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Research Article

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Histological and Physiological Studies on the Effects of Some Energy Drinks on Male Rats

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ABSTRACT

Consumption of Energy drinks (ED) rich in caffeine has been increasing significantly recently between individuals. Health problems related to caffeine such as reproductive diseases were occurred. Therefore, this research aim to study the histological effects of ED on rats' testis, oxidative profile interfering with organ functions, and the possible reversibility of the produced effects. Adult male rats were divided into five groups, these were; Group1 served as control, Groups 2,3,4 and 5 recieved orally either Red Bull or Power horse for 7 weeks. Groups3 and 4 were left for 2 weeks to recover from energy drinks treatment. Blood samples were taken for determination of serum testosterone and tissue malondialdehyde and reduced glutathione were estimated in homogenised testes. Rat testes in ED treated groups showed degenerated germinal epithelium with interstitial edema, dilated congested blood vessels, decreased glutathione, and increased MDA levels. The mean of serum testosterone was also significantly decreased However, testes of recovery Groups had nearly normal histological structure. on conclusion, ED have an effect on testicular structures and functions which were recovered to almost normal when animals were allowed living without further ED. So it should be taken under restricted precautions.

Key words: *Energy Drinks, Testis, Testosterone, Malondialdehyde, Glutathione.*

INTRODUCTION

Nowadays, energy drinks consumers especially young individuals are increasing which lead to Caffeine abuse. The term of Caffeine is the central of nervous system (CNS) that stimulate methylxanthine class (10). Globally, Caffeine recorded as a most consumed psychoactive drug while it has both positive and negative health effects. In addition, some studies show Caffeine negative health effects such as heart problem, psychiatric problems and reproductive diseases [1]. The main mechanism action of Caffeine is focus on the antagonism of adenosine receptors [2]. Thus, the molecular structure of adenosine is like Caffeine, which both have a double bond structure, while Caffeine can occur the adenosine receptor sites [3].

On the other hands, the male reproductive system has been influenced by Adenosine and its antagonists [4]. Resulting of some studies that have been established Sertoli cells (SCs) is where adenosine receptors are presence [5], and where these cells able to modulated via adenosine and its analogues [6]. The responsibility for testis and hence functions that express male phenotype is the somatic SCs [7]. Also, they can metabolize some substrates as glucose which leads to support lactate supply to increase germ cells [8]. The general view of SCs functions is the essential key to occur normal spermatogenesis. However, in testis, first the SCs is produce high rates of lactate then, any following deregulation will lead to oxidative stress (OS) at high level at the end will cause male infertility or subfertility [9].

To sum, the increase studies number on male reproductive system at laboratory animal have been showed the toxic effect of caffeine on it. For example, in adult animals, reduced spermatogenesis because of Caffeine effected on testicular atrophy [10]. The effect extended to testis that become smaller and lighter, while in Leydig cell the production of testosterone was decreased. The overall, Caffeine leads to lose weight and reduce both mass and fat body [11].

The aim of this study was to determine the histological and physiological effects of energy drinks (named Red bull, Power horse) on rat testis. This study is also considered as a trial to throw some light on the possible mechanism by which energy drinks might affect the oxidative profile interfering with organ functions. Nevertheless, the possible reversibility of the produced effects was a main concern in this study.

MATERIALS AND METHODS

Animals

Fifty adult male Sprague-Dawley rats (150 and 200 g) obtained from the Animal House of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, KSA. Rats were housed in filter-top polycarbonate cages (five animals/cage) under standard temperature (24 ± 2ºC), humidity (55 ± 5%) and lighting (12 h: 12h Light: Dark) conditions for 1 week before the experiment to acclimatize. Food formed of Purina rat chow and drinking water supplied ad libitum. All animal procedures conducted in accordance with the Committee of Animal Investigations, Faculty of Medicine, King Abdulaziz University.

Energy drinks

Red Bull

100 ml of Red Bull contains: taurine (400 mg), sucrose and glucose (11.3 g), B12 (0.4mcg), caffeine C8H10N4O2 (32mg), gluconolactone (240 mg), niacin (7.2 mg), B6 (0.8 mg), pantenol (2.4 mg), inositol (20 mg), B2 (0.64 mg), artificial flavoring and sparkling water.

Power Horse

Energy drink (Power Horse ®) containing: Carbonated water, sucrose, glucose, citric acid, Glucuronolactone, Taurine, Caffeine, Inositol, Vitamins (Niacin, Pantothenic Acid, Riboflavin, B6, B12). Natural Flvours: Artficial Flavour(aroma), Colours (Caramel). Energy drink (Power horse ®) containing by 100 ml: Energy 191 kl, protein 0.0 g, Carbohydrates 10.7 g, fat 0.0 g, Vitamins: Niacin 8 mg, Pantothonic acid 2mg, Riboflavin 0.06 g, B6 2mg, B12 2mg. Taurine 0.4 g, Caffeine C8H10N4O2 (32 mg).

Experimental Design and Animal Groups

The rats were randomly assigned to 5 groups: a control group (Group 1) which received water (n=10), induction groups (Groups 2 and 3), which received Red Bull (n=10) or Power horse (n=10) respectively. The Red Bull and Power horse (3.57 mL/kg/d equivalent to a person weighing 70 kg consuming 250 mL per day) [12]. and water were administered by oral gavage. The supplementation continued up to 7weeks. The recovery groups (Groups 4 and 5) which received Red Bull (n=10) or Power horse (n=10) respectively then were permitted to live without further energy drinks intake for further 2weeks. At the end of experiment, rats were sacrificed with an overdose of anesthetic.

Methods

Serum testosterone assay

The serum sample concentrations of testosterone were measured by using enzyme-linked immunosorbent assay as described in the instructions provided with the assay kits (DRG Instruments GmbH,Marburg, Germany).

Tissue processing for light microscopy

At the end of the experiment, rats from each group were killed. The organs were then taken, weighed, and put them in Bouin's fixative. The tissue were then prepared through paraffin technique. Sections (5-6 μm in thickness) were stained with H&E stain general histological structure [13].

Tissue processing for Electron microscopy

For electron microscopy, small tissue blocks (1mm3) were taken, fixed in 2.5% phosphate buffered glutaraldehyde at 4°C for 2 hours and washed three times in the same buffer, each for 15 minutes. They were then postfixed in 1% buffered osmium tetroxide for one hour at room temperature. After dehydration in ethanol samples were embedded in Epon. Ultrathin sections were stained with Toluidine blue and examined [14].

Estimation of malondialdehyde (MDA) (lipid peroxidation marker)

Testes were homogenized with a buffer to obtain 1:10 (wt/vol). MDA was considered as an index of oxidative stress [15]. MDA was measured in a spectrofluorometer, as described by Wasowicz et al., (1993).

Estimation of Reduced Glutathione (GSH)

GSH was measured by the method of Moron et al., (1979). Results were presented as mmol/g [16].

Statistical analysis

All measurments presented are the mean ± deviation. The statistical package for social science (SPSS) version 16 for windows was used for the data analysis. The mean results were compared.

Comparison of variables between groups was performed using One-way analysis of variance (ANOVA). Statistical significances were considered at P-value < 0.05.

RESULTS

Light microscopic examination and Semi thin ultrasructure

Testes in the control group showed normal histological structure (Fig1). In the ultrastructure of semi thin of testicular tissue showed normal seminiferous tubules (ST) with regular outlines and normal shape and arrangement of Sertoli cells with triangular-shaped nuclei, spermatogonia rest on intact basement membrane, large primary spermatocytes in different stages of meiotic division, round and elongated spermatids and numerous mature sperms are present. Regular shape of interstitial tissue with Leydig cells (Fig 2).

Red bull–treated group showed distortions and necrosis, loss of germinal cells and decreased interstitial tissue. Seminiferous tubules with irregular outlines were also detected in this study. In addition to disorganization of germinal epithelium, and interstitial edema were also found. Other ST showed sloughing of epithelial cells with vacuolations into the lumen of ST. Spermatogenic cells appeared with pyknotic nuclei. The interstitial spaces contained Leydig cells with oval darkly stained nuclei (Fig 3).

In the other hand, Power horse–treated group showed shrunken ST with irregular outlines. Degenerated germ cells were sloughed in the lumina of ST. Different germ cell layers appeared with pyknotic nuclei. Scanty seminiferous epithelium with little elongated spermatids towards the lumen. Edema and dilated congested blood vessels are also noticed (Fig 4). In the ultrastructure of semi thin of testicular tissue showed vacuoles in spermatogenic cells, fatty infiltration, and degenerated germ cells. Scanty seminiferous epithelium with little elongated spermatids towards the lumen. Numerous Sertoli cells are close together and resting on thick basal lamina. Ring chromatin of spermatogonia nuclei and few sperms within germ cells. Lipid droplets in interstitial tissue and some of primary spermatocytes with small deeply stained nuclei (Fig 5).

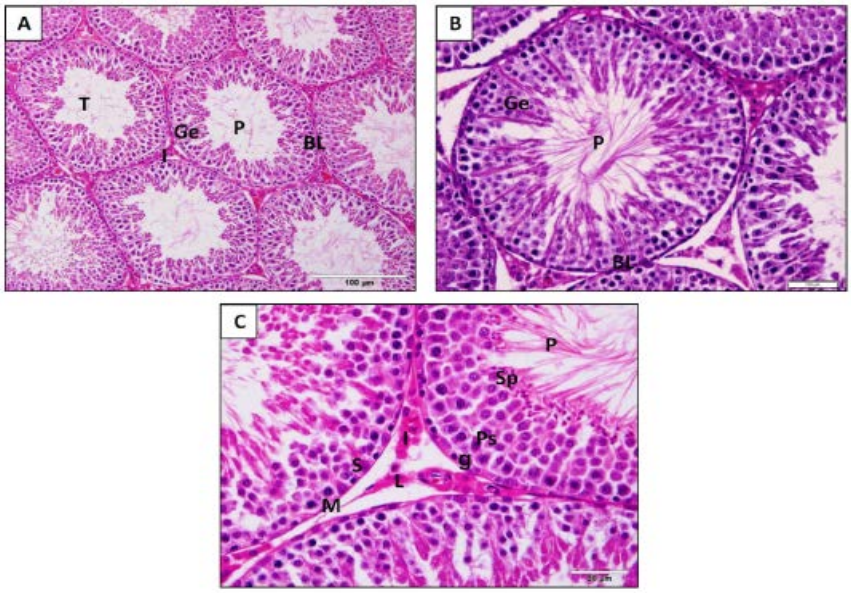

Fig 1. Light microscopic micrograph of testicular tissue from adult rat in control group showing: A- Normal seminiferous tubules (T). B- The seminiferous tubules ensheathed with basal lamina (BL) and containing normal germinal epithelium (Ge), sperms (P) are seen in the lumen. C- Spermatogenic cells; spermatogonia (g), primary spermatocytes (Ps), spermatids (Sp) and sperms (P). Sertoli cells (S) are also seen. The interstitial tissue (I) shows Leydig cells (L). Myoid cells (M) surround the seminiferous tubule. H&E staining. (Original magnification, "A" X200; "B" X400 "C" X1000).

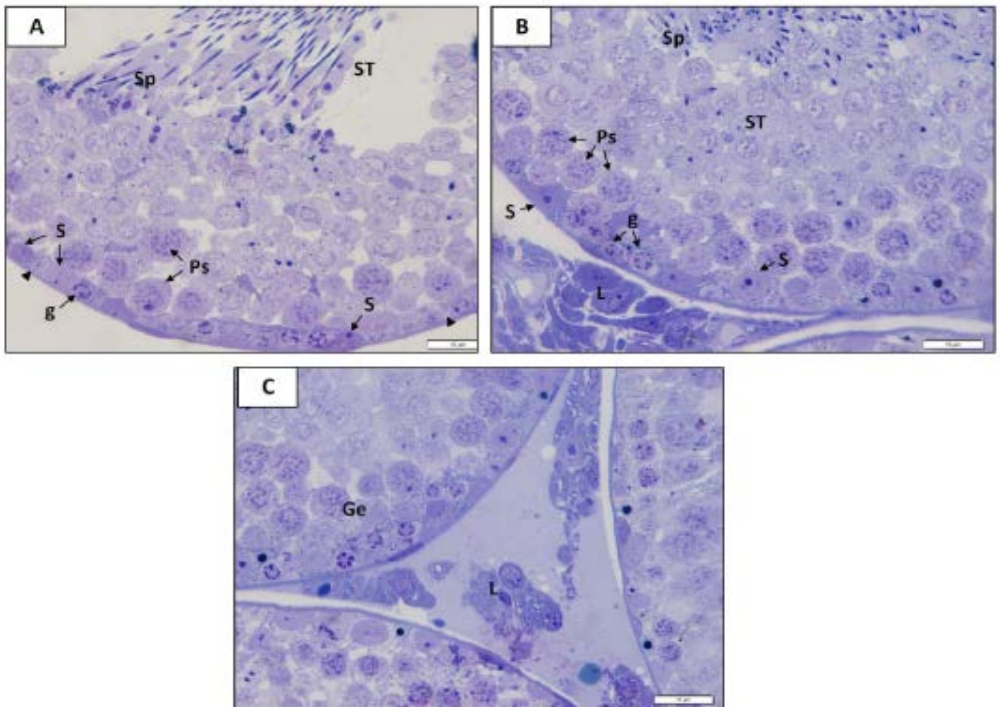

Fig 2. Histological structure micrograph in semi thin sections of seminiferous tubules and testicular tissue from adult rat in control group showing: A- Seminiferous tubules (ST) have normal shape and arrangement of Sertoli cells(S) with triangular-shaped nuclei, spermatogonia(g) rest on intact basement membrane(arrowhead), large primary spermatocytes(Ps) in different stages of meiotic division, round and elongated spermatids (Sp) and numerous mature sperms (S) are present. B- Normal structure of seminiferous tubules (ST) and spermatogoni cells: Sertoli cells(S), spermatogonia(g), primary spermatocytes(Ps), rounded spermatids (Sp). The interstitial spaces in-between the tubules contain Leydig cells (L). C- Multiple rounded seminiferous tubules with regular outlines, lined by layers of germinal epithelium (Ge). Regular shape of interstitial tissue with Leydig cells (L). Touldine blue staining. Scale bar=100 µm

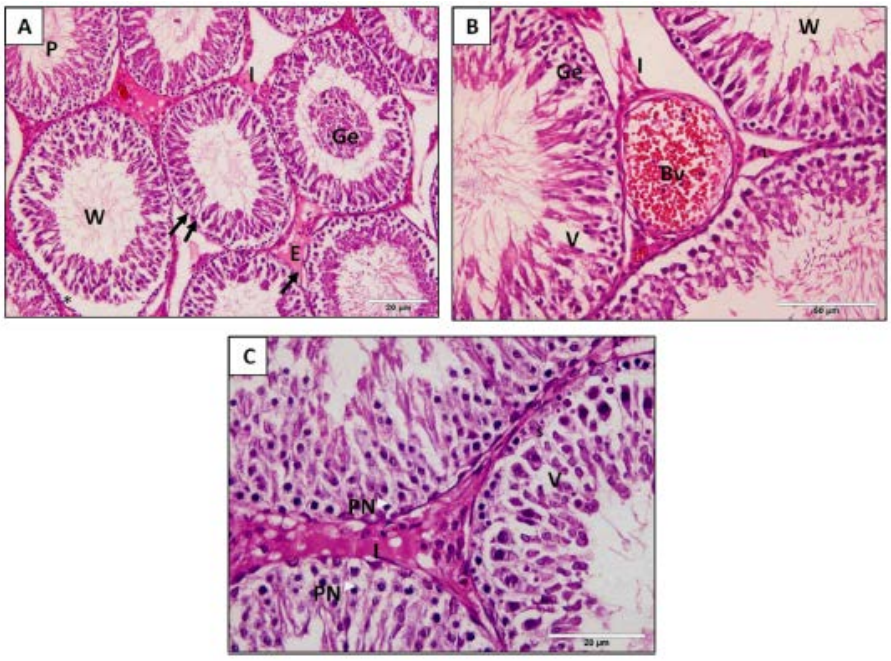

Fig 3. Light microscopic micrograph of seminiferous tubules and testicular tissue from examined rat in group treated with Red bull showing: A- shrunken tubules (double arrows) and the tubules have apparent diminished layers of germinal epithelium. Some tubules are resting on an irregular basement membrane (arrow) and sloughing of germ cell layers (*).The lumina of some seminiferous tubules are filled with degenerated germ cells (Ge), and Edema (E) in the interstitial tissue are seen. B- seminiferous tubules with disorganized germinal epithelium and marked vaculations (V). Congested dilated blood vessels are also seen (Bv). C- distorted seminiferous tubules, degeneration of spermatogenic cells, vacuolation (V), pyknotic nuclei (PN). H&E staining. (Original magnification, "A" X200; "B" X400 "C" X1000).

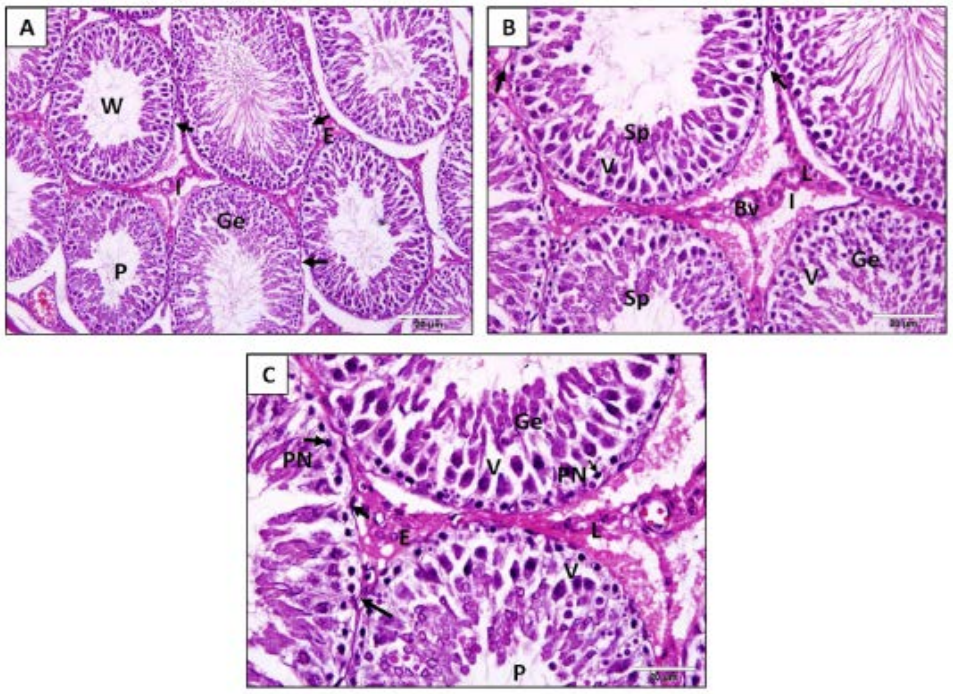

Fig 4. Light microscopic micrograph of seminiferous tubules and testicular tissue from rat in group treated with Power horse showing: A- some tubules appear shrunken and have irregular outlines (arrow). Some lumina contain aggregations of degenerated germ cells (Ge), and others are wide (W) with rare presence of sperm (P). Edema (E) in interstitial spaces (I), are noticed. B- irregular thick and shrunken basal lamina (arrow), the seminiferous tubules with disorganized epithelial lining, vacuoles (V) and lumina of the tubules are filled with degenerated germ cells(Ge). C- irregular basal lamina (arrow), the seminiferous tubules have disorganized germinal epithelium with vaculations (V). pyknotic nuclei (PN) of spermatogonia. Edema (E) in the interstitial tissue, and degenerated the Leydig cells (L). H&E staining. (Original magnification, "A" X200; "B" X400 "C" X1000).

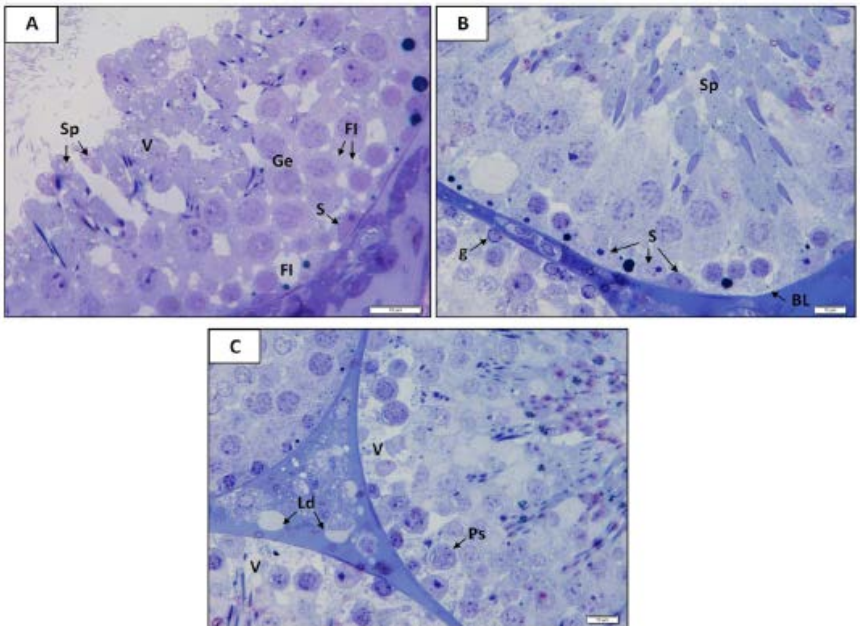

Fig 5. Histological structure micrograph in semi thin sections of seminiferous tubules and testicular tissue from adult rat in treated group with Power horse at 3mg/kg BW/day for 7 weeks showing: A- Vacuoles (V), fatty infiltration(FI), and degenerated germ cells(Ge). Irregular shape of Sertoli cells(S). Scanty seminiferous epithelium with little elongated spermatids (Sp) towards the lumen. B- Numerous Sertoli cells(S) are close together and resting on thick basal lamina(BL). Ring chromatin of spermatogonia nuclei(g), and Irregular elongated sprmatids(Sp). Few sperms (S) within germ cells. C- Vaculation(V) in spermatogenic cells, and lipid droplets (Ld) in interstitial tissue. Some of primary spermatocytes(Ps) with small deeply stained nuclei. Touldine blue staining. Scale bar=100 µm

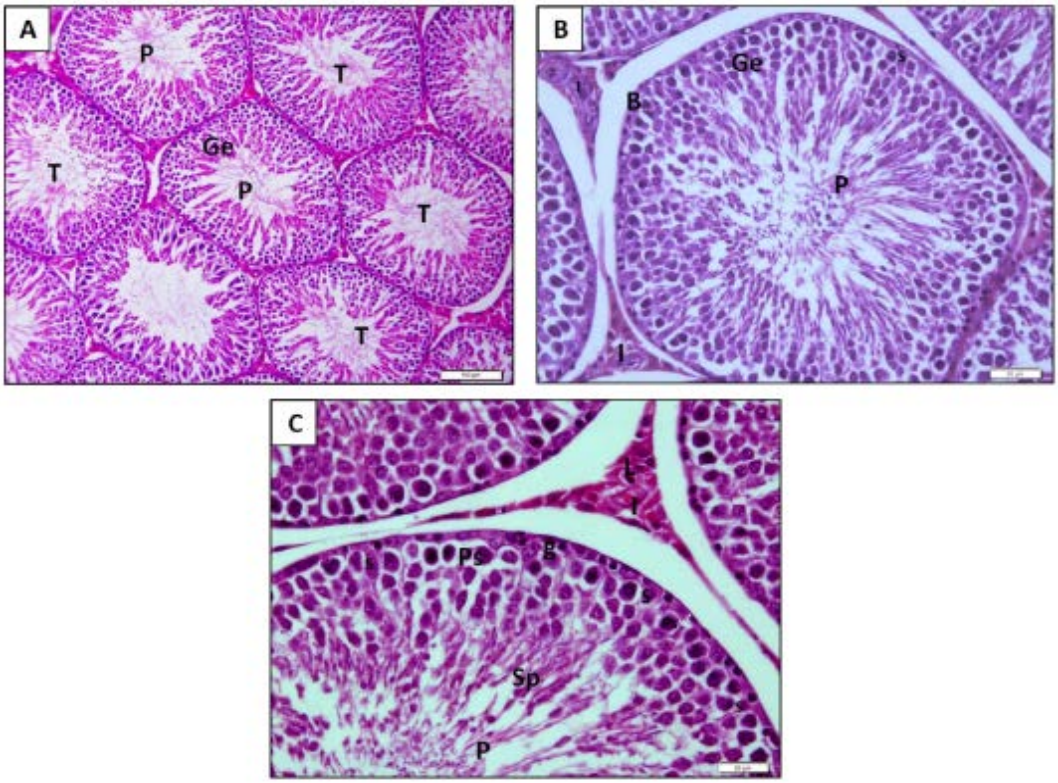

Fig 6. Light microscopic micrograph of seminiferous tubules and testicular tissue from adult rat in recovery group of Red bull showing: apparently normal germinal epithelium and interstitial tissue. H&E staining. (Original magnification, "A" X200; "B" X400 "C" X1000).

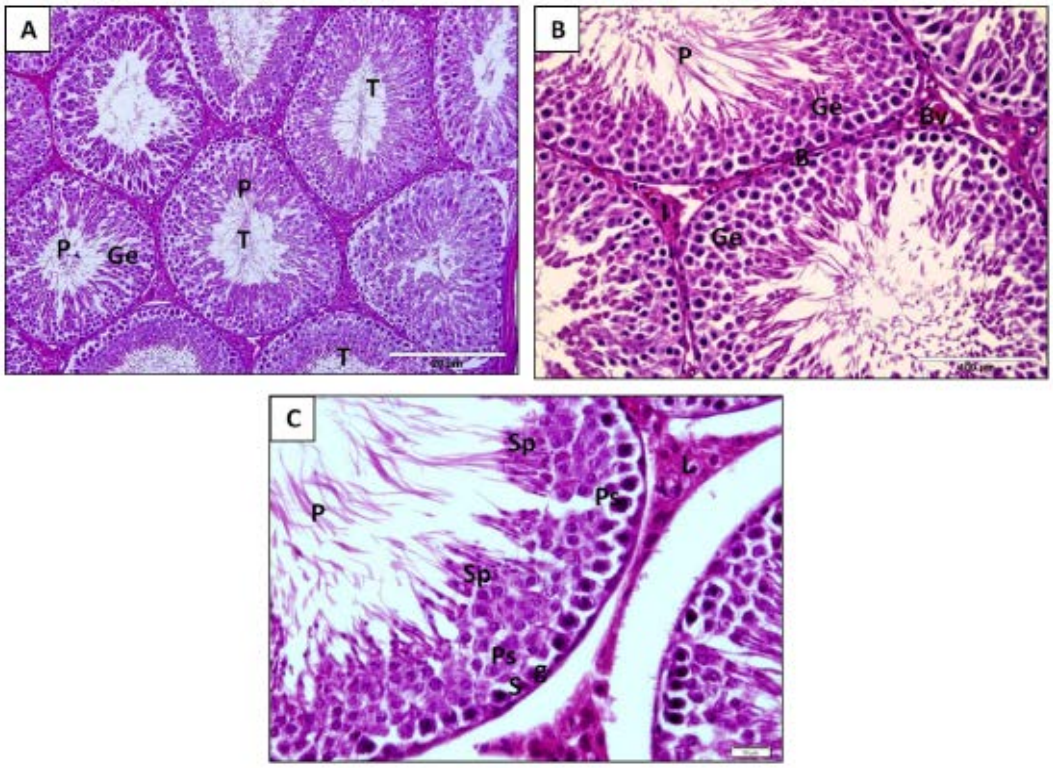

Fig 7. Light microscopic micrograph of seminiferous tubules and testicular tissue from rat in recovery group of the Power horse showing: apparently normal germinal epithelium and interstitial tissue. H&E staining. (Original magnification, "A" X200; "B" X400 "C" X1000).

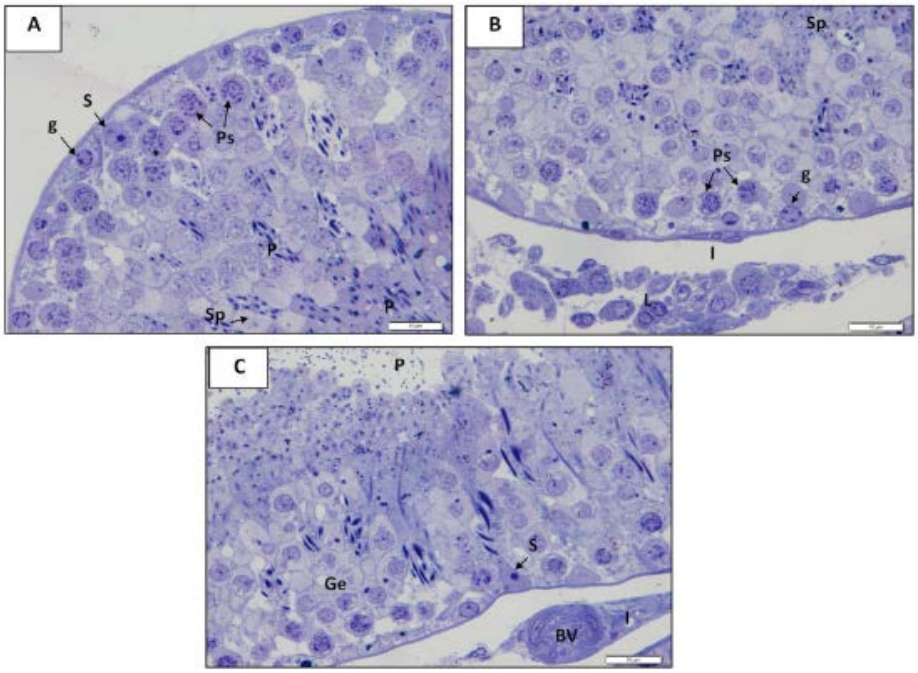

Fig 8. Histological structure micrograph in semi thin sections of seminiferous tubules and testicular tissue from rat in recovery group of the Power horse showing: A- Regular seminiferous tubules contain aggregations of sperms(P). Spermatogonia(g) resting on a thin basement membrane and have small rounded nuclei. Primary spermatocytes(Ps) appear with their large rounded nuclei. Spermatids(Sp) are small rounded cells with rounded nuclei. Sertoli cells(S) are seen between spermatogenic cells. B- Several types of normal spermatogenic cells. Spermatogonia(g) are resting on a thin basement membrane and have small rounded nuclei. Primary spermatocytes(Ps) appear with the large rounded nuclei. Spermatids (Sp) are small rounded cells with rounded nuclei. Clusters of interstitial cells of Leydig(L) with acidophilic cytoplasm are seen in the interstitial space(I). C- ST having nearly regular contour and are lined by stratified germinal epithelium(Ge). Their lumina contain aggregations of sperms(P). Sertoli cells(S) are seen between spermatogenic cells. interstitial space(I) show narrow with clusters cells, and normal blood vessel (BV). Touldine blue staining. Scale bar=100µm

Statistical Results

The statistical analysis illustrated that the mean Glutathione level in tissue homogenate was significantly decreased in Group 2 and 3.However in Group 4 and Group 5, the values were insignificantly changed compared to the control group (G1). The results were summarized in (Table 1 and Fig 9).

In contrary, the mean tissue MDA level was significantly increased in Group 2 and 3, while the change in the recovery groups (4 and 5) was non-significant compared to the control group (G1). Nevertheless the same findings were presented in (Table 1 and Fig 10).

The mean concentrations of the serum testosterone were significantly decreased in Group 2 and 3.However in the recovery groups (4 and 5), there was non-significant change in the testosterone concentration when compared to the control group (G1). The analyzed results were presented in (Table 1 and Fig 11).

Table 1. The mean of the tissue Glutathione and MDA together with the serum Testosterone in different groups

Groups	Glutathione (mmol/L) M + SD	MDA (mmol/L) M + SD	Testosterone (µmol/L) M + SD
G1 (Control group)	32.90 + 2.94	0.365 +.103	0.633 + 0.127
G2 (Red bull)	20.62 + 2.09*	0.57 + 0.098*	0.297 + 0.068*
G3 (Power horse)	24.6 + 3.2*	0.72 + 0.168*	0.222 + 0.020*
G4 (RB Recovery)	31.4 + 1.9#	0.36 + .0811#	0.663 + 0.066#
G5 (PH Recovery)	30.7 + 1.5#	0.43 + 0.109#	0.474 + 0.123#

Results were expressed as mean+/- standard deviation (M + SD).

P value: Significance versus G1 (Control group). * = Significant: $P < 0.05$ and # = Non significant : $P > 0.05$

RB = Red Bull PH = Power Horse

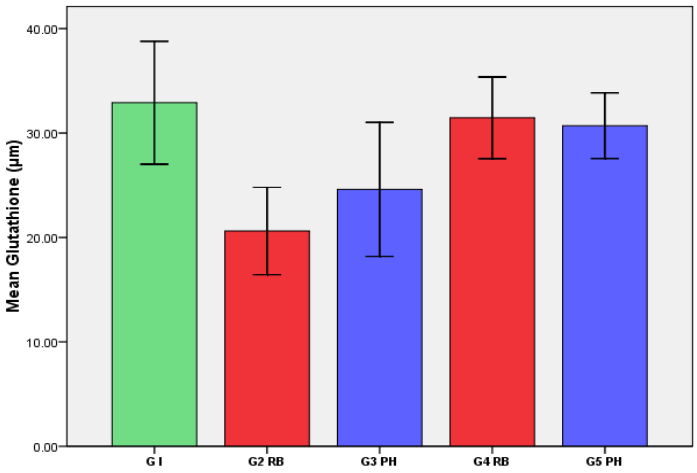

Fig 9. The mean level of homogenate Glutathione in different examined groups

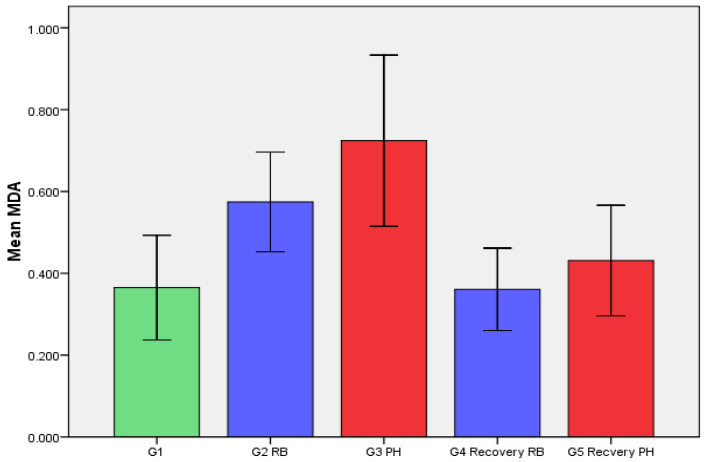

Fig 10. The changes in the mean levels of MDA in different examined groups

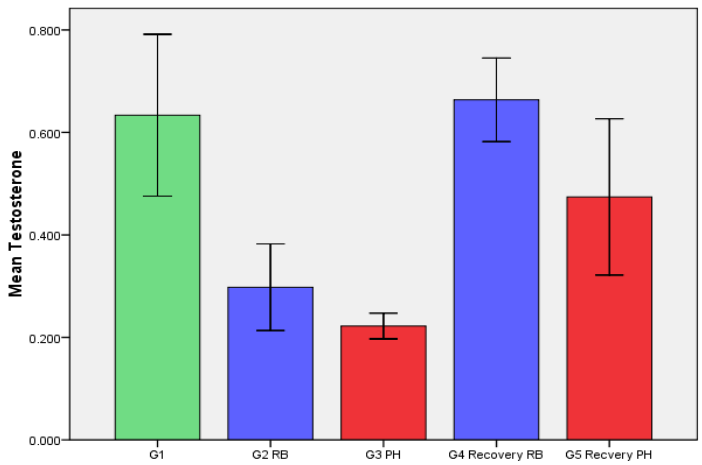

Fig 11. The changes in the mean serum concentration of Testosterone in different investigated groups

DISCUSSION

It is well known that the major function of the testes is spermatogenesis and hormone synthesis [17]. An energy drink is a type of beverage containing stimulant usually including caffeine,which is marketed as providng mental and physical stimulation as energy. .The purpose of this study was conducted to examine the histological effects of

ED (Red Bull and Power Horse) on male rats (Wister rats). Our finding indicate that the ingestion of ED have had various effects.

In our results the treatment of male rats with Red Bull for 7 weeks revealed that the seminiferous tubules appeared shrunken and irregular shape and sloughing germ cells, also wide interstitial space and edema were noticed. Some tubules with multiple distortion, and severe damage and necrosis, spermatogonic cells showed Pyknotic nuclei. lyedig cell appeared in the interstitial space with darkly stained nuclei [18]. Our findings are in agreement with El-shennawy and Abo Elwafa., (2011).

In the present study, in power horse group, some seminiferous tubules were shrunken. Some lumina of ST had aggregations of germ cells with dark nuclei, another one had no sperms. Some ST have vacuolations. Scanty seminiferous epithelium with little elongated spermatids towards the lumen. Pyknotic nuclei of spermatogonia and deformed sertoli cells. Also, irregular shape of interstitial tissue with wide space, clusters cells have dark nuclei, edema are also noticed [19]. These explanation of the present finding were in agreement with Anniballo et al., (2000).

The present study shows that male rats' 7-week continuous exposure to Energy Drinks, namely Red Bull and Power horse, resulted in a number of histopathological changes such as epithelial cell sloughing, atrophic changes and a drop in germ cell count because of cytotoxicity. An evidence of genotoxicity was also observed in the form of degenerative changes in the seminiferous tubules and decrease of spermatozoa in the testis. These findings are in agreement with a study by Thakur et al., (2014) at which the effect of nanoparticles (20µg/kg/day) on the wistar rats was experimented by gavage technique for 90 days [20].

However, (Elsharaky et al., 2010) studied the effect of gossypol acetic acid in a dose of (5, 10, 20 mg/kg BW, respectively) on through intraperitonial injection in male albino rats, they found that there were decreased activity of spermatogenesis and some seminiferous tubules had necrotic cells characterized by pyknotic nuclei among the round spermatids. Also, found a significant decrease in serum levels of testosterone. These finding were in agreement with our results [21].

The present study work are in accordance with Hamza et al., (2015), who studied the effect of sodium floried in a dose of 10.3 mg/kg body weight intraperitoneal for a period of 30 days on male rats, they found that there were maeked alttrations in the histology of testis, such as disorganized epithelium and reduction of the cells of the seminiferous tubules which forming the spermatogenesis, also increasing the lumen diameter, disorganized spermatids, and the seminiferous tubules appeared atrophied and there were vacuolation and stages of necrosis, and also reported that Glutathion(GSA) was shrply decreased [22].

However, other studies by Albadri., (2012) showed that damage of interstitial tissue, atrophy of leydig cells, infiltration of cells, and also atrophy of sertoli cells. In the other hand, they showed significant decrease in plasma testosterone level, when they used khat consumption with dose of (50 mg/kg) body weight for eight weeks. These observations were in agreement with our study.

Our study showed that destruction of the seminiferous tubules and pronounced changes in the epithelium of the tubules, and decrease in it's diameter, decrease of Leydig cells, and the presence of degenerative germ cells when rats were intubed with ED for seven weeks. These finding in agreement with Albadri et al., (3013) who observed impaired germ cells specially spermatogenesis, when used ethanol consumption orally to rats, and their offspring in a dose of (3 g/kg) body weight for four and eight weeks [23].

Previous studies by Marchlewicz et al., (2007), that have reported an increasing of oxidative stress indicators in rat's testis as a result of treatment with lead acetate (PbAc) as 1% aqueous solution for 9 months. Our current studies revealed significantly increasing the concentration of lipid peroxides in testis of male rats that given ED for seven weeks. Our data are similar with these results [24].

In our obtained results showed that was reduced in the testosterone level in rats treated with ED. However, it was found that our results were in disagreement with those found by Tousson et al., (2012), who found high level of testosterone when used (1,4-androstadiene-17b-ol-3- one; Boldenone) injections [25]. In addition, the previous study by Gabr and Shaker., (2006) found that increased in plasma testosterone level in rats which treated by androgenic steroid compared with the untreated rats [26].

On the other hand, Shimomura et al., (2005) found that was significantly reduced in testosterone concentration when used ethinylestradiol in rats [27].

In our results that were treated animals with red bull and power horse observed abnormal histological structure in testis rats such as vacuolation and sloughing of germinal epithelium, abnormal distribution of spermatocytes, and

reduced of sperme in the lumen of seminiferous tubules. These finding was agreement with (Tousson et al., 2012) that injected rabbits with 5 ml/kg BW boldenone undecylenate for 3, 6, 9 weeks.

Our results are agreement with (Groot and Biolatti, 2004), they found that Boldenon (BOL) caused same harmful effect in testicular tissue of these cattle [28]. Also, these results are similar effect in testis of bull which treated with zeranol and estradiol [29].

However, the present investigation are in agreement with those obtained by Silcox et al., (1986) , Rodriguez et al., (1991) in rats testis [30, 31].

Previous studies were investigated that found decreased testicular development and hieght proliferation level in male calves which treated with anabolic steroids [32]. Similarity results were showed by Rodriguez et al., (1991) in testicular tissue of lamb and calf which treated with estradiol and trenbolone. These results are in agreement with our finding.

The present study revealed that the recovery groups from Red Bull and Power Horse had apparently normal histological structure.

CONCLUSION

To conclude, the present investigation showed that both Energy Drinks (Red Bull, and Power Horse) have an effect on the structures and function of testicular tissue in adult male rats especially spermatogonic epithelium.

It should be taken under restricted precautions. We recommended that further studies are needed.

CONFLICTS OF INTEREST

We have no conflict interest to declare.

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