Original Article

Effect of berberis vulgaris fruit extract on Gentamicin induced Histopathological changes in liver of Albino rats

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Objective: To evaluate the effect of the Berberis vulgaris fruit extract (BVFE) on the hepatic cells against cytotoxicity induced by gentamicin.

Methodology: This experimental study was conducted at department of Anatomy, Liaquat National Hospital &Medical College, Karachi, Pakistan for a period of four weeks in March 2020. The severity of liver damage was observed in randomly selected four different visual fields of each slide under light microscopy, using the following parameters: hepatolobular architecture, arrangement of hepatocytes within lobules, presence of congestion, hydropic degeneration & vacuolization, inflammatory infiltrate, mean hepatocyte count and mean nuclear diameter.

Results: There was statistically significant difference in mean hepatocyte count (p = 0.00) while no significant difference in the mean nuclear diameter among various groups.

Conclusion: Berberis vulgaris fruit extract is hepatoprotective against the damaging effects of drugs like gentamicin in albino rats and the protective effects are better at higher doses.

Keywords: Berberis vulgaris fruit extract, gentamicin, albino rats, hepatoxicity.

INTRODUCTION

Liver regulates metabolic functions of the body including detoxification of drugs. Drug induced hepatotoxicity is a potentially fatal and a major clinical challenge for the physicians.[1,2] It is caused by medications, herbal and dietary supplements or other xenobiotics.[3] Drug induced hepatotoxicity can be Intrinsic or Idiosyncratic. Intrinsic refers to liver toxicity induced by a drug in a predictable and dose-related manner while Idiosyncratic is associated with a less consistent dose-toxicity relationship.[4,5] More than 900 drugs, toxins, and herbs have been reported to cause liver injury, among them antibiotics are the most common cause.[6]

Clinical manifestation range from elevation of liver enzymes with or without symptoms to development of severe liver injury leading to acute hepatic failure.[7] Gentamicin is a potent bactericidal agent belonging to aminoglycoside group of antibiotics. It is used against aerobic gram negative bacteria and is a widely used for the prophylaxis and treatment of urinary tract and abdominal infections.[8,9] It is a hepatotoxic and produces tissue injury by free radical damageand by apoptosis.[10,11] It produces marked elevation in the levels of ALT, AST and a decrease in total serum protein and albumin level.[12,13]

Liver damage produced by the hepatotoxic drugs can be reverted or treated by plant extracts.[14] Berberis vulgaris is one such medicinal plant which is used to treat hepatic disorders.[12] There are variety of alkaloids in the various parts of this plant like fruit, leaves, root, the most important of which is berberine; this alkaloid can exert different effects including antioxidant, anti-inflammatory, hypoglycemic, hypotensive, and hypolipidemic.[15] Berberine protects from hepatic tissue destruction by reducing steatosis, necrosis and myofibroblast and inflammatory cell proliferation in hepatocytes.[16]

METHODOLOGY

This experimental study was conducted at department of Anatomy, Liaquat National Hospital &Medical College, Karachi, Pakistan for a period of four weeks in March 2020. The study was approved by the institutional Research & Ethical review committee (Letter No: 0436-2020LNH-ERC). Thirty two male albino rats, weighing 160-230 gm were housed in clean, properly ventilated cages in a temperature-controlled room and had free access to standard animal diet and water throughout the experiment.

The Berberis Vulgaris fruit was purchased from local herbal market of Karachi and confirmed by a botanist. 1000 gm of dried and powdered fruit was soaked in adequate volume of ethanol and water 70:30, stirred in

an orbital shaker and the extract was obtained using percolation method. The extract was filtered and evaporated to obtain semisolid syrup.[17] The rats were randomly divided into four groups (n = 8) and the drugs were administered using animal feeding intubation needle (BVFE) and intra-peritoneally using insulin syringe (gentamicin). Group A (control group) received normal saline daily for 21 days. Group B (gentamicin group) received 80 mg/kg injection gentamicin intra-peritoneally daily for 21 days. Group C (gentamicin + BVFE100) received gentamicin 80 mg/kg intraperitoneally and BVFE 100 mg/kg orally for 21 days. Group D (gentamicin + BVFE200) received gentamicin 80 mg/kg intraperitoneally and BVFE 200 mg/kg orally for 21 days.

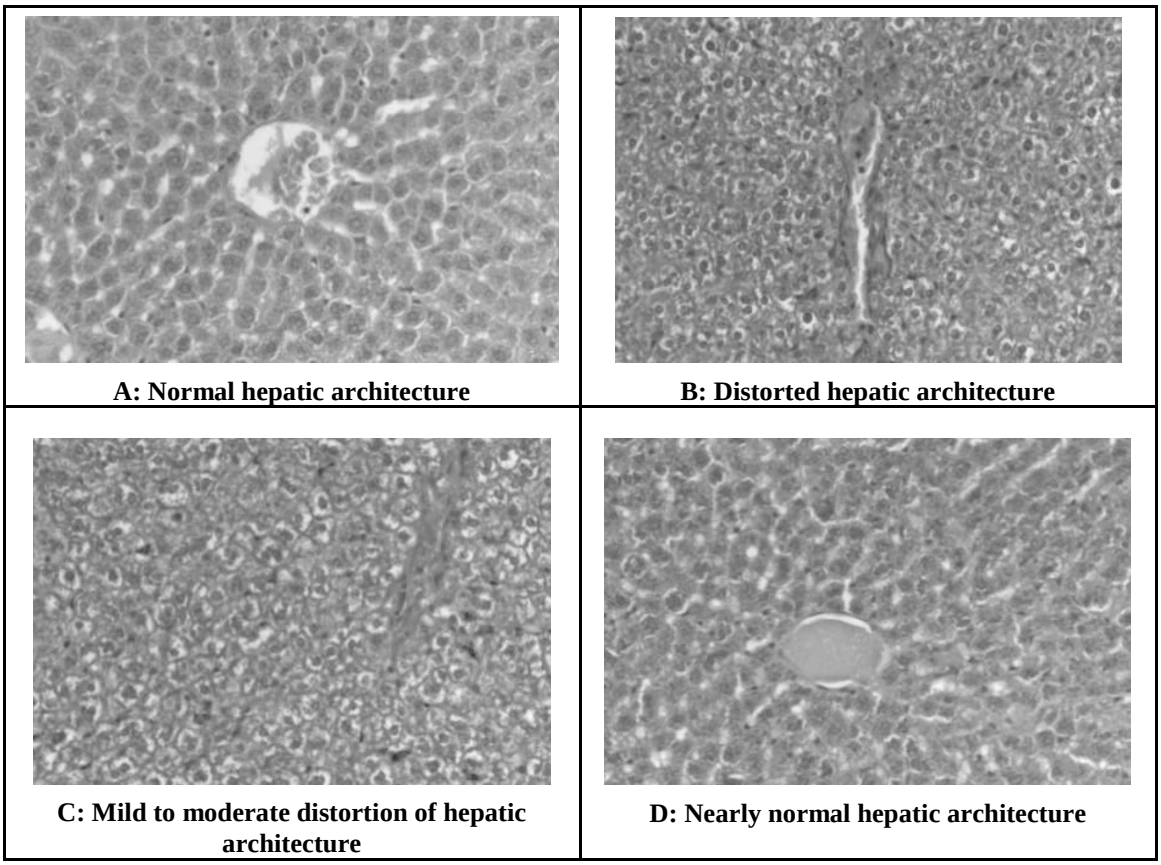
At the end of the experimental period, all animals were weighed for final body weight, anesthetized and sacrificed. Livers were removed after careful dissection, washed with saline and photographed for gross appearance. The specimen was preserved in 10% formalin solution. The specimens embedded in paraffin were cut into 5 micron thick sections, stained with hematoxylin and eosin (H&E) stain, examined under light microscopy and photographed with digital camera using Moticam 1080 HDMI & USB.[18,19]

The severity of liver damage was observed in randomly selected four visual fields of each slide under light microscopy, using the following parameters: hepatolobular architecture, the arrangement of hepatocytes within lobules, presence of congestion, hydropic degeneration & vacuolization, inflammatory infiltrate, mean hepatocyte count and mean nuclear diameter. These microscopic findings were calculated separately and labeled as normal or absent (0), mild (1), moderate (2), and severe (3) for each parameter.[19]

Statistical Analysis: The data were analysed using SPSS 22. Morphometric parameters like hepatocyte count and nuclear diameter of various treatment groups was performed using one-way analysis of variance (ANOVA). Chi square test was used to compare the histopathological changes in the liver tissue.$p<0.05$ was considered significant.

RESULTS

Mean initial weight of groups A, B, C & D was 173.5, 200.37, 200.75 & 174.75 gm, respectively (Table1). Mean liver weight of group A and D was 6.48 ± 0.30 & 6.54 ± 0.59 gm while group B & C was 7.85 ± 1.27 & 7.34 ± 0.59 gm, respectively.



A: Normal hepatic architecture

B: Distorted hepatic architecture

C: Mild to moderate distortion of hepatic architecture

D: Nearly normal hepatic architecture

Fig. 1: Photomicrograph of sections from Liver tissue of different experimental groups' ×400.

Table 1: Statistical comparison of weight between groups at the start of experiment.

Groups	N	Mean	Std. Deviation	sig
Group A	8	173.5000	7.59699	0.016*
Group B	8	200.3750	24.40104	
Group C	8	200.7500	32.49066	
Group D	8	174.7500	10.44373	
Total	32	187.3438	24.31196	

Table 2 shows the comparison of histopathologic features among different experimental groups. The microscopic examination of liver sections of the animals in the control group (Group A) showed normal hepatic architecture, with hepatic lobules containing regularly arranged hepatocytes and no lymphocytic infiltration (Fig. 2-A), while group B (gentamicin) showed moderate to severe changes with loss of normal lobular architecture. There was statistically significant difference in mean hepatocyte count ($p = 0.00$) while no significant difference was found in the mean nuclear diameter among various groups (Table 3).

Table 2: Comparison of histopathological changes in liver within different experimental groups (n = 8).

	Group A Control Group	Group B Gentamicin Group	Group C Gentamicin+ BVFE 100 mg/kg Group	Group D Gentamicin + BVFE 200 mg/kg Group	P-value
Classic hexagonal lobule					
Normal	8 (100%)	0 (0.0%)	0 (0.0%)	2 (25.0%)	
Mild distortion	0 (0.0%)	0 (0.0%)	1 (12.5%)	6 (75.0%)	0.000*
Moderate distortion	0 (0.0%)	3 (37.5%)	5 (62.5%)	0 (0.0%)	
severe distortion	0 (0.0%)	5 (62.5%)	2 (25.0%)	0 (0.0%)	
Hepatocyte in cords					
Normal	8 (100%)	0 (0.0%)	0 (0.0%)	4 (50.0%)	
Mild distortion	0 (0.0%)	0 (0.0%)	1 (12.5%)	3 (37.5%)	0.000*
Moderate distortion	0 (0.0%)	3 (37.5%)	5 (62.5%)	1 (12.5%)	
severe distortion	0 (0.0%)	5 (62.5%)	2 (25.0%)	0 (0.0%)	
Portal vein, Hepatic artery Bile duct					0.001*
Normal	8 (100%)	2 (25.0%)	1 (12.5%)	6 (75.0%)	
Compressed	0(0.0%)	6 (75.0%)	7 (87.5%)	2 (25.0%)	
Mono nuclear cell infiltrate					
None	8 (100%)	0 (0.0%)	1 (12.5%)	5 (62.5%)	
Mild	0 (0.0%)	0 (0.0%)	2 (25.0%)	3 (37.5%)	0.000*
Moderate	0 (0.0%)	5 (62.5%)	4 (50.0%)	0 (0.0%)	
Severe	0 (0.0%)	3 (37.5%)	1 (12.5%)	0 (0.0%)	
Sinusoidal Congestion					
None	8 (100%)	0 (0.0%)	0 (0.0%)	4 (50.0%)	
Mild	0 (0.0%)	0 (0.0%)	4 (50.0%)	2 (25.0%)	0.000*
Moderate	0 (0.0%)	4 (50.0%)	4 (50.0%)	2 (25.0%)	
Severe	0 (0.0%)	4 (50.0%)	2 (25.0%)	0 (0.0%)	
Vacuolated Hepatocytes					
None	8 (100%)	0 (0.0%)	0 (0.0%)	1 (12.5%)	
Mild	0 (0.0%)	0 (0.0%)	4 (50.0%)	7 (87.5%)	0.000*
Moderate	0 (0.0%)	4 (50.0%)	4 (50.0%)	0 (0.0%)	
Severe	0 (0.0%)	4 (50.0%)	0 (0.0%)	0 (0.0%)	

*Significant at 0.05.

Table 3: Mean comparison of Morphometric parameters.

		Mean	Std. Deviation	P-Value
Hepatocyte Count	**Group A**	34.8750	9.14076	0.000*
	Group B	20.1250	2.74838	
	Group C	16.0000	2.50713	
	Group D	26.5000	2.61861	
	Total	24.3750	8.71317	
Nuclear Diameter	Group A	28.0425	3.36721	0.579
	Group B	30.0713	3.77871	
	Group C	30.0950	3.61254	
	Group D	29.1600	2.54000	
	Total	29.3422	3.30394	

ANOVA was applied. *Significant at 0.05.

DISCUSSION

Liver is the main organ at risk along with kidneys, owing to its role in detoxification of drugs.[20] This study confirmed the harmful effects of gentamicin on the liver of albino rats as shown by the histopathologic examination, similar findings were observed in other studies after administration of gentamicin at a dose of 80 mg/kg.[21] There was a marked decrease in the final body weight of the rats receiving gentamicin as compared to control and BVFE – treated groups which is compatible with other studies confirming the damage produced by oxidative stress due to this drug.[22,23]

Gentamicin produces free radicals which generates toxic effects on the cellular structure of liver tissue which was observed in the present study as loss of normal lobular architecture, irregular arrangement of liver hepatocytes and vacuolar degeneration.[24] This is in agreement with the earlier studies reported by Serges et al.[23] Findings of this study are consistent with an earlier study done by Rahimi etal documenting the antioxidant properties of berberine present in BVFE at a higher dose.[16]

Previous studies have shown that rats treated with BVFE showed mild inflammatory changes like mononuclear infiltrate and glycogen deposition within the hepatocytes,[16] which was also observed in the present study. These results are in contrast to a study which showed that BVFE could not cure the damage in the liver tissue of tested rats.[20]

The findings of this study have shown that the appropriate dose of BVFE can accelerate recovery in the parenchyma of the organ in albino rats against gentamicin induced hepatoxicity. Further studies are required at a larger scale to investigate, the mechanism of action of BVFE against gentamicin induced biochemical and histopathologic disturbances in the liver.

CONCLUSION

This study showed that Berberis vulgaris fruit extract was hepatoprotective against the damaging effects of gentamicin in albino rats and the protective effects were better at higher doses.

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Conception and design: Saima Athar, Lubna Faisal, Zia-ul-Islam.
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