Research Article

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Role of Alpha Lipoic Acid in Ameliorating the Histological Alterations of Pituitary-Testicular Axis –induced by Hydrogen Peroxide in Rats

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Abstract

The present study was aimed to investigate the role of alpha-lipoic acid (ALA) as an antioxidant against in ameliorating histological disorders of pituitary- testicular axis- induced by hydrogen peroxide (H2O2) in adult Wistar rats. Forty adult male rats were randomly divided into four equal groups (10 rats /group) and were handled daily as follows for 56 days: Control group (C) were intubated distilled water and received ordinary tap water; group T1 were intubated 60mg/kg B.W of ALA and received ordinary tap water; group T2 were administered H2O2 in tap water at a concentration of 0.05%, while group T3 were intubated 60mg/kg B.W of ALA and received ordinary tap water containing 0.05% H2O2. At the end of the experiment, body weights were recorded, then pituitary and testes were excised for histopathological study and testicular weight was recorded too. Rats administered H2O2 showed a significant decrease in testes weight to body weight ratio accompanied with major histopathological changes of the testes in comparison with other groups including; a significant decrease in the diameter of seminiferous tubules, high of germinal epithelial cell and degenerative changes with incomplete spermatogenesis. Besides, a significant decrease in the number of Leydig's cells in comparison with other experimental groups. Furthermore, pituitary gland of group T2 manifested a severe histological alteration in architecture characterized by atrophy with marked necrotic and degenerative changes. Whereas, rats administered ALA (group T3) shows an improvement of histological changes of pituitary and testicular tissues induced by hydrogen peroxide. In conclusion, the results indicated that alpha-lipoic acid mitigated pituitary-testicular dysfunctions induced by H2O2 through its antioxidant effects via scavenging free radicals.

Keywards: : Hydrogen peroxide, Alpha lipoic acid, Testes, Pituitary gland, Leydig's s cells

دور حمض الفا لايبوك في تخيف حدة التغيرات النسجيه للغدة النخاميه والخصى المستحدثة باستخدام ببيروكسيد الهيدروجين في الجرذان

الخلاصة

هدفj الدراسة الحالية الى معرفة دور حمض الفا الليبويك كمضاد للأكسدة في تخفيف التغيرات النسجيه للغدة النخاميه -الخصى المستحدثة ببيروكسيد الهيدروجين في الجرذان الذكور البالغة. تم أستخدام أربعين من الجرذان الذكور البالغه وقسمت عشوائيا إلى أربع مجاميع متساوية وعوملت يوميا ولمدة 56 يوما على النحو التالي: مجموعة السيطرة (C) اعطيت الماء المقطر بالإضافة إلى مياه الصنبور؛ مجموعة T1 أعطيت 60 ملغم /كغم من وزن الجسم من حمض الفا ليبويك مع مياه الصنبور العادية؛ في حين أعطيت المجموعة T2 ماء الصنبور الحاوي على بيروكسيد الهيدروجين بتركيز 0.05٪ ، أما المجموعة T3 أعطيت 60 ملغم /كغم من وزن الجسم من حمض الفا ليبويك مع ماء الصنبور الحاوي على بيروكسيد الهيدروجين بتركيز 0.05٪ . في نهاية التجربة تم وزن الجرذان ، ثم تم استئصال الغدة النخامية والخصيتين لغرض الدراسة النسجية وتم تسجيل وزن الخصية أيضا. أظهر الفحص النسيجي للخصى لمجموعة T2 أن أعطاء بيروكسيد الهيدروجين تسبب في تغيرات نسيجية بالمقارنة مع المجموعات الأخرى تمثلت بأنخفاض كبير في قطر النبيبات المنوية ، ارتفاع الخلايا الظهارية الجرثومية وعدد خلايا ليديك ، مع وجود التغيرات التنكسية ونطف غير مكتملة . فضلا عن، فقد اظهر الفحص النسيجي للغدة النخامية لمجموعة T2 تغييرات شديدة في المظهر النسجي تميزت بضمور مع نخر ملحوظ وتغيرات تنكسية. في حين أن جرذان المجموعة T3 أظهرت تحسنا كبيرا تمثلت في تقليل الضرر النسيجي للنبيبات المنوية كما تم أصلاح التغيرات المرضية للغدة النخامية لنفس المجموعه من قبل حمض الفا ليبويك والتي أستحدثت بيروكسيد الهيدروجين. في الختام اشارت النتائج الى ان حمض الفا ليبويك يخفف من حدة التغيرات التركيبية-الوظيفية للغدة النخامية-الخصى الناجم عن بيروكسيد الهيدروجين من خلال تأثيره المضاد للاكسدة عن طريق كنس الجذور الحرة.

Introduction

In modern world, involvement of stress has been suggested in the development of human depression (1) . In animals, unpredictable stressors have been shown to induce changes in behavioral parameters, including changes in locomotors and explorative behavior, impairment of feeding, drinking and sexual behavior (2). Normally low levels of reactive oxygen species (ROS) are required for normal sperm functioning and capacitation(3), disproportionate levels of ROS can negatively impact the quality of spermatozoa and impair their fertilizing capacity (4,5). Under pathological condition, excessive production of ROS, exceeds the antioxidant capacity of seminal plasma resulting to induced oxidative stress (OS) by inducing lipid peroxidation (LPO) and/or DNA damage associated with poor sperm functions (6,7,8,9). So it has been considered that OS affect the fertility status and physiology of spermatozoa (10). Hydrogen peroxide (H_2O_2)is non- radical oxidant produced in *vivo* by many reactions via a wide variety of enzymes(11). Recently many studies showed that H_2O_2 is one of the most toxic reactive oxygen species in the mammalian spermatozoa (12,13).

Alpha lipoic acid is found in abundance in animal tissues with high metabolic activity, such as kidney, heart, and liver, and to a lesser extent in fruits and vegetables. All alpha lipoic acid supplied by the diet is transported in the bloodstream to tissues and incorporated into cells. (14). ALA works on the cellular level to help produce energy in the body, as a part as, a coenzyme in the citric acid cycle by preparing the fuel for the mitochondria, and plays a vital role in mitochondrial electron transport reactions required for cellular energy production (15). It has been reported that ALA have powerful antioxidant abilities, equal to that of coenzyme Q 10, vitamin C, and vitamin E (16). Unlike other antioxidants, ALA alpha lipoic acid has ability to neutralize free radicals in intracellular and extracellular environments (17).

Researchers suggested that alpha lipoic acid possess a dual effect of lipid lowering and anti-atherosclerotic properties (18) and could improve insulin resistance and hyperlipidemia associated with high fat diet in mice by scavenging ROS (19). Besides (20) demonstrated that strengthening of endogenous antioxidant capacity of kidney during diabetic nephropathy , through generated a novel genetic antioxidant mouse model with over-or under expression of lipoic acid synthase gene could be an effective strategy for prevention and treatment of diabetic nephropathy. Recently, α-lipoic acid through its antioxidant and steroidogenic properties mitigated testicular toxicity which eventually restored the male reproductive health of carbimazole-exposed rats (21). The present study was aimed to investigate whether the administration of hydrogen peroxide in drinking water affects the pituitary and testicular functions and if so, whether the supplementation of ALA protects the pituitary and testes and its functions in adult male rats.

Materials and Methods

Forty adult male rats were randomly divided into four equal groups (10 rats /group) and were handled daily as follows for 56 days : Group C, rats in this group were intubated distal water plus received ordinary tap water and served as control ; Group T1 , rats in this group were intubated 60mg/kg B.W. of alpha lipoic acid as well as received ordinary tap water ; Group T2, rats were administered H_2O_2 in tap water at concentration of 0.05% and Group T3, rats in this group were intubated 60mg/kg B.W. of alpha lipoic acid and received ordinary tap water containing 0.05% H_2O_2.

At the end of experimental period, animals were weighed by sensitive balance and were anesthetized by intramuscular injection of (Ketamine 90mg/Kg B.W and Xylazine 40mg/kg B.W), abdominal cavity was opened, testes were excised , put in the physiological normal saline and cleaned from adipose and connective tissues. After being cleaned both testes (left and right) were weighed individually by a sensitive balance, then testicular weight /body weight ratio (%) was calculated. For histological studies , testis and pituitary gland were excised, cleared and preserved in neutral-buffered formalin 10%. Then histological sections for testes and pituitary gland were prepared with thickness equal 5-6 µ and stained with Hematoxylin-Eosin stain (H&E) using standard histological protocols according to (22) for histopathological study. Whereas, the diameter of seminiferous tubules and high of

germinal epithelial cells of tubules were by upload all image into a computer by means of a digital camera (MEM 1300) through the microscope. The measurement has been carried out with image J (Java-based image processing program developed at the National Institutes of Health), as well as counting of Leydig's cells was done by reading the cells between each three seminiferous tubules 10 cross-section per rat were recorded for 10 rats each group . Readings were done under 40X magnifications and calculation of the mean number of Leydig's cells cell / µmm2 (23). Statistical analysis of data was conducted on the basis of One-Way Analysis of Variance (ANOVA) utilizing a significant levels of ($P<0.05$). Specific group differences were determined using Least Significant Differences (LSD) as portrayed by (24).

Results and discussion

The ratio of testis weight to the body weight illustrated in figure (1-A) revealed that this ratio was significantly ($P<0.05$) increased in animals that received ALA (T1) compared to other groups, in the same time there were significant ($P<0.05$) decrease in this ratio in group T2 when compared to C and T1 groups. While an increase in this parameter was observed in group T3 when compared with group T2 , but did not reach the limits of significance ($P>0.05$). Figure(1-B) illustrates that rats received 0.5% hydrogen peroxide in drinking water (group T2), showed a significant ($P<0.05$) decrease in the number of Leydig's cells as compared with that in groups C and T1. Whereas, addition of ALA to hydrogen peroxide treated group (T3) , caused remarkably increased in the number of interstitial cells (2.2± 0.37) without reaching a significant ($P>0.05$) level as compared to group T2 (1.5±0.22). Furthermore, the histological sections of testes rats showed non-significant differences ($P>0.05$) in the number of Leydig's cells in rats treated with ALA (groupT1) compared to control group with mean value were (3.3± 0.30) and (2.7± 0.21) respectively.

Results of the present study demonstrate a decrease in testicular weight to body weight ratio in group T2. This finding gives the evidence that H_2O_2 has adverse effect on testicular weight to body weight ratio, may be due to induced-oxidative stress. This results was agreement with (25,26). Besides, this decrement was in parallel with that of decrease in number Leydigs cells may be through the effect of H_2O_2 on testicular tissue. The significant decline of this parameter following H_2O_2 administration may be associated with impaired spermatogenesis caused by reduced testosterone secretion and/or due to change in oxidant/antioxidant status (26,27). Some reports, showed that the decrease in testicular weight was also as the result of reduction of diameter of seminiferous tubules, germinal epithelial cells arrest and inhibition of steroid biosynthesis of Leydig cells (28,29). In addition, an elevation in serum ROS level associated with disruption of antioxidant enzymes may be led to loss of animal appetite (30) could be claimed. The improvement in testicular weight to body weight ratio in rats exposed to ALA plus H2O2 or ALA alone , which is in accordance with (31). It is believed that ALA via its antioxidant property can scavenge several reactive oxygen species and also inhibits the generation of hydroxy radicals that attack sulphur-containing antioxidants and has ability to sustain the levels of protein thiols which act as antioxidant and modulate tissue endogenous antioxidants (31,32). Based on these extraordinary properties, ALA has been shown to protect the male reproductive health against a range of testicular toxicants in experimental animals such as polychlorobiphenyl (33).

Microscopic views revealed no differences was reported in the histological structure of pituitary gland of rats in control group figures (1, 2) On the contrary, group T2, which received H_2O_2 in drinking water , the sections showed atrophy and vaculation in stromal of pituitary gland with a marked necrotic and degenerative changes figures (3,4,5,6,). Furthermore, the results showed a mononuclear and inflammatory cells with fibrosis and debris tissue figures (7,8) as compared to control group. In spite of pathological alterations in group T2 , all aspects of histological structures are well achieved by ALA against hydrogen peroxide of pituitary gland in group T3, characterized by a reduction in degenerative and necrotic changes with no clear lesion figures (9,10). Besides, sections of pituitary gland of group T1 showed no significant differences and similar to those in control group figures (11,12).

Testicular sections of rats received ALA had normal seminiferous tubules with complete spermatogenesis and normal interstitial connective tissue figures (13, 14,) in comparison to control figure (15,16). H2O2 treated rats group T2 exhibited histopathological alterations in thewith degenerative changes of majority of seminiferous tubules characterized with incomplete spermatogenesis and edema figure (17). Besides the majority of seminiferous tubules were almost devoid of spermatids and spermatozoa with vacuolar degeneration of spermatogonia , Sertoli cells and Leydigs cells which evoked on cessation of spermatogenesis figures (18, 19) . Other sections revealed a severe disorganized and degeneration interstitial tissue figure (20). Conversely, the testes of rats treated with H_2O_2 concurrently with alpha- lipoic acid , revealed some slight vacuolization with normal germ cells of the seminiferous tubules and connective tissue figure (21). In addition a numbers of seminiferous tubules showed a marked improvement of spermatogenesis with well-organized figure (22) and presence of sperm in lumen with normal architecture of Leydig's cells evidenced that ALA had a protective effects.

Histopathological alterations of architecture were obvious in sections of rat testes exposure to H_2O_2 in tap water. Similar results were corresponding with (34,35) .As mentioned previously , these findings are well correlate with the H2O2-induced oxidative stress. Moreover, high of germinal cells of seminiferous tubules and Leydig's cells (in the interstitial tissue) were decreased at the end of the experiment. H2O2 accumulation in Leydig's cells, may be important in oxidative stress-induced apoptosis and decreased testosterone production. These results are consistent with (36,37). As it is known, testosterone is necessary for spermatogenesis. Hydrogen peroxide - can caused reducing of testosterone level, which lead to a decrease in testicular weight (as mentioned above) with disturbance the spermatogenesis and structural and functional damages of the cells under the influences of OS , these results are consistent with (38). In accordance to these changes, (39) reported that oxidative stress induced by acrylamide caused cytoskeletal inhibition, could result in diminished uptake of cholesterol by Leydig's cells and consequent reduced testosterone synthesis (40). Besides, accumulation of H2O2 in Leydig's cells, may be important in -induced apoptosis and decreased testosterone production (36).

On the contrary, ALA treatments (group T3) caused decrease the pituitary- testicular toxicity and protected them from oxidative damage. These effects are similar to (39,41) using alpha lipoic acid against pesticides and acrylamide toxicity respectively. The administration of ALA induced significantly reverted back the structural alteration of both testes and pituitary gland to near normal, this might be due to the free radical scavenging activity of ALA manifested by rearrangement of cells of seminiferous tubules and restored activity. Alpha Lipoic Acid giving dual protection via its action on both inside the cell and at the membrane level, thereby, its regulate the metabolism and reduction the incidence of mitochondrial dysfunction and then sufficient amount of ATP production (42). According to available literatures , no studies were done on effect of H2O2 and lipoic acid on pituitary – testicular axis . Therefore, it would be logical to conclusion that exposure to hydrogen peroxide resulted in histological changes of the pituitary and testes of albino rats. These changes are proportional to the duration of exposure. Moreover, it could be concluded, that ALA has a protective role against H2O2 by its antioxidant properties which is reflected on pituitary and testicular histology .

Research Article
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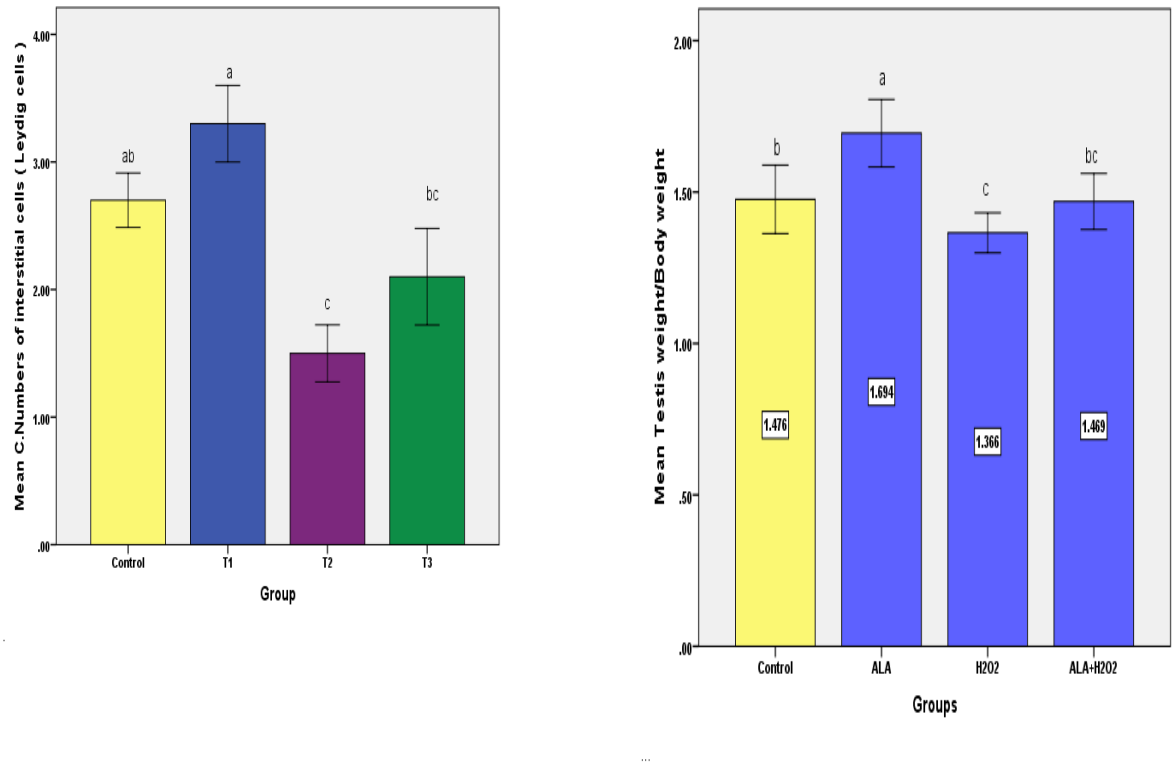

A **B**

Figure (1) Effect of alpha lipoic acid (ALA) and hydrogen peroxide (H_2O_2) for 56 days male rats on (A) testis weight / body weight ratio and (B) number of interstitial cells (Leydig's cells)

Values are expressed as means ± of alpha lipoic acid . n = 7/ group. C : control received drinking tap water. T_1 : gavages alpha lipoic acid (ALA) (60 mg/ kg B.W). T_2 : received 0.5% H_2O_2 in drinking tap water. T_3 : received 0.5% H_2O_2 in drinking tap water plus 60 mg / kg B.W of ALA. Means with different small letters denote significant differences ($p < 0.05$) between groups.

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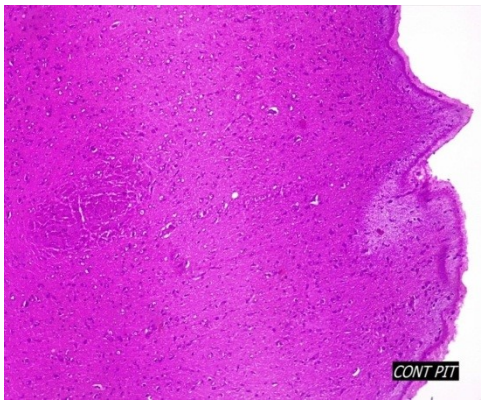

Figure 1. Section in pituitary gland of control animal shows normal structure of the gland (H&E stain 10X)

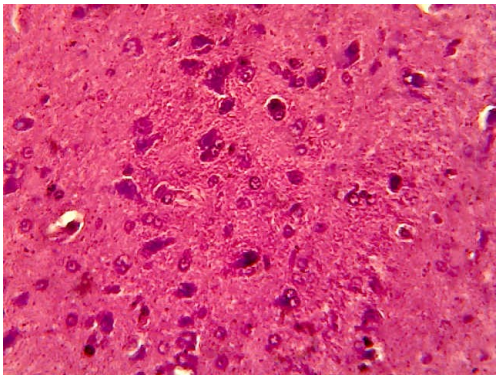

Figure 2. Section in pituitary gland of control animal shows normal structure of the gland (H&E stain 40X)

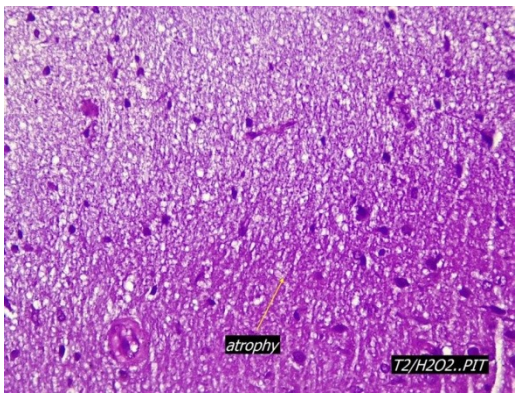

Figure 3. Section in pituitary gland of animal treatment with H2O2 in drinking water shows atrophy (H&E stain 10X)

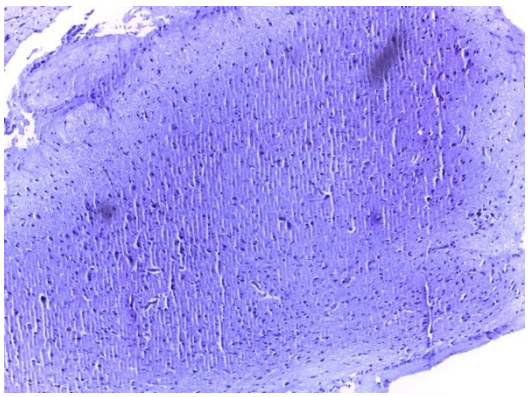

Figure 4. Section in pituitary gland of animal treatment with H2O2 shows necrotic area and cellular debris (10X)

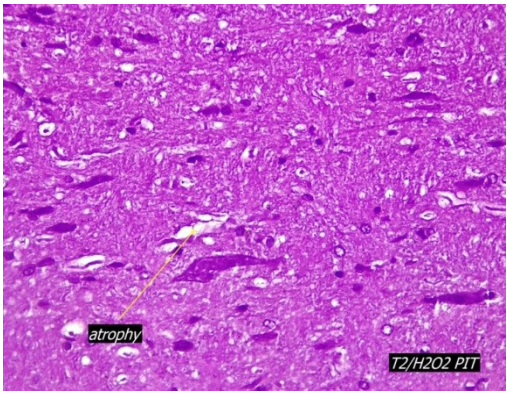

Figure 5. Section in pituitary gland of anima treatmentwith H2O2 in drinking water shows atrophy (H&E stain 40X)

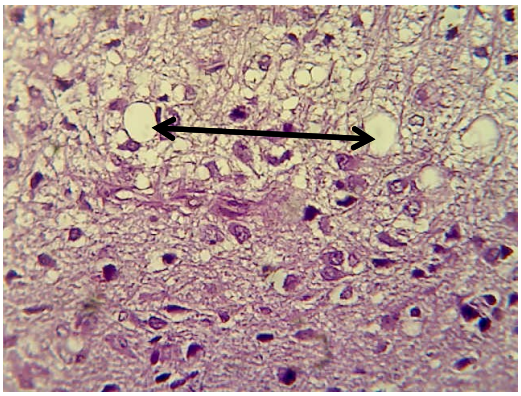

Figure 6. Section in pituitary gland of animal treatment with H2O2 shows vacuolation in the stroma of these gland ⟷ (H&E stain 40X)

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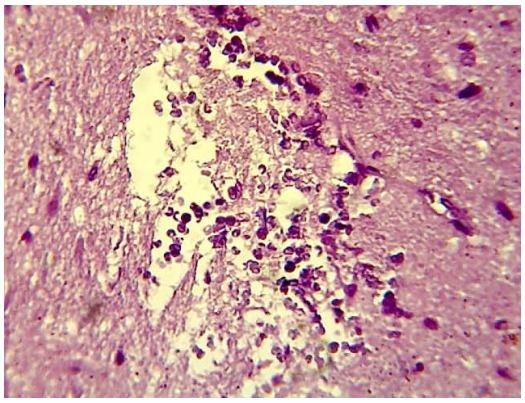

Figure 7. Section in pituitary gland of animal treatment with H2O2 shows necrotic area containing inflammatory cells and cellular debris ⟷ (H&E stain 40X)

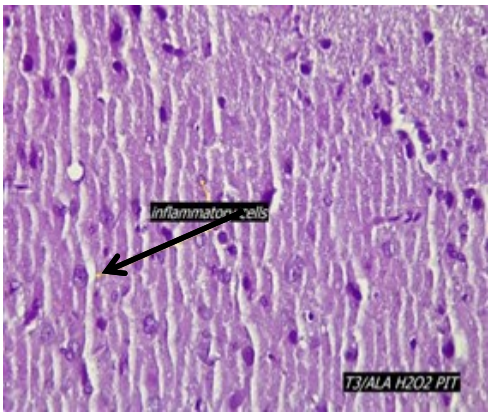

Figure 8. Section in pituitary gland of animal treatment with H2O2 shows cells and inflammatory cellular debris ← (H&E stain 40X)

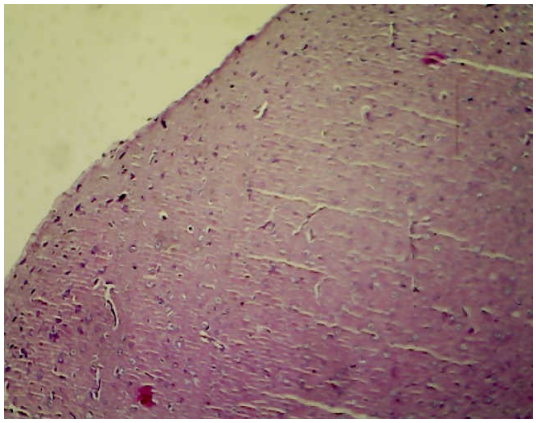

Figure 9. Section in pituitary gland of animal treatment with ALA and H2O2 shows no clear lesions (H&E stain 10X)

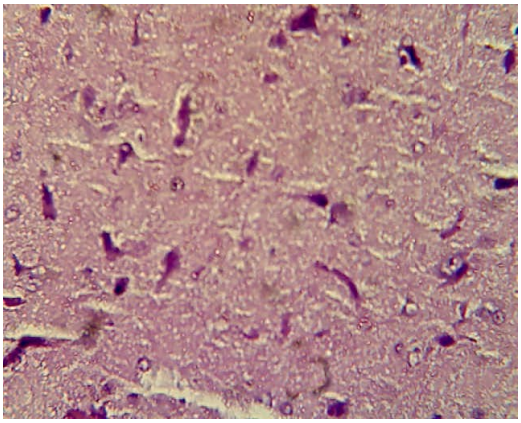

Figure 10. Section in pituitary gland of animal treatment withALA and H2O2 shows no clear lesions (H&E stain 40X

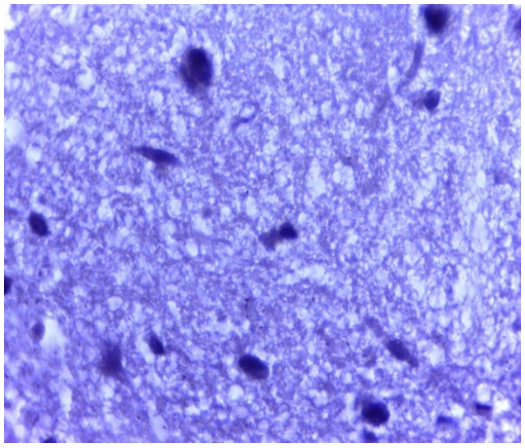

Figure 11. Section in pituitary gland of animal treatment with ALA shows no clear lesions (H&E stain 10X)

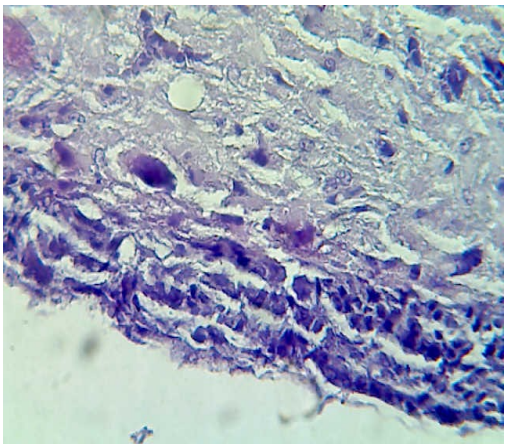

Figure 12. Section in pituitary gland of animal treatment with ALA shows no clear lesions (H&E stain 40X)

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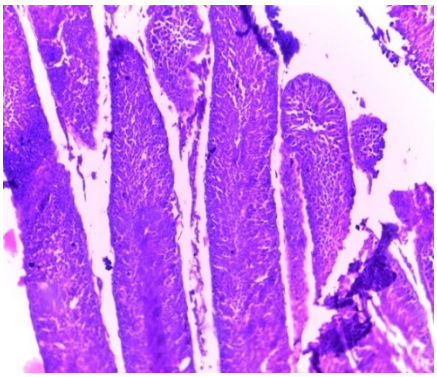

Figure 13. Section in testis of animal intubated with alpha lipoic acid shows (H&E 10X)

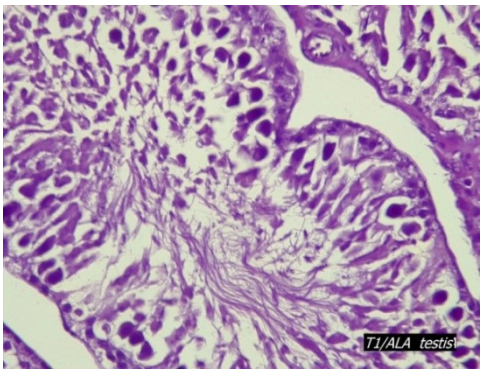

Figure 14. Section in testis of animal intubated with alpha lipoic acid(group T1) shows normal architecture of testicular tissue with well arrangement of seminiferous tubules (H&E 40X)

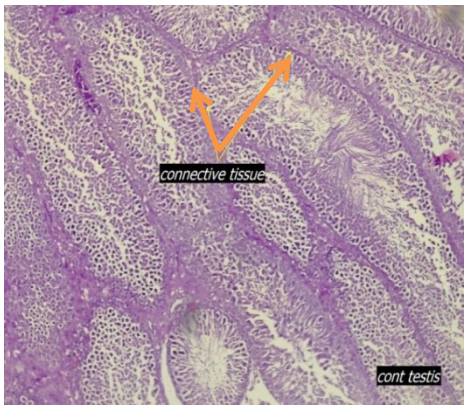

Figure 15. Section of normal rat testis (control group) shows normal arrangement of of seminiferous tubules with complete spermatogenesis and connective tissues

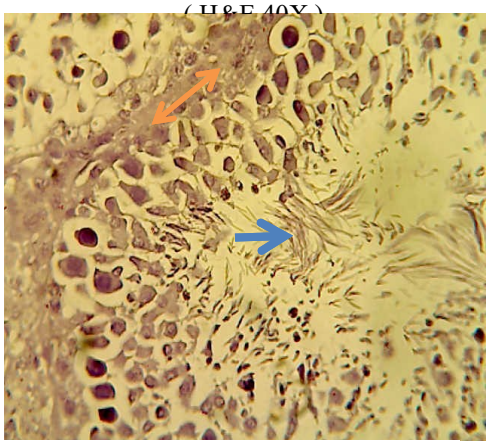

Figure 16. Section in testis of control animal shows normal interstitial tissue, ,sertoli cells , spermatocytes and spermatozoa in the lumen of seminiferous tubule

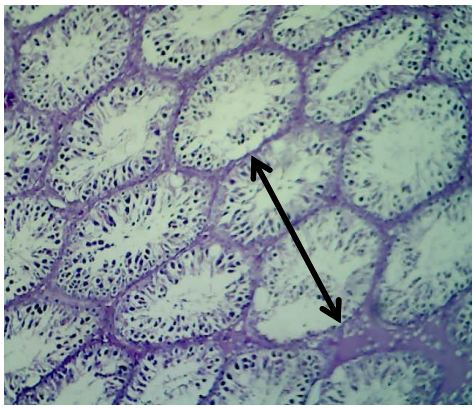

Figure 17. Section in testis of animal treatment with H2O2 in drinking water shows incomplelete spermatogenesis epithelium with edema in the interstitial tissue

(H&E stain 10X)

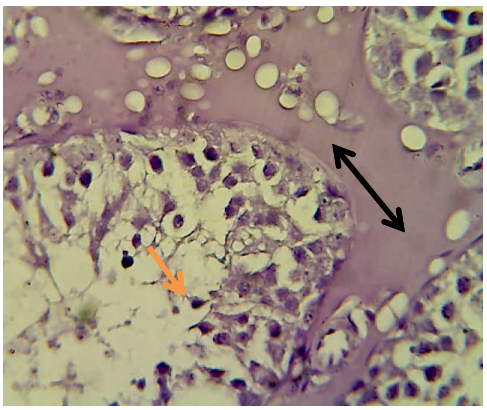

Figure 18 . Section in testis of animal treatment with H2O2 in drinking water (group T2) shows incomplelete spermatogenesis characterized by few numbers of germinal cells with vaculation and edema in the interstitial tissue (H&E stain 40X)

Research Article
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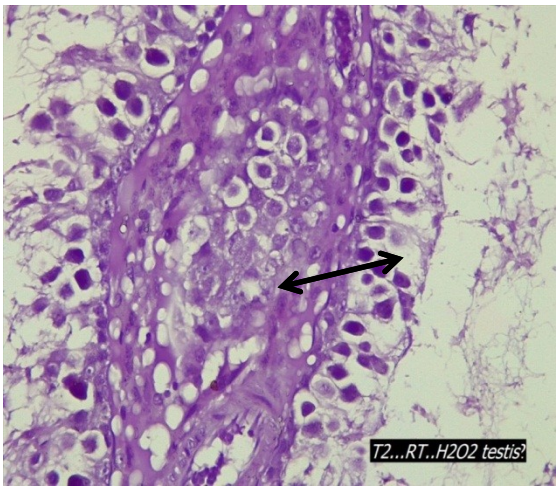

Figure 19. Section in testis of animal treatment with H_2O_2 in drinking water (group T2) shows vacuolation of germinal epithelial layer and , Leydigs cells and degeneration of interstitail tissue ⟷ (H&E stain 40X)

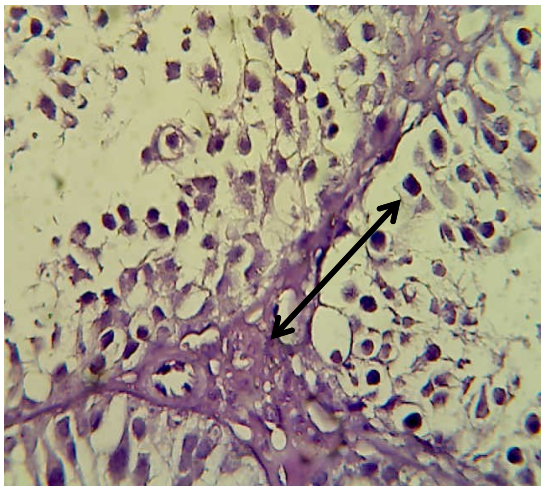

Figure 20. Section in testis of animal treatment with H_2O_2 in drinking water (group T2) shows sever disorganized and some necrotic cells with incompelete spermatogensis appear and degeneration of interstitail tissue ⟷ (H&E stain 40X)

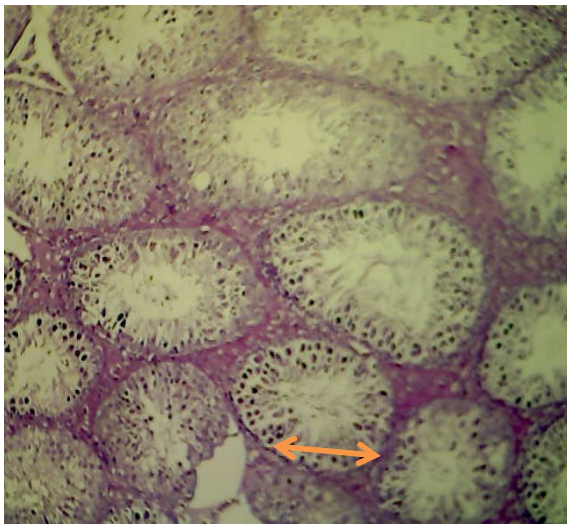

Figure 21. Section in testis of animal treatment with ALA and H2O2 (group T3) shows most of seminiferous tubules revealed slight vaculation of germinal epithalial cells with normal of both of seminiferous ducts and connective tissue ⟷ (H&E stain 10X)

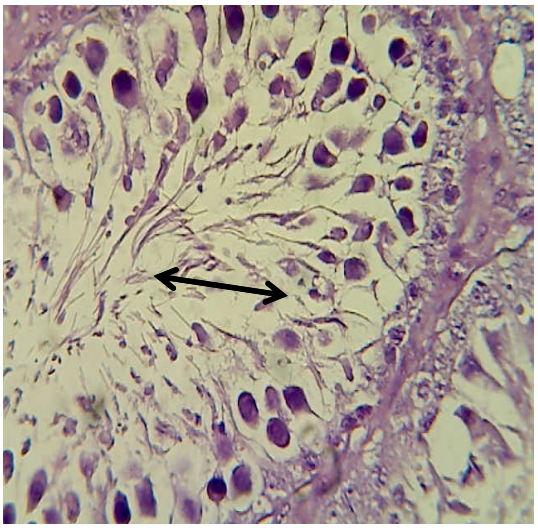

Figure 22. Section in testis of animal treatment with ALA and H_2O_2 (group T3) showed normal spermatogenesis and the seminiferous tubule filled with spermatozoa ⟷ (H&E stain 40X)

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