Kufa journal for Veterinary Medical Sciences Vol.(10) No.(1)

Kufa journal for Veterinary Medical Sciences

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Effect of alpha Lipoic Acid on Some Reproductive Hormones in Adult Male Wister Rats Exposed to Hydrogen Peroxide

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Received date:6May2019 **Accepted:**(449)12Juny2019 **page** (79-87) **Pubished:** 30 Juny2019

Abstract

The present study was aimed to investigate the role of alpha lipoic acid (ALA) on reproductive hormonal profile in adult male Wister rats treated with hydrogen Peroxide. Forty adult male rats were divided randomly 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 tap water ; group T_1 were intubated 60mg/kg B.W of ALA and received tap water; group T_2 administered hydrogen peroxide H_2O_2 in tap water at concentration of 0.05%, while group T_3 were intubated 60mg/kg B.W of ALA and received ordinary tap water containing 0.05% H_2O_2. Blood samples were collected at 0, 28 and 56 days of the experimental periods for measurement of serum follicular stimulation hormone (FSH), lutelizing hormone (LH) and testosterone (T) concentrations. The results revealed that group T2 showed a significant decrease in FSH and testosterone concentrations. While there were significant increase in previous parameters in T3 group compared to T2 group. Whereas, rats in group T3 shows significant improvement in above serum hormonal profile by repairing role of alpha lipoic acid against hydrogen peroxide in (group T2). In conclusion, alpha lipoic acid mitigated the deleterious effect of that-induced H_2O_2 induced pituitary-testicular dysfunction through antioxidant mechanism by free radical scavenging properties.

Key Words*: Alpha lipoic acid, hydrogen peroxide, LH, FSH and testosterone.*

دور حمض الفا الليبويك على صورة هورمونات التكاثر في ذكور جرذان الوستر البالغة المعاملة ببيروكسيد الهيدروجين

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الخلاصة:

الهدف من هذه الدراسة هو التحري عن دور حمض الفا الليبويك على صورة هورمونات التكاثر في ذكور جرذان الوستر البالغة المعاملة ببيروكسيد الهيدروجين (H_2O_2) . تم استخدام 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 بواسطة الفم بالإضافة إلى مياه الصنبور؛ مجموعة T_1أعطيت الجرعة الفعالة من حمض الفا الليبويك (60 ملغم لكل كيلو غرام من وزن الجسم) بواسطة الفموكذلكمياه الصنبور العادية؛ في حين أعطيت المجموعة T_2 ماء الصنبور الحاوي على بيروكسيد

الهيدروجين بتركيز 0.05٪، أماالمجموعة T_3 أعطيت الجرعة الفعالة من حمض الفا الليبويك بواسطة الفم مع ماء الصنبور الحاوي على بيروكسيد الهيدروجين بتركيز 0.05٪ . بعد تصويم الحيوانات جمعت عينات الدم للفترات 0 و 28 و 56 يوما من التجربه لتحديد تركيز الهرمون المحفز للجريباتوالهرمون الليوتيني و هرمون التستوستيرون.
أظهرت النتائج أن المجموعة T_2 أظهرت انخفاض معنوي في تركيز هرمون التستوستيرون وهرمون المحفز للجريبات في الدم. في حين أن جرذان المجموعة T_3 أظهرتزيادة معنويه كبيره فيصورة الهرمونات التكاثرية في الدم مقارنه بالمجموعة T_2. بينماالجرذان ف المجموعة T_3اظهرت تحسن معنوي لهذه الهرمونات بواسطة الدور التصليحي لحامض الليبوليك ضد بيروكسيد الهيدروجين في (المجموعةT_2). الخلاصة ان حامض الليبوليك خفف التأثير الضار لبيروكسيد الهيدروجين على محور النخامية- الخصية من خلال ميكانيكية مقاومة الأكسدة وذلك بواسطة خاصية كسح الجذور الحرة.

Introduction

Clearly, secretion of LH and FSH from the anterior pituitary is controlled by hormonal signals from both the hypothalamus and the testes including: Testosterone, estradiol (E2), dihydrotestosterone (DHT) and inhibin, while testosterone inhibits the secretion of GnRH and gonadotropins, besides inhibin B and follistatin suppress selectively the release of FSH from the pituitary gland, meanwhile activin stimulates this process (**1** & **2**)

Control of both two main functions of the testes are undergo to the central nervous system in a classic endocrine feedback loop with follicle stimulating hormone (FSH) and luteinizing hormone (LH) as the key hormonal signals. LH acts to promote of spermatogenesis through directly affecting on Leydig cells (that are located between the seminiferous tubules) to synthesize and produce testosterone, that is stimulates the maturation of germ cells in seminiferous tubules, whereas FSH affects the Sertoli cells thereby facilitating spermatogenesis. Therefore, **(3)** confirm that the trophic effects of testosterone/FSH on gametogenesis are mediated via somatic Sertoli cells.

Hydrogen peroxide (H_2O_2) is non- radical oxidant produced in *vivo* by many reactions via a wide variety of enzymes, it is a common reactive oxygen species (ROS), which is highly reactive and can interact with nearby molecules, inducing oxidative stress (OS) **(4)**. many studies showed that H_2O_2 is one of the most toxic reactive oxygen species in the mammalian spermatozoa **(5 & 6).** A considerable amount of literature has been published about the role of OS and ROS in the etiology and/or progression of a number of diseases **(7).**

Alpha-Lipoic acid is a naturally occurring nutraceutical, whose therapeutic effect has been related to its antioxidant activity and its ability to ameliorating oxidative damage (**8** & **9)**. 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 (**10**). It has been reported that ALA have powerful antioxidant abilities, equal to that of coenzyme Q 10, vitamin C, and vitamin E (**11**)**.** Unlike other antioxidants, ALA alpha lipoic acid has ability to neutralize free radicals in intracellular and extracellular environments **(12).** Therefore, the current study was designated to explore the protective role of ALA to ameliorating the deleterious effect of H_2O_2 on testicular function.

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 ALA **(24)** as well as received ordinary tap water ; Group T2, rats were administered H_2O_2 in tap water at concentration of 0.05% **(25)** 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**.** Blood samples were collected and transferred to a gel tube without anticoagulant at zero, 28and 56 days of experiment. Sera were isolated estimate follicular stimulating hormone (FSH), Luteinizing hormone (LH) and Testosterone hormone (T) concentrations. Assessment of serum Hormonal using ELISA Technique for FSH, LH and T hormones and optical density (OD value) of each well at once, used a micro-plate reader set at 450 nm. Calculation of results of the ELISA results were calculated depend on the optical density reading for each standard and samples optical density. Then the standard curve was plotted by the mean OD value for each standard on the Y-axis against the concentration on the X-axis and draw a best fit curve through the points on the graph.

Statistics

Statistical analysis of data was done on the basis of tow – way analysis of variance using a significant levels of ($p<0.05$), furthermore LSD test was used to determine specific differences (**13).**

Results

In this study all treatment groups showed non-significant ($p>0.05$) difference in serum FSH concentration compared to control at zero time Figure 1-A. Statistical analysis of the data indicate that the mean value of serum FSH concentration of T_2 group (81.29 ±1.8) and (76.28±1.56) was significantly ($p<0.05$) decrease after 28 and 56 days of treatment periods while in combination ALA with H_2O_2 in drinking water (group T_3) showed a significant ($p<0.05$) increase in serum FSH level at two treatment periods as compared to group T_2. Highest significant ($p<0.05$) decrease in this parameter was observed in T_2 and T_3 groups after 28 and 56 days of the treatment as compared with Zero time.

while there are no significant ($p>0.05$) differences in the mean values of serum LH level between experimental groups during pretreatment period (Zero time), a significant ($p<0.05$) increase in this parameter was observed at days 28 and 56 of the experiment in group T_2 with mean value of (46.29±1.41) and (44.85±1.24) respectively compared with T_1, T_3 and control groups Figure 1-B. Combination of ALA and H_2O_2 caused non-significant ($p>0.05$) differences in serum LH concentration at the end of the experiment comparing to control group. Within the time, non-significant ($p>0.05$) differences were observed in serum LH level at 28 and 56 days of the experiment in control, T_1 and T_3 groups comparing with pretreated period.

Figure 1-C pointed to the mean values of serum testosterone concentration of control and three treated groups. The results showed a significant ($p<0.05$) decrease in serum testosterone concentration in groups T_2 and T_3 as compared with control and T_1 treated groups. The figure also shows non-significant ($p>0.05$) differences in serum testosterone concentration at two treatment periods between control and T_1 groups. With exception to control and T_1 groups, within the time significant ($p<0.05$) decrease in T_2 and T_3 treated groups comparing to the pretreated period.

Discussion

The results that rats received 0.05% H_2O_2 (group T_2) cause significant decrease in testosterone concentration as well as FSH and LH as compared to other treated groups. Similar results were obtained by other investigators (**14**, **15 &16).**

Study the hormonal profile in adult male is critical point to overview the rate of reproductive performance. Steroidogenic of the testes characterized by an intense synthesis and secretion of testosterone. Furthermore, FSH and LH as well as other biochemical activity play an important role in testicular tissue functions (**17).** It is well

known that pituitary gland is highly sensitive to oxidative stress **(18).**

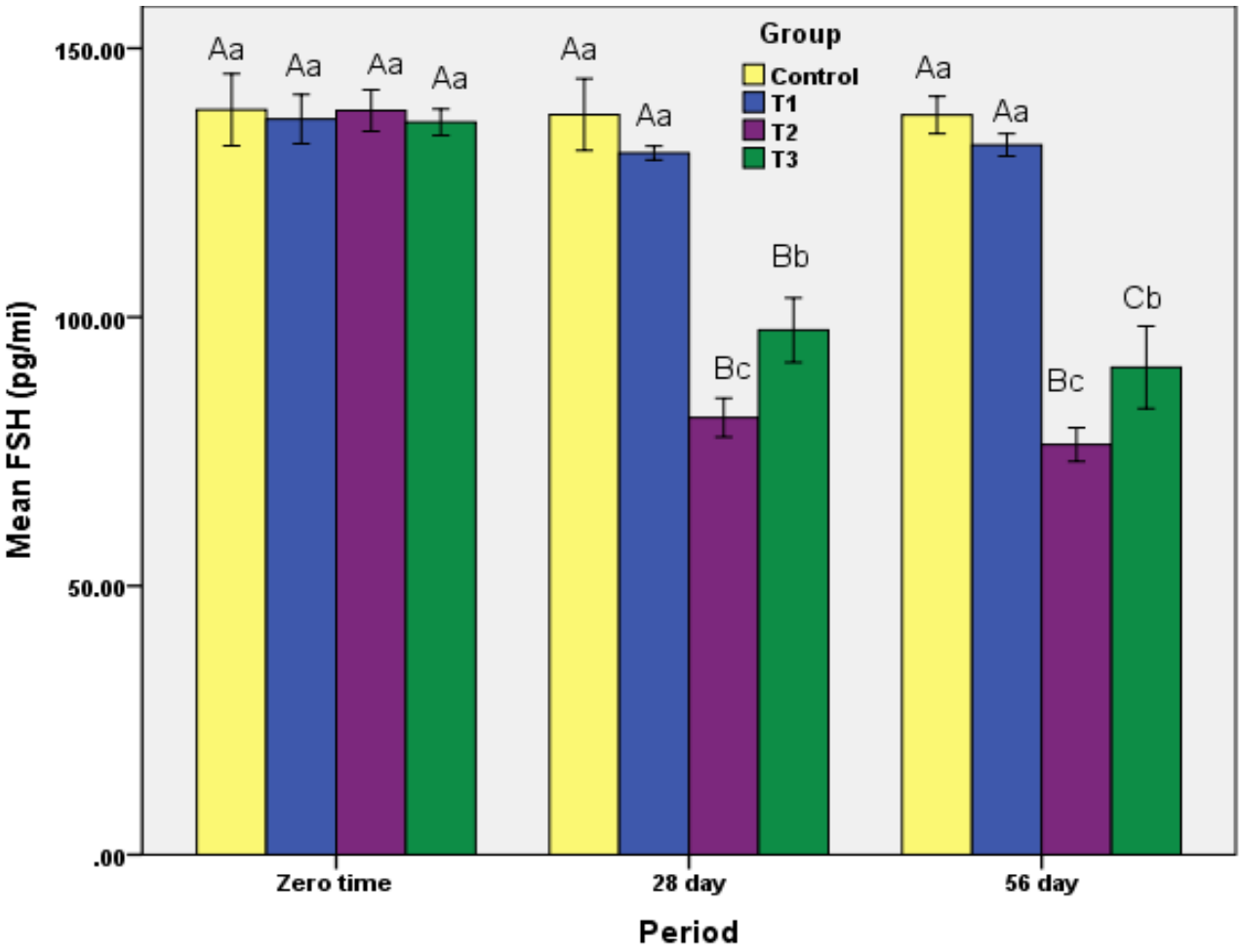
Results of the present study also confirmed that the detrimental effect of H_2O_2 on the pituitary hormones may be through suppressed serum prolactin, LH, FSH, and GH concentrations. This was agreed by other studies (**19)**, **(14)** and **(16).** Moreover, any chemical agent suppressing the secretion of pituitary gonadotropins has produce antispermatogenic and antifertility effects , impaired secretion of LH results in deficient androgen secretion by the testes, inhibition of spermatogenesis and loss of libido (**20**). It is often well known that small amounts of ROS are produced in the Steroidogenic pathway and spermatozoa are necessary for fertilizing capabilities (**21)**, apoptosis and spermatogenic failure (**22**)

Hydrogen peroxide toxicity may be affected pituitary gland causing a reduction in gonadotropins secretion leading to inhibition of steroid biosynthesis by Leydig cells and then decrease in testosterone concentration **(23).** A reduction of testosterone concentration in H_2O_2 treated group was documented in the present study by histological changes of the testis

As shown in the results significant improvement of testosterone, FSH and LH concentrations were observed in rats treated with ALA+ H_2O_2 (group T3) as compared to group T2. So, it could be concluded that that alpha lipoic acid reduced testicular damage that caused by H_2O_2, as evidenced by the significant increase of serum hormonal profile and due to ALA is a multifunctional antioxidant.

Conclusions

From the results and discussion of the current study, it can be concluded that oral intubation of 0.05% H_2O_2 to adult male rats caused significantly decrease in hormonal profile including Testosterone, FSH and LH. On the other hand, intubation of alpha lipoic acid (ALA) possessed antioxidants activity with improvement male reproductive performance against oxidative stressed rats induced experimentally by H_2O_2.



A

Figure.1 (A) Presents effect of alpha lipoic acid (ALA) for 28 and 56 days on serum FSH hormone concentration compared to the zero time of experiment of hydrogen peroxide (H_2O_2) treated male rats

Values are expressed as means ± SE n = 7/ group

C : control received tap water.

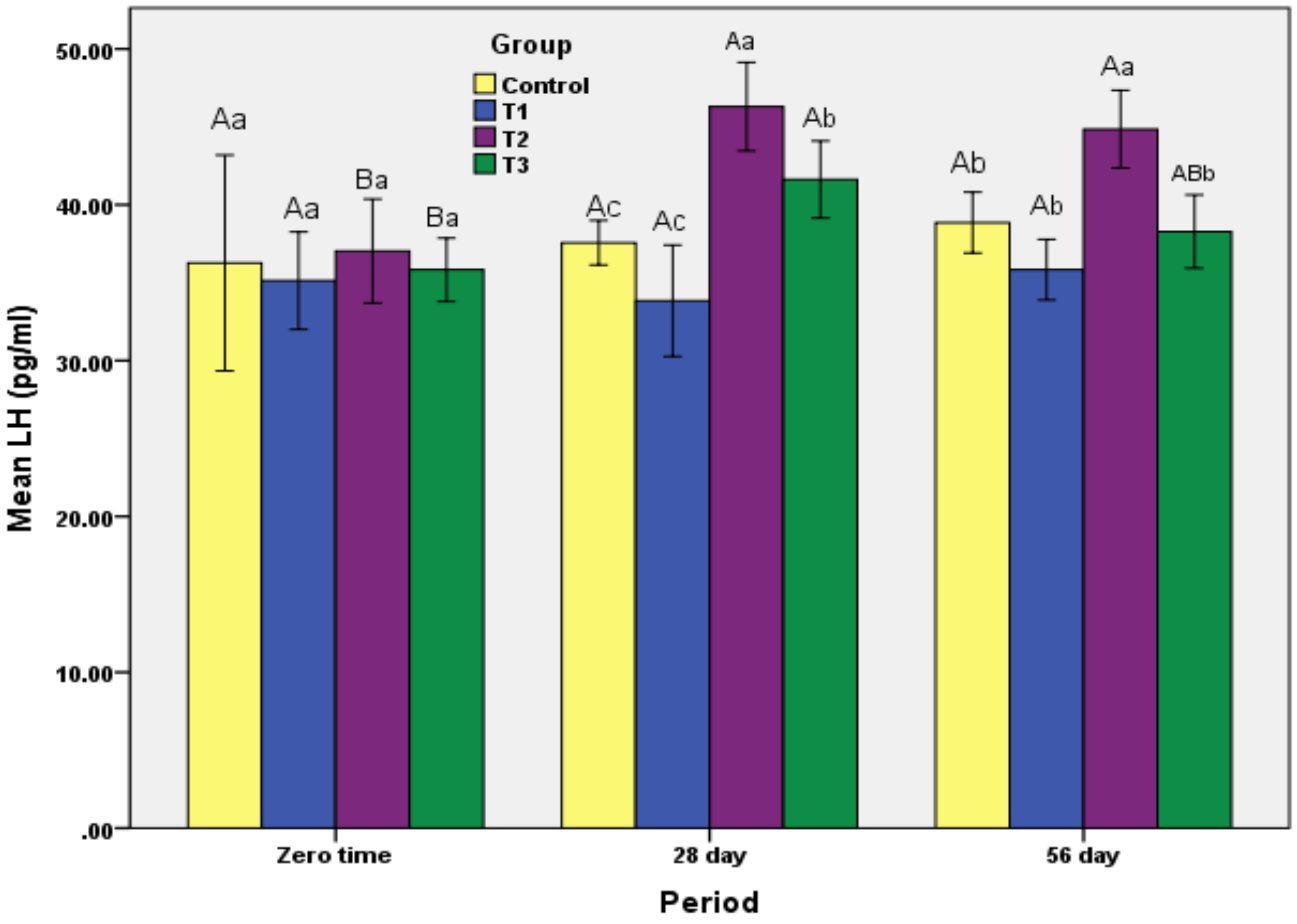
T_1 : gavages alpha lipoic acid (ALA) (60 mg/ kg B.W).

T_2 : received 0.05% H_2O_2 in drinking tap water.

T_3 : received 0.05% 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.

Means with different capital letters denote significant differences ($p<0.05$) within groups.



B

Figure.1 (B) Presents effect of alpha lipoic acid (ALA) for 28 and 56 days on serum LH hormone concentration compared to the zero time of experiment of hydrogen peroxide (H_2O_2) treated male rats

Values are expressed as means ± SE n = 7/ group

C : control received tap water.

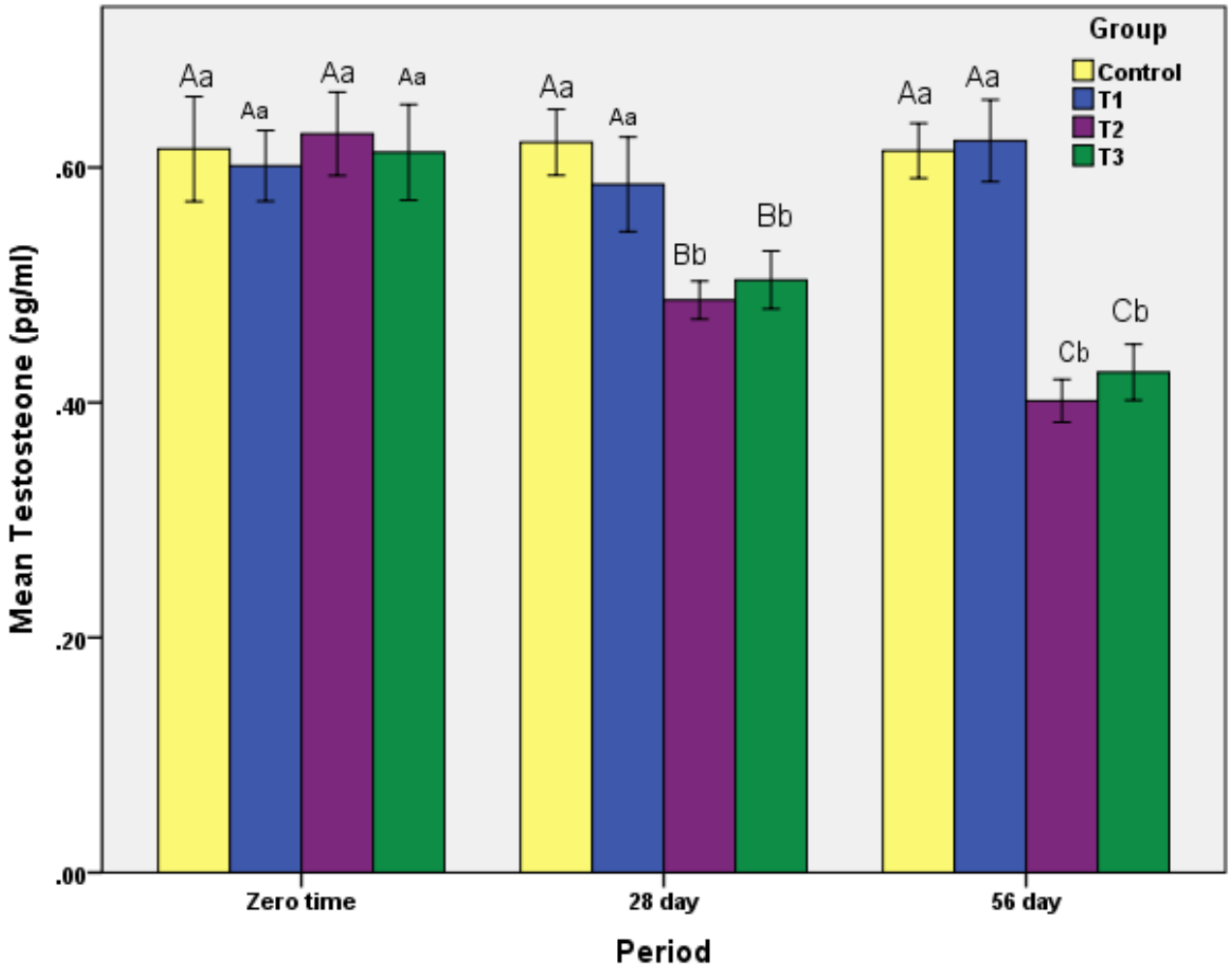
T_1 : gavages alpha lipoic acid (ALA) (60 mg/ kg B.W).

T_2 : received 0.05% H_2O_2 in drinking tap water.

T_3 : received 0.05% 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.

Means with different capital letters denote significant differences ($p<0.05$) within groups.



C

Figure.1 (C) Presents effect of alpha lipoic acid (ALA) for 28 and 56 days on serum Testosterone hormone concentration compared to the zero time of experiment of hydrogen peroxide (H_2O_2) treated male rats

Values are expressed as means ± SE n = 7/ group

C : control received tap water.

T_1 : gavages alpha lipoic acid (ALA) (60 mg/ kg B.W).

T_2 : received 0.05% H_2O_2 in drinking tap water.

T_3 : received 0.05% 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.

Means with different capital letters denote significant differences ($p<0.05$) within groups.

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