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In vivo Antioxidant Activityof Ethanolic Extract of Aerial parts of *Albizia procera roxb* (*benth.*) against Paracetamol induced Liver Toxicity on Wistar Rats

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Abstract
To evaluate the *in-vivo* antioxidant activity of aerial parts of ethanolic extract of *Albizia procera* against paracetamol induced toxicity in rats. Paracetamol treated rats shows significantly reduced the levels of tissue enzymatic antioxidant and non enzymatic antioxidant. The level of TBARS are elevated in paracetamol treated rats (groupII) when compared with control group of rats. The ethanolic extract of *Albizia procera* in paracetamol treated rats were found lowered concentration of TBARS when compared with paracetamol treated rats. Paracetamol induces the oxidative stress in cell by producing reactive oxygen species. After administration of ethanolic extract of *Albizia procera* in paracetamol treated rats showed significant increase in the levels of antioxidant enzyme such as superoxide dismutase (SOD), catalase (CAT) and non enzymatic antioxidant glutathione (GSH) when compared with paracetamol induced rats (groupII). Based on the results, it was concluded that the ethanolic extract of *Albizia procera* have significant *in-vivo* antioxidant activity and can be used to protect tissue from oxidative stress.

Keywords: *Albizia procera*, *in-vivo* antioxidant, Paracetamol, lipid peroxidation.

INTRODUCTION

Free radical induced oxidative damage has long been thought to be the most important consequence of the aging process[1]. Such conditions are considered to be important causative factors in the development of diseases such as diabetes, stroke, arteriosclerosis, cancer, hepatitis and cardiovascular diseases[2-3]. Studies show that these radicals also affect the equilibrium between pro-oxidant sand antioxidants in biological systems, leading to modifications in genomes, proteins, carbohydrates, lipids and lipid peroxidation[4] thus inactivating antioxidant defense. Plant and its products are rich sources of a phytochemicals and have been found to possess a variety of biological activities including antioxidant potential[5].Natural antioxidants are in high demand for application as nutraceuticals, bio pharmaceuticals, as well as food additive because of consumer preference.

Paracetamol is a commonly available drug well known for its analgesic and antipyretic effects. At therapeutic doses, Paracetamol is considered a safe drug and is safely bio transformed and eliminated as non-toxic conjugates of sulfate and glucuronic acid, and a small portion is converted to NAPQI (N-acetyl-p-benzoquinone imine) which is detoxified by glutathione (GSH) and eventually eliminated in the urine or bile [6]. However, during overdose of Paracetamol, the glucuronidation and sulfation routes become saturated and rapid depletion of hepatic GSH levels occurs which causes oxidative stress and the NAPQI thus formed binds covalently to liver proteins[7]. Hepatotoxicity induced by acetaminophen results in prominent elevations of liver marker enzymes and reactive oxygen species (ROS) which further aggravates oxidative stress and are involved in a number disease processes. Therefore, new potential therapeutics for Paracetamol overdose is being routinely investigated in preclinical studies. Conventional medicines used for treatment of liver diseases have adverse side effects and are costlier. So, there is a need to evaluate natural compounds as an effective alternative which are safer and cost effective.

Albizia procera is a tree with an open canopy, up to 30 m tall and trunk of 35 (60 max.) cm in diameter. It is widely distributed from India and Myanmar through Southeast Asia to Papua New Guinea and northern Australia. This plant is used traditionally in anticancer[8], pain, convulsions, delirium, and septicemia[9]. The decoction of bark is given for rheumatism and haemorrhage and is considered useful in treating problems of pregnancy and for stomach-ache, sinus. They are reported to exhibit various pharmacological activities such as CNS activity, cardiotonic activity, lipid-lowering activity, anti-oxidant activity, hepatoprotective activity, hypoglycemic activity, etc[10]. Even through, traditionally, leaves of *Albizia procera* were extensively used for the treatment of variety of wounds[11].Seeds are powdered and used in amoebiasis. It cures urinary tract infections including glycosuria, haemorrhoids, fistula and worm infestation. It also suppresses skin diseases. Fruits of *Albizia procera* acts as astringent and diminishes Kapha and Sukra[12]. In india, leaves are poulticed onto ulcers[13]. Our literature survey revealed that the hepatoprotective activity of ethanolic extract from aerial parts of *Albizia procera* was not investigated; hence these activities have been investigated in the present study.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The aerial parts of *Albizia procera* were collected from Tuliarai, Thirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medicinal Plants Unit Siddha, Government of India. Palayamkottai. The aerial parts of *Albizia procera,* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of Extracts

The above powdered materials were successively extracted with ethanol by hot continuous percolation method in Soxhlet apparatus[14]for 24 hrs. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Animals

Male albino wistar rats each weighing 150-200gmwas procured from the central animal house, RMMCH in Annamalai University at Chidambaram. The animals were maintained on their respective diets and water *ad libitum*, and rats were maintained on a 12 hour light / dark cycle in a temperature regulated room (20-25^{o}C) during the experimental procedures. The experiments were carried out as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethics Committee (IAEC), Annamalai University (Approved number: 160/1999/CPCSEA/ **777**). The animals were cared for according to the guiding principles in the care & use of animals.

Acute toxicity test

Acute toxicity tests were performed according to OECD - 423 guidelines. Albino rats (n = 6) of either sex selected by random sampling technique were employed in this study. The animals were fasted for 4 hr with free access to water only. The ethanolic extract of *Albizia procera* suspended in normal saline: 0.5% carboxy methyl cellulose was administered orally at a dose of 5 mg/kg initially and mortality was observed for 3 days. The mortality was observed in 5/6 or 6/6 animals, and then the dose administered was considered as toxic dose. However, the mortality was observed in less than four rats, out of six animals then the same dose was repeated again to confirm the toxic effect. If mortality was not observed, the procedure was repeated for higher doses i.e. 2000mg/kg.

Experimental Design

Rats were divided randomly into five groups of six animals each and treated for one week (7 days) as follows.

Group-I Animals served as normal control, treated with vehicle (0.5% carboxy methyl cellulose).1ml/kg once daily for 7 days orally.

Group-II Animals served as toxic control, will receive 1ml vehicle for 7 days and on the 5thday paracetamol 2g/kg, b.wt will be given per orally.,

Group III Animals Received Ethanolic extract of *Albizia Procera*(Roxb.)Benth**.** 200mg/kg b.wt,orally daily for 7 days. A single dose of paracetamol 2g/kg body weight will be administered per orally on 5th day .

Group-IV Received Ethanolic extract of *Albizia Procera*(Roxb.)Benth**.** 400mg/kg b.wt, by orally daily for 7 days. A single dose of paracetamol 2g/kg b.wt will be administered p.o on 5th day.

Group-V Received *Silymarin* 25mg/kg b.wt by orally daily for 7 days and a single dose of paracetamol 2g/kg b.wt will be administered p.o on 5th day.

Biochemical Analysis

Dissection and Homogenization

On the 8th day all animals were sacrificed by cervical decapitation. The liver was isolated and weighed accurately and used for the preparation of homogenate. Portions of the tissue from liver were blotted, weighed and homogenized with ethanol (3 volumes). The lipid extract obtained by the method of Folch et al. It was used for the estimation of Thiobarbituric acid reactive substances[15](TBARS). Another portion of the tissues was homogenized with phosphate buffer saline and used for the estimation of reduced glutathione (GSH), superoxide dismutase(SOD), catalase(CAT).

Estimation of Reduced Glutathione

Reduced glutathione (GSH) in the liver was estimated according to the method of Ellman (Ellman G.L, 1959)[16]. Sample (0.75ml) of homogenate was precipitated with 0.75ml of 4% sulphosalicylic acid and centrifuged at 1200g for 15 min at 4^{o}C. The assay mixture contained 0.5ml of supernatant and 4.5ml of 0.01M, DTNB. (5-5'-dithiobis (2-nitro benzoic acid)) in 0.1M, phosphate buffer (pH 8.0). The yellow colour developed was read immediately at 412 nm. The results were expressed as micromole of GSH per milligram of proteins.

Superoxide dismutase activity (SOD)

Superoxide dismutase activity was estimated according to the method of kono (Kono Y, 1978)[17],where in the reduction of nitro blue tetrazolium chloride (NBT) was inhibited by superoxide dismutase and measured at 560 nm spectrophotometrically. Briefly the reaction was initiated by addition of hydroxylamine hydrochloride to the reaction mixture containing NBT and post nuclear fraction of liver homogenate. The results were expressed as units per milligram of protein with one unit of enzyme defined as the amount of SOD required to inhibit the rate of reaction by 50%.

Catalase (CAT)

Catalase activity was assessed by the method of luck (Luck H, 1971)[18], where the breakdown of H_2O_2 was measured at 240nm. Briefly the assay mixture consisted of 3ml of H_2O_2 phosphate buffer (0.0125M; H_2O_2) and 0.05ml of supernatant of liver homogenate and the change in the absorbance was measured at 240nm. The enzyme activity was calculated using the milli-molar extension coefficient of H_2O_2 (0.07). The results were expressed as micromole of H_2O_2 decomposed per min per milligram of protein.

Lipid peroxidation assay (LPO)

Malondialdehyde (MDA), a secondary product of lipid per oxidation reacts with thiobarbituric acid at pH 3.5. The red pigment produced was extracted in n-butanol-pyridine mixture and estimated by measuring the absorbance at 532 nm (Kuldip Singh, 2009)[19].

Statistical Analysis

The values were expressed as mean ± SEM. (n=6). Statistical analyses were performed with one way analysis of variance (ANOVA) followed by Dunnett.'s multiple comparison test by using Graph Pad Instat Software. P value less than 0.05 was considered to be statistically significant. *P<0.05, **<0.01 and ***<0.001, when compared with control and toxicant group as applicable.

RESULTS AND DISCUSSION

The acute toxicity of ethanolic extract of aerial parts of *Albizia Procera* was carried out as per OECD-423 guidelines for safe dose administration to animals and the study was carried out as described in experimental section. The results of acute toxicity study revealed that LD_{50} values of ethanolic extract of aerial parts of *Albizia Procera* were high and apparently showed the safety of extract. The treatment of rat with ethanolic extract of aerial parts of *Albizia Procera* did not change any autonomic or behavioural response in rats. The zero percent mortality for ethanolic extract of aerial parts of *Albizia Procera* was found at the doses of 2000mg/kg. Overall results suggested the LD_{50} value of 2000mg/kg. Hence the therapeutic dose was calculated as $1/10^{th}$ (200mg/kg) of the lethal dose for hepatoprotective and *in-vivo* antioxidant activity.

The activities of tissues TBARS levels in paracetamol treated rats are shown in table 1. Elevated levels of TBARS levels were found in liver of group II rats when compared with group I. TBARS are mainly unstable peroxides, and are by-products of membrane phospholipid peroxidation. Increased concentration of TBARS is thus indicative of enhanced lipid peroxidation and failure of the antioxidant defense mechanism to inhibit free radical generation. The concentration of TBARS was significantly reduced by ethanolic extract of *albizia procera w*ith paracetamol treated rats.

The effect of ethanolic extract of *albizia procera* on tissues superoxide dismutase, catalase and reduced GSH enzyme levels in paracetamol treated rats are shown in table 2. Superoxide dismutase is one of the most important intracellular antioxidant enzymes, present in all aerobic cells has an antitoxic effect against superoxide H anion[21]. Catalase is a haemoprotein and its protects cells from the accumulation of H_2O_2 by dismutating it to form of $H_2O$and O_2 or by using it as an oxidant in which it works as peroxidise. The activities of SOD and Catalase in the tissue like liver significantly lowered in paracetamol treated rats (group II) rats than that of control group I. This effect may be due to clear manifestation of excessive formation of free radicals and activation of lipid peroxidation system resulting in tissue damages[22]. After the administration of ethanolic extract of *albizia procera* with paracetamol treated rats were showed significantly increases the levels of SOD and catalase when compared with group II.

Glutathione is a major non-protein thiol in living organisms ,which plays a central role in coordinating the body's antioxidant process[20]. Decline in the glutathione content in the liver of paracetamol intoxicated rats and its subsequent return towards the near normalcy in paracetamol plus ethanolic extract of *a Albizia procera* administered group also reveal antilipid peroxidative effect of *Albizia procera* .

CONCLUSION

On the basis of the study we conclude that Ethanolic extract of aerial parts of *Albizia procera* possesses *in vivo* antioxidant and lipid peroxidation activity can be employed in protecting tissue from the oxidative stress. The phytoconstituents may be responsible for the inhibition of lipid peroxidation and enhance the antioxidant activities of Ethanolic extract of *Albizia procera.*

Table.1 Effect of ethanolic extract of aerial parts of *Albizia Procera* on tissue TBARS in paracetamol treated rats

TREATMENT	TBARS (Thiobarbituric acid Reactive substances) (moles/ml)
Group – I	189.24±2.82
Group – II	278.54±4.32[a]
Group – III	187.65±3.52[b]***
Group – IV	165.45±3.21[b]***
Group – V	149.21±3.86[b]***

Data are expressed as mean±SEM., *n* = 6 rats per group. P values; *P<0.05; **P<0.01; ***P<0.001; ns= not significant; compared to Paracetamol group. One way ANOVA followed by Dunnett's test. **a**→ Group II compared to Group I; **b**→ Group II compared to Group III, IV and V.

Table.2 Effect of ethanolic extract of aerial parts of *Albizia Procera* on tissue SOD, CAT and GSH in paracetamol treated rats

TREATMENT	SOD (U/L)	CATALASE (mg/ dl)	REDUCED-GSH (mg/dl)
Group – I	45.14±1.79	269.06±3.86	137.27±3.83
Group – II	36.17±1.46 [a]	168.43±2.35[a]	76.19±1.35[a]
Group – III	42.16±1.63[b]*	174.28±3.42[NS]	88.18±3.65[b]*
Group – IV	44.79±1.28[b]**	182.25±2.63[b]*	91.32±2.32[b]**
Group - V	44.13±1.67[b]**	187.20±3.53[b]**	96.98±1.92[b]***

Data are expressed as mean±SEM., *n* = 6 rats per group. P values; *P<0.05; **P<0.01; ***P<0.001; ns= not significant; compared to Paracetamol group. One way ANOVA followed by Dunnett's test. **a**→ Group II compared to Group I; **b**→ Group II compared to Group III, IV and V.

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