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A comparative assessment of artificial and natural energy drinks in the epididymal and testicular milieu

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Abstract. Artificial and natural energy drinks are both taken for increased energy, physical stamina, and alertness, although they differ in composition. This study investigated the effects of artificial and natural energy drinks on the testicular milieu in male pubertal rats.

Eighteen Wistar rats were randomly divided into 3 groups of 6 rats each and all animals had access to food *ad libitum*. Group 1: (control) received water only; Group 2: (artificial energy drink- AED) received AED; Group 3: (natural energy drink- NED) received NED. A dose of 1.41ml/day/150g animal was administered and this lasted for 28 days. Sperm and testicular variables, biochemical parameters, and hormonal assays were carried out.

There were significant decreases in the levels of testosterone, Lactate dehydrogenase, glucose, 3β-Hydroxysteroid dehydrogenase, and 17β- Hydroxysteroid dehydrogenase activities in AED and NED groups when compared to the control group. There was a marked increment in sperm abnormalities in the NED group when compared to AED and control groups. Also, the intake of AED led to an elevated level of Glucose-6-phosphate dehydrogenase compared to the control while a significant reduction was observed in the NED group when compared to the AED group. Artificial and natural energy drinks although consumed for strength and vigor distorted epididymis and testicular integrity via alteration of the testicular metabolism, lowering sperm quality and androgenic hormones in pubertal male Wistar rats.

Keywords: energy drink, jaggery, sperm quality, steroidogenic enzymes, lactate dehydrogenase.

Introduction

The emergence of energy or power drinks has been over three decades now, and they differ from tea, coffee, soft drinks, fruit drinks, and sports drinks. Energy drink as the name implies was invented to build up energy and strength, for alertness, and to improve mood. Studies reveal that these drinks are perhaps targeted at adolescents (Reissig *et al*., 2009), and there are other studies pinpointing the fact that males are more affiliated with energy drinks than females (Friis *et al*., 2014; Dillion *et al*., 2019).

The main ingredients in energy drinks include water, taurine, guarana, glucuronolactone, added sugars, and a high concentration of caffeine which is a nitrogenous compound of the alkaloid family which has concealed physiological effects such as stimulating the central nervous system (Campbell *et al*.,2013; Fields *et al*., 2015). Popular energy drinks could be referred to as artificial energy drinks because they contain added sugars as well as caffeine which varies between 50-505 mg per can or bottle (Keaver *et al*., 2017). These values appear extremely high when compared to a 250ml cup of coffee which contains 80-120mg of caffeine or a cup of tea which contains 60mg of caffeine. Although the safe limits of caffeine consumption remain to be determined, there are suggestions from data that the maximum recommended intake of caffeine per day in adolescents should be 100mg/day and in adults, it could be up to 400mg/day (Heckman *et al*., 2010). Apart from the added caffeine content of energy drinks, other components such as guarana contain caffeine which is not usually mentioned as part of the caffeine contents thus the caffeine content of such drinks might be higher than what is listed on the drink packs thereby causing caffeine toxicity (Duchan *et al*., 2010). There are other constituents added to artificial energy drinks claimed to boost energy and mental alertness and the relationships between these components and caffeine in these drinks are not known (De Sanctis *et al*., 2017).

There exist other types of energy drinks which boost the body's energy needs as well as supply nutrition to the body. These groups of energy drinks appear less popular but are from natural sources and do not contain added sugars or artificial caffeine. They could be referred to as natural energy drinks and examples include kvass and jaggery. Jaggery also referred to as *gur, panela, kokuto, hakura,* and *rapadura* is processed from sugarcane juice. It is a natural sweetener produced without the use of chemicals (Nath *et al*., 2015). It is made up of longer chains of sucrose. It produces heat and gives instant energy to the body (Rao and Singh, 2021). The energy it gives lasts for a long time, and it is harmless to the body (Kumar and Singh, 2019). Jaggery contains large amounts of iron during its preparation, and it, therefore, has anti-anemic properties. It also contains reducing sugars, minerals, vitamins, proteins, and

phenols apart from its sucrose content. Jaggery is one of the products of sugarcane obtained in unrefined form (Kumar and Singh, 2019) and this explains the medicinal and nutritional properties of jaggery brought about by the presence of phenolic compounds. The procedure by which jaggery is made is the term used by the Food and Agriculture Organization of the United Nations (FAO, 1994) to describe a traditional slightly processed sweetener derived from sugar cane.

In recent years, jaggery is being used globally as part of food processes and as an energy drink. The current generation of youths and adolescents appear to prefer energy drinks which keep them "on the go". Energy drinks continue to gain increasing acceptability, especially among male folks. This study was therefore designed to compare the effects of an artificial energy drink with a natural one (jaggery) on male reproductive functions in Wistar rats.

Materials and methods

Energy drinks

An energy drink (commercial name not disclosed) containing 31.5mg caffeine per 100ml, niacin (3.0mg), vitamin B_6 (0.3 mg), vitamin B_{12} (0.3µg) with an energy value of 283kj/67kcal was used for the study. Jaggery sugar (Golden Sugar Company Limited) was also used for the study.

Animals

Ethical approval was obtained from the College of Medicine, University of Lagos Animal Care and Use Research Ethics Committee (CMUL/ACUREC/ 07/21/876). Experimental procedures relating to the use of animals were by the EU Directive 2010/63/EU for animal study and the study conformed with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guideline (2010). Eighteen (18) male Wistar rats (150-170g) were obtained from the Central Animal House, University of Lagos. They had access to rat feed and water and these animals were acclimatized for one week to laboratory conditions before the commencement of the administration of the drinks. The rats were kept in standard laboratory conditions of relative humidity, dark/light cycle, and temperature.

Experimental design

The control (Control) group received distilled water (vehicle) daily. The artificial energy drink (AED) group was administered a dose of 1.41ml/day/ 150g of animal body weight of the energy drink daily for 4 weeks through oral

gavage and the natural energy drink (NED) group was administered 1.4 ml/ 150g body weight of the jaggery daily for 4 weeks through oral gavage. The artificial energy drink was calculated based on the dose equivalent to one can of energy drink (250mL) by a human adolescent while jaggery solution (1.25 w/v) was prepared every day. Both energy drinks were diluted with water. The animals were not restricted to food consumption in any way. The body weight and food intake were measured weekly.

Blood collection and serum preparation

After the last day of administering the energy drinks, the rats were sacrificed using pentobarbital sodium anesthesia (50mg/kg, i.p). Blood samples were collected by cardiac puncture into plain sample bottles and the blood was allowed to stand for 30 min thereafter they were centrifuged at 3000rpm for 5min. The supernatant was decanted from the centrifuged blood and frozen at -20^{0}C. Right testis and seminal vesicle tissues were homogenized in phosphate buffer solution, centrifuged at 4,000rpm, and the supernatant separated and stored at -20^{0}C for analysis.

Biochemical assays

Testosterone, Luteinising Hormone (LH), and Follicle Stimulating Hormone (FSH) were assessed by enzyme-linked immunosorbent assay (ELISA) using ELISA assay kits (Acc-Bind Elisa microwell monoband (USA). Testicular cholesterol level was determined using spectrophotometric methods and laboratory kit reagents (Randox Laboratory Ltd, UK) were used for the analysis, and their absorbance was read using a UV-Vis spectrophotometer (DREL 3000 HACH) while testicular glycogen content was determined by harvesting and cleaning the testes of the animals. Afterward, known weights of testes were homogenized in ice-cold trichloroacetic acid (deproteinizing) solution and incubated for 15 minutes in a water bath. After discarding the precipitate, the supernatant was mixed with sulphuric acid and heated for 5 minutes and the absorbance was read with an ELISA reader (Biobase Bioindustry Co. Ltd., Shandong, China) at 620 nm wavelength. Standard glycogen (Sigma; St. Louis, MO, USA) was also prepared and employed for the standard curve. Glucose-6-phosphate was assayed with a Glucose-6-phosphate kit (Sigma; St Loius, MO, USA). Serum lactate dehydrogenase (LDH) and fructose were measured through enzyme colorimetric procedures with reagents purchased from Randox Laboratory Ltd (Antrim, UK). The catalytic property of LDH leading to reversible oxidation of L-lactate to pyruvate, mediated by the hydrogen acceptor, NAD+, is harnessed as a basis of the measurement of LDH activity.

The rate of production of NADH that changes the optical density of the sample was measured spectrophotometrically at 340 nm. The conversion of pyruvate to lactate or the reverse reaction of oxidation of L-lactate to pyruvate can be monitored spectrophotometrically. The analysis of fructose levels was as follows: 20 µl of seminal plasma was mixed thoroughly with 220 µl distilled water, and later deproteinized with 50 µl of ZnSO4 and 50 µl of NaOH. After 15 min of incubation, it was centrifuged at 2500 rpm and 200 µl of clear supernatant was mixed with Indole reagent followed by 32% hydrochloric acid. The mixture was incubated at 60°C for 20 min and after cooling, readings were taken at 470 nm (Karvonen and Malm, 1955). The terminal fasting blood glucose level of the rats was determined using Accu-Check Active (manufacturer: Roche Diagnostics, Pvt Ltd., Mumbai, Maharashtra, India).

Sperm analysis

The analysis of sperm function was carried out as described in a previous study (Adekunbi *et al*., 2016). Briefly, the caudal epididymis was harvested, and the epididymis was cut into pieces in 1 ml of 37^0C of normal saline solution. A drop of the solution was placed on a glass slide and covered with a coverslip and placed under a microscope at x40. The sperm motility was based upon; oscillatory or stationary, slow progression or rapidly progressive, vibrating movements, and these were expressed in percentages. The sperm morphology was determined as follows: a smear preparation was made (with formal saline) and was stained with 1 percent eosin stain, and it was allowed for 20 -30 minutes to allow staining to occur. A hundred (100) sperms per animal were morphologically examined at x100 magnification, the abnormal sperms were categorized based on the presence of irregular heads, detached tails, midpiece bending, and double tails. The sperm count was done using the enhanced Neubauer hemocytometer and was expressed as million/ml of suspension.

Statistical analysis

Data were presented as means ± SEM. Statistical analysis was carried out by analysis of variance (ANOVA) supported by the Newman-Keuls test when pair-wise comparison was done between the groups. The analysis was done using version 5.0 (GraphPad Software, San Diego California, USA). The level of statistical significance was placed at $p<0.05$.

Results

Effects of artificial and natural energy drinks on body weight and feed intake in male Wistar rats

All animals had increased body weights in their respective groups. There was however a significant reduction in the body weight of the NED group when compared to the control and AED groups respectively (Fig.1).

The food intake in the AED and NED groups was smaller ($P<0.05$) compared to the control group throughout the period of the experiment. The food intake in the natural energy drink (NED) group was also smaller when compared to the artificial energy drinks (AED) group.

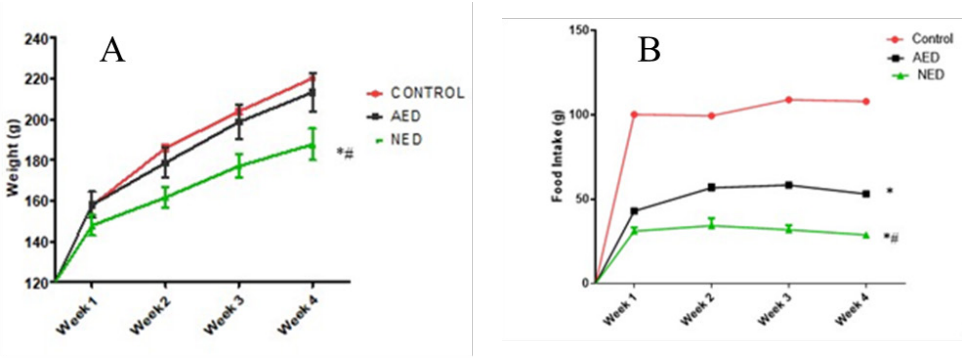

Fig.1. A shows reduced mean body weights of rats administered NED when compared to the control and AED groups. Values are expressed as mean ± SEM of 6 rats per group, *p <0.05 vs control, # p<0.05 vs AED. Natural Energy Drink (NED), Artificial Energy Drinks (AED); **B** shows reduced food intake of rats administered NED when compared to control and AED groups as well as when AED was compared to control. Values are expressed as mean ± SEM of 6 rats per group, *p <0.05 vs control, # p<0.05 vs AED. Natural Energy Drink (NED), Artificial Energy Drinks

Effects of artificial and natural energy drinks on fasting blood glucose level and seminal vesicle fructose in male Wistar rats

There was a significant decrease in the fasting blood glucose level in the AED and NED groups when compared to the control group. There was however no significant difference in the seminal vesicle fructose levels of the AED and NED groups when compared with the control (Fig. 2).

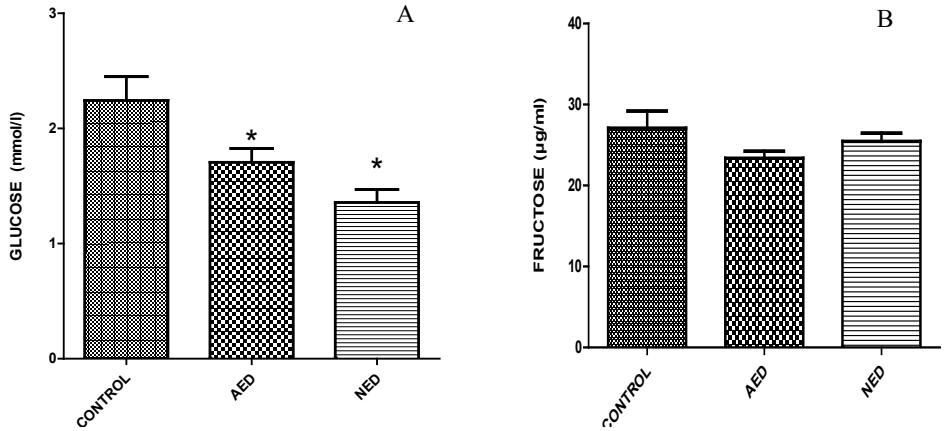

Fig 2. A shows reduced fasting blood glucose levels characterized rats in the AED and NED groups when compared to the control group, while **B** shows there were no changes in the seminal fructose levels. Values are expressed as mean ± SEM of 6 rats per group, *p <0.05 vs control. Natural Energy Drink (NED), Artificial Energy Drinks (AED).

Effects of artificial and natural energy drinks on 3-beta hydroxysteroid dehydrogenase (3β-HSD) and 17-beta hydroxysteroid dehydrogenase (17β-HSD) in male Wistar rats

A significant decrease in the 3-beta hydroxysteroid dehydrogenase (3β-HSD) activity was observed in the AED and NED groups when compared to the control group. Also, a significant decrease in the 17-beta hydroxysteroid dehydrogenase (17β-HSD) activity was observed in the AED and NED groups when compared to the control group. There was, however, a significant increase in the 17-beta hydroxysteroid dehydrogenase (17β-HSD) activity observed in the NED group when compared to the AED group (Fig. 3).

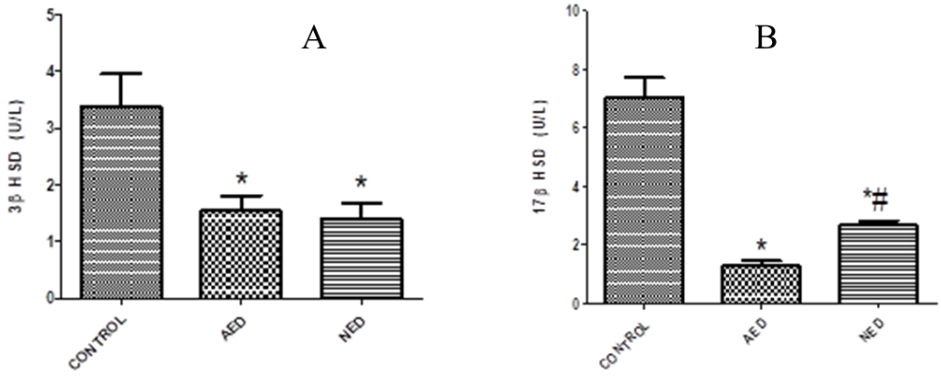

Fig 3. A, B show decreased 3β-HSD and 17β-HSD activities characterized rats in the AED and NED groups compared to the control group and **B**, also shows an increased 17β-HSD activity characterized rats in the NED group when compared to the AED group. Values are expressed as mean ± SEM of 6 rats per group, *p <0.05 vs control, # p<0.05 vs AED. Natural Energy Drink (NED), Artificial Energy Drinks (AED), 3-beta hydroxysteroid dehydrogenase (3β-HSD), 17-beta hydroxysteroid dehydrogenase (17β-HSD).

Effects of artificial and natural energy drinks on Lactate dehydrogenase (LDH) activity and Glucose-6-phosphate dehydrogenase (G6PD), testicular glycogen, and testicular cholesterol levels in male Wistar rats

A significant decrease in the Lactate dehydrogenase activity was seen in AED and NED groups when compared to the control group. There was a significant increase in the Glucose-6-phosphate dehydrogenase (G6PD) level in the AED group compared to the control group. Furthermore, there was a significant decrease in the Glucose-6-phosphate dehydrogenase level in the NED group compared to the AED group. There was no significant difference in the testicular glycogen level of the AED and NED groups when compared to the control. A significant decrease in the testicular cholesterol level was observed in the NED group when compared to the control group as well as in the NED group when compared to the AED group (Fig. 4).

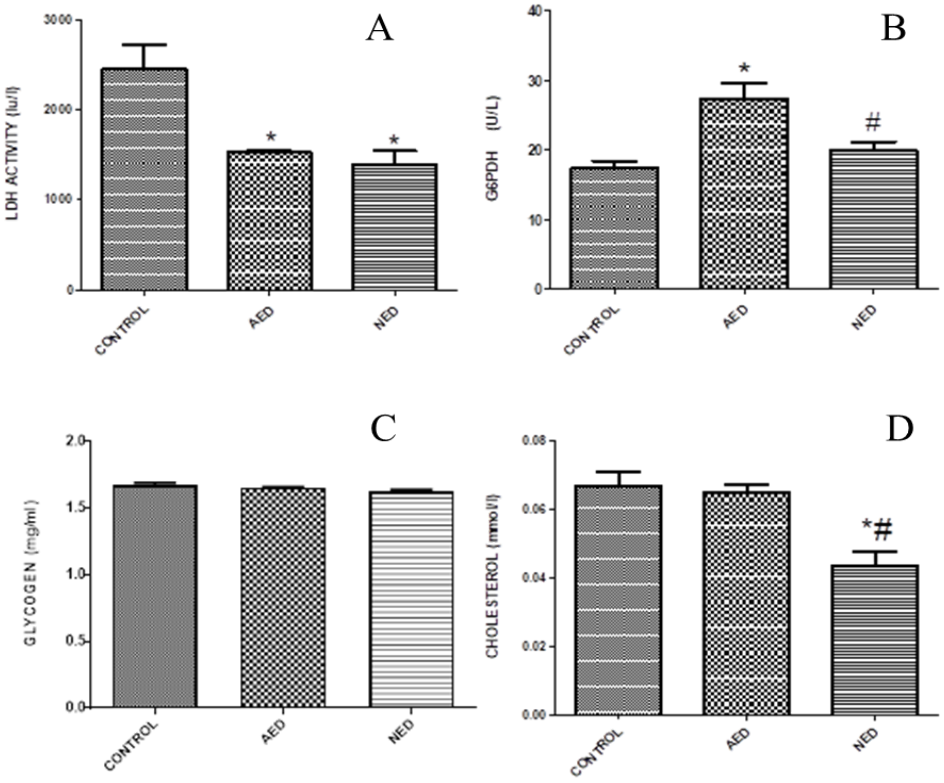

Fig 4. A shows reduced LDH activity characterized rats in AED and NED groups compared to the control group. **B** shows increased G6PD level was observed in animals in the AED group compared to the control group but a decrease in G6PD level was observed in animals in the NED group compared to the AED group. **C** shows no significant difference in the glycogen levels of rats in the AED and NED groups compared to the control group. **D** shows that reduced testicular cholesterol level was observed in the NED group when compared to the control group and also in the NED group when compared to the AED group. Values are expressed as mean ± SEM of 6 rats per group, *p <0.05 vs control, # p<0.05 vs AED. Natural Energy Drink (NED), Artificial Energy Drinks (AED), Lactate dehydrogenase (LDH), Glucose-6-phosphate dehydrogenase (G6PD).

Effects of artificial and natural energy drinks on serum hormonal profile in male Wistar rats

Administration of the artificial energy drink (AED) and the natural energy drink (NED) led to reduced testosterone levels in the AED and NED groups when compared to the control group. There was however a significant

increase in the testosterone level of the NED group when compared to the AED group. A significant decrease in the luteinizing hormone level was observed in the NED group when compared to the control and AED groups. There was however no significant difference in the follicle-stimulating hormone levels of the AED and NED groups when compared to the control (Fig. 5).

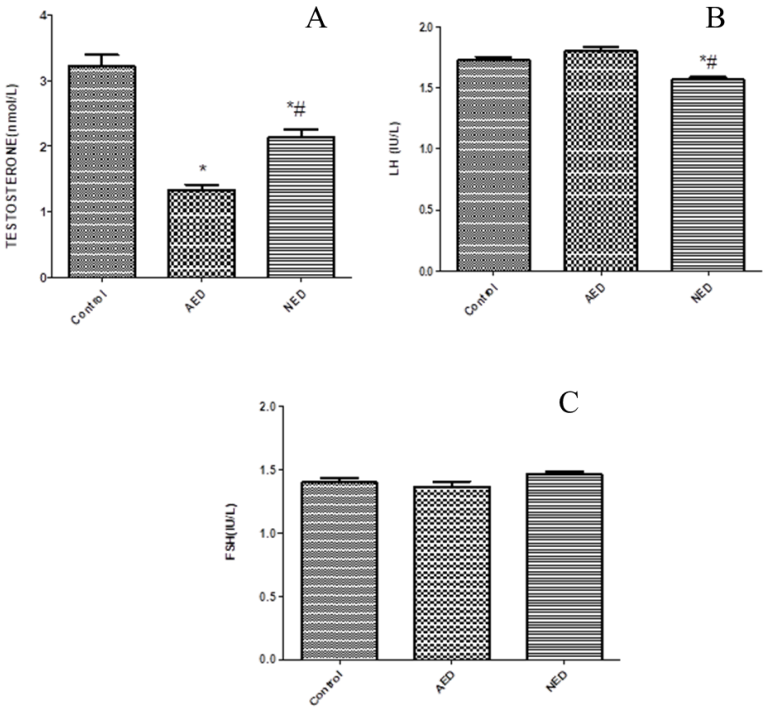

Fig 5. A shows reduced testosterone levels characterized rats in the AED and NED groups when compared to the control group but an increase in the testosterone level characterized rats in the NED group when compared to the AED group; **B** shows reduced LH level was observed in rats in the NED group when compared to the control and AED groups. **C**- shows there was no significant difference in the FSH levels. Values are expressed as mean ± SEM of 6 rats per group, *p <0.05 vs control, # p<0.05 vs AED. Natural Energy Drink (NED), Artificial Energy Drinks (AED), Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH).

Effects of artificial and natural energy drinks on epididymal sperm characteristics in male Wistar rats

Sperm motility was significantly reduced in the NED group when compared to the control and AED groups while there was no significant

difference in the sperm count of the NED and AED groups when compared to the control group. The abnormal sperm morphology significantly increased in the NED group when compared to the control and AED groups (Tab.1).

Table 1. Effects of artificial and natural energy drinks on sperm variables in male Wistar rats**.**

	CONTROL	**AED**	**NED**
Sperm motility (%)	50.20 ±0.92	53.50 ±1.34	45.67±1.56 *#
Sperm count(million/ml)	63.78 ± 3.99	65.62 ± 3.30	61.20 ± 3.49
Abnormal sperm morphology (%)	13.33± 0.84	13.83± 0.98	21.17± 0.75*#

Values are expressed as mean ± SEM of 6 rats per group, *p <0.05 vs control, # p<0.05 vs AED. Natural Energy Drink (NED), Artificial Energy Drinks (AED).

Discussion

This study examined the impact of artificial and natural energy drinks on male reproductive functions. This becomes vital considering the growing concerns about male youngsters' preference for energy drinks. In this study, it appeared that high sucrose consumption is associated with the control of food intake as reported in another study (Adekunbi *et al.,* 2016). The artificial energy drink contains added sugars while the natural energy drink contains natural sugars. Sucrose satiating effects have been observed in previous studies (Anderson and Woodend, 2003; Tappy and Lē, 2009) and it was reported that sucrose satiating effect results in a high feeling of fullness as a result of the sweet taste of sugar (Lavin *et al.,* 2002). It is expected that food intake should match body weight. In this study, animals had increased body weights in their respective groups. There was however a significant reduction in the body weight of the NED group when compared to the control and AED groups respectively. There are conflicting data in the literature concerning sugar consumption on body weight gain in experimental animals; while some authors have reported an increased weight gain in rats, others have reported no significant changes or reductions (Adekunbi *et al.,* 2016; Driescher *et al.,* 2019). In this study, the significant reduction in body weight of the NED group compared to the control and AED groups appear to be a consequence of reduced food intake. Other components in these energy drinks could have roles to play regarding body weight and food intake regulation. The natural drink (jaggery) has been reported to assist in body weight control which could

be the reason for the reduction in body weight of the NED group when compared to the control group (Rao and Singh, 2021). Although the body weight of the AED group was not affected when compared to the control, there was a significant reduction in food intake in the group. Caffeine which is one of the important components of artificial energy drinks improves weight maintenance because of its thermogenic property. It also suppresses appetite and energy intake (Harpaz *et al.,* 2017; Correa *et al.,* 2018). Caffeine intake also decreases food intake in males (Graneri *et al.*, 2021).

There were no signs of systemic toxicity observed in the animals administered both natural and artificial energy drinks, evaluating sperm variables is however an essential step for predicting reproductive toxicity in males (Nallella *et al.,* 2006). According to this study, there was an increased percentage of aberrant sperm morphology in the NED group when compared to control and AED groups, motile sperms were also decreased in the NED group when compared to control and AED groups, while there was no significant difference in the sperm count among groups (Table 1). This result agrees with a study that suggested that a high intake of sucrose might lead to an increase in the percentage of abnormal sperm (Adekunbi *et al.,* 2016) and energy drinks contain high levels of sucrose as well (Jaffé, 2015). Abnormal sperms could also result from likely alterations during spermiation because it is released from the Sertoli cells into the lumen of the seminiferous tubules (Esteves, 2015).

The hypothalamic-pituitary-gonadal (HPG) axis is a standard negative feedback control. As blood testosterone level rises, the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels should decrease. In this study, there was a significant decrease in the testosterone level of the AED and NED groups when compared to the control. The blood testosterone level of the NED group was significantly increased compared to AED. The LH level was significantly decreased in the NED group when compared to the control and AED groups. There was no significant difference in the FSH levels of the groups when compared to the control (Fig 5). The following inferences can be made from these results. Caffeine, a component of artificial energy drinks has been reported to reduce testosterone production (Al-Eryani *et al.,* 2018). The decrease in the testosterone level of the AED group was not affected by LH and FSH levels. This infers that the action of AED may not be through the gonadotropins but directly on the testes. There was an increase in the blood testosterone of the NED group when compared to the AED group which also resulted in the decrease of the blood LH levels in the NED group when compared to the control and AED groups respectively although there was no significant difference in the FSH level of the NED group. The testosterone level

of the NED group unlike the AED group appears to be stimulated perhaps partially by the gonadotropin-releasing hormone (GnRH) which resulted in reduced LH hormone secretion. Jaggery consumption might however be a better option for an energy drink than artificial energy drinks. Testosterone promotes spermatogenesis thus the reduction in testosterone level might be the result of the alterations reported in the sperm variables. The abysmal levels of the hormones may alter reproductive functions and may be a potential causative factor of male infertility consequently.

Lactate dehydrogenase is an indicator enzyme of carbohydrate metabolism needed for germ cell production and differentiation which will eventually result in active glycosylation by the Sertoli cells which secrete lactate as the main energy substrate for spermatids and spermatocytes (Akomolafe *et al.,* 2017). In this study, there was a decrease in LDH activity in AED and NED groups when compared to the control. The low LDH activity would inhibit androgen production (El-Kashoury e*t al.,* 2010) and low sperm action (Akomolafe *et al.,* 2017).

There was a significant increase in the testicular glucose-6-phosphate dehydrogenase (G6PD) level in the AED group when compared to the control. A study reported that an increase in glucose-6-phosphate dehydrogenase (G6PD) level would restore the redox balance in endothelial cells exposed to a high amount of glucose (Zhang *et al*., 2012),. According to this study, however, serum glucose was significantly reduced. The increase in G6PD level thus observed in this study was modulated in an entirely different pathway in the AED group and it was not a result of improved NADPH level which is required in the maintenance of tissue integrity. This scenario is different from the decreased G6PD level observed in the NED group when compared to the AED group. This reduced G6PD level would obstruct spermatogenesis and reduce androgen production (Anuja *et al.,* 2010).

Glycogen is the main source of energy for sperm cells, and it is directly proportional to the steroid hormone level (Govardhan and Changamma, 2014). In this study, there was no significant difference in the testicular glycogen levels in the AED and NED groups when compared to the control. Imbalance in glycogen and fructose contents in the testis and seminal vesicle could lead to germ cell apoptosis or degeneration as well as a decrease in the number of mature and motile spermatozoa (Kamal *et al.,* 1993; Kuramori *et al.,* 2009) as observed in the study.

In this study, the fasting blood glucose levels in both AED and NED groups significantly decreased when compared to the control group. A study also reported reduced fasting glucose after sugar-sweetened beverage consumption (Driescher *et al.,* 2019). Artificial and natural energy drinks have sugars as part of their constituents. Jaggery for instance also contains fructose

which has been reported not to affect blood glucose adversely when consumed in moderate amounts (Gaby, 2005). The animals in this study could probably be in a state of mild hyperglycemia which was why the effect of the glucose level was masked. This is because it has been reported that sucrose in drinking water as opposed to when taken in solid form may lead to adiposity in rodents (Kawasaki *et al.,* 2005). The cause of this however is not clear. It may however be because the level of sucrose, when delivered in water, is much higher than in the solid form (Togo *et al.,* 2019). When rodents consume sugar in drinking water, there is usually increased adiposity and impaired blood glucose homeostasis (Togo *et al.,* 2019). The significant decrease in the fasting glucose level in the AED group is however not in agreement with the study by (Bukhar *et al.,* 2012) that reported that consumption of artificial energy drinks led to a significant rise in serum glucose. The significant increase in the serum glucose levels in this study may be because of the duration of exposure to artificial energy drinks. This study exposed the animals to an artificial energy drink for 4 weeks, whereas in another study the animals were exposed to an artificial energy drink for 6 weeks (Bukhar *et al.,* 2012). The significant increase in the serum glucose levels in these studies may also be because of the age of the male rats used. In this study, pubertal rats were used, whereas, in another study, adult male rats were used (Bukhar *et al.,* 2012). In this study, there was also a significant decrease in the serum glucose level in the NED group compared to the control group. This finding is not in agreement with another study. The authors stated that the consumption of jaggery would lead to an increase in serum glucose (Patel *et al.,* 2011). The significant increase in the serum glucose levels in this study may be because of the duration of exposure to the natural energy drink. This present study exposed the animals to natural energy drinks for 4 weeks, whereas in another study, the animals were exposed to jaggery for 16 weeks (Patel *et al.,* 2011). The reason why the glucose levels in the AED and NED groups were significantly reduced when compared to the control group requires further investigation.

Cholesterol is a known precursor involved in steroidogenesis, a decrease in testicular cholesterol concentration would thus lead to a decreased production of testosterone and will impair fertility (Gatsing *et al.,* 2010). In this study, there was a significant decrease in the testicular cholesterol level in the NED group when compared to the control and AED groups (Fig. 4). The potassium that is present in jaggery is responsible for reducing cholesterol levels (Rao and Singh, 2021). The cholesterol level of the rats in the AED group was not affected because according to some authors the presence of taurine in artificial energy drinks prevents the decrease in testicular cholesterol levels (Chen *et al.,* 2016; Schuchowsky *et al.,* 2017).

In this study, there was a significant decrease in the testicular 3-beta hydroxysteroid dehydrogenase (3β-HSD) activity in both the AED and NED groups when compared to the control (Fig 3). This finding signifies that the consumption of both artificial and natural energy drinks caused a decrease in the activity of this important steroidogenic enzyme, which resulted in a decrease in testosterone synthesis in the Leydig cells of the testes and could thus impair fertility.

There was a significant decrease in the 17-beta hydroxysteroid dehydrogenase (17β-HSD) activity in the testicular homogenate of the AED group when compared to the control group (Fig. 3). This steroidogenic enzyme catalyzes the final step in the biosynthesis of the testosterone pathway (Rebourcet *et al.,* 2020). The finding from this study signifies that the consumption of artificial energy drinks will cause a decrease in the steroidogenesis of testosterone in the Leydig cells. There was a significant decrease in the 17-beta hydroxysteroid dehydrogenase (17β-HSD) activity in the NED group compared to the control group. This finding from the study signifies that the consumption of natural energy drinks could also cause a decrease in testosterone synthesis. In this study, there was a significant increase in the 17-beta hydroxysteroid dehydrogenase (17β-HSD) activity in the NED group compared to the AED group. This finding also shows that natural energy drink consumption could cause a significant increase in 17-beta hydroxysteroid dehydrogenase (17β-HSD) activity when compared to artificial energy drink consumption. Artificial energy drink consumption might cause a larger decline in testicular steroidogenesis of testosterone than natural energy drink consumption.

Conclusions

In conclusion, the results of this study establish that the consumption of both artificial and natural energy drinks would negatively affect testicular-metabolic characteristics, androgenic hormones, and sperm functions. The energy drinks disrupted steroidogenesis and the synthesis of testosterone and gonadotropins and altered sperm quality. The AED and NED thus have anti-gonadal properties.

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