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Attenuation of paracetamol induced hepatotoxicity by *albizia odoratissima* in rats

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Abstract---Hepatotoxicity is a common cause of severe metabolic disorders and even death. Paracetamol (acetaminophen) is widely used as an antipyretic and analgesic, and it produces acute liver damage if administered in excess. Since the conventional treatment of liver diseases is associated with a wide range of adverse effects, botanical agents are commonly used. In this study we examined the therapeutic effect methanolic extract of bark of *Albizia Odoratissima* on

paracetamol induced hepatotoxicity in rats. A total of 30 animals were randomly divided into five groups, and each group consisted of six animals and received the below mentioned treatment for the period of 21 days. Group I (Normal group): Received vehicle only. Group II (Negative group): Received the Paracetamol (PCM) at dose of 3g/kg body weight orally. Group III (Test group -I): Received the Paracetamol (PCM) at dose of 3g/kg body weight orally and Methanolic extract of bark of *Albizia Odoratissima* at dose of 250 mg/kg orally. Group IV (Test group - II): Received the Paracetamol (PCM) at dose of 3g/kg body weight orally and Methanolic extract of bark of *Albizia Odoratissima* at dose of 500 mg/kg orally: PCM + MEAO (250mg/kg). Methanolic extract of *Albizia Odoratissima* attenuated the liver toxicity in rats at doses of 250, 500mg/kg through normalization serum liver markers, correction of hyperlipidemia, and restoration of anti-oxidants in dose dependent manner significantly. Hepatoprotective effect of *Albizia Odoratissima* is due to presence of phenolic compounds and flavonoids as protective agents from liver damage. In future, isolation of active principles and finding of mechanism is need to evaluate the potent protective effect *Albizia Odoratissima*.

Keywords---*albizia odoratissima*, hepatoprotective, paracetamol.

Introduction

The liver plays a vital role in physiological functions that include oxidation, reduction, hydrolysis, conjugations, sulfation and acetylation in detoxification along with carbohydrate, lipid and protein metabolism of the body [1]. Injury to the liver can lead to deterioration of its functions and may culminate in organ failure. The likely risk factors for the development of the liver diseases have been suggested to include pathogenic microorganisms and viruses, hepatotoxins, overdose and duration of drugs, obesity and malnutrition, alcohol, autoimmune disorders, type-2 diabetes, and genetic factors [2]. Hepatotoxicity is a common disease, which leads to serious consequences ranging from metabolic disorders to even death [3]. Liver diseases are one of most serious and common disease in worldwide but, despite tremendous advanced in modern medicine, their prevention and treatment options still remain limited [4]. conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious adverse effects [5]. The herbal drugs are widely used in the treatment of hepatic disorders. The plant extract from the plants are now in great demand in the developing world for primary health. It is considered to be inexpensive and safe to recommend for the treatment of liver disorder [6].

Albizia Odoratissima Benth. (Mimosaceae) is commonly known as 'Black Siris' and 'Ceylon rose-wood. *Albizia Odoratissima* Benth(family: Mimosaceae) commonly known as Black Siris (Hindi), Bhusirisah (Sanskrit) Karmaru (Punjabi), Cinduga (Telugu), Karuvagai (Tamil) and Ceylon rose-wood (Eng.), is a large erect tree distributed in the sub-Himalayan tracts, slopes and valleys up to 1,500 m, common in peninsular India, Assam, West Bengal and also throughout the western ghats of South India. It is a large deciduous tree, 15-25 m tall with dark

grey bark, leaves bipinnate, leaflets 4-15 pairs, obliquely oblong whereas flowers being pale yellowish white, fragrant in terminal heads and fruits are oblong pod, reddish brown at maturity. In traditional Indian Medicine, the bark of *A. Odoratissima* is used in the treatment of leprosy, ulcers and cough. The bark is astringent, acrid, cooling, depurative, expectorant and useful in skin diseases, rheumatism, erysipelas cough, bronchitis, diabetes and burning sensation [7, 8]. Literature review revealed the no scientific data on hepatoprotective activity of bark of *Albizia Odoratissima* Benth. The present study is aimed to evaluate the hepatoprotective activity of methanolic extract of bark of *Albizia Odoratissima* Benth against paracetamol induced hepatotoxicity in rats.

Materials and Methods

Plant Material Collection

Bark of *Albizia Odoratissima* Benth was collected from Seshachalam hills of Tirumala, Chittoor district, Andhrapradesh, India.

Identification and Authentication

Albizia Odoratissima Benth was identified and authenticated by Dr. K. Madhava chetty, Srivenkateswara University, Tirupati, Andhrapradesh, India. (Voucher number: 0807)

Chemicals and Reagents

All the chemicals and reagents used in the study were obtained from the standard suppliers and were of good quality.

Extraction procedure

A weighed quantity (1000 g) of fresh, finely grounded powder was extracted with methanol at 60-70° c by continuous hot percolation using Soxhlet apparatus. The extract then obtained was collected by filtration using a muslin cloth. Thus, obtained filtrate was subjected to solvent evaporation to obtain solid extract, which was weighed and stored in a desiccator.

Qualitative phytochemical screening

The methanolic extract of *Albizia Odoratissima* Benth is subjected to the screening of phytoconstituents like flavonoids, tannins, alkaloids, steroids.

Experimental Animals

Male Wistar rats (180–200 g) were procured from Raghavendra Enterprises (Bangalore, Karnataka, India) and will be maintained on standard pellet diet with water and acclimatized to the departmental laboratory for a week in 12 h light and dark cycle. The study will be approved by Institutional Animal Ethical Committee constituted as per CPCSEA guidelines. All the animal experiments were conducted according to the protocols approved by the Institutional Animal

Ethical Committee (Protocol No: IAEC/VMKVMC/2020) according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Experimental Design

A total of 30 animals were randomly divided into five groups, and each group consisted of six animals and received the below mentioned treatment for the period of 21 days.

Group I (Normal group): Received vehicle (CMC) only

Group II (Negative group): Received the Paracetamol (PCM) at dose of 3g/kg body weight orally

Group III (Test group -I): Received the Paracetamol (PCM) at dose of 3g/kg body weight orally and Methanolic extract of bark of *Albizia Odoratissima* at dose of 250 mg/kg orally

Group IV (Test group – II): Received the Paracetamol (PCM) at dose of 3g/kg body weight orally and Methanolic extract of bark of *Albizia Odoratissima* at dose of 500 mg/kg orally: PCM + MEAO (250mg/kg)

Group V (Standard group): Received the Paracetamol (PCM) at dose of 3g/kg body weight orally and Methanolic extract of bark of *Albizia Odoratissima* at dose of 500 mg/kg orally: PCM + MEAO (500mg/kg)

Statistical Analysis

Results are presented as mean±SEM and analysed by using one way ANOVA with Tukey test as post hoc test. $P < 0.05$ is considered to be significant.

Results and Discussion

The methanolic extract of bark of *Albizia Odoratissima Benth* showed the positive result for flavonoids, tannins, alkaloids, steroids (Table 1). Paracetamol is one of the mostly used analgesics and antipyretics. It is considered to be a safe drug when used at therapeutic levels. However, it is known to cause centrilobular necrosis upon overdose with the potential to progress to liver failure (Davidson & Eastham, 1966; Lee, 2004)

Protective effect of *Albizia Odoratissima* against paracetamol induced hepatotoxicity is not scientifically proven yet. In the current study, Possible protective effect of *Albizia Odoratissima* have been examined in paracetamol induced hepatotoxicity in rats. Paracetamol induced hepatotoxicity is evident from increased levels of biochemical parameters such as SGPT, SGOT, ALP, total bilirubin, total protein, liver volume, liver weight, blood urea, blood creatinine, lipid profile except HDL and decreased levels of anti-oxidants, blood albumin compared to normal group.

Effects of methanolic extract of bark of *Albizia Odoratissima* on Liver function tests

Paracetamol administered group rats showed the significant increased levels of Liver weight ($P < 0.0001$), Liver volume ($P < 0.0001$), SGPT($P < 0.0001$),

SGOT($P<0.0001$), ALP($P<0.0001$), Total bilirubin ($P<0.0001$) compared to normal group rats, indicates for liver damage. Methanolic extract of bark of *Albizia Odoratissima* 250, 500 mg/kg corrected the liver damage in rats through normalization of Liver weight ($P<0.0001$, $P<0.0001$), Liver volume ($P<0.0001$, $P<0.0001$), SGPT ($P<0.0001$, $P<0.0001$), SGOT ($P<0.0001$, $P<0.0001$), ALP ($P<0.0001$, $P<0.0001$), Total bilirubin ($P<0.05$, $P<0.0001$) significantly in dose dependent manner compared to paracetamol administered group, evident for protection from liver damage due to presence of phytochemicals (flavonoids, alkaloids, tannins, phenolic compounds) as like standard drug silymarin (Table 2 & 3)

Effects of methanolic extract of bark of *Albizia Odoratissima* on Lipid profile

Paracetamol administered group rats showed the significant increased levels of triglycerides ($P<0.0001$), total cholesterol ($P<0.0001$), LDL($P<0.0001$), VLDL($P<0.0001$), atherogenic index($P<0.0001$) and decreased levels of HDL ($P<0.0001$) compared to normal rats. Methanolic extract of bark of *Albizia Odoratissima* at dose 250, 500 mg/kg and standard drug silymarin corrected the hyperlipidemia with decrease in triglycerides ($P<0.0001$, $P<0.0001$) total cholesterol ($P<0.0001$, $P<0.0001$), LDL ($P<0.0001$, $P<0.0001$), VLDL ($P<0.0001$, $P<0.0001$), atherogenic index ($P<0.0001$, $P<0.0001$) and raised levels of HDL ($P<0.0001$, $P<0.0001$) in dose dependent manner compared to paracetamol administered group (Table 4).

Effects of methanolic extract of bark of *Albizia Odoratissima* on Lipid peroxidation

Paracetamol administered group rats showed the significant increase in lipid peroxidation ($P<0.0001$) compared to normal rats. Methanolic extract of bark of *Albizia Odoratissima* treated rats at dose 250, 500 mg/kg and standard drug silymarin were showed significant decrease in lipid peroxidation in dose dependent manner ($P<0.0001$, $P<0.0001$) paracetamol administered group (Table 6).

Effects of methanolic extract of bark of *Albizia Odoratissima* on anti-oxidant parameters

Paracetamol administered group rats showed that significant decrease in superoxide dismutase, catalase, reduced glutathione, Glutathione S transferase, Glutathione peroxidase ($P<0.0001$, $P<0.0001$, $P<0.0001$, $P<0.01$, $P<0.0001$ respectively) compared to normal group rats. Methanolic extract of bark of *Albizia Odoratissima* treated rats at dose 250, 500 mg/kg were showed significant decrease in superoxide dismutase, catalase, reduced glutathione, Glutathione S transferase, Glutathione peroxidase ($P<0.0001$, $P<0.0001$, $P<0.0001$, $P<0.0001$, $P<0.0001$, $P<0.0001$, $P<0.01$, ns, $P<0.0001$ respectively) in dose dependent manner compared to paracetamol administered group as like silymarin. As previous studies reported that oxidative stress is a mediator of liver toxicity in various experimental models such as paracetamol, carbon tetrachloride, antitubercular drug and ethanol-induced hepatotoxicity. Methanolic extract of

Albizia Odoratissima treated rats protected from liver damage due to presence of anti-oxidant principles (Table 5).

Effects of methanolic extract of bark of Albizia Odoratissima on blood urea, serum creatinine and albumin

Paracetamol administered group rats showed that significant increase in blood urea, serum creatinine ($P < 0.0001$, $P < 0.0001$ respectively) and significant decrease in blood albumin ($P < 0.0001$). Methanolic extract of bark of Albizia Odoratissima treated rats at dose 250, 500 mg/kg were showed significant decrease in blood urea, serum creatinine ($P < 0.0001$, $P < 0.0001$ respectively) and significant increase in blood albumin ($P < 0.0001$) in dose dependent manner compared to paracetamol administered group and results were similar to silymarin (Table 7).

Conclusion

The methanolic extract bark of Albizia Odoratissima treated rats at dose 250, 500 mg/kg were showed protective effect on paracetamol induced hepatotoxicity in rats dose dependently. Hepatoprotective effect of Albizia Odoratissima due to presence of phenolic compounds and flavonoids as protective agents from liver damage. In future, isolation of active principles and finding of mechanism is need to evaluate the potent protective effect Albizia Odoratissima.

Conflict of interest: The authors declare no conflict of interest

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S. No.	Test	Methanolic Extract of bark of <i>Albizia Odoratissima</i>
1	Alkaloids	+
2	Flavonoids	+
3	Tannins	+
4	Phenolic compounds	+
5	Steroids	+

Table 1: Phytochemical screening of Methanolic extract of bark of *Albizia Odoratissima*

Group	SGPT (U/l)	SGOT(U/l)	ALP(U/l)	TP(U/l)
NC	71.1±2.03	83.8±1.23	133.0±1.66	7.6±0.20
PCM	212.8±2.84****	286.1±5.65****	339.1±24.23****	3.3±0.18****
PCM + MEAO (250mg/kg, P.O)	171.4±2.72####	182.4±2.33####	161.9±3.10####	6.6±0.14####
PCM + MEAO (500mg/kg, P.O)	120.8±2.38####	136.0±1.72####	159.4±2.32####	6.6±0.14####
PCM + Silymarin (50mg/kg, P.O)	91.8±1.52####	96.8±1.17####	142.1±3.33####	7.4±0.24####

Table 2: Effect of methanolic extract of *Albizia Odoratissima* on liver marker enzymes

All values are expressed mean±SEM (N=6). NC indicates normal control group; PCM paracetamol induced hepatotoxic group, Paracetamol induced hepatotoxic group rats treated with Methanolic extract of *Albizia Odoratissima* at dose 250mg/kg/ P.O (PCM + MEAO 250mg/kg, P.O), Paracetamol induced hepatotoxic group rats treated with Methanolic extract of *Albizia Odoratissima* at dose 500mg/kg/ P.O (PCM + MEAO 500mg/kg, P.O). Paracetamol induced hepatotoxic group rats treated with Silymarin at dose 50mg/kg P.O (PCM + Silymarin 50mg/kg P. O).

****P<0.0001 compared with normal control

####P<0.0001 compared with Paracetamol group

Group	Total Bilirubin (mg/dl)	Liver Weight (g/100g)	Liver Volume (ml/100g)
NC	0.4±0.02	3.4±0.05	4.0±0.07
PCM	7.0±0.32****	6.0±0.15****	5.9±0.13****
PCM+ MEAO (250mg/kg, P.O)	6.0±0.15#	4.6±0.06####	4.6±0.06####
PCM + MEAO (500mg/kg, P.O)	4.5±0.25####	3.2±0.13####	3.3±0.18####
PCM + Silymarin (50mg/kg, P.O)	2.0±0.23####	3.3±0.14####	3.3±0.14####

Table 3: Effect of methanolic extract of Albizia Odoratissima on total bilirubin, liver weight and liver volume

All values are expressed mean±SEM (N=6). NC indicates normal control group; PCM paracetamol induced hepatotoxic group, Paracetamol induced hepatotoxic group rats treated with Methanolic extract of Albizia Odoratissima at dose 250mg/kg/ P.O (PCM + MEAO 250mg/kg, P.O), Paracetamol induced hepatotoxic group rats treated with Methanolic extract of Albizia Odoratissima at dose 500mg/kg/ P.O (PCM + MEAO 500mg/kg, P.O). Paracetamol induced hepatotoxic group rats treated with Silymarin at dose 50mg/kg P.O (PCM + Silymarin 50mg/kg P. O).

****P<0.0001 compared with normal control

####P<0.0001 compared with Paracetamol group

#P<0.05 compared with Paracetamol group

Group	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	Atherogenic index
NC	68.2±0.83	85.7±0.89	36.4±0.98	15.3±0.48	27.8±0.83	1.8±0.15
PCM	160.0±2.91****	156.4±0.57****	142.1±1.43****	39.0±0.76****	15.5±0.39****	9.3±0.34****
PCM+MEAO (250mg/kg, P.O)	85.0±1.53####	93.6±1.74####	98.0±1.70####	22.0±0.93####	21.9±0.37### 33.1±0.91####	4.3±0.22####
PCM+MEAO (500mg/kg, P.O)	62.1±3.24####	89.0±1.33####	87.3±1.39####	19.7±0.83####	33.1±0.91####	3.0±0.03####
PCM + Silymarin (50mg/kg, P.O)	68.4±0.79####	87.0±0.83####	50.3±2.68####	16.1±0.80####	30.1±0.84####	2.4±0.23####

Table 4: Effect of methanolic extract of Albizia Odoratissima on lipid profile

All values are expressed mean±SEM (N=6). NC indicates normal control group; PCM paracetamol induced hepatotoxic group, Paracetamol induced hepatotoxic group rats treated with Methanolic extract of Albizia Odoratissima at dose

250mg/kg/ P.O (PCM + MEAO 250mg/kg, P.O), Paracetamol induced hepatotoxic group rats treated with Methanolic extract of Albizia Odoratissima at dose 500mg/kg/ P.O (PCM + MEAO 500mg/kg, P.O). Paracetamol induced hepatotoxic group rats treated with Silymarin at dose 50mg/kg P.O (PCM + Silymarin 50mg/kg P. O).

****P<0.0001 compared with normal control

###P<0.0001 compared with Paracetamol group

Group	Superoxide dismutase (SOD) (U/min/gm tissue)	Catalase (U/min/gm tissue)	Reduced glutathione (μ g/mg protein)	Glutathione S Transferase (μ g/mg protein)	Glutathione peroxidase (μ g/mg protein)
NC	178.0 \pm 2.98	26.6 \pm 0.63	85.8 \pm 1.05	2.2 \pm 0.19	26.8 \pm 0.59
PCM	80.2 \pm 2.03****	8.2 \pm 0.23****	26.9 \pm 1.88****	1.1 \pm 0.18**	11.2 \pm 0.27****
PCM + MEAO (250mg/kg, P.O)	140.2 \pm 2.05####	14.6 \pm 0.75####	59.2 \pm 0.91####	1.3 \pm 0.16 ^{ns}	19.9 \pm 0.79####
PCM + MEAO (500mg/kg, P.O)	162.5 \pm 2.24####	23.3 \pm 1.29####	75.8 \pm 2.93####	2.2 \pm 0.15##	26.7 \pm 0.54****
PCM + Silymarin (50mg/kg, P.O)	176.7 \pm 1.98####	23.7 \pm 0.82####	77.1 \pm 1.18####	2.3 \pm 0.21###	26.2 \pm 0.86####

Table 5: Effect of methanolic extract of Albizia Odoratissima on anti -oxidants

All values are expressed mean \pm SEM (N=6). NC indicates normal control group; PCM paracetamol induced hepatotoxic group, Paracetamol induced hepatotoxic group rats treated with Methanolic extract of Albizia Odoratissima at dose 250mg/kg/ P.O (PCM + MEAO 250mg/kg, P.O), Paracetamol induced hepatotoxic group rats treated with Methanolic extract of Albizia Odoratissima at dose 500mg/kg/ P.O (PCM + MEAO 500mg/kg, P.O). Paracetamol induced hepatotoxic group rats treated with Silymarin at dose 50mg/kg P.O (PCM + Silymarin 50mg/kg P. O). ****P<0.0001, **P<0.01 respectively compared with normal control, ####P<0.0001, ###P<0.001, ##P<0.01, ns - non significant respectively compared with Paracetamol group

Group	Lipid peroxidation (μ mol MDA/ mg protein)
NC	0.2 \pm 0.02
PCM	5.4 \pm 0.14****
PCM (250mg/kg, P.O)	2.8 \pm 0.31####

PCM + MEAO (500mg/kg, P.O)	1.9±0.27####
PCM + Silymarin (50mg/kg, P.O)	0.8±0.07####

Table 6: Effect of methanolic extract of Albizia Odoratissima on Lipid Peroxidation

All values are expressed mean±SEM (N=6). NC indicates normal control group; PCM paracetamol induced hepatotoxic group, Paracetamol induced hepatotoxic group rats treated with Methanolic extract of Albizia Odoratissima at dose 250mg/kg/ P.O (PCM + MEAO 250mg/kg, P.O), Paracetamol induced hepatotoxic group rats treated with Methanolic extract of Albizia Odoratissima at dose 500mg/kg/ P.O (PCM + MEAO 500mg/kg, P.O). Paracetamol induced hepatotoxic group rats treated with Silymarin at dose 50mg/kg P.O (PCM + Silymarin 50mg/kg P. O). ****P<0.0001 compared with normal control, ####P<0.0001 compared with Paracetamol group.

Group	Blood urea (mg/dl)	Serum Creatinine (mg/dl)	Blood Albumin (g/dl)
NC	14.0±0.46	0.6±0.02	6.0 ±0.20
PCM	27.2±0.71****	2.6±0.09****	2.8±0.14****
PCM + MEAO (250mg/kg, P.O)	19.3±1.01####	0.9±0.08####	4.6±0.19####
PCM + MEAO (500mg/kg, P.O)	15.5±0.47####	0.5±0.04####	5.0±0.18####
PCM + Silymarin (50mg/kg, P.O)	13.3±0.56####	0.5±0.02####	6.2±0.09####

Table 7: Effect of methanolic extract of Albizia Odoratissima on Blood urea, serum creatinine, blood albumin

All values are expressed mean±SEM (N=6). NC indicates normal control group; PCM paracetamol induced hepatotoxic group, Paracetamol induced hepatotoxic group rats treated with Methanolic extract of Albizia Odoratissima at dose 250mg/kg/ P.O (PCM + MEAO 250mg/kg, P.O), Paracetamol induced hepatotoxic group rats treated with Methanolic extract of Albizia Odoratissima at dose 500mg/kg/ P.O (PCM + MEAO 500mg/kg, P.O). Paracetamol induced hepatotoxic group rats treated with Silymarin at dose 50mg/kg P.O (PCM + Silymarin 50mg/kg P. O). ****P<0.0001, **P<0.01 respectively compared with normal control, ####P<0.0001, ###P<0.001, #P<0.01, ns – non significant respectively compared with Paracetamol group.