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Impact of Code Red energy drink on the functions and structure of the kidney of Wistar Albino rats: possible therapeutic effects of blueberry ethanolic extract

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Abstract

This study aimed to investigate effects of low and high doses of Code Red (CR) on rat's kidney and blueberry therapeutic actions. Thirty Wistar rats were divided into 5 groups, Group 1 (Control): negative control. Group 2 (Low dose Code Red, LDCR): received LDCR (0.72ml/100 grams/day) orally for 8 weeks. Group 3 (High dose Code Red, HDCR): received HDCR (1.44ml/100grams/day) for 8 weeks. Group 4 (LDCR+Blueberry): received LDCR for 8 weeks then blueberry (250 mg/kg/day) for 6 weeks. Group 5 (HDCR+Blueberry): received HDCR then blueberry. Blood samples were withdrawn at experimental end for kidney functions (urea, uric acid, creatinine), inflammatory markers (interleukin (IL)-6, IL-1β); oxidative stress [glutathione (GSH) malondialdehyde (MDA), superoxide dismutase (SOD)]. Kidney tissue examined at experimental end. Exposure to CR doses of 0.72 and 1.4 ml/100grams/day induced increases in creatinine, urea, uric acid versus control and in high versus low dose. IL-6, IL-1β increased in HDCR versus control. MDA increased, while SOD, GSH decreased by exposure to two CR doses versus controls. Parameters improved after blueberry administration. Structural alterations found in renal tissues after CR ingested that improved by blueberry. In conclusion, Code Red admiration led to renal destruction via inflammation and oxidative stress and improved by blueberry

Keywords: Blueberry, energy drink, histopathological changes, Kidney functions, rats.

1. Introduction

Energy drinks (EDs) are lightly carbonated non-alcoholic drinks that aimed to provide the user energy spurt through supplying with energy enhancing components, particularly caffeine (Tóth et al., 2020). First EDs appearance was in Europe and Asia in 1960 due to customer requirements for dietary supplements that give energy (Reissig et al., 2009). Saudi studies reported that more than half of consumers were young (13 - 35 years old), more than half consumed it for more than a year, and over 40% used to drink more than 3 cans per week (Elsoadaa et al., 2016). Globally, there are an increase in EDs consumption, due to believe that EDs can elevate physical strengths, stimulate faster responses, decreased sleep needs and keeping body in attentive with higher mental concentration state (Tóth et al., 2020).

In markets there are different EDs kinds sold with different names as Power Horse, Burn, Boom Boom, Monster, AMP Energy, Code Red and Red Bull. In Saudi Arabia, Code Red is one of most frequently consumed EDs. The EDs components generally comprising in its contents the following, caffeine, amino acids (taurine, creatine and carnitine), simple sugars (glucose and fructose), herbs (ginseng and ginkgobiloba), plant stimulants (yerba mate, ephedrine and guarana), vitamins B complex and a naturally occurring glucose metabolite (inositol, glucuronolactone and maltodextrin) (Tahmassebi and BaniHani, 2020). Due to vast array of components added in EDs constituents, it is expected that the undesirable effects will be more severe than other beverages that possess only caffeine. Caffeine levels in EDs are between 50 and 505 mg/can, which are much higher than caffeine content of one Coke can (34mg) (Burrows et al., 2013). A typical ED may have up to 300 mg of caffeine, from added caffeine and sources as guarana (Pennay et al., 2011). Caffeine amount of guarana (40–80mg/ gram of extract) is not always cleared in packaging. The adverse side effects of EDs had been reported. Khayyat et al. (2012) reported significant increase of liver enzymes in rats ingested EDs. Ugwuja (2014) found that EDs intake alone or with alcohol associated with significant alterations in total white blood cells count, plasma calcium, potassium, and triglycerides and liver and kidney functions.

Traditional medications by using herbs and medicinal plants are widely used by publics for treating some diseases due to simplicity in application, cheap or availability and low side effects than synthetic drugs. Blueberry (*Vaccinium spp.*) contains active antioxidant like polyphenols as anthocyanins, flavanols, phenolic acids and flavanols and anti-inflammatory as anthocyanins (Johnson et al., 2013). Blueberry supplementation attenuate pro-inflammatory cytokine formation in glial cells of rats brain (Lau et al., 2007), protect rat heart from ischemia (Ahmet et al., 2009), protect kidney from nephropathy (Elks et al., 2011), and thus exerting protective effects against hypertension-induced nephropathy in spontaneously hypertensive rats (Elks et al., 2011). So, blueberry supplementation in diet may have tissue-therapeutic actions in many diseases.

Because of the wide EDs consumption between peoples, it is important to study the harmful impact on health and investigating their potential side effects. So, the target of the current experimental study was to investigate the effects of ingestion of low and high doses of Code Red energy drink for eight weeks the kidney of adult male Wistar albino rats induced by ingestion of low and high doses of Code Red energy drink for eight weeks and to assess potential therapeutic role of oral administration of blueberry ethanolic extract for 6 weeks against renal injury induced by Code Red.

2. Material and methods

2.1. Materials

Blueberry powder was purchased from Xi'an Pincredit Bio-tech Co., LTD, Xian, China. Blueberry (300 g) was extracted three times with 700 mL of ethanol. This experiment was made each time for 3 hours in water bath at 90 °C. Then ethanol extract was filtered through Whatman No.1 filter paper. The extract was dried by evaporated for 20 hours and weighed. The samples were stored at -20 °C until used (Zheng et al., 2013). The energy drink brand name used in present study was "Code Red", J & Sharp Holdings Pty Ltd, USA. It was purchased from a local store in Jeddah, Saudi Arabia.

2.2. Animals

Thirty adult male Wistar Albino rats aged 90-120 days and weighed 80- 120 grams were utilized. The rats were obtained from animal house of King Fahd Medical Research Center, King Abdulaziz University (KAU), Jeddah, Saudi Arabia. The study protocols and ethics were approved by King Abdulaziz University Research Ethics Committee. All experimental steps were incompliance with roles made by National Health Institutes "Guide for Care and Use of Laboratory Animals" (Publication# 85-23). Rats were breed in standard cages at an ambient temperature of (21±1°C) with 12 hours light-dark cycle. The rats get free access to water and eat *ad libitum* on normal commercial chow diet.

2.3. Study design

The rats were housed under standard laboratory atmosphere for one week before experiment start for acclimatization. Any rats showed abnormal behaviors were excluded from the research. Then, rats were sorted into 5 groups (6 rats each), each group put into separate cages, as fallow: Group 1 (Control): negative control, rats intake distilled water and chow diet only. Group 2 (Low dose Code Red, LDCR): received low dose Code Red (0.72 ml/ 100grams/ day) orally for 8 weeks. Group 3 (High dose Code Red, HDCD): received high dose Code Red (1.44 ml/ 100grams/ day) orally for 8 weeks. Group 4 (LDCR + Blueberry): received small dose Code Red orally for 8 **weeks** then stopped and received blueberry (250 mg/kg/ day) extracts (Larrosa et al., 2010) for 6 weeks. Group 5 (HDCR +

blueberry): received high dose Code Red orally for 8 weeks then stopped and received blueberry (250mg/kg /day) extracts for 6 weeks. All treatments were given orally via gavage.

The LDCR (0.72 ml/ 100 grams/ day) was calculated according to the quantity permitted for an adult which is two cans of energy drink that is equal to 500 ml/ day and average body weight of adult man is 70 kilograms. High dose (1.44 ml/ 100 grams/ day) was calculated based on quantity allowed for adult that is four cans of energy drink that is equal to 1000 ml/ day and average body weight of an adult man is 70 kilograms (Backer and Baeissa, 2014). The total body weights were recorded at start and end of the experiments.

2.4. Sample collection

At experimental end, the rats were fasted for 12 hours and blood were collected from retro orbital veins into plain tubes and kept at room temperature for 15 min, then centrifuged for 10 min at 3000 Xg to get serum. Serum aspirated, aliquot and kept frozen at (-20ºC) until usage. Kidney function tests (urea, uric acid and creatinine), oxidative stress biomarkers as glutathione (GSH), superoxide dismutase [SOD and malondialdehyde (MDA)], and inflammatory markers as interleukin (IL)-6 and IL-1β were made in the central laboratory by commercially available kits according to kits protocol.

2.5. *Histology examination*

Following blood withdrawal, rats of all groups were euthanized by cervical dislocation under deep ether anesthesia, the abdomen was opened, and kidneys were excised, opened transversely (left) and longitudinally (right) then fix immediately in 10% formalin and prepared for microscopic study in histopathology laboratory KAUH. Paraffin sections 5 micron thick was stained by hematoxylin and eosin (H&E) for general features.

2.6. Statistical Analysis

The data obtained during the study were analyzed utilizing IBM SPSS Statistics for Windows, version 23 (IBM SPSS, IBM Corp., Armonk, N.Y., USA). Shapiro – Wilk test was utilized to evaluate normal value distribution. Collected value presented as mean +/- standard deviation (SD). Statistical comparisons were made by One-Way analysis of variance (ANOVA) then post hoc test, least significant difference (LSD) to determined significance. P <0.05 was considered statistically significant.

3. Results

3.1. Total body weights and kidney weights

Table (1) showed body weight and kidney weights in different studied groups. The initial body weight showed insignificant changes between different studied groups. The final body weights were significantly lower in groups 2 (LDCR) and 3 (HDCR) (P= 0.004 and P< 0.0001), but significantly elevated in group 5 (HDCR + Blueberry) (P =0.014) versus control. Final body weight was significantly increased in group 4 (LDCR + Blueberry) versus group 2 (LDCR) (P <0.0001) and in group 5 (HDCR + Blueberry) versus group 3 (HDCR) (P<0.0001) indicating the beneficial effects of blueberry on body weight. The percentage changes in body weight were calculated as final body weight - initial body weight/ initial body weight X 100. The percentage changes in body weight was significantly decreased in group 3 versus control and group 2 (P=0.007 and P=0.038) indicated harmful effects of Code Red on body weight. Percentage changes in body weight showed significant increase in group 4 versus group 2 (P=0.022) and in group 5 versus group 3. Kidney weight was significantly decreased in groups 2, 3 and 4 versus control (P <0.0001, P <0.0001 and P=0.042, respectively) but was significant increase in group 4 versus group 2 (P =0.024) and in group 5 versus group 3 (P<0.0001). Kidney index was calculated as kidney weight divided by final total body weight X 100. It's more accurate than kidney weight as it also depends on the surface area of the body. The kidney index was significantly declined only in groups 4 (LDCR + Blueberry) versus control (P =0.039).

Table 1: Comparison of initial and final total body weights, percentage change in weight, kidney weights and kidney index between different studied groups.

Groups / Parameters	Group 1 (Control)	Group 2 (Low dose code red)	Group 3 (High dose code red)	Group 4 (Low dose code red + Blueberry)	Group 5 (High dose code red + Blueberry)
Initial body weights (grams)	104.5±6.06	93.17±19.36	104.17±8.59	97.29±17.10	108.50±5.65
Significance	-	[1]P =0.141	[1]P =0.965; [2]P =0.152	[1]P =0.325; [2]P =0.572	[1]P =0.596; [3]P =0.566; [4]P =0.131
Final body weights (grams)	233.83±33.72	189.50±11.31	171.50±23.72	244.14±27.93	270.17±17.80
Significance	-	[1]P =0.004	[1]P =0.0001; [2]P =0.204	[1]P =0.446; [2]P =0.0001	[1]P =0.014; [3]P =0.0001; [4]P =0.062
Percentage changes (%)	124.27±35.33	109.20±35.54	64.16±12.37	157.33±53.65	149.78±22.17
Significance	-	[1]P =0.471	[1]P =0.007; [2]P =0.038	[1]P =0.108; [2]P =0.022	[1]P =0.226; [3]P =0.0001; [4]P =0.706
Kidney weights (grams)	0.82±0.03	0.64±0.02	0.63±0.02	0.74±0.05	0.90±0.14
Significance	-	[1]P =0.0001	[1]P =0.0001; [2]P =0.742	[1]P =0.042; [2]P =0.024	[1]P =0.076; [3]P =0.0001; [4]P =0.001
Kidney index (%)	0.36±0.05	0.34±0.02	0.37±0.05	0.30±0.03	0.34±0.06
Significance	-	[1]P =0.481	[1]P =0.584; [2]P =0.216	[1]P =0.039; [2]P =0.157	[1]P =0.478; [3]P =0.214; [4]P =0.159

Data were expressed as mean +/- standard deviation. [1]P: significance versus group 1 (Control); [2]P: significance versus group 2 (Low dose code red); [3]P: significance versus group 3 (High dose code red); [4]P: significance versus Group 4 (Low dose code red + Blueberry). Percentage change of body weight was calculated as final body weight minus initial body weight divided by initial body weight then multiplies by 100. Liver index was calculated as liver weight divided by final body weight multiple by 100.

3.2. Kidney function tests

Serum urea and creatinine levels were significantly higher in LDCR, HDCR, LDCR + Blueberry and HDCR + Blueberry groups versus control group (urea: $P<0.0001$for all; creatinine: $P<0.0001$, $P<0.0001$, $P=0.046$ and $P=0.003$, respectively) and in HDCR versus LDCR (urea: $P<0.0001$). Meanwhile, significant decline in serum urea and creatine values were noticed in LDCR + Blueberry versus LDCR ($P<0.0001$) and in HDCR + Blueberry versus HDCR ($P<0.0001$). Meanwhile, significant decrease in serum creatinine levels were noticed in LDCR + Blueberry versus LDCR ($P<0.0001$) and in HDCR + Blueberry versus HDCR ($P<0.0001$). Serum uric acid values were significantly elevated in LDCR and

HDCR versus control group (*P*=0.029 and *P*<0.0001, respectively) and in HDCR versus LDCR (*P*=0.007). Meanwhile, significant decrease in serum urea levels were noticed in HDCR + Blueberry versus HDCR (*P*<0.0001) (Table 2).

Table 2: Comparison of kidney function tests between different studied groups.

Groups / Parameters	Group 1 (Control)	Group 2 (Low dose code red)	Group 3 (High dose code red)	Group 4 (Low dose code red + Blueberry)	Group 5 (High dose code red + Blueberry)
Urea (mg/dl)	14.43±1.43	25.83±4.02	58.33±1.51	21.67±2.50	18.83±1.94
Significance	-	^{1}P <0.0001	^{1}P <0.0001; ^{2}P <0.0001	^{1}P <0.0001; ^{2}P <0.0001	^{1}P <0.0001; ^{3}P <0.0001; ^{4}P =0.058
Uric acid (mg/dl)	5.07±0.59	6.33±1.86	7.93±0.50	6.03±0.33	5.50±0.55
Significance	-	^{1}P =0.029	^{1}P <0.0001; ^{2}P =0.007	^{1}P =0.089; ^{2}P =0.587	^{1}P =0.435; ^{3}P <0.0001; ^{4}P =0.338
Creatinine (mg/dl)	0.65±0.18	1.69±0.11	1.73±0.40	0.92±0.21	1.07±0.11
Significance	-	^{1}P <0.0001	^{1}P <0.0001; ^{2}P =0.754	^{1}P =0.046; ^{2}P <0.0001	^{1}P =0.003; ^{3}P <0.0001; ^{4}P =0.259

Data were expressed as mean +/- standard deviation. ^{1}P: significance versus group 1 (Control); ^{2}P: significance versus group 2 (Low dose code red); ^{3}P: significance versus group 3 (High dose code red); ^{4}P: significance versus Group 4 (Low dose code red + Blueberry).

3.3. Inflammatory markers and oxidation stress activities

Serum IL-6 values were significantly higher in HDCR versus control and LDCR (*P*<0.0001for both) but significantly decreased in HDCR + Blueberry versus HDCR (*P*<0.0001). Serum IL-1β values were significantly elevated in HDCR versus control and LDCR (*P*<0.0001 for both) but significantly decreased in LDCR + Blueberry versus LDCR (*P*=0.013) and in HDCR + Blueberry versus HDCR (*P*<0.0001). Serum MDA values were significantly elevated in LDCR, HDCR, LDCR + Blueberry and HDCR +B groups versus control group (*P*<0.0001, *P*<0.0001, *P*<0.0001 and *P*=0.025, respectively) and in HDCR versus LDCR (*P*=0.006). Meanwhile, significant decrease in serum MDA values were found in HDCR + Blueberry versus HDCR and LDCR + Blueberry (*P*=0.001 and *P*<0.0001, respectively). Serum SOD and GSH levels were significantly lower in LDCR and HDCR versus control (*P*<0.0001 for all) and in HDCR versus LDCR (*P*<0.0001 for all). There were significant increase in SOD and GSH serum levels in LDCR + Blueberry versus LDCR (*P* <0.0001 for both). Serum SOD were significant increase in HDCR + Blueberry versus HDCR (*P* <0.0001) and GSH levels were significantly increased in LDCR + Blueberry versus LDCR (*P* <0.0001) and in HDCR + Blueberry versus control and HDCR (*P*=0.016 *and* *P*<0.0001) (Table 3).

Table 3: Comparison of inflammatory markers and oxidative stress between different studied groups.

Groups / Parameters	Group 1 (Control)	Group 2 (Low dose code red)	Group 3 (High dose code red)	Group 4 (Low dose code red + Blueberry)	Group 5 (High dose code red + Blueberry)
IL6 (pg/ml)	4.65±0.37	4.16±0.65	7.42±0.35	4.80±0.54	5.18±0.86
Significance	-	^{1}P =0.187	^{1}P <0.0001; ^{2}P <0.0001	^{1}P =0.641; ^{2}P =0.076	^{1}P =0.127; ^{3}P <0.0001; ^{4}P =0.254
IL-1β (pg/ml)	14.88±1.40	16.60±1.34	33.24±2.53	13.90±1.55	15.58±1.64
Significance	-	^{1}P =0.111	^{1}P <0.0001; ^{2}P <0.0001	^{1}P =0.313; ^{2}P =0.013	^{1}P =0.486; ^{3}P <0.0001; ^{4}P =0.090
MDA (nmol/ml)	0.25±0.06	0.75±0.12	1.00±0.05	0.74±0.19	0.43±0.16
Significance	-	^{1}P <0.0001	^{1}P <0.0001; ^{2}P =0.006	^{1}P <0.0001; ^{2}P =0.832	^{1}P =0.025; ^{3}P =0.001; ^{4}P <0.0001
SOD (U/ml) antioxidant	178.67±7.09	149.40±7.06	102.80±8.56	178.57±9.69	176.17±14.22
Significance	-	^{1}P <0.0001	^{1}P <0.0001; ^{2}P <0.0001	^{1}P =0.986; ^{2}P <0.0001	^{1}P =0.663; ^{3}P <0.0001; ^{4}P =0.664
GSH (ng/ml) antioxidant	15.42±1.73	8.48±0.94	3.12±1.11	17.81±2.75	18.52±2.60
Significance	-	^{1}P <0.0001	^{1}P <0.0001; ^{2}P <0.0001	^{1}P =0.048; ^{2}P <0.0001	^{1}P =0.016; ^{3}P <0.0001; ^{4}P =0.547

Data were expressed as mean +/- standard deviation. ^{1}P: significance versus group 1 (Control); ^{2}P: significance versus group 2 (Low dose code red); ^{3}P: significance versus group 3 (High dose code red); ^{4}P: significance versus Group 4 (Low dose code red + Blueberry). SOD: superoxide dismutase, GSH: glutathione, MDA: malondialdehyde, IL-6: interleukin (IL)-6, IL-1β and interleukin (IL)-1β.

3.4. Histological results

Histological study was done in the present research to confirm and describe what was found in biochemical analysis regard kidney function of rats receiving low and high dose of code red. Figure (1) showed that kidney sections from control, low dose code red and low dose code red + blueberry extract. G1: control kidney tissue with normal cortical and medullary tissues (renal corpuscles, glomerular capillaries and tubules), G2 (Low dose code red): kidney of rats received low dose code red showed mild to moderate alteration in such structures. High powers cortex: showed small size renal corpuscle with slight distortion of glomeruli capillaries. Focal dilation of kidney tubules that showed small dark degenerated nuclei of the lining epithelium was observed. The medulla also showed groups of dilated tubules and some showed intra- luminal cell debris or casts. Such changes were seen to be ameliorated by administration of blue berry extract given orally for 4 weeks (G4, low dose code red + blueberry).

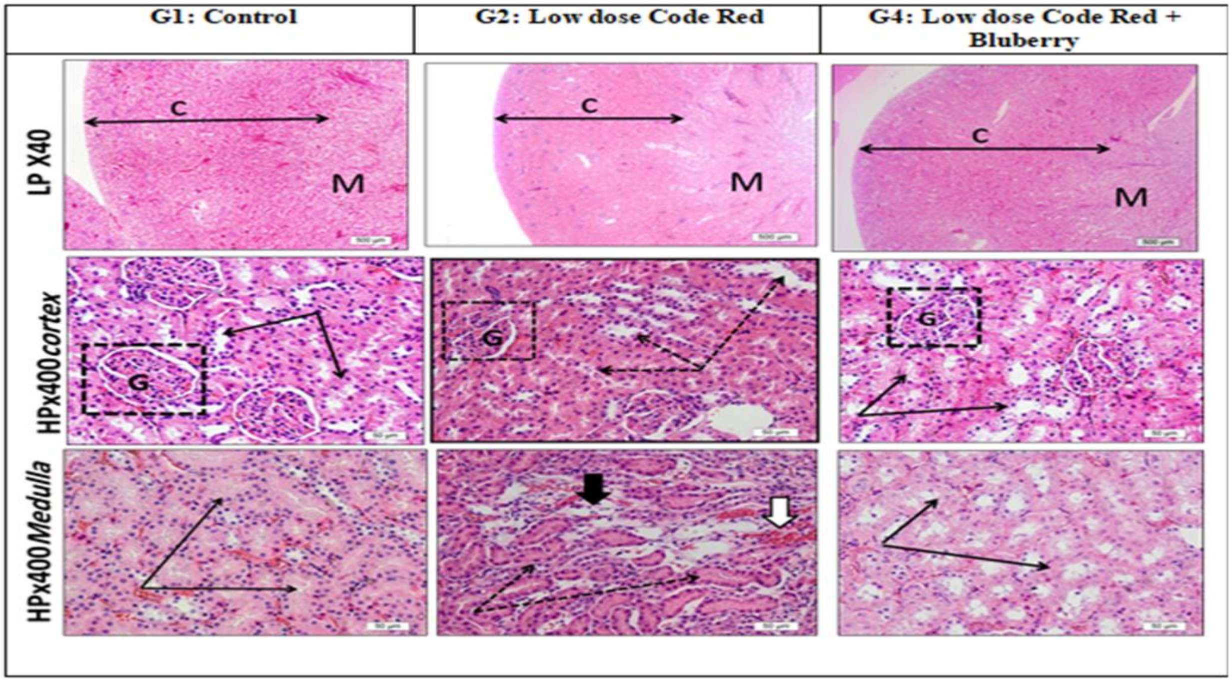

Figure (1): Sections in rat kidney stained by H&E to show Low power of kidney sector X40 High powers X400 of both cortex (C) and medulla (M) of:

G1 (Control group): Low power X40 showed normal thickness (double head arrow) outer cortex (C) and inner medulla (M). High power X400 cortex showed normal size renal corpuscle (dotted square) and glomerular capillaries (G), normal tubules lined with active cuboidal cells (arrows). Medulla with normal size regular shape tubules (arrows) lined by active cuboidal cells with narrow empty clean lumen (arrows).

G2 (Low dose code red group): Low power showed slight decreased in cortex (C) thickness (double head arrows). High powers cortex showed small size renal corpuscle (dotted square) with slight distortion of glomeruli capillaries (G), focal dilation of kidney tubules that showed small dark degenerated nuclei of lining epithelium (arrows). Medulla showed groups of dilated tubules and some showed intraluminal cell debris or casts (arrows).

G4 (Low dose code red + blueberry group): showed preservation of normal structure, cortical thickness, cortical renal corpuscle and glomeruli and medullary tubules.

In kidney sections stained by H&E for general structure rat kidney of G1: normal control group showed normal thickness outer cortex, renal corpuscles and their glomerular capillaries are of normal size and features, medullary tubules showed normal regular shape, narrow lumen and active lining epithelium. Blood vessels around the tubules are normal and non-congested. In contrast administration of high dose of code red energy drink in G3: administration of high dose of code red energy drink was caused decreased cortical thickness, marked scattered regions of hemorrhage in both cortex and medulla, glomerular and peritubular capillaries were congested, both renal corpuscles and tubules showed deformed disorganized arrangement. The nuclei of tubular epithelium looked dark indicating inactivity. The lumens of most tubules were dilated and full with hyaline casts or desquamated degenerated cells. Histological changes were more severe compared to group receiving low dose energy drink. G5: kidney of rats receiving blue blueberry extract in addition to high dose code red showed restoration of normal structure of both renal cortical and medullary tissues (Figure 2).

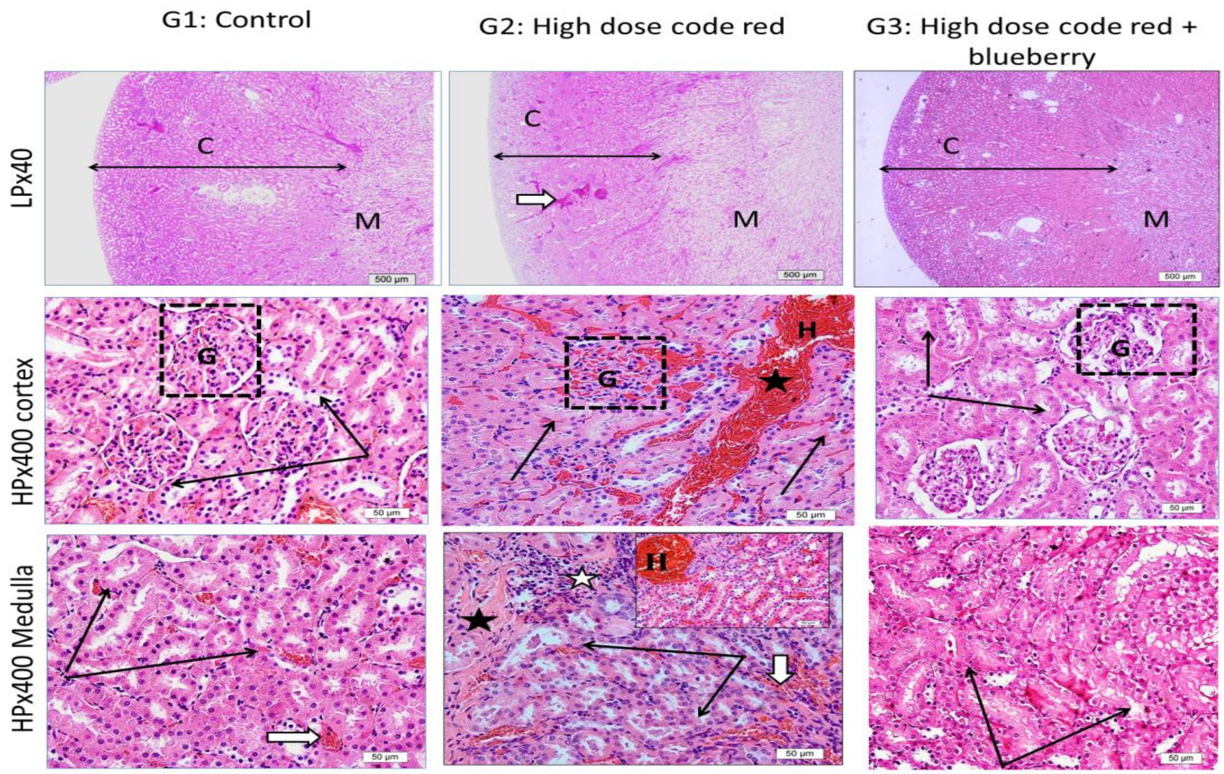

Figure (2): Sections in rat kidney stained by H&E to show low power of kidney sector X40, High powers X400 of both cortex (C) and medulla (M) of:

G1 (control group): Low power X40 showed normal thickness (double head arrow) outer cortex (C) and inner medulla (M). High power X 400 showed cortex with normal size renal corpuscle (dotted square) and glomerular capillaries (G), normal tubules lined with active cuboidal cells (arrows). Medulla with normal size regular shape tubules (arrows) lined by active cuboidal cells with narrow empty clean lumen (arrows). Few capillaries around the tubules could be seen (white arrow)

G3 (High dose Red code group): Low power showed decreased cortex (C) thickness (double head arrows) and regions of hemorrhage (white arrow). High powers showed cortex with massive hemorrhage (H), small size renal corpuscle (dotted square) with congested glomeruli capillaries (G). Kidney tubules were deformed and showed small dark degenerated nuclei of the lining epithelium (arrows). Medulla showed dilated tubules with intra-luminal cell debris or casts (arrows), marked hemorrhage (H) (insert) and congested capillaries between tubules (white arrow), notice presence of inflammatory cells (white star) and fibrosis (black star).

G5 (High dose code red + blueberry group): showed preservation of normal structure, cortical thickness, cortical renal corpuscle and glomeruli and medullary tubules.

4. Discussion

The results of this study showed that administration of low dose (0.72 ml/ 100 grams/ day) as well as high dose (1.44 ml/ 100 grams/ day) of Code Red for 8 weeks results in decreased in percentage changes in body weight in High dose Cod Red versus control and Low dose Code Red group. Meanwhile, administration of blueberry for 6 weeks led to increase in percentage changes in body weight in Low dose Code Red + Blueberry versus Low dose Code Red and in High dose Code Red + Blueberry versus High dose Code Red. In this respect, it was reported that EDs usually utilized for loss of weights. Energy drink companies don't explicitly endorse weight loss as a benefit of their cans. Red Bull leads to “stimulate metabolism.” However, Celsius energy drink aggressively marketed for weight loss. This statement is printed on Celsius can: “Burns Calories!” and “Burn up to 100 calories or more per can!” (Ballard et al., 2010). Meanwhile, Malek *et al*. (2006) found insignificant changes in body weight or body compositions after individuals intake tablets contains common EDs ingredients (as caffeine, guarana, niacin, vitamin B6) or placebo for 8 weeks. The weight loss observed in this study with high dose Code Red may contributed to caffeine contents as caffeine increased markers of thermogenesis and oxidations of fats (Clark et al., 2020). Meanwhile, results of this study showed that kidney index was significantly decreased in Low dose Code Red + Blueberry versus control indicated toxic effects of Code Red on kidney structure as shown below.

The results of this study revealed that administration of low dose as well as high dose of Code Red for 8 weeks results in increased in serum values of urea, uric acid and creatinine versus control group. Moreover, values of uric acid, urea, and creatinine were elevated in high versus low dose Code Red groups indicated more toxicity of high dose Code Red on kidneys. Creatinine, urea and uric acid are metabolic protein by product that increased in affected kidney and appeared in the circulation (AlBasher et al., 2020). This can make sense as renal tubules get in direct approach with toxic harmful substances during their excretion in urine and so led to tubular destructions. Akande and Banjoko (2011b) found that there was an elevation in urea values in rats' administrated low dose (10 mg/kg) and high dose (20mg/kg) of "Power Horse" for 14 days. The action of energy drinks on kidney functions might be due to its caffeine contents. Caffeine affected kidney function via A2A adenosine receptors inhibition that increased expansion of inflammatory reactions in interstitial, enhances proteinuria and changed renal histology and physiology (Khayyat et al., 2014). Ugwuja (2014) found that drinking of low dose (3.75 ml/kg) and high dose (7.5 ml/kg) of Bullet (energy drink) alone or with alcohols (+2 g/Kg alcohol) for one month elevated kidney functions (serum creatinine, uric acid and urea) of rats. Khayyat *et al.* (2014) reported that oral administration of (1.5ml/ 100grams) for 4 weeks of 3 types of EDs (Red Bull, Power Horse and Code Red) induced elevations in serum urea, uric acid and creatinine versus control. Mansy *et al.* (2017) demonstrated that oral administration of ED "Red Bull" at different concentrations (0.4, 1.1 and 2.2 ml/ 100 gram) to rats for 12 weeks resulted in varying degrees of kidney destruction. This was evident in ED-induced significant elevations in serum creatinine, blood urea nitrogen and uric acid values. El-ghazouly (2017) reported that oral administration of "Red Bull" energy drink to rats for 4 weeks resulted in renal damage that evident by elevation of urea and creatinine serum levels. However, some ED-based studies reported findings that were at variance with these results. For example, it had been reported that EDs (Power Horse and Red Bull) (5 ml) orally administered daily for 36 days to rabbits associated with higher plasma total protein and lowered levels of creatinine, albumin and uric acid (Ebuehi et al., 2011). Yet, Akande and Banjoko) (2011a) found insignificant association between caffeine intake and urea and creatinine serum levels in rats treated with high dose (20 mg/kg) and low dose (10 mg/kg) of "Power Horse" for 14 days. These disparities ED effects may be attributed to lack of uniformity in composition of these energy beverages.

The results of this research showed that administration of high dose only of Code Red led to significant elevation in pro-inflammatory cytokines as IL-1β and IL-6 versus control and low dose Code Red group. In this respect, it had been reported that carbohydrate and caffeine consumption before endurance cycling in men significantly increased leukocytosis and IL-6 release (Phillips et al., 2014). Díaz *et al.* (2016) reported that intake of alcohol and EDs mixture for 60 days led to an increase in reactive gliosis, IL-1β, tumor necrosis factor-α, inducible nitric oxide, reactive oxygen species, lipid peroxidation, and nitric oxide in temporal cortex and hippocampus. They also reported immunoreactivity to caspase-3 and decline of synaptophysin in same brain areas. The results suggested that chronic administration of alcohol in addition with ED causes an inflammatory reactions and oxidative stress, which induced cell death via apoptosis in temporal cortex and hippocampus of adult rats. Also, Kang *et al.* (2002) found that intake caffeine in high concentrations leads to an inflammatory reactions and death in neurons of neonatal rats and in cell cultures. In this study, the levels of these cytokines returned to normal after ingestion of blueberry for 6 weeks as blueberry contains anthocyanins that had anti-inflammatory properties (Johnson et al., 2013).

Regarding oxidative stress markers, serum MDA was significantly increased; while antioxidants (SOD and GSH) were significantly decreased after admiration of low and high doses of Code Red in this study. The levels were more affected in high dose versus low dose groups. After administration of blueberry for 6 weeks levels of SOD returned to normal; while levels of MDA decreased and GSH increased but still away from normal levels. Caffeine induces oxidative stress in tissues had been previously reported by Ekaluo *et al.* (2016) who stated that caffeine causes a decrease in antioxidant defense system as SOD, glutathione peroxidase (GPx) and catalase (CAT), followed by elevated in free radical activities and subsequently leading to oxidative stress. Caffeine significantly increased concentration of MDA as a marker for lipid peroxidation which usually accompanied oxidative cellular damage. Mansy *et al*. (2017) revealed that ED "Red Bull" exposure led to increased oxidative stress in rats that was evident by significant decreases in SOD, CAT and GPx activities. These enzymes are important antioxidants which work in concert with non-enzymatic antioxidant system to protect cells from oxidative destruction by free radicals. SOD neutralizes highly reactive superoxide anion by converting it to hydrogen peroxide, which is in turn degraded to water by GPx and CAT (Sharma and Sangha, 2014). The significant reductions in blood levels of these enzymes, especially in rats received medium and high doses (1.1 and 2.2 ml/100 g body weight/day) for 12 weeks of ED, might be due to ED-induced increases in superoxide radical, thereby overwhelming neutralizing capacities of antioxidant enzymes. Exposure of human cells to high levels of caffeine induced a pro-oxidant environment in cells, leading to increased protein oxidation, while low levels of caffeine had no effect on cells antioxidant capacity (Dias et al., 2015). Caffeine significantly increased blood urea nitrogen levels, resulting in activation of xanthine oxidase which in turn, stimulated xanthine oxidation to uric acid, and superoxide anion and H_2O_2 generation. The interaction between H_2O_2 with O_2 produces free radicals. On the other hand, several studies have independently demonstrated the antioxidant properties of many components of ED such as taurine, ginseng, caffeine and guarana (Obochi et al., 2010).

Histological study of the kidney revealed that rats received low dose cod red showed small size renal corpuscle with slight distortion of glomeruli capillaries. Focal dilation of kidney tubular cells with small dark degenerated nuclei of their lining epithelium. The medulla showed dilated tubules and intra- luminal cell debris or casts. Such changes were ameliorated by administration of blueberry for 6 weeks. Administration of high dose of code red caused decreased cortical thickness, marked scattered regions of hemorrhage in both cortex and medulla, glomerular and peritubular capillaries congestion, both renal corpuscles and tubules showed deformed disorganized arrangement. The nuclei of tubular epithelium looked dark indicating inactivity. The tubular lumens were dilated and full with hyaline casts or desquamated degenerated cells. Kidney of rats receiving blue blueberry extract in addition to high dose code red showed restoration of normal structure of both renal cortical and medullary tissues. Worthley *et al.* (2010) reported that renal corpuscles structures were destructed by EDs as revealed by dilatation of glomerular capillaries, closing of urinary space and destruction of filtering membrane. Mubarak (2012) reported that Masson's trichrome-stained sections of kidney cortex of Red Bull -ingested rats revealed marked elevation of collagen fibers bounded congested blood vessel, Bowman's capsules and basement membrane indicating fibrosis. Mubarak (2012) attributed occurrence of fibrosis to caffeine toxic influence. Khayyat *et al.* (2014) studied the impact of some EDs on kidney and detected necrosis of renal tubules and glomeruli, lobulation of glomerular capillaries, intertubular hemorrhage and inflammation areas among tubules, cavitation areas and dilatation of renal tubules. Drinking of large dosage of EDs responsible for kidney injuries due to effects of different EDs ingredients. This explained by the fact that the renal tubules are affected by excreted or cleared toxic chemicals during their removal (Kukner et al., 2007). Eldurssi *et al.* (2019) explained the necrosis of most kidney tubules and glomeruli due to ATP consumption that ended with cell apoptosis. The intertubular inflammation and hemorrhage zones could be microcirculatory disorders that arise from caffeine found in EDs (Khayyat et al., 2014). Khayyat *et al.* (2014) reported that oral administration of (1.5ml/ 100grams) of 3 types of energy drinks for 4 weeks (Red Bull, Power Horse and Code Red) in rats led to necrosis of renal tubules and glomeruli, intertubular hemorrhage and leucocytic infiltrations. Electron microscopic examination revealed ultrastructure changes in nucleous and cytoplasmic organelles in cells of proximal and distal tubules and renal corpuscles. These changes were marked in rats ingested "Power Horse" drink (Khayyat et al., 2014). Mansy *et al.* (2017) reported variations in kidney function parameters of rats exposed to different doses of ED (0.4, 1.1 and 2.2 ml/100 g body weight/day) for 12 weeks were in agreement with the lesions in photomicrographs of these tissues. Lesions were brought about by tissue damage arising from ED-induced oxidative stress. Lesions in kidney tissues were attributed to potential reaction of taurine and caffeine (Berger and Alford, 2009). El-ghazouly (2017) reported that drinking of Red Bull had bad effect on structure of renal cortex of rats. H&E-stained sections of kidney cortex from Red Bull -ingested group revealed glomeruli degeneration with widening of Bowman's space, segmentation of glomerular capillaries, 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 lumina of renal tubules, empty spaces, dilated congested blood vessels and areas of hemorrhage, massive cellular infiltration and acidophilic hyaline material within renal cortex.

In this study, it was observed that blueberry succeeded to some extent to protect the kidney from the adverse side action of Code Red-induced histopathological and funnctional alterations. The Code Red and Blueberry treated group revealed highly significant decrease in serum levels of urea and creatinine, inflammatory markers and oxidative stress markers as compared to the Code Red only treated group.

Elks *et al.* (2011) reported that after 6 and 12 weeks of blueberry ingestion, renal blood flow (RBF) and glomerular filtration rate (GFR) parameters were elevated, estimated renal vascular resistance (RVR) was decreased, kidney free radical formations was decreased, and renal glutathione and catalase values were maintained in blueberry- ingested stroke- prone spontaneously hypertensive rats (SHRSP) versus those of SHRSP ingested control diet. The results of chronic feeding studies showed that total reactive oxygen species, peroxynitrite formation rates and superoxide, were significantly decreased and antioxidant activities were elevated in blueberry- ingested SHRSP than in corn- ingested SHRSP. Blueberry diet protected against oxidative renal destruction by attenuating free radical production and preserving catalase and glutathione levels, and thereby improving blood pressure and renal hemodynamics (Elks et al., 2011). Blueberry is known scavengers of reactive oxygen species/reactive nitrogen species, including superoxide, *in vitro* (Neto, 2007). While results of the current study showed major therapeutic effects of blueberry diet on renal functions and structure, the effects reported prevent pathogenesis rather than treatment disease. In support of this possibility, same blueberry diet has been shown to both confer protection against myocardial ischemia when started before myocardial infarction and protection from more myocardial dysfunction when given 2 weeks after myocardial infarction (Ahmet *et al.*, 2009). These results in myocardium indicate potential for blueberry to be utilized as protective and therapeutic.

5. Conclusions

The findings of this study suggested that oral administration of low and high doses of energy drinks "Code Red" for 8 weeks leads to structural alterations in rat renal tissue especially high dose, which could play an important role in kidney damage. The pronounced reduction in the blood levels of key antioxidant enzymes and increased oxidative stress markers and inflammatory markers suggests that the harmful effects of Code Red were mediated through enhanced reactive oxygen species formation, oxidative stress and inflammatory markers. Blueberry extract had marked therapeutic action against Code Red -induced renal destruction in rats when administrated after Code Red. The therapeutic effect of blueberry is via its antioxidant and anti-inflammatory properties. So, blueberry usage with Code Red is recommended. If animal-to-man extrapolation is permitted, these results call for restraint and caution in Code Red and other energy drinks consumption. Thus, the need for adequate public awareness cannot be over-emphasized.

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