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HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *MADHUCA LONGIFOILIA* LEAVES ON D-GALACTOSAMINE INDUCED LIVER DAMAGE IN RATS

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ABATRACT

Madhuca longifolia is a native plant used in traditionally for treating different disorder. In the present study, *in vivo* hepatoprotective effect of Ethanolic extract of *Madhuca longifolia* leave (EEMLL) extract was evaluated using D-Galactosamine (GalN) induced acute hepatotoxicity in rats. Pretreatment with EEMLL (200mg/kg & 400mg/kg) for seven days significantly reduced the impact of Galactosamine toxicity (400mg/kg,i.p) on the serum markers of liver damage. Furthermore, considering the well-known implication of free radicals in tissue injury, *in vitro* antioxidant properties of the extract were determined with a view to suggest the possible mechanism of activity. Therefore, presented result suggests that EEMLL is potent hepatoprotective agent that could protect liver against the acute injury and this ability might be attributed to its antioxidant potential.

Key Words*: Madhuca longifolia*, Hepatoprotective, D-galactosamine

1. INTRODUCTION

Hepatic disease is a broad term that is used to describe any single number of diseases affecting the liver. Liver disorders, caused by a number of agents such as viruses, alcohol, various drugs etc, are a huge threat to public health owing to its life threatening complications. Our understanding of liver diseases and its causes has highlighted the role of herbal medicine in its treatment. Also the ability of modern medicine to damage the liver has further accentuated the role of herbal medicine in the treatment of liver disorders.

Madhuca longifolia belonging to the family Sapotaceae is an indigenous plant found largely in the central and north Indian plains and forests. Commonly known as Mahua, it is used in the treatment of epilepsy, and inflammations. Presence of a genuine sapogenol protobasic acid and prospagenol in the seed kernels of *Madhuca longifolia* was reported by Yosioka (1974). Analgesic activity of the alcoholic extract was reported by Dinesh Chandra (2001) while the antioxidant and hepatoprotective activity of the leaves of the plant was reported (Samaresh Pal Roy, 2010).

Among the various experimental models of hepatitis, D- galactosamine (D-GalN) is widely used to evaluate hepatoprotective activity and its effects on liver closely resembles to that of viral hepatitis in pathogenesis and toxicity manifestations.

2. MATERIALS AND METHODS

2.1 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.

2.2 Collection & Extraction: Leaves samples of the plant *Madhuca longifolia* were collected from Bardoli village, Gujarat during April, 2011 and was authenticated by Dr. Bimal Shah. The leaves 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, 70% 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 70% ethanol extract yielded a brown semi-solid (16g).

2.3 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 70% 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).

2.4 Preparation of Sample: The standard silymarin and the plant extract were suspended in distilled water using sodium carboxymethylcellulose (sodium CMC, 0.3%). The test and standard drugs were administered orally to the animals with the help of an intragastric catheter.

2.5 Acute toxicity studies: An acute oral toxicity study in rats was carried out as per OECD – 423 guidelines. The study revealed that the 70% ethanolic extract of the leaves of *Madhuca longifolia* was found to be safe at the dose level of 2000 mg/kg body weight (0/3 mice were dead). Hence 2000 mg/kg was considered as the LD_{50} cutoff dose for the 70% ethanolic extract.

Therefore, 1/10 (200mg/kg) and $1/5^{th}$ (400mg/kg) of cut off dose was selected for the further study i.e. for screening of hepatoprotective property.

2.6 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 (Shyamal et al., 2006). 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 & Direct bilirubin.

2.7 Histological studies: Livers were quickly removed and preserved in neutral buffered formalin.

2.8 Data analysis: Quantitative data were expressed as mean ± S.E. 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.

3. RESULTS & DISCUSSION

3.1 Biochemical observations: There is an increase in SGPT levels observed in D-galactosamine treated group (112.49 IU). The extract has shown a dose dependent effect. SGPT levels were restored by 400 mg/kg of 70% ethanolic extract of the leaves (62.52 IU), whereas standard 100 mg/kg silymarin has also shown statistically significant effect (61.91IU). SGOT level has been increased significantly in D-galactosamine treated group (132.78 IU). 400 mg/kg of 70% ethanolic extract of the leaves reduced the elevated levels of SGOT (91.37 IU), which is statistically significant when compared to D-galactosamine treatment. Standard silymarin 100 mg/kg has reduced the SGOT level significantly (78.96 IU). In case of total and direct bilirubin, a dose dependent effect of the extract has observed. 400 mg/kg 70% of ethanolic extract has reduced the elevated levels of total and direct bilirubin by D-galactosamine from 2.25mg/dl and 1.74 mg/dl to 1.32 mg/dl and 0.55 mg/dl respectively. The results of 400 mg/kg 70% ethanolic extract was found to be comparable with the results of 100 mg/kg silymarin on the same marker enzymes. There is an increase in ALP levels observed in D-galactosamine treated group (546.79 U/L). The extract has shown a dose dependent effect. ALP levels were restored to 421 U/L by 400 mg/kg 70% ethanolic extract of the leaves which is statistically significant when compared with D-galactosamine treated group. The restoration by standard silymarin 100 mg/kg was also significant i.e. 371.45 U/L. The 70% EEMLL also restored the elevated total cholesterol when treated with d-galactosamine with a significant manner.

3.2 Histopathological observations: Histology of the liver sections of negative control animals (Group I) showed normal liver architecture with well brought out central vein, well preserved cytoplasm and prominent nucleus and nucleolus (Fig. 1). The liver sections of positive control, only D-galactosamine treated, animals (Group II) showed hepatic cells with severe toxicity characterized by inflammatory cell collection, scattered inflammation across liver parenchyma, focal necrosis and swelling up of vascular endothelial cells (Fig. 2). Silymarin (Fig. 3) exhibited protection from galactosamineinduced changes in the liver (Fig. 4). Pre-

treatment with 70% EEMLL (Fig. 4) appeared to significantly prevent the galactosamine toxicity as revealed by the hepatic cells with well preserved cytoplasm. Pre-treatment with 70% EEMLL also caused marked decrease in inflammatory cells (Fig. 5).

Table No. 1 Effects of 70% EEMLL on liver (weight and kidney) and its biochemical markers in D-galactosamine induced hepatotoxicity

Treatment	Biochemical parameters Mean ± SEM					
	SGOT Units/Liter	SGPT Units/Liter	ALP International Units/Liter	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-galactosamine (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-galactosamine + 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-galactosamine + 70% ethanolic extract (400mg/kg i.p. + 200 mg/kg p.o.)	113.15± 2.17**	92.66± 2.10**	502.54± 5.33**	1.87± 0.02***	1.14± 0.01***	139.52± 2.24***
D-galactosamine + 70% ethanolic extract (400mg/kg i.p. + 400 mg/kg p.o.)	91.37± 1.27***	62.52± 0.85***	421± 5.70***	1.32± 0.04***	0.55± 0.02***	103.9± 1.50***

Values are the mean ± S.E.M. of six rats/ treatment

Significance *P <0.05, **P <0.01 and *** P<0.001, compared to D-galactosamine treatment

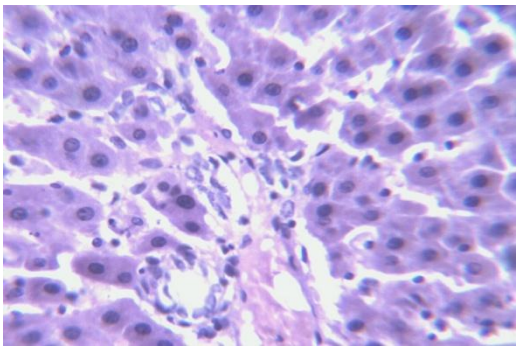

Fig. No. 1Liver architecture of Normal Control

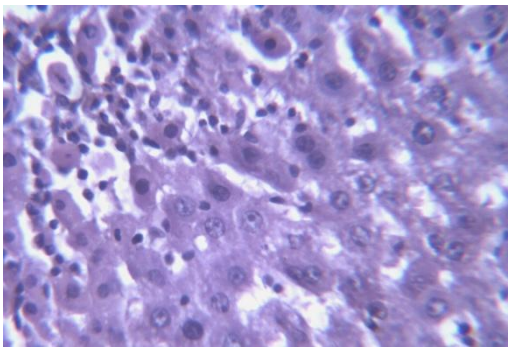

Fig. No. 2 Liver architecture of D-GalN treatment

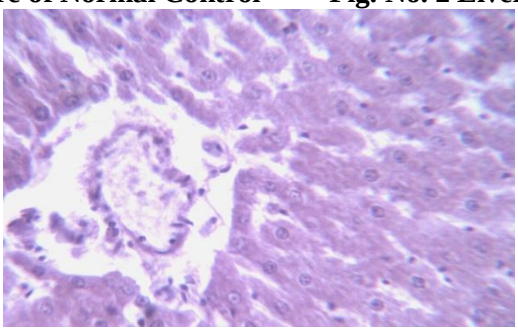

Fig. No. 3. Liver architecture of D-GalN + 100 mg/kg Silymarin treatment

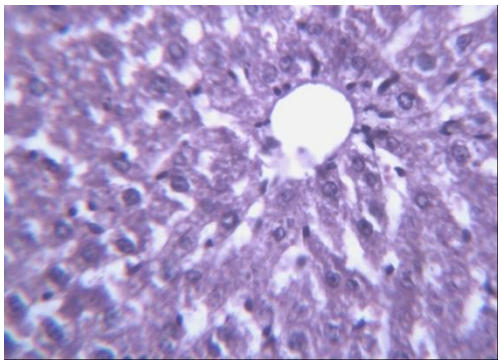

Fig. No. 4 Liver architecture of D-GalN + 200 mg/kg 70% EEMLL

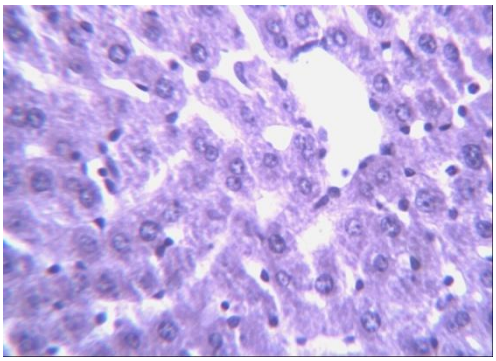

Fig.No. 5 Liver architecture of D-GalN + 400mg/kg of 70% EEMLL

DISSCUSSION

Galactosamine induced experimental model system in rats is recognized to be much like viral hepatitis in humans from both morphological and functional points of view. Galactosamine has great liver specificity because hepatocytes have high levels of galactokinase and galactose-1-uridyltransferase. Galactosamine does not affect other organs. Galactosamine causes hepatic injury with spotty hepatocytes necrosis and marked portal and parenchymal infiltration. Galactosamine also causes depletion of uridine diphosphate (UDP) by increasing the formation of UDP-sugar derivatives, which results in inhibition of RNA and protein synthesis leading to cell membrane deterioration. 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. Elevated serum enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver. Hence significant rise in the transaminases levels could be taken as an index of liver damage. In our study the rise in AST and ALT levels induced by D-galactosamine administration was significantly reduced by 70% EEMLL 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.. Galactosamine induced elevation in serum urea level was also significantly reduced by 70% EEMLL and silymarin pre-treatments.

Galactosamine is reported to produce intensive inflammatory infiltration in the liver parenchyma and peripheral areas. In our study also D-galactosamine administration (Group II) showed severe hepatotoxicity with heavy infiltration of inflammatory cells around portal tract and in the liver parenchymal cells. Pre-treatment with 70% EEMLL and silymarin for 7 days protected the rat livers from D-galactosamine induced histopathological changes.

Above results suggest that 70% EEMLL and silymarin possess significant protection against D-D-galactosamine induced hepatotoxicity in rats. As this experimental model has striking resembles with the human viral hepatitis 70% EEMLL may also be evaluated in clinical settings.

4. CONCLUSION

The present study demonstrates that 70% EEMLL possesses hepatoprotective activity significantly in a dose dependent manner against D-galactosamine induced hepatic injury in rats. In addition, the hepatoprotective property may be attributed to the antioxidant principles which are present in the plant. Further investigation is going on to isolate, characterize and screen the active principles that possess hepatoprotective property.

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