HEPATOPROTECTIVE ACTIVITY OF DALBERGIA SISSOO LEAVES AGAINST PARACETAMOL INDUCED HEPATIC INJURY IN RATS

Suprabha Devi[*1], Vikram Singh[*1], Raj K. Prasad[*1], M. K.Mishra[1]
Shambhunath Institute of Pharmacy, Jhalwa, Allahabad- 210012 (U.P.), India

Abstract

In traditional medicinal trees, *D. sissoo* is a popular species around the world.In very larg numbers distribution in different parts of India. *Dalbergia sissoo* is uses for medicine purpose from many of years and now theire growing demand for natural based medicines, marketed products, pharma industries and cosmetics. *D.sissoo* is hugely growing plant which is using traditionally as anti-inflammatory, antipyretic, analgesic, anti-oxidants, anti-diabetic and as antimicrobial agent. Many chemical constituents have been isolated and sflavanols, tannins, saponins, steroids and terpenoids, carbohydrates. Compounds isolated from *D.sissoo* like an isoflavone, biochanin is a essential therapeutic cancer preventive agent with a different estrogenic property. *D.sissoo* have numaral pharmacological properties however, it is necessary that numbers of clinical and pharmacological studies should be done to determine the undeveloped potency of the plant. The present research was goal to determine the effective hepato-protective activity of ethanol extracts of leaves of *D.sissoo* using *in vivo* paracetamol hepatotoxicity models to evaluate the superstistious use of the plant.

The hepato-protective activity of the ethanol extracts of leaves were studied on male albino wistar rats, liver toxication produce by acetylaminophenol (2.0 gm/kg, p.o.) by determining biochemicals. Number of biochemicals parameters were studied to determine the hepatoprotective property of ethanol extracts in serum like glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), and serum alkaline phosphatase (SALP) , total bilirubin (SB), total protein, and histopathologies in liver were also studied compare with silymarin (100mg/kg, p.o.) as standard hepatoprotective agents were determined to assess the effect of the ethanol extract of leaves of Dalbergia sissoo (100 and 200 mg/kg) on the acetylaminophenol produce hepatic toxication.

The phytochemical determination of the extracts showed presence of Biochanin A, tectoregenin, carbohydrates, proteins, amino acids, tannins compounds, and flavanoids. Pre-treatment of the rats with ethanol extract subjected to acetylaminophenol administration caused a remarkably decrease in the values of SGOT ,SGPT , ALP, and SB almost comparable to the silymarin . The hepatoprotective was proved by histopathologic study of the liver tissues of control group and ethanolic extract treat animal group.

The results indicate that this plant possesses potential hepatoprotective properties and has therapeutic potential for the treatment of liver diseases.

IndexTerms: *Dalbergia sissoo* Linn. Paracetamol(acetylaminophenol); hepatoprotective activity; silymarin; histopathology.

INTRODUCTION

The history of herbal medicines is very old as human civilization . Medicinal plants have principle and cheif source of important phytochemicals, they are used broadly for the evolution of new drugs against many type of deseases and disorders [1-2]. Herbal drugs play an significant role in therapy of different deseases it include hepatopathy (liver disease). Medicinal herbs are used in so much range in the treating of hepatic diseases like hepatitis, cirrhosis, and loss of appetite, some medicinal plants and herbs have proven hepatoprotective potency. Flavanol ,lignin mixture(Silymarin) extract from the milk thistle (*Silybum marianum)* is a popular natural medicine for liver diseases [3].

From the medicinal plants, *D. sissoo* (Family- Fabaceae) (known as Shisham) is a beneficial Indian medicinal plant which has approved with medicinal activity to treat many ailments. *D. sissoo* is present in many parts of India. Dalbergia sissoo is a long tree . It's growth to 25 m in height and 2-3 m in width. [4] Although , review literature data assisted the cultural use of the *D.sissoo* in hepatic ailments are not found and its indefinite mechanism (s) are unknown till now . Medicinal plants have been the part and collection of human population to control disease from the human contemporary. The recent explanation of therapeutic activity of medicinal plants were explained in Rigveda (2500-1800 BC), Charak Samhita and Sushruta Samhita. Herbal drugs are used to cure many diseases from ancient time and a way of many usual type of treatment present in wide range in whole world [1]. Natural drugs are good substances to treat many diseases in growing countries. The normal drugs commonly obtained from plants [2]. The plants used as a source of drugs has been assumed and is an significant substances of the health care system in India also [3].

Literature review says and ethnic information collected from Jaunpur interior area, Uttar Pradesh that the plant *D.sissoo* (shisham) has reported the use of the leaves for the management of hepatotoxicity. Hence, the objective of this study was to ascertain the scientific basis for the use of *D. sissoo* Roxb.(Fabaceae) in the management of hepatotoxicity using paracetamol induced hepatotoxicity in rats. Tannin or dyestuff: *D.sissoo* pods contain 2% tannin.

Lipids: Heartwood yields light brown, viscous, non-drying fixed oil (5.35%), suitable as a lubricant for heavy machinery.

Poison: *D.sissoo* is reported to have pesticidal properties. Aqueous extracts from the leaves, stems and roots inhibit the reproduction, growth and development of the insect pest Utethesia pulchella. Mixed with Azadirachta indica oil cake, sawdust from *D. sissoo* reduces egg laying and increases larval mortality in Melodogyne javanica. Methanol extract from the roots has insecticidal properties, especially against Diacrisia obliqua, Spodoptera litura and Argina cubrania.

Medicine: Oil obtained from the seeds is used to cure skin diseases. The powdered wood, applied externally as a paste, is reportedly used to treat leprosy and skin diseases.

D.sissoo oil also showed strong repellent activity against mosquitoes like Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus, and is also resistant to some wood boring insects. Its aerial parts showed significant bronchodilator and estrogen like activities. Leaf juice is used for eye ailments. The alcoholic extract *D.sissoo* leaves produces significant anti- inflammatory effects in different models of inflammation, without having any side effects on the gastric mucosa. Rural people in India and Nepal use *D.sissoo* leaves to treat animals suffering from non-specific diarrhoea. Leaf extract has been used to treat sore throats, heart problems, dysentery, syphilis, and gonorrhoea. The extract of *D.sissoo* leaves showed antioxidant activity and is almost two times higher than other commonly used antioxidant like selenium and vitamin E. The extract of *D.sissoo* leaves contains a large amount of flavonoids.

Leaf extract has been used to treat sore throats, heart problems, dysentery, gonorhea, syphilis [10] . The plant has many reputed medicinal properties and has been used for years in daily life to treat disease all over the world. This review represent ,therefore ,an effort to given detailed survey of the literature on D.sissoo[11]. It possess many chemical constituents they are dalbergin[12], 4-phynylchromone (dalbergichromene)[13], chromone[14], flavones[15],dalberginone[16].

MATERIAL AND METHODS

Collection of Plant Material

The leaves of *D. sissoo* was collected from the local area of Junpur , Uttar Pradesh, India in the month of August 2016 and Authenticated by Botanical Survey of India , Chatham Lines, Allahabad-211002 and its herbarium file is kept on no. SIP-IAEC/009/09-16.

Fig. 1: D. sissoo plant

Fig. No. 2: D. sissoo leaves

Extraction methods

The leaves was dried under shade, powdered with a mechanical grinder and passed through a 40 – mesh sieve. The successive solvent cold extraction method used to obtain various extracts including petroleum ether, chloroform, ethyl acetate , ethanol and aqueous extracts. The solvents were removed from the extracts under reduced pressure by using a rotary vacuum evaporator (Buchi model, jyoti Lab, Gwalior, India). The percentage of yield of extracts was noted. The brownish extract was obtained and is dissolved in their respective solvents for pharmacological studies.

Preliminary phytochemical screening

The ethanol extracts of leaves was screened for the presence of various phytoconstituents like steroids, alkaloids, glycosides, flavonoids, carbohydrates, amino acids , proteins and phenol compounds.[5]

Animals

Healthy , adult Albino Wistar rats (180-200gm) of either sex were purchased from the Central Drug Research Institute , Lucknow used for study . Housed individually in polypropylene cages, maintained under standard conditions (12h light; and12 hr dark cycle; 23±2^{0}C, 50±5% , relative humidity), they were fed with standard rat pellet diet (Hindustan Lever Ltd; Mumbai , India) and were add libitum. The Institutional Animal Ethics Committee approved the study.

Acute toxicity study

The acute oral toxicity study has to be carried out as per the guidelines set by OECD, revised draft guidelines 423 , received from CPCSEA, ministry of social justice and empowerment, Govt. of India. The animals are randomly selected, marked to permit individual identification , and kept in their cages for at least 5 days prior to dosing to allow for acclimatization to the laboratory conditions .The test substance is administered in a single dose by gavage using a

Stomach tube or a suitable intubation canula. In the unusual circumstance that a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours . Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. [6]

In vivo Hepatoprotective activity

The rats have to be randomized into five groups comprising of six animals in each groups as given below.

groups	Doses
Group I	Normal control rats were given Tween 80 for 4 days
Group II	Hepatotoxicity control rats have been given Tween 80 for 4 days followed by paracetamol (2gm/kg, p.o.) on 3rd day.
Group III	The rats have been given silymarin(100 mg/kg, p.o.) for 4 days followed by paracetamol (2gm/kg, p.o.) on 3rd day .
Group IV	Test rats have been given ethanol extract of leaves of *D. sissoo* (100mg/kg, p.o.) followed by paracetamol (2 gm/kg , p.o.) on 3rd day.
Group V	Test rats have been given ethanol extract of leaves of *D. sissoo* (200gm/kg , p.o.) followed by paracetamol (2 gm/kg,p.o.) on 3rd day.

Assessment of Hepatoprotective Activity

At the end of 5th day, blood was collected by heart puncture and serum was separated for the estimation of alanine aminotransferase (ALT) , aspartate aminotransferase (AST) , alkaline phosphatase (ALP), total bilirubin, total cholesterol,etc. and the liver was isolated from the rats of all the groups and kept in 10% formalin solution and hence send for histopathological investigation[7].

Statistical Analysis

Hepatoprotective activity

The data was represented as mean ± SD. Results were analysed by one way Anova followed by Tukey-Kramer Multiple Comparison test using GraphpadInstat (trial) 3.0.10.0 software. Results were expressed as the mean± SD. Statical analysis of the data group means were compared by one way analysis of variance (ANOVA) followed by Tukey- Kramer Multiple Comparison test. P<0.0001 was considered extremely significance.

Table 1: *Phytochemical study of the extracts of leaves of Dalbergia sissoo*

+ = Presence ; - = Absence

Phytoconstituents/ Extracts	Petrolium ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
Alkaloids	-	+	-	-	-
Glycosides	-	+	+	-	+
Flavanoids	-	+	+	++	-
Steroids	-	-	-	+	-
Tannins	-	-	-	+	+
Carbohydrates	-	-	-	+	-
Saponins	-	-	-	+	+
Terpinoids	+	-	-	+	-

RESULTS

Table 1 shows the results of Phytochemical study was performed on the ethanol extracts of the leaves of *D. sissoo* and the presence of various phytoconstituents such as alkaloids , ccarbohydrate , flavonoids , steroids , tannins, and Terpenoids, Saponins were observed.

Hepatoprotective activity

Table 2 shows the results of hepatoprotective activity of ethanol extracts of leaves of *D. sissoo* on paracetamol induced hepatotoxicity in rats. The hepatic enzymes SGOT, SGPT, ALP, bilirubin, total protein in serum was significantly increased in paracetamol treated animals when compared to normal. The ethanol extracts of leaves of *D.sissoo* of low and high dose significantly reversed the levels of SGOT, SGPT, ALP, bilirubin , total protein respectively when compared to paracetamol alone treated rats. So the animals treated with ethanol extract of leaves of Dalbergia sissoo showed statistically significant ($p<0.0001$) protection against paracetamol induced hepatotoxicity in rats , which is comparable to the reference compound Silymarin . Thus silymarin (100mg/kg) treated animals showed significant decrease in SGOT, SGPT, ALP, bilirubin, total protein.

Histopathological Study

The histopathology study of liver was also performed which showed hepatoprotective effect. The hepatoprotective effect of *D.sissoo* leaves was confirmed by histopathological examination of the liver tissue of control and treated animals. The histological architecture of liver sections of healthy rats showed normal cellular architecture with distinct hepatic cells and sinusoidal space, Fig. 1. In the liver section of the rats intoxicated with paracetamol Fig. 2 , there was disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis, while rats treated with silymarin and intoxicated with paracetamol showed less disarrangement and degeneration of hrpatocyte Fig .3. The histopathological profile of the rat treated with ethanol extract showed no visible changes confirming the safety of the extract at selected higher and lower doses (4,5) and the liver section of the rats treated with higher and lower doses of ethanol extract and toxicated with paracetamol showed moderate hepatoprotective activity, Fig. 6,7.

Histopathological studies

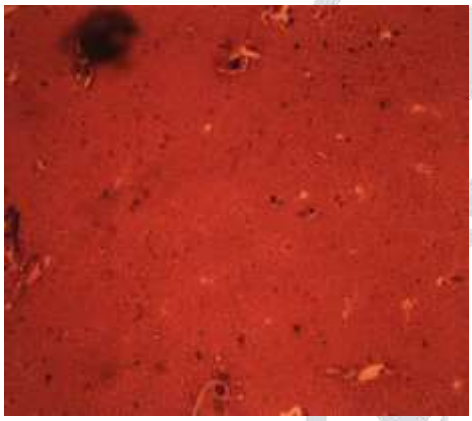

Fig 3: Normal gp. Showing Normal cellular architecture With distinct sinusoidal space And central vein

Fig 4: Toxic control gp showing severe necrosis of hepatocytes.

Fig. 5: Standard silymarine (100mg/kg, p. o.) Treated gp . showing less disarrangement Of hepatocytes as well as marked regeneration activity

Fig.6: Ethanol high dose (200mg/kg) showing normal architecture with moderate hepatocyte generation

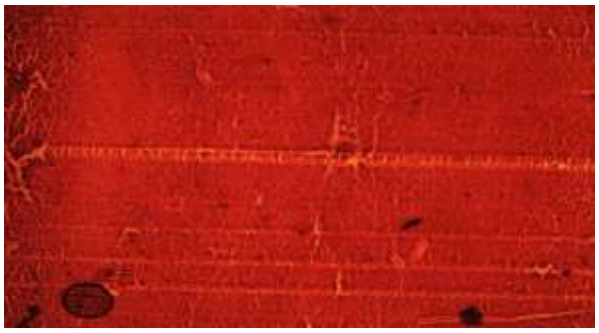

Fig. 7: Ethanol low dose (100mg/kg)showing normal architecture With moderate hepatocyte degeneration

Table 2: *Effect of D. sissoo on serum marker enzymes (SGOT, SGPT, and ALP) ,Total bilirubin ,total protein l on paracetamol induced hepatotoxicity in rats*

Groups (n=6)	Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Bilirubin(mg/dl)	Total Protein(gm/dl)
I	Normal control	26.95±0.2881****	64.25± 0.4505****	120.38± 0.4262****	0.4567± 0.0388****	7.08± 0.2483****
II	Paracetamol 2.5 gm/kg	37.32± 0.4262****	74.3± 0.2828****	180.23± 0.3615****	0.79± 0.052****	5.95± 0.6058****
III	Silymarin100 mg/kg+ paracetamol	29.33± 0.4802****	54.4833± 0.3764****	100.2833± 0.3869****	0.66± 0.0907****	7.18± 0.3869****
IV	Ethanol extract 100 mg/kg+ paracetamol	19.18± 0.3656****	23.63± 0.2160****	80.08± 0.3430****	0.014± 0.0057****	6.83± 0.2160****
V	Ethanol 200 mg/kg+ paracetamol	23.22± 0.2639****	39.1± 0.2828****	70.25± 0.4231****	0.587± 0.0216*****	7.08± 0.2317****

Values are expressed as mean ±SD for (n= 6) rats in each group , when compared to control **p<0.0001

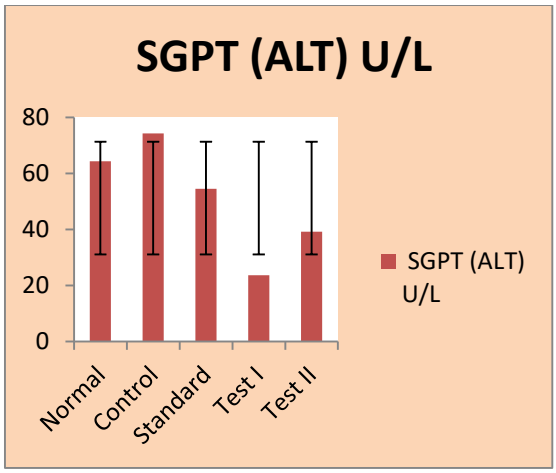

Fig. 8: SGPT level decreased

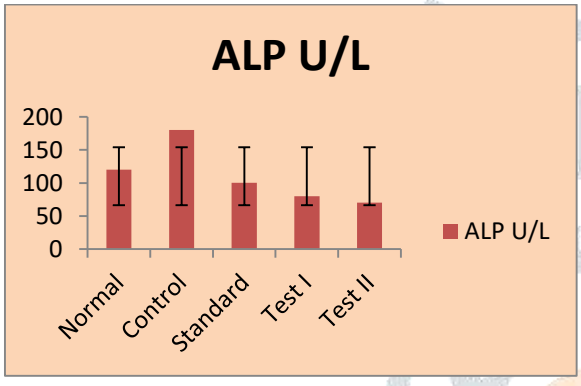

Fig. 9: ALP level decreased

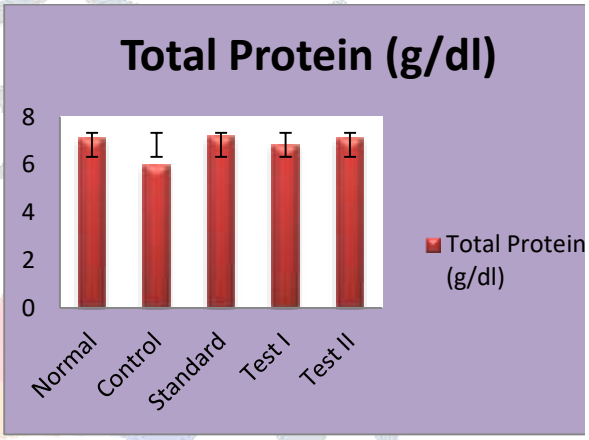

Fig. 10: Protein level increased

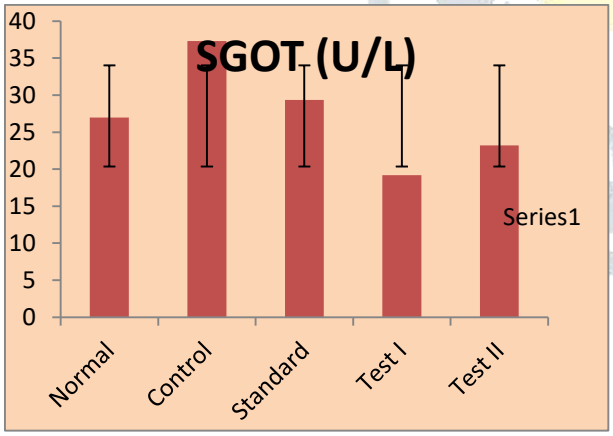

Fig. 11: SGOT level decreased

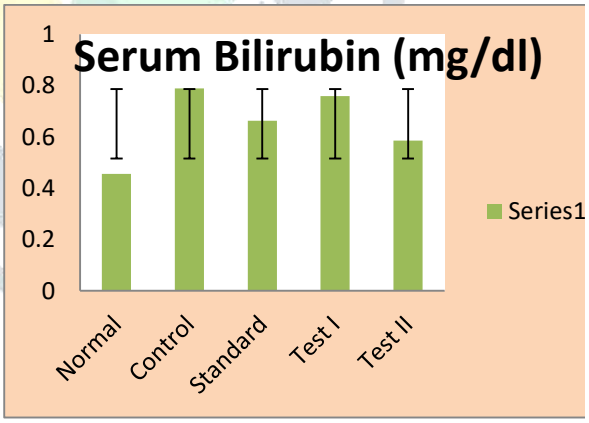

Fig. 12: Serum Bilirubin is decreased

DISCUSSION

The estimation of enzymes in the serum is a useful quantitative biochemical marker of the extent and type of hepatocellular damage. 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.

The rat treated with an overdose of paracetamol developed significant hepatic damage, which was observed by a increase level of serum hepatic enzymes (SGPT and SGOT). Biochemical parameters demonstrate significant increase of serum enzymes in the

toxic control groups in the present study. Histopathological profile also show a major damage in the same groups. Thus , it clearly states that, toxicity is due to either of the above mechanisms such as depletion of glutathione store or free radical generation or lipid peroxidation.

Administration of *D. sissoo* leaves ethanol extracts (100mg/kg and 200mg/kg p.o.) after paracetamol treatment resulted in a significant reduction ($p>0.10$) of paracetamol- induced elevation of SGPT and SGOT and appears to be protective in reducing the injurious effect of paracetamol.

Fig. 12 : shows the Bilirubin level decreases with ethanolic ethanolic extraction of leaves.

In present study the histopathological studies also shows normal, toxic , toxic control (silymarin treated), ethanol higher and lower dose treated hepatocytes of liver.

Histo pathology also describe the less cell damage is due to the effect of D.sissoo ethanolic extract .

All gps of L.F.T. graphs of all serum enzymes Fig. : 8,9,10,11,12 shows the very significant effect of ethanolic extract *D.sissoo* leaves.

In conclusions, the ethanol extracts of leaves of Dalbergia sissoo exhibited protective effect against paracetamol – induced hepatotoxicity and possess anti-lipid peroxidative and free radial scavenging activities.

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