

Screening of hepatoprotective activity of *Madhuca longifolia* bark on D-Galactosamine induced hepatotoxicity in rats.

Samaresh Pal Roy^{1,2*}, T. Kannadasan², Ramji Gupta^{1,2}

Department of Pharmacology, *¹Shree Dhanvantary Pharmacy College, Kim, Surat, Gujarat, India

²Anna University, Chennai, Tamilnadu, India

Abstract

In this present investigation the ethanolic extract of *Madhuca longifolia* bark (EMLB) was screened for hepatoprotective activity against D-galactosamine (d-GalN) induced hepatotoxicity in rats. Wistar rats were divided into five groups (n=6). Hepatotoxicity in rats was achieved by intraperitoneal injection 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 EMLB (200mg/kg B. wt. and 400 mg/kg B. wt.) 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 *Madhuca longifolia* bark possesses hepatoprotective activity against D-GalN induced hepatotoxicity in rats.

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

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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 [1]. GalN hepatitis is induced by a multiple step mechanism [2]. In particular, the peroxidation of endogenous lipids has been shown to be a major factor in the cytotoxic action of GalN [3]. GalN-induced oxidative damage is generally attributed to the formation of the highly reactive hydroxyl radical (OH[•]), stimulator of lipid peroxidation and source for destruction and damage to the cell membrane [4].

In the absence of reliable modern hepatoprotective drugs, there are a number of traditional medicines recommended for treatment of liver diseases. Many herbs reportedly possess hepatoprotective activity such as *Silybum marianum* [5], *Tridax procumbens* [6], *Strychnos potatorum* [7], *Andrographis paniculata* [8], *Picrorhiza kurroa* [9], and *Aquilegia vulgaris* [10].

Madhuca longifolia belonging to the family Sapotaceae is an indigenous plant found largely in the central and north

Indian plains and forests [11]. Commonly known as Mahua, it is used in the treatment of epilepsy, and inflammations. Presence of a genuine saponin protobasic acid and prospagenol in the seed kernels of *Madhuca longifolia* was reported by Yosioka [12]. Analgesic activity of the alcoholic extract was reported [13] while antihyperglycemic activity [14] and anti-inflammatory activity [15] of the extract of *Madhuca longifolia* bark was also studied. The plant possesses remarkable antioxidant and hepatoprotective potential against carbon tetra chloride and d-galactosamine induce hepatic injury [16,17].

This study aimed to conduct a scientific experiment using the ethanolic extract of *Madhuca longifolia* bark 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

Leaf samples of the plant *Polyalthia longifolia* were collected from Bardoli, Surat, Gujarat, during January 2013 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, 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 libitum*. 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, the negative control group, received the vehicle (normal saline). Group II served as positive control group and 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 [18].

Group-I : Normal Saline (1ml/kg, p.o)

Group-II : d-GalN (400mg/kg, i.p)

Group-III: Silymarin (100mg/kg p.o.)+d-GalN (400mg/kg, i.p)

Group-IV: EEMLB (200 mg/kg p.o.) + d-GalN (400mg/kg, i.p)

Group-V : EEMLB (400mg/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 hours 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 [19-22].

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 and examined for histopathological changes (200×) under a compound microscope [23].

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.

Results

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 EEMLB 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). The results are shown in (Table-1).

Table 1. Effects of EEMLB 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 mg/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

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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 + EEMLB (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 + EEMLB (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***

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

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

Histopathological Observation in D-Galactosamine induced hepatotoxicity

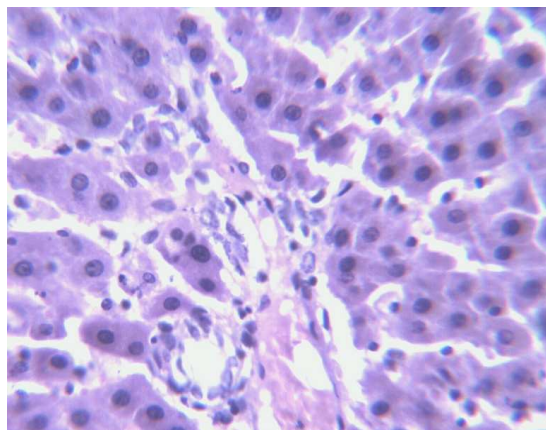


Figure 1. Liver architecture of Normal Control

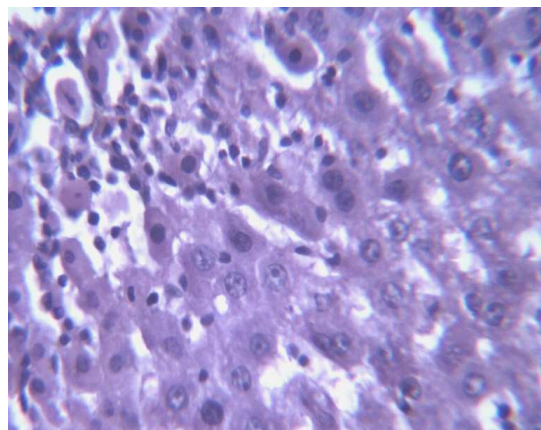


Figure 2. Liver architecture of d-GalN treatment

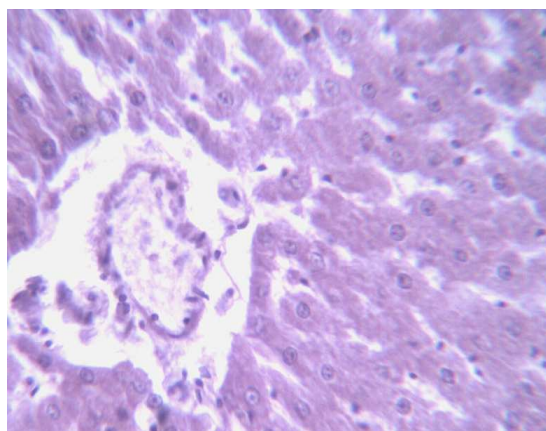


Figure 3. Liver architecture of d-GalN + 100 mg/kg Silymarin treatment

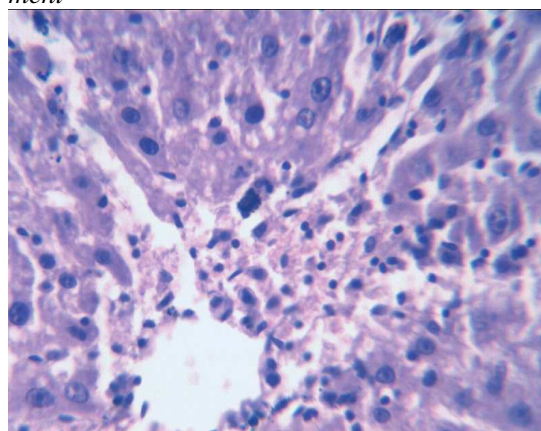


Figure 4. Liver architecture of d-GalN + 200 mg/kg of EEMLB

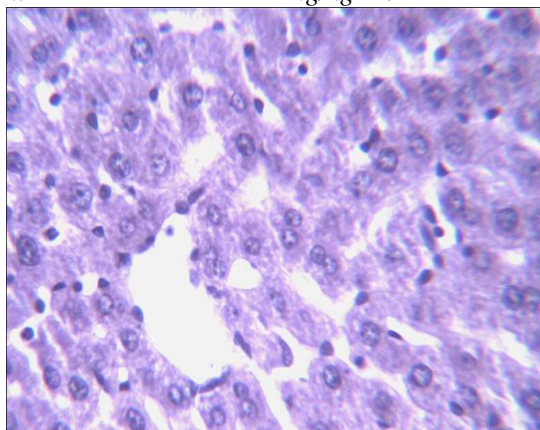


Figure 5. Liver architecture of d-GalN + 400mg/kg of EEMLB

Discussion

Any disease/disorder is associated with cell injury due to the generation of free radicals like superoxide anion ($O_2\bullet$), NO radical, $NOO\bullet$, $OH\bullet$ and H_2O_2 radical. Free radicals damage the cell membrane and cellular constituents like DNA etc. Though the free radicals are generated even in health, human beings possess the inbuilt natural mechanism to scavenge the generated free radicals. The inbuilt scavenging systems constitute the glutathione, superoxide dismutase (SOD), catalase. But during prolonged stressful conditions the free radicals produced cannot be handled by our inbuilt mechanism alone.

Thus released free radicals react with the membrane polyunsaturated fatty acid (PUFA) and oxidise them to lipid peroxides. This lipid peroxidation damages membrane protein as well as the lipids. Thereby, the integrity of the 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, and total and direct Bilirubin increased significantly in group treated with d-GalN comparing to normal control and it is an obvious indication of hepatic insult [24-26].

Study of any herbal medicine becomes more significant when it ameliorates some diseases conditions. In the present investigation, hepatoprotective effects of the ethanolic extract of *Madhuca longifolia* bark was studied based on d-GalN induced liver hepatitis. The hepatoprotective effect of *Madhuca longifolia* has been shown in earlier studies. In our study the rise in SGOT, SGPT, ALP, and bilirubin levels induced by D-galactosamine administration was significantly reduced by EEMLB 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 factor in hepatoprotective activity [27,28]. 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 *Madhuca longifolia* bark 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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***Correspondence to:**

Samaresh Pal Roy
Department of Pharmacology
Shree Dhanvantary Pharmacy College
Kim, Surat, Gujarat
India