Chemoprotective Effect of *Anogeissus Latifolia* Bark Extract on Paracetamol- Induced Hepatotoxicity.

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Summary

This study was undertaken to investigate the chemoprotective activity of ethanol extract of the bark of *Anogeissus latifolia* (ALE) against Paracetamol induced hepatotoxicity in rat model. The ethanol extract of ALE at 100 mg and 300 mg/kg and Liv-52 (a reference heptoprotective agent) at 2 ml/kg for seven days. The effect of extract on serum amino transferase (ALAT), serum aspartate aminotransferase (ASAT) alkaline phospate (ALP) and histopathology were studied. Ethanol extract of ALE showed a dose dependant significant reduction in the levels of ASAT, ALAT and ALP that was altered because of paracetamol intoxication in rats. Histopathological observations confirmed the beneficial roles of ALE and Liv-52. The ethanol extract of ALE was also investigated for antioxidant activity; by lipid peroxidation method. From these results, it was suggested that extract of ALE colud protect from paracetamol-induced Heptotoxicity by its antioxidative effects.

Keywords: *Anogeissus latifolia*, Chemoprotective, Hepatotoxicity**,** Antioxidant, Liv-52.

Introduction

Some plants have been found, scientifically, to possess hepatoprotective activity and the underlying mechanism of action involves their antioxidant property (Gupta *et al*., 2004a). Free radicals or oxidative injury now appears to be the fundamental mechanism behind a number of human diseases and disorders (Atawodi, 2005), including liver disorders. Many compounds, including clinically useful drugs, can cause cellular damage through metabolic activation of the chemical to highly reactive compounds such as free radicals, carbenes and nitrenes (Gupta *et al.,* 2004b). Now the Paracetamol related hepatotoxicity is the most common cause of the potentially devastating clinical syndrome of acute liver failure in many western countries (Schiodt *et al.,* 1999). Plants have always provided solutions to such devastating chemical effects

possibly by their antioxidant potential (Gupta *et al.*, 2004a: Gupta *et al.*, 2004b: Kukonguiriyapan *et al.*, 2003). The plant under investigation, namely *Anogeissus latifolia*, is reported to have antioxidant activity (Shanta Mehrotra et al, 2004), and hence, can be succesfuly used in oxidative stressed conditions like liver damage.

Anogeissus latifolia Wall. (Combretaceae), is a large or moderate sized tree characteristic of dry deciduous forests and common throughout India. The plant is traditionally used for the treatment of dysentery, snake bite, leprosy, diabetes, wounds and ulcers, skin diseases including hepatopathy (Anonymous, 1985). Different phytochemicals have been isolated and characterized from the bark of this tree which include ellagic acid, flavellgic acid, gallic acid, chebulagic acid, quercetin and myricetin (Deshpande et al, 1976; Reddy

et al, 1965). Other known chemicals include Sitosterol, rutin, myricetin, procyanidin along with gallotannins, shikimic acid, alanine and phenyl amine (Bhakuni et al, 1971). Almost all of the phytocontituents of the plant *A.latifolia*, are known antioxidants and hence justify its selection for the present study.

Material and Methods

Plant material and extraction

The Bark of *A.latifolia* was collected from Chikmagalur, Karnataka (India) during the month of May 2006. The plant was authenticated by Botonical Survey of India, Coimbatore, Tamil Nadu (Authentication no. BSI/SC/5/23/06-07/Tech.880). The bark was dried under shade and powdered coarsely. The powdered material (250 g) was extracted with n-Hexane to remove the fatty substances (defating); the marc

was further extracted exhaustively with 70% ethanol in a soxhlet apparatus and filtered. The extract was concentrated under reduced temperature and pressure to get dry residue [(26.8 g) (Pushpangadan et al 2005)]. Preliminary qualitative analysis of the extract showed the presence of Carbohydrates, Glycosides, Tannins, Phenolic compound and Flavonoides.

Chemicals

All chemicals and solvents used were obtained from S.D. Fine Chemicals, Mumbai, Loba Chemie Indo Austranel Co., Mumbai, Ranbaxy laboratories Ltd., Punjab, Sigma Fine Chemicals, Mumbai and Hi media Laboratories, Mumbai, India. For biochemical estimations, kits were procured from Ecoline, E. Merck Ltd., M.I.D.C., Taloja. Liv-52 syrup was procured from market, manufactured by Himalaya Drug Company, Bangalore.

Autoanalyser

Microlab 100 manufactured by M/s Vital Scientific N.V., The Netherlands, was used to estimate biochemical parameters for Aspartate aminotransferase (ASAT), Alanine aninotransferase (ALAT), Alkaline Phosphatase (ALP).

UV Spectrophotometer

UV Spectrophotometer manufactured by Shimadzu was used to estimate lipid peroxidation.

Animals

All animals were maintained under standard laboratory conditions (22-28^{o}C, 60-70% relative humidity, 12 h dark/ light cycle) and were fed with standard rat pellet (M/s Hindustan Lever Ltd., Bangalore, India), and *water ad libitum*. The experiments were conducted as per the guide line of

CPCSEA, Chennai, India. (Aproval No.JSSCP/IAEC/M.PHARM/Phy.Pharm/03/2006-07)

Preparation of drug solution.

Required quntities of ethanol extract of *Anogeissus latifolia* (ALE) was suspended in 0.5% Sodium Carboxy methyl cellulose (CMC), to get 100 mg and 300 mg/ml solutions for oral administration. Liv-52 syrup (used as standard) was administered orally as itis at a dose of 2ml/kg body weight.

Preparation of hepatotoxicant.

Required quantity of Paracetamol was suspended in 0.5% CMC to produce 0.15 g/ml suspension and administered orally.

Chemoprotective studies

30 Female albino Wistar rats, weighing about 180-220 g were divided into 5 groups of six animals each. Group I served as

solvent control (normal animals), group II, as toxicant control received 0.5% CMC (2 ml/kg), group III, as positive control received Liv-52 (2 ml/kg), while Group IV and V received the freshly prepared ALE suspended in 0.5% CMC at two different dose levels, viz., 100 mg/kg and 300 mg/kg body weight. The animals were treated for 7 days and on the 7th day, one hour after dosing, Paracetamol (1.5 g/kg body weight) was administered to all the groups except Group I. After 24 hours the animals were anaesthetized and blood was collected by sino-orbital puncture for the assessment of the biochemical markers. Blood was allowed to clot at room temperature and serum separated by centrifugation at 4^{o}C at 2500 rpm and was subjected to various biochemical estimations like ASAT, ALAT and ALP. The animals were sacrificed later and the liver was perfused and excised. Part of

the liver was stored in 10 % Formalin saline for histopathological studies and the remaining portion was frozen at -70ºC and used for the estimation of lipid peroxidation.

Liver TBARS and protein estimation

TBARS was used as an index of lipid peroxidation and measured by the method of Niehaus and Samuelson (1968). Total protein in the tissue homogenate was estimated (Lowry et al, 1951). The levels of TBARS were expressed as nmol MDA/mg protein.

Histopathological studies

The liver stored in 10% Formalin saline was used to prepare paraffin sections of 5-10 µ thickness and stained with haemotoxylin and eosin, mounted in neutral DPX medium to observe histopathological changers (Mukherjee, 1988).

Statistical analysis

The statistical analysis was carried out by one way analysis of variance (ANOVA).The values are represented as mean ± S.E.M. Comparision of mean values of different groups treated with different dose levels of formulation and positive control with normal were estimated by Turkey's Multiple Comparision Test. P< 0.05 was considered significant.

Results and Discussion

The effect of ethanolic extract of *Anogeissus latifolia* on serum transaminases, alkaline phosphatase and lipid peroxidation in Paracetamol intoxicated rats are summarized in Table 1. Single administration of Paracetamol to vehicle control rats produced significant (*P*<0.001) elevation of liver marker enzymes in plasma namely, ASAT (174.1 ± 4.81) and ALAT (88 ± 3.95) when compared to normal control (109.1 ±

4.31, 59.43 ± 3.41). ALE at a dose of 300 mg/kg showed significant (P<0.01) reduction of ASAT (132 ± 8.10) and ALAT (64 ± 2.60) levels when compared to Paracetamol intoxicated rats. The level of ALP was also very significantly (347± 19.59; P<0.001) reduced as against toxicant control. ALE at a dose of 100mg/kg body weight also showed significant reduction in ASAT 138.4 ± 10.48 (P<0.05), ALAT 71.0 ± 3.70 (P<0.01) and ALP 438.2 ± 13.72 (P<0.001) levels when compared to Paracetamol administered rats. Liv-52 also showed significant reduction in ASAT 129 ± 11.89 (P<0.01), ALAT 69.83 ± 5.61 (P<0.05) and ALP 347±12.46 (P<0.001) levels respectively.

Lipid peroxidation was significantly elevated following Paracetamol administration (P<0.001, 122.08 ± 4.46) when compared to normal control rats (48.01 ± 5.8). ALE at 300

mg/kg showed significant reduction in lipid peroxidation (56.97 ± 3.06, P<0.01) when compared to toxicant control. ALE at 100 mg/kg showed significant (85.96 ± 3.10, P<0.01) reduction in lipid peroxidation. Liv-52 at 2 ml/kg also showed significant (P<0.001) reduction in lipid peroxidation 80.46 ± 11.06 when compared to Paracetamol administered rats.

Fig.1 shows histomorphology of liver of the untreated control, the Paracetamol treated (Fig. 2) shows gross necrosis of Hepatocytes characterized by nuclear pyknosis and karyolysis. Treatment with *Anogeissus latifolia* extract at both dose levels 100mg and300mg/kg, Plate No. 4 and 5 respectively reversed to a large extent the hepatic lesions with fewer necrotic zones produced by Paracetamol. Positive control liver treated with Liv 52 (Fig. 3) shows protected liver tissue with normal Hepatocytes with sinusoidal dilation only.

The bark of *Anogeissus latifolia* was selected to evaluate its chemoprotective effect in paracetamole induced hepatotoxic rat model. The plant selection was guided by its utility profiles in the traditional systems of medicine. Litrature survey of the plant futher suggested that, the bark has been evaluated scientifically for its antioxidant and wound healing activities (Shanta Merhotra et al, 2004). A survey into it's chemoprofile supported its antioxidant potential. There was, however, no evidence of any scientific studies on its hepatoprotective or chemoprotective actions. The qualitative chemical examination showed the presence of Carbohydrates, Glycosides, Phenolic compounds, Flavonoides and Tannins. The TLC studies confirmed the presence of these phytoconstituents along with Rutin and quercetin. The presence of poly phenols and flavonoids support its

antioxidant potential. Rutin and Quercetin are well established antioxidants. Rutin is reported to possess protective effect against Paracetamol and CCl_4 –induced hepatotoxicity in rats (Khalid et al, 2002). The drug consists of Gallic acid which is also an antioxidant suggested that it is a rich source of antioxidants and that it may exert a cumulative antioxidant effect producing favarouble action in chemoprotection of liver.

Paracetamol gets converted into a toxic reactive intermediate called N-acetyl-p-benzoquinone imine (NAPQI) (Whitcomb and Block,1994) following metabolism by a number of isozyms of Cytochrome P-450 (CYPs).The massive production of reactive species leads to depletion of protective physiological moieties (Glutathione and alpha tocopherol, etc,) ensuing wide spread propogation of the alkylation as well

as peroxidation, causing damage to the macromolecules in vital biomembranes (Mitra et al, 2000; Hong et al, 1992; Dixon, et al., 1971). An obvious sign of hepatic injury is the leaking of cellular enzymes into the plasma due to the disterbance caused in the transport functions of the hepatocytes (Zimmerman and Seeff, 1970) When liver cell plasma is damaged, a number of enzymes normally located in cytosol are released into the blood, thereby causing increased enzyme levels in the serum. In our study, the significant elevation of marker enzymes following single dose administration Paracetamol indicated serious toxicity produced by the chemical. ALE at 100mg and 300mg/kg showed dose dependent but significant reduction in the levels of ASAT, ALAT and ALP levels that were elevated as a result of Paracetamol (1.5g/kg) assault. ALE at 100mg and

300mg/kg showed significant reduction in lipid peroxidation suggesting antilipid peroxidation activity (Table 1). The ehanol extract of Anogiesus latifolia thus showed a promising effect against paracetamol challenge in this model. All bichemical findings were positively supported by the histopathological results.

Table 1. Effect of *Anogeissus latifolia* on plasma biochemical parameters and lipid peroxidation in liver on Paracetamol intoxicated rats.

Treatment (n=6)	ASAT (U/l)	ALAT (U/l)	Alkaline Phosphatase (U/l)	Lipid peroxidation (nmoles/100mg protein)
Normal	109.1 ± 4.31	59.43 ± 3.41	320.3 ± 8.12	48.01±5.84
Paracetamol (1.5 g/kg)	174.1 ± 4.81$^{a\,\mathbf{a}\,\mathbf{a}}$	88 ± 3.95$^{a\,a\,a}$	549.4 ± 10.91$^{a\,a\,a}$	122.80 ± 4.46^{aaa}
Liv52 (2 ml/kg)	129 ± 11.89$^{b\,b}$	69.83 ± 5.61^{b}	447.3 ± 12.46$^{b\,b\,b}$	80.46 ± 11.06^{bbb}
ALE (100 mg/kg)	138.4 ±10.48^{b}	71 ± 3.70^{b}	438.2 ± 13.72$^{b\,b\,b}$	85.96 ± 3.10^{bb}
ALE (300 mg/kg)	132 ± 8.10$^{b\,b}$	64 ± 2.60$^{b\,b}$	347 ± 19.59$^{b\,b\,b}$	56.97 ± 3.06^{bbb}

Values are expressed as mean ± S.E.M of n=6 (n=number of animals), a $P < 0.05$, a a $P < 0.01$, a a a $P < 0.001$ Vs Normal, b $P < 0.05$, b b $P < 0.01$, b b b $P < 0.001$ Vs paracetamol

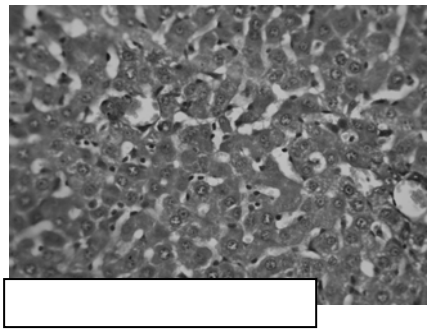

Fig. 1 Control group shows normal liver

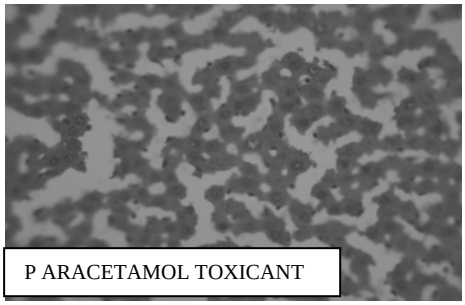

Fig. 2. Shows gross necrosis of Hepatocytes Histology characterized by nuclear pyknosis and karyolysis.

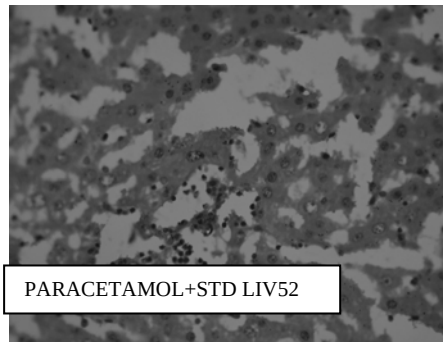

Fig. 3. Shows protected liver tissue with Hepatocytes with sinusoidal dilation

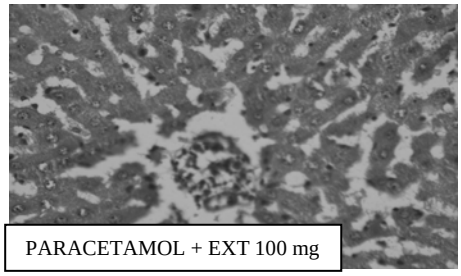

Fig. 4. Reversed to a Lesions with fewer necrotic zones

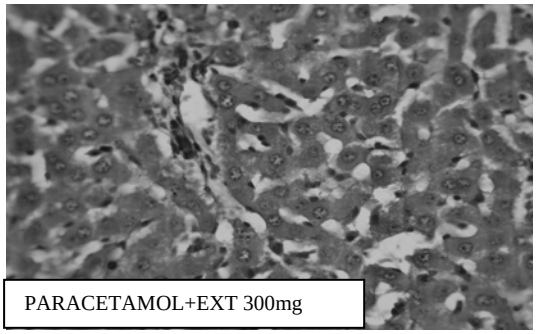

Fig. 5. Reversed to a large extent the hepatic Lesions with fewer necrotic zones

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