Original Article

The Effect of Energy Drinks and Their Withdrawal on the Renal Cortex of Adult Male Albino Rats. Histological, Ultrastructural and Morphometric Study

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ABSTRACT

Introduction: Energy drinks are beverages which are consumed by youngsters in large quantities during exercises and studying. Many studies have shown that energy drinks have numerous negative consequences on many organs in heavy consumption.
Aim of the Work: This research aimed to study the effect of energy drinks and their withdrawal on the renal cortex of adult male albino rats.
Materials and Methods: Thirty adult male albino rats were divided equally into 3 groups; a control group (C), an experimental group (T) that was given 2.2ml\100gm\day of the energy drink orally for 8 weeks and a recovery group (R) that was given the energy drink for the same dose and duration as group T and was left for another 8 weeks after cessation of administration of the energy drink. Renal cortex specimens were prepared for the light and electron microscopes.
Results: Experimental group T revealed disturbed architecture of the renal cortex as shrunken glomeruli with disorganized tubular cells in the form of vacuolated cytoplasm and deeply stained pyknotic nuclei. Weak PAS reaction or even loss were seen in the brush border of some proximal convoluted tubules (PCTs). Ultrastructural examination showed podocytes with irregular nucleus, fusion and effacement of their feet processes and focal glomerular basement membrane thickening. In contrast, minimal regression and persistence of most changes was observed in the recovery group R.
Conclusi**on:** The used energy drink for long duration induced irreversible damaging effects on the renal cortex of adult male albino rats.

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Key Words: Electron microscopy, energy drinks, light, renal cortex.

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INTRODUCTION

Energy drinks are flavored beverages. They consist of many ingredients mainly large amount of caffeine. They contain a larger amount of caffeine 3–5 times than any other gaseous beverages). Energy drinks contain additives such as taurine, vitamins complex, ginseng and sugar[1,2].

Caffeine in a moderate amount can be tolerated by healthy people. Heavy consumption of caffeine due to drinking large amounts of energy drinks might have a lot of negative influences as seizures, palpitation, stroke and sudden death[3,4].

Energy drinks are often consumed by young individuals especially during exercising sports and studying. Prevalence of energy drinks is due to its significance to increase attention, energy and improve physical and cognitive performance[2,5,6].

Many studies have shown that energy drinks have numerous negative consequences on many organs such as liver, heart, pancreas, gastric mucosa and duodenum[6,7,8].

The kidney is an important organ that is highly susceptible to toxic injury by noxious chemicals because it receives about 20-25% of the cardiac output[9]. So, this work evaluates influence of intake of these energy drinks on structure of rats' renal cortex and possibility of recovery.

MATERIALS AND METHODS

Materials

Energy drink: The used drink was the red bull. It contains high quantities of caffeine, taurine, vitamins complex and sugar. It was obtained from a regional market and administered orally once daily via an intragastric tube in a dose of 2.2 ml\100 gm for 8 weeks. This dose is equivalent to six cans in human that equal 1540 ml of this drink[10].

Animals and experimental design

This work involved 30 adult male albino rats weighing about 200-250 grams for each. All the animals were kept in clean, well-ventilated cages and fed the same commercial

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laboratory meal and water. They were given a week to adjust to their new surroundings before beginning the experiment. This experiment was approved by Local Research Ethical Committee, Quality Assurance Unit, Faculty of Medicine, Tanta University (Code No: 32872/01/19). The animals were divided into three main groups:

I. **Control group (C):** It consisted of 10 rats that were subdivided into two equal subgroups, Control -ve that were kept without treatment and Control +ve that were given 2.2ml\100gm\day of distilled water orally using a gastric tube for 8 weeks. They were used to study the normal histological structure of the renal cortex.

II. **Experimental group (T):** It included 10 rats that were given 2.2ml\100gm\day of the energy drink orally using a gastric tube for 8 weeks.

III. **Recovery group (R):** It included 10 rats that were given 2.2ml/100gm/day of the energy drink for 8 weeks by a gastric tube, then the rats were left untreated for another 8 weeks for possibility of recovery.

Specimens processing and staining

At the ending of the experimental periods (8 weeks in groups C&T and 16 weeks in groups C&R), the animals were sacrificed after intraperitoneal sodium thiopental injection for general anesthesia (30 mg/kg)[11,12]. The abdominal wall was incised and the kidneys were dissected and washed with saline. Specimens from the renal cortex were prepared for light and electron microscopic study.

For light microscopic study, the specimens from the cortex of the right kidney were taken and promptly fixed in a 10% buffered formol saline solution for 24 hours, dehydrated as the alcohol level rises, then two changes of xylol were used to clear it. Paraffin embedding was done after 2 hours of impregnation in pure paraffin at 55oC. Lastly, the rotatory microtome was used to cut 5 micron sections. (Leica Biosystems, China) to be stained with Hematoxylin and Eosin (H&E) for histological examination and Periodic Acid Schiff (PAS) reagent for histochemical examination[13].

For electron microscopic study

The cortex of the left kidney specimens was cut into 1 mm3 pieces using a sharp razor blade and were fixed in 2.5 percent phosphate buffered glutaraldehyde (pH 7.4), post-fixation was done with 1% phosphate-buffered osmium tetroxide, and then dehydrated in ascending grades of alcohol. After dehydration the specimens were immersed in acetone before being imbedded in epoxy resin. Then polymerization was performed until the blocks were very hard for sectioning. Uranyl acetate was used to stain ultrathin slices (75nm) and counter stain with lead citrate[14]. Ultrathin sections were investigated using JEOL-JEM-100 SX electron microscope.

Morphometric study

The following parameters were measured using Image J software (National Institute of Health, Bethesda, Maryland, USA):

Thickness of the glomerular basement membrane (GBM)

Ten EM images (X 3000) from each group were used. Measurements were taken between the endothelial cells of the glomerular capillaries as they attach to the basement membrane and the outer lining of the glomerular basement membrane's lamina rara externa, which are present below the cytoplasmic membranes of podocytes' feet processes with the exclusion of areas of tortuosity to prevent tangential plane of sections[15].

Area percentage (Area %) of the PAS positive reaction

For each group at a magnification power of (X 400) 5 randomly selected microscopic fields were measured.

Statistical analysis

SPSS software (SPSS Inc., version 13, USA) analyzes the data, which are subjected to ANOVA and Scheffe test comparing different groups to control. Obtained information was then expressed as (mean,SD). Results finally considered to be significant when $P < 0.05$ and highly significant if $P < 0.001$ and non-significant if $P > 0.05$.

RESULTS

Histological results

The examined H&E-stained sections of the control subgroups (C1) & (C2) illustrated the normal structure of the renal cortex. It showed three main structures: Malpighian corpuscles (MC) with regular continuous Bowman's capsules, proximal and distal convoluted tubules (PCTs &DCTs) (Figure 1).

Sections of renal cortex obtained from experimental group (T) demonstrated severe disturbance of the histological architecture. Many glomeruli were sclerotic or shrunken. Others appeared having a wide Bowman's space (Figure 2). Some glomeruli appeared segmented or hypercellular with narrowing or focal obliteration of the capsular space (Figures 2,3). The convoluted tubules revealed extensive damage in the form of degeneration of their lining cells with cytoplasmic vacuolation and cellular debris inside their lumen and many disorganized tubules with dilated lumen (Figure 4). Besides all these changes, there were mononuclear cellular infiltration (Figure 5).

Renal cortical sections from the recovery group (R) showed minimal regression of some of the microscopic lesions and persistence of others. Many glomeruli appeared vacuolated, congested, shrunken, segmented and hypercellular. Regarding the convoluted tubules, many disorganized tubules with dilated lumen appeared and vacuolated cytoplasm of their lining cells with pyknotic nuclei were seen (Figure 6). There was also mononuclear cellular infiltration (Figure 7).

Histochemical results (PAS)

Control subgroup C1&C2 illustrated a strong PAS positive reaction in the basement membrane of Bowman's capsule's parietal layer and the basement membrane of the convoluted tubules. (Figure 8) as well as the brush borders of PCTs (Figure 9).

Experimental group (T) appeared with focal loss of the reaction from the basement membrane of the parietal layer of Bowman's capsule and focal increase in other areas (Figure 10). Moreover, a weak reaction appeared in the brush border of many PCTs with focal interruption in some areas and also a weak reaction of the basement membrane of many tubules (Figure 11).

The renal cortical sections obtained from the recovery group (R) showed focal loss of the reaction in the basement membrane of the parietal layer of Bowman's capsule and focal increase of the reaction in other areas. Also, a weak reaction appeared in the brush border of PCTs with focal interruption in some areas (Figure 12).

Electron microscopic results

Ultrathin sections obtained from all control subgroups showed normally appeared podocyte with euchromatic central nucleus. The cell body of podocytes gave major processes (primary processes). Secondary processes arose from primary processes and terminated by feet-like expansions on the basal lamina of the capillary wall (Figure 13). Cells of the PCTs were resting on a thin basement membrane and had basal plasma membrane infoldings with mitochondria that were arranged in the basal half of the cell in palisading arrangement. The cells also had a single large rounded euchromatic nucleus that were present nearly in the central region. Their cytoplasm contained lysosomes and pinocytotic vesicles with numerous thin long apical microvilli that gave its characteristic striated border (Figure 14). Cells of the DCTs were resting on a thin basement membrane having a basal plasma membrane infoldings with mitochondria that were arranged in the basal half of the cell in palisading arrangement. The cells had a rounded or ovoid nucleus with less extended chromatin (Figure 15).

Experimental group (T) revealed massive ultrastructural destructive changes in the renal corpuscles, proximal and distal convoluted tubules. Podocytes had fusion and effacement of their secondary feet processes. Moreover, some podocytes had shrunken irregular shaped nucleus with clumped peripheral chromatin. The glomerular basement membrane had focal irregular thickening. (Figure 16). Most cells of the PCTs showed shrunken hyperchromatic nucleus and swollen vacuolated mitochondria with loss of their cristae and their normal basal palisade arrangement. Additionally, many cytoplasmic vacuoles, dilated RER and dense bodies were detected with loss of the apical microvilli (Figures 17,18). While some cells of the DCTs showed dark shrunken nucleus with clumped chromatin, dilated perinuclear space, dilated RER and cytoplasmic vacuoles (Figure 19).

In recovery group (R), most structural changes had persisted. Podocyte showed shrunken nuclei with areas of fusion and effacement of their feet processes and focal thickness and irregularity of the glomerular basement membrane (Figure 20). Disrupted PCTs with focal loss of apical microvilli and loss of the normal basal palisading arrangement of mitochondria with numerous dense bodies (Figure 21). While a few DCTs showed disarrangement of the mitochondria, numerous dense bodies and cytoplasmic vacuoles with irregular basement membrane (Figure 22).

statistical results of morphometric data

Mean PAS area %: Experimental group (T) showed a statistically highly significant decrease in PAS positive reaction when compared to control group and recovery group (R) showed a statistically significant decrease when compared with control group and a non- significant change when compared with Experimental group (T) (Table 1, Histogram 1).

Mean GBM thickness revealed a highly significant increase in experimental group (T) when compared to the control group. Moreover, the recovery group (R) showed a statistically significant increase in the mean thickness of the glomerular basement membrane when compared with control group but a significant decrease when compared with experimental group (T) (Table 2, Histogram 2).

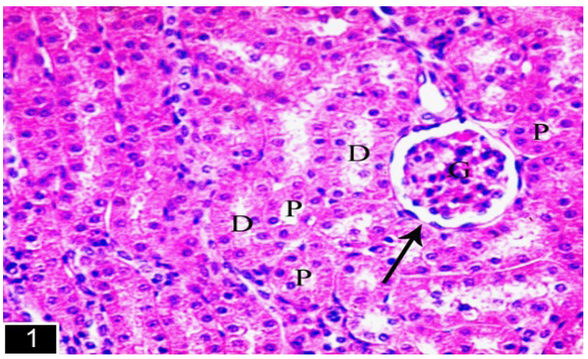


Fig. 1: is showing a renal corpuscle (G) with a regular continuous Bowman's capsule (→), proximal convoluted tubules (P), and distal convoluted tubules (D). (H&E, control, x 400)

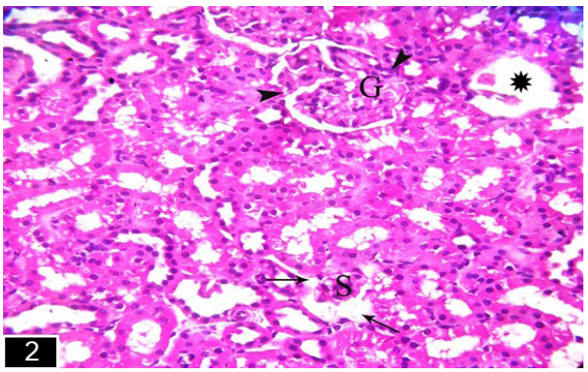


Fig. 2: is showing segmented glomerulus (G) with focal adhesions between the capsule and glomerulus (►). Another glomerulus is shrunken (S) with loss of continuity of the lining of the parietal layer of the capsule (→) and nearly empty corpuscles (*). (H&E, experimental T, x 400)

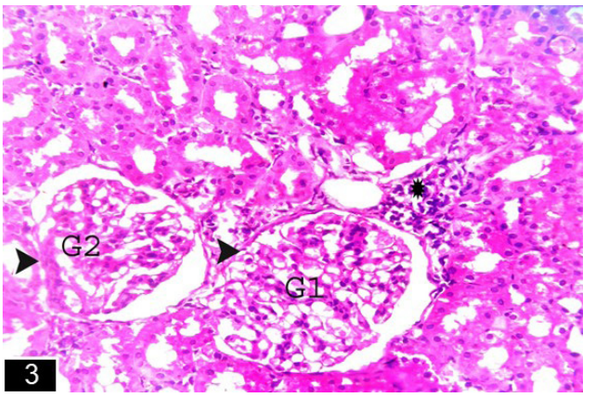


Fig. 3: is showing hyper cellular (G1) and segmented glomeruli (G2) with focal obliteration of their spaces and focal adhesion between the glomeruli and their capsules (►). Mononuclear cellular infiltration (*) is also noticed. (H&E, experimental T, x 400)

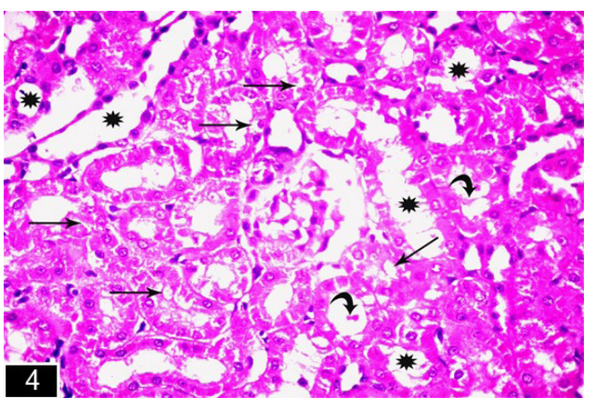


Fig. 4: is showing many convoluted tubules with vacuolation of their cells (→) and dilatation of their lumen (*). Notice luminal debris (curved arrow). (H&E, experimental T, X400)

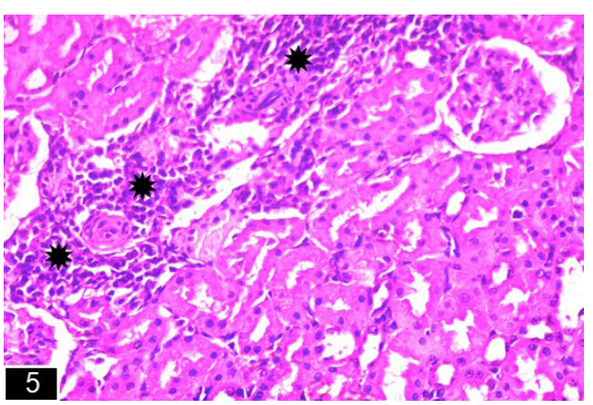


Fig. 5: is showing severe mononuclear cellular infiltration (*). (H&E, experimental T, x 400)

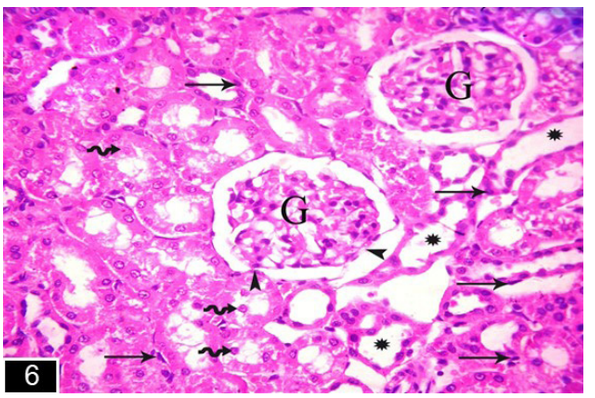


Fig. 6: is showing vacuolated glomeruli (G) with focal adhesion between the glomerulus and Bowman's capsule (►). Some tubules are dilated (*). Noticed disorganized and vacuolated tubular cells (wavy arrow) and deeply stained nuclei (→). (H&E, recovery R, x 400)

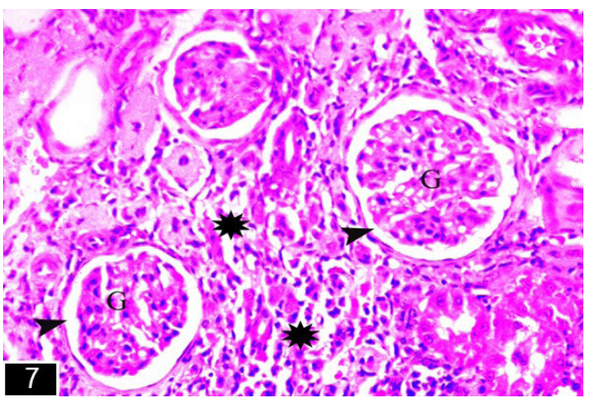


Fig. 7: is showing vacuolated glomeruli (G) with nearly normal Bowman's space (►). Notice mononuclear cellular infiltration (*). (H&E, recovery R, x 400)

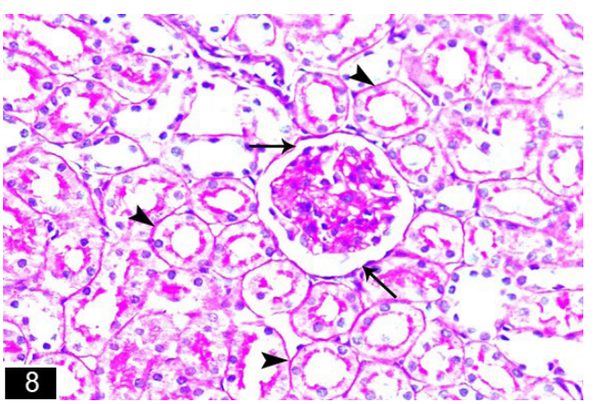


Fig. 8: is showing a strong PAS reaction of the basement membrane of the parietal layer of Bowman's capsule (→) and also of the convoluted tubules (►). (PAS, control, x 400)

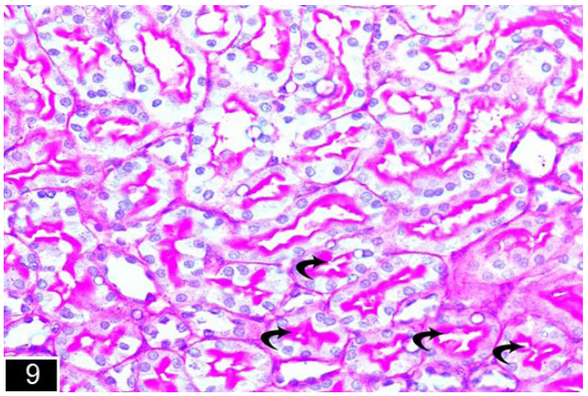


Fig. 9: is showing a strong reaction in the brush border of PCTs (curved arrow). (PAS, control, x 400)

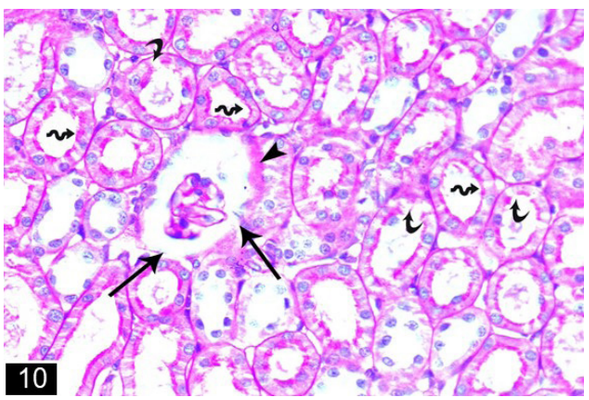


Fig. 10: is showing focal loss of PAS reaction from the basement membrane of the parietal layer of the Bowman's capsule (→) and focal increase of the reaction in other areas (►). Notice a weak reaction in the brush border of many PCTs (wavy arrow) with focal loss in some parts (curved arrow). (PAS, experimental T, x 400)

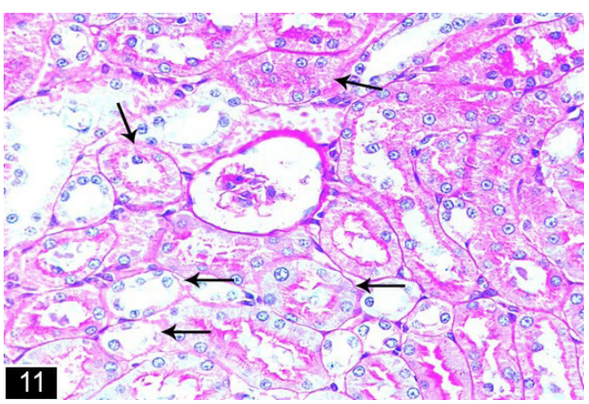


Fig. 11: is showing a weak reaction in the basement membrane of some convoluted tubules (→). (PAS, experimental T, x 400)

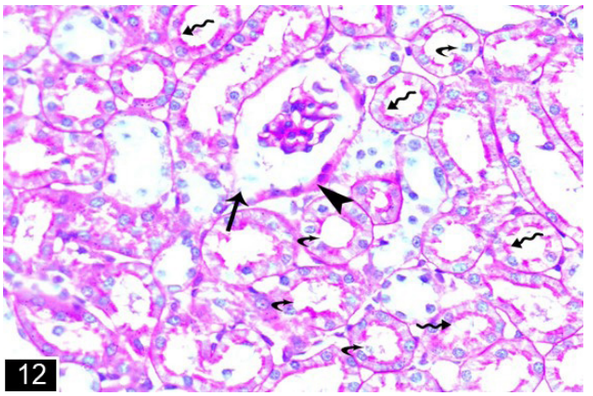


Fig. 12: is showing focal loss of PAS reaction from the basement membrane of the parietal layer of Bowman's capsule (→) and focal increase in other areas (►). Notice a weak reaction in the brush border of many PCTs (wavy arrow) with focal loss in some parts (curved arrow). (PAS, recovery R, x 400)

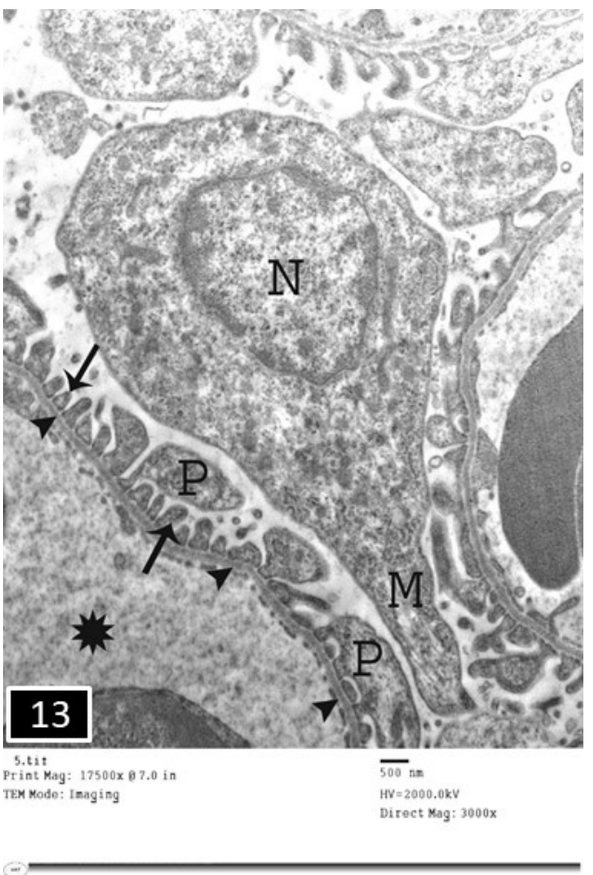


Fig. 13: is showing normally appeared podocyte with euchromatic central nucleus (N), major process (M), minor process (p) and the feet processes (→). It also shows a glomerular blood capillary (*) and the basement membrane (►). (MC, control, x3000)

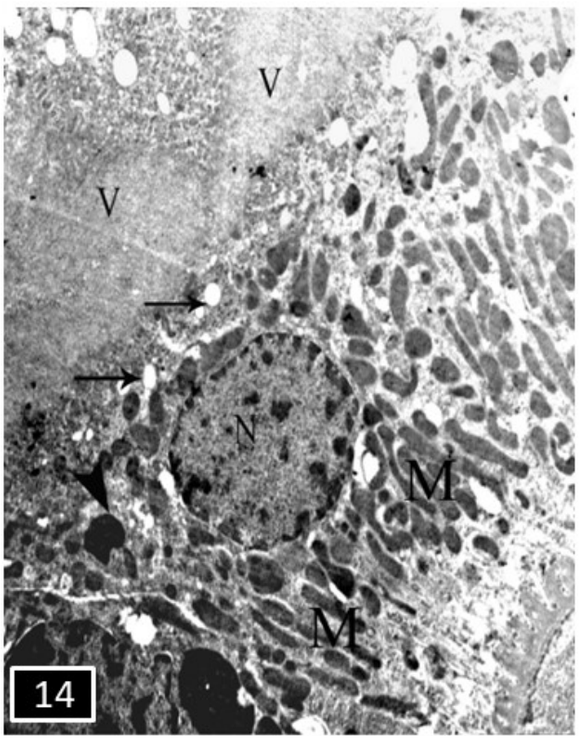


Fig. 14: is showing a PCT cell having euchromatic central nucleus (N), apical microvilli (V), pinocytotic vesicles (→), lysosomes (►) and basal plasma membrane infoldings with nearly normal palisading arrangement of mitochondria (M). (PCT, control, x1500).

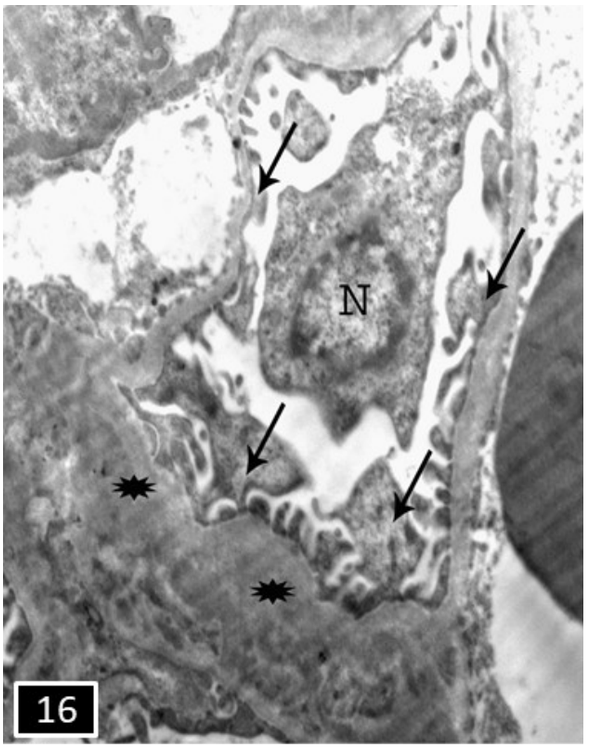


Fig. 16: is showing sever thickening of the glomerular basement membrane (*). A podocyte shows a shrunken nucleus with clumped peripheral chromatin (N). Swollen feet processes of the podocytes (→) are also noticed. (MC, experimental T, x3000)

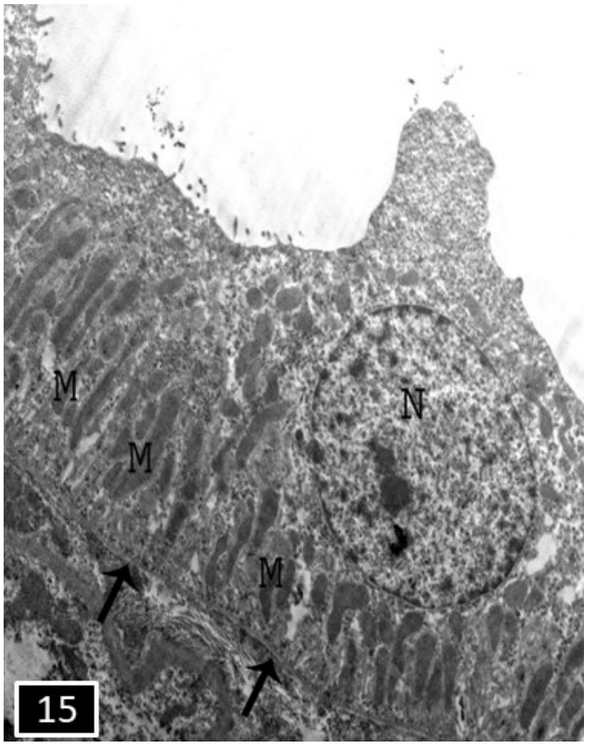


Fig. 15: is showing a DCT cell rests on a thin regular basement membrane (→), a large euchromatic nucleus (N) and normal arrangement of mitochondria (M). (DCT, control, x1500)

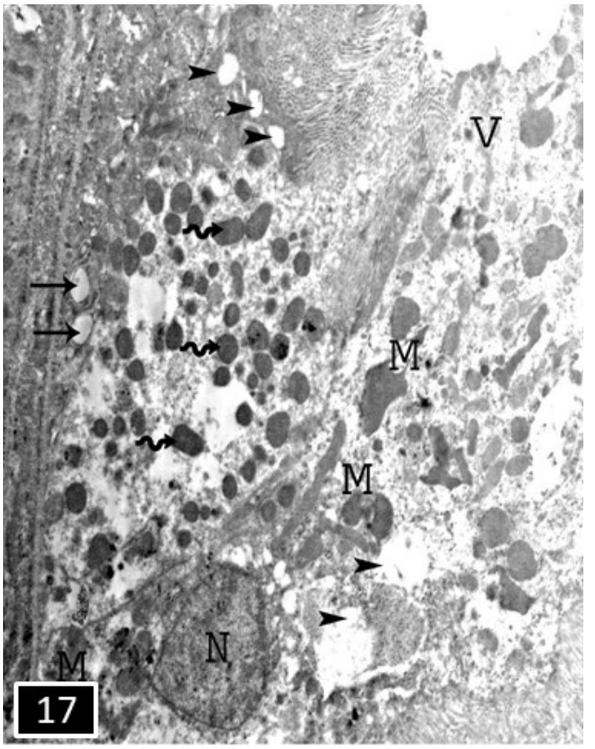


Fig. 17: is showing disrupted apical microvilli (V), a shrunken nucleus (N), loss of mitochondrial arrangement (M), numerous dense bodies (wavy arrow), dilated RER (→) and cytoplasmic vacuoles (►). (PCT, experimental T, x1500)

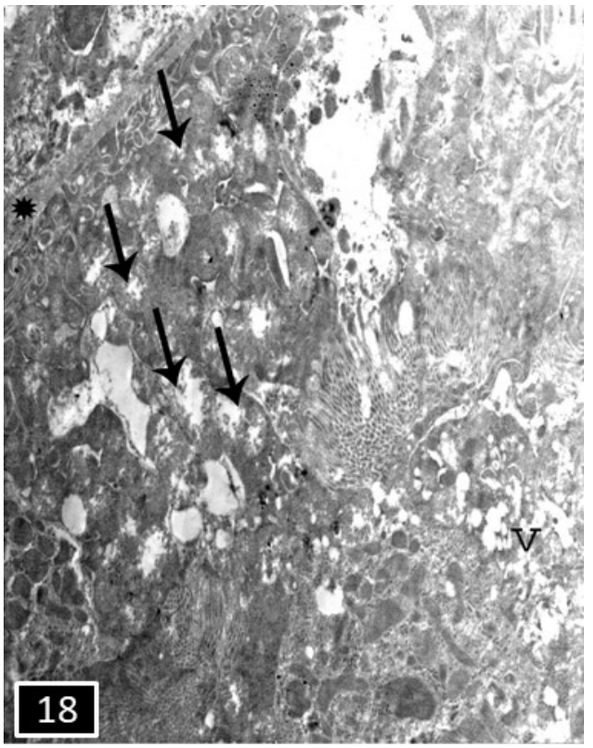


Fig. 18: is showing loss of mitochondrial arrangement with swollen vacuolated mitochondria with distortion of their cristae (→), and vacuoles of the cytoplasm (V). Notice the presence of focal thickening of basement membrane (*). (PCT, experimental T, x1500)

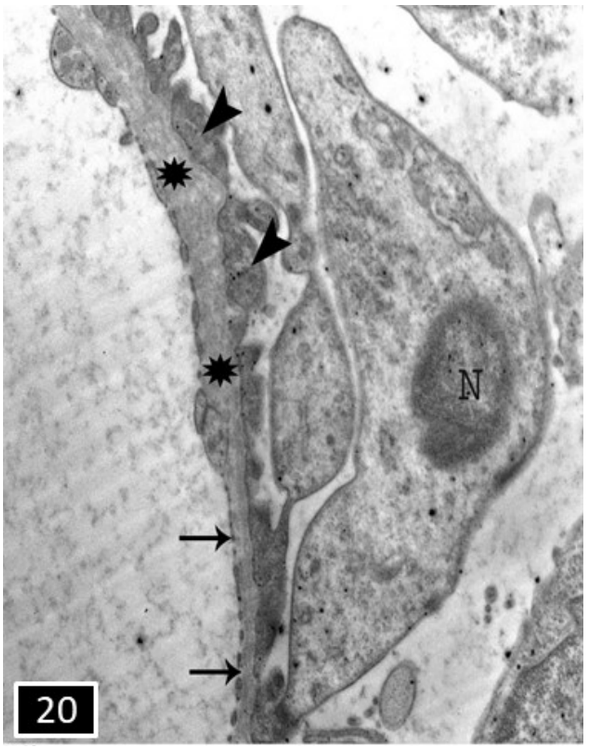


Fig. 20: is showing a podocyte with a shrunken nucleus (N) and focal thickness of GBM (*). Notice some fused feet processes of podocytes (►). It also shows normal fenestrated endothelium (→). (MC, recovery R, x3000)

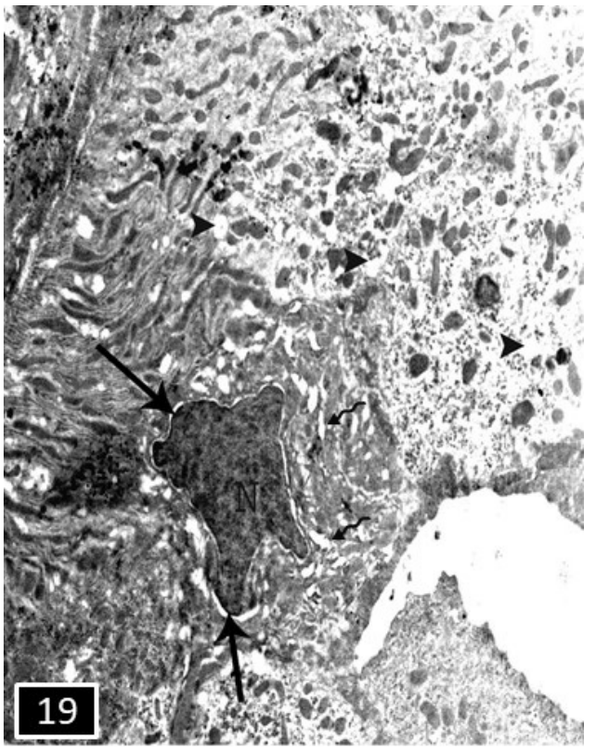


Fig. 19: is showing an irregular dark shrunken nucleus (N), dilated perinuclear space (→) and RER (wavy arrow) and cytoplasmic vacuoles (►). (DCT, experimental T, x1500)

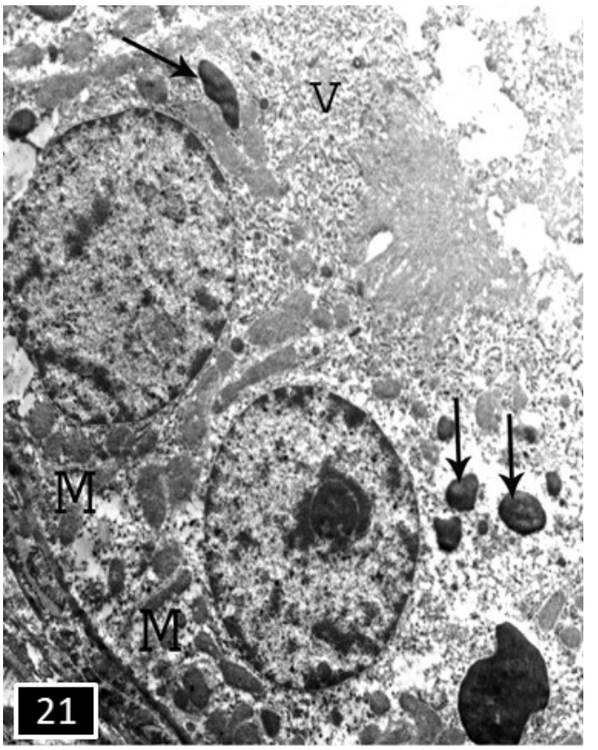


Fig. 21: is showing focal loss of apical microvilli (V) and numerous dense bodies (→). Notice abnormal mitochondrial arrangement (M). (PCT, recovery R, x1500)

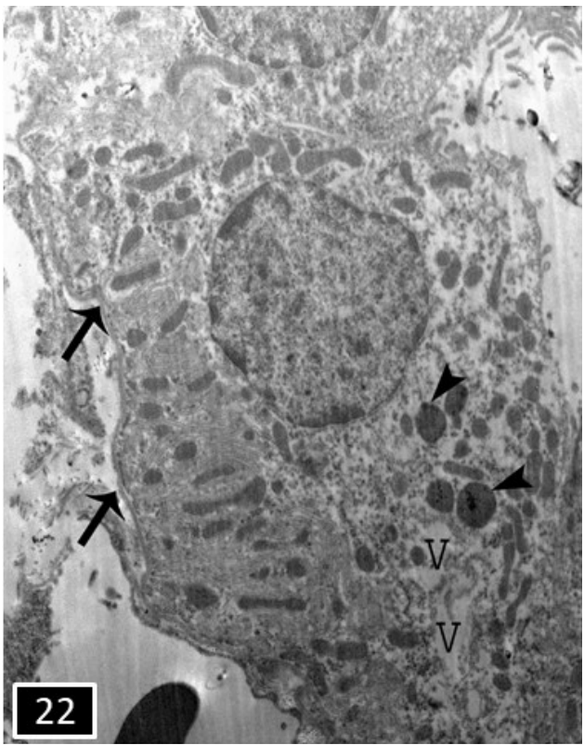


Fig. 22: is showing cytoplasmic vacuoles (V) and dense bodies (►). It rests on an irregular basement membrane (→). (DCT, recovery R, x1500)

Table I: Mean PAS area %.

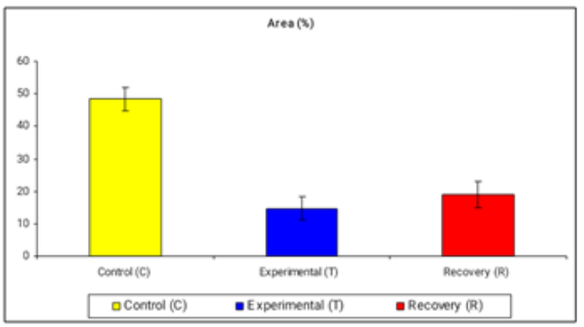
Area (%)	Control (C)	Experimental (T)	Recovery (R)
Range	42.94 – 55.69	10.41 – 22.54	12.12 – 25.23
Mean ± SD	48.35 ± 3.63	14.74 ± 3.66	18.90 ± 4.07
F test		233.379	
P value		0.001**	
	C & T	C & R	T & R
T test	20.602	17.072	2.408
P value	0.001**	0.001**	0.027*

SD: standard deviation * Significant *p value* < 0.05 ** Highly significant *p value* < 0.001

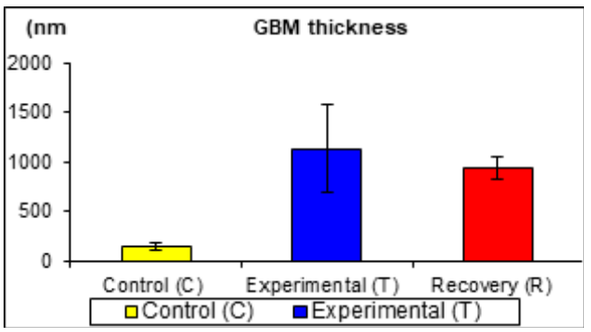
Table II: Mean GBM thickness.

GBM thickness (nm)	Control (C)	Experimental (T)	Recovery (R)
Range	105.41 – 197.17	468.16 – 1684.25	762.93 – 1075.03
Mean ± SD	143.94 ± 30.17	1134.49 ± 442.07	930.19 ± 111.27
F test		39.314	
P value		0.001**	
	C & T	C & R	T & R
T test	7.069	21.566	1.417
P value	0.001**	0.001**	0.173*

SD: standard deviation * Significant *p value* < 0.05 ** Highly significant *p value* < 0.001



Histogram I: Mean PAS area %.



Histogram II: Mean GBM thickness.

DISCUSSION

Kidneys are metabolically active organs that maintain the extracellular fluid. They are also vulnerable to substances that disrupt metabolism. They are responsible for secretion of many toxic substances and elimination of a high number of free radicals which contribute to a process of oxidative stress through secretion of cytokines, interleukin (IL-1) and tumor necrosis factor (TNF-α) that may result in kidney damage[9,16]. Energy drinks have high amounts of caffeine which has a diuretic effect on the body leading to loss of fluid in urine[17,18]. So, the goal of our work was to determine how these energy drinks affected rats' renal cortex and whether they could be recovered after their withdrawal.

Caffeine is present in energy drinks in high levels in several concentrations Varied from 50 to 505 mg per can (32:134mg per 100ml)[3]. The average consumption of caffeine in healthy adults is about 180 mg per day and therefore, any increase in this concentration may lead to harmful health conditions and severe toxic symptoms[19].

This study showed that the used energy drink in a dose of 2.2ml/100gm/day orally for 8 weeks induced histological changes in rats' renal cortex. On the other hand, the recovered group showed minimal regression of some changes with persistence of others after cessation of administration of the energy drink for the same period.

The present study was in line with Memudu *et al*., (2020)[20] who studied the effect of the caffeinated energy drink on the renal cortex of Wister rats. They stated that the consumption of the energy drink led to several alteration in the histoarchitecture of the renal cortex which is attributed to oxidative stress and lipid peroxidation. Previous research studied the effects of the energy drink on the glomerular parameters and its conservation by vitamin D3. They found that the extent of kidney injury is related to caffeine concentration in the energy drink resulting in increased superoxide production[21].

Persistent changes of the renal cortex in the recovery group coincided with results found by Bano *et al*., (2020)[22] who studied the effects of withdrawal of the caffeinated energy drink on histological parameters of kidneys of albino rat. They stated that the histological alterations in kidneys are irreversible after withdrawal of these caffeinated energy drinks.

The present work showed mononuclear cellular infiltration in experimental group. The same changes were found by Salih *et al*., (2018)[23] during studying the histopathological effect of energy drink on rabbits' heart, kidney, liver and brain. Mononuclear cellular infiltration following excessive energy drink consumption was attributed to a high amount of caffeine that cause inhibition of A2 Adenosine receptors followed by acceleration of interstitial inflammation leading to disruption of the renal structure[24].

Many Malpighian corpuscles in the present work showed shrunken or segmented glomeruli with widening of Bowman's space. These alterations were explained by previous research to be due to renal vasoconstriction in response to different nephrotoxic substances. Shrinkage of some glomeruli was also supposed to be due to oxidative stress[25,26].

The glomerular sclerosis was attributed to activation of parietal epithelial cells (PrECs) which border the Bowman's capsule. These PrECs proliferate and extend on to the glomerular tuft of capillaries and compress the glomerular capillaries by producing excess matrix. They added that parietal epithelial cells proliferate and accumulate through the capsular space forming pseudocrescents[27].

Hypercellularity of the glomeruli observed in this research may be resulted from increase of the number of cells in the mesangial area in the form of agglomerates (mesangial hypercellularity) or by cell aggregates that is present inside the capillary lumen (endocapillary hypercellularity)[28].

On the other hand, these results against Akande and Banjoko, (2011)[1] who studied biochemical and histological influence of the energy drinks on the kidney and liver of rats. They stated that no changes occurred after intake of energy drink. In the present work, visceral epithelial cell injury could explain the adhesions between the glomerular tuft of capillaries and Bowman's capsule[29].

In the current research, many convoluted tubules of the experimental group showed several alterations of their normal histological structure due to intake of the energy

drink. They showed cytoplasmic vacuolations of the tubular cells, disorganized tubular cells with extrusion into the tubular lumen as well as cellular debris inside the lumen and deeply stained nuclei of some tubular cells. These findings appeared to be similar to results of Alduweesh *et al*., (2020)[25]. They stated that these findings may be due to increased permeability of cell membranes in reaction to cell injury, resulting in an increase in intracellular water, producing cytoplasmic vacuolation. Also, it may be attributed to the impairment of the mitochondrial uptake of oxygen and release of the cytotoxic lysosomal enzymes into the cytoplasm in addition to increased calcium influx from the extracellular compartment to intracellular one and stimulation of calcium dependent phospholipases that finally lead to tubular vacuolar damage[30].

In the present work, many cells of the renal tubules appeared vacuolated with pyknotic nuclei. This was attributed to the energy drink's high caffeine concentration. and the interaction between it and other ingredient as sodium benzoate leading to nuclear changes and fatty degeneration of tubular cells[31]. Pyknotic nuclei could also be the result of nuclear changes caused by a nonspecific DNA disruption. It caused irreversible chromatin clumping into a solid basophilic mass in the cells, which eventually went into necrosis or apoptosis[32]. Cytoplasmic vacuolation of tubular cells may be also explained by cellular edema which resulted from disturbance in cellular calcium due to the high sensitivity of the calcium pumping ATPase which is present in both plasma membrane and endoplasmic reticular membrane[33].

Additionally, desquamation of some tubular cells with nuclear extrusion inside their lumen and their damage may be attributed to the oxidative stress through the imbalance of free radical generation with antioxidant defense system[34].

The present work showed tubular dilatation and presence of some debris inside tubular lumen, tubular atrophy, extrusion of tubular epithelial cells inside the lumen and even loss of the brush border. Similar findings were observed with other researchers who attributed tubular damage to direct contact of tubules with toxic chemicals during elimination and excretion of these drinks. They explained tubular necrosis to be due to ATP depletion that finally resulted in cell death. Carbon dioxide that is present in a large amount in energy drinks damages mitochondrial membrane, change in ATP production and causes hypoxia which decreases amount of blood to cells and leads to cell death and necrosis. Tubular cell necrosis led to widen the intertubular space[5,35].

Desquamation of tubular cells may be also explained by alterations in cell microfilaments and changes on the surface attachment proteins which led to alteration of cell adhesion[36]. The observed tubular necrosis may be due to ATP depletion[37].

The PAS-stained sections obtained from the experimental group revealed absence of PAS reaction of the brush border of some PCTs and interruption of their basal laminae that led to decrease area percentage of PAS stain in the experimental group compared to the control. These findings were attributed to reactive oxygen metabolites (ROM) generation which lead to rapid loss of integrity of the cytoskeleton. These findings can be explained by the process of necrosis and apoptosis which lead to loss of the apical brush boundary and separation of the tubular cells from its basement membrane leaving an area of denuding basement membranes[38].

Electron microscopic examination of the renal cortex of the experimental group showed damage to the renal glomeruli with glomerular basement membrane focal thickening and congestion of the capillaries. Podocytes showed cytoplasmic vacuolation, RER dilatation, irregular shaped nucleus and focal effacement and fusion of feet processes. In addition, cells of some convoluted tubules showed cytoplasmic vacuolation, irregular shaped nuclei, shrunken hyperchromatic nuclei, dilated RER, dense bodies and focal loss of the basal palisading arrangement of mitochondria with abnormal shapes as swollen, fused and vacuolated mitochondria. Additionally, some cells of PCTs showed focal loss of the apical microvilli. So, electron microscopic results confirmed the light microscopic data.

Similar results were detected by other researchers who stated that the irregular glomerular basement membrane thickening may be due to the large surface area of the glomerular capillaries that are liable to large amounts of toxins and immunological complexes in the blood. They found the cytoplasm of the tubular epithelial cells had cytoplasmic vacuoles, damaged mitochondria, numerous lysosomes and loss of apical microvilli. They added that all of these changes could be attributed to tubular cells necrosis secondary to contact with toxins[39].

Energy drinks produce disruption of actin cytoskeleton of the cells which reflect the possible injury caused by these drinks. Similarly, dilated RER led to increase ER stress which initiate caspase activation and apoptosis which lead to mitochondrial damage via reactive oxygen species production. These changes could be explained by high preservative toxic effects of energy drinks that cause cell damage and DNA damage by a process of lipid peroxidation (oxidative stress)[40]. The effacement and fusion of feet processes and podocyte deformity may be caused by ROS overproduction which resulted in cytoskeletal changes such as actin disaggregation[41].

The mitochondrial damage observed in this study may be attributed to the increase in mitochondrial generation of ROS that led to their swelling and therefore breakdown of outer mitochondrial membrane causing release of intermembrane proteins and enhancing cell death. The excess reactive oxygen species can damage the cell as it can cause many alterations on protein as peptide chain fragmentation, change of electrical charge of proteins and certain amino acids which are oxidised by specific proteases resulting in enhanced vulnerability to destruction and proteolysis[42]. The mitochondrial degeneration may be

also due to increased water influx that leads to separation between inner and outer mitochondrial membrane and then degeneration[43].

The focal loss of the apical microvilli of the tubular cells and necrosis may be attributed to decrease in the alkaline phosphatase activity. This is an enzyme marker of the brush border membrane of PCTS. The decrease in its activity may be due to deep hypotonic circulatory arrest[42]. The tubular cell damage with loss of apical microvilli may be also attributed to lipid peroxidation and inflammation[44].

Moreover, irregular shrunken and pyknotic nuclei were also found in our work. These changes can be caused by high amounts of reactive oxygen species which lead to lipid peroxidation and subsequent reaction with DNA resulting in DNA damage[41]. The nuclear changes were also explained as a part of necrosis of the cells[44].

Numerous electron dense bodies, lysosomes and chromatin condensation were also observed in some degenerated tubular cells. This agreed with previous researchers who attributed these changes to the alteration of structure of cytoskeleton of many cells as energy drinks disrupt actin cytoskeleton of those cells. These changes may be explained also by autophagy of damaged cytoplasmic debris or degenerated organelles[5,40,45]. These heterogeneous electron-dense bodies were illustrated to be secondary lysosomes and was approved to be an indicator of intracellular degeneration of macromolecules[43,46].

CONCLUSION

From our research, it could be concluded that energy drinks for long duration have a damaging effect on the rats' renal cortex which are irreversible with persistence of most histological changes after cessation of its consumption. So, they should be used with caution in a regulated manner.

RECOMMENDATIONS

- Usage of energy drinks should be limited with good awareness of that.
- More research must be performed to illustrate the influence of energy drink on the renal cortex in different periods and the possibility of recovery.
- More work is needed to prove the influence of energy drinks on the renal medulla.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير مشروبات الطاقة وسحبها على القشرة الكلوية فى ذكرالجرذ الأبيض البالغ . دراسة هستولوجيه والتركيب الدقيق وقياسية شكليه

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المقدمة: مشروبات الطاقة هي المشروبات التى يتم استهلاكها على نطاق واسع من قبل الشباب أثناء التمارن والدراسه. أثبتت العديد من الدراسات أن مشروبات الطاقة لها العديد من التأثير ضار على العديد من الأعضاء عند استهلاكها المفرط.

الهدف: اجرى هذا العمل لدراسة تأثير مشروبات الطاقة وسحبها على القشرة الكلوية فى ذكر الجرذ الأبيض البالغ.

مواد وطرق البحث: ثلاثون من ذكور الجرذان البيضاء البالغةتم تقسيمهم بالتساوي إلى ثلاث مجموعات. مجموعة ضابطة (س) ، مجموعة تجريبية (ت) اعطيت ٢,٢مل لكل ١٠٠جم من مشروب الطاقه مرة واحدة يوميا عن طريق الفم لمدة ثمانيه أسابيع ومجموعة التعافي (ر) التي أعطيت مشروب الطاقة بنفس الجرعة والمدة التي تم تناولها للمجموعة ت ثم ايقاف تناوله لمدة ثمانيه اسابيع اخرى. عينات القشرة الكلوية تمت معالجتها للفحص بالمجهر الضوئي والإلكتروني.

النتائج: أظهرت المجموعة التجريبيه ت تغييرات في تركيب القشرة الكلوية حيث تتقلص الكبيبات مع خلايا أنبوبية غير منظمة. وتفاعل ايجابى ضعيف بواسطه كاشف شيف الأحمرى الدورأو حتى فقدانه في حدود الفرشاة لبعض النبيبات الملتويه القريبه. وأظهر الفحص البنائي خلايا رجلاء بها نواة غير منتظمة ، واندماج وانصهار لأقدامهم الزائدة زيادة فى سمك الغشاء القاعدى للطبقة الجدارية لكبسولة بومان. في المقابل ، لوحظ تراجع بسيط مع استمرار لمعظم التغييرات في مجموعة التعافى ر.

الإستنتاج: مشروب الطاقة المستخدم لفترة طويله تسبب في إحداث آثار ضارة لا رجعة فيها على القشرة الكلوية للفئران البيضاء.