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ORIGINAL ARTICLE

Impact of alcohol on male reproductive hormones, oxidative stress and semen parameters in Sprague–Dawley rats

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KEYWORDS

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Oxidative stress;
Luteinizing hormone;
Alcohol;
Infertility

Abstract *Objective:* To investigate the impact of alcohol on the reproductive hormones, oxidative stress and semen parameters.

Design: This is an experimental animal study.

Materials and methods: Adult male Sprague–Dawley rats weighing between 170 and 200 g received 30% v/v ethanol at a concentration of 2 g/kg body weight for a period of 4 weeks, 8 weeks and 16 weeks. Parameters tested include: testosterone, estrogen, luteinizing hormone (LH), follicle stimulating hormone (FSH), gonadotropin hormone releasing hormone (GnRH), malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), sperm count and sperm motility.

Results: After the 4 week study, there was a significant decrease ($p < 0.05$) in estrogen levels, sperm count and sperm motility. Testosterone levels also decreased while MDA levels increased significantly. After the 8 week study, testosterone levels decreased significantly, LH and FSH also decreased but GnRH levels increased significantly. MDA and SOD levels increased significantly but sperm count and sperm motility decreased significantly compared to controls. After the 16 week study, testosterone and GnRH levels decreased significantly compared to controls. MDA levels increased significantly while sperm count and motility decreased significantly.

Conclusion: Acute and chronic administration of alcohol depletes testosterone levels, increases oxidative stress and decreases semen parameters. This impact of alcohol on testosterone levels is mediated by direct testicular toxicity and by altering the hormone feedback system in the pituitary gland and the hypothalamus.

1. Introduction

Infertility is the inability of a couple to conceive after a year of regular unprotected sexual intercourse (1). Treatment and management of infertility has become of global concern as

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the need to have children is of great priority in families. It is reported that about 15% of couples of reproductive age are infertile and about 50% of these cases are male related (2,3).

The problem of infertility is that it produces stress, a couple failing to achieve the expected goal of reproduction, experiences the feelings of frustration and disappointment. These psychological stresses will lead to generalized increased oxidative stress levels (3). A strong body of evidence suggests that small amounts of reactive oxygen species (ROS) are necessary for spermatozoa to acquire fertilizing capabilities however, it has been reported that stress has a negative effect on sperm concentration, motility and morphology (4,5). It has also been reported that about 25–80% of males with infertility record high levels of ROS (6–8).

Chia et al. (9), reports that about 42% of men with infertility cases consume alcohol. This presents alcohol as a potent precursor of male factor infertility. Moreover, researchers have reported an association between alcohol consumption and decreased testosterone levels (10,11). Testosterone is a hormone produced by the Leydig cells in the interstitium of the testes (12). Its synthesis is triggered by a negative feedback to the anterior pituitary gland which releases luteinizing hormone which in turn is regulated by gonadotropin releasing hormone (GnRH) of the hypothalamus (13). There must be a balanced interplay between these hormones from the hypothalamus, pituitary gland and the testis for a successful germ cell development (14). Decrease in sperm density, eventually leading to azoospermia, has been found to be associated with raised follicle stimulating hormone (FSH), LH and normal or low testosterone level (15).

It is still uncertain if alcohol reduces semen parameters by acting directly on testicular tissue or via the hypothalamic–pituitary–gonadal (HPG) axis. This study is targeted at distinguishing the path of action by which alcohol reduces semen parameters.

2. Materials and methods

2.1. Chemicals

Thirty percent (30% v/v) of ethanol prepared from absolute ethanol (99.86%) with substance identification number 1170 manufactured by James Burrough (F.A.D. Ltd. UK) was used at a concentration of 2 g/kg body weight.

2.2. Animal experiments

Thirty-six adult male Sprague–Dawley rats weighing between 170 and 200 g were procured from the Nigerian Institute of Medical Research (NIMR) located in Yaba, Lagos. The animals were housed in the animal holdings of the Laboratory Animal Center, College of Medicine, University of Lagos, in well ventilated plastic cages with 12:12 light–dark cycles at 27 ± 1 °C. All procedures guiding the use of the animals were in accordance with the standard international guidelines on the use of animals for research. Approval for the study was obtained from the Departmental Ethics Committee and also granted by the Experimental Ethics Committee on Animals Use of College of Medicine, University of Lagos, Nigeria. The animals for the experiments were randomly divided into 3 groups to represent the 3 phase duration. The first phase lasted 4 weeks, the second, lasted 8 weeks while the third lasted 16 weeks. In each phase the animals were divided into 2 subgroups: A and B. The mode of administration for all groups was through gastric gavage. Subgroup A represented control that received distilled water while subgroup B represented animals that received 30% v/v of alcohol. At the end of each of the phases, the rats were sacrificed after which blood was collected for biochemical analysis and the testes harvested for histological studies.

2.3. Parameters investigated

2.3.1. Reproductive hormones

The blood specimens from the animals were collected via ocular puncture of orbital vein into plain sample bottles, centrifuged at 3000*g* and assayed for testosterone, LH, FSH, Estrogen and GnRH levels using the enzyme-linked immunosorbent assay kits.

2.3.2. Biochemical parameters estimation

2.3.2.1. Testicular malondialdehyde. Malondialdehyde (MDA) levels in the testicular tissue were measured by the method developed by Ohkawa et al. (16). This is based on the measurement of thiobarbituric acid malondialdehyde absorbance. The tissue MDA levels were expressed as *nmol/ml* tissue.

2.3.2.2. Superoxide Dismutase (SOD) activity. Superoxide Dismutase activity was determined by modifications of methods described by Beauchamp and Fridovich (17) and Sun and Zigma (18) using its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm. The reaction mixture (3 ml) containing 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of the homogenate and 0.03 ml of epinephrine in 0.005 N HCl was used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min.

2.3.3. Semen parameters

2.3.3.1. Sperm count. The sperm count was determined using the Neubauer improved hemocytometer. Epididymal fluid ratio of 1:20 was prepared by adding 0.1 ml of fluid to 1.9 ml of water. The dilution was mixed thoroughly and both sides of the counting chamber were scored and the average taken. Spermatozoa within five of the red blood cell squares including those which lie across the outermost lines at the top and right sides were counted, while those at the bottom and left sides were left out. The number of spermatozoa counted was expressed in millions/ml (19).

2.3.3.2. Sperm motility. The cauda epididymis of the rats were incised and a drop of epididymal fluid delivered onto a glass slide, covered by a 22 × 22 mm cover slip and examined under the light microscope at a magnification of ×100 while evaluating different fields (20). For the purpose of this study, motility was classified as either motile or non-motile (21). After assessing different microscopic fields, the relative percentage of motile sperm was estimated and reported to the nearest 5% using the subjective determination of motility (19).