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Original Research Article

Comparative aphrodisiac and toxicity effect of energy drinks consumption in male wistar rats

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Abstract

Energy drink is a type of drink containing sugar and stimulant compounds, usually caffeine or natural stimulants majorly from plant sources. This study evaluates the comparative aphrodisiac and toxicity effect of energy drinks consumption in male wistar rats. Determination of aphrodisiac potential following the oral administration of graded doses (0.5, 1.0, and 1.5 ml/kg) of red-bull, Orijin bitters, and monkey tail. Viagra (Sildenafil citrate) and distilled water served as positive and negative controls, respectively. Sexual behavioural parameters (mounting and intromission frequencies, mounting, intromission, and ejaculatory latencies) were observed. Serum testosterone and cholesterol concentrations were progressively monitored on days 1, 7, 14, and the acute toxicological evaluation of the various energy drinks based on any onset of mortality and behavioural changes. The results showed that red bull increased significantly mounting frequency. A significantly decreased in mounting and intromission latencies in dose-dependent manner, particularly on days 1 and 14. The Orijin bitters revealed a prolonged ejaculatory latency. Testosterone and cholesterol concentrations were also increased as the dose increased, particularly on days 1 and 7. The lowest dose of 0.5 ml/kg showed the best aphrodisiac effect for the Mockite energy drink. The toxicity studies showed that there were no acute behavioural changes with zero mortality. These findings, therefore, validated the claim of the local use of energy drinks as an aphrodisiac in males.

Keywords: Aphrodisiac, Energy drink, Orijin bitters, Monkey tail, Testosterone, Red bull

1 Introduction

Energy drinks were first reported in Europe and Asia in 1960 because of customer dietary supplements that give energy [1]. Consumption of energy-drink globally has increased by 14% (i.e., 1.5 billion liters higher) between 2007 and 2011 and grown by a mean of 10% yearly from 2007 to 2011. More than half of young adults consume a minimum of one can of energy drink monthly, and about 6% use energy drinks daily [2]. Most energy drinks contain natural products such as guarana, ginseng, taurine, 50 to 505 mg of caffeine, and 35 grams of processed sugar per 8-oz serving. The amounts of guarana, taurine, and ginseng found in popular energy drinks are far below the amounts expected to deliver therapeutic benefits or adverse events. However, the other ingredients with potential interactions such as between taurine and other amino acids and between caffeine and some herbal extracts can create synergistic effects, especially side effects. On the other hand, caffeine and sugar are present in amounts known to cause various adverse health effects [1].

Centres for Disease Control and Prevention reported that high school students and young adults consume energy drinks almost at the same rate as soda [1]. Indeed, the energy drink consumption rate might be higher than estimated levels in this self-reporting survey since such surveys usually have a high probability of underestimation. Locally, a refined herbal derivative of these energy drinks is in high demand. Bitters originally was developed for medicinal, digestive, and flavouring purposes. Manufacturers promoted and sold the bitters as a medical cure. The flavouring bitters are usually added to different alcoholic drinks and cocktails to add different flavours to a drink [3]. Aphrodisiacs with a healthy lifestyle can achieve a better sexual life [4]. Sexual feelings are an inevitable part of life. Sex is the most cherished, indispensable, and integral part of every individual and can be a cradle of pleasure and satisfaction. There has been erroneous information, unawareness, fear, and pessimistic outlook as far as sex is concerned. In developing countries, the inability to afford

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modern medical healthcare has forced patients to seek traditional medical attention. The use of herbs in the treatment of ailments in Africa is an age-old practice. Man's continuous reliance on herbs for therapeutic and nutritional benefits come to stay. Many plant extracts are traditionally in use to improve sexual performances [5]. Myths and misconceptions are widespread and are passed on from one generation to another. These sexual myths can result in sexual dysfunctions, misery, silent suffering, distressed interpersonal relationships, and even divorce. Sexual ignorance is a social disease and can be solved via compulsory all-inclusive sex education, which can boost awareness and improve society [4]. However, aphrodisiac has been implicated in treating / managing these arrays of sexual disorders [6–9]. Most aphrodisiacs can amplify sensual experience facets such as light, touch, smell, taste, and hearing. This improved sensual consciousness leads to sexual stimulation and inclination [11]. However, a significant constraint in understanding the link between energy drinks and the aphrodisiac potentials and adverse effects of their consumption is inadequate knowledge about the active aphrodisiac component and toxicity effect of the various compounds present in them.

Based on reported cases of energy drinks associated with health problems and the well-established physiological effects of the active ingredients of energy drinks, it is very likely that their compositions may be responsible for the observed aphrodisiac and adverse effects seen [4]. Hence, this study explores the aphrodisiac and toxic effects of acute prolonged intake of energy drinks on hepatic and renal tissues in rats.

2 Materials and methods

The brand of energy drinks used in the present study was "Red bull," a product of Rauch Trading AG, Switzerland (manufactured for Red Bull GmbH, Austria), Orijin Bitters, a product of Diago/Guinness Nigeria PLC, and Monkey tail local cocktail. These drinks were purchased from the Market Square store in Benin City, Nigeria, except Monkey's tail, which was purchased from Warri, Delta State, Nigeria.

2.1 Drugs, assay kits, and other reagents

Estradiol benzoate and progesterone were purchased from Sigma-Aldrich from China and the USA. Sildenafil citrate was obtained from a community pharmacy outlet in Benin City, Edo State. The testosterone assay kit was procured from Monobind Inc., USA, while every other chemicals used were of analytical grade.

2.2 Acute toxicity study

Twenty-five (25) male rats, as stated in the mating behavioural study earlier comprising of wholly randomised animals of five groups comprising five rats, were used. These animals were monitored for two hours for any behavioural changes such as hyperactivity, sedation, salivation, diarrhoea, accelerated breathing, tail posture, and convulsions after administering the energy drinks (at the respective doses (0.5 ml/kg, 1.0 ml/kg, and 1.5 ml/kg), distilled water and standard drug (viagra) across all the groups. The mortality or lethality was noted after 24 h, and the Lethal Dose (LD_{50}) was determined. These observations continued for 14 days for any delayed signs.

2.3 Experimental design

Two hundred and twenty-five (75) male and thirty 60 non-oestrus female Wistar rats of 140–270 g and 145–260 g body weights respectively were used. The animals were acquired from the animal house of the Department of Anatomy, Faculty of Basic Medical Sciences, University of Benin, Nigeria. The animals were kept in clean wooden cages placed in the well-aerated animal house of the Department of Biochemistry, Faculty of Life Sciences, for acclimatisation with peak conditions (temperature, 25 °C; photoperiod, 12 h of natural light and 12 h of dark). The animals were permitted free access to water and fed with standard commercial pellets. The cages were cleaned daily throughout the work, and animals monitored daily for general health and weighed weekly. No adverse events were noticed before and during the experiment. The 225 male rats were wholly randomised into five groups of 15 and given appropriate treatment orally. Group A was given (2 ml of distilled water) while group B, C, and D were given 0.5, 1.0, and 1.5 ml/kg body weight, respectively, of Red bull, Orijin bitters, and Monkey tail energy drinks. Group E was given the standard drug, sildenafil citrate (5 mg/kg). The oral administration was carried out using an orogastric tube. All animals used in this study were handled following the international guiding principles for biomedical research involving animals as outlined by the Council for International Organization of Medical Sciences and the International Council for Laboratory Animal Science [12].

2.4 Mating behavioural study

The mating behavioural tests from the groups (in a completely randomised manner), five male rats each were selected and observed for sexual behaviour after their daily doses on days 1, 7, and 14 across the varying doses of energy drinks between 19.00 and 03.00 h under a faint light in the Laboratory following the modification of the previously published procedures [14]. The female animals were artificially brought into oestrus (heat) by the successive administration of estradiol benzoate (10 μg/100 g body weight) and progesterone (0.5 mg/100 g body weight) through subcutaneous injections, 48 hr, and 4 hr respectively before pairing. Female rats were only allowed mating during the oestrus phase since this process. The receptive female rats were introduced to the male rats 30 min after administering the drinks at the respective doses to the male rats in a locally special fabricated wooden cage with glass doors and coloured lights. A pairing of the female rats with the male rats across various doses, including controls in the ratio 1:1 (1 female to 1 male), was done. Also, mating behaviour commenced after 10 min of placing the paired animals in the cage and recorded with a video

camera on a tripod stand and discontinued if the male fails to manifest sexual interest. Furthermore, replacement of any female animal that does not show receptivity by another artificially 'heated' female. The occurrence of events and phases of mating after the video recording were analysed, and the frequencies and phases determined. The parameters of male sexual behaviour that after 35 min observation period include: Mount frequency (M.F.) and intromission frequency (IF) are the number of mounts and intromissions from the time of introduction of the female until ejaculation. Mount latency (M.L.) and intromission latency (I.L.) are the time interval (s) between the introduction of the female and the first mount or intromission by the male, and ejaculation latency (E.L.) is the time interval (s) between the first intromission and ejaculation [14].

2.5 Test for libido

There was an assessment of the level of sexual desire of the male rats by the protocol outlined in [13] and libido test carried out using the mounting and intromission frequencies as well their latencies of the mating behavioural test during the 1st, 7th, and 14th day.

2.6 Serum preparation

Yakubu *et al*. [13] defined the modified serum preparation technique involving collecting blood at 1¼ hours after giving the energy drinks, the standard drug (Viagra), and distilled water on days 1, 7, and 14. Under chloroform anaesthesia, with sterile forceps and scissors, the stomach was cut open to expose the internal organs of the animals. Blood was collected via cardiac puncture using a 5 ml syringe and needle per animal into the appropriately labelled clean lithium heparin (to collect plasma) and non-coagulant (plain) (to collect serum) sample bottles and kept at a temperature between 23 °C and 25 °C for ten minutes to clot. The bottles were centrifuged at 3000 rpm for ten minutes using a laboratory centrifuge. The sera and plasma collected were later aspirated with Pasteur pipettes into dry plain bottles and utilised within 12 h of preparation for biochemical assays.

2.7 Determination of serum testosterone

The serum testosterone concentrations were determined quantitatively using the microplate enzyme immunoassay kit for total testosterone concentration in human serum described in the manufacturer's test procedure (Accu-Blind ELISA Microwells, Product code: 3725–300, Monobind Inc., USA).

2.8 Determination of plasma cholesterol

Röschlau *et al*. [18] method for determining total cholesterol levels using enzymatic kits from Randox Laboratories Limited United Kingdom was employed.

2.9 Data analysis

Presentation of data as mean ± SEM of five replicates was done. One-way ANOVA comparing means of different groups and a Duncan multiple range test to analyse differences among different means and the interaction between the variables also performed.

3 Results

The mounting frequency (M.F.) increased on days 7 and 14 as the dose increased, as shown in Fig. 1.

Orijin bitters' effect on M.F. was notably increased at 0.5 ml on day 7 and 1.5 ml on day 14 compared to the positive control (Viagra). The negative control (distilled water) at different doses across the days was not significantly different from each other and significantly different from the positive control (Viagra) and all other energy drinks across the varying doses and days.

Monkey tail at 1.5 ml dose at day one had an increased mounting effect compared to even the positive control (Viagra) and all other energy drinks. On different days for the Red bull, each of the dose groups was not significantly different from the other with an almost consistent range of mounting frequency. The Orijin bitters group revealed an increase in terms of mounting frequency on day 7 (0.5 ml) from the other days.

The Red bull exerted the most significant effect on Intromission frequency (IF) on days 1 and 14 as the dose decrease, while on day 7, Orijin bitters had the highest effect.

The Orijin bitters and the positive control group (5 mg/kg of Viagra) were significantly different ($P < 0.01$) from all other groups on day 1 and 14 while on day 1 (1.5 ml), it was the monkey tail and the Viagra group that were significantly different ($P < 0.01$) from the other dose groups. The highest dose group on day 7 was found significantly different ($P < 0.01$) from the same dose groups on days 1 and 14.

All dose groups on each of days 1 and 7 were observed not significantly different from each other. The negative control (distilled water) and Orijin bitters were significantly different from the other dose groups on day 14 (1.5 ml). However, there was an increase in mount latency (ML) on days 1 and 14 as the dose increases.

As observed, the depiction effect of various energy drinks on ejaculatory latency was noticeable. All dose groups on each day 1 and 7 were observed not significantly different. On day 14, the Orijin bitters group was significantly different from the other groups.

Increased ejaculatory latency (E.L.) on day 1 and 14 as the dose increases with reverse on day 7 was witnessed. There was a decrease in ejaculatory latency on days 1 and 14 as the doses increase against an increase observed on day 7.

Also, the extract increased cholesterol concentrations (Fig. 5), corroborating the increased testosterone concentrations observed.

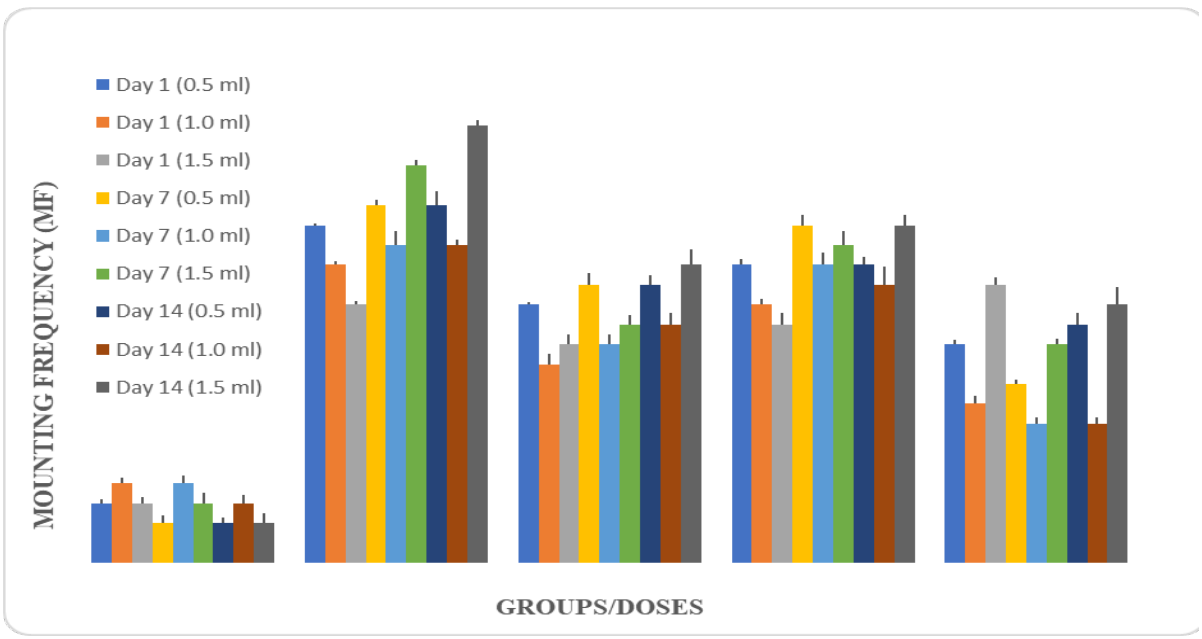

Figure 1. Effects of varying doses of energy drinks across the various days on mating behaviours mounting frequency.

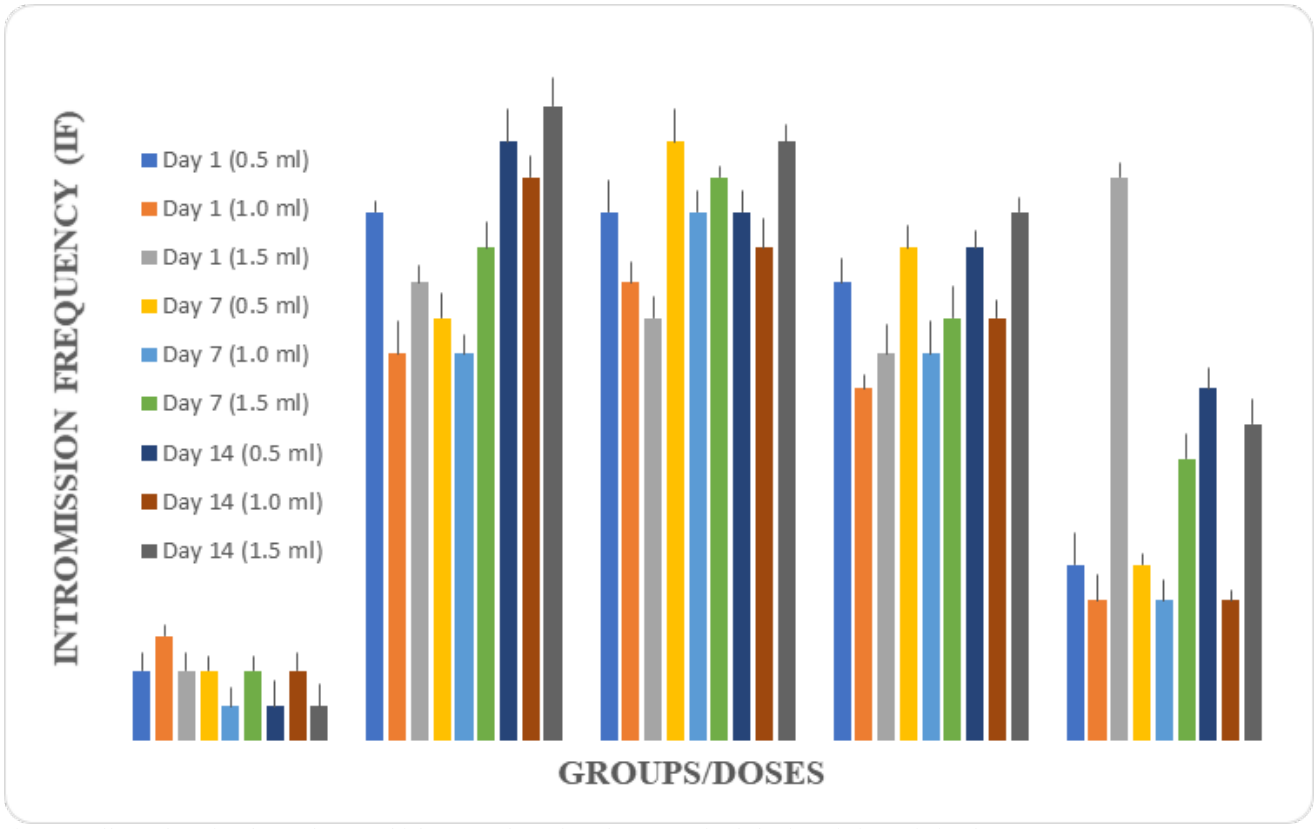

Figure 2. Effects of varying doses of energy drinks across the various days on mating behaviours in intromission frequency

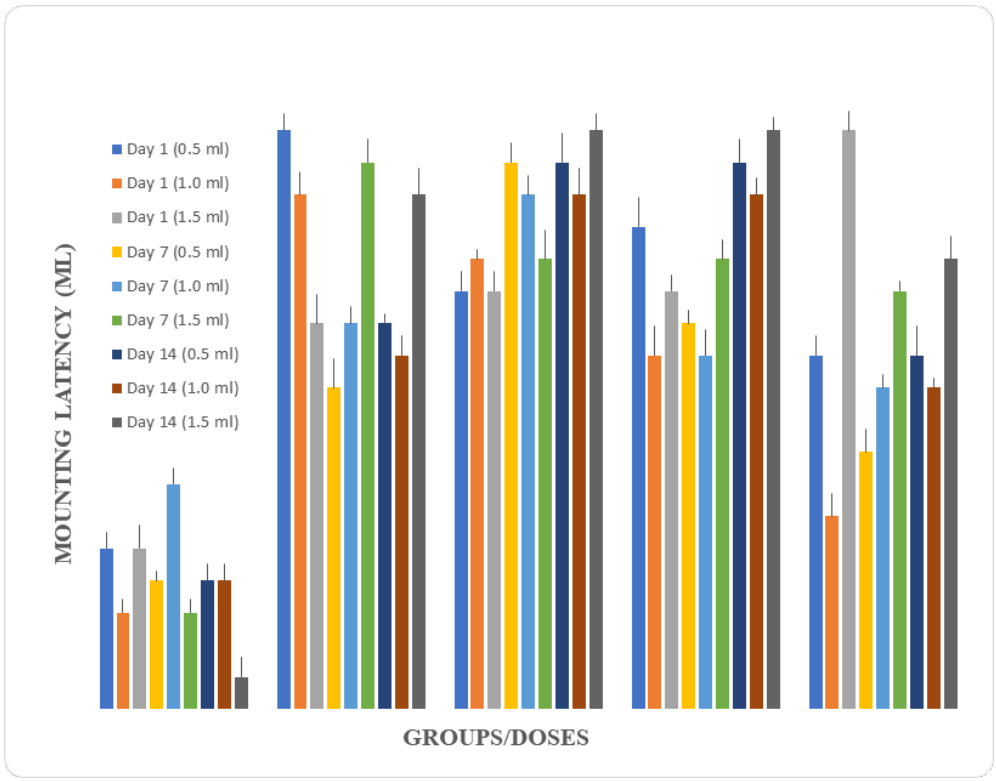

Figure 3. Effects of varying doses of energy drinks across the various days on mating behaviours in mounting latency

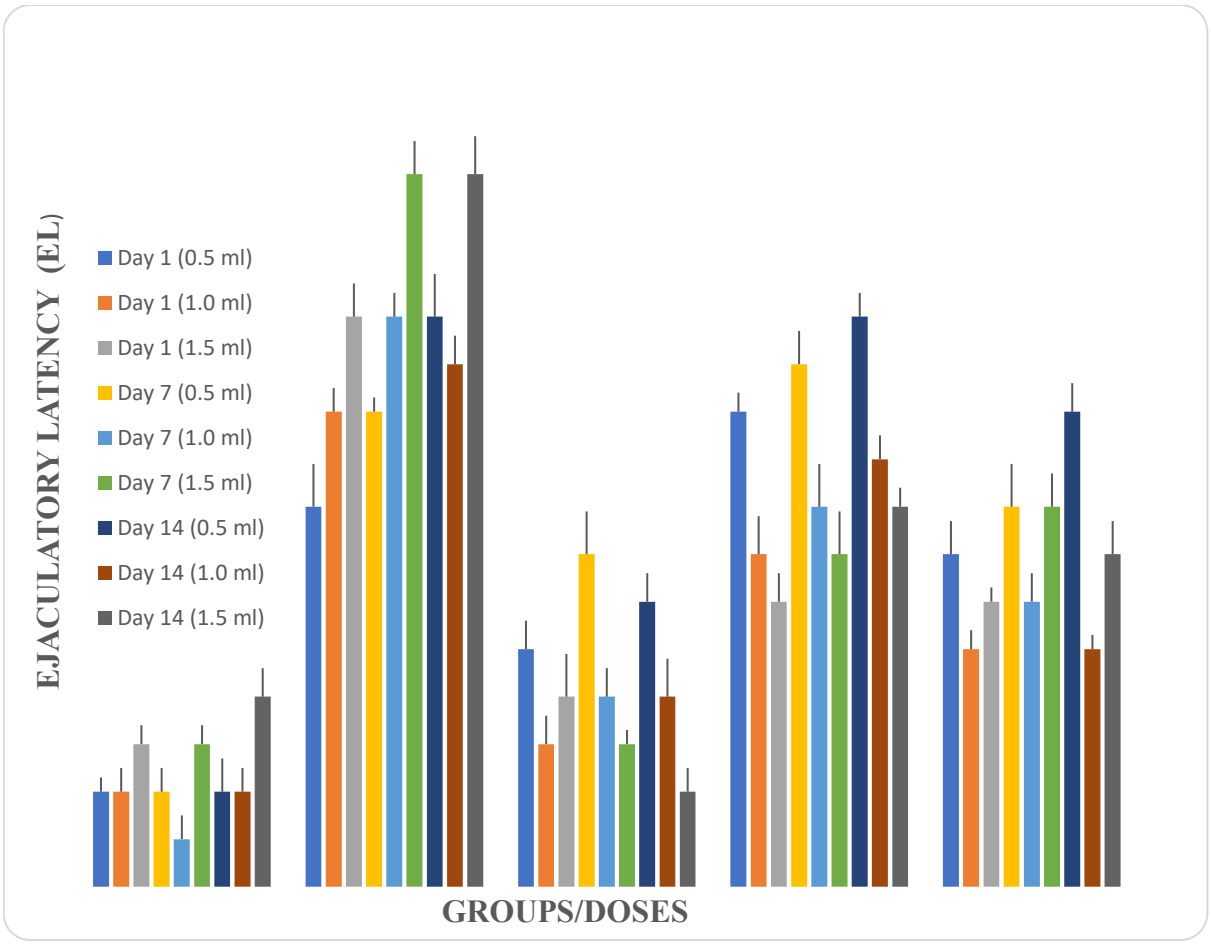

Figure 4. Effects of varying doses of energy drinks across the various days on mating behaviours in ejaculatory latency

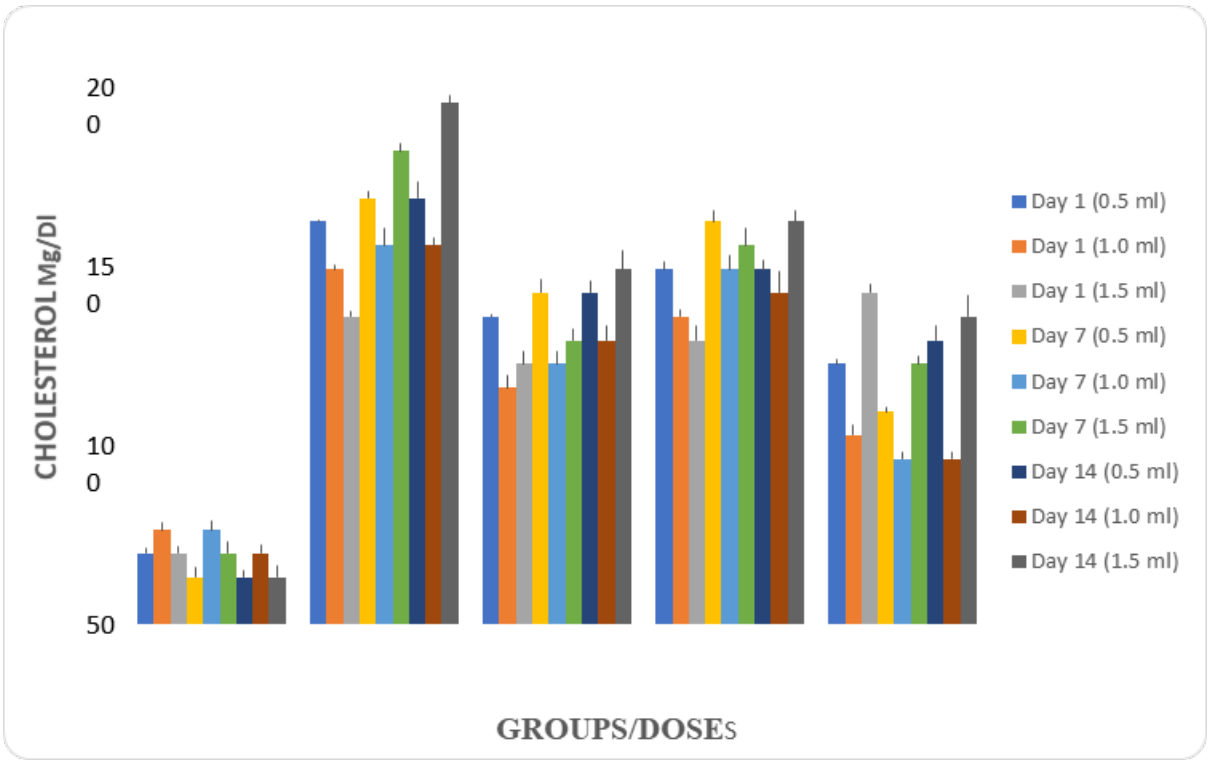

Figure 5. Effects of varying doses of energy drinks across the various days on mating behaviours in cholesterol expression translates into increased libido.

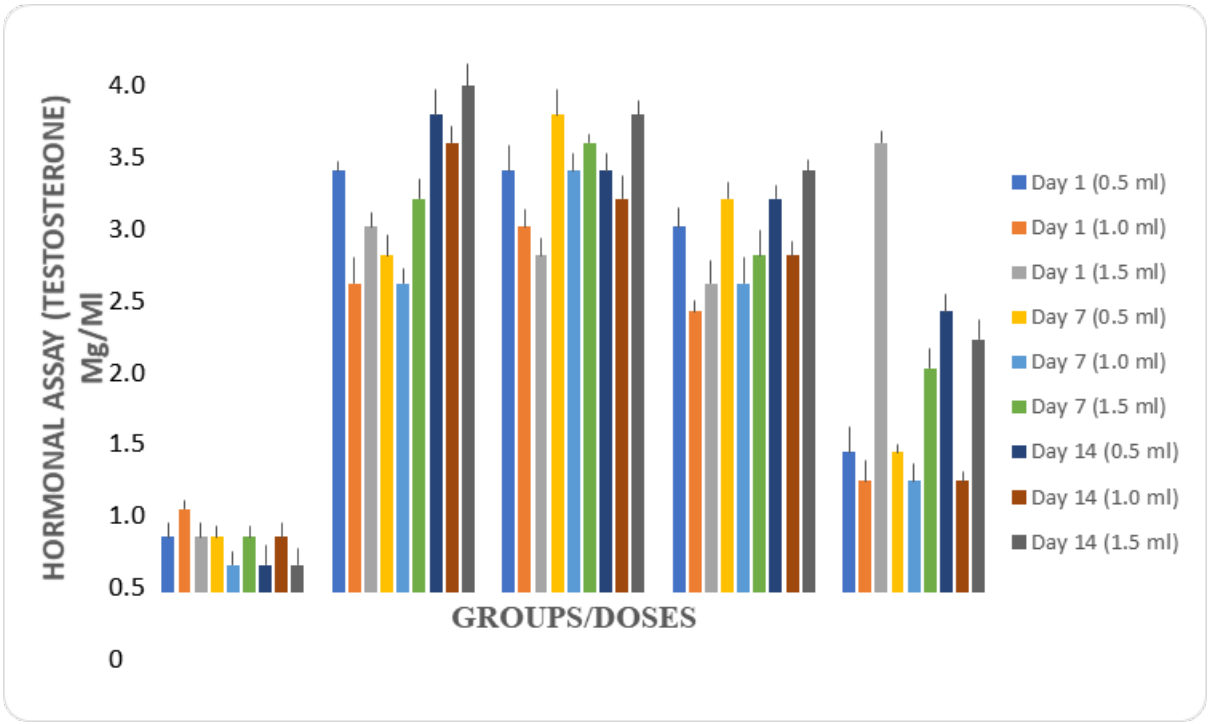

Figure 6. Effects of varying doses of energy drinks across the various days on mating behaviours in testosterone

4 Discussions

An animal model for the initial screening to determine the aphrodisiac potential of a test drug is an accepted model. Also, using rat models to mimic and understand the possible effects of consumed substances is quick and straightforward [31]. It can also be said to be used to evaluate the aphrodisiac and stimulating activity on penile erection against erectile dysfunction [31]. Sexual behavioural parameters such as mount and intromission frequencies are indices of sexual vigour, libido and potency [28, 30]. The mating behavioural analysis revealed that the orijin bitters and red bull increased mount and intromission frequencies compared with the negative control group. However, the effect was less than that of viagra (Figs. 1 and 2). It also decreased the rate of mount and intromission latencies in the male rats (Figs. 3 and 4) and prolonged the ejaculatory latencies (Fig. 4) on day 1, 7 and 14. These significant increases in mounting frequencies (M.F.) and intromission frequencies (IF) indicate arousal. Also, corresponding decreases in mount latency (ML), intromission latency (I.L.) indicate arousal of male rats. It also reflects enhanced performance, motivation and vigour. These findings agree with an earlier report by Alabi *et al*. [20], Yakubu and Akanji [16], Gbankoto *et al*. [18] on the significant changes in ML and I.L. Also, the prolonged ejaculatory latency (E.L.) by the orijin bitters indicates that the sexual function of the male rats was enhanced (prolonged duration of coitus), suggesting an aphrodisiac activity. These findings, which are similar to the report by Erhabor and Idu 2017 [21] Fouche *et al*. [17], further support the activity of orijin bitters in enhancing sexual function. However, the highest dose of 1.5 ml/kg of the red bull had a reversed activity/inhibition on sexual behavioural parameters on day 1 and 14 and agreed with the findings of Lieberman [22]; Rogers *et al*. [23]. They observed the same reverse inhibition at the highest dose in their respective studies, which may be due to sedation as animals showed no form of sexual interest. Reports reveal that androgens are essential modulators of male sexual behaviour, including erection and libido. These androgens may act both at the central and peripheral nervous system levels Salih *et al*. [24]. Testosterone is one of the main androgens in the male gonads produced by the testis' interstitial Leydig cells [25]. Reports reveal that testosterone enhances sexual function and libido. It also improved the intensity of orgasm and ejaculations [26, 27]. An increase in testosterone led to a moderate but corresponding increase in sexual desire or libido [28]. Also, the red bull 1 ml/kg and monkey tail consumption resulted in the highest testosterone concentration on day 1 and 14. In contrast, the 1.5 ml/kg of the monkey tail on day 1 produced the highest concentration of testosterone level at the initial consumption (Fig. 6). This might have accounted for the profound effect on sexual and masculine behavioural parameters on the first day of the experiment. Reports suggest that cholesterol is a requirement for regular activity of the testicles. Cholesterol is also a known precursor in the synthesis of steroids, including bile acids, steroid hormones and vitamin D [29, 30]. Yakubu *et al*. [7, 12] reported that an increase in testicular and serum cholesterol concentrations led to a corresponding increase in the aphrodisiac activity of a medicinal plant. This increase in cholesterol concentrations results in increased production of testosterone. All animals across the various groups showed no significant adverse acute toxicological effect attributed to the acute consumption of the various energy drinks. Also, adverse changes in behaviour were absent, indicating that physical and clinical signs were unremarkable. The intake of food and water was regular, suggesting that the animals had a normal appetite. There was no mortality during the entire period of the study. Therefore the lethal dose (LD_{50}) of the extract is more significant than 1.5 ml/kg since up to this dose with no record of death. These findings agree with the previous report by Gatsing *et al*. [29] reported 0 % mortality.

5 Conclusion

Overall, this study showed that the various energy drinks evaluated have aphrodisiac potential, lending credence to its acclaimed use and abuse as an aphrodisiac agent in Nigeria. The lowest dose of 0.5 ml/kg of the orijin bitters presented the best aphrodisiac effect. The monkey tail has a functional capacity to increase testosterone and cholesterol concentrations at a regulated average dose of 1.0 ml. Possible mechanisms of action for its aphrodisiac property and the ability of the orijin bitters to relax the corpus cavernosum muscle of the penile organ as another possible mechanism of action. These findings, therefore, give backing to the acclaimed local use of energy drinks as an aphrodisiac in males.

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