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Screening of hepatoprotective activity of *Mimusops elangi fruit* on d-galactosamine induced hepatotoxicity in rats

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ABSTRACT

In this present investigation the ethanolic extract of *Mimusops Elangi fruit* (EEMEF) was screened for Hepatoprotective activity against D-galactosamine (d-GalN) induced hepatiotoxicity in rats. Wistar rats were divided into five groups (n=6). Hepatotoxicity in rats was achieved by intraperitoneal dose of 400mg/kg of d-GalN for five days. Silymarin (100mg/kg) was given as reference standard. The degree of protection against liver toxicity was determined by measuring the serum biochemical parameters viz. SGPT (serum alkaline phosphatase), SGOT (serum glutamine oxaloacetate transaminase), ALP (alkaline phosphatase**),** Total Cholesterol and Bilirubin (Total and Direct). The result shows that d-GalN has enhanced the levels of SGPT, SGOT, ALP, Total cholesterol and bilirubin. Pretreatment with EEMEF (200mg/kg B. wt. and 400 mg/kg B. wt.) has brought back the altered levels of biochemical markers to the near normal levels. Histopathological studies also confirmed the hepatoprotective activity of these extracts when compared with d-GalN treated groups. It can be concluded from the result that the extract of *Mimusops Elangi fruit* possesses hepatoprotective activity against d-GalN induced hepatotoxicity in rats.

Key Words*: Mimusops Elangi fruit*, Hepatoprotective, D-galactosamine

INTRODUCTION

Hepatitis induced by D-galactosamine shows many metabolic and morphological aberrations in the livers of experimental animals similar to those observed in human viral hepatitis. A large evergreen tree found in the Deccan Peninsula and Andaman Island, and frequently cultivated in gardens for ornament; it is grown also as an avenue or shade tree throughout the greater parts of India. It has made important contribution to the field of science from ancient times as also to modern research due to its large number of medicinal importance (Nadkarni, 1996).

Fruit and seed of *Mimusops Elangi* showed presence of Quercitol, ursolic acid, dihydro quercetin, quercetin, β - d glycosides of β sitosterol, alpha- spinasterol. This study aimed to conduct a scientific experiment using the ethanolic extract of *Mimusops Elangi fruit* at different doses from previous report to investigate its hepatoprotective activity and possible mechanism in D-galactosamine induced rat hepatic injury.

MATERIALS AND METHODS

Chemicals: D-Galactosamine (d-GalN) was purchased from Merck India Ltd., Mumbai, India. 5, 5-dithiobis-2-nitrobenzoic acid was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai. Ecoline assay kits for serum aspartate aminotransferase (ASAT), alanine amino transaminase (ALAT), alkaline phosphatase (ALP), total Cholesterol (TC), Total & Direct bilirubin (TB) were obtained from Merck Ltd., Ambemath, India and Silymarin from Ranbaxy India Ltd., New Delhi. All the other chemicals used were of analytical grade.

Collection & Extraction: *Fruit* samples of the plant *Mimusops Elangi* were collected from Bardoli, Surat, Gujarat. It was authenticated by Dr. Bimal Shah. The Fruit of the plant were shade dried at room temperature and were then pulverized. The coarse powder obtained was successively extracted with various organic solvents in the increasing order of their polarity (petroleum ether, chloroform, ethanol & water) in a soxhlet extractor for a period of 24 – 28 hours. The extracts were then concentrated to dryness in a rotavapor under reduced pressure and controlled temperature. The ethanol extract yielded a brown semi-solid (16g).

Animals: Both sex Wistar rats (150 – 200g) were selected for the study. They were housed in polypropylene cages in an air-conditioned area at 22˚C ± 3˚C and 59 to relative humidity with 12 hour light & dark cycle. All the animals had free access to standard diet and clean water *ad libitium.* The experiments were conducted according to the Institutional Animal Ethics Committee (IAEC) regulations approved by the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental protocol: The rats were grouped randomly into five groups, each containing six animals. Group I served as negative control received the vehicle (normal saline). Group II served as positive control received the

vehicle (normal saline). Group III was treated with standard drug silymarin at 100 mg /kg body weight. Group IV and V were treated with plant extract at the dose levels of 200 and 400 mg/kg body weight respectively for five days.

Group-I : Normal Saline (1ml/kg, p.o)
Group-II : d-GalN (400mg/kg, i.p)
Group-IV : Silymarin (100 mg/kg p.o.) + d-GalN (400mg/kg, i.p)
Group-III : EEMEF (200 mg/kg p.o.) + d-GalN (400mg/kg, i.p)
Group-IV : EEMEF (400 mg/kg p.o.) + d-GalN (400mg/kg, i.p)

On the fifth day of the treatment, animals of all groups except group I received a single dose of d-GalN in distilled water at 400 mg/kg body weight intraperitoneally after two hour of their respective treatment. After 24 hours of d-GalN administration blood was collected retro-orbitally under light ether anesthesia. Immediately, after blood withdrawal all groups were sacrificed. Liver samples were also collected for histological and biochemical estimations. The blood samples were allowed to clot for 30 – 40 minutes. Serum was separated by centrifugation at 37˚C and was used for estimation of various biochemical parameters like SGOT, SGPT, ALP, Total Cholesterol, Total & Direct Bilirubin (Mishra, 1967).

Histological studies: Small pieces of liver fixed in 10% buffered neutral formalin were processed for embedding in paraffin. Sections of 5–6 µm thickness were stained with hematoxylin and eosin, examined for histopathological changes (200×) under a microscope (Sharma, 2000).

Data analysis: Quantitative data were expressed as mean ± S.E.M and all statistical comparisons were made by means of one-way ANOVA test followed by Tukey's test. *P*-Values less than 0.05 were considered statistically significant while *P*-values less than 0.01 were considered extremely significant.

RESULTS AND DISCUSSION

Biochemical observations: Administration of d-GalN resulted in a significant rise in the levels of SGPT, SGOT, ALP, Total Cholesterol and Bilirubin (Total and Direct) when compared to the vehicle treated group (Group-I). The extract of EEMEF treatments significantly (P<0.001) reversed the levels of elevated biochemical parameters in dose dependent manner (Group IV & V). The results indicated that the effect of test extract on biochemical markers was found to be almost comparable than the reference standard, Silymarin (Group III).

Table.1.Effects of EEMEF on biochemical markers in D-galactosamine induced hepatotoxicity

Treatment	Biochemical parameters Mean ± SEM					
	SGOT U/L	SGPT U/L	ALP IU/L	Total Bilirubin mg/dl	Direct Bilirubin mg/dl	Total Cholesterol g/dl
Negative Control (1ml vehicle)	59.58± 1.79	38.1± 0.76	298.93± 5.60	0.68± 0.01	0.22± 0.01	72.33± 1.21
Positive Control D-GalN (400mg/kg i.p.)	132.78± 2.38	112.49± 2.67	546.79± 13.04	2.25± 0.06	1.74± 0.02	182.25± 2.20
D-GalN + Standard (Silymarin) (400mg/kg i.p.+ 100 mg/kg p.o.)	78.96± 1.42***	61.91± 1.77***	371.45± 9.93***	1± 0.05***	0.44± 0.01***	92.5± 1.56***
D-GalN + EEMEF (400mg/kg i.p.+ 200 mg/kg p.o.)	118.85± 1.45***	93.23± 1.45***	513.11± 2.17***	1.88± 0.03***	1.15± 0.01***	141.65± 0.72***
D-GalN + EEMEF (400mg/kg i.p.+ 400 mg/kg p.o.)	96.29± 2.33***	67.67± 1.82***	420.91± 3.30***	1.29± 0.02***	0.58± 0.01***	105.31± 1.41***

Histopathological observation: The results of histopathological studies of normal rat liver showed normal hepatocytes, sinusoids. Liver section of rat treated with d-GalN exhibited severe necrosis, disappearance of hepatocytes and areas of inflammation with increased sinusoidal spaces. Liver section of rat treated with EEMEF (200mg) and d-GalN exhibited mild degree of necrosis, reduced sinusoidal dilation and less inflammation. Liver section of rat treated with EEMEF (400mg) and d-GalN showed normal hepatocyte appearance with normal sinusoids with no inflammation. Liver section of rat treated with silymarin (100mg) and d-GalN exhibited normal hepatocytes.

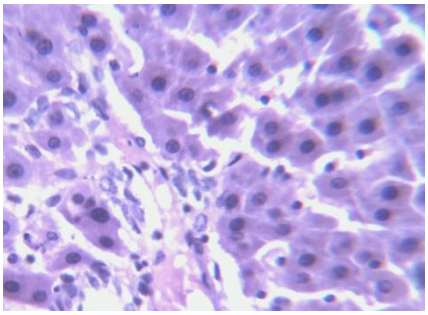

Figure.1.Liver architecture of Normal Control

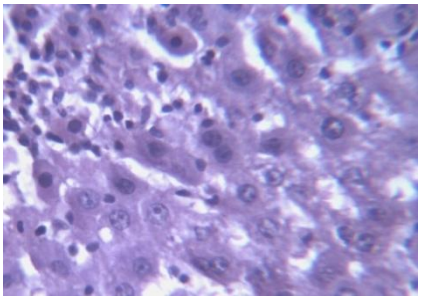

Figure.2. Liver architecture of d-GalN treatment

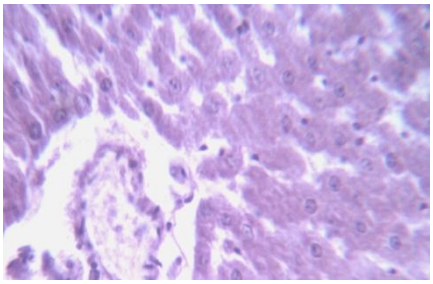

Figure.3. Liver architecture of d-GalN + 100 mg/kg Silymarin treatment

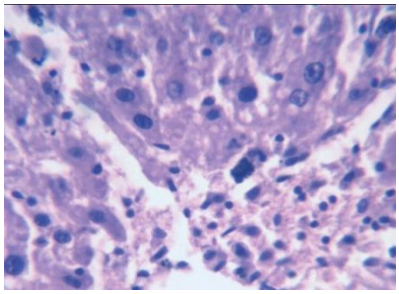

Figure.4.Liver architecture of d-GalN + 200 mg/kg of EEMEF

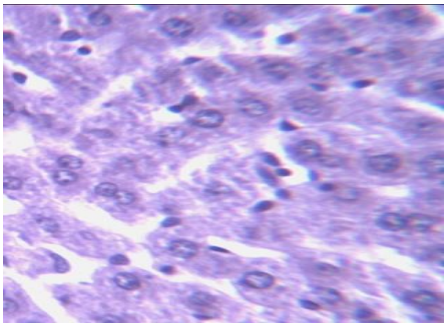

Figure.5.Liver architecture of d-GalN + 400mg/kg of EEMEF

Any disease/disorder is many times associated with cell injury due to the generation of free radicals. The free radicals damage the cell membrane, cell vital constituent like DNA etc. Though the free radicals are generated, the human beings possess inbuilt natural mechanism to scavenge the generated free radicals. The inbuilt scavenging systems are the glutathione, superoxide dismutase (SOD), catalase. But during prolonged stressful conditions produces free radicals which cannot be handled by our inbuilt mechanism alone. The various free radicals that are released in to the body are superoxide anion ($O_2\bullet$), NO radical, $NOO^{\bullet}$, $OH^{\bullet}$ and H_2O_2 radical. Thus released free radicals react with the membrane polyunsaturated fatty acid (PUFA) and oxidise them to lipid peroxides. This lipid peroxidation damage membrane protein as well as the lipids. Thereby, the integrity of membrane is lost. Therefore it is considered that the extent of lipid peroxidation is directly proportional to cell damage. In addition the free radicals may also attack DNA and causes tissue damage.

Galactosamine administration in rats disrupts the membrane permeability of the plasma membrane causing leakage of the enzymes from the cell, which leads to elevation in levels of serum enzymes (Mitra et al., 2000). It is apparent that the levels of SGPT, SGOT, ALP, Total Cholesterol, Total and direct Bilirubin increased significantly in group treated with d-GalN comparing to normal control.

Study of any herbal drug becomes more significant when it ameliorates some diseases conditions. In the present investigation, hepatoprotective effects of the ethanolic extract of *Mimusops Elangi* Fruit were studied based

on d-GalN induced liver hepatitis. The hepatoprotective effect of *Mimusops Elangi* Fruit has been shown in earlier studies. In our study the rise in SGOT, SGPT, ALP, Bilirubin levels induced by D-galactosamine administration was significantly reduced by EEMEF pre-treatment suggesting that its hepatoprotective activity might be due its effect against cellular leakage and loss of functional integrity of the cell membrane in hepatocytes. Histopathological studies also support the protective effects of the plant. Flavonoids, a polyphenolic derivative could be the major contributory factors in hepatoprotective activity. It seems the protective activity of the plant may be due to strengthening the inbuilt antioxidant system and the antioxidant principles in the plant. However further studies are needed to completely establish the mechanism of hepatoprotective effect of the plant.

CONCLUSION

Based on the present study, it can be concluded that ethanolic extract of *Mimusops Elangi* fruit have potent hepatoprotective activity in a dose dependent manner. Further, isolation of active principles will be advantageous to produce novel bioactive constituents from these extracts which may possess more significance in the treatment of liver diseases and to elucidate its exact mechanism of action. Attempts are being made to isolate and characterize the active principle to which the hepatoprotective activity can be attributed.

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