

Original Research Article

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

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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-group, Treatment 1 was offered 100% water. Treatments 2-5 were offered 25%, 50%, 75% and 100% Red Bull[®] respectively. Sperm head abnormality 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 haemoglobin 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.

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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 a ~~burst~~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, ~~guarana~~guarana, 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 peoplesuch 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).

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. 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~~alter male reproductive function has not been investigated extensively (Nawrot *et al.*, 2003). Data from *in vitro* studies suggest that caffeine has variable,

Comment [D1]: This sentence should be in the previous paragraph.

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). 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.

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 (Nawroth *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 cup(s) (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).

The effect of caffeine on behaviour of mice was studied by Red Bull Company. Different concentrations of 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.

2.0 MATERIALS AND METHODS

2.1 Animal Husbandry

Comment [D2]: This sentence should be revised. Is there some doubt that haematological parameters analysis could be a suitable methodology for the present study? The focus of the present study is not to validate this methodology; thus, authors should cite studies that support this methodology.

Comment [D3]: The exactly same paragraph was written previously.

Comment [D4]: What means this abbreviation?

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^R Energy Drink (RBED), 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 0 g, carbohydrates 11.3g, fat 0 g, with vitamins as % recommended daily allowance. The pH was 4.3.

Comment [D5]: What the percentages of these substances?

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 19-29 grammes/grams. The disparity in weight of mice in each group was ± 2.0 grammes/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	1:1
4	75	25	0.75:0.25
5	100	0	1:0

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

2.4 Sperm Morphology Count

Mice were 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.

Comment [D6]: How many mice were euthanized each time and in each treatment?

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~~ 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.

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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 behavior. This was carried out during the last week of the experiment.

Comment [D7]: One week or five days?

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 Dunnett t-Test, respectively. On days 7 and 28, all the means of the normal sperm heads for the four treatments were significantly different ($P < 0.05$) from the control. On day 14, only Treatment 5 was significantly different at $P < 0.05$ 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 $P < 0.05$ from Control. The multiple comparison of treatment means with the control for the normal sperm counts showed significance difference at $P < 0.05$ 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 [D8]: 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; *= Significant at $P < 0.05$

Table 3: Multiple comparisons of Treatment means with Control using Dunnett 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	Treatment				
time (Days)	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 [D9]: This table is not necessary. All this information is already showed in table 2.

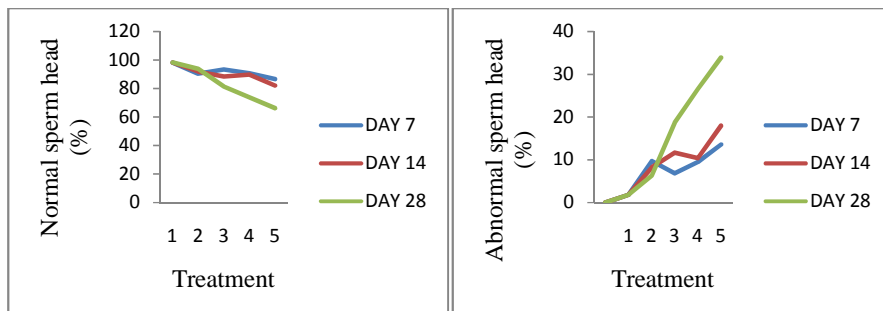


Fig. 1: Percent normal sperm head with increasing concentration of Red Bull intake **Fig. 2:** Percent abnormal sperm head with increasing concentration of Red Bull intake

Comment [D10]: These figures could be altered. It would be more understandable if lines indicated treatments and the x axis indicated the days.

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

Comment [D11]: Table 3 or 4?

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), $\times 10^9/L$						
Exposure time (Days)	Treatments					Standard ^b
	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	3.2-12.7
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						
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	11.8-14.9
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), %						
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	36.72-46.8
28	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						
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	31.8-34.7
28	30.8±0.0	31.3±0.0*	30.2±0.6	29.2±0.1*	29±0.2*	
Platelets $\times 10^9/L$						
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*	766-1657
28	479.7±48.1	331±46.5	464±1.2	459.3±33.9	350.7±25.3	
Lymphocyte, $\times 10^9/L$						
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	60-95
28	74.3±4.3	87.3±1.6	70.3±0.4	65.7±2.1	80.3±3.3	
Neutrophil, %						
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	7-31
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), $\times 10^9/L$						
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	20-3.7
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 \pm SEM, n=3 for Control, and 2 for other Treatments; * = Significant at $P < 0.05$;

^bStandard Source: <http://en.aml-vet.com/animal-species/mouse/hematology>

Comment [D12]: Different from Control or from Standard?

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

White blood cell (WBC), $\times 10^9/L$		Treatments									
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											
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), %											
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											
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 $\times 10^9/L$											
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, $\times 10^9/L$											
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		-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	0.333	0.795	1.000	0.500	-2.000	0.295
MID (A combination of monocytes, eosinophils and basophils), $\times 10^9/L$											
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

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

*=Significant at $P < 0.05$

Comment [D13]: This table is not necessary. All this information is already showed in table 4.

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 $P<0.05$

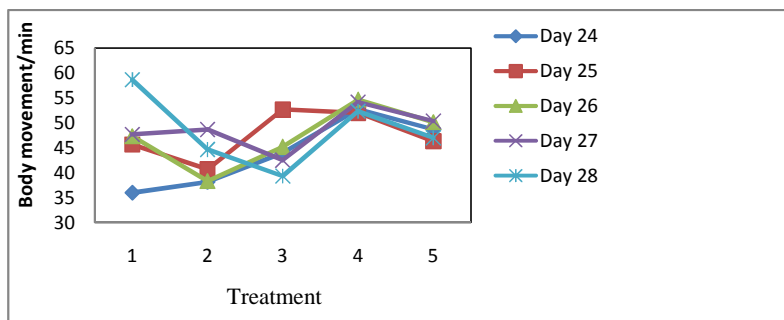


Figure 3: Variation in body movement with increasing concentration in Red Bull intake

Comment [D14]: The figure has the same information of the table; thus, it can be removed.

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 $P < 0.05$. This could imply that treatment levels and exposure of male mice to RBED caused significant reduction in normal sperm counts. There was an inverse relationship between increasing concentration and normal sperm counts. This implies that high concentration and prolonged use of Red Bull caused a reduction in normal while increasing the incidence of abnormal sperm heads. A 21-81% abnormality in sperm head that was significant ($P < 0.05$) with 100% RBED administration could imply the reproductive risk associated with prolonged use of this beverage.

Comment [D15]: This sentence is not supported by data showed in table 2. Besides, ANOVA followed by Dunnet's test allow the comparison of treatments with a control; to indicate some relationship between RBED concentration and normal sperm counts a correlation (Pearson or Spearman) or linear regression analyses should be performed.

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. 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). At what stage in spermatogenesis this incidence occurred was not studied in this work. Since 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. Moreover, 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 abnormality was significant at $P < 0.05$ with 100% RBED administration. The high pH of RBED (4.3) could have contributed to the sperm head abnormalities.

Comment [D16]: 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 megalothad spermatozoa with intracytoplasmic sperm injection. Human Reproduction.

Comment [D17]: This sentence is too vague.

Comment [D18]: Is 4.3 pH high? Besides, what is the relationship between pH and sperm head abnormalities?

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

White blood cell, neutrophils, MID, platelets and mean corpuscular haemoglobin concentration (MCHC) showed means that differed significantly ($P < 0.05$) 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. It 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 (<http://www.nlm.nih.gov/medlineplus/ency/article/003647.htm>); 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 (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.

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

The initial significant difference to higher values in body movement and lower values at the end of the study period might seem that the drink was not affecting 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).

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

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Comment [D20]: Is aloe vera an antidote for RBED effects? This sentence is not clear.

Comment [D21]: The authors did not discuss this finding. How could RBED affect those blood parameters?

Comment [D22]: This sentence needs clarification.

common energy drinks in the Nigerian market is necessary so as to sensitize users with the attendant side effects.

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