

HEPATOPROTECTIVE ACTIVITY OF *DALBERGIA SPINOSA* (ROXB) AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

P.Thirupathy Kumaresan^{1*}, Arunjosph, Gururajesh U, Renugadevi, C Sathish Kasula

Department of Pharmacology, Arulmigu kalasalingam college of Pharmacy,
Krishnankoil 626126, vir dhunagar Dist, Tamilnadu, India

*Corresponding Author Email: thirukumaresan70@yahoo.com

ABSTRACT

Objective: To study the hepatoprotective activity of methanol and aqueous extracts of *Dalbergia spinosa* leaves against paracetamol induced liver damage in rats. **Methods:** Hepatotoxicity was induced by paracetamol and the biochemical parameters such as serum glutamic pyruvic transaminase (sGPT), serum glutamic oxaloacetic transaminase (sGOT) and serum alkaline phosphatase (sALP), serum bilirubin (SB) and histopathological changes in liver were studied along with silymarin as standard hepatoprotective agents. **Results:** The phytochemical investigation of the extracts showed presence of steroids and flavonoids. Pre-treatment of the rats with methanol and aqueous extract prior to paracetamol administration caused a significant reduction in the values of sGOT, sGPT, sALP and sB ($P < 0.01$) almost comparable to the silymarin. The hepatoprotective was confirmed by histopathological examination of the liver tissue of control and treated animals. **Conclusions:** From the results it can be concluded that *Dalbergia spinosa* possesses hepatoprotective effect against paracetamol-induced liver damage in rats. The present study was conducted to evaluate the hepatoprotective activity of a alcoholic extract of flowers *Dalbergia lanata* against paracetamol induced liver damage in rats. The alcoholic extract of flowers of *Dalbergia spinosa* (100 and 200 mg/kg) was administered orally to the animals with hepatotoxicity induced by paracetamol (3gm/kg). Silymarin (25mg/kg) was given as reference standard. All the test drugs were administered orally by suspending in 0.5% Carboxy methyl cellulose solution. The plant extract was effective in protecting the liver against the injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and bilirubin. It was concluded from the result that the alcoholic extract of possesses hepatoprotective activity against paracetamol induced hepatotoxicity in rats.

KEY WORDS

Paracetamol, hepatoprotective, hepatotoxicity and *Dalbergia spinosa*

INTRODUCTION

Nowadays liver disease is a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects¹. In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders².

In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. *Dalbergia spinosa* Roxb. (Common name: Nechitanchedi in Tamil, belonging to the family, Fabaceae. It is widely distributed in mangrove forest, Chidhambaram and coastal parts of Peninsula and Benga³. It is a large shrub with a tendency to climb,

4. with oblanceolate shaped leaf, flowers are yellowish strand and pods are compressed, generally the parts used are leaves and roots, to treat for inflammations, urinary problems, pain and fever⁴⁵⁶⁷. The plant has been reported for its chemical constituent's isoflavone, and possess spermicidal, cardiovasculars Isoflavone was isolated from *Dalbergia spinosa* leaves⁸. The study was conducted to establish the traditional use of *Dalbergia spinosa* as hepatoprotective against paracetamol induced hepatotoxicity in rats.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing between 150 – 220 gm were used for this study. The animals were obtained from animal house, A. K College of Pharmacy, Krishnankoil, Virudhunagar Dist Tamilnadu, India. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of $24 \pm 2^\circ \text{C}$ and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Amurth Pvt Ltd, Bangalore). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC) and were in accordance with the guidelines of the IAEC. Research J. Pharm. and Tech. 1(4): Oct.-Dec. 2008

Plant Material:

The fresh plants were collected in rural areas of Krishnankoil, Virudhunagar District, Tamilnadu. The plant was identified by Dr Stepan, Botanist American College Madurai, and voucher specimen was deposited in the Department of Pharmacology, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil. After authentication, the plants were collected during winter season jan 2009 and shade

dried and milled into coarse powder by a mechanical grinder.

Preparation of Extract:

The coarse powder plant material was extracted with ethanol: water (1:1) by using soxhlet apparatus. The solvent were removed under reduced pressure to get semisolid mass. Standard methods were used for preliminary phyto chemical screening of the extract was performed to know the phytoconstituents in the extract⁹; it was found that the extract contains alkaloid, flavonoids, glycosides, steroid, and tannins.

Hepatoprotective Activity:

A total of 24 animals were equally divided into 4 groups of six each. Group – I served as normal control received 0.5% (CMC) carboxy methyl cellulose solution (1 ml/kg) once daily for 3 days. Group – II served as paracetamol control, administered with paracetamol (3 gm/kg) as single dose on day 3. Group III received, *Dalbergia spinosa* extract (200 mg/kg) once daily for 3 days. Group – IV served as reference control, received Silymarin (25 mg/kg) once daily for 3 days. Group III and IV received paracetamol (3gm/kg) as single dose on day 3, thirty minutes after the administration of *Dalbergia spinosa* and Silymarin respectively. All the test drugs and paracetamol were administered orally by suspending in 0.5% CMC solution. After 48 h of paracetamol feeding, the blood was collected by direct cardiac puncture under light ether anesthesia and serum was separated for the estimations alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)¹⁰,¹¹ and bilirubin¹²

Statistical Analysis:

The values were expressed as mean $\pm$ SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' - test. P values < 0.05 were considered significant.

Table 1: Effect of flower of *Dalbergia spinosa* Linn extracts on Paracetamol induced hepatotoxicity in rats

Groups (n)	Dose mg/kg	Total Bilirubin (mg %)	Total Protein (gm %)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	Vehicle	1.53 ± 0.053	8.38 ± 0.12	36.16 ± 3.954	315.83 ± 24.88	74.5 ± 8.03
Paracetamol	3 ml	2.16 ± 0.11	6.98 ± 0.05	542.16 ± 43.04	738.666 ± 0.02	169.83 ± 7.58
Silymarin	100	0.93 ± 0.07**	7.71 ± 0.06***	260.33 ± 23.22*	319.5 ± 6.307***	147.16 ± 16.91*
Benzene extract	100	1.25 ± 0.051**	7.1 ± 0.05***	111.66 ± 4.16**	155.5 ± 10.88***	102.33 ± 3.34*
Alcoholic extract	200	1.2 ± 0.057*	7.1 ± 0.07***	160.66 ± 3.676*	196.83 ± 23.26***	189.16 ± 5.05*

Values are expressed as Mean ± SEM, n = 6 rats in each group.

* P < 0.01, ** P < 0.001, *** P < 0.05 compared to standard group.

RESULT

The results of hepatoprotective activity of alcoholic extract of *Dalbergia spinosa* on Paracetamol treated rats are shown in Figure I and II. The hepatic enzymes ALT, AST, ALP and bilirubin in serum was significantly (P < 0.001) increased in paracetamol treated animals when compared to control. The alcoholic extract of *Dalbergia spinosa* treatments significantly (P < 0.01) reversed the levels of AST, ALP and bilirubin (P < 0.01) and ALT (P < 0.001) when compared to paracetamol alone treated rats. Silymarin (25 mg/kg) treated animals also showed significant decrease in AST (P < 0.01), ALT, ALP and bilirubin (P < 0.001) levels when compared to paracetamol alone treated rats.

DISCUSSION

The hepatotoxicity is caused by the reaction metabolite of Paracetamol ie N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione depletion. It is a well-known antipyretic and analgesic agent, which produces hepatic necrosis at higher doses¹³. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome¹⁴ or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity^{15, 16}. Normally AST and ALP are present in high concentration in liver. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the blood increases. ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non hepatic diseases is unusual. ALT is more

selectively a liver paranchymal enzyme than AST¹⁷. Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and Bilirubin which are enzymes originally present higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage¹⁸. The elevated level of these entire marker enzymes observed in the group II, paracetamol treated rats in this present study corresponded to the extensive liver damage induced by toxin. The reduced concentrations of ALT, AST and ALP as a result of plant extract administration observed during the present study might probably be due in part to the presence of flavonoids. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotinoids, glycosides, flavanoids, organic acids, lipids, alkaloids and xanthines¹⁹. Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. Decrease in serum bilirubin after treatment with the extract in liver damage induced by paracetamol indicated the effectiveness of the extract in normal functional status of the liver.

CONCLUSION

The alcoholic extract has shown the ability to maintain the normal functional status of the liver. From the above preliminary study, we conclude that the alcoholic extract of *Dalbergia spinosa* is proved to be one of the herbal remedies for liver ailment.

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*Corresponding Author:

Thirupathy Kumaresan*

Department of Pharmacology
Arulmigu Kalasalingam college of Pharmacy
Krishnankoil 626126,
Virudhunagar Dist,
Tamilnadu, India