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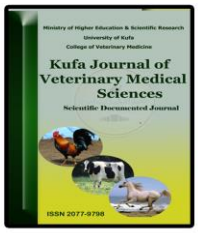

The effect of different concentration of Ethyl alcohol on the seminiferous tubules of the mature rabbits.Histo-physiological study

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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 the eighteen mature male rabbits were divided randomly into three groups ,each group have six animals first and second treated group were injected subcutaneously (S/C) four milliliter ethyl alcohol (15%), eight milliliter ethyl alcohol (25%) respectively for period (six) weeks as one dose every 48 hours, The third group was the control which treated with distil water at the same doses of treated groups . The result showed :-

1.First group was revealed increase in mean diameter of seminiferous tubules (250.75 ± 7.8) micrometer, and decrease in testosterone level (18.85 ± 10.52) microgram/milliter. as compared with the control group(distil water).

2. The first and second treated groups were observed impairment in spermatogenic process due to reduction of the testosterone level.

3. 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 occurs in the spermatogenic cells in the first and second treated groups when compared with control group.

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

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الخلاصة:

أجريت الدراسة الحالية للتقصي عن تأثير تراكيز مختلفة من الكحول الأثيلي في النبيبات المنوية لذكور الأرانب البالغة. اذ استعملت ثمانية عشر من ذكور الأرانب البالغة, قسمت إلى ثلاثة مجاميع بصورة عشوائية. حقنت مجموعة المعاملة الأولى تحت الجلد جرعة من الكحول الأثيلي وبمقدار (4) مليليتر وبتركيز (15%).حقنت مجموعة المعاملة الثانية (ثمانية)مليليتر كحول أثيلي بتركيز (25%) تحت الجلد ولمدة امتدت الى ست أسابيع, بواقع جرعة واحدة كل (48)ساعة. حقنت مجموعة السيطرة تحت الجلد بماء مقطر وبنفس مقدار جرعة الكحول الأثيلي لمجموعة المعاملة الأولى والثانية وكانت النتائج:

1. لوحظت زيادة في معدل أقطار نبيبات ناقلة المني (7.8 ± 250.75)مايكروميتر, ونقصان في مستوى هرمون الشحمون الخصوي (10.52 ± 18.85) مايكروغرام/مليليتر لمجموعة المعاملة الأولى مقارنة مع مجموعة السيطرة.
2. لوحظ على مجموعة المعاملة الأولى والثانية, عطل في عملية نشأة النطف نتيجة لانخفاض مستوى هرمون الشحمون الخصوي.
3. زيادة معنوية وبمستوى احتمالية ($P<0.05$) في معدل أقطار النبيبات الناقلة للمني وبقيمة إحصائية لمجموعة المعاملة الثانية والتي وصلت الى(6.83 ± 280)مايكروميتر.
4. حدثت تغيرات اضمحلالية وتنخر في الخلايا المنشئة للنطف في مجموعة المعاملة الأولى والثانية عند مقارنتها مع مجموعة 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) milliliter (15%) ethyl alcohol, The second treated group was injected S/C (eight)milliliter (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. morphometeric 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 spermatosytes ,spermatids and spermatozoa, 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 diameters. Also our results were appeared degenerative alteration in the some spermatogenic cells, and necrotic changes in some primary spermatocytes, spermatids and sperms(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 I 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 Albadri *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 $P < 0.05$

2	Treated group inject (8)ml with Ethyl alcohol (25%)	280 ± 6.83 A**	More significant A at P < 0.05
3	Control group	209.42 ± 6.8 2o4 ± 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 ±

	parameters	Mean ± SD Level of testosteron	Statical analysis
1	Treated group injected (4) ml S/C with ethyl alcohol (15%)	18.85 ± 10.52 a*	Significant a at P < 0.05
2	Treated group injected (8)ml S/C with ethyl alcohol (25%)	14.68 ± 2.56 A**	More significant A at P < 0.05
3	Control group	23.65 ± 5.44 19.54 ± 1.5	

Small letter less significant
Capital letter more significant.

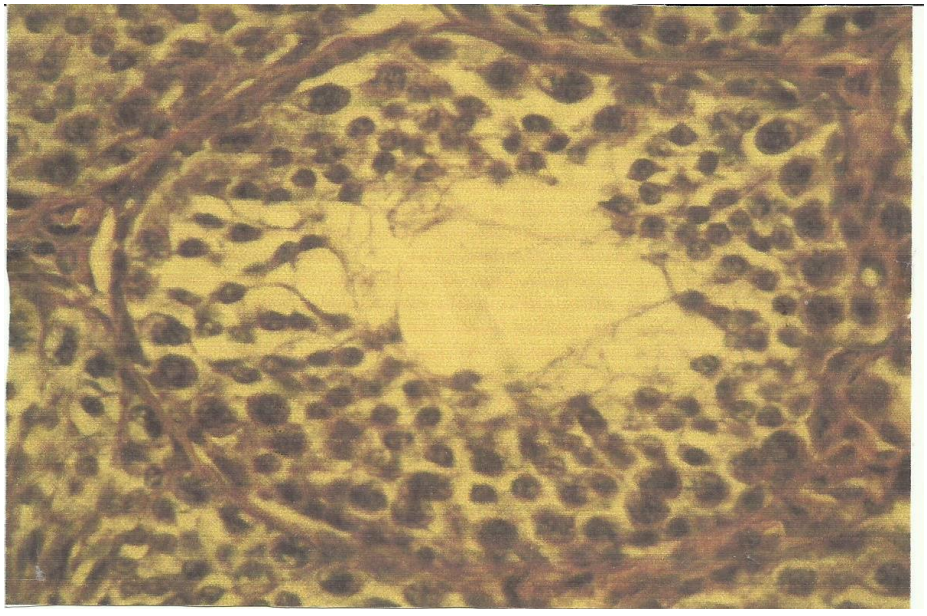

Figure 1: Rabbit seminiferous tubule (control group) consist of spermatogenic cells and Sertoli cells, spermatogonia are rest on the basement membrane, which Composed of collagen and elastic fibers that surround by myoid cells Hematoxlin – Eosin stain. 250 X.

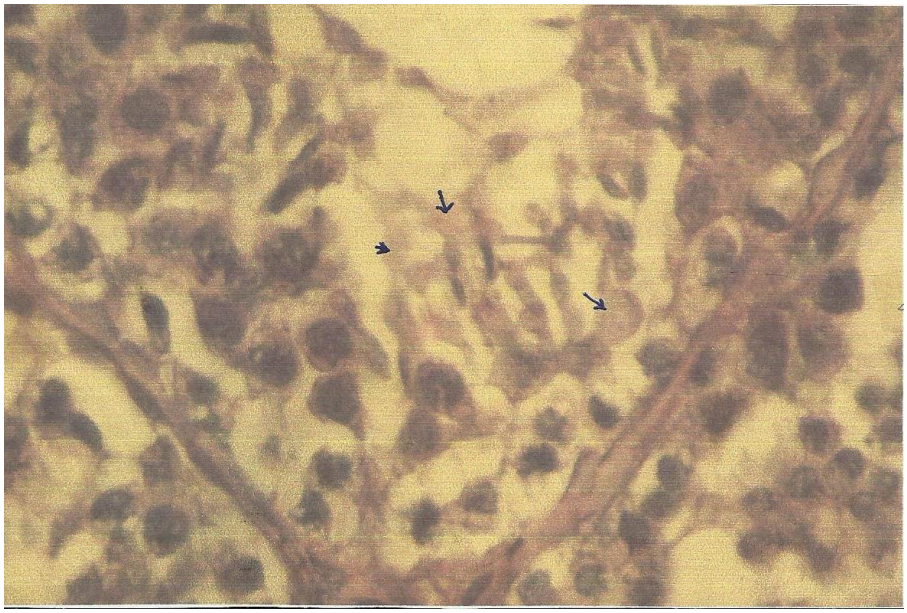

Figure 2: Rabbit seminiferous tubules treated with (15%) ethyl alcohol were appeared Degenerative and necrosis changes (small arrow) in some spermatogonia, Primary spermatocytes, spermatids, spermatozoa and sertoli cells.Hematoxylin And Eosin. 450X.

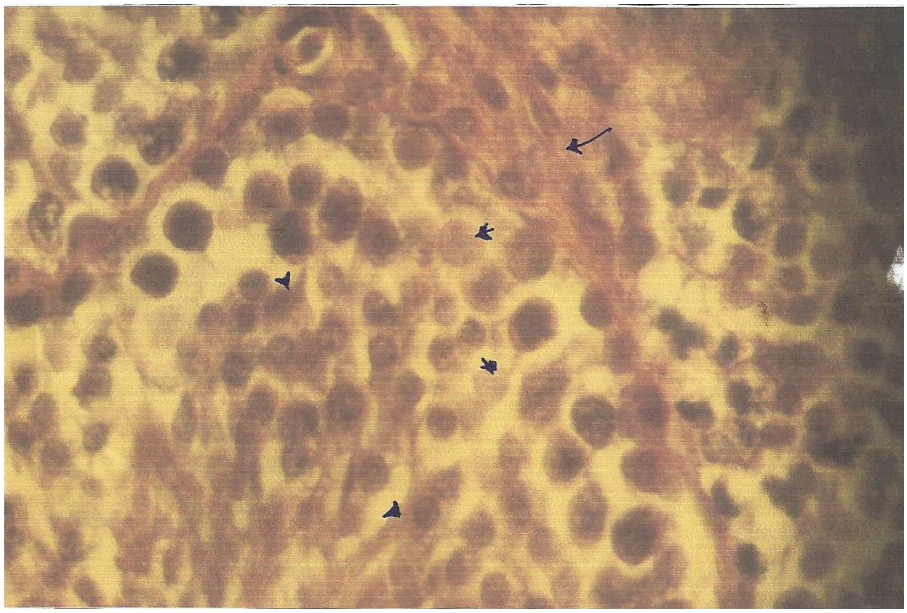

Figure 3: show the rabbit seminiferous tubules were adminstrated with (25%) ethyl Alcohol , damage in the testicular tissue which included degenerative Alteration, necrosis (small arrows) in the spermatogenic cells and haemorrahge In the interstitial tissue (long arrow).Hematoxylin and Eosin.450X.

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