Original Article

Hepatoprotective Effects of Apple Cider Vinegar: A Histological Study in Albino Rats

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ABSTRACT

Objectives: To find out the protective effects of apple cider vinegar (ACV) on histology of the liver of albino rats.

Methodology: It was an experimental study conducted at Experimental Research Lab (Animal House) of Post Graduate Medical Institute (PGMI) and Department of Anatomy & Department of Pathology of King Edward Medical University, Lahore. Forty male albino rats were divided by lottery method into 4 groups of 10 animals each. Group 1, control group (CG) was administered food and distilled water 0.5ml/kg body weight. Group 2, the energy drink group (EDG) was given 7ml/kg body weight energy drink. Group 3, apple cider vinegar group (ACVG) was given 2ml/kg body weight apple cider vinegar. Group 4, (ACVG + EDG) was given 2ml/kg body weight apple cider vinegar and 7ml/kg body weight energy drink. All the doses were given by oral gavage once a day for 30 days. Apple cider vinegar was diluted in distilled water in a 1:5 ratio.

Results: Toxicity was produced by oral administration of energy drinks while hepatoprotectivity was observed by co-administration of apple cider vinegar along with the hepatotoxic agent. All slides of control group 1 showed normal architecture of the liver. In group 2, most animals showed a total loss of architecture of the hepatic lobule with congestion and an increase in the diameter of all vessels. Portal triad showed moderate inflammatory infiltrate and nuclei in this group showed pyknotic changes. Animals of group 3 showed dilated central vein with mild periportal inflammation and few dilated vessels in the portal triad. The mean diameter of hepatocytes in this group was 19.90±1.79µm. Routine hematoxylin and eosin (H & E) staining of group 4 showed mild periportal inflammation and few dilated vessels in the portal triad, vacuolization was present, and the mean diameter of hepatocytes was 22.00±1.35µm.

Conclusion: The dose of 1.3ml/150gm body weight/day of energy drink is hepatotoxic to rats at the histological level. Apple cider vinegar when given to rats for a month has a protective role in hepatotoxicity induced by energy drinks in rats as evident by hepatocellular damage at the histological level. Thus ACV offered partial protection to the liver against damage by energy drinks.

Keywords: *Apple cider vinegar. Energy drink. Apple cider vinegar group. Hepatoprotection. Albino Rats.*

INTRODUCTION

Energy drink consumption has become popular in recent years.[1] The consumption around the world has been doubled from the year 2006 to 2012.[2] These drinks are popular among students and athletes and contain glucuronolactone, amino acids mainly taurine, creatine, plant-based guarana, simple sugars like glucose and fructose, various herbs like ginseng and biloba.[1,2] Caffeine is the main ingredient of these drinks which acts as a psychological and physiological stimulant by stimulating the central nervous system.[3] Researchers mentioned that different brands of energy drinks contain different amounts of caffeine.[4] The consumption of such caffeinated energy drinks has shown damaging effects on the levels of liver enzymes in the serum of rats.[5]

Vinegar is an acidic solution that has been in use for different purposes for centuries. Apple cider vinegar (ACV) is the most popular vinegar that has been used by humans. Research has shown positive effects of ACV on many health issues, including high blood pressure, diabetes, skin ailments, heart ailments, high cholesterol, digestive and immune system problems.[6] It is an important detoxifying agent. It oxidizes and thins the blood. Apple cider vinegar neutralizes any poisonous substances that enter the body and kills destructive microbes that may be present in specific foods. A good quality ACV contains amino acids and antioxidants in variable amounts.[7] Acetic acid is present in abundant amounts in ACV along with flavonoids and polyphenols. The healthful benefits of polyphenols are due to their antioxidant, anti-allergic and anti-inflammatory properties.[8]

The liver is the primary organ directly involved in the metabolism of the substances and chemicals entering through the gastrointestinal tract, thus is most vulnerable to the harmful and toxic effects of whatever we eat or drink.[9]

Since the use of energy drinks has become very popular recently, their harmful effects are backed by many studies. An effort is required to find a substance that gives protection to the liver against these drinks. Apple cider vinegar has been shown to have antioxidant properties, thus, it might prove to exhibit a protective effect on the liver against the deleterious effects of energy drinks.[10] So, the present study was designed to evaluate the hepatoprotective effects of ACV on the liver of albino rats.

METHODOLOGY

It was an experimental study conducted on forty adult male albino rats after taking ethical approval from the ethical review board of the institute. The study was

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conducted at Experimental Research Lab (Animal House) of Post Graduate Medical Institute (PGMI) and Department of Anatomy & Department of Pathology of King Edward Medical University, Lahore.

Male albino rats of 8-12 weeks age, weighing 130-160g were divided by lottery method into four groups, with ten animals in each group. Animals of these groups were housed in separate stainless steel cages. The animals were maintained on an ad libitum standard chick diet and distilled water and were allowed to acclimatize for a week before starting the experiment. All animals were weighed before and at the end of the experiment.

Group 1 (CG) animals were administered food and distilled water 0.5ml/kg/day for 30days. Group 2 (EDG) animals were given energy drink in a dose of 7ml/kg body weight[11] that is, 1.3 ml/150 gm weight of rat. Group 3 (ACVG) animals were given ACV once a day in a dose of 2ml/kg body weight diluted with distilled water at a ratio of 1:5,[12] approximately 0.3ml/150gm weight of rat. Group 4 (ACVG + EDG) animals were given the same doses of both liquids, 2ml/kg ACV & 7ml/kg ED, once a day for 30 days. The doses were given once daily by oral gavage for 30 days in each group.

At the end of the experiment, the animal was anesthetized by chloroform inhalation and sacrificed on the 30th day. The doses were given once daily by oral gavage for 30 days in each group. An incision was made in the midline, exposing the abdominal viscera. The liver was grasped gently with the left hand and pulled out. The portal vein, hepatic artery, and bile duct were ligated after exposure. Once the liver was free from all ligaments and vessels, it was removed from the body. All the specimen were preserved in 10% formalin solution, tissue processing was done in an automatic tissue processor followed by sectioning and hematoxylin and eosin (H & E) staining.

STATISTICAL ANALYSIS

The collected information of the four groups was entered into computer software Statistical Package for Social Sciences (SPSS) version 21 and analyzed through it. All the data was collected on proformas. Post-Hoc Tukey test was applied to observe the mean differences among the groups significantly. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

All slides of control group 1 showed normal architecture of the liver. The portal triad comprised a branch each of the portal vein, hepatic artery, and bile duct. Branch of portal vein was lined with flattened endothelium with wider lumen containing erythrocytes as compared to the branch of the hepatic artery that had thick walls and rounded lumen also lined with flattened endothelium while the bile duct was lined with cuboidal epithelium (Figure 1).

In group 2, most of the animals showed total loss of architecture of the hepatic lobule. All blood vessels showed congestion and an increase in diameter. Portal triad showed moderate inflammatory infiltrate with a predominance of lymphocytes. Nuclei in group 2 showed pyknotic changes (Figure 2).

Histological preparations of animals of group 3 showed dilated central vein with congestion at the center. Mild periportal inflammation and few dilated vessels in the portal triad were also seen (Figure 3).

Animals of group 4 stained with H & E staining showed preservation of general architecture of hepatic lobules having central vein lined with flattened epithelium at the center and radiating cords of hepatocytes of one or two cell thickness. Hepatocyte cords enclosed sinusoids that were lined with continuous flattened epithelium and contained specialized stellate cells called Kupffer cells towards their lumen (Figure 4). Routine H & E staining showed mild periportal inflammation and few dilated vessels in the portal triad. Pyknosis and congestion were also seen in both group 3 and group 4.

Cytoplasmic Changes

Cytoplasmic changes were present in all animals of group 2, 3, and 4 while absent in all of group 1. The overall difference was significant with a p-value <0.001 and groups 3 and 4 also had a significant difference from groups 1 and 2 with a p-value <0.001 (Table 1).

Inflammatory Changes

The inflammatory changes around the portal triad were found in groups 2, 3, and 4 while absent in group 1. In group 2, 50.0% had moderate and 50.0% had severe inflammation. In group 3 all 10 animals had moderate inflammation while in group 4, 40.0% had moderate and 60.0% had severe inflammation. The overall difference was found significant with a p-value <0.001 (Table 2).

Changes Around The Central Vein

Changes around the central vein were found present in all animals of groups 2 and 3 while absent in all animals of groups 1 and 4. Congestion and pyknosis were present in all 10 animals of group 2, 3, and 4 while absent in all animals of group 1. The overall difference and difference between groups were significant with p-values <0.001 (Table 3).

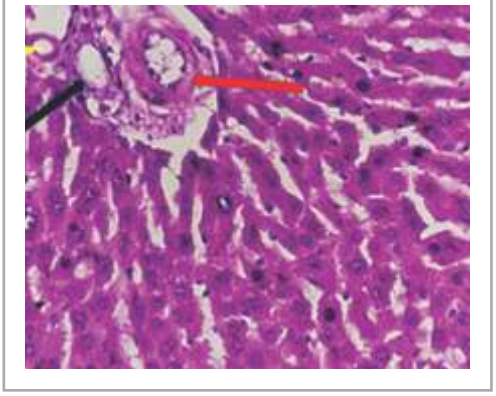

Figure 1: Photomicrograph from Histological Preparation of Rat Liver from Group 1 Showing Cuboidal Cell Lined Bile Duct (Yellow Arrow), Portal Vein (Black Arrow) Lined by Flattened Cells, Hepatic Artery (Red Arrow) Filled with Blood. No Periportal Inflammation seen on H & E Staining (Magnification 40×10 X=400)

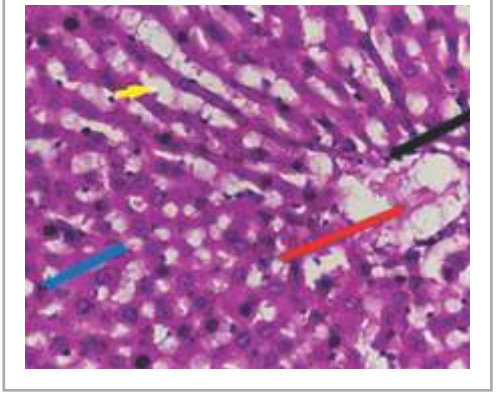

Figure 2: Photomicrograph from Histological Preparation of Rat Liver from Group 2 Showing Dilated Central Vein (Red Arrow) filled with Blood. Lymphocytic Aggregation around Central Vein (Black Arrow). Distorted Sinusoids (Yellow Arrow) with Kupffer Cells are Visible. Pyknotic Nuclei Seen (Blue Arrow) on H & E Staining (Magnification 40×10 X=400)

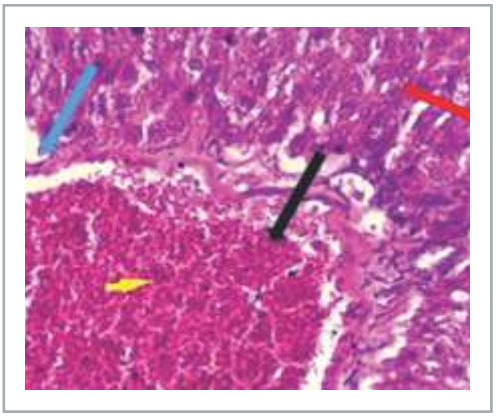

Figure 3: Photomicrograph from Histological Preparation of Rat Liver in Group 3 Showing Congestion (Yellow Arrow) with Dilated Central Vein (Black Arrow), Marked Vacuolization (Blue) with Mild Pyknosis (Red Arrow) is also seen on H & E Staining (Magnification 40×10 X=400)

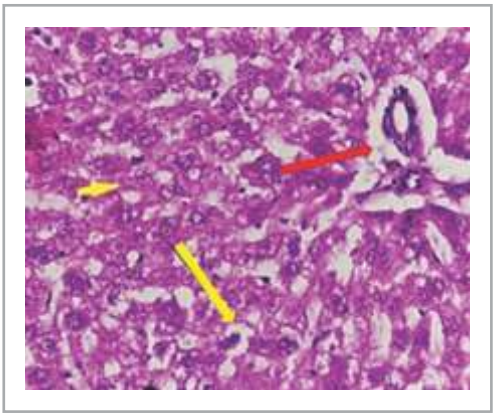

Figure 4: Photomicrograph from Histological Preparation of Rat Liver in Group 4 Showing Reduced Portal Inflammation (Red Arrow) on H & E Staining (Magnification 40×10 X=4)

Table 1: Status and Comparison of Cytoplasmic Changes Among Four Groups

Groups	Cytoplasmic Changes (Vacuolization)					
	Present		Absent		Total	
	n	%	n	%	n	%
Group 1(Control)	0	0.0	10	100.0	10	100.0
Group 2 (Energy Drink)	10	100.0	0	0.0	10	100.0
Group 3 (Apple Cider Vinegar)	10	100.0	0	0.0	10	100.0
Group 4 (ED +ACV)	10	100.0	0	0.0	10	100.0

Table2: Inflammatory Changes around Portal Triad in Study Groups

Groups	Inflammatory Changes Around Portal Triad									
	Absent		Mild		Moderate		Severe		Total	
	n	%	n	%	n	%	n	%	n	%
Group 1(Control)	10	100.0	0	0.0	0	0.0	0	0.0	10	100.0
Group 2 (Energy Drink)	0	0.0	0	0.0	5	50.0	5	50.0	10	100.0
Group 3 (Apple Cider Vinegar)	0	0.0	0	0.0	10	100.0	0	0.0	10	100.0
Group 4 (ED +ACV)	0	0.0	0	00.0	4	40.0	6	60.0	10	100.0

Table 3: Changes around the Central Vein, Congestion and Pyknosis among Four Groups

Groups	Changes Around the Central Vein (n)		Congestion (n)		Pyknosis (n)	
	Absent	Present	Absent	Present	Absent	Present
Group 1(Control)	10	0	10	0	10	0
Group 2 (Energy Drink)	0	10	0	10	0	10
Group 3 (Apple Cider Vinegar)	0	10	0	10	0	10
Group 4 (ED +ACV)	10	0	0	10	0	10

DISCUSSION

Energy drinks are being consumed abundantly all over the world. These drinks are marketed among young people to improve their physical and mental performance such as concentration, attention, and alertness.[5] It is believed to be due to the presence of a combination of different stimulants including caffeine, guarana, ginseng, vitamins, taurine, amino acid derivatives such as carnitine, ribose, carbohydrates, and sugar derivatives, including glucuronolactone.[13,14] Although with their potential beneficial effects, massive consumption of energy drinks has been shown to result in life-threatening toxicity.[7]

The use of energy drinks has been reported to cause damage to the liver tissue in male wister albino rats as mentioned by Khayat et al. and various histological changes indicated the loss of functional integrity and hepatocellular damage to the liver were also seen in this study.[7] There are claims that this hepatocellular damage can be prevented by various herbs and natural liquids like vinegars[15,16], but no scientific evidence was available on hepatoprotection by apple cider vinegar on hepatotoxicity induced by energy drinks. Therefore, the current study was designed to provide scientific proof.

In the present study, changes around the central vein, inflammation around the portal triad, pyknosis, and leukocytic infiltration were observed in our experimental groups after histological damage of the liver by energy drinks (Figure 2, Table 3 & 4). Our findings coincide with the results of Gheith et al., who observed in 2017 that the histological sections of the liver showed architectural distortion after energy drink intake with pyknosis, necrosis leukocytic infiltration, and vacuolization.[4] Similar findings were observed by Harb et at. in 2016 in a case report.[17]

In another study, liver sections of rats treated with energy drinks for 4 weeks were examined that showed leukocytic infiltration and congestion of blood sinusoids. The polyhedral shape of hepatocytes was lost with the appearance of pyknotic nuclei. The normal liver architecture was distorted with marked vacuolization and necrosis of most hepatocytes.[7] These findings coincide with the histological examination of slides of the rat so four energy drink treated group which has shown congestion, vacuolization and inflammation around the portal triad.

In the present study, the histological structure of the liver was damaged by the intake of energy drinks for 30 days. The mean diameter of hepatocytes was observed smallest for group 2 while the largest for group 4 (Table 1). When compared among groups the difference was found significant. Congestion, pyknosis, changes around the central vein, and inflammation around the portal triad was observed in all experimental groups

(Figure 2, 3 & 4). Pyknosis was marked in the energy drink group while marked congestion in the central vein was observed in the ACV group (Table 4). Our findings coincide with the results of another study in which histological sections of the liver showed architectural distortion after energy drink intake at a dose of 3-6ml/day was observed. According to their results, the hepatic lobules were not distinctly demarcated although the hepatocytes were seen to radiate as single plate or cells from the central vein towards the portal tracts. There were moderate intracytoplasmic vacuoles in hepatocytes, and sinusoids were distorted. With high doses of energy drink (6-12 ml/day), hepatocytes increased in size and showed intracytoplasmic vacuolation.[14]

The present study showed lesser pyknosis, congestion, and changes around the central vein in group 4 given ACV and ED (Figure 3 & 4, Table 4). These findings coincide with the results of another study. They reported that the group treated with nicotine and apple cider vinegar in a dose of 2ml/kg body weight showed somewhat normal liver structure and normal appearance of the hepatocytes with decreased vacuolization when compared with the nicotine treated group. Also, the ACV-treated group restored more or less the normal size of the nucleus.[18] Bouazza et al. also observed that animals given apple vinegar showed few inflammatory infiltrates on liver sections.[19]

The current study observed partial protection by ACV to the liver against damage by energy drinks. Further studies are recommended to determine the beneficial as well as harmful effects of different doses of ACV on various organs and systems to determine an appropriate effective dose.

CONCLUSION

The dose of 1.3ml/150gm body weight/day of energy drink is hepatotoxic to rats at the histological level. Apple cider vinegar when given to rats for a month has a protective role in hepatotoxicity induced by energy drinks in rats as evident by hepatocellular damage at the histological level. Thus ACV offered partial protection to the liver against damage by energy drinks.

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