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AMELIORATING EFFECT OF ALLICIN ON REPRODUCTIVE FUNCTIONS IN CYCLOPHOSPHAMIDE TREATED MALE RATS

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Abstract

Allicin, which is one of the main biologically active compounds derived from garlic, has been shown to exert various pharmacological activities is considered to has therapeutic potential effect for many pathologic conditions, and provides broad antioxidant activity to reduce free radical damage. The aim of the present study is to investigate the positive role of allicin in reducing the side effects induced by Cyclophosphamide drug in reproductive system of male rats.

In this experiment, fifty adult male Wister rats were used, about four month old, with average weight about(162.5±13gm) were divided randomly into five equal groups (10 animals for each group) and treated for 60 consecutive days as following : The first group (C) was given 1 ml distilled water orally as a control group. The second group (T1) was given allicin orally in a dose of 50 mg\kg B .W. The third group (T2) was given cyclophosphamide orally in a dose of 10 mg /kg /B .W .The fourth group (T3) was given allicin orally (50mg/kg/B. W) for 30 days then given cyclophosphamide orally (10 mg /kg B. W/ day)for 30 days. The fifth group (T4) was given cyclophosphamide orally (10 mg/kg B.W/day)for 30 days then given allicin orally (50 mg/kg B.W/day for 30 day.

At the end of the experiment all animals were sacrificed and blood samples were collected from the heart and serum samples were isolated for assessment of MDA and CAT. Testis from all animals were removed for estimation of Testis weight and semen parameters.

The results of this study were revealed a significant differences ($P \leq 0.05$) represented by the increase in sperms concentration, percentage of sperms motility, percentage of sperms viability weight of testis and CAT concentration in T1 group as compared with other groups also, increased in T3 and T4 groups as compared with T2 group, while decreased in T2 group. The percentage of abnormal sperm and concentration of MDA decreased significantly in T1 group as compared with other group, and decreased in T3 and T4 group compared with T2 group, while increased in T2 group. We could be concluded that allicin in dose of 50 mg/kg/B.W has both preventive and therapeutic role in ameliorating cyclophosphamide toxicity in adult Wister male rats.

Key words : Allicin, Cyclophosphamide, Semen parameters, MDA, CAT.

Introduction

Cyclophosphamide is a chemotherapy drug used to treat different types of cancer, including lymphomas, leukemia, myeloma, lung cancer and breast cancer (Moore, 1991). Cyclophosphamide is an alkylating agent that has an effective anticancer activity. It introduces alkyl radicals into DNA strands of cells and stops cancer cells from upward (Young *et al*., 2006; Shanafelt *et al*., 2007). It has also an immunosuppressive effect suppress the body's natural immune response and used to treat some autoimmune diseases. Cyclophosphamide is also used to treat severe rheumatoid arthritis, minimal change disease and multiple sclerosis granulomatosis with polyangiitis (Makhani *et al*., 2009).

Cyclophosphamide may cause vomiting, nausea, diarrhea, stomach ache, loose of appetite (Singh *et al*., 1991), hyperpigmentation of the skin, bone marrow suppression (Lohrmann, 1984), gonadal suppression, hypogammaglobulinemia, hemorrhagic cystitis (blood in the urine with painful voiding), increased urinary frequency, urinary bladder cancer, myelodysplasia, alopecia (loss of hair), pulmonary fibrosis (Malik *et al*., 1996), fast heartbeat (Flyod *et al*., 2005).

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to a series of reactions that may damage cells (Bjelakovic *et al*., 2013). Antioxidants such as ascorbic acid (vitamin C) or thiols terminate chain reactions (Jiang *et l*., 2010). Antioxidants mainly include two different groups of substances: industrial chemicals which are added to products to prevent oxidation and natural chemicals found in foods and body tissue which are said to have beneficial health effects (Vertuani *et al*., 2004).

There are two major types of antioxidants in living cells: enzymatic antioxidants and non enzymatic antioxidants (Sies, 1997; Herrera and Barbas, 2001). Recent trends in controlling and treating diseases tend to favor natural antioxidant compounds rather than synthetic ones. Allicin is the principle active component of garlic oil (*Allium sativum*), which is present in the form of alliin in the whole garlic bulb (Lawson, 1996). The intact garlic cloves contain alliinase and alliin in different parts rather than allicin (Zhang *et al*., 2017). However, when the clove is cut or crushed, alliinase and alliin interact to form allicin which is responsible for the strong odor of garlic and the antibiotic properties of garlic (Alpers, 2009).

Allicin was first isolated and studied in the laboratory by Cavallito and Bailey in 1944 (Gruhlke and Slusarenko, 2014). It plays a vital role in decreasing the infection, aging and cardiovascular disease (Powolny and Singh, 2008), cholesterol and blood pressure. Also, it helps to inhibit coagulation and provides broad antioxidant activity to reduce free radical damage (Alpers, 2009).

The purpose of the present study is to investigate the positive role of allicin in reducing the side effects induced by cyclophosphamide drug in reproductive system of male rats by studying of semen parameters.

Materials and Methods

1. Experimental animals

In this experiment fifty adult male Wister rats were used, about four month old, with average weight about (162.5±13gm) obtained from animal house in college of veterinary Medicine at university of AL-Qadisiyah. The animals housed in well ventilated wire-plastic cages with dimensions 40×60 cm and reared under controlled conditions about 12hour light and 12 hour dark at 22^{0}C. The animals were allowed to acclimatize for 14 days before experimentation.

2. Experimental design

Fifty adult male Wister rats were divided randomly into five equal groups (10 animals for each group) and treated for 60 consecutive days as following:

1. Control group (C),was given 1 ml distilled water orally.

2. The first treated group (T1) was given allicin orally in a dose of 50 mg\kg B .W once daily dissolved in 1 ml distilled water for 60 day (Ashry *et al*., 2013).

3. The second treated group (T2) was given cyclophosphamide orally in dose of 10 mg/kg /B.W once daily dissolved in 1 ml distilled water for 60 day (Wtwt *et al*., 2012).

4. The third treated group (T3) was given allicin orally (50mg/kg/B.W) for 30 days then given orally cyclophosphamide 10 mg /kg B. W/day for 30 days.

5. The fourth treated group (T4)was given cyclophosphamide orally (10 mg/kg B.W/day) for 30 days then given orally allicin (50 mg/kg B.W/day) for 30 day.

3. Animals Sacrificing

Body weight has been recorded weekly because of administration of doses of allicin and cyclophosphamide depending on body weight. Twenty four hours after last administration all animals were anaesthised by mixing of Ketamine and xylazin (9mg/kg/B.W, 10mg/kg/B.W) respectively intraperitonial, to sacrified then blood samples were collected from the heart to obtain the serum of animals for assessment of MDA and CAT. Testis samples from all animals were removed and have been reserved in formalin 10% for histological study. While, another testis was took for estimation of sperm parameters.

4. Collection of blood samples

Blood was collected from each animal directly from the heart by using 5 ml disposable syringe ,then putting in gel and clot activator tube and left at room temperature until clotted, then it were centrifuged at 5000 rpm for 15 minutes, the serum was aspirated from the tube and stored at -20^{0}C untile used for analysis (Shittu *et al*., 2006).

5. Laboratry measurements

The animals were investigated for the following parameters :

5.1. Semen parameters which were include: Estimation of :

a. Concentration of sperms .

b. Total motility percentage .

c. Live sperms percentage.

d. Percentage of abnormal sperms.

5.2. Weight of testes

5.3. Estimation of catalase (CAT) and malondialdehyde (MDA) levels in serum.

a. Sperms concentration

The sperms were calculated according to method adopted by Hinting (1989). The tail of epididymis was put in 1 ml of normal saline and then dissected to 200 small pieces by microsurgical scissors and served at 37ºC to be examined by taking drop of mixture by Pasture pipette and put the drop on slide and covered by cover slide and then the number of sperms was calculated in 10 microscopic field by using objective lens 40X of microscope. Then, the concentration of sperms (sperm/ milliliter) was calculated from mean of calculated sperms in ten field and then the mean multiply by 1 million.

b. Total Sperms Motility

Sperms were taking from epididymal content obtained by maceration of the tail of epididymis on a dry, clean and warm slide; mixing well with a drop of warm normal saline and directly examined under (10X) objective lens of a light microscope for determination of motility percentage of spermatozoa according to Cheng *et al.* (2006).

The percentage of motility sperms =

$$\frac{Number\ of\ sperms\ of\ particular}{Total\ number\ of\ sperms} \times 100$$

c. Percentage of Abnormal Sperms

The abnormalities of sperms were detected according to Alizadeh *et al*. (2010). The smear was prepared by putting 50ml from epididymal tail mixture on slide, and then adding drop of eosin –nigrosin stain and mix for 30 second, and then spread by another slide, and after drying of smear, examined by oil lenses (X100) and 200 sperms were calculated and the abnormal sperms were detected according to the following equation.

Percentage of abnormal sperms =

$$\frac{Number\ of\ abnormal\ sperms}{Total\ number\ of\ sperms} \times 100$$

d. Percentage of live Sperms

A drop of epididymal content taking from each rats was blend with equal drop of eosin-nigrosin stains preparatory in according to Bjorndahl *et al.* (2003), examined by oil lenses (X100) and 200 sperms were calculated the dead sperms stained head, while a life sperms don't stained after calculating of sperms the following equation will be :

Percentage of live sperms =

$$\frac{Number\ of\ lives\ sperms}{Total\ number\ of\ sperms} \times 100$$

5.2. Estimation of testes weight : The testes were dissected out freed from adherent tissues, and weighed by using sensitive balance.

5.3. Assessment of MDA concentration

By using the Thiobarbituric acid (TBA) method of Buege and Aust for determination of serum MDA, in which MDA reacts with TBA to give a pink color that is read at (535 nm) (Buege and Aust, 1978).

5.4. Determination of catalase (CAT) concentration

According to Aebi (1974) and Kakkar *et al*. (1984), CAT activity was assayed by measuring the degradation rate of H_2O_2, where its disappearance rate was monitored spectrophotometrically at 230 nm.

6. Statistical analysis

A computerized program, the statistical package for social sciences (SPSS), was used to analyze data. The data were expressed as means ± standard errors (SE). Differences between group means were estimated using a one-way analysis of variance (ANOVA) with least significant difference LSD was detected to compare between groups, and Results were considered statistically significant at $P < 0.05$ (Joda, 2008).

Results

1. The effect of Allicin on semen parameters in Wister male rats treated with Cyclophosphamide

1.1. Concentration of sperms (million)

Table 4-1 demonstrated there was a significant increase ($P \leq 0.05$) in sperms concentration in T1 group (112.44 ± 4.46 milion/ml) as compared with other groups, while there was a significant decrease in sperms concentration in T2 group (61.57 ± 1.5milion ml) as compared with other groups. And there were significant differences represented by the increase in sperms concentration in T3 and T4 groups (81.7 ± 0.54), (79.77 ± 1.99) respectively as compared with T2 group, and there was no a significant difference ($P \leq 0.05$) between T3 and T4 groups.

1.2. Percentage of Sperms Motility (%)

Table 4-1 revealed no significant differences ($P \geq 0.05$) in sperms motility between T1 and C groups. Also there was a significant decrease ($P \leq 0.05$) in sperms motility

in T2 groups (49.9 ± 1.1) as compared with other groups. And there was a significant increase in sperms motility in T3 and T4 groups (83.6 ± 1.16),(77.4 ± 1.51) respectively as compared with T2 group , and there was a significant difference between T3 and T4 groups.

1.3. Percentage of sperms abnormalities (%)

Tables 1-4 showed a significant increase ($P \leq 0.05$) in percentage of abnormal sperms in T2 group (22.63 ± 0.89) when compared with T1 group and control group .And there was a significant decrease in percentage of abnormal sperms in T1 group (9.38 ± 0.65) as compared with other group. At the same time, there was a significant decrease in percentage of abnormal sperms in T3 and T4 groups (14.54 ± 0.58), (15.5 ± 0.31) respectively when compared with T2 group, and there was no significant difference ($P \geq 0.05$) between T3, T4 groups, and no significant difference ($P \geq 0.05$) between T3 and C group.

1.4 Percentage of Live Sperms (%)

Table 4-1 revealed a significant increase ($P \leq 0.05$) in the percentage of life sperms in T1 group (89.31 ± 0.84) in compression to other groups, while T2 group (54.51±2.07) appeared a significant decrease as compared with other groups. Also there was a significant difference ($P \leq 0.05$) represented by increasing in the percentage of live sperms in T3 and T4 groups (77.05 ± 1.98), (76.82 ± 1.78) respectively as compared with T2 group, and there was no significant difference ($P \geq 0.05$) between T3 and T4 groups.

2. Weight of testes (mg)

Table 2 demonstrated that average of testes weight in T1 group tend to increase which reached to (1.23 ± 0.01), while average of testes weight decrease significantly ($P \leq 0.05$) in T2 group (0.72 ± 0.04) in compared with other group. Also there was a significant increase in weight of testes in T3 and T4 groups (1.09 ± 0.03), (0.92 ± 0.04) respectively when compared with T2 groups, and there was a significant difference ($P \leq 0.05$) between T3 group and T4 group, also there was no significant difference ($P \geq 0.05$) between T3 and C group.

3. Concentration of MDA (µmol/L)

Table 2 showed that T2 group appeared a significant increase ($P \leq 0.05$) in concentration of MDA (2.54 ± 0.034) when compared with other groups, while there was a significant decrease in concentration of MDA in T1 group (1.21 ± 0.005). Also, there was a significant decrease in concentration of MDA in T3 group and T4 group (1.31 ± 0.011), (1.23 ± 0.192) respectively as compared with T2 group. Likewise there were no significant differences ($P \geq 0.05$) between T1, T3, T4 and C group.

4. Concentration of CAT (U/L)

Table 2 demonstrated there was a significant increase ($P \leq 0.05$) in CAT concentration in T1 group (0.85 ± 0.008) as compared with other groups, while there was a significant decrease in CAT concentration in T2 group (0.28 ± 0.016) as compared with other groups. And there was a significant difference represented by the increase of CAT concentration in T3 and T4 groups (0.75 ± 0.005), (0.69 ± 0.006) respectively as compared with T2 group, and there was a significant difference in CAT concentration between T3 and T4 groups represented by the increase of CAT concentration in T3 when compared with T4 group.

Discussion

1. The effect of Allicin on Semen Parameters in Wister male rats treated with Cyclophosphamide

Cyclophosphamide (CP) uses to treat cancer malignancies and acts as an immunosuppressive agent. CP is mutagenic and induces many side effects such as alteration of male spermatozoa leading to sterility. The metabolism of CP produces two active metabolites, phosphoramide mustard and acrolein. Some studies have reported that CP can induce germ cell toxicity by generating ROS (Sikka, 2001). ROS induce a significant reduction in semen quality by decreasing sperm count and motility. They can also increase sperm defects and impairment of antioxidant synthesis such as SOD,CAT, GPX, SOD,GSH (Hatamoto *et al*., 2006).

The results of the present study of CP treated rats (T2) showed that CP induced significantly decrease in sperm count and motility in compression with the control group. Selvakumar *et al*. (2006) found that CP increased MDA and hydrogen peroxide levels as well as decreased activities of SOD, GPx and GR in the mitochondrial fraction of testes. Lear *et al*. (1992) showed that chronic administration of CP resulted in an increased lipid peroxidation in rats, increased lipid peroxidation associated with continuing oxidative stress lead to severe damage at plasma membrane level (Fischer *et al*., 2003; Vernet *et al*., 2004). As it is mentioned previously CP results in severe lipid peroxidation by elevating the oxidative stress (Ordoudi *et al*., 2009). Spermatozoa are rich in polyunsaturated fatty acids which are susceptible to peroxidative damage (Chi *et al*., 2008). The decrease in sperm count is an important factor leading to male infertility (Meistrich and Brown, 1983). Selvakumar *et al*. (2006) reported that the decrease in sperm counts is

due to the generation of ROS by CP and the consequential elimination of sperm cells at different stages of development. Toxic side effects of CP on spermatocytogenesis and spermiogenesis and so on germinal epithelium destruction could be the result of decrease of sperm generation (Meistrich, 1986).

CP causes DNA strand damage which may be associated to affect on sperm production (Meistrich, 2009). Besides, cyclophosphamide can lead to decreased plasma testosterone and change in the count of germ cells (Das *et al*., 2002). This affects steroid hormones and finally results in disturbed spermatogenesis process therefore this lead to reduction in number sperms. The first sign of increased ROS content is remarkable reduction in sperm motility (Iwasaki and Gagnon, 1992). The increased levels of ROS considerably influences sperm enzymatic content and increases phospholipids peroxidation, which ultimately reduces fluidity of cell membrane and sperm motility (Agarwal *et al*., 2003). Generation of ROS by CP decomposes sperm plasma membrane and is therefore responsible for loss of sperm motility (Aitken and Clarkson, 1987), which is presumably caused by a rapid loss of intracellular ATP leading to damage in sperm flagellum. Activity of Na^+-K^+-ATPase is highly sensitive to ROS. Thus, depletion of Na^+-K+-ATPase can be a good reason for the reduction of sperm motility. Both decreased sperm motility and increased sperm DNA damage can result from high levels of reactive oxygen species produced by leukocytes in semen (Aitken *et al*., 1998).

Recently, Maremanda *et al*. (2014) demonstrated that CP administration significantly increased the oxidative stress, sperm DNA damage and reduced sperm count and motility. Similarly, this study suggested the increase percentage of abnormal sperm and percentage of dead sperm in CP administrated group (T2). The increase in sperm abnormalities indicates that CP induced DNA damage in germ cells leading to altered sperm morphology. Sikka (2004) has reported that peroxidation of critical thiol groups in protein can alter the structure and function of spermatozoa. During the maturation process, a part of the sperm cytoplasm removes as residual bodies by Sertoli cells, otherwise the cytoplasmic droplets will be remain at the middle piece of sperm. Thus, the sperms with cytoplasmic droplets did not complete their maturation process (Gil-Guzman *et al*., 2001). Therefore, we can assume that CP exerts its degenerative impact partly via influencing the Sertoli cells physiologic function, which could be partly attributed to CP-induced oxidative stress. The existence of morphologic abnormalities and decreased sperm viability has been associated with ROS production (Codrington *et al*., 2004; Aziz *et al*., 2004). Cyclophosphamide causes reduction of 17β-Hydroxysteroid dehydrogenases (17β-HSD), which is an enzyme that catalyzes the interconversion of androstenedione to teoststerone and a reduction of its activity can decrease spermatid count per testis, sperm count per epididymis, daily sperm production/gram testis, sperm motility, and significantly increased abnormal sperm rates (Abarikwu *et al*., 2012).

Also, defects in the flagella, changes in motility and morphology of spermatozoa are likely associated with infertility. According to previous studies, the rate of ROS generation is directly related to the number of dead or abnormal spermatozoa (Iwasaki and Gagnon, 1992). These results indicate that CP induced cytotoxicity in sperm cells and this is in agreement with reports of Elangovan *et al*. (2006) and Oyagbemi *et al.* (2016).

Our results demonstrated that animals, which were treated with allicin (T1) showed improvement in sperms quality (viability, motility and concentration) and decrease in sperms abnormality as compare with control group. Biologically active compounds derived from *A. sativum*, have been shown to exert various pharmacological activities and are considered to have therapeutic potential effect for many pathologic conditions (Rivlin, 2001).

Allicin (diallyl-thiosulfinate) is the most biologically active compound of *A. sativum*. It certainly acts as a "physiological antioxidant (Gruhlke and Slusarenko, 2014). Whole garlic and aged garlic extract exhibit direct antioxidant effects and enhance the serum level of two antioxidant enzymes, catalase and glutothion peroxidase (Popov *et al*., 1994). Decreasing the percentage of abnormal sperms may be due to ability of allicin to scavenge endogenous ROS, which can damage cell structure like nucleic acids , carbohydrates, proteins, lipids, and change their functions (Birben *et al*., 2012). Furthermore, the antioxidant activity of allicin is very necessary in mitotic divisions of germ cells in seminiferous tubules and then produce normal sperms without abnormalities. Allicin protect sperm plasma membrane from oxidative stress by ROS.

The reproductive hormones (ICSH, SSH and testosterone) have cooperative effects on spermatogenesis, ICSH stimulate leydig's cells to produce testosterone hormone and SSH stimulate sertoli cells to synthesis androgen binding protein which carries testosterone to the target sites in the spermatogonia and epididymis for development and maturation of sperms (Pineda and Dooly, 2003).

Aqueous extract of *Allium sativum* treatment

ameliorated the pituitary-testicular injury and dysfunction in Wister rats Induced reproductive disturbances (Ayoka *et al*., 2016). This result leads to conclude that allicin have improver effect on sperm quality by stimulating the testis and epididymis or hypothalamic-pituitary-testis axis through stimulating testosterone hormones secretion and enhanced the serum level of antioxidants or by exhibit direct antioxidant effect.

Also our results showed that the treatment with allicin confers cytoprotection against the deleterious effects were caused by CP preventing cellular damage in animals that were treated with allicin before cyclophosphamide (T3) and animals that treated with allicin after cyclophosphamide (T4) as compared with cyclophosphamide treated group (T2). Endogenous and exogenous antioxidants may protect cells and tissues from destructive effects of ROS and other free radicals. Previous studies reported that sperm disorders can be improved by exogenous antioxidants/ROS scavengers. Some studies have reported that garlic improves male sexual function and has beneficial effect in the recovery of testicular functions (Hammami and El May, 2013). Allicin has ameliorating effect reproductive hormones which are responsible for improving sperms production and induced increasing the blood levels of antioxidant enzymes (SOD, CAT, Gpx) (Dhanarasu, 2015). The increase in these enzymes reduced the mitochondrial-generated reactive radicals from causing oxidative stress and cellular damage (Flora *et al*., 2009). It is important to note that administration of allicin may has brought about increased antioxidant activities, which protect against oxidative damage to DNA, proteins and membrane lipids. Furthermore, it can prevent and/or detoxify intermediate metabolites of chemical carcinogens and stimulate the immune responses. This probably protected the sperm cells and leydig cells from depletion due to lipid peroxidation caused by ROS within the testes. This could be the reason why the allicin treated groups (T3, T4) had improved sperm quality as compared to the CP-treated group (T2). It could be concluded that administration of allicin, reduces CP-induced oxidative stress by decreasing lipid peroxidation and activating antioxidant enzymes in the testes .

2. The effect of Allicin on weight of testesin Wister male rats treated with Cyclophosphamide

The results of the present study showed a significant increase in weight of testis in T1 group, which treated with allicin when compared with other groups. The reason of this increasing may be the result of the beneficial effect of allicin on gonadotropin hormones. Also, allicin stimulates testosterone production which is essential for normal growth of testis. Therefore, this leads to increase in numbers of leydig's cells, sertolicells, spermatogonia cells and spermatocytes (Pineda- and Dooly, 2003). Allicin is the most biologically active compound of garlic (*A. sativum*), which induces spermatogenesis (Chauhan *et al*., 2007). Also, it can be concluded that the increase in weight of testis returned to increase in spermatogenesis, and number of testicular cells. Moreover, allicin prevents cellular damage by decreasing lipid peroxidation and this leads to protect the testicular cells and sperms from oxidative stress.

In T2 group which was treated with cyclophosphamide the results revealed there were significant decrease in weight of testis when compared with other groups because of toxic side effects of CP on spermatocytogenesis and spermiogenesis and so on germinal epithelium destruction which cause decrease of sperm generation (Meistrich, 1986). Also cyclophosphamide causes reduction in reproductive hormones which induce reducing in sperm concentration and number of testicular cells, and this leads to decrease in weight of testis. The weight of the testis largely depends on the mass of the differentiated spermatogenic cells, a reduction in the organ weight may be attributed to decreased sperm production (Katoh *et al*., 2002). Other studies had also been reported that chronic low dose administration of cyclophosphamide can decrease the weights of reproductive organs in male rats, cause atrophy in certain organs such as testis and epididymis, and hence affect fertility rate (Lu *et al*., 2015; Das *et al*., 2002).

Our results showed that treatment with allicin after treatment with cyclophosphamide (T3) and before cyclophosphamide (T4) a significant increase in weight of testis when compared with T2 group. This protective effect of allicin can be explained by the fact that it prevents cellular damage occurring as a result of oxidative stress in spermatogenic cells of seminiferous tubules and leydig cells. Our results showed that treatment with allicin confers cytoprotection against the deleterious effects caused by cyclophosphamide preventing cellular death. allicin acts as a free radical scavenger and restores the antioxidants system in cells such as superoxide dismutase, catalase, glutathione peroxidase (Popova and Popove, 2005).

Also, our results indicated there was a significant difference between T3 and T4 groups represented by increasing in weight of testis in T3 group when compared with T4 group this increase return to increase in sperms

Table 1 : The Effect of Allicin on Semen Parameters in Wister Male Rats Treated with Cyclophosphamide.

Parameters / Groups	Sperms concentration (million/ml)	Percentage of total motility (%)	Percentage of abnormal sperms(%)	Percentage of sperms viability(%)
C group	**A**89.34 ± 0.78	**A**90 ± 53	**A**13.39 ± 0.53	**A**84.49 ± 1.14
T1 group	**B**112.44 ± 4.46	**A**91.2 ± 0.62	**B**9.38 ± 0.65	**B**89.31 ± 0.84
T2 group	**C**61.57 ± 1.5	**B**49.9 ± 1.1	**C**22.63 ± 0.89	**C**54.51 ± 2.07
T3 group	**D**81.76 ± 0.54	**C**83.6 ± 1.16	**AD**14.54 ± 0.58	**D**77.05 ± 1.98
T4 group	**D**79.77 ± 1.99	**D**77.4 ± 1.5	**D**15.5 ± 0.31	**D**76.82 ± 1.78
LSD 0.05	6.942	3.162	1.873	4.896

Numbers = mean ± Standard Error (S.E). Different litters = Significant Differences ($p<0.05$).
C= Control group, drenched orally with distilled water for (60) days.
T1= Drenched orally with allicin (50 mg /kg B.W/day) for (60) days.
T2= Drenched orally with cyclophosphamide (10 mg/kg B.W/day).
T3= Drenched orally with allicin (50 mg/kg/B.W/day)for (30) days then with cyclophosphamide (10 mg/kg/B.W/day) for (30) days.
T4= Drenched orally with cyclophosphamide (10 mg/kg/B.W/day) for (30) days then with allicin (50 mg/kg/B.W/day) for (30) days.

Table 2 : The Effect of Allicin on testes weight and concentration of MDA and CAT in Wister male rats treated with Cyclophosphamide.

Parameters / Groups	Weight of testes(mg)	Concentration of CAT(U/L)	Concentration of MDA(µmol/L)
C group	**AC**1.15 ± 0.01	**A**0.83 ± 0.005	**A**1.23 ± 0.005
T1 group	**A**1.23 ± 0.01	**B**0.85 ± 0.008	**A**1.21 ± 0.005
T2 group	**B**0.72 ± 0.04	**C**0.28 ± 0.016	**B**2.54 ± 0.034
T3 group	**C**1.09 ± 0.03	**D**0.75 ± 0.005	**A**1.31 ± 0.011
T4 group	**D**0.92 ± 0.04	**E**0.69 ± 0.006	**A**1.23 ± 0.192
LSD 0.05	0.107	0.0014	0.809

Numbers = mean ± Standard Error (S.E). Different litters = Significant Differences ($p<0.05$).
C= Control group, drenched with distilled water orally for (60) days.
T1= Drenched with allicin orally (50 mg /kg B.W/day) for (60) days.
T2= Drenched with cyclophosphamide orally (10 mg/kg B.W/day).
T3= Drenched with allicin orally (50 mg/kg/B.W/day)for (30) days then with cyclophosphamide (10 mg/kg/B.W/day) for (30) days.
T4= Drenched with cyclophosphamide orally (10 mg/kg/B.W/day) for (30) days then with allicin (50 mg/kg/B.W/day) for (30) days.

concentration ,sertolicells,spermatogonia cells and spermatocytesin T3 group.

3. The effect of allicin on concentration of MDA and CAT in Wister male rats treated with cyclophosphamide

In table 2, animals of T1 group revealed there was a significant increase in catalase (CAT) enzyme level in serum and a significant decrease in level of malondialdehyde (MDA) as compared with other groups.

MDA is a product of lipid peroxidation in the body and it is used as a standard in assessing free radicals damage in the blood and MDA level is an index for pathologic lipid peroxidation of sperm (Verma and Kanwar, 1999). The decreased concentration of MDA in the blood in this study is an indication of an increased activity of antioxidant enzymes in the blood. This finding is similar with that of Zhang *et al*. (2017). Previous study showed that use of allicin significantly reduced the content of MDA, and increased SOD and GSH-Px activities compared with control group (Gong-chen *et al*., 2014).

Catalase is one of the endogenous antioxidant enzymes. It reacts very efficiently with hydrogen peroxide (H_2O_2) to form water and molecular oxygen . Allicin acts as a free radical scavenger and restore the antioxidants system in cells such as superoxide dismutase, catalase, glutathione peroxidase (Popova and

Popove, 2005), this explain the reason of increase the level of catalase and decrease of MDA level with allicin treatment.

In T2 group which was treated with cyclophosphamide there was a significant decrease in level of CAT and increase in level of MDA in serum in compared with other groups. Excessive production of free radicals or ROS is mainly responsible for peroxidation of cell membrane lipids and other unsaturated lipids (especially LDL) the terminal product of lipid peroxidation n is MDA. (Chlubek *et al*., 2003). Cyclophosphamide can prevent the activities of catalase and peroxidase and increase lipids peroxidation in testicular tissue (Nayak *et al*., 2016), MDA generated with peroxidation by reactive oxygen species of fatty acids and lead to irreversible cell damage (Montjean *et al*., 2010). This explain why increase in MDA level and decrease CAT level with cyclophosphamide treatment.

In T3 and T4 group the results of this study revealed there was a significant increase in catalase (CAT) enzyme level in serum. Likewise, a significant decrease in level of malondialdehyde (MDA) as compared with T2 group, due to the protective effect of allicin on lipid peroxidation and enzymatic antioxidants in damaged cells (Dhanarasu, 2015). The increase in these enzymes reduced the mitochondrial-generated reactive radicals from causing oxidative stress and cellular damage (Flora *et al*., 2009). It is important to note that administration of allicin may has brought about increased antioxidant activities, which protect against oxidative damage. It could be concluded that administration of allicin, reduces CP-induced oxidative stress by decreasing lipid peroxidation and activating antioxidant enzymes in the testes. Also our results indicated there was a significant difference between T3 and T4 groups represented by increasing in CAT level in T3 group when compared with T4 group.

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