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Afr. J. Biomed. Res. Vol. 21 (January, 2018); 87- 90

Research article

Haematological Effects of Leaf Extract of *Moringa oleifera* Lam in Normal and Myelo-suppressed Wistar Rats

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ABSTRACT

Haematological effects of leaves extract of *Moringa oleifera* Lam were investigated in normal and myelo-suppressed Wistar rats. Acute toxicity and phytochemistry of *Moringa oleifera* were determined. Wistar-rats (n=35), aged 2 to 3 months, weighing 120 - 170 grams were divided into 5 groups of 7 rats per group, labeled A to E. Groups A and B were induced intraperitoneally with 3mg/kg body weight (b.wt) of cyclophosphamide for 7 days to achieve myelo-suppression. Groups A, B, C and D were orally administered with graded-doses of the extract (A=150, B=300, C=150 and D=300 mg/kg body weight) for 14 days. Group E served as control. Blood samples (3.0 ml) were collected on days 8 and 15 from each rat through the retro orbital plexus of the median canthus into tri-potassium ethylene diamine tetra acetic acid containers and analyzed using haematological auto analyzer (Sysmex KX-21N) following manufacturers guideline. The acute toxicity test of *Moringa oleifera* leaves revealed an oral LD50 of 3000 mg/kg body weight. Its phytochemical analyses revealed flavonoids ++, alkaloids ++, saponins +, tannins +, proteins ++, carbohydrate +, reducing ssugars +, steroids ++ and terpenoids +. On days 8 and 15 respectively, haemoglobin, haematocrit and total white blood cells of groups C, D and RBC of group D increased significantly ($p < 0.05$) when compared with the control. Groups A and B revealed dose- and time-dependent non-significant increases ($p > 0.05$) in haemoglobin, haematocrit, red blood cells and total white blood cells on day 15 when compared with day 8.

Keywords: *Moringa oleifera*, toxicity, phytochemical, myelo-suppression.

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Received: April, 2017; Revised Version Accepted: August, 2017

Abstracted by:
Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION

In various parts of the globe, medicinal plants have remained a significant alternative source of drugs for majority of populations that experience inadequate contacts with orthodox healthcare facilities (Omonkhelin *et al*., 2009). Due to poverty and underdevelopment in many parts of Nigeria and Africa at large, the use of such medicinal plants is leading a major role, and in most cases they are explored indiscriminately without adequate clinical and laboratory diagnosis and monitoring of treatments or relapses or toxicity as the case may be.

The uses of many chemotherapy drugs lead to some degree of myelosuppression (Ozkan *et al*., 2005). Myelo-suppression is characterized by the decrease in bone marrow cellularity, frequency and content of stem and progenitor cells. Granulocyte-macrophage progenitors are the most important suppressed group among haematopoietic cells resulting in neutropenia (Ozkan *et al*., 2005).

Moringa oleifera (M. Oleifera) is the most widely cultivated species of a monogeneric family, the moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicine and industrial uses (Anwar *et al*., 2007). The leaves of *M. oleifera* have been reported to be a valuable source of both macro and micro nutrients and is now found growing within tropical and sub-tropical regions worldwide, congruent with the geographies where its nutritional benefits are most needed. *Moringa oleifera* is rich in simple sugar, rhamnose, glucosinolates and isothiocyanates (Bennett *et al*., 2003; Fahey 2001). The leaves of *M. oleifera* contain thiamine, riboflavin and niacin. *Moringa oleifera* leaves are excellent source of protein, vitamins A, B-complex and C as well as minerals. All parts of *M. oleifera* are used in the treatment of ailments such as diabetes, scurvy, intestinal worms, diarrhoea, headache, earache, toothache, skin rashes and abrasion, wounds, ulcers, bronchitis, anaemia, sore

throats, rheumatism, lower back pain, liver and spleen problems, asthma, gout, lumbago, epilepsy, rabies, prostate and bladder problems warts, tumors, tuberculosis and so on (Caceres *et al*., 1990). They are exceptionally high in calcium and iron (Anwar *et al*., 2007). The hypocholesterolemic effects of crude extract of leaf of *M. oleifera* Lam in high fat diet fed wistar rats have equally been revealed (Ghasi *et al*., 2000).

There is paucity of data on the haematological effects of the leaves extract of *M. oleifera*. The aim of this study was to investigate the haematological effects of *M. oleifera* in normal and myelo-suppressed Wistar rats. The specific objectives were to determine the acute toxicity and the haematological effects of *M. oleifera* in Wistar rats.

MATERIALS AND METHODS

Collection of plant materials: The leaves of *Moringa oleifera* were obtained from the forest in Pocket Layout in Enugu South Local Government Area of Enugu State, Nigeria. It was identified and authenticated by a taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria Nsukka Campus, Nigeria and a voucher specimen kept in the herbarium for future reference.

Animal housing: Wistar rats (n = 35), were purchased and housed in the animal house of the College of Medicine, University of Nigeria Enugu Campus. The rats were acclimatized for two weeks and were fed with commercially available rat feed and had access to feed and water *ad libitum*.

Preparation of extract: Two hundred (200) grams of grinded, shade dried *M. oleifera* leaves were soaked in 2.5 litres of methanol for 48 hours with two- hourly vigorous shaking. The mixture was filtered using whatman No. 1 filter paper and evaporated to dryness on a rotary evaporator (Model 349/2 Carting Ltd). Then 10 grams of the concentrated crude extract was reconstituted with 100 ml of distilled water to get extract concentration of 100mg/ml ready for use.

Experimental design: Wistar-rats (n = 35), aged 2 to 3 months, weighing 120 - 170 grams were divided into 5 groups of 7 rats per group, labeled A to E. Groups A and B were induced intraperitoneally with 3mg/kg body weight of cyclophosphamide for 7 days to achieve myelo-suppression. Groups A, B, C and D were orally administered with graded-doses of the extract (A = 150, B = 300, C = 150 and D = 300 mg/kg bodyweight) for 14 days. Group E served as control. Blood samples (3.0 ml) were collected on days 8 and 15 from each rat through the retro orbital plexus of the median canthus into tri-potassium ethylene diamine tetra acetic acid containers for complete blood count and analyzed using haematological auto analyzer (Sysmex KX-21N) following manufacturers guideline. .

Statistical analysis: The Statistical Package for Social Science computer software version 20 was used for data analysis. The results of the tests were analyzed using analysis of variance and student's t-test at 95% confidence interval with p value less than 0.05 been considered as significant.

RESULTS

The acute toxicity test of *Moringa oleifera* leaves revealed an oral LD_{50} of 3000 mg/kg body weight. The phytochemical analyses of *Moringa oleifera* leaves revealed flavonoids ++, alkaloids ++, saponins +, tannins +, proteins ++, carbohydrate +, reducing ssugars +, steroids ++ and terpenoids + (table 1). On days 8 and 15 respectively, haemoglobin, haematocrit and total white blood cells of groups C, D and RBC of group D increased significantly ($p < 0.05$) when compared with the control. However, groups A and B revealed dose- and time-dependent non-significant increases ($p > 0.05$) in haemoglobin, haematocrit, red blood cells and total white blood cells on day 15 when compared with day 8 (tables 2 and 3).

Table 1:
The phytochemical constituents of the leaves extract of *Moringa oleifera*

Constitutents	Extract
Flavonoids	++
Antraquinone glycosides	-
Anthracene glycosides	-
Alkaloids	++
Saponins	+
Tannins	+
Resins	-
Proteins	++
Carbohydrate	+
Reducing sugars	+
Hydrolysis test for glycosides	-
Cyano-genetic glycosides	-
Fat and oils	-
Steroids	++
Terpenoids	+

Key: (-) Absent, (+) Present, (++) Moderately present

DISCUSSION

Despite the numerous researches conducted on *M. oleifera*, there is paucity of data on the haematological effects of the leaves extract of *M. oleifera*. During its use, it may have a good or adverse effects on haematological variables. Hence, it becomes necessary to investigate the myelo-protective activity of crude methanol leaves extract of *M. oleifera* in myelo-suppressed and normal Wistar rats. The myelo-protective activity is observed by judging the changes in haematological parameters of CP-induced anaemia model (Ufelle *et al*., 2011).

Cyclophosphamide was used to induce myelo-suppression in Wistar rats. Cyclophosphamide belongs to the nitrogen mustard subclass of alkyl ting agents under cytotoxic drugs used in the treatment of lymphomas, some forms of leukaemia and some solid tumors (Shanafelt *et al*., 2007). Cyclophosphamide have been known to act by slowing or stopping cell growth (Shanafelt *et al*., 2007).

Table 2:
Mean and standard deviation of haematological parameters of myelo-suppressed, normal and control wistar rats on day 8 of extract administration

Groups/ Variables	A Myelo-suppressed/ 150mg/kg b.wt Extract	B Myelo-suppressed/ 300mg/kg b.wt extract	C Normal/ 150mg/kg b.wt extract	D Normal/ 300mg/kg b.wt extract	E Control
Hb (g/dl)	5.8 ± 0.5	9.7 ± 0.8	12.3 ±1.2*	13.2 ±1.5*	11.5± 0.6
Hct (%)	21 ± 1	29 ± 2	38 ± 3*	42 ± 5*	0.35 ± 2
RBC (x10^9/L)	1.5 ± 0.3	3.2 ± 0.8	4.3 ±1.2	5.1 ± 0.5 *	4.7 ±1.5
MCHC (g/dl)	27.62 ± 0.5	33.45 ± 0.7	32.37 ± 0.4	32.38 ± 0.5	31.08 ± 0.2
MCH (Pg)	38.67 ± 0.8	30.31± 0.2	28.60 ± 0.6	26.67 ± 0.8	24.47 ± 1.2
MCV (Fl)	140 ± 2.5	90.63 ± 0.5	88.37± 1.3	82.35 ± 0.6	78.72 ± 1.5
TWBC (x10^9/L)	2.2 ±1.0	3.5 ± 0.8	4.7 ± 0.5*	5.5 ± 1.2*	4.2 ± 0.6
Neut (%)	37 ± 2	42 ± 3	55 ± 3	61 ± 2	55 ± 3
Lymp (%)	60 ± 3	54 ± 1	43 ± 2	36 ± 2	42 ± 2
Mono (%)	2 ± 1	2 ± 1	1± 1	2 ± 1	2 ± 1
Eosi (%)	1 ± 1	2 ± 1	1± 1	1 ± 1	1 ± 0.5

Table 3:
Mean and standard deviation of haematological parameters of myelo-suppressed, normal and control wistar rats on day 15 after oral administration of the extract

Groups/ Variables	A Myelo-suppressed/ 150mg/kg b.wt Extract	B Myelo-suppressed/ 300mg/kg b.wt extract	C Normal/ 150mg/kg b.wt extract	D Normal/ 300mg/kg b.wt extract	E Control
Hb (g/dl)	8.5 ± 0.5	10.8 ± 0.8	12.9 ±1.2*	13.8 ±1.5*	11.5 ± 0.6
Hct (%)	23 ± 1	29 ± 2	39 ± 3*	42 ± 5*	35 ± 2
RBC (x10^9/L)	2.2 ± 0.3	3.7 ± 0.8	4.6 ±1.2	5.5 ± 0.5 *	4.7 ±1.5
MCHC (g/dl)	36.96 ± 0.2	37.24 ± 0.4	33.94 ± 0.2	32.28 ± 0.5	31.08 ± 0.4
MCH (Pg)	38.67 ± 0.5	30.31 ± 0.3	28.60 ± 0.5	26.67 ± 0.2	24.47 ± 0.5
MCV (Fl)	153 ± 2.8	90.63 ± 1.5	88.37 ± 1.2	82.35 ± 0.8	78.72 ± 0.3
TWBC (x10^9/L)	3.5 ±1.0	4.2 ± 0.8	5.3 ± 0.2*	6.1 ± 0.8*	4.2 ± 0.6
Neut (%)	45 ± 2	50 ± 3.0	53 ± 3.0	56 ± 2.0	55 ± 3.0
Lymp (%)	52 ± 3	47 ± 1.0	45 ± 2.0	40 ± 2.0	42 ± 2.0
Mono (%)	1± 1	2 ± 1.0	1± 1.0	2 ± 1.0	2 ± 1.0
Eosi (%)	2 ± 1	1 ± 1.0	1± 1.0	2± 1.0	1 ± 0.5

The observed high LD_{50} of *Moringa oleifera* leaves indicates that the leaves of *Moringa oleifera* may not to toxic and will be safe for consumption. The observed numerous phytochemical constituents of *Moringa oleifera* indicate that this extract may possess medicinal properties and will be very useful in herbal therapy of some ailments.

The observed significant increases in haemoglobin, haematocrit and total white blood cells of the normal groups C, D and RBC of group D indicates haematopoietic potentials of the extract. These findings may be attributed to the observed phytochemicals. However, the observed dose- and time-dependent non-significant increases on day 15 in haemoglobin, haematocrit, red blood cells and total white blood cells in the myelo-suppressed groups A and B when compared with day 8 indicates that the extract may posses myelo-protective as well as haematinic activities. It was dose-dependent because the increases occur at higher extract dose. It was time-dependent because the increases occur at longer duration of extract administration. It was not significant probably because the cyclophosphamide effects did not fade out completely during the study period.

The blood films of the myelo-suppressed groups A and B showed moderate anisocytosis and poikilocytosis on day 8 but moderate anisocytosis and poikilocytosis on day 15 indicating haematinic potentials of the extract. However, the blood films of the normal groups C and D showed normocytic and normochromic red blood cells indicating haematopoietic potentials of the extract. The observed leucocytosis in both myelo-suppressed and normal groups indicates immune stimulatory potentials of the extract. This immune stimulatory potential by the extract agrees with the findings of previous

researchers that it has anti microbial activity (Caceres *et al*., 1990).

In conclusion, the normal and myelo-suppressed rats revealed increases in haemoglobin, haematocrit, red blood cell and total white blood cell count on days 8 and 15. This result pattern has demonstrated that the crude methanol leaves extract of *M. oleifera* may posses' myelo-protective, haematopoietic and haemopoietic potentials when orally administered to myelo-suppressed and normal wistar rats. However, further studies using column chromatography is recommended in order to characterize the leaves extracts and hence, find out the active components.

Acknowledgment

The authors wish to acknowledge the staff of the Department of Plant Science and Biotechnology, University of Nigeria Nsukka Campus, and the Animal House of College of Medicine University of Nigeria Enugu Campus for their valuable contribution to the success of the study.

Conflict of interest statement

The authors declared that they have no conflicts of interest.

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