Original Research Article

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

OLALERU, F.[a] and ODEIGAHP.G.C.[b]

[a]Department of Zoology, University of Lagos

[b]Department of Cell Biology and Genetics, University of Lagos

Corresponding Author: OLALERU, FATSUMA,

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 constituent, caffeine on reproductive and health related issues. To test the reproductive, health, and other effects of energy drink, increasing concentration of Red Bull® was offered *ad libitum* to adult male mice for twenty eight days. The Control, Treatment 1 was offered 100% water. Treatments 2-5 were offered 25%, 50%, 75% and 100% Red Bull®, respectively. Sperm head abnormally counts and haematological parameters were carried out at 7, 14, and 28 days of exposure, while body movements studies of the mice were conducted 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. The percent abnormal sperm head for Treatments 1-5 were 1.82, 8.07, 12.40, 15.46 and 21.81 respectively. Haematological parameters that were most affected were the mean corpuscular haemaglobin concentration and platelets. The number of body movements increased with increasing concentration of Red Bull®. These results 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: Energy Drink, Red Bull®, Sperm morphology, Haematological parameters.

1 INTRODUCTION

Energy drinks refer to beverages that contain caffeine in combination with other ingredients such as taurine, guaraná, and B vitamins, and that claim to provide its consumers with extra energy (European Commission on Food Safety, 1999). They are non-alcoholic, often lightly carbonated beverages designed to give the consumer burst energy by the addition of a number of energy enhancing ingredients, most notably caffeine. Depending on the brand, energy drinks may also contain other additives such as B vitamins, taurine, ephedrine, carbonated water, á, glucuronolactone, maltodextrin, inositol, carnitine, creatine and ginseng (Akande and Banjoko, 2011).

Energy drinks are becoming popular in many countries. In the United States for instance, the market for energy drinks has grown rapidly, with 6 billion units consumed in 2010 (Wolk *et al*., 2012) This popularity might be due to the aggressive advertisements in the mass media which generally target the young people such as students (Malinauskas *et al*., 2007). In Nigeria energy drinks are gaining wide use 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). 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.

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 comprehensive literature review, Pennington *et al*. (2010) reported jitteriness, nervousness, dizziness, the inability to focus, difficulty concentrating, and insomnia as the specific effects that were reported by adolescents that used energy drinks. Wolk *et al*. (2012) stated that caffeine is the main active ingredient in energy drinks, and excessive consumption by children and adolescents may acutely cause caffeine intoxication, resulting in tachycardia, vomiting, cardiac arrhythmias, seizures, and death.

Ingested caffeine has been reported to have the capacity to cross the blood-testis barrier. Caffeine consumption as a factor that could alter male reproductive function has not been investigated

extensively (Nawrot *et al*., 2003). Data from *in vitro* studies suggest that caffeine has variable, dose-related effects on human sperm motility, number and structure. Men who drank one or two cups (of unknown volume) of coffee per day had increased sperm motility and density compared with subjects who drank no coffee. However, men who drank more than two cups per day had decreased sperm motility and density (Dlugosz and Bracken, 1992).

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). It was used in this study to test for the effects of Red Bull.

The effect of caffeine on behaviour of mice was studied by Red Bull Company. Different concentrations of Red Bull® energy drink (RBED) were orally administered to six weeks old male and female mice. The study which lasted for 13 weeks showed that mice activities increased with increasing concentration of RBED (European Commission on Food Safety, 2003).

Although energy drinks has been implicated to cause agitation, nervousness, anxiety and various health relation conditions in children and adolescents, there are few local researches on the effects of these beverages on reproduction, haematology and behavior. The objectives of this study were to investigate the effects of Red Bull® energy drink on sperm morphology, haematological parameters and behaviour on adult male mice.

2.0MATERIALS 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 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® energy drink, a product of Austria was purchased from retail outlets. The ingredients stated on the label 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 0g, carbohydrates 11.3g, fat 0 g, with vitamins as % recommended daily allowance. The pH was 4.3.

Experimental animals were exposed to Red Bull® through their drinking water, which was offered *ad lib* and changed daily.

2.3 Treatment Arrangement

The 36 adult male mice were weighed and randomly divided into treatments. Each treatment (except the Control that had four) had a total of eight mice sub-divided into four per cage. Mice weighed between 19-29grams.The disparities in weight of mice in each group were ± 2.0 grams.

The mice were fed *ad libitum* with pelleted feed and given Red Bull® energy drink mixed with water in the concentrations shown on Table 1. The feed was composed of crushed corn, wheat middling, fish meal, ground nut cake, brewers' yeast, bone meal, oyster shell, sodium chloride, antibiotic and antioxidant.

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

Treatment	% Red Bull	% Water	Red Bull®:Water Ratio
1 (Control)	0	100	0:1
2	25	75	0.25:0.75
3	50	50	0.5:0.5
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

For each treatment, one mouse was euthanized for epididymes collection by cervical dislocation. The sperm from excised epididymes were stained and examined under Olympus compound

microscope for sperm head abnormality. The sperm head morphology described by Otubanjo and Mosuro (2001) was used as guide.

2.5 Blood Sample Collection and Full Blood Count

Blood was collected from the mice by using the retro-orbital method as described on http://vetmed.duhs.duke.edu/GuidelinesforRetroOrbitalBloodCollection.html. Venous blood from the orbital sinus was collected into tubes containing ethylene diamine tetra acetic acid (EDTA) anticoagulant. The technique described by Jain (1986) was used for determining the haematological parameters. HaematologyAnalyser (BC 2800 Model) was used 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

The method described by Abalaka and Auta (2010) was used in studying the mice behaviour. Number of body movements: lifting of head, walking, climbing, and eating per minute was used to determine behaviour. This was carried out on the last five days of the study.

2.7 Data Analyses

Descriptive statistics in the form of graphs and tables, and inferential statistics were used in interpreting the data. Analysis of variance was carried out on the sperm head counts (normal and abnormal). Post- hoc test for significant values was conducted using Dunnet. The mean of the haematological parameters were compared with the average of the range of values of mice haematology data, obtained from http://en.aml-vet.com/animal-species/mouse/hematology, using t-test.

3 RESULTS

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

Seven of the abnormal sperm heads as described by Otubanjo and Mosuro (2001) were observed with amorphous and pin heads being the most common. Amorphous heads were irregular round

shaped, while the pin heads had tiny round shapes. Both types of sperm heads did not have acrosome.

Tables 2 and 3 show the normal and abnormal sperm head counts and a multiple comparison of the means using Dunnet t-Test; respectively. 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 was significantly different at $P \leq 0.05$ from the Control. The means of the abnormal sperm heads for all the Treatments on day 14, and on day 28 for Treatment 3 were significantly different at $P \leq 0.05$ from Control. The multiple comparison of treatment means with the control for the normal sperm counts showed significance difference at $P \leq 0.05$ for Treatments 2,3,4 and 5 on day 7 and 28 and Treatment 5 on day 14.

Figures 1 and 2 show the respective percent of normal and abnormal sperm head count with increasing exposure time to and concentration of Red Bull intake. The normal sperm head counts decreased with increasing exposure period to and concentration of RBED. The abnormal sperm heads increased with longer exposure to and increasing concentration of RBED

Comment [D1]: The percentages were different at P < 0.05? The significance is not presented on the figures.

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

Normal Sperm head count					
Exposure	**Treatments**				
Time (Days)	**1**	**2**	**3**	**4**	**5**
7	1114.25±31.4	208±30.9*	120.5±35*	184.25±55.8*	312±96.4*
14	1114.25±31.4	1227±230.7	1152.75±78.8	1113.25±32.3	574.5±24.4*
28	1114.25±31.4	564±156.9*	617.75±158.7*	152.75±41.5*	418±106*
Abnormal Sperm head count					
7	20.25±8.9	20.25±6.3	8.25±4.2	17.5±7.6	42.25±16.3
14	20.25±8.9	100.5±23.5*	134.25±36.8*	115.5±28.8*	103.25±25.3*
28	20.25±8.9	35.5±5.7	115.5±12.2*	40.5±14.4	141.75±46.9

Data are presented as Mean±SEM, n= 4; *= Significantat $P \leq 0.05$ when Treatment means were compared with Control.

Table 3: Multiple comparisons of Treatment means with Control using Dunnet t-Test of normal and abnormal sperm heads of adult male mice exposed to different durations and concentrations of Red Bull energy drink

Normal Sperm head count					
Exposure time (Days)	**Treatment**				
	1	**2**	**3**	**4**	**5**
7	-	--906.25*	--993.75*	--930.00*	--802.25*
14	-	112.75	38.50	--1.00	--539.75*
28	-	--550.25*	--496.50*	--961.50*	--696.25*
Abnormal Sperm head count					
7	-	2.50	--9.50	--0.25	24.55
14	-	80.25	114.00*	95.25	83.00
28	-	15.25	95.25*	20.25	121.50*

*= Mean Difference is significant from Control group at $P<0.05$

Comment [D2]: This table is not necessary. All this information is already showed in table 2.

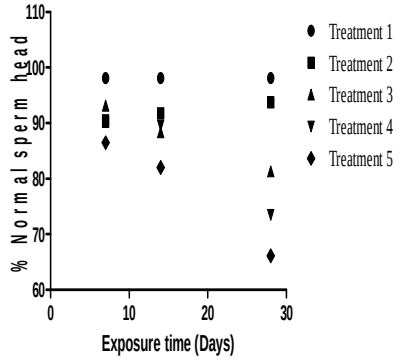

Fig. 1: Percent normal sperm head with increasing concentration of Red Bull intake

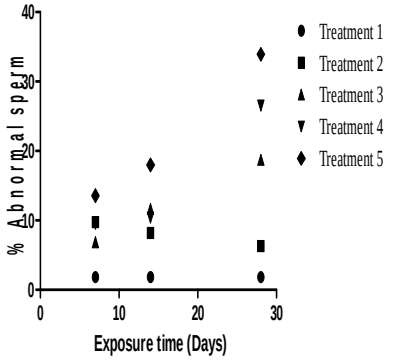

Fig. 2: Percent abnormal sperm head with increasing concentration of Red Bull intake

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 4 shows the mean ±SEM values for the eight blood parameters tested. Treatment means that were significantly different at $P<0.05$ from the Control were indicated with asterisk (*) sign. Haemaglobin, packed cell volume and lymphocytes did not have any significant difference.

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

Table 4 shows the treatment means juxtaposed with a Haematological Standard for mice. Table 5 shows the comparison of these treatment means with the average of the standard. Treatment means when compared with standard were not significant for white blood cells and haemoglobin, whereas platelets and MCHC were significant for period of exposure to and concentration of RBED intake.

Table 4: Effect of duration of exposure to and concentration of Red Bull® on the haematological parameters of adult male mice

White blood cell (WBC), x 10^9/L					**Standard[b] 3.2-12.7**
Exposure time (Days)	**Treatments**				
	1	**2**	**3**	**4**	**5**
7	9.07±1.0	5.10±0.0	3.70±0.7*	8.30±4.1	6.50±0.5
14	9.07±1.0	12.20±2.3	8.80±0.3	7.30±0.3	8.43±0.6
28	9.07±1.0	7.40±0.7	11.60±0.3	8.30±0.6	10.90±1.0
Haemoglobin (Hgb), g/L					**11.8-14.9**
7	14.5±1.1	13.6±0.0	13.2±0.1	13.1±0.1	13.7±0.3
14	14.5±1.1	13.9±0.2	14±0.2	13.1±1.6	12.9±0.6
28	14.5±1.1	12.6±1.2	14.9±0.4	14.6±0.1	13.8±0.6
Packed Cell Volume (PCV), %					**36.72-46.8**
7	47.5±4.0	46.0±0.1	44.9±0.3	44.7±0.8	43.4±0.7
14	47.5±4.0	46.4±0.4	46.5±0.4	38.7±5.0	45.9±1.1
	47.5±4.0	37.2±3.8	48.3±2.3	49.3±0.4	49.1±2.3
Mean Corpuscular Haemoglobin Concentration (MCHC), g/dL					**31.8-34.7**
7	30.8±0.0	29.6±0.1*	29.4±0.1*	29.1±0.4*	30±0.0*
14	30.8±0.0	30.2±0.2	30.1±0.1*	30.4±0.1	29.0±0.6
28	30.8±0.0	31.3±0.0*	30.2±0.6	29.2±0.1*	29±0.2*
Platelets x10^9/L					**766-1657**
7	479.7±48.1	295±9.8*	297.3±12.7*	283.7±67.0*	371.7±31.5
14	479.7±48.1	362.7±10.6	404.7±4.5	311.7±42.4*	290±22.1*
28	479.7±48.1	331±46.5	464±1.2	459.3±33.9	350.7±25.3

Lymphocyte, x10 ^{9}/L						**60-95**
7	74.3±4.3	65.3±2.1	75±1.2	81±1.2	83.7±1.6	
14	74.3±4.3	79.7±5.3	74.3±2.8	88.3±3.3	82±3.7	
28	74.3±4.3	87.3±1.6	70.3±0.4	65.7±2.1	80.3±3.3	
Neutrophil, %						**7-31**
7	14.3±1.2	22.3±1.6*	17.7±1.6	13±2.5	12.7±0.9	
14	14.3±1.2	14±4.9	17.7±0.4	7.7±1.8	11±2.5	
28	14.3±1.2	8.7±1.6	20±1.2	23.7±2.8*	12±2.5	
MID (A combination of monocytes, eosinophils and basophils), x10^{9}/L						**0-3.7**
7	11.3±1.8	12.3±0.4	7.3±0.4	6±1.2*	3.7±0.9*	
14	11.3±1.8	6.3±0.3	8±2.5	4±1.2*	7±1.2	
28	11.3±1.8	4±0.0*	9.7±0.9	10.7±0.9	7.7±0.9	

Data are presented as Mean± SEM, n=3 for Control, and 2 for other Treatments; *= Significant at $P < 0.05$ with Control. [b]**Standard Source:** http://en.aml-vet.com/animal-species/mouse/hematology

Table 5: Pair-wise two tailed t-Test of haematological parameters of mice with standard values

Comment [D3]: This table is not necessary. All this information is already showed in table 4.

White blood cell (WBC), x 10^^{9}/L	**Treatments**				**Standard[b]**	**3.2-12.7**
Exposure time (Days)	**1**	**2**	**3**	**4**	**5**	
	t-value *P*	**t-value *P***	**t-value *P***	**t-value *P***	**t-value *P***	
7	1.172 0.362	aa	-3.500 0.177	0.385 0.766	-2.188 0.273	
14	1.172 0.362	1.574 0.360	2.222 0.269	-1.111 0.467	0.235 0.853	
28	1.172 0.362	-0.136 0 .914	8.875 0.071	0.441 0.736	2.300 0.261	
Haemoglobin (Hgb), g/L						**11.8-14.9**
7	1.319 0.318	aa	-1.500 0.374	-2.500 0.242	0.571 0.670	
14	1.319 0.318	2.400 0.251	2.500 0.242	-0.462 0.725	-0.357 0.782	
28	1.319 0.318	-0.828 0.560	3.111 0.198	5.750 0.110	0.929 0.524	
Packed Cell Volume (PCV), %						**36.72-46.8**
7	1.775 0.218	20.700 0.031*	8.543 0.074	3.463 0.179	1.635 0.349	
14	1.775 0.218	8.880 0.071	11.600 0.055	-0.164 0.896	2.600 0.234	
28	1.775 0.218	-0.647 0.634	2.724 0.224	15.480 0.041*	2.186 0.273	
Mean Corpuscular Haemoglobin Concentration (MCHC), g/dL						**31.8-34.7**
7	aa	-36.500 0.017*	-76.000 0.008*	-10.625 0.008*	aa	
14	aa	-12.400 0.051	-13.500 0.020*	-13.750 0.020*	-6.923 0.091	
28	aa	aa	-4.400 0.142	-26.667 0.024*	-13.833 0.046*	
Plateletes x10^^{9}/L						**766-1657**
7	-18.644 0.003*	-76.042 0.008*	-58.645 0.011*	-10.982 0.058	-21.481 0.030*	
14	-18.644 0.003*	-64.962 0.010*	-146.364 0.004*	-16.971 0.037*	-34.463 0.018*	
28	-18.644 0.003*	-15.114 0.042*	-498.667 0.001*	-18.458 0.034*	-27.435 0.023*	
Lymphocyte, x10 ^{9}/L						**60-95**

7	0.898	0.464	-5.200	0.121	-1.333	0.410	2.000	0.295	2.750	0.222
14	0.898	0.464	0.000	1.000	-0.571	0.670	2.375	0.254	0.667	0.626
28	0.898	0.464	5.250	0.120	-14.000	0.045*	-4.400	0.142	0.375	0.722
Neutrophil, %										**7-31**
7	-2.135	0.166	2.000	0.295	-1.000	0.500	-1.667	0.344	-6.000	0.222
14	-2.135	0.166	-0.500	0.705	-3.000	0.205	-4.200	0.149	-2.333	0.626
28	-2.135	0.166	-5.500	0.114	O.333	0.795	1.000	0.500	-2.000	0.295
MID (A combination of monocytes, eosinophils and basophils), x10^3/L										**0-3.7**
7	6.527	0.023*	21.300	0.030*	11.300	0.056	2.433	0.248	2.150	0.277*
14	6.527	0.023*	9.300	0.068	1.717	0.336	1.767	0.328	3.767	0.165
28	6.527	0.023*	aa		8.150	0.778	9.150	0.069	6.150	0.103

a= The t-test cannot be computed because the standard error of the difference was 0.

*=Significantat$P$<0.05

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

Table 6 shows the effect of RBED on the body movements of adult male mice. Fig. 4 is a graphic representation of the values. The body movements means of mice on Treatments 4 and 5 of were significantly higher (P<0.05) from Control on day 24. On day 26, mice on 25% and 75% Red Bull showed significantly (P≤0.05) lower and higher values respectively from the Control. However, Treatments 2, 3 and 5 were significantly lower (P<0.05) from Control on day 28.

Table 6: Effect of Red Bull on body movement of mice

Exposure time (Days)	Mean±SEM value for each group based on % concentration of Red Bull drink				
	1	**2**	**3**	**4**	**5**
24	36±1.3	38.2±1.8	44±5.3	52.7±2.3*	48.7±4.5*
25	45.7±2.7	40.7±4.9	52.7±5.0	52±5.1	46.3±4.7
26	47.3±2.1	38.3±1.7*	45.2±4.1	54.7±1.8*	50.2±3.7
27	47.7±1.8	48.7±2.9	42.5±3.3	54.2±3.3	50.3±3.1
28	58.7±1.3	44.7±3.6*	39.3±5.1*	52.3±3.6	47±4.2*

SEM= Standard Error of Mean; *= Significantly different from Control at P≤0.05

4 DISCUSSION

4.1 Effects of exposure time to and concentration of Red bull on adult mice's sperm head morphology

Normal sperm head counts were highest for mice on 0% RBED. This was followed by 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. When the treatment means on days 7 and 28 were compared with control, Dunnet t-Test showed that there was significant at $P \leq 0.05$. This could imply that treatment levels and exposure of male mice to RBED caused significant reduction in normal sperm counts. A 21-81% abnormality in sperm head that was significant ($P \leq 0.05$) with 100% RBED administration could imply the reproductive risk associated with prolonged use of this beverage. Fertility test with mice that produced the highest percentage of abnormal sperm morphology was not conducted. It is possible that abnormal sperm head morphology reflects abnormality in spermatogenesis with resultant embryos having low potential for establishing a normal pregnancy (Kahraman *et al*., 1999). The mice were 8 weeks of age at the commencement of the 4 weeks study. The abnormality could have occurred at some stage in the 5 weeks spermatogenesis cycle.

Amorphous and pin heads were the major forms of abnormalities in sperm morphology encountered. These did not have acrosome needed for the penetration of ovum during fertilization. Fertility in males depends on normal linear progressive sperm motility and normal morphology with several compounding factors related to diet, lifestyle, stress and socioeconomic affecting semen quality (Dada *et al*., 2001). Changes in sperm head shape might be correlated to changes in the motility and egg penetrating capacity of the sperm (Pagulayan and Gutay-Baoanan, 1993). Kahraman *et al*., 1999 reported a low fertility and pregnancy rates in a study where megaloheads sperm was used for intracytoplasmic sperm injection. This shows that sperm head defects could cause some levels of infertility in men. In addition, the amorphous and pin heads observed in this study were similar to those reported by Otubanjo *et al*., (2007) where the abnormalities were attributed to induced mutagenicity of the test drug, ivermectin. The criterion for a positive response or mutagenicity, according to them, is based on evidence of statistically significant occurrence of abnormal sperm at $P<0.05$ and reproducibility in separate experiments. In this study sperm head abnormally was significant at $P<0.05$ with 100% RBED administration.

Comment [D4]: This finding is the major one related to sperm assessment. However, it was poorly discussed. A discussion regarding to the causes of amorphous and pin head sperm is necessary. I suggest that the authors read the article: Kahraman et al (1999).Fertility of ejaculated and testicular megalohead spermatozoa with intracytoplasmic sperm injection.Human Reproduction.

Comment [D5]: Is 4.3 pH high? Besides, what is the relationship between pH and sperm head abnormallties?

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 ($P \leq 0.05$) with the Control. The significant

difference of platelets and MCHC for all the treatments when compared with standard haematological values implies that they were the most affected by Red Bull concentration and exposure time in adult male mice. These results were similar to that obtained with Red Bull® by Khayyat *et al*., 2014. They tested three brands of energy drinks: Power Horse®, Red Bull® and Code Red® on Wistar albino rats for four weeks, with blood parameters analysed at two or four weeks. Increase in WBC might imply activation of the immune system, a normal cell-mediated immune response (Khayyat *et al*., 2014). Energy drinks have been linked to increased platelet aggregation and decreased endothelial function in healthy young adults (Higgins *et al*., 2010). Destruction of red cells reflects failure of hepatocellular functions that could be caused by caffeinated energy drink (Akande and Banjoko, 2011).

Significant decrease in platelets could also imply that continuous use of RBED could lead to thrombocytopenia, a condition whereby not enough platelets are made in the bone marrow, are destroyed while in the blood stream, spleen or liver (Khayyat et al., 2014); and anaemia due to the low MCHC, a red blood cell index. *Aloe vera* seems to produce outcomes that may seem as antidote to this effect of RBED (Channa *et al*., 2014). The significantly high values of MID (white blood cell differentials made up of monocytes, eosinophils and basophils) could imply that RBED intake could have triggered the production of these blood cells.

Comment [D6]: Is aloe vera an antidote for RBED effects? This sentence is not clear.

Comment [D7]: The authors did not discuss this finding. How could RBED affect those blood parameters?

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

There was an initial significant difference ($P<0.05$) in number of body movements of mice on the higher values of RBED. However, there was no consistent trajectory in the body movements over duration of exposure to and concentration of RBED. This outcome might seem that the drink did not affect the animals' activity rates. In a 13-week study Red Bull Company conducted where 6 weeks old mice of both sexes were orally offered RBED at 0%, 33%, 50% and 100% *ad libitum*, increased activity was recorded in all the treatment groups compared with controls. Frequency of the activity was similar in the first and last months of the study, showing that tolerance did not develop over time (European Commission on Food Safety, 2003).

Comment [D8]: This sentence needs clarification.

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 Red Bull energy drink, on sperm morphology and haematology, as a likely cause of thrombocytopenia and anaemia.

Despite the warning on the label: "Not recommended 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 sperm head could reduce the reproduction potential of male adults, testing of common energy drinks in the Nigerian market is necessary so as to sensitize users with the attendant side effects.

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