Original Research Article

SHORT TERM EFFECTS OF ENERGY DRINK ON SPERM MORPHOLOGY, HAEMATOLOGICAL PARAMETRES AND BEHAVIOUR OF ADULT MALE MICE

Abstract

With the rising popularity of energy drink among Nigerian youths and males especially, there is the need to investigate the possible side effect of its major stimulant, caffeine on diverse health related issues in general and reproductive outcome in particular. To test the mutagenic and other effects of energy drink, Red Bull energy drink was offered *ad libitum* to adult male mice assay, in increasing concentration of the drink for twenty eight days. Sperm head abnormaly and haematological parameters were carried out at 7, 14, and 28 days of exposure, while body movements studies of the mice were done on the last five days of energy drink exposure. The study showed that mice offered 75% concentration of Red Bull for 14-28 days had significantly ($P< 0.05$) negative effects on sperm head abnormalities. Haematological parameters that were most affected were the mean corpuscular haemaglobin concentration (MCHC) and platelets. This could imply that exposure to energy drink should be at low concentration and not for long period so that it does not have negative reproductive, haematological and behavioural outcomes.

Key Words: Mutagen, Energy Drink, Red Bull, Sperm morphology, Haematological parameters.

1 INTRODUCTION

Energy drinks, non-alcoholic carbonated drinks designed to boost energy and categorized under Food and Beverage Industry are gaining wide use in Nigeria especially among youths, sportsmen and adult males. They are marketed with catchy names that convey strength, power, speed, sexuality and often with appropriate music (Akande and Banjoko, 2011).

Like all soft drinks, energy drinks contain little nutritional value and high amount of sugar. What really differentiates energy drinks from soft drinks is that they also contain significantly higher doses of caffeine. Common energy drinks contain around 160 – 300mg caffeine per 500 ml, while coffee contains 40 – 80mg/cup and tea 20-60mg/cup. Depending on the brand, energy

drinks may also contain other additives such as B vitamins, taurine, ephedrine, carbonated water, guarana, glucuronolactone, maltodextrin, inositol, carnitine, creatine and ginseng.

A variety of physiological and psychological effects have been attributed to energy drinks and their ingredients. Two studies reported significant improvements in mental and cognitive performances as well as increased subjective alertness (Howard and Marczinski, 2010). In a web survey conducted by Serfert *et al* (2011), it was reported that energy drinks were consumed by 30% to 50% of adolescents and young adults. Frequently containing high and unregulated amounts of caffeine, these drinks have been reported in association with serious adverse effects, especially in children, adolescents, and young adults with seizures, diabetes, cardiac abnormalities, or mood and behavioral disorders or those who take certain medications. Of the 5448 US caffeine overdoses reported in 2007, 46% occurred in those younger than 19 years.

Nehlig and Debry (1994) reported that the mutagenic potential of coffee and caffeine has been demonstrated in lower organisms, but usually at doses several orders of magnitude greater than the estimated lethal dose for caffeine in humans. They concluded that the chances of coffee and caffeine consumption in moderate to normal amounts to induce mutagenic effects in humans are almost nonexistent. They also stated that caffeine seems to potentiate rather than to induce chromosomal aberrations, transforms sublethal damage of mutagenic agents into lethal damage and that coffee and caffeine are also able to inhibit the mutagenic effects of numerous chemicals. In the Philippines, Red Bull was banned because of the suspected effects on infertility in men.

In a comprehensive literature review, Pennington *et al* (2010) stated that specific effects that have been reported by adolescents that used energy drink included jitteriness, nervousness, dizziness, the inability to focus, difficulty concentrating, and insomnia. In another report from the United States, students that took energy drink on their way to school were observed to be restless and had destructive tendencies. As regards the psychological effect of energy drink, two studies reported significant improvements in mental and cognitive performances as well as increased subjective alertness (Howard and Marczinski, 2010).

Full blood count is a frequently used laboratory test performed to support the diagnosis of several diseases: anaemia, certain cancers, infections, acute hemorrhagic states, allergies and immunodeficiency disorders or used in periodic health examination and preoperative evaluation

(George and Parker, 2003). Thus it is probable that the ailing conditions associated with overdose of energy drink stimulants (caffeine, ginseng, guarana and taurine) could be detected from haematological parameters.

Even though the caffeine in energy drink has been implicated to cause infertility in men, agitation, nervousness and anxiety in children and adolescents, the few local researches done were on their biochemical and histological effects on tissues and organs (Akande and Banjoko, 2011) with little on mutagenesis and haematology. The objectives of this study were to look at the short term effects of "Red Bull" energy drink on sperm morphology, haematological parameters and behaviour on adult male mice.

2 MATERIALS AND METHODS

2.1 Animal Husbandry

Thirty six (36), eight weeks old adult Albino male mice, (*Mus musculus*) bioassay model were purchased from a stock raised in the Zoology Laboratory, University of Lagos. They were acclimatized to their new cages for a period of four days (in the same Laboratory) during which they were fed pelletized food purchased from a reputable source and given water *ad libitum*. Mice were chosen as a model for this study because according to Pagulayan and Gutay-Baoanan (1993) their spermatogenesis is similar to that of man.

2.2 Test Substance

Red Bull^{R} Energy Drink (RBED), a product of Austria was purchased from retail outlets. The stated ingredients were: water, sucrose, glucose, acidity regulator (sodium citrates), carbon dioxide, taurine (0.4%), glucuronolactone (0.24%), caffeine (0.03%), inositol, vitamins (niacin, pantothenic acid, B6, B12), flavourings, colours (caramel, riboflavin). Each 100 ml contained: Energy 192 kj (45 kcal), protein 0 g, carbohydrates 11.3g, fat 0 g, with vitamins as % recommended daily allowance.

Experimental animals were exposed to it through their drinking water, which was changed daily.

2.3 Treatment Arrangement

The 36 adult male mice were weighed and randomly divided into treatment groups. Each treatment group (except the Control that had four) had a total of eight mice sub-divided into four per cage. The disparity in weight of mice in each group was ± 2.0 grammes. The mice were fed with pelleted food and given *ad libitum* Red Bull energy drink mixed with water in the concentrations shown on Table 1.

Table 1: Test substance, Red Bull and water ratios, in volume/volume

Treatment	% Energy Drink	% Water	Ratio
1 (CONTROL)	0	100	0:1
2	25	75	0.25:0.75
3	50	50	1:1
4	75	25	0.75:0.25
5	100	0	1:0

On days 7, 14, and 28 epidydimes and blood samples were collected from the mice for sperm morphology count and haematology parameter tests respectively.

2.4 Sperm Morphology Count

Mice were sacrificed for epididymes collection by cervical dislocation. The sperm from excised epididymes were stained and examined under electron microscope for sperm head abnormality; these were compared with a work done in a similar environment and reported by Otubanjo and Mosuro (2001).

2.5 Blood Sample Collection and Full Blood Count

Retro-orbital method as described on http://vetmed.duhs.duke.edu/GuidelinesforRetroOrbitalBloodCollection.html was used in blood collection from the mice. Veinous blood from the orbital sinus was collected into tubes containing ethylene diamine tetra acetic acid (EDTA) anticoagulant. Blood was analysed with Haematology Analyser (BC 2800 Model) to determine haemoglobin (Hgb), white blood cells (WBC.), packed cell volume (PCV) and mean corpuscular hemoglobin concentration (MCHC). Differential blood parameters such as monocytes, eosinophils and basophils (termed MID in this work), neutrophils, lymphocytes, and platelets were also determined.

2.6 Body Movements

Animal behaviour was determined through the number of body movements per minute, during the last week of the experiment. Counted body movements were lifting of head, walking, climbing, and eating.

2.7 Data Analyses

The mean and standard deviation of the sperm head counts (normal and abnormal), blood parameters and body movement were analysed using Microsoft Excel. Means that differed with the Control were compared using Student–t two tail tests.

3 RESULTS

3.1 Red Bull Energy Drink Effect on Sperm Morphology of Adult Male Mice

Plate 1 showed photograph of normal, while plates 2-4 showed abnormal sperm head morphologies. Black arrow was used to show the sperm head depicted. Seven of the abnormal sperm heads as described by Otubanjo and Mosuro (2001) were observed with amorphous and pin heads being the most common.

Table 2 showed the normal and abnormal sperm head counts and their deviation from mean. On days 7 and 28, all the means of the normal sperm heads for the four treatments were significantly

different ($P \leq 0.05$) from the Control. On day 14, only Treatment 5 (100% energy drink) was significantly different at $P \leq 0.05$ from the Control. The means of the abnormal sperm heads for all the Treatments were significantly different at $P \leq 0.05$ from Control on day 14, and on day 28 for Treatment 3 (50% energy drink).

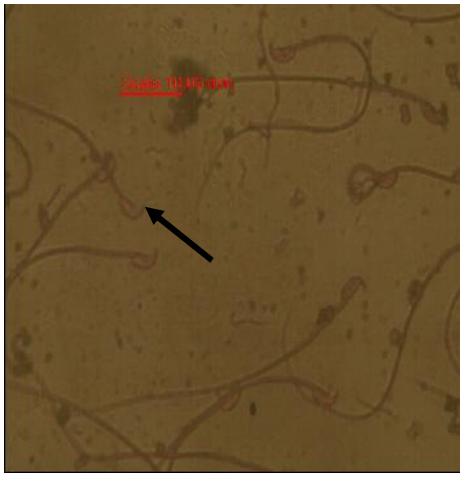

Plate 1: Normal mouse sperm head of Trt 1 at day 28 (Mag. x 200)
Note: Trt= Treatment on all the Plates

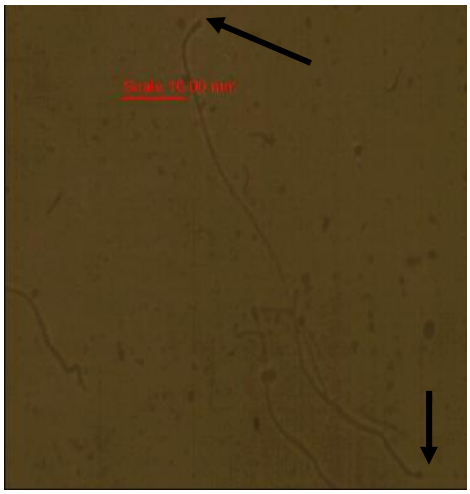

Plate 2: Pin sperm head of Trt II mouse after 14 days of exposure to Red Bull (Mag.x 200)

Plate 3: Pin head sperm of Trt IV mice on day 14 of exposure to Red Bull (Mag. x200)

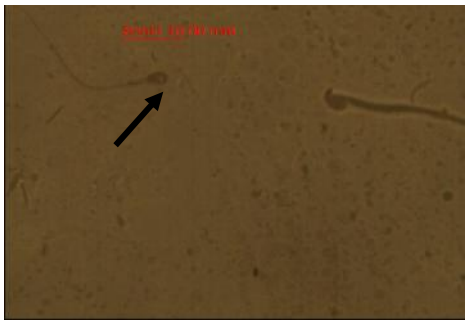

Plate 4: Amorphous sperm head of Trt V after 14 days of exposure to Red Bull (Mag. x 200)

Table 2: Concentration and duration of exposure to Red Bull effect on mice sperm head count

Normal Sperm head count					
Period of exposure (Days)	**Mean±SD value for each group based on % concentration of Red Bull drink**				
	1	**2**	**3**	**4**	**5**
7	1114.25±62.8	208±61.8*	120.5±70.0*	184.25±111.5*	312±192.8*
14	1114.25±62.8	1227±461.4	1152.75±157.5	1113.25±64.6	574.5±48.8*
28	1114.25±62.8	564±313.7*	617.75±317.4*	152.75±82.9*	418±211.9*
Abnormal Sperm head count					
7	20.25±17.8	20.25±12.6	8.25±8.3	17.5±15.2	42.25±32.6
14	20.25±17.8	100.5±46.9*	134.25±73.5*	115.5±57.5*	103.25±50.6*
28	20.25±17.8	35.5±11.3	115.5±24.3*	40.5±28.7	141.75±93.7

SD= Standard Deviation; *= Significantly different from the Control group at $P \leq 0.05$

3.2 Red Bull Energy Drink Effect on Haematological parameters of Adult Male Mice

Red Bull effect on different blood parameters varied with their concentration and duration of use (Table 3), which shows the mean ±SD values for the eight parameters tested. Treatment means that were significantly different at $P \leq 0.05$ from the Control were indicated with asterisk (*) sign. Haemaglobin, packed cell volume and lymphocytes did not have any significant difference.

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159 **Table 3:** Effect of Red Bull on the haematological parameters/factors of mice

White blood cell (WBC), x 10^^{9}/L					
Period of exposure (Days)	**Mean±SD value for each group based on % concentration of Red Bull drink**				
	1	**2**	**3**	**4**	**5**
7	9066.7±1650.3	5100±0.0	3733.3±1270.2*	8266.7±7043.7	6466.7±923.8
14	9066.7±1650.3	12166.7±3926	8800±519.6	7300±519.6	8433.3±981.5
28	9066.7±1650.3	7433.3±1270.2	11633.3±461.9	8266.7±981.5	10900±1732.1
Haemoglobin (Hgb), g/L					
7	14.5±1.5	13.6±0.0	13.2±0.1	13.1±0.1	13.7±0.4
14	14.5±1.5	13.9±0.3	14±0.3	13.1±2.3	12.9±0.8
28	14.5±1.5	12.6±1.7	14.9±0.5	14.6±0.2	13.8±0.8
Packed Cell Volume (PCV), %					
7	47.5±5.6	46.0±0.2	44.9±0.4	44.7±1.1	43.4±1.0
14	47.5±5.6	46.4±0.6	46.5±0.5	38.7±7.1	45.9±1.6
28	47.5±5.6	37.2±5.4	48.3±3.2	49.3±0.6	49.1±3.3
Mean Corpuscular Haemoglobin Concentration (MCHC), g/dL					
7	30.8±0.0	29.6±0.1*	29.4±0.1*	29.1±0.5*	30±0.0*
14	30.8±0.0	30.2±0.3	30.1±0.1*	30.4±0.2	29.0±0.8
28	30.8±0.0	31.3±0.0*	30.2±0.9	29.2±0.2*	29±0.3*
Platelets x10^^{9}/L					
7	479.7±68.0	295±13.9*	297.3±17.9*	283.7±94.7*	371.7±44.5
14	479.7±68.0	362.7±15.0	404.7±6.4	311.7±60.0*	290±31.2*
28	479.7±68.0	331±65.8	464±1.7	459.3±47.9	350.7±35.8
Lymphocyte, x10 ^{9}/L					
7	74.3±6.1	65.3±2.9	75±1.7	81±1.7	83.7±2.3
14	74.3±6.1	79.7±7.5	74.3±4.0	88.3±4.6	82±5.2
28	74.3±6.1	87.3±2.3	70.3±0.6	65.7±2.9	80.3±4.6
Neutrophil, %					
7	14.3±3.8	22.3±2.3*	17.7±2.3	13±3.5	12.7±1.2
14	14.3±3.8	14±6.9	17.7±0.6	7.7±2.9	11±3.5
28	14.3±3.8	8.7±2.3	20±1.7	23.7±4.0*	12±3.5
MID (A combination of monocytes, eosinophils and basophils), x10^{9}/L					
7	11.3±2.5	12.3±0.6	7.3±0.6	6±1.7*	3.7±1.2*
14	11.3±2.5	6.3±0.6	8±3.5	4±1.7*	7±1.7
28	11.3±2.5	4±0.0*	9.7±1.2	10.7±1.2	7.7±1.2

160 SD= Standard Deviation; *= Significantly different from the Control group at $P \leq 0.05$

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3.3 Comparison of Haematological parameters of Adult Male Mice exposed to Red Bull Energy Drink and Haematological Standards

Table 4 showed a comparision of the Treatment means with two Haematological Standards for mice. Apart from MID which had higher values than the two reference standards; all the other parameters had values close to the standards.

Table 4: Blood factors of mice exposed to Red Bull compared with haematology standards

Blood Factors	1	2			3			4			5			Standard	
		Days			Days			Days			Days				
		7	14	28	7	14	28	7	14	28	7	14	28	1	2
PCV	47.5	46.0	46.4	37.2	44.9	46.5	48.3	44.7	38.7	49.3	43.4	45.9	49.1	39-49	36.72-46.8
Hgb	14.5	13.6	13.9	12.6	13.2	14.0	14.9	13.1	13.1	14.6	13.7	12.9	13.8	10.2-16.6	11.8-14.9
Platelet	479.7	295	363	331	293	405	464	284	312	459	372	290	350	160-410	766-1657
MCHC	30.8	29.6	30.2	31.3	29.4	30.1	30.2	29.1	30.4	29.2	30.0	29.0	29.0	-	31.8-34.7
MID	11.3	12.3	6.3	4.0	7.3	8.0	9.7	6.0	4.0	10.7	3.7	7.0	7.7	0.3-3	0-3.7
Lymphs	74.3	65.3	79.7	87.3	75	74.3	70.3	81.0	88.3	65.7	83.7	82.3	80.3	55-95	60-95
Neut	14.3	22.3	14.0	8.7	17.7	17.7	20.0	13.0	7.7	23.7	12.7	11.0	12.0	-	7-31
WBC	9.7	5.1	12.2	7.4	3.7	8.8	11.6	8.3	7.3	8.3	6.5	8.4	10.9	6-15	3.2-12.7

Standard 1, Source: http://www.ahc.umn.edu/rar/refvalues.html

Standard 2, Source: http://en.aml-vet.com/animal-species/mouse/hematology

3.4 Red Bull Energy Drink Effect on Adult Male Mice Body Movement

Table 5 showed the means of body movements of adult mice offered different concentrations of Red Bull and compared with the Control group. Only mice on 75% and 100% Red Bull had significantly different means, at $P \leq 0.05$, from control on day 24 of the test period. On day 26, mice on 25% and 75% Red Bull showed significant difference ($P \leq 0.05$) from the Control.

Table 5: Effect of Red Bull on body movement of mice

Period of exposure/ Days	Mean±SD value for each group based on % concentration of Red Bull drink				
	1	**2**	**3**	**4**	**5**
24	36±3.1	38.2±4.5	44±12.9	52.7±5.6*	48.7±11.0*
25	45.7±6.7	40.7±11.9	52.7±12.3	52±12.5	46.3±11.6
26	47.3±5.1	38.3±4.2*	45.2±10.1	54.7±4.5*	50.2±9.0
27	47.7±4.4	48.7±7.1	42.5±8.1	54.2±8.1	50.3±7.5
28	58.7±3.1	44.7±8.7*	39.3±12.5*	52.3±8.7	47±10.3*

SD= Standard Deviation; * =Significantly different from the Control group at $P \leq 0.05$

4 DISCUSSION

4.1 Effects of concentration and exposure time of Red bull on mice sperm head morphology

Normal sperm head counts were highest for mice on 25% Red Bull on day 14 of exposure, while the lowest was observed for mice on 75% and on day 14. Abnormal sperm head counts were lowest on Control followed by 25% and 75% on day 28. This could imply that high concentration and prolonged use of Red Bull caused a reduction in normal while increasing the incidence of abnormal sperm heads. Amorphous and pin heads were the major forms of abnormalities in sperm morphology encountered during the study. These did not have acrosome needed for penetration of ovum during fertilization.

Other substances have been reported to cause abnormal sperm heads. Odeigah (1997) recorded increasing percentage of abnormal sperm heads with increasing concentration of formaldhyde treated rats. Pagulayan and Gutay-Baoanan (1993) reported the incidence of variant abnormal shapes of acrosome that was dose dependant with almost 4 fold increment when Malathion was used on mice. They alluded that changes in head shape may be correlated to changes in the motility and penetrating capacity of the sperm, with balloon types as the most critical, because of the absence of hook which is vital for the entry of sperm to egg.

The higher values of sperm head abnormalities of mice exposed to varying concentrations and exposure time of Red Bull indicates that the substance might have caused damage to the pre-meiotic stage of spermatogenesis which is the period when deoxy ribonuceic acid (DNA) synthesis occurs Odeigah (1997).

4.2 Effects of concentration and exposure time of Red Bull on mice haematological factors

White blood cell, neutrophils, MID, platelets and mean corpuscular haemaglobin concentration (MCHC) showed means that differed significantly (≤ 0.05) with the Control. This implied that platelets and MCHC values were the most affected by Red Bull concentration and exposure time in adult male mice. Low platelets could be caused by drug toxicity, while low MCHC, a red blood cell index, could lead to anaemia. White blood cell differentials (monocytes, eosinophils and basophils) termed MID in this work had values higher than the two standards.

Ashaolu *et al* (2011) reported significant effect (≤ 0.05) compared to control, in haematological parameters of rats given different concentrations of monosodium glutamate (MSG). At 14 days, MCHC of mice rats fed MSG at 5.5 kg/kg body weight had means that significantly differed from control. The reported high levels of neutrophils and lymphocytes implied a compromised immune status, while low values in packed cell volume, haemaglobin, red blood cells and mean corpuscular haemoglobin concentration were indicative of anaemic condition. Ajagbonna *et al* (2006) in Ashaolu *et al* (2011) showed that ingestion of a drug could alter the normal range of haematological parameters.

Okochi *et al* (2003) did report a positive increase in levels of haemaglobin, packed cell volume and red blood cell; while white blood cell and lympocytes decreased in trypanosome infected rats that were treated with African Herbal Formula.

4.3 Effect Red Bull Energy Drink on Body Movement of Adult Male Mice

Only mice on 75% and 100% Red Bull had significantly different means from control on day 24 of the test period. On day 26, mice on 25% and 75% Red Bull showed significant difference from the control. This implied that activity rate of adult male mice reached peak on 75% Red Bull. Forbes *et al* (2007) reported that during repeated cycling tests in young healthy adults an energy drink significantly increased upper body muscle endurance. In laboratory studies, caffeine at a dose of about 6 mg/kg body weight (e.g., 490 mg for a 180-lb person) has often proved effective at enhancing exercise performance lasting from 1-120 min (Graham, 2001).

5 CONCLUSION

There is an inherent challenge in extrapolating this result from mice studies to humans. However, the result did indicate the potential health risks associated with regular and prolonged use of energy drink, Red Bull on spermatogenesis and haematology. More information is needed on behavior, and the effect of energy drinks on oogenesis in female mice.

Despite the warning on the label: "Not reommended for children and persons sensitive to caffeine", there is no monitoring and control over this group accessing it. Public regulatory and health agencies should be proactive in taking measures that would protect the vulnerable group. As damage to deoxy ribonucleic acid (DNA) is the fundamental mechanism of induced mutation, mutagenecity testing of common energy drinks in the Nigerian market is necessary since many young people and non alcohol users may feel at home with them without knowing the attendant side effects.

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