THE IMPACT OF CHRONIC ALCOHOL CONSUMPTION ON SEX HORMONES AND SEMEN PARAMETERS IN MALE RABBITS

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ABSTRACT

Background: *It is not clear what concentration of alcohol constitutes male fertility risk.*

Aim: *This study evaluates the impact of chronic alcohol consumption on sex hormones and semen indices in albino rabbits fed with different concentrations of alcohol.*

Materials and methods: *The animals (aged 10-15 months) were divided into 5 groups of 6 animals each; groups 1, 2, 3, and 4 were fed with 4%, 12%, 20% and 40% alcohol respectively at 5ml/Kg body weight for 6 weeks, while group 5 served as control. Serum sex hormones were assayed using automated chemistry analyzer (MINI VIDAS). Semen analysis histomorphological studies were done using standard methods. The means were compared using Students' T-test and Pearson correlation was used to correlate the measured parameters with concentrations of alcohol administered.*

Results: *Results showed that serum testosterone, LH and FSH were significantly lower (P<0.001) in all alcohol treated groups than control. Sperm count and motility were also significantly lower (p<0.001) in all treated groups than control. Testosterone (r= -0.761, P<0.001), FSH (r = -0.775, P<0.001), LH (r= -0.778, P< 0.001), sperm count (r= -0.880, P<0.001) and motility (r= -0.911, P<0.001) correlated negatively with increasing concentrations of alcohol. The histomorphological examination of the testes and epididymis in groups 2, 3 and 4 showed moderate to severe histomorphological changes.*

Conclusion: *Chronic ingestion of alcohol in high percentages had deleterious effect on the male sex hormones, testes and epididymis which could affect fertility potential. A careful use of high concentrations of alcohol is suggested.*

Key words: *Alcohol, testosterone, LH, FSH, fertility.*

INTRODUCTION

Alcohol is second to nicotine as the most commonly used drug in most societies and may contribute to organ damage and infertility in the male chronic consumers. Alcohol abuse has constituted some public health challenges all over the world.[1] Alcohol may exert a powerful influence on the development of male infertility, since about 42% of males with infertility were reported to be chronic alcohol consumers[2] which may suggest that excessive alcohol is one of the many causes of infertility.[3] There is an increasing body of evidence suggesting considerable decline in sperm counts and volume. A meta-analysis of 61 studies from 1938 to 1991 showed that there was a 58% decline in sperm count and 20% decline in sperm volume in the last 50 years was observed.[4,5] Recent studies had evaluated the effect of a fixed concentration of alcohol on sex hormones by feeding experimental animals (rats) with 30% v/v ethanol at a dose of 2g/kg body weight for 4, 8 and 16 weeks.[6,7] Some authors reported that exaggerated intake of alcoholic beverages account for their toxic/side effects.[8,9] It is however not clear what constitutes exaggerated alcohol consumption since several studies on alcohol toxicity either had used fixed concentration of alcohol, different volumes or relied on self-reported volumes in human studies.

Infertility may be due to abnormalities of the reproductive system that impairs the body's ability to perform the basic function of reproduction.[10] Infertility is not just a woman's concern but a problem with the male could be the sole cause, or a contributing cause of infertility in about 40 percent of infertile couples.[10] Male infertility accounts for 40-50% of infertility among couples and is mainly due to deficiencies in the semen and semen quality. The quality of semen could be used as an important measure of a male's ability to produce off springs. It has been reported that men who drink alcohol regularly are more likely to have erection problems. Long term alcohol consumption has been implicated in permanent impotency.[11] In both men and women, the hormones regulating reproduction form a complex and finely controlled system that affects many cells in the body. The male reproductive system consists of three parts: the brain region called the hypothalamus, the anterior pituitary and the testes.[12] Alcohol is able to diffuse into all tissues and affects their vital functions because of its amphiphilic property.[8,9]

There is controversy regarding the effect of alcohol consumption on fertility, whereas some studies have shown a relationship between alcohol and infertility, it is not however clear what concentration of alcohol consumption is detrimental to fertility.[13-15] Some have also reported that consumption of low concentrations of alcohol may have no deleterious effect on fertility, but moderate consumption on the other hand may have slight alterations in sperm maturity, while chronic consumption may lead to impaired sperm development in 20% of cases.[16] But the concentrations of alcohol that could be described as low, moderate or high has not been well defined. This study seeks to evaluate the impact of chronic alcohol consumption on sex hormones, semen indices and testicular morphology in albino rabbits fed with fixed volume but different concentrations of alcohol over a period of time.

MATERIALS AND METHODS

Thirty male adult albino Rabbits *Oryctolaguscuniculus* aged 10 to 15 months weighing between 1.2 to 1.9 kg were used for this study. The rabbits were purchased at the cattle market Aduwawa, Benin City and they were housed in the animal house at Pharmacology Department, University of Benin, Benin City between January and March 2015. The animals were allowed to acclimatize for a period of two weeks and were treatment for six weeks. The animals were provided with water and commercially available feeds *ad libitum*, the feed was supplied by Bendel feed and flower mill, Benin City. The alcohol for treatment remained in the laboratory under natural condition of temperature (21 °C - 22°C). At the end of the acclimatization period the animals were divided into five groups of six animals each.

The experiment consisted of administering 5ml/kg body weight of 4%, 12%, 20% and 40% ethanol obtained from the dilution of 43% ethanol. The animals were divided into five groups of six animals each: group 1 was treated with 4% ethanol, group 2 12%, group 3 20% and group 4 received 40% ethanol while group 5 received no alcohol treatment (control).

The alcohol was administered by gavage once daily (in the morning) for six consecutive weeks. Animals were sacrificed thereafter and blood, semen samples and tissues were collected from the rabbit.

Ethical consideration

The experimental protocol was reviewed and approved by the ethical committee (EC/FP/015/11, dated 2nd October, 2015) of the Department of Pharmacology, University of Benin, Nigeria.

Blood Collection: Blood samples were collected from the animals using cardiac puncture (10). The animals were anaesthetized using chloroform, each animal was placed on its back on a solid surface; a 'v shaped' cut was made in the abdomen so as to allow easy access to the heart, a 25G needle and a 20ml syringe were used to collect blood directly from the heart. Blood samples were collected into plain specimen bottles and were allowed to clot for about six hours at room temperature. The specimens were centrifuged at 3000rpm for 5minutes, the serum was separated into another plain container and kept frozen at -20°C until was analyzed.

Semen Collection

Semen analysis was performed on all animalsat the end of the treatment periods. The semen was collected directly from the epididymis after the animals were sacrificed. The epididymis was incised and epididymal fluid collected and was used for semen analyses using the Neubauer counting chamber.[17]

Histomorphological evaluation

After the animals were sacrificed, the testes and epididymis were harvested and immediately fixed in 10% neutral buffered formalin solution. The tissues were taken to the histopathology laboratory at the University of Benin Teaching Hospital. Using standard operating procedures, tissues were dissected and introduced into labelled tissue cassettes. The thickness of tissues did not exceed 3-5mm. Tissues were then processed using automatic tissue processor (Leica

TP2010, Germany) for 18hours. The processor passed the tissues through the four stages of tissue processing namely: fixation, dehydration, clearing (dealcoholisation) and impregnation (infiltration). The tissues were then embedded in paraffin wax using the Leica automatic tissue embedder and sectioned to get ultra-thin sections of 5microns using the thermos scientific semi-automated rotary microtome (Leica Microsystems, Nussloch, Germany). Tissues were floated out from the thermos scientific digital floating bath on frosted end pre-labeled slides and dried on the thermos scientific digital slimline hot plate. Tissues were further dried in the hot air oven overnight and stained with Haematoxylin and Eosin stains to show the general tissue structure. Stained slides were mounted in DPX and allowed to dry before viewing under the microscope using x10 and x40 magnification.

Sample Analysis

Male sex hormones (testosterone, FSH and LH) were assayed using automated chemistry analyzer (MINI VIDAS, Marcy I'Etoile, France). Semen analysis was done according to WHO standard manual while histomorphological studies were conducted using light microscopic technique.

Statistical Analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS) version 20.0 (Chicago-IL, USA). Results were expressed as Mean ± Standard deviation, Student's t-test was used to compare the means of two groups while One way Analysis of Variance (ANOVA) was used for comparing means to determine if there was significant difference between more than two groups. Pearson Correlation coefficient was used for determining relationship between measured parameters and increasing concentration of alcohol.

RESULTS

The results are as shown in tables 1, 2, 3and 4 while figures 1 and 2 show the histomorphological changes in both right and left testis of control and treated rabbits. The animals were all weighed before and after the completion of the experiment before they were sacrificed. There was a significant reduction in the weight of the rabbits fed with 12%- 40% alcohol but the rabbits that were fed with 4% alcohol ($P<0.058$) and controls ($P<0.000$) had significant weight gain (table1). Compared with the control, animals fed with 12-40% alcohol had significantly decreased ($P<0.001$) levels of LH, FSH and testosterone (table 2), while there was no significant different between those fed with 4% alcohol and the control. The sperm count and motility of the rabbits fed with 12%- 40% alcohol were also significantly reduced ($P<0.001$) when compared with the control (table 3). There was no significant decrease in the count and motility of the animals fed with 4% alcohol compared with controls. There was a negative correlation between increasing concentrations of alcohol and serum levels of FSH ($r= -0.775$; $P< 0.000$), LH ($r= -0.778$; $P<0.000$) and testosterone ($r= -0.761$; $P< 0.000$) (Table 4). Negative correlation was observed between increasing concentrations of alcohol and sperm count ($r= -0.880$; $P<0.000$) and motility ($r= -0.911$; $P<0.000$) (table 4).

Table 1: Comparison of weight of rabbits before and after alcohol treatment (Mean ± Standard deviation).

	Mean initial weight (kg)	*Mean weight after treatment (kg)*	*p-value*
4% Alcohol	1.55(0.05) (1.45-1.65)	1.67(0.15) (1.38-1.96)	0.058
12% Alcohol	1.68(0.1) (1.48-1.88)	1.63(0.10) (1.43-1.83)	0.542
20% Alcohol	1.68(0.07) (1.53-1.83)	1.48(0.07) (1.33-1.63)	0.01
40% Alcohol	1.82(0.07) (1.67-1.97)	1.48(0.05) (1.38-1.58)	0.001
Control	1.28(0.10) (1.08-1.48)	1.78(0.12) (1.54-2.03)	0.001

Standard deviation and confidence interval are in parenthesis.

Table 2: Serum testosterone, LH and FSH levels in animals fed with different concentrations of alcohol and controls (mean(SD))

	4% Alcohol	*12% Alcohol*	*20% Alcohol*	*40% Alcohol*	*Control*	*p*
FSH(mU/mL)	$0.33(0.1)^{ab}$ (0.23-0.43)	$0.23(0.02)^{bc}$ (0.18-0.28)	$0.21(0.05)^{bc}$ (0.11-0.31)	$0.15(0.02)^{c}$ (0.10-0.2)	$0.39(0.14)^{a}$ (0.24-0.54)	0.001
LH(mU/mL)	$0.26(0.05)^{ab}$ (0.21-0.31)	$0.19(0.05)^{bc}$ (0.09-0.29)	$0.15(0.05)^{c}$ (0.05-0.25)	$0.12(0.00)^{c}$ (0.12-0.12)	$0.31(0.04)^{a}$ (0.21-0.41)	0.001
Testosterone (ng/mL)	$1.68(0.73)^{a}$ 0.95-2.63)	$0.89(0.4)^{ab}$ (0.01-1.77)	$0.44(0.17)^{b}$ (0.10-0.78)	$0.41(0.05)^{b}$ (0.36-0.46)	$1.71(0.39)^{a}$ (0.93-2.49)	0.001

Means with different superscripts indicate levels of significant: c=p=0.05; b=p<0.001;a=p>0.05. Standard deviation and confidence interval are in parenthesis.

Table 3: Effect of different concentrations of alcohol on semen count and motility

	Group 1 4% Alcohol	*Group 2 12% Alcohol*	*Group 3 20% Alcohol*	*Group 4 40% Alcohol*	*Control 0%Alcohol*	*P value*
Count (x10⁶/mL)	$90.33(10.4)^{a}$ (69.5-111.1)	$83.00(24.1)^{b}$ (34.8-131.2)	$35.33(13.3)^{c}$ (8.58-62.1)	$17.00(6.1)^{c}$ (4.7-29.3)	$95.33(13.3)^{a}$ (68.6-122)	0.001
Motility (%)	$75.00(7.88)^{a}$ (59.2-90.8)	$58.00(2.84)^{c}$ (52.3-63.7)	$29.33(4.3)^{c}$ (20.7-38.0)	$20.00(3.8)^{c}$ (12-27.6)	$73.33(10.7)^{a}$ (51.9-94.7)	0.001

Means with different superscripts indicate levels of significant: b=p=0.05; c=p<0.001.Standard deviation and confidence interval are in parenthesis.

Table 4: Pearson correlation coefficient of measured variables with increasing concentrations of alcohol.

PARAMETERS	R – VALUE	P – VALUE
FSH	-0.775**	0.001
LH	-0.778**	0.001
TESTOSTERONE	-0.761**	0.001
COUNT	-0.880**	0.001
MOTILITY	-0.911**	0.001
WEIGHT	-0.623**	0.001

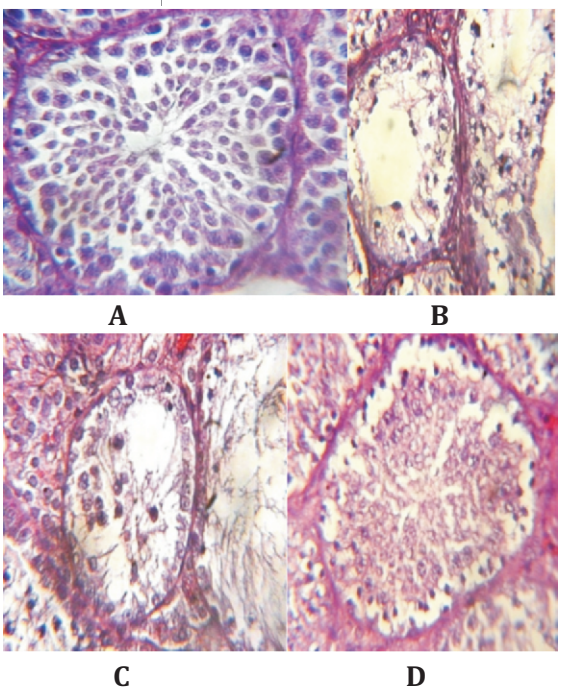

Figure 1: (A) Cross section of the right testis of control rabbit showing the stratified seminiferous epithelium revealing some visible cells of the spermatogenic series. (B) Group 4 right testis showing hypocellularity, reduction in cells of the spermatogenic series as a result of degeneration, sloughing and shortening of seminiferous epithelium. An

area of vascular haemorrhage in the testis is observed. (C)group 3 left testis showing disruption to the seminiferous tubules epithelium. (D)sections of the seminiferous tubules are fairly circular or oval in outline, transverse and longitudinal sections respectively with prominent stratified seminiferous epithelium revealing some visible cells of the spermatogenic series, no histological disruption was seen. (x400 magnification, Haematoxylin and Eosin stained).

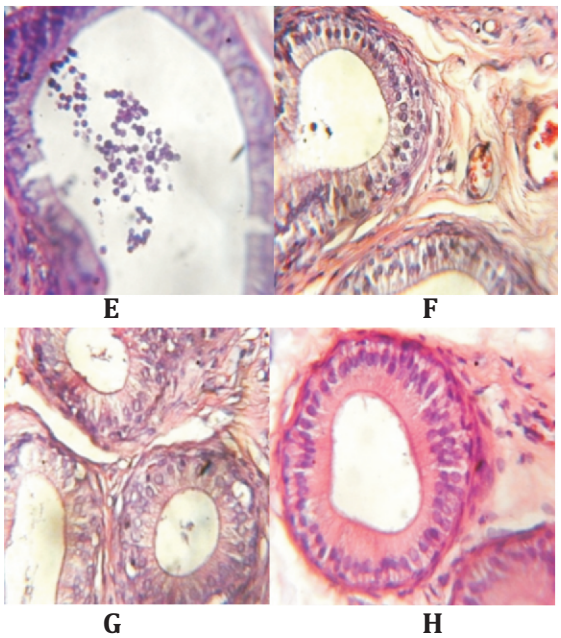

Figure 2: (E Control epididymis showing some tubules are filled with spermatozoa, normal features with epithelium well defined, (F) Group 4 left epididymis showing visible atrophy, there is observable epithelial disruption and an area of vascular haemorrhage, (G) group 3 left epididymis showing marked atrophy of the cellular content, decrease in size of the seminiferous tubules and reduced spermatogenesis is observed. (H)group 1 left epididymis showing normal epithelial. (x400 magnification, Haematoxylin and Eosin stained).

DISCUSSION

It was observed from this study that chronic consumption of alcohol resulted in weight loss, decreased levels of testosterone, LH, FSH, sperm count and motility when compared with control. This observation is consistent with previous studies.[18-21] Contrary to earlier belief that alcohol may be used as efficiently as fat and carbohydrate as energy source, it is now known that the progressive substitution of carbohydrate with alcohol in an otherwise balanced diet leads to decrease in body weight.[22] It was reported that the addition of 90g of alcohol to the daily diet increased the daily energy expenditure by 7% and inhibit lipid oxidation by the ingestion of additional alcohol to 50% of calorie.[22] One of the proposed mechanisms responsible for the apparent loss of alcohol derived energy is the existence of isoenzymes of alcohol dehydrogenase (ADH), which is a cytolic enzyme responsible for alcohol metabolism. In order to bring about the oxidation of ethanol, ADH converts its cofactor nicotinamide adenine dinucleotide (NAD+) to NADH. The energy rich molecule donates electron to the electron transport chain in the mitochondria membrane, which in turn lead to the synthesis of adenosine triphosphate (ATP). Because the ADH-mediated ethanol oxidation is located in the cytoplasm, the NADH formed cannot pass through the mitochondria membrane. Therefore, the cellular redox potential is altered when ethanol is metabolized.[23] Chronic alcohol intake does not only affect micronutrient uptake in the small intestine but may also impair the absorption of

macronutrients. Studies have indicated that absorption of amino acids, lipid and glucose in the intestine is markedly impaired when alcohol is chronically consumed.[24-25] Alcohol consumption and potential changes in dietary habits have also been reported.[26] There was no significant decrease in body weight in animals in group 2 but a significant ($p<0.000$) increase in body weight of animals in the control group was observed (table 1).

It is not clear whether the impact of alcohol administration on male fertility potential is dependent on the concentration of alcohol consumed. Previous study had reported a decrease in male sex hormones in experimental animals fed with fixed concentration (30% v/v) of alcohol and the measured sex hormones were determined at three different phases such as 4, 8 and 16 weeks.[27] Decreased levels of sex hormones in both acute and chronic alcohol administration were observed[27] The present study evaluated the effects of chronic consumption of different concentrations of alcohol on sex hormones and testicular cells. Our data indicated that chronic consumption of alcohol in concentrations above 4% resulted in decreased sex hormone levels.

Testosterone and FSH are required for the successful germ cell development in the testes. In normal physiological condition, decreased level of testosterone causes activation by feedback mechanism via the long loop of the hypothalamus leading to the release of gonadotropin releasing hormone (GnRH) which then stimulates the anterior pituitary to secrete LH. LH stimulates the secretion of testosterone and FSH.[3,7,15] From the data presented in this study, it shown that testosterone, LH and FSH were lower than normal indicating that chronic alcohol consumption above 4% suppressed the synthesis of the measured hormones at the levels of the hypothalamus, the anterior pituitary and the testes. In addition, the histomorphological studies of the testes and epididymal cell showed that adverse morphological changes occurred in rabbits fed with 12-40% alcohol while those fed with 4% alcohol and the control group had no observable morphological abnormality. This study therefore has identified the mild, moderate and high concentrations of alcohol that may be harmful to male fertility potentials. In human studies, low levels of testosterone were reported in both moderate and chronic alcohol consumption,[21,28] this corroborates the findings in this study that alcohol has a direct toxic effect on the testis which led to decreased seminiferous tubular function (figures 1 and 2). The observed decreased in LH and FSH levels in these studies implied that alcohol acted not only on the testes but also on the hypothalamic-pituitary axis.[29] The observed results are not consistent with some authors who reported increased levels of LH and FSH in alcohol consumers. They opined that feedback mechanism of decreased testosterone on the hypothalamic–pituitary–gonadal (HPG) axis resulted in an increased level of FSH and LH.[2,27,30-32] The significant decrease in sperm count and sperm motility of 12-40% alcohol treated animals in this study is an indicator of decreased levels of sex hormones and impaired testicular function.

CONCLUSION

This study has demonstrated that chronic consumption of alcohol above 4% could induce male infertility by acting directly on the hypothalamic-pituitary-testicular axis

thereby reducing the testosterone, FSH and LH levels and sperm indices of the experimental animals. A careful use of high concentrations of alcohol is suggested.

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REFERENCES

1. Kumar P, Clark M. Alcohol. Kumar and Clark clinical medicine., *W. B. Saunders, London, UK* 2002: 250-1.
2. Chia SE, Lim ST, Tay SK, Lim ST. Factors associated with male infertility: a case control study of 218 infertile and 240 fertile men. BJOG 2000;**107**: 55–61.
3. Emokpae MA, Uadia PO, Omale- Itodo A, Orok TN. Male infertility and Endocrinopathies in Kano, North Western Nigeria. *Annals Afri Med* 2007; **6**: 64- 7.
4. Sinclair S. Male infertility: Nutritional and Environmental considerations. *Altern Med Rev* 2000;**5**:28-38.
5. Keskin MZ, Budak S, Gubari S, Durmaz K, Yoldas M, Celik O, Aksoy EE, Libey YO. Do Cigarette and alcohol affect semen analysis? *Arch Ital Urol Androl* 2016; 88**(1)**:56-9.
6. Parasuraman S, Raveendran R, Kesavan A. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother*2010; **1**: 87–93.
7. Dosumu OO, Akinola OB,Akang EN. Alcohol-induced testicular oxidative stress and cholesterol homeostasis in rats – the therapeutic potential of virgin coconut oil. *Middle East Fertil Soc J.* 2010;**17**:122–8.
8. Condorelli RA, Calogero AE, Vicari E, La Vignera S. Chronic consumption of alcohol and sperm parameters; our experience and the main evidences. *Andrologia* 2015; **47**:368-79.
9. Alvarez S. Do some additions interfere with fertility? *Fertil Steril* 2015;**103**:22-6.
10. American Society for Reproductive Medicine. Infertility: an overview, a guide for patients. *Fertil Steril* 2013; **3**: 3-15
11. Hart RJ, Doherty DA, McLanchlan RI, Walls ML, Keelan JA, Dickson JE, Skakkebaek NE, Norman RJ, Handelsman DJ. Testicular function in a birth cohort of young men. *Hum Reprod* 2015;**30**:2713-24.
12. Brugh VM, Lipshultz LI. Male factor infertility. *Medical Clinics of North America.* 2004;**88**: 367–85
13. Pourentezari M, Talebi AR, Mangoli E, Anvari M, Rahimipour M. Addiction deleterious effects of alcohol consumption on sperm parameters and DNA intergrity in diabetic mice. *Andrologia* 2016;**48**:564-9.
14. Sharma R, Biedenharn K, Fedor J,Agarwal A. A Lifestyle factors and reproductive health: Taking control of your fertility. *Reprod Biol Endocrinol* 2013;11: 66.
15. Uadia PO, Emokpae MA. Male infertility in Nigeria: A neglected reproductive health issue requiring attention. *J. Basic Clin. Reprod. Sci.* 2015;**4**: 45-53
16. Ren J, Banan A, Keshavarzian A, Zhu Q, LaPagliaN,McNulty J. Exposure to ethanol induces oxidative damage in the pituitary

gland. *Alcohol* 2005;**35**:91-101.
17. Martinez M, Macera S, de Assis GF, Pinheiro PFF, Almeida CCD, Tirapelli LF, et al. Structural evaluation of the effects of chronic ethanol ingestion on the testis of Calomyscallosus. *Tissue Cell* 2009;**41**:199–205.
18. Rooney KL, Domar AD. The impact of life-stylebehaviours on infertility treatment outcome. *Curr Opin Obstet Gynecol* 2014;**26**:181-5.
19. Shafi H, Esmaeilzadeh S, Delavar MA, Haydari FH, Mahdinejab N, Abedi S. Prevalence of varicocele among primary and secondary infertile men: Association with occupation, smoking and drinking alcohol. *N Am J Med Sci* 2014; **6**:532-5.
20. Guthauser B, Boitrelle F, Plat A, Thiercelin N, Vialard F. Chronic excessive alcohol consumption and male fertility: a case report on reversible azoospermia and a literature review. *Alcohol Alcohol* 2014;**49**:42-4.
21. Osinubi A, Daramola AO, Noronha CC, Okanlawon AO,Ashiru OA. The effect of quinine and ascorbic acid on rat testes. *West Afr. J. Med.* 2007;**26**: 217–21.
22. Wegnerberger S, Kanuri G, Bergheim I. Alcohol drinking patterns and nutrition in Alcoholic liver disease In: Trends in Lacoholic liver disease Research-Clinical and Scientific aspects. Ichiro Shimizu (ed). In Tech publisher, *Rijeka, Croatia.* 2012 pp.1-23.
23. Van Haaren MRT, Hendriks HFJ. Alkoholstoffwechsel. In: Alkohol und AlkoholfolgekrankheitenGrundlagen – Diagnostik – Therapie, Singer, M.V., Teyssen, *S. Springer Berlin Heidelberg,* 1999 pp.95-107.
24. Wang N, Tikellis G, Sun C, Pezic A, Wang L, Wells JC, Cochrane J, Ponsonby AL, Dwyer T.The effect of maternal prenatal smoking and alcohol consumption on the placenta-to-birth weight ratio. *Placenta.* 2014;**35**:437-441
25. Sohrabvand F, Jafari M, Shariat M, Haghollahi F, Lotfi M. Frequency and epidemiologic aspects of male infertility. *Acta Med Iran* 2015;**53**:231-235.
26. Alshahrani S, Agarwal A, Assidi M, Abuzenadah AM, Durairajanayagam D, Ayaz A, Sharma R, Sabanegh E. Infertile men older than 40years are at higher risk of sperm DNA damage. *Reprod Biol Endocrinol* 2014;**12**:103.doi: 10.1186/1477-7827-12-103
27. Suter PM. Alkohol und Ernährung. In: Alkohol und Alkoholfolgekrankheiten Grundlagen – Diagnostik – Therapie, Singer, MV, Teyssen S (ed). *Springer Berlin Heidelberg* 2005 pp.326-348.
28. Oremosu A.A, Akang EN. Impact of alcohol on male reproductive hormones, oxidative stress and semen parameters in Sprague–Dawley rats. *Middle East FertilSocJ* 2014;**20**: 114–8.
29. Hoek JB, Pastorino JG. Ethanol, oxidative stress and cytokine induced liver cell injury. *Alcohol* 2002;**27**: 63–8.
30. Emanuele M, Tentler J, Emanuele N, Kelley M. In vivo effects of acute EtOH on rat α and β luteinizing hormone gene expression. *Alcohol* 1991;**8**: 345–8.
31. Sengupta S, Ray R, Shetty K, Desai N. Pituitary gonadal functioning in male alcoholics in an Indian psychiatric hospital. *Alcohol* 1991; **26**: 47–51.
32. Mendelson JH, Mello NK, Ellingboe J. Effects of acute alcohol intake on pituitary–gonadal hormones in normal human males. *J PharmacolExpTher* 1977; **202**: 676–682.