นิพนธ์ต้นฉบับ • Original Article

พิษกึ่งเฉียบพลันของเอธานอลต่อการทำงานของระบบสืบพันธุ์ของหนูเพศผู้

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Subacute Toxicity of Ethanol on the Function of Male Rat Reproductive Tract

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หลักการและเหตุผล: เอธานอลเป็นปัจจัยหนึ่งที่ทำให้ระบบสืบพันธุ์ทำงานผิดปกติ ข้อมูลส่วนใหญ่ได้จากการศึกษาผลของเอธานอลในขนาดสูงแบบเฉียบพลันและแบบเรื้อรัง อย่างไรก็ตาม ความรู้เกี่ยวกับพิษกึ่งเฉียบพลันเมื่อได้รับเอธานอลซ้ำ ๆ กันนั้นยังมีน้อยมาก

วัตถุประสงค์: เพื่อศึกษาเกี่ยวกับพิษกึ่งเฉียบพลันของเอธานอลต่อระบบสืบพันธุ์เพศชายและกลไกการออกฤทธิ์

วิธีการศึกษา: หนูพันธุ์ Sprague Dawley เพศผู้จะถูกแบ่งเป็นกลุ่มควบคุมและกลุ่มทดลอง หนูกลุ่มควบคุมจะได้รับ 0.9% NSS และกลุ่มทดลองจะได้รับเอธานอลขนาด 0.5, 1, 2, 5 และ 10 กรัมต่อน้ำหนักตัวหนึ่งกิโลกรัมวันละครั้งโดยการฉีดเข้าใต้ผิวหนังเป็นเวลา 5 วัน จากนั้นจะถูกนำมาศึกษาดูการเปลี่ยนแปลงต่อไปนี้; น้ำหนักและการเปลี่ยนแปลงทางจุลกายวิภาคของอัณฑะ, ปริมาณ, การเคลื่อนไหว และความอยู่รอดของอสุจิ, ปริมาณ lipid peroxidation และระดับ serum testosterone

ผลการศึกษา: การได้รับเอธานอลซ้ำ ๆ กันเป็นเวลา 5 วันตั้งแต่ 2 กรัมต่อน้ำหนักตัวหนึ่งกิโลกรัมจะลดปริมาณและการเคลื่อนไหวของอสุจิ การเปลี่ยนแปลงของตัวแปรดังกล่าวไม่ขึ้นกับขนาดของเอธานอล นอกจากนี้ยังทำให้การสร้างอสุจิหยุดในระยะ spermatocyte เอธานอลมีผลเพิ่ม lipid peroxidation ในอัณฑะแต่ไม่ทำให้ระดับ serum testosterone เปลี่ยนแปลง

สรุป: เอธานอลมีผลทำลายโครงสร้างที่เกี่ยวข้องกับการสร้างและการเคลื่อนไหวของอสุจิ กลไกการเปลี่ยนแปลงส่วนหนึ่งน่าจะเกิดจากการเพิ่ม lipid peroxidation ในอัณฑะ การได้รับเอธานอลขนาดปานกลางซ้ำๆ กันแม้ไม่นานก็ทำให้การทำงานของระบบสืบพันธุ์เสื่อมลงได้

Background: Ethanol had been recognized to be a factor inducing male reproductive dysfunction. Most of the information concentrate on the acute single large dose and chronic effect of ethanol. To date, little is known concerning subacute toxicity of repetitive doses of ethanol.

Objectives: To study subacute toxicity of ethanol on the male reproductive system and determine the possible mechanism (s).

Method: Male Sprague - Dawley rats were divided into 2 groups; control and experimental groups. The control group received 0.9 % NSS while the experimental groups received ethanol at various doses; 0.5, 1, 2, 5 and 10 g/kg BW. once daily for 5 days via subcutaneous route. Then they were determined weight and histology of testes, amount, motility and viability of sperms, levels of lipid peroxidation and serum testosterone.

Results: Ethanol treatment at doses of 2, 5 and 10 g/ kg BW decreased sperm amount and sperm motility significantly. However, the effect of ethanol did not show a dose dependent manner. The spermatogenic arrest also occured at the spermatocyte level. Ethanol also increased the level of lipid peroxidation in testes but it did not produce a significant change in serum testosterone level.

Conclusions: Ethanol increased the level of lipid peroxidation in testes. This process may be partly responsible for the damage of structures involved in spermatogenesis and motility of sperm. The present study showed that repetitive doses of ethanol even at moderate concentration was also able to impair reproductive function.

Key words: ethanol, sperm amount, sperm motility, spermatogenic arrest

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Introduction

The effect of ethanol on various aspects of reproductive physiology are well documented. Chronic ethanol abuse was reported to induce amenorrhea and infertility in woman (1-3) and hypogonadism, feminization, sexual impotence in man (2, 4-6). Clinical symptoms of ethanol induced reproductive dysfunction in male also included testicular atrophy and gynaecomastia (7). In addition, it also altered reproductive hormone homeostasis (2, 4-6). Prolactin level was elevated (7, 8) while testosterone levels were either reduced (9-13) or unaffected (14-18). Short - term alcohol administration (less than 4 hours duration) did not alter testosterone concentration but longer administration (days to weeks) resulted in a sustained fall in this hormone level (19).

To date, most studies of ethanol induced infertility have been conducted both clinically and laboratory animals. However, most of these investigations have focused on the acute especially single large dose and chronic effects of ethanol, few data are available concerning subacute toxicity of ethanol. Therefore, the present study was conducted to evaluate several indices of reproductive tract in rats that have been exposed to subacute toxicity of ethanol and the possible mechanism underlied these changes.

Materials and methods

Animals:

Young adult male Sprague - Dawley rats (age 8 week olds; 180-200 g) obtained from Animal Center, Faculty of Medicine, Khon Kaen University, were used as experimental model. They were housed 5 per cage, maintained in 10:14; light: dark cycle and given access to food and water ad libitum.

Experimental design:

The animals were divided into ethanol treated and control groups. The ethanol treated group was treated with ethanol at various doses 0.5, 1, 2, 5 and 10 g/kg BW. once daily via subcutaneous injection for 5 days while control group was treated with normal saline in the same pattern. All injections were performed between 7.30-8.30 am. After the last injection, they were killed by cervical dislocation. The testes were removed, weighed and determined amount of sperms, sperm motility, sperm viability and morphologic alteration of testes. The blood was drawn from abdominal aorta for the determination of serum testosterone and corticosterone.

Determination of sperm amount, sperm motility and sperm viability:

Testes, epididymis and vas deferens were dissected and removed the connective tissue and pads. The epididymis was scored longitudinally with blade then the cauda epididymis and vas deferens were squeezed with pairs of forceps and transfered the sperm suspension into Eppendorf tube containing 1 ml of KRB - HEPES (Krebs Ringer Bicarbonate solution with HEPES, pH 7.4) that kept at 37 °C. The sperm suspension was washed with 0.5 ml KRB-HEPES containing 0.6% BSA and centrifuged at 500 g or 1500 rpm at 30 °C for 10 minutes. Then the sperm pellet was resuspended with 1 ml of KRB-HEPE-BSA (Krebs Ringer Bicarbonate solution with HEPES and 0.6% BSA, pH 7.4) and used for the determination of sperm amount, sperm motility and sperm viability. The sperm amount and sperm motility were determined using haemocytometer under light microscope at 10 times magnification. The sperm motility was counted as percent of motility sperms. In the determination of sperm amount, the sampling was random, fields were determined in a predetermined sequence in the four corners center of the grid squares.

The sperm suspension was stained with trypan blue in PBS (phosphate buffer saline) pH 7.4 and studied under light microscope at 40 times magnification in order to determine the sperm viability.

Morphometric analysis:

The testes were isolated and fixed with Bouin's fixative. After fixation, the testes were sectioned and stained with hematoxylin and eosin according to method of Clark et al, 1973. Testicular morphology was studied under light microscope at 40 times magnification.

Determination of lipid peroxidation:

Testes were promptly excised after decapitation, weighed and chilled in ice - cold 0.9% NaCl. They were homogenized in 4 ml of 1.15% KCl using a glass Potter - Elevhjem homogenizer. Then they were determined lipid peroxide level according to method of Ohkawa et al (1979).

Protein determination:

Testicular homogenate was determined protein concentration by caolorimetric method of Lowry et al (1951).

Determination of serum testosterone level:

The level of testosterone in serum obtained from abdominal aorta was determined by radioimmunoassay using TESTO- CT2, an available commercial radio-

immunoassay kit (Cis Bio International, France) according to method of Goldzieger et al. (1976). All samples were measured in a single assay. The detection limit was 0.1 nmol/l.

Results

Daily administration of ethanol at various doses; 0.5, 1, 2, 5 and 10 g/kg BW subcutaneously for 5 days did not produce significant changes in both the viability of sperms (figure 1) and weight of testes (figure 2). The weights of left and right testes also did not show significant difference. However, the amount of the testicular sperms and the motility of sperms were decreased significantly (figure 3 and 4). The significant reduction of the amount of sperms was firstly observed after ethanol treatment at dose of 2 g/kg BW. The reduction of sperm amount showed a maximum effect at dose of 5 g/kg BW and no further reduction upon increasing dose. The increase doses (5 and 10 g/kg BW) did not show significant difference in amount of sperms from that of dose 2 g/kg BW.

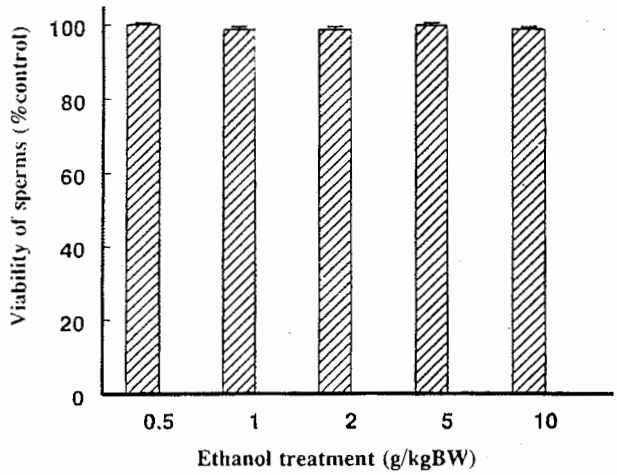

Figure 1 The effect of repetitive doses of ethanol on viability of sperms (% control)

After ethanol treatment at dose of 1 g/kg BW the motility of sperm decreased minimally, though it was statistical significance (figure 4). The increase doses (5 and 10 g/kg BW) did not show any further decrease in sperm motility from that of dose 2 g/kg BW. According to these changes, we can conclude that the subacute effects of ethanol to reduce sperm amount and motility at this range of doses was not depend on dose of ethanol.

The testicular morphology also showed that ethanol treatment at dose of 2 g/kg BW. reduced the

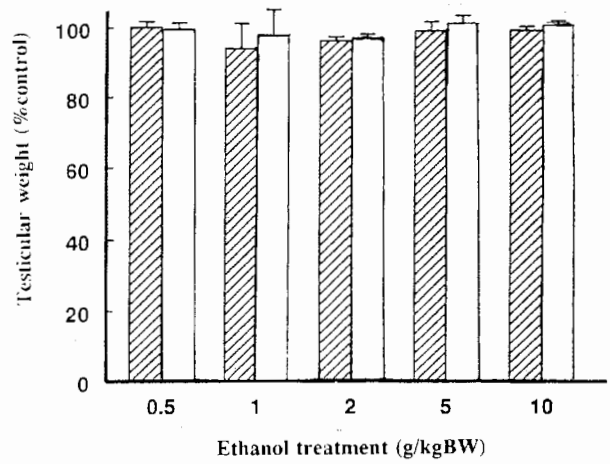

Figure 2 The effect of repetitive doses of ethanol on testicular weight (% control) ▨ left testis, □ right testis

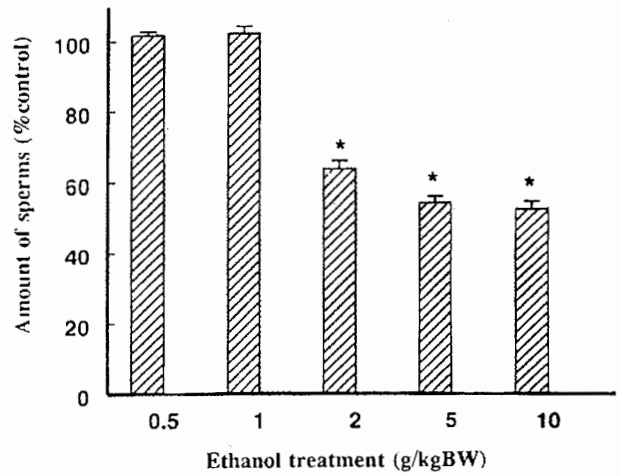

Figure 3 The effect of repertitive doses of ethanol on amount of sperms (% control), * p-value<0.001

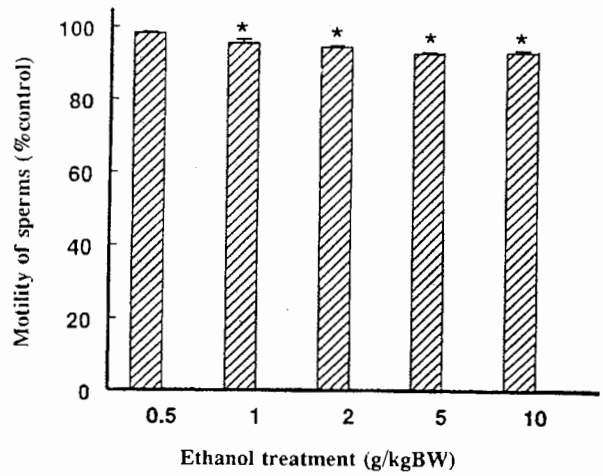

Figure 4 The effect of repetitive doses of ethanol on motility of sperms (% control), *p-value<0.001

spermatocyte layers and amount of spermatids also decreased (figure 5). The level of malondialdehyde (MDA), a product of lipid peroxidation process, in the testes also increased significantly after ethanol treatment at this dose (figure 6).

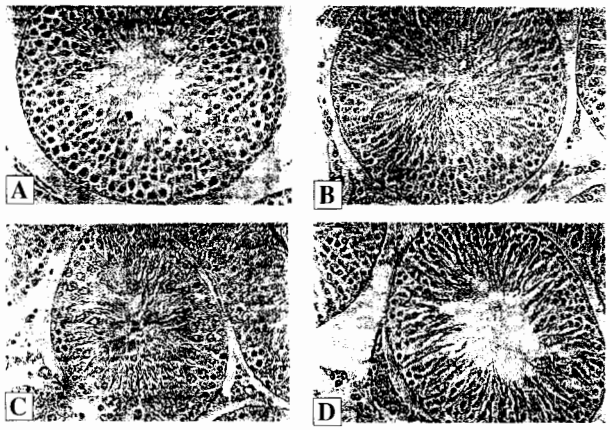

Figure 5 The effect of repetitive doses of ethanol on testicular morphology; control, A: 2 g/kgBW, B: 5 g/kgBW, C and 10 g/kgBW, D

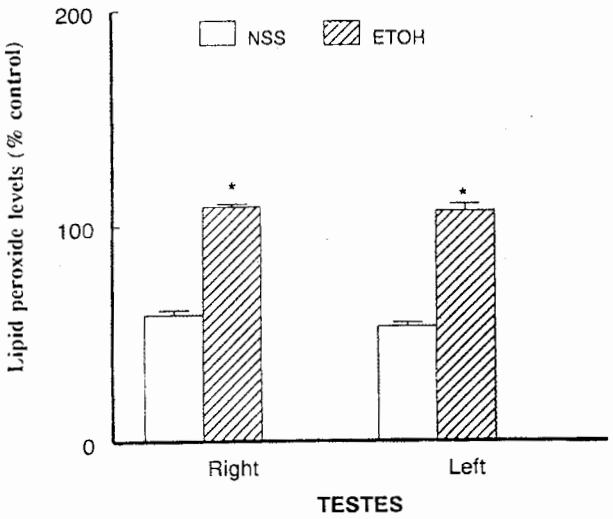

Figure 6 The effect of repetitive doese of 2 g/kgBW ethanol on lipid peroxide levels (% control) in right and left testes, *p-value<0.05

In this experiment we also determined the effect of ethanol on testosterone level but it did not show significant difference from that of control group (data have not been shown).

Discussion

Few studies have been directed toward the evaluation of subacute toxicity of repetitive doses of ethanol on male reproductive system. The present study utilized an animal model describing the adverse effect of subacute toxicity of ethanol. In agreement with previous studies, ethanol treatment reduced the amount of sperms significantly (19) . This study showed that there are many possible mechanisms to reduce the amount of sperms. One possible cause is due to the toxic effect of ethanol to interrupt the complex process of germ cell differentiation leading to the formation of spermatozoa or spermatogenic arrest (20) . The spermatogenic arrest should occur at different levels. However, this study showed that ethanol induced spermatogenic arrest at spermatocyte level. There are many possible factors which may contribute important roles in this process such as the reduction in the levels of follicle stimulating hormone (FSH), testosterone, seminiferous growth factors especially transforming growth factor β, insulin liked growth factor I (IGF I) and the level of vitamin A (20).

FSH and testosterone have been recognized for a long time to initiate and regulate the spermatogenesis (21-22). In this experiment ethanol failed to disturb the level of testosterone so synthetic processes of this hormone should not be the principal target of ethanol in this case . The previous studies showed that acute ethanol treatment usually decreased testosterone level but the testosterone level in chronic ethanol treatment had been reported both decrease and unaffected (7, 9, 18, 23-25) . The discrepancy may be due to the adaptation of rats either via the increased metabolism of ethanol or its active metabolites with tissue or via decreased sensitivity of testicular cell producing testosterone to ethanol is unknown (26). The role of FSH on the toxicity of ethanol in this case is still unknown because we do not measure its level. Ethanol may possibly reduce FSH level and result in the reduced spermatogenesis (27).

Seminiferous growth factors such as TGF β and IGF I were postulated to involve the regulation of testicular spermatogenesis (28, 29). The concentration of IGF I was highly correlated with the amount of sperms (28). The reduction in plasma IGF I level was reported to reduce in low sperm count man (29). TGF β was also reported to involve in the regulation of steroid including testosterone metabolism. It acted indirectly as a differentiation factor by inhibiting growth (20). Ethanol may possibly reduced the level of the two seminiferous growth factors mentioned above. However, the precise action is still required further investigation.

Vitamin A deficiency was also induced spermatogenic arrest at the preleptotene stage of spermatogenesis (20). The conversion of vitamin A to bioactive

retinol in testes was inhibited by ethanol so the reduction in active retinol induced by ethanol may be one factor contributing role in the spermatogenic arrest induction.

In addition to the effects mentioned earlier, reactive oxygen free radical also played important role in physiology and pathology of sperm (30). This compound was reported to have both positive and negative effects on sperm functions. Low concentrations of the compound were reported to be necessary for the sperm capacitation and hyperactivation while high concentrations of this compound were reported to inhibit mitochondria and initiate chain reaction of lipid peroxidation process and result in membrane injury.

The result from this study showed that ethanol increased the level of lipid peroxidation in testes. This was correspondence with the previous studies. Ethanol was reported to increase free radical levels (31-34). The increased free radicals induced by ethanol in turn increased the lipid peroxidation and resulted in the damage of epididymis including Sertoli cell which in turn resulted in the decrease in spermatogenesis.

Ethanol also decreased protein synthesis (35) and this included androgen binding protein (ABP) which contributed significant role in creating high androgen concentration in the vicinity of certain meiotic germ cells (20). This condition was also essential for spermatogenesis so the effect of ethanol may probably occur partly via this mechanism.

Ethanol treatment also deteriorated the motility of sperms significantly. The probable mechanisms may be due to 1) the toxic effect of ethanol on energy production as mentioned earlier 2) the interference in the function of microtubules (36) either by the disturbance in the polymerization process or by the damage of this structure induced by ethannol 3) The reduction in the level of IGF I . (37, 38). However, the precise action is still required further investigation.

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