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Toxicity Screenings of *Ficus platyphylla* Stem Bark in Rats

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Abstract: Preparations of *Ficus platyphylla* Del. Holl. (Moraceae) have been used in the Nigerian traditional medicine for the management of epilepsy, psychosis, depression, pain and inflammation for many years and their efficacy is widely acclaimed among the Hausa communities of northern Nigeria. Here, we evaluated the acute and sub-acute toxicological properties of the methanol extract of *F. platyphylla* stem bark in rats, in order to determine its safety and to complement earlier efficacy studies on this widely used medicinal plant. Signs of toxicity, body weight changes, relative organs weight, feed and water consumption were monitored following 28 days of daily oral administration of graded doses of the extract in rats. Effects of the extract on haematological and biochemical parameters were also examined. The oral LD_{50} of the extract was estimated to be >5000 mg kg^{-1}. The body weights of treated rats increased progressively, but the changes were not significantly different from the control groups. The extract neither produces significant changes in feed and water consumption nor affected the relative organs weight. Although, some insignificant variations were observed in haematological and biochemical indices, these important parameters were normal and within acceptable limits. No lesions or pathological changes of the organs attributable to treatment with the extract were observed from the pathological examinations. Present results provided evidence on that short-term administration of the methanol extract of *F. platyphylla* at doses lower than 1000 mg kg^{-1} is safe in rats and may not exert severe toxic effects.

Key words: *Ficus platyphylla*, acute toxicity, sub-acute toxicity, haematology, biochemistry

INTRODUCTION

The World Health Organization estimated that about 80% of the people that are living in developing countries use exclusively traditional medicines to treat their health problems. In developed countries more recently, interest has risen in the value of plants as sources of new drug candidates and in herbal medicines for healthy lifestyles. Traditional medical practitioners make claims of successful treatments of diseases using a variety of herbal medicines often with little supporting evidence. A proper scientific evaluation of these herbal medicines with pertinent emphasis on established pharmacological and toxicological paradigms is imperative in order to determine their efficacy and safety.

Ficus platyphylla Del. Holl. (Moraceae) is a deciduous plant locally known as "gamji" and widely distributed throughout the savannah region of the West African coast. Preparations of the plant have been used in the Nigerian traditional medicine for management of insomnia, epilepsy, psychosis, depression, pain and inflammation for many years and their efficacy is widely acclaimed among the Hausa communities of northern Nigeria (Audu, 1989). The cold water extract, decoction or powder of the stem or root bark are usually taken orally, while the powder is often mixed with food and eaten, or placed in burning charcoal and inhaled (Audu, 1989). Our previous studies revealed that the plant contain biologically active substances with potential central nervous system, antinociceptive, anti-inflammatory, and gastrointestinal activities in rodents (Gamaniel *et al*., 2000; Amos *et al*., 2001, 2002; Chindo *et al*., 2003, 2008, 2009).

Despite the diversified utilizations, widely acclaimed and proven efficacy of preparations of *F. platyphylla* stem bark in the Nigerian traditional medicine, the safety profile of this important medicinal plant has not been elucidated. The aim of the present study is to evaluate the acute and sub-acute toxicities of the methanol extract of

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its stem bark in rats in order to complement earlier efficacy studies on this widely used medicinal plant.

MATERIALS AND METHODS

Plant material: The plant material was collected from Zaria, Kaduna State, Nigeria. The plant was identified and authenticated by Mallam I. Muazzam of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. A voucher specimen (No. 4035) was deposited at NIPRD Herbarium for future reference.

Preparation of extract: The stem bark of *F. platyphylla* was chopped, cleaned, air dried for 10 days and milled into coarse powder using pestle and mortar. Extraction was carried out by cold maceration of 500 g of the coarse powder with 2.5 L of 70% v/v methanol for 72 h, with constant shaking using the GFL shaker (No. 3017GBh, Germany). The resultant mixture was filtered using Whatman filter paper (No.1) and the filtrate was concentrated to dryness *in vacuo* at 40°C using rotary evaporator to give a yield of 25% w/w of the extract. Aliquot portions of the extract were weighed and dissolved in distilled water for use in the study.

Animals: Wistar rats of either sex, obtained from Animal Facility Centre (AFC) of NIPRD were used for the studies. The animals were kept in plastic cages and housed under standard conditions of temperature, relative humidity and light/dark cycles (12/12 h). They were fed with standard diet (Ladokun Feeds, Plc, Ibadan, Nigeria) and water *ad libitum*. These animals were approved for use by the AFC committee after reviewing the protocol for good laboratory practice and animal handling, which is in compliance with the National Institutes of Health Guide for the Care and use for Laboratory animals (Publication No. 85-23, revised 1985).

Acute toxicity studies: The oral median Lethal Dose (LD_{50}) of the methanol extract of *F. platyphylla* stem bark was determined in rats by a modified method of Lorke (1983). The study was carried out in two phases. In the first phase, eighteen rats of either sex randomly divided into three groups of six rats each were, respectively administered 10, 100 and 1000 mg kg^{-1} of the extract orally. In the second phase of the study, 2000, 4000 and 5000 mg kg^{-1} of the extract was administered orally to another fresh set of three groups of six rats per group. In each of the two phases of the study, the rats were kept under the same conditions and observed for signs of toxicity and mortality for the first critical 4 h and thereafter daily for 7 days (Aniagu *et al*., 2004; Salawu *et al*., 2009). The oral median lethal dose is determined as the geometric mean of doses that caused 0 and 100% mortality (if any), respectively Lorke (1983).

Sub-acute toxicity studies: Sub acute toxicity study was carried out in accordance to WHO (1992) and OECD (1995) guidelines. Forty eight Wistar rats of either sex deprived of food for 24 h, were randomly divided into six test groups of eight animals per group (n = 8). The test groups received graded doses (50-1000 mg kg^{-1}) of the extract, feed, and water; while the control group received feed and water. The treatment continued for 28 days. All the animals were observed daily for clinical signs and mortality once before dosing, during dosing and up to 4 h after dosing throughout the 28 days' treatment period. The body weight of each rat was measured at specific times using a sensitive balance, once during the acclimatization period, once before commencement of dosing (D1) and once every 7 days (D7, D14, D21, D28) during the dosing period. The amounts of feed and water consumed were measured daily as the difference between the quantity of feed and water supplied each day and the amount remaining post 24 h.

At the end of treatment period (28 days), animals were humanely anaesthetised with Ketamine and blood collected for sera preparation by cardiac puncture. The essential organs including the hearts, livers, lungs, spleen, kidneys, brains, uteruses and testes were surgically dissected out and weighed in grams. The Relative Organ Weight (ROW) was then calculated as follows:

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of rat on sacrifice day (g)}} \times 100$$

Haematological and biochemical analysis: Blood samples obtained at the end of the 28-day treatment period were analysed immediately after blood collection using the Human Automated Haematology System Analyser (HumaCount Plus; Human Gesellschaft fur Biochemica and Diagnostica MBH, Wiesbaden, Germany). Parameters analysed include haemoglobin, packed cell volume, White Cell Count (WBC), red blood cell count, Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), platelets (PLT) and differential leukocyte counts (neutrophils, basophils, eosinophils and lymphocytes).

For biochemical analysis, a portion of the blood sample from each animal was dispensed into plain bottles, allowed to clot and centrifuged at 3500 rpm for 10 min. The sera was separated, stored at -4°C and used for evaluation of biochemical parameters, which include alanine transaminase (ALT), aspartate transaminase (AST) levels and alkaline phosphatase (ALP) levels, albumin, total and direct bilirubin, serum urea nitrogen and

creatinine. Serum biochemical examinations were carried out with an automated chemistry analyzer (Cobas Mira, Roche) using commercial kits obtained from Randox Laboratories, UK.

Histopathological study: Histopathological investigation was done according to methods described in the literature (Vasilescu *et al*., 1955; Akdogan *et al*., 2003; Abd-Elhamid, 2004; Rosidah *et al*., 2009). Briefly, small blocks of tissues were taken from the brain, liver, kidney, heart and spleen and fixed in Bouin's fluid for 16-24 h, then dehydrated through changes of an ascending grade of alcohol (70, 90, 96% and absolute). The tissues were cleared in xylene and embedded in paraffin. Sectioning was done at 6 μm as thickness using a rotary microtome and dried overnight in the oven at 37°C. Tissue slices were processed according to the method described by Lison (1960) and stained with haematoxylin and eosin.

Statistical analysis: Data were expressed as Mean±SEM Data were analyzed by one-way ANOVA followed by Dunnett's post hoc test for multiple comparisons and $p<0.05$ was considered significant.

RESULTS

Acute toxicity studies in rat: Lethal effects were not observed after the oral administration of the methanol extract of *Ficus platyphylla* stem bark (1250, 2500 and 5000 mg kg^{-1}). No behavioural changes were observed during the observation period. The oral LD_{50} of the methanol extract of *Ficus platyphylla* stem bark was estimated to be greater than 5000 mg kg^{-1}.

Sub-acute toxicity studies in rat: The sub-acute oral administration of graded doses (50-1000 mg kg^{-1} daily) of the methanol extract of *Ficus platyphylla* stem bark resulted in insignificant changes in the body (Fig. 3) and relative organ weights (Fig. 4) of the treated rats compared to control. The extract neither produces significant changes in water nor food consumption in treated rats compared to control group (Fig. 1, 2). Both the control and rats treated with the methanol extract of *Ficus platyphylla* stem bark appeared uniformly healthy at the end of the experiment as well as throughout the 28 day period.

Haematological and biochemical parameters in rats: The haematological parameters analyzed include the Red Blood Cell Count (RBC), Haemoglobin Concentration (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), platelets, White Blood Cell Count (WBC) and white blood cell differential count (Fig. 5, 6). Although, some insignificant variations

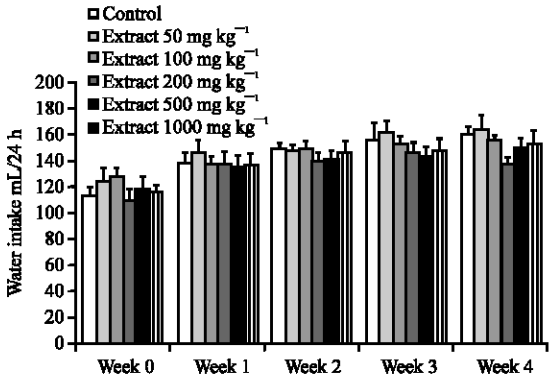

Fig. 1: Effects of extract on water intake. Data are expressed as the Mean±SEM, All values in the test groups are not significantly different from control group

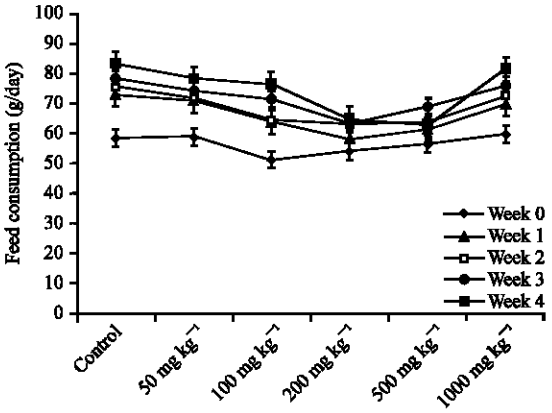

Fig. 2: Effects of extract on feed intake. Data are expressed as the Mean±SEM, All values in the test groups are not significantly different from control group

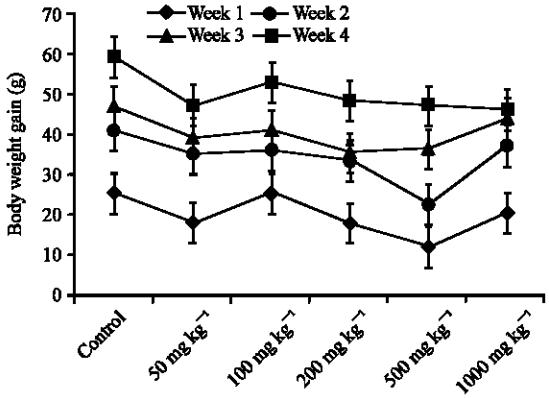

Fig. 3: Effects of extract on body weight gain in rats. Data are expressed as the Mean±SEM, All values in the test groups are not significantly different from control group

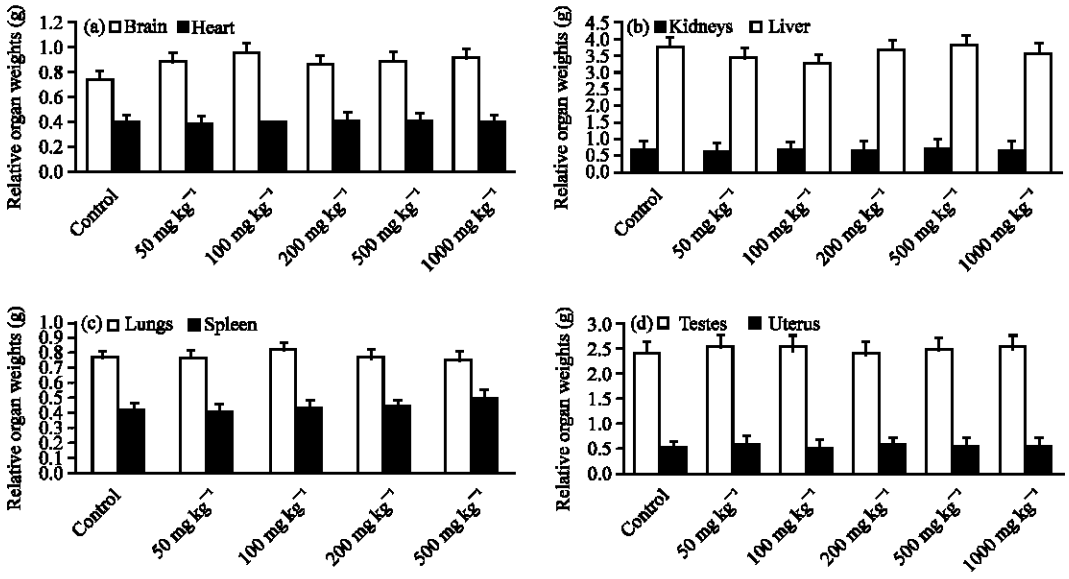

Fig. 4 (a-d): Effects of the effects of the extract on relative organs weights, Data are expressed as the Mean±SEM. All values in the test groups are not significantly different from control group, Effects on (a) Brain and heart, (b) Kidneys and liver, (c) Lungs and spleen and (d) Testes and uterus

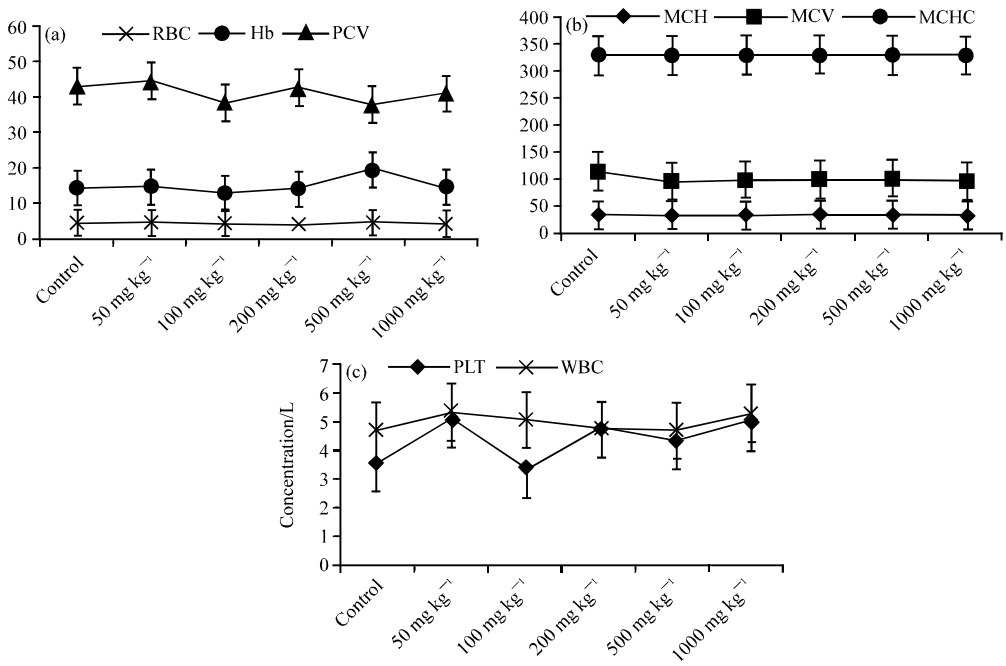

Fig. 5 (a-c): Effect of extract on haematological parameters, (a) Red blood cell count {RBC ($\times 10^{12}$ L^{-1})}, hemoglobin {Hb (g dL^{-1})}, Packed cell volume {PCV (%)}, (b) Mean corpuscular hemoglobin {MCH (pg $cell^{-1}$)}, Mean corpuscular volume {MCV (μm^3)}, Mean corpuscular hemoglobin concentration {MCHC (g L^{-1})}, (c) Platelets {PLT ($\times 10^9$ L^{-1})}, White blood cells {WBC ($\times 10^9$ L^{-1})}, Data are expressed as the Mean±SEM, All values in the test groups are not significantly different from control group

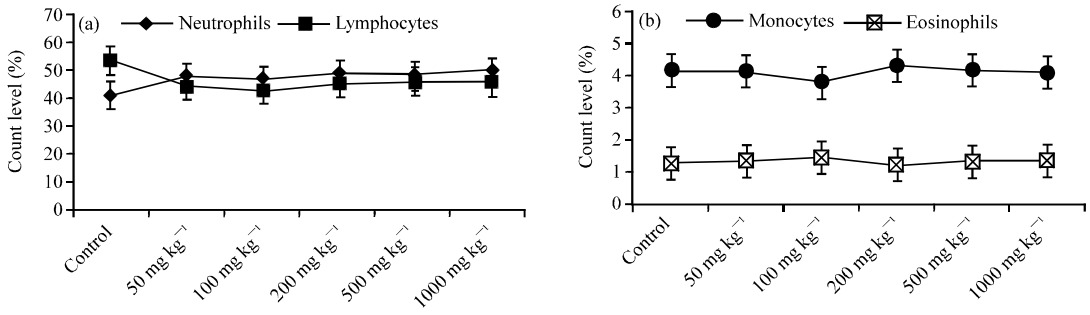

Fig. 6 (a-b): Effects of extract on WBC differential counts. Data are expressed as the Mean±SEM, All values in the test groups are not significantly different from control group, (a) Effects on neutrophils and lymphocytes, (b) Effects on monocytes and eosinophils

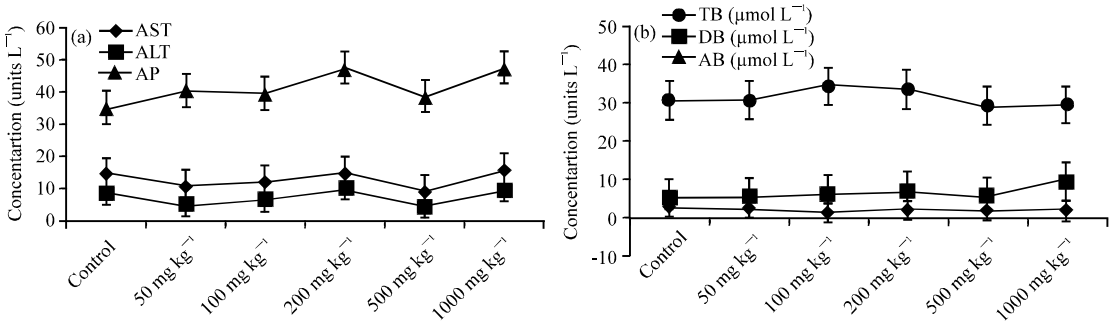

Fig. 7 (a-b): Effects of extracts on liver function parameters. Effects on (a) Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AP) and (b) Total bilirubin (TB), direct bilirubin (DB) and albumin (AB), Data are expressed as the Mean±SEM, All the values in the test groups are not significantly different from control group and within the normal range

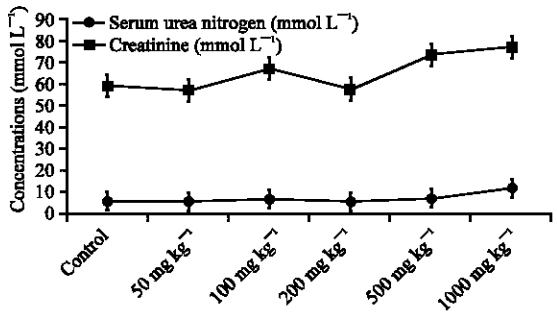

Fig. 8: Effects of extract on serum urea nitrogen and creatinine, Data are expressed as the Mean±SEM, All values in the test groups are not significantly different from control group and within the normal range

were observed in haematological indices, these important parameters were normal and within acceptable limits throughout the experimental period in both treated and control groups.

The biochemical studies showed insignificant differences between treated and control groups in all the parameters analyzed (Fig. 7, 8).

Histopathological study: No lesions or pathological changes of the organs attributable to treatment with the extract were observed from the pathological examinations of the organs (data not shown).

DISCUSSION

Herbal medicines, which are believed to be harmless, are gaining popularity in developing countries. These remedies are "natural" and commonly used for self-medication without supervision. Although, medicinal plants may cause several biological activities in humans, very little is known regarding the potential toxicity for many of these bioactive substances, such as *F. platyphylla*. The present study revealed that the methanol extract of *F. platyphylla* stem bark is relatively non-toxic when administered orally to rats.

The acute toxicity study indicated that the extract at a dose of 5000 mg kg^{-1} caused neither visible signs of toxicity nor mortality in rats, suggesting its safety. The extract with an LD_{50} >5000 mg kg^{-1} p.o. is considered to be non-toxic. This analysis is based on the toxicity classification proposed by Loomis and Hayes (1996) and Rosidah *et al*. (2009) viz., that substances with an LD_{50} values from 500 to 5000 and 5000 to 15,000 mg kg^{-1} body weight are regarded as slightly toxic and practically non-toxic, respectively. Generally, reduction in body weight gain and relative organ weights is a simple and sensitive index of toxicity after exposure to potentially toxic substances (Teo *et al*., 2002). In the present study, the methanol extract of *F. platyphylla* stem bark did not significantly affect body or relative organ weight as compared to the control group, which suggests that the extract did not hinder rat growth.

The haematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status for both animals and humans (Adeneye *et al*., 2006). After 28 days of treatment, with *F. platyphylla*, there were no treatment-related changes in the haematological parameters between the control and treatment group, indicating that the methanol extract of *Ficus platyphylla* stem bark does not affect haematopoiesis and leucopoiesis in rats. The orally administrated doses of the extract were not-toxic and did not interfere with the production of circulating red blood cells, white blood cells and platelets. In addition, there were no significant differences in biochemical parameters of the groups treated compared to the control. The lack of significant alterations in the levels of ALT, AST, creatinine and BUN are good indicators of liver and kidney functions (El Hilaly *et al*., 2004), which suggests that sub-acute administration of the methanol extract of *F. platyphylla* stem bark did not alter the hepatocytes and kidneys of the rats. In the present study, histopathological evaluation of the sub-acute oral ingestion of the methanol extract of *F. platyphylla* stem bark did not adversely affect the morphology of rat organs, which corroborate the results from biochemical analysis. Oral administration of 1000 mg/kg of the extract daily for 28 days was well tolerated by the treated rats and this dose is substantially higher than doses that demonstrated central nervous system, antinociceptive, anti-inflammatory and gastrointestinal activities in rodents (Gamaniel *et al*., 2000; Amos *et al*., 2001, 2002; Chindo *et al*., 2003). Extrapolation of these results to humans suggests that the methanol extract of *F. platyphylla* stem bark should be relatively safe for usage at doses between 50 and 1000 mg kg^{-1}.

In conclusion, the oral administration of graded doses (50-1000 mg kg^{-1}) of the methanol extract of *F. platyphylla* stem bark to rats for 28 days did not result in death and was not associated with adverse effects reflected in the general condition, growth, body and relative organ weights, haematology, or clinical biochemistry values and did not result in histopathological abnormalities. According to these results, the methanol extract of *F. platyphylla* stem bark could be categorized as a No-Observed-Adverse-Effect Level (NOAEL) crude drug that acts harmlessly under the current normal usage (Copplestone, 1988), which is considered to be of no toxicological concern (WHO, 1987). The short-term oral administration of this important medicinal plant at doses lower than 1000 mg kg^{-1} may not exert severe toxic effects.

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