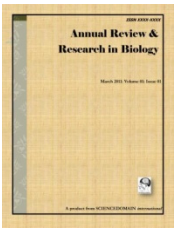

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Assessment of Biochemical Effect of "Power Horse" Energy Drink on Hepatic, Renal and Histological Functions in Sprague Dawley Rats

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Research Article

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ABSTRACT

Objective: Energy drinks are popular and widespread and raising concerns about implications on human health. Hepatological, histological and renal function tests of Sprague-Dawley albino rats were investigated in rat liver, brain and kidney by administering "power-horse" energy drink.
Methodology: For this study twenty healthy adult female rats (142 – 148g) were divided into 4 groups with 5 rats in each group and they were treated as follows: Control group was given water only after acclimatization for 28 days when food and water were freely available to the four groups. Low dose group (administered energy drink of 10mg/kg body weight) and high dose group (administered energy drink of 20mg/kg body weight). Recovery groups received high dose of energy drink (20mg/kg body weight) for 14 days and allowed a recovery phase of 7 days thereafter when they received water and standard diet. Rats were sacrificed and blood samples collected through orbital sinus and cardiac puncture. Liver, brain and kidney tissues for all the groups were harvested. Liver and renal function parameters were analyzed while liver; brain and kidney were histologically examined.
Results: Serum alanine amino transferase (ALT) and alkaline phosphatase (ALP) activities increased significantly ($p<0.05$) in the experimental groups compared with the control (49.83±0.38 U/L, 582.33±9.06 U/L vs. 44.40±0.60 U/L, 331±4.90 U/L) while the activities of ALT and AST in the recovery group reduced, although not significantly ($P>0.05$) compared with the high dose group. Urea concentrations in the experimental groups increased ($P<0.05$) significantly compared with the control (10.10±0.15mmol/L vs.3.66±0.10 mmol/L). There was no significant difference ($P>0.05$) in the concentrations of creatinine in the experimental groups compared with the control group (44.20±00 mmol/L vs. 44.20±02 mmol/L). Serum Na and HCO_3^{2-} in the experimental groups increased ($P<0.05$) significantly when compared with the control group (141.07± 0.56, 28.03±0.09 vs. 136.62± 0.72, 23.15±0.65).

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Conclusion: Data of the present study indicate that “Power Horse” consumption has adverse effects on the liver and therefore requires caution in its consumption.

Keywords: Power Horse; caffeine; energy; drinks; histology; rat;

1. INTRODUCTION

Energy drinks are non-alcoholic, often lightly carbonated beverages that are designed to give the consumer a burst of energy by the addition of a number of energy enhancing ingredients, most notably caffeine. These are commonly available in grocery stores, bars and night clubs, super markets, vending machines, usually displayed alongside soft drinks, juices and sports drinks. These are manufactured in small, bullet-shaped cans. These drinks usually do not emphasize energy derived from the calories they contain, energy drinks are usually formulated to give the consumer an energy jolt by using a combination of methylxanthines, B vitamins complex, and exotic herbal ingredients (Alford et al., 2001). Energy drinks commonly include caffeine, other plant based stimulants (guarana, ephedrine, yerba mate), simple sugars (glucose, fructose), amino acids (taurine, carnitine, creatine), herbs (various forms of ginseng, ginkgo biloba), maltodextrin, inositol, Glucuronolactone (a naturally occurring glucose metabolite).Some contain high levels of sugar, while most brands also offer an artificially sweetened version. Often manufacturers add a very small dose of a powerful stimulant such as carnitine, but the doses of this add-ins are usually so small that any added "boost" is purely psychological. Despite exotic formulations, generally the energy boost in these drinks is delivered via the whopping dose of common caffeine. Energy drinks should not be confused with sports drinks such as Gatorade or PowerAde, which are intended to replenish electrolytes, sugars, water and other nutrients and are usually isotonic (containing the same proportions as found in the human body).

Energy drinks are marketed to consumers as stimulants hence they often have catchy names that convey strength, power, speed, sexuality and often include appropriate background music (e.g., Power horse, Red bull, Full throttle, Dare devil, Cocaine e.t.c). Generally speaking, energy drinks are marketed towards young people, such as students pulling all-nighters (while studying, driving long distances), partying (Malinauskas et al., 2007). The main ingredient of energy drinks associated with diuresis and fluid-electrolyte balance is caffeine, although taurine is also associated with osmoregulation, detoxification and bile acid conjugation. Caffeine stimulates renal glomerular filtration and inhibits reabsorption of sodium within nephrons thereby stimulating an increased sodium and water excretion. Effects of taurine on renal and liver functions are inconclusive, with the majority of researchers examining energy drinks attributing effects to caffeine alone (Childs and de Wit, 2008; Scholey and Kennedy, 2004; Smit and Rogers 2002; Alford et al., 2001).

Energy drinks are typically attractive to young people. Approximately 65% percent of its drinkers are between the ages of 13 and 35 years old, with males being approximately 65% of the market (Mintel energy drink report, 2006). A 2008 state-wide Patient Poll conducted by the Pennsylvania Medical Society’s Institute for Good Medicine found that: 20 percent of respondents ages 21–30 had used energy drinks in high school or college to stay awake longer to study or write a paper; 70 percent of respondents knew someone who had used an energy drink to stay awake longer to study or work (Newswise, 2008). Energy drinks are also popular as drink mixers.

The complete physiological response to energy drinks is still to be determined; however changes in heart rate and blood pressure have been associated with chronic intake of energy drinks (Bichler et al., 2006). It was reported that caffeine increased creatinine, urea (Portolés et al., 1985; Tofovic et al., 2007), AST (Aspartate transferase), ALT (Alanine transferase) in serum of rats (Cheul Do et al., 1997).Caffeine is the major active ingredient in energy drinks; it is also present in a host of other beverages (Coffee, tea, Hershey's bar etc.). However, the effects experienced after intake of energy drink and a caffeine containing beverage e.g. coffee are quite different. This study therefore investigated the suspected synergistic relationship between ingredients of energy drinks.

2. MATERIALS AND METHODS

2.1 Animals

Twenty female albino Sprague-Dawley rats weighing 142 – 148 grams were collected from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria. They were housed in wooden cages and allowed to acclimatize for four weeks. The rats were fed with rat chow and water ad libitum and under natural light and dark rythms prior to commencement of the study. The rats were divided into four groups of five rats each and "power horse" energy drink was administered as described in the study protocol.

2.2 Sample collection and study protocol

The several cans of energy drink, 'power horse' manufactured by Vitalis – Group, Austria were purchased within Lagos Metropolis, Nigeria. The energy drink was administered with gastric gavage as follows: Group 1 (Control): The group was administered 1.0ml water only. Group 2 (Low dose) was given 3.1-6.3ml of energy drink daily for 21 days. Group 3 (High dose) was given 6.3-12.5ml of energy drink for 21 days while Group 4 (Recovery) was given 6.3-12.5ml of drink based on their body weights for 14 days after which they were given water and the standard diet from 15^{th} – 21^{st} day.

Table 1. Volume (ml) of energy drink administered to animals in the low dose (10mg/kg) and those in the high dose group (20mg/kg)

Body Weight (g)	Low Dose (10mg/kg)	High Dose (20mg/kg)
100	3.1	6.3
110	3.4	6.9
120	3.8	7.5
130	4.1	8.1
140	4.4	8.8
150	4.7	9.4
160	5.0	10.0
170	5.3	10.6
180	5.6	11.3
190	5.9	11.9
200	6.3	12.5

2.3 Collection of Blood and Biochemical analysis

The rats were sacrificed by decapitation and blood was taken from each rat by cardiac puncture and allowed to clot. Serum samples were extracted by centrifuging the clotted blood at 3000g for 10min in a Beckman Model T – 6 refrigerated centrifuges. The serum samples were used for biochemical analyses.

Serum electrolytes (Na^+, K^+, CL^- and HCO_3^{2-}) levels were determined using automated Medical Easylyte sodium /potassium analyser. Serum urea, creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine amino transferase (ALT) activities were determined using Reflotron Plus Strips (Tietz, 1995; Bergemeyer and Horder, 1986 and Heins et al., 1995).

2.4 Histology (Akande et al., 2010)

The rat liver, kidney and brain from each group were fixed in 10% formol saline for 72 hours. The organs were dehydrated in graded alcohol, cleaned in xylene and embedded in paraffin. The resulting blocks were exhaustively sectioned. The sections were randomized, while the selected sections were stained in haemotoxylin and eosin. The slides were then examined at magnifications of X 400 under optical microscope.

2.5 Statistical analysis

Statistical analysis was carried out using the analysis of variance (ANOVA) followed by the student Newman – Keuls post hoc test (Graph pad prism 5.0) to identify differences between individual mean. Statistical significance was set at $P<0.05$.

3. RESULTS AND DISCUSSION

Table 2 shows the effects of the administration of the caffeinated energy drinks on the serum AST, ALT and ALP activities. The results show that, energy drink consumption significantly increased the AST, ALT and ALP levels in all the test groups when compared with the control group, while the activities of these enzymes in the recovery group reduced, although not significantly when compared with the high dose group. Only the ALP activities of the recovery group showed a significant increase when compared with the high dose group. These results are in agreement with Cheul Do et al., (1997) who reported that there is an increase in the concentration of AST, ALT in serum of rats after treatment with caffeine.

Table 2. Effects of caffeine concentration in Red horse energy drink on Liver functions enzymes in rats

Groups	Aspartate transferase (AST) (U/L)	Alanine transferase (ALT)(U/L)	Alkaline Phosphatase (ALP) (U/L)
Control group	51.00 ± 0.45	44.4 ± 0.60	331 ± 4.90
Low dose group (10mg/kg)	53.65 ± 1.26*	49.2 ± 0.34*	431.25 ± 7.79*
High dose group (20mg/kg)	68.67 ± 0.38*	49.83 ± 0.38*	582.33 ± 9.06*
Recovery group (20mg/kg)	67.7 ± 0.70*	49.73 ± 0.32*	471.33 ± 2.96*

*Data are represented as Mean ± S.E.M (n=5). Significant * P<0.05*

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Table 3 shows the effects of caffeinated energy drinks on the levels of urea and creatinine. Urea concentration in the test groups increased significantly when compared with the control group, this is in disagreement with other researchers who reported that there is no relationship between caffeine and the concentration of urea in serum of rats (Cheul Do et al., 1997). There was a significant decrease in the concentration of urea of the recovery group when compared with the high dose group. There was no significant difference in the concentration of creatinine of the test groups when compared with the control group.

Table 3. Effects of concentration of caffeine contained in Red horse energy drink on renal functions in rats

Groups	Urea (mmol/L)	Creatinine (mmol/L)
Control group	3.66 ± 0.10	44.2 ± 0.00
Low dose group (10mg/kg)	6.41 ± 0.12*	44.2 ± 0.00
High dose group(20mg/kg)	10.1 ± 0.15*	44.2 ± 0.00
Recovery group (20mg/kg)	9.00 ± 0.06*	44.2 ± 0.00

*Data are represented as Mean ± S.E.M (n=5). Significant * P<0.05*

Table 4 showed the significant differences between the concentrations of the serum electrolytes. The concentrations of sodium, bicarbonate and potassium of the low dose group and the high dose group showed significant increases when compared with the control group while the concentration of sodium and chloride in the serum of rats in the recovery group did not differ significantly when compared with the control. Chloride concentration showed no significant difference between the test groups and the control group.

Table 4. Effects of concentration of caffeine contained in Red horse energy drink on concentration of serum electrolytes in rats

Groups	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)
Control group	136.62 ± 0.72	5.51 ± 0.10	104.86 ± 0.45	23.15 ± 0.65
Low dose group (10mg/kg)	139.50 ± 0.51*	6.46 ± 0.04*	104.95 ± 0.20	27.10 ± 0.32*
High dose group (20mg/kg)	141.07 ± 0.56*	6.63 ± 0.06*	107.23 ± 0.91	28.03 ± 0.09*
Recovery group	136.03 ± 0.09*	6.46 ± 0.04*	104.13 ± 0.19	27.07 ± 0.12*

*Data are represented as Mean ± S.E.M (n=5). Significant * P<0.05*

Histological sections in the control group showed pale staining and fibrillary cerebral matter. There were clear hollows around the neuronal cell bodies and widening of spaces around blood vessels (Photo 1). Sections of cerebellum show a light granular cell layer and intervening Purkinje cell layer. The overlying meninges are unremarkable (Photo 1).

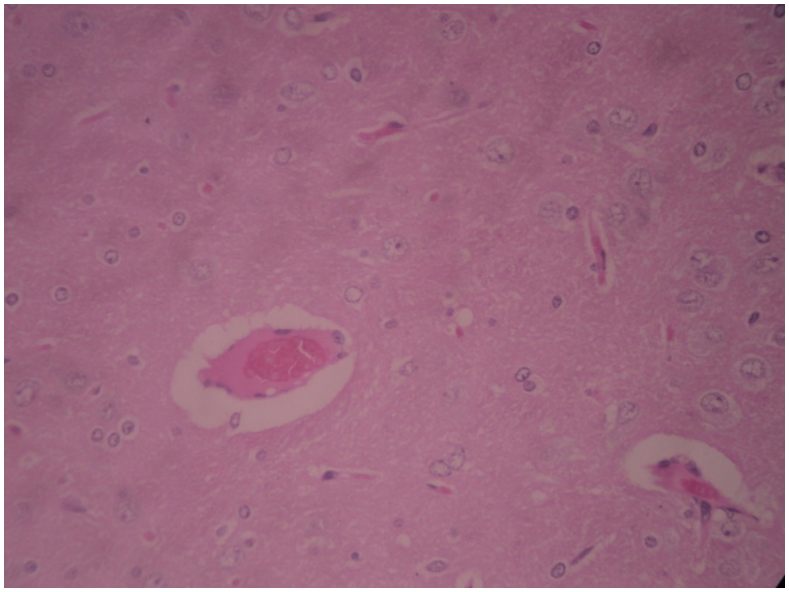

Photo 1. Photomicrograph of the brain tissues of rats administered water In the control group (X400)

Histological sections of renal cortex showed glomeruli with normal cellularity and the Bowman's capsule, matrices between capillaries and capillaries were normal. The medulla consists mainly of tubules lined by cuboidal cells with brightly pink eosinophilic cytoplasm. The arteries, arterioles and veins as well as the interstitium (spaces between tubules) were unremarkable (Photo 2).

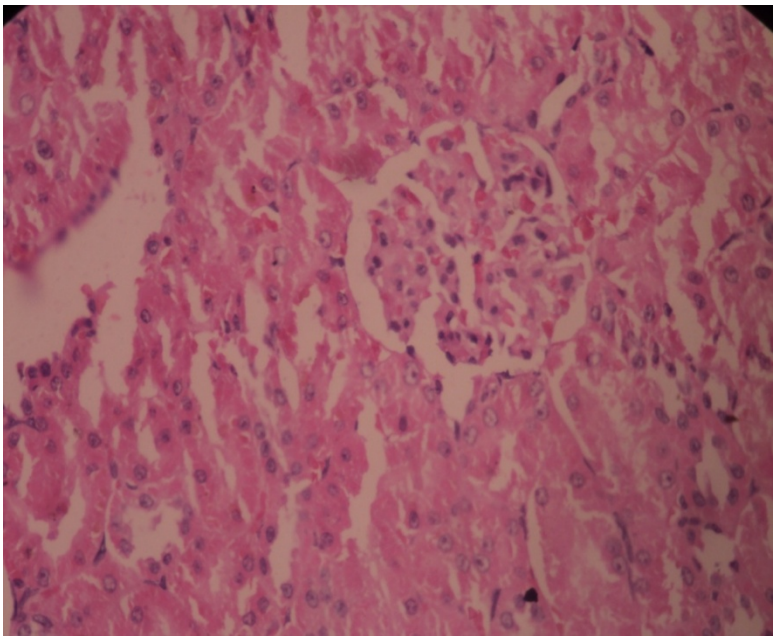

Photo 2. Photomicrograph of kidney tissues of rats administered water in the control group (X 400)

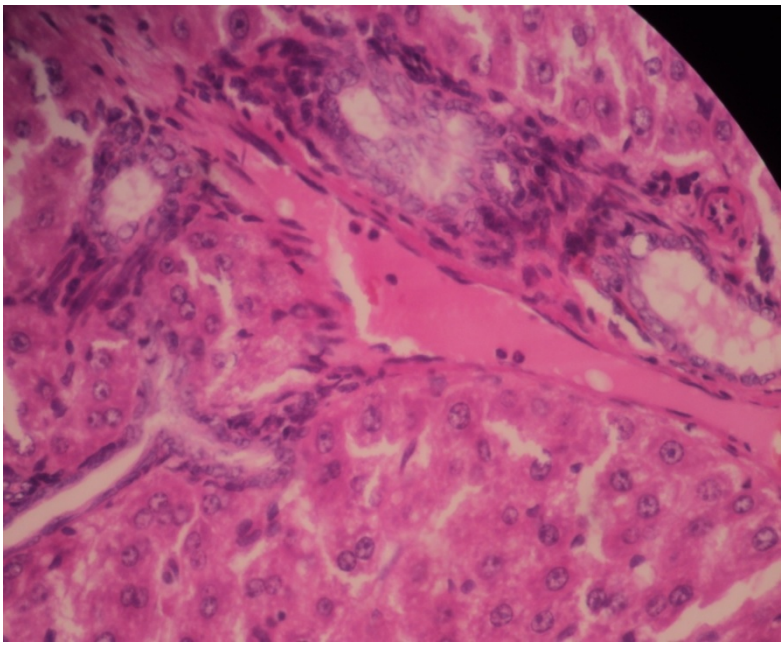

Photo 3. Photomicrograph of hepatocytes of rats administered water in the control group (X 400)

Histological sections of liver tissues showed well preserved hepatic architecture. The hepatic lobules were outlined at the edges by portal tracts (collection of portal arteries, portal vein and bile canaliculi) that were unremarkable. The hepatocytes plates and the intervening sinusoids were not congested. No necrosis was observed (Photo 3).

Histological sections of the brain tissues in the low dose group showed pale staining and fibrillary cerebral matter. There were clear hollows around the neuronal cell bodies and widening of spaces around blood vessels. Sections of cerebellum show a light granular cell layer and intervening Purkinje cell layer. The overlying meninges are unremarkable. Histologic sections of the liver showed mild architectural distortion. The hepatic lobules were not distinct. The hepatocytes were arranged in lobules. They were seen to radiate as single plate or cells from the central vein towards the portal tracts. The hepatocytes showed moderate intracytoplasmic vacuoles, sinusoids were distorted but no necrosis was seen (Photo 4).

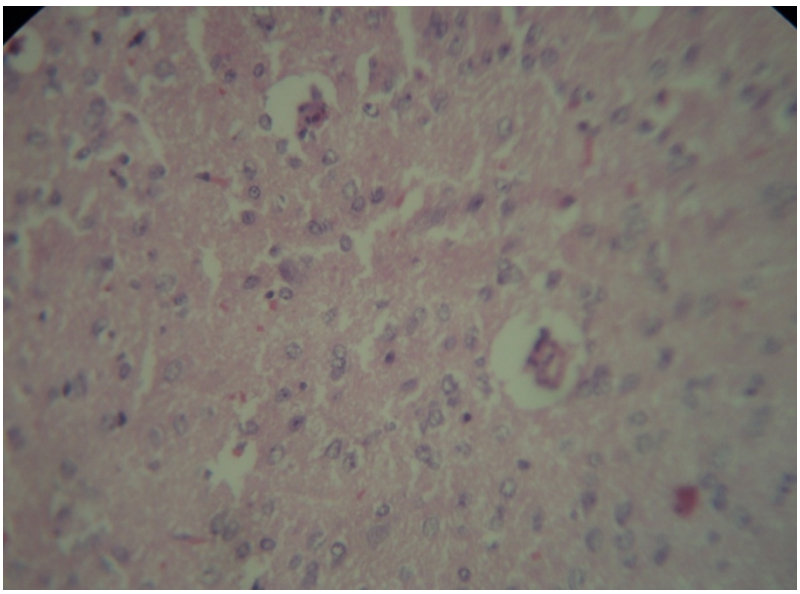

Photo 4. Photomicrograph of brain tissues of rats in the low dose group (X 400)

Histological sections in the recovery group showed pale staining and fibrillary cerebral matter. There were clear hollows around the neuronal cell bodies and widening of spaces around blood vessels. Sections of cerebellum show a light granular cell layer and intervening Purkinje cell layer. The overlying meninges are unremarkable (Photo 5).

Histological sections in the high dose group showed pale staining and fibrillary cerebral matter. There were clear hollows around the neuronal cell bodies and widening of spaces around blood vessels. Sections of cerebellum show a light granular cell layer and intervening Purkinje cell layer. The overlying meninges are unremarkable. Sections of the liver showed moderate architectural distortion. They were seen to radiate as parallel single plates of cells from the cerebral vein towards the portal tracts which were difficult to identify, the hepatocytes appear increased in size and show intracytoplasmic vaculation.

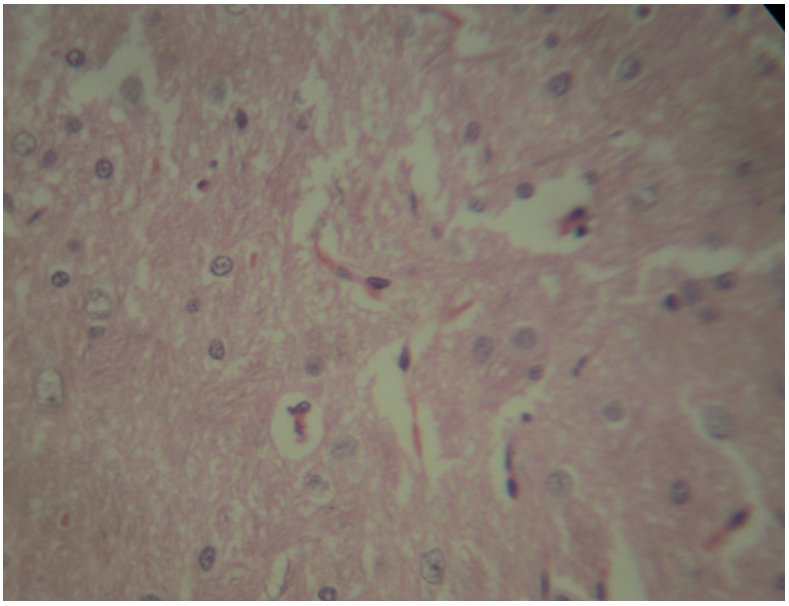

Photo 5. Photomicrograph of section of the brain of an animal in the recovery group (X 400)

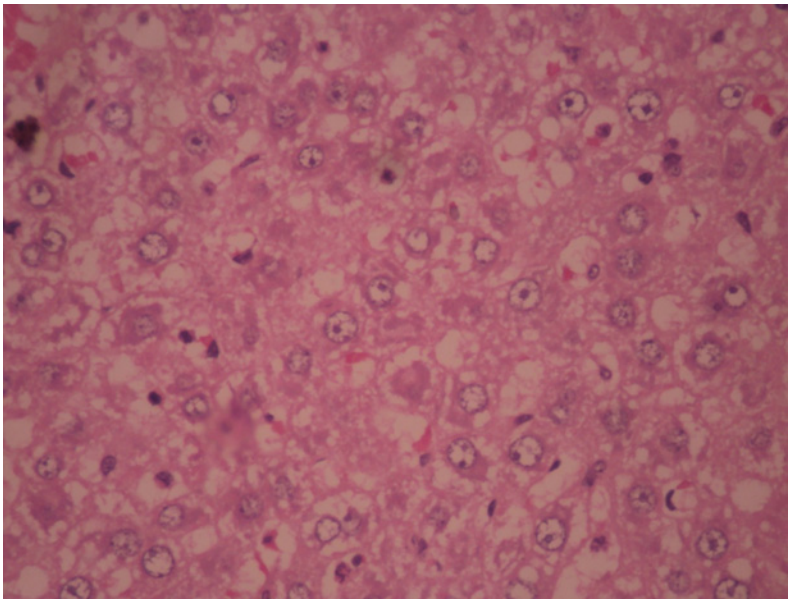

Photo 6. Photomicrograph of hepatocytes of rats in the high dose group (X 400)

Cytoplasm had disappeared. Sinusoids were distorted, no necrosis was seen. Sections showed moderate architectural distortion. The hepatocytes showed mixed morphology that is some cells were regenerating (losing some of the vacuoles). Some of the hepatocytes showed moderate intracytoplasmic vaculation along cell membrane. Other hepatocytes showed granular eosinophilic cytoplasm with little or no vacuoles. No necrosis was seen (Photo 6).

The histopathological study of the brains and kidneys of the rats in the test and control groups appeared normal with no irregularities or abnormalities. The histopathological study of the hepatocytes of rats in the low dose and the recovery group showed distortion from the normal architecture of the liver, the hepatocytes had increased in size, the cytoplasm appears to be diminishing and the emergence of vacuoles was observed. The hepatic lobules were not distinct and no necrosis was observed. The hepatocytes of an animal in the recovery group showed moderate architectural distortion, the hepatocytes showed mixed morphology i.e. some cells were regenerating, thereby there was a gradual loss of the vacuole and reappearance of the cytoplasm around the cells were observed (Photos 7-8).

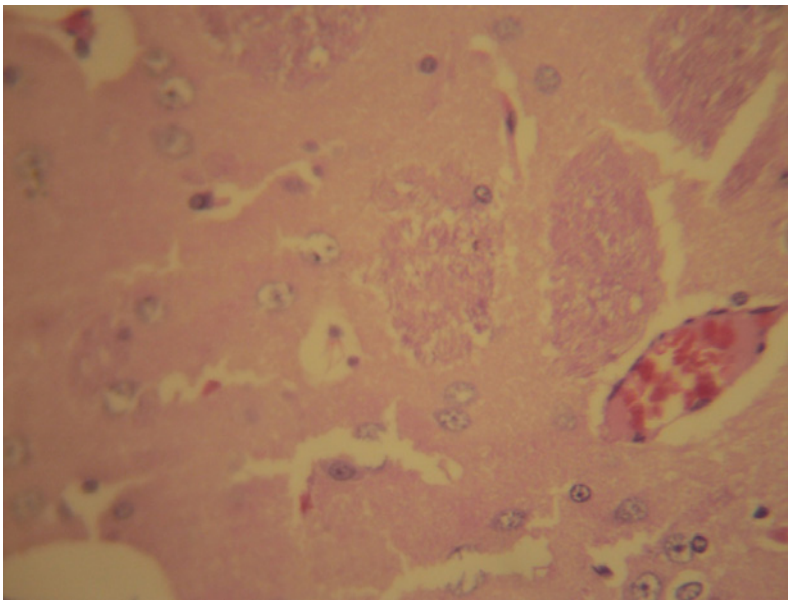

Photo 7. Photomicrograph of brain tissues of rats in the high dose group (X 400)

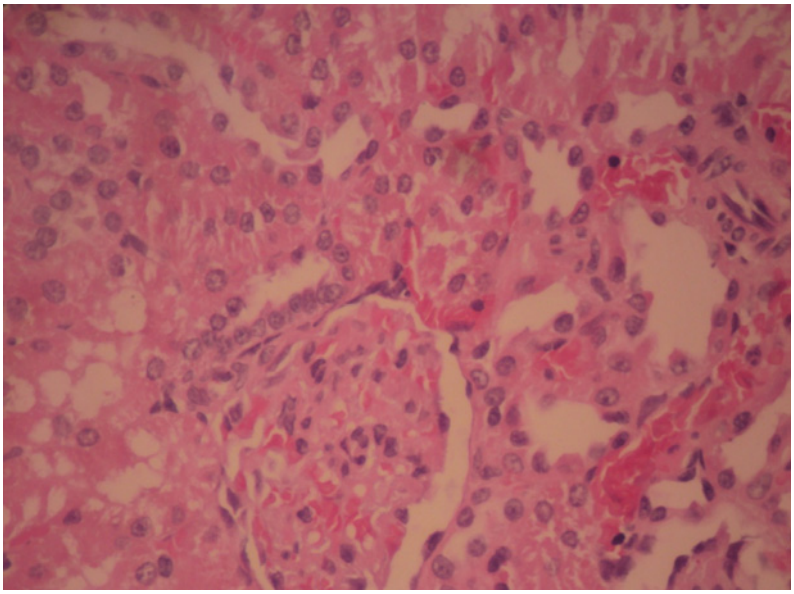

Photo 8. Photomicrograph of kidney tissues of rats in the high dose group (X 400)

4. CONCLUSION

The study has shown that caffeinated energy drinks consumption has damaging effects on the hepatocytes. However, the damage done by excessive consumption of caffeinated energy drink is reversible as observed in the results of the blood chemistry analysis and the histopathological study of the organs of animals in the recovery group.

Further research is recommended on the effects of excessive consumption over a longer period of time (say 2 months), with a longer recovery phase to determine if a total recovery is possible on cessation. Research should also be done on effects of sugar and sugar free energy drinks on liver functions to determine if the energy got is as a result of the glucose and the metabolite glucuronolactone. Also a review should be done using a caffeine and taurine energy drink, and only caffeine energy drink in order to elucidate a possible synergy between caffeine and taurine.

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