

HISTOLOGICAL EFFECTS OF *EUGENIA JAMBOLANA* SEED EXTRACT ON LIVER OF ADULT ALBINO RATS

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Background: The therapeutic value of *Eugenia jambolana*, commonly known as 'Jamun' in Hindi, has been recognized in different system of traditional medicine for the treatment of various conditions. Its seeds are used for the treatment of diabetes mellitus and hyperlipidemia by reducing the lipid levels in the body; this action is presumed to be due to blocking the action of enzyme 3-hydroxyl methyl glutaryl (HMG-CoA reductase in the liver. Herbal drugs are getting into use with the notion that these are relatively harmless; the practice has shown that many of them also have toxic effects. Since hardly any work is available on the toxic aspect of *Eugenia Jamblana*, the present study was planned to see the effect of ethanolic extract of *Eugenia Jamblana* on liver using albino rats as an experimental model.

Methods: The animals were divided into three groups A, B and C. Group A served as a control and received only distilled water comparable to the experimental animals calculated according to their body weight, where as B and C served as experimental groups. 100 and 200 mg of ethanolic extract of *Eugenia Jamblana* was dissolved in one ml of distilled water each and was given orally for 30 days/kg body weight. **Results:** liver enzyme ALT and gamma GT were significantly raised when compared to the control group, *p*-value being <0.05. Histological studies showed ballooning degeneration of hepatocytes, focal areas of hepatocytes necrosis with lymphocytic infiltration, providing supportive evidence for biochemical findings indicative of functional derangement. The effect of the extract was not dose dependent. Statistical analysis using ANOVA and chi-square showed statistically significant difference when the values from experimental animals were compared with those from the control, indicating that the ethanolic extract of *Eugenia Jamblana* seed possesses hepatotoxic effect.

Conclusion: The ethanolic extract of *Eugenia jambolana* seed extract is toxic to liver as evident by derangement in liver enzyme levels and disturbed liver histology.

Keywords: *Eugenia Jambolana*, Statins, Hepatotoxicity

INTRODUCTION

Eugenia Jambolana is the member of Myrtaceae family. It is also known in Hindi as jamun, jambo, jambul, jamhool, in English as black plum, purple plum, black berry and, nerudu in Telugu.¹

In Pakistan it is used as the main constituents in many traditional medicines for the treatment of diabetes mellitus. It is present in Dianex² and in Diabecon³ herbal medicines. *Eugenia jambolana* is reported to have hypolipidemic effect; it reduces blood cholesterol, triglycerides and free fatty acids.¹ This effect was reported to be due to presence of flavonides, saponins and glycoside in the extract⁴ which in turn decreased the activity of enzyme 3-HMG Co-A reductase in liver, responsible for cholesterol biosynthesis.⁴ Statins, a lipid lowering drug, also act by reducing the activity of the same enzyme⁵, but are reported to be toxic to liver, producing an increase in the level of liver enzymes^{6,7} and derangement in histological architecture by causing centrilobular necrosis, cholestasis, infiltration with mononuclear cells and fibrosis.⁸ The mechanism of action of *Eugenia jambolana* and statins is same. Since there is hardly any work reported on hepatotoxicity of *Eugenia jamboana*, the present study was, therefore, planned to evaluate deleterious effects of *Eugenia jambolana* seeds using rats as an experimental animals.

MATERIAL AND METHODS

Ethanolic extract of *Eugenia jambolana* seeds was prepared by getting fruits of *Eugenia jambolana* (jamun) from fruit shop. Pulp was removed from the seeds and washed several times with distilled water to remove the traces of pulp; the seeds were then dried at room temperature, kernel of the seeds was separated from the seed coat, powdered in an electrical grinder and stored at 5 °C. Hundred gm of kernel powder was suspended in 250 ml of distilled water and allowed it to stand over night in refrigerator; it was then sieved through several layers of muslin cloth. The filtrate (water extract) was discarded. The residue was extracted with 95% ethanol using sox halation wherein ethanol was evaporated in a rotatory evaporator at 40–50 °C. The yield of kernel was 3.2 g/100 g of seed powder.⁴

Eighteen male albino rats of 6–8 weeks age, 150–200 gm of weight were procured from National Institute of Health, Islamabad. They were housed in experimental research laboratory of University of Health Sciences, Lahore, under controlled room temperature (23–25 °C), humidity (60%) and light and dark cycles of 12 hours each. They were fed on standard rat diet and water ad libitum, allowed to acclimatize for one week, weighed before the start of experiment and

subsequently at weekly interval. The animals were randomly divided into three groups A, B and C, having 6 rats each. Group A served as control, received distilled water orally for thirty days comparable to that given to the experimental animals, and experimental groups B and C were given ethanolic extract of *Eugenia jambolana* seeds orally at doses of 100 and 200 mg/kg respectively, daily each dissolved in 1 ml distilled water.

Blood samples from each group were collected by cardiac puncture in vacuum tubes and allowed to stand, for one hour to separate the serum, using test tube stand. The tubes were centrifuged at the speed of 3000 revolution/min, the clear serum was collected with the help of a clean dropper in plastic tubes and stored in freezer at -20 °C for testing on a later date; the tubes were properly labelled. Serum Alanine aminotransferase and Gamma glutamyl transferase levels were measured by using commercially available kits of HUMAN Company.

The histological preparations of liver were stained using Eosin and Haematoxylin for general histological study, Periodic Acid Schiff and Diastase techniques for the demonstration of glycogen in the liver sections.

The information from three groups was entered into computer software Statistical Package for Social Sciences (SPSS) version 15 and analyzed through it. The qualitative and quantitative measurements were compared between the groups for differences. Any difference in the qualitative measurements was tested by fisher exact test and for quantitative value the ANOVA test was applied. The *p*-value of 0.05 or less was considered as statistically significant.

RESULTS

The mean value of glutamyl transferase (Gamma GT) of group A, B and C were 73.36±1.37, 96.88±4.39 and 96.39±4.73 U/l respectively. Statistically significant difference was observed when group A was compared with groups B and C, *p*-value in both cases being <0.001; there was, however, no statistically significant differences were observed between groups B and C (*p*>0.92).

The mean values of Alanine aminotransferase of group A, B and C were 49.65±4.53, 57.16±5.25 and 111.6±17.02 U/l respectively. Difference among the groups was statistically significant when groups A and C and, B and C were compared together, (*p*<0.002), while no statistically significant difference was observed between groups A and B, (*p*>0.62).

The liver of animals in the control group showed typical hepatolobular architecture, consisting of central vein with radiating cords of hepatocytes separated by sinusoids; portal areas composed of portal vein, hepatic artery and bile duct were situated at the periphery. The hepatocytes were polygonal in shape, with central, lightly stained nucleus and clear nucleolus, few binucleated cells were also present, and the cytoplasm was regularly distributed without vacuulations (Figure-1). In group B the general hepatolobular architecture of the liver was deranged, there was a loss of radial arrangement of hepatocytes and sinusoids, Number of binucleated cells was increased (Figure-2), Inflammatory cells, especially lymphocytes, were found infiltrating around portal track, i.e., periportal inflammation (Figure-3), areas of necrosis were found around the central vein, centrilobular necrosis. Examination of preparations obtained from the animals of group C, showed comparable changes to group B; it, therefore, appeared that the effects of *Eugenia jambolana* extract on the liver were not dose dependent.

Table-1: Mean value of serum enzymes (U/L) in groups (Mean±SE)

Groups	Group A	Group B	Group C	<i>p</i>
Alanine aminotransferase	49.65±4.53	57.16±5.25	111.6±17.02	0.002*
Gamma GT	73.36±1.37	96.88±4.39	96.39±4.73	0.001*

**p*<0.05 is statistically significant

Table-2: Shows comparison of liver functions tests among the group

Dependent Variable	(I) Groups	(J) Groups	Mean Difference (I-J)	Std. Error	<i>p</i>
GAMMA-GT	Group A	Group B	-23.51983*	5.39303	0.001*
		Group C	-23.02433*	5.39303	0.001*
	Group B	Group A	23.51983*	5.39303	0.001*
		Group C	0.49550	5.39303	0.928
	Group C	Group A	23.02433*	5.39303	0.001*
		Group B	-0.49550	5.39303	0.928
ALT	Group A	Group B	-7.51667	15.01009	0.624
		Group C	-62.01667*	15.01009	0.001*
	Group B	Group A	7.51667	15.01009	0.624
		Group C	-54.50000*	15.01009	0.002*
	Group C	Group A	62.01667*	15.01009	0.001*
		Group B	54.50000*	15.01009	0.002*

*Statistically significant

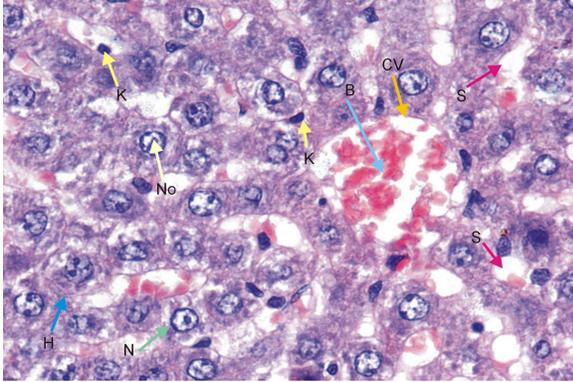


Figure-1: A photomicrograph of liver section from group A

Central vein (CV) in the centre of hepatic lobule filled with blood (B). Hepatocytes (H), arranged in form of cords, are rounded to polyhedral in shape and radiate peripherally; they show nucleus (N) with clear nuclear membrane and nucleolus (No), cords are separated by sinusoids (S) with Kupffer cells (k), H&E stain, $\times 200$.

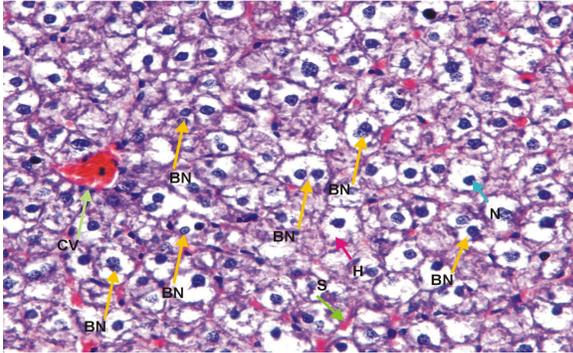


Figure-2: A photomicrograph of liver section from group B

Deranged architecture of liver, ballooning of hepatocytes (H) with hyperchromatic nuclei (N), nucleoli are not clearly seen, multiple binucleated cells (BN), sinusoidal (S) arrangement are also seems to have been deranged, central vein (CV) filled with blood H&E stain $\times 200$.

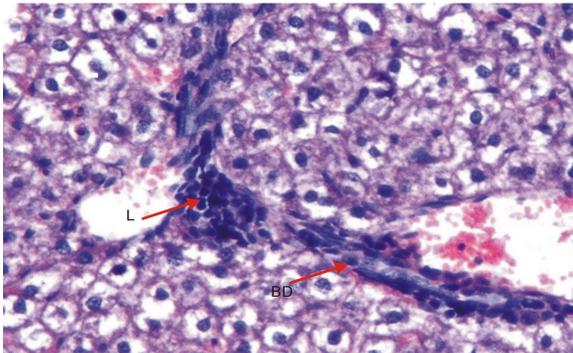


Figure-3: Photomicrograph of liver section from group C

Periportal inflammation consisting of collection of lymphocytes (L) around the bile duct (BD), H&E stain, $\times 200$.

DISCUSSION

Liver enzymes are the primary markers of liver damage and were observed to have increased at the

end of the experimental period indicating deleterious effect on the function of the liver which also showed histological changes. *Eugenia jambolana* seed extract, increased the liver enzymes, ALT and gamma GT, which was statistically significant ($p=0.002$ and 0.001 respectively), when group A was compared with that of groups B and C respectively, indicating toxic effect on the liver functions; the finding is comparable with that reported earlier after the use of statins⁹, Dai-Saiko-To (a Chinese herb)¹⁰, *Polygonum multiflorum* (a herb)¹¹. *Eugenia jambolana* seed extract decreased the lipid levels in rats, by reducing the activity of HMG-CoA reductasae enzyme in liver.⁴ Our findings are comparable to those reported earlier after using statins (lipid lowering drugs).¹²⁻¹⁶ The pattern of hepatic damage by statins was different in these reports, giving various pictures of cholestatic, toxic and autoimmune hepatitis.^{12,14,15} Liver lesions with the use of statins were found in both animals and in humans.^{5,15} In our study it was observed that the general architecture of liver in the experimental groups was damaged, possibly on account of hepatocytic swelling. This finding was comparable to Garba (2006) who used aqueous extract of Hoechst stem bark and reported cloudy swelling of hepatocytes.¹⁷ It was reported that herbs caused ballooning of hepatocytes and Kupffer cells hyperplasia.¹⁰ The size of hepatocytes after the use of *Eugenia jambolana* seed extract was significantly increased, when the size of hepatocytes from the control was compared with those of the experimental groups, the difference was statically significant, ($p<0.000$); the finding was comparable to that reported by Banerjee (2001), who studied the effect of garlic in a dose of 1000 mg/kg/day¹⁸ and noticed hepatocytic swelling after its use in rats.

Eugenia jambolana seed extract caused statistically significant necrosis in the experimental animals and when the findings of the control were compared with those in the experimental animals, the difference was found to be statistically significant, ($p<0.000$). The comparable findings were reported after using aqueous extract of stem bark of hoest,¹⁸ *polygonum multiflorum*,¹¹ turmeric¹⁹ and garlic in various studies¹⁹. The histological preparations from the Rats of the experimental groups showed lymphocytic infiltration around the bile duct, periportal inflammation. This finding was although, statistically not significant ($p>0.119$), was conformed by the use of statin,¹⁶ germander and shou-wo-pian¹¹ for graying of hair.

CONCLUSION

The ethanolic extract of *Eugenia jambolana* seed extract is toxic to liver as evident by derangement in liver enzyme levels and disturbed liver histology.

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