

Full Length Research Paper

Phytochemical screening and effect of ethanol root extract of *Microdesmis puberula* on some haematological and biochemical parameters in normal male albino Wistar rats

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Microdesmis puberula Hook f. ex. Planch (Pandaceae) is one of the understudied medicinal plants whose pharmacological properties are beginning to be elucidated. In the present study, ethanol root extract of *M. puberula* was used to assess its effects on some biochemical and haematological parameters in male rats. The root extract was administered in graded doses of 200, 400 and 600 mg/kg body weight to groups II, III and IV, respectively while the control group received 1 ml of Tween 80 solution. Phytochemical screening of the extract revealed the presence of saponins, cardiac glycosides, deoxysugars, alkaloids and terpenes. The results of acute toxicity study showed that the extract has a wide margin of safety. The extract did not exhibit any significant effect ($p > 0.05$) on haematological parameters such as packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and red blood cell (RBC) count. Alanine aminotransferase (ALT) and aspartate aminotransferase did not show any significant difference ($p > 0.05$) between control and treatment groups. There was a significant ($p < 0.05$) increase in serum cholesterol, low density lipoprotein-cholesterol (LDL-C), triacylglycerols (TGs) and significant ($p < 0.05$) decrease in high density lipoprotein-cholesterol (HDL-C) as a consequence of the administration of this extract. Generally, the results indicate that there were no serious adverse effects associated with the use of this extract except in the case of lipid profile studies.

Key words: *Microdesmis puberula*, ethanol extract, phytochemicals, haematological/biochemical parameters, toxicity.

INTRODUCTION

Microdesmis puberula Hook. F. ex Planch (Pandaceae) is a dioecious plant that can grow up to a height of about 6 m if not harvested or prematurely cut (Dounias, 2008). It occurs predominantly from Eastern Nigeria down to DR Congo and Uganda. In Nigeria, its local names include Mkpiri or Mbugbo in Igbo; Idi-apata in Yoruba and Ntabit in Ibibio language (Esonu et al., 2004).

It has been reported that various parts of *M. puberula*

(stem bark, leaves and roots) are used for several medicinal purposes (Dounias, 2008; Okany et al., 2012). In Eastern Nigeria, the roots of this plant are used in the treatment of gonorrhoea and erectile dysfunction (Ajibesin et al., 2008; Zamblé et al., 2006; Roumy et al., 2008). The analgesic and antistress properties of *M. puberula* have recently been reported (Okany et al., 2012). Several polyamine derivatives were earlier identified

in the hydromethanolic root extract of this plant (Roumy et al., 2008; Zambé et al., 2006).

Despite the reported uses of *M. puberula* in traditional medicine, there are no reported studies addressing the issue of safety in the administration of this plant extract. Therefore, the present study was designed to evaluate the effect of the root extract on some hepatic, renal and haematological parameters in normal rats.

MATERIALS AND METHODS

Plant

Samples of *M. puberula* were uprooted from the bushes around the University of Uyo main campus, and were authenticated at the Department of Botany of the same university. A voucher specimen (31 L) was deposited at the departmental herbarium.

Preparation of root extract

The roots were washed, air-dried for two weeks and ground into a powder using a mechanical grinder. About 200 g of the powdered sample were soaked in 500 ml of 80% ethanol in a conical flask and were covered with aluminium foil. The mixture was stirred intermittently and allowed to stand for a period of 48 h. The resulting decoction was filtered and the filtrate was subjected to complete solvent evaporation using a regulated hot plate at a temperature of 40 to 50°C. The extract obtained was packaged in an air tight container and was stored below 4°C until required.

Phytochemical screening

Phytochemical screening of the extract was carried out using standard procedures as described by Sofowora (1993) and Trease and Evans (2002).

Determination of median lethal dose (LD₅₀)

The LD₅₀ of the root extract was determined by the method of Lorke (1983). Twelve albino mice with average weight of 25.3 g were used. In the first phase, nine mice were divided into three groups of three animals per group and were respectively treated with the extract at doses of 100, 500 and 1000 mg/kg body weight orally. They were observed for signs of toxicity within 24 h period. In the second phase of the experiment, three mice were divided into three groups of one mouse each and were treated with the extract at doses of 1600, 2900 and 5000 mg/kg body weight orally. The LD₅₀ was calculated as the geometric mean of the maximum dose with 0% mortality and the minimum dose producing 100% mortality.

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

where D₀ is the maximum dose producing 0% mortality and D₁₀₀ is the minimum dose producing 100% mortality.

Experimental animals

Male albino rats (210 to 220 g) were obtained from the animal house facility of the Faculty of Basic Medical Sciences, University of Uyo, Uyo. The animals were housed in a well ventilated experimental section of the animal house and maintained under

standard conditions of 12 h light and dark cycle with room temperature at 25±3°C and 33 to 60% humidity. The animals were maintained on standard pellet diet (Pfizer Livestock Company Ltd, Aba, Nigeria) and allowed to acclimatize for a period of two weeks after which they were divided into four groups of six rats per group. Group I served as control whereas groups II, III and IV received oral doses of 200, 400 and 600 mg/kg body weight of the extract, respectively.

Administration of the extract was carried out between the hours of 10 am and 12 noon daily. The experiment lasted 14 days.

Sample collection

The animals were anaesthetized with chloroform vapour 24 h after the last administration of the extract. Blood samples were collected by cardiac puncture using sterile syringes and needles. The blood samples were divided into two portions. One portion (about 2 ml) was transferred into sample bottles containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant and used for the assessment of haematological parameters. The other portion of blood was collected in sterile plain tubes without anticoagulant and allowed to clot. Serum was obtained by centrifugation at 3000 g for 5 min using a bench top centrifuge (MSE Minor, England). The serum samples were stored frozen until required for analyses.

Determination of haematological parameters

Haematological parameters were determined within 2 h of sample collection using Mindary™ Differential BC 5300 Automated Hematologic Analyser at the Haematology Unit of the University of Uyo Teaching Hospital.

Determination of serum enzymes

The serum enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were carried out using the RANDOX kit (Randox Laboratories Ltd, England).

Determination of lipid profile

Estimation of total serum cholesterol was carried out using the cholesterol oxidase-phenol-aminophenazone (CHOD-PAP) method and high density lipoprotein-cholesterol (HDL-C) by polyethylene glycol cholesterol oxidase-phenol-aminophenazone (PEG-CHOD-PAP) method using reagent kits supplied by Randox Laboratories, England.

Triacylglycerols (TGs) were determined using glycerol phosphate oxidase-phenol aminophenazone (GPO-PAP end point assay) method using diagnostic reagent kit (Randox Laboratories, England). LDL-cholesterol was calculated from the equation:

$$\text{Serum LDL-cholesterol (mg/dl)} = \text{Total cholesterol} - (\text{HDL} + \text{TG}/5)$$

Estimation of blood urea nitrogen (BUN) and creatinine

These were determined using reagent kits supplied by Randox Laboratories Ltd, England.

Statistical analysis

Data obtained were expressed as means ± standard deviation (SD). Statistical analysis was carried out using the one way analysis of

Table 1. Phytochemical profile of ethanolic root extract of *M. puberula*.

Constituent	Inference
Tannins	-
Phlobatannins	-
Saponins	+
Flavonoids	-
Anthraquinones	-
Cardiac glycoside	++
Deoxy sugar	++
Terpenes	+
Alkaloids	+

-- Absent; + = present; ++ = highly present.

Table 2. Effect of the ethanolic root extract of *M. puberula* on haematological indices in normal male Wistar rats.

Group	RBC ($\times 10^6 \mu\text{l}$)	PCV (%)	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)	WBC ($\times 10^3 \mu\text{l}$)
I (control)	8.64 \pm 0.09	40.70 \pm 2.68	14.45 \pm 0.14	52.15 \pm 0.63	16.73 \pm 0.18	32.10 \pm 0.10	8.25 \pm 0.65
II (200 mg/kg)	7.87 \pm 0.30	40.80 \pm 0.26	14.34 \pm 0.28	54.38 \pm 0.37	17.68 \pm 0.11	32.53 \pm 0.19	11.17 \pm 0.80 ^a
III (400 mg/kg)	8.17 \pm 0.23	41.55 \pm 1.78	14.42 \pm 0.15	52.77 \pm 0.60	17.27 \pm 0.19	32.68 \pm 0.31	18.40 \pm 0.46 ^{a,b}
IV (600 mg/kg)	8.15 \pm 0.21	41.20 \pm 2.88	14.07 \pm 0.13	53.58 \pm 0.80	17.30 \pm 0.38	32.18 \pm 0.33	15.00 \pm 0.52 ^{a,b,c}

Values represent mean \pm SEM (n = 6); ^aSignificantly different (p < 0.05), compared with group I; ^bSignificantly different (p < 0.05), compared with group II; ^cSignificantly different (p < 0.05), compared with group III.

variance (ANOVA). Turkey's multiple range tests was used to determine the significance of difference between means. Statistical significance was accepted at p < 0.05.

RESULTS AND DISCUSSION

The phytochemical screening of the ethanolic root extract of *M. puberula* (Table 1) revealed the presence of trace or moderate amounts of saponins, cardiac glycosides, deoxy sugars, terpenes and alkaloids. Okany et al. (2012) has also reported the presence of flavonoids, saponins and cardiac glycosides in the methanolic stem wood extract of the plant. Alkaloids constitute a diverse group of secondary metabolites in plants with pharmacologic activities (Roberts and Wink, 1998). The cardiac glycosides increase the force of contraction of the heart and are valuable in the treatment of heart failure (Stryer, 1995). Terpenes are important as components of essential oils e.g. menthol (Holme, 2004). Deoxy sugars are components of several bioactive compounds (Kennedy and White, 1983). Saponins have the properties of protein precipitation, cholesterol binding and haemolysis of red blood cells (Sofowora, 1993).

The LD₅₀ of the extract was found to be higher than 5000 mg/kg body weight orally. Evaluation of LD₅₀ has been described as an important step in the toxicological investigation of an unknown substance (Lorke, 1983). The classification of acute systemic toxicity based on LD₅₀ values as recommended by the Organization for

Economic Cooperation and Development (OECD) is as follows: very toxic, ≤ 5 mg/kg; toxic $>5 \leq 50$ mg/kg; harmful, $>50 \leq 500$ mg/kg; no label, $>500 \leq 2000$ mg/kg (Walum, 1998). Lorke (1983) noted that LD₅₀ values greater than 5000 mg/kg are of no practical interest. Hence, the LD₅₀ value obtained in the present study shows that the extract is non toxic when administered via oral route. It is therefore considered safe for use by humans. Okany et al. (2012) had also observed that the stem wood extract of this plant has a wide margin of safety when administered orally. Similarly, Zamblé et al. (2007) reported that the aqueous root extract of *Microdesmis keayana*, a closely related species to *M. peberula*, did not cause any mortality or changes in behaviour at a dose of 2000 mg/kg body weight.

Table 2 shows the effects of the extract on haematological parameters. There was a marginal dose dependent increase in packed cell volume (PCV) which was not significantly different from control. Similarly, the results for red blood cell (RBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were found not to be significantly different from the control. This indicates that the extract does not possess any haematotoxic effect. An important condition for safety in the administration of medicinal plants is the need for such plant extracts to maintain normal haematological state in animals or to reverse any existing negative haematological status (Uboh et al.,

Table 3. Effect of the ethanolic root extract of *M. puberula* on ALT, AST, total protein and albumin levels in normal male Wistar rats.

Group	ALT (U/L)	AST (U/L)	Total protein (g/dl)	Serum albumin (g/dl)
I (control)	18.69 ± 1.56	33.11 ± 2.07	58.11 ± 4.30	24.08 ± 2.69
II (200 mg/kg)	18.23 ± 2.30	33.46 ± 3.22	56.98 ± 4.32	23.88 ± 2.56
III (400 mg/kg)	18.75 ± 3.96	33.68 ± 3.98	57.66 ± 4.89	23.98 ± 2.63
IV (600 mg/kg)	18.75 ± 4.42	34.33 ± 2.01	58.14 ± 4.24	25.86 ± 2.52

Values represent mean ± SEM (n = 6); p>0.05 in all treatment groups.

2010).

There was a significant ($p < 0.05$) increase in WBC count across all treatment groups. Such increase has been attributed to various factors such as infection and inflammatory response (Tegui et al., 2007). Other factors which have been implicated include excitement, fear, pain or apprehension in animals in the course of the experiment (Mbaya et al., 2008). However, Igwe et al. (2011) surmised that increase in WBC count is a normal immune response to assault on the animal system by drug administration.

The results obtained for the effect of the extract on serum enzymes, total protein and albumin levels in the rat are shown in Table 3. There was no significant change in the activities of serum enzymes assayed. The values obtained for total protein and albumin compared favourably with the control.

ALT and AST play important roles in diagnostic enzymology (Naik, 2010). ALT is widely accepted as a more specific marker of hepatocellular damage, because it occurs in the cytosol whereas AST has cytosolic and mitochondrial forms and is present in various tissues including the heart, skeletal muscle, kidneys, brain, pancreas and blood cells (Batzakis and Briere, 1979). The aminotransferase enzymes leak into circulation when liver cells or their membranes are damaged. Consequently, the estimation of their activities in serum serves as a useful quantitative marker of hepatic injury (Giboney, 2005). In view of the fact that the root extract did not produce any significant changes in serum enzyme activities, it can be inferred that the extract did not inflict hepatocellular injury on the experimental animals (Kew, 2000).

Plasma proteins are synthesized in the liver and the synthetic functions of the liver is compromised in hepatocellular damage. Hence, determination of serum protein including albumin is one method of assessing liver function. In the present study, there was no significant alteration in serum total protein and albumin. Hence, the synthetic function of the liver is preserved during the administration of the extract.

The effect of the extract on BUN and creatinine is presented in Table 4. Estimation of BUN and creatinine are screening tests for renal function. These metabolites are usually eliminated from the body through glomerular filtration. An increased plasma creatinine and urea imply

Table 4. Effect of the ethanolic root extract of *M. puberula* on serum creatinine and BUN in normal male Wistar rats.

Group	Serum creatinine (mg/dl)	BUN (mg/dl)
I (control)	1.80 ± 0.46	22.60 ± 4.39
II (200 mg/kg)	1.62 ± 0.33	20.59 ± 2.55
III (400 mg/kg)	1.30 ± 0.11	17.26 ± 3.49
IV (600 mg/kg)	1.18 ± 0.51	18.61 ± 5.30

Results presented as mean ± SEM (n = 6); p > 0.05 in all treatment groups.

the impairment of renal function. The serum level of these metabolites is usually parallel to the severity of renal malfunction. The present study did not demonstrate any significant alterations in BUN and creatinine and this suggests the absence of nephrotoxicity as a consequence of the administration of the extract.

In lipid profile studies, the parameters of interest include serum cholesterol, TGs, low density lipoprotein cholesterol (LDL-C) and HDL-C. Alterations in these lipid fractions provide useful information concerning the status of lipid metabolism as well as predisposition to atherosclerosis and its associated coronary disease (Singh et al., 2012).

The effects of the ethanolic root extract of *M. puberula* on lipid profile are shown in Table 5. Serum cholesterol, LDL-C and TGs were found to increase significantly ($p < 0.05$) in a dose-dependent fashion as compared to the control. Increase in serum cholesterol could be attributed to an enhanced β -oxidation resulting in increased levels of acetyl coenzyme A (CoA), a key substrate in the biosynthesis of the cholesterol (Naik, 2010). High blood cholesterol is an important risk factor for cardiovascular disease (Treasure et al., 1995). Consequently, the observed increase in serum cholesterol is detrimental to the health of the animals.

TGs are the major storage forms of fatty acids. Increase in serum concentrations of TGs as observed in the present study could be attributed to increased lipolysis which may ultimately deplete the body store of fatty acids. It has been reported that patients with cardiovascular disease exhibit high serum levels of TGs (Singh et al., 2012). Hence, the result of the present study calls for caution in the administration of the root

Table 5. Effect of the ethanolic root extract of *M. puberula* on serum lipid profile of male Wistar rats.

Group	Total cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	Triglycerides (mg/dl)
I (control)	76.68 ± 2.74	57.46 ± 2.92	15.50 ± 0.22	104.36 ± 2.18
II (200 mg/kg)	83.66 ± 1.94 ^a	45.35 ± 2.55 ^a	30.88 ± 1.54 ^a	113.87 ± 2.39 ^a
III (400 mg/kg)	87.48 ± 3.20 ^a	41.21 ± 1.80 ^a	42.00 ± 2.80 ^a	113.78 ± 2.46 ^a
IV (600 mg/kg)	112.93 ± 2.40 ^a	39.31 ± 1.72 ^a	51.94 ± 3.93 ^a	127.82 ± 2.68 ^a

Values represent mean ± SEM (n = 6). ^aSignificantly different (p < 0.05) from control.

extract of *M. puberula*. The present study has also shown that serum levels of HDL-C decreased significantly (p < 0.05) in a dose-dependent fashion at the end of experimental period.

The results of the present study has shown that administration of ethanol root extract of *M. puberula* does not have any significant toxic effect on liver and kidney functions as well as on haematological parameters in male rats. The observed alterations in serum lipid profile could predispose the animals to atherosclerosis, hence the need for caution in the use of this plant root extract for medicinal purposes.

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