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STUDY EFFECT OF HYDROGEN PEROXIDE ON TESTIS TISSUE AND THE EXTENT ABILITY OF DRUGS GLUTATHIONE AND AQUOUS EXTRACT OF *NIGELLA SATIVA* TO RESTRAIN

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ABSRACT

The current study was investigated effect hydrogen peroxide on number sperm and precentage sperm abnormalltes and in testis histological. The results of the study showed a significant decreased in the number sperm and significant increased in precentage sperm abnormalltes in group terated by hydrogen peroxide (group 2) compare with control group at probability level P≤0.05. there are also showed result significant decreased in spermatide, primary spermal and Spermatogina with bleeding and disintegration of intercalary tissue in testis tissue in group 2 compare with control group. showed terated animale by Glutathion and aquous extract of *Nigella Sativa* with hydrogen peroxide (group 3&4) respectively significant increased in number sperm and significant decreased in precentage abnormalltes sperm compare with group2 at probability level P≤0.05. there are also showed result significant increased in spermatide, primary spermal and spermatogina with absence bleeding and suion intercalry tissue in testis tissue in group 3&4 compare with group2.

KEYWORDS: H2O2, Glutathione, *Nigella Sativa.*

INTRODUCTION

Hydrogen peroxide is a clear, colourless liquid which is completely miscible with Water The industrial manufacture of hydrogen peroxide can be traced back to its isolation in 18 18 by L. J. Thenard. Thenard reacted barium peroxide with nitric acid to produce a low concentration of aqueous hydrogen peroxideby reaction barium peroxide with nitric acid and it is formed by reaction with barium chloride with hydrochloric acid.[1,2,3]

Although hydrogen peroxide was toxic material but Many mammalian cell types also produce H2O2 in response to a variety of extracellular stimuli, with the H2O2 so produced serving as a signaling molecule that regulates various biological processes. Stimulation of cells with various agonists thus induces H2O2 production, and blockage of H2O2 accumulation results in inhibition of signaling by such stimulants.[4,5]

The spermatozoa produced few amount of Reactive Oxygen Species (ROS) in specific physiological condition which are necessary for capacitation, acrosome reaction and fertilization.[6] There are a balance between the ROS production and defense mechanism of Antioxidants in male reproductive tract, although may be increased the production of ROS , or the reduction of Antioxidants should be caused to Oxidative Stress.[7,8] While the large amount of ROS produced by immature sperm and leukocytes caused harmful effects on normal spennatozoa as a result of lipid oxidation activity.[9] The seminal fluid contains molecules with high molecular weight and low molecular weights called Antioxidants or Scavengers system protect the seminal fluid from the ROS.[10] The Oxidative Stress considered a very important factor in male infertility, because the increasing of Oxidative Stress related negatively with normal sperm parameters.[11]

MATERIALS AND METHODS

Study Desigen

This study was designed to investigated effects of a hdrogen peroxide on testis tissue in male laboratory albino mice Mus musculus L and the extent ability of drugs Glutathione and aquous extract of Nigella Sativa to restrain.

The mice were divided into four groups (each of contain six male mice)

1-The first group (control group): Intraperitonelly(i.p) injected with 0.1ml of physiological saline.

2- The second group: i.p injected with o.1ml of hydrogen peroxide of a concentration 0.5% for 20day.

3- the third group : i.p injected with 0.1 ml of hydrogen peroxide today for 20day and terated by Glutathion of aconcentration 100mg/kg for 10day .

4- the fourth group : i.p injected with 0.1 ml of hydrogen peroxide of a concentration 0.5% for 20 day and terated by extract of *Nigella Sativa* of aconcentration 100mg/kg.

Extraction of the *Nigella Sativa*

Dependence of[12] method to extraction of the *nigella sativa* seed.

Calculate the Number of Sperm

For calculated the number of sperm used[13] Method, as follow

1- The left epididymis was took then cutting its to several small pieces and placed in test tube.
2- To each tube 2 mL of a solution of formalin saline was added (prepared by dissolving 5gm of sodium bicarbonate in 100 mL of formalin).
3- 0.1 mL of 5% dye Eocene was added (attend by dissolving 5 gm of Eocene dye in the 100 mL of D.W).
4- Tubes centrifuged at 1500 rpm for 5 min.

The number of sperm calculate the calculation of red blood cells by used the Haemocytometer as follow.

The number of sperm (million sperm / 1 mL) = number of sperm in the five squares × 10^4

Determine Abnormalities in the Sperm of Male mice Laboratory

Method was used for determination of abnormalities by,[14] as follows

1. The right epididymis cut to several pieces and placed in test tubes .
2. To each tube added (5) mL of NaCl brine and left for 15 minutes.
3. A drop of the resulting solution put on a glass slide and left to dry .
4. Slides stained with eocene dye concentration of 1% (dissolving attend (1 gm) of powder dye in the Eocene (100 mL) of distilled water) for 10 min and left to dry.
5. Slides washed with fresh tap water to remove the dye being a certain percentage, and left to dry and then the slides became ready for microscopic examination

Calculated (100) sperm per slide, in chronological order was divided into

1. Sperm with normal appearance: a sperm that show the whole parts of the head and a piece of intermediate and guilt .
2. Sperm with abnormal appearance: the sperm, which lost one of its parts, or the sperm that got the superficial or volumetric changes in the light that identified the percentage of deformed sperm.

Histological study

The testes and epididymis were subjected to fixation in 10% formaldehyde solution, dehydration in ethanol, embedded in paraffin wax, sectioned on 5µ and stained with haematoxylin and eosin according to[15] method.

Statistical Analysis

Statistical analysis were performed by using the soft ware SPSS version 17.0, the results were expressed as mean ± standard deviations (mean ± SD). One way ANOVA was used to compare parameters in different studied groups. P-values ($P < 0.01$) were considered statistically significant.

RESULTS

1.Effect of hydrogen peroxide and glutathion and *Nigella Sativa* on the Number and Abnormalities of Sperm. The results of the current study showed significant decreasing ($P \leq 0.05$) in the count of sperm in group (2,3,4) compared to the control group but there was a significant increase ($P \leq 0.05$) in the abnormalities of sperms in group tow compared to the control group. at this times, there was a significant increase ($P \leq 0.05$) in the count of sperm in group (3&4) compared to the group two, while there was a significant decreased ($p \leq 0.05$) in the abnormalitise of sperm in group (3&4) compared to group two.

Table1: showen the effect of hydrogen peroxide and glutathion and Nigella Sativa on number and abnormalities of sperm

the number and abnormalitis of sperm group	the number of sperm $\times 10^4$	abnormalitise sperm
Control group (0.1ml)normal saline	a 825.66±11.61	a 12.07 ± 0.57
Two group (0.1ml) hydrogen peroxide	b 446.66± 27.02	b 21.16 ± 1.07
Three group (0.1ml)H_2O_2+ (0.2ml)glutathion	c 674.83 ± 29.19	a 15.66 ± 1.05
Fourth group (0.1ml)H_2O_2+ (0.2ml)*Nigella Sativa*	d 605.83 ± 17.84	a 15.83 ± 2.52
LSD	80.65	3.65

2. Effect of hydrogen peroxide and glutathion and Nigella sativa on spermatogenisis

The results of the current study showed significant decreasing ($P \leq 0.05$) in spermatogena, primary spermal and spermatide in group (2) compared to the control group but there was a nonsignificant decreased ($P \leq 0.05$) in spermatide and primary spermal in group (3&4)

compared to the control group at this times ,there was a significant decreased (P≤0.05) in spermatogena in group (3&4) compared to the control group, while there was a significant increase (p≤0.05) in the spermatide and primary spermal in group (3&4) compared to group two but there was a non significant increase in spermatogena in group two.at this time showen in figure testis tissue bleeding and dismitegration of inercalary tissue in group 2 compared with control group.

Table2: showen the effect of hydrogen peroxide and glutathion and *Nigella Sativa* on spermatogenisis

Parameter Group	spermatogina	primary spermal	spermatide
Control group (0.1ml)normal saline	a 50.16±1.99	a 42.50 ± 1.76	a 40.83 ± 1.70
Two group (0.1ml) hydrogen peroxide	b 28.33± 3.48	b 37.16 ± 1.24	b 23.66 ±2.91
Three group (0.1ml)H_2O_2+ (0.2ml)glutathion	b 37.83 ± 2.70	a 40.66 ± 2.34	a 36.16 ± 4.79
Fourth group (0.1ml)H_2O_2+ (0.2ml)*Nigella Sativa*	b 31.50 ± 2.36	a 36.83 ± 5.10	a 33.33 ± 6.44
LSD	9.36	10.39	15.20

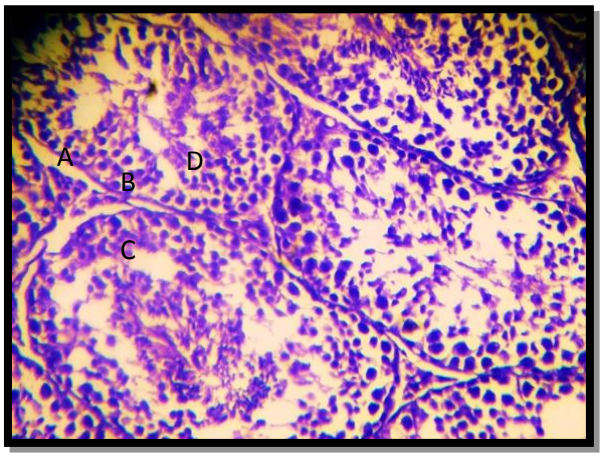


Figure (1): Cross section in mouse testis tissue on control group (E&H) 200X A.Spermatide B.primary spermal C. Spermatogena D.intercalary tissue

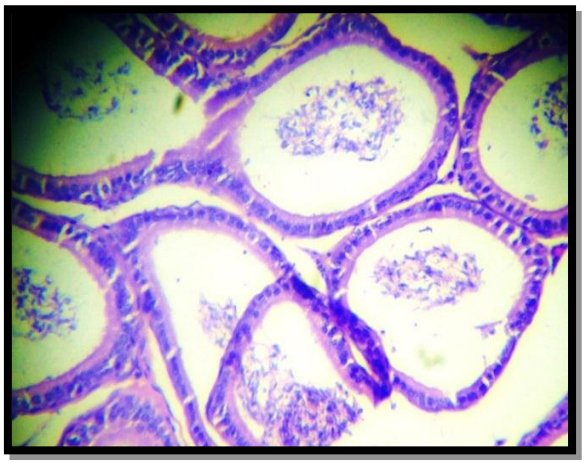
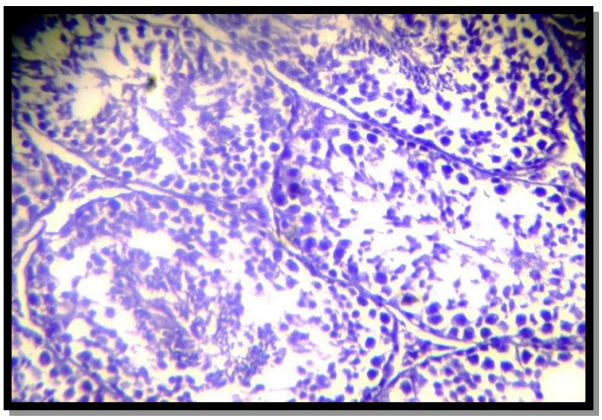
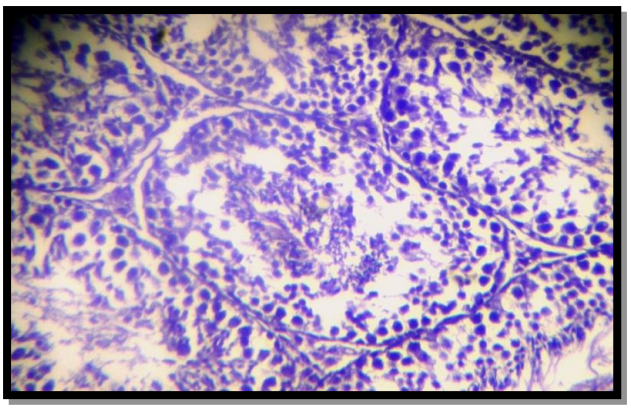


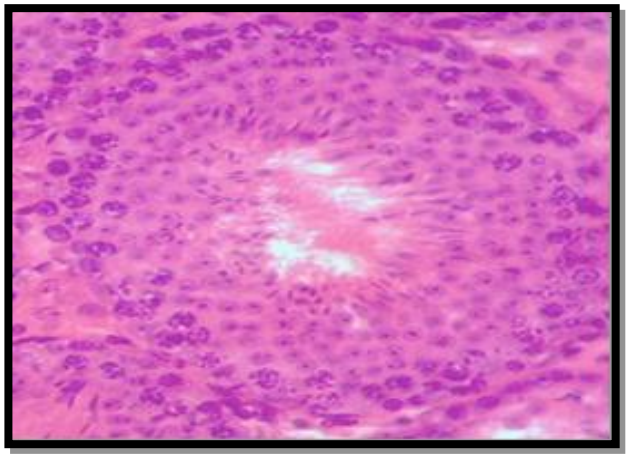
Figure (2): Cross section in mouse testis tissue on tow group (E&H) 200X shwoen dismtegration of intercalary tissue and decreased spermatide and primary spermal and spermatogena



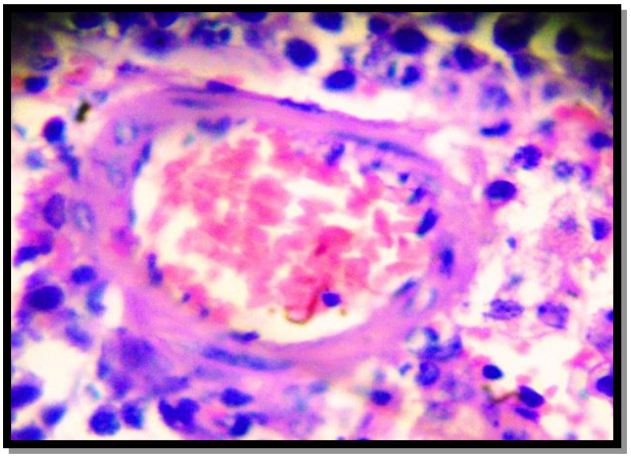
Figure(3): Cross section in mouse testis tissue on three group (E&H) 200X shwoen suion of intercalary tissue and increase number of spermatide and primary spermal and spermatogena



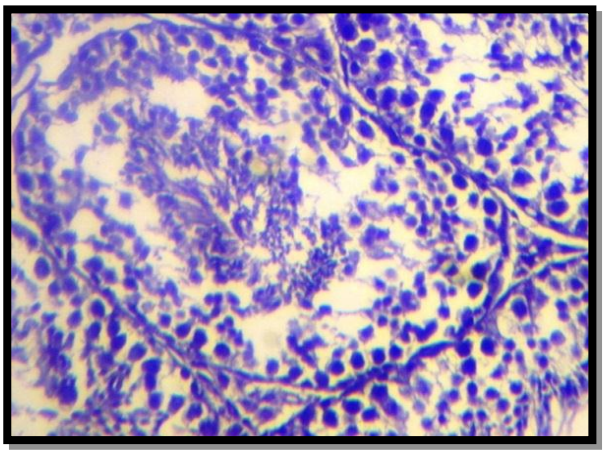
Figure(4): Cross section in mouse testis tissue on four group (E&H) 200X shwoen start suion of intercalary tissue and increase number of spermatide and primary spermal and spermatogena



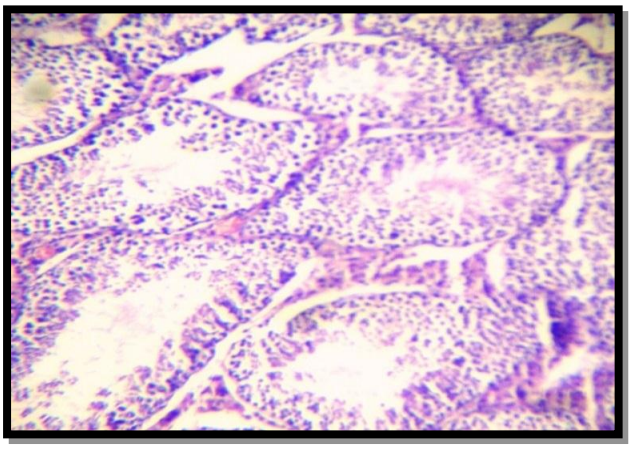
Figure(5): Cross section on seminiferous tubuls (E&H) 200X showen all stage spermatogenisis in control group



Figure(6): Cross section on seminiferous tubuls (E&H) 200X showen bleeding inside seminiferouse tubules in tow group



Figure(7): Cross section on seminiferous tubuls (E&H) 200X showen all stage spermatogenisis in three group



Figure(8): Cross section on seminiferous tubuls (E&H) 200X showen all stage spermatogenisis in four group

DISCUSSION

The current result showed significant decreased in number of sperms and there was significant increas in the abnormalitise ($P \leq 0.05$) in second group compared to the control group.

May be caused decreased in number of sperms is the effect of hydrogen peroxide on stroli cells that play an important role in spermatogenisise,[16] pointed the hydrogen peroxide directly effects on stroli cells and also effect on the cellular structure to spermatide leading to decreased in number of sperms and increase in the abnormalitise.

Also, we see that the decreased is happening in average conceneration of sperm and increase the precentage in abnormalitise of sperm may be due to the effect of the free radical on interstitial cella (Leydig cell) and inhabition of secretion of the male hormone (testosterone hormone),[17] referanced that the treatment of animals by hydrogen peroxide causes the production of free radicals in the body that caused oxidative stress on leydig cell and inhabition level of the teststerone hormone mainaly catalyst to prouduction sperms.

On other hand, the free radicals generated from the addition of hydrogen peroxide may interefere with energy prouduction and metabolism cause decreased ATP conceneration in sperm which cause decreased in number sperm,[19] pointed in his study that the hydrogen peroxide caused decreased ATP conceneration in sperm which leads to lower energy and inability to function ,causing the death of sperm.

The decrement in the number of sperm and increase the proportion abnormalities of sperm due to the effect of hydrogen peroxide on the mitochondria and DNA sperma,[20] mointed that the free radicals responsible for decreased in the number of sperm and increase the proportion abnormalities of sperm because inhabition mitochondria function and manufacture DNA leads to change in the structure of the sperm.

Also, may be caused decreased in the number of sperm and increase proportion abnormalitise of sperm to the role of hydrogen peroxide in the oxidation of membrans sperm. may[18] pointed that the sperms are surrounded by membrane rich bilateral fat that interact with hydrogen peroxide proccess lipid peroxidatin.

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