Histological sections of the testes also showed the presence of necrosis in the testicular tissues, as well as unclear stages of sperm formation and a decrease in the number of mature sperms. As for the diabetic group treated with the aqueous extract of Malva Parviflora seeds, the diabetic group treated with Insulin Clargine, and the group with the disease treated with the aqueous extract of the Malva Parviflora seed with Insulin Clargine, they showed an improvement and an increase in the concentrations of SSH, ICSH, Testosterone and the antioxidants SOD, and CAT and GSH, and the decrease in lipid peroxidation (MDA) in the blood serum, as well as the improvement of the parameters of the sperm represented by an increase in the number and motility, and the number of abnormalities decreased, and the histological sections showed a clear improvement in the testes tissues.

Introduction

Approximately 90% of male diabetic patients suffer from sexual disorders represented by impotence, with a decrease in sexual desire, erectile dysfunction and ejaculation, which can lead to infertility in them (Al-Roujeaie et al., 2017). High blood sugar leads to an increase in reactive oxygen species (ROS) where free radicals lead to the oxidation of proteins, fats and DNA in the sperm due to the absence of free radical scavenging enzymes in the sperm and it depends on the protection of the reproductive system, the antioxidant (Sakkas and Alvarez, 2010) Also, oxidative stress can cause DNA damage in testicular and epididymal tissues, whether epithelial or connective, leading to poor sperm quality (Rains et al., 2011; Vlassara et al., 2013). Oxidative stress is one of the causes of irregular secretion of sex androgens from male sex hormones in the hypothalamic-pituitary axis with its effect on semen quality and apoptosis in spermatozoa (Muratori et al., 2015). All of these factors negatively affect the quality of sperm in terms of number, shape and motility, which can lead to infertility. Some medicinal plants are effective against oxidative stress and its harmful effects (Hajian, 2014; Baradaran, 2017). Malva pariflora, is a wonderful gift of nature to mankind, and it belongs to the family Malvaceae. As it is used as human food by cooking its green parts, in addition to that, it is considered as a medicinal

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supplement for various diseases (Dugani et al., 2016). This plant spreads in the subtropical areas of the Mediterranean Basin, Europe, Central Asia and the Middle East, and it grows in uncultivated areas and on both sides of the roads (Sharifi et al.,2020). Malva pariflora is one of the most promising medicinal plants in the field of complementary medicine, which possesses a wide range of biological activities, including its antioxidant, antimicrobial, anti-inflammatory and antihypertensive activity (Farhan et al., 2012; Dugani et al., 2016).

Materials and Methods

Collect the seeds of Malva parviflora

Malva parviflora seeds were collected in the spring of 2021 from rural areas in Nineveh Governorate in northern Iraq, after which they were purified from impurities and dried in the open air to get rid of moisture, then placed in sterilized opaque plastic boxes to preserve samples until use.

Preparation of the aqueous extract

The extract is prepared by grinding the seeds and then weighing 50 g of ground seed powder and placing it in 500 ml of distilled water in a glass conical flask of 1000 ml capacity and placed in a Stirrer Magnetic stirrer at a speed of 70 revolutions per minute for an hour in order to mix the seed powder with water in a perfect way, then the mouth of the beaker is closed with a piece of plastic to prevent contamination and the beaker is covered with a black opaque cover, then the flask is left in the Shaker incubator device for 24 hours, then the mixture is filtered by layers of medical gauze to get rid of the solid parts of the filtrate and then filtered again with 10n Whatman filter paper, then the aqueous extract is dried by cooling under vacuum pressure and using a Lyophilizer device, the samples are placed after drying in opaque glass bottles with tight-fitting plastic caps and then placed in the refrigerator until use (Riose et.al 1987).

The animals used in the study

Animals were obtained from male laboratory albino rats at the age of 12-61 weeks from the animal house of the College of Veterinary Medicine, Tikrit University, with weights ranging from 190-230 grams, the animals were placed in plastic cages with dimensions 40-60-30 cm and with a floor consisting of sawdust, which was changed every three days during the experiment period, with continuous cleaning of the cages. The health of the animals was confirmed by the veterinarian specialized in the animal house. Water and the appropriate diet approved by the Animal House were provided, consisting of 35% yellow corn, 35% wheat, 20% soybeans, 10% animal protein concentrate. The animals were subjected to the appropriate environmental conditions of temperature 23±3 °C, with a light cycle divided into 12 hours of light and 12 hours of darkness.

The distribution of the animals in the experiment

The experiment started on the 22nd of December to the 21st of January and lasted for a month. After confirming that the animals were infected, they were distributed into seven groups in each group, five animals, 20 infected animals and 15 healthy animals, taking into account the homogeneity of weights in each group. The dosage of Insulin Clargine was certified according to previous studies (Stammberger and Essermeant, 2012) and (Ameen et al., 2015). Insulin Clargine is injected intraperitoneally with an Insulin injection pen that has a regulated IU indicator for the Insulin dose approved in the study. The needles are changed at each injection dose to one rat as follows:

1- First group: the control group, which is given regular feeding consisting of the diet and drinking water throughout the experiment period during the month.
2- Second group: the infection control group, in which diabetes mellitus is developed by injecting Alloxan under the skin at a concentration of 130 mg/kg, which is given the appropriate diet and water throughout the experiment period.
3- Third group: a group treated with aqueous extract of Malva pariflora at a concentration of 100 mg/kg by oral tube feeding every 24 hours during the duration of the experiment.
4- Fourth group: A group treated with Insulin Clargine at a concentration of 5 IU/kg by subcutaneous injection every 48 hours in order to avoid a sharp drop in glucose in the blood.
5- A group of diabetic patients induced with Alloxan at a concentration of 130 mg/kg, which was treated with aqueous extract of Malva pariflora seeds at a concentration of 100 mg/kg by tube feeding every 24 hours.
6- A group with diabetes mellitus induced by Alloxan at a concentration of 130 mg/kg, which was treated with Insulin Clargine at a concentration of 5 international units/kg every 24 hours.
7- A group of diabetic patients induced with Alloxan at a concentration of 130 mg/kg, which was treated with Insulin Clargine at a concentration of 5 IU/kg every 24 hours with aqueous extract of Malva pariflora at a concentration of 100 mg/kg every 24 hours.

Obtaining blood serum for biochemical tests

After completing the experiment, the animals fasted for 24 hours, then the animals were anesthetized with Chloroform, after they were anesthetized, the blood was drawn by heart stab using 5 ml medical syringes, then the blood was drained into gel tubes, then the tubes were placed in a centrifuge at 3000/min to obtain the serum, the blood serum was placed in

Eppendorf tubes and frozen at -20 °C until biochemical tests were performed.

Estimation of the concentrations of antioxidants and antioxidants in the blood serum

SOD Estimate

The concentration of superoxide dismutase was estimated by the method: Modified Photochemical Nitroblue Tetrazolum NBT Sodium cyanide is used as a peroxidase inhibitor, this method is based on estimating the enzymatic activity of superoxide dismutase indirectly through the instantaneous change in the optical density of formazine, which resulted from the oxygen reduction of Nitro blue tetrazolium(NBT), the result of irradiation generated from blood serum (Brown and Goldstein, 1983), as it is the decrease in the optical density of formazine is an indication of the increase in the activity of oxide dismutase enzyme.

MDA Estimate

To estimate the concentration of MDA in the serum, the method that depends on the interaction of the effect between thiobarbituric acid (TBA) and that depends on the method of measuring the concentration of MDA was used, which represents one of the products of lipid oxidation on the reaction between lipid peroxides such as MDA with TBA in a medium that depends on the pH function of the complex product with a pink color (Muslih et al., 2002).

CAT Estimate

The level of catalase enzyme (CAT) is estimated by the reaction of ammonium metavanadate NH_4VO_3 with hydrogen peroxide H_2O_2 in acidic conditions, by reducing vanadium V to III. Hydrogen peroxide is a strong oxidizing agent that when NH_4VO_3 is oxidized, a red-yellow complex called peroxovanadium $NH_4VO(O_2)SO_4$, which is absorbed at the wavelength of 452 nm (Hadwan and kadhum, 2018).

GSH Estimate

The concentration of glutathione in the serum was estimated according to the method of Elllman's reagent by reacting the sulfhydryl group present in the glutathione tri-peptide with Ellman's reagent in an alkaline medium to produce a yellow-colored complex consisting of nitro benzoic acid and a disulfide compound, the concentration of glutathione depends on the intensity of the color in the sample according to (AL-zamely, 2001).

Estimation of the concentration of the hormones SSH, ICSH and Testosterone in the blood serum

The level of hormones concentration in the serum is measured by the AIA-360 TOSOH device of Japanese origin of 2019 version with the use of its kit from the same manufacturer of the device, the device contains 25 holes numbered sequentially in which samples are placed, with a place to put the detector next to the holes in the device.

Get Sperm

To obtain sperms, the tail of the epididymis is excised, then 2 ml of warm 0.9% physiological solution is added to the epididymis, then mashed with a sharp blade and left for 5 minutes so that the sperms can exit the epididymis procedure and diffuse into the solution, then 20µ of the mash is withdrawn by the inserted micropipette and injected into the sperm counting chamber at the designated injection site where the sample spreads to all parts of the counting chambers by capillary action, the counting slides are pre-warmed with a glass counting slide transfer case into the incubator at 37 °C to maintain sperm viability. Sperm parameters are checked using CASA Computer- Assisted semen analysis (machine name SW3702) from Sanwe, Japan.

Preparation of tissue sections

The testicles are removed and then fixed in 10% formalin in 100 ml plastic numbered containers for 24 hours (Bancroft and Gamble, 2008), then the organs are gently washed with tap water so that the organs are not affected and for several times, and then we perform the following sequential operations (Dehydration- Clearing - Infiltration- Embedding - Sectioning -Staining- Mounting).

Statistical Analysis

Significant differences were found using the Anova one-way test, and the two differences were confirmed using the standard deviation, then finding the significant differences for the averages by Duncan's multiple range test at the probability level ($p \leq 0.05$ (Bruning and Kintz, 1987).

Results

Results of the level of concentrations of antioxidants and oxidants

Table (1) shows a significant decrease in the level of concentration of antioxidants GSH, CAT and SOD and a significant increase in the level of concentration of lipid peroxide (MDA) in the experimental group with Alloxan diabetes at the probability level ($p \leq 0.05$) compared to the healthy control group, as for the diabetic group treated with aqueous extract of Malva pariflora seeds, the diabetic group treated with Insulin Clargine, and the diabetic group treated with aqueous extract of Malva pariflora seeds with Insulin Clargine, they showed a significant increase in the values of antioxidants GSH, CAT and SOD, and a significant decrease in the level of MDA lipid peroxide concentration in comparison to the experimental diabetic group, and the healthy group treated with aqueous extract of Malva pariflora seeds showed a significant increase in the antioxidant values GSH, CAT and SOD without a significant change in the level of MDA lipid peroxide concentration compared to the healthy group, while

the group treated with Insulin Clargine showed a significant decrease in the level of the concentration of antioxidants GSH, CAT and SOD and a significant increase in the level of the concentration of lipid peroxide (MDA) compared to the healthy group.

Table (1) shows the level of values of antioxidants and oxidizing factors				
StandardsGroups	GSH (µmol/l)	CAT (U/ml)	SOD(U/ml)	MDA(µmol/l)
Healthy control	9.980±0.79B	1.260±0.06bc	9.990±0.72ab	4.866±1.11d
Injured control	6.688±1.03D	0.906±0.13d	7.922±1.06d	8.506±1.00a
Healthy +extract of Malva	11.290±1.18A	1.314±0.20b	10.278±0.69a	4.600±0.73d
Insulin Clargine Healthy +	8.282±0.56C	0.942±0.09d	9.138±0.68c	6.442±0.46b
Injured + extract of Malva	8.970±0.88C	1.470±0.23a	9.498±0.50bc	5.246±0.83cd
Injured + Insulin Clargine	10.130±1.00B	1.246±0.18c	8.642±0.99cd	5.700±1.06c
Injured + extract of Malva +Insulin Clargine	9.776±1.28B	1.224±0.07c	9.074±0.69c	4.566±0.40d

Sex hormones

Table (2) shows a significant decrease in the concentration level of the sex hormones Testosterone, SSH and ICSH in the group affected with experimental diabetes with Alloxan at the probability level ($p \leq 0.05$) compared to the healthy control group, as for the group suffering from diabetes and treated with aqueous extract of Malva pariflora seeds, and the group with experimental diabetes and treated with Insulin Clargine, and the group suffering from experimental diabetes and treated with aqueous extract of Malva pariflora seeds with Insulin Clargine showed a significant increase in testosterone, SSH and ICSH in comparison with the diabetic group, the healthy group treated with aqueous extract of Malva pariflora seed also showed a significant increase in the concentration of Testosterone with a significant decrease in the concentration of ICSH compared to the healthy control group, while the healthy group treated with Insulin Clargine showed a significant decrease in the level of ICSH, SSH and Testosterone hormones compared to the healthy control group.

Table (2) shows the concentrations of Testosterone, SSH and ICSH			
StandardsGroups	Testosterone(ng/dL)	SSH (mIU/ml)	ICSH(mIU/ml)
Healthy control	475.4±31.2B	1.642±0.29A	0.472±0.12A
Injured control	164.6±18.2F	1.326±0.08C	0.250±0.05C
Healthy +extract of Malva	580.4±84.0A	1.682±0.25A	0.370±0.07B
Insulin Clargine Healthy +	419.6±13.5C	1.404±0.19Bc	0.372±0.10B
Injured + extract of Malva	279.4±36.1E	1.666±0.32A	0.422±0.12Ab
Injured + Insulin Clargine	291.6±36.9De	1.576±0.15Ab	0.340±0.06B
Injured + extract of Malva +Insulin Clargine	333.2±31.2D	1.604±0.08A	0.464±0.11A

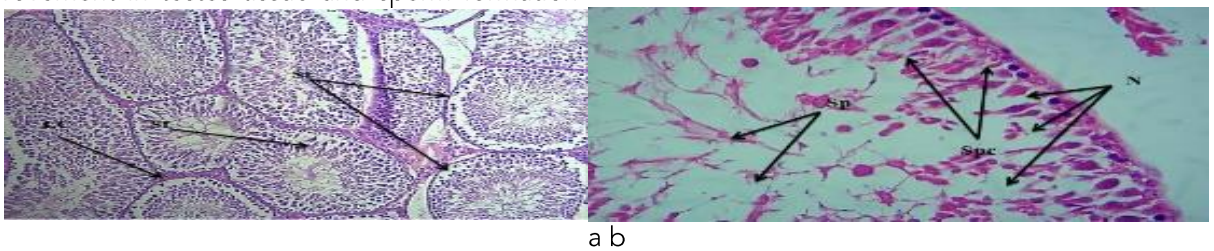
Sperm criteria

Table (3) shows a significant decrease in the concentration of sperm, movement and normal, and a significant increase in non-motile or slow-moving and distorted sperms in the group with experimental diabetes at the probability level ($p \leq 0.05$ compared to the healthy control group, as for the diabetic group treated with aqueous extract of Malva pariflora seeds, the diabetic group treated with Insulin Clargine , and the diabetic group treated with aqueous extract of Malva pariflora seeds with Insulin Clargine, they showed a significant increase in sperm concentration, motility and normal with a significant decrease in sperm count. immobile or slow-moving and distorted compared to the diabetic group, the healthy group treated with aqueous extract of Malva pariflora seeds also showed a significant increase in sperm concentration with no significant change in movement, normal and non-motile or slow and distorted sperms in comparison with the healthy control group, while the healthy group treated with Insulin Clargine showed a significant increase in the concentration, distorted sperms, and non-motile or slow-moving sperms, with a significant decrease in the normal and natural motility of the sperm compared to the healthy control group.

Table (3) shows the criteria for sperm, the number of sperm, the concentration of sperm, deformity, normal, motility, slow or not moving					
Standards Groups	Number) 10^6(	Movement%	the slow or not moving%	Deformity%	Normal%
Healthy control	113.67±12.5B	63.87±8.09A	36.13±6.09d	8.80±1,51e	91.20±1.51a
Injured control	21.67±4.51E	15.20±3.60D	84.80±9.60a	22.40±1.41a	77.60±1.41 e
Healthy +extract of Malva	133.33±7.09A	71.90±9.66A	28.10±8.66d	7.63±1.36e	92.37±1.36a
Insulin Clargine Healthy +	124.30±15.2Ab	39.20±6.99Bc	60.80±6.99 bc	18.30±1.74b	81.70±1.74d
Injured + extract of Malva	72.67±17.01D	49.60±7.50B	50.40±7.50c	12.30±4.82d	87.70±4.82b
Injured + Insulin Clargine	86.00±7.00Cd	36.30±9.24C	63.70±9.24b	16.20±3.51 bc	83.80±3.51cd
Injured + extract of Malva +Insulin Clargine	94.70±18.1C	43.90±7.77Bc	56.10±7.77bc	14.67±4.76cd	85.33±4.76bc

Testicle tissue

The results showed pictures of cross-sections of tissues (b) that the use of Alloxan in the development of diabetes mellitus in male rats led to the presence of necrosis in the testicular tissues, as well as unclear stages of sperm formation and a decrease in the number of mature sperms. The results of cross-sectional images of tissues (e) of a group of Alloxan-induced diabetic rats that were treated with aqueous extract of Malva pariflora seeds showed a significant improvement in testes tissue and sperm formation stages in animals with induced diabetes. The results of cross-sectional images of tissues (f) in the group of rats with diabetes and treated with Insulin Clargine showed a clear improvement in the parameters of the testicular tissue and the stages of sperm formation inside it. While the pictures of cross-sections of tissues (d) in the healthy group that was used with Insulin Clargine in its treatment showed the presence of blood congestion in the testicular tissue with the stages of sperm formation within the tubule and the naturally mature sperm.



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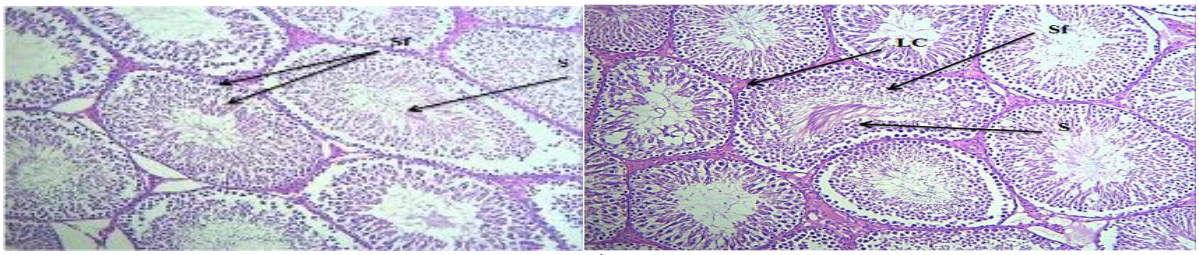

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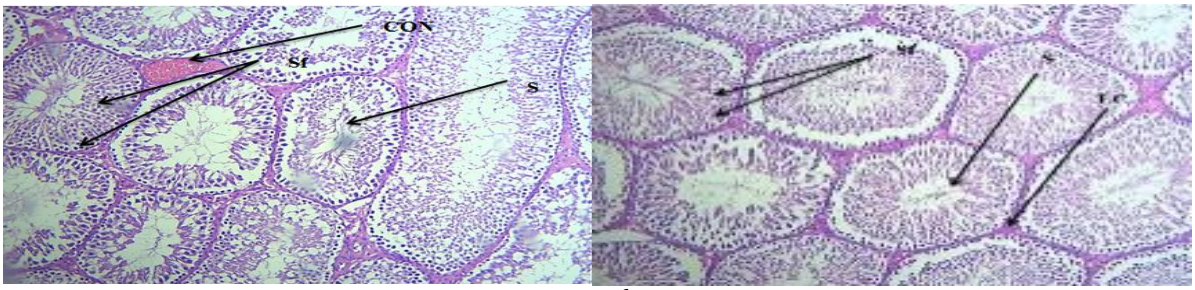

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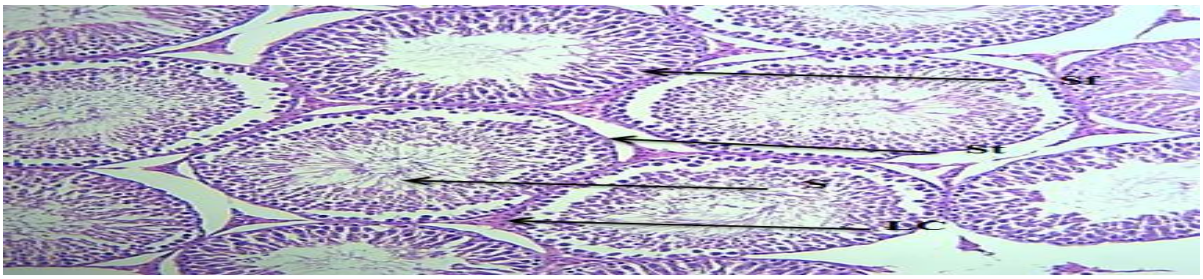

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Picture (a) a section of the testis of the control group showing the seminiferous tubules (St) and the stages of spermatogenesis within the tubule (Sf) and the Leydig cells(LC). H & E 100X, picture (b) A section of rat testis from a group of experimental diabetic rats showing testicular tissue necrosis (N) as well as unclear spermatogenesis (spc) and lack of mature sperm count (SP), H&E 400X, picture (c) section of the testes of the group treated with Malva pariflora seed extract only showing the stages of sperm formation within the tubule (Sf) and the mature sperm (S normally). H & E 100X, Picture (d) section of the testes of the group treated with Insulin only showing the stages of sperm formation within the tube (Sf) and mature sperm (S) normally with congestion (CON). H & E 100X, picture (e) a section of the testes of the diabetic group treated with Malva pariflora seed extract only showing the stages of sperm formation within the tubule (Sf), the normally mature sperm (S), and Leydig cells (LC). H & E 100X, picture (f) a section of the testes of the group affected by diabetes and treated with Insulin only, showing the stages of sperm formation within the tubule (Sf), the mature sperm (S), and Leydig cells (LC). H & E 100X, picture (g) a section of the testis of a group of diabetics treated with Insulin and Malva pariflora seed extract showing the seminiferous tubules (St) and the stages of spermatogenesis within

the tubule (Sf) and the Leydig cells (LC) and the mature sperm (S). H & E 100X.

Discussion

The results of this study showed in Table (1) that the animals in which diabetes was induced by Alloxan had a significant decrease in the values of the antioxidants SOD, GSH and CAT and a significant increase in MDA, compared to the control group, where these results agreed with the findings of many Studies including (Sekiou. et al., 2019) Alloxan causes an increase in the level of glucose in the blood serum, stimulating the generation of more free radicals as a result of the destruction that occurred in the beta cells of the pancreas (Bonnefont-Rousselot et al., 2004). The hyperglycemic factor stimulates the rise of more free radicals and because free radicals cause damage and destruction of cells and their membranes (Haghani et al., 2016; Macdonald Ighodaro et al., 2017). Free radicals cause an increase in the level of MDA in the serum, which is an indication of increased lipid peroxidation and production of malondial aldehyde as a result of the effect of free radicals on the oxidation of fats in cell walls (Al-Karagoly, 2007). The results showed that animals with induced diabetes treated with aqueous extract of Malva pariflora seeds had a significant increase in the antioxidant values of SOD, GSH and CAT, and a decrease in the level of lipid peroxidation represented by the value of MDA, the reason for this is that the Malva pariflora seeds contain multiple phenolic compounds capable of scavenging free radicals (Chatenet, 2008)) leading to an improvement in CAT, GSH, and SOD values, and a decrease in MDA compared to the infection control group, the results also showed that diabetic animals treated with Insulin Clargine had a significant increase in the values of the levels of antioxidants GSH, CAT and SOD, and a significant decrease in the values of MDA, Insulin Clargine lowers the level of glucose in the blood serum, thus reducing the level of free radicals and reducing the oxidative stress caused by them, thus reducing the level of fat peroxidation in the body (MDA). The administration of Insulin Clargine in a group of healthy animals led to a relative increase in MDA values with a decrease in SOD, GSH and CAT compared to the control group, Insulin Clargine causes weight gain due to the accumulation of fat represented in white adipose tissue, leading to an increase in cholesterol in the blood (Yang et al., 2014), as high cholesterol levels cause an increase in the rate of free radicals, leading to a decrease in antioxidants and a high rate of fat oxidation (Mohamed, 2010) and this is what the results of this study showed.

The results of the study showed in Table No. (2) that Alloxan-induced diabetes led to a decrease in the concentrations of SSH, ICSH and Testosterone hormones compared to the healthy group, the decrease in the level of testosterone may be due to oxidative stress caused by the generation of free radicals as a result of diabetes mellitus induced by Aloxan, which causes a decrease in the concentration of testicular androgen, another cause of testosterone deficiency may be poor utilization of serum glucose by Leydig cells in the testes that produce the hormone, or a decrease in the sex hormones ICSH and SSH, which have the function of stimulating the Leydig cells to secrete testosterone (Navarro-Casado et al. al.,2010), the results of this study show that the male sex hormones ICSH and SSH were also decreased in the group of animals with diabetes induced by Alloxan in comparison with the healthy control group, whereas, the reason for this decrease could be due to the interference of diabetes with the hypothalamic-pituitary axis (Soliman et al., 2019). Also, many researchers indicated that a decrease in the concentrations of ICSH and SSH hormones in the blood serum is associated with a decrease in the level of the hormone Insulin, and the reason is that the hormone Insulin has a strong relationship with the pituitary gland and its vital activity in the synthesis and secretion of ICSH and SSH (Jangir et al.,2022). The group of animals that were treated with the aqueous extract of hibiscus seeds showed an improvement in the level of concentrations of male sex hormones SSH, ICSH and Testosterone compared to the affected control, the Malva pariflora seed contains many antioxidant compounds represented by phenols and flavonoids (Abdel-Ghani et al., 2013; Jaradat et al., 2021), which have an important role in scavenging free radicals and preserving testicular tissues from damage as well as protecting an area Hypothalamus with pituitary gland from the effect of free radicals generated by the high level of glucose in the blood serum (Elangovan et al.,2021). The group of diabetic rats treated with aqueous extract of Malva pariflora seeds did not show a significant improvement in the level of testosterone compared to the healthy control group, the newly developed diabetes can lead to programmed destruction and death of testicular cells and tissues, including testosterone-producing Leydig cells and Sertoli cells that nurture sperm as a result of the activity of free radicals in the animal body in general and the testicle in particular (Hosseinipour et al., 2019). As for the drug Insulin Clargine, it showed an improvement in the level of androgens through the role of Insulin in reducing the level of glucose in the serum, accompanied by a reduction in the types of free radicals generated due to oxidative stress formed due to the high level of glucose in the blood serum (Rochette et al., 2014). The group of healthy rats dosed with aqueous extract of Malva pariflora seeds showed an increase in the level of testosterone hormone. The study of (Azubuike et al., 2018) indicated that there is a joint effect between the flavonoids that are present in Malva pariflora seeds and the increase in the concentration of testosterone in the blood serum, it was also mentioned that Querecetin is one of the effective flavonoids, capable of increasing sperm production and raising the level of testosterone (Taepongsorat et al., 2008), in a group of healthy

animals, Insulin administered to a group of healthy animals causes a decrease in the concentrations of androgens in the blood serum, Insulin Clargine can lead to an increase in serum cholesterol, which affects the testicles, causing the generation of more free radicals and the accumulation of the rate of oxidative cell membranes, causing the accumulation of oxidized fats and MDA peroxidation in its tissues (Liu et al., 2020). The use of Insulin Clargine with aqueous extract of Malva pariflora seeds in a group of Alloxan-induced diabetic rats showed a synergistic role in improving the values of sex hormones in the blood serum.

The results of this study showed in Table (3) that Alloxan-induced diabetic rats had a decrease in the vital parameters of sperm represented by the number, motility and normal shape, and a high percentage of deformed, not move or slow-moving when compared to the healthy control group, Oxidative stress disrupts the reproductive function of the testicles and epididymis, and therefore antioxidants must be enhanced in the face of the harmful effects of free radicals (Shrilatha, 2007), the types of free radicals represented by reactive oxygen species destroy the membranes of sperm cells by oxidizing fats, as the sperm membranes contain a large amount of polyunsaturated fatty acid PUFA (Agarwal et al., 2017), fatty acids are an important catcher of free radicals in cells and their membranes thus interact with them, causing damage to the sperm DNA (Shoorei et al., 2019). The Malva parviflora seeds showed an improvement in the quality of the sperm parameters by preserving the testicular cells from damage, as the study of (Samie et al., 2018) indicated that the compound Querecetin present in the Malva parviflora seeds may improve testicular weight in rats with experimental diabetes. Treatment of Alloxan-induced diabetic rats with insulin Clargine improved the sperm parameters represented in number, movement and normal shape, thus protecting the testicular tissues from the danger of free radicals, the use of Alloxan in the development of diabetes in male rats causes testicular tissue necrosis as well as unclear sperm formation stages and a decrease in the number of mature sperm, this study agreed with (Roy et al., 2015) and (Ifegwu et al.,2021), studies indicate that oxidative stress can cause DNA damage in testicular and epididymal tissues, whether epithelial or connective, leading to poor sperm quality (Rains et al., 2011; Vlassara et al., 2013). The group of Alloxan-induced diabetic rats treated with aqueous extract of Malva parviflora seeds showed a significant improvement in testicular tissue and sperm formation stages in animals with induced diabetes, the Malva parviflora seeds are rich in flavonoids and phenols, which contribute to increasing the activity of antioxidants and inhibiting free radicals, thus preserving cell membranes in the genitals from damage caused by the excessive activity of free radicals (Elangovan et al.,2021). The group of Alloxan-induced diabetic rats treated with Insulin Clargine showed a clear improvement in the parameters of the testicular tissue and the stages of sperm formation inside them, indicated the study of (Feng et al.,2001), the hormone insulin stimulates the insulin-like growth factor IGF-1, which promotes the proliferation of Leydig cells in the testis by signals of the hormone ICSH, the use of insulin Clargine in the doses of healthy rats led to the presence of blood congestion in the testicular tissue with the stages of sperm formation within the tubule and the naturally mature sperm, the presence of blood congestion may be attributed to the fact that insulin Clargine can lead to the accumulation of fat represented by white adipose tissue and high cholesterol in the blood serum (Yang et al., 2014), thus generating free radicals and the accumulation of oxidative fats that cause blood congestion.

Conclusions

The aqueous extract of Malva parviflora seeds showed ability against Alloxan-induced oxidative stress on hormonal criteria represented by sex hormones, antioxidants, sperm parameters and testicular tissues.

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