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EVIDENCES OF HEPATOPROTECTIVE AND ANTIOXIDANT EFFECT OF DALBERGIA *PANICULATA* IN ACETAMINOPHEN INDUCED HEPATOTOXICITY IN RATS

Geeta Soren*[1], Srinivas Rao N[2], Sridhar Y[2] & Karunakar Reddy[2]

[1]Depatment of Pharmacology, M.N.R. Medical College, Sangareddy, Telangana.

[2]Dept. of Pharmacology, S.R.R. College of Pharmaceutical Sciences, Telangana, India.

*Corresponding Author Email: geetasoren@yahoo.com

ABSTRACT

Aim: *To evaluate the hepatoprotective and antioxidative activity of methanolic leaf extract of Dalbergia paniculata in acetaminophen induced hepatotoxicity in rats.* ***Methods:*** *Male wistar rats (150-200g) were divided into six groups. Group I – Normal Control, Group II – Toxic Control, Group III –Standard, Group IV to VI received Methonolic leaf extract of D. Paniculata at 250, 500 and 1000 mg/kg, p.o respectively. Hepatoprotective activity was evaluated by liver function tests, antioxidative activity by TBARS method and was also sectioned for histopathological examination.* ***Results:*** *The methonolic leaf extract of D. Paniculata produced dose dependent inhibition of increased levels of SGOT, SGPT, ALP, Bilirubin and MDA in Acetaminophen induced hepatotoxicity rats. Histopathological alterations are significantly different in all the groups.* ***Conclusion:*** *The Dalbergia Paniculata methonolic leaf extract shows significant hepatoprotective and antioxidative activity.*

KEY WORDS

Hepatoprotectivity, Antioxidativity, Acetaminophen, Hepatotoxicity, Dalbergia Paniculata.

INTRODUCTION

Evolution of multiple chronic disorders requires various forms of medicinal agents for treatment. However practicing polypharmacy drastically increases risk of hepatic injury. As liver is the vital organ that regulates metabolism, secretion, storage and detoxifying functions in humans. Any damage to the liver or impairment of its functions leads to injurious effects. Damage or injury to the liver caused by a drug, chemical or other agent is called hepatotoxicity. Liver diseases (like jaundice) are the common ailments affecting mankind, though no remedy is available in allopathy to date [1,2, 3,4].

The principle cause of acetaminophen induced hepatic damage is lipid peroxidation and decreased activities of antioxidants, enzymes and generation of free radicals [3]. Acetaminophen is a widely used analgesic and antipyretic drug that has a good safety profile when used at the recommended dose, however at higher doses can lead to liver damage .The characteristic histological lesion is centrilobular or zone 3 necrosis that may develop into sub massive or massive hepatic necrosis. The zone 3 location of the initial histological change is a consequence of the location in this area of cytochrome P_{450} enzyme that is involved in metabolizing the drug (CYP2E1) [3,4].

In spite of advances in synthetic drugs in recent years for many ailments, some of the drugs of plant origin have still retained their importance in disorders like jaundice, asthma, diabetes etc. Some of the important marketed ayurvedic formulations which are useful as

hepatoprotective agents are hepatoguard, liv52, livomin, nirosil, stimuliv, sorbilin etc [4,6,7].

In the present study the attention was focused on evaluating the antioxidant activity and hepatoprotective activity of methanolic extract of leaf of *Dalbergia paniculata* in acetaminophen induced liver damage in rats.

MATERIALS AND METHODS

Animals

Healthy male albino rats weighing between 150-200 g were used for the study. The animals were procured from Sainath agencies, Hyderabad and the animals were kept in polypropylene cages. Animal house were maintained under standard hygienic conditions, at 25 ± 2^{0}C, humidity (60 ± 10 %) with 12 hrs day and night cycle, with food and water *ad libitum*. The experimental protocols were approved by the Ethical Committee in Animal Experimentation study procedures.

Drugs

All drug suspensions were prepared for the different groups with 3% (W/V) aqueous suspension of gum acacia as vehicle.

Preparation of the Methanolic Extract of *Dalbergia paniculata* (MEDP):

The fresh leaves of *Dalbergia paniculata* were collected and kept for shade drying. Dried leaf material was powdered using mechanical grinder and passed through sieve # 60 to get the powder of desired coarseness. Powdered material was preserved in an air tight container for further use. A weighed quantity (150gms) of the powder was subjected to continuous hot extraction using methanol as a solvent in Soxhlet Apparatus. The extract was evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to give an extract sample. Percentage yield of methanolic (95%) extract of *Dalbergia paniculata* was found to be 17.5 % w/w.

Standard Hepatoprotective

Silymarin (SILY) powder (obtained from Micro Labs Ltd., Bangalore, India) was used to make the suspension in doses of 200 mg/kg.

Hepatotoxin

Acetaminophen powder (I.P.) (obtained from Bharat Chemicals, Tarapur, Gujarat, India) was used to make the suspension in a dose of 2 g/kg BW for the respective groups.

Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines. The dose level of up to 2000mg/kg, No mortality or any other autonomic or behavioral responses such as tremors, convulsion, salivation, diarrhea, lethargy, sleep and/or coma were observed.

Methods

The experiment was carried out on 36 healthy albino rats for 10 days. Before starting the experiment, the animals were allowed to acclimatize to the laboratory environment for 1 week.

Grouping and Treatment Schedule

The Male wistar rats weighing 150-200 g were used. The over night fasted animals were divided into six groups (n=6) as follows,

- Group I: Normal control (Normal saline - 5 ml/kg BW/day orally)
- Group II: Experimental control (Acetaminophen 2g/kg, p.o – 5mlVehicle/kg BW/day)
- Group III: Standard Silymarin (200 mg/kg + Acetaminophen 2g/kg p.o in 5 ml vehicle)
- Group IV: MEDP (250mg/kg + Acetaminophen 2g/kg p.o in 5 ml vehicle)
- Group V: MEDP (500mg/kg+ Acetaminophen 2g/kg p.o in 5 ml vehicle)
- Group VI: MEDP (1000mg/kg+ Acetaminophen 2g/kg p.o in 5 ml vehicle)

Dosing

The drug suspensions and the vehicle were administered per orally by an intragastric feeding tube at a uniform volume of 5 ml/kg BW.

Biochemical Estimations

On Day 10th after overnight fasting all the animals were anesthetized with anesthetic ether and blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary tube and collected in plain sterile centrifuge tubes and allowed to clot. Serum was separated by centrifugation at 7000 rpm for 15 min. at 5°C. The separated serum was used for estimation of AST and ALT (Reitman's and Frankel method), ALP (pNPP Kinetic Method), TB (Mod. Jendrassik and Grof's Method), Direct Bilirubin, Indirect Bilirubin, and Lipid peroxidation (thiobarbituric acid).

Histopathological Examination

The rats were then sacrificed under deep ether anesthesia and the liver samples were excised and washed with normal saline. A record of each liver was made, regarding size and shape, color and presence or absence of any nodule. Then, the livers were fixed immediately in 10% formalin solution. A paraffin embedding technique was carried out and sections were taken at 5-mm thickness, stained with hematoxylin and eosin and examined microscopically for histopathological changes.

Statistical Analysis

The results, obtained from the LFT were presented as mean and standard error of mean (SEM) for each group (mean ± SEM). All groups were subjected to one-way analysis of variance (ANOVA), which was followed by Bonferoni's test to determine the intergroup variability. P-value of <0.01 (highly significant) as our desired level of significance.

RESULTS

Effect of methanolic extract of *Dalbergia paniculata* in Acetaminophen induced hepatotoxic Rats:

Present study revealed that the serum levels of SGOT, SGPT, ALP, Bilirubin (Total & Direct) and MDA were significantly increased (p< 0.001) in Acetaminophen treated (Group-II) rats when compared with normal control. Group-III rats treated with Silymarin (200mg/kg, p.o) produced significant reduction (p< 0.001) in SGOT, SGPT, ALP, Bilirubin, (Total, Direct & Indirect), and MDA levels when compared with Acetaminophen toxic control(Table:1, Table:2, Table:3). In Groups IV,V&VI treated with methanolic extract of leaf of *D.paniculata* at doses of 250, 500 and 1000mg/kg, p.o respectively, there is significant decrease in SGOT, SGPT, ALP, Bilirubin (Total, Direct & Indirect) and MDA levels when compared with Group-II (Acetaminophen treated) rats.

Table: 1 Effect of Methanolic extract of *D.paniculata* on Serum SGOT, SGPT, ALP levels in Acetaminophen induced hepatotoxicity in Rats

Treatment groups	SGOT (IU/L)	SGPT(IU/L)	Alkaline Phosphotase (IU/L)
Group I	48.79 ± 5.85	53.41 ± 6.86	183.6 ± 19.8
Group II	129.70 ± 5.23$^{\#}$	139.86 ± 5.99$^{\#}$	410.3 ± 45.8$^{\#}$
Group III	59.27 ± 3.71**	61.40 ± 5.66**	221.4 ± 39.9**
Group IV	111.94 ± 3.87*	118.96 ± 4.93*	380.9 ± 39.9*
Group V	80.09 ±7.71**	83.21 ± 5.88**	317.6 ± 18.5**
Group VI	70.47 ± 9.36**	65.90 ± 5.18**	247.6± 27.9**

Data represent mean ± S.E.M (n = 6).

$^{\#}$ p < 0.0001 compared to normal control; **p<0.001and *p<0.05 compared to Toxic Control.

Table: 2 Effect of Methanolic extract of *D.paniculata* on Serum Bilirubin (Total, Direct & Indirect) levels in Acetaminophen induced hepatotoxicity in Rats

Treatment groups	TOTAL BILIRUBIN (mg/dl)	DIRECT BILIRUBIN (mg/dl)	INDIRECT BILIRUBIN (mg/dl)
Group I	O.85 ± 0.08	0.29 ± 0.01	0.56 ± 0.07
Group II	2.76 ± 0.36$^{\#}$	1.16 ± 0.08$^{\#}$	1.60 ± 0.28$^{\#}$
Group III	1.14 ± 0.09**	0.34 ± 0.03**	0.79 ± 0.10**
Group IV	2.45 ± 0.65*	0.92 ± 0.07*	1.52 ± 0.58*
Group V	2.04 ± 0.47**	0.75 ± 0.11**	1.28 ± 0.36**
Group VI	1.55 ± 0.16**	0.56 ± 0.08**	0.98 ± 0.07**

Data represent mean ± S.E.M (n = 6).
$^{\#}$ p < 0.0001 compared to normal control; **p<0.001and *p<0.05 compared to Toxic Control.

Table: 3.Effect of methanolic extract of *D.paniculata* on liver homogenate MDA levels in Acetaminophen induced hepatotoxic rats

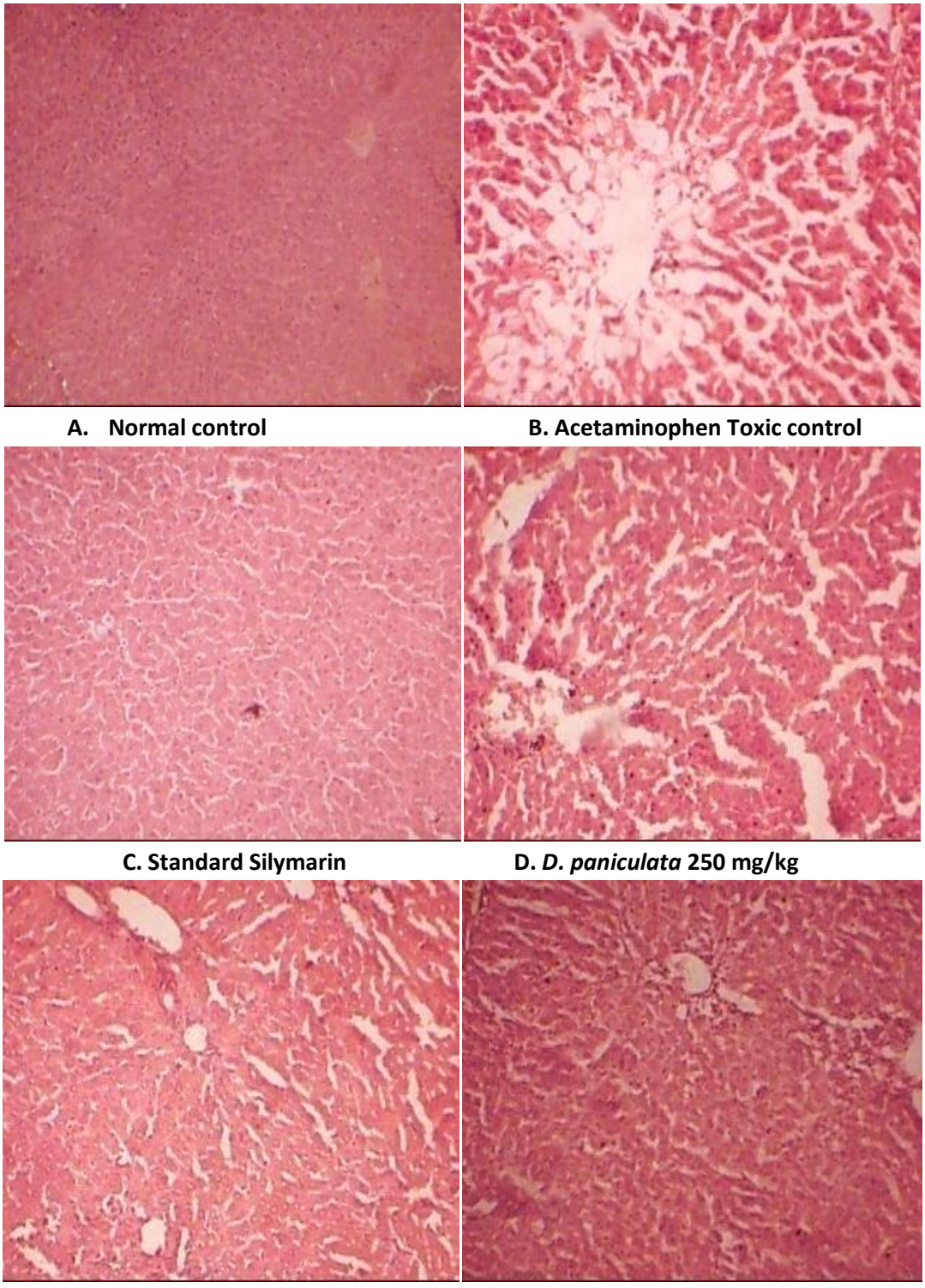
Group(n=6)	Treatment	MDA(nmol/mg)
I	Normal control	1.41
II	Toxic control	16.6$^{\#}$
III	Standard	2.2**
IV	Dalbergia *paniculata* 250 mg/kg	3.71**
V	Dalbergia *paniculata* 500mg/kg	3.23**
VI	Dalbergia *paniculata* 1000mg/kg	2.35**

Data represent mean ± S.E.M (n = 6). $^{\#}$ p < 0.0001 compared to normal control;
**p<0.001 compared to Toxic Control

Histopathological Examination

Fig: 1 shows the histopathology of Liver sections of normal control, toxic control, Silymarin treated and methonolic extract of *D.paniculata* treated groups respectively. Normal control group shows normal hepatic cells, whereas administration of acetaminophen in toxic control caused gross necrosis and periportal infiltration and the architecture was partly distorted. Treatment with Silymarin showed regenerative changes with occasional mitotic activity, almost near normal. The MEDP 250mg/kg, treated group showed focal areas of necrosis and the architecture was partly distorted. Whereas MEDP-500, 1000mg/kg, treated group showed regenerating hepatocytes and showed no evidence of inflammatory infiltrate and necrosis.

Figure. 1:



A. Normal control **B. Acetaminophen Toxic control**

C. Standard Silymarin **D. *D. paniculata* 250 mg/kg**

***E. D. paniculata* 500mg /kg** **F. *D. paniculata* 1000mg /kg**

DISCUSSION AND CONCLUSION:

Acetaminophen induced hepatic injuries are commonly used models for the screening of hepatoprotective drugs and the extent of hepatic damage is assessed by the level of released cytoplasmic alkaline phosphatase and

transaminases (SGOT and SGPT) and Bilirubin in circulation [14].

The present study also revealed that the given dose of acetaminophen (2g/kg, p.o) produced significant ($p<0.001$) elevation in SGOT, SGPT, Alkaline phosphatase, Bilirubin (TB & DB) and MDA levels indicating an impaired liver function. This results supported by *Rao et al.,* 2010; Dubey *et al.,* 2008. The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and tocopherols etc.) and ensuing wide spread propagation of the alkylation as well as peroxidation, causing damage to the macromolecules in vital biomembranes [9,10 - 14].

The investigation further reveals that the methanolic extract of leaf of *Dalbergia paniculata* Roxb. had been effective in offering protection, which is comparable to silymarin. MEDP when administered to the rats exhibited protection against acetaminophen induced liver injuries as manifested by the reduction in toxin mediated rise in serum enzymes after 10 days of treatment.

Acetaminophen, an analgesic and antipyretic agent is safe in recommended dose but produces hepatic necrosis when ingested in very large doses. It is established that at these relatively large doses acetaminophen is biotransformed in to reactive metabolite N-acetyl p-benzoquinoneimine (NAPQI) by cytochrome P-450 mixed function oxidase. Acetaminophen toxicity is enhanced by NAPQI formation or reduction in the antioxidative capacity of the liver [9, 10-14].

Lipid peroxidation is a complex and natural deleterious process. The significant increase observed in levels of lipid peroxides in acetaminophen intoxicated liver shows free radical induced liver damage [10]. It can be suggested that the hepatoprotection afforded by the methanolic extract of *D.paniculata* Roxb. may be ascribed to one or more of these factors [8].

Some *Dalbergia* species are known to possess antimicrobial, antioxidant, anti-inflammatory, and antidiarrhoeal activities [2]. Isoflavanoids, neoflavonoids, Glycosides, Terpenoids, Sterols, Furans & Other miscellaneous compounds which are present in *Dalbergia paniculata* Roxb. Literature review reveals that these chemical constituents possess antioxidant activity. These antioxidants can inhibit all the deleterious oxidative changes involved in liver damages [2,6,8].

Phytochemical investigations revealed that roots, leaves, seeds, and bark of *Dalbergia paniculata* Roxb. showed presence of flavonoids, isoflavonoid glycosides, neoflavonoid glycosides, terpenoids, sterols, furans & other miscellaneous compounds. The literature has already documented the antioxidant and hepatoprotective value of flavonoids, glycosides, tannins, coumarinoligans [2, 6, 8]. Thus, it appears that the hepatoprotection offered by *Dalbergia paniculata* Roxb. extract may be related to its free radical scavenging activity.

The IC_{50} value for standard Ascorbic acid was found to be 37.59 µg/ml., whereas the IC_{50} value for methanolic extract of *Dalbergia paniculata* Roxb. were found to be 38.40 µg/ml. MEDP exhibited strong antioxidant activity which is very nearer to Ascorbic acid.

Histopathology of liver sections of normal, toxic control, Silymarin, MEDP- 250, 500,1000mg/kg treated groups respectively are shown in Figure 1. Normal control shows normal hepatic cells, whereas administration of Acetaminophen in toxic control caused gross necrosis and periportal infiltration and the architecture was partly distorted. Treatment with silymarin showed regenerative changes with occasional

mitotic activity. The MEDP 250 mg/kg treated group showed focal areas of necrosis and the architecture was partly distorted. Where as MEDP-500,1000mg/kg treated group showed regenerating hepatocytes and showed no evidence of inflammatory infiltrate. The overall structure of rat liver was near normal.

From the above all *in-vitro* and *in-vivo* preclinical experiments it was found that *Dalbergia paniculata* Roxb. has good hepatoprotective activity when compared with respective standard drugs. *D.paniculata* possesses strong antioxidant activity which is very nearer to standard Ascorbic acid. Orally administered extract of *D. paniculata* produced significant decrease in SGOT, SGPT, ALP, Bilirubin (Total & Direct) and MDA levels & showed recovery against the toxic effects of acetaminophen. This is because of antioxidant activity, the activity of the extracts is found to be dose dependant and protects liver against oxidative damage and could be used as an effective hepatoprotector.

Hepatoprotective activity of *D. paniculata* could be due to the presence of flavonoids, glycosides which possess hepatoprotective activity against acetaminophen induced liver damage in rats. The hepatoprotective effect of the extract was further concluded by the histopathological examinations of the liver sections, which reveals that the normal liver shape was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with MEDP and intoxicated with acetaminophen the normal cellular shape was retained as compared to silymarin, thereby confirming the hepatoprotective effect of *D. paniculata.* It should be notified that a thorough evaluation is necessary to elucidate full pharmacological profile of *D. paniculata* to develop as a good therapeutic molecule.

In light of our study, here we have clearly demonstrated that Dalbergia *paniculata* was effective to protect the liver damage induced by acetaminophen and also possess antioxidative property. The use of medicinal plant products to treat hepatotoxicity will result in positive clinical outcome in patients who receive several medicines due to chronic disease condition.

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***Corresponding Author:**
geetasoren@yahoo.com