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Full Length Research Paper

Histological and Immunohistochemical study on the Effect of the Energy Drink "Red Bull" on the Renal Cortex of Adult Male Albino Rats and the Possible Protective effect of *Nigella Sativa* oil

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Abstract

Introduction: *General health and wellbeing of youth and adolescents are threatened due to drinking energy drinks and the phenomenon is expanded day by day between younger ages. Several warnings have been issued regarding the potential adverse effects of energy drinks including nephrotoxicity. The anti-inflammatory and antioxidant properties of N. sativa are accountable for its renoprotective effects.* ***Aim:*** *This study aimed to investigate the histological and immunohistochemical alterations in the renal cortex of male rats induced by ingestion of Red Bull (RB) energy drink and to estimate the probable protective action from treating with Nigella sativa oil (NSO) against Red Bull-induced renal injury.* ***Material & Methods:*** *Forty adult male albino rats were classified randomly into four experimental groups (n=10 /group): group I was kept as control, group II (treated with NSO), group III (given RB) and group IV (treated with RB + NSO). Blood samples were collected at the end of the treatments, for biochemical analysis of kidney function, then the kidney samples were obtained and prepared for histological and immunohistochemical studies.* ***Results:*** *administration of RB elevated significantly the serum levels of urea and creatinine. The histological results of RB-treated group revealed glomerular degeneration, distortion and dilatation of renal tubules, dilated congested blood vessels, cellular infiltration and increased collagen fibers deposition. The immunohistochemical results revealed marked increase in cox-2 and caspase-3 expressions in the renal tissue of RB-treated group. Co-administration of Nigella sativa oil with Red Bull revealed minimal changes.* ***Conclusion:*** *The energy drink Red Bull had toxic effect on the kidney. Nigella sativa oil had marked protective effect against RB-induced renal damage in rats when co-administrated with RB.*

Key words: *Red Bull, Nigella sativa oil, kidney, cox-2, caspase-3.*

Introduction

Nowadays, there are increasing in the demand of non-alcohlic beverages which is called energy drinks among the youths. Energy drinks are lightly carbonated non-alcoholic beverages that are aimed to provide the user a spurt of energy through supplying with a number of energy enhancing components , particularly the caffeine (Alford et al., 2001). Globally, there are an increase in the consumption of energy drinks, due to the believe that the energy drinks can stimulate faster responses, elevate the physical strengths, and weakening sleep needs and keeping the body in a state of attentive higher mental concentration of the organism (Finnegan, 2003 and Ferreira et al., 2004). Most of energy drinks consumers are young adults, physically active subpopulations, teenagers, athletes, where these drinks are sold as natural substitutes that upsurge fun and improve physical and cognitive performance such as concentration, attention, and alertness (Kaminer, 2010).

In the markets there are different kinds sold with different trade names of energy drinks such as Power horse, Monster, Boom Boom, Burn, AMP Energy and Red Bull. In Egypt, Red Bull is one of the most frequently consumed energy drink. The manufacturing company's entitlement that consumption of Red Bull will afford the customer with high energy and enhanced performance, both physically and mentally (Frances et al., 2010). The components of energy drinks generally comprising in its contents the following,simple sugars (fructose ,glucose), caffeine, amino acids (creatine, carnitine, taurine,), plant stimulants (ephedrine, yerba mate, guarana), herbs (e.g. ginkgobiloba, ginseng), vitamins B complex and a naturally occurring glucose metabolite (glucuronolactone, inositol, maltodextrin) (Alford et al., 2001 and Malinauskas et al., 2007).

Due to the vast array of components added in the constituents of energy drinks, it is expected that theundesirable effects will be more severe than other beverages which contain only the caffeine (Gunja and Brown, 2012). A study has shown that the caffeine levels in

EDs are between 50 and 505 mg/ can, which are much higher than the caffeine content of one can of Coke (34 mg) (Burrows et al., 2013). Several warnings have been issued regarding the potential adverse effects of energy drinks including hepatotoxicity, nephrotoxicity, neurologic complications, alterations in the cardiovascular system and changes in the structure and function of secretory gland (Khayyat et al., 2014). Due to the wide spread of drinking energy drinks among youths , the health hazards effects accompanied drinking of undefined doses or over doses for long time will be more harmful , which may be returned to its contents from caffeine or caffeine-like effect contents. The toxicity of over dose symptoms of caffeine are appear in vascular and GIT systems, as anxiety, palpitations, high systolic pressure, hypertension, convulsions, vomiting, nausea, , metabolic acidosis andhypocalcemia(WHO, 2005), and in scarce cases, death (Kerrigan and Lindsey, 2005 and Starling, 2011).

Traditional medications by using medicinal plants and herbs are widely used by the public's for treating some diseases due to simplicity in application, cheap or it is availability and low side effects than synthetic drugs .These medicinal plants contains several ingredients possessing antibacterial, anti-inflammatory and antioxidant activities (Hajhashemi et al., 2004). One of these herbs is the black seed or *Nigella sativa*, is one of the family Ranunculaceae, which is growing in North Africa, southern Europe and Southwest Asia (El-Tahir & Bakeet , 2006 and Aljabre et al., 2015). In many countries around the world such as Arabian countries, India, Iran and Europe, Black sees and their oil are occasionally used in treating any illnesslikefever, hypertension, asthma, dizziness, diabetes, tumor, inflammation, bronchitis, cough, flu, headache, gastrointestinal disturbances, eczema, painful menstruation in women and impotence in males(Ali and Blunden, 2003).

The pharmacological action of Nigella sativa may be attributed to its contents of essential oils and other constituents, where it contains Thymoquinone (30- 48%), P-cymene (7-15%), Carvacrol (6-12%), 4-terpineol (2 -7%), Tanethole (1-4%) and Sesquiterpene (1-8%). (Burits & Bucar, 2000 and Padhye et al., 2008). Darakhshan et al., 2015, reported that Thymoquinone (TQ) has different pharmacological effects such as anti-inflammatory, antimicrobial, antioxidant, antidiabetic, anti-tumor and immunomodulatory effects. The nephroprotective activity of *N. sativa* has been reported previously by many authors in both clinical (El-Shamy et al., 2011) and experimental (Yaman & Balikci, 2010 and Hadjzadeh et al., 2012) studies. The antioxidant and anti-inflammatory activities of *N. sativa* are considered the key factors accountable for its renoprotective and hepatoprotective effects (Salem, 2005).

Because of the wide consumption of energy drinks between the peoples, it is important to study the harmful impact on health and investigating their potential side effects. So, the target from the current work was to throw the light on the histological and immunohistochemical alterations in renal cortex of adult male albino rats induced by ingestion of Red Bull energy drink and to assess the potential protective role of *Nigella sativa* oil (NSO) against the renal injury induced by the energy drink.

Materials and Methods

Chemicals

Energy drink

The brand of energy drinkused in the present study was “Red Bull” (RB). Red Bull cans (250 ml) were purchased from a local market in Shibin Elkom city (Egypt).

Nigella sativa oil (NSO)

NSO was purchased from the Kahira Pharmaceutical and Chemical Industries Co. (Cairo, Egypt).

Animals

Forty adult male albino rats weighing 180–200 g were used in this study. Strict care and hygiene were maintained to keep them in normal and healthy conditions. they received a balanced diet with free access to water. All animal procedures were performed according to approved protocols and in accordance with the recommendations for the proper care and use of laboratory animals.

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

Group I (control group): Animals of this group were left without any treatment.
Group II (NSO-treated group): Animals of this group were givenNigella sativa oil orally by a gastric tube at a dose of 1 ml/Kg b.wt. daily for 4 weeks.
Group III (RB-treated group): Animals of this group were given Red Bull orally by a gastric tube at a dose of 1.5 ml/100g b.wt. daily for 4 weeks.
Group IV (RB and NSO-treated group): Animals of this group were given orally Red Bull at a dose of 1.5 ml/100g b.wt. + Nigella sativa oil at a dose of 1 ml/Kg b.wt. daily for 4 weeks. The dose of Red Bull was estimated on the basis of a previous study (Khayyat et al.,2014 a) to mimic estimated high human consumption level of 1050 ml (about four cans). The dose of *Nigella sativa* oil was estimated on the basis of a previous study (Mohammed et al., 2014).

Methods

24 h after the last dose, blood samples were collected to measure the levels of serum creatinine and urea of all groups for statistical analysis, then the animals were scarified by cervical decapitation. Kidney samples were obtained and cleaned by normal saline and

then fixed in 10% formal saline and processed in the usual way to obtain the ordinary paraffin blocks. Sections of 4µm thick were cut and subjected to the following studies.

(1) Histological study: using Hematoxylin & eosin (H&E) stain for routine histological examination and Masson trichrome for detection of collagen **(Kiernan, 2015)**.

(2) Immunohistochemical study:

Four µm thick sections were deparaffinised, rehydrated, and endogenous peroxidase activity was blocked with 3% H2O2 in methanol. Sections were pre-treated in citrate buffer (pH 6.0), and were incubated with rabbit polyclonal antibodies against cyclooxygenase-2 (COX-2) and caspase-3 (Thermo Scientific, USA, dilution 1:1000). The sections were incubated with biotinylated goat anti-polyvalent, then with streptavidin peroxidase and finally with diaminobenzedine as chromogen. Slides were counterstained with hematoxylin, and were examined under light microscope.

Morphometric study

The diameters of both proximal convoluted tubules and distal convoluted tubules were measured using a digital image analysis system (image J software Open source, contributors worldwide) for quantitative histomorphometry. Measurements were performed with ten non overlapping fields from five H&E-stained sections belonging to five animals (at magnification 400) from each group.

Statistical analysis

All data (data for serum creatinine, serum urea and the diameters of both proximal & distal convoluted tubules) were analyzed statistically using SPSS, version 22 (SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean ± SD and analyzed by using one-way analysis of variance test followed by post-hoc test for comparison between all groups. Differences were regarded as non significant if *P* values were > 0.05, significant if *P* values were p < 0.05 and highly significant if *P* values were < 0.01.

Results

Biochemical results

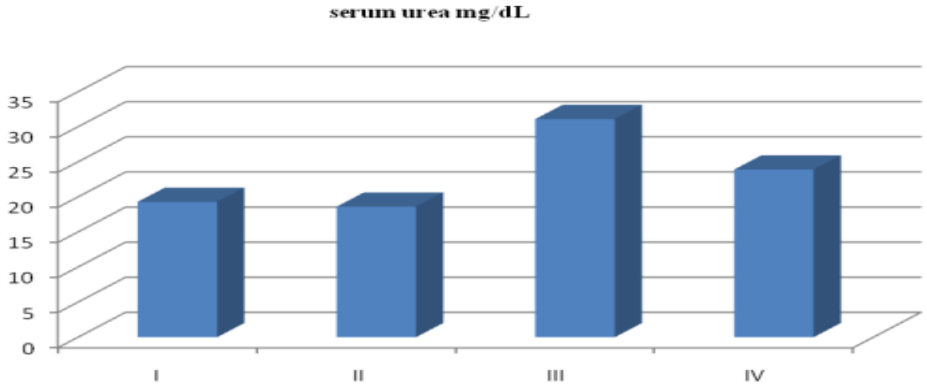
Serum urea and creatinine levels

The serum levels of urea and creatinine in the NSO-treated group (group II) wasnon-significantly differ(P>0.05)than that of control rats (group I). Oral administration of Red Bull for four weeks (group III) elevated significantly (P<0.01) the serum levels of urea and creatinine as matched with the control and NSO-treated groups (group I & II). However, the serum levels of urea and creatinine in the RB and NSO-treated group (group IV) was significantly declined (P<0.01) as matched with the RB-treated group [Table 1 and Histograms 1&2].

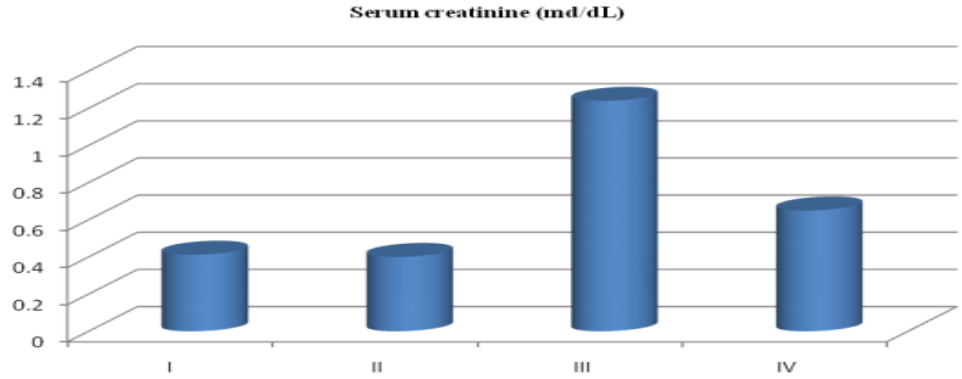
Table 1. Levels of serum urea and creatinine in the control and experimental groups

	Group I	Group II	Group III	Group IV	P.value
Serum urea (mg/dL) Mean ± SD	19.3±1.68	18.6±1.95	31.1±2.63	23.9±2.13	(P1>0.05) * (P2<0.01) *** (P3<0.01) *** (P4<0.01) ***
Serum creatinine (mg/dL) Mean ± SD	0.41±0.03	0.40±0.02	1.24±0.11	0.65±0.05	(P1>0.05) * (P2<0.01) *** (P3<0.01) *** (P4<0.01) ***

P1: Group I V Group II; P2: Group I V Group III; P3:Group II V Group III; P4: Group III V Group IV
*Non significant * (P> 0.05) ; Significant** (P<0.05) ; Highly significant*** (P<0.01)*



Histogram 1. Levels of serum urea in the control and experimental groups



Histogram 2. Levels of serum creatinine in the control and experimental groups.

Histological results

Haematoxylin and Eosin stain

By using H&E-stained slides of the control group (group I) showing normal structure after examined histologically.It showed that the cortex of the kidney was composed of renal corpuscles, proximal and distal convoluted tubules (DCT). The renal corpuscle was composed of glomerulus (a tuft of capillaries) bordered by Bowman's capsule. The Bowman's capsule was composed of an inner visceral layer (had modified epithelial cells known as podocytes) and outer parietal layer (lined by simple squamous epithelium) which were separated by Bowman's space (urinary space). . The proximal convoluted tubules (PCT) had narrow lumena and lined by a single layer of pyramidal cells. The pyramidal cells contain rounded basal nuclei and an acidophilic granular cytoplasm, indistinct cell boundaries, and an apical brush border. The distal convoluted tubules exhibit a wide lumena and lined by cubical cells that lacked a brush border. Their cells had central rounded nuclei and a faint acidophilic cytoplasm (Fig. 1).The NSO-treated group(group II) showed a picture comparable to that of the control rats (Fig. 2). The sections fromRB-treated group (Gr. III) revealed many histopathological changes as compared to control group. The glomeruli showed variable degrees of affection. Some glomeruli showed segmentation of their tufts of capillaries which appeared dilated and congested. Other glomeruli showed marked degeneration with widening of Bowman's space. There was marked distortion and dilatation of renal tubules with cytoplasmic vacuoles and pyknotic nuclei in their lining cells. Some tubules showed loss of their nuclei. Other tubules revealed sloughing necrotic cells inside their lumina. Empty spaces were observed within the renal cortex. There were dilated congested blood vessels and areas of hemorrhage within the renal cortex. Also, massive cellular infiltration and acidophilic hyaline material were observed within the renal cortex (Fig. 3, 4, 5, 6 & 7). Sections from RB and NSO-treated group (group IV) revealed nearly normal histological structure of the renal cortex except for few tubules with few cytoplasmic vacuoles in their lining cells. Also, the glomeruli appeared nearly normal except for slight congestion of glomerular capillaries and widening of Bowman's space. Minimal cellular infiltration was observed in between tubules (Fig. 8).

Masson's trichrome

Masson's trichrome-stained sections of the renal cortex of the control rats revealed slightquantities of collagen fibers bordering Bowman's capsules, basement membrane of renal tubules and glomerular capillaries (Fig. 9). The NSO-treated ratsnot showing a significant alteration as matched with control group rats. The sections fromRB-treated group (group III) revealed marked growth of collagen fibers round the congested blood vessels, Bowman's capsules and basement membrane of the renal tubules (Figs. 10 &11), while sections from RB and NSO-treated group(group IV) revealed moderate amount of collagen fibers around basement membrane of the renal tubules and glomerular capillaries (Fig. 12).

Immunohistochemical results

- *Immunohistochemical staining forcox-2*

Immunohistochemical staining of sections of the renal cortex from the control and NSO-treated groups(groups I and II) revealed weak to mild positive immune-reactivity for cox-2 in the renal tubules and the renal corpuscles (Fig. 13 & 14).In RB-treated group (group III), strong to very strong positive immune-reactivity forcox-2 was found in the renal tubules, the renal corpuscles, and at the site of cellular infiltration (Fig. 15 & 16), while sections from RB and NSO-treated group(group IV) revealed moderate positive immunoreactivity for cox-2 in the renal tubules and the renal corpuscles (Fig. 17).

- *Immunohistochemical staining for caspase-3*

Immunohistochemical staining of sections of the renal cortex from the control and NSO-treated groups(groups I and II) revealed negative cytoplasmic immune-reactivity for caspase-3 in some renal tubules. Other renal tubules and the renal corpuscles showed minimal positive cytoplasmic immune-reactivity for caspase-3 (Fig. 18 & 19).In RB-treated group (group III), very strong positive immune-reactivity for Caspase-3 was found in the renal tubules and the renal corpuscles (Fig. 20), while sections from RB and NSO-treated group (group IV) revealed moderate positive immune-reactivity for Caspase-3 in the renal tubules and the renal corpuscles (Fig. 21).

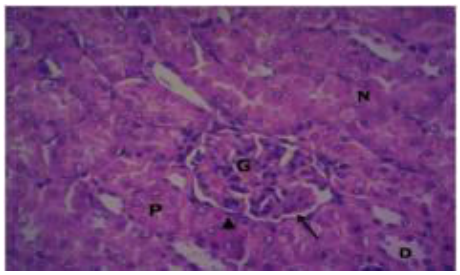


Fig 1: A photomicrograph of a section of the renal cortex of the control group (I) showing normal architecture of the renal corpuscle which is composed of the glomerulus (G) and surrounded by Bowman's space (arrow). The parietal layer of Bowman's capsule is lined by simple squamous epithelium (arrow head). Proximal convoluted tubules (P) are lined by pyramidal cells with a narrow lumen. Distal convoluted tubules (D) are lined by cubical cells with a wider lumen. Notice the vesicular nuclei (N) of the lining cells of tubules. **H&E × 400**

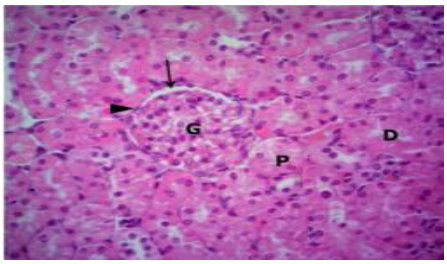


Fig 2: A photomicrograph of a section of the renal cortex of NSO-treated group (II) showing a picture similar to control. The renal corpuscle which is composed of the glomerulus (G) surrounded by Bowman's space (arrow). The parietal layer of Bowman's capsule is lined by simple squamous epithelium (arrow head). Proximal convoluted tubules (P) are lined by pyramidal cells with a narrow lumen. Distal convoluted tubules (D) are lined by cubical cells with a wider lumen. **H&E × 400**

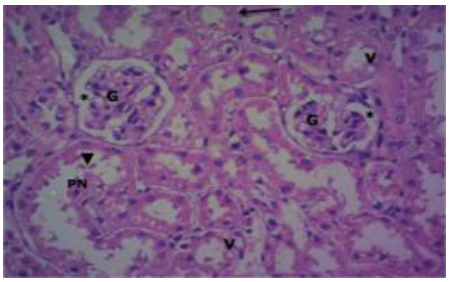


Fig 3: A photomicrograph of a section of the renal cortex of RB-treated group (III) showing glomeruli (G) with segmentation of their tufts of capillaries which appear dilated and congested. widened Bowman's space is observed (*). There is marked distortion and dilatation of renal tubules with cytoplasmic vacuoles (V) and pyknotic nuclei in their lining cells (PN). Some tubules show loss of their nuclei (arrow). Other tubules show epithelial shedding inside their lumina (arrow head). **H&E × 400**

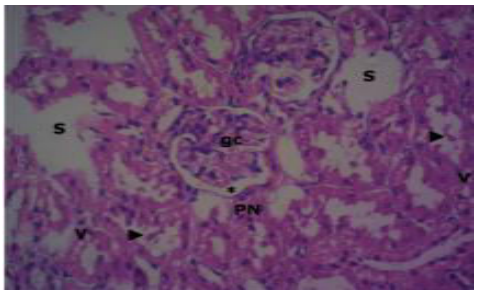


Fig 4: A photomicrograph of a section of the renal cortex of RB-treated group (III) showing empty spaces (S) within the renal cortex. There is marked distortion of renal tubules with cytoplasmic vacuoles (V) and pyknotic shrunken nuclei (PN) in their lining cells. The dilated lumens of tubules contain sloughing necrotic cells (arrow heads). Dilated congested glomerular capillaries (gc) and widened Bowman's space (*) are observed. **H&E × 400**

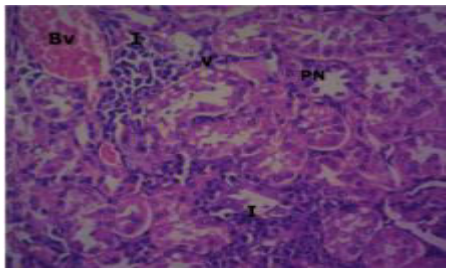


Fig 5: A photomicrograph of a section of the renal cortex of RB-treated group (III) showing massive cellular infiltration (I) within the renal cortex. Dilated congested blood vessel (Bv) is observed. Notice the distortion of renal tubules with cytoplasmic vacuoles (V) and pyknotic shrunken nuclei (PN) in their lining cells. **H&E × 400**

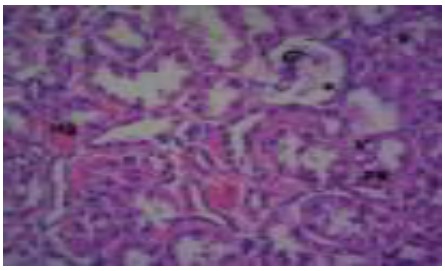


Fig 6: A photomicrograph of a section of the renal cortex of RB-treated group (III) showing markedly degenerated glomerulus (G) with widening of Bowman's space (*). Areas of hemorrhage (Hg) are observed in between the tubules which show cytoplasmic vacuoles (V) and pyknotic shrunken nuclei (PN) in their lining cells. **H&E × 400**

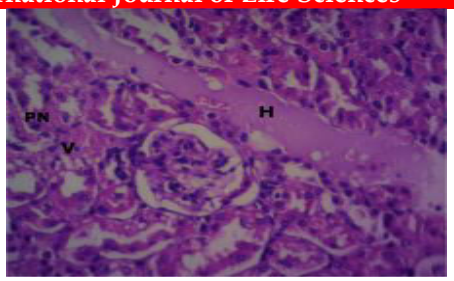


Fig 7: A photomicrograph of a section of the renal cortex of RB-treated group (III) showing acidophilic hyaline material (H) in between the tubules which show cytoplasmic vacuoles (V) and pyknotic shrunken nuclei (PN) in their lining cells. **H&E × 400**

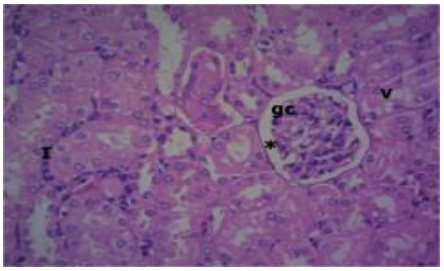


Fig 8: A photomicrograph of a section of the renal cortex of RB and NSO-treated group (IV) showing normal appearance of renal tubules except for few tubules with few vacuoles (V). The glomerulus appears nearly normal except for slight congestion of glomerular capillaries (gc) and widening of Bowman's space (*). Notice minimal cellular infiltration (I) in-between tubules. **H&E × 400**

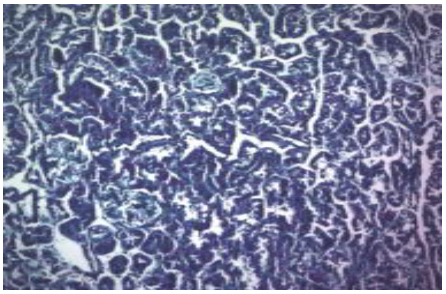


Fig 9: A photomicrograph of a section of the renal cortex of the control group (I) showing minimal amounts of collagenous fibers around Bowman's capsule, glomerular capillaries and basement membrane of renal tubules. **M.T. x 200**

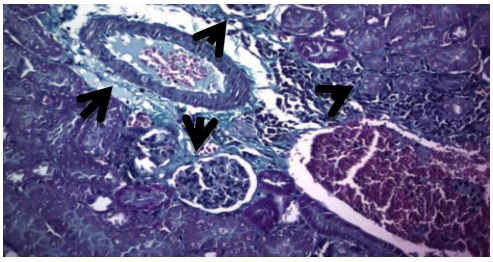


Fig 10: A photomicrograph of a section of the renal cortex of RB-treated group (III) showing marked increase of collagen fibers around a congested blood vessel, basement membrane of the renal tubules and Bowman's capsules (arrows). **M. T. x 200**

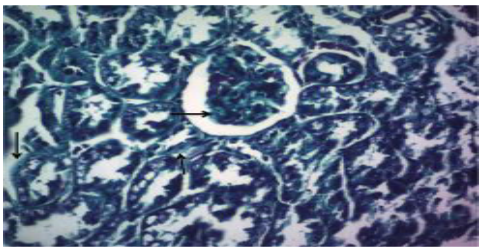


Fig 11: A photomicrograph of a section of the renal cortex of RB-treated group (III) showing marked increase of collagen fibers around basement membrane of the renal tubules and glomerular capillaries (arrows). **M. T. x 400**

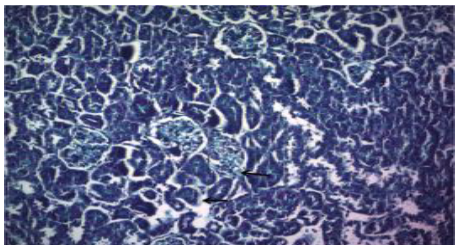


Fig 12: A photomicrograph of a section of the renal cortex of RB and NSO-treated group (IV) showing moderate amount of collagen fibers around basement membrane of the renal tubules and glomerular capillaries (arrows). **M.T. x 200**

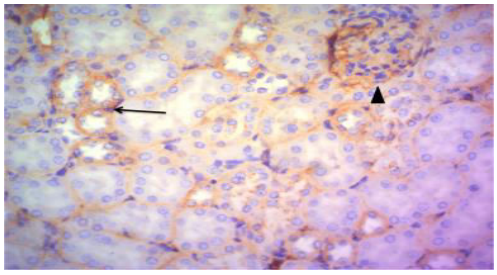


Fig 13: A photomicrograph of a section of the renal cortex of the control group (I) showing weak to mild positive immunoreactivity for cox-2 in the renal tubules (arrow) and the renal corpuscle (arrow head). **cox-2x400**

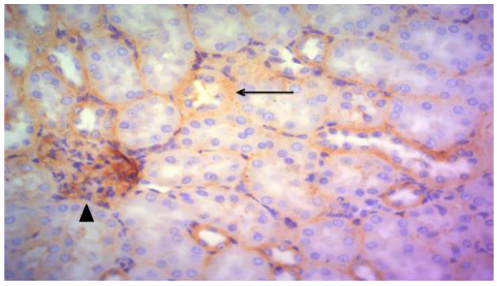


Fig 14: A photomicrograph of a section of the renal cortex of NSO-treated group (II) showing weak to mild positive immunoreactivity for cox-2 in the renal tubules (arrow) and the renal corpuscle (arrow head).**cox-2 x 400**

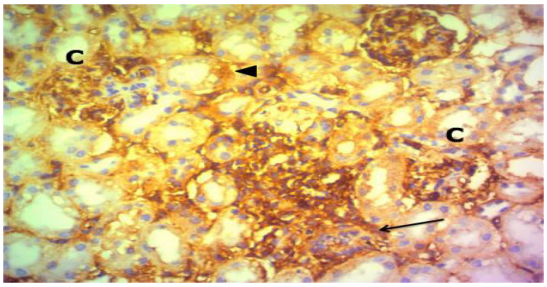


Fig 15: A photomicrograph of a section of the renal cortex of RB-treated group (III) showing strong positive immunoreactivity for cox-2 in the renal tubules (arrow head), the renal corpuscle (C), and at the site of cellular infiltration (arrow).**cox-2x400**

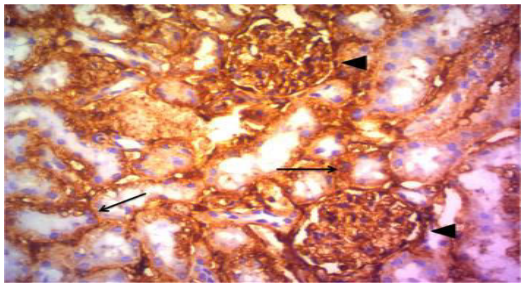


Fig 16: A photomicrograph of a section of the renal cortex of RB-treated group (III) showing very strong positive immunoreactivity for cox-2 in the renal tubules (arrows) and the renal corpuscle (arrow heads).**cox-2x400**

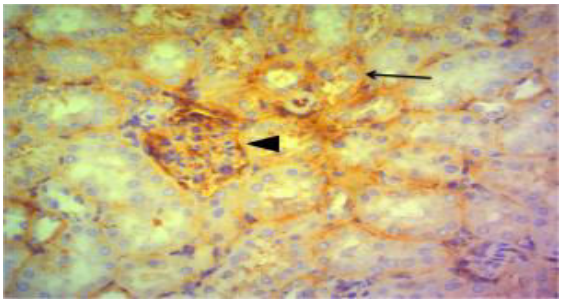


Fig 17: A photomicrograph of a section of the renal cortex of RB and NSO-treated group (IV) showing moderate positive immunoreactivity for cox-2 in the renal tubules (arrow) and the renal corpuscle (arrow head).**cox-2x400**

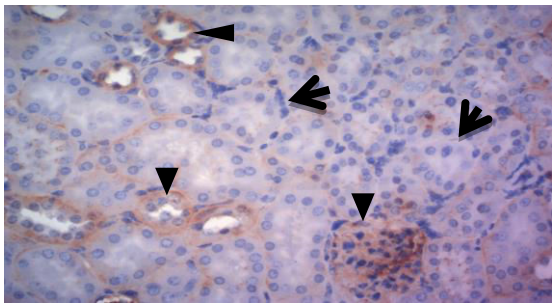


Fig 18: A photomicrograph of a section of the renal cortex of the control group (I) showing negative cytoplasmic immunoreactivity for caspase-3 in some renal tubules (arrows). Other renal tubules and the renal corpuscle show weak positive cytoplasmic immunoreactivity for caspase-3 (arrow heads). **Caspase-3 x400**

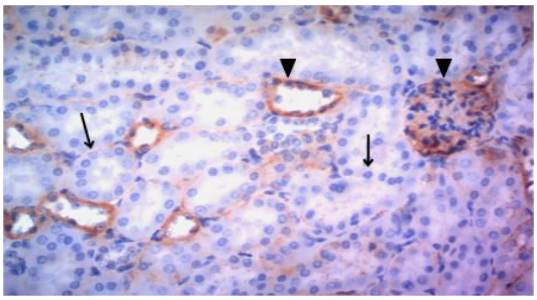


Fig 19: A photomicrograph of a section of the renal cortex of NSO group (II) showing negative cytoplasmic immunoreactivity for caspase-3 in some renal tubules (arrows). Other renal tubules and the renal corpuscle show weak positive cytoplasmic immunoreactivity for caspase-3 (arrow heads). **Caspase-3 x400**

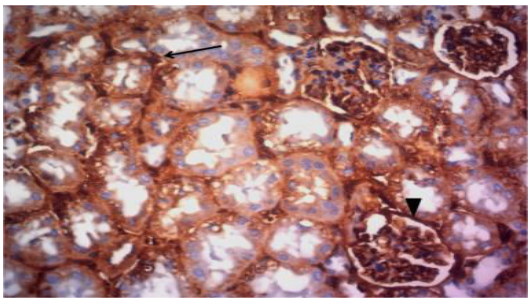


Fig 20: A photomicrograph of a section of the renal cortex of RB-treated group (III) showing very strong positive cytoplasmic immunoreactivity for caspase-3 in the renal tubules (arrow) and the renal corpuscles (arrow head). **Caspase-3 x400**

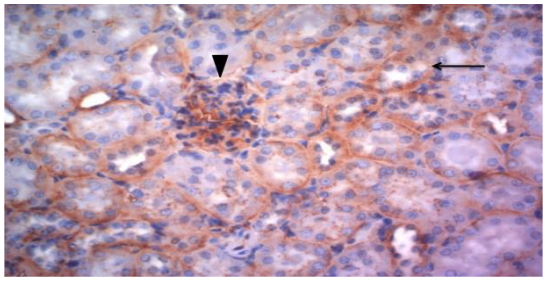


Fig 21: A photomicrograph of a section of the renal cortex of RB and NSO-treated group (IV) showing moderate positive cytoplasmic immunoreactivity for caspase-3 in the renal tubules (arrow) and the renal corpuscle (arrow head). **Caspase-3 x400**

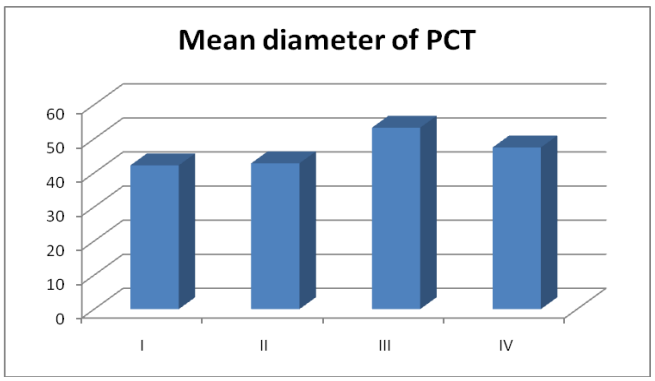
Morphometric results

The diameters of the proximal and distal convoluted tubules of the NSO-treated group (group II) not showing a significant variation(P>0.05) in comparison with the control rats (group I). Whereas, a significant increase was recorded in the diameters of proximal and distal convoluted tubules, in the RB-treated rats (Group III) than the control rats. Also, there was a significant decrease in the mentioned criteria in the RB and NSO-treated rats (Gr. IV) as compared to the RB-treated rats (Gr. III) as shown in table 2 and histograms 3&4].

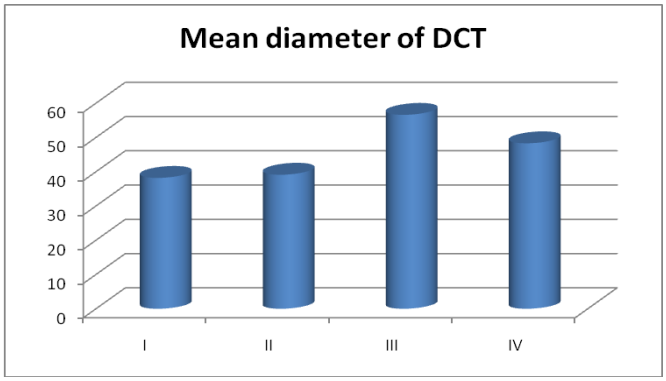
Table 2. The diameters of the proximal and the distal convoluted tubules in the control and experimental groups

	Group I	Group II	Group III	Group IV	P.value
The diameter of the proximal convoluted tubules Mean ± SD	42.2±2.76	42.8±2.30	53.24±3.79	47.44±2.70	(P1>0.05) * (P2<0.01) ** (P3<0.01) ** (P4<0.01) **
The diameter of the distal convoluted tubules Mean ± SD	38.2±2.86	39.12±2.47	56.6±3.21	48.3±2.49	(P1>0.05) * (P2<0.01) *** (P3<0.01) *** (P4<0.01) **

P1: Group I V Group II; P2: Group I V Group III; P3:Group II V Group III; P4: Group III V Group IV
*Non significant * (P> 0.05) ; Significant** (P<0.05) ; Highly significant*** (P<0.01)*



Histogram 3. The diameters of the proximal convoluted tubules in the control and experimental groups.



Histogram 4. The diameters of the distal convoluted tubules in the control and experimental groups.

Discussion

In the currentinvestigation, the oral administration of Red Bull energy drink to rats for 4 weeks resulted in renal damage. This was evident in the elevation of the serum levels of urea and creatinine in RB-treated group. This was in harmony with the result of Khayyat et al., 2014b who found that the Red Bull energy drink promptedrises of renal biomarkers creatinine, uric acid and urea. Also, Ugwuja, 2014found drinking of Bullet alone (energy drink) with or without alcoholselevated the kidney function parameter tests (creatinine, uric acid and urea) in the serum of rats. He stated that, both creatinine and urea are metabolic protein byproduct, which are noticed in affected kidney and appear in the circulation. Moreover, Akande and Banjoko, 2011 reported that there is an increase in urea concentration in rats treated with power horse. The effect of energy drinks on renal function could be attributed to caffeine which is one of the main ingredients of energy drinks. This was confirmed by several authors (Portoles et al., 1985, Tofovic et al., 2002; Tofovic et al., 2007 and Abd El-Moneim et al., 2009)who showed that caffeine can elevate creatinine and urea concentration in the blood serum .The mode of action of caffeine to elevate kidney function criteria may be via inhibition of A2A adenosine receptors, which hastens the expansion of inflammatory reactions in the interstitial, enhances proteinuria and alters renal histology and physiology as has been reported by Khayyat et al., 2014 b.

Moreover, caffeine induces oxidative stress in tissues as has been previously reported by Ekaluo et al., 2016 who stated that Caffeine causes a decrease in antioxidant defense system (SOD, GPx and CAT), followed by elevated in the free radical activities and subsequentlyleading to oxidative stress. Previous studies suggest that Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) are the foremost injurious ROS scavengers in vital organs (Fujii et al., 2003). In addition, Caffeine significantly increased concentration of Malondialdehyde (MDA) as a marker for elevated activity of lipid peroxidation which usually accompanied

oxidative cellular damage, therefore, an increase in the level of MDA cab be used as a biomarker for presence of oxidative stresses (Ekaluo et al., 2016).

In the current work, drinking of Red Bull as one of energy drinks had a sever bad effect on normal histological structure of the renal cortex of rats. H&E-stained sections of renal cortex from RB-treated grouprevealed many histological changes including glomeruli degeneration with widening of Bowman's space, segmentation of glomerular capillaries which appeared dilated and congested, marked distortion and significant dilatation of renal tubules with presence of cytoplasmic vacuoles and pyknotic nuclei in their lining epithelial cells, sloughing necrotic cells inside the lumina of renal tubules, empty spaces within the renal cortex, dilated congested blood vessels and areas of hemorrhage within the renal cortex, massive cellular infiltration and acidophilic hyaline material within the renal cortex. These data are in harmony with the finding of Khayyat et al., 2014 b in their study on impact of some energy drinks on the kidney structure and they detected necrosis of renal tubules and glomeruli, lobulated glomerular capillaries, intertubular hemorrhage as well as inflammation areas among the tubules, cavitation areas and dilatation of renal tubules.

Shide and Chandrasekaran, 2011: postulated that drinking of large dosage of energy drinks may be responsible for inducing of kidney injuries due to effect of different ingredients in the drink. This was explained by the fact that the renal tubules are affected by the excreted or cleared toxic chemicals during their elimination or withdrawal (Kukner et al., 2007). Shimizu et al.(1996) explained the observed necrosis of most renal tubules and glomeruli which may be attributed to the depletion of ATP, which lastly ended with cell apoptosis. The intertubular inflammation and hemorrhage zones which were detected in the current work might be attributed to microcirculatory disorders that arise from the caffeine are found in the energy drinks (Khayyat et al., 2014 b). Mubarak, 2012 recorded hemorrhage areas in the submandibular salivary glands in rats treated with Red bull.

Masson's trichrome-stained sections of the cortex of kidney of RB-treated ratsrevealed marked elevation of collagen fibers bounded congested blood vessel, Bowman's capsules and basement membrane of the renal tubules indicating fibrosis. This confirmed the findings of Mubarak, 2012 in her study on influence of Red Bull energy drink on salivary glands, she detected sever fibrosis of the connective tissue septa with thickening of the connective tissue capsule. Mubarak, 2012, attributed occurrence of fibrosis to the toxic influence of caffeine to the wound healing property due to elevated depositions of fibrin on the underlying connective tissue. Cyclooxygenase-2 (COX-2) is the inducible key enzyme for the prostaglandin (PG) biosynthetic pathway. Prostaglandins (PGs) synthesized by COX-2 having an important role in the processes of inflammation and appearance of inflammatory symptoms such as pain ,fever and dysfunction(Smith, 1996).

Adegboyega and Ololade, 2004 reported that in the cortex of normal kidneys, COX-2 was expressed in the endothelial cells of glomeruli, arterioles and arteries, in the ascending limb of the loop of Henle (medullary rays) and macula densa in the form of obviously elevated numbers of mitogenic, inflammatory, and physical stimuli (Komers et al., 2001). In the present study, COX-2 immunoexpression in the renal cortex was markedly increased following Red Bull consumption reflecting the presence of inflammation. This was in agreement with the result of Ahmed, 2016 who detected strong COX-2-immunostaining in cytosol of almost all interstitial cells of testis of RB-treated rats.

In our study, the expression of the cytoplasmic immunoreactivity caspase-3 was highly positive in the renal tubules and glomeruli of RB-treated group. This was in harmony with the result of Ayuob and ElBeshbeishy, 2016 who reported that the administration of the power horse, one of the energy drinks to rats resulted in a strong cytoplasmic caspase-3 reaction in pancreas and stomach. In this study, it was observed that Nigella sativa oil (NSO) succeeded to some extent to protect the kidney from the adverse side action of the RB-induced histopathological and biochemical alterations. The RB and NSO-treated group revealedhighly significant decrease in serum levels of urea and creatinine, more preserved histological structure, less collagen fibers depositionas compared to the RB-treated group. This was in agreement with Mohammed et al., 2014 who stated that administration of NSOdecreased significantly the toxic effects of cadmium-induced injury in the kidney tissues, and can translated by dropping in both urea and creatinine in the serum following NSO treatment. Also, Mohammed et al.,2014 recorded that treatment with NSOresulted in elevation in the level of GSHwhile, MDA level was declined , in addition to improvement of or reduction in DNA fragmentation in the kidney tissue in coordinate with Sayed-Ahmed and Nagi, (2007) stated that treatment with thymoquinone (TQ) a derivative from NSOlead to a significant elevation in GSH and decrease in creatinine and blood urea nitrogen due to potent anti-oxidant activity, against gentamycin-induced nephrotoxicity. Moreover, Mohammed et al.,2014 stated that giving of NSO with cadmium produce a significant protective action as it ameliorate the oxidative stresses in kidney tissues and resumedCAT and SOD levels nearly to the normal status in comparison with cadmium-treated rats.

In addition, Fouad et al., 2016 reported that TQ, exerts obvious antioxidant action , suppresses lipid peroxidation, and scavenges reactive oxygen radicals. In addition, the RB and NSO-treated rats revealed reduction in the COX-2 expression in the glomeruli and the renal tubules as compared to the RB-treated group. This could be attributed to anti-inflammatory effect of TQ (a compound derived from NS) as has been previously reported by Fouad et al., 2016 who stated that TQ reduces the release of inflammatory cytokines, and inhibits COX-2 in the kidney thus decreasing the production of inflammatory prostaglandins. Moreover, NSO could specifically reduce cytoplasmic immunoreactivity for caspase-3 in the RB and NSO-treated groupas compared to the RB-treated group. This confirmed the results of Fouad et al., 2016 who reported that TQ decreased the cadmium-induced expression of caspase-

3, an executioner of cell apoptosis in the kidney tissue. This was in agreement with previous studies, which showed that TQ provided a significant anti-apoptotic effect by inhibiting caspase-3 activity (Fouad and Jresat, 2015). Therefore, it could be stated that NSO protected against RB-induced renal cell apoptosis. The reduced caspase-3 activity observed with NSO treatment may be due to its antioxidant, and anti-inflammatory activities.

Conclusion

The present findings demonstrated that the energy drink "Red Bull" had toxic effect on the kidney. Nigella sativa oil had marked protective effect against RB-induced renal damage in rats when co-administrated with RB. The protective effect of NSO is via its antioxidant and anti-inflammatory properties. So, the use of NSO with RB is recommended.

References

Abd El-Moneim M, Afify M, AbouElalla F, Hassan A. Short and long term effect of caffeine on liver, kidney as well as glucose, insulin, triglycerides and cholesterol on normal rats. Australian Journal of Basic and Applied Sciences 2009;3(4):3259-65.

Adegboyega PA, Ololade O. Immunohistochemical expression of cyclooxygenase-2 in normal kidneys. ApplImmunohistochemMolMorphol. 2004 Mar;12(1):71-4.

Ahmed, AM. Expression of transcription factor NF-KAPPA B/P65 and cyclooxygenase-2 (COX-2) in testicular damage induced by Red Bull energy drink in rat. International Journal of Advanced and Applied Sciences, 3(10) 2016, Pages: 49-56.

Akande IS. and Banjoko OA. (2011). Assessment of Biochemical Effect of "Power Horse" Energy Drink on Hepatic, Renal and Histological Functions in Sprague Dawley Rats. Annual Review & Research in Biology. 1(3): 45-56.

Alford, C., Cox, H., Wescott, R. (2001). The effects of Red Bull energy drink on human performance and mood. Amino Acids, 21, 139-150.

Ali BH, Blunden G. Pharmacological and toxicological properties of Nigella sativa. Phytother Res. 2003 Apr;17(4):299-305.

Aljabre SH, Alakloby OM, Randhawa MA: Dermatological effects of Nigella sativa. J DermatolDermatolSurg 2015; 19: 92–98.

Ayuob N, ElBeshbeishy R. Impact of an Energy Drink on the Structure of Stomach and Pancreas of Albino Rat: Can Omega-3 Provide a Protection? PLoS One 2016;11(2): e0149191.

Burits M, Bucar F. Antioxidant activity of Nigella sativa essential oil. Phytother Res. 2000 Aug;14(5):323-8.

Burrows T, Pursey K, Neve M, Stanwell P. What are the health implications associated with the consumption of energy drinks? A systematic review. Nutr Rev 2013; 71: 135–148.

Darakhshan S, Pour AB, Colagar AH and Sisakhtnezhad S. "Thymoquinone and its therapeutic potentials," Pharmacol. Res., vol. 95-96C, pp. 138-158, 2015.

Ekaluo UB, Uno UU, Edu NE, Ekpo PB, Etta SE. 2016. Effect of Trévo Dietary Supplement on Caffeine Induced Oxidative Stress in Albino Rat Models. The Pharmaceutical and Chemical Journal. 3(2):92-97.

El-Shamy KA, Mosa MM, El-Nabarawy SK, El Qattan GM. Effect of Nigella sativa tea in type 2 diabetic patients as regards glucose homeostasis, liver and kidney functions. J ApplSci Res 2011;7:2524-34.

El-Tahir KE, Bakeet DM: The black seed Nigella sativa linnaeus – a mine for multi cures: a plea for urgent clinical evaluation of its volatile oil. J Taibah UnivSci 2006; 1: 1–9.

Ferreira SE, Hartmann Quadros IM, Trindade AA, Takahashi S, Koyama RG, Souza-Formigoni ML. Can energy drinks reduce the depressor effect of ethanol? An experimental study in mice. PhysiolBehav. 2004;82(5):841–7.

Finnegan D. The health effects of stimulant drinks. Nutr Bull. 2003;28(2):147–55.

Fouad, A.A., I. Jresat, I. "Thymoquinone therapy abrogates toxic effect of cadmium on rat testes," Andrologia, vol. 47, pp. 417-426, 2015.

Fouad AA, Alwadaani HA and Jresat I. Protective Effect of Thymoquinone against Nephrotoxicity Induced by Cadmium in Rats. World Academy of Science, Engineering and Technology International Journal of Animal and Veterinary Sciences Vol:10, No:2, 2016.

Frances RR, Tyler DG, Narjes B, Nicole H, April M, et al. Effect of Red Bull energy drink on cardiovascular and renal function. Amino Acids. 2010; 38:1193–1200.

Fujii, J., Luchi, Y., Matsuki, S. & Ishii, T. Cooperative function of antioxidants and redox systems against oxidative stress in male reproductive tissue. Asian Journal of Andrology, 2003, 5:231-242.

Gunja N, Brown JA. Energy drinks: health risks and toxicity. Med J Aust. 2012; 196(1): 46–9.

Hadjzadeh MA, Keshavarzi Z, TabatabaeeYazdi SA, GhasemShirazi M, Rajaei Z, Khajavi Rad A. Effect of alcoholic extract of Nigella sativa on cisplatin-induced toxicity in rat. Iran J Kidney Dis 2012;6:99-104.

Hajhashemi V, Ghannadi A, Jafarabadi H. Black cumin seed essential oil, as a potent analgesic and anti-inflammatory drug. Phytother Res. 2004 Mar;18(3):195-9. doi: 10.1002/ptr.1390.

Kaminer Y. Problematic use of energy drinks by adolescents. Child AdolescPsychiatrClin N Am., 2010; 19(3):643-50.

Kerrigan S, Lindsey T. Fatal caffeine overdose: two case reports. Forensic Science International 2005;153(1):67-9.

Kiernan JA. Histological and histochemical methods; theory and practice. 5th ed Oxford, UK: Butterworth Heinemann; 2015. pp. 238–310.

Khayyat,L.I. , Essawy,A.E., Al Rawy,M.M., and Sorour,J.M. Comparative study on the effect of energy drinks on haematopoietic system in Wistar albino rats. Journal of Environmental Biology, Vol. 35, 883-891, September 2014 a.

Khayyat,L. , Essawy,A., Sorour,J. and Al Rawy,M. Impact of Some Energy Drinks on the Structure and Function of the Kidney in Wistar Albino Rats. Life Sci J 2014 b;11(10):1131-1138.
Komers R, Lindsley JN, Oyama TT, Schutzer WE, Reed JF, Mader SL, Anderson S. Immunohistochemical and functional correlations of renal cyclooxygenase-2 in experimental diabetes.J Clin Invest. 2001 Apr;107(7):889-98.
Kukner, A.; Colakoglu, N.; Kara, H.; Oner, H.; Ozogul, C.; Ozan, E. (2007). Ultrastructural changes in the kidney of rats with acute exposure to cadmium and effects of exogenous metallothionein. Biol.TraceElem.Res. 119,2,137-146.
Malinauskas, BM., Aeby, VG., Overton, RF., Carpenter-Aeby, T. and Barber-Heidal, K. (2007). A survey of energy drink consumption pattern among college students. Nutr. J. 6: 1-7.
Mohammed ET, Hashem KS and Abdel Rheim MR. Biochemical study on the impact of Nigella sativa and virgin olive oils on cadmium-induced nephrotoxicity and neurotoxicity in rats. J Invest Biochem. 2014; Vol 3, Issue 2.
Mubarak, R. (2012). Effect of red bull energy drink on Rat's submandibular salivary glands (Light and Electron microscopic study). J. Amer. Sci. 8(1):366-372.
Padhye S, Banerjee S, Ahmad A, Mohammad R, Sarkar FH. From here to eternity-the secret of Pharaohs: Therapeutic potential of black cumin seeds and beyond. Cancer Ther. 2008 Sep;6(b):495-510.
Portoles, M., Minana, M., Jorda, A. and Grisolia, S. (1985): Caffeine-induced changes in the composition of the free amino acid pool of the cerebral cortex. Neurochemical Research, 10(7): 887-895.
Salem ML. Immunomodulatory and therapeutic properties of the Nigella sativa L. seed. IntImmunopharmacol 2005;5:1749-70.
Sayed-Ahmed MM, Nagi MN. Thymoquinone supplementation prevents the development of gentamicin-induced acute renal toxicity in rats. ClinExpPharmacolPhysiol 2007;34:399-405.
Shide,E. and Chandrasekaran,V. (2011). The Effects of Energy Drinks on the Structure and Function of Epithelial Cells and Fibroblasts.www.stmarys-ca.edu..
Shimizu, S.; Eguchi, Y.; Kamiike, W.; Waguri, S.; Uchiyama, Y.; Matsuda, H.; Tsujimoto,Y. (1996). Retardation of chemical hypoxia-induced necrotic cell death by Bcl-2 and ICE inhibitors: Possible involvement of common mediators in apoptotic and necrotic signal transductions. Oncogene,12, 2045-2050.
Smith WL., Garavito RM., Dewitt DL. Prostaglandin endoperoxide H synthases (Cyclooxygenases) -1 and -2. J. Biol. Chem., 1996, 271, 33157.
Starling S. Energy drinks safety questioned by German agency. Avail-51 2011.
Tofovic, S., Kost, C., Jackson, E. and Bastacky, A. (2002): Long term caffeine consumption exacerbates renal failure in obese, diabetic, ZSF1(fa-fa) rats. Kidney International, 61: 1433-1444.
Tofovic, S., Salah, E., Jackson, E. and Melhem, M. (2007). Early renal injury induced by caffeine consumption in obese, diabetic ZSF1 rats. Renal Failure, 29, 891-902.
Ugwuja, E (2014): Biochemical Effects of Energy Drinks Alone or in Combination with Alcohol in Normal Albino Rats. Advanced Pharmaceutical Bulletin, 2014, 4(1), 69-74.
WHO. WHO Basic Analytical Toxicology 2005 [Available from: http://www.who.int/ipcs/publications/training_poisons/basic_analytical_tox/en/index.html.
Yaman I, Balikci E. Protective effects of Nigella sativa against gentamicin-induced nephrotoxicity in rats. ExpToxicolPathol 2010;62:183-90.