HISTOLOGICAL EFFECTS OF CAFFEINATED SOFT DRINK AND ENERGY DRINK ON THE HEPATOCYTE OF THE ADULT RATS

SAIRA MUNAWAR[1], MUHAMMAD SUHAIL[2], GODFREY PAUL WILLIAM[3]

ABSTRACT

Objective/Background: Ever rising trend of consuming soft drinks and energy drinks has raised concerns about their safety. Hence, this study was designed to observe the potential adverse effects of most commonly consumed soft drink, Coca Cola (containing caffeine) and energy drink, Red Bull(caffeine and taurine) on the morphology and histology of hepatocyte of rats.

Material and Methods: 45 albino rats were divided into three equal groups, control group A and experimental groups B and C. 11ml/kg body weight of distilled water, Coca Cola and Red Bull were given to groups A, B and C respectively for 8 weeks. Later the rats were sacrificed and livers were dissected and preserved in formaline for histological examination.

Results: Hepatocytes of both experimental groups B and C were rounded and enlarged in comparison to control group with p value of <0.01. Both group B and C had swelling and ballooning of hepatocyte due to hydropic degeneration and appeared vacuolated in comparison to control group (p value <0.001). Hepatocyte of experimental groups had greater diameter in comparison to control (p value <0.001). However, the coca cola receiving experimental group had an average higher diameter in comparison to group receiving Red Bull (p value 0.01).

Conclusion: Both, caffeinated drinks (soft and energy drinks) have adversely affected morphology as well as histology of hepatocyte of adult albino rats. However, the Coca Cola group was affected more in comparison to Red Bull group probably due to its hepatoprotective constituent, taurine in addition to caffeine (which was present in both drinks), somehow reducing its toxic effect.

INTRODUCTION

Both, caffeinated soft drinks as well as energy drinks are nonalcoholic sweet beverages.[1] they are consumed as a style statement or for recreational purposes enhancing physical and mental performance.[2] Meager FDA regulation has increased the consumption globally.[3]

The most commonly consumed caffeinated soft drink is Coca Cola, its constituents mainly include sucrose (fructose and glucose), caramel coloring, caffeine, phosphoric acid, lime extract, coca extract, citric acid, vanilla, cinnamon and nutmeg with trace amounts of orange.[4,5] Whereas on the other hand, most commonly consumed energy drink is Red Bull, which contains caffeine, taurine, glucuronolactone, inositol, niacin, pantenol, vitamin B2, B6, B12, citric acid, caramel color, artificial flavor and sparkling water.[6] 0.12mg and 0.32 mg caffeine is present in each ml of Coca Cola and Red Bull respectively. In addition, taurine is also present in Red Bull in quantity of 4mg in each ml.

Researchers have found soft drink consumption to increase serum triglyceride levels, lipogenesis, adiposity and fatty liver[7, 8]. Their prolong usage was reported to cause malignant mammary tumor and pancreatic carcinoma.[9] Obesity, insulin resistance and inflammation were also found to be associated with soft drink consumption.[1]

Cases of hepatitis, jaundice, hypertension, myocardial infarction and sudden death were reported with energy drink consumption.[3,10,11] Energy drink consumption by rats disturbed their liver architecture as well.[2]

MATERIAL AND METHODS

After acclimatization, 45 albino rats were used as sample. Food and water was made available ad libitum along with maintenance of light and dark cycle.

Lottery method was used to randomly divide the rats into three equal groups of 15 each. The group A served as control group. 11ml/kg body weight of distilled water, Coca Cola and Red Bull per day, were given through orogastric tube to group A, B and group C respectively for 2 months.

After 2 months, the dissected and formaline fixed livers were sliced to minute pieces and later paraffin blocks were prepared from each lobe .Any additional

abnormal area was also sectioned. Spencer and Bancroft procedure for tissue processing was used.[12]

5 µm thick sections were cut by using rotary microtome and were stained with haematoxylin and eosin. Using light microscopy, control group was compared with experimental groups for various parameters.

The micrometer of objective as well as the eyepiece was superimposed, for measuring the diameter (both vertical and transverse) of hepatocyte at 40 x (fig. 1) . Afterwards, average diameter was calculated for each hepatocyte. Five different fields were observed for calculation of diameter of hepatocyte and then the mean was taken.

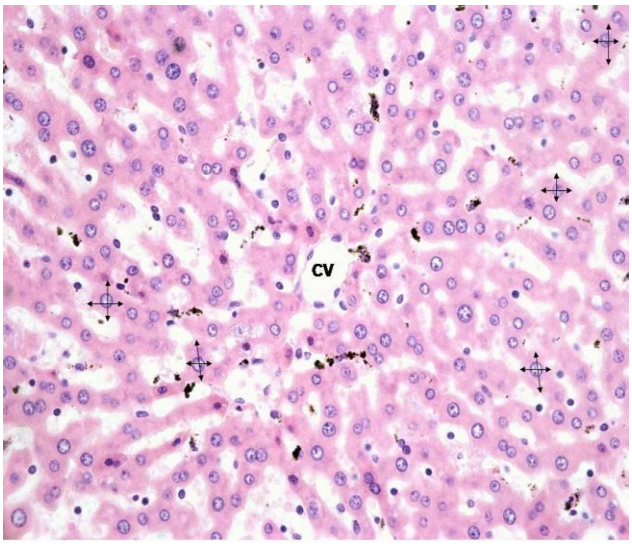

Fig.1: Measurement of hepatocyte diameter (H&E, 40x).

Data was analyzed by SPSS version 22.0. Chi square test was used to analyze the shape of hepatocytes and vacuolization. Whereas, ANOVA and Tukey's tests were used to compare the mean diameter of hepatocyte, considering P-value <0.05 to be statistically significant.

RESULTS

1. Shape of Hepatocyte:

Hepatocytes of control group, showed normal polygonal shape, rounded nuclei with prominent nucleoli (fig.2) while, in affected areas of experimental groups receiving Coca Cola and Red Bull, hepatocytes were founded to be rounded and enlarged (fig.3, 4) . The comparison among control and experimental groups was statistically significant with p value <0.001 however, there was no statistical significance between the two experimental groups (table.1).

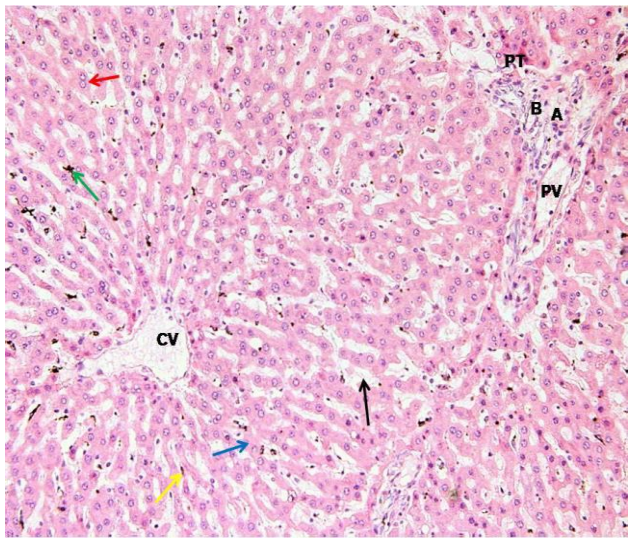

Fig.2: Centrilobulor vein (CV) and portal triad (PT) with portal vein (PV), hepatic artery (A) and bile ductule (B) of control group. Cords of hepatocytes (Blue arrow) with intervening hepatic sinusoids (Black arrow) lined with endothelial cells (Yellow arrow), kupffer cell (Green arrow) and Binucleated hepatocytes (Red arrow) can also be seen (H&E, 20x).

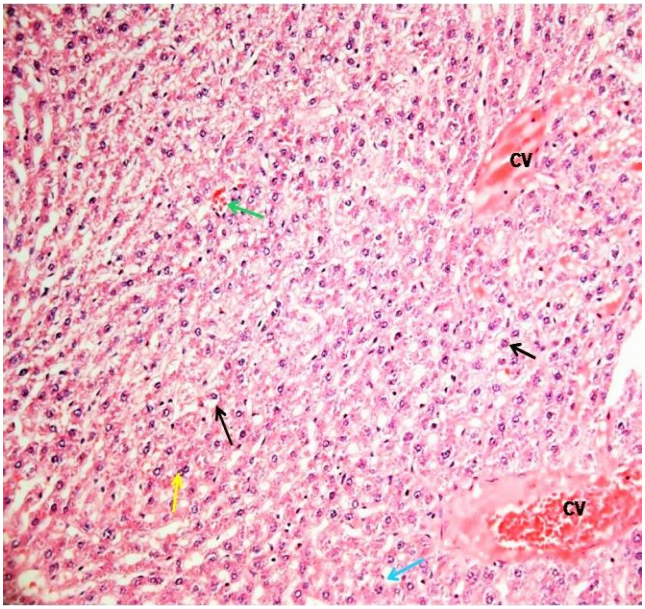

Fig 3: Rounded and vacuolated of hepaocytes (Black arrows) of Coca Cola group. Centrilobular vein (CV) and binucleated hepatocytes (Yellow arrow) and sinusoids lined by endothelial cells (Blue arrow) showing RBC's (Green arrow) (H&E, 20x).

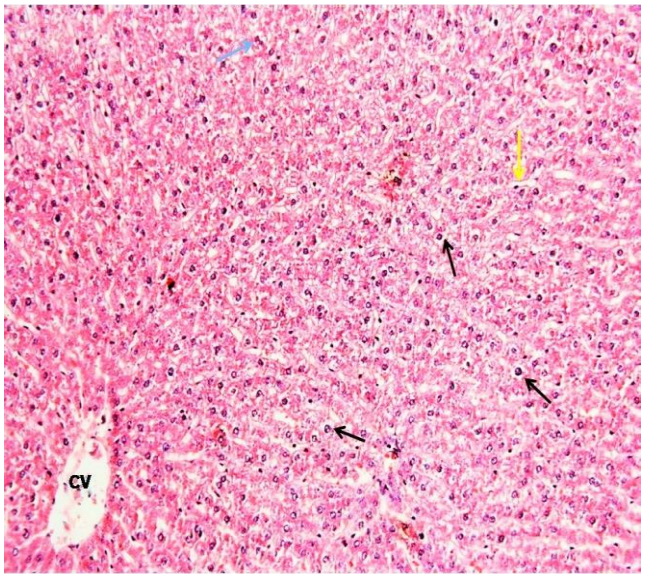

Fig.4: Rounded and vacuolated hepaocytes (Black arrows) of Red Bull group. Centrilobular vein (CV) and binucleated hepatocytes (Blue arrow) and sinusoids (Yellow arrow) (H&E, 20x).

Table 1: Multiple Comparison of Shape of Hepatocyte of Rats in Control Group A, and Experimental Groups B And C

Group I	Group J	Chi-square	Df	p-value
A	B	26.13	1	<0.001
	C	26.13	1	<0.001
B	C	…	….	….

2. Vacuolization / Hydropic Change in Hepatocytes:

Hydropic degeneration with cytoplasmic vacuolization was found in hepatocytes of both experimental groups receiving Coca Cola and Red Bull (fig.3,4). It was statistically significant (p-values <0.001) in comparison to control and the difference between the two experimental was not statistically significant, p-value 0.388 (table2).

Table 2: Multiple Comparison for Hydropic Changes/Vacuolization of Hepatocytes of Rats in Control Group A, and Experimental Groups B And C

Group I	Group J	Chi-square	Df	p-value
A	B	19.55	1	< 0.001
	C	12.15	1	< 0.001
B	C	0.75	1	0.388

Diameter of Hepatocyte:

12.17 ± 1.85 µm, was the mean diameter calculated for hepatocytes of control group, 24.5 ± 1.51 µm for Coca Cola group and 22.64 ± 1.60 µm for Red Bull group. Both experimental groups, had significantly greater diameter in comparison to control group (p-values <0.001). Coca Cola group also had greater mean diameter of hepatocyte in comparison to Red Bull group which was statistically significance (p-value 0.010) (table.3 &4)

Table 3: Comparison of Diameter of Hepatocyte of Rats in Control Group A, and Experimental Groups B And C (One Way Anova)

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	1327.1	2	663.5	240.9	<0.001
Within Groups	115.7	42	2.8		
Total	1442.7	44			

Table 4: Multiple Comparison of Diameter of Hepocyte of Rats in Control Group A, and Experimental Groups B And C (Tukey's Test)

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	p-value
A	B	-12.34	0.61	<0.001
	C	-10.48	0.61	<0.001
B	C	1.86	0.61	0.010

DISCUSSION

The key ingredients of Coca Cola as well as Red Bull are metabolized in liver so their effects on hepatocytes were observed. Hepatocytes of Coca Cola and Red Bull group were rounded in comparison to control group in this study. Similarly, Khayyat et al reported rounded hepatocytes when given different energy drinks.[13] In acute and sub acute hepatic injury, the hepatocytes become rounded indicating necrosis at early stage.[14]

Hepatocytes appear swollen, ballooned and vacuolated due to hydropic degeneration in experimental groups in comparison to control group.

Akandae and Banjoko also reported vaacuolization with power horse energy drink on liver[2], Mubarak in submandibular gland[15] and Amnah in testis.[16]

Hydropic swelling is the outcome of impeded regulation of cell volume. Incompetent Na+ K pump, or hindrance with ATP synthesis or augmented permeability to sodium, any of these can be the cause of defective regulation of cell volume.[17] Caffeine alters sodium potassium ATPase activity[18]as well as phosphodiestrase enzyme[19] causing dilated, vesicular RER and swollen mitochondria.[13,20]

Hepatocytes of the experimental groups, receiving Coca Cola and Red Bull had greater diameter in comparison to control. Furthermore, Coca Cola group had greater diameter in comparison to Red Bull group, which was statistically significant. An increase in size of hepatocytes, given various energy drinks was reported by Bukhar et al.[20]

CONCLUSION

Consuming caffeinated soft drink (Coca Cola) and taurine containing caffeinated energy drinks (Red Bull) have adversely affected the histology of rat liver**.** Both Coca Cola and Red Bull have negatively affected the rat liver histology but more in Coca Cola group**.** This would have been the result of taurine, which is hepatoprotective and has decreased the hepatoxicity of caffeine.

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