Australian Journal of Basic and Applied Sciences, 7(8): 455-460, 2013
ISSN 1991-8178

Phytochemical Screening and Effect of Ethanol Root Extract of *Microdesmis Puberula* on Some Haematological and Biochemical Parameters in Normal Male Albino Wistar Rat

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Abstract: *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. 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 1ml 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 effects ($p>0.05$) on haematological parameters such as PCV, Hb, MCV, MCHC and RBC count. ALT and AST 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, LDL-C, TGs and significant ($p<0.05$) decreased HDL-cholesterol 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, toxicity, lipid profile, haematological/biochemical parameters.

INTRODUCTION

Microdesmis puberula Hook. F. ex Planch (Pandaceae) is a dioecious plant that can grow up to a height of about six metres 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*, 2006); Zamblé *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.

MATERIAL AND METHODS

Plant Material:

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 (31L) was deposited at the Departmental Hebarium.

Preparation of Root Extract:

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

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Phytochemical Screening:

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

Determination of Median Lethal Dose (LD_{50}):

The LD_{50} of the root extract was determined by the method of Lorke (1983). Twelve albino mice with average weight 25.3g 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 1000mg/kg body weight orally. They were observed for signs of toxicity within 24 hours period. In the second phase of experiment, three mice were divided into three groups of one mouse each and were treated with the extract at doses of 1600, 2900 and 5000mg/kg body weight orally. The LD_{50} 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 D_0 = maximum dose producing 0% mortality
D_{100} = minimum dose producing 100% mortality

Experimental Animals:

Male albino rats (210-220g) 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 hours light and dark cycle, room temperature 25 ± 3^{o}C and 33-60% 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 1 served as control whereas groups II, III and IV received oral doses of 200, 400 and 600mg/kg body weight of the extract respectively.

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

Sample Collection:

The animals were anaesthetized with chloroform vapour twenty four hours 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 2ml) was transferred into sample bottles containing 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 3000g for five minutes 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 two hours of sample collection using Mindary Differential BC 5300Automated 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 HDL-cholesterol 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:

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

Estimation of Urea and Creatinine:

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

Statistical Analysis:

Data obtained were expressed as means ± SD. Statistical analysis was carried out using the one way analysis of 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). Saponnins have the properties of protein precipitation, cholesterol binding and hemolysis of red blood cells (Sofowora, 1993).

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

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

Legend: - = absent; + = present; ++ = highly present

The LD_{50} of the extract was found to be higher than 5000 mg/kg body weight orally. Evaluation of LD_{50} 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 LD_{50} values as recommended by the Organization for Economic Cooperation and Development (OECD) is as follows; very toxic, $\leq$ 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 LD_{50} values greater than 5000 mg/kg are of no practical interest. Hence, the LD_{50} 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 *M. 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 PCV which was not significantly different from control. Similarly, the results for RBC, Hb, MCV, MCH and MCHC were found not to be significantly different from control. This indicates that the extract does not possess any haematoxic 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*, 2011).

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

Group	RBC ($x10^6$ µl)	PCV (%)	Hb (g/dl)	MCV (fl)	MCH (pg)	MHC (g/dl)	WBC ($x10^3$ µl)
I (control)	8.64 ± 0.09	40.70 ± 2.68	14.45 ± 0.14	52.15 ± 0.63	16.73 ± 0.18	32.10 ± 0.10	8.25 ± 0.65
II (200mg/kg)	7.87 ± 0.30	40.80 ± 0.26	14.34 ± 0.28	54.38 ± 0.37	17.68 ± 0.11	32.53 ± 0.19	11.17 ± 0.80[a]
III (400mg/kg)	8.17 ± 0.23	41.55 ± 1.78	14.42 ± 0.15	52.77 ± 0.60	17.27 ± 0.19	32.68 ± 0.31	18.40 ± 0.46[a,b]
IV (600mg/kg)	8.15 ± 0.21	41.20 ± 2.88	14.07 ± 0.13	53.58 ± 0.80	17.30 ± 0.38	32.18 ± 0.33	15.00 ± 0.52[a,b,c]

Legend: Values represent mean ± SEM (n = 6)
a = significantly different ($p<0.05$), compared with group I
b = significantly different ($p<0.05$), compared with group II
c = significantly different ($p<0.05$), compared with group III

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 (Teguia *et al,* 2007). Other factors which have been implicated include excitement, fear, pain or apprehension in animals in the course of experiment (Mbaya *et al*, 2008). However, Igwe *et al* (2011) surmise 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.

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 (µl)	AST (µl)	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 (200mg/kg)	18.23 ± 2.30	33.46 ± 3.22	56.98 ± 4.32	23.88 ± 2.56
III (400mg/kg)	18.75 ± 3.96	33.68 ± 3.98	57.66 ± 4.89	23.98 ± 2.63
IV (600mg/kg)	18.75 ± 4.42	34.33 ± 2.01	58.14 ± 4.24	25.86 ± 2.52

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

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 extract.

The effect of the extract on BUN and creatinine is presented 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 the impairment of renal function. The serum level of these metabolites usually parallels 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 extract.

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

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

Results presented as mean ± SEM (n = 5); $p>0.05$ in all treatment groups.

In lipid profile studies, the parameters of interest include serum cholesterol, triacylglycerol (TGs), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (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 compared to control. Increase in serum cholesterol could be attributed to an enhanced β-oxidation resulting in increased levels of acetyl CoA, a key substrate in the biosynthesis of 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 animals.

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 (200mg/kg)	83.66 ± 1.94[a]	45.35 ± 2.55[a]	30.88 ± 1.54[a]	113.87 ± 2.39[a]
III (400mg/kg)	87.48 ± 3.20[a]	41.21 ± 1.80[a]	42.00 ±2.80[a]	113.78 ± 2.46[a]
IV (600mg/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)
a = significantly different ($p<0.05$) from control.

Triacylglycerols (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

triacylglycerols (Singh *et al*, 2012). Hence the result of the present study calls for caution in the administration of the root extract of *M. peberula*.

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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