Methanol Stem Bark Extract of *Ficus platyphylla* Protects Against Carbon Tetrachloride-Induced Liver Damage in Wistar Rats

1C. J. Ugwah-Oguejiofor, 1S. G. Ibrahim, 2H. E. Mshelia, 3U. Mohammed and 1I. M. Adebisi

1Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
2Department of Pharmacognosy and Ethnomedicine, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria
3Department of Morbid Anatomy and Forensic Medicine, Usmanu Danfodiyo University and Teaching Hospital, Sokoto, Nigeria

[Corresponding Author: E-mail: nenyen789@yahoo.com]

**ABSTRACT**

*Ficus platyphylla* (Gutta percha tree) is used in Nigerian traditional medicine for treating pain and psychosis. This study evaluated the protective effect of methanol stem bark extract of *Ficus platyphylla* (MEFP) against carbon tetrachloride (CCl₄)-induced liver injury in Wistar rats. Rats were allotted into six groups namely normal control, negative control, standard drug (silymarin) and extract treated (100, 200 and 400 mg/kg p.o. respectively) groups. Treatment was administered for 7 days. Animals in groups II-VI received a single dose of CCl₄ (1:1) p.o. in olive oil at 1.5 ml/kg body weight after last treatment. Experimental rats were sacrificed after 24 hours of CCl₄ administration and blood samples collected for determination of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities. Supernatant of liver homogenate was used to determine activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Results indicate increased activities (p < 0.05) in CAT, SOD and GPx in samples of MEFP treated rats while levels of ALT and AST in serum samples of treated groups differ significantly (p<0.05) when compared with negative control group (Group III). Treatment with methanol stem bark extract of *Ficus platyphylla* may have offered protection against CCl₄ induced liver damage in experimental rats.

**Keywords:** *Ficus platyphylla*, Gutta percha Tree, Liver Damage, Carbon Tetrachloride, Wistar Rats

**INTRODUCTION**

The liver is the body organ saddled with the task of metabolism and detoxification of drugs and xenobiotics (Ugwah-Oguejiofor and Ugwah, 2018; Alrashood et al., 2020). Injury to the liver may cause anomalies in its general metabolic functions resulting in various liver disorders. These disorders could be transient or life threatening. Certain medicines and chemicals such as excess alcohol intake, intake of certain drugs, exposure to various toxin and pollutants have been implicated in diseases resulting from liver injury (Iqbal et al., 2018; Huang et al., 2020). Liver diseases are the fifth most common cause of death and the second major cause of death amongst all digestive diseases (Agada et al., 2020). They cause approximately 2 million deaths annually worldwide (Asrani et al., 2019).

In Nigeria, its incidence is high with varying degree of prevalence reported in different geopolitical areas across the country. According to the World Health Organisation (WHO) data published in 2018, death from liver disease in Nigeria was placed at 60,044 or 3.10% of total deaths and thereby ranking Nigeria the second in the world (Stanaway et al., 2018). This causes a challenge to the already burdened health care delivery in the country. Currently, there are no approved, effective drugs for treating liver diseases (Chen et al., 2019), and the effectiveness of the non-pharmacological approaches are minimal (Svistunov et al., 2018). Therefore, it is imperative that urgent medical attention be given to the search for pharmacological agents for the treatment of liver disease. A variety of medicinal plants are proposed for use in the treatment of liver toxicity. *Ficus platyphylla* (Gutta percha tree) is one of...
such plants whose stem bark extract is used traditionally for the treatment of liver diseases (Kankara, et al., 2018; Nnamudi et al., 2020). *Ficus platyphylla* Del. Holl (Moraceae), commonly referred to as the Gutta percha tree, is a large deciduous tree widely distributed in the savannah regions along the West African coast. It is known as *gamji* among the Hausas in Northern Nigeria. Various preparations of the plant are used to treat several diseases including psychosis, infertility, malaria, and CNS disorders (Ugwah-Oguejiofor et al., 2011; Chindo et al., 2016). Previously, studies on this plant have revealed its central nervous system effect, antimalarial and gastrointestinal activities (Shittu et al., 2011; Chindo et al., 2014). Its methanol stem bark extract (MEFP) has been investigated for several pharmacological activities. *Ficus* species have been shown to possess hepatoprotective effect against human hepatocellular carcinoma cells (Al-Musayeib et al., 2017). In this study, the aim was to examine the protective action of MEFP in CCl₄-induced liver toxicity in Wistar rats.

**MATERIALS AND METHODS**

**Plant Collection and Identification**

*Ficus platyphylla* stem bark was obtained from Sokoto south Forestry Zone 1, Sokoto State, Nigeria, in the month of March 2017. Initial identification and authentication of the plant was done by Dr. H. E. Mshelia of Faculty of Pharmaceutical Sciences, Usman Danfodiyo University, Sokoto. A voucher specimen PCG/UDUS/MORA/003 was safely kept in the herbarium of the same Faculty for future reference.

**Preparation of Plant Material and Extraction**

The stem bark of *Ficus platyphylla* plant was cleaned and air dried to constant weight. It was then pulverized mechanically using mortar and pestle into a dry powder and weighed. The powder (500 g) was extracted with 1.5 L of 70% methanol using cold maceration method for 48 hours. The resultant mixture was filtered, and the filtrate evaporated to dryness in an oven set at 40°C. The percentage yield was calculated as follows:

% yield = \( \frac{W_2}{W_1} \times 100 \)

Where \( W_2 \) = Weight of extract (g); \( W_1 \) = Weight of plant material (g)

**Chemicals and Reagents**

Some chemicals and reagents used for this study include methanol, L-Ascorbic acid, DPPH, Molisch reagent, Tris buffer (pH 7.7), L-Aspartate, MDH, LDH, Sodium hydroxide, Sodium Azide, 2-Oxaloacetate, and L-Alanine. Kits for biochemical analyses of some liver marker enzymes were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, MO, USA).

**Determination of Free Radical Scavenging Activity of Ficus Platystyllyla Methanol Stem Bark Extract**

The ability of MEFP to scavenge 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) was determined following the method of Goyal et al. (2010). A stock concentration of 125 mg/ml MEFP in methanol was prepared. Serial dilution was carried out to obtain various concentrations (6.25, 3.13, 1.56, 0.78 and 0.39 mg/ml). Exactly 1 ml of freshly prepared DPPH was added to the test tubes containing the different concentrations of MEFP mixed and incubated at 20 °C for 30 min. The absorbance for both MEFP and standard ascorbic acid were taken at 517 nm and the percentage inhibition calculated using the relationship:

% of radical Scavenging activity = \( \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100 \)

**Determination of Total Phenolic content of Ficus Platystyllyla Methanol Stem Bark Extract**

The total phenolic content of MEFP was determined using Folin-Ciocalteu reagent (Kalantar et al., 2018). After preparation of different concentrations of MEFP, 0.5 ml of Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 0.4 ml of 7.5% sodium carbonate were added. The tubes were mixed gently for 15 s and allowed to stand for 30 min at 40 °C for colour development. The absorbance of the resulting colour complex was measured at 765 nm. The percentage yield was calculated as follows:

% yield = \( \frac{W_2}{W_1} \times 100 \)

Where \( W_2 \) = Weight of extract (g); \( W_1 \) = Weight of plant material (g)
The total phenol content was expressed in milligrams of gallic acid equivalents per gram of MEFP.

**Experimental Animals**

Male Wistar rats, weighing 166-188 g were acquired from ABU-Zaria, animal facility centre. They were allowed to acclimatize for 2 weeks in well-built cages kept in a hygienic environment with housing condition maintained at 25 ± 2 °C at 12-hour day/night cycles before starting the experiment. The animals were allowed access to feed and water *ad libitum*. The study protocol was approved by the animal research ethical committee of the Department of Pharmacology and Toxicology UDUS (PTAC/Fp/(ME)/OT/26-20). The care and handling of the animals were according to the established public health guidelines (NIH, 1985).

**Grouping of Experimental Rats and Induction of liver injury**

Six experimental groups comprising 6 rats each were grouped as follows: Group I served as the normal healthy (positive) control group and was given 5 ml/kg of normal saline. Groups II and III were the negative and standard drug treated groups and received 5 ml/kg normal saline and 100 mg/kg silymarin, respectively (Ugwah-Oguejiofor and Ugwah, 2018). Groups IV – VI were administered 100 mg/kg, 200 mg/kg and 400 mg/kg MEFP, respectively. Treatment lasted for 7 days after which a single dose of 1.5 ml/kg CCl₄ in olive oil (1:1) p.o. was given to the rats in Groups II-VI. After 24 hours of CCl₄ administration, rats were sacrificed and blood samples collected. The livers were harvested, weighed and divided into two parts. One part was fixed in 10% phosphate-buffered neutral formalin and used for histopathological assessment while the other part was homogenised, and prepared for tissue antioxidant enzyme assays.

**Biochemical Assays**

The collected blood samples were centrifuged at 4000 x g for 10 minutes. Sera obtained were pipetted into labelled tubes for estimation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes using the method of Reitman and Frankel (1957). For antioxidant enzyme assays, preserved liver tissue as described was rinsed with phosphate buffered saline (PBS) solution (pH 7.4) to remove traces of blood cells and clots. The tissue was homogenised in 10 ml of cold buffer (50 mM Tris-HCl pH 7.5, 5 mmol EDTA, and 1 mmol DTT) per gram tissue. The homogenate was centrifuged at 4000 x g for 15 minutes. Estimation of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were carried out using the supernatant. The tests were carried out using the respective kits and following manufacturer’s specifications.

**Histopathological Examination of liver Tissues of Experimental Rats**

The fixed liver tissues were dehydrated in an ascending series of alcohol, cleared in xylene, and embedded in paraffin wax (60°C melting point). They were sectioned serially (5 µm thick) and stained using haematoxylin and eosin dyes (H&E). The sections were then mounted on a photomicroscope (Carl Zeiss Microscope) and examined at Magnification x 100.

**Data Analysis**

Data were presented as mean ± standard error of mean (SEM). The results were analysed using GraphPad Prism version 6 software. Multiple comparison of means amongst groups were made using one-way analysis of variance (ANOVA) Tukey post hoc test. Differences at p<0.05 were considered statistically significant. IC₅₀ value for both MEFP and ascorbic acid were calculated using GraphPad Prism version 6.

**Results**

**Yield and Total Phenolic Content of *Ficus Platypylila* Methanol Stem Bark Extract**

A percentage yield of MEFP was observed to be 11.4% w/w while the total phenolic content was found to be 7.99 mg/ml expressed as gallic equivalent (GAE/ g extract).

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Effect of MEFP on DPPH Scavenging Activity
The MEFP DPPH scavenging activity showed that there was absorption band at 517 nm in visible region and DPPH solution decolourized as the colour changes from deep violet to light yellow. The percentage inhibition of MEFP and ascorbic acid are presented in Table 1. While the IC$_{50}$ of MEFP was 3.77 µg/mL, that of ascorbic acid was 12.88 µg/mL.

<table>
<thead>
<tr>
<th>CONCENTRATION (mg/mL)</th>
<th>% INHIBITION FOR MEFP</th>
<th>% INHIBITION FOR ASCORBIC ACID</th>
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<tbody>
<tr>
<td>6.25</td>
<td>91.51</td>
<td>97.37</td>
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<tr>
<td>3.13</td>
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<td>0.78</td>
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<td>0.39</td>
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MEFP IC$_{50}$ = 3.77 µg/mL; Ascorbic acid IC$_{50}$ = 12.88 µg/mL

Effect of MEFP on Liver Function Parameters
Administration of CCl$_4$ in the rats produced an increase in ALT and AST (Figure 1). Pre-treatment with MEFP at doses of 100 and 400 mg/kg inhibited the CCl$_4$–induced oxidative stress by significantly (p<0.05) decreasing the serum levels of ALT and AST when compared to the negative (CCl$_4$) control group.

Effect of MEFP on Liver Antioxidant Enzymes Activities
Administration of CCl$_4$ produced lower activity of CAT. MEFP administration at all dose levels caused an increase in CAT activity in the liver enzymes assay which was significantly (p<0.05) higher than the negative control group (Figure 2). The extract in a dose dependent manner caused a rise in the SOD activity which was initially decreased as a result of CCl4 administration. The
significant increase was more at the doses of 200 and 400 mg/kg than at 100 mg/kg treatment groups when compared with the negative control group (Figure 3). Pretreatment of the animals with MEFP at all dose levels showed a significant (p<0.05) increase in hepatic GPx activity with respect to the negative control group (Figure 4).

**Figure 2:** Effect of methanol extract of stem bark of *Ficus platyphylla* (MEFP) on catalase (CAT) activity
*p* < 0.05 compared with CCl₄ group, n=6

**Figure 3:** Effect of methanol extract of stem bark of *Ficus platyphylla* (MEFP) on superoxide dismutase (SOD) activity
*p* < 0.05 compared with CCl₄ group, n=6

**Figure 4:** Effect of methanol extract of stem bark of *Ficus platyphylla* (MEFP) on glutathione peroxidase (GPx) activity
*p* < 0.05 compared with CCl₄ group, n=6
Histopathology of the liver of MEFP treated rats
The histopathological examination of the liver tissues of both the controls and treated animals are shown in Figures 5 A-F. The liver of rats in the positive control group showed hepatocytes with normal architecture (Figure 5A), whereas the liver of rats in the CCl₄ control group, showed distorted hepatic architecture as evident by intense ballooning and necrosis of hepatocytes (Figure 5B).

**Figure 5**: Micrograph section of hepatic tissue of MEFP treated rats exposed to CCl₄ intoxication
Key: (A) (Group I) Normal saline treated group - well preserved hepatic architecture, portal tract (Long arrow) and normal hepatocyte (short arrow); (B) (Group II) CCl₄ treated group - Distorted hepatic architecture evidence by Ballooning +++ (Long arrow) and Necrosis of the hepatocytes +++ (arrow); (C) (Group III)Silymarin treated group for 7 days: Preserved hepatic architecture, portal tract (long arrow) and sinusoid (short arrow) (D) (Group IV) CCl₄ + methanolic stem bark extract of Ficus platyphylla 100 mg/kg for 7 days: Ballooning ++, hepatocytes vacuoles ++ (micro vesicular steatosis) (short arrow), preserved architecture and portal tract (long arrow) (E) (Group V) CCl₄ + methanolic stem bark extract of Ficus platyphylla 200 mg/kg for 7 days: Ballooning + (short arrow), vacuole ++ (micro vesicular steatosis) (F) (Group VI) CCl₄ + methanolic stem bark extract of Ficus platyphylla 400 mg/kg for 7 days: Preserved hepatic architecture Ballooning +, vacuoles ++ Magnification x 100.
+ = Low; ++ = Moderate; +++ = High

**DISCUSSION**
The study showed that exposure to CCl₄ (1.5 ml/kg) caused an elevation in the serum AST and ALT enzymes levels and a reduction in the activities of the hepatic antioxidant liver enzymes as observed in samples obtained from the negative control group (Group II). CCl₄ causes acute oxidative injury to the liver leading to oxidative damage and leakage of AST and ALT in the serum (Kaur et al., 2006; Ihedioha et al., 2019). Treatment with MEFP probably protected liver hepatocytes as levels of AST and ALT were significantly (p < 0.05) reduced when compared with that of the negative control group. Activities of serum ALT and AST are considered markers for the estimation of liver injury (Liu et al., 2017; Yan et al., 2019). Findings in this study suggest that MEFP treatment may have preserved the integrity of hepatic cellular membrane against CCl₄ toxicity. This result is consistent with findings on hepatoprotective properties of Ficus species (Singab et al., 2010; Al-Musayeib et al., 2017; Sheidu et al., 2020).
This result suggests MEFP hepato-protection sustained antioxidant enzymes activities thereby minimizing incidences of liver injury due to CCl₄ exposure. Catalase and glutathione peroxidise enzymes decompose H₂O₂ to molecular oxygen (O₂) and water (H₂O), thereby preventing the production of free radicals (Bachar et al., 2012; Wang et al., 2017). Similarly, the normal activities of SOD observed in samples obtained from the MEFP treatment groups show the potential of the extract to mediate the activities leading to oxidative stress such as production of superoxide ions (Kanhar and Sahoo, 2019) and other peroxides that cause cell membrane damage in CCl₄ intoxication.

Histopathological examinations of sections of liver tissues of the negative control group show intense ballooning and necrosis of hepatocytes, an indication of CCl₄ damage. However, cell architecture of sections prepared from liver of rats that received 100, 200 and 400 mg/kg treatment of MEFP compared with silymarin standard drug treated group which appears to have minimal ballooning but well preserved portal tract. The preserved histological architecture of hepatocytes in the groups that received treatment supports the biochemical findings which indicate MEFP protects against CCl₄ damage. Constituents of the plant extract may have played a role in the observed hepatoprotective potential of Ficus platyphylla. The rich phenolic content of MEFP may have prevented inflammatory response and oxidative damage to hepatocytes (El-hawary et al., 2019) as observed in this study. Phytochemicals such as flavonoids with antioxidant potential and free radical-scavenging ability contribute to the hepatoprotective potential of plants (Lawal et al., 2014) probably through neutralizing effects of electron transport to free radicals and activation of antioxidant enzymes (Alam and Sharma, 2020).

CONCLUSION
From the present study, the elevated levels of liver antioxidant markers and histopathological examinations of the liver suggest MEFP has a protective role against CCl₄ toxicity The radical scavenging activity of the extract may partly be responsible for the hepatoprotective mechanism of the plant. Further studies are needed to isolate and characterise the active principle responsible for this activity.

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REFERENCES


