

Impact of Some Energy Drinks on the Structure and Function of the Kidney in Wistar Albino Rats

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Abstract: Three kinds of energy drinks (Power horse, Red bull and Code red) were used to study their possible adverse effects on the structure and function of the kidney in Wistar albino rats. Animals were divided into four groups: group 1 served as control, while animals in groups 2, 3 and 4 each were orally administered with a type of the energy drinks daily for 4 weeks. After two and four weeks of treatment, blood samples were collected for biochemical analysis of kidney function, while small pieces of kidney were removed and quickly fixed to carry out light and electron microscopic investigations. Results of the present study showed that the energy drinks induced an elevation of renal biomarkers urea, uric acid and creatinine. The elevation in these parameters was time dependant and the Power horse was more effective in its action followed by Red bull and to less extend Code red. Histopathological observations of kidney tissue in energy drinks administered animals, revealed distinct pathological lesions as represented by necrosis of renal tubules and glomeruli as well as intertubular hemorrhage and leucocytic infiltration. Moreover, electron microscopic results showed marked ultrastructure alterations in the nucleous and cytoplasmic organelles in the cells of proximal and distal tubules as well as in the renal corpuscles. These alterations were more obvious in rats given power horse. The different action of the energy drinks on kidney function could be attributed to the different mixture of their ingredients.

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1. Introduction

The popularity of energy drinks and the growth in their consumption among adolescents and young adults have brought worries regarding general health and well being of these consumers. Adolescents and young adults are often uninformed about the content of energy drink. (Rath, 2012). Most brand often contain caffeine, Taurine, Guarana, Ginseng, B vitamins, Ginko Biloba, L-carnitine, sugars, Antioxidants, Glucuronolactone, Yerba Mate, Creatine, Acai Berry, Milk Thistle, L-theanine, Inositol and artificial sweeteners (Kavita *et al.*, 2008; Bigard, 2010). The media and case studies have linked harmful effects with the consumption of energy drink whereas few researches have examined the consequences of individual ingredients and effect of the ingredients combined (Sefeit *et al.*, 2011).

Several warnings have been issued regarding the potential adverse effects of energy drinks.

Alterations in the cardiovascular and haematopoietic system (Ragsdale *et al.*, 2010; Higgins *et al.*, 2010; Worthley *et al.*, 2010; Khayyat *et al.* 2014), neurologic complications (Babu *et al.*, 2011; Wolk *et al.*, 2012) and changes in the structure and function of secretory glands (Mubarak, 2012) were reported.

According to Ebuehi and colleagues (2011), consumption of energy drink was associated with

higher total protein, triglyceride, HDL and LDL but lower ALT, AST, creatinine, uric acid and albumin. This is in contrast with Bukhar *et al.* (2012) and Khayyat *et al.* (2012) who recorded high significant elevation of liver enzymes in animals treated with energy drinks. Also, Ugwuja (2014) reported that, consumption of energy drink alone or in combination with alcohol is associated with significant alterations in some biochemical parameters such as total white blood cell count, plasma potassium, calcium, renal functions, liver enzymes and plasma triglycerides

Conflication of the results of the previously mentioned researchers might have been because of the various ingredients contained in different brands of energy drink and as well the amount of these ingredients consumed during a study as Stinke *et al.* (2009) pointed out.

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 structure and function of the kidney in Wistar albino rats.

2. Material and Methods

Forty male Wistar albino rats, weighting 120 ± 10 gm were used, conditioned in standard metallic cages (5 rats per cage) and kept in a temperature-controlled environment ($24 \pm 2^{\circ}\text{C}$) with an alternating

12 h light – dark cycle. They were acclimatized to the laboratory conditions, fed standard rat chow and water was available *ad libitum*.

Animals were maintained and experimental procedures complied with the guide for care and use of laboratory animals.

Experimental design:

Several cans of three types energy drinks: Power horse, Red Bull and Code red were used in this experiment. They have been bought from local market at Makkah Governorate, Makkah, Saudi Arabia.

The animals were divided into 4 groups of 10 rats each and treated as follows:

Group 1: Animals of this group were given 3 ml distilled water and served as control.

Group 2: Animals of this group were orally administered with 1.5 ml/100g b.wt of Power horse daily for 4 weeks.

Group 3: Animals of this group were orally administered with 1.5 ml/100g b.wt of Red bull daily for 4 weeks.

Group 4: Animals of this group were orally administered with 1.5 ml/100g b.wt of Code red for 4 weeks.

After two and four weeks of treatment, five animals from each group were killed by cervical dislocation, quickly dissected and liver was removed.

Histological and Ultrastructural studies:

Kidney from control and treated animals were removed, cut and fixed in buffered formalin, dehydrated in ethanol, cleared in xylene and embedded in paraffin. Sections were stained with haematoxylin and eosin and examined with light microscope.

For transmission electron microscopy: small slices of kidney were immediately fixed in 4F1G in phosphate buffer (pH7.2) for 3 hours at 4°C and post-fixed in 2% OSO_4 in the same buffer at 4°C for 1-2 hours. The specimens were dehydrated through graded series of ethanol, embedded in epon-araldite mixture and polymerized at 60°C. Ultrathin (50 nm) sections from selected areas were cut with glass knives on LKB ultramicrotome. They were double stained with uranyl acetate and lead citrate and examined by jeol 100CX electron microscope.

Biochemical assays

For biochemical study, blood samples were collected from each rat via cardiac puncture method and allowed to clot. The serum was rapidly separated by centrifuging the clotted blood at 3000g for 10 min in a Beckman Model T-6 refrigerated centrifuge and processed for determination in clean and dry tubes.

Sera were stored at -20 °C until assayed for the biochemical parameters. Creatinine, urea and uric acid were estimated using the methods of Henry (1974), Patton and Crouch (1977) and Caraway (1955), respectively.

Statistical analysis:

Data were expressed as mean $\pm$ SD of five replicates and were subjected to one way analysis of variance (ANOVA) followed by student's T test. Results were considered statistically significant at $P < 0.05$.

3. Results

Histological and ultrastructure results:

Light microscopic examination of kidney sections of the control rats showed normal histological structure of renal tubules as well as renal corpuscles (Fig.1a). However, kidney tissue of rats given power horse, red bull and code red for 4 weeks revealed distinct pathological lesions as represented by necrosis of renal tubules and glomeruli (Fig.1b of power horse group & Fig.1c of Red bull group). Hydropic changes in epithelium and intertubular hemorrhage as well as inflammation areas were noticed among the tubules (Figs.1b & 1c). Several renal tubules appeared dilated (Fig.1d of code red group). Meanwhile, some glomeruli had lost their normal circular shape and converted into shrunken, abnormally structures (Figs.1b, 1c & 1d).

The ultrastructure of the proximal tubular cells in control rats demonstrate long densely packed apical microvilli collectively formed a brush border and regular basement cell membrane with well-developed basal infoldings. Large spherical, basal nuclei with one or more dense nucleoli appeared surrounded by numerous electron dense mitochondria. Lysosomes, pinocytotic vesicles, rough endoplasmic reticulum cisternae can be observed throughout the cytoplasm (Fig.2a).

The distal tubular cells of the control rats revealed regular trilaminar basement membrane with many basal infoldings. The microvilli zone is less developed than in the proximal compartment with wider lumen. The cells showed spherical apical nucleus with a central or peripheral electron-dense nucleolus. Moreover, elongated and rounded-shaped mitochondria, few lysosomes and short rough endoplasmic cisternae are seen within their cytoplasm (Fig.3a).

Electron micrographs of the renal corpuscles of control rats showed the glomerular capillary wall, basal membrane, podocytes and podocyte pedicels in normal structure (Figs.4a & 4b).

Electron microscopic observations showed that energy drinks, after 4 weeks, induced obvious renal ultrastructural alterations.

Some cells of proximal tubules showed irregularly-shaped nuclei with loose chromatin materials, while others appeared with pyknotic nuclei (Fig.2b of Power horse group, Fig.2d of Red bull group & Fig.2e of Code red group). Swollen, pleomorphic mitochondria, irregular lysosomes containing dense material, pinocytotic vesicles and free ribosomes were numerous in many cells (Figs. 2c & 2e). In addition the basement membranes of the proximal tubular cells, rich in electron-dense mitochondria, thickened and the intracytoplasmic foldings were dilated (Fig.2e).

The distal tubular cells revealed, also, marked ultrastructural changes. They appeared with irregular and pyknotic nuclei (Fig.3b of Power horse group; Fig.3c of Red bull group & Fig.3d of Code red group). Their cytoplasm contained numerous elongated mitochondria and many electron-lucent vacuoles (Fig.3d). There was an irregularity of the basement membrane of some distal tubular cells (Figs.3b & 3c), while other cells showed cytoplasmic bulges toward the lumen from the apical cytoplasm (Fig.3d).

Moreover, The renal corpuscles revealed dilatation of the glomerular capillaries with thickened undulated basement membrane (Fig.4c of Power horse group; Fig. 4d of Red bull group & Fig.4e of Code red group). The urinary space of the Bowman's capsule was extremely closed in many areas (Fig. 4c). Some podocytes were exfoliated (Fig.4e). In addition, there was an increased fusion of podocyte pedicles in certain areas (Fig. 4f).

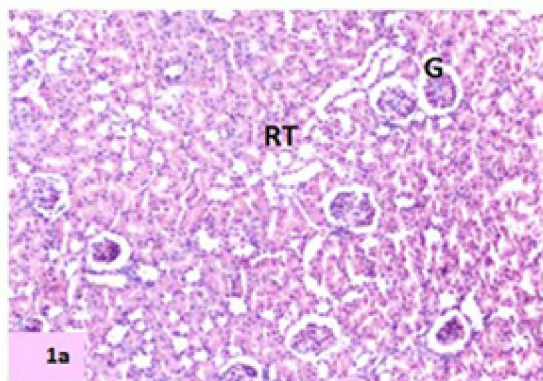


Fig.(1a): Light micrograph of kidney section from control rat showing normal renal tubules (RT) and glomeruli (G). H&E . x100

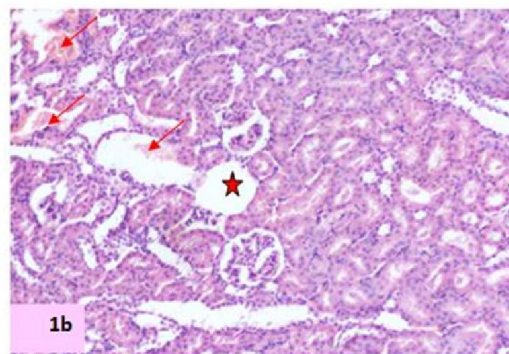


Fig.(1b): Light micrograph of kidney section from rats given power horse for 4 weeks showing necrotic lesion in glomeruli and renal tubules. Note the presence of cavitation areas (star) and hemorrhage (arrows). H&E. x100

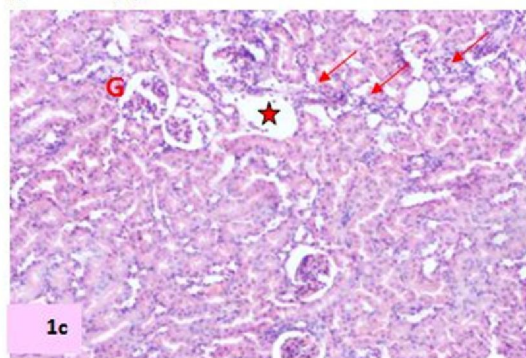


Fig.(1c) : Light micrograph of kidney section from rats given red bull for 4 weeks showing shrinkage and lobulated glomerular capillaries (G). Cavitation area, star; inflammation ,arrows. H&E. x100

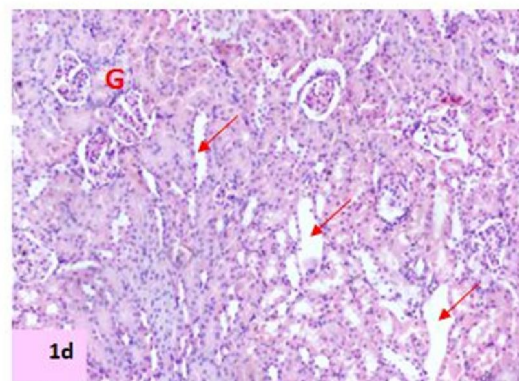


Fig.(1d): Light micrograph of kidney section from rats given code red for 4 weeks showing Dilatation of some renal tubules (arrows) and abnormal glomeruli (G).H&E. x100

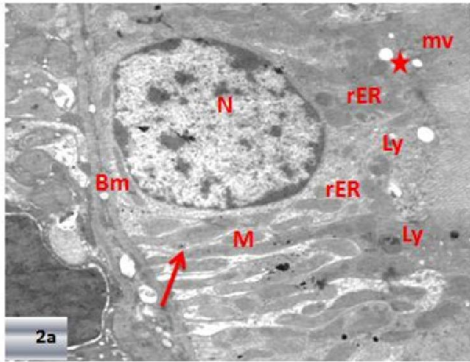


Fig.(2a):Electron micrograph of kidney rat from control group showing the proximal tubular cell with apical microvilli (mv), regular basement membrane (Bm), basal infoldings (arrow), nucleus (N), numerous mitochondria (M), lysosomes (Ly), pinocytotic vesicles (*), rough endoplasmic reticulum (rER). x3000

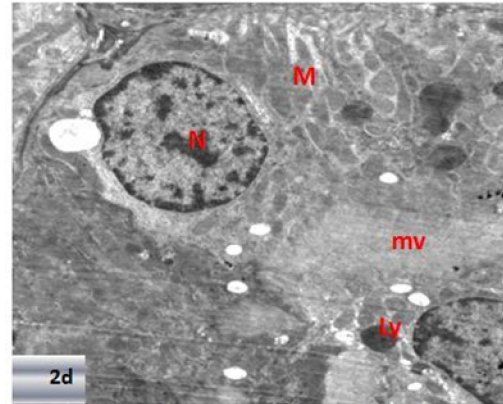


Fig.(2d): Electron micrograph of kidney from rats given red bull for 4 weeks showing proximal tubular cell with irregular nucleus (N), bizarre mitochondria (M), lysosomes (Ly) and microvilli (mv). X3000

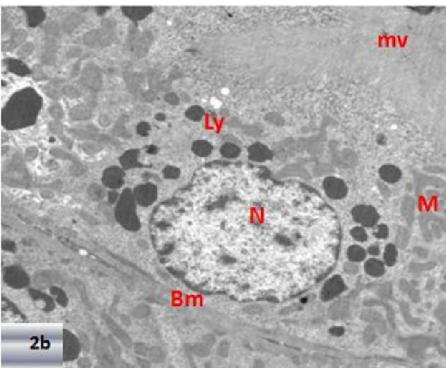


Fig. (2b): Electron micrograph of kidney from rats given power horse for 4 weeks showing proximal tubular cell with their microvilli (mv) and basement membrane (Bm). Note an irregular nucleus (N), mitochondria (M) and large number of lysosomes (Ly). x2500

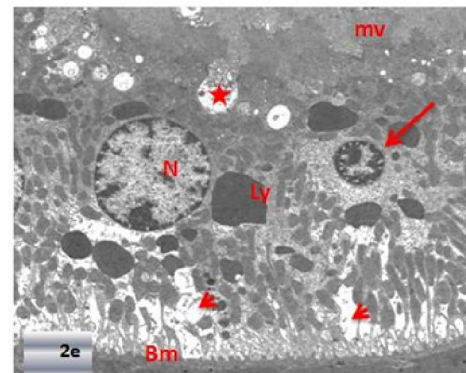


Fig. (2e): Electron micrograph of kidney from rats given code red for 4 weeks showing proximal tubular cell with pyknotic nucleus (arrow) and dilated intracytoplasmic foldings (head arrows). Basement membrane, Bm; lysosomes , Ly; microvilli, mv; pinocytotic vesicles ,* . x2000

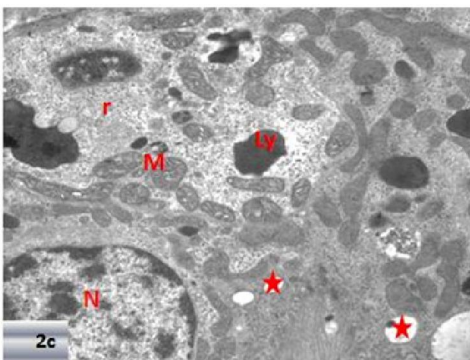


Fig.(2c): Electron micrograph of proximal tubular cell from rats given power horse for 4 weeks. The cytoplasm contains numerous pleomorphic mitochondria (M), irregular lysosomes (Ly), pinocytotic vesicles (*) and free ribosomes (r). x4000

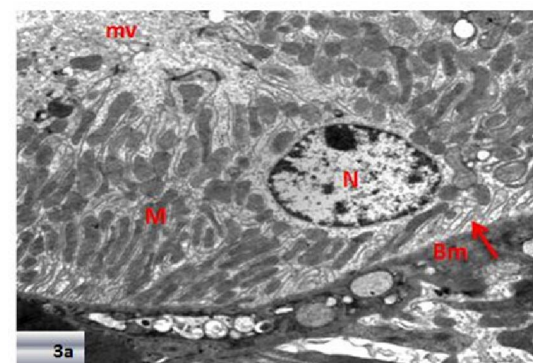


Fig.(3a): Electron micrograph of kidney rat from control group showing the distal tubular cell. Bm, basement membrane ; basal infoldings, arrow ; microvilli, mv ; nucleus,N ; mitochondria, M. x 2500

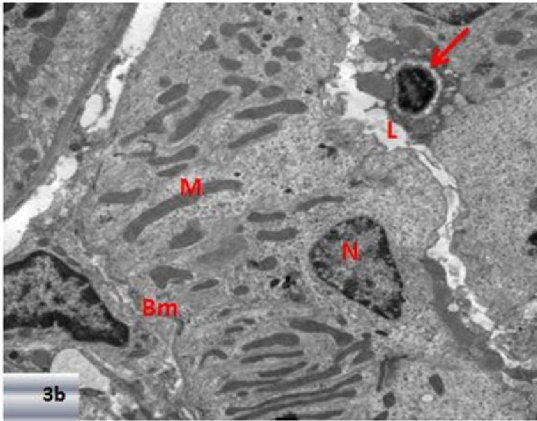


Fig. (3b): Electron micrograph of kidney from rats given power horse for 4 weeks. The distal tubular cells appeared with irregular (N) and pyknotic (arrow) nuclei. Numerous elongated mitochondria, M; basement membrane, Bm; lumen, L. x2500

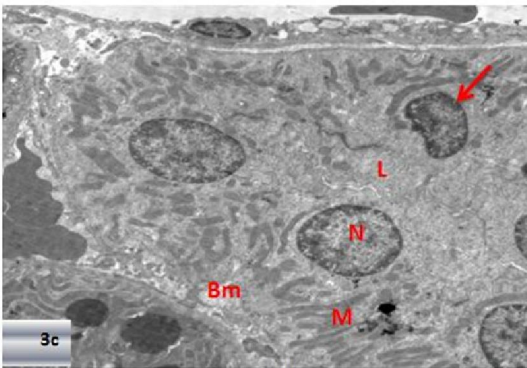


Fig. (3c): Electron micrograph of kidney from rats given red bull for 4 weeks showing the distal tubular cells with pyknotic nuclei (arrow). Basement membrane, Bm; mitochondria, M; nucleus, N; lumen, L. x2000

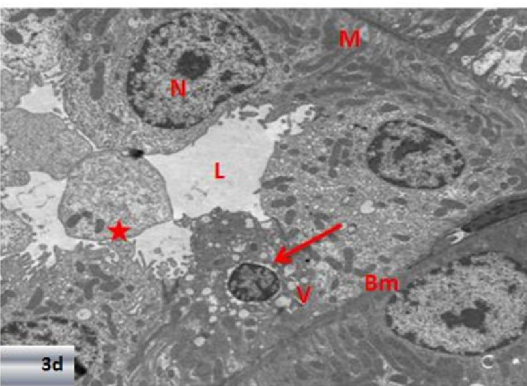


Fig. (3d): Electron micrograph of kidney from rats given code red for 4 weeks. Note the tubular cells showed pyknotic nuclei (arrow) and many vacuoles (V). Cytoplasmic bulge toward the lumen, *; basement membrane, Bm; mitochondria, M; nucleus, N; lumen, L. x2000

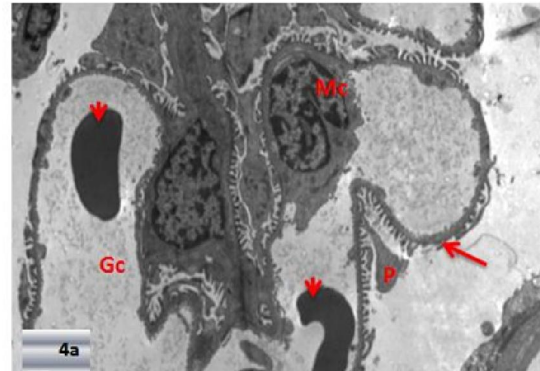


Fig. (4a): Electron micrograph of kidney rat from control group showing the renal corpuscles. Note the glomerular capillaries (Gc), RBCs (head arrows), mesangial cell (Mc), podocytes (P) and podocyte pedicels (arrow). x1500

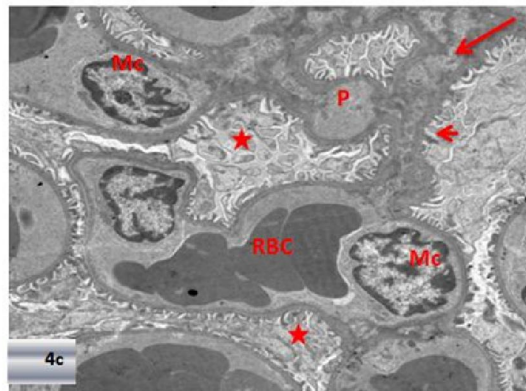


Fig. (4c): Electron micrograph of kidney from rats given power horse for 4 weeks showing thickening and undulation in the basement membrane of the glomerular capillaries (arrow). Note the urinary space appeared closed in many areas (*). mesangial cell, Mc; podocyte, P; podocyte pedicels, head arrow. x 2000

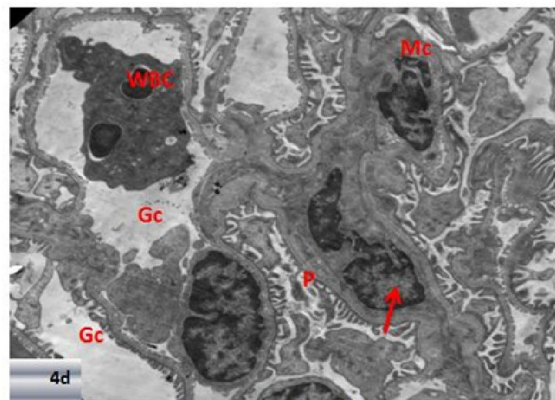


Fig. (4d): Electron micrograph of kidney from rats given red bull for 4 weeks showing the dilatation in glomerular capillaries (Gc) and thickening of their walls. Mesangial cell, Mc; podocyte, P; podocyte pedicels, arrow. x2500

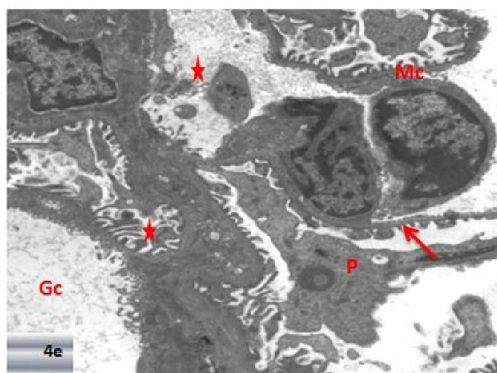


Fig.(4e): Electron micrograph of kidney from rats given code red for 4 weeks. Note the undulated, thick basement membrane of the glomerulus (Gc) and exfoliated podocytes (*). Mesangial cell, Mc; podocyte, P; podocyte pedicels, arrow. x2500

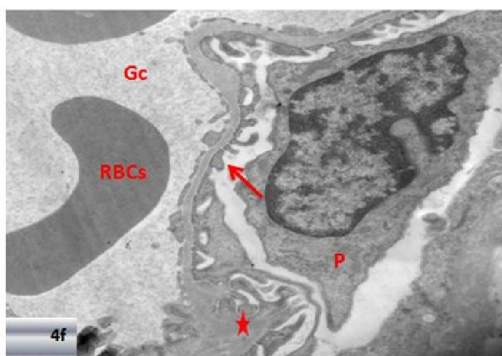


Fig.(4f): Electron micrograph of kidney from rats given code red for 4 weeks showing the fusion of podocyte pedicels (*). Glomerular capillaries, Gc; podocyte, P; podocyte pedicels, arrow. x5000

Biochemical results:

Data in figures (5-7) show the effect of Power horse, Red bull and Code red on the serum levels of urea, uric acid and creatinine.

Oral administration of Power horse for two & four weeks significantly ($P < 0.05$) increased the urea level as compared to control. Red bull and Code red induced marked elevation in urea only after four weeks of treatment. On the other hand, uric acid activity was slightly increased after treatment rats for two weeks with Power horse, Red bull or Code red. However, after 4 weeks, only animals treated with Power horse and Red bull showed marked elevation in uric acid when compared to controls. A significant increase in creatinine value was recorded in experimental animals after two & four weeks of treatment with Power horse. Red bull caused significant ($P < 0.05$) elevation in creatinine value firstly after four weeks of administration. There is no significant difference in creatinine concentration in animals treated with Code red for two or four weeks.

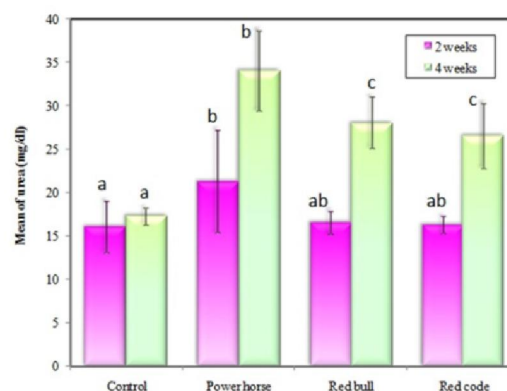


Figure (5): Effect of administration of Power horse, Red bull and Code red on urea concentration in rats. The values are expressed as means $\pm$ S.E (n = 5).

No common letters indicate significant difference at $P < 0.05$

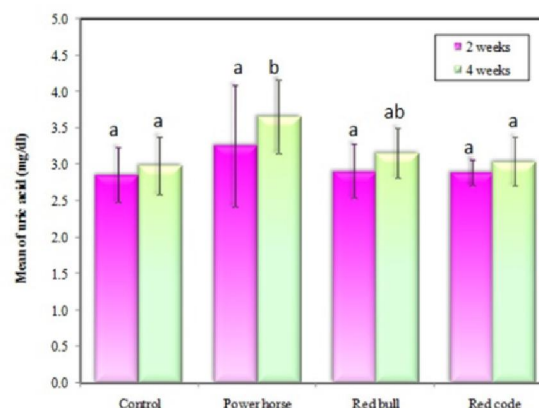


Figure (6): Effect of administration of power horse, Red bull and Code red on the level of uric acid in rats. The values are expressed as means $\pm$ S.E (n = 5).

No common letters indicate significant difference at $P < 0.05$.

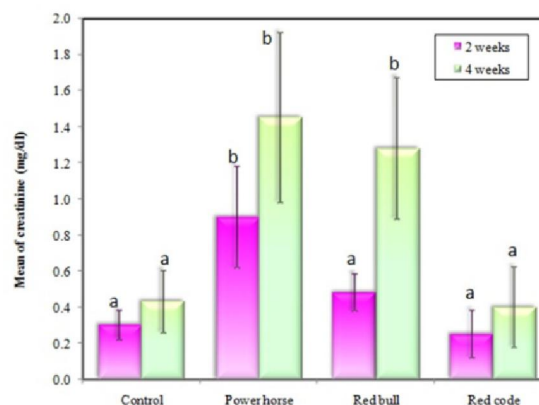


Figure (7): Effect of administration of power horse, Red bull and Code red on creatinine value in rats. The values are expressed as means $\pm$ S.E (n = 5).

No common letters indicate significant difference at $P < 0.05$

4. Discussion

The present study showed that administration of energy drinks caused renal toxicity that demonstrated by an elevation of urea, uric acid and creatinine and this elevation was time dependant. This is in agreement with the results of Ugwuja (2014) who reported that, consumption of energy drink Bullet® alone or with alcohol resulted in higher urea, uric acid and creatinine in the serum of rats. He mentioned that, both urea and creatinine are products of protein metabolism, which are accumulated in the blood when the kidneys are affected.

Also, Akande and Banjoko (2011) who reported that there is an increase in urea concentration in rats treated with power horse. The authors attributed the effect of energy drinks on renal function to caffeine which is one of the main ingredients of energy drinks. Many researchers showed that caffeine can increase the serum urea and creatinin concentration (Portoles *et al.*, 1985, Tofovic *et al.* 2002 & 2007, Abd El – Moneim *et al.*, 2009). This elevation may be attributed to that, caffeine through inhibition of A2A adenosine receptors, accelerates the development of interstitial inflammation, augments proteinuria and changes renal function and structure. Tofovic *et al.* (2000) reported that caffeine produced severe tubulointerstitial damage including tubular atrophy, presence of proteinaceous material, tubular dilatation, interstitial inflammation and interstitial fibrosis, as well as increased glomerulosclerosis and adversely affects renal function in rats.

The results of the present study indicated that oral administration of energy drinks induced histopathological and ultrastructural alterations in the kidney tissue of rats after 4 weeks of treatment.

Light microscopic examination revealed marked degenerative lesions in many renal tubules and renal corpuscles. Shide and Chandrasekaran (2011) proposed that if kidney tissue is exposed to a high enough concentration of energy drink ingredients, it could possibly be disrupted. This is justifiable since the renal tubules are in contact with toxic chemicals during their excretion and elimination (Kukner *et al.*, 2007). The observed necrosis of most renal tubules and glomeruli may be related to the depletion of ATP, which finally leads to the death of the cells (Shimizu *et al.*, 1996). The intertubular hemorrhage and the inflammation areas which were recorded during this study might be attributed to microcirculatory disturbances that developed due to the caffeine content of the energy drinks. Mubarak (2012) recorded hemorrhage areas in submandibular salivary glands in rats treated with Red bull.

In the present study, electron micrographs illustrated the matching between the ultrastructural changes occurred in cells of both proximal and distal

tubules. Both tubular cells appeared with pyknotic nuclei, thickened basement membrane and dilated intracytoplasmic foldings. Mubarak (2012) stated that such changes might be due to the preservatives added to the energy drinks as sodium benzoate. Moreover, the increase in the amount of lysosomes in epithelial cells of the proximal tubules, presence of intertubular inflammatory cells and cytoplasmic bulges in some distal cells could be related to the alteration of cytoskeleton structure in these cells (Caglar *et al.*, 2003). Shide and Chandrasekaran (2011) mentioned that, energy drinks showed disruptions of the actin cytoskeleton networks in kidney cells. On the other hand, the presence of membranous structure and lipofuscin granules in the cytoplasm of some proximal tubular cells may reflect the probable injury caused by energy drinks. Similar alterations have been reported due to the use of many chemicals which had a direct nephrotoxic action (Inkielewicz & Krechniak, 2003; Tofovic *et al.*, 2007; Akande & Banjoko, 2011; Sorour & Al-Rawi, 2011).

In the current study, the structure of the renal corpuscles were also affected by energy drinks. The observed dilatation of the glomerular capillaries, closing of the urinary space and increase in the filtering membrane disorders seemed to emphasize Na/H₂O balance disorder in the tissue and change in the ultrafiltration (Onarlioglu, *et al.*, 1998). This might be due to different reactions of taurine associated with other active ingredients of the energy drinks as caffeine (Tofovic *et al.*, 2007; Mubarak, 2012). In addition, degeneration of podocytes and fusion of pedicels were striking noticed. Çağlar *et al.* (2003) suggested that this degeneration may develop as a result of loss of electrical load in pedicels.

In conclusion, the findings of this study suggested that energy drinks may cause irreversible structural changes in rat renal tissue, which could play an important role in renal dysfunction.

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