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Effect of ethanolic leaf extract of *Moringa olifera* leaf on haematological and biochemical parameters of wistar rats

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ABSTRACT

Evaluation of the effect of ethanolic leaves extract of Maringa oleifera on Haematological and certain Biochemical parameters was studied. The study assessed Total protein, Albumin, Billirubin for biochemical and PCV, Hb and WBC for haematological indices. Twenty five (25) albino rats of wistar strain (100-175g) were used for the studies and were divided into 5 groups of five (5) rats per each. Group A served as control and was treated with distil water of treatment equivalence, group B, C,D, and E were treated with various concentration the extract as follows; viz: 100mg/kg, 200mg/kg, 300mg/kg, 1g/kg respectively. The administration of the extract lasted for twenty eight (28) days period after which the animals were sacrificed and blood obtained for biochemical and haematological analysis. The result of the study reveals that the ethanolic leaf extract of M. Oleifera lowers seruim bilirubin, but increase albumin, and Total protein levels in all treated groups compared with the controls all at ($p<0.05$) . More so, similar increased was also recorded for Haemoglobin (Hb) and Hematocrit but no significant ($p<0.05$) changes was observed for WBC in all treatment groups. The leaves extract of M. oleifera administered orally prove to be biochemically and haematologically save.

Keywords: Haematologcal, Biochemical, *Moringa olifera* leaf.

INTRODUCTION

Moringa oleifera, or the horse radish tree (Florida) is a tropical plant species that is an indigenous tree in northern India and Pakistan. It has been introduced throughout the tropics and subtropics and has become popular in many African countries. *Moringa oleifera* goes by many names, which depends on locality and/or their uses. It is known as drum stick tree in the Sudi region in India, the Ben oil tree in Haiti, and as Nèbèday in Senegal. In Asia, the fruits are the most important part of *Moringa oleifera* while the leaves are preferred in Africa (Lowell, 1989).

In the Philippines, the leaves of the *Moringa* are cooked and fed to babies; it is called mother's best friend or *Malungga*, while the leaves are used for salad and in soups. In Sudan, the flowers are made into paste and fried; the

leaf powder is put in the diet of children and pregnant/ lactating women, while whole, pounded or the seed cake, a residue from oil extraction have long been used to purify water(Lowell, 1989). In Nigeria seeds are eaten like groundnuts or added locally to sauces for their bitter taste. The seed oil known as “Ben oil” or “Behen oil” can be used for cooking, in hair dressing as a lubricant , in perfume industry as a base for fragrance and as volatile compounds in perfumes. Moringa acid oil, consisting of fatty acids from the seeds oil, is used as a lubricant and in soap making.

Almost all parts have traditional medicinal applications; especially their uses as an anodyne, antineoplastic, antispasmodic and disinfectant (bactericidal, fungicidal) are wide-spread. The bark excretes a white-reddish gum called Ben gum or Moringa gum). Nutritionally, Proximate evaluation reveals that the leafy tips of Moringa oleifera contains per 100g edible portion: water 78.7%, energy 268kg (61 kcal), protein 3.1g, fat 1.1g, carbohydrate 3.3g, total dietary fibre 2.0g, magnesium 117mg, phosphorus 112mg, iron 20mg zinc 0.6mg, thiamine 0.3mg, vitamin A 256IU, calcium 125mg, riboflavin 0.7mg, niacine2.2mg, folate 40mg, ascorbic acid 51.7mg (Olsen, 1987). The raw fruits contains per 100g edible portion: water 88.2g, energy 155kj (37kcal), protein 2.1, fat 0.2g, carbohydrate 8.5g, total dietary fibre 3.2g, calcium 20mg, magnesium 45mg, phosphorus 50mg, zinc 0.4mg, vitamin A 74 IU, thiamine 0.05mg, riboflavin 0.07mg, niacin 0.6mg, folate 41mg, ascorbic acid 141.0mg while the dry seeds contain on average: protein 29%, fibre 7% and oil 36-42% of the total fatty acid content oleic acid 65-75%. Behonic acid 90%, palmitic acid 90%, stearic acid 7%, and small amount of lignoceric acid and myristic acid (Olsen, 1987). The oil is clear and odourless and does not get rancid quickly. Seeds of *Moringa oleifera* contain a glucosinolate that hydrolyses to yields 4-(α- L-rhamnosyloxy) - benyl isothiocynate, an active bactericide and fungicide. The seeds of *Moringa oleifera* yield a lower amount (4-5% of dry weight) of gluco-inolate than those of *Moringa stenopetala* (8-10% of dry weight) and should therefore be used at a higher dosage (Lowell, 1989).

Moringa trees have been used to combat malnutrition, especially among infants and nursing mothers. Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. Moringa is especially promising as a food in the tropics because the tree is in full leaf at the end of the dry season when most foods are typically scarce. Moringa leaves have been reported to contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges, and more potassium than bananas, and that the protein quality of Moringa leaves rivals that of milk and eggs. The nutritional properties of Moringa are now so well known that there seems to be little doubt of the substantial wealth benefit to be realized by consumption of Moringa leaf powder in situation where starvation is imminent (Lowell, 1989). Pharmacologically, *Moringa oleifera* has been reported to be useful in the treatment, prevention of disease or infection either by using dietary or tropical administration of Moringa preparation such as (e.g. extracts, decoction, poultices, creams, oils, emollients, salves, powders and porridges) (Palada, 1996).It is useful to review the claims that have been made and to assess the quality of evidence available for the more well- documented claims. Wide spread claims of medicinal effectiveness of various Moringa tree preparation have encouraged authors at the John Hopkins university to further investigate some of these possibilities. A plethora of traditional medicine references attest to its curative power and scientific validation of these popular uses is developing to support at least to some of the claims. Moringa preparation have been cited in the scientific literature as having antibiotic, anti inflammatory, hypocholesterolemic, anti anaemic and hypoglycaemic activities, as well as having considerable efficacy in water purification by flocculation, sedimentation, antibiosis and even reduction of schitosome cercariae titer (Olsen,. 1987). Research on Moringa for which the existing scientific evidence appears to be particularly strong introduces antibiotics and cancer prevention (Kurup,1954., Das,1957). *Moringa* species have long been recognized by folk medical practitioners as having value in tumour therapy (Seyit, 2000), Dorothy Cullman and others examined compound -4(-L-rhamnopyranosyloxy) benzyl isothiocynate and its cognate isothiocynate for their cancer preventive potential (Fahey,2004). The consumption of variety of local herbs and vegetables by man is believed to contribute significantly to the improvement of human health in terms of prevention and cure of disease because plants have long served as a useful and rational source of therapeutic agents (Singh, 1975). However, since not much information is available about haematopoietic potentials or toxicity of *Moringa oleifera*, *this study was undertaken to unravel it haematopoetic properties.*

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

Fresh horseradish leaves were purchased at Sabon Gari market in Kano State. The leaves were dried in an airy room for about 3 days of drying, away from direct sunlight to avoid possible damage to their phyto-constituents. 40g of

the dried leaves was soaked in 250ml of ethanol, shaken for 10minutes and then allowed to stay in refrigerator for 48 hours at 4^{o}C. The mixtures were first filtered with cheese cloth, then with WhatMan No 1 filter paper (24cm). The filtrates were separately concentrated *in vacuo* using Rotary Evaporator (Model RE52A, China) to 10% of its original volume at 37o C - 40o C. These were concentrated to complete dryness in water bath in order to obtain the crude extract (Won *et al.*, 2005)

Laboratory Animals

Twenty (28) albino rats of wistar strain (100-175g) were obtained from the animal holding unit, Department of biochemistry department, university of Port Harcourt, Nigeria and were allowed acclimatization period of fourteen (14) days in well ventilated room with a temperature and relative humidity of 29±2^{o}c and 70% respectively. They were maintained with commercial rat chow (Vital Feeds LMT) and water *ad libitum*. The animals were housed in a cage and were exposed to 12 hour light-dark cycle and handled according to standard protocol. At the end of the acclimatization period, they were divided into four groups of six (6) rats per group. Group A served as control and was treated with distil water of treatment equivalence, group B, C,D,E were treated with 100mg, 200mg, 300mg and 1g/kg of the crude extract of *Moringa oleifera*. The administration of the extract lasted for twenty eight (28) days period after which the animals sacrificed after 24hrs after the last administration in chloroform saturated chamber in accordance with the guidelines of the European Convention for the Protection of Vertebrate animals and other scientific purposes –ETS-123 (European Treaty Series, 2005).

Determination of Haematological and Biochemical Parameters

Whole blood was collected from the heart by cardiac puncture using sterile syringe and needle. The whole blood samples were put in Ethylene di-amine tetra acetate (EDTA) treated sample tubes. The packed cell volume or the haematocrit was determined by the method of Baker and Silverton, 1985). White blood cell count (WBC) was determined by the method of Baker and Silverton, 1985 while Haemoglobin (Hb) was determined by the principle of Alexander and Griffins (1999). More so, biochemical assay was carried as follows, Bilirubin by the method described by Jendrassik and Grof (1938), total proteins assay was conducted by the method of Tiez (1995) while serum albumin levels was examined as described by Grant, (1987).

Statistical Analysis

The results of the proximate analysis and anti-nutrient screening were analysed for statistical significance by one way ANOVA (F- ratio) (Welkowitz, 1976) and student 't' test were applicable values at ($p<0.05$) were regarded as significant in comparison with appropriate control. All data were expressed as means of ± SEM.

RESULTS

The results of assessment of effect ethanolic leaf extract of *Moringa olifera on some haematological and biochemical parameters* of wistar ratsis presented in table 1 and 2 respectively based on 100mg, 200mg, 300mg and 1g/kg body weight of the extract.

TABLE 1 Effect of crude extract of *M.oleifera* on hematological parameters of wistar rats

Groups	Dose	HEAMATOLOGICAL PARAMETERS		
		PCV (%)	HB (%)	WBC (10^{9}/l)
A (Control)	-------	35.00±0.82	11.67±0.27	1.60±0.07
B	100mg/kg	31.90±1.29 ^{b}	10.66±0.42^{b}	1.68±0.09^{a}
C	200mg/kg	33.00±0.82 ^{b}	11.01±0.27^{b}	1.20±0.14 ^{b}
D	300mg/kg	35.75±0.95 ^{a}	11.92±0.32^{a}	1.13±0.10 ^{b}
E	1g/kg	37.25±0.96 ^{b}	12.42±0.33^{b}	1.48±0.13 ^{a}

Values are means ± standard deviation (n=6 for each group)
a= values are not significantly different from control at P≤ 0.05
b= values are significantly different from control at P≤ 0.05

Statistical evaluation reveals that the white blood cells counts of group B (1.68±0.09), E (1.48±0.13) which received 100mg and 1g/kg body weight of the extract showed no significant ($P<0.05$) change while those of group C (1.20±0.14) and D (1.13±0.10) were significantly ($P<0.05$) higher all when compared with the control (1.60±0.07). However, a significant ($p<0.05$) decreased was recorded for HB level in group B (10.66±0.42) and C (11.01±0.27) while significant ($p<0.05$) increased was observed for group D (11.92±0.32) and E (12.42±0.33) all when compared with the control (11.67±0.27). Beside, similar trend as observed for HB was also recorded for Haematocrit (PCV).

More so, for the biochemical parameters, statistical analysis showed that the ethanolic leaf extract of *Moringa olifera* recorded significant ($p<0.05$) increase for total proteins levels for all treatment groups; B (62.69±0.56), C (62.13±0.14), D (61.17±1.01), and E (57.80±3.67) all compared with the control (49.78±0.21). Similar trend was also observed for albumin and billirubin in all treatment groups when compared with the control ($p<0.05$).

TABLE 2 Effect of crude extracts of *M.oleifera* on serum biochemical parameters of wistar rats

Groups	Dose	BIOCHEMICAL PARAMETERS		
		Total Protein (g/l)	Albumin(g/l)	Bilirubin (mg/l)
A (Control)	--------	49.78±0.21	25.68±1.17	21.73±0.76
B	100mg/kg	62.69±0.56[b]	38.19±0.32[b]	87.49±1.35[b]
C	200mg/kg	62.13±0.14[b]	35.55±1.30[b]	83.67±1.27[b]
D	300mg/kg	61.17±1.01[b]	32.87±1.43[b]	82.09±1.39[b]
E	1g/kg	57.80±3.67[b]	26.31±0.87[b]	79.54±1.72[b]

a = values are means ± standard deviation (n=6 for each group)
b = percentages increase or decrease of parameters as compared with the control ($P \leq 0.05$).

DISCUSSION

The effect of ethanolic leaves extract of M. oleifera on haematological parameters was evaluated, analysed and interpreted. The assessment of haematological parameters is a biomarker for evaluating the haematotoxic potential of the extract in area of pharmacognosy (Aboyade, *et al*., 2009). Thus, the significant ($p< 0.05$) increase recorded for PCV and Hb levels at 300mg and 1g/kg body weight following the administration of ethanolic leaf extract of *M. oleifera* suggest that the extract contain some bioactive constituents or phytoconstituents which should have imposed or boosted haematopoietic activities. It is also supported by the fact that *M. Oleifera* leaf is rich in terms of nutritional value. Studies have shown that the leaf of *M*.oleifera is an outstanding source of vitamin A, B, C and also among the best plant source of minerals like iron and it is reportedly prescribed for anaemia and lactating mothers in the Philippines and also an excellent source of protein (Martin, 2000). Albumin is used as an indicator of liver impairment, reduced absorption or protein loss (Sacher and McPherson, 2000). Moringa oleifera leaf has repairing effect on the liver due to their nutritional properties such as the presence of essential amino acid like methonine and cysteine (Ramachandram, 1980; Oliveira *et al*, 1999) and thus boosting the total proteins and albumin level Ekam et al (2012). This study also agrees with the findings of lowell, (1989) that *Moringa* leaf have hypocholesterolemic, hypotensive and anti-anaemic activities. This was clearly observed with the 300mg and 1g concentration dose of *M. Oleifera* per kg body wieght. The biochemical examination of the serum total bilirubin showed very significant decreased in the level of treated groups and also increased in PCV and Hb levels when compared with the control group. This was in agreement with the finding of Yan Jun, (2004). Conclusively, from the research findings, we can conclude that oral administration of ethanolic leaf extract of *Moringa oleifera* might have a erythropoietic potential or that the extract aids the incorporation of haemoglobin in the RBC. Hence, *Moringa oleifera* leaf appears to be aid full in the management anaemic conditions since it possess erythropoietic and hepato protective potentials.

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