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Full Length Research Paper

Haematological and Serum Biochemical Variables in rats Treated with Ethanol Extract of the Root of *Moringa oleifera*

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ABSTRACT

The haematology and serum biochemical effects of oral administration of the ethanolic extract of the root of *Moringa oleifera* at 50, 100 and 150 mg/kg were investigated in 30 mated female Wistar rats. The rats were assigned into five groups of six rats each. Group A was given 50mg/kg of the extract; group B, 100mg/kg; group C, 150 mg/kg; group D, 0.2ml of corn oil; and group E, 0.2ml of distilled water. Groups D and E, corn oil and distilled water treatment groups respectively, served as the controls while groups A, B and C were the treatment groups. There was no significant difference (P>0.05) between the two control groups and the treatment groups for the RBC, WBC, PCV, MCV, MCH and MCHC and Hb. However, the mean lymphocyte values for groups B and C were significantly different (P<0.05) from those of group A as well the two control groups. The total protein, albumin, globulin and A/G ratio showed no significant difference (P>0.05) between the two control groups and the treatment groups. There were no significant differences (P>0.05) in the values of AST, ALP, creatinine, urea, GGT, glucose, cholesterol and ALT between the treatment groups and the control groups. It can therefore be concluded that oral administration of ethanolic extract of the root of *M. oleifera* is harmless to the rats since no adverse effects were detected in haematological and serum biochemical investigations

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Keywords*: Moringa oleifera*, haematology, serum biochemistry, female rats

INTRODUCTION

Historic documentations of the importance of medicinal plants date back to many thousand years and current trends indicate that medicinal plants are used against a wide range of health problems such as cough, cold, cataract, constipation and many other ailments (Rasonavivo *et al*., 1992; Jimenez *et al*., 2003). The tree, *M. oleifera* (*Moringaceae*), is cultivated widely around the world (Odee, 1998) and used for various purposes one of which is as a feed supplement to livestock (Martin, 2007; Fadiyimu *et al.*, 2010).

Moringa oleifera was widely used by the ancient Romans, Greeks and Egyptians as animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed-cake), green manure (from leaves), gum (from tree trunks), honey (flower nectar), medicine (all plant parts), pulp (wood), rope (bark) and water purification, powdered seeds (Fuglie, 1999; Zanu *et al*., 2012).

Several therapeutic benefits of *M. oleifera* had been documented; these include antibiotic (Fahey *et al*., 2002; Haristoy *et al.*, 2005); anticancer (Bharali *et al.*, 2003); antiulcerogenic effects (Akhtar and Ahmad, 1995); analgesic (Rao and Ojha, 2003) and antiurolithiatic activities (Bennett *et al*., 2003).

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Research reports on the effect of the leaves and seeds of *M. oleifera* on haematology and serum biochemistry had been documented (Hisham *et al*., 2012; Ajibade *et al*. 2012). Aqueous extract of roots and barks depresses the central nervous system of rats; has antihepatotoxic effects in rats (Kumar and Pari 2003). It also induces anti-implantation activity in the mice and causes foetal resorption at late pregnancy (Shukla *et al*., 1988). The aqueous extract of the root has also been found to induce biochemical alteration in female genital tracts of ovariectomised rats (Shukla *et al*., 1989).

Despite the fact that the roots of *Moringa oleifera* are frequently used in phytotherapy, very little research has been done on its effects on female reproduction with respect to haematology and serum biochemistry. This study is therefore designed to investigate the haematological and serum biochemical effects of the ethanolic extract of *Moringa oleifera* root on cycling female Wistar rats.

MATERIALS AND METHODS

Collection and Preparation of Aqueous Ethanolic Extract

The roots of *M. oleifera* were air dried and pulverized before the commencement of the ethanol extraction. The extraction was carried out as described by Njar *et al*. (1993). The pulverized roots weighing 250 g were exhaustively extracted with distillated ethanol by means of cold extraction and extract evaporated *invacuo*. The root extract of *M. oleifera* was concentrated *invacuo* using a rotary evaporator at 40°C. The solvent (distillated ethanol) remaining in the extract was finally removed by placing the root extract in porcelain dishes in temperature-controlled oven to give a residue (extract). The extract weighed 1.5 g and was reconstituted with 20mls of corn oil to give a concentration of 75mg/ml.

Experimental animals

Thirty sexually matured female Wistar rats were used for the study. They were kept in the Animal House, Faculty of Veterinary Medicine, University of Ibadan. Commercial rat feed pellets and water was given ad libitum. The rats were stabilized for five weeks before they were introduced to males of proven fertility at the ratio of three females to one male overnight. The following morning, the females rats were observed for vaginal plugs to ensure that mating had taken place before the commencement of the treatment protocol.

Treatment Protocol

The animals were divided in five groups of six animals each. Group A consisted of animals with an average weight of 150 g and they were treated with a dose of 50mg/kg of extract per animal daily for five days. Group B consisted of animals with an average weight of 150g and they were treated with a dose of 100mg/kg of extract per animal daily for five days. Group C consisted of animals with an average weight of 175g, received 150mg/kg of extract per animal daily for five days. Group D with animals of an average weight of 195 g served as the control group 1 and the animals were treated with 0.2ml of cornoil each daily for five days. Group E which served as control two; has animals with an average weight of 200 g and were treated with 0.2ml of distilled water each daily for five days.

Blood Sampling

Blood samples were collected from the orbital sinus of the rats into clean test tubes containing EDTA 24 hours after the last treatment. Haematological parameters were determined as described by Guyton and Hall (2006). Drops of whole blood were used to fill some heparinised microhematocrit capillary tubes to determine packed cell volume (PCV), and hemoglobin (Hb). Whole blood was also used to make three air dried blood smears. The smears were stained with Wright's stain and examined for red blood cell (RBC), white blood cell (WBC), differential WBCs (lymphocytes, heterophil, monocytes) and platelet estimate, while Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Mean corpuscular volume (MCV) were calculated. Blood samples were also collected for biochemical analysis, centrifuged at 3000 rpm for ten minutes to isolate the serum. Total protein, albumin, globulin, creatinine, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and blood urea nitrogen were determined by use of automated analysers as described by Meyer and Harvey (1998).

Data analysis

All data were expressed as means and standard deviation, comparison was by the student t test using the Graphpad Prism version 4.00 for Windows, Graphpad Software. Significance was reported at $P < 0.05$.

RESULTS

The mean and standard deviation (SD) values of the effect of ethanolic extract of the root of *Moringa*

oleifera on the haematological parameters of the female Wistar rats are given in Table 1. There was no significant difference (P>0.05) between the two control groups and the treatment groups for the PCV, Hb, and RBC. Also, the MCV, MCH and MCHC all showed no significant difference (P>0.05). There was no significant difference (P>0.05) for the WBC values among the treatment groups. Lymphocyte values for groups B and C were significantly different (P<0.05) from those of group A as well those of the two control groups. These differences were such that the values for groups B and C were higher than those of A and control groups. For the neutrophil values, there was a significant difference (P<0.05) between the control groups and treatment groups B and C such that the values for these treatment groups B and C were lower than those of the control groups and group A. There were however no significant differences (P>0.05) with the values obtained for monocyte and eosinophil across the five groups. The means of the platelet counts across the groups showed no significance difference (P>0.05).

The mean and standard deviation (SD) values of the effect of ethanolic extract of the root of *Moringa oleifera* on the serum biochemical parameters of the female Wistar rats are given in Table 2. The total protein, albumin, globulin and A/G ratio showed no significant difference (P>0.05) between the two control groups and the treatment groups.

Table 1.
Effects of the ethanol extract of *Moringa oleifera* roots on the haematological parameters of the female Wistar rats

Parameters	Group A	Group B	Group C	Group D	Group E
PCV (%)	46.6±1.52[a]	46.5±1.52[a]	50.0±2.10[a]	49.7±2.80a	51.5±3.73[a]
Hb	15.3±0.581[a]	14.9±0.668[a]	16.0±0.763[a]	16.0±1.22[a]	16.2±1.26[a]
RBC	7.81±0.590[a]	7.67±0.323[a]	8.54±0.243[a]	8.22±0.569[a]	8.61±0.227[a]
MCV (fl)	59.5±3.04[a]	60.6±1.00[a]	58.6±2.81[a]	60.5±1.99[a]	59.8±3.94[a]
MCH (pg)	19.7±1.45[a]	19.5±0.42[a]	18.7±0.99[a]	19.6±1.02[a]	18.8±1.51[a]
MCHC (%)	32.8±1.16[a]	32.1±0.66[a]	32.0±1.23[a]	32.4±0.84[a]	31.4±0.87[a]
WBC (10^9 L)	6.0±1140[a]	6.48±3.00[a]	6.25±2.14[a]	7.58±2.67[b]	7.12.±1.87[b]
PLT (L)	112.00±8.32[a]	113.83.±2.64[a]	120.00±2.92[a]	112.50±16.32[a]	118.50±23.84[a]
LMP (%)	72.6±3.85[a]	81.0±4.00[b]	81.8±7.25[b]	75.5±6.83[a]	70.0±7.01[a]
NTP (%)	23.6±4.28[b]	15.2±3.92[a]	17.2±5.78[a]	21.3±6.31[b]	25.0±1.21[b]
MNT (%)	2.40±0.894[a]	1.33±0.816[a]	1.33±1.21[a]	0.833±0.753[a]	2.33±1.21[a]
ESP (%)	1.40±1.14[a]	2.50±1.52[a]	3.00±1.79[a]	2.33±1.37[a]	2.67±1.21[a]

Means with different superscript within rows are significantly different (P<0.05)
PCV = Packed cell volume; Hb= haemoglobin concentration; RBC = red blood cell count; MCV= mean corpuscular volume MCHC = mean corpuscular haemoglobin concentration; WBC = white blood cell count; PLT = Platelet; NTP = Neutrophil; MNT = Monocyte; ESP = Eosinophil

Table 2.
Effects of the ethanol extract of *Moringa oleifera* roots on the serum chemistry of the female Wistar rats (mean ± SD).

Parameters	Group A	Group B	Group C	Group D	Group E
TP	7.43±0.468[a]	7.97±0.862[a]	8.50±0.447[a]	8.57±0.383[a]	8.43±0.225[a]
Albumin	4.58±0.279[a]	4.60±0.494[a]	4.93±0.314[a]	4.92±0.214[a]	4.60±0.283[a]
Globulin	2.85±0.197[a]	3.08±0.859[a]	3.57±0.258[a]	3.65±0.339[a]	3.83±0.441[a]
A/G	1.55±0.0548[a]	1.67±0.606[a]	1.35±0.266[a]	1.32±0.147[a]	1.20±0.210[a]
AST	43.0±4.98[a]	44.5±2.07[a]	45.5±1.76[a]	42.8±3.43[a]	43.7±2.07[a]
ALT	31.0±2.10[a]	28.2±3.06[a]	28.5±2.43[a]	29.2±2.32[a]	29.3±3.50[a]
ALP	94.2±21.4[a]	92.5±20.3[a]	86.2±20.6[a]	78.7±4.41[a]	86.8±18.4[a]
UREA	14.2±1.47[a]	14.0±1.90[a]	14.7±1.75[a]	14.3±1.37[a]	14.5±1.87[a]
Creatinine	14.2±1.47[a]	0.667±0.207[a]	0.600±0.141[a]	0.550±0.105[a]	0.68±0.197[a]
GGT	2.70±0.522[a]	3.03±0.641[a]	3.02±0.571[a]	3.35±0.989[a]	2.95±0.883[a]
Glucose	127±19.7[a]	122±5.53[a]	121±6.21[a]	124±8.57[a]	123±4.15[a]
Cholesterol	77.5±19.7[a]	70.8±25.0[a]	71.0±20.8[a]	60.3±15.5[a]	91.3±27.0[a]

Means with different superscripts within rows are significantly different (P<0.05).
TP = total protein; A/G= albumin/globulin; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; GGT=gamma-glutamyl transpeptidase

There were no significant differences (P>0.05) in the values of the liver enzymes AST and ALT between the treatment and control groups. ALP value also showed no significant difference (P>0.05) between the treatment groups and the control groups. There was no significant difference (P>0.05) for the creatinine value between the treated groups and the control group. The urea value also showed no significant difference (P>0.05) between the treatment groups and the control groups. The GGT enzyme showed no significant difference (P>0.05) between the treatment group and the control group. There was no significant difference between the glucose and the cholesterol values between the treatment groups and the controls.

DISCUSSION

Moringa oleifera has gained popularity as a life-saving nutritional power plant that can feed the needy. It is commonly used to treat cardiac disorders, diabetes and infections as a means of natural therapy without the users fully comprehending the effects on their haematology and serum biochemistry (Lipipun *et al.*, 2003). Findings from this study have shown that there are no significant differences in the sum of the means of the PCV, Hb and Red blood cells across the groups (P>0.05) at the dose rate of 50,100 and 150mg/kg. The present study shows that *M. oleifera* ethanolic root extract induces no significant changes in the RBC, Hb, and PCV values of rats. The absence of significant changes on these blood indices may therefore suggest that the extract is safe in the rats with no deleterious effect on the haematological parameters. This observation is in agreement with the report of Jahn (1988) who reported that the aqueous extract of the seed of *M. oleifera* did not induce any toxic effect in Wistar rats. It also agrees with the findings of Ajibade *et al*., (2012) where *Moringa oleifera* seed extract was used at high doses of 400, 800 and 1600mg/kg with no resultant negative effect on the haematology of the female Wistar. The decreased neutrophil concentration observed in the rats might have resulted from the suppression of leucopoiesis in the bone marrow which may have consequential effects on the immune and phagocytic activity of the blood cells of the animals (Afolayan and Yakubu, 2009). At the doses used in this study, there was no significant difference (P>0.05) on the platelet count. This signifies that the blood coagulation factors were not impaired at the doses used. However, Ajibade *et al*. (2012) recorded reduction in platelet counts with increase in the dose rate using leaf extract of *M. Oleifera*.

Measurement of serum biochemical parameters can be especially useful to help identifying the target organs of toxic effects as well as the general health status of animals, and it is advocated to provide early warning of potentially deleterious changes in stressed organisms (Sacher & Mcpherson, 2000). The ethanolic extract of the root of *M. oleifera* on the serum biochemistry of the female Wistar rat at the doses used did not show any obvious changes in the animals. This suggests that the ethanolic extract of the root of *Moringa oleifera* has no toxic effect on the serum biochemistry of the female Wistar rats. This is at variant with the findings of Ajibade *et al.* (2012) that there were significant increases in the liver enzymes when high doses of *M. oleifera* seed extracts were used. Ferreira (2004) also recorded that the seed extract of *M. oleifera* is hepatoprotective at doses of 200 and 400mg/kg in Wistar rats. Kumar and Pari (2003) also recorded that the root of Moringa has a hepatotoxic effect. Bharali *et al*. (2003) reported that the oral administration of the hydroalcoholic extract of *M. oleifera* drumsticks enhanced levels of hepatic enzymes involved in detoxification of xenobiotic substances, such as carcinogens and plant venomous compounds. The findings from this study showed no adverse effect on the urea concentration in serum of the experimental rats. This is in agreement with the report of Ferreira (2004) on the effect of the seed extract of *M. oleifera* on the serum biochemical parameters of *Rattus noviegicus*.

In conclusion, oral administration of ethanolic extract of the root of *M. oleifera* is harmless to the rats since no adverse effects were detected in haematological and serum biochemical investigations. However, further studies involving the histopathological observations of the liver of Wistar rats treated with the ethanolic extract of the root of *M. oleifera* are necessary to confirm this hepatoprotective action

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