ORIGINAL ARTICLE

Histological Effects of Caffeinated Energy Drink Consumption and Its Withdrawal on Kidneys of Experimental Rats

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ABSTRACT

Objective: The present research was carried out to observe withdrawal effects of energy drinks, whether these histomorphological changes are reversible or not.

Study Design: Laboratory based experimental study.

Place and Duration of Study: The research was carried out from 1st July to 30th August 2019 at national institute of health Islamabad.

Materials and Methods: Total thirty adult male albino rats were divided into 3 groups by simple random sampling, with ten rats in each group. Group I was control group, while energy drink group II received 3.57ml/kg body weight red bull corresponding to one can of energy drink (250ml) in humans orally for eight weeks. Rats in withdrawal group III received energy drink for first four weeks followed by normal diet and water for last four weeks. After eight weeks, rats were sacrificed and their right kidneys were removed. Slides were prepared using hematoxylin eosin and Periodic Acid Schiff Stain, results were analyzed by SPSS.

Results: The results showed that use of energy drink for 8 weeks resulted in increase in weight of kidneys along with histological alterations in renal cortex of rat kidneys. Grade 4 (severe) congestion, hemorrhage, loss of brush border and necrosis was observed in energy drink group II. Withdrawal of energy drink in group III resulted in weight of kidneys near to control group along with significant reduction in congestion, hemorrhage, loss of brush border and necrosis grades from grade 4 to grade 3 and 2 with $P \leq 0.05$.

Conclusion: Caffeinated energy drinks are having damaging effects on kidneys of albino rats and these histological changes caused by caffeinated energy drinks in this duration of study and in low doses corresponding to one can of energy drink (250ml) in humans are reversible.

Key Words: *Caffeinated, Energy Drink, Histological, Kidneys, Withdrawal.*

Introduction

Energy drinks (EDs) were first introduced in UK in 1929 as hospital drink; in 1980 they were promoted for replenishing lost energy. They are now available in > 140 countries as part of a multi- billion dollar business.[1] In 1960s they appeared in Asia and Europe.[2] There are diverse types of energy drinks available in Pakistan. First and most trendy caffeinated energy drink to be introduced is red bull.

Target of these caffeinated drinks market is mainly youth. In Pakistan majority of energy drink users are youngsters with age group of 13-35 years. EDs claim to provide burst of energy by using a combination of caffeine (chief active ingredient), guarana, yerba mate, taurine, glucose, fructose and glucuronolactone.[3] Caffeinated energy drinks (EDs) contain higher levels of caffeine along with other ingredients that are not commonly found in sodas and juices, marketed as providing mental and physical stimulation especially in youth. They differ from soft or sport drinks due to their unique composition.[4]

Caffeine present in these drinks is up to 500 mg per 20 oz. (600 mL) serving that is 15 times the amount of caffeine present in a 12-ounce (360 mL) serving of cola. Beverages that contains guarana actual levels of caffeine are much higher than levels mentioned on the label.[5] Youth using EDs is unaware of these high

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levels of caffeine and its adverse effects that is alarming.

The kidneys are the organs that filter waste products from the blood. Kidneys are predominantly vulnerable to ischemic and toxic damage. Many studies have depicted adverse health effects of EDs, one of them is hastened progression of renal micro vascular impairment and chronic kidney disease. [6] Literature illustrates that exposure of rats to energy drink leads to kidney damage causing renal vascular congestion, hemorrhage of interstitial tissue, focal atrophy and degeneration of lining epithelium of Proximal and Distal convoluted tubule.[7] There is limited data available regarding withdrawal effects of these drinks, therefore present study was conducted to observe nature of renal damage and to observe changes after withdrawal of these drinks whether these changes are reversible or not.[1]

Materials and Methods

This laboratory based experimental study was conducted from 1st July to 30th August 2019 by mutual collaboration with national institute of health (NIH) and Islamic international medical college (anatomy department) after approval from ethical review committee (ERC).

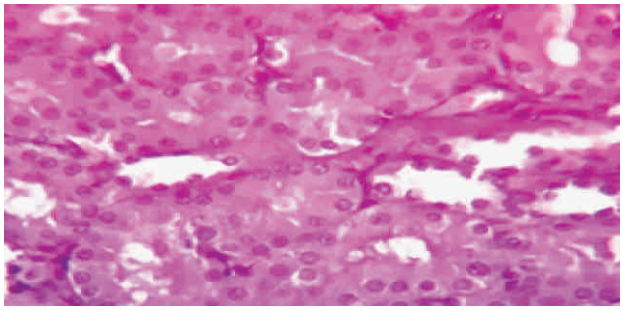
Thirty adult male healthy albinos Sprague Dawley rats (*n*=10/group) weighing 250±10 gm were used in experiment. They were housed at animal house of NIH Chak Shahzad Islamabad and were acclimatized to laboratory surroundings with free food and water access under natural dark and light rhythms prior to commencement of study. Female rats and rats with any disease or pathology were excluded. Total thirty animals were divided into three groups each group having ten rats selected by simple random sampling and treated in this way:

Control Group I (*n*=10)	Received normal diet and water for eight weeks.
Energy Drink Group II (*n*=10)	Received 3.57ml/kg red bull orally for eight weeks.
Withdrawal Group III (*n*=10)	Received 3.57ml/kg red bull orally for first four weeks followed by routine diet in next four weeks.

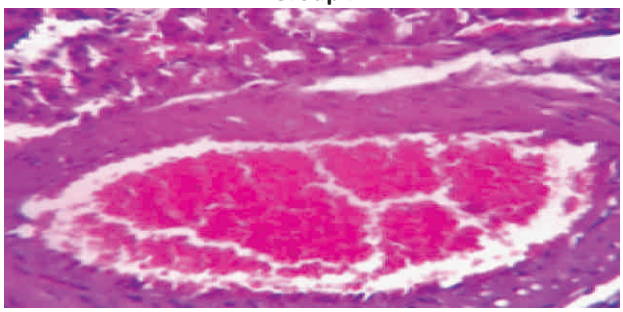
After 8 weeks of completion of experimental study rats were dissected and kidneys were removed. After fixation and embedding transverse sections of 5 µm thickness were obtained. Staining was done with Hematoxylin and eosin and Periodic Acid-Schiff stain. The slides were examined under light microscope in X10, X40 Power. Parameters observed were weight of kidneys, congestion, hemorrhage, loss of brush border, and necrosis. Using SPSS version 21 non parametric data was analyzed by means of chi square test. A *p* value of equal or less than 0.05 was considered as significant value.

Results

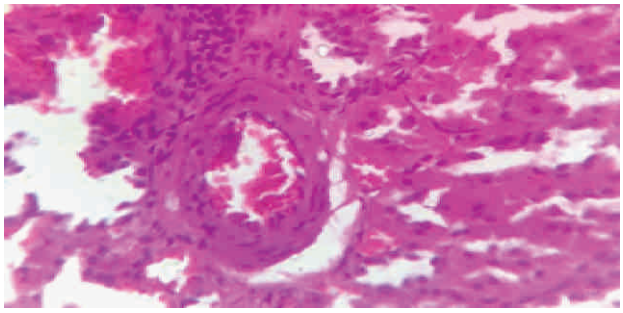
Renal cortical parenchyma appeared to be normal in 100% rats in control group I, while 25% rats showed minimal congestion (grade 1). In ED group severe congestion (grade 4) was noted in 62.5% of rats. In withdrawal group 62.5% lesions were moderate (grade 3) showing reversal of histological alterations caused by EDs (Fig: 1, Table I).



Group I



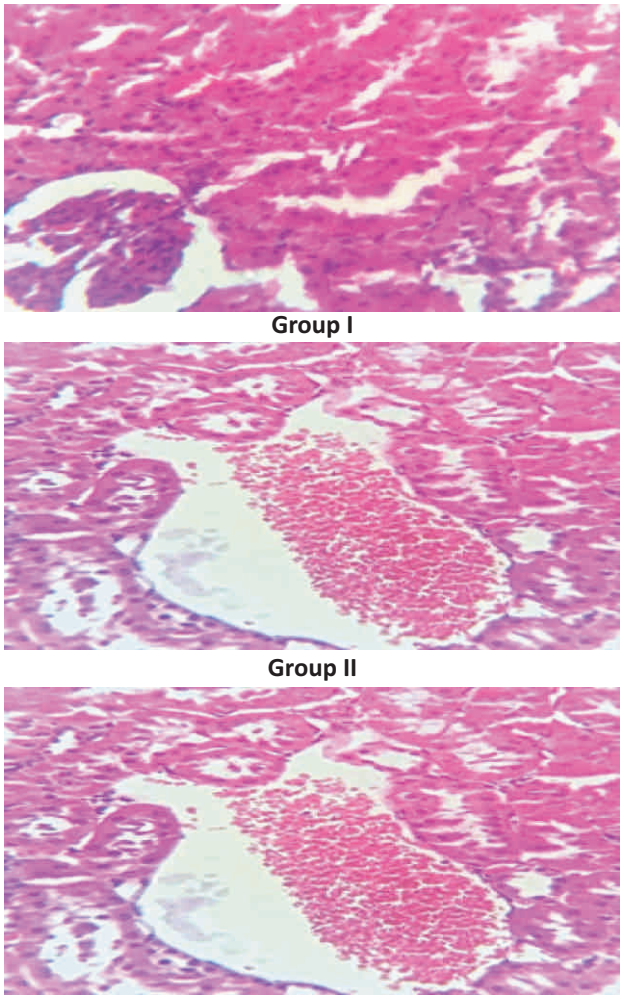
Group II



Group III

Fig 1: Distribution of Renal Cortical Parenchymal Congestion Among Different Groups, Group I K1R5 Showing No Congestion, Group II K1R2 Showing Severe Congestion And Arrows Indicating Moderate Congestion n Group III K1R7 (H&E, X400).

In 100% rats of control group I no hemorrhage was observed in renal cortex while in ED group severe hemorrhage (grade 4) was noted in 75% of rats. In withdrawal group 75% lesions were mild (grade 2) showing significant reduction in hemorrhage grade when compared to ED group (Fig: 2 and Table I).



Group I

Group II

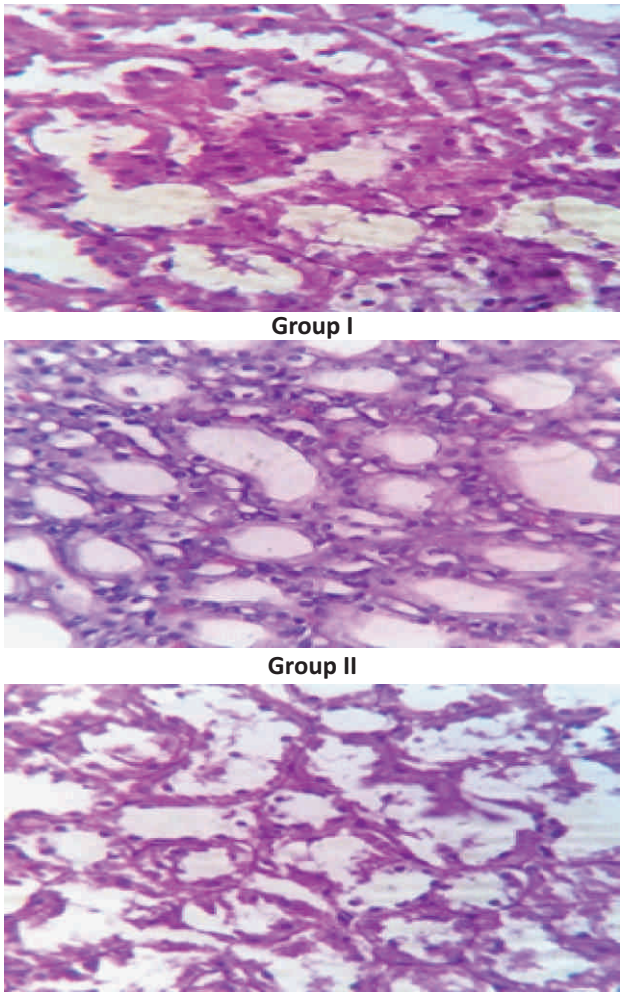
Group III

Fig 2: Distribution of Hemorrhage In Renal Cortical Parenchyma of Different Groups, Group I K1R2 Showing No Hemorrhage, Group II K1R8 Showing Severe Hemorrhage and Arrows Indicating Mild Hemorrhage In Group III K1R9 (H&E, X400).

Table I: Distribution of Grades of Congestion and Hemorrhage In Control and Experimental Groups of Albino Rats By Chi-Square Test

Grades	Congestion			Hemorrhage		
Grade 0	100%*	0%	0%	100%	0%	0%
Grade 1	0%	0%	0%	0%	0%	12.5%
Grade 2	0%	0%	25%	0%	12.5%	75% *
Grade 3	0%	37.5%	62.5%*	0%	12.5%	12.5%
Grade 4	0%	62.5%*	12.5%	0%	75%*	0%
***p* value**	0.000*			0.000*		

Renal cortex appeared to be normal in 100% rats in control group I. In ED group severe (grade 4) loss of brush border was observed in PCT of 87.5% rats. In withdrawal group mild loss of brush border in 50% of rats was seen showing significant reversal of histological alteration after withdrawal of ED. (Fig: 3, Table II)



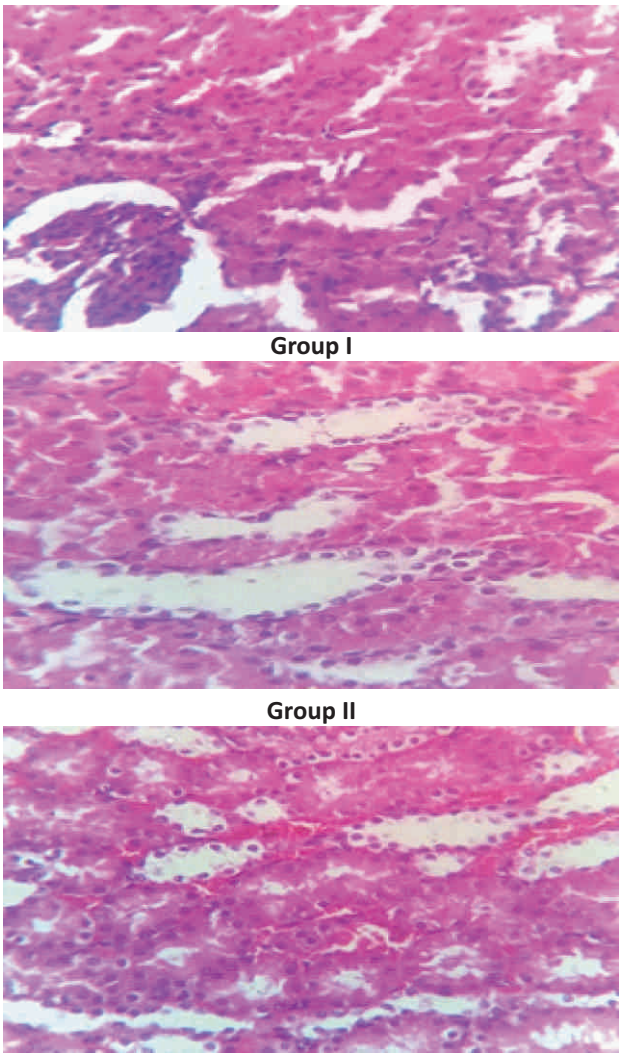
Group I

Group II

Group III

Fig 3: Distribution of Loss of Brush Border In PCT Of Different Groups, Group I K1R5 Showing Prominent Apical Brush Border, Group II K1R9 Showing Severe Loss of Brush Border In PCT And Arrows Indicating Mild Loss of Brush Border In Group III K1R1 (PAS, X400).

In control group 100% rats showed normal renal cortical architecture. In ED group severe (grade 4) necrosis was observed in 100% of rats. In group 3 moderate (grade 3) necrosis was seen in 75.5% rats showing significant reduction in grades of necrosis when compared to energy drink group. (Fig: 4 and Table II)



Group I

Group II

Group III

Fig 4: Distribution of Necrosis In Renal Tubules of Different Groups, Group I K1R3 Showing No Necrosis, Group II K1R5 Showing Severe Tubular Necrosis and Arrows Indicating Moderate Necrosis In Group III K1R7 (H&E, X400).

Table II: Distribution of Grades of Loss of Brush Border and Necrosis in Control and Experimental Groups of Albino Rats By Chi-Square Test

Grades	Loss of brush border			Necrosis		
Grade 0	100%*	0%	0%	100%	0%	0%
Grade 1	0%	0%	12.5%	0%	0%	12.5%
Grade 2	0%	0%	50%*	0%	0%	12.5%
Grade 3	0%	12.5%	37.5%	0%	0%	75%*
Grade 4	0%	87.5%*	0%	0%	100%*	0%
***p* value**	0.000*			0.000*		

p value ≤ 0.05

Discussion

This study investigated the reversal of renal histomorphological features after withdrawal of ED in kidneys of male rats. In this study we observed no congestion with normal renal cortical parenchyma in control group. In ED group severe congestion in renal cortical parenchyma was observed. In withdrawal group, moderate congestion was seen. Impairment of venous outflow due to inflammatory mediators results in a localized increase in blood to different areas of kidney, which is demonstrated histologically as congestion.[8] Taiwo et al documented congestion after administration of energy drink in different doses to rabbits.[9] He documented that changes observed were reversible in 28 days duration of study.[9] Similarly results has been supported by many other studies.[10,11,12]

In our study severe hemorrhage was observed in ED group. In rats of group III mild hemorrhages was seen. Hemorrhage observed was due to the effect of inflammation leading to expansion of blood vessels, as result vessels rupture and blood flows out. A previous study showed similar findings after administration of ED 1ml/animal/day orally for 4 weeks.[13] Comparable findings were observed in liver of rats after administration of high dose of EDs.[14] Akande also proved that damaged caused by ED was reversible in 28 days duration.[15]

The present study showed severe loss of brush border of PCT in ED group, while in withdrawal group mild loss of brush border was observed that showed reversal of histological changes caused by red bull after withdrawal of ED. This might be explained by vulnerability of cellular membranes to toxins, leading to decrease cellular production of ATP and accumulation of reactive oxygen species causing cell damage and detachment of epithelial cell from basement membrane. Similar results were shown in albino rabbits by Salih, results were ascribed to high level of caffeine in EDs.[16]

Most common cause of acute kidney injury is acute tubular necrosis that causes death of tubular epithelial cells leading to renal failure. In group III moderate necrosis was seen which was significantly reverted after withdrawal of ED.[17]Coagulative pattern of necrosis was observed in various experimental groups which is more common in toxic or ischemic injury.[18]The observed necrosis may be due to carbon dioxide present in energy drinks that damages membranes of mitochondria, change in ATP production, hypoxia and cell deah.[13]In another

study after administration of red bull, severe necrosis was observed in seminiferous tubules of male rats.[19]

In this study we observed the severity of renal injury in relation to time duration for which EDs were used. In ED group significant difference was observed in weight of kidneys, congestion, hemorrhage, loss of brush border and necrosis. This indicates that EDs leads to renal damage that is duration or time dependent, since more damage was observed in ED group when compared to group III, it showed reversal of these histological features after withdrawal of ED. Our findings are consistent with the results of study that proved that effects of caffeine on cell survival are highly time and dose dependent; in low doses it increases cell survival and at higher doses it increases super oxide production.[20,21] Also the study proved that chronic caffeine intake has age dependent effects on brain.[22]

Conclusion

In conclusion caffeinated energy drinks are having damaging effects on kidneys of rats, besides that with low doses, corresponding to one can of ED (250ml in humans) and with this duration of study histomorphological changes caused by caffeinated EDs are reversible.

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