Middle East Fertility Society Journal (2014) **19**, 221–228

Middle East Fertility Society

Middle East Fertility Society Journal

www.mefsjournal.org
www.sciencedirect.com

ORIGINAL ARTICLE

Alcohol induced testicular damage: Can abstinence equal recovery?

O.O. Dosumu *, **A.A.A. Osinubi, F.I.O. Duru**

Department of Anatomy, Faculty of Basic Medical Sciences, College of Medicine, University of Lagos, Nigeria

Received 12 April 2013; accepted 28 January 2014
Available online 26 March 2014

KEYWORDS

Alcohol;
Testis;
Oxidative stress;
Abstinence;
Testosterone;
Seminiferous epithelium

Abstract Drinking continues to be a major problem in many parts of the world. Significant effects on testicular morphology and function in animals as well as man have been well described. To further explore the impact of chronic ethanol exposure on the testes, we designed this study specifically to define whether or not there was complete recovery after abstinence by examining reproductive hormones, testicular histomorphometry, testicular antioxidants as well as semen parameters after ethanol exposure.

Sexually mature male Sprague–Dawley rats were randomly divided into control, abstinent and non-abstinent groups. Alcohol was administered orally at 7 ml/kg body weight per day thrice in a week for 2, 4 and 8 weeks. Control animals received an equivalent amount of distilled water. Histological analysis of the seminiferous tubules of the animals in the non-abstinent group showed severe reduction of cells of the spermatogenic series, hypocellularity, tubular atrophy and significant reductions in the tubular diameter and cross-sectional areas ($p < 0.001$). Testicular weight, sperm count and motility, were also significantly reduced ($p < 0.001$) while testicular malondialdehyde (tMDA) levels increased significantly ($p < 0.001$). Hormonal assay showed significant reductions in the levels of testosterone (TT) ($p < 0.05$) while luteinizing hormone (LH) and follicle stimulating hormone (FSH) remained unchanged. In the recovery or abstinent groups (group III), despite weeks of abstinence from alcohol, the groups still demonstrated high levels of tMDA, low sperm count and motility and significantly reduced ($p < 0.001$) testicular diameter and cross sectional area values. However, increased TT levels and non-severe reduction in the seminiferous epithelium observed in these groups showed signs of epithelial regeneration and probable recovery tendencies.

Abbreviations: tMDA, testicular malondialdehyde; tGSH, testicular glutathione; LH, luteinizing hormone; FSH, follicle stimulating hormone; TT, testosterone; ROS, reactive oxygen species; TBARS, thiobarbituric acid-reactive substances.

* Corresponding author. Tel.: +234 803 370 1857.
E-mail address: olufunkedosumu@gmail.com (O.O. Dosumu).

Peer review under responsibility of Middle East Fertility Society.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.mefs.2014.01.003

In conclusion, the present study shows that total alcohol abstinence following chronic ethanol administration failed to reverse completely alcohol-induced testicular damage.

1. Introduction

The consumption of alcohol has long been part of everyday life in many societies and it will continue to be so in the future. However, the World Health Organisation (67) has found that alcohol consumption represents the third largest risk factor for disease burden in high-income countries, behind only smoking and hypertension, both of which are also associated with alcohol misuse.

Ethanol has been reported to be among the most widely abused drug which can suppress reproductive function and sexual behaviour in laboratory animals and humans. Alcohol abuse has been considered as one of the problems associated with poor semen production and sperm quality (1,57). Both chronic and acute consumption of alcohol has been reported to cause fertility disturbances such as low sperm count and motility, reduced serum/plasma testosterone level, testicular atrophy and irregularity in the diameter of the seminiferous tubules in men and laboratory animals (62,38,39,15). In addition, Martinez et al. (39) reported histological abnormalities in testicular tissue of alcoholic animals. These include intense intercellular spaces, irregular diameter of seminiferous tubules and high amount of necrotic cells in the lumen compared with controls. Epididymal sperm motility also decreased in ethanol-treated rats.

In men, low levels of testosterone have been repeatedly associated with both moderate consumption and chronic alcohol abuse (24,49,50,51,52). In addition, serum TT has negatively been associated with the duration of alcohol abuse (38). Forquer et al. (23) have also reported significant reductions in androgen levels following ethanol intoxication and withdrawal in males.

Undoubtedly, ethanol consumption produces a significant decrease in the percentage of motility, concentration (38) and normal morphology in human and animal spermatozoa (4,42). Previous studies have shown that alcohol ingestion followed by herbal treatment modalities showed good recovery tendencies with testicular parameters almost restored to normalcy (15). However, it remains to be determined whether normalcy can be restored within the testicular milieu following prolonged periods of abstinence without treatment. Hence, the present study was carried out to determine the effects of chronic administration of ethanol followed by abstinence on the testes of adult rats.

2. Materials and method

2.1. Chemicals

Thirty percent ethanol (17) prepared from absolute ethanol (99.86% v/v) with substance identification number 1170 manufactured by James Burrough (F.A.D. Ltd. UK) was used for the study.

2.2. Animal experiments

Adult male Sprague–Dawley rats weighing (150–170 g) were used for the study. Animals were procured from the Nigerian Institute of Medical Research (NIMR). The animals were housed in the Anatomy Department Animal Control Room in well ventilated plastic cages with 12:12 light–dark cycle at 27 ± 1 °C. Rats were randomized into nine groups of five animals each. The mode of administration for all groups was through gastric intubation, and animals in the treatment groups received 7 ml/kg body weight of 30% ethanol per day, thrice in a week (17). All animals were largely divided into three categories: I (control), II (abstinent) and III (non-abstinent). All group I rats served as control and received distilled water; group II rats were subdivided into groups a, b, c and received ethanol for 2, 4 and 8 weeks respectively; group III rats were also subdivided into groups a, b, c and fed ethanol for 2, 4 and 8 weeks respectively followed by the same corresponding number of weeks of abstinence. At the end of the treatment period, the rats were sacrificed after which blood and tissues (testes) were collected for the various assays. All experimental protocols followed the guidelines approved by the Ethics Committee of the College of Medicine, University of Lagos, Nigeria.

2.3. Parameters investigated

2.3.1. Semen analysis

The cauda epididymis of the rats was 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 (68). For the purpose of this study, motility was classified as either motile or non-motile/dead (44). 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 (31).

The sperm count was determined using the Neubauer improved haemocytometer. 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 (31).

2.3.2. Biochemical estimations

The lipid peroxidation products were estimated by measuring TBARS and were determined by (43). Nonenzymatic antioxidants such as reduced glutathione and catalase were estimated by Ellman (19) and Sinha (54) respectively.

2.3.3. Hormone determination

The serum levels of TT, FSH and LH were measured using commercially available enzyme-linked immunoassay kit (Diagnostic automation Inc, CA) according to the manufacturer's instructions.