Toxic effect of nicotine on sperm Morphology in adult male albino rats: Protective potential of Thymus vulgaris Aqueous Extract

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Abstract:

Smoking is one of the most problems that cause oxidative stress and affect the health of humans. That affected Fertility in males which nicotine has a toxic effect on spermatogenesis. Thyme extract is shown to have some beneficial effects such as antioxidant properties. Thus, this study aimed to evaluate the antagonistic ability of thyme extract against the toxic effect of the nicotine on epididymal sperm defects in male adult rats, there were divided into four groups; G1: normal saline, G2: 0.6mg/kg nicotine, G3: 0.5g/kg thyme extract, G4: 0.6mg/kg nicotine and 0.5g/kg thyme extract. Treatments lasted for 28 days. Sperms morphology was assessed for fertility. It was significantly (P<0.05) increased in the nicotine-treated group, compared with control (P<0.05). However, co-administration of nicotine with thyme extract significantly decreased the values of sperm defects as compared to that observed in nicotine group (P<0.05). The findings suggest that the thyme extract possibly ameliorates the deleterious effects of nicotine on some reproductive indices in male albino rats.

Key words: Nicotine, Thymus vulgaris, sperm, Morphology, Rat.
تشير النتائج إلى أن مستخلص الزعتر المائي ربما يقلل من الآثار الضارة للنيكوتين على بعض مؤشرات الخصوبة في ذكور الجرذان البيضاء.

الكلمات المفتاحية: النيكوتين، زعتر، حيوان المنوي، الشكل الظاهري، الجرذ.
Introduction:

More than seventy million people suffer from infertility worldwide.[1] Globally, male factors account for at least 50% of all infertility cases.[2] Cigarette smoking is one of the factors that contribute to male infertility.[3,4] Tobacco smoke contains nicotine, carbon monoxide, carcinogens, and irritant substances[5] and nicotine is considered the main chemical in tobacco that is responsible for engendering tobacco use and dependence.[6] Studies have shown that nicotine is detrimental to the male reproductive system[7,8] Several reproduction studies report that smoking can cause decreased semen quality, reproductive system dysfunction, hormone disturbances, spermatogenesis, sperm maturation, and spermatozoa function [9, 10,11]. The effect of nicotine on the male reproductive system is a rising concern, as a previous study indicated that nicotine caused a decrease in the number of spermatocytes. In addition, cigarette smoking disrupts the anterior pituitary gland, spermatids[12], and testicles mechanism, affecting the formation of gonadotropins (GnRH) include Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) that works during spermatogenesis. Nicotine treatment causes a significant decrease in the mean serum FSH and testosterone. The imbalance mechanism of reproductive hormones will eventually affect the mechanism of spermatogenesis in testis [13] However, There are many mechanisms cellular mechanisms through which nicotine can affect different organs, several studies have shown higher oxidative damage and reduced antioxidant status in smokers versus nonsmokers. Moreover, protection of different antioxidants against nicotine exposure[14,15] suggest the involvement of oxidative stress as an important mechanism in nicotine-induced side effects, ultimately leads to impaired sperm morphology, a decline in the progressive sperm, and increased sperm death [16,17].

However, modern research has shown that phytogenic compounds or bioactive compounds of plant origin are rich in antioxidants, such as flavonoids, phenols, terpenes, polysaccharides, saponins, alkaloids, vitamins, and trace elements (18 ,19). These antioxidants directly or indirectly exert their effects on the body’s antioxidant system by eliminating excessive free radicals and thus protecting the body [20]. (Thymus vulgaris), is a species of flowering plant, an aromatic and medicinal plant in the mint family Lamiaceae [21] with a wide distribution throughout Asia and especially in Iran. Aromatic plants have been studied extensively
because a rich source of natural antioxidants is available in their essential oils or diverse extracts [22]. Thyme extract has some constituents such as thymol, carvacrol, flavanoid, caffeic acid, and labiatic acid, showing antibacterial, antimycotic, and antioxidative(23), showing that the phenolic compound of thyme extract has a strong antioxidant activity. These active components had the best oxidative status [24] Studies have shown that this plant, with its high antioxidant content, can protect sperm against toxic chemicals.[25] To date, there appears to be no study on The role of thymol in improving sperm parameters induced by the reproductive toxicity of nicotine in rats as an animal model. That is why this study was designed.

Materials and methods :

Animals :
In this study, 20 male rats weighing 200-220 grams were purchased from Animal House at the Faculty of Veterinary Medicine- Omar Mukhtar University. The animals were housed in a room with a temperature of 22±2°C under controlled environmental conditions within a 12-hour light/ dark cycle and had free access to water and food. The ethical and humane principles were observed during the experiments [26].

Chemicals

Nicotine Preparation
Nicotine hydrogen tartrate (95% nicotine) was used in the study (Sigma, USA). It is partially soluble in water and freely soluble in saline.

Thyme aqueous extraction
Dried leaves of thyme (Thymus vulgaris) were purchased from the market in AlbidaLibya and were identified by Herbarium, Department of Botany, Omar Al Muhtar University. The leaves of thyme were grounded into powder using an electrical grinder. One hundred grams of the fine-powder were subjected to extraction with boiling distilled water in a covered flask and left for 30 minutes. After that, the extract was cooled and filtered using Whatman filter paper to remove the particulate material then. The filtrate was dried in a vacuum (Department of plant protection, Omar Al-Mukhtar University). The required doses were then weighted and reconstituted in 5 ml of distilled water a minute ago before oral administration. [27]
Methods

Experimental design

The study was performed on 20 adult male albino rats, divided into four groups; each group consisted of 5 rats as follows:

Control group: Animals were injected intraperitoneally with 0.9% saline water once daily.

Group II: received 0.6 mg/kg body weight of nicotine by intraperitoneal (ip) injection to induce reproductive damage.

Group III: was provided as a thyme control group receiving 0.5 g/kg per day thymol dissolved in 0.20 mL normal saline orally.[29]

Group IV: were received NIC at doses of 0.6 mg/kg per day; (ip), dissolved in 0.20 mL normal saline, plus 0.5 g/kg per day Thyme, orally. The experiment period was 28 days.

Collection of epididymal sperm smears:

The sperm smears were obtained from the caudae epididymis of the testes of adult control and treated males. The caudae epididymis were cut into small pieces in 1 cc. saline solution. Sperm smears were obtained from the resulting suspension. They were stained by eosin. A binocular microscope with _40 eyepieces and _100 oil immersion objective lenses were used for this study. Abnormality sperm were recorded randomly.

Statistical analysis

Data analysis was performed using one-way analysis of variance (ANOVA) along with a post hoc multiple comparison test (least square difference) and the data were expressed as mean and standard error of the mean (SEM) at the significance level of $P<0.05$.

Results

Table 1 and Fig. 1 shows that the frequency of abnormally shaped sperms in the testes of male albino rats. Total sperm defect was significantly ($p<0.05$) increased in Nicotine group compared with control. It was however significantly ($p<0.05$) decreased in N+thymol group compared with Nicotine group. Sperm head defect was significantly ($p<0.05$) decreased in
N+thymol group compared with Nicotine group. Sperm tail defect was significantly (p<0.05) increased in Nicotine group compared with control. It was significantly (p<0.05) decreased in N+thymol group compared with Nicotine group. head +tail defect was no significantly (p<0.05) increased in Nicotine group compared with control.

**Table 1: Comparison of sperm morphology in the different experimental groups.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>control</th>
<th>Thymol</th>
<th>Nicotine</th>
<th>Nicotine+thymol</th>
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</thead>
<tbody>
<tr>
<td>Head defect (%)</td>
<td>1.00±0.00</td>
<td>1.50±0.20</td>
<td>3.50±0.78</td>
<td>2.00±0.00</td>
</tr>
<tr>
<td>Tail defect (%)</td>
<td>1.00±0.28</td>
<td>1.00±50.00</td>
<td>3.00±0.35</td>
<td>1.75±0.00</td>
</tr>
<tr>
<td>head +tail defect (%)</td>
<td>0.50±0.00</td>
<td>0.00±0.00</td>
<td>1.25±0.00</td>
<td>1.30±0.50</td>
</tr>
<tr>
<td>Total defect (%)</td>
<td>2.00±0.00</td>
<td>1.75±0.30</td>
<td>8.00±0.00</td>
<td>3.50±0.00</td>
</tr>
<tr>
<td>Normal sperm (%)</td>
<td>98.00±0.26</td>
<td>98.35±0.00</td>
<td>92.00±0.30</td>
<td>96.00±0.00</td>
</tr>
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Values are expressed as mean ± SEM, n = 5.

*p<0.05 vs. control; a = p<0.05 vs. Nicotine
Figure (1): Different types of mice sperms abnormalities induced by Nicotine (A) Normal sperm (B) double head (C) hook head (D) amorphous head sperm (E) bent neck (F) Absence of tail (G) wheel tail (H) double head and tail sperm
Discussion

Researchers have reported the detrimental effect of cigarette smoking on male fertility [30, 31]. Previously, it had been shown that nicotine was a major toxic for reproductive health and had toxic influences on sperm count and motility in adult ICR mouse (32), because it induces oxidative stress and tissue damages leading to the fact that cigarette smoke can directly or indirectly by interference reproductive toxicity with spermatogenesis.

The main content possessed by cigarettes is nicotine [33]. Cigarette smoke is a source of free radicals [34]. Free radicals are generated due to incomplete combustion [35]. The mechanism of decreased sperm function under oxidative stress has been shown to involve excessive ROS [36]. This study was conducted to evaluate the effect of thymol on sperm motility, viability, count morphology, in nicotine-induced reproductive toxicity of male rats. Results in this study about the effect of nicotine is agreement with earlier reports of [37-39]. The observed reduction in sperm indices may be due to the action of nicotine in generating reactive oxygen species in the testes [40]. Oxidative stress (OS) has been identified as one of the many mediators of male infertility by causing sperm dysfunction. Augmented production of ROS overwhelms the body's antioxidant defenses. While small amounts of ROS are required for normal sperm functioning, disproportionate levels can negatively impact the quality of spermatozoa and impair their overall fertilizing capacity. They attack DNA, lipids, and proteins; alter enzymatic systems; produce irreparable alterations; cause cell death; and ultimately, lead to a decline in the semen parameters associated with male infertility [41]. Administration of thymol significantly improved the evaluated indices in nicotine-treated rats, that were observed in nicotine-treated rats. Studies have shown that thymol in nicotine-treated rats improve both sperm quality and quantity [42, 43]. The beneficial effect of Thyme is mostly due to its antioxidant potentials. It is a lipid soluble antioxidant which plays a major protective role against oxidative stress and prevents the production of lipid peroxides by scavenging free radicals which are toxic byproducts of many metabolic processes in biological membranes [44]. Earlier studies have reported that induction of testicular oxidative stress led to the increase in apoptosis of germ cells and consequent abnormal spermatogenesis [45]. Nicotine administration can damage sperm DNA and cell membrane as well as induce apoptosis in testicular cells [46]. While, administration thymol inhibited the...
apoptosis of testicular tissue through reduction of the testicular expression of caspase-3 [47].

T. vulgaris seems to play a role in elevating sperm motility and viability by promoting the sperm antioxidant defense system, including superoxide dismutase, glutathione peroxidase, and catalase.[48]. Furthermore, thyme extract, which show anti-oxidant activity, has an inhibitory effect on lipid peroxidation, which could decrease the strength of inflammatory response [49]. Previous researcher claimed that nicotine involved in inhibiting testosterone production through its effects on acetylcholine receptors on cell membrane [50] (Kasson and Hsueh, 1985). A drop in the testosterone level will lead to infertility of males due to its major role in spermatogenesis [51]. Thym vulgaris extract containing apigenin, as a flavonoid compound, regulates the secretion of LH by Leydig cells and increases the serum testosterone level. [47], this improve the structure of the seminiferous tubules aijured [52], this will improve the quality of semen. This study provides data on the adverse effects of nicotine on sperm quality. It is also providing evidence that Thymus vulgaris is potentially useful in improving the fertility of male rats by increasing the sperm count and number of normal sperm morphology. It is important in the future to study the mechanism on the adverse effect of nicotine on spermatogenesis as well as the beneficial effect of Thymus vulgaris in improving male fertility.

Conclusion

This study indicated that nicotine reduced sperm defect and thyme extract had a protective effect against the toxic effect of nicotine. It can be suggested that thyme can be used as supplement to improve fertility among male smokers due to the nicotine abuse.
Reference


4- Wong, WY.; Thomas, CM.; Merkus, JM.; Zielhuis, GA.; Steegers-Theunissen, RP. 2000. Male factor subfertility: possible causes and the impact of nutritional factors. Fertil Steril, 73: 42-435


<table>
<thead>
<tr>
<th></th>
<th>Authors</th>
<th>Title</th>
<th>Journal/Source</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Oyeyipo, IP.;  Raji, Y.;  Bolarinwa, AF.</td>
<td>Nicotine alters male reproductive hormones in male albino rats: The role of cessation.</td>
<td>J. Hum Reprod Sci. 6(1):40-4.</td>
<td>2013</td>
</tr>
<tr>
<td>14</td>
<td>Rachid Mosbah; Mokhtar Ibrahim Yousef; Alberto Mantovani</td>
<td>Nicotine-induced reproductive toxicity, oxidative damage, histological changes and haematotoxicity in male rats: The protective effects of green tea extract.</td>
<td>Experimental and Toxicologic Pathology,67(3):253-259.</td>
<td>2015</td>
</tr>
<tr>
<td>17</td>
<td>Viloria, T.; Meseguer, M.; Martínez-Conejero, JA.; O’Connor, JE.; Remohi, J.; Pellicer, A.; Garrido, N.</td>
<td>Cigarette smoking affects specific sperm oxidative defenses but does not cause oxidative DNA damage in infertile men.</td>
<td>Fertil Steril, 94(2):631–637.</td>
<td>2010</td>
</tr>
<tr>
<td>20</td>
<td>Chaves, N.; Santiago, A.; Alias, J.C.</td>
<td>Quantification of the antioxidant activity of plant extracts, analysis of sensitivity and hierarchization based on the method used.</td>
<td>Antioxidants (basel) 9(1):76.</td>
<td>2020</td>
</tr>
</tbody>
</table>


26 -WMA DECLARATION OF HELSINKI – ETHICAL PRINCIPLES FOR MEDICAL RESEARCH INVOLVING HUMAN SUBJECTS. Helsinki, Finland 48th 1996.


33-Rachid Mosbah; Mokhtar Ibrahim Yousef; Alberto Mantovani.2015. Nicotine-induced reproductive toxicity, oxidative damage, histological changes and haematotoxicity in male rats: The protective effects of green tea extract, Experimental and Toxicologic Pathology, 67 259-253 : (3 )


