



Hepato Protective Activity of Various Extracts of *Indigofera barberi* Gamble against D-Galactosamine Induced Toxicity In Rats

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Abstract: Medicinal plants have been found to possess hepatoprotective activity to varying degrees. The aim of the present study is to investigate the hepatoprotective effects of *Indigofera barberi* Gamble (aerial part of the plants extracts) in D-Galactosamine induced hepatic toxicity in Wistar albino rats. The rats received a single dose of D-Galactosamine (400 mg/kg, i.p) to induce hepatotoxicity; *Indigofera barberi* Gamble petroleum ether, chloroform and ethanol extracts (200 mg/kg, p.o) individually and Silymarin (25 mg/kg, i.p.) were administered after the injection of D-Galactosamine. It was found that D-Galactosamine induced hepatic damage resulted in a significant increase in the activity of AST, ALT, ALP, TB and LDH ($p < 0.01$); decrease in total protein. *Indigofera barberi* Gamble ethanolic extracts treatment attenuated the protective activity against D-Galactosamine induced hepatotoxicity in rats similar that of standard Silymarin. Whereas animal pretreatment with petroleum ether and chloroform extracts of *Indigofera barberi* did not change above mentioned parameters significantly. Thus the present study provides a scientific rationale for the traditional use of this plant in the management of liver diseases.

Keywords: D-Galactosamine, Silymarin, *Indigofera barberi*, Liver toxicity, ethanolic extract.

Introduction

Hepatitis is a common disease in the world especially in the developing countries. Despite, substantial progress in the treatment of liver diseases by oral hepatoprotective agents, search for newer drugs continues because the existing synthetic drugs have several limitations. Hence, there are many researchers in traditional medicines attempting to develop new drugs for hepatitis⁽¹⁾. D-Galactosamine (DGaIN) induced hepatic injury in animals mimics the sequences of events in human hepatitis and is widely used in the screening of hepatoprotective drugs. The advantage of this model is that DGaIN potentiates the toxic effects of lipopolysaccharide (LPS) and produces fulminate hepatitis within a few hours. DGaIN is an amino sugar selectively metabolized by hepatocytes, which depletes the Uridine phosphate pool, resulting in the inhibition of mRNA and protein synthesis⁽²⁾. These processes may lead to cellular damage and inflammation, resulting in histological and biochemical picture that closely resembles viral hepatitis. Upon stimulation with LPS, liver macrophages secrete various pro-inflammatory cytokines, leading to hepatic necrosis and decreased levels of antioxidant enzymes and the scavenging of free radicals and thus these model provides a practical tool for the evaluation of drugs and compounds that interfere with hepatic apoptosis and inflammatory liver injury⁽³⁾. *Indigofera barberi*

is a small shrub which belongs to the family *Fabaceae* and is distributed in India. The whole plant is medicinally important and many reports claim to cure several diseases in traditional system of medicine. In particular it is used for various ailments including liver disorders and tumors, wounds, skin disease, jaundice, anthelmintic, nephroprotective and diabetes.^(4,5) However there are no report of hepatoprotective activity of *Indigofera barberi* has been found in literature. Therefore, the present study was planned to investigate the effect of various extracts of whole plant *Indigofera barberi* in D-Galactosamine induced hepatic injury in Wistar rats.

Materials and Methods

Preparation of *Indigofera barberi* Gamble extract:

The whole plants of *Indigofera barberi* were collected from the forest region of Talakona (Nelakona region) of Chittoor dist, Andhrapradesh, India, in the month of November-2012. The plant material was identified and authenticated from the Plant anatomy research centre, Chennai, Tamilnadu, India. Shade dried whole plants were powdered to moderately coarse grade. Petroleum ether, chloroform and ethanolic extracts of whole plant were obtained by using Soxhlet extractor. The extraction was continued for 12 cycles. The extracts were

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concentrated under reduced pressure under controlled temperature (40-50°C) using rotavapour. The petroleum ether, chloroform and ethanol extracts were obtained as a greenish black (1.24% w/w), green (3.14% w/w) and reddish black (3.28% w/w) in color. All the extracts were semisolid in nature, they were kept in an air tight container at 4°C for future use. Suspension of each extract was freshly prepared using 0.1% Tween 80, for experimental use.

Animals:

The institutional animal ethics committee (Register No.160/1999/CPCSEA), Annamalai University, Annamalai Nagar, India; approved the experimental design. *Albino* (Wistar) female rats of 180-220gm (weighing) were used for the study. Animals were housed in well ventilated room (temperature 23±2°C, humidity 65-70% and 12h light/dark cycle) at Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. Animals were fed with standard pellet diet and water *ad libitum*. As per the standard practice, the rats were acclimated 15 days before the commencement of the experiment. They were fed on healthy diet and maintained in hygiene environment in the animal house. All studies were conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms and the National Institute of Health guidelines "Guide for the Care and use of Laboratory Animals".

Drugs and Chemicals:

Silymarin was purchased from Micro labs Ltd., Bangalore, India; Tween 80 was purchased from S.D. Fine Chemicals Ltd., Mumbai, India; D-Galactosamine (DGaIN) was purchased from Merck India, Ltd., Mumbai, India; and other solvents/reagents were of analytical grade.

Experimental protocol:

Rats were randomly divided into six groups of six animals each. Group I served as normal control and received normal saline

1ml through orally for 21 days. Group II served as toxic control and received normal saline 1ml through orally for 21 days. Group III were treated with 25mg/kg of Silymarin orally for 21days, Group IV were treated with petroleum ether extract of *Indigofera barberi* (PEEIB) at a dose of 200mg/kg suspended in 0.1% Tween 80 through orally, Group V were treated with chloroform extract of *Indigofera barberi* (CEIB) at a dose of 200mg/kg suspended in 0.1% Tween 80 through orally, Group VI were treated with ethanolic extract of *Indigofera barberi* (EEIB) at a dose of 200mg/kg suspended in 0.1% Tween 80 through orally, Group II to group VI received DGaIN (400 mg/kg, i.p.) on 21st day. After 24 h of DGaIN administration animals in all the groups were humanely sacrificed using Ketamine and 5ml of blood was withdrawn by cardiac puncture and allowed to clot for 30mins at room temperature. The serum was separated by using refrigerated centrifuge and used for the assay of marker enzymes viz Aspartate amino transferase (AST)⁽⁶⁾, Alanine aminotransferase (ALT)⁽⁶⁾, Alkaline Phosphatase (ALP)⁽⁷⁾, Total protein (TP)⁽⁷⁾, Total bilirubin (TB)⁽⁸⁾ and lactate dehydrogenase (LDH)⁽⁷⁾ by using Secomam semi auto analyzer.

Statistical analysis:

The Statistical analysis was carried out by one way analysis of variance (ANOVA) followed by NewmannKeul's multiple range tests. The values are represented as Mean ± SEM. Probability value of P < 0.01 was determined to be statistically significant.

Results and Discussion

The objective of the study was to witness the reports in traditional system of medicine by pretreatment with petroleum ether and chloroform and ethanolic extracts of *Indigofera barberi* for the usage of hepatoprotective activity against DGaIN induced hepatotoxicity in rats. Changes associated with DGaIN induced liver damage are similar to that of acute viral hepatitis. Hence, DGaIN mediated hepatotoxicity was chosen as the experimental model^[9].

Table.1: Results of the evaluation of hepatoprotective activity of various extracts of *Indigofera barberi* Gamble against D-Galactosamine – induced hepatic injury in experimental rats

Group. No.	TREATMENT DOSE (mg/Kg)	AST (IU/mL)	ALT (IU/mL)	ALP (IU/mL)	TP (gm/dl)	TB (mg/dl)	LDH (U/L)
I	Normal control	78.65±	42.40±	62.25±	6.05±	0.45±	306.40±
		3.85	2.40	2.05	0.68	0.10	8.48
		*a	*a	*a	*a	*a	*a
II	Toxic control (DGalN)	406.15±	386.45±	306.12±	3.08±	1.15±	508.17±
		12.30	9.68	9.28	0.21	0.32	16.18
		*b	*b	*b	*b	*b	*b
III	Treatment - Silymarin 25mg/kg	146.3±	156.05±	196.42±	5.68±	0.56±	396.08±
		4.05	5.75	5.85	0.55	0.10	9.75
IV	Treatment -PEEIB 200mg/kg	365.50±	322.65±	288.12±	3.58±	0.96±	485.18±
		10.60	12.06	8.90	0.30	0.22	16.10
V	Treatment - CEIB 200mg/kg	358.42±	306.42±	272.26±	3.48±	0.90±	472.20±
		11.25	10.52	9.42	0.27	0.27	15.68
		*b	*b	*b	*b	*b	*b
VI	Treatment -- EEIB 200mg/kg	170.60±	176.85±	202.12±	5.45±	0.61±	408.20±
		11.25	8.20	6.45	0.27	0.11	11.68

*a – values are significantly different from Normal control at $P < 0.01$; *b – values are significantly different from Toxic control (G2) at $p < 0.01$.

Significant increase in ($P < 0.01$) Serum AST, ALT, ALP, TB and LDH and significant decrease in total protein levels were observed in animals treated with DGalN 400mg/kg (Group II) ($P < 0.01$) as compared to normal control group (Group-I). Pretreatment with ethanolic extract of *Indigofera barberi* (EEIB) at a dose 200mg/kg, orally for 21 days decreased the levels of above indices like AST, ALT, ALP, TB, LDH and increased levels of TP significantly ($P < 0.01$) in group VI. Whereas animal pretreatment with petroleum ether and chloroform extracts of *Indigofera barberi* did not change above mentioned parameters significantly. Silymarin pretreatment produced significant decrease in ($P < 0.01$) serum AST, ALP, ALP, TB, LDH and significant increase in TP at ($p < 0.01$) in group III.

D-Galactosamine also causes depletion of Uridinephosphate (UDP) by increasing the formation of UDP-sugar derivatives, which results in inhibition of RNA and protein synthesis leading to cell membrane deterioration^[10]. D-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^[11]. Elevated serum enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane in liver^[12]. In our study the increase in AST, ALT, ALP, LDH levels and decrease in TP, TB levels induced by DGalN administration was significantly normalized by EEIB pre-

treatment not by PEEIB and CEIB suggesting that significant hepatoprotective activity which might be due its effect against cellular leakage and loss of functional integrity of the cell membrane in hepatocytes. D-Galactosamine is reported to produce intensive inflammatory infiltration in the liver parenchyma and peripheral areas. In our study also D-Galactosamine administration showed severe hepatotoxicity with heavy infiltration of inflammatory cells around portal tract and in the liver parenchymal cells. Pretreatment with EEIB and Silymarin for 21 days protected the rat livers from D-Galactosamine induced hepatotoxicity.

Conclusion

The EEIB found to have significant hepatoprotective activity by DGalN induced hepatic toxicity. The probable action could be due to stabilization of the hepatocellular membrane which might be used in the treatment of viral hepatitis. Thus the present study provides a scientific rationale for the traditional use of this plant in the management of liver diseases.

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