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Comparative study on the effect of energy drinks on haematopoietic system in Wistar albino rats

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Abstract

Energy drinks have become popularized and the market value for these drinks is continually growing. Therefore, the present study aimed to evaluate the effect of three popular kinds of energy drinks (Power Horse, Red Bull and Code Red) on certain hematological parameters and on the ultrastructure of blood cells in male Wistar albino rats. Animals were treated orally with Power Horse, Red Bull and Code Red respectively for 4 weeks. Blood samples were taken after two and four weeks for determination of haematological indices. Ultrastructure examination of blood cells was carried only after 4 weeks of treatment. The results indicated significant reduction ($P<0.05$) in red blood cell count, haemoglobin content, haematocrit value , blood platelets count and neutrophils in animals treated with Red Bull and Power Horse and these changes were time dependant. Insignificant changes were recorded in rats administered with Code Red. On the other hand, ultrastructural alterations, including both nucleus and cytoplasm of peripheral blood cells, were recorded in all treated animals but they were more pronounced in animals received Red Bull and Power Horse. It is concluded that energy drinks have serious detrimental impacts on haematopoietic system of male rats.

Key words

Blood cells, Energy drinks, Haematological parameters, Ultrastructure, Wistar rats

Introduction

There are several energy drink brands available in the worldwide market, and the market for these drinks has grown rapidly in the Arabic world. Energy drinks mostly contain caffeine, taurine, l-carnitine, carbohydrates, glucuronolactone, vitamins and other herbal supplements like ginseng and guarana (Malinauskas *et al*., 2007; Heckman *et al*., 2010; Yunusa and Ahmad, 2011). Majority of these energy drinks are targeted at athletes, teenagers and college students who consume large doses of these drinks in the hope to increase their energy level or compensate lack of sleep (Miller, 2008; Attila and Cakir,2011).

Research suggests that energy drink formulations, in addition to increasing energy utilization, may also improve mood, enhance physical endurance, reduce mental fatigue and increase reaction time (Reissig *et al*., 2009; Heckman *et al*., 2010). Beck *et al*. (2006) reported that caffeine-containing supplement may be an effective supplement for increasing upper body strength and therefore could be useful for competitive and recreational athletes who perform resistance training. According to the manufactures , the stimulating effects of these drinks are due to interaction between various ingredients (Van den Eynde *et al*., 2008).

Despite their potential beneficial effects, massive consumption of energy drinks result in life-threatening toxicity. Additives such as guarana, yerba mate, cocoa and kola nut may increase the caffeine content of energy drinks (Seifert *et al*., 2011). Reissig *et al*. (2009) mentioned that different brands of energy drinks contain caffeine ranging from 50 mg to 550 mg per can or bottle. On the other hand, Kavita *et al*. (2008) reported that, caffeine content of these products is presently unregulated and rapid growth in the consumption of these supplements has resulted in increasing reports of caffeine poisoning.

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Several warnings have been issued regarding the potential adverse effects of energy drinks including hepatotoxicity and nephrotoxicity (Vivekanandarajah *et al*., 2011; Bukhar *et al*., 2012; Khayyat *et al*., 2012), neurologic complications (Iyadurai and Chung, 2007; Babu *et al*., 2011; Wolk *et al*., 2012) alterations in the cardiovascular system (Ragsdale *et al*., 2010; Higgins *et al*., 2010; Worthley *et al*., 2010) and changes in the structure and function of secretory glands (Mubarak, 2012).

The current work aimed at studying the adverse effects of three popular kinds of energy drinks (Power Horse, Red Bull and Code Red) on the haematopoietic system in Wistar Albino rats.

Materials and Methods

Forty male albino rats weighting 120 ± 10 gm were used. They were housed in standard metallic cages (5 rats per cage) and kept in a temperature-controlled environment (24 ± 2 C) with an alternating 12 hr light–dark cycle. Standard commercial rodent diet was supplied. Free excess of water was provided ad-libitum. The animals were allowed to acclimatize under laboratory conditions for one week before the experiment. All the experiments were done in compliance with the Guide for the Care and Use of Laboratory animals.

Experimental design : The energy drinks Power Hours, Red Bull and Code Red were bought from local market at Makkah Governorate, Makkah, Saudi Arbia. The major constituents of these energy drinks are caffeine, guarana, taurine, ginseng, vitamins and carbohydrates (Van den Eynde *et al*., 2008).

The animals were divided into 4 groups of 10 animals each . Animals of the first group were kept on normal diet and water and served as control. While animals in the other three groups were orally administered for four weeks with a single daily dose (1.5 ml/100g b.w) of Red Bull, Power Horse and Code Red respectively. After two and four weeks, rats from both control and experimental groups were anaesthetized with ether (Sigma, MO) and decapitated. The peripheral blood samples were collected via cardiac puncture method into blood collecting tubes containing few crystals of EDTA as an anticoagulant.

Haematological studies : Anticoagulated blood samples were used for haematological examination. Routine haematological parameters and a complete blood count was carried out using an automated 18-parameter haematology analyzer (ABX Micros 60, Horbia ABX, France).

Ultrastructural studies : Following centrifugation of blood sample, plasma was removed gently with a pipette to avoid disturbing the buffy coat. About 2 ml of 4F1G (Formaldehyde and Glutaraldehyde) buffered with 0.1 M phosphate buffer (Sodium phosphate dibasic) was added drop by drop to the buffy coat . The buffy coat was allowed to stand for 18 hr at 4°C. 1 mm slices of the plug were cut into smaller pieces. Specimens were then postfixed in 2% OsO_4 (osmium tetroxide) at 4°C for 2 hr, and dehydrated in graded series of ethanol, and then embedded in Epon-araldite mixture in labeled beam capsules. LKB ultramicrotome was used to obtain ultrathin sections (50 nm thick) which were picked upon 200 mesh naked copper grids. Grids were double stained with uranyl acetate for ½ h and lead citrate for 20-30 min. Scoping the grids was achieved by using Jeol 100 CX TEM.

Statistical analysis : Data were expressed as mean ± SD of five replicates and were subjected to one way analysis of variance(ANOVA) followed by students T test. Results were considered statistically significant at $P<0.05$.

Results and Discussion

Data in Table 1 show that administration Red Bull, resulted in a significant ($p < 0.05$) decrease in erythrocytic count, Hb content and PCV value as compared to control. This drop was apparent from 2nd week and became more pronounced after 4 weeks. However, treatment with Power Horse or Code Red induced a significant decrease in the value of these parameters firstly after 4 weeks. In addition, mean cell volume (MCV), mean cell haemoglobin concentration (MCHC) and mean cell haemoglobin (MCH) were more affected with Red Bull administration.

Depletion of RBCs count and Hb content leads to iron deficiency anemia which is characterized by a microcytic hypochromic blood picture (Ballinger, 2007). Depression in RBCs count and Hb content recorded in the present work could be attributed to disturbed hematopoiesis, destruction of erythrocytes, and reduction in the rate of their formation and/or their enhanced removal from circulation. Karmarker *et al*. (2ooo) mentioned that, reduction in haematocrit, red blood cells and haemoglobin might be attributed to the hyperactivity of bone marrow, which leads to production of red blood cells with impaired integrity that are easily destroyed in the circulation. Decrease in hemoglobin content could not only be due to decrease in red blood cells count but also due to impaired biosynthesis of hem in bone marrow (Abdel Aziz and Zabut, 2011). Destruction of red cells reflects failure of hepatocellular functions that could be caused by caffeinated energy drink consumption (Akande and Banjoko, 2011; Bukhar *et al*., 2012). Changes in corpuscular volume, corpuscular hemoglobin and corpuscular hemoglobin concentration were consistent with changes in red blood cell counts and hemoglobin levels. These changes may be correlated with some pathological changes developed in blood-forming organs, or with the destruction of red blood cells, or with both factors (Abdel Aziz and Zabut, 2011).

Treatment with Red Bull, Power Horse or Code Red caused insignificant decrease in total WBC count at the 2nd

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Table 1 : Effect of administration of Power Horse, Red Bull and Code Red on haematological parameters of Wistar albino rats

	Time	Control	Power Horse	Red Bull	Code Red
RBCs (10^6 mm^{-3})	**2wks**	$4.85^{a} \pm 0.36$	$4.53^{a} \pm 0.29$	$3.76^{b} \pm 0.41$	$4.68^{a} \pm 0.28$
	4wks	$4.57^{a} \pm 0.02$	$3.65^{b} \pm 0.49$	$3.29^{b} \pm 0.13$	$4.12^{a} \pm 0.29$
p		0.002*	0.095	0.028*	0.010*
Hb (g dl^{-1})	**2wks**	$14.25^{a} \pm 1.04$	$13.78^{a} \pm 0.80$	$11.05^{b} \pm 1.21$	$14.10^{a} \pm 0.87$
	4wks	$13.27^{a} \pm 0.35$	$10.73^{b} \pm 1.44$	$9.93^{b} \pm 0.38$	$12.13^{a} \pm 0.85$
p	0.002*	0.086	0.031*	0.010*	
PCV (%)	**2wks**	$49.50^{a} \pm 2.08$	$45.50^{ab} \pm 3.87$	$44.50^{b} \pm 2.38$	$45.75^{ab} \pm 3.86$
	4wks	$46.0^{a} \pm 2.16$	$32.75^{b} \pm 2.63$	$32.75^{b} \pm 2.36$	$37.25^{c} \pm 3.50$
p	<0.001*	0.766	0.032*	0.001*	
MCV (fl)	**2wks**	$122.58^{a} \pm 18.64$	$98.43^{b} \pm 9.29$	$97.82^{b} \pm 9.05$	$102.38^{b} \pm 4.69$
	4wks	$99.32^{a} \pm 9.76$	$93.60^{a} \pm 5.41$	$90.2^{a} \pm 5.49$	$90.28^{a} \pm 4.15$
p	0.048*	0.594	0160	0.201	
MCH (g dl^{-1})	**2wks**	$31.08^{a} \pm 1.48$	$29.63^{a} \pm 0.61$	$29.40^{a} \pm 1.67$	$30.95^{a} \pm 2.09$
	4wks	$30.73^{a} \pm 1.99$	$29.30^{a} \pm 0.43$	$22.06^{b} \pm 14.47$	$30.60^{a} \pm 10..21$
p	0.061	0.323	0.881	0.809	
MCHC (g dl^{-1})	**2wks**	$32.70^{a} \pm 1.91$	$31.50^{a} \pm 1.76$	$28.33^{b} \pm 3.23$	$32.60^{a} \pm 1.40$
	4wks	$30.10^{a} \pm 2.91$	$28.80^{a} \pm 1.36$	$24.40^{b} \pm 3.64$	$30.25^{a} \pm 2.88$
p	0.172	0.795	0.013*	0.206	

Data represented as mean ±S.E. (n=5); p : p value for student t-test between the two periods of treatment in each group; Different superscripts within each raw indicate statistical significant differences between groups at 0.05; *: Statistically significant at p > 0.05

Table 2 : Effect of administration of Power Horse, Red Bull and Code Red on WBC total and differential counts and blood platelets of Wistar albino rats

	Time	Control	Power Horse	Red Bull	Code Red
Total WBCs	**2wks**	$13937.50^{a} \pm 462.50$	$12325.0^{a} \pm 746.52$	$11975.0^{a} \pm 625.0$	$12725.0^{a} \pm 920.48$
(10^3/mm^3)	**4wks**	$12975.0^{a} \pm 1119.80$	$13900.0^{a} \pm 612.37$	$13950.0^{a} \pm 170.78$	$13075.0^{a} \pm 597.74$
p	0.471	0.154	0.046*	0.761	
Platelets	**2wks**	$219.50^{a} \pm 11.65$	$184.5^{b} \pm 2.90$	$192.50^{b} \pm 6.91$	$207.25^{ab} \pm 9.64$
	4wks	$204.25^{a} \pm 11.34$	$170.25^{b} \pm 4.70$	$170.0^{b} \pm 7.48$	$186.75^{ab} \pm 10.43$
p	0.548	0.151	0.065	0.001*	
Differential WBC	**2wks**	$53.75^{a} \pm 2.25$	$47.25^{b} \pm 1.93$	$50.0^{ab} \pm 1.68$	$49.0^{ab} \pm 1.08$
count %Neutrophils	**4wks**	$58.0^{a} \pm 3.0$	$46.0^{b} \pm 1.87$	$51.0^{ab} \pm 1.47$	$50.75^{ab} \pm 3.09$
p	0.300	0.658	0.670	0.612	
Stab forms	**2wks**	$2.0^{a} \pm 0.41$	$3.0^{ab} \pm 0.41$	$5.25^{c} \pm 0.63$	$3.75^{bc} \pm 0.48$
	4wks	$2.0^{a} \pm 0.41$	$3.0^{a} \pm 0.41$	$5.0^{b} \pm 0.41$	$2.75^{a} \pm 0.48$
p	1.000	1.000	0.750	0.190	
Lymphocytes	**2wks**	$38.25^{a} \pm 2.14$	$43.75^{a} \pm 2.50$	$39.50^{a} \pm 1.32$	$41.75^{a} \pm 1.25$
	4wks	$35.0^{a} \pm 1.78$	$46.75^{b} \pm 1.55$	$39.25^{ac} \pm 0.85$	$40.25^{c} \pm 2.06$
p	0.287	0.346	0.879	0.556	
Monocytes	**2wks**	$5.0^{a} \pm 0.41$	$4.25^{a} \pm 0.48$	$4.0^{a} \pm 0.71$	$4.50^{a} \pm 0.65$
	4wks	$3.50^{a} \pm 0.65$	$3.0^{a} \pm 0.41$	$4.0^{a} \pm 0.41$	$4.50^{a} \pm 0.96$
p	0.097	0.094	1.000	1.000	
Eosinophils	**2wks**	$1.0^{a} \pm 0.41$	$1.75^{a} \pm 0.48$	$1.25^{a} \pm 0.25$	$1.33^{a} \pm 0.33$
	4wks	$1.50^{a} \pm 0.65$	$1.25^{a} \pm 0.48$	$0.75^{a} \pm 0.25$	$1.75^{a} \pm 0.63$
p	0.537	0.488	0.207	0.623	
Basophils	**2wks**	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	4wks	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
p	-	-	-	-	

Data represented as mean ±SE (n=5); p : p value for student t-test between the two periods of treatment in each group; Different superscripts within each raw indicate statistical significant differences between groups at 0.05; *: Statistically significant at p > 0.05

week, followed by a slight elevation after 4 weeks (Table 2). In differential WBC count, treatment with Red Bull or Code Red for 2 weeks significantly increased the stab forms , while other types of WBCs were slightly affected. However, treatment with Power Horse for 2 weeks significantly decreased the neutrophils, while all the other types remained comparable to control. After 4 weeks

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of treatment, Red Bull administration induced slight decrease in neutrophils and eosinophils, while other types were elevated especially stab forms which were markedly increased. In animals treated with Power Horse, neutrophils were significantly decreased, while lymphocytes were markedly elevated. Marked changes of lymphocytes and monocytes were recorded after 4 weeks of treatment with Code Red (Table 2). Elevation of WBC count might indicate activation of immune system, a normal cell–mediated immune response and increase in lymphocytes and monocytes could be attributed to the action of caffeine that stimulates hemopoietic system to release more of these cells (El-Demerdash, 2004). Bassini-Cameron *et al.* (2007), demonstrated that caffeine treatment during exercise leads to a greater degree of leukocytosis, lymphocytosis and muscle damage.

The present results showed a significant decrease in number of blood platelets after administration of Red Bull and Power Horse for 2 or 4 weeks (Table 2). However, a slight insignificant thrombocytopenia was recorded after treatment with Code red. Energy drinks having been linked to significant alterations in the cardiovascular system including increased platelet aggregation and decreased endothelial function in healthy young adults (Higgins *et al*., 2010). Worthley *et al*.(2010) in a controlled trial of young adults found that consumption of

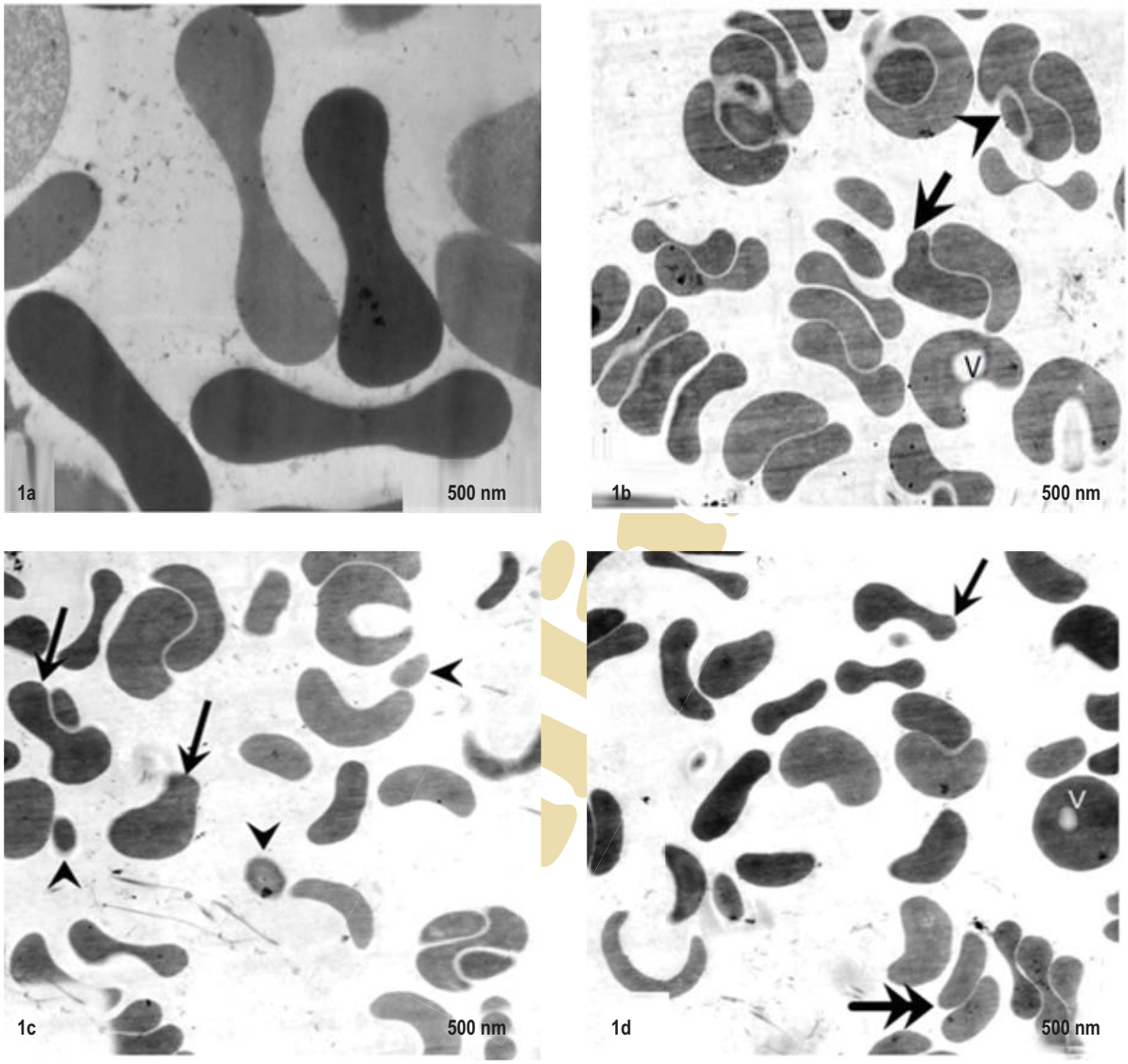

Fig 1 : Electron microscopic structure of erythrocytes of rats (a) control, (b) Red Bull, (c) Power Horse, (d) Code Red. Arrows point at cells with crenations, double arrow indicates hypo-chromatic erythrocytes, arrow heads indicate fragmented cells, V: vacuoles

sugar-free energy drink (80mg caffeine, 1000mg taurine, 600mg glucuronolactone) resulted in a significant increase in platelet aggregation and mean arterial pressure and a significant decrease in endothelial function.

At electron microscopic level erythrocytes of control rats appeared with dense homogenous appearance due to their high haemoglobin content. They were non-nucleated and devoid of typical cytoplasmic organelles (Fig. 1a).

Erythrocytes in rats treated with Red Bull or Power Horse were hypochromic and showed poikilocytosis and anisocytosis. They were observed with marked crenation, vacuolation and fragmentation (Fig. 1b and c). Administration of Code Red induced poikilocytosis in erythrocytes that had a moderate dense homogeneous appearance. Some crenatted and vacuolated hyperchromatic cells were recorded (Fig.1d). Sherlock and Dooley (1993) reported that destruction of red cells reflects failure of hepatocellular functions and various morphological

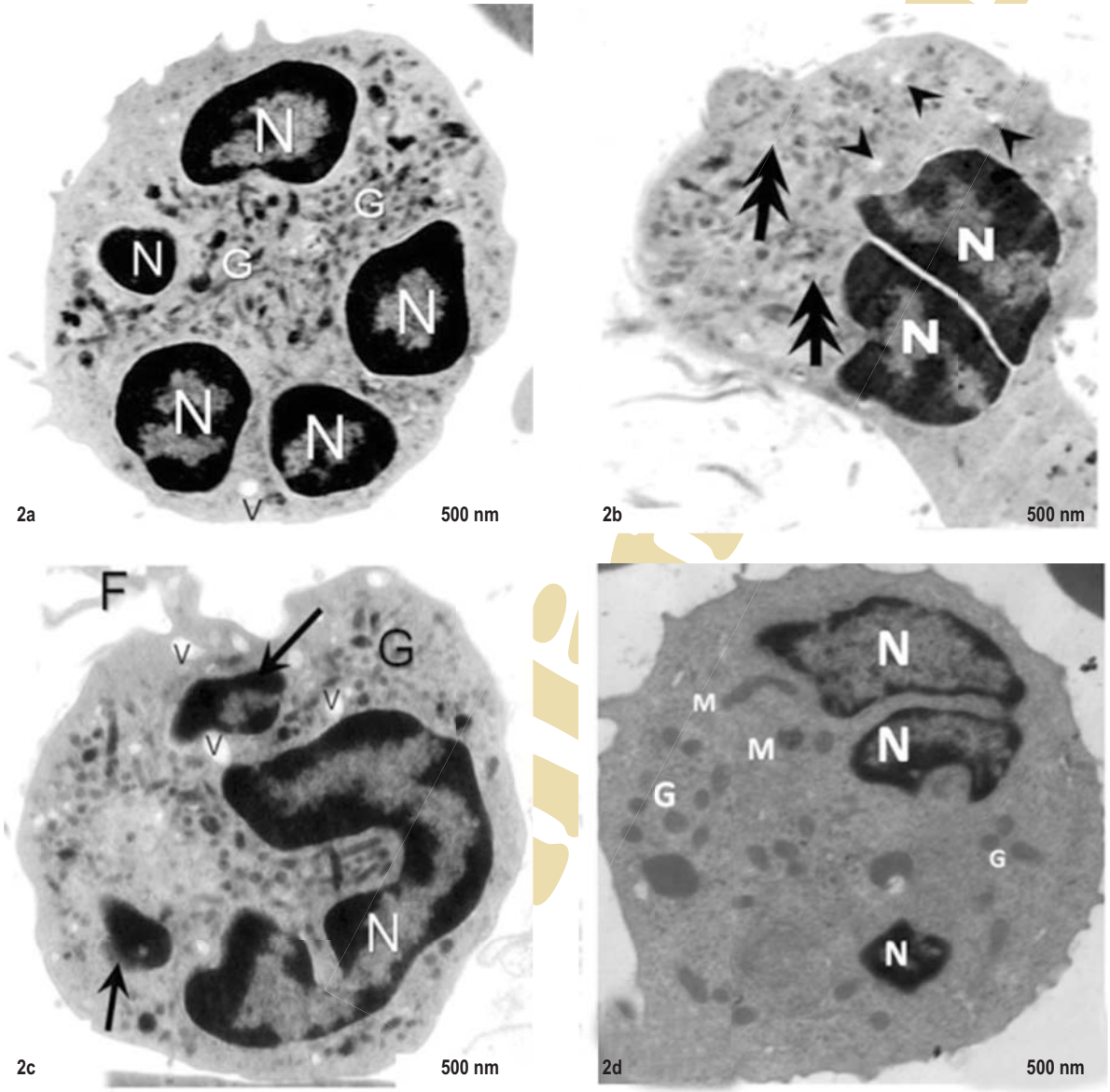

Fig 2 : Electron microscopic structure of monocytes of rats (a) control, (b) Red Bull, (c) Power Horse, (d) Code Red. Arrows point at nuclear fragments, double arrows indicate degenerated granules, arrow heads point at degenerated cytoplasmic areas , F: filopodia G: polymorphic cytoplasmic granules, M: destructed mitochondria , N: hyper-chromatic nuclear lobes, V: vacuoles

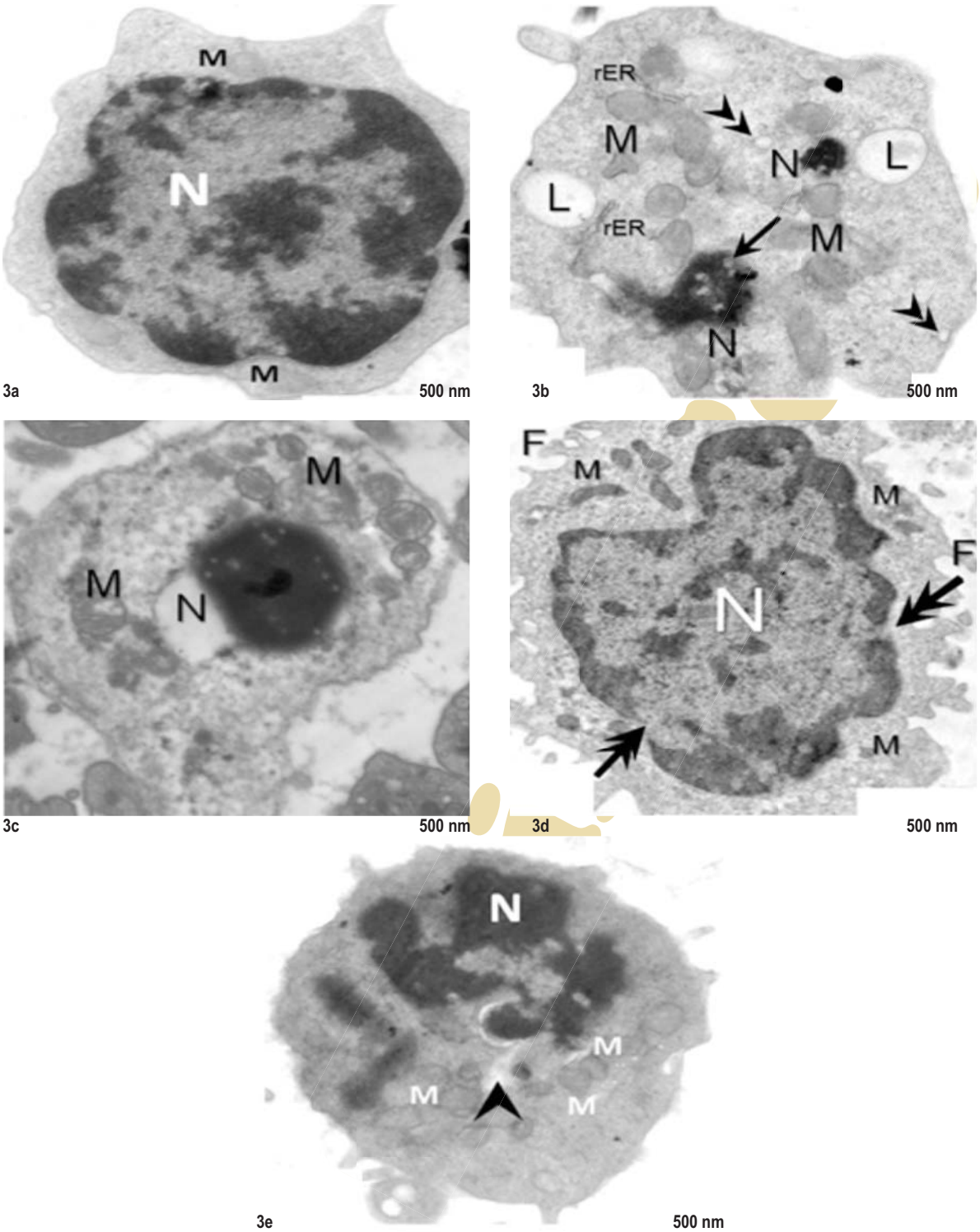

Fig 3: Electron microscopic structure of lymphocytes of rats (a) control, (b) Red Bull, (c) Power Horse, (d) Code Red. Arrow points at intranuclear inclusions, double arrows indicate dilated nuclear pores, arrow head indicates degenerated cytoplasmic area, double arrow heads point at vesiculated endoplasmic reticulum, F: filopodia, L: Lipid droplets, M: destructed mitochondria, N: nucleus, rER: rough endoplasmic reticulum

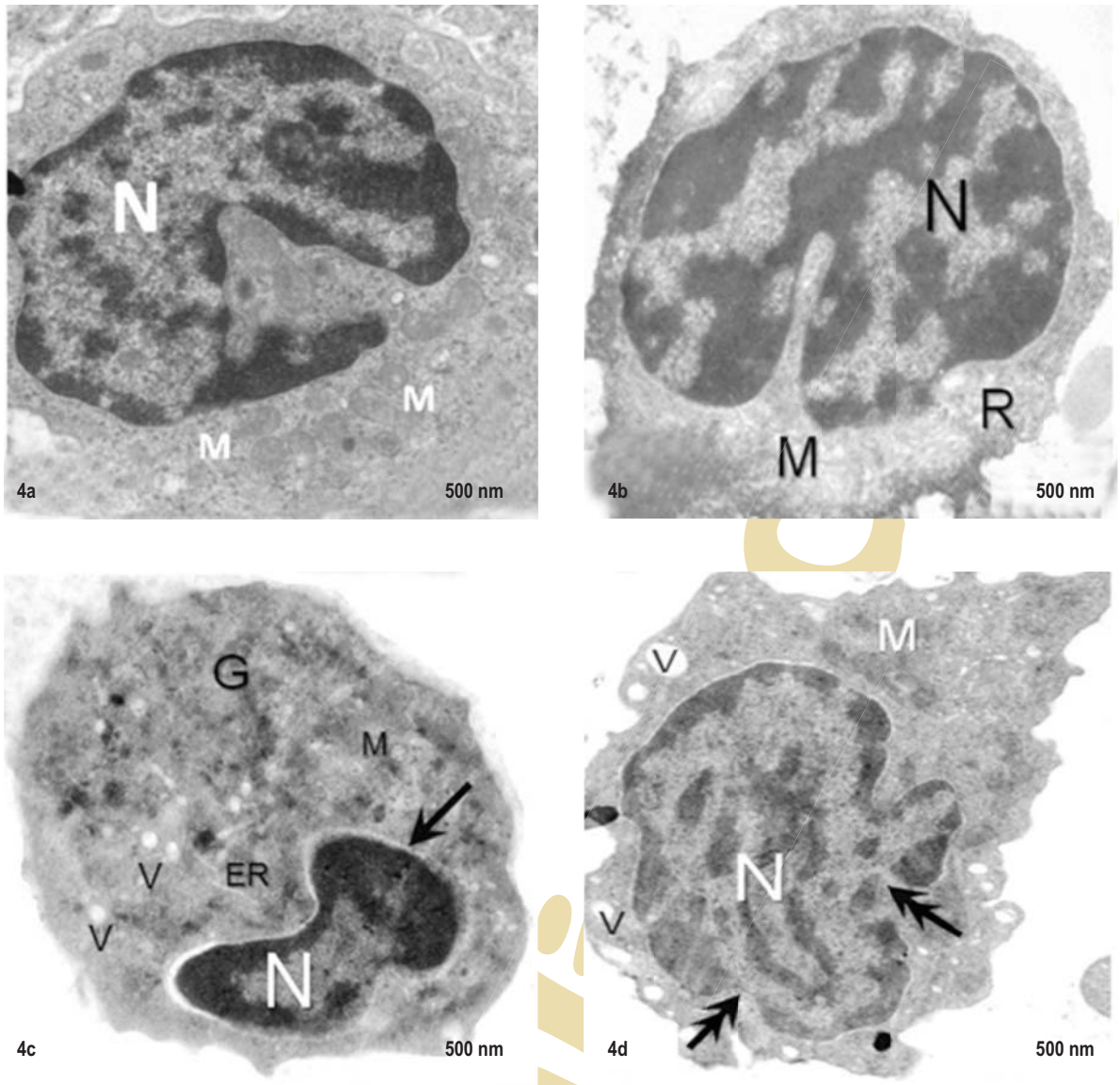

Fig. 4 : Electron microscopic structure of monocytes of rats (a) control, (b) Red Bull, (c) Power Horse, (d) Code Red. Arrow points at dilated nuclear envelope, double arrows point at dilated nuclear pores, ER: endoplasmic reticulum, G: destructed cytoplasmic granules, M: degenerated mitochondria, N: Nucleus, V: vacuoles.

abnormalities in RBCs are probably due to the changes in the membrane cholesterol and phospholipid content and/or ratio. Failure in hepatocellular functions induced by energy drinks was previously dimonstrated by Bukhar *et al*. (2012) and Khayyat *et al*. (2012).

Neutrophils in control rats appeared with more than one nuclear lobe (Fig. 2a). Heterochromatin was located peripherally and the central area of nuclear lobe was occupied chiefly by euchromatin. The cytoplasm of neutrophils was found containing small and large spherical dense granules . Rats treated with Red Bull, neutrophils appeared with irregular contour, bilobed hyperchromatic nucleus, degenerated granules and degenerated cytoplasmic areas (Fig. 2b) . Band neutrophils with fragmented nucleus and vacuolated cytoplasm containing polymorphic granules were recorded in the blood of rats administered with Power Horse (Fig. 2c). However, in animals given Code Red, peculiar neutrophils with euchromatic bilobed nucleus , pycknotic nuclear fragments and degenerated mitochondria with indistinct cristae were noticed (Fig. 2d). Hypersegmented neutrophils were frequently noticed in patients with megaloblastic anemia (Sinha *et al*., 2006).

Lymphocytes in control rats are typically round in shape with round nuclei showing distinct heterochromatin and euchromatin (Fig. 3a). In rats treated with Red Bull, lymphocytes appeared with segmented or distored hyperchromatic nuclei containing numerous intranuclear inclusions and cytoplasm containing degenerated rER and mitochondria with lysis and disorganized cristae (Fig. 3b and c). Lymphocytes with indented nucleus, dilated nuclear pores and dense destructed mitochondria were represented in rats treated with Power Horse (Fig. 3d). However, animals given Code Red showed lymphocytes with pycknotic destructed nucleus and cytoplasm containing degenerated areas and mitochondria with less distinct cristae (Fig.3e).

Monocytes in control rats were found to be large cells with large kidney shaped nuclei. Heterochromatin formed a definite, irregular band aligning the nuclear envelope and was haphazardly mixed with euchromatin in the central part of nucleus (Fig. 4a). These cells appeared with deeply indented nucleus and obvious disintegrated mitochondria in Red Bull-treated rats (Fig.4b). Monocytes with hyper-chromatic eccentric nuclei and vacuolated cytoplasm were observed in animals treated with Power Horse (Fig. 4c). However, rats administered with Code red, monocytes exhibited eccentric deeply indented nucleus , dilated nuclear pores and vacuolated cytoplasm with destructed mitochondria (Fig. 4d).

Similar ultrastructure alterations in leucocytes have been described in experimental animals intoxicated with different chemicals and drugs (El-Mofty *et al*., 2000; Essawy *et al*., 2010) and the hyper-segmented neutrophils recorded in the current study were frequently noticed in patients with megaloblastic anemias (Sinha *et al*., 2006). These pathological alterations could be signs of toxicity and may be attributed to the preservatives added to energy drinks such as sodium benzoate, and/ or to the toxic action of the overdose of caffeine content (Mubarak ,2012). Reissig et al., (2009) reported that, adverse reactions and toxicity from high-energy drinks stem primarily from caffeine content. Data on caffeine-related toxicity have been reported from poison centres in the United Kingdom and United States (Waring *et al*.,2009).

The present results showed that Red Bull was more effective in its action on hematopoietic system, followed by Power Horse while Code Red was less effective. Different action of energy drinks could be due to different mixture of their ingredients. From the data obtained in this work, it is suggested that energy drinks have serious detrimental impact on hematopoietic system. This could be attributed to caffeine overdose and other bioactive ingredients that have hazards.

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