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Original Article

Energy Drink (Fearless) Effect on Sperm Parameters and Testicular Histology of Wistar rats

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Abstract

BACKGROUND AND AIM: In Nigeria, commercial energy drinks like Fearless are the most widely consumed. Nevertheless, there is currently no evidence linking the effects of Fearless energy drinks on seminal parameter. The purpose of this study was to investigate the effects of energy drinks like Fearless on the testis.

METHODOLOGY: Twenty adult male Wistar rats weighing averagely 140-160g were divided into four groups of five rats each. Group A was the control and received 5ml/kg of distilled water. For three weeks, the following doses of the Fearless energy drink was given to groups B, C, and D via oral gavage: 2.5, 5, and 7.5 mL/kg/day, respectively. After treatment, the animals' were sacrificed and their testis were weighed. The sperm parameters (motility, count) were evaluated, and the testis were processed for histological studies.

RESULTS: Results showed that there was a statistically significant decrease ($p < 0.05$) in the sperm count and motility in the ED (Fearless) groups (Group B 619±65.76 and 69.5±2.5, Group C 602±70.01 and 62.0±10.00, Group D 514±26.5 and 55.0±.6.19) respectively when compared to group A (662±10.01 and 92.5±2.5). There was an increase in the body weight in the treated groups, but no difference was observed with respect to relative weights of the testis. Groups B and C showed moderate effect on the testicular tissue with moderate spermatogenic arrest. Group D, exhibited scattered appearance of seminiferous tubules with loss of spermatogenic germ cells, marked degeneration of interstitial cells and congested thick vessels in the interstitial tissue.

CONCLUSION: Fearless energy drink induced dose dependent negative changes on sperm quality and morphology of the testis .of Wistar rats

Keywords:
Fearless Energy drink, Testis, Sperm count, Sperm morphology

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INTRODUCTION

Energy drinks (EDs) are a kind of bottled beverages that may or may not include additional dietary supplements in addition to caffeine and a few well-known brands of energy drinks are Predator, Komando, Red Bull, Bang Energy, Monster, Rockstar and 5 Hour Energy (Costantino *et al.*, 2023). Energy drinks provide a customer with a "energy boost" by combining stimulants and energy boosters (Alsunni, 2015). The primary component of most energy drinks is caffeine (Kudema *et al.*, 2023). Energy drink also contains a variety of other chemicals, including sucrose, glucuronolactone, taurine, maltodextrin, guarana, ginseng, yerba mate, acai berry, and ginkgo biloba, as well as natural extracts. Vitamin C, Vitamin D, and Vitamins B are micronutrients that are present; including vitamins B2 (riboflavin), B3 (niacin), B6 (pyridoxine, pyridoxal, and pyridoxamine). Also present are Inositol B8 and B12, calcium, zinc Iron, chromium, molybdenum, manganese, macronutrients (carbohydrates and protein) artificial sweeteners such as sucralose and aspartame (Ariffin *et al.*, 2022). There have been documented health benefits including enhanced physical performance, better mood and attitude, enhanced memory and attention and a good source of vitamin B (Ariffin *et al.*, 2022). As a result, the beverages advertise that they will increase focus, energy, endurance, and sports performance (Al-Shaar *et al.*, 2017). A number of detrimental effects on health while using caffeine have been documented, including cancer, ischemic stroke, headaches, epileptic seizures, hallucinations, anxiety,

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depression, gastrointestinal and renal effects, dental effects, obesity and type II diabetes (Athanasiadis *et al.*, 2023). Energy drink producers have recently turned their attention from athletes to teenagers and young adults (Jagim *et al.*, 2023). College students have been heavily marketed to about EDs' capacity to regulate mood, boost alertness, lessen weariness, enhance physical performance, and lower elevated levels of perceived stress (Barcelos *et al.*, 2020). The safety of these items is currently the subject of serious questions because numerous reports have linked energy drinks to adverse health effects (Nadeem *et al*., 2021). Energy drinks' high levels of sugar, caffeine, and aspartame are primarily to blame for the majority of health problems associated with their intake (Ariffin *et al.*, 2022). International research has demonstrated a drop in men's semen quality, which may be the reason of the decline in male fertility (Mann *et al*., 2020). A growing number of researchers are examining the relationship between sperm traits and lifestyle choices like eating, drinking, smoking, being overweight, or engaging in physical activity (Kumar *et al.*, 2022).

Regarding the effect of energy drink intake on the general population's infertility, there is a lot of misinformation out there (Akaev & Adilov, 2021).

Fearless energy drink are non-alcoholic energy boosting beverages that provides limitless benefits at work, at training or sport. The ingredients used in making fearless energy drink are water, carbohydrate (12 g sugars), flavoring, carbon dioxide, taurine, citric acid E 330, acidity regulator (sodium citrate E 331), inositol, caffeine (0.031%), niacin (3 mg), colors (tartrazina E102 and sunset yellow FCF E110), vitamin B12 (0.3µg), vitamin B6 (0.3 mg), fiber (<0.5 g), protein (<0.5 g), fat (<0.5 g), salt (0.02 g), ginseng extract and energy value (283 KJ) (Iheanacho *et al*., 2022). The side effect of fearless energy drink includes; nervousness, insomnia, vomiting, change in blood pressure, weight gain and also increases the risk of type 2-diabetes (Iheanacho *et al*., 2022). But there is no proof that drinking energy drinks like Fearless has any effect on a man's ability to reproduce. It is critical to draw a link between the use of energy drinks (such as Fearless) and infertility. Furthermore, research on the male reproductive system in lab animals who use caffeine has revealed decreased spermatogenesis (Grandner *et al*., 2014). The purpose of this study is to assess the impact of energy drink (Fearless) on the motility, concentration, and testicular morphology of male Wistar rats.

MATERIALS AND METHOD

Animals

A total of 20 male Wistar rats (Rattus norvegicus) weighing between the ranges of 140-160 g, was obtained from Iyke Animal farm, located at Nnewi Anambra State, and bred in the experimental house of The Faculty of Basic Medical Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli Campus. They were acclimatized for 14 days. Grower mesh (Sander feeds) and tap water was provided throughout the experimental period.

Experimental protocol

The rats were randomly assigned to four groups of five. Using an orogastric tube, the experimental groups were given daily oral dosages of the fearless energy drink in the following manner: Group A was given 5 ml of distilled water orally once a day as a control. For three weeks, Group B was given 2.5 ml, Group C was given 5 ml, and Group D was given 7 ml of fearless energy drink. The experimental rats were weighed weekly using an Emperor Camry weighing scale. All procedures involving animals were performed in accordance with the guidelines guiding the use and care of laboratory animals and approved by the Departmental Committee on the Use and Care of animals (National Research Council, 2011).

Termination of the experiment

Before and after Fearless energy drink was administered to determine the morphological observations, all animals were weighed using a sensitive weighing balance. Animals in all the groups were sacrificed by cervical dislocation. Their testes were removed and weighed. The relative testicular weight was recorded and processed for testicular histology. The caudal epididymis was removed from the testis and processed for the epididymal sperm profile.

Sperm parameters

The cauda epididymis was removed and incisions of about 1mm was created; and these was suspended in 1 ml of Ham-F-10 solution. Estimating motility was done at room temperature, in the range of 24 to 28°C. Each spermatozoon found was evaluated and the microscopic field was methodically inspected. For the purposes of this study, motility was defined as either active motility or sluggish motility. After repeating the same process, the average reading was obtained (Yuan *et al*., 2010). The Neubauer enhanced hemocytometer was utilized to ascertain the number of sperm. A dilution ratio of 1:20 from each well-mixed sample was used to dilute 50µl of epididymal spermatozoa suspended in physiological saline with 950µl diluent. As long as the difference between the two counts did not exceed 1/20 of their sum (i.e., less than 10% difference), the haemocytometer's two chambers were scored and the average sperm count was determined. The sample dilution was re-mixed, another hemocytometer was set up, and counting

began when the two counts did not agree by 10%. The count was done three times on each of the samples taken from each epididymis in order to reduce error. An observation was constituted as the mean of all the six counts, three from each side, from single rat. To examine sperm morphology, smear was prepared from the samples and was stained and investigated by the Papanicohaou staining method, it was grouped into normal and abnormal sperm (Zare *et al*., 2010)

Histopathological Studies

The right-side testes from the control and experimental rats were removed and weighed. After fixation of testes with Bouin's fluid at room temperature for 24h, routine tissue preparation was done. Briefly, the tissues were transferred to 70% alcohol, dehydrated by passing through ascending grades of alcohol, after which the tissues were cleared in xylene and finally embedded in paraffin wax. Using a rotary microtome, 5μm thickness sections were cut and stained with Haematoxylin-Eosin (H&E) protocol. The stained slides were observed in a research microscope.

Statistical analysis

The statistical analysis was completed using GraphPad Prisms (version 6.0) (GraphPad Software, USA). Data was analyzed using ANOVA followed by Bonferroni Post Hoc test and values were considered significant at *p<0.05*.

Results

Body and Testicular weight

At the end of the experiment, body weight showed significant increase in groups B, C and D respectively, when compared to the control group A. While the relative testicular weight did not show any statistically increase in the groups B, C and D when compared to the group A as showed in Table 1

Table 1: Effect of energy drink (Fearless) on body and testicular weight

Groups	Initial body weight (g)	Final body weight (g)	Relative Testicular weight (g)
A	157.2±22.80	163.2±21.06	1.3±0.05
B	149.8±22.20	171.5±21.50*	1.6±0.20
C	156.6±29.40	174.2±28.20*	1.5±0.11
D	148.7±28.20	170.3±27.50*	1.4±0.31

Values represented as M± SD. N=5: *(*P*<0.05) when compared to control group A;

Values were considered significant at *p<0.05*. MEAN±SD.

Sperm count and motility

The sperm count of the animals administered energy drink (Fearless) (Groups B, C and D) showed a dose dependent significant (P≤0.05) when compared to control group A. The sperm motility of the animals administered energy drink (Fearless) (Groups B, C and D) showed a statistically significant decrease respectively when compared to control group A as shown in Table 2

Table 2: Effect of Fearless energy drink on sperm motility and sperm count

Groups	Total sperm count (x10^6/mls)	Normal sperm cells (%)	Abnormal sperm cells (%)	Active motility (%)	Sluggish motility (%)
A	662±10.01	90.5±5.0	9.5±6.1	92.5±2.5	7.5±2.5
B	619±65.76*	80.3±0.0	19.7±3.4	69.5±2.5	30.5±1.8
C	602±70.01*	72.5±2.5	27.5±5.0	62.0±0.0	38.0±2.5
D	514±26.5 *	75.2±4.0	24.8±7.5	55.0±.6.2	45.0±2.5

Values represented as M± SD. N=5: *(*P*<0.05) when compared to control group A

Histological Findings

Figure 1 showed Group A with normal seminiferous tubules having basement membrane with regular outlines, intact complete germ cell layers thickness, with most showing mature spermatozoa, besides the appearance of Leydig cells with its normal population and histological features and appearance. The Groups administered with energy drinks (Fearless) showed marked histological alterations that were dose-dependent. Group B showed mild effect on the testicular tissue with mild spermatogenic arrest. Group C showed moderate effect on the testicular tissue with moderate spermatogenic arrest. Group D showed scattered appearance of seminiferous tubules with marked degeneration, interstitial cells looked sparse and degenerated, in addition to highly congested thick vessels in the interstitial tissue.

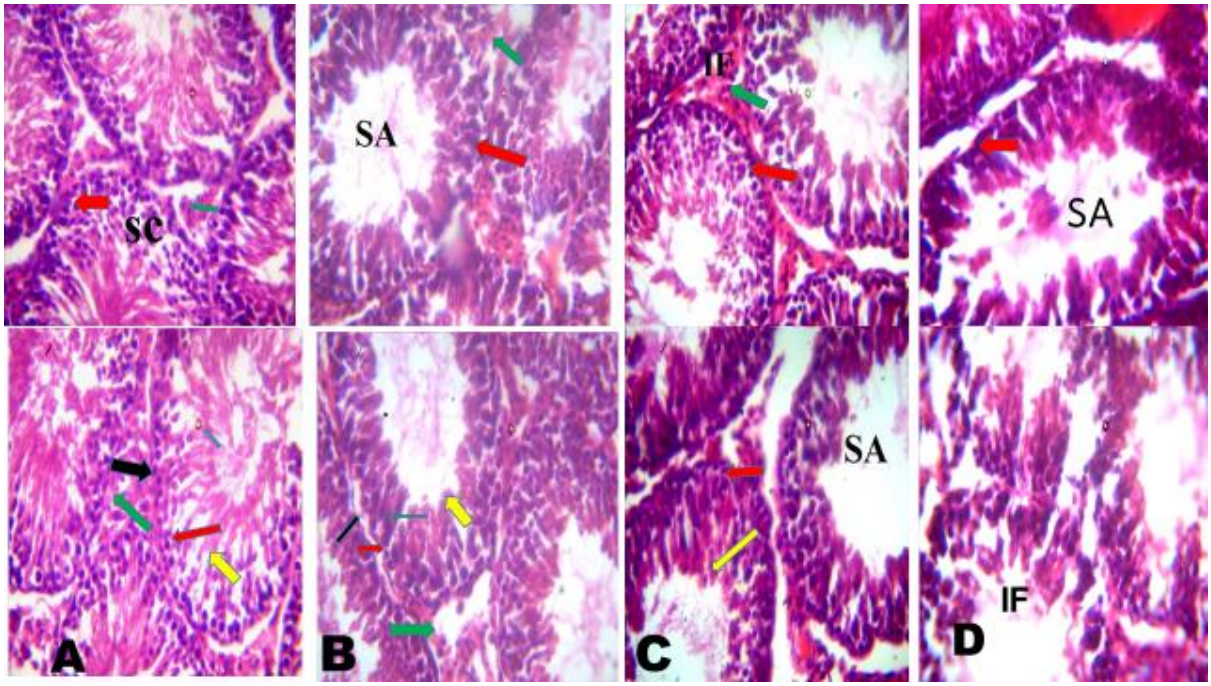

Figure 1: Paraffin sections from adult Wistar albino rat testis stained by H&E and photographed at high powers (×400). Group A control. Group B 2.5ml, Group C 5ml, Group D 7ml. Seminiferous tubule having basement membrane (black arrow), sertoli cell (blue arrow), spermatogonia (red arrow), mature spermatids (yellow arrow), Leydig cells (green arrow), spermatogenic arrest (SA), interstitial fibrosis (IF).

Discussion

After taking Fearless energy drink for 21 days, the rats' body weight increased, but there were no appreciable variations in the testicular weights. Therefore, the study suggests that the treated animals' markedly increased body weight was caused by their prolonged and continuous energy drink consumption. The caffeine content of the energy drinks (Fearless) may have contributed to the outcome by making people sleepy, as suggested by (Grandner *et al*., 2014). The high rate of catabolism brought on by the high insulin availability induced by the energy drink sweeteners may also be the cause of the study's reported significant weight gain. This would increase the rate at which fat is stored in adipose tissues (Adjene *et al*., 2014). This validates a previous theory that suggests consuming more sweeteners may raise the chance of gaining weight due to a drop in satiety and an insufficient decrease in energy intake to make up for the loss intake (Malik & Hu, 2022). This research supports the hypothesis that the obesity and overweight epidemic may be fueled by the use of artificial sweeteners, such as those found in energy drinks (Fowler *et al*., 2008; Graneri *et al*., 2021). Additionally, Adjene *et al*., (2014) observed that adult Wistar rats' body weights may increase as a result of long-term energy drink usage. Nonetheless, some stated that the body weight of the rats who drank energy drinks did not alter significantly (Ugwuja, 2014).

Long-term energy drink usage has been shown to adversely affect sperm concentration (Schuchowsky *et al*., 2017).. Our results may have been influenced by the gonadotoxic qualities of the energy drink (Fearless,) as there was a decrease in both sperm count and motility following oral administration. This outcome was consistent with that of .Schuchowsky *et al*., (2017), who observed that using energy drinks reduced the concentration of sperm. Caffeine has been shown to have preferential effects on Sertoli and spermatogonia cells, which can harm spermatids and impair their ability to differentiate and produce mature spermatozoa. Therefore, the high quantity of caffeine in the energy drink would have the impact of reducing the concentration of sperm either directly, indirectly, or through the endocrine system (Ricci *et al*., 2017). The rats in the study that had energy drank (Fearless), displayed significant dose-dependent changes in their histology. The control group's testicular cytoarchitecture was found to be unaltered. Group C testicular tissue displayed severe spermatogenic arrest and interstitial fibrosis, whereas the low-dose Fearless (group B) testicular tissue displayed minor spermatogenic arrest. Group D, which consumed the most energy drink (Fearless), displayed severe spermatogenic arrest, pyknotic testicular cell and hemorrhage.

The sloughing of germ cells and the reduced size of the seminiferous tubules were observed. Genotoxicity was demonstrated by degenerative changes in the seminiferous tubules and a decrease in spermatozoa in the testis. As a result, the energy drink's many

ingredients, including taurine, guarana, and caffeine, may be linked to these hazardous symptoms (Jouda *et al*., 2019), According to Dias *et al*., (2015), caffeine alters the Sertoli cells' glycolytic and oxidative properties, which may impair a man's ability to procreate. These explanations for the current findings were consistent with previous studies that shown significant histological changes in the testicles of male adult rats following excessive and prolonged consumption of Red Bull energy drink (Ahmed, 2016).

El-ghazouly *et al*., (2017), reports that many histological abnormalities, such as epithelial cell sloughing, atrophic changes, and a decrease in germ cell count due to cytotoxicity, were observed in male rats exposed to Red Bull and Power Horse energy drinks constantly for seven weeks. A high dose of caffeine (200 mg/kg body weight) has been shown to negatively impact the histoarchitecture of the seminiferous tubules in the testis. This leads to a notable reduction in spermatogenic cells, atrophic cells with necrosis, increased spermatid degeneration, and almost no spermatozoa (Bassey *et al.*, 2011; Ekaluo *et al*., 2016). Caffeine also has the effect of making the testicles lighter and smaller, as well as decreasing the amount of testosterone produced by Leydig cells (Park *et al*., 2015). According to a study, male fecundability was found to be correlated with consumption of energy drinks and caffeinated cola (Ricci *et al*., 2017). Thus, our study demonstrated that the usage of the energy drink (Fearless) resulted in changes to the testis' histology and seminal parameters.

Conclusion

The results of this investigation demonstrated that the testes are poisoned when exposed to Fearless energy drinks. There should be moderation and caution when consuming energy drinks.

Conflict of interest

The author has nothing to disclose and has no competing exist.

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Author's contribution

Nwakanma AA and Idaguko CA has planned, designed, completed the statistical analysis and wrote the manuscript. Okafor SR and Amanjide FO carried out the experiment work and the Laboratory tests. All the work of this article was under the supervision of Nwakanma AA and Idaguko CA, all the others read and approved the final manuscript.

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