PROTECTIVE EFFECT OF NIGELLA SATIVA OIL ON TESTICULAR MORPHO-PHYSYLOGICAL CHANGES INDUCED BY CHRONIC ALCOHOLISM IN ALBINO RATS

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INTRODUCTION

Alcoholism is an illness caused by consumption of alcohol beverages at a level that interferes with physical or mental health, and social, family, or occupational responsibility. People with alcohol dependence have to increase markedly amounts of alcohol to achieve intoxication or the desired effect (Thomas, 2003).

Alcoholism affects 15 to 20 million Americans and affects three to four times as many family members, coworkers, and friends. It is involved in half of all fatal automobile accidents and is responsible for more than 100,000 deaths annually, representing about 5% of all deaths in the United States. Fetal alcohol syndrome is the third leading cause of birth defects with accompanying mental retardation (Michigan society for medical research, 2003).

Acute and chronic alcoholism carry the potential to damage many organs of the body, including the heart, brain, liver, kidney, digestive system and reproductive system (erectile dysfunction and cessation of menstruation). When alcohol was given, its level in the body increased and produced the intoxication effects. The undesirable toxic effects include damage of Leydig cells and spermatogenic lineage cells (Michigan society for medical research, 2003).

Zinc is essential for normal growth and reproduction and it has a beneficial effect on the process of tissue repair and wound healing (King, 1990). Zinc is a constituent of antioxidant, superoxide dismutase. It is also needed for proper maintenance of vitamin E level in the blood and aids in the absorption of vitamin A (Bert and Falchuk, 1993). It exerts a protective effect and possibly preventing cellular damage caused by oxidative stress (Marchesini et al., 1996).
Zinc metalloenzymes include 200 related enzymes for which zinc is needed that participate in the metabolism of lipids, carbohydrates, synthesis of protein and nucleic acids. Zinc has a metabolic role under various physiological and pathological conditions (Vallee, 1998). Both zinc and copper are cofactors in metalloenzymes Cu²⁺, Zn²⁺, superoxide dismutase, and Zn²⁺ competes Cu²⁺ for the binding sites (Sharonova et al., 2000).

Copper is highly toxic in excess, and results in cellular damage. Reduction of ceruloplasmin oxidase activity results in free radical release and this may enhance oxidative damage of cells (Eagon et al., 1999).

Selenium is an important component of the antioxidant enzyme glutathione peroxidase (Anumann et al., 1997) Glutathione peroxidase stimulation of catalytic cycle, in synergism with vitamin E. it also enhances the immune system in elderly (Clarrk et al., 1998).

Black seed (Nigella sativa), a member of the Rununculaceae family, is one of the native plants that are widely distributed in Egypt. Nigella sativa and its constituents have been widely used in folk medicine and experimental animal studies (Hassan, and El Dakhakhny, 1992). In Arabian folk medicine, it is used for treatment and prevention of many diseases like bronchial asthma, diabetes, rheumatism and hypertension. Also, Nigella sativa was used to elevate the immunity of the individual (El - Kadi et al., 1987). Nigella sativa seeds and its constituents have antioxidant action (capable of scavenging oxygen derived free radicals), anti tumor activity, digestive stimulant, and an immune modulating effect. Also, it has been reported that Nigella sativa has a cyto-protective effect on tissues exposed to cytotoxic agents (El - Kadi et al., 1987; Hassan. and El Dakhakhny, 1992).

The present work was done to investigate the morphophysiological changes of albino rat testicles exposed to experimental chronic alcohol intake for 30 days and also, to detect if oral administration of Nigella sativa oil has any role in prevention of these effects, if any.

MATERIAL AND METHODS

The present study was carried out on 40 adult male albino rats weighing 250 - 300 gm. The animals had free access of water and standard diet of milk and bread. The rats were divided into four equal groups.

- **Group 1**: Is the control group.
• **Group 2**: Received oral Nigella sativa oil obtained from (Pharco-Pharmaceutical- Alexandria - Egypt), daily in a dose of 0.88 gm / kg. B.W. (*El-Dakhakhny et al.*, 2000) for 30 days.

• **Group 3**: Animals were given oral 40% ethanol (ethyl alcohol) daily in a dose of 3 ml / kg. B.W. (*Gamal*, 2001).

• **Group 4**: Animals were given oral 40% ethanol in a dose of 3 ml / kg. B.W. daily for 30 days and at the same time rats were administered Nigella sativa oil orally in a dose of 0.88 gm / kg. B.W. daily.

After 30 days all rats were weighed before decapitation. After decapitation, blood samples were taken and centrifuged to separate serum. Serum is used to estimate Testosterone according to (*Stlay et al.*, 1996). Leutinizing hormone (LH) and Follicular stimulating hormone (FSH) according to (*Kullin and Santier*, 1996). For determination of trace elements (Zn$^{+2}$, Cu$^{+2}$, Se$^{+2}$), microanalysis of energy dispersive X-ray florescence technique (EDXF), and Neutron activation analysis technique (NAAT) were used (*El Nimer et al.*, 1996; *El Nimer et al.*, 1996).

The testicles were dissected, weighed, immediately fixed in Bouin's solution, and then prepared for histological examination.

**RESULTS**

The results of the present work showed that:

**General Examination**:

Rats of group (1), and (2) showed insignificant increase in body weight, normal amount of food consumption and no deaths during the period of the experiment. Animals of group (3) showed increased mortality rate (20%), marked reduction in food intake and significant loss of body weight (p value < 0.05) as compared to other groups (1, 2). The animals of group (4) showed less mortality rate (10%) and insignificant reduction of body weight (Table : 1) (Fig. 1). There was slight increase in the percentage of testicular weight in relation to body weight in rats of group (3). The percentage of testicular weight in relation to the body weight (after 30 days) of the control group was 2.1%, while in alcohol treated group was 2.7% and alcohol plus Nigella treated group was 2.1% (Fig. 2).
Blood Examination (Hormonal analysis):

The present work showed that chronic oral alcohol administration for 30 days (group 3) resulted in significant decrease in Testosterone, LH and FSH levels compared with the control group (1) and Nigella Sativa oil treated rats (group 2), (P < 0.05). In group (4), there was insignificant decrease in Testosterone level compared to the control group (P > 0.05) but a significant reduction was observed as regard FSH and LH (P < 0.05). Also, there was a significant reduction of trace elements, (Zn⁺², Cu⁺², Se⁺²), in rats administered alcohol in comparison to control group (P < 0.05), which improved after intake of Nigella sativa with alcohol in comparison to alcohol treated group, (P < 0.05) (Table 2) and (Figs. 3 - 8).

Histological Examination:

Testicular morphology of rats in group (2), which administered Nigella Sativa oil, resembled that of the control group (group; 1), (Figs. 9 - a & 9 - b).

Testicular pattern of rats in group (3), which was given alcohol only showed that the capsular blood vessels were dilated and congested (Fig. 10 - a). The seminiferous tubules were widely separated from each other and revealed reduction in the diameter (Fig. 10 - b). There was partial separation of the basement membrane of some seminiferous tubules. Also, there was a significant decrease in the number of sperms, as well as spermatogenic lineage cells. Other tubules were nearly empty from spermatogenic cells. The number of spermatogonia was markedly increased in the highly affected tubules. In some tubules, the spermatogenic cells were arrested at primary spermatocyte stage, others were arrested at spermatid stage, (Fig. 11 - a). The seminiferous tubules showed thickening of the basement membrane (Fig. 11 - b). Vacuoles were seen within the seminiferous tubules (Fig. 12 - a). The interstitial tissue revealed scanty number of Leydig cells, hyalinization, edema, dilated and congested blood vessels (Fig. 12 - b).

Testes of animals in group (4), which was treated with alcohol and Nigella sativa oil at the same time, showed more or less the same testicular morphology as in groups (1 & 2) as in (Fig. 13).
Table 1: Shows the body weight in grams before and after 30 days, of the control group, alcohol treated group, and alcohol plus Nigella sativa treated group.

<table>
<thead>
<tr>
<th>Item</th>
<th>Mean</th>
<th>SD</th>
<th>T test P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Before</td>
<td>275.8</td>
<td>18.6</td>
<td>0.14*</td>
</tr>
<tr>
<td>Control After</td>
<td>287.2</td>
<td>16.9</td>
<td></td>
</tr>
<tr>
<td>Alcohol Before</td>
<td>261.7</td>
<td>26.7</td>
<td>0.04*</td>
</tr>
<tr>
<td>Alcohol After</td>
<td>233.3</td>
<td>26.8</td>
<td></td>
</tr>
<tr>
<td>Alcohol + Nigella</td>
<td>256.7</td>
<td>39.9</td>
<td>0.13*</td>
</tr>
<tr>
<td>Before</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol + Nigella</td>
<td>299.3</td>
<td>42.6</td>
<td></td>
</tr>
</tbody>
</table>

* insignificant p value compared to control.
* Significant value as (p < 0.05).

Table 2: Shows the effect of oral Nigella sativa oil on serum testosterone, LH, FSH, Zn²⁺, Cu²⁺, and Se²⁺ levels in alcohol administrated albino rats.

<table>
<thead>
<tr>
<th>Item</th>
<th>Testosterone (ng / ml) Mean (6) SE + / - T</th>
<th>LH (m.i.u. / ml) Mean (6) SE + / - T</th>
<th>FSH (m.i.u. / ml) Mean (6) SE + / - T</th>
<th>Zinc (ug / 100ml blood) Mean (6) SE + / - T</th>
<th>Copper (ug / 100ml blood) Mean (6) SE + / - T</th>
<th>Selenium (ug / 100ml blood) Mean (6) SE + / - T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.79</td>
<td>3.43</td>
<td>9.68</td>
<td>74.7</td>
<td>39.2</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>0.1</td>
<td>0.26</td>
<td>0.59</td>
<td>0.47</td>
<td>0.009</td>
</tr>
<tr>
<td>Nigella</td>
<td>4.99</td>
<td>3.51</td>
<td>10</td>
<td>75.8</td>
<td>39.1</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>0.15</td>
<td>0.04</td>
<td>0.22</td>
<td>0.78</td>
<td>0.62</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>0.09*</td>
<td>0.8*</td>
<td>0.9*</td>
<td>0.39*</td>
<td>0.12*</td>
<td>1*</td>
</tr>
<tr>
<td>Alcohol</td>
<td>4.21</td>
<td>2.95</td>
<td>7.51</td>
<td>26.1</td>
<td>25.1</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>0.13</td>
<td>0.07</td>
<td>0.4</td>
<td>0.39</td>
<td>0.41</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>3.8**</td>
<td>4**</td>
<td>4.6**</td>
<td>69**</td>
<td>22.5**</td>
<td>20**</td>
</tr>
<tr>
<td>Alcohol + Nigella</td>
<td>4.69</td>
<td>3.11</td>
<td>8.2</td>
<td>45.8</td>
<td>36</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>0.05</td>
<td>0.1</td>
<td>0.58</td>
<td>0.44</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>1.64*</td>
<td>2.9**</td>
<td>5.4**</td>
<td>35**</td>
<td>4**</td>
<td>11**</td>
</tr>
</tbody>
</table>

T*: insignificant p value compared to control.
T**: Significant p value compared to control (p < 0.05). SE: Standard error.
Cor bel
I;'ig, (l) : IilxIy weighl of thc tlillerent groups hr'lirrc irnd nfter lhe sludy.

Fig. (1) : Body weight of the different groups before and after the study.

Fig. (2) : Percentage of testicular weight / body weight.
Fig. (3): Serum testosterone level.

Fig. (4): Serum LH level.
Fig. (5) : Serum FSH level.

Fig. (6) : Serum zinc level.
Fig. (7) : Serum copper level.

Fig. (8) : Serum selenium level.
Fig. (9 - a) : Testicular morphology of a control rat.
(H. & E.; x 200)

Fig. (9 - b) : Testicular pattern of a Nigella Sativa oil treated rat (group 2).
(H. & E.; x 200)
Fig. (10 - a) : A testis from rat administered alcohol alone shows dilated and congested capsular vessels.

(II. & E.; x 100)

Fig. (10 - b) : A testicle of a rat from group (3) shows lymph edema in between seminiferous tubules.

(II. & E.; x 200)
Fig. (11 - a): An alcohol administered rat testicle shows partial separation of the basement membrane of the seminiferous tubule.

(H. & E.; x 200)

Fig. (11 - b): The testicular morphology of an alcohol treated group reveals thickening of the basement membrane of the seminiferous tubules.

(H. & E.; x 100)
Fig. (12 - a): Vacuoles are seen in the seminiferous tubule of the testis of an animal from group (3). The affected tubules show decreased number of spermatogenic cells.

(H. & E.; x 200)

Fig. (12 - b): A testis of a rat from group (3) reveals decreased number of Leydig cells, hyalinization, edema and dilated congested blood vessels of the interstitial tissue.

(H. & E.; x 200)
DISCUSSION

Alcohol is the most widely drug abuse in the United States and other countries. Unlike most other drugs, alcohol carries the potential to damage many organs of the body including the heart, brain, liver, digestive system and the reproductive system. Essential information about alcoholism had come from studies with animals showing sensitivities and responses to alcohol similar to those of humans, so animal models were selected to the present work. Albino rats were chosen because they have many of the same biological and behavioral reactions to alcohol as humans, as regarding the male reproductive system (Salonen et al., 1992).

The present study revealed increased mortality rate between rats administered alcohol alone (group 3) but decreased in rats received alcohol and Nigella sativa oil at the same time (group 4). Increased mortality rate was most properly due to malnutrition, liver disease and multi-organ affection resulted from alcoholism. A decrease in body weight and food intake was significant in group (3). This might be for decreased appetite. This was in agreement with Gamal (2001) who reported that rats received alcohol for one month lost weight because alcohol stimulated leptin secretion. Leptin, is the hormone that regulates appetite, discovered in 1994. A slightly increased percentage of testicular weight in relation to body weight in alcohol
treated group was due to reduction in body weight and increased testicular weight due to edema and congestion of the testicles.

This work showed that chronic alcohol intake caused a profound testosterone suppression associated with decreased LH and FSH levels. Moreover, acute and chronic alcohol intake were associated with low levels of hypothalamic LHRH and pituitary LH in adult and pubertal male rat and inhibited testosterone secretion by the testes as well.

Also, chronic alcohol abuse produced sexual dysfunction and impaired sperm production in both human and animals (Yan and Jaffe, 1991; Bielawski and Abel, 1997; Hiney et al., 1999; Emanuele et al., 2001). The mechanisms of this alcohol-induced damage have not been fully explained. Several possible mechanisms were described.

Testicular opioids when produced within the testes suppressed testosterone synthesis. One opioid, beta endorphin, had been increased with acute and chronic alcohol consumption. Similarly, beta endorphin produced in the hypothalamus resulted in decreased LHRH. In addition, opioids might increase apoptosis (programmed cell death). Apoptosis at the gonadal level would result in the death of Leydig cells as well as spermatogenic cells, leading not only to low testosterone level but also, to diminished sperm production (Nanji and Hiller, 1997; Yin et al., 1999).

Another cause for alcohol's harmful effects on testosterone production was decreased production of nitric oxide (NO). This NO is a vasodilator synthesized in the testes by nitric oxide synthetase enzyme. Inhibition of this enzyme by alcohol decreased production of NO. Thus there is no vasodilatation which resulted in accumulation of waste products that caused damage to Leydig cells and subsequent reduction in testosterone secretion (Azad et al., 1991).

Alcohol consumption might induce oxidative damage either by enhancing the production of toxic compounds called free radicals (byproducts of alcohol metabolism) or by decreasing the level of antioxidants (Polavarapu et al., 1998). Alcohol administration increased alcohol level in the blood. The body began to detoxify it by its conversion into acetaldehyde then into acetate. Acetaldehyde bound the red blood corpuscles' membrane making them more rigid and prevented them from easy passage into smaller capillaries. This reduced the oxygen supply to most of the cells of the body including the testicles.

Hypoxia in its turn reduced the ability of the cytoplasmic protein tubulin to assemble into microtubules. these microtubules provided a structural support for the cells. Without these microtubules, the cells gradually atrophied and died off (Adams
et al., 1992). Also, alcohol induced elevated levels of pituitary prolactin and cytokines which might be responsible for suppression of testosterone secretion (Ogilvie and Rivier, 1997).

When testosterone level decreased, LH level did not increase as would be expected. This might be due to inability of the pituitary gland to respond appropriately to declined testosterone implied that alcohol had a central effect on the interaction between the nervous system and the endocrine system (Cooke et al., 1991).

Studies in alcohol-fed rats had established that decreased LH level resulted from impairment in both LH production and LH secretion. Alcohol could disrupt the functioning of the LHRH receptors or its interaction with LHRH, resulting in diminished LH release. Alcohol might affect the activity of LH molecule, rendering it less capable of stimulating hormone production in the testes. Therefore, alcoholic's deleterious effects on LH function were qualitative as well as quantitative (Emanuele et al., 1993).

The secretion of FSH was reduced by alcohol intake, but the exact mechanism was unknown; it might be due to increased level of estradiol. This estradiol enhanced the release of beta endorphin which suppressed the secretion of FSH (Hikim and Swerdloff, 1999).

Mark (1993), in contrast to the results of this study, reported that acute and chronic alcohol administration caused profound low testosterone level accompanied by normal LH and FSH levels. He supposed that the damaging effect of alcohol was localized to the testes and it had no central role.

Low levels of testosterone in adult men had been associated with a variety of medical problems including accelerated osteoporosis, decreased muscle and prostate functions, anemia, altered immune function and decreased reproductive ability (Cooke et al., 1991).

Dilatation and congestion of the capsular and interstitial tissue blood vessels detected in this study were due to allergy caused by the toxic effect of alcohol itself, or its end products of metabolism. This toxicity also caused damage of the spermatogenic cells as it might affect the blood testis barrier. There was increased number of spermatogonia in highly affected seminiferous tubules. This was because spermatogonia proliferated to compensate decreased number of spermatogenic cells (Mark, 1993). Separation of the basement membrane of the seminiferous tubules noticed in the present study was most probably due to oxidative injury resulted in disarrangement and ultimately destruction of hemidesmosomes that joined the spermatogonia to the basement membrane (Mark, 1993). An adequate protein diet is
important for maintaining cell structure as well as its cell membrane proteins. Alcohol affects protein nutrition by causing impaired and delayed digestion of proteins into amino acids in the small intestine and the liver (John, 2002; Emanuele and Nicholas, 2003).

The present study showed a reduction of the blood levels of Zn$^{+2}$, Cu$^{+2}$, and Se$^{+2}$ after alcohol administration. The testicular damage could be explained by zinc and selenium deficiency. Zinc exerts a protective effect on the activity of spermatogenic cells, possibly by preventing cellular damage caused by the oxidative stress (Marchesini et al., 1996).

The results of the present work revealed that administration of Nigella sativa oil for 30 days to alcohol administrated albino rats produced a significant improvement of physiological and morphological changes of the testicles if compared with albino rats administered alcohol alone. This might be due to the anti-oxidant properties of Nigella sativa oil. Also, Nigella sativa might enhance the production of nitric oxide which is a vasodilator that increases the blood supply to the testicles and other parts of the gonadal axis to remove the harmful waste products produced from alcohol metabolism (El-Kadi et al., 1987; Hassan and El Dakhakhny, 1992).

It appeared that alcoholic's damaging effects on reproduction were mediated at all three levels of male reproductive unit (hypothalamus, pituitary and gonads). Also, it is clear that Nigella sativa oil improved markedly these effects.

**SUMMARY**

The present work was designed to evaluate the protective effect of Nigella sativa oil on the morph-physiological changes of albino rat testes submitted to experimental chronic alcoholism. The rats were divided into four equal groups. Group (1) is the control group, group (2) was administered Nigella sativa oil alone, group (3) was administered alcohol for 30 days and group (4) was administered alcohol and Nigella Sativa oil at the same time for 30 days. All rats were decapitated after 30 days for blood examination and the testicles were dissected and prepared for histological examination.

The study revealed an increase in the mortality rate (20%) and significant reduction of the body weight in group (3) compared to other groups ($P < 0.05$). Blood examination showed a significant reduction in testosterone, LH and FSH levels ($P < 0.05$) in the same group. There was also a significant reduction in blood level of
trace elements, Zn$^{2+}$, Cu$^{2+}$, and Se$^{2+}$, ($P < 0.05$). These changes, improved in rats administered Nigella sativa oil and alcohol at the same time group (4).

Moreover, histological examination of the testes of rats administered alcohol alone showed marked morphological changes in the form of decreased diameter of the seminiferous tubules, partial separation of the basement membrane of some seminiferous tubules, decreased number of Leydig cells and spermatogenic lineage cells. Oral administration of Nigella sativa oil with alcohol (group 4), caused improvement and reduction of the testicular changes. The diameter of the seminiferous tubules increased with increased number of both spermatogenic cells and Leydig cells. The hormonal level of Testosterone, LH and FSH showed improvement in relation to alcohol treated group, ($P < 0.05$). In addition, the level of trace elements Zn$^{2+}$, Cu$^{2+}$, and Se$^{2+}$ showed significant increase in relation to group (3) ($P < 0.05$).

It is concluded that chronic alcohol intake affected the hypothalamo - pituitary - gonadal axis; a system of endocrine glands and hormones involved in male reproduction. Also, it is recommended to give Nigella sativa oil as a treatment and protective to the toxic effects of alcohol.

REFERENCES


المشخص العربي

دراسة تأثير زيت حبة البركة (الحبة السوداء) على التغيرات التي يحدثها الاستعمال المزمن للكحول على الخصية وبعض الهرمونات في الفئران البيضاء

نافحة مجموعة مكونة على الروبي * روسياء على الشربيني**

قسم البستولوجي - كلية الطب - جامعة القاهرة
قسم الفسيولوجي - كلية الطب - جامعة طنطا**

يهدف هذا البحث إلى دراسة تأثير حبة البركة (الحبة السوداء) على التغيرات البستولوجية والفسيولوجية التي يحدثها الاستعمال المزمن للكحول في خصية الفئران البيضاء. وقد أجري البحث على أربع مجموعات متساوية كالتالي:

المجموعة الأولى وهي المجموعة الضابطة والمجموعة الثانية وقد أعطت زيت حبة البركة عن طريق الفم بجرعة 88. ملجم يوميا لمدة ثلاثون يوما. والمجموعة الثالثة أعطت أثيل الكحول فقط بتخفيض 40% لمدة ثلاثون يوما عن طريق الفم والمجموعة الرابعة أعطت الكحول زيت حبة البركة في نفس الوقت وبعد ثلاثين يوما تم نيب الفئران وجمعت عينات دم لتحليلها وأخذت الخصية وحضرت للدراسة البستولوجية.

وقد دلت نتائج هذا البحث على أن هناك تغيرات في الخصية في الفئران التي تعاطت الكحول وحدها وكانت في مصورة نقصان قطر أنابيب الخصية وعدد الخلايا المبطنة لها وكذلك عدد خلايا الديدج وكذلك هذه التغيرات مصحوبة بنقص نسب دالله إستئصال في مستوى الهرمونات مثل التستوستيرون وهرمون LH وهرمون FSH وقياس نسبة بعض العناصر وهي الزنك واللزاق والسولينيوم.

- 193 -
وقد تحسنت كثيرا هذه التغييرات بإعطاء زيت حبة البركاء عن طريق الفم في نفس الوقت مع الكحول.

ويوصى هذا البحث بتجنب تناول الكحول لما له من آثار مدمرة على الخصوبة وعلى الهرمونات التي تفرزها والتي تتحكم في أدائها كما يوصى باستعمال حبة البركاء يومياً وبالجرعة المحددة بما لها من فوائد منها تحسن الآثار الجانبية للكحول.

مجلة التشريح المصرية 36 (2)، يوليو 2002 - 194 -