JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Testicular and epididymal toxicity inducedby high fat diet (HFD), alcohol, and their combination in male Wistar rats

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Abstract : Obesity and alcohol abuse are life style diseases and both significantly involved in several reproductive pathologies contributing to infertility in men. The aim of this experiment was to study the independent and combinative effect of HFD and alcohol on bodyweight, relative reproductive tissues, sperm parameters, circulatory testosterone levels, testicular steroidogenic enzyme activities, antioxidant capacity in testes and epididymis in male wistar rats. A total of twenty-four male Wistar rats were randomly divided into 4 groups: (1) Control group (CON) fed with a commercial standard diet; (2) HFD, high fat diet group fed with a commercial beef chow containing 60% crude fat for 10 weeks; (3) Alcohol alone was given orally at 2 g/kg bw/day; (4) Co-administration of alcohol (2 g/kg) and HFD. Our resulted showed that rats in HFD group exhibited increased body weight gain compared to control. Further, markedly increase bodyweight gain in co- administration of HFD combined alcohol rats. On the other hand, there was statistically significantly decreasein bodyweight gain between alcohol alone treated group compared to control ($p< 0.05$). Moreoversignificant increase in relative weight of somatic tissues such asliver, kidney and brain was observed in HFD or co- administration of HFD combined alcohol compared to control rats. The relative weights of testes, epididymis, seminal vesicles, and prostate glands were significantly decreased in alcohol alone or combined HFD exposure rats compared to control rats ($p< 0.05$). Exposure to HFD or alcohol significantly decreased daily sperm production, motile-, viable- and HOS-tail swelled sperm, 3β- and 17β- hydroxysteroid dehydrogenase activity levels in testes of rats compared to control ($p< 0.05$). Similar trend was observed in co-administrated with HFD plus alcohol rats. Our results also showed that administration of rats with HFD or alcohol alone and combined alcohol and HFD caused a significant decrease of antioxidant enzyme (SOD, CAT, GSH-Px, and GR) activities and remarkable reduction in MDA content in testes, as well as in epididymis indicating that HFD alone or alcohol treated alone or alcohol plus HFD can increase oxidative stress in testes and epididymis. These findings suggest that HFD or alcohol has adverse effects on spermatogenesis and steroidogenesis. In addition, co- administrated HFD and alcohol rats showed higher reproductive toxicity than individual treatments. Finally, we conclude that consumption of alcohol combined with high calories diet leads to male infertility in rats.

IndexTerms **- Alcohol, Obesity, Reproductive Toxicity, High fat diet**

I. INTRODUCTION

Various lifestyle factors/habits adversely affect male fertility which includes alcohol intake, unhealthy diet, sedentary behaviour which leads to obesity.Numerous studies have identified that obesity induced testicular oxidative stress resulted in damage of testicular structure and function such as spermatogenesis and steroidogenesis, impairment of sperm quality parameters and decreased fertilization capacity of sperm [1].It was noticed that animals fed with high fat diet (HFD) resulted increase in body weight and gonadal fat thereby causes hypercholesterolemia which effects on decrease sperm count [2,3,4]. In few other studies, severalauthors concluded that reduction in circulating testosterone levels was linked with a decreased quality of sperm and also decrease activity levels of testicular 3 β- and 17 β-HSD [5,6]. It was also reported that mice fed with HFD resulted in deterioration of testicular histology due to hyperlipidaemic testes. In this finding rat testes observed that basement membrane of seminiferous tubules (involved in spermatogenesis) appeared to thickened and wrinkled that may causes reduced in the rate of sperm production [7,8].However, alcoholcan also causes alteration in the male reproductive tract of human and rodent models. Studies have found evidence of testicular lesions, decreased testicular weight, morphological alteration in testis, diminished steroidogenic enzyme activities, testosterone deficiency, testicular atropy, sexual dysfunction [9,10,11,12,13,14].The animals treated for 8 weeks with ethanol at a dosage level at 3 g/kg, exhibited reduced testosterone [15]. Although a large number of studies have reported separately on the deleterious effects of HFD or alcohol on reproduction, there have been no reports dealing particularly with the high fat diet induced obesitycombined with alcohol intake on reproduction in male wistar rats. Therefore, we have examined the effect of HFD diet alone or alcohol alone and combined effects on body weight gain, relative body weight of somatic and reproductive tissues, sperm parameters, serum testosterone level and related with testicular steroidogenic marker enzymes of 3β- and 17β- hydroxysteroid dehydrogenase(HSD) activity levels and epididymal and testicular antioxidant system in male Wistar rats.

II. MATERIAL AND METHODS

2.1 Procurement and maintenance of animals

Male Wistar rats with a body weight of 190±10 g (12 week old) were purchased from an authorized vendor (Sri Ragavendra Enterprises, Bengaluru, India). All rats were housed (4 per cage) in clean polypropylene cages (18" x 10'x 8") containing paddy husk as bedding material and were provided with tap water *ad libitum* and standard rodent chow purchased from Sai Durga Agencies, Bengaluru, India. Rats were acclimiatized for one week before being used for experimentation and maintened at temperature 22-25°C; 12:12 hr light:dark cycle. The experiments were carried out in accordance with guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Governement of India, Ministry of Social Justice and Empowerement, Government of India [16]. All the procedures were approved by the Insitutional Animal EthicalCommittee.

2.2 Experiment Design

Animals were randomly divided into four groups with six animals each and the details of groups are under: Group 1: Control rats given standard rodent chow diet
Group 2: Rats fed with high fat diet (HFD) containing 60% crude fat for 60 days Group 3: Rats given 2g/kg/bw/day for 60 days
Group 4: Rats administred with HFD combined with alcohol for 60 days.

The dose of alcohol was based on the published literature by Hari Priya *et al.*,[10]High fat diet (HFD) were purchased from National Centre for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition (NIN), Hyderabad, Telangana, India. One kilo of high fat diet (HFD) contains (g/kg) casein 422 g, cystine 3 g, starch 172 g, cellulose 50 g, groundnut oil 25 g, tallow 90 g, mineral oil 37 g, and Vitamin mix 10 g.

2.3 Necrospy and Determination of body weight and tissue somatic indices

The initial body weight on the first day of experiment and the final body weight on prior to necropsy of the experiment were determined and used to calculate body weight gain over the 60-day period of treatment.Afterthat,the animals were were fasted overnight before being euthanized by either anaesthesia and cervical dislocation on the day following the last treatment. Tissues such as brain, liver, kidney, testes, epididymis (caput, corpus and cauda), vas deferens, seminal vesicles and prostate glands were quickly removed, cleared of adhering connective tissue, weighed separately by using a Schimadzu electronic balance (Model No: BL-220H; Kyoto, Japan). Tissue somatic indices (TSI) were calculated by using the following formula:

TSI = [weight of the tissue (g) / body weight of the animal (g)] x 100

Testes were used for determination of daily sperm production (DSP) and biochemical studies whereas epididymides were used for the determination of sperm analysis and biochemical studies. Further, serum was obtained by centrifuged with 3,000 x g for 10 min and stored at -20°C until used for determination oftestosterone concentrations.

2.4 Sperm parameters

2.4.1 Daily sperm production (DSP)

DSP was evaluated according to established method. Briefly, the testes were decapsulated and homogenized in 0.9% of physiological saline solution containing 0.05% Triton X-100 solution using glass Teflon homogenizer. Then staining by trypan blue, elongated spermatids with unbroken nuclei were counted in the haemocytometer was observed under light microscope. Moreover, obtained values were subsequently used to compute DSP by formula : number of spermatids x Dilution factor / weight of testes x moment divisor (6.1days)

2.4.2 Epididymal sperm analysis

The tail of right epididymitis was placed on clean 30 mm dishes containing with 2.0 ml of M199 containing 0.5% fetal bovine serum and minced with a fine scissors to allow spermatozoa to swim out for 10 -15 min at 37 °C. After incubation for 10 in at room temperature, supernatant was separated from epididymal tissue-fluid mixture.Sperm suspensions were evaluated for sperm counts, viability, motility and morphology following the procedure of as described below.

2.4.3 Sperm counts

Sperm were collected from right caudal (tail) part of the epididymis in 2.0 ml of M199 medium with 5 mg/mL fetal bovine serum. The sperm were counted by using Neubauer Chamber of haemocytometer [17]. Sperm count was expressed as number of sperm / ml of solution

2.4.4 Sperm motility

The sperm was allowed to diffuse into M199 with Hanks' and L-glutamine containing 0.5% fetal bovine serum. The sperm sample was incubated in a glass chamber for 5 min. at 37 °C and further diluted with M199 solution (1:3). The motility of approximately 150-200 sperm each sample in a Neubauer haemocytometer was observed with an optical microscope. The results expressed as percentage of the motile sperm of the total spermcounted [17].

2.4.5 Sperm viability

The ratio of live to dead spermatozoa was determined using trypan blue (1% w/v) staining [18]. Briefly, 0.2 ml of undiluted sperm used for counting was incubated with 0.2 ml of trypan blue solution for 15 min at 37ºC. A drop of the suspension was placed in a Neubauer haemocytometer chamber under a cover slip, allowed to settle for 1 min and observed under Olympus microscope (Model No: BX41TF). The number of stained and unstained spermatozoa was scored in 20 separate fields. The spermatozoa which were not stained with trypan blue were considered as viable. Sperm viability was expressed as a percentage of viable sperm of the total sperm counted.

2.4.6 Sperm functional test (Hypo-Osmotic Swelling Test)

Sperm membrane integrity was determined using the hypo-osmotic swelling (HOS) test [19]. The viable sperm are exposed to hypo-osmotic medium containing 1.35 fructose and 30.735 g sodium citrate in 100ml of distilled water. There is an influx of fluid causing the tail to coil was observed under light microscope. One ml of hypo-osmotic swelling (HOS) solution was taken and incubated at 37 °C for 30 min. To this add 0.1 ml of semen suspension and counted for initial coiling. After that, solution was incubated at 37 °C for 30 min and then two drops of the above mixture were placed on a glass slide, cover slipped it and the sperm with coiled tail were counted under phase contrast microscope (Olympus Model No: BX41TF) at 100X.The percentage of sperm with coiled tail was calculated by subtracting the number of initially tail coiled sperm from the finally tail coiled sperm.

2.5 Assay of testicular steroidogenic marker enzymes

The activity levels of 3β- and 17β- hydroxysteroid dehydrogenase (HSD) were measured in testicular homogenate [20]. The reaction mixture in a volume of 2.0 ml of contained 100 µM of sodium pyrophosphate buffer (9.0), 0.5 µM cofactor NAD for 3- βHSD, 0.5 µM NADPH for 17 β-HSD, 0.08 µM substrate (dehydroepiandrosterone for 3-βHSD, androstenedione for 17β-HSD) and 100 µM of coenzyme. Furthermore, the reaction was carried out in a quartz cuvette of 1.0 cm path length at 23°C. Change in absorbance at 340 nm was measured at 20 seconds for 3 minutes in an ultraviolet (UV)-Visible double-beam spectrophotometer (UV- 1700, Shimadzu, Japan). The enzyme activity was expressed as nM of NAD converted to NADH / mg protein / min for 3β-HSD and as nM of NADPH converted to NADP / mg protein / min for 17β-HSD.

2.6 Determination of serum testosterone concentrations

Blood from each rats was collected by cardiac puncture and allowed to clot at 37 °C for 1h. The serum was obtained by centrifugation at 3,000 x g for 10 min. Serum from each sample was then immediately frozen at -20 °C until assayed. The serum testosterone levels were determined by an ELISA method using a DRG testosterone ELISA kit (ELISA EIA-1559, 96 wells kit, DRG instrument, GmbH, Marburg, Germany). The assay was performed according to the user manual. The sensitivity, intra- and inter assay variation coefficients of kit were 0.083 ng/ml, 3.34 - 4.16 % and 4.73 - 9.94 % respectively.

2.7 Estimation of testicular and different regions of epididymis (caput, corpus and cauda) antioxidantenzymes activities

A 10% homogenate was prepared with glass-teflon homogenizer in ice-cold Tris buffer (2 mM, pH 7.4, contains 0.25 M sucrose) followed by centrifugation at 800 x g for 20 min at 4°C and clear supernatant was used for enzyme assays. Lipid peroxidation (LPx) was estimated in terms of malondialdehyde (MDA) content and determined by using the thiobarbituric acid[21]. The LPx levels were expressed as µmol of MDA formed/g tissue. Superoxide dismutase (EC 1.15.1.1)activity was assayed[22]. The SOD activity levels were expressed in nmoles pyrogallol oxidized/mg protein/min. Activity of Catalase(EC 1.11.1.6) was also assayed [23]. The activity of the enzyme was expressed in nmoles of H_2O_2 metabolized/mg protein/min. Glutathione peroxidise (EC 1.11.1.9) activity was measured[24]. The activity was expressed as nmoles NADPH oxidized/mg protein/min. Glutathione reductase(EC 1.6.4.2) was assayed according to the method[25]. The activity was expressed in terms of nmoles NADPH oxidized/mg protein/min.Protein content in the enzyme source was determined[26].

2.8 Statistical Analysis

The statistical analysis was performed by SPSS 20.0 software (SPSS Inc., Chicago, IL, United State). Data were analyzed by One-way analysis of variance followed by *Post hoc* Tukey comparison test to identify differences in means among treatments. Data were assumed to be statistically significant at $P < 0.05$.

III. RESULTS

3.1 Body weight and Tissue indices

Table 1. High fat diet (HFD) and/or alcohol induced changes on body weights (g) adult male wistar rats

Parameter	CON	HFD	AL	HFD+AL
Initial body weight (g)	205.83±3.2	210.00±6.2* (-2.02)	195.17±3.5* (-5.17)	190±6.9* (-7.69)
Final body weight (g)	336.50±5.5	397.17±8.1* (-18.02)	311.75±9.3* (-7.35)	413.33±8.8* (-22.83)
Weight gain (g)	130.67±6.8	187.03±5.4* (-43.13)	116.58±0.1* (-10.78)	223±16.0* (-70.65)

CON = Control diet group; HFD = high fat diet; AL = alcohol; HFD + AL = high fat diet and alcohol intake. Data are shown as mean ± S.D (Standard Deviation) of 6 individuals. Significant difference was indicated by * at $p< 0.05$ compared CON vs HFD, AL, HFD+AL (treatment groups).

Table 1 shows the mean initial and final body weights, weight gain of the rats in different groups. Rats fed with HFD had significantly higher body weight gain than the control rats fed with the normal diet ($p< 0.05$). Further, abnormal increase in body weight gain was observed in co-administrated HFD combined alcohol intake rats. Conversely, alcohol treated rats showed a decrease in the body weight gain as compared with control rats ($p < 0.05$). However, high fat diet feeding induced liver enlargement, increasing the relative weight of somatic tissues of liver ($F = 6.685$; df = 3, 20; $p<0.05$),andkidney($F = 24.970$; df = 3, 20; $p< 0.05$), brain ($F = 33.2$; df = 3, 20; $p< 0.05$) were also increased when compared to control diet. Conversely, a significant decrease in relative weights of testes ($F = 8.682$; df = 3, 20; $p< 0.05$), caput epididymis ($F = 5.554$; df = 3, 20; $p< 0.05$),
corpus epididymis ($F = 1.009$; df = 3, 20; $p<0.05$), cauda epididymis ($F = 18.669$; df = 3, 20; $p< 0.05$), vas deferens ($F = 3.496$; df = 3, 20; $p< 0.05$), seminal vesicles ($F = 3.052$; df = 3, 20; $p< 0.05$) and prostate gland ($F = 1.828$; df = 3, 20; $p< 0.05$), penis ($F = 517$; df = 3, 20; $p< 0.05$)was observed in experimental animals when compared to control animals (Table 2).

Moreover, increased relative weights of somatic tissues of liver (-9.52% and 58.75% compared to HFD and alcohol alone treated rats), kidney (65.26% and 201.92% compared to HFD and alcohol alone treated rats), brain (7.46% and 20% compared to HFD and alcohol alone treated rats) was observed in rats treated with both HFD and alcohol, when compared to either HFD or alcohol alone treated rats. In constrast, decrease in reproductive tissues indices of testis (-36.30% and -21.87% compared to HFD and alcohol alone treated rats), caput epididymis (-13% and -21.62% compared to HFD and alcohol alone treated rats), corpus epididymis (-16.66% and -16.66% compared to HFD and alcohol alone treated rats), cauda epididymis (-16.66% and -30.00% compared to HFD and alcohol alone treated rats), seminal vesicle (-46.66% and -40% compared to HFD and alcohol alone treated rats),prostate gland (-28.57% and -23.07% compared to HFD and alcohol alone treated rats), and penis (-33.33% and -20% compared to HFD and alcohol alone treated rats) was observed in rats treated with both HFD and alcohol, when compared to either HFD or alcohol alone treated rats (Table 2).

Table 2. High fat diet (HFD) and/ or alcohol induced changes on tissue somatic indices (g%) of adult malewistar rats

Parameter	CON	HFD	AL	HFD+AL
Liver	3.2±0.06	3.6±0.44* (-12.5)	2.74±0.13* (-14.37)	4.35±1.21* (-35.93)
Kidney	0.63±0.12	0.95±0.33* -50.79	0.52±0.16* (-17.46)	1.57±0.26* (-149.2)
Brain	0.64±0.14	0.67±0.13* (-4.68)	0.60±0.20* (-6.25)	0.72±0.31* (-12.5)
Testis	2.3±0.10	1.57±0.57* (-31.73)	1.28±0.13* (-44.34)	1.00±0.43* (-56.52)
Caput Epididymis	1.16±0.13	1.00±0.01* (-13.79)	1.11±0.22* (-4.31)	0.87±0.08* (-25)
Corupus Epididymis	0.08±0.05	0.04±0.02* (-50)	0.03±0.02* (-62.5)	0.01±0.01* (-25)
Cauda Epididymis	0.19±0.02	0.06±0.02* (-68.42)	0.10±0.05* (-47.36)	0.7±0.26* (-268.42)
Seminal vesicals	0.50±0.28	0.45±0.09* (-10)	0.40±0.06* (-20)	0.24±0.10* (-52)
Prostate gland	0.16±0.05	0.14±0.02* (-12.5)	0.13±0.02* (-18.75)	0.3±0.29* (-87.5)
Penis	0.14±0.06	0.12±0.04* (-14.28)	0.13±0.02* (-7.14)	0.3±0.29* (-114.28)

CON = Control diet group; HFD = High fat diet; AL = alcohol; HFD + AL = high fat diet and alcohol intake. n=6 per group. Data are shown as mean ± S.D (Standard Deviation). Significant difference was indicated by * for $p< 0.05$ compared CON vs HFD, AL, HFD+AL (treatment groups).

3.2 Steroidogenesis and testosterone levels

The effect of HFD, alcohol, and HFD + alcohol on steroidogenic enzyme activity levels in testicular tissue was shown in Table 3. The average activity level of 3β-HSD in the testes of control rats is 20.60 ± 1.74. A significant decrease was observed in 3β-HSD activity in the testis of rats exposed to HFD alone (-35.92%), alcohol alone (-21.99%) and co-administration of HFD and alcohol (-65.87%) when compared to that of controls (Table 4, Fig. 4a; $p < 0.05$). Similar trend was observed in 17β-HSD activityalso in the testis of rats exposed to HFD alone (-48.86%), alcohol alone (-39.59%) and co-administration of HFD and alcohol (- 60.82%) relative to control rats.However, the decrease in the enzyme activities of 3β- (-46.74% and -56.24% compared to HFD and alcohol alone treated rats) and 17β-HSD (-22.39%) and -99.35% compared to HFD and alcohol alone treated rats) is much more pronounced in alcoholic rats treated with HFD (Table 3). The mean serum concentration of testosterone was significantly (F = 9.801; df = 3, 20; $p< 0.05$) lower in the HFD control and alcohol-exposed groups when compared with the control group. Additionally, further reduction in serum testosterone levels (-26.08% and -33.03% compared to HFD and alcohol alone treated rats) was observed in HFD-treated alcoholic rats (Table 3).

Table 3. Effect of High fat diet (HFD) and/or alcohol on 3β- and 17β-HSD activity levels in the testis and serum levels of testosterone in adult male wistar rats

Parameter	Contorl	HFD	Alcohol	HFD+Alcohol
Testosterone (ng/mL)	2.83±0.61	1.67±0.51 (-40.98)	2.45±0.57 (-13.42)	1.09±0.69 (-61.48)
3β-HSD (nmol of NADPH converted to NADP/mg protein/min)	20.60±1.74	13.20±0.82 (-35.92)	16.07±0.74 (-21.99)	7.03±0.35 (-65.87)
17β-HSD (nmol of NADPH converted to NADP/mg protein/min)	16.72±0.88	8.55±1.46 (-48.86)	10.10±1.19 (-39.59)	6.55±1.33 (-60.82)

CON = Control diet group; HFD = high fat diet; AL = alcohol; HFD + AL = high fat diet and alcohol intake. Data were expressed as mean ± Standard Deviation (S.D), n= 6 individuals per group. * $p< 0.05$. *Compare with respective CON vs treatment groups was done using one way ANOVA followed by *post hoc* test of tukey multiple comparsion test.

3.3 Sperm parameters

The sperm parameters such as daily sperm production (F = 84.515; df = 3, 20; $p< 0.05$) and sperm count (F = 918.556; df = 3, 20; $p <0.05$), sperm motility (F = 852.124; df = 3, 20; $p <0.05$), sperm viability (F = 1286.389; df = 3, 20; $p <0.05$), HOS (F = 381.088; df = 3, 20; $p <0.05$) sperm in HFD or alcohol treated rats were significantly decreased when compared to control rats. (Table 4).

Table 4. Effect of High fat diet (HFD) and/ or alchol on sperm production in testis and sperm quantity and quality in cauda epididymis of adult male wistar rats

Parameter	CON	HFD	AL	HFD+AL
DSP (millions/g testis)	19.83±3.10	9.13±0.60* (-53.95)	9.03±0.74* (-54.46)	5.62±0.58* (-71.65)
Sperm count (millions/mL)	72.23±1.01	41.02±1.03* (-43.2)	43.47±1.87* (-39.81)	22.07±2.37* (-69.44)
Motile Sperm (%)	75.29±2.86	41.37±0.94* (-45.05)	40.18±0.12* (-46.63)	31.50±1.18* (-58.16)
VilableSperm (%)	74.73±1.48	47.15±0.55* (-36.9)	51.30±1.37* (-31.35)	32.11±1.20* (-57.03)
HOS-tail coiled sperm(%)	71.65±4.97	35.21±0.41* (-50.85)	37.61±0.43* (-47.5)	25.40±0.72* (-64.54)

CON = Control diet group; HFD = High fat diet; AL = alcohol; HFD + AL = high fat diet and alcohol intake. DSP = Daily sperm production; HOS-tail coiled sperm = Hypo-osmotic swelling (HOS). Data were expressed as mean ± Standard Deviation (S.D), n= 6 individuals per group. * $p< 0.05$. *Compare with CON vs treatment groups was done using one way ANOVA followed by post hoc test of tukey HSD multiple comparsion test.

Sperm analysis showed difference between HFD and alcohol treated rats. Furthermore, decline in the daily sperm production (-38.44% and -37.76% compared to HFD and alcohol alone treated rats, sperm count (- 46.19% and -49.22% compared to HFD and alcohol alone treated rats), motile sperm (-23.85% and -21.60% compared to HFD and alcohol alone treated rats),viable sperm (-31.89% and -37.40% compared to HFD and alcohol alone treated rats), HOS-tail swelled sperm (-27.86% and -32.46% compared to HFD and alcohol alone treated rats) was observed in rats co-administrated with HFD and alcohol when compared to either HFD or alcohol treated rats was shown in Table 4.

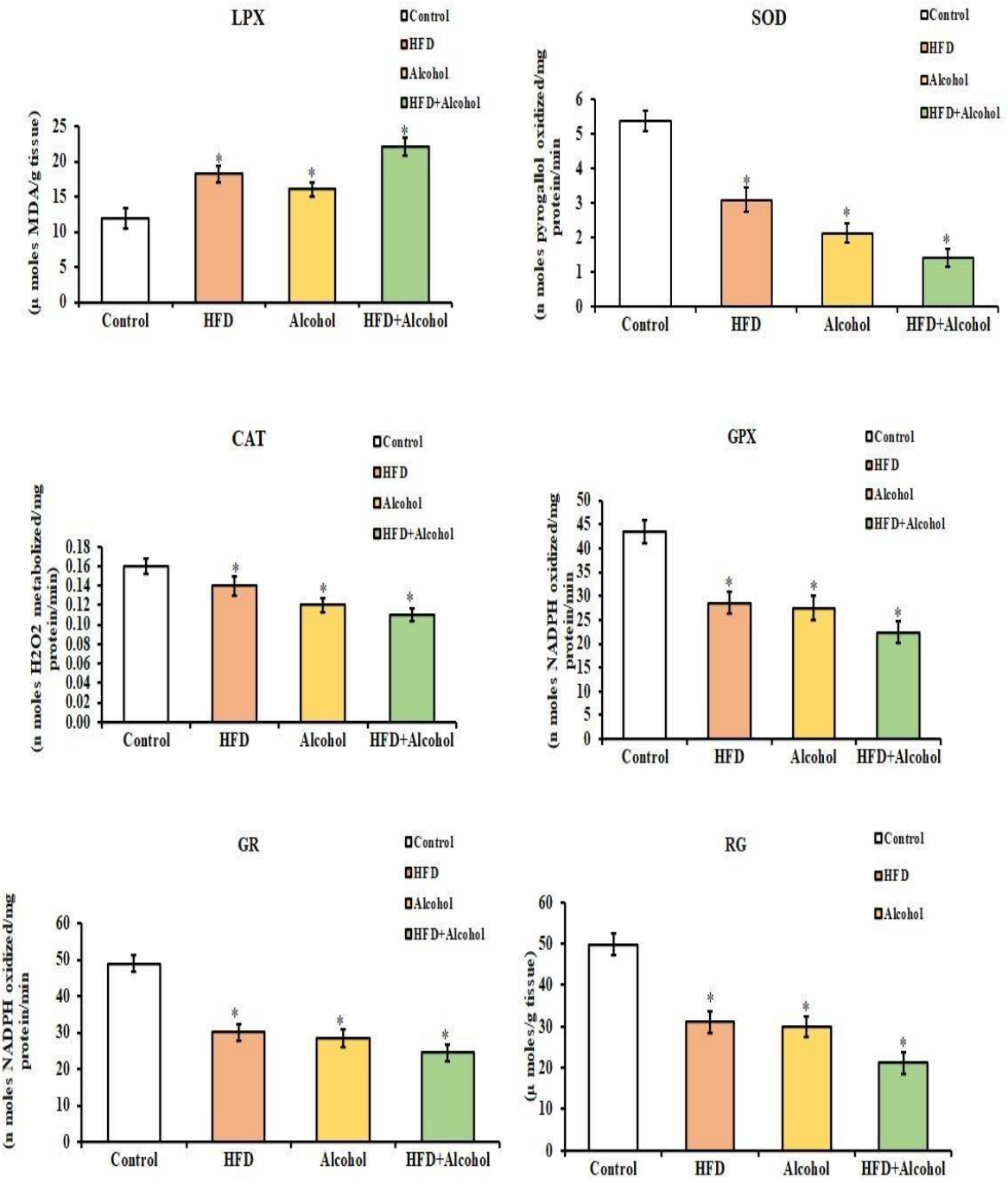
3.4 Epididymal and testicular antioxidant activities

The activity levels of antioxidant enzymes such as superoxide dismutase (F = 41.106 for testis; F = 14.937 for caput epididymis; F = 80.426 for corpus epididymis; and F = 165.169 for cauda epididymis; df = 3, 20; p <0.05*)*, catalase (F = 15.074 for testis; F = 95.418for caput epididymis; F = 99.054 for corpus epididymis; and F = 149.333 for cauda epididymis; df = 3, 20; p <0.05*)*, glutathione peroxidase (F = 4085.187 for testis; F = 132.817 for caput epididymis; F = 731.754 for corpus epididymis; and F = 998.657 for cauda epididymis; df = 3, 20; p <0.05*)*, glutathione reductase (F = 1379.639 for testis; F = 605.364 for caput epididymis; F = 80.216 for corpus epididymis; and F = 28.587 for cauda epididymis; df = 3, 20; p <0.05*)*, reduced glutathione (F = 124.077 for testis; F = 115.062 for caput epididymis; F = 289.263 for corpus epididymis; and F = 48.631 for cauda epididymis; df = 3, 20; p <0.05*)* were significantly decreased in the testis, caput epididymis, corpus epididymis, and cauda epididymis of HFD or alcohol administered rats when compared with the control rats (Figures 1-4).

Co- administration of HFD and alcohol resulted in further decrease in the activity levels superoxide dismutase (-54.36% and -33.80% compared to HFD and alcohol alone treated rats intestis, -45.65%and-22.48%comparedtoHFDandalcoholalonetreatedratsincaputepididymis 37.03% and -26.08% compared to HFD and alcohol alone treated rats in corpus epididymis, and –53.15% and -32.85% compared to HFD and alcohol alone treated rats in cauda epididymis), catalase (-37.58% and -45.86% compared to HFD and alcohol alone treated rats in testis; -23.25% and -10.94% compared to HFD and alcohol alone treated rats in caput epididymis;
-15.90% and – 12.2% compared to HFD and alcohol alone treated rats in corpus epididymis, and -91.98% and - 92.61% compared to HFD and alcohol alone treated rats in cauda epididymis), glutathione peroxidase (-18.20% and -14.86% compared to HFD and alcohol alone treated rats in testis; - 43.87% and -17.24% compared to HFD and alcohol alone treated rats in caput epididymis, - 66.17% and -60.35% compared to HFD and alcohol alone treated rats in corpus epididymis; and – 27.99% and -19.08% compared to HFD and alcohol alone treated rats in cauda epididymis) and glutathione reductase (-18.20% and -14.86% compared to HFD and alcohol alone treated rats in testis; -46.58% and -32.94% compared to HFD andalcoholalonetreated rats in caputepididymis;- 65.61%and-60.44%comparedtoHFDandalcoholalonetreatedratsincorpusepididymis;and -20.27% and -27.84% compared to HFD and alcohol alone treated rats in cauda epididymis) when compared to HFD or alcohol alone treated rats.

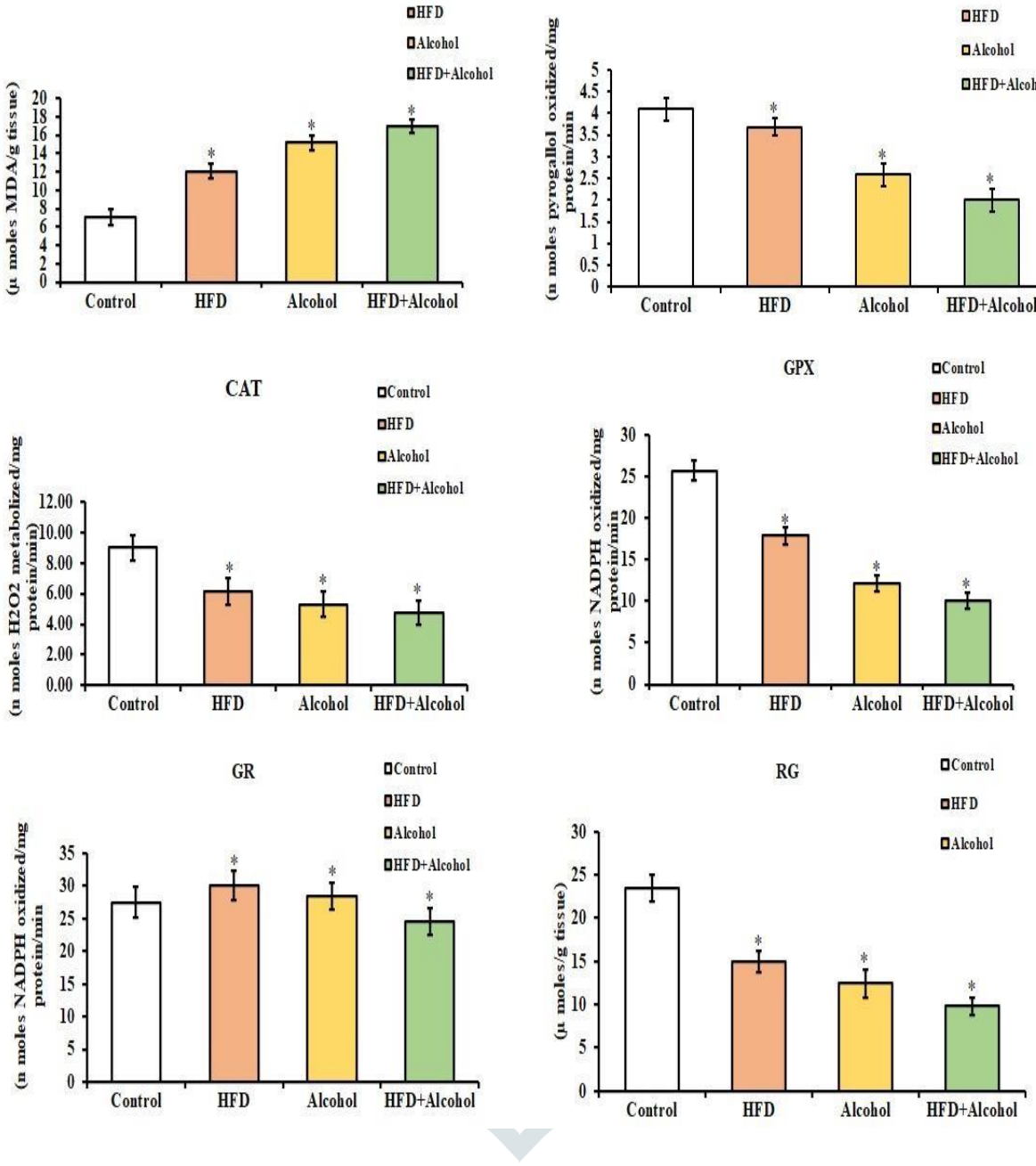
Lipidperoxidation(F=63.933intestis,F=62.954incaput,F=45.022 in corpus, and F = 147.594 in cauda epididymis; df = 3, 20; p <0.05*)* were significantly elevated in testis, caput, corpus, and cauda epididymal regions of HFD or alcohol treated rats (Tables 5-8). Further, co-administration of HFD and alcohol resulted in a further increase in lipid peroxidation (21.66% and 38.27% in the testis, 41.12% and 12.19% in caput epididymis; 36.43% and 63.20% in corpus epididymis; 70.98% and 98.67% in cauda epididymis, compared to HFD and alcohol alone treated rats) when compared to HFD or alcohol treated rats. Additional reduction in glutathione level was observed in testis (-31.98% and -29.41% compared to HFD and alcohol alone treated rats), caput epididymis (-34.55% and -21.17% compared to HFD and alcohol alone treated rats), corpus epididymis (-21.51% and -34.96% compared to HFD and alcohol alone treated rats), and cauda epididymis(-70.98% and - 98.67% compared to HFD and alcohol alone treated rats) of rats co- administered with HFD and alcohol (Figure 1-4).

Figure 1. Effect of high fat diet (HFD) and / or alcohol on pro- and anti-oxidant system in the testis ofadult male wistar rats.



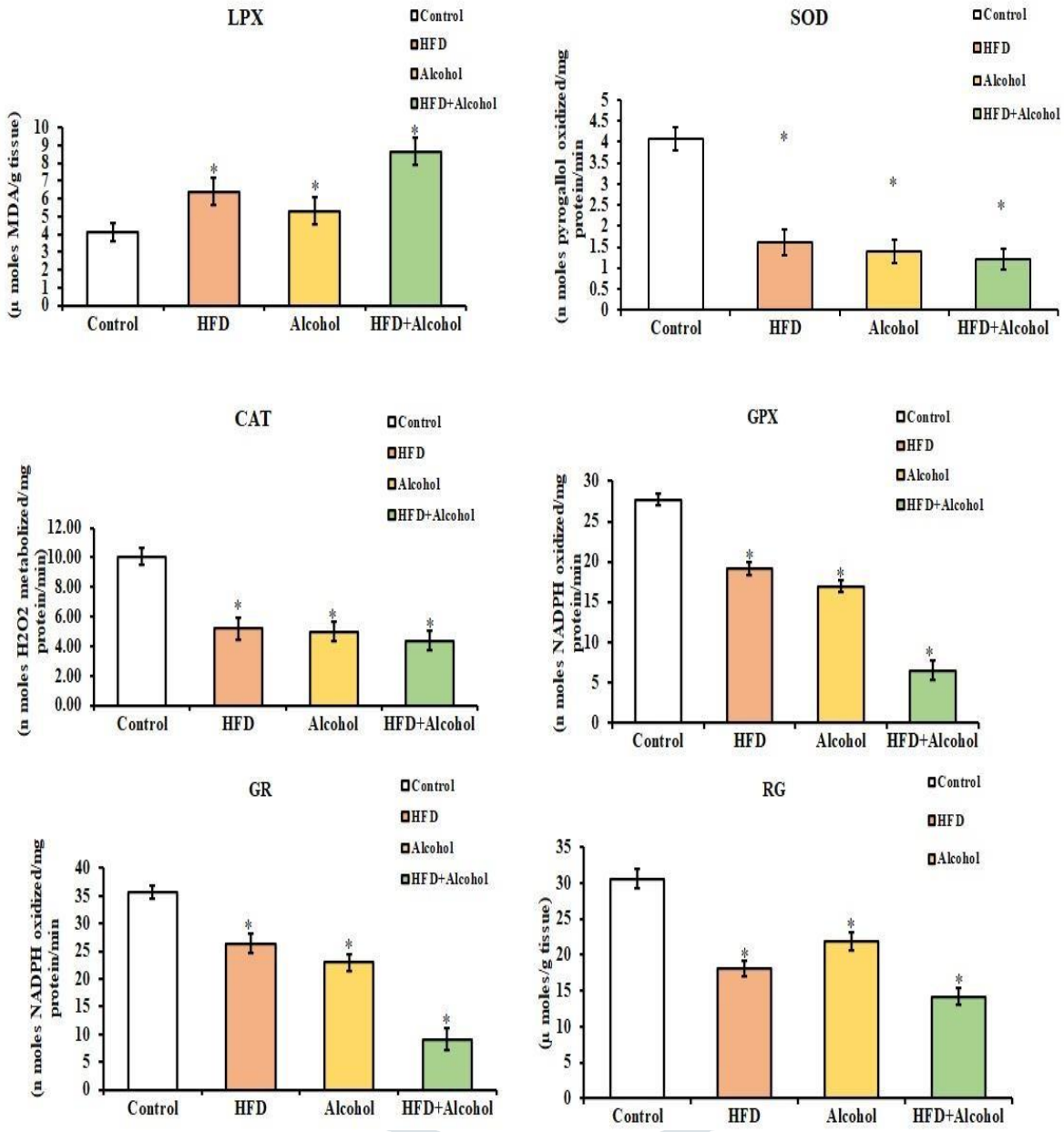
Data were analyzed by One-way ANOVA followed by Tukey HSD *post hoc* test. Bars are Mean ± standarddeviation (n = 6); Asterisks (*) indicates significant difference between control and treament groups at $p < 0.05$.

Figure 2. Effect of high fat diet (HFD) and / or alcohol on pro- and anti-oxidant system in the caput ofadult male wistar rats.



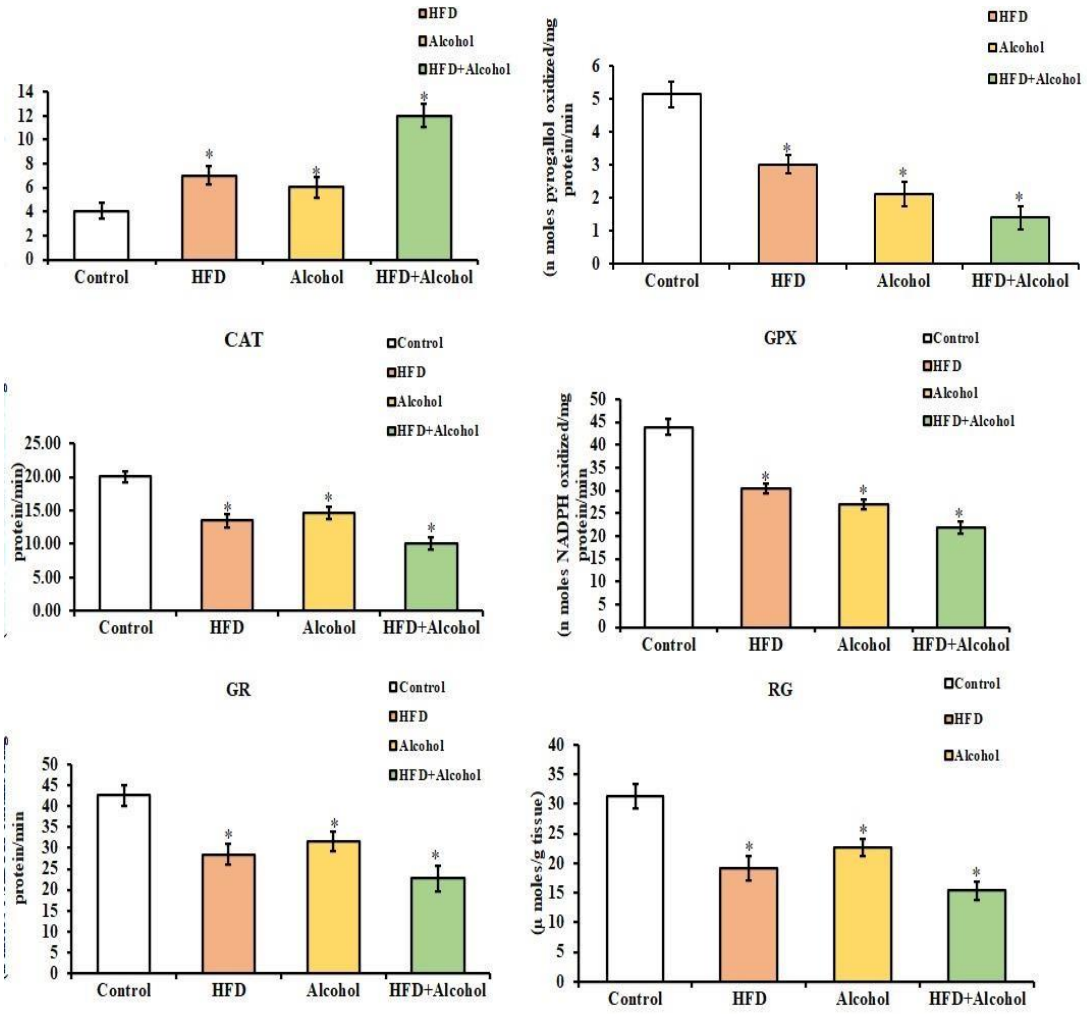
Data were analyzed by One-way ANOVA followed by Tukey HSD *post hoc* test. Bars present Mean ± standarddeviation (n = 6); Asterisks (*) indicates significant difference between control and treament groups at $p < 0.05$.

Figure 3. Effect of high fat diet (HFD) and / or alcohol on pro- and anti-oxidant system in the corpus of adult male wistar rats



Data were expressed as the mean ± Standard Deviation (S.D) (n=6). Data were analyzed by the one-way ANOVA followed by Tukey *post hoc* test using the compare mean feature in SPSS (SPSS Inc., Chicago, IL, United State). Asterisks (*) indicates significant difference between control and treated groups at $p < 0.05$.

Figure 4. Effect of high fat diet (HFD) and / or alcohol on pro- and anti-oxidant system in the cauda of adult male wistar rats.

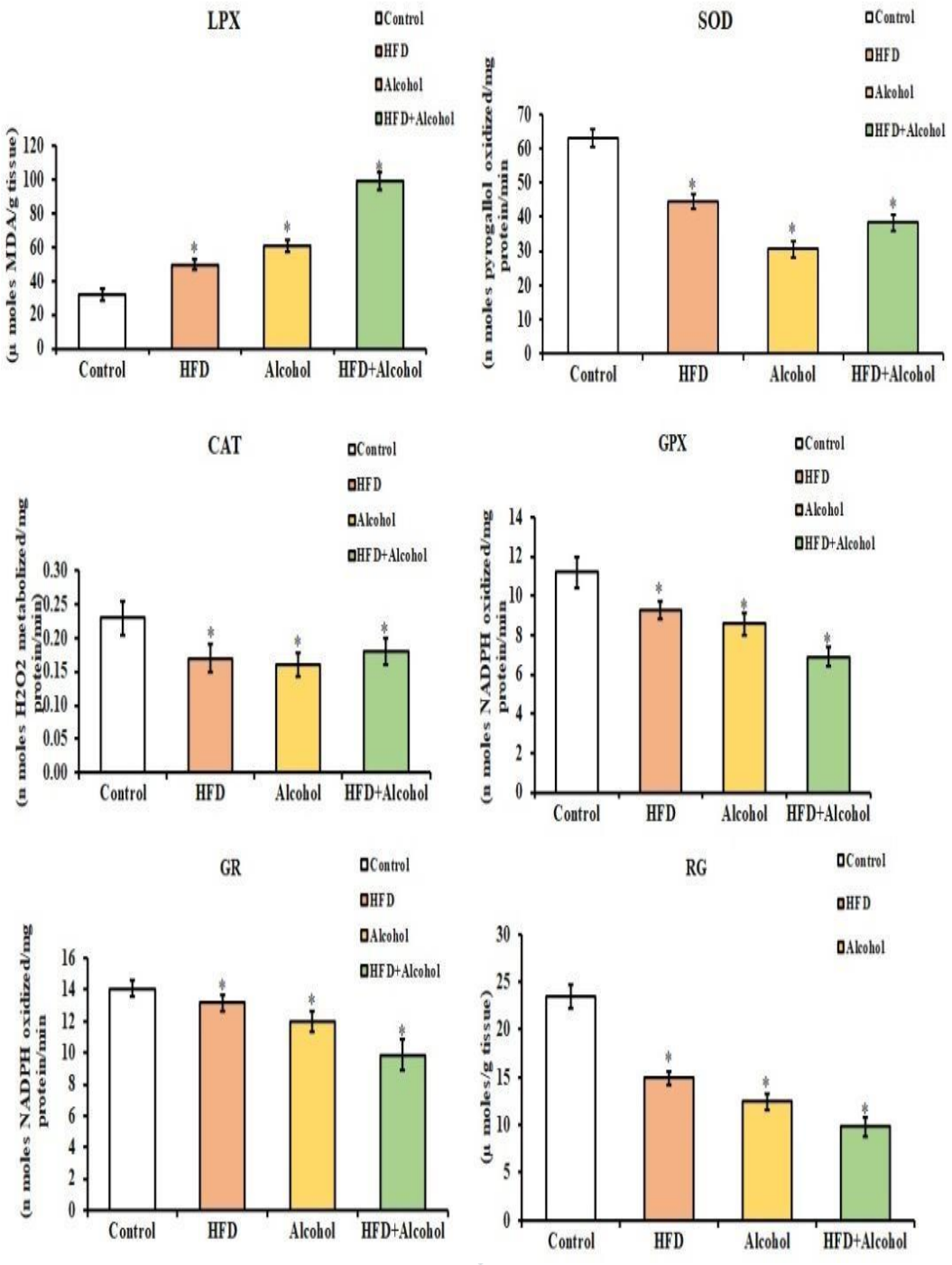


Data were expressed as the mean ± Standard Deviation (S.D) (n=6). Data were analyzed by the one-way ANOVA followed by Tukey *post hoc* test using the compare mean feature in SPSS (SPSS Inc., Chicago, IL, United State). Asterisks (*) indicates significant difference between control and treated groups at $p < 0.05$.

3.5 Antioxidant activities in liver and kidney

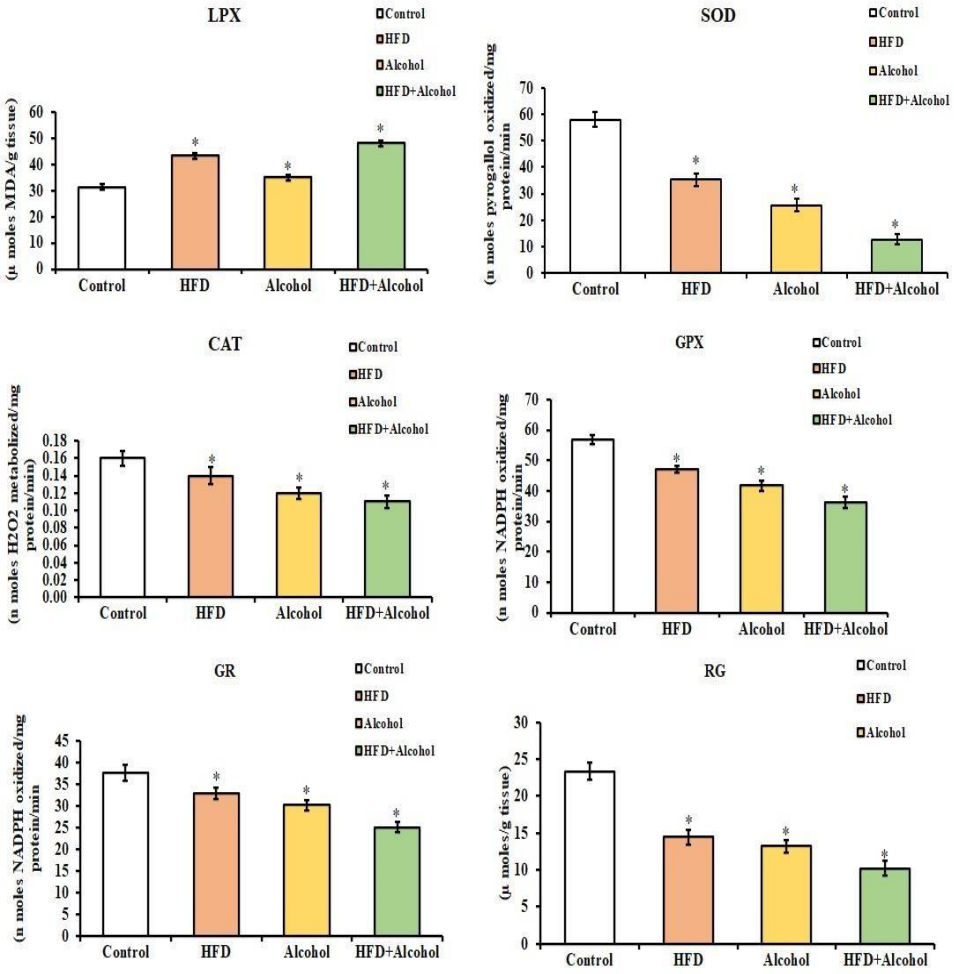
The activity levels of antioxidant enzymes such as superoxide dismutase (*F* = 453.187liver;*F*= 135.605;df = 3, 20; *p* <0.05*)*, catalase (*F* = 11.544 for liver;*F*= 42.274 for kidney;df = 3, 20; *p* <0.05*)*, glutathione peroxidase (*F* = 56.155 for liver;*F*= 67.063 for kidney;df = 3, 20; *p* <0.05*)*, glutathione reductase (*F* = 26.199 for liver;*F*= 321.406 for kidney;df = 3, 20; *p* <0.05*)* were significantly decreased in the liver of HFD rats or alcohol administered rats when compared with the control rats.On the other hand, lipid peroxidation (*F*=6257.13inliver;*F*=396.178inkidney;df = 3, 20; *p* <0.05)were significantly elevated in liver and kidney of HFD treated rats or alcohol treated rats (Figure 5-6).Further, co-administration of HFD and alcohol resulted in a further decrease in the activity levels superoxide dismutase (-13.93 % and 25.36 % compared to HFD and alcohol alone treated rats in liver; -63.85 % and % compared to HFD and alcohol alone treated rats in kidney) catalase (5.88 % and 12.5% compared to HFD and alcohol alone treated rats in liver; 13.93 % and 25.36 % compared to HFD and alcohol alone treated rats in kidney), glutathione peroxidase (-25.5% and -19.3% compared to HFD and alcohol alone treated rats in liver; 23% and -13.5 % compared to HFD and alcohol alone treated rats in kidney) and glutathione reductase (-25.09 % and -17.91% compared to HFD and alcohol alone treated rats in liver; 23.81% and -16.81% compared to HFD and alcohol alone treated rats in kidney). Additional reduction in glutathione level was observed inliver (-34.55%and -21.17% compared to HFD and alcohol alone treated rats) andkidney (-34.55 % and 21.17 % compared to HFD and alcohol alone treated rats in kidney)(Figure 5-6).

Figure 5. Effect of high fat diet (HFD) and / or alcohol on pro- and anti-oxidant system in the liver of adult male wistar rats.



Data were expressed as the mean ± Standard Deviation (S.D) (n=6). Data were analyzed by the one-way ANOVA followed by Tukey *post hoc* test using the compare mean feature in SPSS (SPSS Inc., Chicago, IL, United State). Asterisks (*) indicates significant difference between control and treated groups at $p < 0.05$

Figure 6. Effect of high fat diet (HFD) and / or alcohol on pro- and anti-oxidant system in the kidney of adult male wistar rats.



Data were expressed as the mean ± Standard Deviation (S.D) (n=6). Data were analyzed by the one-way ANOVA followed by Tukey *post hoc* test using the compare mean feature in SPSS (SPSS Inc., Chicago, IL, United State). Asterisks (*) indicates significant difference between control and treated groups at $p < 0.05$

IV. DISCUSSION

Previous studies have reported adverse consequences of alcohol alone or high fat diet alone consumption on male reproductive system[27, 28]. The data in the present study addressed the concern of possible interaction and resultant effect of high fat diet and alcohol on male reproductive toxicity was to evaluate this effect in male wistar rats.

The present work has shown that rats exposed with HFD alone (contains high calorie lard-based diet - 60% kcal from fat) or combined with alcohol exhibited significant higher body weight and relative weights of somatic tissues liver, kidney and brain of rats compared to control rats. These finding suggest that HFD alone treated rats exhibited higher bodyweight and also gain in organ weight in liver, kidney, brain indicaing that index of obesity. Conversely, the relative weights of the testis and accessory reproductive organs decreased significantly in experimental groups as compared to control. The morphology and functional integrity of the testis and accessory sex organs are dependent on availability of androgens. Decrease in the serum level of testosterone may be a reason behind the significant decline observed in the weights of testis and accessory sex organs in rats subjected to high fat diet. The decrease in the weights of testis and accessory sex organs is more pronounced in rats subjected to high fat diet or combined alcohol.

Moreover, administration of alcohol alone caused a reduction in the body weight, relative weights of testes, and accessory sex organs compared to control. The results are in agreement with the findings of earlier reports [29] who observed that ethanol treated rats depressed the body weight in male rats compared with untreated rats. In constrast, some authors showed that rats exposed to alcohol (ethanol: 2g/kg) did not alter body weight gain. This disparity finding suggests thatHFD alone or alcohol alone effect on body weight gain, relative weight of somatic and reproductive organs [30,31]. However, HFD-induced obese rats combined alcohol intake resulted in vulnerable targets for reproductive organs which leads to reproductive toxcity. Thus, these novel effect may be synergetic effect.

The data also revealed that a statistically significant reduction in the daily sperm production (DSP) and lower sperm motility, sperm viability, altered sperm morphology in the rats treated with HFD alone compared to control. Our results are consistent with the earlier reports that HFD induces obesity which deteriorated sperm quality and suppressed spermatogenesis [28]. However, some studies demonstrated that HFD treated rats resulted in more fat accumulation in the scrotum causes increase ROS levels resulting in impairment of spermatogenesis [31]. Several toxicology studies proved that alterations in sperm parameters of animal exposed to alcohol treated rats. It was found that rat exposed ethanol 25% v/v (3 g/kg) for 30 days which leads to decrease in daily sperm production, sperm mobility, sperm count in the testis and corpus, caput epididymis [29,32]. In the same manner, we also found that rats exposed to alcohol dose of 2.0 g/kg/bw/day could significantly reduce

testicular daily sperm production, epididymal total, sperm motile and sperm viability. In addition, there was a statistically significant reduction in the numbers of swelled sperm exposed to hypo- osmotic solution in the rats exposed to alcohol compared to the control group. Co-administration of HFD plus alcohol treated rats also displayed same trend of alterated of sperm parameters compared to control. This finding is novel and significant as it demonstrates that HFD induced obesity combined alcohol intake can adversely affect sperm parameters. Their combination also exhibited a synergistic inhibitory effect resulted in poor sperm quality.

In the present study, there was a statistically significant decrease in the activity levels of 3β- and 17β- HSD in the testes, associated with significantly reduced serum testosterone level in rats fed on HFD, indicating a probable inhibition of androgen synthesis in experimental rats. Likewise the previous reports [28,33], who found that decreased levels of testosterone in serum,and 3β- and 17β- HSD in testes. This observation supported by Myunggi who suggest that HFD treated rats showed testosterone deficiencystimulate increase adiposity which causes regulation of sex hormone production [34]. Furthermore, decreased serum testosterone levels and activity levels of 3β- and 17β- HSD in the testes was observed in rats exposed alcohol. These results are consisent with earlier studies [35,36] who concluded that acute administration of ethanol dose at level range from 1.5 -2.0 g/kg resulted in decreased serum testosterone level in male Sprague-Dawley (SD) rats. However, in co-administred rats further reduction of level of testosterone in serum and lower activity levels of 3β- and 17β- HSD in the testes. This novel observation suggests the synergetic effect of alcohol and HFD on spermatogenesis, inducing oxidative toxicity in testis and epididymis.

In this study, we observed that HFD or alcohol treatment caused a significant decrease in activities of superoxidase dimustase, catalase, glutathione peroxidase, glutathione content with an increase in the levels of lipid peroxidation in the testes and epidiymides[27,28]. Interestingly, a similar observation was made in liver and kidney tissues[37,38].In addition, the present also investigated that obese rats combined with alcohol intake was able to further increase the oxidative stress in testicular and epididymal tissues as manifested from increased MDA and ROS levels, suppressed antioxidant enzymes (SOD, CAT, GSH-Px, and GR) activities in the testicular tissue, different parts of epididymis and also in liver and kidney. These results suggest that HFD induced obese rat combined with alcohol intake resulted in increased oxidative stress in the liver, kidney, testis and epididymis, which leads to impaired metabolic functions in liver,kidney and reproductive tissues and thereby suppressed spermatogenesis and steroidogenesis in the testes.

REFERENCES

[1]. J. M. Rippe, S. Crossely, R Ringer, Obesity as a chronic disease: modern medical and lifestyle management, J Am Diet Assoc. 1998, 98, S9-S15.

[2]. H. Ashrafi, K. Ghabiji, A. Alihemmati, A. Jouyban. The effect of quince leaf (Cydonia oblonga miller) decoction on testes in hypercholesterolemic rabbits. Afr. J. Tradit, Complementary Altern. Med. 2013, 10, 277-282.

[3]. M. Pinto-Fochi, E. Pytlowanciy, V. Reame, A. Rafacho, D. Ribeiro, S. Taboga, R. Goes, A high-fat diet fed during different periods of life impairs steroidogenesis of rat Leydig cells. Reproduction. 2016, 152, 795-808.

[4]. M. Gomez-Elias, T. Caceres, M. Giaccagli, V. Guazzone, G. Dalton, A. De siervi, V. Da Ros, Association between high-fat diet feeding and male fertility in high reproductive performance mice. Sci. Rep. 2019, 9

[5]. A. A. Mac Donald, G.P. Herbison, M. Showell& C. M. Farquhar. The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. Human Reproduction Update 16 293-311.

[6]. C. D. B. Fernandez, F. F. Bellentani, G. S. A. Fernandes, J. E. Perobelli, A. P. A. Favareto, A. F. Nascimento, A. C. Cicogna& W. D. G. Kempinas. Diet-induced obesity in rats leads to a decrease in sperm motility. Reproductive Biology and Endocrinology 9, 32.

[7]. R. Uygur, M. Yagmurca, O. A. Alkoc, A. Genc, A. Songur, K. Ucok, O. A. Ozen, Effects of quercetin and fish n-3 fatty acids on testicular injury induced by ethanol in rats. Andrologia, 625, 1-14.

[8]. K. Zhang, Z. Ly, X, Ji, D. Huang, Melatonin prevents testicular damage in hyperlipidaemic mice, Andrologia, 2012, 44: 230-236

[9]. S. F. P. Mesquita, M. F. Barbieri, E. V. Fernandes, F. G. Andrade, N. R. Arrebola, Effects of swimming on the testicular histomorphology of alcoholized rats. Acta Sci Health Sci. 2011, 35, 37-42.

[10]. P. Hari Priya, B. P. Girish and P. S. Reddy, Restraint stress exacerbates alcohol-induced reproductive toxicity in male rats, Alcohol, 2014, 48, 781-786.

[11]. S. D. F. P. Mesquita, M. F. Barbieri, E. V. Fernandes, F. G. Andrade and N. R. Arrebola, Effects of swimming on the testicular histomorphology of alcoholized rats, Acta. Scie. Health Sci., 2012, 35, 37-42.

[12]. R. Harikrishnan, P. A. Abhilash, S. Syam Das, P. Prathibha, S. Rejitha, F. John, S. Kavitha and M. Indira, Protective effect of ascorbic acid against ethanol-induced reproductive toxicity in male guinea pigs, Br. J. Nutr., 2013, 110, 689-698.

[13]. O. O. Dosumu, A. A. A. Osinubi and F. I. O. Duru, Alcohol induced testicular damage: Can abstinence equal recovery?, Middle East Fertil. Soc. J., 2014, 19, 221-228.

[14]. A. K. Pandey, N. Sapkota, A. Tambi and P. M. Shyangwa, Clinico-demographic profile, sexual dysfunction and readiness to change in male alcohol dependence syndrome inpatients in a tertiary hospital, Nepal Med. Coll. J., 2012, 14, 35-40.

[15]. Uygur, R., Yagmurca, M., Alkoc, O.A., Genc, A., Songur, A., Ucok, K., Ozen, O.A., 2013. 624 Effects of quercetin and fish n-3 fatty acids on testicular injury induced by 625 ethanol in rats. Andrologia 1–14.

[16]. CPCSEA, CPCSEA guidelines for laboratory animal facility, Indian J. Pharmacol., 2003, 35, 257-274 [17]. M. A. Belsey, K. S. Moghissi, R. Eliasson, C. A. Paulsen, A. J. Gallegos and M. R. Prasad, Laboratory manual for the examination of human semen and semen cervical mucus interaction, Press Concern,Singapore, 1980.

[18]. P. Talbot and R. S. Chacon, A triple stain technique for evaluating normal acrosome reaction of human sperm, J. Exp. Zool., 1981, 215, 201-208.

[19]. R. S. Jeyendran, H. H. Van-Der-Ven and L. J. D. Zaneveld, The hypo-osmotic swelling test: An update, Arch. Androl., 1992, 29, 105-116.
[20]. H. U. Bergmeyer, Methods of enzymatic analysis, ed. H. U. Bergmeyer, Academic Press, New York, 1974,pp. 447-489.
[21]. H. Ohkawa, N. Ohishi and K. Yagi, Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction, Anal. Biochem., 1979, 95, 351-358.
[22]. S. Marklund and G. Marklund, Involvement of superoxide anion radical in autooxidation of pyragallol and a convenient assay for superoxide dismutase, Eur. J. Biochem., 1974, 47, 469- 474.
[23]. A. Claiborne, Handbook of methods for oxygen radical research, ed. R. Greenwald, CRC Press, Florida, 1985, pp. 283–284.
[24]. D. E. Paglia and W. N. Valentine, Studies on quantitative and qualitative characterization of erythrocyte glutathione peroxidase, J. Lab. Clin. Med., 1967, 70, 158-169.
[25]. I. Carlberg and B. Mannervik, Purification and characterization of the flavoenzyme glutathione reductase from rat liver, J. Biol. Chem., 1975, 250, 5474-5480.
[26]. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem., 1951, 193, 265-275
[27]. G. E. Siervo, H. R. Vieira, F. M. Ogo, C. D. Fernandez, G. D. Goncalves, S. F. Mesquita, J. A. Anselmo- Franci, R. Cecchini, F. A. Guarnier, G. S. Fernandes, Spermatic and testicular damages in rats exposed to ethanol: influence of lipid peroxidation but not testosterone, Toxicology, 330: 1-8.
[28]. Er Hui Wang, Zeng Li Yu, Yong Jun Bu, Peng Wei Xu, Jin Yan Xi and Hai Yan Liang, Grape seed proanthocyanidin extract alleviates high-fat diet induced testicular toxicity in rats. RSC Adv., 2019, 9, 11842-11850
[29]. Oliva, S.U., Messias, A.G., Silva, D.A., Pereira, O.C., Gerardin, D.C., Kempinas, W.G., 2006. Impairment of adult male reproductive function in rats exposed to ethanol since puberty. Reprod. Toxicol. 22 (4), 59
[30]. H. A. Jeng and S. M. Bocca, Influence of exposure to benzo[a]pyrene on mice testicular germ cells during spermatogenesis, J. Toxicol., 2013, 2013, 387850
[31]. A. Ashok, D. Sulagna, Obesity, Male infertility,2020, 497-508.
[32]. V. Srikanth, T. Malini, P. Govindarajulu, K. Balasubramanian, Effects of ethanol ingestion on epididymal glycosidases and fertility in the rat. Int. J. Andol., 21, 343-35.
[33]. E. M. Pinto-Fochi, E. Z. Pytlowanciv, V. Reame, A. Rafacho, D. L. Ribeiro, S. R. Taboga, R. M. Goes. A high-fat diet fed during different periods of life impairs steroidogenesis of rat Leydig cells. Reproduction, 2016, 152: 795-808.
[34]. B. Myunggi, J. Jin Young, J. P. Seung, P. Y. Seon, L. Jin Oh, L. S. Jae, M. N. Haque and H. Jeong Lee, Testosterone deficiency caused by castration increases adiposity in male rats in a tissue-specific and diet- dependent manner
[35]. T. J. Cicero, R. D. Bell, E. R. Meyer, T. M. Badger, Ethanol and acetaldehyde directly inhibit testicular steroidogenesis. J. Pharmacol. Exp. Ther, 1980, 213: 228-233
[36]. C. Rivier, Alcohol rapidly lowers plasma testosterone levels in the rat: evidence that a neural brain-gonadal pathway may be important for decreased testicular responsiveness to gonadotropin. Alcohol Clin Exp Res. 1999, 23: 38-45.
[37]. J. Kanokwan, J. Nattharat, P. Charinva, W. Chatuphonprasert, A high-fat, high-fructose diet induces antioxidant imbalance and increase the risk and progression of nonalcoholic fatty liver disease in mice, Scientifica, 2016, 10.
[38]. R. S. Vijaya kumar, D. Surya, N. Nalini, Antioxidant efficacy of black pepper (Piper negrum L.) and piperine in rats with high fat diet induced oxidative stress. Redox Rep. 2004, 105-10.