Effect of crude polyphenol extracted from black olive fruit (*Olea europae*) on male reproductive system of rats

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Summary

The current study was conducted to evaluate the effect of olive polyphenols (200 mg/kg) on mature reproductive performance of male rats subjected to oxidative stress by 1%hydrogen peroxide. Twenty rats were divided randomly into four equal groups and treated for 30 days to control group C which were orally adminstrated ordinary tap water, while the first group (G1) was given 1% H_2O_2 in drinking water, the second group (G2) was treated orally alcoholic extract of polyphenol in olive (200mg/kg) by oral intubation. The third treated group (G3) was treated orally H_2O_2 and black olive fruit- polyphenol. Blood samples were collected after 30 days for measuring, the serum concentration of testosterone, follicular stimulating hormone (FSH) and leuitinizing hormone (LH). At the end of experiment, the animals were scarified and testis were removed, weighed as well as section from them were taken for histopathological study and the epididymis were taken for counting dead and live sperms. The results showed a significant increase in the weight of the testis in G2 group as compared to the control and significant decrease in the percentage of live sperms in (G1) group and significant increase in the percentage of dead sperms in H_2O_2 treated G1 group and control group. The results show significant differences in testosterone hormone concentration between treated groups and control group while there was known significant increased in concentration of luteinizing hormone between treated groups and control group. There was a significant decrease in FSH in treated (G1) group comparing with control group. The histological study showed edema and thickness of basement membrane of seminiferous tubules with incomplete spermatogenesis process in treated (G1) group, there were no lesions observed in other treated groups. Finally, the outcomes of of this study documented the advantageous effect of crude polyphenolic compounds of olive against the harmful effect of H2O2 on reproductive system of adult males rats.

Keywords: Olive fruit, 1% H_2O_2, hydrogen peroxide, polyphenol, FSH, LH, Testosterone.

Introduction

Phytoestrogens are polyphenolic non-steroidal plant compounds with estrogen –like biological activity (1). Phenolic compounds are beneficial for health, a large number of studies have identified cellular targets that could be involved in the health promoting actions of dietary plant phenolic compounds. Epidemiological evidence indicates that diets rich in fruits and vegetables promote health and attenuate or delay the onset of many diseases (2- 4). Among phytoestrogen plants, black olive fruits which contain many compositions include water (50%), protein (1.6%), oil (22%), carbohydrate (19.1%), cellulose (5.8%), inorganic substances (1.5%) and phenolic compounds (1–3%). Other important compounds present in olive fruit are pectin, organic acids, and pigments (5) Organic acids show metabolic activity and are intermediate products resulting from formation and degradation of other compounds (6). Both lipophilic and hydrophilic phenolics are distributed in olive fruit. The main lipophilic phenols are cresols while the major hydrophilic phenols include phenolic acids, phenolic alchohols, flavonoids and secoiridoids; they are present in almost all parts of the plant but their nature and concentration varies greatly between the tissues (5 and 7). Phenolic acids are named as secondary aromatic plant metabolites that are commonly distributed throughout the plant kingdom (8 and 9) The main phenolic alcohols

of olives include oleuropein β- (3,4-dihydroxyphenylethanol) or hydroxytyrosol and *p*-droxyphenylethanol (tyrosol) (10 and 11). Flavonoid compounds in olive are mainly comprised of flavonol glycosides such as luteolin 7-*O*-glucoside, rutin, apigenin 7-*O*-glucoside, anthocyanins, cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside (12 and 13). Olive polyphenols have good bioavailability, which is in accordance with their antioxidant efficacy (14). Have cancer chemopreventive activity (15). The unripe olive fruit extract has been shown to possess the calcium channel blocking activity, considered to be responsible for its effectiveness in cardiovascular disorders like hypertension (16). Similarly, olive fruit extract has been shown to contain a combination of laxative and anti diarrheal activities mediated through the presence of cholinergic and calcium channel blocking constituents, respectively (17 and 18). The positive effect of polyphenol extracted from black olive oil on fertility is the aim of this study.

Materials and Methods

The extraction of polyphenolic compounds from olive fruits was carried out according to (19). Twenty mature 3-5 months adult albino Wister male rats were randomly divided into four groups designated and treated as follows for 30 days:- Animals in group one had free access to food and water and served as control (C), group two (G_1) animals were subjected to ad- libitum supply drinking water containing 1% H_2O_2 (35% of hydrogen peroxide solution was diluted with water), group three (G_2) animals were received 200mg /kg B.W. of crude poly phenolic compounds dissolved in distilled water(this dose was choice according to study of polyphenol) (20) , group four (G_3) animals were subjected to ad- libitum supply drinking water containing1% H_2O_2 and received 200mg/ kg B.W. of crude polyphenolic extract of *olea europae* and. At the end of experiment, animals were sacrificed. Tissue specimens and blood samples were taken for analysis. Blood samples were collected by heart puncture technique, serum collection by centrifugation (3000 rpm) for 15minutes and frozen at –20 C° until analysis. Serum samples were used for measuring the serum (testosterone, FSH and LH) were measured by Radio-immunoassay (RIA) kit, semen were collected to calculate the percentage of dead and alive sperms by (21). Testes had removed and weighed according to body weight ratio as in the following equation: Testicular wt-to-body wt ratio= (wt. of testis (gm) / wt. of animal (gm) ×100. After that testes preserved in formalin 10% for histological preparation according to (22). The histological studying involved measurement the diameter and the epithelial thickness of seminiferous tubules using ocular and stage micrometer at power 40 according to (23 and 24). Differences between experimental groups were statistically evaluated using one way analysis of variance (ANOVA) (25). The outcomes of this study documented the advantageous effect of crude polyphenolic compounds of olive apposite the harmful effect of hydrogen peroxide on reproductive system of adult males rats.

Results and Discussion

Serum hormones levels: The mean values of LH, FSH and testosterone concentrations in the control animals groups, H_2O_2 + polyphenol and in group of polyphenol compounds only which compared with group of H_2O_2 illustrates in table (1). Exposure of rats to 1%H_2O_2 orally for 30 days caused a significant decrease ($P\leq0.05$) in serum FSH and testosterone concentration and there′s no differences in serum LH concentration as compared to control group. Also, the results showed a significant decrement ($P\leq0.05$) in serum testosterone in group G2 (polyphenol) and G3 (H_2O_2 + polyphenol) as compared to the G1 (H_2O_2), which refer to the effect of olive fruits that reduced cholesterol (26) an important for synthesis of sex hormones especially testosterone (because it considered as precursor for testosterone synthesis) through its action on leydig cells (27 and 28). The physiological concentrations of testosterone, LH and FSH play an important role in spermatogenesis (29), so a significant decrease of these hormone may cause a decrease in the number and function of somatic and germinal cells of testis followed by a testis weight reduction, meanwhile polyphenol treated group showed increased the weight of the test

is due to the role of polyphenols and flavonoids to increase consumption of food (30). Experimental studied demonstrated that H_2O_2 lead to generation free radicals and produced cell injury (31), also exposure of animal to H_2O_2 cause impairs spermatogenesis by induction of oxidative in testicular mitochondria (32). There is no significant differences in the level of LH hormone in H_2O_2+ polyphenolic compounds treated group. There is no significant difference in the level of FSH hormone in polyphenol and H_2O_2+polyphenol treated group.

Table, 1: Effect of polyphenols extracted from black olive fruit (*Olea Europea*) on the concentration (mmol/ml) of testosterone, LH, FSH hormones on G1 (1% H_2O_2 treated)-male rats and (G2) animals group treated with 200mg/kg B.W. polyphenol and (G3) group of rats treated with 1% H_2O_2 and 200mg/kg polyphenol. (M±S.E .n=5).

Group / Hormone Conc.	Control Mean±SE	G1 Mean±SE	G2 Mean ±SE	G3 Mean±SE
LH. (mmol/ml)	0.426±0.1 A	0.376±0.07 AB	0.32±0.04 B	0.276±0.05 BC
FSH. (mmol/ml)	13.35±1.6 A	8.5±0.9 B	11.22±1.2 AB	12.6±2.3 AB
Testosterone (mmol/ml)	21.9±0.5 A	4.4±0.4 B	1.7±0.09 C	1.9±0.09 C

Different capital letters denote significant differences between groups at (P≤ 0.05).

Weight of the left and right testis: Significant increase ($P \leq 0.05$) in the weight of the left and right testis of the rats administrated polyphenol compounds was observed as compared with control group and other groups as showing in (Table, 2). Such increment might be due to the presence of gonadotropin like substance present in olive (33 and 34), as well as presence of flavonoids and polyphenol compounds which may be increased their food intake and body weight with corresponding increase the weight of testis (26 and 35).

Table, 2: Effect of polyphenols extracted from black olive fruit (Olea Europea) on the weight of left and right testis to body weight on 1% H_2O_2 treated-male rats and animals group treated with 200mg/kg B.W. polyphenol and group of animals treated with 1% H_2O_2 and 200mg/kg polyphenol. (M ± S.E. n=5)

Group / Weight	Control Mean±SE	G1 Mean±SE	G2 Mean ±SE	G3 Mean±SE
Left testis	0.478±0.01 B	0.44±0.02 AB	0.586±0.04 A	0.33±0.01 C
Right testis	0.47±0.02 AB	0.47±0.03 AB	0.52±0.003 A	0.31±0.03 B

L.S.D. =0.06, Capital letters denote differences between groups.

Thickness and diameter of seminiferous tubules: Thickness of seminiferous tubules decreased significantly ($P \leq 0.05$) in animals treated with 1% H_2O_2 and H_2O_2+polyphenol compounds as compared with control group, while the diameter of seminiferous tubules decreased significantly ($P \leq 0.05$) in animals treated with polyphenol compounds and polyphenol+H_2O_2 (Table, 3). However, seminiferous diameter and thickness decrement may be attributed to the reduction in testosterone, FSH and LH hormones concentrations (Table, 1) similar results were reported by (36 and 37).

Table, 3: Effect of polyphenols extracted from black olive fruit *(Olea Europea)* on the thickness and diameter of the somniferous tubules on (G1)1% H_2O_2 treated-male rats and (G2)animals group treated with 200mg/kg B.W. polyphenol and (G3) group of animals treated with 1% H_2O_2 and 200mg/kg polyphenol. (the units mm)

Group / Tubules Semniferou	Control Mean±SE	G1 Mean±SE	G2 Mean±SE	G3 Mean±SE
Thickness of seminefrous tubules (mm)	1.138±0.02 A	0.742±0.02 B	1.042±0.07 AB	0.77±0.03 B
Diameter of somniferous tubules(mm)	3.45±0.09 A	3.26±0.04 A	3.02±0.09 B	2.56±0.09 C

L.S.D. =0.1, L.S.D=0.22 , n=5 each group, Capital letters denote differences between groups

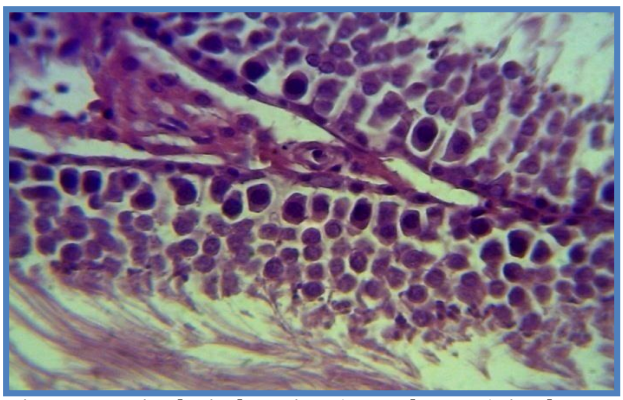
Dead and alive Sperms: The results (Table, 4), demonstrated the effect of 1% H_2O_2 and polyphenol on sperm viability percentage of live sperms. There was a significant ($P \leq 0.05$) decreased in group received 1% H_2O_2 or 1% H_2O_2+ polyphenol as compared with control, this may be due to free-radical mediated oxidative stress and sperm damage (38). Beside, high reactive oxygen species (ROS) levels in seminal plasma have been associated with inhibition of sperm function due to peroxidation of membrane polyunsaturated fatty acids (39). The protective role of polyphenolic compounds to correct the adverse effect of hydrogen peroxide had been documented (40). Mean while there′s no differences in percentage of alive sperms in G2 (polyphenol treated group). The table also showe significant increase ($P \leq 0.05$) of dead sperm in group received 1% H_2O_2 as compared with control group indicating the toxic effect of hydrogen peroxide (41). Rats in G3 which intubated 1% H2O2+polyphenolic compounds showed significant ($P \leq 0.05$) decrement in dead sperms compared with G1 may be due to antioxidant activity of polyphenol compounds that reduce the oxidative stress induced by hydrogen peroxide. There′s no differences in percentage of alive sperms in G2 indicating its improving effect damage but not on healthy rats.

Table, 4: Effect of polyphenols extracted from black olive fruit *(Olea Europea)* on the percentage of dead and alive sperms on 1% H_2O_2 treated-male rats and animals group treated with 200mg/kg B.W.polyphenol and group of animals treated with 1% H_2O_2 and200mg/kgpolyphenol (M±S.E.n=5)

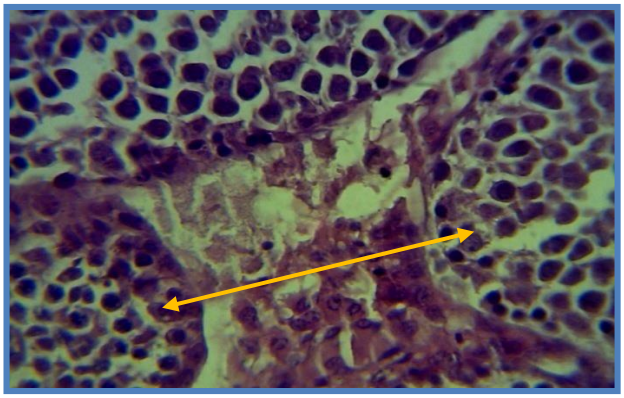
Group / Sperm	Control C Mean ±SE	1%H2O2 G1 Mean±SE	Polyphenol G2 Mean ±SE	1%H2O2+Polyphenol G3 Mean ±SE
Dead sperm	34.35±2.0 AB	62.22±3.1 A	39.53±0.85 AB	43.86±1.89 B
Alive sperms	65.65±2.0 A	37.77±3.1 C	60.47±0.85 AB	56.14±1.89 B

L.S.D =5.6, Capital letters denote differences between groups.

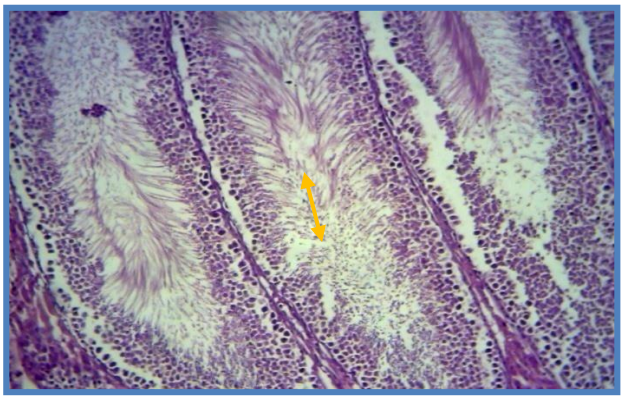
Histopathological changes in testicular tissue: According to the control (Fig. 1), the microscopic examination of rats testis received polyphenolic compounds of black olive fruit (200mg/kg) show a proliferation of leydig cells (Fig. 2), and normal spermatogenesis where the seminiferous tubules filled with sperm in center of lumen (Fig. 3). This may be due to increase the weight of testis as a result of benefits of polyphenolic compounds (26), as well as increased in Testosterone, LH and FSH. The histopathological examination of testis rats received 1% H_2O_2 showed thickness of basement membrane of seminiferous tubules with incomplete spermatogenesis process and cellular debris in their lumen (Fig. 4), other (Fig. 5 and 6) showed impairment and degeneration of spermatogenesis with protein materials and cellular degeneration in the seminiferous tubules which vaculation. This result due to the oxidative stress of H_2O_2 (41).



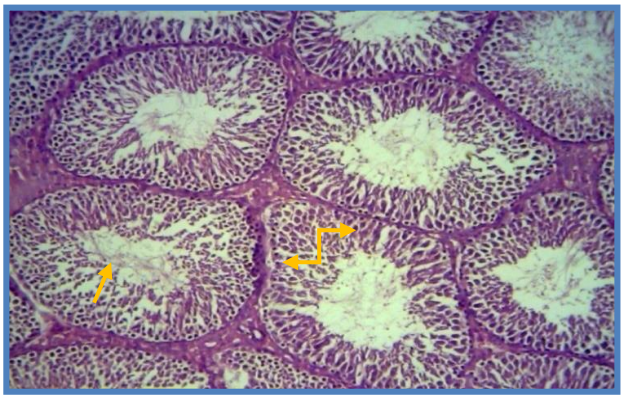
Figure, 1: Histological section (control group) in the rat testis at day 30 post treated with tap water (H and E stain 40x).



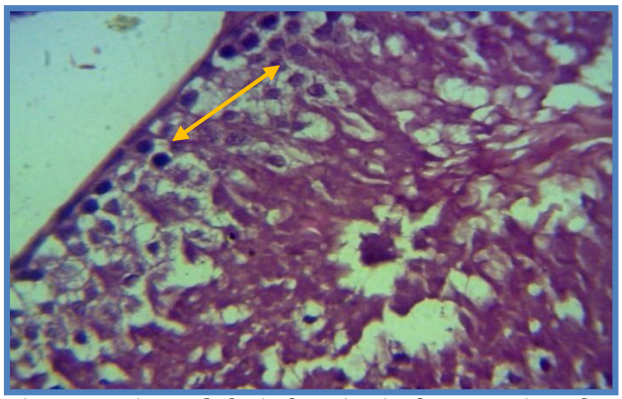
Figure, 2: Histopathological section in the rat testis at day 30 post treated with polyphenol shows proliferation of leydig cells. ⟷ (H and E stain 40X)



Figure, 3: Histopathological section in the rat testis at day 30 post treated with polyphenol shows normal spermatogenesis in seminepherous with sperm in certain of lumen ↔ seminiferous tubules (H and E stain 40X)

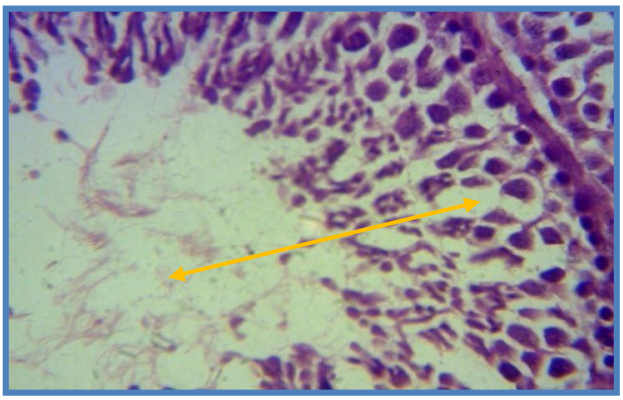


Figure, 4: Histopathological section in the rat testis at day 30 post treated with 1% H2O2 shows thickness of basement membrane of seminiferous tubules ↔ with incomplete spermatogenesis process and cellular debris in their lumen → (H and E stain 40X)

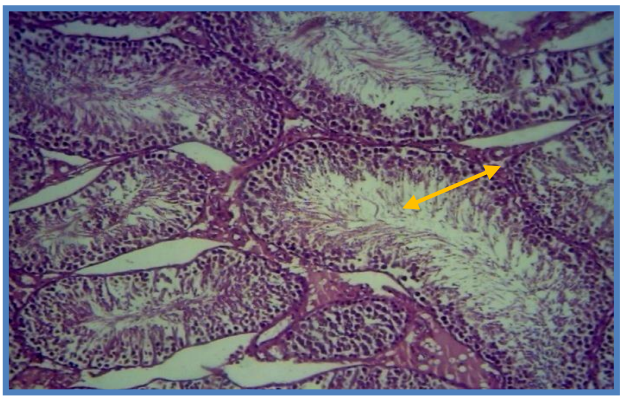


Figure, 5: Histopathological section in the rat testis at day 30 post treated with 1%H2O2 shows degeneration of spermatogenesis with protein materials and cellular degeneration in the seminiferous tubules which vaculation ↔ (H and E stain40x).

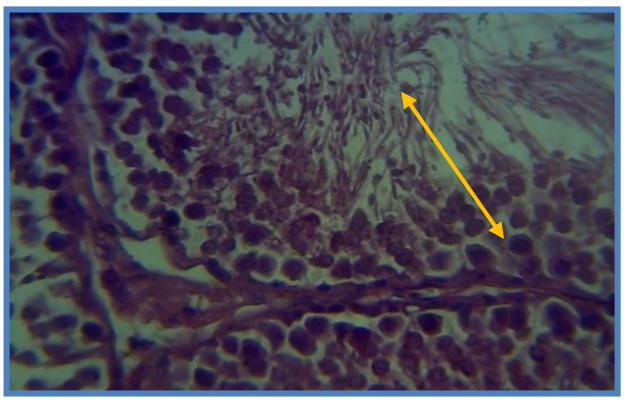
The histological section of rats testis treated with polyphenolic compounds of olive fruit+1% H_2O_2 show normal spermatogenesis in seminiferous with sperm in certain of lumen and moderate thickness of basement membrane (Fig. 7), however there is proliferation of leydig cells in the lumen of seminiferous tubules (Fig. 8), this due to the protective role of polyphenolic compounds (42 and 43), as well as free radical scavenging abilities of polyphenolic compounds due to this result (30 and 44).



Figure, 6: Histopathological section in the rat testis at day 30 post treated with 1%H2O2 shows impairment spermatogenesis and empty lumen of the seminiferous tubules ↔ (H and E stain 40X)



Figure, 7: Histopathological section in the rat testis at day 30 post treated with 1% H2O2 and polyphenol show normal spermatogenesis in seminiferous with sperm in certain of lumen semitubules and moderate thickness of basement membrane ↔ (H and E stain 40X).



Figure, 8: Histopathological section in the rat testis at day 30 post treated with 1% H2O2 and polyphenol show proliferation of leydig cells ↔ (H and E stain 40X).

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تأثير المتعدد الفينولي الخام المستخلص من ثمار الزيتون الأسود في الجهاز التناسلي الذكري للجرذان

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الخلاصة

صممت هذه الدراسة لمعرفة تأثير متعدد الفينولي للمستخلص الكحولي من الزيتون الأسود بتركيز 200 ملغم/كغم في الجهاز التناسلي لذكور الجرذان البالغة السليمة والمعاملة ببيرو كسيد الهيدروجين 1%. تم تقسيم عشرون جرذي إلى أربعة مجاميع متساوية وعوملت لمدة 30 يوم كالأتي: مجموعة السيطرة (C) أعطيت ماء الشرب العادي ،مجموعة معاملة ببيرو كسيد الهيدروجين 1%(G) وقد أعطيت ماء الشرب العادي المضاف إليه ببيرو كسيد الهيدروجين (H_2O_2). مجموعة معاملة بالمستخلص الكحولي لمتعدد الفينول بتركيز 200 ملغم/ كغم عن طريق الفم (G2) ومجموعة معاملة بالمستخلص الكحولي لمتعدد الفينول مع بيرو كسيد الهيدروجين ورمز إليها G3 وبنفس التراكيز السابقة. تم سحب الدم من الحيوانات بعد 30 يوم لغرض قياس تركيز الهرمونات التالية: هرمون الشحمون الخصوي، الهرمون المحفز للجر يبات، الهرمون اللوتيني، يعدها تم التضحية بالحيوانات لدراسة أوزان الخصى نسبة إلى وزن الجسم واخذت نماذج من ذيل البربخ لحساب النسبة المئوية للنطف الحية والميتة، كما وأخذت نماذج من الخصى لدراسة التغيرات النسيجية. أظهرت النتائج ارتفاعا معنويا في أوزان الخصى للمجموعة المعاملة G2 مقارنة مع مجموعة السيطرة وانخفاضا معنويا في النسبة المئوية للنطف الحية للمجموعة G1 وارتفاعا معنويا في النسبة المئوية للحيامن الميتة لنفس المجموعة كما وأظهرت النتائج فروق معنوية في تركيز هرمون الشحمون الخصوي بين المجاميع المعاملة مقارنة مع مجموعة السيطرة. لم تظهر النتائج اي زيادة معنوية في تركيز الهرمون اللوتيني لكل المجاميع المعاملة مقارنة مع مجموعة السيطرة بينما نلاحظ انخفاض واضح في تركيز هرمون المحفز للجريبات للمجموعة G1 مقارنة مع مجموعة السيطرة. بينت نتائج الفحص النسجي حدوث وذمة وتثخن في جدار الغشاء القاعدي للنبيب المنوي واحتقان الأوعية الدموية وعدم اكتمال عملية تكوين النطف في المجموعة المعاملة ببيرو كسيد الهيدروجين. لم تلاحظ أي افات مرضيه في المجاميع المعامله الأخرى. إستنتج إن التاثير المفيد لمركبات المتعدد الفينول الخام لثمار الزيتون الاسود ضد التاثير الضار لبيروكسيد الهيدروجين في وظائف الجهاز التناسلي في ذكور الجرذان البالغة.

الكلمات المفتاحية: ثمار الزيتون، 1% H_2O_2، بيروكسيد الهيدروجين، متعدد الفينول، الهرمون المحفز للجريبات، الهرمون اللوتيني، هرمون التيستوستيرون.