Role of alcoholic extract of Roket (Eruca sativa) leaves on male reproduction of experimentally induced-oxidative stressed rats

Baraa Najim AL-Okaily and Ahmed Jasim Nowfel
Department of Physiology and Pharmacology, College of Veterinary Medicine, Baghdad University, Iraq.
E-Mail: Baraanajim@yahoo.com
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Summary

This study conducted to find out the protective role of ethanolic extract of Eruca sativa leaves against the deleterious effect of hydrogen peroxide on some aspect of male reproduction in adult rats. Forty adult male rats were randomly assigned into four equal groups as follows: control group received tap water (untreated); group T₁ were received tap water containing 0.5% H₂O₂; group T₂ were received tap water containing 0.5% H₂O₂ plus administration of 300 mg/kg. B.W. ethanolic extract of Eruca sativa leaves and group T₃ administration ethanolic extract of Eruca sativa leaves only at the same dose of group T₂. All treatments continued for 60 days. At the end of the experiment, samples of tests and epididymis tissues were taken to prepare histological sections for measurement the diameter of seminiferous tubules, thickness of epithelial cells of seminiferous tubules and histological examination of testes and epididymis. The results in Group T₁ showed a significant decrease in the diameter and thickness of epithelial cells of seminiferous tubules, but these parameters clarified a significant increase in T₂ and T₃ groups as compared with T₁ group. Histological sections of testis and epididymis in group T₁ revealed incomplete spermatogenesis, cell debris, vaculation of Sertoli cells and view sperms in the lumen of seminiferous tubules and epididymis. Besides, normal obvious histological architecture of seminiferous tubules and epididymis with complete spermatogenesis were shown in sections of testis and epididymis of T₂ and T₃ groups’ as compared to T₁. In conclusion, hydrogen peroxide may impair spermatogenesis, furthermore, the results confirm the protective role of E. sativa leaves extract against oxidative stress induced by H₂O₂ in rats.

Keywords: Hydrogen peroxide, Eruca sativa leaves, Testis, Epididymis, Seminiferous tubules.

Introduction

A considerable amount of literature has been published about the oxidative stress (OS) and reactive oxygen species (ROS) play an important role in the etiology and/or progression of a number of human diseases (1). Under pathological conditions, the uncontrolled production of ROS exceeds the antioxidant capacity of the seminal plasma, resulting in oxidative stress which play an important role in male infertility by causing sperm dysfunction (2 and 3). ROS plays a crucial role in several reproductive development and maturation (4 and 5). When the level of ROS exceeds above the normal, it could damage spermatozoa by inducing lipid peroxidation (LPO) on DNA damage (6 and 7) and are associated with poor sperm function (8). The common causes of male infertility include varicocele, genital tract infection, radiation, chemotherapy, erectile dysfunction, gene mutations and aneuploidy (9 and 10). Rocket (Eruca sativa) has a wide spread medicinal use; it is considered as a medicinal plant with many reported properties, including its strong aphrodisiac effect known since Roman times (11 and12). Traditionally, it is used as astringent, diuretic, digestive, emollient, tonic, depurative, laxative (13 - 15), antimicrobial (16), antihyperlipidemic, antihyperglycemic and antiephrotheliatic (17) and commonly used food additive to improvement rumen degradation (18). Other researchers (15) explained that rocket contain a group of anticancer compounds known as glucosinolates; these compounds exert antioxidant activity (19 - 21). Whereas, (22 and 23) proved that the presence of saponine and alkaloids in rocket extract would cause a significant increase in sperm activity. Some evidence suggested that the ethanolic extract of Eruca sativa plant has androgenic activity or stimulate testicular steroid production which increases spermatogenesis in male mice (24). Therefore, the objective of this experiment aimed at investigating the
association of preventive role of ethanolic extract of *Eruca sativa* leaves with oxidative stress induced by H$_2$O$_2$ on some aspect of male reproduction in adult rats.

**Materials and Methods**

The fresh leaves of *Eruca sativa* was purchased from the local market of Baghdad. The fresh leaves of *Eruca sativa* were dried by air, grounded into a fine powder grinder weighing 100 gm then put it in a volumetric conical flask 1000 ml of 70% Ethyl alcohol was added on the powder, after that the mixture was shacked by using magnetic stirrer apparatus for 24hr, the mixture was filtered and then was filtered again using Whatman (No.1) filter paper. The filtrated mixture was concentrated by using incubator on 40°C for 72hr. The yield equal of 10 gm., the extract was stored in a dark sterile screw bottle at 4°C until used (25).

Forty healthy adult males Wistar rats, weighed 200-275 gm. were used and housed in an animal house/ Department of Physiology and Pharmacology/ College of Veterinary Medicine/ Baghdad University. The animals were kept at 22 -25°C, with 12h light/dark cycle. Animals were allowed freely access to water and pellets along the experimental period. After acclimatization two weeks, rats were randomly divided into four groups (10 rats each) as follows: Control group, animals in this group received tap water (untreated); group T$_1$ received tap water contain 0.5% H$_2$O$_2$ (26); group T$_2$ received tap water containing 0.5% H$_2$O$_2$ and administrated orally 300 mg/kg, B.W. ethanolic extract of *Eruca sativa* leaves and group T$_3$ received ethanolic extract of *Eruca sativa* leaves only at the same dose of group T2. All treatments continued for 60 days. At the end of the experiment, animals were sacrificed and samples of testes and epididymis tissues were taken and fixed in neutral formalin solution. Histological sections were prepared at 5 μm and stained with hematoxylin and eosin according to (27) for histopathological examination of testis and epididymis including: measurement of diameter of seminiferous tubule, thickness of epithelial cells of seminiferous tubules and histophotological examination of testes and epididymis has been carried out with image (Java-based image processing program developed at the National Institutes of Health). Analysis of data was carried out using One-Way Analysis of Variance (ANOVA) followed by LSD (28).

**Results and Discussion**

The mean value of thickness and diameter of seminiferous tubules in testes of four experimental groups were illustrated in (Table, 1). The histological sections of rat testes received 0.5% H$_2$O$_2$ (Group T$_1$) exhibited a significant (P<0.05) decrease in thickness of epithelial cells and diameter of seminiferous tubules as compared to control, T$_2$ and T$_3$ groups. Also the increase of space between the seminiferous tubules was observed in T$_1$. Whereas, in H$_2$O$_2$ – *Eruca sativa* treated (T$_2$) group there was a significant (P<0.05) remarkable elevation in thickness of germ cells and diameter of tubules compared to T$_1$. Moreover, the values of T$_2$ tended to normalize that of the control. While, in the group T$_3$, the appearance of all seminiferous tubules was normal, healthy and there were no differences observed between animal at T$_3$ and control as compared to each other.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Group T$_1$</th>
<th>Group T$_2$</th>
<th>Group T$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness of epithelial Cell of seminiferous tubules (mm)</td>
<td>2.84±0.14</td>
<td>1.90±0.17</td>
<td>2.51±0.28</td>
<td>3.31±0.21</td>
</tr>
<tr>
<td>Diameter of seminiferous tubules (mm)</td>
<td>8.99±0.34</td>
<td>6.25±0.22</td>
<td>8.30±0.30</td>
<td>10.66±0.41</td>
</tr>
</tbody>
</table>

Capital letters denote differences between groups, P<0.05.
Histological sections obtained from rat testis exposed to 0.5% hydrogen peroxide (group T₁) at the end of the experiment revealed decrease in the thickness of basement membrane of seminiferous tubules (Table, 1 and Fig. 1), incomplete spermatogenesis, cell debris, vaculation of Sertoli cells and sperms in the lumen of seminiferous tubules (Fig. 2) comparing to control group (Fig. 3), while testes of rats treated with 0.5% H₂O₂ plus ethanolic extract of (300 mg/kg B.W) E. sativa (T₂) showed complete spermatogenesis with presence of large number of sperms in the lumen of seminiferous tubule and active Leydig cells (Fig. 4). Normal obvious histological architecture of seminiferous tubules and complete spermatogenesis were shown in sections of testis of group T₃ (Fig. 5), as well as, the absence of main differences between seminiferous tubules morphology and thickness of basement membrane in comparison to control (Table, 1 and Fig. 1).

Rats of group T₁ showed few sperms in the lumen of epididymis with round dark nuclei cells (Fig. 6) as compared to control group (Fig. 7). Whereas, the epididymis of group T₂ showed normal structure and the lumen filled with sperms (Fig. 8 and 9) as compared with control group (Fig. 7). Meanwhile, the histopathological sections of epididymis of group T₃ showing normal structure and the lumen compact with sperms (Fig. 10) comparing to group T₁ (Fig. 6).

Exposure of rats to hydrogen peroxide (group T₁) results in the appearance of thickness of basement membrane of seminiferous tubules (Table, 1) with incomplete spermatogenesis and cellular debris (Fig. 2 and 4) as well as few sperms in the lumen of epididymis with round nuclei cells (Fig. 8). It has been proven that H₂O₂ induces oxidative stress in animal models, by forming potent ROS and nitrogen–oxygen species (29 and 30).
Oxidative stress augmented production of ROS overwhelms the body’s antioxidant defenses (31-33). Therefore, the increase in thickness of basal membrane in seminiferous tubules which accompanied with incomplete spermatogenesis may be due to an increase in production of ROS that damage normal spermatozoa by inducing LPO and DNA damage (6 and 34). Several investigators showed that severe oxidative stress would causes damage to DNA, proteins, and enzymes, including lipid peroxidation enzymes. Such degeneration leads to cell death (35-37) thereby demonstrating the pathological role of ROS on the male reproductive system (38 and 39). Research suggested that the oxidation of the proteins leads to the loss of function or to the degradation in the peroxisomes, whereas the lipid peroxidation affects the biological function of the membrane. However, the most serious damage is detected at DNA level, as this may lead to mutation of genes, inducing translation of defective proteins, in addition to alteration in
gene expression and eruption of apoptosis (40).

Whereas, other studies (37 and 41) explained that the most of the deleterious effects of H$_2$O$_2$ on tissues, including lipid peroxidation, depend on the conversion of the compound into OH$^\bullet$, which is catalyzed by iron and copper through the Fenton reaction in cells.

Furthermore, (42 and 43) explained that testicular oxidative stress, will lead to an increase in germ cell apoptosis and subsequent hypospermatogenesis. Such stress conditions can cause changes in the dynamics of testicular microvascular blood flow, endocrine signaling, and germ cell apoptosis. Many investigators have demonstrated that testicular testosterone production is acutely reduced in a number of conditions associated with ROS production and oxidative stress in the testis (42, 44 and 45). It is also true that steroidogenesis itself produces ROS, largely from mitochondrial respiration and the catalytic reactions of the steroidalogenic cytochrome P450 enzymes (46-48). The ROS produced by spermatogenesis, if unchecked by intracellular antioxidants, can also damage mitochondrial membranes and contribute to the inhibition of subsequent steroid production (49).

From the data obtained in the current study, oral administration of Eruca sativa leaves extract for 60 days to male rats in groups T$_2$ show an ameliorating the histopathological changes in testis and epididymis which is created by H$_2$O$_2$. Meanwhile, testis and epididymis of rats in group T$_3$ orally administrated Eruca sativa extract show the same histological architecture as in the control group which was compatible with (50) who explained that low dose of Eruca sativa seed oil caused an increase the diameter of seminiferous tubules, may be due to the high rate of proliferation of haploid cell resulting in stimulation of spermatogenesis and increase sperm count. It has been proven that Eruca sativa leaves extract may be capable in improving healing sperm parameter and fertility with increasing the diameter of seminiferous tubules (51). As explained earlier, the presence of bio-active isthiocyranates (ITCs) in ES extract which results from glucosinolate upon myrosinase hydrolysis (15) have antibacterial properties and induce metabolizing enzymes such as glutathione-s-transferase, NADPH, which play an important role in detoxification and protection against oxidative stress (16 and 52).

The results of the current study ascertained that ethanolic extract of E. sativa leaves alleviated the harmful effect of oxidative stress induced by H$_2$O$_2$. Thus, it can be suggested that the component of E. sativa leaves extract have the potential to improve male reproductive functions attributed to its antioxidant and androgenic properties.

**References**


دور المستخلص الكحولي لأوراق نبات الجرجير في الجهاز التكاثري لذكور الجرذان المستحدث فيها الكرب التأكسدي تجريبياً
образ نجم العلي و أحمد جاسم نوفل
فرع الفسلجة والأدوية، كلية الطب البيطري، جامعة بغداد، العراق.
E-Mail: Baaanajim@yahoo.com

الخصائص تقديم

الدراسة هدفت إلى لتحري عن دور المستخلص الكحولي لأوراق نبات الجرجير في الحماية من الأذى الكرب التأكسدي في الجهاز التكاثري لذكور الجرذان لمواقع مختلفة. استخدمت 40 ذكر جردن البالغ من أوزانها 200-275ش. تم قسمتهن إلى أربعة مجموعات متساوية: المجموعة الأولى: المجموعة التي استخدمت ماء الحنفية، المجموعة الثانية: (T1) التي أعطيت ماء الحنفية محتوى 4.2% بيروكسيد الهيدروجين، أما المجموعة الثالثة (T2) فقد أعطيت ماء الحنفية محتوى 4.2% بيروكسيد الهيدروجين مع تجريع 0.44مل/كمتر من الوزن الجسم من المستخلص الكحولي لأوراق نبات الجرجير، المجموعة الرابعة (T3) جرعت 0.44مل/كمتر من وزن الجسم من المستخلص الكحولي لأوراق نبات الجرجير. وعمرت جميع المجموعات لمدة 60 يوماً وفي نهاية التجربة ضخحي بالحيوانات وأخذت عينات من الخصية والبربخ، فضلاً عن قياس خصوبة النثبات مع أقطار النثبات المنوية. أوضحت النتائج أن قياسات الناتجة تمثل دقة في فص حزام الخصية والنثبات المنوية، بوجود فجوات واسعة بين النتائج المنوية والنباتات المنوية للبيروكسيد الهيدروجين (T1)، بوجود تغيرات مشابهة في معدلات النثبات المنوية للبيروكسيد الهيدروجين (T1) للسعة تصل إلى 4% بزيادة نصف فص حزام الخصية ونسبة النثبات المنوية. وسمك الخصية ونسبة النثبات المنوية. وسمك الخصية جميعها في مجموعتي T1 و T2 بالمقارنة مع مجموعتين T1 و T3. كما أن اقتران النثبات المنوية والبيروكسيد الهيدروجين (T1) مع وجود فجوات واسعة بين النتائج المنوية، ووجود حزام الخصية ونسبة النثبات المنوية والبيروكسيد الهيدروجين (T1) مع وجود فجوات واسعة بين النتائج المنوية، ووجود حزام الخصية ونسبة النثبات المنوية ونسبة النثبات المنوية للبيروكسيد الهيدروجين (T1) مع وجود فجوات واسعة بين النتائج المنوية. واستنتج الدراسة أن بيروكسيد الهيدروجين مضاعف عملية تصنيع النثبات المنوية في مجموعتي T1 و T3. ونسبة النثبات المنوية والبيروكسيد الهيدروجين (T1) مع وجود فجوات واسعة بين النتائج المنوية، ووجود حزام الخصية ونسبة النثبات المنوية والبيروكسيد الهيدروجين (T1) مع وجود فجوات واسعة بين النتائج المنوية. واستنتج الدراسة أن بيروكسيد الهيدروجين مضاعف عملية تصنيع النثبات المنوية في مجموعتي T1 و T3. ونسبة النثبات المنوية والبيروكسيد الهيدروجين (T1) مع وجود فجوات واسعة بين النتائج المنوية، ووجود حزام الخصية ونسبة النثبات المنوية والبيروكسيد الهيدروجين (T1) مع وجود فجوات واسعة بين النتائج المنوية.

الكلمات المفتاحية: بيروكسيد الهيدروجين، أوراق الجرجير، الخصية، البربخ، النثبات المنوية.