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EFFECT OF ALCOHOL ON SEXUAL DYSFUNCTIONS AND HORMONAL PROFILES IN MALE WISTAR RATS

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ABSTRACT

Alcohol can affect the hypothalamo-pituitary gonadal axis leading to impotence and sterility by various mechanisms of action. Until recently, alcohol induced liver disease was considered to be primarily responsible for sexual dysfunction in alcoholic men. Ethanol impairs testosterone production by being a direct testicular toxin and by interfering with pituitary luteinizing hormone secretion. In chronic alcohol fed rats, testicular atrophy is associated with reduced plasma testosterone levels by reducing the number of LH binding testicular receptors and also by interfering with the enzymes involved in testosterone biosynthesis. Healthy Male Wister rats were selected for this study, exposure of alcohol with 6% alcohol in feeding bottles orally for 90 days, compared to normal – control rats, the parameters were studied are Mounting Index (MI), Total Sexual Behaviour (TSB), Sperm count, Serum Testosterone by RIA methods, histopathology of target organs and Pituitary were observed under special Histochemical staining: AB-BR-OFG method. In this present study Total sexual behavior (TSB) in normal rats showed: 210.0 ± 0.56 compared to alcohol exposed rats: 260.0 ± 0.96, Sperm count in normal rats: 61.33 ± 0.47 and alcohol exposed rats: 55.33 ± 0.30, Serum

testosterone levels in normal rats: 4.62 ± 0.05 and in alcohol exposed rats: 2.44 ± 0.02, histopathological evaluations showed drastic toxic effects in alcohol exposed rats and the control group were showed compact cellular architecture. **Conclusion:** The increased blood acetaldehyde levels leads to impaired testicular functions and behavioral stress leading to sexual dysfunctions with reduced hormonal profiles.

KEYWORDS: Alcohol, Serum testosterone, Sexual dysfunctions, Total Sexual Behavior (TSB), Sperm count, histopathological profiles.

INTRODUCTION

Alcohol exerts profound effects on the reproductive and neuroendocrine axis in the adult male rats of several species.[1,2,3,4] Numerous studies indicate that alcohol exerts at least two effects on the HPG axis, a direct effect on testicular steroidogensis, and a suppression of LHRH and LH, which results in further depressions in LH-dependent testicular steroidogenesis.

Which of these two mechanisms predominate under in vivo conditions in the intact animal or human has not been conclusively resolved, but there seems to be little doubt that alcohol exerts significant acute and chronic effects on reproductive behavior and endocrinology.[5]

Direct toxic effects of alcohol on cardiac and skeletal muscles leading to the nutritional disorder such as fatty cirrhosis of liver have been reported.[6,7] Oxidative stresses may be a potential mechanism of hormetic effects of calorie restriction (CR) on acute ethanol-induced liver injury.[8] Acetaldehyde exposure increased free acrolein levels. An increase of acrolein by acetaldehyde was spermine oxidase (SMO) dependent.[9]

Alcohol is directly toxic to the testes, causing reduced testosterone levels in men as reported in Alcohol Alert, NIAAA.[10] In another report, Testosterone production is depressed by alcohol's toxicity.[11] Chronic alcohol and nicotine administration also lead to changes in the numbers of nAChRs. At least one subtype of nAChR may help protect cells against alcohol-induced neurotoxicity.[12] Since potential mechanisms for alcohol's damage leading to adverse affects all three parts of the hypothalamic-pituitary-gonadal (HPG) axis.[13]

Hormonal reactions associated with alcohol consumption are related to alcohol metabolism, alcohol-related cell damages. Chronic alcohol use in male rats has also shown to affect their reproductive ability and the health of their offspring. Low levels of testosterone (i.e.,

hypogonadism) was reported in adult men associated with a variety of medical problems including accelerated osteoporosis, decreased muscle and prostate function, anemia, altered immune function, and decreased reproductive ability.[14,15,16,17,18]

Significant health problems were reported in those who experience diminished testosterone levels only in adulthood.[19,20] parental alcohol consumption has an adverse effect on their reproductive ability and offspring health.[21,22] Sexual changes observed in chronic alcoholic men are the result of alcohol abuse per se.[23] Ethanol impairs testosterone production and also interferes with pituitary luteinizing hormone secretion.[24]

Alcohol causes testicular damage probably due to toxic effects of its intermediate acetaldehyde. Mechanistic in vitro studies on the testosterone production by isolated testes revealed that ethanol acts at least in part directly on the testes to affect this hormone production.[25] In chronic alcohol fed rats, testicular atrophy is associated with reduced plasma testosterone levels. Alcohol interferes with testosterone synthesis by reducing the number of LH binding testicular receptors[26] and also by interfering with the enzymes involved in testosterone biosynthesis[27,28,29] Alcohol exerts profound effects on the reproductive neuroendocrine axis in the adult male of several species.[30,31,32] Many studies indicate that alcohol exerts a direct effect on testicular steroidogensis and a suppression of LHRH and LH.[33,34]

Therefore, this present study focuses to correlate hormonal changes with sexual behavioral response along with pathological significance of target organs which could be an ideal way to assess possible sexual dysfunctions in the male Wister rats.

MATERIAL AND METHODS

Healthy Male Wister rats were selected from the Approved Breeding Centre in Bangalore and acclimatized to the experimental room of the Department of Zoology for 15 days; all experimental protocols were approved by IAEC for animal experiment. The rats were maintained strictly under Standard GLP lab conditions.

All experimental procedures were carried out for 90 days / chronic exposure for all the animals on day 91st blood was collected for testosterone assay between 12.00 noon and 2.00 pm, followed by behavioral observations with Necropsy, and target organs were collected

including Pituitary & Gonads for Histopathological studies in 10% Buffered Neutral Formalin.

Experimental group classification

Group 1: Control rats (normal), received in addition to standard laboratory diet 10ml/kg body weight of filtered drinking water will be administered once a day orally for 90 days (n=10).

Group 2: Alcohol exposed rats, received in addition to standard laboratory diet, instead of drinking water 6% alcohol in feeding bottles orally for 90 days (n=10).[35]

Mounting Index (MI)

This was done in a specially designed box. Two female rats at proestrus were kept in the box and one male rat was introduced into the box. The rats were identified by picric acid markings. After a 15 min acclimatization period mounting was observed for 45 min and the number of mounts counted.

Total Sexual Behaviour (TSB)

This was also assessed after following 15 min acclimatization in the mounting box with two female rats per male rat. Male sexual behaviour such as genital grooming and sniffing at females was visually monitored and recorded.[36]

Both mount test and TSB were done at the beginning and at the end of the experimental period

Sperm count

Single cauda of the epididymis was excised and punctured. The fluid was collected in an RBC pipette and diluted with phosphate buffered saline (pH 7.1). The sperm count was done on a Neubauer's chamber.[37]

Serum Testosterone

These were assessed at the end of the experimental period in all animals by RIA methods.[38, 39]

Histology/Histopathology

Histological sections of Pituitary under special staining- (Histochemistry) were observed (AB-BR-OFG method): 20 HPF/animal. BR (bromine water), AB (alcian blue), OFG (Orange g-acid fuchsin, light green) method[40] for basophile cells producing LH, FSH and ACTH. In addition sections of target organs i.e., liver, adrenals, testis and pituitaries

were stained with haematoxylin and eosin and their histopathological profiles were recorded.

Before conducting the Study, LD_{50} is evaluated in mice (n=10) according OECD guidelines: the Dose tried were 200 mg / kg to 2000 mg/kg b.wt, while finnays programme statistical significance found out as 1.0 g/kg b.wt (ref: Wiberg: LD_{50} in young 10.6 and old rats 7.06 g / kg respectively).

All the results were subjected to statistical analysis by an unpaired Student's t-test. The minimum level of statistical significance was set at $p<0.05$.

RESULTS

Table No. 1: Total Sexual Behavior

Total Sexual Behaviour	
Male rats	TSB: Genital grooming and sniffing at females
Normal (Control)	210.0 ±0.56*
Experimental (Alcohol exposed)	260.0 ±0.96*
n=10 Mean ± SEM	* P values: * < 0.05

Table No. 2: Mounting Index (MI)

Mounting Index	
Male rats	MI: Experimental male mounting on lordosis two female rats at proestrus
Normal (Control)	8.05 ±0.21*
Experimental (Alcohol exposed)	9.95 ±0.68*
n=10 Mean ± SEM	* P values: * < 0.05

Table No. 3 Sperm Count (10^6 ml $^{-1}$)

Sperm Count	
Male rats	SC : Single cauda of the epididymis
Normal (Control)	61.33 ±0.47*
Experimental (Alcohol exposed)	55.33 ±0.30*
n=10 Mean ± SEM	*P values: * < 0.05

Table No. 4 Testosterone (ng/ml)

Testosterone	
Male rats	Testosterone : male rats
Normal (Control)	4.62 ± 0.05*
Experimental (Alcohol exposed)	2.44 ± 0.02*
n=10 Mean ± SEM	* P values: * < 0.05

Table No. 5 Percentage cell type counts in pituitaries (values are Mean ± SEM)

Cell Types	Staining recognition	Normal rats	Alcohol exposed rats
ACTH	Basophil (S)	0.495* ±1.40	0.715* ±1.26
FSH, LH	Basophil (R)	0.445* ±0.86	0.275* ±1.32

P values: * < 0.05 AB-BR-OFG method (Bancroft and Cook, 1984)[40]

The nervous system (CNS) is susceptible to the deleterious effects of alcohol. Increase in total sexual behaviour and mount test were recorded in the study indicated the aggressive behaviour of chronic alcohol exposed rats.

Total Sexual Behavior (TSB) for control rats: 210.0 ± 0.56 and alcohol exposed rats: 260.0 ± 0.967 showed increased response (table -1). Mounting index (MI) for control and alcohol exposed rats was 8.05 ± 0.215 and 9.95 ± 0.68 respectively, showed increased response (table -2). Sperm count were recorded for the control and alcohol exposed rats 61.33 ± 0.47, 55.33 ± 0.30 respectively. Decreased (table -3)

Serum testosterone was recorded for the control and alcohol exposed rats 4.62 ± 0.05 and 2.44 ± 0.02 respectively (table -4). All the above mentioned parameters in alcohol exposed rats decreased than the normal.

Microscopic observations of pituitaries in chronic alcohol exposed rats showed more Percentage of active ACTH than FSH and LH, i.e: ACTH in control and alcohol exposed rats 0.495 ± 1.40 and 0. 715 ± 1.26 respectively, FSH and LH was control and alcohol exposed rats 0.445 ± 0.86 and 0.275 ± 1.32 respectively (table -5).

Histopathological evaluations of target organs of alcohol exposed group showed several changes like less vascularity, more degenerative hyperplasia and inflammations than normal rats.

Histopathological evaluations of target organs revealed, in Pituitaries: G1: Normal morphology of pars distalis zone with clear structural compactness and mild vacuolations are observed.

G2: Less vascularity and high degree of degenerative vacuolations in pars distalis, in Testis: GI: Majority of seminiferous tubules showed active spermatogenesis with normal morphology. G2: Less active spermatogenesis with dilatation of the luminal spaces, focal and patchy, proliferations of interstitial spaces in seminiferous tubules.

In Adrenals: G1: Both cortex and medulla showed evidence of normal distribution of zones with normal architecture. G2: Cortical and medullary hyperplasia, dilated vascular spaces in the medullary zone, compressed zona reticularis, in Liver: G1: Essentially normal architecture around the central vein with normal morphology of hepatocytes was observed. G2 Hyperemia with distension of sinusoidal spaces and granularity of cytoplasm, hepatocytes with toxic vacuolations of nuclei are evident.

DISCUSSION

The present study revealed the alcohol dysfunctions on the hypothalamo-pituitary - gonadal axis, the chronic alcohol exposure caused altered behaviour and hampering metabolic status of male reproductive system, a reduction in Sperm Counts, serum testosterone production were below normal and pituitary cell type- counts i.e., ACTH will be increased with declined hormonal cell types FSH, LH**.** This is responsible for androgen hormone secretion. Sexual behaviour also affected by alcohol exposures leading to target organ pathological status.

The Histopathological profile of pituitary showed marked degenerative vocuolations in par distalis, in testicular tissue showed atrophic changes and less active spermatogenesis with proliferative inflammations, in adrenals, both cortical and medullary zones showed hyperplasia and compressed zona raticularis, increase of liver distension and compressed zona raticularis, in case of liver distension of sinusoidal spaces with inflammatory changes and hepatocytes with toxic vocuolations of nuclei are evident.

Alcohol (High Dose) significantly proved to cause decreased libido, increased erection, impaired ejaculation and hormonal changes,[41] in most studies plasma testosterone levels are decreased by chronic ethanol treatment, suggesting an effect on the lydig cells. These statements were proved in this study, while correlating Testosterone hormonal levels, the drastic reduction were evident nearly half to that of normal. Microscopic data reveals that when seminiferous tubules of testes showed Lydig cells drastic reduction.

Histochemistry of pituitary gland under special staining showed significant changes in FSH, LH cellular population's decreased in alcohol exposed rats. Recent ultra structural studies have shown intracellular changes in lydig cells subsequent to chronic ethanol administration.[42]

The precise mechanism of ethanol-induced testicular dysfunction remains to be elucidated; however, large doses of ethanol have been shown to reduce the number of LH binding sites of Lydig cell membranes and several enzymatic steps in the biosynthesis of testosterone appear to be inhibited by ethanol and acetaldehyde.[43]

The seminal vesicle/prostate complex in rats is also affected by chronic ethanol treatments[44] noted a diminution in the depth of the epithelial lining of the seminal vesicles and an associated reduction in the overall weight of the complex. The aggressive and altered mode of behaviour is due to chronic intake of alcohol, hangover or psycho-neurotic depression because of long term alcohol exposure further the effects of alcohol leads to hamper toxicity with reduction in sperm counts.[45]

Serum testosterone levels reduced half of the normal, in pituitary cells ACTH production seems to be more with lesser FSH, LH production. Similarly histopathological approach in target organs were also reveal i.e., less vascularity, degenerative changes, inflammatory response were evident than normal.

The effects of ethanol ingestion on sperm monosaccharides and fertility were reported,[46] despite the immediate effects of alcohol on health while under the influence of depression, memory loss, and impaired reactions.[47] Research implies that even moderate alcohol consumption may induce genetic damage and increase the occurrence of physical and mental handicaps in a child due to alcohol's effects on the egg and sperm.[48]

However, studies with Young (i.e., pubertal) male rats indicate that both acute and chronic alcohol Exposure result in profound testosterone suppression accompanied by lower or normal LH and FSH levels.[49] Whereas some studies have reported that the secretion of LHRH is reduced after acute alcohol consumption and other studies have reported no effect,[50] the ability of the male hypothalamus to synthesize this important hormone appears to be unaltered by alcohol at any dose. The degree of reproductive impairment varies with amount of ethanol ingested and the duration ethanol exposure.[51]

Future Investigation in alcohol exposure studies could be focused on mechanisms of alcohol-induced oxidative damage and apoptosis in the testes, the consequences of paternal alcohol exposure for their offspring, and the effects of alcohol use on leptin and male reproduction. In addition, practical prevention of testicular suppression with naltrexone and Nitric oxide inhibition are promising therapeutic interventions.

Alcohol use disorders (AUDs) lead to alterations in central nervous system (CNS) with impaired learning and memory leading to cell proliferation, apoptosis, and DNA-repair in neural stem cells (NSCs), and the expression of p53-signaling genes.[52] Alcohol dependence produces plasticity in these neuropeptide systems, reflecting a recruitment of those systems during the transition to alcohol dependence.[53]

Moderate alcohol consumption may have protective effects on apoptotic cell death after traumatic brain injury. Protective effects of moderate ethanol consumption might be related to inhibition of lysosomal protease release and nitric oxide production.[54] Alcohol consumption during adolescence has long-term sexually dimorphic effects on anxiety behavior and mood disorders.[55]

Ethanol has been known to suppress reproductive activity in laboratory animals and humans through the inhibition of luteinizing hormone (LH) release by reduction of gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus. Suppressed GnRH mRNA levels in the hypothalamus of ethanol-treated rats strongly demonstrated that hypothalamus is the major action site of ethanol on the HPG axis. Decreased serum LH level may affect the steroidogenesis in the testis, through the inhibition of StAR gene expression that induces dysfunctions of reproductive activity.[56]

CONCLUSIONS

Alcohol has a direct toxic effect on testicular tissue as well as an inhibitory effect on the hypothalamic release of LHRH and FSHRH and also cortical hypertrophy leading to ACTH hyper secretions. Testicular atrophy, liver and pituitary toxic vacuolations, cessation of spermatogenesis process with declined lydig and sertoli cells in gonads were clearly observed as a possible hampering aspects in the chronic alcoholic abuse.

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