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Alcohol-induced histopathology and reproductive hormone imbalance in rats.

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ABSTRACT

The present investigation aim to study the effects of alcohol on reproductive hormones and gonads in rats. Method: 25% ethanol at a dose of 0.015ml/g body weight was given intraperitoneally for six weeks, at an interval of four days to adult male and female Sprague-Dawley rats. At the end of the treatment period, the treated animals were sacrificed at the end of the treatment, and the testes and ovaries were collected for histopatholigical analysis, as well as haematological and seven analysis. Results: Alcohol caused a significant ($p<0.05$) decrease in the level of male and female reproductive hormones: testosterone, estrogen and progesterone in exposed rats, as compared to the controls as well as the litter size and litter weight. The quality of the semen was adversely affected, so also the quality of the histology of the gonads. Conclusion: Alcohol produced infertility in treated rats by adversely affecting the gonads and reproductive hormones.

Key words: Alcohol, ovaries, testes, testosterone, progesterone, estrogen.

INTRODUCTION

Alcohol is a drug, and all drugs can be toxic if abused or misused[17]. The effects of alcohol as with any other drug vary in individuals. It depends on the amount taken, how quickly the alcohol is consumed and whether the alcohol is consumed with other drugs[12]. The effects of alcohol also depend on factors such as age, weight, sex and general health status[3]. All these effects are as a result of the alcohol present in the blood stream, known as the Blood Alcohol Concentration (BAC).

Alcohol has been known to have number of deleterious effects on the body. Alcohol can dissolve in cell membranes and as a result, alter the functions of many cells[5]. Hipkiss *et al* (1998) also discovered that ethanol has affinity for fat and water, based on its hydrogen bonds and alkyl groups. It can thus alter the fluidity of the membranes of the body cells. This can in turn, change constituents such as proteins of the cell membrane to function and interact properly. These modifications affect cellular functions and as a result, have the ability to affect almost all tissues, organs and systems in the body.

Chronic use of alcohol abuse is the singular, most frequent cause of illness and death from liver disease[4]. Alcohol is directly toxic to the epithelial lining of the gastrointestinal tract, leading to esophagitis, gastritis and ulcers. Alcohol has direct toxic effects on the cardiac muscle. Long term use of alcohol increases heart disease[1] as alcohol consumption can cause degeneration of the myocardium, congestive heart failure and arrhymias. Acute alcohol intake affects the release of hormones from the hypothalamus and pituitary[8]. Alcohol can thus, impair the regulation of blood sugar levels[9]. It inhibits the secretion of luteinising hormone, thus producing lower circulating testosterone levels in males[7] and in females, lower levels of estradiol and progesterone[6]. Reduced levels of testosterone in males result in fertility disturbance due to low sperm count and impaired motility[19]. In females, alcohol intake causes ovarian failure, as well as irregularities in the sexual cycle.

The aim of the present study is to investigate alcohol mediated infertility as it relates to changes in reproductive hormones and reproductive organs.

MATERIALS AND METHODS

Animals

Female and male adult rats of the Sprague-Dawley breed weighing an average of 134.0g to 184.3g and age between 3 – 4 months were used in this study. The rats were purchased from the animal house of the College of Medicine, Ambrose Alli University, Ekpoma, Edo State. The rats were housed in polycarbonate cages, and fed ad libitum on rat chow (Pfizer). The rats also had adequate supply of drinking water. They were left to acclimatise for two weeks before the commencement of the study. Only healthy animals were used. A total of twenty rats were used.

EXPERIMENTAL

The study included a male and female experiment. The test rats were given 25% ethanol intraperitoneally at a dose 0.015ml/g body weight. The rats were left together to mate. Pregnancy in the rats was confirmed by the presence of spermatozoa in vaginal plug got from the female rats.

Semen analysis

Semen collection

The caudal epididymis was exposed and cut into several small pieces, about 1cm long. These pieces were placed in a specimen bottle containing 1ml of physiological saline. The saline was homogenised to enable the spermatozoa swim. The specimen bottles and their contents were incubated at 37^0C for fifteen minutes. This was done in other to enable the sperm become motile from the caudal epididymis.

Determination of Sperm Motility

The classification by Linder *et al* was used in the assessment for sperm motility. In each assessment, four fields were scanned to accommodate one hundred successive spermatozoa which were classified yielding a percentage of each motility.

A drop of well mixed liquefied semen was placed on a clean, glass slide and was viewed under a light microscope, using a 40x objective lens. The above procedure was repeated twice for both the control specimen, and the treated specimen. A mean value was determined in each case.

Determination of Sperm Density

The sperm density was assessed by the method of Wyrobeck and Bruce (1975). Semen was diluted to one in twenty (1 in 20) by filling a calibrated test tube with well mixed liquefied semen up to the 1ml mark. Next, sodium bicarbonate-formalin diluting fluid was added up to the 20ml mark and mixed very well. With the aid of a Pasteur pipette, the counting chamber of an improved Neuber counting chamber was filled with the diluted semen. A period of three to five minutes was allowed to elapse in order to allow the spermatozoa to settle with the aid of a light microscope, using a 10x objective lens, the number of spermatozoa in an area of two large squares were counted. The number of spermatozoa in 1ml of fluid was calculated by multiplying the number counted by 100,000.

Histology of gonads

The testes and ovaries were fixed in formalin, dehydrated in ethanol and embedded in molten paraffin wax. The sections were cut at 5µm and stained with haematoxylin and eosin. The sections were viewed under light microscope.

Hormone assay

Serum testosterone, progesterone and oestrogen were assayed from frozen samples by ELISA method using microwell ELISA kit.

Table - 1: Weight of testes of rats used and weight of male rats Mean ± SEM

Rats	Weight of Testes (g)	Weight of Rats (g)
Control	1.08 ± 0.05	184.3 ± 4.29
6-weeks Treatment	2.13 ± 0.74*	162.4 ± 2.26*

* P< 0.05, compared with the mean control values

Statistical analysis

Data were expressed in means ± SEM. Student's 't' test was used for statistical comparison. P<0.05 was considered significant.

RESULTS

The mean weight of the testes of the rats in the 6-weeks treated group showed values significantly higher than the means control weights of testes. A histological section of the testes in the control group shows seminiferous tubules that have normal basement membrane. The germinal epithelium is intact. The lumen is packed with maturing and mature spermatozoa. This is indicated by the lumen being densely stained. The interstitial spaces are prominent. The testes from the 6-weeks group showed disruption of the basement membrane, as well as disruption of the germinal epithelium. The interstitial spaces are not as distinct as in the control. In the case of ovary, the control ovary showed normal graafian follicles while the treated ovaries showed shrunken graafian follicles. The testosterone, oestrogen and progesterone

Table -2: Testosterone, estrogen and progesterone values in control and alcohol treated rats. Mean ± SEM

Rats	Testosterone (nmol/l)	Estrogen (pg/ml)	Progesterone (ng/ml)
Control	63.4 ± 1.76	30.4 ± 0.63	1.26 ± 0.10
6-Weeks	21.5 ± 1.36*	22.2 ± 1.76*	0.84 ± 0.17*

*P<0.05, compared with the mean control values.

Table - 3: Percentage motile sperms, percentage dead sperms, mean sperm density. Mean ± SEM

Semen analysis	Control rats	6 Weeks treatment
% motile sperms	62 ± 4.24	24 ± 4.36*
% dead sperms	38 ± 4.55	76 ± 1.14*
Sperm density (10^6/ml)	208 ± 13.10	186 ± 2.55*

*P<0.05, compared with the mean control values.

Table - 4: Litter size and litter weight in control and alcohol treated rats. Mean ± SEM.

Group of rats	Litter size (male)	Litter size (female)	Litter Weight(g) Male	Litter Weight(g) Female
Control	8 ± 4	8 ± 4	9.6 ± 4	9.9 ± 4
6-weeks Treatment	5 ± 4*	5 ± 4*	5.8 ± 4*	4.5 ± 4*

*P<0.05, compared with the mean control values.

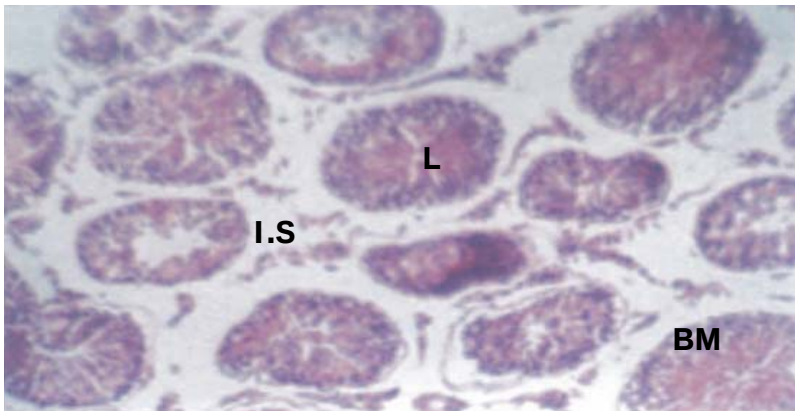

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Fig. - 1: Histology of control testes

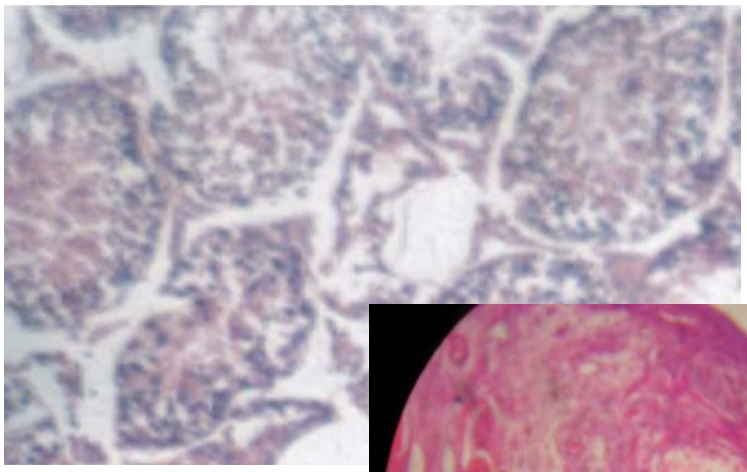
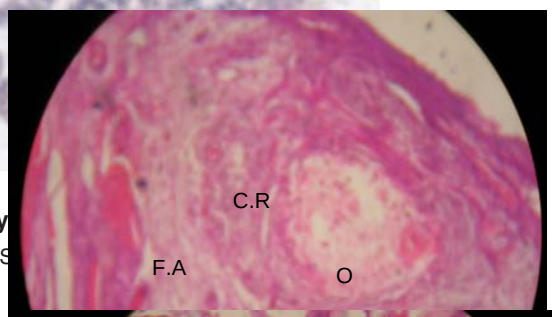


Fig. - 2: Histology

B.M = Basement Membrane; I.S



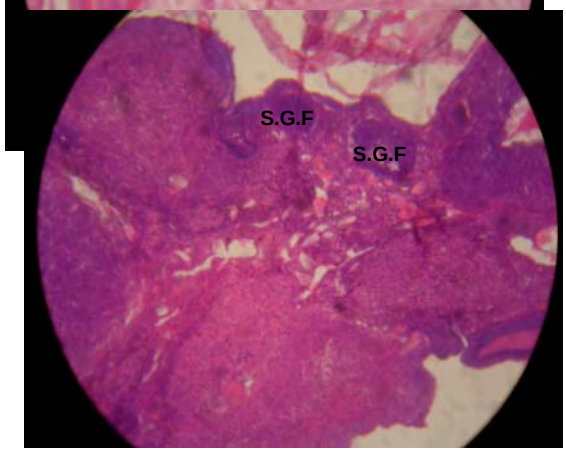


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Fig. - 3: Histology of control ovary

C.R = Corona Radiata; F.A = Follicular Antrum; O = Oocyte

Fig. - 4: Histology of treated ovary

S.G.F = Shrunken Graafian Follicle

values in the alcohol treated rats were reduced. The percentage motile sperms and the mean sperm density in treated rats reduced while the percentage dead sperms in the treated rats increased. The litter size and litter weight in the treated rats also reduced.

DISCUSSION

The mean weight of the tests of the rats in the test group was significantly ($p < 0.05$) greater than that of the control (Table1). In the histological section of the treated rats, the lumen of some of the seminiferous tubules appears clear (Fig1). This is an indication of a reduction in sperm activity. The seminiferous tubules are composed of somatic cells (myoid and sertoli cells) and germ cells (spermatogonia, spermatocyes and spermatids)[5]. reported an alteration in the sertoli cell function of the seminiferous tubules, with a resultant derangement of the germ cell – sertoli cell configuration of the germinal epithelium of the seminiferous tubules[5].

Also reported alcohol to be a "barrier – breaker" as it results in a loss of blood – testes barrier integrity. This could account for the disruption in the germinal epithelium and basement membrane (Fig. -1) seen in the treated tests[11] also suggested that spermatogenesis is disrupted at the spermiogenic stage, probably due to the derangement of the germ cell configuration. The result is a reduction in sperm activity as indicated by the clear lumen of the seminiferous tubules of the testes of the treated male rats (Fig1). The interstitial spaces of the testes of the treated rats are not as distinct as in the control, an indication of inflammation of the testes known as orchitis.

The histology of the treated ovaries showed shrunken graafian follicles (Fig 2). Alcohol is very toxic to the gonads, and is capable of causing irregular sexual cycles, and sexual cycles without ovulation. This is as a result of its toxic effect on the graafian follicles, thereby causing the graafian follicles to shrink. It causes a reduction in the growth of the ovarian follicles[16].

Alcohol also adversely affects the testosterone, estrogen and progesterone levels of the treated rats as seen in (Table 2). In males, leutinizing hormone and follicle stimulating hormone stimulates the synthesis and release of oestrogen and progesterone by the granulosa cells, and corpus luteum in the ovaries. Ethanol is known to be a strong leydig cell toxin[7]. It interferes with testosterone production by leydig cells and hence, the reduction in testosterone production[17]. This is consistent with the findings of this study as seen in Table 2. Ethanol blocks the last stage in the biosynthetic pathway of testosterone that is, the conversion of androstendione to testosterone. In females, there is a decrease in plasma leutinising hormone level. This decrease is as a result of alcohol inhibiting the synthesis and release of leutinising hormone releasing hormone (LHRH). The resultant effect is a decrease in the synthesis and release of estrogen and progesterone[15]. These finding are consistent with the reduced estrogen and progesterone values in Table 2.

Sperm count and motility are indices of spermatogenesis and sperm maturation respectively. An assessment of these parameters provides evidence on the effects of ethanol on male fertility and fecundity. (Table 3) showed that the sperm count, as well as the motility of the treated male rats was significantly less than those of the control rats. Ethanol inhibits 5a – reductase in the liver[4]. Inhibition of this enzyme in the epididymis has been shown to reduce the fertilizing capacity of the spermatozoa. However, the mechanism is unknown[2]. Alcohol also may interfere with normal sperm structure and movement by inhibiting the metabolism of vitamin A, which is essential for sperm development[13].

The above resulted in a significant decrease in fertility, which is manifested by the reduction in litter size and litter weight seen in Table 4. Also, the shrunken effect of ethanol on the graafian follicles resulted in a decrease in the litter size recorded in the female treated rats (Table 4).

Generally, it is pertinent to conclude that alcohol could induce infertility in exposed subjects.

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