

Comparative Haematological and Serum Biochemical Evaluation of Rats Fed with Hydro-ethanolic Leaves Extracts of *Moringa oleifera* and *Telfairia occidentalis*

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Abstract: The study was carried out to compare the effect of hydro-ethanolic leaves extract (MOLHE) and (TOLHE) of *Moringa oleifera* and *Telfairia Occidentalis* respectively on some haematological and serum biochemical indices. A total of fifty wistar rats of both sexes (110-255g) were randomly allotted into five groups: A to E (n = 5). Group A (control) received water treatment equivalence. Groups B and D; C and E received 200mg/kg and 300mg/kg of TOHLE; MOLHE orally for 7 and 14 days respectively. Animals in group B and D significantly increased packed cell volume (PCV), Haemoglobin (Hb) and neutrophil levels. Group C had no significant effect on haematological indices while group E treated with 300mg/kg MOLHE showed dose dependent significant increases in PCV, Hb and lymphocyte levels in comparison with the control. Animals of both extracts recorded insignificant changes in white blood cells (WBC) compared to control. Animals in groups B and C significantly ($p < 0.05$) showed increased total protein concentration at 200mg/kg while serum total cholesterol (TC) was

insignificantly ($p>0.05$) lowered in all the treatment groups compared to control. Both leaves extracts could supplement total protein requirement, lower TC level and boost haemopoietic activities. Comparatively, TOLHE and MOLHE indicated insignificant difference in biochemical parameters analysed. However, significant ($p<0.05$) difference and increment in PCV, Hb and neutrophil levels were evident in the group treated with 200mg/kg TOLHE. Thus TOLHE may be haematologically better and /or useful in the cure of anaemia than MOLHE. In contrast, MOLHE enhanced lymphocytosis and could boost immune system more than TOLHE.

Keywords: *Moringa oleifera*, *Telfairia occidentalis*, leaves extracts, comparative effects

Introduction

Green leafy vegetables are particularly important in promoting health because of their rich sources of nutrients [1]. It has been reported that consumption of plant foods are associated with numerous health benefits rooted in their various physiological effects as a result of their phytochemical and nutritional constituents [2]. Thus consumption of natural plant foods and the use of nutritional therapy and phytotherapy to improve health, prevent and treat diseases are highly recommended [3]. *Telfairia occidentalis* Hook f. and *Moringa oleifera* Lam, have unique nutritional and medicinal benefits and are widely employed in folkloric medicine to treat different health conditions including malnutrition and nutrient deficient anaemia in most underdeveloped and developing parts of the world. *Telfairia occidentalis* is a plant commonly called fluted pumpkin or guard. *Telfairia occidentalis* belongs to the tribe of Joliffiae of the subfamily Cucurbitaceae [4]. The plant is perennial, dioecious, drought tolerant and usually low thrillished and thrives well in humid climate and well drained soil. It has simple, dark green viewed leaves that are as wide as 18cm and long as 35cm [5]. Ghanaians, Cameroonians, Serria Leoneans and Nigerians are the leading producers of fluted pumpkin in

West Africa. The plant is believed to have originated from the Igbos in the eastern Nigeria [6]. Fluted pumpkin leaves is good source of mineral such as Mn, Ca, Fe, Zn, K, Co, Cu, Mg; protein, vitamin A, B₂, B₄, B₁₂, C, and B₃ which are essential in human and animals. The iron content in fluted pumpkin leaves is the nutritional factor for its extensive use of the leaf extraction as blood tonic against fatigue and anaemia [7]. *Telfairia occidentalis* leaves extract has hepatoprotective, anti-inflammatory; cholesterolemic properties [8]. *Telfairia occidentalis* leaf meal (TOLM) had significant effect on haematological indices in animal model [9].

However, several studies have also been documented on the nutritive, therapeutic and prophylactic potentials of *M. oleifera* leaf meal. *Moringa oleifera* Lam is the most widely cultivated species of the genus, moringa or drumstick tree is the English common name. *Moringa oleifera* (MO) is a member of the family moringaceae, a perennial angiosperm plant [10]. MO is a native of the sub-Himalaya northern part of India and is cultivated across tropical and subtropical countries of the world including Nigeria. It is a fast growing evergreen deciduous tree, which can reach a height of 10-12m and trunk, a diameter of 45cm; with fragile branches and the leaves that build up

feathery foliage [11]. MO leaves are uniquely rich in trace elements, essential amino acids, and antioxidants such as vitamin C, flavonoids, and β -carotene [12]. Leaves extract has anti-inflammatory, anti-hyperlipidemic properties; improves humoral and cellular immunity and boosts haematological indices [13,14]. However, leaves extracts of *M. oleifera* and *T. occidentalis* are often being employed in combating malnutrition and nutrient associated anaemia [15]. This current study aimed at evaluating and comparing the effects of *T. occidentalis* and *M. oleifera* hydro-ethanolic leaves extracts on haematological and serum biochemical parameters.

Materials and Methods

Plant Material and Extracts

Preparation

The fresh leaves of *Telfairia occidentalis* was bought at Oja Oba market, Owo, while the fresh leaves of *Moringa oleifera* was collected in the department of Biological Sciences, College of Natural and Applied Sciences, Achievers University, Owo, Ondo State. Both plant leaves were authenticated by a Biochemist in the department of Biological Sciences, College of Natural and Applied Sciences, Achievers University, Owo, Ondo State, Nigeria. The both leaves were washed with clean water, separately shade-dried and reduced to a powdery form by grinding using electric blender, model EM-242. In separate set up 169.4 g of *M. oleifera* powdered sample and 123.7g of *T. occidentalis* were soaked in 1000ml and 770ml of 50% ethanol (hydro-ethanol) for 48 h at 4 °C respectively. After which it was sieved with a white cloth and then with Whatman No 1 filter paper (24cm). The filtrates were concentrated to complete

dryness in water bath at 37 °C – 40 °C to obtain a semisolid extract of 61.42g (36.3%) *M. oleifera* leave hydro-ethanolic extract (MOLHE), and 31.3g (25.3%) *T. occidentalis* leave hydro-ethanolic extract (TOLHE). The extracts were stored at 4 °C and used for the study.

Study Animals and Experimental

Design

A total of fifty adult wistar rats of both sexes, range from 110g -255g; with average weight of 158.3g were used in the study. The rats were obtained and maintained in the Animal House of the department of Biological Sciences, College of Natural and Applied Sciences, Achievers University, Owo, Ondo State, Nigeria. They were allowed to acclimatise for two weeks. Males were separated from the females to avoid possible pregnancy. The animals were housed in wire mesh cages under standard conditions (Temperature, 25-28 °C, 12 h light-dark cycles) and fed with commercial rat pelletized diet (vitafeed Ltd., Ibadan, Nigeria) and had access to water ad libitum. After the acclimatization period, the animals were allotted into two parts. Each part was divided into five groups A, B, C, D and E of five rats each. Group A served as the control and received only 1 ml distilled water treatment equivalence while group B, C, D and E were the treatment groups. Group B and D received 200mg/Kg and 300mg/Kg of the TOLHE while group C and E also received 200mg/Kg and 300mg/Kg of the MOLHE respectively. Both extracts were administered orally for 7 and 14 days. On the 8th and 15th day, rats were anaesthetised with chloroform and samples were collected for some haematological and serum biochemical parameters.

The study was generally conformed to the guidelines of the National Institute of Health for laboratory animal care and use [16] and in accordance with the principles of good laboratory procedure [17].

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 for haematological assay while lithium heparin sample tubes were for biochemical assay. The packed cell volume (PCV), White blood cell count (WBC), neutrophil and lymphocyte were determined by the method of Baker and Silverton [18] while Haemoglobin (Hb) was determined by the cyanomethaemoglobin method described by Cheesbrough [19]. Also, for biochemical assay, total proteins assay was done by the method of Tiez [20] and serum albumin levels assay as described by Grant [21]. Total cholesterol and triglyceride were determined by appropriate commercial kits (Randox laboratories, UK).

Data Analysis

All statistical analyses were assessed using SPSS statistical version 20.0 Software Package. Results were expressed as mean \pm SEM. Differences among the groups were analyzed by

one-way analysis of variance (ANOVA). Paired samples t-test was

used for comparison. Values were considered statistically significant at $p < 0.05$.

Results

The effects of MOLHE and TOLHE on haematological indices are shown in Tables 1 and 2 while serum biochemical parameters are in Tables 3 and 4. Results showed that group C treated with 200mg of MOLHE within 7 days recorded insignificant ($P > 0.05$) increase in PCV and Hb level compared to the control in Table 1. Also insignificant ($P > 0.05$) decrease in PCV and increase in Hb level were observed in 14 days compared to control (Table 2). Following the administration of 300mg MOLHE for 7 and 14 days, group E showed dose dependent significant ($p < 0.05$) increases in PCV, Hb levels and lymphocyte levels compared to the control groups in tables 1 and 2.

On the other hand, group B treated with 200mg of TOLHE significantly ($P < 0.05$) increased PCV but insignificant increase in Hb level within 7 days compared to the control (Table 1). But progressive significant ($p < 0.05$) increases in PCV and Hb level were recorded in 14 days. Tables 1 and 2 showed significant increase in neutrophil levels in group B throughout the experimental periods compared to control. Group D significantly ($p < 0.05$) increased PCV only within 7 days however, both PCV and Hb level were significantly ($p < 0.05$) increased in 14 days when compared with control in Tables 1 and 2.

Table 1: Effect of MOLHE and TOLHE on some haematological parameters for 7 days

Groups	Dose	PCV (%)	Hb (g/dl)	WBC (10 ⁹ /L)	Neut. (%)	Lymph.(%)
A(Control)	--	35.3±1.5	11.8±0.5	6.13±1.1	18.8±7.7	80.5±8.0
B(TOLHE)	200mg/Kg	39.0±3.0*	13.0±1.0	3.88±1.2	28.3±9.6*	70.3±9.6
C(MOLHE)	200mg/Kg	36.5±1.6	12.2±0.5	4.01±1.0	26.3±7.7	73.8±7.7
D(TOLHE)	300mg/Kg	40.0±4.2*	13.4±1.4	3.60±8.6	15.5±3.0	83.5±3.0
E(MOLHE)	300mg/Kg	39.3±0.9*	15.8±5.0*	5.71±1.0	19.5±2.1	90.5±2.1*

PCV = Packed cells volume, Hb = Haemoglobin concentration, WBC = white blood cells count, Neut. = Neutrophils level, Lymph. = Lymphocytes level

*indicates significant (p < 0.05) mean difference from control.

Table 2: Effect of MOLHE and TOLHE on some haematological parameter for 14 days

Groups	Dose	PCV (%)	Hb(g/dl)	WBC(10 ⁹ /L)	Neut. (%)	Lymph.(%)
A(Control)	--	39.0±0.4	12.1± 0.2	6.76±1.9	27.5±7.3	71.3±7.4
B(TOLHE)	200mg/Kg	45.5±1.3*	15.2±0.4*	8.69±1.4	39.0±5.8*	62.5±5.6
C(MOLHE)	200mg/Kg	38.5±0.7	12.9±0.3	8.51±6.6	29.5±2.1	69.3±2.8
D(TOLHE)	300mg/Kg	43.8±1.1*	14.6±0.8*	8.01±3.1	28.8±7.9	70.8±7.9
E(MOLHE)	300mg/Kg	44.0±1.7*	14.7±0.5*	9.31±2.9	21.5±5.7	79.5±5.0*

Groups B and C recorded significant (p<0.05) increase in total protein levels within 7

days, while group C also recorded 40% increase in albumin level with p<0.05 in 14 days treatment all compared to control (Tables 3 and 4). Total

cholesterol levels were insignificantly lowered (p>0.05) in all the treatment groups compared to the control in tables 3 and 4. Triglyceride level was insignificantly lowered in group B only compared to control in table 4.

Table 3: Effect of MOLHE and TOLHE on some serum biochemical parameters for 7 days

Groups	Dose	TP (g/dl)	ALB (g/dl)	TC (Mmol/L)	TRIG. (Mmol/L)
A(Control)	--	67.7±3.1	41.8±1.5	1.9±0.1	1.4±0.0
B(TOLHE)	200mg/Kg	72.1±2.2*	42.8±2.0	1.6±0.1	1.6±0.0
C(MOLHE)	200mg/Kg	72.5±1.9*	43.6±1.6	1.7±0.1	1.5±0.6
D(TOLHE)	300mg/Kg	69.9±3.6	41.9±0.8	1.7±0.0	1.4±0.0
E(MOLHE)	300mg/Kg	69.8±4.4	41.4±1.8	1.8±0.2	1.4±0.5

Table 4: Effect of MOLHE and TOLHE on serum biochemical parameters for 14 days

Groups	Dose	TP (g/dl)	ALB (g/dl)	TC (Mmol/L)	TRIG. (Mmol/L)
A(Control)	--	69.4±1.0	41.1±1.8	2.0±0.0	1.3±0.1
B(TOLHE)	200mg/Kg	72.6±2.9	42.4±1.5	1.8±0.1	1.1±0.2
C(MOLHE)	200mg/Kg	70.1±2.2	45.1±1.7*	1.0±0.2	1.4±0.1
D(TOLHE)	300mg/Kg	69.6±3.5	42.7±1.5	1.9±0.1	1.5±0.1
E(MOLHE)	300mg/Kg	72.4±1.4	41.9±1.5	1.9±0.8	1.5±0.1

TP= Total protein, ALB= Albumin, TC= Total cholesterol; TRIG= Triglyceride

*indicates significant ($p < 0.05$) mean difference from control.

Discussion

The leaf extracts of *Telfairia occidentalis* (TO) and *Moringa oleifera* (MO) are rich in protein; essential amino acids, vitamins, and minerals. The iron content in MO and TO is the nutritional factor for the extensive use of the leaves extraction as blood tonic to treat anaemia [7,22]. Iron is a necessary component of haemoglobin and myoglobin for oxygen transport. Malnutrition is the most common and wide spread cause of nutritional anaemia, such as iron deficiency anemia: a public health problem and serious problem among pregnant women and children in the developing countries. Cobalt, a constituent of Vitamin B₁₂, is essential for the maturation of erythrocyte. Vitamins B₁₂ and folate are haemopoietic factors. While ascorbic acid aids in iron absorption, copper involves in iron utilization and haemoglobin formation [23]. Thus Chandra *et al.* [24] observed that supplementation of haematinics (Cu, Fe, Co, folate and vitamin B₁₂) resulted in the removal of primary causes of nutritional anaemia and subsequent treatment promotes erythropoiesis in

rats. MO and TO leaves are rich in natural haematinics.

Thus the significant increases in PCV and Hb levels observed in this study following oral administration of *Telfairia occidentalis* may be due to its rich nutritional contents such as essential amino acids, vitamins and trace elements [9]. This finding is consistent with the observation of Obeagu *et al.* [9] when rats were fed with 200mg/kg and 300mg/kg of fluted pumpkin leaves for 7 and 14 days. However, the insignificant ($p > 0.05$) increase in WBC also observed in this study is inconsistent with the significant ($p < 0.05$) increase reported by Eseyin *et al.* [8]. Neutrophil is involved in cellular immunity and fights against bacterial infection. Rich nutritional content including copper and zinc might also contribute to the significant ($p < 0.05$) increase in neutrophil level recorded in this study [25].

Despite the reported high iron content in MO leaves, oral administration of 200mg/kg (MOHLE) resulted in insignificant ($p > 0.05$) changes in haematological indices compared to

control. This observation is also different from the significant ($p<0.05$) haematological values recorded in group treated with similar dose 200mg/kg TOHLE. The effect may be caused by the low iron bioavailability due to the polyphenolic compounds that exist widely in MO leaves, flowers and seeds [13]. Phenol has inhibitory effect that relates to its structure; association with galloyl and catechol groups which chelates iron and form non-bio-available polyphenol-iron complexes [27]. However the dose dependent significant increase in PCV, lymphocyte level and haemoglobin concentration following the administration of 300mg/kg MOHLE could be explained by an increase in ascorbic acid and beta-carotene, the non-heme-iron enhancers; protein intake that provides amino acids to porphyrin, globin and transferrin synthesis [28]. These significant ($p<0.05$) increments in PCV and Hb levels agree with the findings of Ujah *et al.* [15]. Again, the insignificant increase in WBC observed in this study is inconsistent with the significant increase reported by Ujah *et al.* [15]. Copper is considered to have strong effect on the immune system and is required for antibody development and lymphocytes replication [26]. However, the significant increase in lymphocytes levels observed in this study is in support of the work done by Gupta *et al* [14] that MO leaves extract contain bioactive phytochemical constituent that could enhance lymphocytosis and thus improves cellular immunity. Results also recorded statistically insignificant ($p>0.05$) decrease in total cholesterol levels in all the treatment groups, as well as triglyceride in the group that received 200 mg/kg of TOLHE) may be haematologically better and /or in the cure of anaemia

MOHLE compared to control. Cholesterol is a key component of cell membranes and also necessary for the formation of hormones such as estrogen, testosterone and vitamin D. High low-density lipoprotein (LDL) cholesterol in the body is implicated in atherosclerosis. MO leaves contain phytosterol such as Beta-sitosterol and can reduce intestinal uptake of dietary cholesterol [29]. Consequently, the prevention of intestinal uptake of dietary cholesterol by beta-sitosterol and quercetin might be the mechanism of the lowered cholesterol levels compared to control observed in this study. This finding agrees with the observation of Eseyin *et al.* [8]. Significant ($p<0.05$) increase in albumin level was also observed in 200mg/kg (MOHLE) when compared with the control. The increment in the total protein levels in both plants extracts compared to control may be due to the high content of crude protein presence in MO and TO leaves. This increment is in line with the previous findings reported by Ujah *et al.* [15] that MO leaves extract is a good source of supplementary protein in animals and humans.

Conclusion

From the findings of this study, *Telfairia occidentalis* and *Moringa oleifera* Lam. leaves extracts could boost haemopoietic activity, supplement protein requirement, and lower serum cholesterol level. Comparatively, the MOLHE and TOLHE indicated no significant difference in the biochemical parameters analysed. However, significant ($p<0.05$) difference and increment in PCV, Hb and neutrophil levels were evident in low dose 200mg/kg TOLHE. This suggests that *Telfairia occidentalis* leaves extract (than *Moringa oleifera* leaves extract (MOLHE). In contrast, MOLHE

enhanced lymphocytosis while TOLHE did not; thus MOLHE could boost immune system more than TOLHE. We therefore recommend that *Telfairia occidentalis* and *Moringa oleifera* Lam. leaves be consumed simultaneously for

effective nutritional and therapeutic benefits. More studies are strongly recommended to compare the effect of these two plants on haematological and serum biochemical parameters.

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