Original Article

Hepatoprotective Effects of Apple Cider Vinegar on Gentamicin Induced Toxicity in Albino Rats

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ABSTRACT

Objective: To assess the morphological effects of Apple cider vinegar on hepatotoxicity caused by gentamicin in male adult albino rats.

Study Design: Experimental study.

Place and Duration of Study: This study was conducted at the Department of Anatomy, Postgraduate Medical Institute (PGMI), Lahore, Pakistan for a period of 21 days starting on June 1st, 2019.

Materials and Methods: This experimental study comprised 30 male adult albino rats divided into three groups, A, B and C with 10 rats in each group. Group A was control and it received distilled water 4ml/kg/day via intra-peritoneal route for 21 days. Group B received only Gentamicin 100mg/kg/day from day 10th-21st intraperitoneally. Groups C received Apple cider vinegar 2ml/kg/day via oral gavage for first 10 days then along with Gentamicin 100mg/kg/day intraperitoneally for next 11 days upto day 21st. Rats were sacrificed on 22nd day. Histological parameters of liver i.e., size of hepatocyte, hepatocyte vacuolization and central vein congestion were studied. The results were analyzed by SPSS version 22.0.

Results: Hepatocyte size in group B was significantly higher when compared with group A and C (p value < 0.001). However, no significant difference was observed in the hepatocyte size between the group A and C. Hepatocyte vacuolization was absent in all rats of control group A but present in all rats of group B. While it was present in 3 rats (30%) of group C. No rat of group A showed congested central vein but all rats of group B showed congested central vein. Only 1 rat (10%) of group C showed congestion of central vein of liver.

Conclusion: From the foregoing results, it is clear that gentamicin can induce many histological changes in the liver causing severe toxicity that is reflected in the parameters i.e., size of hepatocyte, central vein congestion and hepatocyte vacuolization. It also demonstrates that apple cider vinegar protects against its hepatotoxicity on molecular level through its antioxidants pathways.

Key Words: Apple cider vinegar, gentamicin, hepatocyte vacuolization, micrometry.

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INTRODUCTION

Liver is the chief organ of drug metabolism. It consists of hexagonal hepatic lobules.[18] These lobules have a central vein and hepatocytes radiates from it in the form of cords, with associated thin-walled sinusoids that drain blood from the portal venule towards the central vein.[2] The fenestrations in endothelial cells of sinusoids allow the exchange of portal blood with the adjacent hepatocyte. The liver, mainly hepatocytes, is involved in metabolism of drugs, amino acids, lipids and glycolysis.[2]

Gentamicin is derived from Micromonospora purpurea and is prescribed to treat infective endocarditis, sepsis, meningitis, peritonitis, bacterial conjunctivitis and infections caused by gram-negative bacteria.[3] Despite the beneficial effects, it inhibits the non-enzymatic and enzymatic antioxidants in liver thereby elevating the levels of Reactive Oxygen Species (ROS). It results in enhanced oxidative stress and damage to lipids in membrane, cellular proteins and nucleic acids leading to liver injury.[8]

Apple cider vinegar contains beta-carotene and flavinoids which reduces oxidative stress, act as antioxidant phytochemical and decrease the level of Reactive oxygen species (ROS).[19] Vinegar also contains vitamins, mineral salt, amino acids, phenols and organic acid.[6] These compounds have a vast window of pharmacological functions, such as acting as an antioxidant, antidiabetic[17] and cholesterol lowering agents.[7]

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MATERIALS AND METHODS

This study was conducted at the Department of Anatomy, Postgraduate Medical Institute (PGMI), Lahore, Pakistan for a period of 21 days starting on June 1st, 2019.

30 adult male albino rats were procured from animal house of PGMI. The healthy rats of 8-10 week of age with weight range of 180-220g were selected. They were properly acclimatized and kept in well ventilated and temperature maintained house at 24±2ºC, humidity 55 ± 5% and dark & light cycles, each cycle of 12 hours. Rat chow and water was given to the animals ad libitum.

Dissection and Tissue Sampling: At the end of experimental period, on 22nd day, 24 hours after the administration of last dose of the agent, each rat was anaesthetized. Skin was cut by giving a midline incision. Liver was identified in the right upper abdominal region and excised carefully. The other half was fixed using 10% NBF (Neutral Buffered Formalin).

Histological Techniques

Tissue Preservation: The liver of each animal was placed in neutral buffered formalin. Tissue was processed for up to 18 hours by using the automatic tissue processor (HISTOTOUCH III-USA). For embedding, liquid paraffin was then poured onto the tissue piece to make tissue block. By using microtome, sections of 3 µm thickness were obtained and stained with Hematoxylin and Eosin.

Histological Parameters

Quantitative:

1. Size of hepatocyte.

Qualitative:

1. Hepatocyte vacuolization.
2. Central vein congestion.

Histological Examination: For assessment and measurement of histological parameters, light microscope (Leica DM 1000) was used to examine the prepared tissue sections, using magnifications of 10X as well as 40X.

Micrometry (Size of hepatocyte): For measuring this parameter, an ocular micrometer was inserted into the eyepiece of Leica 1000 DM microscope and tissue sections were examined under 40X magnification. The ocular micrometer was calibrated with 40X objective lens as to precisely focus the engraved linear scale on its surface. After removing stage micrometer, ocular micrometer was used for measuring diameter of hepatocytes.[4] 3 hepatic sections from each of the 30 albino rats were observed. 5 or more hepatic lobules with central vein were identified in cross-sectional view (Fig.1); thus 450 in total of hepatic lobules with central veins were examined and the mean diameters of hepatocyte were noted down.

Qualitative Parameters

Central vein Congestion: Central vein of each hepatic lobule was assessed at magnification power 40X of objective lens using Leica microscope, DM 1000. 5 central veins in hepatic lobules in each section were examined and 3 sections from each animal were taken. Thus the central veins of 450 in total of hepatic lobules were recorded.[15] The mean was calculated in each rat using SPSS 22 in each group.

Hepatocyte Vacuolization: Following H&E staining, slides were meticulously observed at 10X for the presence of vacuolization of hepatocytes.[20]

Statistical Analysis: The analysis of gathered data was done by applying SPSS 22.0 (Statistical Package for Social Sciences). Mean ±S.D was given for quantitative variables like diameter of hepatocytes in liver. Kruskal Wallis test was used for the comparison of the hepatocyte size (µm²) among groups. The mean differences of qualitative variants, i-e central vein congestion and hepatocyte vacuolization parameters were determined by Fisher's exact test. *P*-value of ≤0.05 was considered as statistically significant.

RESULTS

Size of Hepatocyte (µm²): The mean hepatocyte size (µm²) in all groups was determined using micrometry. It was found that the hepatocyte size (µm²) in all groups were significantly different (p value < 0.001).

Table No.1: Experimental groups of animals, mode of intervention and dosage of drug

Groups	Intervention and Dosages	Number of Animals (N)	Method of Administration	Duration of Dosage	Day of Sacrifice
Group A	4ml/kg/day of distilled water	10	Intraperitoneally	21 days	22nd
Group B	Initially 4ml/kg/day distilled water for 10 days then gentamicin 100mg/kg/day for next 11 days up to day 21	10	Intraperitoneally	21 days	22nd
Group C	Apple cider vinegar 2ml/kg/day for 1st 10 days then both Apple cider vinegar 2ml/kg/day plus gentamicin 100mg/kg/day for next 11 days up to day 21	10	Gentamicin intraperitoneally and Apple cider vinegar Orally by gavage tube	21 days	22nd

Table No.2: Comparison of hepatocyte size (µm²) among groups

Variable	Group A Mean ± SD	Group B Mean ± SD	Group C Mean ± SD	P-value#
Hepatocyte size (µm²)	14.5 ± 0.80	22.8 ± 1.67	14.8 ± 0.65	< 0.001*
	14.2 (14.2 – 15.21)	23.3 (22.3 – 23.3)	15.0 (14.2 – 15.2) [a]	

#Kruskal Wallis test, Median (IQR) *p value ≤ 0.05 is regarded as significant statistically

Table No.3: Distribution of hepatocyte vacuolization among groups

Hepatocyte Vacuolization	Group A n (%)	Group B n (%)	Group C n (%)	p-value
Absent	10 (100.0%)	0 (0.0%)	7 (70.0%)	< 0.001*
Present	0 (0.0%)	10 (100.0%)	3 (30.0%)	

Fisher's exact test
*p value ≤ 0.05 is considered statistically significant

Table No.4: Distribution of central vein congestion among groups.

Central Vein Congestion	Group A n (%)	Group B n (%)	Group C n (%)	p-value
Absent	10 (100.0%)	0 (0.0%)	9 (90.0%)	< 0.001 *
Present	0 (0.0%)	10 (100.0%)	1 (10.0%)	

Fisher's exact test
*p value ≤ 0.05 is considered statistically significant

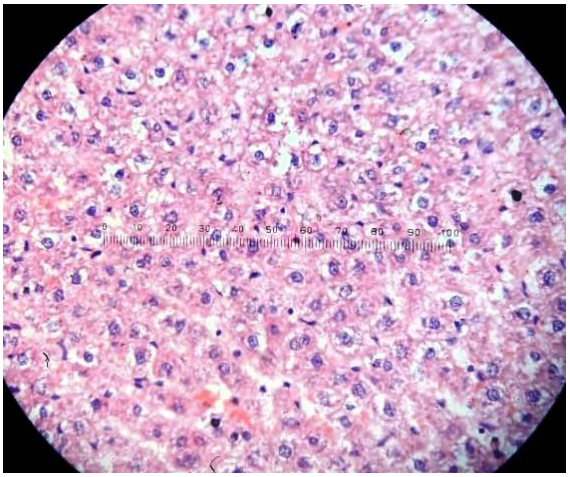

Figure No.1: Micrometery of the hepatocyte

Hepatocyte vacuolization: Hepatocyte vacuolization in all rats of control group A was absent. In group B, vacuolization in hepatocytes all rats were present while in group C, hepatocyte vacuolization was present in only 3 rats (30.0%).

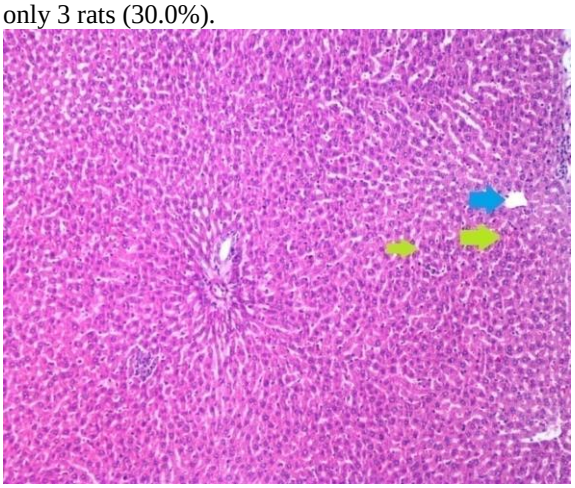

Figure No. 2: Photomicrograph of section of liver from Control group A showing central vein (blue arrow) and hepatocytes (green arrow) radiating from central vein. H&E stain 10X.

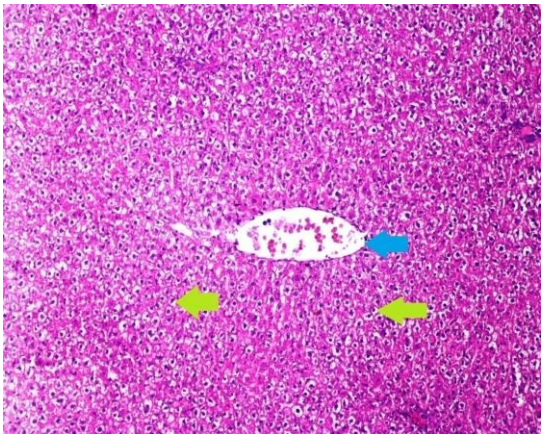

Figure. No.3: Photomicrograph of section of liver of gentamicin treated Group B showing vacuolization of hepatocytes (green arrow) and congested central vein (blue arrow). H&E stain 10X.

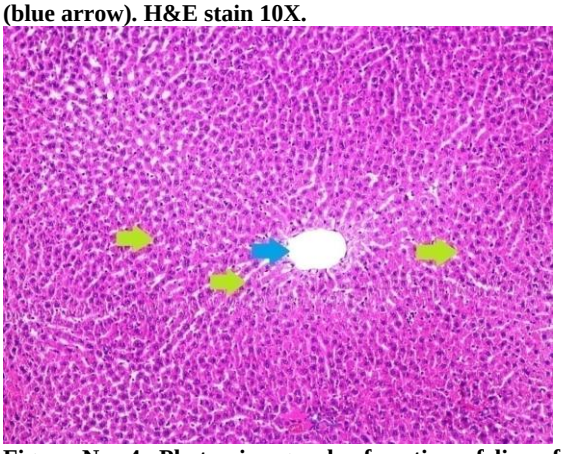

Figure No. 4: Photomicrograph of section of liver from Group C treated with gentamicin and apple cider vinegar showing hepatocytes (green arrow) and central vein (blue arrow). The liver architecture is preserved, no vacuolization is seen and central vein is lined by epithelium. H&E stain 10X.

Central vein congestion: Central vein congestion in all rats of control group A was absent. In group B, central vein congestion in all rats was present while in group C, central vein congestion was present in only 1 rat (10.0%). Tables 1-4 and Figures 1-7 be seen.

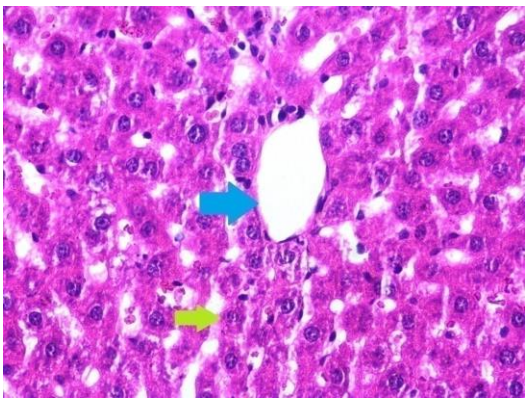

Figure No. 5: Photomicrograph of section of liver from Control Group A that shows central vein (blue arrow) and hepatocytes (green arrow). H&E stain 40X.

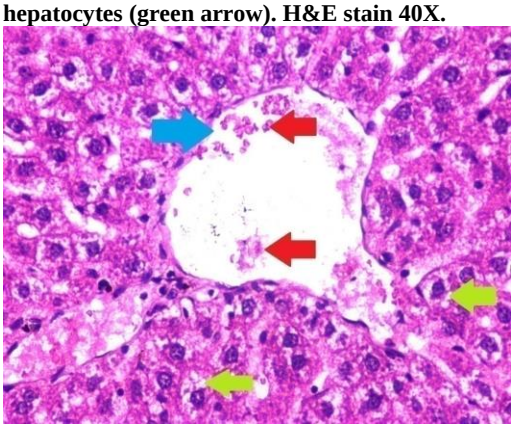

Figure No. 6: Photomicrograph of section of liver from Group B treated with gentamicin that shows congested central vein (blue arrow) and hepatocytes (green arrow). Epithelium of central vein (blue arrow) is disrupted and inflammatory and red blood cells (red arrow) are seen in dilated central vein. H&E stain 40X.

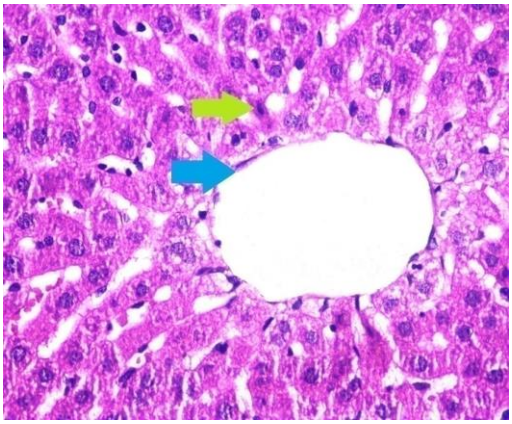

Figure No. 7: Photomicrograph of section of liver from Group C treated with gentamicin and apple cider vinegar showing central vein (blue arrow) and hepatocytes (green arrow). The liver architecture is preserved; central vein is lined by epithelium. H&E stain 40X.

DISCUSSION

Gentamicin enhances the oxidative stress in liver leading to generation of ROS (reactive oxygen species) that cause inflammation and cell necrosis.[14]

Apple cider vinegar (ACV) decreases serum lipid peroxidation and serum catalase activity while up regulates endogenous superoxide dismutase (SOD) activity in rats exposed to chronic restraint stress.[1]

A statistically significant increase in the mean hepatocyte diameter (µm) of gentamicin treated group B was observed as compared to group A. Control and group C showed no significant disparity. The increase in diameter of hepatocyte in group B is due to damage of cell organelles by Reactive oxygen species leading to accumulation of fatty vacuoles in hepatocytes. This is in agreement with the results of studies carried out by Hassan et al. (2018) and Nale et al. (2012).[10,12] The diameter of hepatocytes of group C is near to control group A showing the protective effect of apple cider vinegar on liver hepatocytes by acting as an antioxidant and preserving the liver architecture. These findings were same as the data available by the previous studies by Omar et al. (2015) and Bouazza et al. (2016).[15,5]

Hepatocytes were observed for accumulation of fatty vacuoles in all groups. Hepatocyte vacuolization was not observed in any of the control group A rats. However, hepatocyte vacuolization was visible in all rats of group B and only 3 rats of group C. This is mainly due to generation of reactive oxygen species by gentamicin leading to damage of hepatocyte DNA and protein structure, thus damaging the organelles leading to accumulation of fatty vacuoles in hepatocyte.[12] These results are in accord to the study carried out by Hassan et al. (2018) which showed the presence of vacuoles in hepatocytes after treatment with 80mg/kg gentamicin for 15days.[10] While apple cider vinegar treated group C showed minimal vacuolization showing less hepatocyte damage. The main mechanism involved is through its antioxidant activity as well as its hypolipidemic effect.[9] Nazıroğlu et al. (2014) studied the role of apple cider vinegar in regulating the serum lipid profile in ovariectomized mice which were on high cholesterol diet and showed that apple cider vinegar normalizes the liver profile such as AST, ALT and lipid profile.[13]

Liver sections of all the rats in group A, B and C was observed for histopathologic changes in the central vein with respect to its epithelial lining and presence or absence of blood/inflammatory cells in its lumen. All observed sections of rats of control group A showed normal histology of central vein. There was damage to the epithelial lining of central vein as well as red blood cells and inflammatory were present in its lumen in all the observed sections of gentamicin treated rats of group B confirming for central vein congestion while only 1 rat of apple cider vinegar treated group C showed central vein congestion. The mechanism involved is formation of reactive oxygen species that cause damage to the endothelium of blood vessels and accumulation of blood cells as well as inflammatory cells.[11] While apple cider acts as an antioxidant to protect the endothelial lining of blood vessels.[15] These

findings are similar to the studies carried out by Omer et al. (2016) and Jannat et al. (2018).[16,11]

CONCLUSION

From the foregoing results, it is clear that gentamicin can induce many histological changes in the liver causing severe toxicity that is reflected in the parameters i.e. size of hepatocyte, central vein congestion and hepatocyte vacuolization.

Author's Contribution:

Concept & Design of Study:	Shumaila Sohail
Drafting:	Masooma Ahmad, Huma Jawad
Data Analysis:	Haseeb Ahmed Awan, Wardah Yaseen, Fatima Jawad
Revisiting Critically:	Shumaila Sohail, Masooma Ahmad
Final Approval of version:	Shumaila Sohail

Conflict of Interest: The study has no conflict of interest to declare by any author.

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