

EFFECTS OF CAFFEINATED ENERGY DRINK WITHDRAWAL ON HISTOLOGICAL AND BIOCHEMICAL PARAMETERS OF ADULT ALBINO RAT KIDNEYS

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ABSTRACT

Objective: The present study investigated withdrawal effects of caffeinated energy drink on renal histological and biochemical parameters of adult albino rats.

Material and method: Thirty adult male rats weighing 250 ± 10 gm were equally divided into 3 groups. Rats in control group I received normal diet and water for 8 weeks. Rats in energy drink group II received 3.57ml/kg body weight red bull daily by gavage for 8 weeks. Withdrawal group III received 3.57ml/kg body weight red bull for 4 weeks daily, followed by normal diet and water for next 4 weeks. Blood samples were taken through tail vein at end of 4 and 8 weeks for determination of serum urea and creatinine levels. After completion of 8 weeks' rats were sacrificed, kidneys were removed and slides were prepared using Periodic acid-Schiff stain. Readings were taken through image j software and results were analyzed by SPSS.

Results: On histological examination decrease in diameter of glomerulus along with widening of bowman capsule space was observed. In withdrawal group, the histological changes were not reverted to normal since no significant difference was observed when compared to energy drink group with $P \geq 0.05$. Amongst the biochemical parameters, serum urea levels were increased significantly in the energy drink group. In the withdrawal group, serum urea levels were not reduced at the end of 8 weeks, results were significant when compared with control group with p value 0.000. The results were not significant in case of serum creatinine levels.

Conclusion: Histological and biochemical changes in adult albino rats kidneys are not reversible after withdrawal of caffeinated energy drinks.

Keywords: caffeinated, energy drink, withdrawal, glomerular, biochemical parameters

This article may be cited as: Bano SS, Ali S, Ayub S, Ahmed A, Bajwa I. Effects of caffeinated energy drink withdrawal on histological and biochemical parameters of adult albino rat kidneys. *J Med Sci* 2020 April;28(2):107-111

INTRODUCTION

Energy drinks are soft drinks that contains sugar along with stimulant, usually caffeine, besides other ingredients¹. Attitudes and practices concerning energy drink consumption and the prevalence of side effects among medical students. Twenty-two percent of respondents were regular users, particularly men ($p < .0005$). They are advertised in providing energy, physical and mental alertness. Due to their high caffeine levels these drinks have been associated with adverse health effects². Kidneys are one of vital organs of our body, having excretory function

in removal of toxic and metabolic compounds. Overload of toxins from certain compounds can lead to inflammation that can affect kidney function leading to renal damage and failure³.

Nowadays trends are changing and we are moving from fresh juices towards fizzy drinks and energy drinks are widely used by youth during different routine activities. Red bull is pioneer and most widely used brand among youth⁴. Red bull contains caffeine, taurine, guarana and vitamin B12 series⁵. Amount of caffeine present in 250ml of a can of red bull is 150 mg/L. Data proves that if beverage contains amount of caffeine in doses of 150 mg/L the message of high caffeine content must appear on label⁶. In Pakistan youth is more towards use of these drinks, majority of the users are male and they are unaware of amount of caffeine contained in energy drinks⁷. Pakistan regulatory food authority banned the sale of caffeinated energy drinks, in spite of that these are freely available in stores without age group limitations.

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Date received: 25-02-2020

Date revised: 02-05-2020

Date accepted: 05-05-2020

Caffeine is socially acceptable psychoactive drug, having addictive properties⁸. Physical and psychological dependence of caffeine occurs with persistent consumption of caffeine-containing drinks or foods⁹. Children and adolescents are group that is more sensitive to effects of caffeine contained in these caffeinated energy drink¹⁰ the Internet, MD Consult, and CINAHL. The popularity of energy drinks and the rapid growth of their excessive consumption among adolescents and young adults have brought about great concern in regards to overall health and well-being. Caffeine, which is readily available to minors, is the most commonly used psychoactive substance in the world and imposes a potentially harmful influence on health, academic performance, and personal adjustments. Teens and young adults account for nearly \$2.3 billion of energy drink sales. Adolescents and young adults are often unaware that various products, such as energy drinks, herbal medications, and various other medications that promote alertness, contain caffeine. When these products are taken together, caffeine toxicity and severe adverse effects can occur. Practitioners need to be aware of the consequences of energy drink consumption and be prepared to provide appropriate patient education. ©2012 The Author(s). Active component in red bull, that is caffeine, disrupts redox homeostasis and leads to production of reactive oxygen species (ROS). These reactive oxygen species inhibits different cellular antioxidants and can lead to oxidative stress in renal parenchymal cell besides other organs of body¹¹. Damage by this oxidative stress can also lead to histological as well as biochemical alterations in kidneys that deteriorate kidney functions and can lead to acute renal failure^{12,13} including a recent hypoglycemic episode. Rechallenge was not attempted.

MATERIAL AND METHODS

Thirty healthy adult male, albinos, Sprague Dawley rats, weighing 250 ± 10 grams, selected by simple random sampling were used in this experimental study. Female rats and animals with disease and pathology were excluded. The animals were kept in metallic cages at animal house Islamabad with suitable laboratory environment and were acclimatized for four weeks. Thereafter they were equally divided into three groups with ten rats in each group.

Dose calculation: Dose of energy drink used in this study were equivalent to 250-ml can ingested by a 70-kg individual. Dose of 3.57 ml/kg corresponds to 3.75 ml/1000gm or 0.00375m/gm of rat. The weight of rat was 250gm so dose for one rat was almost equal to 0.94ml. Doses were measured by 5cc plastic syringe and were given orally by gavage.

Control group I: Rats in this group were given normal diet and water daily for a period of eight weeks

Energy drink group II: Rats in this group received daily doses of red bull for eight weeks

Withdrawal group III: Rats in this group received daily doses of red bull for first four weeks, followed by normal diet and water for next four weeks

Blood sample collection: Blood samples were taken through tail vein at day 1 and day 28. Thereafter, samples were taken by cardiac puncture at end of experimental study (day 56). Blood samples were centrifuged and serum extracted was used for biochemical analysis of serum urea and creatinine levels.

Histological analysis: At end of experimental study all animals were sacrificed. Kidneys were removed and immediately fixed in 10% formalin for 48hrs. Dehydration was done with graded series of alcohol; tissue was cleared in xylene and embedded in paraffin. Transverse sections of 5 μm thickness were stained with Periodic acid-Schiff (PAS) stain. All slides were examined under light microscope at 40X. Glomerular parameter measurements were taken by zooming in image 50% in image j software.

Statistical analysis: Data was analyzed in SPSS version 22. One-way analysis of variance (ANOVA) was applied for mean comparison of quantitative variables followed by Post hoc Tukey test for multiple comparisons of these groups. A p value of equal or less than 0.05 was considered as significant.

RESULTS

Assessment revealed that serum creatinine was elevated at 5.5 mg/dL, from a baseline of 0.9 mg/dL. An interview revealed a 2- to 3-week history of daily ingestion of 100 to 120 oz of Red Bull energy drink. Resolution of renal dysfunction occurred within 2 days of discontinuation of Red Bull and persisted through 10 months of follow-up.

The mean diameter of glomerulus in control group was $161 \pm 0.94 \mu\text{m}$, which was reduced to $121 \pm 1.02 \mu\text{m}$ in energy drink group, showing a significant difference (p value = 0.000). In withdrawal group, mean diameter of glomerulus was $134.1 \pm 1.00 \mu\text{m}$ after 8 weeks. There was significant difference in the mean diameter of glomerulus in the withdrawal group when compared with the control group with p value 0.000 (fig:1). Bowman space was increased significantly from $13.7 \pm 0.26 \mu\text{m}$ in the control group to $43.8 \pm 0.75 \mu\text{m}$ in the energy drink group. In withdrawal group, mean bowman space was $34.4 \pm 0.70 \mu\text{m}$, result was significant when compared to control group (table 1 and 2). Serum urea levels were increased significantly in energy drink group at day 56. Serum urea levels were reduced in withdrawal group at day 56, results were significant when compared with control group with p value 0.000, no significant difference was observed in case of creatinine levels in withdrawal group at day 56 when compared with control group (table 3 and 4).



Figure 1: Photomicrograph of histological section at 40X on PAS staining showing normal diameter of glomerulus(D)and bowman space(BC) in control group I, energy drink group II showing shrinkage of glomerulus and widening of bowman space, withdrawal group III also showing shrinkage of glomerulus and bowman space widening.

Table 1: Comparison of mean diameter of glomerulus and bowman space among control and experimental groups of albino rats (N=30)

Groups	Diameter of glomerulus (μm)		Bowman space (μm)	
	Mean	SEM	Mean	SEM
Control	161.5	0.94	13.7	0.26
Energy drink	121.0	1.02	43.8	0.75
Withdrawal	134.1	1.00	34.4	0.70
P value	0.000*			

Table 2: Multiple comparison of mean diameter of glomerulus and bowman space among control and experimental groups of albino rats

Groups	Diameter of glomerulus in μm		Bowman space in μm	
	Mean difference	p value	Mean difference	p value
control vs energy drink group	40.5*	.000*	30.1*	0.000*
Control vs withdrawal group	27.4*	0.00*	20.7*	0.000*
Energy drink vs withdrawal group	13.1	.000*	9.40	0.000*

Table 3: Comparison of mean levels of serum urea (mg/dl) in albino rats (N=30) at day 28 and day 56.

Groups	Serum urea levels at day 1	Serum urea levels at day 28	Serum urea levels at day 56
Control	0.52 \pm 28.50	0.52 \pm 28.50	1.2 \pm 34.10
Energy drink		0.00 \pm 29.00	0.67 \pm 41.40
Withdrawal		1.57 \pm 31.40	0.57 \pm 39.20
P-value	0.00		

Table 4: Comparison of mean levels of serum creatinine (mg/dl) in albino rats (N=30) at day 28 and day 56

Groups	Serum creatinine levels at day 1	Serum creatinine levels at day 28	Serum creatinine levels at day 56
Control	0.00 \pm 0.28	0.00 \pm 0.28	0.00 \pm 0.29
Energy drink		0.00 \pm 0.28	0.01 \pm 0.31
Withdrawal		0.00 \pm 0.30	0.01 \pm 0.31
P-value	0.00		

DISCUSSION

It could be presumed from results of this study that high caffeine content in energy drinks is having damaging effects on histological and biochemical parameters

of adult albino rats' kidneys. Administration of red bull for period of 8 weeks resulted in shrinkage of glomerulus and widening of bowman space. This was evident that these histological alterations were not reversible at end of

8 weeks. The histological alterations in present study are consistent with results of Sajjad et al, who used soft drinks in his experimental study and observed widening of Bowman space and shrinkage of glomerulus on kidneys of albino rats¹⁴. Taiwo observed similar adverse effects of red bull on histological and biochemical parameters of liver and kidneys of rabbits¹⁵.

The present study showed that serum urea levels increased progressively till 8 weeks by the use of red bull. Changes observed were not reverted to normal after withdrawal of energy drink, although no significant difference was observed in case of serum creatinine levels. Similar increase in serum urea and creatinine levels has been reported when rats were given energy drink in different doses¹⁶. Ugwuja used energy drinks in low and high doses alone and in combination with alcohol, observed effects were alterations in renal and hepatic biochemical parameters¹⁷.

According to Mansy et al, chronic consumption of energy drinks for 12 weeks increases creatinine and uric acid levels besides alterations in liver function tests, the observed effects were due to free radical production and oxidative stress¹⁸. Study conducted by Ogunlabi et al. suggested that chronic energy drink intake alters liver and kidney functions of rats¹⁹. The results of this study are consistent with results of another study conducted on red bull that showed significant increase in urea and creatinine levels after administration of drink²⁰.

The results of our study are contrary to Masoud et al who observed no changes in serum urea levels after 35 days of energy drink and khat administration, results might be due to short duration of study¹⁷. Results of this study are against the results of Akande et al who observed same biochemical findings in kidneys and liver of rats when they were given energy drinks and changes observed were reversible in 14 days duration of study²¹. In another study heavy intake of cola beverages for three months caused no change in urea and creatinine levels, in that study glomerular morphology was also unaltered²². Hajra studied withdrawal effects of sting on behavior of male rat pups and observed effects were impaired memory, anxiety and depression²³. The results of our study demonstrated that renal histological and biochemical alterations are not reversible after withdrawal of caffeinated energy drink in this duration of study.

CONCLUSION

Histological and biochemical changes in adult albino rats' kidneys are not reversible after withdrawal of caffeinated energy drinks. Since these caffeinated energy drinks have deleterious effects on kidney system; therefore, it should be used with caution. There should be check by government of Pakistan on free availability of these drinks especially for youth.

RECOMMENDATIONS

Further studies are recommended to observe long term effects of energy drinks and their reversal with prolonged duration.

There should be check by government of Pakistan on free availability of these drinks as they are hazardous and their sale should be limited.

Regarding amount of caffeine in energy drinks and their psychological dependence awareness programs are necessary.

To ban sale of energy drinks especially in children and youngsters, legislations should be made by government of Pakistan.

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CONFLICT OF INTEREST: Authors declare no conflict of interest

GRANT SUPPORT AND FINANCIAL DISCLOSURE: NIL

AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under

Bano SS: Manuscript writing, slides examination, experiment conduction

Ail S: Topic selection, guidance in writing and experiment conduction, supervisor

Ayub S: Covered biochemical aspect in manuscript, sample collection, tests, result and data collection

Ahmad A: Data collection, discussion

Bajwa I: Statistical analysis and result interpretation

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.