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RESEARCH ARTICLE

Adverse Effects of Excessive Use of Some Beverages on Male Albino Rats

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ABSTRACT

Beverages are non-alcoholic drinks designed to induce stimulation by the addition of active compounds, particularly high levels of caffeine. They are currently promoted as agents that boost energy (both mental and physical capabilities), This study aims to assess the side effects of energy drinks, such as Red Bull and Red Strong, on numerous physiological parameters and histological characteristics of the liver and kidney in male albino rats. Fifteen rats were allotted into three groups: group (1) consumed distilled water as the control group, group (2) drank Red Strong energy, and group (3) drank Red Bull energy, administered orally by gavage once a day for 8 weeks, with each group receiving 2.0 mL/100 g of body weight in the energy drink. The outcome shows that the body weight gain increased significantly induced in high long-term energy drinking groups and elevated the liver function enzymes, including alanine transaminase, aspartate transaminase, total serum bilirubin, and alkaline phosphatase. Furthermore, it adversely affects renal function through increased urea, creatinine, uric acid, and decreased glomerular filtration rates. Furthermore, adverse influences on reproduction organs by a decline in testosterone and sperm properties. Histological studies showed alteration structure in energy-drinking groups such as degenerative kidney tubules, hemorrhage, shrinkage of the glomerulus and the dilated sinusoid, degenerative hepatocyte, and inflammation in the liver. The presented study showed that the high consumption of beverages (Red Strong and Red Bull) have adverse effects on the liver and kidneys of male albino rats.

Keywords: Energy drinking, Red Strong, Red Bull, caffeine, liver enzyme

INTRODUCTION

Beverages are alcohol-free drinking, which primarily consists of significant amounts of caffeine and other chemical compounds.[1] In addition, there seems to be a substantial increase in energy drink usage among young adults and athletes in recent years. However, there are worries over the potential health hazards that may arise from excessive usage.[2] The beverages enhance mental performance[3] by encouraging wakefulness, helping people stay alert, improving their mood, reducing sleepiness and fatigue, and improving driving performance.[4] Grocery stores and online platforms present an extensive selection of over 50 distinct brands of energy drinks. Moreover, their compositions are significantly different.[5] The fundamental characteristics of all these beverages are the high content of caffeine and compounds, depending on the particular brand.[6] Research indicates that for most consumers, between 15 and 30 years old, over 40% have reported a daily consumption of more than two cans.[7] Stimulant drinks frequently contain caffeine, L-carnitine, taurine, D-glucuronolactone, guarana, and vitamin B complex.[8] Reports demonstrated that prolonged and excessive ingesting of energy drinks has harmful impacts on the broad spectrum of bodily organs. They potentially result in the Initiation of multi-system organ failure for instance harm to the digestive organs.[9] Physiological consequences encompass a range of illnesses including anxiety, diarrhea, dehydration, diuresis, vomiting, and nervousness, as well as more serious results such as rhabdomyolysis and acute renal injury,[10] cardiovascular illnesses such as acute mania, tachycardia, bronchodilation elevated blood pressure, ventricular fibrillation and stroke,[4] and seizures of the neurological system.[11] Gastrointestinal disturbances include stomach acid secretion,[10] *Diabetes mellitus* with future acute pancreatitis, hepatomegaly, and hypertriglyceridemia.[12] Furthermore, pregnant women who consume excessive amounts of caffeine are at an increased hazard of experiencing late miscarriages

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and stillbirths.[13] Energy drinks containing caffeine are advertised as a means to alleviate tiredness and enhance vitality and attentiveness.[14] The purpose of this study is to assess the impact of caffeine-rich energy beverages (Red Bull and Red Strong) on several physiological indicators and the histological characteristics of male rats.

MATERIALS AND METHODS

Beverages

The beverages brands applied in the current study were Red Strong, a product of Karwanchi for soft drinks (Iraq-Kirkuk), and Red Bull, a product of Rauch Trading AG, Switzerland (manufactured for Red Bull GmbH, Austria). Strong Red and Red Bull purchased from the local market in Erbil city.

Animal Feeding and Environments

Fifteen healthy adult male albino rats (*Rattus norvegicus*) average weight of 150 ± 20 g were kept in a special cage in the animal house center. The rats were under special standard conditions, including a temperature and light, respectively, 25 ± 2°C, and a 12-h light/12-h dark cycle.

Experimental Design of Animal and Blood Collection

The rats were randomly assigned to three groups, with five in each group. Group (1): Control group (distiller water), group (2): Strong red energy drinking, and group (3): Red Bull energy drink; the Red Bull and Red Strong group were orally administrated with 2.0 mL/100 g body weight of energy drinking for 8 weeks. The doses for all groups were administered orally through gavage once daily. The groups had *ad libitum* access to tap water. The body weights of rats were recorded (every week) to calculate the body weight gain during the experiment for 8 weeks. The rats received an overnight fast before the day during which the blood sample was collected. Then, the blood was drawn by puncturing the heart and collected in non-heparinized tubes for a biochemical test, then centrifuged to prepare serum.

Biochemical Analysis

The biochemical parameters included renal function tests (urea, creatinine, uric acid, sodium, potassium, chloride, total serum protein, and glomerular filtration rate) and liver function tests (Alanine aminotransferase [ALT], aspartate aminotransferase [AST], total serum bilirubin [TSB], and alkaline phosphatase [ALP]). The analyses were carried out using the colorimetric method with Roche diagnostic kits from Germany, applying the Auto analyzer biochemistry Cobas Integra 400 plus (Made in Switzerland).

Histopathological Analysis

The liver and kidney of each dissected animal were extracted and preserved in a 10% neutral buffered formalin solution. The preserved tissue was then processed to create slices, which were stained (hematoxylin and eosin). Finally, we applied the light microscope to observation and distinguish histological section.

Analysis of Sperm Motility

The Sperm motility was measured by counting 200 sperm cells per rat in at least 10 sample fields. This was done using a warmed slide (37°C) and a warmed coverslip (37°C) with a microscope that had a heated stage. The magnification used was (400×).[15]

Sperm Count

The procedure was performed by applying phosphate buffer saline which involved diluting the sperm suspension with Phosphate buffer (1:20). Then counted in the WBC position[16] of the Neubauer hemocytometer (eight Squares). Subsequently, the sperm cells were enumerated utilizing a binocular microscope with each square having dimensions of 1 mm^2. The resulting count was subsequently multiplied by a factor of 5×10^4 to get the total quantity of spermatozoa per million/epididymis.[15]

Statistical Analysis

The data were submitted for statistical evaluation using the statistical package Graph Pad Prism 9. The experiment that was conducted was a One-way analysis of variance and Tukey's test for comparing means between various groups. Results were applied comparison of the means and standard error (Mean ± standard error [SE]) for all groups and measured as statistically significant at $P \leq 0.05$. If the value has the same symbols (*) and (*) = non-significant, but different symbols (*) and (#) = significant.

RESULTS

The fluctuation in body weight gain of male albino rats is shown in Table 1. According to the current study, the body weight of the Red Strong group exhibited a considerable increase in comparison to the control group. There were non-significant differences between the participants in the Red Bull group. Furthermore, after being given beverages, the proportion of weight gain increased in both groups.

The renal function test findings showed a significant difference between the Red Strong and Red Bull groups in the mean ± SE of serum urea, creatinine, uric acid, and Glomerular Filtration rate (GFR) [Figure 1]. In particular, urea, creatinine, uric acid, and potassium levels were significantly increased than in the control group, and GFR was reduced considerably. Nonetheless, the groups had no significant variation in TSP, sodium, potassium, and chloride serum levels as shown in Figure 2.

Activities of enzymes in the liver are shown in Figure 3, which demonstrates the changes in liver function status in the induced groups. It shows that serum ALT and ALP levels in the Red Strong group were considerably elevated compared to group 1 but not significantly higher than in the Red Bull group. The beverage administered group (Red Strong and Red Bull) also increased their serum AST levels considerably. Nevertheless, there was no significant change in the serum TSB levels across all groups.

The influences of beverage and alternation of the reproductive system in rats were shown in Figure 4. The mean

Table 1: (Mean±SE) Effects beverages on body weight in male albino rats

Group	Parameters (Mean±SE)			
	Initial weight	Final weight	Weight gain	Weight gain%
Control	162.80±7.88	301.76±14.38	138.96±8.754*	85.5±4.57
Red strong	182.00±7.95	356.70±11.41	174.70±7.70	96.53±4.84
Red bull	167.60±4.01	329.30±6.50	161.70±8.10*#	96.87±5.73

The symbols (*) and (#) = significant, SE: Standard error

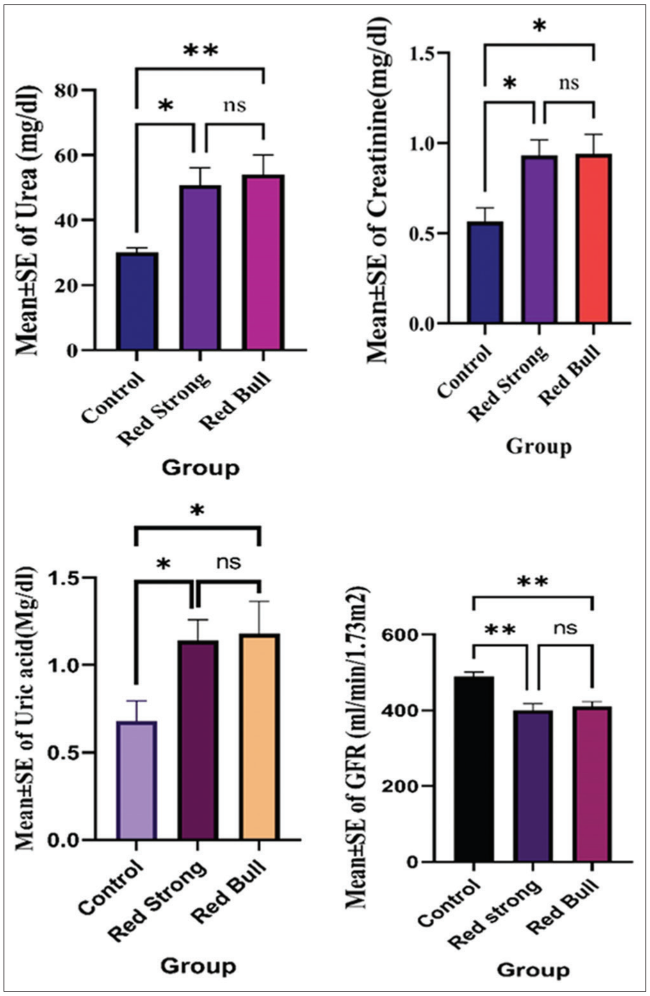

Figure 1: (Mean ± standard error) variations in the level of serum urea, creatinine, uric acid, and GFR in the control and beaverage administered group (Red Strong and Red Bull). GFR: Glomerular Filtration rate

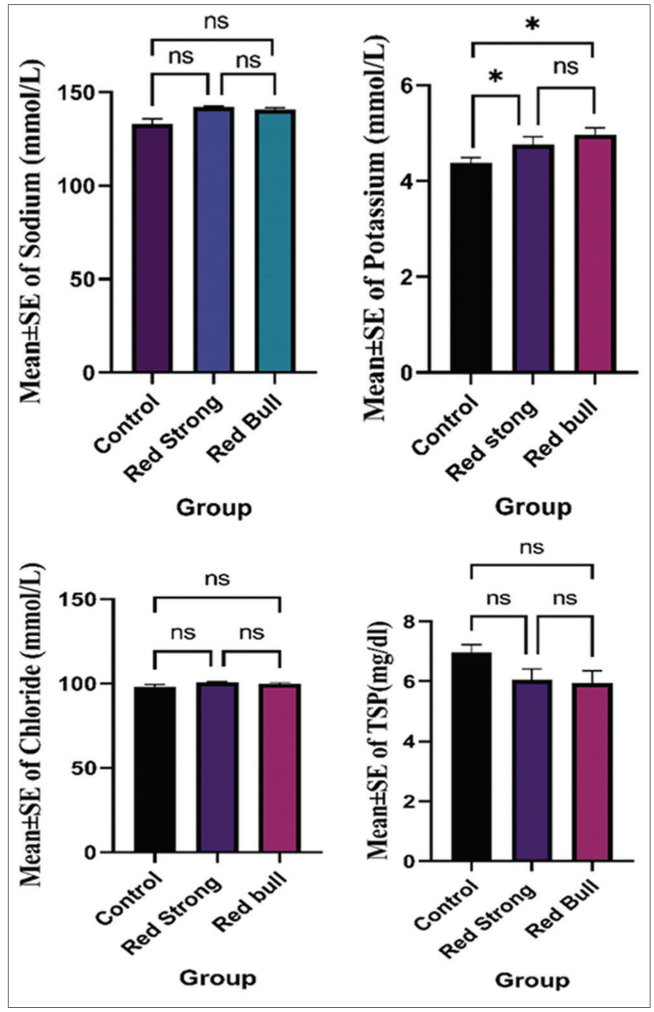

Figure 2: (Mean ± standard error) Variations in the level of sodium, potassium, chloride, and TSP in the control and beaverage administered group (Red Strong and Red Bull). TSP: Total serum protein

concentrations of serum testosterone, total sperm count, and motility significantly declined in groups 2 and 3 compared to the control group.

Histological Findings

The general architecture section through the kidney of the control group showed normal glomerulus and kidney tubules [Figures 5 and 6]. However, the degenerative kidney tubules, hemorrhage, shrinkage, and degenerative of the glomeruli, huge inflammation between urinary tubules with the absence of urinary space between glomeruli no space in kidney tubules, no urinary space, are observed in the kidney of beverage administered group [Figure 7 and 8]. Moreover, Fibrotic aggregation, with the absence of urinary space between kidney tubules, shrinkage, and degenerative of the glomeruli, is observed in the kidney of the beverage administered group, Red Bull [Figures 9 and 10]. The liver section of the control group rats appeared to have a well-defined central vein, hepatocyte, and sinusoid [Figures 11 and 12]. Sections of the liver in the Red Strong groups showed dilated sinusoids, degenerative hepatocytes, Strong fibrotic area, and Inflammation [Figures 13 and 14]. The liver sections of Red

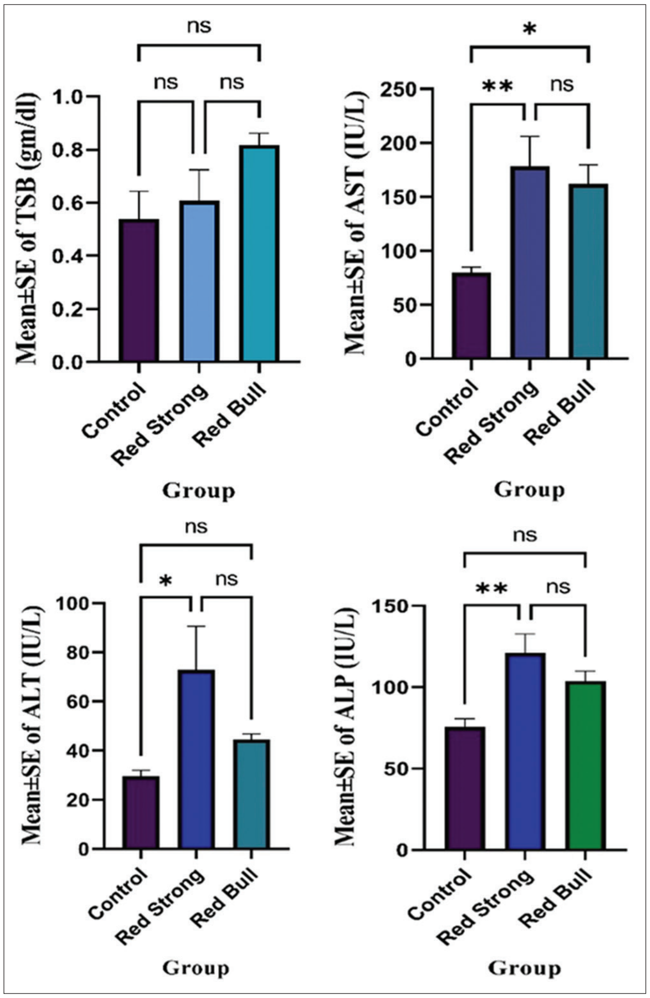

Figure 3: (Mean ± standard) Level of TSB, ALT, AST, ALP in control and beverage administered group (Red Strong and Red Bull). TSB: Total serum bilirubin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase

Bull groups illustrated dilated central veins, sinusoids, fat droplets, and inflammations [Figures 15 and 16].

DISCUSSION

Beverage is commonly consumed by adolescents and is known for its high caffeine content, which enhances energy levels and mental and physical abilities.[5] The body weight increased in groups of rats that induced energy drinking. The group that consumed the energy-inducing drink had significantly higher levels of glucose and glycogen, according to a study by Munteanu *et al*.[17] showed a substantial rise in the glucose and glycogen levels in the group that had the energy-inducing drink. The increase in body weight is associated with the composition of energy drinks, which are composed of various components.[18] Consuming sugar-sweetened beverages has been demonstrated to lead to an increase in body weight and, ultimately, the development of obesity.[19] This would increase the fat storage rate in adipose tissues.[20]

The outcomes of the present study illustrated that high caffeine content in beverage has damaging effects on many biochemical parameters, the level of renal function abnormality among induced energy drinking groups by increased urea, creatinine, uric acid, and decreased GFR, This results agree with Ugwuja[21] who conducted research on rats and found that drinking energy drinks were linked to significantly higher levels of urea, uric acid and creatinine. The level of caffeine is one of the essential components of all energy drinks.[22] Although it has a stimulating impact, it cannot serve as an alone energy source. The symptoms include psychomotor agitation, affective sleeplessness, tachycardia, arrhythmia, elevated arterial blood pressure, and nausea.[23] On the other hand, in the present study, the serum potassium level increased significantly in the consumption of energy drinks. Potassium plays a crucial role in maintaining proper heart function, and its deficiency can result in changes to circulatory system function or cardiac abnormalities.[24]

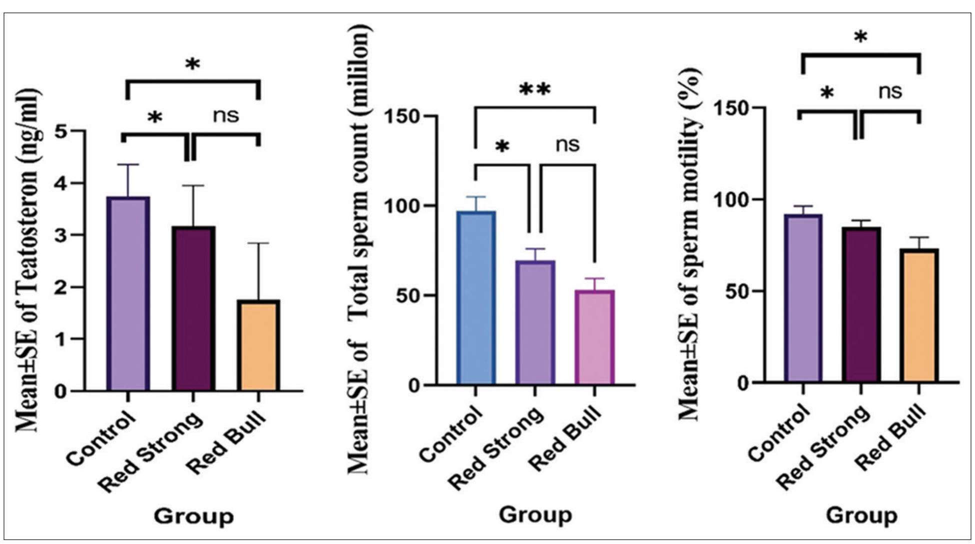

Figure 4: (Mean ± standard) The testosterone level, total sperm count, and motility in the control and beverage administered group (Red Strong and Red Bull)

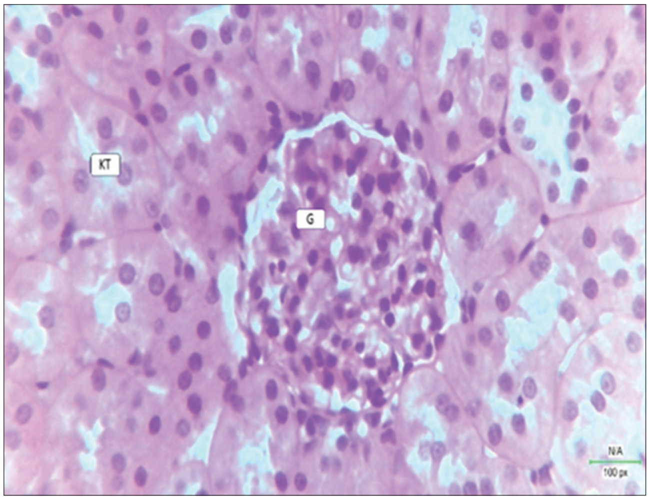

Figure 5: Histological analysis of kidney from control group albino rats: Control group, G: glomerulus, KT: Kidney tubules. (Hematoxylin-Eosin stain, 400×)

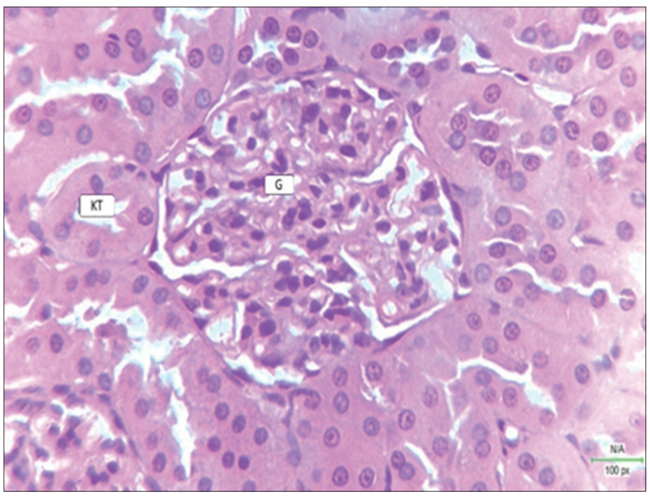

Figure 6: Histological analysis of kidney from control group albino rats: G: glomerulus, KT: Kidney tubules. (Hematoxylin-Eosin stain 400×)

The enzymatic level in hepatic male rats increased by induced high-beverage compared to control groups. These results agree with that illustrated by administering an energy drink orally to rats, resulting in elevated liver enzymes, including ALT, AST, and ALP, in their blood. This is a dependable indication of liver damage induced by toxic substances.[21,25] It was also demonstrated by Mukhiddinovna[7] that consuming energy drinks for 3 months damages the liver and kidneys by altering liver function tests and raising urea, creatinine, and uric acid levels. This change attributes these changes to oxidative stress and free radical production.[26] A 22-year-old lady presented with symptoms of a mild fever, abdomen pain, nausea, and vomiting. Upon investigation, it was discovered that she had been ingesting 10 cans of energy drinks per day for 2 weeks, resulting in abnormal liver test results. The laboratory tests revealed significant increases in ALT and AST levels, although bilirubin, ALP, and GGT levels were within the normal range.[27]

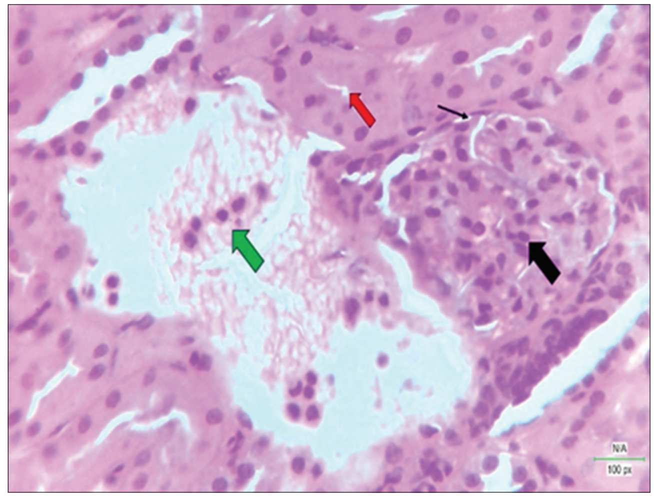

Figure 7: Histological analysis of kidney from Red Strong group albino rats: Thick Black arrow represents degenerative and shrinkage, Fine Black arrow represents no urinary space. Red arrow represents no space in kidney tubules, green arrow represents degenerative of kidney tubules (Hematoxylin-Eosin stain, 400×)

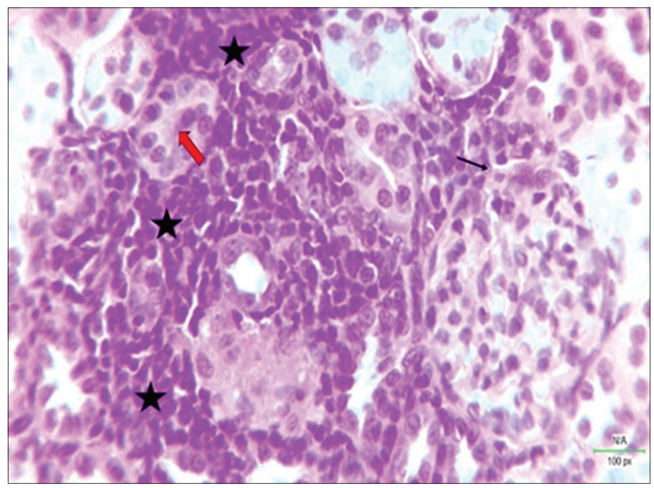

Figure 8: Histological analysis of kidney from Red Strong group albino rats: D: Red Strong Group. Star represents inflammation, Fine Black arrow represents no urinary space, and Red arrow represents no space in kidney tubules (Hematoxylin-Eosin stain, 400×)

The current research investigates the influence of energy beverages on the reproductive system of male rats. Among the sperm parameters, sperm concentration and motility mainly exhibited a detrimental response to the consumption of energy drinks a reduction in both sperm count and motility following oral administration.[20] Our study conducted with other research showed the gonadal toxic and pro-oxidant qualities of energy drinking, caffeine specifically affects Sertoli cells and spermatogonia while causing minimal damage to spermatids, differentiating cells, and mature spermatozoa.[19] The effects may be related to histological problems. Nwakanma *et al.*[20] evidence of genotoxicity was observed in germ cell sloughing and decreased seminiferous tubule size by the presence of degenerative in the seminiferous tubules by alterations and reduction in the number of spermatozoa in the testis. In

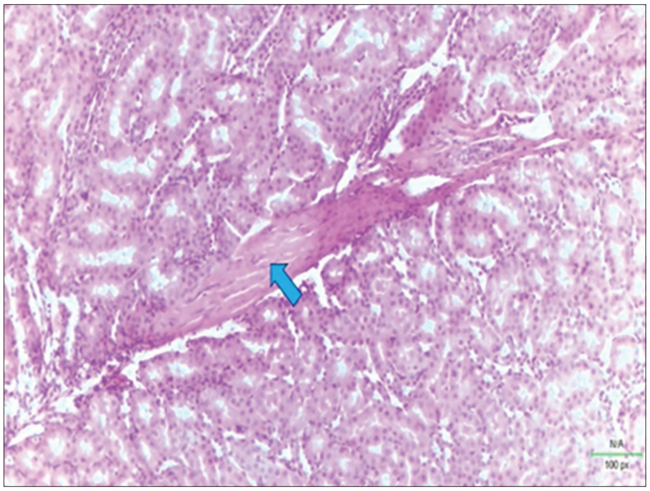

Figure 9: Histological analysis of kidney from Red Bull group albino rats: Blue arrow represents Fibrotic area. (Hematoxylin -Eosin stain,100×)

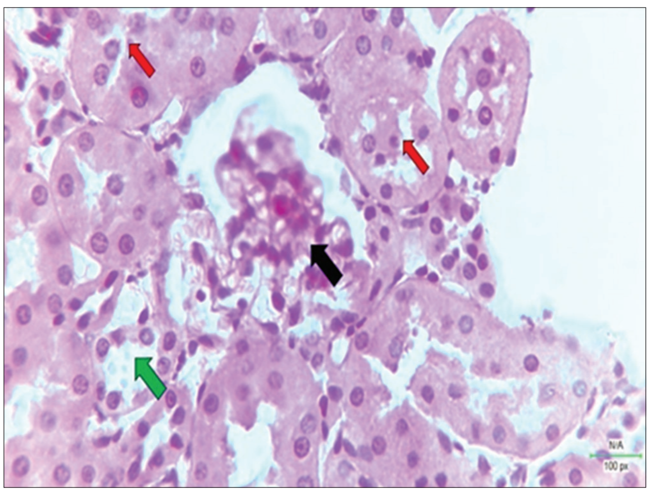

Figure 10: Histological analysis of kidney from Red Bull group albino rats: Thick black arrow represents degenerative and shrinkage, Red arrow represents no space in kidney tubules, green arrow represents degenerative of kidney tubules (Hematoxylin-Eosin stain,400×)

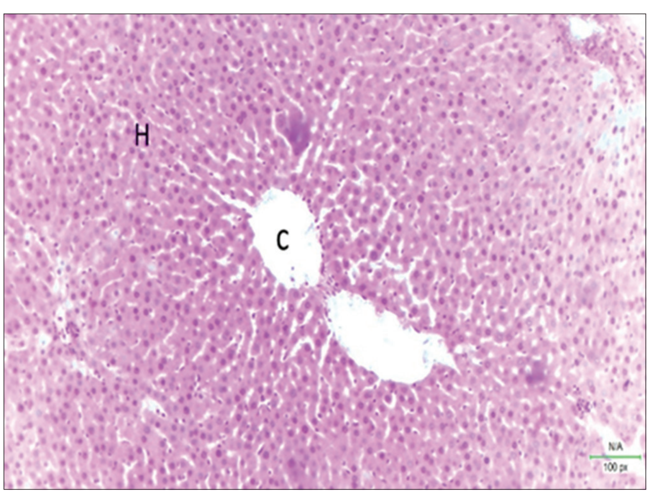

Figure 11: Histological analysis of liver from control group albino rats: C: Central vein, H: Hepatocyte, S: Sinusoid. (Hematoxylin-Eosin stain,100×)

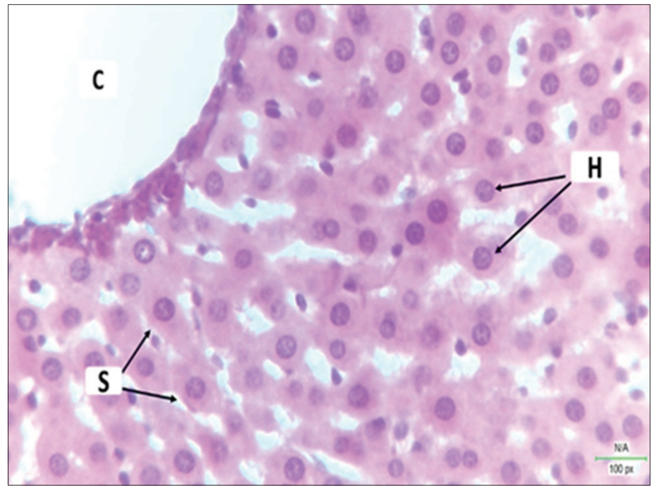

Figure 12: Histological analysis of liver from control group albino rats: C: Central vein, H: Hepatocyte, S: Sinusoid. (Hematoxylin-Eosin stain,400×)

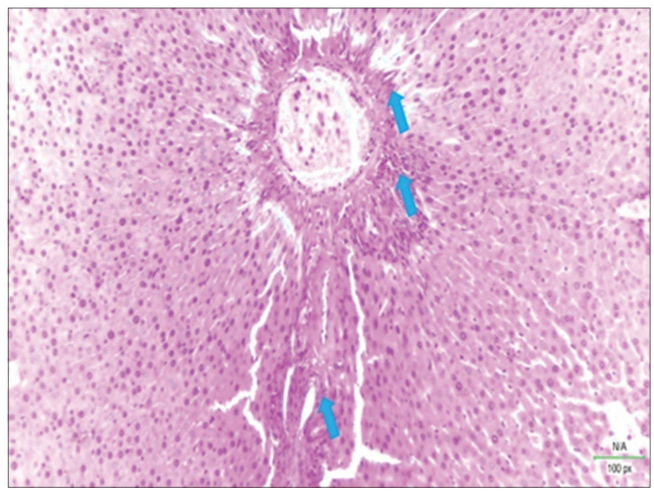

Figure 13: Histological analysis of liver from Red Strong group albino rats: the blue arrow represents the fibrotic area. (Hematoxylin-Eosin stain,100×)

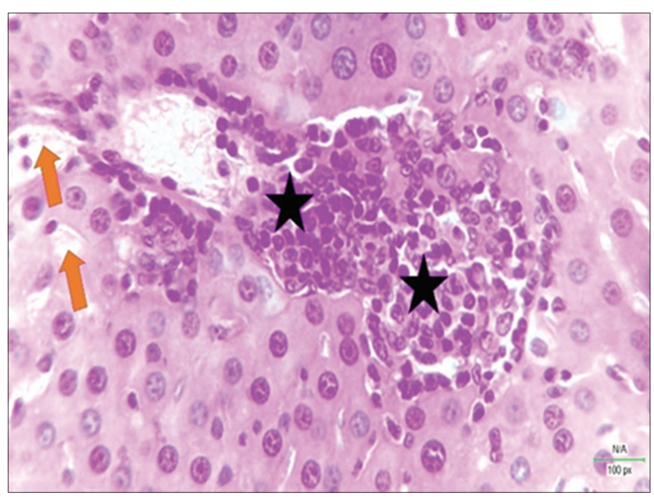

Figure 14: Histological analysis of liver from Red Strong group albino rats: Red Strong Group Star represents inflammation, Orang arrow represents dilation (Hematoxylin-Eosin stain,400×)

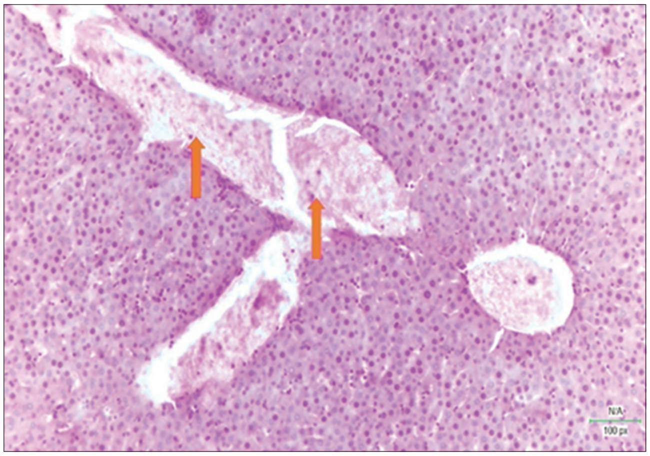

Figure 15: Histological analysis of liver from Red Bull group albino rats: Orang arrow represents dilation (Hematoxylin-Eosin stain,100×)

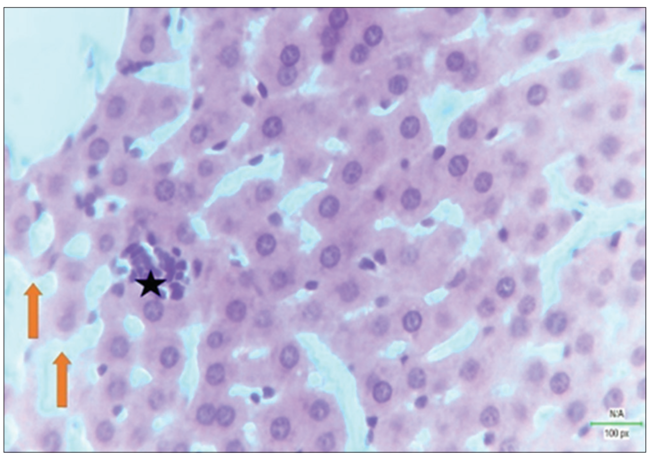

Figure 16: Histological analysis of liver from Red Bull group albino rats: Star represents inflammation, Orang arrow represents dilation (Hematoxylin-Eosin stain,400×)

addition, caffeine also induces testicular atrophy by reducing Leydig cell-mediated testosterone synthesis, resulting in decreased testicular weight and size.[28] They increase the concentration of oxidative stress such as lipid peroxides in the testis of male rats who are given energy for 7 weeks.[29]

The histological study showed abnormal kidney structure due to high consumption of beverages, such as degenerative kidney tubules, hemorrhage, shrinkage of the glomerulus, and no space in kidney tubules. The study of Mazzali *et al*.[30] illustrated that renal function was mildly decreased by mild tubulointerstitial injury along with monocyte infiltration in hyperuricemic rats with mild vascular disease involving the pre-glomerular arteriole. A case report documents the occurrence of acute tubular necrosis in the kidneys of a young adolescent following the consumption of energy drinks, which resulted in an elevation of creatinine levels.[31] Degeneration and hemorrhage of the kidney by caffeine and energy drinking.[32] Furthermore, the dilated sinusoid, degenerative hepatocyte, and fat droplet showed in energy-drinking groups. In another study, rats received an energy drink (1.5 mL/kg) for 14 and the observed changes included the infiltration and aggregation of inflammatory cells with hepatocyte necrosis.[33]

CONCLUSION

Long-term beverage or high-concentration consumption interferes negatively with liver and kidney functions and impacts the reproductive systems of male albino rats. The beverage consumption (Red Strong and Red Bull) has adverse effects, including increased hepatic enzymes and alteration in renal function. Furthermore, interferes negatively with testosterone levels, sperm count, and motility. Hence, it causes increased weight gain in induced groups. Moreover, histological abnormality architecture or damage was observed in the hepatic and renal male rats.

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