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HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *EUGENIA JAMBOLANA* LEAF ON LIVER DAMAGE CAUSED BY PARACETAMOL IN RATS

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ABSTRACT

Ethanolic extract of *Eugenia jambolana* leaves were investigated for their Hepatoprotective potential against liver injury induced by paracetamol in rats. The liver damage was induced in male albino rats (200 - 220gm) by administering paracetamol (2 gm/kg) for 7 days and extent of damage was studied by assessing the biochemical parameters such as Total bilirubin, Direct bilirubin, Indirect bilirubin, Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), Alkaline phosphatase (ALP), Total protein (TP), Albumin and Globulin. Histopathological changes of liver sample were compared with respective control. Treatment of rats with ethanolic extract (200mg/kg) for 7 days resulted in significant hepatoprotection for total protein (6.6 g/dl) only, while at 400 mg/kg, the restoration was significant for enzyme markers, viz. SGOT, SGPT, ALP (22.5 U/L, 18.5 U/L, 58.5 U/L) in paracetamol-treated rats. The lethal dose (LD50) of the plant was greater than 3000 mg/kg. The ethanolic extracts of *Eugenia jambolana* leaves have protected liver from paracetamol damage against induced liver injury.

KEYWORDS

Hepatoprotective, Paracetamol, Serum glutamate oxaloacetate transaminase and Alkaline phosphatase.

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INTRODUCTION

Medicinal plants are the oldest type of healthcare known, and have been used throughout history by many cultures. Many of the modern medicines are produced indirectly from medicinal plants, for example aspirin[1]. Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian

medicine. Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. Many food crops have medicinal effects, for example garlic. Medicinal plants are resources of new drugs. It is estimated there are more than 250, 000 flower plant species [2].

The liver is an essential body organ that forms an important barrier between the gastrointestinal blood, which contains large amounts of toxins and antigens, and the body. Around 60% of the liver is formed by liver cells, hepatocytes, which are radially grouped into thick unicellular layers around the terminal hepatic veins, forming the smaller anatomic units of the liver, the classic lobes. The liver produces a large amount of hormones, enzymes, and performs several functions essential to life. It is also the organ responsible for cleansing of toxins from the bloodstream, by turning them into removable substances. Detoxification is essential as toxins may damage both liver cells membranes and the cell membranes of other organs. Glutathione, the main detoxifying and antioxidant agent of the liver, is essential for enhancing the removal of toxins and prevents cell damage. The term liver disease refers to many diseases and disorders that may cause impaired liver function can make the liver a decrease of its functions. The dysfunction may be primary, but the liver is often secondarily affected by disorders of other organ systems, since it is involved in many metabolic and detoxifying processes. This is especially due to toxics agents produced by the diseases of the gastrointestinal tract [3]. Usually, more than 75% or three quarters of liver tissue needs to be affected before decrease in function occurs. The *Eugenia jambolana* (Family: Myrtaceae) is well known for its medicinal properties in indigenous medicine in India[4].

MATERIAL AND METHODS

Preparation of ethanolic extracts of *Eugenia jambolana* leaves

The leaves was separated from plant and it was washed with absolute ethanol to avoid the microbial growth, the leaves were dried at open air under the shade, cut in to small pieces and powdered mechanically, then 50 gm of powder *Eugenia jambolana* leaves was extracted with 250ml ethanol in a soxhlet apparatus for 72 hrs. The extract obtained was concentrated by recovery of ethanol. The concentrated product was used as ethanolic extract of leaves of *Eugenia jambolana* [5, 6].

Experimental animals

Male Wistar Albino rats, weighed 200 ± 20 g were utilized for this study. They were housed in polypropylene cages under standard laboratory conditions (12-h light/ 12-h dark cycle, 21 ± 2 ºC, and relative humidity 55-70 %).

Experimental design

The rats were divided into six groups (A to F), each group consisting of six rats.

Group A - Served as normal control and received subcutaneous administration of 5% tween 80 at a dose of 5 ml/kg on alternate days, for one week.

Group B - Rats were subcutaneously injected with paracetamol (2g/kg) in a suspension of 5 % tween 80 at a dose of 5 ml/kg body weight, on alternate days, for a week and were considered as diseased control.

Group C - Rats were subcutaneously injected with paracetamol (2g/kg) in a suspension of 5 % tween 80 at a dose of 5 ml/kg body weight and Silymarin at a dose of 50 mg/kg body weight was treated orally for a week.

Group D - Rats were subcutaneously injected with paracetamol (2g/kg) in a suspension of 5 % tween 80 at a dose of 5 ml/kg body weight and extract of *Eugenia jambolana* leaves at the dose of 100 mg/kg was treated orally for one week.

Group E - Rats were subcutaneously injected with paracetamol (2g/kg) in a suspension of 5 % tween 80 at a dose of 5 ml/kg body weight and extract of *Eugenia jambolana* leaves at the dose of 200 mg/kg was treated orally for one week.

Group F - Rats were subcutaneously injected with paracetamol (2g/kg) in a suspension of 5 % tween 80 at a dose of 5 ml/kg body weight and extract of *Eugenia jambolana* Leaves at the dose of 400 mg/kg was treated orally for one week.

Acute toxicity study as per OECD guideline 425

In the assessment and evaluation of the toxic characters of the substance, determination of acute oral toxicity is usually an initial step. It provides information of health hazards likely to arise from a short-term exposure by the oral route. Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24h. Data from an acute study may serve as a basis for classification and labeling. LD oral, is a statistically derived single dose of a substance that can be expected to cause death in 50% of animals when administered by the oral route. The LD value 50 expressed in terms of test substance per unit weight of test animal (mg/kg). If there is no reversal before reaching the selected upper (2000 or 5000 mg/kg) limit dose, then a specific number of animals are dosed at the limit dose [7].

RESULTS

Phytochemical investigation on a tropical tree *Eugenia jambolana* leaves

The preliminary phytochemical screening like Saponins, Tannins, Amino acids, Proteins, Glycosides, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids was done with the ethanolic extract of *Eugenia jambolana* leaves according to the procedure. In the above chemical test the ethanolic extract of *Eugenia jambolana* gives positive results for Saponins, Tannins, Amino acids, Proteins, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids except glycosides. The results of preliminary test of ethanolic extract of *Eugenia jambolana* leaves were shown in Table No.1.

Hepatoprotective activity of *Eugenia jambolana* leaves

The results of paracetamol - induced hepatotoxicity are shown in Table No.2. Animals treated with a four subcutaneous dose of paracetamol developed significant liver damage in inducer control, as evident from a significant increase in the serum activities of Total Bilirubin, Direct bilirubin, Indirect bilirubin, SGOT, SGPT, Albumin, Globulin, and Alkaline phosphate concentrations and a decrease in the total protein compared with normal control rats, indicating acute hepatocellular damage and biliary obstruction. The restoration level of elevated serum enzyme markers and increase in protein level was observed in silymarin-treated animals. The elevated levels of serum markers were reduced in the animal groups treated with ethanolic extracts *Eugenia jambolana* leaves. Among the three graded doses of ethanolic extract, 200 mg/kg showed a significant hepatoprotection for total protein (6.6 g/dl) only, while at 400 mg/kg, the restoration was significant for enzyme markers, viz. SGOT, SGPT, ALP (22.5 U/L, 18.5 U/L, 58.5 U/L) and for the nonenzyme marker, viz. Bilirubin in addition to total protein (0.4 mg/dl and 6.2 g/L, respectively) exhibited significant restoration of serum markers for all the parameters studied, indicating its effectiveness.

Histopathological study of *Eugenia jambolana* leaves

The histopathological studies of control, paracetamol, silymarin and test sample-treated liver sections are shown in Figure No.1. Histological studies of control animals showed normal hepatocyte whereas the liver section of paracetamol -treated animals showed intense centrilobular, necrosis, vacuolization and macro vesicular fatty changes. The liver section of standard drug and silymarin-treated animals showed normal hepatocyte, which is comparable with the liver section of normal animals. The animals treated with different doses of the ethanolic showed recovering of hepatocyte, especially in the 400 mg/kg bw-treated group with minimal inflammation and near-normal architecture possessing higher hepatoprotective activity as compared with the ethanolic extract treated at 100 mg/kg which shows a moderate number of recovered hepatocyte with a small amount of necrosis, vacuolization and macro vesicular fatty changes. However, the liver sections of the animals treated with ethanolic extract of *Eugenia jambolana leaves* exhibited significant liver protection against paracetamol, as evident by the presence of normal hepatic cords, absence of

necrosis and fatty infiltration, supplementing the protective effect of ethanolic extract of *Eugenia jambolana leaves* which is comparable to the standard drug and control animals liver sections.

Acute toxicity study as per OECD guideline 425

Acute toxicity test at 3000mg/kg of leaf extracts of *Eugenia jambolana leaves* produced no mortality after 24 hours of observation. The median lethal dosage (LD50) of the ethanolic leaves extract was greater than 3000 mg/kg body weight. The extract did not produce any grossly negative behavioral changes such as excitement, restlessness, respiratory distress, convulsions or coma. However, a reduction in body weights of the rats was observed. The reduction in weight may be due to reduced fluid and water intake, which may be secondary to feeling of fullness and loss of appetite after administration of the extract. Despite the above side effects, the very high value of the LD50 indicated that the extract of *Eugenia jambolana leaves* is practically non-toxic. The effect of *Eugenia jambolana* extracts at three dose levels (100, 200 and 400 mg/kg) and paracetamol on serum marker enzymes, total protein, albumin and bilirubin in paracetamol-induced hepatic injury. Hepatic injury induced by paracetamol caused significant ($p < 0.05$) rise in SGOT, SGPT, ALP, cholesterol and bilirubin, but decreases in levels of total proteins and albumin were observed. Administration of the plant extract and paracetamol at three dose levels resulted in recovery as indicated by the decrease in the hitherto increase of the serum parameters, produced by paracetamol. The effect was almost similar to that produced by silymarin treatment.

DISCUSSION

The paracetamol induced liver damaged rats on treatment with the ethanolic crude extract at the dose 400 mg/ kg showed significant changes and restored the biochemical parameters, thereby indicating their protection against the injurious effects of paracetamol, which may be due to the inhibitory effects on cytochrome P450 resulting in the hindrance of the formation of hepatotoxicity free radicals[8, 9]. Thus, the histological study supports the hepatoprotective activity of the *Eugenia jambolana* from hepatic toxic effect of paracetamol and silymarin. Our results demonstrate a very good protective effect of *E. jambolana* leaves extract against paracetamol induced liver injury, which is probably due to the presence of antioxidant potential of tannins, flavonoids and anthraquinones present in the extract [10, 11].

Table No.1: Phytochemical screening results of *Eugenia jambolana* leaves

S.No	PHYTOCONSTUTIENT	RESULT
1	SAPONINS	+
2	TANNINS	+
3	AMINO ACIDS	+
4	PROTEINS	+
5	GLYCOSIDES	-
6	CARDIAC GLYCOSIDES	+
7	ALKALOIDS	+
8	CARBOHYDRATES	+
9	FLAVONOIDS	+

PRESENT = (+), ABSENT = (-)

Table No.2: Effect of ethanolic extract of *Eugenia jambolana* leaves on paracetamol induced hepatotoxicity in rats

Groups	Total Bilirubin (mg/dl)	SGOT(U/L)	SGPT(U/L)	ALP(U/L)	Totalprotein(g/dl)	Albumin (g/dl)	Globulin (g/dl)
Normal Control	0.33±0.07	35.00±9.90	24.50±0.71	57.50±3.54	6.60±0.85	4.00±0.90	2.60±0.57
Paracetamol induced Diseased control	0.65±0.09**	75.50±2.12**	69.50±7.78**	112.50±10.61***	7.90±0.14**	5.90±0.80**	2.90±0.14
Paracetamol +Silymarin (50 mg/kg, p.o)	0.33±0.07##	45.00±8.49#	28.50±16.26#	65.00±21.21#	6.25±0.35#	3.90±0.14#	2.25±0.35#
Paracetamol +EJ LEAF (100 mg/kg, p.o)	0.55±0.07*	50.00±7.07*,#	40.50±2.12*#	76.00±5.66#	6.55±0.07#	3.80±0.28#	2.75±0.35
Paracetamol +EJ LEAF (200 mg/kg, p.o)	0.40±0.09	42.00±2.83#	30.50±6.36#	59.50±7.78#	6.50±0.42#	4.10±0.14#	2.40±0.28
Paracetamol +EJ LEAF (400 mg/kg, p.o)	0.35±0.06#	21.00±2.83*, #	17.00±2.83#	58.00±8.49#	6.10±0.14#	4.00±0.28#	2.10±0.14##

Data represent means±SD (n=6), One Way ANOVA followed by Dunnett's multiple comparison tests.*p<0.05, **p<0.01, ***p<0.001 when compared with normal control group, #p<0.05, ##p<0.01 when compared with diseased control.

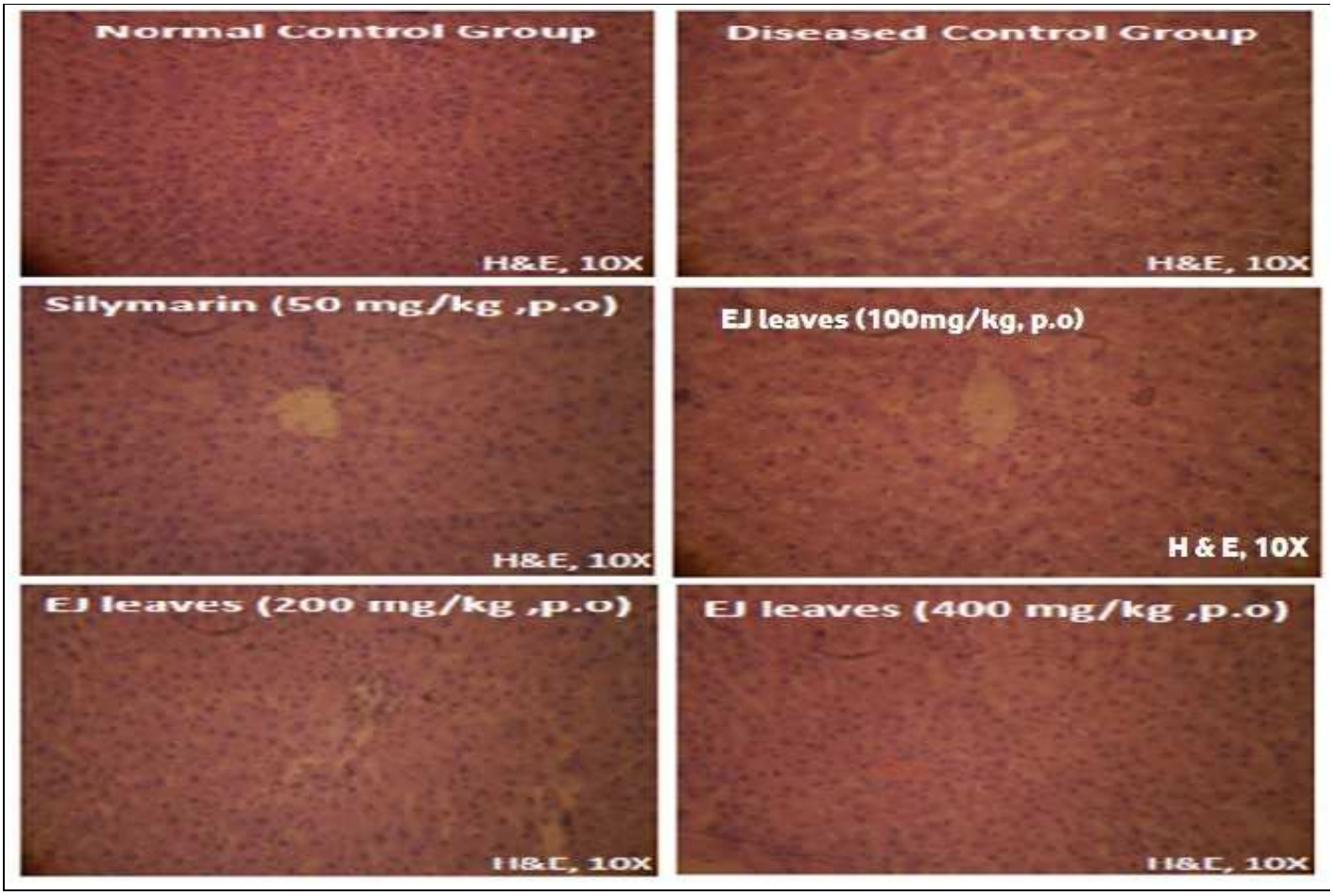

Figure No.1: Histology changes in liver tissues (H&E Staining) with 10X magnification in rats

CONCLUSION

In this experimental model, the ethanolic extract *Eugenia jambolana* leaves (100, 200, 400 mg/kg) with reference standard Silymarin (4mg/kg) significantly effective in abnormalities of enzyme profile in experimental rats. The leaves have a potential to be used in the treatment of hepatoprotectivity. Data on the short and long term adverse effects of *Eugenia jambolana* leaves ingestion needs to be collected.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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