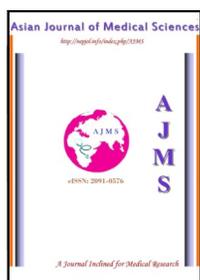


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Deleterious Effects of Ethanol on Hematological Parameters and Fertility in Albino Rats

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Abstract

Objective: Hematological disorders including anaemia are prevalent in alcoholics. The study was an attempt to find out the toxic effects of ethanol on blood values and semen parameters of albino rats.

Material & Methods: 15 male albino rats with body weight (bwt) of 190 - 220 gm were used for the 2-phase study. 25% ethanol was administered via oral cannula to a group of 5 male rats each at daily dose of 0.6ml/200gm bwt respectively for 3 days during phase I. Phase II was a recovery study involving 5 male rats exposed to dose regimen as in phase I, and sacrificed after 3-day withdrawal of treatment. The control group of 5 male rats was given sterile water ad-libitum. Each animal was weighed before sacrifice to obtain difference in bwt relative to its basal value. Blood samples were collected by cardio-puncture from the rats for hematology and serum testosterone at the end of each phase. Semen parameters were determined and compared with controls.

Results: Ethanol caused significant reduction ($P < 0.05$) in the hematological profile as well as in the serum testosterone and semen parameters of the animals. Discontinuation of the drug use however showed gradual recovery of the depressed indices of the semen, serum testosterone and blood parameters.

Conclusion: The ethanol could induce reversible changes in hematological profiles and semen parameters of rats, and by extension man. Hence, the study supported the use of alcohol with caution especially in infertile men and those prone to anemic tendencies.

Key Words: Albino rats; ethanol; semen parameters; hematology

1. Introduction

The male contribution to infertility among couples worldwide has been estimated to be about 33%.¹ In Nigeria, the male partners' contribution to sub fertility is estimated to be about 54% based on semen analysis alone.¹

Estimate of couples who desire but fail to have children range from 10 to 12%.² Male reproductive toxins are encountered in many ways; industrially and environmentally, therapeutically and self administered as recreational drugs.³ Despite the above, little attention is placed on male infertility in developing countries because of the widely erroneous belief that infertility is a female

problem.⁴

In the past, studies have shown no specific trend in serum hormone profiles of infertile males.⁵ While some workers reported normal serum follicular stimulating hormone (FSH) and luteinizing hormone (LH) levels, others have reported increased levels of these gonadotropins and a decreased level of testosterone in infertile males compared with fertile ones.^{5,6}

Many studies have been undertaken to determine the temporal relationship between alcohol and fertility.^{3, 7-9}

The aim of this study was to determine the effects of ethanol on sperm parameters (sperm motility, sperm count and sperm viability) and serum testosterone level, as well as on hematological parameters in male albino rats and to extrapolate the findings to human beings

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hence assist in solving the problem of infertility attributed to alcoholism in man.

2. Material and Methods

2.1. Animals

15 male albino rats of the Wistar strain (190-220 gm) obtained from the Animal house of Department of Physiology, University of Ibadan, Nigeria were used for the study. The animals were fed with standard rat cubes (Ladokun Feeds Nig. Ltd.) and water ad libitum. They were housed five animals per cage in the animal house of Department of Physiology, Olabisi Onabanjo University, Ikenne at room temperature under photo-period controlled environment where they were acclimatized for a period of seven days.

2.2. Experimental procedure

The study was divided into two phases involving the use of drug and sampling in the first phase; as well as drug administration, recovery period followed by sample collection in the second phase. Absolute ethanol was obtained from Dr. Ademowo OG of University College Hospital, University of Ibadan, Ibadan, Nigeria and was diluted to 25% ethanol. The drug was administered orally to the rats daily for 3 days.

Phase 1 entails the use of five male rats that were treated with 0.6ml/200gm/body weight each of the 25% ethanol for 3 days.

Phase 2 was a recovery study involving five male rats that were each given 0.6ml/200gm body weight of 25% ethanol for three days and allowed to recover from the treatment for another three days.

There was a group of five male rats given sterile water throughout the study, and these serve as the control (C) in both phases. The animals were sacrificed in the morning 24.00 hours after each phase.

2.3. Analytical procedure

The rats were weighed prior to treatment and at the end of each phase to obtain differential weight gains (if any) relative to basal values. The animals were anaesthetized with 25% urethane administered at a dosage of 0.6ml per 100g body weight.

Determination of Hematological parameters: Blood sample was collected via cardio-puncture for analyses. Hematology was done according to standard methods.¹⁰

Determination of sperm motility: A simple classification system proposed by the World Health Organization, which provides the best possible assessment of sperm motility,

was used.¹¹

Determination of sperm count: The new improved Naubauers counting chamber was used in the determination of sperm count. The semen was extracted from cauda epididymis of the rats.¹² Drops of semen were placed in the chamber and a cover slip was applied. This was placed under light microscope and spermatozoa counted on each of the five squares.

Determination of serum testosterone: Serum obtained from the blood collected via cardiac puncture was used to measure the level of testosterone using the Enzyme Immuno Assay (EIA) technique as previously described.¹²

2.4. Statistics

All calculations were done using the SPSS-V11 statistical software package 13 for analysis of the data. The data were presented as Means \pm Standard deviation (SD), and statistical analyses carried out using the Student's t-test and ANOVA. Differences were considered to be of statistical significance at an error probability of less than 0.05 ($P < 0.05$).

3. Results

Effect of ethanol on body weight: There was no significant change in body weights of ethanol treated rats when compared with the controls (data not shown). This trend was also observed in the recovery group.

Effect of ethanol on hematological parameters: There was significant reduction in all the hematological parameters measured after the administration of 0.6ml/200gm body weight of 25% ethanol (table 1). Drug withdrawal resulted in gradual restoration of the hematological values.

Table 1: Effects of ethanol on hematological parameters

Groups	PCV (%)	Hb (x 10g/L)	WBC (10^9 /L)	RBC (10^{12} /L)
Control	57.00 \pm 1.22	20.00 \pm 0.63	3.0 \pm 0.6	7.85 \pm 0.86
Test	50.00 \pm 1.00 [*]	16.35 \pm 0.58 [*]	1.14 \pm 0.73 [*]	4.75 \pm 0.37 [*]
Recovery	53.33 \pm 1.22 [*]	18.42 \pm 0.65 [*]	2.04 \pm 0.6 [*]	5.92 \pm 0.93 [*]

*Values are significantly lower at $p < 0.05$

Effect of ethanol on sperm parameters (sperm counts, viability and motility): Administration of 0.6ml/200gm body weight of 25% ethanol significantly reduced ($p < 0.05$) the progressive sperm motility, sperm count and sperm viability when compared with the controls (Table 2). Drug withdrawal resulted in gradual restoration of sperm parameters (Table 2).

Table 2: Effects of ethanol on semen parameters

Groups	Sperm motility (%)	Sperm count (10 ⁶ /mL)	Sperm viability (%)
Control	92.00 ± 1.22	96.60 ± 0.60	94.00 ± 1.05
Test	45.00 ± 2.00*	59.00 ± 1.18*	55.55 ± 0.15*
Recovery	77.00 ± 1.25*	80.00 ± 1.06*	72.50 ± 1.22*

*Values are significantly lower at p < 0.05

Effect of ethanol on serum testosterone level: Serum testosterone levels of ethanol treated rats significantly reduced (p<0.05) when compared to the controls. However there was an appreciable increase in serum testosterone levels of rats in the recovery group. (Table 3).

Table-3: Effects of ethanol on serum testosterone level

Groups	n mol/L
Control	2.00 ± 0.05
Test	0.08 ± 0.04*
Recovery	1.35 ± 0.03*

*Values are significantly lower at p < 0.05

4. Discussion

The present study demonstrated that ethanol had an adverse effect on hematological parameters and spermatogenesis. This investigation revealed that administration of ethanol to rats significantly reduced (p<0.05) the blood profile of the animals. The non significant change in body weights of the ethanol treated rats may be because of the short duration of ethanol administration.

The study also indicated that ethanol significantly reduced (p<0.05) the sperm parameters (sperm motility, sperm count and sperm viability) of the rats. This interference in spermatogenesis caused reduction in sperm density and this is in agreement with work of Dare et al.³ The study also suggested that ethanol has inhibitory effect on sperm motility. The deleterious effect of ethanol may be because it is a barrier breaker.^{14,19}

Ethanol may acts at several subcellular sites to reduce fertility and one of such sites could be at the point of testosterone production by Leydig cells hence reduction in its production as seen in table-3. This reduced production is in agreement with works of Robert et al, and Mendelson et al.^{15, 16, 19}

Ethanol at levels commonly seen in the blood of chronic alcohol ingesting men had been shown to inhibit the activity of 17 alpha-hydroxyprogesterone aldolase (which is the hormone that forms dehydrotestosterone from 17 alpha-hydro progesterone in a concentration dependent manner).^{17,19,20}

The present study indicated that ethanol impaired spermatogenesis in male rats hence consumption of alcohol should be discouraged in man because it is capable of reducing male reproductive capacity. Ethanol also induced anemia hence people prone to anemic tendencies should abstain from any form of alcohol.

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