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Effect of *Ficus capensis* Ethanol Extract on Haematological and Biochemical Markers in Male Wistar Rats

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ABSTRACT

The leaves of *Ficus capensis* plant was screened for hematological, safety and serum enzyme activities in male albino Wistar rats. Fifteen rats were used for the study, they were grouped into five groups of 3 rats each. Group 1 was the negative control group and received dimethyl sulphoxide (DMSO). Groups 2, 3, and 4 were the treatment groups received 500, 600 and 800 mg/kg body weight of the *F. capensis* extract, respectively. Group 5 was the positive control group which received anaemic drug, cyclophosphamide. The rats were dosed for 14 days, thereafter was sacrificed and blood collected by cardiac puncture for analysis. All results in treatment groups were compared with the negative control at statistical confidence of 95% ($p<0.05$). The positive control was used as guide to compare the established anaemic condition. Haematological parameters, packed cell volume (PCV), red blood cell count (RBC), Haemoglobin, total leukocyte count, decreased significantly ($p<0.05$) as the dose of the extract increased from 500 to 800 mg/kg body weight. Differential leukocyte count indicated neutrophilia by statistical increase of neutrophils at ($p<0.05$), and lymphopeania by statistical decrease of lymphocytes at ($p<0.05$). Mean corpuscular volume (MCV) increased indicating macrocytic anaemia and mean corpuscular haemoglobin concentration (MCHC) was normal indicating normochromic anaemia. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), Albumin, Globulin and Total protein (TP) increased as dose of *F. capencis* extract increased. There was an effect of *F. capensis* extract on haematological and biochemical indices which indicated neutrophilia, lymphopeania, macrocytic normochromic anaemia and mild haematological and tissue damage at highest dose.

Keywords: Safety, Serum enzyme, *Ficus capensis*, Haematology, Cyclophosphamide.
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INTRODUCTION

Plants are source of drugs and used for nutritional purposes with some having some toxic side effects. There is growing awareness in alternative therapies and curative use of plants as natural products. For the past fifteen years there has been the search for phytochemicals by native and plant origin for pharmaceutical and nutritional purposes (Oktay et al., 2003) but without the thought of their safety. However, some plants have been found to possess anti-nutritional and toxic factors such as alkaloids, saponins, tannins and glycosides (Bonsi et al., 1995; Ekpenyong et al., 2012). Compounds in *F. capensis* plants have been identified using gas chromatography-mass spectrometry (GCMS) analysis (Igwe et al., 2016) and other plants phytocompounds (Igwe et al.,2015; Puruschotaman and Ravis, 2013). There have been reports of the presence of toxic phytochemicals in plants (Argheore et al., 1998; Ekpenyong et al., 2012), hepatotoxicity of some plant

extracts on mice (Igie et al.,1995), ability of the plant to inhibit and even reverse carbon tetrachloride induced toxicity in rats (Babalola et al., 2001).

This study is therefore designed to examine the actual effect of ethanol extract of *F. capensis* leaves on liver, kidney, lungs, in rats using some serum enzymes as biochemical markers. Tissue cells contain characteristics enzymes which enter the blood only when the cells to which they are confined are damaged or destroyed. The presence in the blood of significant quantities of these specific enzymes indicates a possible site of tissue damage.

The increase or decrease of these enzymes is of diagnostic importance and can be used to ascertain their relationship to metabolic problem (Ophardt, 2003). Specific enzymes assayed for in serum biochemistry are ALT, AST ALP, Gama glutamyl transferase (GGT), Iditol dehydrogenase (ID), glutamate dehydrogenase. *F. capensis* thumb belongs to Family-Moraceae,(Lumbile and Mogotsi,2008), a genius of plants collectively known as figs or fig tree. It is an evergreen tree that is widely distributed in tropics, both leaves and roots have been used for leprosy (Nguyi, 1988; Dafalla, 2005; Oyeleke et al., 2008; Karmen et al.,1955). The leaves are used as vegetable with a reported blood-boosting effect (Otitoju et al., 2014) and antisickling effect of red blood cell (Umeokoli et al., 2013), for treatment of anaemia (Sandabe and Kwari, 2000). Nutritional constituents of *F. capensis* includes flavanoids, reducing sugar, saponins, tannins, anthraquinone, starch, protein, lipid, glycosides (Umeokoli et al., 2015). Mineral and proximate composition showed the presence of iron, cobalt, copper, protein, carbohydrate, crude fibre and ash content (Ihedioha et al., 2015). Compounds identified in *F. capensis* essential oil in higher percentage are; carvacol (65.78%), caryophyllene (29.81%), caryophyllene oxide (25.70%) (Francois et al., 2010) which made *F. capensis* to be classified as phenolic compounds. In the GC-MS work of Igwe et al. (2015a), some of the compounds identified in *F. capensis* are; benzene1,2,3 triol (38.7%), sorbic acid (38.36%), hexadecanoic acid (12.63%), mequinol (0.83%). Locally, *F. capensis* is used for treatment of anaemia in southeast Nigeria (Umeokoli et al., 2015). There are specific enzymes which when present in the blood in significant quantities indicate the probable site of damage. They include aldolase, creatinine, phosphokinase, gamma glutanyl transfarase, lipase, ALT, AST, (Karmen et al.,1955; Ophardt, 2003). The two major proteins found in the blood are albumin and globulin. These proteins can be measured individually or combined into single test called TP which measures all proteins in the sample (Kristina and Margo,2017).

The protein in plasma includes albumin, globulin, fibrinogen and other clotting factors. Serum proteins are made up of albumins and globulins only (Doumas and Peters, 1997). Testing these substances provide information about the organ or tissue as well as the metabolic state of the animal (Doumas and Peters, 1997). Elevation above normal range indicates disease condition (Larry,2005). There are many liver enzymes but the two that appear in most profiles are ALT and ALP (Karmen et al., 1955; Ophardt, 2003). In kidney function, urea (also blood urea nitrogen BUN) and creatinine are by-products of protein and muscle metabolic breakdown, respectively and are excreted entirely by kidney, (Doumas and Peters, 1997). Elevation above normal range indicates kidney and muscle disease condition. The enzyme creatinine kinase, AST and ALT apart from being muscle enzymes are used to assess liver function (Bong et al., 2008; Ophardt, 2003). The most important measure of red blood cell status is done by checking the PCV, MCV, mean corpuscular haemoglobin (MCH) and MCHC (Anosa, 1977) and can be used to classify anaemia (Butensky et al., 2008). MCV which is the average volume of red cells in a specimen can be elevated or decreased in accordance with average red blood cell size. Low MCV indicates microcytic (small average RBC size), normally indicates normocytic (normal average RBC size), high MCV indicates macrocytic (large average RBC size), (Choladda et al., 2015). MCH which is the average mass of haemoglobin per red blood cell in a sample and its value diminishes in hypochromic anaemia (Medline, 2009). MCHC which is the average concentration of haemoglobin in RBC if low indicates hypochromic anaemia (Choladda et al., 2015). In this study, the aim is to screen for the safety of *F. capensis* in organs using some serum enzymes as biochemical markers in male Wistar rats.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of *F. capensis* (Plate 1) were collected from the University environment in Umudike, Nigeria and was identified by Prof. M. C. Dike at the Taxonomy section of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria.

Preparation of Plant Extract

The identified leaves of *F. capensis* was shade dried for 10 days and pulverized to a coarse powder using a mechanical grinder. The plant extract was prepared using Soxhlet method described by Jensen (2007). Thirty-five grams (35g) of coarse powdered sample was introduced into the extraction chamber of the Soxhlet extractor using ethanol as solvent. Temperature was maintained at 70°C throughout the extraction period of 48 h. At the end of the extraction period the extract was concentrated using oven at 30°C to obtain dried extract which was weighed and kept in a well labelled sterile specimen bottle. Different doses of 500, 600 and 800 mg/kg body weight were prepared and administered to rats in group 2, 3, and 4, respectively. These doses were

Plate 1. Picture of *Ficus capensis* leaves.

calculated from a stock solution dissolved in distilled water.

Experimental Animals

Wistar rats (125 to 135 g) about seven weeks old were purchased from College of Vet Medicine laboratory animal house and ethical approval obtained from the College of Vet Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria. Approval was in line with guidelines for the care and use of laboratory animals as given by the National Research Council (1985). The rats were allowed to acclimatize for two weeks to their diets and water, thereafter were divided into different groups. All rats had access to food and water *ad libitum.*

Design of Study

Fifteen rats were used for the study, they were grouped into five of 3 rats each. Group 1 was the negative control group and was administered DMSO. Groups 2, 3, and 4 were the treatment groups which received 500, 600 and 800 mg/kg body weight of the *F. capensis* extract, respectively. Group 5 was the positive control group which received a known anaemic drug, cyclophosphamide. The rats were dosed for 14 days, thereafter was sacrificed by cardiac puncture and blood collected for analysis. The effect of *F. capensis* extract was checked on haematological parameters and serum enzymes activities.

Haematology and Biochemical Investigation

Haematological investigations performed by manual methods include RBC, white blood cell count (WBC) platelet count (PLT), differential count (DC) Hemoglobin concentration (Hb), PVC or hematocrit as described by Cole (1986). PCV was measured by the micro-hematocrit method using capillary tubes while RBC and WBC were measured manually using an improved Neubauer counting chamber. The differential counts were measured manually using a thin blood film stained with Leizhman stain. Hemoglobin concentrations were determined by cyanomathemoglobin method (Kachmar,1970). Using RBC, PCV and Hb concentration Hb, MCV, MCH, MCHC were calculated using standard formulae in hematological textbooks (Lewis et al., 2006; Hoffbrand and Moss, 2011).

$$MCV = \frac{PCV}{RBC} \times 10 \quad fl$$

$$MCH = \frac{Hb}{RBC} \times 10 \quad pg$$

$$MCHC = \frac{Hb}{PCV} \times 100 \quad g/dl$$

Biochemical investigation was performed using ELISA reagent kits. ALT, ALT and AST was measured using serum enzyme levels to determine liver, heart and kidney state (Doumas and Peters, 1997; Karmen et al., 1955; Rietman and Frankel, 1957). Albumin, globulin and TP were determined. Biuret method as described by Lubran (1978) was used to determine TP, albumin by Doumas et al. (1971); Doumas and Peters (1997). Samples were analysed immediately to avoid artifactual changes (Ihedioha and Onwubuche, 2007).

Statistical Analysis

Data obtained were analysed using Statistical Package for Social Sciences (SPSS) version 20. All values were expressed as mean ± Standard error. Data were further subjected to one-way analysis of variance (ANOVA) to compare the different doses of 500, 600 and 800 mg/kg body weight with the control. Duncan post-hoc statistics was used to separate significant means. The statistical confidence was set at 95% ($p<0.05$).

RESULTS

Table 1, shows the effect of *F. capensis* extract on the haematological parameters in experimental rats. PCV (51.00 ± 0.57) in negative control group reduced to 47.00

Table 1: Effect of *F.capensis* extract on the haematological parameters in rats.

Parameters	DMSO (-ve Control)	500 mg/kg extract	600 mg/kg extract	800 mg/kg extract	Cyclophosphomide (+ve Control)
PCV (%)	51.00 ± 0.57^{ab}	46.00 ± 0.57^{c}	53.66 ± 2.84^{a}	47.00 ± 0.57^{bc}	37.00 ± 0.57^{d}
RBC ($\times10^{6}$/µl)	9.11 ± 0.04^{a}	8.10 ± 0.02^{c}	8.55 ± 0.57^{b}	7.25 ± 0.57^{d}	6.06 ± 0.44^{e}
Hb (g/dl)	17.00 ± 0.19^{b}	15.33 ± 0.19^{c}	19.00 ± 0.19^{a}	15.66 ± 0.19^{c}	12.33 ± 0.19^{d}
TLC ($\times10^{3}$/ µl)	20.56 ± 0.19^{a}	9.16 ± 0.14^{c}	11.57 ± 0.23^{bc}	14.53 ± 2.48^{b}	4.70 ± 0.11^{d}
MCV (fl)	56.04 ± 0.45^{d}	56.45 ± 0.27^{d}	66.66 ± 0.22^{a}	64.82 ± 0.28^{b}	60.98 ± 0.52^{c}
MCH (pg)	18.66 ± 0.14^{d}	18.90 ± 0.17^{d}	22.20 ± 0.05^{a}	21.60 ± 0.11^{b}	20.33 ± 0.17^{c}
MCHC (g/dl)	33.33 ± 0.00	33.35 ± 0.02	33.33 ± 0.00	33.33 ± 0.00	33.33 ± 0.00

Values presented as mean ± S.E; different superscript represents significant difference at p<0.05. PCV = Pack Cell Volume; Hb= Haemoglobin; RBC = Red Blood Cell; TLC= Total Leukocyte Count; MCV= Mean Corpuscular Volume; MCH= Mean Corpuscular Haemoglobin; MCHC= Mean Corpuscular Haemoglobin Concentration.

Table 2: Effect of *F. capensis* extract on differential leukocyte count in rats.

Parameters	DMSO (-ve Control)	500 mg/kg extract	600 mg/kg extract	800 mg/kg extract	Cycloposphomide (+ve Control)
Neutrophils (%)	5.40 ± 0.69^{b}	4.77 ± 0.13^{b}	7.63 ± 0.08^{b}	12.66 ± 2.24^{a}	4.40 ± 0.11^{b}
Eosinophils (%)	0.00 ± 0.00^{b}	0.27 ± 0.10^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Basophils (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocyte (%)	15.16 ± 0.31^{a}	4.12 ± 0.11^{b}	3.69 ± 0.29^{b}	1.86 ± 0.24^{c}	0.30 ± 0.00^{d}
Monocyctes (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values presented as mean ± S.E; different superscript represent significant difference at p<0.05.

± 0.57 (in group 4); RBC 9.11.00 ± 0.04 reduced to 7.25 ± 0.57; Haemoglobin 17.00 ± 0.19 reduced to 15.66 ± 0.57. Total leukocyte count (TLC) progressively reduced from 20.65 ± 0.19 to 14.53 ± 2.48. MCV was increased from 56.00 ± 0.45 to 64.82 ± 0.28 indicating macrocytic condition and normal MCHC of 33.33 ± 0.00 indicating normochromic anaemic state. Table 2 shows the effect of *F. capensis* extract on the differential leukocyte count in experimental rats. The differential leukocyte count indicated marked neutrophilia, 5.4± 0.69 was increased to 12.66 ± 2.24 and lymphopeania 15.16 ± 0.31 reduced to 1.86 ± 0.24. Eosinophil, Basophils, Monocytes were not significantly affected when compared with the control group. For clinical biochemical parameters, *F. capensis* extract demonstrated elevated levels of the serum enzymes in a dose dependent fashion. ALP was increased from 60.63 ± 0.23 to 74.00 ± 0.57; AST was increased from 73.83 ± 3.08 to 135.63 ± 0.55 and ALT increased from 22.23 ± 0.26 to 51.53 ± 0.36. Table 3 shows the effect of *F. capensis* extract on some biochemical parameters in rats. Albumin, Globulin and TP increased as dose of *F. capensis* extract increased. Albumin 2.86 ± 0.03 increased to 5.00 ± 0.11; Globulin 2.36 ± 0.21 increased to 5.16 ± 0.03 and TP 7.1 ± 0.05 increased to 11.6 ± 0.11. This effect was dose-dependent.

DISCUSSION

In this study, the effect of varying concentration of ethanolic leaf extract of *F. capensis* on heamatological and biochemical markers in rats was evaluated. The extract of *F. capensis* causeda significant reduction in heamatological parameters in the treated rats. Table 1 shows the dose of 800 mg/kg b.w. showed the highest reduction indicating that the extract could be safe at lower dose but could be toxic at doses higher than 800 mg/kg b.w. This is in agreement with the finding of Oyewole and Oladele (2010), although the extract used in the study was *F. exasperata.* MCV increased indicating macrocytic anaemia, MCHC was normal indicating normochromic anaemia. Cyclophosphamide (+ve control) established anaemia by reducing the values of the haematological parameters (PCV, RBC, Hb concentration and TLC) and was used for comparison with the DMSO (-ve control) and treatment groups. There was no significant change in differential leucocyte counts except the neutrophilia and lymphocytopenia which occurred in dose-dependant manner. The extract of *F. capensis* revealed increase in serum enzymes (ALT, AST and ALP) which was not significant but a significant reduction at high dose of 800 mg/kg b.w. ALT and AST are measured clinically as diagnostic tool to assess hepatocellular injury and health status of the liver (Omeodu et al., 2008). Measurement of these enzymes is significant in clinical and toxicological studies as change in their activities are indicative of tissue damage by toxicants. Increase in serum level of these enzymes may be due to leakage from the tissues into the blood system as a result of the destruction of their cellular membranes (Azza et al., 2012). Exposure to chemical compounds in the structure, function, metabolic transformation and concentration of biomolecules,

Table 3: Effect of *F. capensis* extract on some biochemical parameters in rats.

Parameters	DMSO (-ve Control)	500 mg/kg extract	600 mg/kg extract	800 mg/kg extract	Cyclophosphomide (+ve Control)
ALP (IU/L)	60.63 ± 0.23^{d}	70.46 ± 0.36^{c}	72.26 ± 0.88^{b}	74.00 ± 0.57^{a}	50.30 ± 0.05^{e}
AST (IU/L)	73.83 ± 3.08^{e}	104.43 ± 0.33^{c}	123.40 ± 0.57^{b}	135.63 ± 0.55^{a}	96.26 ± 0.31^{d}
ALT (IU/L)	22.23 ± 0.26^{d}	42.70 ± 0.60^{c}	47.86 ± 0.38^{b}	51.53 ± 0.36^{a}	48.13 ± 0.90^{b}
Albumin (g/dl)	2.86 ± 0.03^{d}	3.20 ± 0.05^{c}	3.96 ± 0.03^{b}	5.00 ± 0.11^{a}	2.63 ± 0.08^{e}
Globulin (g/dl)	2.36 ± 0.21^{d}	3.33 ± 0.17^{c}	3.90 ± 0.05^{b}	5.16 ± 0.03^{a}	1.70 ± 0.05^{e}
TP (g/dl)	7.10 ± 0.05^{d}	8.63 ± 0.08^{c}	10.73 ± 0.12^{b}	11.60 ± 0.11^{a}	3.66 ± 0.08^{e}

Values presented as mean ± SEM; different superscript represent significant difference at $p<0.05$. AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; TP= Total protein; ALP = Alkaline phosphatase.

enzymes and even metabolic pathways. These may lead to alteration of various biochemical mechanisms and physiologic conditions (Murray et al., 2000), increase in the liver enzymes are seen in liver disorders, hepatic toxicity, liver cirrhosis, and hepatitis, while decrease indicates B_6 deficiencies, acute renal disease, and insufficient liver function((Deshpande et al.,1998; Miyakoshi et al., 2004; Mohanty et al., 2006). From the results obtained in this study, it could be inferred that the extract of *F. capensis* at the various doses cause an increase on the liver marker enzymes evaluated but effect was highest and significant ($p<0.05$) at high dose (800 mg/kg body weight) which could have affected haematological and biochemical parameters. Albumin, Globulin and TP increased as dose of *F. capensis* extract increased. Albumin 2.86 ± 0.03 increased to 5.00 ± 0.11; Globulin 2.36 ± 0.21 increased to 5.16 ± 0.03 and TP 7.1 ± 0.05 increased to 11.6 ± 0.11 (Table 3). The blood proteins, albumin (ALB) and TP increased could be suggestive of inflammation as the plant extract may have stimulated synthesis of certain blood proteins by the hepatocytes (Kachmar,1970).

CONCLUSION

Consumption of *F.capensis* at low dose (500 mg/kg b.w.) and moderate dose (600 mg/kg b.w.) can enhance haematological parameters and could be safe for the liver, kidney and heart muscle but the extract may be toxic to these organs when consumed at high dose (>800 mg/kg b.w.). Thus, the plant extract of *F. capensis* induced macrocytic normochromic anaemia, neutrophilia and lymphopaenia. There was haematological decrease (PCV, RBC, HB and TLC) at high dose and tissue damage because of the increase in ALT, AST and ALT in the treated groups. All these changes induced by *F.capensis* suggests that the extract is not safe at high dose.

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