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Phytochemical Screening, Acute and Sub-chronic Toxicity Studies of Aqueous Stem Bark Extract of *Ficus polita*

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Ficus polita Vahl is a tropical African evergreen shrub or small tree belonging to the family Moraceae, and usually growing up to 15 metres tall. Extracts from different part of the plant had been demonstrated to exhibit various medicinal activities. Qualitative phytochemical screening of aqueous stem bark extract of *F. polita* was determined. Acute (LD_{50}) and sub-chronic oral toxicity studies of the extract of *F. polita* were evaluated in *wistar* albino rats. Phytochemical screening of

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the extract revealed the presence of alkaloids, polyphenols, flavonoids, glycosides, anthraquinones, saponins, tannins, fats and oils, terpenoids, carbohydrates, starch, gums and mucilages, and proteins. The LD_{50} of this extract was estimated to be more than 5000 mg/kg. Oral administration of the extract (1000, 2000, 3000, and 4000 mg/kg body weight) revealed significant difference ($p<0.05$) in haematological parameters RBC, haemoglobin, PCV, MCV, MCH, and lymphocytes, in some of the treated doses. There was no significant increase ($p>0.05$) in MCHC, WBC, platelets, and PDW, in some of the treated doses when compared with control. Analyses of serum total protein, albumin, creatinine, urea, sodium, and chloride revealed significant changes ($p<0.05$) in some of the treated doses compared to their controls. However, no significant differences ($p>0.05$) were observed in serum liver enzymes (AST and ALT), total bilirubin, direct bilirubin, and potassium of some of the treated doses when compared to their controls. These results suggest that the aqueous stem bark extract of *F. polita* is rich in phytochemicals, and may be considered relatively safe at the tested doses.

Keywords: Ficus polita; toxicity; aqueous; stem bark; extract.

1. INTRODUCTION

Plants, either as traditional preparations or pure active principles, have always been among the common sources of medicines [1]. Over 5000 plants are known to be used for medicinal purposes in Africa, but only a few have been studied [2]. A great number of Nigerian higher plants are traditionally noted for their medicinal properties, but regrettably only few have so far been studied for their active constituents. The use of herbal remedies can be attributed to their perceived efficacy and the fact that they are a cheap source of medicines [3].

There is an assumption that the use of plants in traditional medical practice for treatment of various ailments is harmless and safe because they are derived from natural sources [4]. This assumption is based on the common belief that herbs are by nature safer and gentler than orthodox drugs and plant-based medicine have been used in the treatment of diseases over many centuries [5].

However, herbal preparations assumed to be safe may contain contaminants such as pathogenic microbes [6], heavy metals [7] and aflatoxins [8] due to the manner in which they are prepared. In addition, they are often administered over an extended period during disease management without consideration of long term toxic effects [9]. Moreover, many studies have reported various toxic effects of herbal medicines, such as hepatotoxicity [10] and nephrotoxicity [11].

F. polita Vahl is a tropical African evergreen shrub or small tree belonging to the family Moraceae, and usually growing up to 15 metres tall, and sometimes to 40 meters tall. The plant is commonly known as Hartblaarvy, Heart-leaved fig, polish fig, rubber plant, wild rubber fig, wild rubber tree [12]. The leaf extracts had been demonstrated to exhibit antimalarial activity [13]. Extracts of methanol and dichloromethane from the plant had also been reported to exhibit anti-inflammatory activities [14]. The methanolic extract of the root had been found to possess antimicrobial activities and several compounds had been isolated from it [15].

Investigation of phytochemicals and determination of efficacy and safety of herbal remedies is necessary as many people use them for self - medication. It should, therefore, be emphasised that the traditional use of any plant for medicinal purposes warrants the safety of such plant. Plants in folk medicine should, therefore, be evaluated for safety or toxicity and necessary recommendations be made on their use. Therefore, the current study was aimed at investigating the phytochemical composition, and determining the acute (LD_{50}) and sub-chronic toxicity of the aqueous stem bark extract of *F. polita*. For the sub-chronic toxicity test, haematological indices, as well as liver and kidney function parameters of the tested rats were assessed.

2. MATERIALS AND METHODS

2.1 Study Animals

Male and female albino rats weighing between 100 - 200 g were purchased from animal house of Biological Science Department; Bayero University, Kano. The animals were housed in well-ventilated cages in the animal house of Biological Science Department of Bayero University Kano. The rats were allowed to

acclimatize for one week prior to the experiment and had free access to food and clean water.

2.2 Collection and Preparation of Plant Material

Stem bark of *F. polita* were obtained from Kofar Marusa New Lay-out, Katsina, Nigeria. The plant was identified and voucher specimen (BUKHAN 0104) was deposited at the Herbarium Unit of the Department of Plant Biology, Bayero University Kano, Nigeria. Pieces of the stem bark were air dried under shade for two weeks and ground into powder using a mortar and pestle. The dry powder (500 g) was soaked in 5 litres of distilled water, and vigorously shaken, for 48 hours, at room temperature. The mixture was filtered with muslin cloth and later with Whatman Number 1 Filter Paper. The filtrate (extract) was concentrated to dryness in an oven at 45℃. Percentage yield was calculated, and the extract was stored in a plastic container until required. The dried extract was reconstituted in distilled water, at different concentrations, until required for use.

2.3 Phytochemical Screening

Phytochemical tests were carried out by using the standard methods of [16-21].

2.4 Lethal Mean Dose (LD_{50}) Determination

The limit test procedure described by Organisation for Economic Cooperation and Development (OECD) guidelines was adopted [22]. Five (5) rats of either sex, selected by random sampling, were used for the study. The animals were fasted overnight, providing only water, after which the aqueous stem bark extract, was administered orally at a dose level of 5000 mg/kg body weight to each rat at 48 hours interval. Each rat was kept under observation for the first 4 hour and within the period of the 48 hour. Behavioural changes (weakness, difficulty in movement, sedation, hyperactivity, hair loss, reduced response to sound, loss of appetite, grooming), body weight, and mortality were observed for a period of 14 days.

2.5 Sub-chronic Toxicity Studies

A total of 25 rats, divided into five (5) groups of five (5) rats each, were daily administered with different concentrations (based on LD_{50}) of the extract for 28 days. Control group received distilled water, while the other four groups received 1000, 2000, 3000, and 4000 mg/kg body weight of the aqueous stem bark extract, respectively. The rats were fasted overnight. On the 29^{th} day, weight were taken, and the rats were humanely sacrificed. Blood samples were taken in EDTA containers and plane containers, for haematological and biochemical analyses, respectively.

2.6 Haematological Analysis

Haematological analyses were performed using automated haematological analyser (Cell Dyn 3500). The blood parameters analysed were RBC, haemoglobin, PCV, MCV, MCH, MCHC, WBC, lymphocytes, platelets, and PDW.

2.7 Biochemical Analysis

Biochemical analyses were performed on sera obtained after centrifugation of the whole blood. Standardised diagnostic kits (Labkit®) (Randox) and Teco diagnostic kits were used for spectrophotometric determination of the following biochemical parameters: Aspartate aminotransferase and Alanine aminotransferase [23], Bilirubin [24], Albumin [25], Total protein [26], Urea [27], Sodium, Potassium and Chloride [28], and Creatinine [29].

2.8 Statistical Analysis

Results were expressed as mean ± standard error. The data collected were subjected to one-way Analysis of Variance (ANOVA) using Graphad Instat, Version 3.02, Benferoni, (San Diego, USA). Statistical significance was considered at $P<0.05$.

3. RESULTS

Table 1 shows the phytochemical screened from the extract. The result revealed the presence of alkaloids, polyphenols flavonoids, glycosides, cardiac glycosides, anthraquinones, saponins, tannins, fats and oils, terpenoids, carbohydrates, starch, gums and mucilage, and proteins.

The LD_{50} (acute toxicity test) of the aqueous stem bark extract of *F. polita* was estimated to be more than 5000 mg/kg.

Table 2 present the effect of administration of aqueous stem bark extract of *F. polita* on

haemotological parameters The results in showed significant difference ($p<0.05$) for RBC (3000 mg/kg dose), haemoglobin (3000 mg/kg dose), PCV (3000 mg/kg dose), MCV (3000 mg/kg dose), MCH (3000 mg/kg dose), and lymphocytes (3000 mg/kg dose) when compared to their control doses. There was no significant difference ($p>0.05$) for MCHC, WBC, platelets, and PDW.

Table 3 shows the effect of aqueous stem bark extract of *F. polita* on biochemical parameters. The results in showed significant difference ($P<0.05$) for total protein (2000 and 3000 mg/kg doses), albumin (1000, 2000 and 4000 mg/kg doses), creatinine (1000 mg/kg dose), urea (1000, 2000, and 4000 mg/kg dose), sodium (4000 mg/kg dose), and chloride (1000, 2000, 3000, and 4000 mg/kg doses) when compared to their control doses. There was no significant difference ($P>0.05$) for AST, ALT, total bilirubin, direct bilirubin, and potassium.

Table 1. Phytochemical screening of the aqueous stem bark extract of *F. polita*

Phytochemicals	Inference
Alkaloids	+
Polyphenols	+
Flavonoids	+
Glycosides	+
Cardiac glycosides	+
Anthraquinones	+
Saponins	+
Tannins	+
Fats and oils	+
Terpenoids	+
Steroids	-
Anthocyanins	-
Emodins	-
Coumarins	-
Chalcones	-
Carbohydrates	+
Starch	+
Gums and Mucilages	+
Proteins	+

Key: (+) Present (-) Absent

Table 2. Effect of aqueous stem bark extract of *F. polita* on haemotological parameters

Parameters	Control	Aqueous stem bark extract of *F. polita* (mg/kg)			
		1000	2000	3000	4000
RBC ($X10^6$ cells /mm^3)	6.38±0.01	6.84±0.36	5.97±0.98	4.54±0.03*	6.81±0.31
Haemoglobin (g/dl)	11.0±0.09	12.77±0.19	8.00±1.30	7.57±0.08*	11.37±0.36
PCV (%)	41.75±0.13	45.47±1.71	37.05±3.90	28.35±0.05*	42.45±1.20
MCV (μM^3/red cells)	61.11±0.31	62.75±0.70	63.62±0.67	64.32±0.12*	63.05±0.79
MCH (pg/red cells)	16.15±0.09	16.80±0.14	16.85±0.39	16.95±0.17*	16.77±0.17
MCHC (g/dl/red cells)	26.55±0.30	26.62±0.14	26.25±0.35	26.67±0.36	26.52±0.04
WBC ($X10^6$ cells /mm^3)	8.60 ± 0.28	7.18 ± 0.17	5.62±1.68	6.35±1.08	8.22± 0.43
Lymphocytes (%)	76.45±2.79	65.12±0.49	66.70±1.09	82.72±5.17*	69.18±0.06
Platelets ($X10^3$ cells/mm^3)	574.00±69.50	564.50±0.64	565.25±7.71	569.17±4.62	573.71±2.83
PDW (%)	10.40±0.12	10.45±0.49	10.17±0.04	11.83±0.70	10.63±0.56

*Values are mean ± SEM (n=5), Comparisons are between control and tests, *= Significant different (P<0.05), RBC= Red blood cells, PCV= Packed cell volume, MCV= Mean corpuscular volume, MCH= Mean corpuscular haemoglobin, MCHC= Mean corpuscular haemoglobin concentration, WBC= White blood cells, PDW= Platelets distribution width*

Table 3. Effect of aqueous stem bark extract of *F. polita* on biochemical parameters

Parameters	Control	Aqueous stem bark extract of *F. polita* (mg/kg)			
		1000	2000	3000	4000
AST (u/l)	112.83±2.10	110.29±3.32	111.31±2.13	109.91±2.32	110.31±3.11
ALT (g/dl)	46.90±2.90	47.32±1.32	47.96±2.11	46.32±2.34	45.31±4.11
Total Bilirubin (µmol/l)	1.27±0.01	1.29±0.02	1.31±0.02	1.28±0.03	1.31±0.01
Direct Bilirubin (µmol/l)	1.15±0.01	1.12±0.02	1.16±0.01	1.26±0.01	1.14±0.02
Total Protein (g/dl)	6.81±0.01	7.37±0.21	7.90±0.62*	7.91±0.13*	7.11±0.27
Albumin (g/dl)	1.38±0.05	1.62±0.02*	1.57±0.08*	1.46±0.07	1.54±0.13*
Creatinine (mg/dl)	32.02±0.02	30.04±0.47*	32.47±0.29	33.24±1.15	33.51±0.36
Urea (mg/dl)	9.94± 0.49	8.27± 0.16*	8.99±0.18*	9.65±0.16	7.99±0.25*
Sodium (mEqL/L)	68.29±0.02	69.96±0.60	68.06±0.92	67.68±0.46	66.07±0.81*
Potassium (mEqL/L)	2.85±0.02	2.83±0.01	3.00±0.01	2.76±0.14	2.76±0.07
Chloride (mEqL/L)	90.89±2.08	68.33±0.05*	82.27±0.05*	113.20±1.90*	77.98±0.80*

*Values are mean ± SEM (n=5), Comparisons are between control and tests, * = Significant different (P<0.05), AST= Aspartate aminotransferase, ALT= Alanine aminotransferase*

4. DISCUSSION

Secondary metabolites are biosynthesized in plants for different purposes including growth regulation, inter and intra-specific interactions and defense against predators and infections [30]. Plants are recognised for their ability to produce a wealth of these secondary metabolites and mankind has been using many species for centuries to treat a variety of diseases [31]. Many of these natural products have been shown to demonstrate interesting biological and pharmacological activities and are used as chemotherapeutic agents or a starting point in the development of modern medicine [32]. However, some plant extracts could be inherently dangerous, containing naturally occurring toxins, which may be cytotoxic or carcinogenic [33].

In the acute toxicity study (LD_{50}), a dose of 5000mg/kg body weight for the tested animals produced no mortality after 72 hours of observation and up to 14 days period. It also had no adverse effects on the behavioural responses of the tested rats after 14 days of observation. Neither mortality nor weight loss occurred. In screening drugs, determination of LD_{50} is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. It is an initial assessment of toxic manifestations and is one of the initial screening experiments performed with all compounds. Data from the acute toxicity study may; (a) Serve as the basis for classification and labeling; (b) Provide initial information on the mode of toxic action of a substance; (c) Help arrive at a dose of a new compound; (d) Help in dose determination in animal studies; (e) Help determine LD_{50} values that provide many indices of potential types of drug activity.

Haematological indices provide physiological information on the blood picture and the reticuloendothelial system [34], and are therefore used in the diagnosis of diseases and evaluation of the toxic effects of exogenous compounds including plant extracts and drugs [35]. RBC, PCV and haemoglobin are associated with the total population of the red cells, while MCV, MCH, and MCHC are related to individual RBC.

Iron deficiency is the major cause of hypochromic microcytic anaemia, in which case there may be low RBC, heamoglobin and PCV [36]. Thus, the rats administered with 3000 mg kg body weight of the extract may be iron deficient. Moreover, Chaudhari et al. [37] reported iron – chelating capabilities of several phenolics and flavonoids from natural sources. In macrocytic anaemia, there is a decrease in the absolute number of RBC per unit volume. In this case the individual cell is larger in volume and diameter and contains more haemoglobin. As a result, MCV and MCHC are elevated. This form of anaemia is seen in folic acid and vitamin B_{12} deficiencies [38]. Thus, the rats administered with 3000 mg/kg dose of the extract may be deficient in folic acid and vitamin B_{12}. Since only rats administered with 3000 mg/kg dose of the extract significantly differed with the control group, the significant difference observed may not be associated with the extract.

White cells and its indices play a vital immune function. They attack and destroy foreign particles, cell waste materials and bacteria. The lymphocytes help to specifically recognise a diverse range of antigens, differentiate and mature to functional capacity, respond to the antigens and establish immunologic memory [39]. The increased lymphocytes seen in the rats administered with 3000 mg/kg dose demonstrated that the extract boosted the immune system of the affected rats.

Biochemical parameters are useful in toxicity studies by providing information about *in vivo* effects of test substances [40]. Liver is considered the key organ in the metabolism, detoxification, and secretory functions in the body, and its disorders are numerous with no effective remedies [41]. Liver plays a key role in many metabolic processes of not only itself but of other tissues as well. Severe hepatic injury, as a result of metabolism of some of the toxic phytochemicals found in medicinal plants and failure of the metabolic products to be eliminated by the liver may be associated with marked distortion of its functions [42]. Liver enzymes, aspartate and alanine aminotransferases (AST and ALT) are involved in amino acid metabolism. Large amounts of AST are present in the liver, kidney, cardiac muscle, and skeletal muscle [43]. Serum ALT and AST are always found to increase in liver cell damage and the greater the degree of the liver damage the higher the activities of both enzymes [44]. The result (Table 3) showed no significant effects on ALT and AST at all treatment doses.

Bilirubin, a metabolic breakdown product of haem derived from senescent red blood cells [45]. Bilirubin is a useful index of the excretory

function of the liver, in addition to its being a useful tool in assessment of haemolytic anaemia. Raised serum levels of direct and total bilirubin is an indication of an impaired hepatic excretion [46].

The assay of total protein alone may not portray the true picture of the metabolic state of an individual since the concentration of individual proteins do not rise or fall in parallel with one another. Albumin is the most abundant of the plasma proteins with the physiological role of maintenance of osmotic pressure, transportation of endogenous and exogenous substance and serving as protein reserve. The ability of the liver to synthesise albumin is diminished if the synthetic function of the organ is affected [47]. Rapid reduction in serum albumin is therefore apparent in hepatitis and liver cirrhosis. In the present study, the extract did not cause a significant reduction in total protein and albumin. This suggests that sub-chronic administration of aqueous stem bark extract of *F. polita* has no hepatotoxic effects in rats.

Kidney is the major excretory organ in the body, kidney function is affected by a number of factors, which may ultimately result in its failure [48]. Renal function indices such as urea, creatinine, electrolytes, and uric acid can be used to evaluate the functional capacity of the nephrons of study animals and are considered as good indicators of kidney function. Similarly, the serum concentrations of electrolytes, uric acid and creatinine could give an insight into the effect of plant extract on the tubular and or glomerular part of the kidney [49]. Therefore, the nontoxic effect of the aqueous stem bark extract at almost all the doses investigated on the renal function indices may suggest that the normal functioning of the nephrons at the tubular and glomerular levels were not affected. However, a significant decrease in creatinine (1000 mg/kg dose) may not be due to the extract. A significant decrease in urea (1000, 2000, and 4000 mg/kg doses) could be as a result of water overload. Moreover, plasma urea concentration is less reliable than creatinine as an index of glomerulus filtration rate.

Increase or decrease in the levels of electrolytes within serum may be a consequence of the hypo or hyper functioning of the concerned organ or tissue. Sodium, potassium, and chloride are examples of clinical electrolytes used in assessing kidney function [50]. Sodium is the major cation of the extracellular fluid where it regulates acid-base equilibrium and protects the body against excessive fluid loss. Hypernatraemia though rare, may occur in dehydration and steroid hormone administraton. Hyponatraemia, on the other hand, is more common and may be due to chronic sodium losing nephropathy. Loss of gastrointestinal secretion through diarhoea or vomiting, loss from skin as a result of burns, loss from kidneys through the use of diuretics and metabolic loss through starvation or diabetic ketoacidosis [51]. The significant reduction in sodium (4000 mg/kg dose) may be due to metabolic loss through starvation, but not due to the extract. Potassium is the major intracellular cation with similar roles to those of sodium. Excessive renal loss of potassium is associated with diuresis, renal loss as a result of potassium losing nephropathy or as a result of renal tubular acidosis. Other causes of hypokalaemia include excessive loss without adequate replacement as in chronic diarrhoea, malabsorption syndrome, perspiration and chronic fever, chronic stress, poor dietary habit, Cushing's syndrome, hyperaldosteronism, liver disease with ascites, use of some drugs such as indomethacin, phenylbutazone and steroid hormone [52]. Hyperkalaemia is usually encountered frequently in renal failure, improper use of K^+ sparing diuretics, hypoaldosteronism, insulin deficiency associated hyperglycaemia, Addison's disease, and massive tissue destruction. Potassium is used as the most convincing electrolyte marker of renal failure. The combination of decreased filtration and decreased secretion of potassium in distal tubule during renal failure cause increased plasma potassium [53]. Hyperkalaemia is the most significant and life-threatening complication of renal failure [54]. From the present study, there was no significant difference in potassium, but chloride, in all the treated doses.

5. CONCLUSION

From these findings, it is suggested that the aqueous stem bark extract of *F. polita* is rich in phytochemicals that may be responsible for some of the reported pharmacological activities of this plant. It is shown that it will take more than 5000 mg/kg dose of aqueous stem bark extract of *F. polita* to kill 50% of the tested albino rats. The aqueous stem bark extract of *F. polita* is safe, at the tested sub-chronic doses, and well tolerated for the 28 days study period. Thus, the extract has potential for safe use as herbal medicine. The nontoxicity observed in these studies may be connected to the fact that the

secondary metabolites that my likely cause toxic effects may be absent from the extract, or if present, may be in a very negligible nontoxic levels.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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