The effect of different concentration of Ethyl alcohol on the seminiferous tubules of the mature rabbits. Histo-physiological study

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Abstract

This study was applied to investigate the effect of different concentration of ethyl alcohol on seminiferous tubules in adult male rabbits. The study was conducted on eighteen mature male rabbits divided randomly into three groups, each group having six animals. The first treated group was injected subcutaneously (S/C) four milliliters of ethyl alcohol (15%), eight milliliters of ethyl alcohol (25%) respectively for a period of six weeks as one dose every 48 hours. The third group was the control group treated with distilled water at the same doses as the treated groups. The results showed:

1. The first group showed an increase in mean diameter of seminiferous tubules (250.75 ± 7.8) micrometer, and a decrease in testosterone level (18.85 ± 10.52) microgram/milliter as compared with the control group (distilled water).
2. The first and second treated groups observed impairment in spermatogenic process due to a reduction in the testosterone level.
3. A significant increase (P<0.05) in the mean diameter of seminiferous tubules in the second treated group that reached to (280 ± 6.85) micrometer when compared with control group.
4. Degenerative changes and necrosis were observed in the spermatogenic cells in the first and second treated groups when compared with the control group.

تأثير تراكيز مختلفة من الكحول الأثلي على النبيبات ناقلة المني في الأرانب البالغة . دراسة نسيجية - وظيفية

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كلية التقنيات الصحية والطبية/كوفا

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الخلاصة:

أجريت الدراسة الحالية للكشف عن تأثير تركيزات مختلفة من الكحول الأثيلي في النبيبات المنوية لذكور الأرانب البالغة. استخدمت 18 ذكرًا من الأرانب البالغة، تقسُطت إلى ثلاثة مجموعات بصورة عشوائية. حقن المجموعة الأولى تحت الجلد جرعة الكحول الأثيلي بتركيز (15%) وحجم (4 ملليлитر) لمدة ستة أسابيع. حقن المجموعة الثانية تحت الجلد جرعة الكحول الأثيلي بتركيز (25%) وحجم (8 ملليлитر) لمدة ستة أسابيع. حقن المجموعة الثالثة تحت الجلد بماء مقطر ونفس مقدار جرعة الكحول الأثيلي. ن comparer مع المجموعة السيطرة.

النتائج:
1. ظهرت زيادة في معدل أقطار النبيبات المنوية (7.85 ± 7.8 ميكرومتر) وانخفاض في مستوى هرمون الشحمون الخصوي (18.85 ± 10.52 ميكروغرام/ملليتر) للمجموعة الأولى مقارنة بالمجموعة السيطرة.
2. ظهرت تحذيرات في نشأة النطف نتيجة لانخفاض مستوى هرمون الشحمون الخصوي.
3. ظهرت تغيرات ضمحلية ونخر في الخلايا المنشأة للنطف في المجموعة الأولى والثانية عند مقارنتها مع المجموعة السيطرة.

Introduction:

Most investigation about alcohol effects on male reproduction have been carried out in rats and rabbits, because the rats and rabbits model mimics the human male reproductive system. Work had demonstrated that both acute and chronic alcohol exposure were associated with low levels of hypothalamic luteinizing hormone releasing factor and pituitary luteinizing hormone (LH), in adult (1,2) and pubertal male rat, and further investigations had suggested that alcohol inhibits testosterone secretion by the testes as well (3). Low level testosterone in adult men have been concerned with a different of medical problems involving accelerated osteoporosis, decrease muscle and prostate function, changed in immune function, anemia and reduce in reproductive abilities(4,5,6,7,8). It is well known that chronic alcohol abuse produces sexual dysfunction and impairs sperm production in both human and mammals(9). Both acute and chronic alcohol exposure induce cell necrosis as well as apoptosis, and oxidative stress plays essential in both processes (10). Therefore, the aim of this study was to determine the effect of different concentration of ethyl alcohol on the seminiferous tubules in the adult male rabbits.

Material and methods:

Eighteen mature male rabbits was divided randomly into three groups, six animals for each group. The first treated group was injected S/C (four milliliters) of 15% ethyl alcohol. The second treated group was injected S/C (eight milliliters) of 25% ethyl alcohol for period extended six week, as one dose every 48 hours. The third group was control group injected subcutaneously (S/C) with distil water for period six week at same doses of first and second treated groups. The testis were carefully dissected from scrotum, the histological specimens of both testis (left and right) were fixed in 10% formalin and dehydrated in serial graduation of ethyl alcohol (70%, 80%, 90%, 100%) and then the tissue specimens were clarified in xylol and embedded in wax paraffin and the
tissue blocks were cut serially at six micrometers thickness, after that the histological sections were de – waxed and stained with Hematoxylin and Eosin stain(11), for general histological purposes and histological changes due to the effect of ethyl alcohol in different doses. Morphometric measurements by using ocular micrometer to evaluate the diameter of seminiferous tubules, and hormone assay to measure the testosterone level by using ELIZA in treated and control groups.

**Results and Discussion**

Testes of adult rabbits consist of seminiferous tubules, each seminiferous tubule composed of spermatogonia resting on the basement membrane, primary and secondary spermatocytes spermatids and spermatooza, as well as sertoli cells which extend from basement membrane into lumen of tubule, and their processes that supports spermatogenic cells (Figure 1), among the seminiferous tubules there are Leydig’s cells that produce testosterone. The present study was revealed an increase in mean diameter of seminiferous tubules (250.75± 7.8) micrometer, there was a significant difference at (P < 0.05) in the seminiferous tubules diameter of the first treated group when compared with control group (209.42±6.8)(table 1) micrometer. This findings were corresponding with other studies(12,13), they mentioned, the effect of ethanol in the treated mice for period (four–eight) weeks which led to decline in the seminiferous tubules diameter. Also our results were appeared degenerative alteration in the some spermatogenic cells, and necrotic changes in some primary spermatocytes, spermatids, and spermatozoa (figure 2). No significant differences at (p < 0.05) in testosterone level in the first treated group (18.85±10.52)µg/millimeter when compared with control group (23.65±5.44) µg/ml (table 2). These observations were similar to previous studies(14,15) where they recorded decrease in the testosterone level and impairment in the spermatogenic process as well as reduce in the normal percent of spermatozoa and sertoli cells damage. The second treated group was showed histo-pathological changes which involved deattachment of spermatogonia from basement membrane, necrosis and degenerative change. That occur in some spermatogenic cells such as primary spermatocytes, spermatids, spermatozoa, and sertoli cells (figure 3). From another hand some Leydig’s cells cytoplasm was observed degenerative changes which led to decline in the testosterone level, that reached to (14.68 ± 2.56)µg/ml when compared with control group (19.54±1.5)µg/ml (table 2).

The mean diameter of seminiferous tubules in the second treated group was injected (eight)ml,(25%) ethyl alcohol for period six weeks, mean diameter of seminiferous tubule was men that, the rabbit pancreas is located reached to (280± 6.83) micrometer when compared with control group (204±3.84) micrometer (table 1). These investigations were agreement with previous workers (16,17) they reported, the ethanol that inhibit the synthesis process of steroid compounds in the testis and led to infertility. Our findings in the histological changes aspects were similar with recent study was carried out by Albadi et al., 2013(18). They study the affect of (25%) ethanol on the male rats for period ranged (four–eight) weeks, which led to histological changes that represented by necrosis and degeneration in the spermatogenic cells, as well as damage in the testicular tissue.

| Table 1: Mean diameter (SD±) of seminiferous tubules in the treated rabbits With different concentrations of ethyl alcohol and control. Measurement by micrometer. |
|-------------------------------------------------|-------------------------------------------------|------------------------------------------------|
| parameters                                      | Mean diameter of Seminiferous tubules SD±      | Statically analysis                              |
| 1 Treated group inject(4)ml with Ethyl alcohol (15%) | 250.75 ± 7.8 a*                                 | Significant a at P < 0.05                         |

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More significant A at P < 0.05

Treated group inject (8)ml with Ethyl alcohol (25%)
280 ± 6.83 A**

Control group
209.42 ± 6.8
204 ± 3.84

SD ± standard deviation
Small letter less significant.
Capital letter high significant.

Table 2: Testosterone assessment in the rabbits treated groups injected S/C
With Different concentrations of ethyl alcohol, and control group. Measurement by µg/ml (microgram/milliliter) mean – SD ±

<table>
<thead>
<tr>
<th>parameters</th>
<th>Mean ± SD Level of testosterone</th>
<th>Statical analysis</th>
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<tbody>
<tr>
<td>1 Treated group injected (4) ml S/C with ethyl alcohol (15%)</td>
<td>18.85 ± 10.52 a*</td>
<td>Significant a at P &lt; 0.05</td>
</tr>
<tr>
<td>2 Treated group injected (8)ml S/C with ethyl alcohol (25%)</td>
<td>14.68 ± 2.56 A**</td>
<td>More significant A at P &lt; 0.05</td>
</tr>
<tr>
<td>3 Control group</td>
<td>23.65 ± 5.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>19.54 ± 1.5</td>
<td></td>
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</tbody>
</table>

Small letter less significant
Capital letter more significant.

Figure 1: Rabbit seminiferous tubule (control group) consist of spermatogenic cells and Sertoli cells, spermatogonia are rest on the basement membrane, which Composed of collagen and elastic fibers that surround by myoid cells Hematoxlin – Eosin stain. 250 X.
Figure 2: Rabbit seminiferous tubules treated with (15%) ethyl alcohol were appeared Degenerative and necrosis changes (small arrow) in some spermatogonia, Primary spermatocytes, spermatids, spermatozoa and sertoli cells. Hematoxylin And Eosin. 450X.

Figure 3: show the rabbit seminiferous tubules were administrated with (25%) ethyl Alcohol, damage in the testicular tissue which included degenerative Alteration, necrosis (small arrows) in the spermatogenic cells and haemorrhage In the interstitial tissue (long arrow). Hematoxylin and Eosin. 450X.
Reference:


patterns with biochemical measures.

