Hepatoprotective evaluation of *Anogeissus latifolia*: *In vitro* and *in vivo* studies

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Abstract

AIM: To evaluate the hepatoprotective activity of a hydroalcoholic extract of the bark of *Anogeissus latifolia; in vitro* in primary rat hepatocyte monolayer culture and *in vivo* in the liver of Wistar rats intoxicated by carbon tetrachloride (CCl₄).

METHODS: In the *in vitro* study, a primary hepatocyte monolayer culture was treated with CCl₄ and extract of *Anogeissus latifolia*. Hepatoprotective activity was demonstrated in the CCl₄ damaged primary monolayer culture. In the *in vivo* study, the hepatoprotective activity of a hydroalcoholic extract of *Anogeissus latifolia* was analyzed in liver injured CCl₄-treated rats. Biochemical parameters including serum transaminases [aspartate aminotransferase (AST) and alanine aminotransferase (ALT)] and alkaline phosphatase (ALP) in serum were analyzed. The biochemical findings were supplemented with histopathological examination of rat liver sections.

RESULTS: *In vitro*: primary hepatocyte monolayer cultures were treated with CCl₄ and extract of *Anogeissus latifolia*. A protective activity could be demonstrated in the CCl₄ damaged primary monolayer culture. *In vivo*: Hydroalcoholic extract of *Anogeissus latifolia* (300 mg/kg) was found to have protective activity in rats with CCl₄-induced liver damage as judged from serum marker enzyme activity.

CONCLUSION: The above findings lead to the conclusion that the hydroalcoholic extract of *Anogeissus latifolia* is hepatoprotective. Hence, we suggest that the inclusion of this plant in the management of liver disorders is justified.

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Key words: *Anogeissus latifolia*; Hepatoprotective; Carbon tetrachloride

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INTRODUCTION

Oxidative stress has been implicated in the pathogenesis of acute and chronic liver injury in a variety of pathophysiological conditions such as hepatotoxic exposure, intrahepatic cholestasis, alcoholic liver injury, liver ischemia and viral hepatitis[1-4]. Over-production 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 processes 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\[6\]. 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\[6-8\].

*Anogeissus latifolia* Wall (Combretaceae) is a large 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 hepatopat\[9\]. 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\[10\]. A variety of substances which might contribute to hepatoprotective activity have been identified in extracts of *Anogeissus latifolia* including tannins, gallic acid, ellagic acid and flavonoids such as lutin and quercetin, which are potential antioxidants\[11,12\]. The bark of the plant is also reported to possess several biological activities such as antiulcer, antimicrobial and wound healing activities. Gastroprotective potential of *Anogeissus latifolia* extract 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\[13,14\]. The bark of the plant was standardized for the presence of chemical constituents such as gallic acid and ellagic acid (0.95% w/w and 0.25% w/w, respectively) using High Performance Thin Layer Chromatography (HPTLC) by Govindrajan et al.\[15\]. Further, we identified and quantified the other constituents of the bark, quercetin and rutin (1.875% w/w, 0.1617% w/w, respectively) using HPTLC; these are reported as potent antioxidants and hepatoprotective agents\[16,17\]. Antioxidant action has been reported to play a crucial role in hepatoprotection\[18\]. The hydroalcoholic extract of *Anogeissus latifolia* is reported to have hepatoprotective activity in paracetamol-induced toxicity in a rat model\[19\]. Thus, the present study was therefore undertaken to investigate the hepatoprotective activity of hydroalcoholic extract of *Anogeissus latifolia in vitro* and *in vivo* against CCl\(_4\)-intoxicated rats.

MATERIALS AND METHODS

Materials

Plant material and extraction: Bark of *Anogeissus latifolia* was collected from Chikmagalure, Karnataka, South India during the month of May. It was authenticated by Botanical survey of India, Coimbatore, Tamilnadu, India (No. BSI/SC/5/23/06-07/tech.880).

The bark was shade-dried and powdered coarsely. The coarse powder (250 g) obtained was treated with n-hexane to remove the fatty substances; the bark was further submitted to exhaustive lipid extraction with 70% ethanol in Soxhlet apparatus and filtered. The extract was concentrated under reduced temperature and pressure to obtain dry residue (26.8 g)\[18\].

Chemicals: All chemicals and solvents used were obtained from S.D. Fine Chemicals, Mumbai, Loba Chemie Indo Austranal Co., Mumbai, Ranbaxy laboratories Ltd., Punjab, Sigma Fine Chemicals, Mumbai and Hi media Laboratories, Mumbai, India. For various 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.

Animals: Healthy, adult female albino rats of Wistar strain, weighing 180-220 g were obtained from the animal house of J.S.S College of Pharmacy, Ooty, India. The animal house was well ventilated and the animals were exposed to 12 h day and night cycles at a temperature of 20 ± 2°C. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and standard rat pellet obtained from M/s Hindustan Lever Ltd., Bangalore, India (CPCSEA-JSSCP/IAEM/PHY.PHARM/2006-07).

Autoanalyser and UV spectrophotometer

Microlab 100, manufactured by M/s Vital Scientific N.V., The Netherlands, was used to estimate biochemical parameters for aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), UV-160 Spectrophotometer, manufactured by Shimadzu Corporation, Japan, was used to estimate total phenol content, total flavonol content and lipid peroxidation.

Methods

Estimation of total phenolic content: Phenolic compounds are commonly found in both edible and inedible plants and they have been reported to have multiple biological effects, including antioxidant activity\[20\]. Total phenol was determined using the Folin-Ciocalteu method. This test is based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. After oxidation a green blue complex is measured at 750 nm. The total phenol content of a tested material is related to its antioxidant activity\[21\].

Estimation of total flavonol content: Total flavonol content was determined by the method of Wolsky\[22\]. This involved an aluminum chloride colorimetric method. The principle of this method is that aluminum chloride forms an acid stable complex with the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminum...
chloride forms acid labile complexes with the ortho-dihydroxyl groups in the A or B ring of flavonoids[29].

**Preparation of drug solution:** The 70% ethanolic extract of *Anogeissus latifolia* was suspended in 1% carboxy methyl cellulose (CMC), for oral administration. The concentrations of extract selected were 100 mg and 300 mg/kg body weight. Liv-52 syrup was administered orally at 2 mL/kg body weight.

**Carbon tetrachloride-induced hepatotoxicity:** It is emphasized that hepatotoxins which cause acute hepatitis should have close resemblance with viral hepatitis, clinically, biochemically and histologically. Certain drugs are responsible for chronic hepatic disease. Chemically-induced hepatic injury for experimental studies should be severe enough to cause cell death or to modify hepatic functions. The mechanism of acute hepatic injury depends upon the chemical compounds used to induce toxicity. Carbon tetrachloride (CCl4) is one of the most powerful hepatotoxins in terms of severity of injury. It causes toxic necrosis leading to biochemical changes, having clinical features similar to those of acute viral hepatitis[26,27]. CCl4 at a dose of 0.5 mL/kg was dissolved in olive oil (1:1) and 0.1 mL was administered for each 100 g of rat body weight intraperitoneally.

**Standard Liv-52 Syrup:** Liv-52 is a poly herbal formulation introduced in 1954 as a specially formulated ayurvedic herbal remedy for the treatment of viral hepatitis and has been widely prescribed for infective hepatitis ever since[28]. It is an ayurvedic formulation available as tablets and syrup containing the following herbs: Capparis spinosa, Cichorium intybus, Solanum nigrum, Terminalia arjuna, Cassia occidentalis, Achillea millefolium, Tamarix galica and Phyllanthus amarus.

**In-vitro hepatoprotective activity**

**Hepatotoxin and test substances:** For *in vitro* studies, CCl4 (0.1 mol/L), was used to produce submaximal toxicity in isolated rat hepatocytes. The test solutions were administered at dose levels of 125, 250 and 500 μg/mL. Liv-52 was used as a positive control at a dose level of 250 μL/mL. All the substances were dissolved in DMSO[29].

**Isolation of rat hepatocytes:** The rat hepatocytes were isolated according to the method of Seglen et al[30]. The livers were isolated under aseptic conditions and placed in HEPES (N-2-hydroxyethylpiperazine-N-2-ethanesulphonic acid) buffer I containing HEPES (0.01 mol/L), NaCl (0.142 mol/L) and KCl (0.0067 mol/L), pH 7.4. The livers were cut into small pieces and then incubated with a second buffer containing HEPES (0.1 mol/L), NaCl (0.0667 mol/L), KCl (0.0067 mol/L) and 0.5% Collagenase type IV, pH 7.6, for about 45 min at 37°C in an incubator with constant shaking. Hepatocytes were obtained after filtration and cold centrifugation (4°C, 200 rpm for 2 min, three times) and suspended in HEPES buffer I. The viability of the hepatocytes was assessed by trypan blue (0.2%) exclusion method[30].

**Primary cultures of rat hepatocytes:** The method of Tingstrom and Obrink[31] with slight modifications was used for the culturing of rat hepatocytes. The freshly isolated viable hepatocytes were suspended in culture medium RPMI-1640 supplemented with calf serum (10%), HEPES and gentamycin (1 μg/mL). These cells (approximately 1-2 × 10^6/mL) were then seeded into culture bottles and incubated at 37°C in an atmosphere of 5% CO2 in a carbon dioxide incubator. Upon incubation for 24 h the hepatocytes formed a monolayer. The newly formed cells were round and most appeared as individual cells. These cells were 95%-96% viable as confirmed by trypan blue exclusion test.

**Hepatic cytotoxicity testing:** The hydroalcoholic extract of *Anogeissus latifolia* was tested for hepatic cytotoxicity at 250, 500 and 1000 μg/mL on isolated rat hepatocytes. After 24 h of incubation at 37°C in a CO2 incubator, the percentage viability of hepatocytes was tested using trypan blue exclusion[31].

**Hepatoprotective activity:** Twenty-four hours after the establishment of the monolayer of hepatocytes, the medium was decanted and the culture was washed with HEPES buffer-I and finally the hepatocytes were suspended in Buffer-I. The hepatic cytotoxicity was induced with CCl4 (0.1 mol/L). Triplicate hepatocyte suspensions (0.1 mL) from different cultures were distributed into various culture tubes labeled as control, toxicant, standard (Liv-52 + toxicant) and test (test sample + toxicant). The control group received 0.1 mL of vehicle (30% DMSO) and toxicant groups received 0.1 mL of CCl4, while the test groups received 0.1 mL of respective test solutions (250, 500 and 1000 μg/mL) followed by 0.1 mL (0.1 mol/L) of hepatotoxin. The standard groups received 0.1 mL of Liv-52 (250 μL/mL) followed by hepatotoxin. The contents of all culture tubes were made up to 1 mL with HEPES buffer I. The contents of all the tubes were mixed well and incubated in a CO2 incubator for 24 h at 37°C. In test and standard groups the hepatocytes were preincubated with respective solutions for 30 min and then exposed to hepatotoxin. After incubation, hepatocyte suspensions were collected to assess cell damage. Cell viability was evaluated by trypan blue exclusion method. Hepatocyte suspensions were centrifuged at 200 rpm. The leakage of the enzymes ALT and AST secreted outside the cells was determined from the supernatant.

**Assessment of hepatoprotective activity:** The effect of different extracts on liver protection was determined by measuring an increase in the percentage of viable cells in that group of cells incubated with extracts, compared with the control and toxicant-alone groups. Reversal of toxin-induced elevations in the level of enzymes was also considered to assess hepatoprotective activity. Kits procured from Ecoline, E. Merck Ltd., using an auto-analysers, carried out the biochemical estimations (Table 1).

**In vivo acute toxicity studies:** Acute oral toxicity was induced according to the Organization for Economic Co-
Operation and Development (OECD) 423 guidelines procedure[33]. Healthy, young adult Wistar albino rats of weight variation not exceeding ± 20% of the mean weight were selected. The animals were fasted for 4 h with free access to water only. *Anogeissus latifolia* was administered orally at a dose of 5 mg/kg initially. Mortality, if any, was observed for 3 d. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher doses (50, 300, 2000 mg/kg) of *Anogeissus latifolia* were employed for further toxicity studies. *Anogeissus latifolia* did not produce any behavioral changes and mortality up to the dose of 3000 mg/kg body weight. Hence, 1/10th of this dose, i.e. 300 mg/kg (high dose) was used for the study.

**In-vivo hepatoprotective activity and estimation of biochemical parameters**

**Experimental design:** Twenty four albino Wistar rats, weighing about 180-220 g were divided into 4 groups of six animals each. Group I served as solvent control (normal animals). Group II served as CCl₄ toxicant control and received 1% CMC, 2 mL/kg b.w. Group III served as positive control and received Liv-52, 2 mL/kg b.w., while Group IV received the freshly prepared *Anogeissus latifolia* suspended in 1% CMC at a dose level of 300 g/mL b.w. The animals were treated for 7 d and on the 7th d after one hour of dosing, the toxicant CCl₄ (500 μL/kg i.p.) was administered to all the groups except Group I. After 24 h, the animals were anesthetized and blood was collected by sino-orbital puncture for the assessment of various enzyme activities. The blood was centrifuged at 2000 rpm for 10 min. The serum was separated and was used for various biochemical estimations such as AST, ALT, 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. The remaining was frozen at -70°C and was used for the estimation of lipid peroxidation.

**Estimation of lipid peroxidation by thiobarbituric acid reactive substances (TBARS):** The level of lipid peroxidation in liver homogenate was determined by the method of Niehaus and Samuelson[34]. Malondialdehyde and other thiobarbituric acid reactive substances were quantified by their reactivity with thiobarbituric acid in acidic conditions. The reaction generates a pink colored chromophore, which can be read in a colorimeter at 535 nm.

**Statistical analysis**

The statistical analysis was carried out by one-way analysis of variance (ANOVA). The values are represented as mean ± SE. Comparison of mean values of different groups treated with different dose levels of extracts and positive controls were estimated by Tukey’s Multiple Comparison Test. *P* < 0.05 was considered significant.

**RESULTS**

**Hepatic cytotoxicity**

When normal hepatocytes were treated with the extracts under test conditions, there were no alterations in the values of percentage viable cells as compared to the control at the dose level up to 1000 μg/mL, indicating that the extracts were not toxic to the cells.

**Effects against CCl₄-induced toxicity**

Incubation of hepatocytes with CCl₄ (0.1 mol/L) resulted in 65% depletion in viability of hepatocytes. Similarly an elevation of about 268.45% and 267.06% of ALT and AST levels were observed, respectively, upon intoxication with CCl₄. Hepatocytes treated with *Anogeissus latifolia* showed a concentration-dependant (100-1000 μg/mL) protective effect by restoring the viability of hepatocytes (36.05%-83.87%) levels, while the positive control Liv-52 showed good protective effect by restoring viability (86.7%). ALT (97.5%) and ALT (92.8%). The maximum protection was seen with 1000 μg/mL of *Anogeissus latifolia*. Results are represented in Table 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Viable cells (%)</th>
<th>ALT (IU/L)</th>
<th>AST (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell control</td>
<td>93.88 ± 0.81</td>
<td>18.70 ± 0.46</td>
<td>21.53 ± 0.63</td>
</tr>
<tr>
<td>Toxicant (1 mol/L CCl₄)</td>
<td>33.06 ± 114</td>
<td>50.20 ± 0.61</td>
<td>57.50 ± 0.94</td>
</tr>
<tr>
<td>Std Liv-52 (250 μg/mL)</td>
<td>85.84 ± 11.96 (86.7%)</td>
<td>19.46 ± 0.35 (97.59%)</td>
<td>24.10 ± 0.37 (92.85%)</td>
</tr>
<tr>
<td>ALE (250 μg/mL)</td>
<td>57.63 ± 1.58 (40.39%)</td>
<td>41.33 ± 0.62 (28.16%)</td>
<td>44.53 ± 1.25 (36.05%)</td>
</tr>
<tr>
<td>ALE (500 μg/mL)</td>
<td>77.60 ± 0.57 (73.72%)</td>
<td>38.50 ± 0.45 (37.14%)</td>
<td>32.80 ± 0.65 (68.66%)</td>
</tr>
<tr>
<td>ALE (1000 μg/mL)</td>
<td>81.24 ± 1.67 (79.20%)</td>
<td>22.23 ± 0.50 (88.48%)</td>
<td>27.33 ± 0.69 (83.87%)</td>
</tr>
</tbody>
</table>

Values in brackets indicate percentage protection against toxicant. Significant reduction compared to hepatotoxic (*P* < 0.05). ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Table 1: Effect of *Anogeissus latifolia* extract (ALE) on CCl₄-induced toxicity in rat hepatocytes (mean ± SE)

*P* < 0.001; *P* < 0.01; *P* < 0.05
(P < 0.01), AST 117 ± 6.7 IU/mL (P < 0.01) and ALP 258 ± 15.54 IU/mL (P < 0.01) levels when compared to CCl₄-administered rats. Liv-52 also showed significant reductions in ALT 71.4 ± 5.6 IU/mL (P < 0.01), AST 111.7 ± 8.7 IU/mL (P < 0.01) and ALP 258 ± 15.54 IU/mL (P < 0.01) levels when compared to CCl₄-administered rats (Table 2).

**Lipid peroxidation**

Lipid peroxidation was significantly elevated following CCl₄ administration (14.8 ± 1.3 nmol, P < 0.001) when compared to normal control (4.1 ± 0.5 nmol) (Figure 1). _Anogeissus latifolia_ at a dose of 300 mg/kg, b.w., resulted in significant (P < 0.01) reductions in lipid peroxidation (6.1 ± 0.5 nmol) when compared to toxicant control. Liv-52 at 2 mL/kg b.w., showed significant (P < 0.01) reductions in lipid peroxidation (5.4 ± 0.6 nmol) when compared to normal control (4.1 ± 0.5 nmol) (Figure 1).

**Histopathology**

The protective effect of the hydroalcoholic extract of _Anogeissus latifolia_ was further confirmed by histopathological examination of the control (Figure 1A), CCl₄-treated and extract-treated groups. The liver of CCl₄-treated rats (Figure 1B) shows damaged liver cells and ballooning changes of the hepatocytes. The histopathological pattern of the livers treated with extracts at 100 mg and 300 mg/kg (Figure 1C) shows mild feathery changes, little ballooning degeneration of hepatocytes along with normal hepatocytes. Positive control liver treated with Liv 52 (Figure 1D) shows a normal lobular pattern with minimal pooling of blood in the sinusoidal spaces. The present study reveals the hepatoprotective activity of the hydroalcoholic extract of _Anogeissus latifolia_ against well-known hepatotoxin CCl₄.

**DISCUSSION**

The bark of _Anogeissus latifolia_ was selected to evaluate its antihepatotoxic effect in preclinical models on the basis of its utility profile in the traditional system of medicine. Subsequently, a survey of literature suggested that it has been used for different diseases including inflammation, diabetes, diarrhoea and skin diseases, as well as hepatopathy. The bark has been evaluated scientifically for its antioxidant and wound healing activity. There was, however, no evidence of any scientific studies on its hepatoprotective action. A qualitative chemical examination showed the presence of carbohydrates, glycosides, phenolic compounds, flavonoids and tannins. The presence of polyphenols and flavonoids supports its antioxidant potential. Total phenol content and total flavonol content was estimated in the extract, and found to be 64.43% and 43.9 mg/g of extract respectively. Since the bark has been reported to contain quercetin and rutin[9], we estimated the quantity of these substances in the extract by HPTLC and this was found to be 1.875% w/w, and 0.1617% w/w respectively. The drug also contains gallic acid. The high percentage of quercetin, rutin and gallic acid in the extract justifies the potent antioxidant activity[13,15,16] 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[35,36]. 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 hepatotoxicity induced by CCl₄ is due to its metabolite CCl₃·, a free radical that binds to lipoprotein and leads to peroxidation of lipids of the endoplasmic reticulum[37]. The ability of a hepatoprotective drug to reduce the injurious effects, or to preserve the normal hepatic physiological mechanisms which have been disturbed by a hepatotoxin, is an index of its protective effects. Although serum enzyme levels are not a direct measure of hepatic injury, they show the status of the liver. The lowering of enzyme levels is a definite indication of hepatoprotec-

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**Table 2** Effect of _Anogeissus latifolia_ on plasma biochemical parameters and lipid peroxidation in CCl₄-intoxicated rats liver (n = 6, mean ± SE)

<table>
<thead>
<tr>
<th>Treatment (μL/kg)</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Lipid peroxidation nmol of MDA/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>88.2 ± 5.44</td>
<td>54 ± 2.7</td>
<td>249.5 ± 18.2</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>CCl₄ (500 μL/kg)</td>
<td>254.9 ± 19.5</td>
<td>222.8 ± 10.14</td>
<td>328.5 ± 25.36</td>
<td>14.8 ± 1.3</td>
</tr>
<tr>
<td>Liv-52 (2 mL/kg)</td>
<td>111.7 ± 8.7</td>
<td>71.4 ± 5.6</td>
<td>255 ± 24.1</td>
<td>5.4 ± 0.6</td>
</tr>
<tr>
<td>ALE (300 mg/kg)</td>
<td>117 ± 6.7</td>
<td>66.2 ± 6.1</td>
<td>258 ± 15.54</td>
<td>6.1 ± 0.5</td>
</tr>
</tbody>
</table>

*P < 0.01, *P < 0.001 vs normal; *P < 0.01 vs carbon tetrachloride. ALP: Alkaline phosphatase.
tive action of the drug. The serum ALT, AST, and ALP levels are reliable markers of liver function\(^\text{[18]}\). In our study, an increase in LPO level in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms resulting in excessive free radical. In CCl\(_4\)-induced hepatitis, administration of *Anogeissus latifolia* at 300 mg/kg b.w. produced significant reductions in ALT, AST, ALP levels and lipid peroxidation. Thus it could be suggested that *Anogeissus latifolia* has hepatoprotective activity in this model, a concept which was further supported by the histopathological results. The reactive species-mediated hepatotoxicity can be effectively managed upon administration of agents possessing antioxidant\(^\text{[19]}\), free radical scavenger\(^\text{[20]}\) and anti-lipid peroxidant\(^\text{[21]}\) activities. The inhibitors of cytochrome P450 isoenzymes (CYPs) are known to reduce the toxicity of CCl\(_4\).\(^\text{[12]}\). Rutin and quercetin, which are constituents of *Anogeissus latifolia* extract, have been reported to inhibit CYPs\(^\text{[22]}\) and might have contributed favorably toward the observed hepatoprotection. *Anogeissus latifolia*, being a potent antioxidant, free radical scavenger, contributed favorably in this regard towards the observed hepatoprotection. The *in vitro* and histopathological studies are direct evidence of efficacy of this drug as a hepatoprotectant. Thus, the presence of rutin, quercetin and other antioxidants in *Anogeissus latifolia* may be the contributing factor towards its hepatoprotective activity and justifies the folkloric use of the plant in liver diseases.

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