National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Molecular, biochemical, and pathological impacts of energy drinks on renin–angiotensin–aldosterone pathway in Wistar rats

Tamer Ahmed Ismail[1,2], Mohamed Abdo Nassan[3], Adel Qlayel Alkhedaide[1], Mohamed Mohamed Soliman[1,4], Dalia Ibrahim Mohamed[5]

[1]Laboratory Technology Department, Turabah University College, Taif University, Saudi Arabia, [2]Departmentof Physiology, Faculty of Veterinary Medicine, Zagazig University, Egypt, [3]Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, Egypt, [4]Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt, [5]Department of Biochemistry, Animal Health Research Institute, Zagazig Branch, Egypt

Correspondence to: Mohamed Soliman, E-mail: mohamedsoliman8896@yahoo.com

Received: April 02, 2018; **Accepted:** April 17, 2018

ABSTRACT

Background: Energy drinks (EDs) are widespreaded among young adolescents and adults to enhance physical and mental performance with numerous public health hazards. **Aims and Objective:** This study was conducted deeply to explore the mechanism by which EDs affect blood pressure (BP) and health status of the kidney. **Materials and Methods:** To examine impacts of EDs on rats, 60 male Wistar rats were divided into four groups. Control group was given water only; the remaining rats were administered orally red bull, code red, and power horse at a dose of 10 mg/kg once daily. After 60 days, rats were sacrificed and blood samples collected. Kidney tissues for all groups were harvested. Serum was collected for examining biochemical parameters related to kidney functions. Reverse transcription polymerase chain reaction was carried to examine the molecular changes in genes implicated in BP regulation and renin–angiotensin–aldosterone system (RAAS) pathway. Finally, histopathological examination for the kidney was investigated. **Results:** At biochemical level, consumption of EDs showed a significant ($P < 0.05$) increase in serum levels of glucose, urea, creatinine, uric acid, and phosphorus as compared with control group. Significant increases ($P < 0.05$) in expression of renin, angiotensin-converting enzyme (ACE), angiotensin II (Ang II), angiotensin type 1 receptors, desmin, erythropoietin (EPO), nitric oxide synthase-1, transforming growth factor β1, and kidney injury molecule-1 in rats administered EDs for 2 months as compared with control group. However, ACE2, angiotensin type 2 receptors, MAS receptor, and beta-2 macroglobulin (B2m) were not changed in ED-administered groups compared with control rats. **Conclusion:** Marketed EDs have initial hazardous effects on renal function and certainly increase BP through high caffeine content with the concern of the amount of sugar added in these drinks.

KEY WORDS: Energy Drinks; Blood Pressure; Renin–angiotensin–aldosterone System; Kidney

INTRODUCTION

Energy drink (ED) consumption has been increasing dramatically in the past few decades by young adolescents and adults.[1] EDs are extremely marketed for their energy-boosting effect to enhance physical and mental performance.[2]

Caffeine is one of the major active constituents of EDs and approximately forms 3–5 times the amount of caffeine contained in any other gaseous beverages. Caffeine concentration varies from product to other of these beverages.[2] Physiologically, caffeine effects include increased heart rate and nervousness. High caffeine consumption might lead to irritability, insomnia, tremor and seizures, hypertension, cardiac arrhythmias, and palpitations.[3] In addition to caffeine, EDs also contain taurine, a sulfur-containing amino

Access this article online

Website: www.njppp.com

Quick Response code

DOI: 10.5455/njppp.2018.8.0411517042018

acid, found in most mammalian tissues that enhance the effects of caffeine.[4] Moreover, the high sugar content (10–13%) of the EDs leads to obesity and diabetes.[5] The adverse health hazards associated with ED remains controversial among scientists.[6]

Renin–angiotensin–aldosterone system (RAAS) is a hormonal cascade that plays a critical role in blood pressure (BP) regulation and body fluid homeostasis.[7] Dysregulation of the RAAS underlies certain disorders such as pathogenesis of cardiovascular and renal disorders.[8]

Angiotensin II is the most important bioactive peptide of RAAS, exerts pivotal roles in regulating cardiovascular function such as vascular tone in normal physiological condition, as well as in the pathogenesis of hypertension and other cardiovascular dysfunctions.[9] It acts directly on vascular smooth muscle as a potent vasoconstrictor. In addition, it could affect heart rate and cardiac contractility through its action on the sympathetic nervous system. Moreover, angiotensin II affects renal sodium and water reabsorption through its ability to stimulate the zona glomerulosa cells of adrenal cortex to synthesize and secrete aldosterone.[10]

Ang II is formed by angiotensin-converting enzyme-1 (ACE1) and acts on Ang type 1 receptors (AGT-R1) to exert its evident physiological and pathophysiological effects. This synthetic cascade of Ang II has been considered to be the hypertensive axis (ACE 1/Ang II/AGT-R1) of the RAAS. ACE 2 cleaves Ang II to Ang-(1–7), which binds the Mas receptor (Mas R) to exert effects opposite of those produced by Ang II. This has been viewed as the antihypertensive axis (Ace2/Ang-[1–7]/MasR) of the RAAS.[11]

Aldosterone (ALD) is a major regulator of BP and controls water homeostasis and electrolyte balance. However, high levels of adrenal ALD secretion can cause hypertension. Higher ALD level was also associated with the incidence of hypertension even within the normal BP range.[12]

The risks of heavy consumption of EDs among young people have largely gone a major health threat and are expected to become a significant public health problem in the future. Therefore, this study was conducted deeply to explore the mechanism by which EDs affect BP and health status of the kidney through biochemical analysis of serum levels of glucose, urea, creatinine, uric acid, calcium (Ca), and phosphorus. In addition, molecular expression of certain genes related to renal physiology and mechanisms for BP regulation in kidney was examined. For instance, measuring the mRNA expression of renin, ACE, Ang II, Angiotensin type 1 receptors, desmin, EPO, kidney injury molecule-1 (KIM-1), nitric oxide synthase-1 (NOS1), transforming growth factor β1 (TGFB1), and B2 M was done in renal tissues. This work was visually confirmed through histopathological examination of renal tissues of experimental animals.

MATERIALS AND METHODS

Materials

Adult male Wistar rats were purchased from King Fahd Center for Scientific Research, King Abdel-Aziz University, Saudi Arabia. Agarose and ethidium bromide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Kits for glucose, creatinine, urea, uric acid, calcium, and phosphorus were purchased from Bio-diagnostic Co., Dokki, Giza, Egypt. Code Red, Power Horse, and Red Bull were purchased from Taif markets in Saudi Arabia. The deoxyribonucleic acid (DNA), 100 bp ladder, was purchased from MBI, Fermentas, Thermo Fisher Scientific, USA. Trizol for RNA extraction and oligo dT primer were purchased from QIAGEN (Valencia, CA, USA).

Animals, Experimental Design and Sampling

This study was approved by the Ethical Committee of the Dean of Scientific Affairs, Taif University, Saudi Arabia (project number 5599/38/1). 60 male Wistar rats, 6 weeks old, weighing 150 g were used for this study. Rats were kept under observation for 1 week for appropriate acclimatization. Rats were kept under conditions of controlled temperature (25 ± 2°C) and relative humidity of 50 ± 10% with a 12 h/12 h day-night cycle in laboratory animal unit, College of Applied Medical Sciences, Turabah, Taif University. Animals gained free access to food and water *ad libitum* for 1st week. Next, rats were allocated into four groups, control without any treatment; Red Bull group; Code Red group, and Power Horse group.

Rats administered orally Red Bull, Code Red, and Power Horse at a dose of 10 mg/kg equivalent to 7.5 mL/kg once daily for 60 days.[13,14] At the end of experimental design, all rats were sacrificed after anesthetization by diethyl ether inhalation. Blood and kidney were collected from slaughtered rats in sterilized vacutainer tubes. Serum was extracted after centrifugation of clotted blood for 10 min at ×4000 g and kept at −20°C till assays. For gene expression, kidney tissues were kept in Trizol reagent at −80°C for ribonucleic acid (RNA) extraction. For histopathological examination, sections from kidney were inserted in 10% neutral-buffered formalin at room temperature for 24 h.

Biochemical Assessments

Serum concentrations of glucose, urea, creatinine, uric acid, calcium, and phosphorus were measured spectrophotometrically using Cobas 6000 Roche according to the manufacturer's instructions.

RNA Extraction and cDNA Synthesis

Total RNA was extracted from kidney tissue of experimental rats based on a previous study.[15] Kidney samples were flash frozen in liquid nitrogen and subsequently stored at −80°C in 1 mL trizol

(QIAGEN Inc., Valencia, CA). Frozen samples were homogenized, and then, 0.3 mL chloroform was added to the homogenate. The obtained mixtures were shaken for 30 s and then centrifuged for ×16,400 g at 4°C for 15 min. The supernatant was transferred to new tubes, and the same volume of isopropanol was added to the samples, shacked for 15 s, and centrifuged at 4°C and ×16,400 g for 15 min. The RNA pellets were washed with ethanol 70%, dries up, and then dissolved in diethylpyrocarbonate (DEPC) water. RNA purity and concentration were determined spectrophotometrically at 260 nm. The RNA integrity was confirmed in 1.5% agarose stained with ethidium bromide. The ratio of the 260/280 optical density of all RNA samples was 1.7–1.9.

For cDNA synthesis, mixture of 4 μg total RNA and 0.5 ng oligo dT primer (Qiagen Valencia, CA, USA) in a total volume of 11 μL sterilized DEPC water was incubated in the Bio-Rad T100TM Thermal cycle at 65°C for 10 min for denaturation. Then, 2 μL of ×10 RT-buffer, 2 μL of 10 mM dNTPs, and 100 U Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase (SibEnzyme Ltd. Ak, Novosibirsk, Russia) were added, and the total volume was completed up to 20 μL by DEPC water. The mixture was then reincubated in the thermal cycler at 37°C for 1 h, then at 90°C for 10 min to inactivate the enzyme.

Semi-quantitative Reverse Transcription-polymerase Chain Reaction (RT-PCR) Analysis

Specific primers for examined genes [Table 1] were designed using Oligo-4 computer program and synthesized by Macrogen (Macrogen Company, GAsan-dong, Geumcheon-gu, Korea). PCR was performed as explained before.[15] As a reference, expression of glyceraldehyde-3-phosphate dehydrogenase mRNA was examined [Table 1]. PCR products were electrophorized on 1.5% agarose gel stained with ethidium bromide in tris-Borate-EDTA buffer. PCR products were visualized under ultraviolet light and photographed using gel documentation system. The intensities of the bands were quantified densitometrically using ImageJ software version 1.47 (http://imagej.en.softonic.com/).

Histopathological Examination

Kidney specimens from sacrificed rats were preserved in 10% buffered neutral formalin for 24 h for fixation followed by washing, then trimming, dehydration in ascending grades of alcohol, and then clearing in xylene. Embedding and mounting in paraffin followed by cutting into sections of 5-micron thickness were done. Staining was performed by normal routine stain (H and E).[16]

Statistical Analysis

Results were shown as means ± standard error of means (SEM). Data were analyzed using analysis of variance and *post-hoc* descriptive tests by SPSS software version 11.5 for Windows with $P < 0.05$ regarded as statistically significant. Regression analysis was performed using the same software.

RESULTS

Effect of EDs Consumption for 2 Months on Biochemical Parameters of Wistar Rats

As shown in Table 2, consumption of Red Bull, Code Red, and Power Horse for 2 months showed a significant ($P < 0.05$) increase in serum levels of glucose, urea, creatinine, uric acid, and phosphorus as compared with control group. However, serum calcium level showed a significant decrease in EDs administered rats as compared with control groups.

Effect of EDs Consumption for 2 Months on mRNA Expression of Renin, ACE, and ACE2 in the Kidney of Wistar Rats

Red Bull, Code Red, and Power Horse consumption for 60 days significantly upregulated gene expressions of both renin and ACE ($P < 0.05$) as compared with control group [Figure 1]. However, ACE2 gene expression in renal tissues of all examined groups exhibited no significant changes and was similar to control rats.

Effect of EDs Consumption for 2 Months on mRNA Expression of AGT-II, AGT-R1, and AGT-R2 in Kidney of Wistar Rats

As presented in Figure 2, gene expressions of AGT-II and AGT-R1 were significantly upregulated ($P < 0.05$) in Red Bull, Code Red, and Power Horse administered rats compared to control group. However, there was no significant change observed in gene expression of AGT-R2 in all groups administered EDs.

Effect of EDs Consumption for 2 Months on mRNA Expression of EPO and Desmin in the Kidney of Wistar Rats

In addition to renin–angiotensin pathway examination, the current study has investigated the effect of EDs on the mRNA expression levels of certain genes associated with normal kidney function and stability such as desmin and renal hypoxia marker erythropoietin (EPO). The expressions of desmin and EPO were significantly upregulated ($P < 0.05$) in Red Bull, Code Red, and Power Horse administered rats as compared with control group [Figure 3].

Effect of EDs Consumption for 2 Months on mRNA Expression of NOS-1 and Transforming Growth Factor β1 (TGF-β1) in the Kidney of Wistar Rats

Expression of NOS-11 that participates on renin expression was significantly increased ($P < 0.05$) in the three groups administered EDs as compared with control group [Figure 4]. Moreover, expression of mRNA of the profibrotic cytokine and TGFβ1 was

significantly elevated ($P < 0.05$) in the ED-administered groups as compared to control group [Figure 4].

Effect of EDs Consumption for 2 Months on mRNA Expression of MAS-II, KIM-1, and B2-M in the Kidney of Wistar Rats

The mRNA expression of MAS-II receptor was not changed in the ED-administered rats. However, KIM-1, an early marker of renal proximal tubule damage, was significantly upregulated

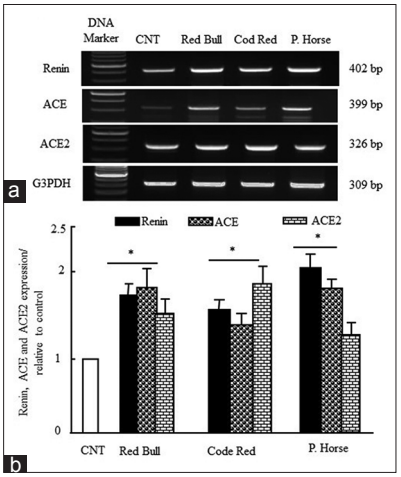

Figure 1: Effect of energy drinks consumption on renin, ACE, and ACE2 expression. Total RNA was extracted from kidney tissues, and the expressions of renin, ACE, and ACE2 were analyzed by semi-quantitative RT-PCR analysis. Values are means ± SE of 15 different rats. *P < 0.05 versus control group. Upper panels (a) are mRNA expression of examined genes. Lower columns (b) are densitometric analysis of renin, ACE, and ACE2 expression stated in upper panels

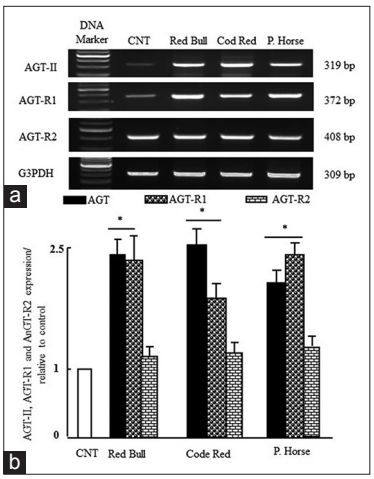

Figure 2: Effect of energy drinks consumption on AGT-II, AGT-R1, and AGT-R2 expression. Total RNA was extracted from kidney tissues, and the expressions of AGT-II, AGT-R1, and AGT-R2 were analyzed by semi-quantitative RT-PCR analysis. Values are means ± SE of 15 different rats. *P < 0.05 versus control group. Upper panels (a) are mRNA expression of examined genes. Lower columns (b) are densitometric analysis of AGT-II, AGT-R1, and AGT-R2 expression stated in upper panels

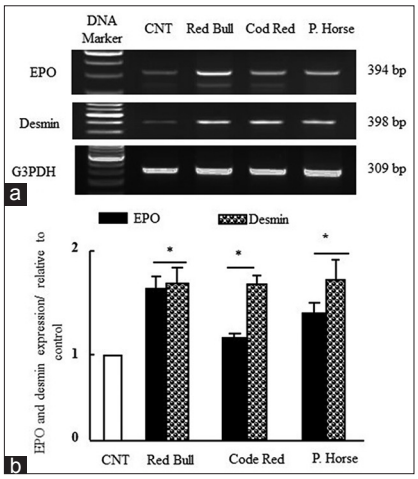

Figure 3: Effect of energy drinks consumption on EPO and desmin expression. Total RNA was extracted from kidney tissues, and the expressions of EPO and desmin expression were analyzed by semi-quantitative RT-PCR analysis. Values are means ± SE of 15 different rats. *P < 0.05 versus control group. Upper panels (a) are mRNA expression of examined genes. Lower columns (b) are densitometric analysis of EPO and desmin expression stated in upper panels

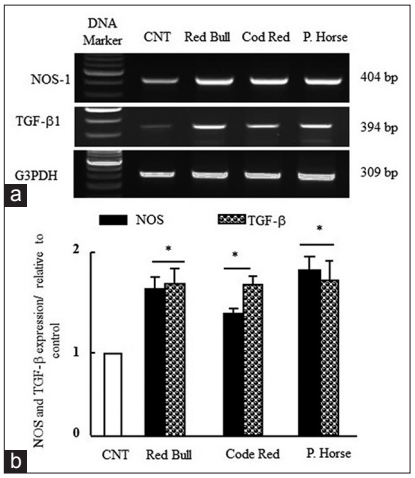

Figure 4: Effect of energy drinks consumption on NOS-1 and transforming growth factor β1 (TGF-β1) expression. Total RNA was extracted from kidney tissues, and the expressions of NOS-1 and TGF-β1 expression were analyzed by semi-quantitative RT-PCR analysis. Values are means ± SE of 15 different rats. *P < 0.05 versus control group. Upper panels (a) are mRNA expression of examined genes. Lower columns (b) are densitometric analysis of NOS-1 and TGF-β1 expression stated in upper panels

($P < 0.05$) in Red Bull and Power Horse administered groups but not in Code Red group [Figure 5]. B2-M was not altered in Red Bull and Power Horse-administered groups compared to control rat. However, Code Red administered rats showed upregulation in B2-M mRNA [Figure 5].

Effects of EDs Consumption for 2 Months on Kidney Histopathology in Wistar Rats

The kidney of control rats showed normal histological structure with normal glomeruli and normal tubules

[Figure 6a]. Meanwhile, the kidney of Red Bull-administered rats revealed moderate hydropic degeneration of tubular epithelium [Figure 6b]. The kidney of Code Red-administered rats showed congestion of periglomerular blood vessels [Figure 6c]. Finally, the kidney of Power Horse-administered rats showed eosinophilic casts inside tubular lumina [Figure 6d].

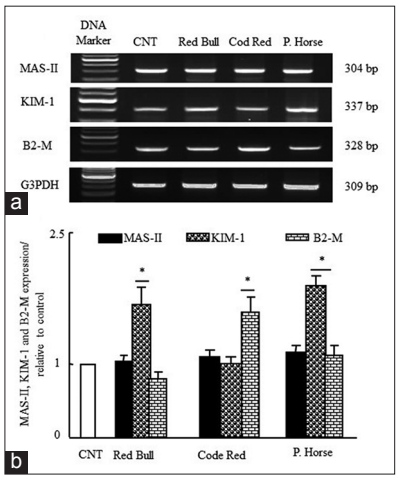

Figure 5: Effect of energy drinks consumption on MAS-II, KIM-1, and B2-M expression. Total RNA was extracted from kidney tissues, and the expressions of MAS-II, KIM-1 and B2-M expression were analyzed by semi-quantitative RT-PCR analysis. Values are means ± SE of 15 different rats. *P < 0.05 versus control group. Upper panels (a) are mRNA expression of examined genes. Lower columns (b) are densitometric analysis of MAS-II, KIM-1 and B2-M expression stated in upper panels

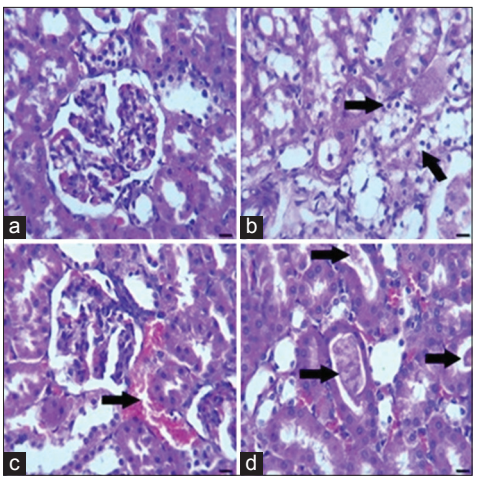

Figure 6: Histopathological examination of rat kidney after energy drinks consumption for 2 consecutive months (a) kidney of control rats showed normal histological structure with normal glomeruli and normal tubules. (b) Kidney of Red Bull-administered rats revealed moderate hydropic degeneration of tubular epithelium (arrows). (c) Kidney of Code Red-administered rats showed congestion of periglomerular blood vessels (arrow). (d) Kidney of Power Horse-administered rats showed eosinophilic casts inside tubular lumina (arrows). Scale bar = 20 μm

DISCUSSION

Current findings confirmed that EDs consumption induced alterations in renal biomarkers and mRNA expression of genes related aldosterone/angiotensin pathways and affected renal histology.

Energy beverages are being well-marketed products in both local and international markets under the claim that these drinks are healthy and good alternatives of bad products spread over the market. This kind of beverages contains several natural compounds that are useful to limited extent. Caffeine is one of ingredients in these drinks which added in high concentrations stimulating central nervous system activation and increasing intracellular calcium ion influx.[17] Taurine is an amino acid derivative that is synthesized naturally in human body. It is derived from homocysteine and essential for central nervous system, cardiac function, retina, and skeletal muscles.[18] Taurine is naturally produced in a concentration between 40 and 400 mg/day while added into EDs in more than 1000 mg/dL. Up to date, there is no clear evidence for neither benefits nor negative effects of taurine supplements. Wilhelm *et al.* have recommended that young people should not consume these EDs as long as there is no positive effect of such beverages with health risk possibility.[19]

Several published reports have concentrated on acute physiological effects of EDs consumption in either young or adults participants. Certain EDs consumed at a high volume significantly increase the QTc interval and systolic BP by over 6 ms and 4 mmHg, respectively.[20] EDs contain caffeine and other energy promoting ingredients such as glucose, taurine, vitamins, minerals, and some herbal extracts**.** Caffeine has proven psychomotor and cardioactive effects, and a combination of caffeine with other energy boosting substances could further augment these effects.[21] Most of these reports have considered the possible caffeine overdose toxicity and concluded that there is no acute negative health effect after consuming such beverages. Therefore, the current study is concentrated deeply on the chronic genetic and pathophysiology effects of EDs consumption on RASS pathway in experimental animals.

Table 2 showed that 2 months consumption of Red Bull, Code Red, and Power Horse clearly increased serum levels of glucose, urea, creatinine, uric acid, and phosphorus with low calcium serum levels corresponding to control group. These data might tell that ED consumption disturbs reabsorption function of kidney and that might be related to BP increase as a result of a high dose of caffeine content in drinks that examined. The presented data are in constituent with the previous report that found elevated plasma urea, uric acid, and creatinine in rats administered EDs in comparison to control suggested renal involvement.[14] Both urea and creatinine are products of protein metabolism, which are accumulated in the blood when the kidneys are affected.

Table 1: PCR conditions of examined genes

mRNA expression	Forward primer (5'-3')	Reverse primer (5'-3')	PCR cycles and Annealing Temperaturte
Renin (402 bp)	TGGATCAGGGAAGGTCAAAG	CCCTCCTCACACAACAAGGT	35 cycles, 60°C 1 min
ACE (399 bp)	CACTGGAGCCTGATCTGACA	AGGGTGCCACCAAGTCATAG	35 cycles, 59°C 1 min
ACE2 (326bp)	GCTAAACATGATGGCCCACT	TACATTTCGTTGTCGGTCCA	35 cycles, 59°C 1 min
AGT-II (319 bp)	CTGTGAAGGAGGGAGACTGC	ACCCCCTCTAGTGGCAAGTT	35 cycles, 59°C 1 min
AGT-R1 (372 bp)	CAGAGGACCATTTGGGCTAA	TGACCTCCCATCTCCTTTTG	35 cycles 60°C 1 min
AGT-R2 (408 bp)	CCCTAAAAAGGTGTCCAGCA	CCAGCAGACCACTGAGCATA	35 cycles, 60°C 1 min
EPO (394bp)	AGTCGCGTTCTGGAGAGGTA	TGCAGAAAGTATCCGCTGTG	37 cycles, 60°C 1 min
Nos-1 (404 bp)	GACAACGTTCCTGTGGTCCT	TCCTTGAGCTGGTAGGTGCT	35 cycles, 60°C 1 min
TGF-1 b (394 bp)	TACAGGGCTTTCGCTTCAGT	TGGTTGTAGAGGGCAAGGAC	35 cycles, 60°C 1 min
Mas-RII (304 bp)	ATGCACACAAACCACCAGAA	GCCGAAAATCATGAAGAGGA	35 cycles 60°C 1 min
KIM-1 (337 bp)	CGCAGAGAAACCCGACTAAG	AATCTCCCAGGAGCTGGAAT	35 cycles, 60°C 1 min
B2-M (328 bp)	GCAGCCTAGCAGTTCAATCC	CCCTACTCCCCTCAGTTTCC	35 cycles, 59°C 1 min
Desmin (398bp)	TCACAATCACCTCTTTGTGGTC	CAGCACCTTCCAGTTCTCTCTT	35 cycles, 54°C 1 min
G3PDH (309 bp)	AGATCCACAACGGATACATT	TCCCTCAAGATTGTCAGCAA	30 cycles, 52°C 1 min

PCR cycle of respective genes are shown, while temperature and time of denaturation and elongation steps of each PCR cycle were 94°C, 30 s and 72°C, 60 s, respectively. ACE: Angiotensin-converting enzyme, ACE2: Angiotensin-converting enzyme-2, AGT-II: Angiotensin-2, AGT-R: Angiotensin receptor subtypes, KIM: Kidney injury molecule, B2-M: Beta-2 macroglobulin, NOS-1: Nitrous oxide synthase-1, TGF-β1: Transforming growth factor beta-1, MAS-II: Mas receptor-1, EPO: Erythropoietin

Table 2: Effect of ED consumption for 2 months on biochemical parameters of Wistar rats.

Group\ Parameter	Control	Red Bull	Code Red	Power Horse
Glucose (mg/dL)	85±3.4	126±2.3*	183±10.4*	215±17.1*
UREA (mg/dL)	20±3.8	55.3±5.0*	51.3±2.3*	48±9.6*
Creatinine (mg/dL)	0.2±0.03	0.9±0.06*	0.98±0.03*	0.83±0.03*
Uric acid	1.1±0.05	2.8±0.07*	3.5±0.09*	2.9±0.01*
Calcium	8.8	5.3±0.1*	5.1±0.8*	5.4±0.9*
Phosphorus	7.6	11.2±0.1*	12.1±0.2*	10.9±0.2*

Values are means±standard error (SEM) for 15 different rats per each treatment. Values are statistically significant at *P<0.05 versus control, ED: Energy drinks

Moreover, drinking one can of ED contains approximately 25–40 g of glucose which might lead to hyperglycemia. Hyperglycemia is considered one of the cardiotoxics and is an important risk factor for acute myocardial infarction and other cardiovascular diseases.[1]

On molecular scale, Figures 1 and 2 demonstrated that ED consumption significantly upregulates the mRNA expression of renin, ACE, AGT-II, and AGT-Rs. These results are nicely consistent with biochemical analysis data explaining the direct effect of such beverages on RASS pathway and ultimately BP increase. Furthermore, these data are parallel with previous studies that showed upregulation in expressions of renin, ACE, and AGT-II.[22] Moreover, increased AGT-R1 expression may be another mechanism that contributes to hypertension. Previous studies have reported an increase in expression of renal AGT-R1 in an animal model of hypertension.[23] Current findings provide evidence of a complex regulatory mechanism between ACE2 and the MAS receptor. The physiological function of ACE2 is to produce AGT (1–7) from AGT-II, which then binds to Mas and stimulates vasodilation.[24] As presented, Figures 1, 2, and 5 showed no change in mRNA expression of ACE2, AGT-R2, and MAS-II receptor, respectively, in renal tissues as compared with control group. Our results are in line with the previous report that stated that AGT-R1 receptor was upregulated; therefore, the effects of increased Ang II production in the adult kidney may still be largely vasoconstrictive.[7]

Visually, renal tissues of Red Bull-administered rats revealed moderate hydropic degeneration of tubular epithelium [Figure 6b]. However, renal tissues of Code Red-administered rats showed congestion of periglomerular blood vessels [Figure 6c], while the histopathological study of Power Horse-administered rats revealed eosinophilic casts inside tubular lumina [Figure 6d].

The histopathological study findings and both molecular and biochemical data were nicely validated through other gene regulation studies that related to renal function and health. Figures 3-5 clearly show that gene expression of desmin, renal hypoxia marker EPO, NOS1, TGFβ1, and KIM-1 was upregulated in rats drank energy beverages for 2 months with no changes in the expression of these genes in control group. Significant increases in mRNA of desmin, indicating glomerular (podocyte) damage, and it further indicates renal damage. Tubular system is likely to be affected, as we found

increased expression of KIM-1, a sensitive marker of tubular damage. Afferent and efferent arteriole, vascular tone, and renin expression are regulated by NOS1-derived nitric oxide.[25] NOS1 mRNA levels were upregulated in our study. This study concluded that EDs may be very harmful on renal function and increased BP and kidney biomarkers-related genes.

CONCLUSION

Consumption of EDs has deleterious effect on renal activity and affected genes associated with hypertension and must be consumed with caution.

FUNDING AND ACKNOWLEDGMENTS

The authors would like to acknowledge and thank the Deans of Scientific Research Affairs in Taif University, Saudi Arabia, for financial support of this study (Project number 5599-38-1).

REFERENCES

1. Peacock A, Martin FH, Carr A. Energy drink ingredients. Contribution of caffeine and taurine to performance outcomes. Appetite 2013;64:1-4.
2. Mangi MA, Rehman H, Rafique M, Illovsky M. Energy drinks and the risk of cardiovascular disease: A review of current literature. Cureus 2017;9:e1322.
3. Kim WS, Choi CK, Yoon SH, Kwon JY. Usual dose of caffeine has a positive effect on somatosensory related postural stability in hemiparetic stroke patients. Ann Rehabil Med 2014;38:775-83.
4. Bouckenooghe T, Remacle C, Reusens B. Is taurine a functional nutrient? Curr Opin Clin Nutr Metab Care 2006;9:728-33.
5. Seifert SM, Schaechter JL, Hershorin ER, Lipshultz SE. Health effects of energy drinks on children, adolescents, and young adults. Pediatrics 2011;127:511-28.
6. Wassef B, Kohansieh M, Makaryus AN. Effects of energy drinks on the cardiovascular system. World J Cardiol 2017;9:796-806.
7. Williamson CR, Khurana S, Nguyen P, Byrne CJ, Tai TC. Comparative analysis of renin-angiotensin system (RAS)-related gene expression between hypertensive and normotensive rats. Med Sci Monit Basic Res 2017;23:20-4.
8. Atlas SA. The renin-angiotensin aldosterone system: Pathophysiological role and pharmacologic inhibition. J Manag Care Pharm 2007;13 8 Suppl B:9-20.
9. Mehta PK, Griendling KK. Angiotensin II cell signaling: Physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol 2007;292:C82-97.
10. Weir MR, Dzau VJ. The renin-angiotensin-aldosterone system: A specific target for hypertension management. Am J Hypertens 1999;12:205s-13.
11. Xu P, Sriramula S, Lazartigues E. ACE2/ANG-(1-7)/Mas pathway in the brain: The axis of good. Am J Physiol Regul Integr Comp Physiol 2011;300:R804-17.
12. Calhoun DA. Aldosteronism and hypertension. Clin J Am Soc Nephrol 2006;1:1039-45.
13. Diaz A, Trevino S, Guevara J, Munoz-Arenas G, Brambila E, Espinosa B, *et al*. Energy drink administration in combination with alcohol causes an inflammatory response and oxidative stress in the hippocampus and temporal cortex of rats. Oxid Med Cell Longev 2016;2016:8725354.
14. Ugwuja E. Biochemical effects of energy drinks alone or in combination with alcohol in normal albino rats. Adv Pharm Bull 2014;4:69-74.
15. Alkhedaide A, Soliman MM, Salah-Eldin AE, Ismail TA, Alshehiri ZS, Attia HF. Chronic effects of soft drink consumption on the health state of wistar rats: A biochemical, genetic and histopathological study. Mol Med Rep 2016;13:5109-17.
16. Ananthaneni A, Namala S, Guduru VS, Ramprasad VV, Ramisetty SD, Udayashankar U, *et al*. Efficacy of 1.5% dish washing solution and 95% lemon water in substituting perilous xylene as a deparaffinizing agent for routine h and e staining procedure: A short study. Scientifica (Cairo) 2014;2014:707310.
17. Nehlig A, Daval JL, Debry G. Caffeine and the central nervous system: Mechanisms of action, biochemical, metabolic and psychostimulant effects. Brain Res Brain Res Rev 1992;17:139-70.
18. Ripps H, Shen W. Review: Taurine: A "very essential" amino acid. Mol Vis 2012;18:2673-86.
19. Wilhelm P, van Diepen MA, Nieuwenhuis L, Boulogne TL. The effect of energy drinks on the cognitive performance of adolescents. Tijdschr Psychiatr 2013;55:57-62.
20. Shah SA, Occiano A, Nguyen TA, Chan A, Sky JC, Bhattacharyya M, *et al*. Electrocardiographic and blood pressure effects of energy drinks and panax ginseng in healthy volunteers: A randomized clinical trial. Int J Cardiol 2016, 218:318-323.
21. Mapanga RF, Joseph D, Symington B, Garson KL, Kimar C, Kelly-Laubscher R, *et al*. Detrimental effects of acute hyperglycaemia on the rat heart. Acta Physiol (Oxf) 2014;210:546-64.
22. Lee HA, Cho HM, Lee DY, Kim KC, Han HS, Kim IK. Tissue-specific upregulation of angiotensin-converting enzyme 1 in spontaneously hypertensive rats through histone code modifications. Hypertension 2012;59:621-6.
23. Collett JA, Hart AK, Patterson E, Kretzer J, Osborn JL. Renal angiotensin II type 1 receptor expression and associated hypertension in rats with minimal SHR nuclear genome. Physiol Rep 2013;1:e00104.
24. Tan Z, Wu J, Ma H. Regulation of angiotensin-converting enzyme 2 and Mas receptor by Ang-(1-7) in heart and kidney of spontaneously hypertensive rats. J Renin Angiotensin Aldosterone Syst 2011;12:413-9.
25. Komers R, Lindsley JN, Oyama TT, Allison KM, Anderson S. Role of neuronal nitric oxide synthase (NOS1) in the pathogenesis of renal hemodynamic changes in diabetes. Am J Physiol Renal Physiol 2000;279:F573-83.

How to cite this article: Ismail TA, Nassan MA, Alkhedaide AQ, Soliman MM, Mohamed DI. Molecular, biochemical, and pathological impacts of energy drinks on renin–angiotensin–aldosterone pathway in Wistar rats. Natl J Physiol Pharm Pharmacol 2018;8(8):1140-1146.

Source of Support: Nil, **Conflict of Interest:** None declared.