

# HEPATOPROTECTIVE ACTIVITY OF ANOGEISSUS LATIFOLIA LEAF AND BARK EXTRACTS AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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## KEYWORDS

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## ABSTRACT

The study was designed to evaluate the hepatoprotective activity of *Anogeissus latifolia* leaf and bark extracts by using solvents viz. petroleum ether, chloroform and methanol. Hepatotoxicity was induced in Wistar rats by intraperitoneal injection of CCl<sub>4</sub> (0.1mL/kg/day for 10 days). The standard drug used was silymarin. Various extracts were given in the dose of 300mg/mL of all the extracts except chloroform leaf and methanolic bark which were prepared @ 200mg/mL, for 14 days. The hepatoprotective effect of these extracts was evaluated for liver function biochemical parameters (total bilirubin, serum protein, alanine aminotransaminase, aspartate aminotransaminase and alkaline phosphatase activities) and histopathological studies of liver. Treatment with methanolic leaf and bark extract significantly prevented the functional, physical, biochemical and histological changes induced by CCl<sub>4</sub> indicating the recovery of hepatic cells. The results suggest that methanolic leaf and bark extract of *Anogeissus latifolia* possesses potential hepatoprotective activity.

## INTRODUCTION

Some plants have been found, scientifically, to possess hepatoprotective activity and the underlying mechanism of action involves their antioxidant property (Anonymous, 1985). Free radicals or oxidative injury now appears to be the fundamental mechanism behind a number of human diseases and disorders (Atawodi, 1971). Oxidative stress has been implicated in the pathogenesis of acute and chronic liver injury in a variety of pathophysiological conditions such as hepatotoxin exposure, intrahepatic cholestasis, alcoholic liver injury, liver ischemia and viral hepatitis (Atawodi, 1971; Stehbens, 2003; Jaeschke *et al.*, 2003 and McDonough, 2003). Overproduction of reactive oxygen species (ROS) and nitrogen species (RNS), along with significant decrease of antioxidant defense in these pathological conditions, impairs various cellular functions through the process of lipid peroxidation, protein oxidation and nucleic base oxidation. Lipid peroxidation causes changes in the physical and chemical properties of cellular membranes, thus altering their fluidity and permeability, leading to impairment in membrane signal transduction and ion exchange, resulting in swelling,

cytolysis and finally cell death. The oxidation of proteins and DNA also relates directly to cellular dysfunction and death (Jaeschke *et al.*, 1988). Accordingly, effects of antioxidants or free radical scavengers have been widely tested for the prevention and treatment of acute and chronic liver injuries. In some of the studies, antioxidants have shown beneficial effects, specifically for prevention and treatment of chronic liver injury (Kukongviriyapan *et al.*, 2003; Gupta *et al.*, 2004a, b).

*Anogeissus latifolia* (Roxb) Wall. ex. BEDD (Family Combretaceae) is a large tree or moderate sized tree characteristic of dry deciduous forests and available throughout India. The plant is traditionally used for the treatment of dysentery, snakebite, leprosy, diabetes, wounds and ulcers and skin diseases, in addition to hepatopathy (Anonymous, 1985). The hydroalcoholic extract is reported to have antioxidant activity. It has been studied for total antioxidant activity, hydrogen donating ability nitric oxide, superoxide scavenging activity and hydrogen peroxide decomposition activity, Integral antioxidative capacity has been determined by chemiluminescence assay. It has also been studied in a lipid peroxidation assay with a thiobarbituric acid reactive

substances (TBARS) method using rat liver homogenate (Govindarajan *et al.*, 2004). A variety of substances which might contribute to hepatoprotective activity has been identified in extracts of *Anogeissus latifolia* including tannins, gallic acid, ellagic acid and flavanoids such as lutein and quercetin, which are potential antioxidants (Reddy *et al.*, 1965; Deshpande *et al.*, 1976; Aruoma *et al.*, 1993; Festa *et al.*, 2001; Boyle *et al.*, 2000; Boots and Haenen, 2008). The bark of the plant is also reported to possess several biological activities such as antiulcer, antimicrobial, antihyperglycemic, anthelmintic and wound healing activities (Govindarajan *et al.*, 2006a; Boots and Haenen, 2008; Parvathi *et al.*, 2009; Parvathi *et al.*, 2009). Gastroprotective potential of *Anogeissus latifolia* has been studied in aspirin, cold-resistant stress (CRS), pylorus ligated and ethanol-induced ulcers. The status of the antioxidant enzymes, superoxide dismutase and catalase, has also been studied in CRS-induced ulcers (Govindarajan *et al.*, 2006; Boots and Haenen, 2008). The present study was undertaken to investigate the hepatoprotective activity of sequential extracts of *Anogeissus latifolia* leaf and bark in CCl<sub>4</sub> induced hepatotoxicity in rats.

## MATERIALS AND METHODS

### Plant material

The stem bark and leaf of *Anogeissus latifolia* were collected from Thyavarekoppa forest area of Shivamogga District, Karnataka. The plant was authenticated by comparing with the herbarium voucher specimen deposited at Kuvempu University Herbaria Ku/sd/Tk/206, Shankarghatta. The materials were air dried under shade, powdered mechanically and stored in the airtight containers. About 1kg of the powdered materials was refluxed successively with the solvents *viz.* petroleum ether, chloroform and methanol in a soxhlet extractor for 48h in four batches of 250g each. Every time before extracting with next solvent the marc was dried at room temperature. The extracts were pooled together and concentrated in vacuum using rotary flash evaporator (Buchi, Flawil, Switzerland).

### Animal selection

The animals were procured from the Central Animal House, IISc, Bangalore. Healthy adult Wistar albino rats of either sex of 2-3 months old and weighing 150-200gm were used for study. These animals were maintained at standard housing conditions (temperature 27 ± 1°C; relative humidity 60 ± 5%) and were fed with commercial diet (Hindustan Lever Ltd., Bangalore) and water *ad libitum*, during the experiment. The institutional animal ethical committee (IAEC/144/1999/CPCSEA) permitted the study.

### Acute toxicity studies

The acute toxicity studies were carried out as per stair case method (Ghosh, 1984). 50 albino mice of either sex weighing 20-25g and 90 days were used to determine LD<sub>50</sub> of various extracts. The mice were divided into 5 groups of 10 each and were administered with aliquot doses of the extracts orally (1000, 1500, 2000, 2500 and 3000mg/kg body weight). Mortality was not noticed upto 3000mg/kg for all the extracts except for chloroform leaf and methanol bark, wherein 100% mortality was observed at the dose of 2500 and 3000 mg/kg.

Accordingly the LD<sub>50</sub> of the extracts was found to be 3000mg/Kg body weight for all the extracts except chloroform leaf and methanol bark which was noticed at 2000mg/Kg body weight. 1/10<sup>th</sup> of this dose was selected as the therapeutic dose for the evaluation of hepatoprotective activity. 1% Tween - 80 was used as the vehicle to suspend various fractions and administered orally.

Nine groups of animals containing six each were used for the study. The animals of Group I served as the control and received the vehicle 1% Tween - 80 w/v at a dose of 1mL/kg/day p.o. for 14 days. Group II - IX received 0.1mL/kg/day i. p. of CCl<sub>4</sub> (E-Merck, Mumbai, India) for 10 days (Jaiprakash *et al.*, 2006). The standard drug Silymarin (Ranbaxy Lab, Dewas) was administered to Group III animals @ 100mg/kg/day p.o. for 14 days. Group IV-IX was treated with various extracts p.o. for 14 days as follows.

Group IV: Pet ether leaf extract (300mg/kg body weight).

Group V: Chloroform leaf extract (200mg/kg body weight).

Group VI: Methanol leaf extract (300mg/kg body weight).

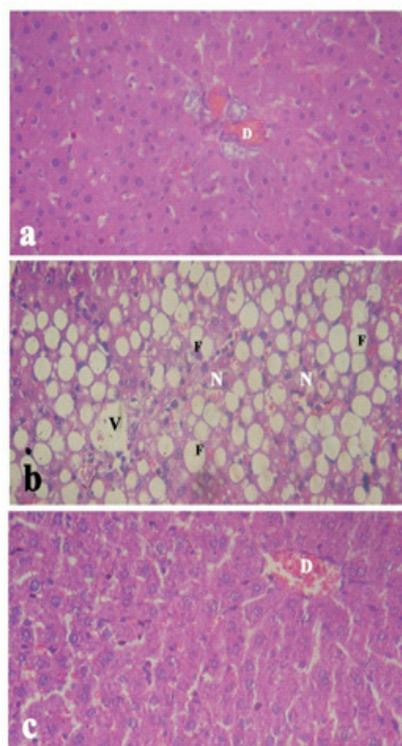
Group VII: Pet ether bark extract (300mg/kg body weight).

Group VIII: Chloroform bark extract (300mg/kg body weight).

Group IX: Methanol bark extract (200mg/kg body weight).

### Assessment of hepatoprotective activity

All the animals were sacrificed on day 14 under light ether anesthesia. The blood samples were collected separately by

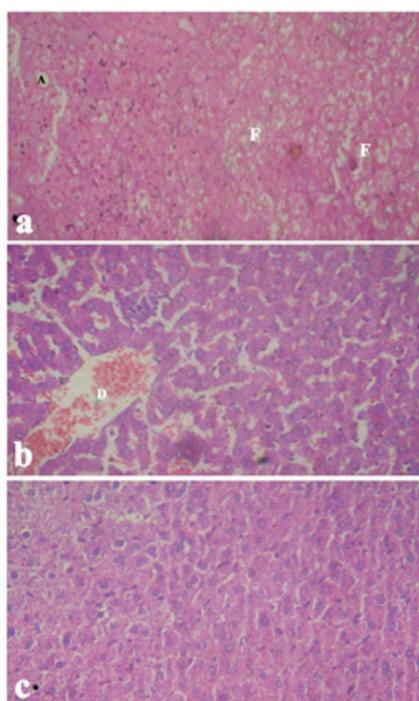


**Figure 1:** a. Section of the liver tissue of control animals showing normal histology, portal triad (Hepatic duct- D) (H and E, 100 X); b. Section of the liver tissue of animals treated with CCl<sub>4</sub> showing the Necrosis (N), Vein (V) and Fatty vacuoles (F) (H and E 100X); c. Section of the liver tissue of Silymarin treated animals showing normal hepatocytes (Hepatic duct D) (H and E 100X).

**Table 1: Effect of *Anogeissus latifolia* leaf and bark extracts against carbon tetrachloride induced hepatotoxicity in rats**

Groups (n)	Total Protein (g/dl)	Bilirubin (g/dl)	AST (IU/l)	ALT (IU/l)	ALP (IU/l)
Control	9.75 ± 0.17	0.52 ± 0.09	52.30 ± 1.15	146.63 ± 5.79	176.24 ± 5.80
CCl <sub>4</sub> treated	5.69 ± 0.09	2.61 ± 0.27	1072.14 ± 13.17	2062.17 ± 28.82	513.04 ± 13.20
Standard	8.74 ± 0.64**	0.54 ± 0.05**	90.14 ± 2.89**	300.29 ± 28.86**	354.13 ± 7.88**
Petroleum ether leaf	6.63 ± 0.18**	0.99 ± 0.17**	103.59 ± 2.63*	377.00 ± 12.97	371.47 ± 5.83
Chloroform leaf	5.99 ± 0.58	1.60 ± 0.21	114.53 ± 5.76	379.94 ± 7.11	378.51 ± 2.62
Methanol leaf	7.76 ± 0.58**	0.72 ± 0.01**	99.16 ± 2.78**	354.76 ± 28.87**	358.40 ± 2.88**
Petroleum ether bark	6.42 ± 0.28*	1.21 ± 0.30*	110.17 ± 2.89*	368.09 ± 9.31*	369.83 ± 13.17
Chloroform bark	7.10 ± 0.58*	1.76 ± 0.23	198.66 ± 18.78	700.30 ± 28.88	492.69 ± 27.77
Methanol bark	8.23 ± 0.57**	0.59 ± 0.03**	94.80 ± 2.88**	343.1 ± 12.27**	361.54 ± 5.78**

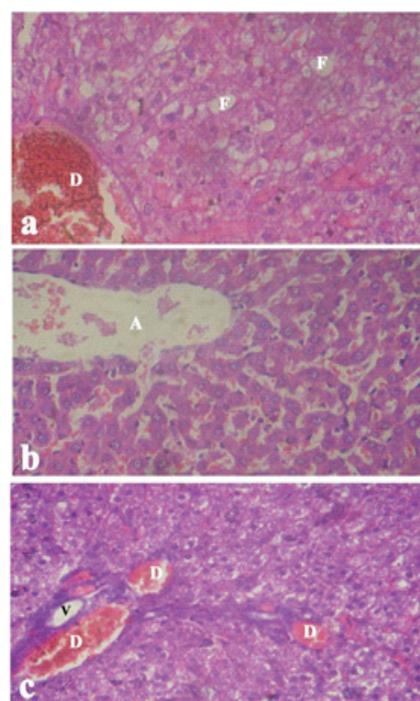
Values are expressed as mean ± SE, n = 6, \* $p < 0.05$  and \*\* $p < 0.01$  v/s CCl<sub>4</sub> treated group.



**Figure 2:** a. Section of the liver tissue of animals treated with petroleum ether Leaf extract of *Anogeissus latifolia* (300mg/kg body weight) showing the absence of Necrosis [Fatty vacuoles (F)] [H & E 100X]; b. Section of liver tissue of animals treated with chloroform Leaf extract of *Anogeissus latifolia* (200mg/kg body weight) showing the absence of Necrosis, very less Fatty vacuoles (F) and Hepatic duct (D) (H & E 100X); c. Section of the liver tissue of animals treated with Methanol Leaf extract of *Anogeissus latifolia* (300mg/kg body weight) showing the absence of necrosis, no Fatty vacuoles (F), (H and E 100X).

carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30min at 37°C. The clear serum was separated at 2500rpm for 10min and biochemical investigations were carried out to assess liver function viz., total bilirubin (Mallory and Evelyn, 1937), total protein (Kingsley and Frankel, 1939) serum transaminases (Reitman and Frankel, 1957) and serum alkaline phosphatase (Bessey et al., 1964) (Raichem, San Diego, CA 92111-1203).

After draining the blood, liver samples were excised, washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48h and then with bovine solution for 6h. Paraffin sections were taken at 5mm thickness processed in alcohol-xylene series and was stained with alum hematoxyline



**Figure 3:** a. Section of the liver tissue of animals treated with petroleum ether Bark extract of 300mg/kg body weight showing the absence of Necrosis (N), Fatty vacuoles (F) and Hepatic duct (D) [H & E 100X]; b. Section of liver tissue of animals treated with chloroform Bark extract of 300mg/kg body weight showing the absence of Necrosis (N), Artery (A) (H and E 100X); c. Section of the liver tissue of animals treated with Methanol Bark extract of 200mg/kg body weight showing the absence of necrosis, no Fatty vacuoles (F), Hepatic duct (D) and Vein (V) (H& E 100X).

and eosin (Galigher and Koyloff, 1971). The sections were examined microscopically for histopathological changes.

#### Statistical analysis

The results are expressed as mean ± SEM of six animals from each group. The data were evaluated by one-way ANOVA. Comparison of mean values of different groups treated with different extracts and positive control were estimated by Tukey's Multiple Comparison Test.  $p < 0.05$  was considered significant and  $p < 0.01$  as highly significant.

#### Preliminary phytochemical Screening

Standard methods (Thorn et al., 1977) were used for preliminary phytochemical screening of the individual fractions to know the nature of phyto-constituents present in

the leaves viz. steroids, flavanoids, alkaloids, saponins, tannins, glycosides etc.

## RESULTS

The effect of various extracts of *Anogeissus latifolia* on total protein, bilirubin, serum transaminases and alkaline phosphatase in toxicity induced rats are summarized in Table 1. Administration of  $\text{CCl}_4$  showed significant increase in ( $p < 0.01$ ) elevation of liver marker enzymes in serum namely AST ( $1072.14 \pm 13.17$ ), ALT ( $2062.17 \pm 28.82$ ), ALP ( $513.04 \pm 13.20$ ) and total bilirubin ( $2.61 \pm 0.27$ ) when compared to normal control respectively. Methanolic leaf and bark extracts at a dose of 300 and 200mg/kg body weight showed significant ( $p < 0.01$ ) reduction of AST ( $99.16 \pm 2.78$ ,  $94.80 \pm 2.88$ ), ALT ( $354.76 \pm 28.87$ ,  $343.1 \pm 12.27$ ), ALP ( $358.40 \pm 2.88$ ,  $361.54 \pm 5.78$ ) and total bilirubin ( $0.72 \pm 0.01$ ,  $0.59 \pm 0.03$ ) levels respectively when compared to  $\text{CCl}_4$  intoxicated rats. Petroleum ether leaf extract also showed significant reduction in AST ( $103.59 \pm 2.63$ ) ( $p < 0.01$ ) and total bilirubin level ( $0.99 \pm 0.17$ ) only while, petroleum ether bark extract showed significant ( $p < 0.05$ ) reduction in AST ( $110.17 \pm 2.89$ ), ALT ( $368.09 \pm 9.31$ ) and total bilirubin level ( $1.21 \pm 0.30$ ) when compared to  $\text{CCl}_4$  intoxicated rats. Standard drug silymarin showed significant ( $p < 0.01$ ) reduction in the levels of AST ( $90.14 \pm 2.89$ ), ALT ( $300.29 \pm 28.86$ ), ALP ( $354.13 \pm 7.88$ ) and total bilirubin ( $0.54 \pm 0.05$ ). However, chloroform bark and leaf did not show significant reduction for any of the parameters.

Total protein was significantly reduced following  $\text{CCl}_4$  administration ( $5.69 \pm 0.09$ ) when compared to normal control rats. Methanolic leaf and bark extract at a dose of 300 and 200mg/kg body weight showed significant ( $p < 0.01$ ) increase in total protein ( $7.76 \pm 0.58$ ,  $8.23 \pm 0.57$ ) levels when compared to  $\text{CCl}_4$  intoxicated rats. Both petroleum ether leaf and bark also showed significant raise in total protein level ( $6.63 \pm 0.18$  and  $6.42 \pm 0.28$ ) ( $p < 0.01$ ). However among the chloroform extracts, only bark showed significant raise ( $7.10 \pm 0.58$ ) in total protein ( $p < 0.05$ ). Standard drug silymarin showed significant ( $p < 0.01$ ) rise in the levels of total protein ( $8.74 \pm 0.64$ ).

Histological profile of the control animals showed normal hepatocytes (Fig. 1a). Group II animals treated with  $\text{CCl}_4$  exhibited intense centrilobular necrosis, vacuolization and macrovesicular fatty changes (Fig. 1b). The sections of liver taken from the animals treated with standard drug silymarin showed the hepatic architecture, which was similar to that of control (Fig. 1c). The animals treated with methanolic leaf and bark extracts exhibited significant liver protection against the toxicant as evident by the presence of normal hepatic cords, absence of necrosis and lesser fatty infiltration (Fig. 2c, 3c.). However moderate accumulation of fatty lobules was noticed in the sections of animals treated with the Petroleum ether leaf and bark extracts (Fig. 2a, 3a.). No recovery from the damage was observed in case of chloroform leaf and bark extracts (Fig. 2b, 3b). Further, among the bark extracts highest hepatoprotective activity was noticed in methanol followed by petroleum ether. Similar effect was noticed in leaf extracts also. From the data it is also evident that, among all the sources of extracts methanolic bark proved to be more effective when

compared to methanolic extracts from leaf in terms of hepatoprotective activity.

## DISCUSSION

The bark of *Aogeissus latifolia* was selected to evaluate its antihepatotoxic effect in  $\text{CCl}_4$  induced hepatotoxic rat model. A survey of literature suggested that it has been used for different diseases including inflammation, diabetes, diarrhea and skin diseases, as well as hepatopathy. The bark has been evaluated scientifically for its antioxidant and wound healing activity. A qualitative chemical examination showed the presence of carbohydrates, glycosides, phenolic compounds, flavanoids and tannins. The presence of polyphenols and flavanoids supports its antioxidant potential. The high percentage of quercetin, rutin and gallic acid in the extract justifies the potent antioxidant activity (Aruoma *et al.*, 1993; Boyle *et al.*, 2000; Boots and Haenen, 2008) which results in the hepatoprotective potential of the extract. Quercetin and rutin are reported to be potential therapeutic agents as they reduce oxidative DNA damage, lipid peroxidation and quench free radicals (Alfanasev *et al.*, 1989; Noroozi *et al.*, 1998). The drug, thus, is a rich source of various antioxidant chemicals which may exert a cumulative antioxidant effect producing favourable actions in various disease conditions such as hepatopathy, diabetes, inflammation and wound healing.

The  $\text{CCl}_4$  has been used as a tool to induce hepatotoxicity in experimental animals (Rechnagl, 1983; Okuno *et al.*, 1986). The hepatotoxicity induced by  $\text{CCl}_4$  due to metabolite  $\text{CCl}_3$  a free radical that binds to lipoprotein and leads to peroxidation of lipids of the endoplasmic reticulum (Rechnagla, 1967). This toxic chemical caused peroxidative degradation in the adipose tissue resulting in fatty infiltration of the hepatocytes. The increase in the levels of serum bilirubin reflected the depth of jaundice and the increase in serum transaminases and alkaline phosphatases was the clear indication of cellular damage and loss of functional integrity of the cell membrane (Sarswath *et al.*, 1993).

The lowering of enzyme levels is a definite indication of hepatoprotective action of the drug. The serum AST, ALT and ALP levels are reliable markers of liver function (Mulanar *et al.*, 1955). In our study, the significant elevation of marker enzymes following administration of  $\text{CCl}_4$  indicated serious toxicity produced by the chemical. In  $\text{CCl}_4$  induced hepatitis, administration of leaf and bark methanolic extracts of *Aogeissus latifolia* produced significant reduction in AST, ALT and ALP activities and total bilirubin levels while exhibiting a significant increase in total protein. Thus it could be suggested that, *Anogeissus latifolia* possess hepatoprotective activity in this model, a concept which was further supported by the histopathological studies.

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