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RESEARCH ARTICLE

Chronic intake of energy drinks affects changes in kidney function biomarkers in a diabetes mellitus animal model

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Abstract

Background: Energy drinks are a food supplement consisting of multivitamins, macronutrients, taurine, and caffeine. Their excessive consumption is suspected to be a risk factor for chronic kidney failure. **Objective:** This study aimed to determine the effect of energy drinks on kidney function in rat models with diabetes mellitus (DM). **Method:** Thirty-two experimental male Wistar rats were injected with alloxan 150 mg/kg intraperitoneally to induce DM and then randomly divided into four groups. The positive control group was treated with drinking water. ED1, ED2, and ED3 groups were respectively treated with energy drinks coded EDK, EDH, and EDE, with a dose equivalent to caffeine 25 mg/kg twice a day for 15 days. All energy drinks used are distributed in the Asian region, especially in Southeast Asia. Urinalysis, haematology, and kidney histopathology evaluations were carried out. **Result:** Energy drinks affect BUN, serum creatinine, sodium, and potassium. In the histopathological observation of kidney tissue, there was a significant difference in the value of damage between the control group and the ED3 group. **Conclusion:** Energy drinks significantly increase the risk of impaired kidney function in diabetes mellitus rat models.

Introduction

Kidney stones (nephrolithiasis) are the critical cause of chronic kidney disease morbidity and affect up to 15% of the worldwide population (Moftakhar *et al*., 2022). Kidney stones grow and develop from crystals that form over a long period without treatment. Kidney stones are formed from (1) calcium (75-85%), which reacts with oxalate, phosphate, carbonate, and citrate; (2) uric acid (5-10%); (3) struvite stones, which come from calcium, magnesium, and ammonium phosphate (10-15%); and (4) cystine stones originating from cystine, which reacts with lysine, arginine, and ornithine (1%) (Moftakhar *et al*., 2022; Siener, 2021). In addition, chronic kidney failure is triggered by diabetes mellitus with nephropathy complications characterised by albuminuria (Miranda-Díaz *et al*., 2016).

Nowadays, a large number of packaged drinks on the market has changed people's lifestyles, so they do not drink enough water and consume lots of these packaged drinks, mainly containing fructose, caffeine, taurine, tartrate, and electrolytes, one of which is an energy drink (Mansy *et al*.,2017; Schifferstein, 2020). Energy drinks are non-alcoholic beverages designed to provide energy by adding energy-boosting ingredients, especially caffeine (Mansy *et al*., 2017).

Energy drinks are available in shops and supermarkets, usually next to soft drinks and fruit juices (Houghtaling *et al*., 2021). These energy drinks are formulated to provide additional energy through a combination of methylxanthines, vitamins, and herbal ingredients (Mansy *et al*., 2017). They generally consist of caffeine, plant-based stimulants (guarana, ephedrine), sugar (glucose, fructose), amino acids (taurine, carnitine, creatine), herbs (ginseng, ginkgo biloba), and soda

(citric acid and sodium bicarbonate) (Mansy *et al*., 2017; Higgins *et al*., 2018).

The main content of energy drinks, namely caffeine, is related to the diuresis effect and fluid-electrolyte balance (Temple *et al*., 2017). Caffeine stimulates renal glomerular filtration and inhibits sodium reabsorption, triggering increased sodium and water excretion (Sawie *et al*., 2023). In addition, caffeine increases bone calcium resorption, causing osteoporosis, and increases the excretion of calcium through the kidneys, which accelerates the formation of kidney stones, resulting in excessive pain reactions due to damage in the surrounding tissue cells (Lai *et al*., 2022).

This study aims to explore the effect of chronic intake of energy drinks on the kidney function of experimental animals with diabetes mellitus (DM).

Methods

Experimental animals

Thirty-two male Wistar rats, 8-10 weeks old, weighing about 120-160 grams with a healthy physical condition, were used in this study. Seven days before the study, the animals were acclimatised to the environment. The room for rearing was set at an air temperature of 23 ± 1°C, while the lighting was arranged in such a way as to change light and dark every 12 hours (Rahmadi *et al*., 2022).

Ethical considerations

This research was conducted at the Animal Laboratory Research Center at the Faculty of Pharmacy, Airlangga University. All protocols in this study were approved by the research ethics committee of the Faculty of Veterinary Medicine, Airlangga University (Animal Care and Use Committee).

Treatment materials

The treatment materials and reagents employed included three energy drink brands, two of which were in solution form (coded EDK and EDH) containing 1,000 mg of taurine, 50 mg of caffeine, and a combination of B vitamins, ginseng extract, inositol, and sorbitol in every 150 ml. The third energy drink (coded EDE) was in powder and contained 1000 mg of taurine, 50 mg of caffeine, a combination of B vitamins, royal jelly, ginseng extract, and aspartame in every 4.6 g of powder. EDE was dissolved in water to a volume of 150 ml.

Experimental protocol

A single injection of 150 mg/kg of alloxan dissolved in 0.9% NaCl was administered intraperitoneally to induce DM. At 72 hours post-injection and the end of the treatment, random blood glucose was measured to determine whether the rats had DM (>200 mg/dL) using the Photometer (Robert Riele GmbH & Co KG, Germany) (Qinna & Badwan, 2015; Puspitasari & Dira, 2022).

Rats positive for diabetes mellitus were then randomly divided into four treatment groups (n = 8 per group). The positive control group was given tap water, ED1 was given EDK, ED2 was assigned EDH, and ED3 was given EDE. The treatment was administered orally twice per day (morning and evening) for 15 days. The dose administered was 7.716 ml/kg, equivalent to 2.572 mg/kg, adjusted based on the caffeine content of 50 mg in 150 ml of energy drinks.

Sampling of urine, blood, and kidney

On day 15, after the last treatment, all rats were put back into the metabolic cage for 24 hours, and urine was collected for 24 hours. Furthermore, ± 2 ml blood was taken directly through the heart with a cardiac puncture technique. Subsequently, the rats were sacrificed by cutting the aorta. The left kidney was extracted and put into a 10% formalin buffer tube.

Urinalysis

Analysis of urine volume and creatinine levels was carried out from samples collected through enzymatic reactions. Part of the reaction results, as much as 200 µl, were injected into the urine analyser.

Renal histopathology analysis

Left kidneys were collected and fixed with 10% formalin. Sections were cut with a microtome with a thickness of 5 µm. The layer was then placed on a glass slide and stained with hematoxylin-eosin (Rahmadi *et al*., 2021). Kidney damage was assessed with the following scoring system:

- Score 0: There is no damage to the kidney tissue (no accumulation of inflammatory cells, no widening of the tubular lumen, no accumulation of debris cells in the lumen, or no widening of the Bowman's space in the glomerulus);
- Score 1: if at least 1 of the above criteria was found;
- Score 2: if at least 2 of the above criteria were found;
- Score 3: if at least 3 of the above criteria were found.

Data Analysis

Urinalysis data (urine/creatinine) and haematological data (BUN, serum creatinine, sodium, potassium, and chloride) were statistically analysed by one-way ANOVA with Tukey's post hoc test. The scoring results for the renal histopathology assessment were analysed using the Kruskal Wallis test with Dunn's post hoc test.

Results

DM induction

A single intraperitoneal injection of alloxan at a dose of 150 mg/kg succeeded in inducing a DM rat model as evidenced by the random mean blood sugar results obtained in each subject 72 hours after the injection; at the end of the treatment, the range of values was 337-451 mg/dL (>200 mg/dL), as shown in Table I (Qinna & Badwan, 2015; Puspitasari & Dira, 2022).

Table I: Random blood glucose profile of each group of rats. Data are shown as mean ± SEM (n = 8).

Groups	Random blood glucose (mg/dl)	
	72 hours after alloxan injection	End of treatment
Negative control	433.00 ± 148.33	408.33 ± 189.91
ED1	407.63 ± 100.86	309.38 ± 145.18
ED2	451.88 ± 114.36	303.50 ± 154.03
ED3	447.43 ± 141.52	337.00 ± 197.30

Effect of energy drinks on urinalysis

In general, the consumption of energy drinks does not affect the urinary creatinine profile. As for urine volume in the control and treatment groups showed an average value ranging from 14.13-40.17 ml/24h (Table II), higher than the urine volume of healthy rats. Based on the literature, the urine volume of healthy rats ranges from 0.98 ± 0.12 ml/100g/8h (Junior *et al*., 2009).

Table II: BUN, serum creatinine, sodium, potassium, and chloride level profile of each group of rats. Data are shown as mean ± SEM (n = 8).

Groups	Parameters						
	Urine volume (ml)	Urine creatinine (mg/dl)	BUN (mg/dl)	Serum creatinine (mg/dl)	Sodium (mmol/l)	Potassium (mmol/l)	Chloride (mmol/l)
Control	40.17 ± 12.99	19.89 ± 8.95	24.20 ± 1.65	0.76 ± 0.06	135.50 ± 0.50	5.23 ± 0.34	98.83 ± 0.70
ED1	23.75 ± 5.57	19.49 ± 2.97	24.71 ± 3.52	0.50 ± 0.06*	134.83 ± 0.17	5.69 ± 0.13	95.86 ± 0.40
ED2	14.13 ± 3.17	12.02 ± 5.20	71.38 ± 8.79**	0.49 ± 0.05*	0.49 ± 0.05*	4.30 ± 0.20*	103.00 ± 1.39
ED3	31.86 ± 14.06	14.60 ± 5.26	27.33 ± 2.25	0.49 ± 0.09*	0.49 ± 0.09*	4.72 ± 0.08	100.00 ± 1.59

*$p < 0.05$ *vs* Control; **$p < 0.01$ *vs* Control

Effect of energy drinks on haematological parameters

Based on the results obtained, energy drinks increased the BUN value in the energy drink treatment group (ED2) compared to the control group ($i < 0.01$). In addition, all energy drinks also triggered changes in serum creatinine values compared to the control group ($p < 0.05$). Furthermore, the energy drink (ED2) also resulted in changes in sodium and potassium values compared to the control group ($p < 0.05$ (Table II).

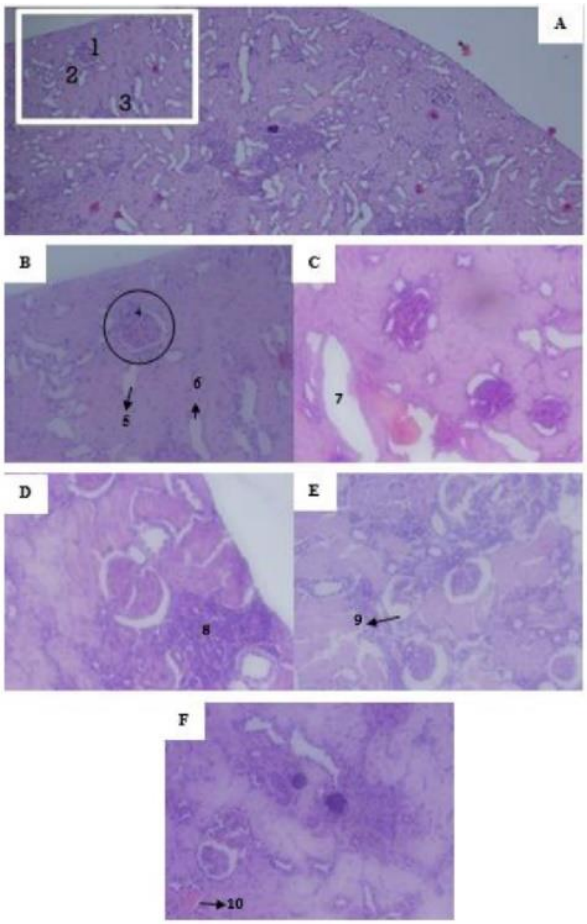
Effect of Energy Drink on Kidney Histopathology

Histopathological observations showed that energy drinks significantly damaged kidneys (Figure 1). Based on the statistical analysis, the difference in kidney damage between the control group and ED3 was significant and higher in ED3 (Table III).

Table III: Scoring results for renal histopathology assessment of each group of rats. Data are shown as mean ± SEM (n = 8).

Groups	Kidney damage score
Control	0.93 ± 0.04
ED1	0.98 ± 0.15
ED2	1.14 ± 0.15
ED3	1.74 ± 0.19*

*$p < 0.05$ *vs* Control



A: Renal tissue with 40x magnification, 1) glomerulus 2) proximal tubule 3) distal tubule; Kidney tissue with magnification 400x B: Control group, 4) glomerulus 5) proximal tubule 6) distal tubule; C: ED1 group, 7) tubular lumen dilation; D: ED2 group, 8) accumulation of inflammatory cells; E: ED3 group, 9) Bowman's space dilation; F: control group, 10) accumulation of cell debris in the lumen.

Figure 1: Representative histopathological feature of a rat's kidney after administration of energy drinks for 15 days.

Discussion

This study examined the effect of chronic intake of energy drinks on kidney function in DM rats. Some of the parameters evaluated included urinalysis, haematology, and histopathology of the kidneys. The day before they were sacrificed, the experimental animals were put into metabolic cages to collect urine for 24 hours to measure the creatinine parameter. The normal rat urine volume was 0.98 ± 0.12 ml/100g/8h (Junior *et al*., 2009), while the urine volume of experimental animals in this study ranged from 14.13 to 40.17 ml/24h, higher than the urine volume of normal rats, indicating polyuria in the experimental animals. In this case, polyuria was caused by two factors: the injection of alloxane to produce the DM model and the intake of caffeine contained in energy drinks. The mechanism of polyuria in DM is that high blood glucose levels cause severe dehydration of the body cells due to osmotic pressure, which causes fluid in the cells to come out. The exit of glucose in the urine will induce a state of diuresis because the osmotic effect of glucose in the tubules prevents the reabsorption of fluid. The overall effect is a massive fluid loss in the urine, leading to polyuria (Bhasin & Velez, 2016).

Caffeine in energy drinks causes diuresis by inhibiting electrolyte reabsorption in the proximal tubule and dilution segment without changes in glomerular filtration or renal blood flow (Fenton *et al*., 2015). A 24-hour urine sample collected is then examined for urine creatinine. This study found that energy drinks did not affect urinary creatinine, as indicated by the absence of significant differences between the control and treatment groups. Even so, there was a decrease in urine creatinine values in all treatment groups compared to the control group. Creatinine is freely filtered by the glomeruli and secreted by the kidney's proximal tubules. There is almost no reabsorption of creatinine by the kidneys. If glomerular filtration is reduced, the blood level will be high. Hence, the creatinine level in the blood and urine reflects the glomerular filtration rate (GFR) (Iacobelli & Guignard, 2021). Previous studies showed that creatinine excretion in the urine was decreased in patients with kidney failure (Moreno-Gómez-Toledano *et al*., 2022).

In addition to testing the urine sample, blood samples were also tested to evaluate BUN and creatinine. Based on previous studies, normal BUN levels in rats are 15-21 mg/dl (Johnson-Delaney, 1996). Compared to normal levels, the BUN levels of the four treatment groups were above normal, especially between the control group and the ED2 group. The second parameter examined from the blood sample was serum creatinine. The findings showed that serum creatinine levels of the three treatment groups were significantly lower than the control. BUN and serum creatinine are screening tests for kidney function. BUN and serum creatinine reflect GFR, but the relationship between BUN and serum creatinine with GFR is not a straight line but a parabolic curve. Serum BUN and creatinine values remain within the normal range until >50% of renal function is impaired. However, within that range, double the normal BUN and serum creatinine (e.g., BUN increasing from 8 to 16 mg/dl or serum creatinine rising from 0.6 to 1.2 mg/dl) indicates a 50% reduction in GFR. Therefore, this test may be biased in the early stages of kidney disease. At all stages of renal insufficiency, serum creatinine is a more reliable indicator than BUN. This condition is because BUN may be affected by food and physiological conditions

unrelated to kidney function. For example, patients with congestive heart failure and normal kidneys generally have a BUN of 50 to 70 mg/dl and a serum creatinine below 1.2 mg/dl. Despite the many limitations of BUN, the examination, in combination with serum creatinine, is a functional screening test for evaluating kidney disease (Manoeuvrier *et al*., 2017). Therefore, a histopathological analysis of the kidney is needed to confirm any suspicion of impaired kidney function.

Furthermore, blood samples taken from experimental animals were also examined for electrolyte parameters, including sodium, potassium, and chloride. Sodium and chloride ions are freely filtered in the glomerulus, so the concentration of these ions in the filtrate is the same as their concentration in the blood (Watson & Austin, 2021). The kidneys then reabsorb large amounts of salt in the proximal tubule and loop of Henle. The remaining fraction is reabsorbed under tight regulation in the distal tubule and collecting ducts to maintain an accurate salt balance (Alexander & Dimke, 2022). Potassium is the principal intracellular cation. The kidneys excrete only 5-15% of filtered potassium to maintain potassium balance. Like sodium, potassium is freely filtered in the glomerulus but undergoes a different process in the tubules (Oldridge & Karmarkar, 2015). Sodium ions are reabsorbed throughout the nephron, and any excreted sodium is non-reabsorbed (Vallon & Thomson, 2020). Conversely, almost all filtered potassium is reabsorbed before the filtrate reaches the collecting tubule (Vallon & Thomson, 2020). In kidney failure, potassium accumulation is caused by reduced nephrons excreting potassium (Yamada & Inaba, 2021). In addition, the decreased ability of the kidneys to excrete sodium causes inadequate excretion of sodium. Hyperkalemia usually shows decreased urinary potassium secretion. The cause is the movement of potassium out of cells or renal retention (kidney failure) (Palmer & Schnermann, 2015). But in this study, the average potassium levels for the control group, ED2, and ED3, were below the normal potassium levels (5.4-7.0 mmol/l) (Johnson-Delaney, 1996). However, the sodium data was below the normal level range (143-156 mmol/l), which means that the experimental animals experienced hyponatremia (Johnson-Delaney, 1996), which reflects hypo osmolality unless there is an excess of other osmolytes in the plasma. Hyponatremia results from excess water intake and renal retention (renal failure and oedema) (Oldridge & Karmarkar, 2015; Vallon & Thomson, 2020). In this study, the experimental animals were DM models, and excess glucose was one of the causes of hyponatremia.

Lastly, to ensure that the abnormal levels of these parameters lead to impaired kidney function, it was essential to analyse histopathological slices of the kidneys with HE staining. Mild kidney damage occurred in the control group because the experimental animals had DM. Furthermore, DM has implications for various chronic complications responsible for multiple morbidity and mortality, including diabetic nephropathy. These complications reflect a complex pathophysiology in which numerous genetic and environmental factors determine susceptibility and progression to chronic renal failure or end-stage renal disease (ESRD). Furthermore, there was a significant difference in kidney damage between the control group and the energy drink treatment group (ED3), indicating more severe kidney damage in the treatment group than in the control group.

Energy drink ingredients, mainly caffeine, taurine, and sweeteners, potentially cause kidney damage. Caffeine is a non-selective adenosine receptor antagonist. At low concentrations, adenosine constricts the afferent arterioles through the A1 receptors, whereas at higher concentrations, it activates the A2 receptors and dilates the efferent arterioles. Activation of both types of receptors results in a marked reduction of intraglomerular pressure and prevention of glomerular hyperfiltration. Blockade of adenosine receptors has the opposite effect. Therefore, inhibition of adenosine receptors by caffeine can impair kidney function and exacerbate kidney damage in obese and diabetic patients (Peleli & Carlstrom, 2017). One of the three energy drinks used in this study contained aspartame as a sweetener. In the body, aspartame is metabolised into its constituent components, i.e. aspartic acid (40%), phenylalanine (50%), and methanol (10%). The absorption of methanol in the body is accelerated into formaldehyde, formic acid, and diketopiperazine, which will accumulate nucleic acids, proteins, and lipids. Formaldehyde causes damage to the kidneys and other organs. Such damage is due to slow excretion from the body and use that exceeds the limits (Czarnecka *et al*., 2021).

Further research is necessary to confirm the findings in this study by comparing the treatment group to the normal group without DN induction and investigating the direct effect of energy drinks on healthy conditions. There is also a need to investigate the challenges and opportunities associated with exploring the impact and underlying mechanism of each single compound present in energy drinks on changes in kidney function biomarkers.

Conclusion

Based on the findings obtained in this study, consuming energy drinks (especially the energy drink coded EDE) influenced the results of haematological examinations, i.e. BUN, serum creatinine, sodium, and potassium. The histopathological observation of the kidney demonstrated that energy drinks lead to significant kidney damage. The energy drinks coded EDH and EDH also produced several changes in kidney function biomarkers but not as much as EDE. Thus, energy drinks have a detrimental effect on the kidney function of the DM rat model.

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