Original Article ISSN: 0976-5700

Evaluation of male albino rat potentiality via Antioxidant and Steroidogenic enzyme activity in testis under chronic ethanol intoxication

Hari Prasad S[1], Shanmugam B[2], Veera Nagendra Kumar D[3], Shanmugam K.R.,[4] Venkata Subbaiah G[5*], Sathyavelu Reddy K[5]

[1]Department of Zoology, Sathyavedu Government Degree College, Andhra Pradesh, India

[2]Department of Zoology, NCERT, Shillong, Meghalaya, India

[3]Department of Zoology, Government College for Men (A), Kadapa, India

[4]Department of Zoology, Government Degree College, Nellore, India

[5]Division of Molecular Biology, Ethanopharmacology, Department of Zoology, Sri Venkateswara University, A.P. India

Corresponding author: doctorsvenkat@gmail.com

Abstract

In recent days, sexual potential **Antioxidant and Steroidogenic enzyme activity** abnormalities in males have been a major concern worldwide Behind this reason, several factors may be influenced such as smoking, drug abuse, stress and alcohol consumption. This study aimed to investigate the potentiality of male rats under ethanol intoxication on rat testis at different doses. The experimental studies were carried out on 40 male rats which were grouped into five, therefore Normal control (NC) received 0.9% NaCl; the second to fourth groups received orally

20% of ethanol ranging in doses from 2 g/kg body weight, 4 g/kg and 6 g/kg (b.w) respectively for two month period. Antioxidant enzymes, Superoxide dismutase (SOD), catalase (CAT) and steroidogenic enzymes such as 3β, 17 β hydroxysteroid dehydrogenase activities measured. 6 g/kg b.w. The dose of ethanol has shown a highly toxic effect on the antioxidant enzymes and steroidogenic enzyme activity which was significantly decreased. In conclusion, we found that a 6g/kg dose of alcohol showed drastic changes in the testis under chronic exposure and it was also suggested that chronic high doses of alcohol abuse could affect the potentiality of testis that lead to induced infertility.

Keywords

Ethanol, Testis, Antioxidant enzymes, Steroidogenic enzymes, lipid peroxidation

Introduction:

The World Health Organization has found that there are about 2 billion people consume alcoholic beverages Worldwide. Among those 76.3 million with diagnosable alcohol disorders and it was associated with morbidity and mortality (WHO Global Status Report, 2012). Chronic alcohol consumption has been implicated in the induction of oxidative stress as a consequence of the imbalance between pro-oxidants and antioxidants (Pushpakiran *et al.,* 2004). Metabolism of alcohol through the enzymatic system varies depending on the dose and time of alcohol consumption. During chronic high doses of alcohol consumption, the alcohol metabolized mostly via the P450E1 cytochrome system than alco

Hol dehydrogenase forms acetaldehyde a highly reactive toxic substance. This could enhance the xanthine oxidase activity which in turn modulates the free radical generation (Z imatkin & Deitrich, 1997). Reactive oxygen species (ROS) has played a main role in inducing oxidative tissue damage in different organs such as the liver, kidney, heart, brain and testis cause to provoking the disease due to a decline in the antioxidant enzyme activity and increased lipid peroxidation (Mallikarjuna *et al.,* 2010; Shanmugam *et al.,* 2011; Venkata subbaiah *et al.,* 2014; Mallikarjuna *et al.,* 2011; Nordmann *et al.,* 1990).

Chronic alcohol consumption has been reported to suppress reproductive function and sexual behaviour in laboratory animals and humans (Grattagliano et al., 1997, Rosemblumet al., 1989). Mechanistic *in vitro* studies on testis activity under ethanol intoxication confirmed that ethanol affects testosterone hormone production (Badr *et al.,* 1977; Cobb *et al.,* 1978). Chronic ethanol ingestion substantiated that ethanol acts as a primary testicular toxin owing to gonadal dysfunction cropping up in humans (Van Tniel *et al.,* 1975). Testicular dysfunction has been seen under ethanol intoxication which may be due to increased oxidative stress in the testis by alterations in antioxidant enzyme activity and increased lipid peroxidation (Olufunke *et al.,* 2012).

Despite the numerous studies on the effect of alcohol on male reproduction in animals and humans, what exact dose of alcohol ingestion to induce infertility without life-threatening in animals and

humans has not been well investigated yet. In this connection, the present study is intended to investigate the exact dose of alcohol ingestion for testicular dysfunction.

Materials and Method

Procurement of Chemicals:

In the present study, all Analytical Grade chemicals were used (AR) and obtained from the following scientific companies: Sigma (St. Louis, MO, USA), Fisher (Pittsburgh, PA, USA), Merck (Mumbai, India), Qualigens (Mumbai, India).

Care and Maintenance of Experimental Animals:

Wistar strain male albino rats weighing 250 ± 20 g were used for the study. The rats were housed in polypropylene cages (18"x 10" X18") under hygienic conditions with a photoperiod of 12 hours light and 12 hours dark. The rats were fed with standard diet pellets (Hindustan Lever Ltd, Mumbai) and water *ad libitum*. The experiments were carried out in accordance with the guidelines and protocols of the commitment of India (CPCSEA, 2003) and approved by the Institutional Animal Ethical Committee resolution No 10(i)/a/CPCSCA/IAEC/ SVU/KSR-KALA date 15/11/2010.

Experimental groups

Forty male albino rats were used in this study. The rats were randomly divided into four groups, 10 animals in each group. Group-I, received 0.9% NaCl and was considered as normal control (NC), Group II-IV rats received 20% ethanol at various dosages in orderly 2g, 4g and 6g/kg b.w. for a month period.

Isolation of Biological component

After a month period, the animals were sacrificed and testes were isolated and kept at -80^{0}C for further biochemical analysis. Apart from the biochemical assays remaining tissue was used for histopathological examination.

Body weight determination

Animals' body weights were taken weekly once for a month period. Body weights were expressed in grams.

Biochemical assessment

Assessment of antioxidant defense system

SOD activity was assayed in the mitochondrial fraction by the method of Misra and Fridovich (1972) the samples were read at 480 nm for 4 min in a Hitachi U-2000 spectrophotometer. Activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 U per milligram of protein.

CAT activity was determined at room temperature by using the modified version of Aebi (1984) and the samples read at 240 nm for 1 min in a spectrophotometer. The CAT was expressed in µm of hydrogen peroxide degraded /mg protein/min.

Assessment of steroidogenic enzyme activity

3 β-hydroxy steroid dehydrogenase activity was determined in the testicular microsomal fraction according to the method described by Bergmayer (1974). Tissues were homogenized, in 10 % ice-cold Tris HCL buffer (P^H 7.4) and centrifuged at 16000 X g for 10 min at 4^0Ccentrifuge (REMI, C-30). The reaction mixture of 2.0 ml contained 100 µmol of sodium pyrophosphate buffer (P^H 9.0) and 0.5 µ mole cofactor NAD, 0.08 µ moles of the substrate (dehydroepiandrosterone) and 100 µl enzyme sources. The colour absorbance read at 340 nm in 20 s intervals for 3 min.

The activity of 17 β-hydroxyl steroid dehydrogenase was determined in the testicular microsomal fraction according to the method described by Bergmayer (1974). Tissues were homogenized in ice-cold 10%Tris- HCl buffer (P^H 7.4) and centrifuged at 16000 X g for 10 min at 4^o c. The reaction mixture of 2.0 ml contained 100 µ moles of sodium pyrophosphate buffer (P^H 9.0) and 0.5 µ mole cofactor NADPH, 0.08 µ moles of the substrate (androstenedione) and 100 µl enzyme sources. The absorbance read at 340 nm in 20 s intervals for 3 min. The enzyme activities were expressed as µ mole of NAD converted to NADH/mg protein/min. Protein content in the enzyme source was estimated by using the method of Lowry et al (1951), and bovine serum albumin (BSA) was taken as standard.

Histopathological study

For qualitative analysis of testicular histology, after completion of the treatment period, the animals were sacrificed and one testis from each group animal was fixed in 10% of formaldehyde for three days. The fixed testes were embedded in paraffin wax and blocks were prepared. Testicular sections were collected from each testis at 5µm thickness using a rotary microtome and stained with haematoxylin and eosin. Histologic analysis was performed using light microscopy. Microphotographs were prepared under low and high magnification.

Statistical analysis:

Statistical analysis of data was represented as mean ± SD. All the statistical analyses were carried out by SPSS software. Dunnett's multiple comparison test and one-way analysis of variance (ANOVA) were used to assess the differences. P Values <0.05 were considered highly statistically significant.

Results

Effect of alcohol on Antioxidant enzymes activity

SOD activity significantly increased in 2 g (15.806 ± 0.346) and 4 g /kg (11.076 ± 0.157) of alcohol dose when compared to control (9.519 ± 0.762), whereas in 6 g of alcohol dose shown (7.277 ± 0.406) a significant (P<0.05) decrease when compared to control and other alcohol dosages (Fig-1).

CAT activity significantly decreased in all alcohol-treated groups 2 g, 4 g, (0.219 ± 0.013, 0.157 ± 0.007) whereas 6 g/kg of alcohol dose shown prominently decreased (0.112 ± 0.005) when compared to the control group (0.375 ± 0.016) and other alcohol dosages (Fig-2).

Effect of alcohol on Steroidogenic enzymes activity

3 β-hydroxy steroid dehydrogenase (3- βHSD) activity significantly ($P<0.05$) decreased with the increase of alcohol dosage 2 g,4 g, 19.34 ± 4.8 and 15.18 ± 4.9 respectively whereas 6 g/kg of alcohol dose 12.45 ±3.9 prominently decreased when compared to control 23.12 ± 5.3 and other alcohol dosage (Figure-3). Similarly, 17-β hydroxy steroid dehydrogenase (17- β HSD) activity also significantly ($P<0.05$) decreased with the increase of alcohol dosage 2 g and 4 g 14.56 ± 3.9 and 10.23 ± 2.6 respectively whereas 6 g/kg of alcohol dose shown prominently decreased the activity 9.87 ± 2.3 when compared to control 19.14 ± 3.6 and other alcohol dosages. (Figure-4)

Histopathology

Histological changes of the testis were observed in normal control and 2g, 4g, and 6g of ethanol dosage groups. Normal control rats showed (Fig- 4A) normal structure of seminiferous tubules and interstitial connective tissue whereas in 2g ethanolic rats showed (Fig-4B) mild degeneration of interstitial connective tissue and seminiferous tubules, 4g rats showed (Fig- 4C) degeneration of interstitial connective tissue (DICT), vaculation in seminiferous tubules and 6g of ethanolic rats shown (Fig-4E) complete degeneration of seminiferous tubules (CST), Leydig cells (CDL) and spermatogonia (CDS) (Fig-1). Testis damage increased with the increase of ethanol dosage in animals and it caused impotency

Discussion:

The present study revealed that chronic high doses of alcohol abuse insult the potentiality of the testis in rats resulting in downregulation of the antioxidant defence system, and decreased steroidogenic enzyme activity. Furthermore, the present study found that the alterations in testicular structure influenced the testicular activity under chronic alcohol intoxication.

Ethanol-induced oxidative damage inflicted by exceeding ROS is considered an important promoting cell injury despite the cells have been protected by virtue of an intricate antioxidant defence system (Poonam Lodhi *et al.,* 2014). It has been demonstrated that ethanol can alter the defence system and increase tissue damage in the liver and kidney (Shanmugam et al., 2011). In the present study, SOD activity significantly increased in 2g, and 4g of ethanol dosage when compared to the control while 6g of ethanol dose showed decreased SOD activity when compared to the control. Ethanol-induced free radicals could enhance the SOD activity by increasing of transcription of SOD mRNA to overcome the free radical-mediated damage, this may be happening in the testis at 2g,3g, and 4g of ethanol dose (Kaur *et al.,* 2006). In contrast our results from a previous study which demonstrated that 2g of ethanol dose decreased the SOD activity in the liver may be low levels of SOD occur in this tissue unlike the testis (Gu *et al.,* 1996). However, chronic high doses of ethanol (6 g /kg) decreased the SOD activity may be due to increased ROS interaction with the enzyme proteins resulting in the inactivation enzyme (Mallikarjuna *et al.,* 2009).

Catalase enzyme detoxifies the H_2O_2 into water and O_2. Chronic ethanol ingestion could decrease the CAT activity in liver and kidney tissue (Shanmugam *et al.,* 2011). These findings are consistent with other reports which demonstrated that CAT activity decreased in acute ethanol intoxication in

testis with dose and time depending manner (Schlorff *et al.,* 1999). The present findings are also consistent with previous reports that CAT activity significantly decreased with the increase in ethanol dose. This reduced CAT activity may be due to a feedback inhibition or oxidative inactivation of enzyme protein resulting in excess ROS generation materialized during ethanol intoxication (Piegeolet and Corbisier, 1990).

Testosterone hormone is an important steroidogenic hormone for the development of sperms in seminiferous tubules and sexual behaviour in adulthood (Wilson, 2001). Leydig cells of the testis are responsible for the production of testosterone in the presence of two main distinct steroidogenic enzymes such as 3β, 17 β hydroxysteroid dehydrogenase (3β, 17 β HSD) by using co-factors NAD^+ and NADPH. 3β HSD has catalysed the conversion of pregnenolone to progesterone and 17 β HSD converts the progesterone to testosterone (Tang, *et al.,* 1998; Ge *et al.,* 1998). An increase of glucocorticoid and cortisol levels in serum was associated with a decrease the testosterone under chronic stress conditions (Yazawa *et al.,*1990). In our results, 3β, 17 β hydroxysteroid dehydrogenases activity significantly decreased at high doses of ethanol (6g/kg b.w) when compared to other dosages of ethanol and normal control groups which account for reduced testosterone levels and spermatogenesis, this may be due to a high dose of ethanol increase the production of acetaldehydes which are accountable to diminish the NAD+/NADPH ratio and competed with the process involved in testosterone production thereby preventing testosterone production (Ellingboe *et al.,* 1979, Gordon *et al.,* 1980) Cicero and Bell (1980) also reported that alcohol and its metabolite acetaldehyde effects on the conversion of androstenedione to testosterone. Chronic high doses of ethanol may down-regulate the steroidogenic acute regulatory

(StAR) protein expression which contributes to decreased steroidogenic enzyme activity under ethanol-induced oxidative stress conditions (Sugawara *et al.,* 1996; Caron *et al.,* 1997).

Spermatogenesis is commonly assessed by the sperm count since it gives the cumulative result of all stages in sperm production and it is highly associated with fertility (Meistrich *et al.,* 1982). In our study, sperm count significantly decreased with the increase in alcohol dose and it drastically declined at the high dose of 6 g/kg when compared to another dosage. Previous studies in animals and humans have demonstrated that chronic binge alcohol ingestion could disturb the spermatogenic function in males (Pajarinen *et al.,* 1996). Another important study has also reported that chronic alcohol ingestion initiated apoptosis in both spermatogonium and spermatocytes (Zhu *et al.,* 2000; Bamac *et al.,* 2005). These findings supported our results, that chronic binge alcohol consumption decreased sperm production and may damage the spermatogonium resulting in increased lipid peroxidation or apoptosis initiated in the spermatogonium (Ganaraja *et al.,* 2008), another possible mechanism may also appear during chronic binge alcohol ingestion reflected the Sertoli cell functions probably by producing damage to some of the proteins required for sperm cell production that the Sertoli cells provide (Zhu *et al.,* 1997). Furthermore, consequences of chronic high-dose alcohol intoxication may induce a reduction in levels of testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) not only hampers their normal morphological development and maturation of spermatozoa, and it also slows the sperm production by testicular germ cells (Emanuele and Emanuele, 1998).

Studies have examined rat sperm motility as the reproductive endpoint and sperm motility assessments are an integral part of some reproductive toxicity test guidelines (Morrissey *et al.,*

1988; Toth *et al.,* 1991). Ethanol-impaired epididymis sperm motility and fertility in rats may result in amendments in the activity of spermatoglycosidase and reduced testosterone levels (Srikanth *et al.,* 1999). Our results strongly supported previous studies showing that sperm motility significantly decreased with the increase of ethanol dose while it was higher at 6 g/kg ethanol dose. The production of high amounts of reactive oxygen species under chronic alcohol ingestion may reduce sperm motility due to changes in cytoskeleton structure on the axonemals within the sperm tail which are essential for sperm motility. Ethanol may affect mitochondria to produce ATP required for the movement of the flagella of sperm cells (Hoek *et al.,* 2002) Hence, a reduced or impaired mitochondrial function will impede sperm motility as observed in the alcohol-treated group.

Chronic binge alcohol consumption imposed oxidative tissue damage resulting in amendments of antioxidant enzyme activity and increased lipid peroxidation. Similar consequences were observed in our study in histopathological examination of testis at the high dose of (6 g/kg) chronic alcohol ingestion. 6 g of chronic alcohol ingestion showed completely diminished seminiferous tubules structure, Leydig cells, spermatogonium and congestion were observed in the testis, these dramatic changes were initiated at 4 g of ethanol dose and it was higher at 6 g of ethanol dose.

Reference:

(1) World Health Organization, WHO Global Status Report on Alcohol. Assessed 16th November,2012.URL(http://www.who.int/entity/substance-abuse/publication/global-status-report-2004 overview.pdf); (2004).

(2) Pushpakiran G, Mahalakshmi K, Anuradha CV. Taurine restore ethanol-induced depletion of antioxidant tenuative stress in rat tissues. *Amino Acids* (2004) 27(1) : 91-96

(3) Zimatkin SM, and Deitrich RA. Ethanol metabolism in the brain. *Addiction Biology* (1997) 2:387-399.

(4) Malikarjuna K, Shamnmugam KR, Nishanth K, Ming-Chieh W, Chia-Hua K, Sathyavelu Reddy K .Alcohol-induced deterioration in primary antioxidant and glutathione family enzymes reversed by exercise training in the liver of old rats. *Alcohol* (2010) 26 (13):1-7

(5) Shanmugam KR, Mallikarjuna K, Sathyavelu Reddy K. Effect of alcohol on blood glucose and antioxidant enzymes in the liver and kidney of diabetic rats. *Indian J Pharmacol* (2011) 43 (3):333-358.

(6) Mallikarjuna K, Somasekhara Reddy N, Shanmugama KR, Chia-Hua, K and Sathyavelu Reddy K. Protective Effect of Endurance Exercise Training on Brain Antioxidant System in Old Age Rats treated with Alcohol: A Comparison with young age rats. *Adv Med Bio*. (2011) 6: 342-351

(7) Nordman R, Rribiere C, Rouach, H. Ethanol-induced lipid peroxidation oxidative stress in extra hepatic tissues. *Alcohol Alcohol* (1990)25:231-237.

(8) Venkata subbaiah G, Ravi S, Shanmugam B, Sathyavelu Reddy K .Combined In-Vivo and In-Silico Studies Shed Insights into the deterioration of Oxidative Damage with Vitamin E Supplementation in Ethanol- Induced Myocardial Infarction in Rats. *RJPBS* (2014) 5(3): 371-382

(9) GrattaglianoI, Vendemiale G, Errico E, Bolognino AE, ,Lillo F, Salerno M, Altomare E. Chronic ethanol intake induces oxidative alterations in rat testis. *J.Appl.Toxicol* (1997) 17: 307-311.

(10) Rosenblum FR, Gavaler JS, VanThiel, DH. Lipid peroxidation mechanism oral alcohol-induced testicular injury. *FreeRad.Biol.Med*. (1989) 7: 569-577.

(11) Badr FM, Bartke A, Dalterio S, Bulger W. Suppression of testosterone reproduction by ethyl alcohol. Possible mode of action. *Steroids* (1977) 30: 647-655.

(12) Cobb CF, Ennis MF, VanThiel DH, Gavaler JS, Lester R. Acetaldehyde and ethanol are direct testicular toxins. *Surg Forum* (1978) 29:641-644.

(13) Van Tniel DH, Gavaler JS, Lester R, Goodman MD. Alcohol induced testicular atrophy: an experimental model for hypogonadism occurring in chronic alcoholic men. *Gastroenterology* (1975): 69 (2): 326-32

(14) Olufunke O, Dosumu, Oluwole B, Akinola, Edidiong N. Akang. Alcohol-induced testicular oxidative stress and cholesterol homeostasis in rats – The therapeutic potential of virgin coconut oil. Middle East Fertil Soc J (2012) 17: 122–128.

(15) Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem*. (1972) 247:3170–3175.

(16) Aebi H. Catalase in vitro. *Methods. Enzymol*. (1985)105:125–126; 25.

(17) Ohkawa H, Ohishi N, Yagi, K. Assay for lipid peroxides in animals and tissue by thiobarbituric acid reaction. *Anal. Biochem* (1979) 95:351–358

(18) Bergmayer HU. Methods of enzymatic analysis. In: Bergmayer, H.U. (Ed.),Verlag Chemie Weinheim. *Academic Press, New York* (1974) 447–489.

(19) Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* (1951) **193:** 265–75.

(20) Belsey MA, Moghissi KS, Eliasson R, Paulsen CA, Callegos AJ, Prasad MR. Laboratory manual for the examination of human semen and semen-cervical mucus interaction. Singapore: Press concern. (1980). p. 10.

(21) Murgatroyd PR, Van de Ven MLHM, Goldberg GR, Prentice AM. Alcohol and the regulation of energy balance: overnight effects on diet-induced thermo genesis and fuel storage. *Br J Nutr* (1996) 75:33-45.

(22) Sonko BJ, Prentice AM, Murgatroyd PR, Goldberg GR, van de Ven ML, Coward WA. Effect of alcohol on postmeal fat storage. *Am JClinNutr* (1994)59:619-25.

(23) Fan AZ, Russellm M, Stranges S. Association of life time alcohol drinking trajectories with cardio metabolic risk. *J cline Endocrinil Metab* (2008) 93:154-61.

(24) Oesteress S, Hillk KG, Hawkin JD Guo J, Catalano R F and Abbott RD. Adolescent heavy episodic drinking trajectories and healthy young adulthood. *J Stued Alcohol* (2004) 65:204–12.

(25) Retana –Marques S, bolinilla-Jaime H, Vazquez- Palacios G, Dominguez-Salazar Martinez-Garcia R, Velazquez-Montezuma J. Body Weights Gain and dimual differences of corticosteron changes in response to acute and chronic stress in rats. psychoneuro endocrinology (2003) 28:207-27

(26) Addolorato G, Capristo E, Greco A, Stefanini GF and Gasbarrini G. Energy expenditure, substrate oxidation, and body composition In subjects with chronic alcoholism: new findings from metabolic assessment. *Alcohol. Clin.Exp.Res.* (1997) 21: 962–967.

(27) Novelli ELB , Diniz YS Galhardi C M G, Ebaid M X, Rodrigues H G, Mani F, Fernandes AAH, Cicogna AC and Novelli Filho, JLVB. Anthropometrical parameters and markers of obesity in rats. *Laboratory Animals* (2007) 41: 111–119.

(28) Klonoff-Cohen H, Bleha J, Lam-Kruglick P. A prospective study of the effects of female and male caffeine consumption on the reproductive endpoints of IVF and gamete intra-Fallopian transfer. *Hum Reprod*. (2002)17:1746–1754

(29) Lodhi P Tandan N Singh N Kumar D and Kumar M. Camellia sinensis (L.) Kuntze Extract Ameliorates Chronic Ethanol-Induced Hepatotoxicity in Albino Rats. *JEvid Based Complementary Altern Med* (2014) (http://dx.doi.org/10.1155/2014/787153)

(30) Kaur P, Kaur G, Bansal MP. Tertiary-butyl hydroperoxide induced oxidative stress and male reproductive activity in mice: Role of transcription factor NF-kappa B and testicular antioxidant enzymes. *Reprod Toxicol* (2006) 22:479-84.

(31) Gu W, Hecht NB. Developmental expression of glutathione peroxidase, catalase, and mananese superoxide dismutase mRNAs during spermatogenesis in the mouse. *J Androl* (1996) 17:256-62.

(32) Mallikarjuna K, Nishanth K, Hou CW, Kuo CH, and Reddy, KS. Effect of exercise training on ethanol-induced oxidative damage in aged rats (2009). *Alcohol* 43: 59-64.

(33) Schlorff EC, Husainand SK, Somani M. Dose and Time Dependent Effects of Ethanol on Antioxidant in rat testes. *Alcohol* (1999)18:203–14

(34) Piegeolet E. and Corbisier P. Houbion A Lambert D Michiels C Raes M Zachary MD Remacle J: Glutathione peroxidase and superoxide dismutase and catalase inactivation by peroxides and oxygen derived free radicals. *Mech. Age Dev* (1990) 51: 283-297

(35) Ganaraja B, Crystal D, Vijayalakshmi BM Nayanatara AK, Ramesh Bhat M , Ramaswamy C Use of vitamin C on effect of ethanol induced lipid peroxidation in various tissues, sperm count and morphology in the Wistar rats. *J Chinese Clin Med* (2008) 3:1-5.

(36) Sikka SC. Relative impact of oxidative stress on male reproductive function. *Curr Med Chem.*(2001) 8:851-862.

(37) Emanuele NV, La Paglia N, Steiner J, Colantoni A, Van Thiel DH and Emanuele MA Peri pubertal paternal EtOH exposure . *Endocrine* (2001)14:213-219.

(38) Nordman R, Ribibere C, Rough H. Ethanol induced lipid peroxidation and oxidative stress in extra hepatic tissues. *Alchol Alcohol* (1990)125:231-237.

(39) Wilson J.D. Prospects for research for disorders of the endocrine system. *J. Am. Med. Assoc.* (2001) 285: 624-627.

(40) Tang PZ, Tsai-Morris CH, Dufau ML. Regulation of 3beta-hydroxysteroid dehydrogenase in gonadotropin-induced steroidogenic desensitization of Leydig cells. *Endocrinology* (1998) 139:4496-4505.

(41) Guo R, Ren J. Alcohol and acetaldehyde in public health: from marvel to menace. *Int. J. Environ. Res. Publ. Health* (2010) 7:1285–301

(42) Yazawa H, Sasagawa I, ishigooka, M and Nakada. Effect of immobilization stress on testicular germ cell apoptosis in rats. *Hum Reprod* (1999) 4(7): 1806-1810

(43) Gordon GG, Vittek J, Southern AL, Munnangi P, Lieber CS. Effect of chronic alcohol ingestion and the biosynthesis of steroids in rat testicular homogenate in vitro. *Endocrinology* (1980) 106:1880-1885.

(44) Cicero TJ, Bell RD. Effects of ethanol and acetaldehyde in the biosynthesis of testosterone either rodent testes. *Biochem Biophys Res Commun* (1980) 94:814-819.

(45) Caron KM, Clark BJ, Ikeda Y, Parker KL. Steroidogenic factor 1actsatall levels of the reproductive axis. *Steroids* (1997) 62:53-56.

(46) Meistrich ML, Finch M, daCunha MF, Hacker U, Au WW. Damaging effects of fourteen chemotherapeutic drugs on mouse testis cells. *Cancer Res*. (1982) 42:122–31

(47) Pajarinen J, Karhunen PJ, Savolainen V, Lalu K, Penttilä A and Laippala P. Moderate Alcohol Consumption and Disorders of Human Spermatogenesis. *Alcohol Clin Exp Res* (1996) 20 (2):332-7.

(48) Zhu Q, Meisinger J, Emanuelle NV Emanuele MA, LaPaglia N, Van Thiel DH. Ethanol exposure enhances apoptosis within the testes. *Alcohol ClinExp Res* (2000) 24 (10):1550-6.

(49) Bamac Y, Colak T, Bamac B, Ercin C, Ozturk B, Ozbek A. Comparative study on apoptosis in the testes of normal and alcoholic rats. *Saudi Med J* (2005) 26:928-33.

(50) Zhu Q, Van Thiel DH and Gavaler JS. Effect of ethanol on rat Sertoli Cell function: Studies in vitro and in vivo. *Alcohol Clin Exp Res* (1997); 21:1409-17

(51) Emanuele MA and Emanuele NV. Alcohol's effects on male reproduction. Alcohol Health Res World (1998); 22:195-218.

(52) Toth GP, Stober JA, Zenick H, Read EJ, Christ SA, Smith MK. Correlation of sperm motion parameters with fertility in rats treated sub chronically with epichlorohydrin. *J Androl*. (1991); 12:54–61.

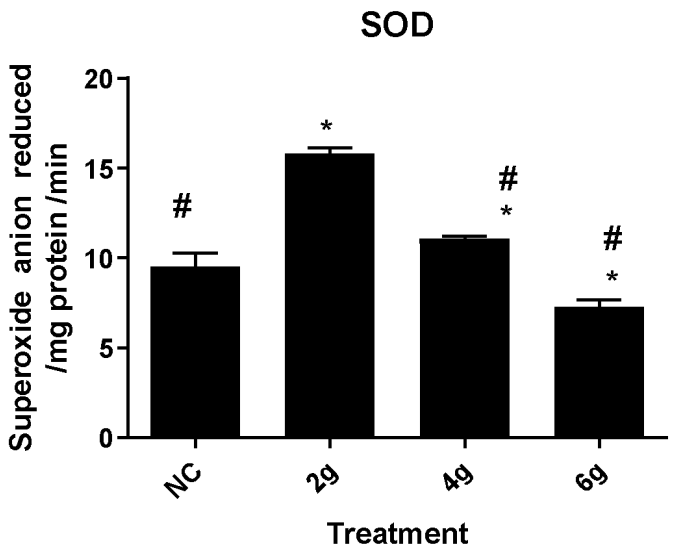
(53) Srikanth V, Malini T, Arunakaran J, Govindarajulu P, Balasubramanian K. Effects of ethanol treatment on epididymal secretory products and sperm maturation in albino rats. *J Pharmacol ExpTher* (1999) 288:509-15.

(54) Montes JF, Estrada G, Lopeztejero MD, Garciavalero J. Changes in the enterocyte cytoskeleton in newborn rats exposed to ethanol in utero. (1996) *Gut* 38:846-852.

(55) Hoek JB, Cahill A, Pastorino JG. Alcohol and mitochondria: a dysfunctional relationship. *Gastroenterology* (2002)122:2049–63.

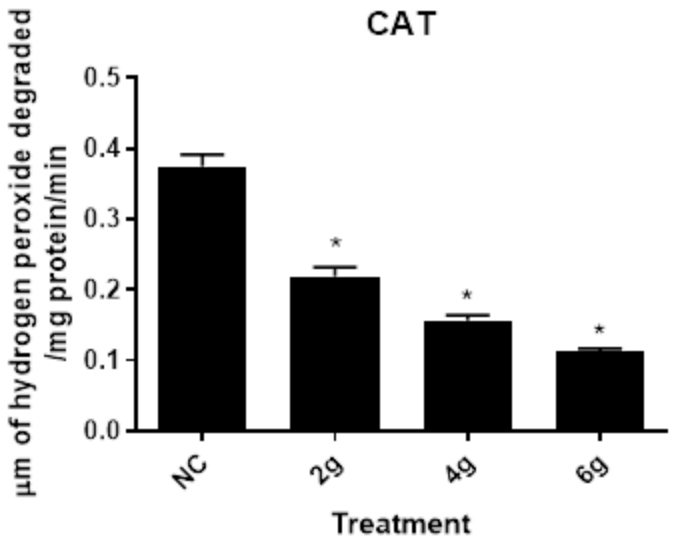
Legends:

Figure-1



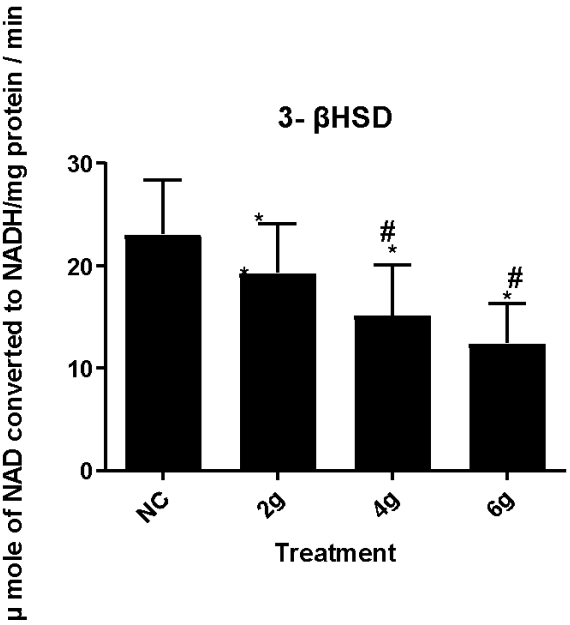
Effect of alcohol at different dosages 2 g, 4 g and 6 g on Superoxide dismutase (SOD) activity in male rats. All the values are means ± SD of six individual observations.* P<0.05 compared with normal control, #P<0.05 compared with 2 g of alcohol dosage.

Figure-2



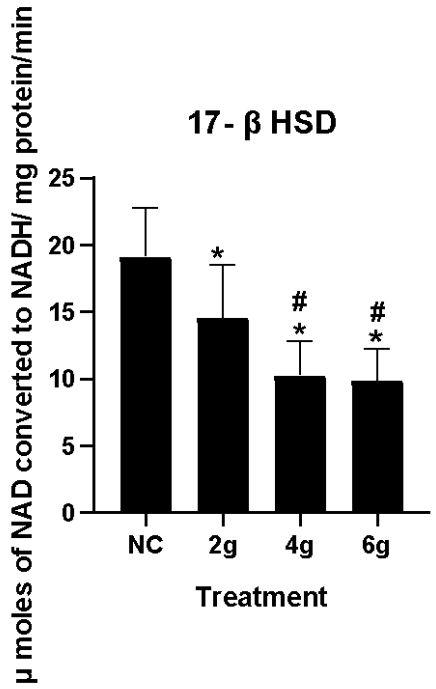
Effect of alcohol at different dosages 2 g, 4 g and 6 g on Catalase (CAT) activity in male rats. All the values are means ± SD of six individual observations.* $P<0.05$ compared with normal control, $^{\#}P<0.05$ compared with 2 g of alcohol dosage.

Figure-3



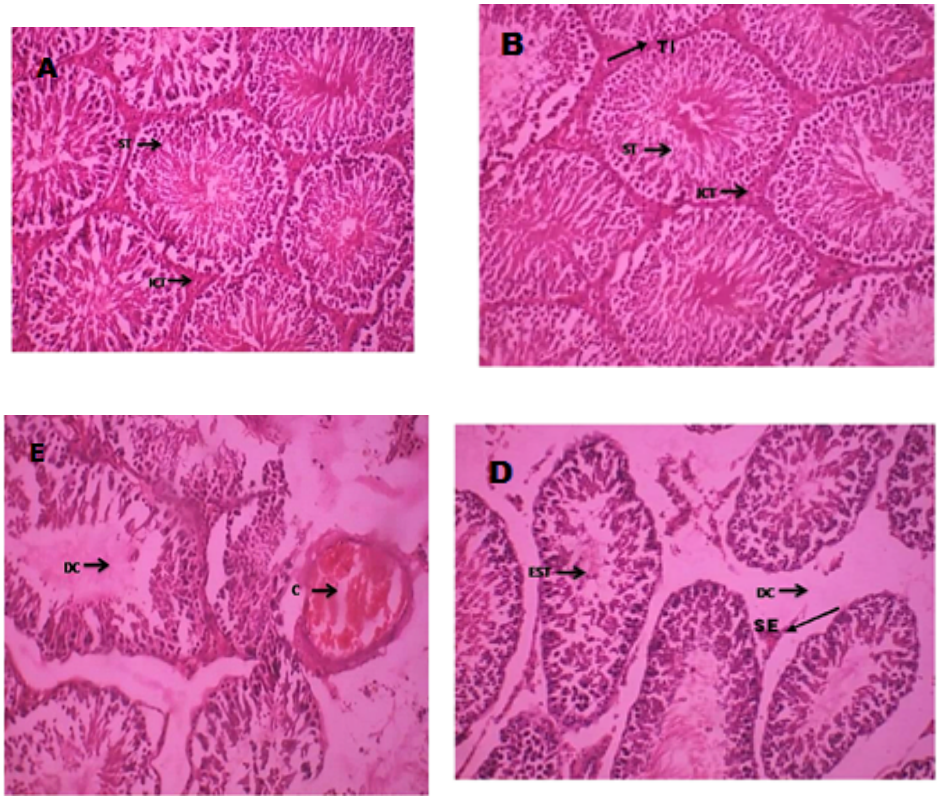
Effect of alcohol intoxication at different dosages on 3β hydroxysteroid dehydrogenases activity in male rats. All the values are means ± SD of six individual observations. * P<0.05 compared with normal control. # P<0.05 compared with 2 g of alcohol dosage.

Figure-4



Effect of alcohol intoxication at different dosages on 17 β hydroxysteroid dehydrogenases activity in male rats. All the values are means ± SD of six individual observations. * P<0.05 compared with normal control. # P<0.05 compared with 2 g of alcohol dosage.

Figure - 5



Photomicrographs of the testis (40x) of normal control (4A) and various dosages of 2 g, 4 g and 6 g/kg alcohol ingestion groups (4B,4C,4D). A-Normal control rats shown: Normal structure of seminiferous tubules (ST), Interstitial (IN), B- 2 g alcohol ingestion group shown: thinking of Interstitial Connective Tissue (ICT), mild degeneration of seminiferous tubules; C- 4 g ethanol dose group shown: degeneration of interstitial connective tissue (DICT), Vacuoles formation in seminiferous tubules; D-6 g ethanol dose shown: complete degeneration of seminiferous tubules (CST), Leydig cells (CDL)and spermatogonium (CDS).