

Synergistic Effects of Leaves Extracts of *Moringa oleifera* and *Telfairia occidentalis* on Some Haemogram and Serum Protein Indices of Adult Wistar Rats

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Abstract

A combined administration of *Moringa oleifera* and *Telfairia occidentalis* leaves are increasingly been employed in herbal medicine in recent time. This study aimed at investigating the synergistic effect of hydroethanolic leaves extracts of *M. oleifera* (HLMO) and *T. occidentalis* (HLTO) on serum protein and haematological parameters of rats. Twenty adult wistar rats of both sexes (185 – 250 g) were randomly distributed into four groups of five rats per group. Group 1 (control) received distilled water treatment equivalence, groups 2, 3 and 4 received 300 mg / kg body weight of HLTO, HLMO and MOTO (HLMO and HLTO equal ratio of 1:1) orally for 14 days respectively. Rats were fed with standard feed and clean water *ad libitum* and were sacrificed 24 hours after the last treatment. Results obtained showed significant increase ($p < 0.05$) in packed cell volume and haemoglobin concentration in group 2 and 4 compared to the control. Significant increase ($p < 0.05$) in lymphocyte was observed in group 3. Total WBC count and albumin increased slightly in all the treatment groups when compared with control. There were significant decreases ($p < 0.05$) in total serum protein and globulin in group 4 compared with control. Our findings therefore, suggest that the synergy of the extracts could better enhance erythropoietin and haemoglobin production than individual extract. The duo extracts modulated serum total protein exempting globulin in rats.

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

1.0 INTRODUCTION

The use of herbal preparations predates recorded history and forms the origin of much of modern medicine (Andrew *et al.*, 2001). Presently, there is increasing interest in herb combination to achieve extra medicinal benefits for a number of diseases instead of the one-drug-one-target paradigm (Chun-Tao *et al.*, 2013). It is believed that the body is continually striving for balance, while herbal preparation supports the balancing process and helps treat a variety of health conditions which in some cases may have fewer side effects than some conventional medicine (Moquin *et al.*, 2009). Herbal preparations can be used either as single herb, combination of herbs or combination of herb (s) and drugs (Chun-Tao *et al.*, 2013). Several plants such as *Azadirachta indica*, *Alstonia boonei*, *Terminalia catappa*, *carica papaya*, *Picralima nitida*, *Pentadethra macrophylla*, *Phyllanthus niruri*, *Euphorbia hirta* and *Newbouldia* have been studied, validated and used in combination to treat many ailments such as drug-resistant malaria (Chuckkwuma, 2015). However, in southeastern Nigeria, the use of *Moringa oleifera* and *Telfairia occidentalis* leaves in combination in complementary and alternative medicine for claim of improved nutritional and medicinal benefits is gaining popularity.

Telfairia occidentalis Hook f. is a perennial, dioecious, drought tolerant and usually low thrilled which thrives well in humid climate and well drained soil (Emeka and Onyechi, 2009). The plant is a member of Cucurbitaceae subfamily; well known as fluted pumpkin in English. In Nigeria, fluted pumpkin is known by different names such as 'Ugu' in Igbo, 'Ubong' in Efik/Ibibio; 'Iroko' in Yoruba (Badifu and Ogunsina, 1991). Also, in South-eastern Nigeria where the plant is believed to have originated from, the fresh leaves of *Telfairia occidentalis* are squished and the dark green extraction mixed with milk is taken as blood tonic (Burket, 1968). *T. occidentalis* leaves contain vitamins, minerals (Mn, Ca, Fe, Zn, K, Co, Cu, Mg), proteins and fatty acids (Fasuyi, 2006; Idris, 2011) while phytochemical analysis also revealed saponin, glycosides, oxalate, alkaloids, tannins, phytate and flavonoid content (Akubue, 1980). Leaves extract of *T. occidentalis* has been found to stimulate anti-inflammatory response, production of alpha and gamma globulin, lower blood glucose level and enhance haemopoietic activities (Eseyin *et al.*, 2000b; Nwozo *et al.*, 2004; Idris, 2011; Obeagu *et al.*, 2014).

Moringa oleifera Lam. (MO) also known as horseradish or drumstick is a native of the sub-Himalaya, northern part of India. It is cultivated across tropical and subtropical countries of the world (Anwar *et al.*, 2007). MO belongs to the family of Moringaceae; the most widely cultivated species among the 13 species of the genus, moringa (Fuglie, 2000). The plant is mostly grown in Central and South America, India, Indonesia, Malaysia and African countries including Nigeria (Kumar *et al.*, 2010). It is a fast growing evergreen deciduous plant, with fragile branches and leaves that form feathery foliage of tripinnate leaves (Olson, 2002). The plant has nutritional, therapeutic and industrial benefits (Khalalla *et al.*, 2010). However, the leaves contain different anti-oxidants, eight essential amino acids and minerals such as iron, calcium, phosphorus, zinc, potassium and copper (Fuglie, 2006; Price 2007). Vitamins (A, B, C, D, and E) content of *M. oleifera* leaves is more than that found in a variety of foods such as carrots, milk, and oranges (Zarkada *et al.*, 1997). Phytochemical analysis also revealed

flavonoid, phytate, saponin, tannin and alkaloid in their respective proportion (Amaglo *et al.*, 2010). *Moringa oleifera* leaves extract has anti-hyperglycemic, anti-inflammatory and haemopoietic properties (Paris and Kumar, 2002; Ndong *et al.*, 2007; Ujah *et al.*, 2013). It also improved humoral and cellular immunity (Gupta *et al.*, 2010). Moreover, leaves of *Moringa oleifera* Lam. and *Telfairia occidentalis* Hook f. have some unique nutritional and therapeutic potential. Presently, attention has so much being directed to the use of both plants leaves in combination for claims of extra and improved nutritional and medicinal benefits. Meanwhile, some herbs when in combination could have antagonistic or complementary effect. The present study investigated synergistic effect of hydroethanolic leaves extracts of *Moringa oleifera* and *Telfairia occidentalis* (MOTO) on serum protein and haematological indices of wistar rats.

2.0 MATERIALS AND METHODS

2.1 Plant Material and Extracts Preparation:

The fresh leaves of *Telfairia occidentalis* were bought at Oja Oba market, Owo, Ondo State while *Moringa oleifera* leaves were obtained in the department of Biological Sciences, College of Natural and Applied Science, Achievers University Owo. Both plants leaves were authenticated by Botanist in the department of Biological Sciences, Achievers University Owo, Ondo State, Nigeria.

The leaves of *Moringa oleifera* and *Telfairia occidentalis* (fluted pumpkin) were properly washed in clean water. Fluted pumpkin leaves were detached from the stalks and cut into pieces. Both leaves were air-dried at room temperature for twelve days. The dried plants leaves were pulverized with an electric blender (Model EM-242) and 169.40 g of *Moringa oleifera* and 123.70 g of *Telfairia occidentalis* powdered samples were soaked in 1000 ml and 770 ml of 50% ethanol (hydro-ethanol) for 48 hours at 4°C. The concoctions were sieved with WhatMan No 1 filter paper (24 cm). The filtrates were concentrated to complete dryness in water bath at 37° C - 40° C to semisolid of 61.42 g (36.3%) of hydro-ethanol leaves extract of *Moringa oleifera* (HLMO) and 31.3 g (25.3%) of hydro-ethanol leaves extract of *Telfairia occidentalis* (HLTO). These stocks were stored at 4° C in refrigerator until required.

2.2 Experimental animals

Twenty male wistar rats weighing between 185 – 250 g were obtained and maintained in Animal Holding of the department of Biological Sciences, Achievers University Owo, Ondo State. They were randomly distributed into four groups of five animals per group. The animals were kept in cages in a room at a temperature ranged from 26 - 30° C with a 12 hour light-dark cycle for two week before the commencement of the experiment to allow the animals acclimatize with the new environment. The animals were fed with standard pellets (Vitafeed Ltd; Ibadan, Nigeria) and clean water *ad libitum*. Maintenance and treatment of animals were in accordance with the principle of the “Guide for care and use of laboratory animals in research and teaching” prepared by the National Academy of Science and published by the National Institute of Health (NIH, 1985) publication 86 - 23 revised in 1985. The ethical clearance was obtained from Ethical and Research Committee, Achievers University, Owo. The rats in Group 1 (control) received distilled water treatment equivalence. Group 2 received 300 mg / kg of leave extract of *Telfairia occidentalis* (HLTO). Group 3 received 300 mg / kg of leaves extract of *Moringa oleifera* (HLMO) while group 4 received 300 mg / kg of MOTO (HLTO and HLMO combined in ratio 150 mg / kg; 150mg / kg) orally for two weeks.

2.3 Blood collection

At the end of the experimental period (14 days), animals were allowed to fast over night. Rats were humanely sacrificed by cervical dislocation and blood samples were collected via cardiac puncture into Ethylene di-amine tetra acetate (EDTA) sample tubes for haematological assay and lithium heparin sample tubes for biochemical assay. The packed cell volume (PCV); White blood cell count (WBC) were determined by the method of Baker and Silverton (1985) and Haemoglobin concentration (Hb) by the cyanomethaemoglobin method described by Cheesbrough (2004). Serum total proteins and albumin assays were carried out using methods described by Tiez (1995) and Grant (1987) while globulin value was obtained by subtracting albumin from total protein. Serum from blood was obtained by centrifugation at 2000 rpm.

2.4 Data Analysis

All statistical analyses were assessed using Software Package for Social Sciences (version 20.0). Results were analyzed and expressed as Mean \pm Standard Error Mean (SEM). Differences among the groups were analyzed by one-way analysis of variance (ANOVA) and comparison by paired t-test. Values were considered statistical significant at $p < 0.05$.

3.0 Results

The haematological analysis showed that group 2 (HLTO) and group 4 (MOTO) recorded statistically significant increases ($p < 0.05$) in packed cell volume (PCV) and haemoglobin concentration (Hb) when compared with the control (Table 1). There was a statistically significant increase ($p < 0.05$) in lymphocyte count; slight increases in PCV and Hb in group 3 when compared with the control group (Table 1). There was a slight decrease in Neutrophil count in groups 3 and 4; the variation in group 2 was not statistically significant when compared with control group. Changes in white blood cell count in all the treated groups were not statistically significant ($p > 0.05$) when compared with the control group (Table 1).

In table 2, there was slight increase in albumin in all the treated groups when compared with the control group, while total serum protein and globulin mean values in group 2 and 3 were not significantly raised ($p > 0.05$). Unlike group 4 which recorded statistically significant decreases ($p < 0.05$) in total serum protein and globulin levels when compared with control.

Table 1: Effects of oral administration of HLTO, HLMO and MOTO on some haematological parameters for 14 days

Haematological Parameters	Groups(n=5)			
	Group 1 (Control)	Group 2 (HLTO)	Group 3 (HLMO)	Group 4 (MOTO)
PCV (%)	38.50±0.41	43.75±1.11 ^a	42.02±1.73	44.00± 2.10 ^a
Hb (g/dl)	12.55±0.50	14.58±0.76 ^a	14.00±0.58	14.68± 0.77 ^a
WBC (10 ⁹ /L)	6.76±1.88	8.01±3.12	9.31±2.91	9.45± 0.41
NEUT (%)	27.50±7.33	28.75±7.86	21.50±5.66	25.25±1.27
LYMP (%)	71.25±7.43	70.75±7.92	79.50±5.05 ^a	73.50±0.43

PCV = Packed cells volume, Hb = Haemoglobin concentration,
 WBC = white blood cells count, NEUT = Neutrophil, LYMP = Lymphocyte
 HLMO = Hydroethanolic leaves extract of *Moringa oleifera*
 HLTO = Hydroethanolic leaves extract of *Telfairia occidentalis*
 MOTO= combined HLMO and HLTO

^a indicates significant ($p < 0.05$) increase value compared to control.

Table 2: Effect of oral administration of HL TO, HLMO and MOTO on serum protein for 14 days

Biochemical Parameters	Groups (= 5)			
	Group 1 (Control)	Group 2 (HLTO)	Group3 (HLMO)	Group 4 (MOTO)
TSP (g/dl)	69.40±1.04	69.55±3.49	72.43±1.39	61.30±1.43 ^b
ALB (g/dl)	41.13±1.82	42.65±1.51	41.88±1.49	42.90±1.26
GLO (g/dl)	28.28±1.50	26.91±3.24	30.80±4.40	18.40±1.87 ^b

TSP= Total serum protein, ALB = Albumin, GLO = Globulin

^b indicates significant ($p < 0.05$) decrease value compared to control.

4.0 Discussion

According to the reported of Spinella (2002) the synergistic effect of a number of herbal components is actually much than the sum of each the individual components. This principle is employed in traditional African medicine, for instance, a decoction from a combination of herbs is commonly used for the management of chronic ailments (Item *et al.*, 2012). John and Rashid (2012) also reported that when herbs are combined, the resultant effect is generally more powerful in improving patient benefit than when the herbs are taken alone. Similarly, Item *et al.*, (2012) observed an enhanced anti-diabetic efficacy for diabetes mellitus when *Veronia amygdalina* Del. and *Azadirachta indica* were combined. Sabiu *et al.*, (2014) also noted that complementary effect of *Telfairia occidentalis* and *Veronia amygdalina* leaves is effective enough to ameliorate hepatic damage

in garlic induced hepatotoxicity. Although, contrary to Aghara (2014) who reported antagonistic effect of combined aqueous leaf extracts of *Moringa oleifera* and *Telfairia occidentalis* on erythropoietic activities in animal model. The combined effect of the extracts of *Telfairia occidentalis* and *Moringa oleifera* may have accounted for the increase in PCV and HB in treatment groups as observed in our study. The extracts of both *Telfairia occidentalis* and *Moringa oleifera* contain natural haematinic agents such as iron, folate, vitamins B₁₂, and copper as have been reported by Ajayi *et al.* (2000) and Ujah *et al.* (2011). Iron is a component of haemoglobin (Hb) and its deficiency results in reduction of red blood cells (RBCs) and/ or hypochromic microcytic anaemia. Copper is an essential element, necessary for iron utilization and haemoglobin formation (Davis and Mertz, 1987). Vitamin B₁₂ is essential for the maturation of RBC; stimulates erythropoiesis (Chandra *et al.*, 2000).

It could be presumed that the combined extracts has literally increased the concentration of some other component like tannin and saponin to a multiple fold, thereby exceeding the desirable amount in the rats; consequently, reduces vitamin B₁₂ absorption in the rats (Doss *et al.*, 2011). The effect of oral administration of HLMO for two weeks might probably be responsible for this haematological changes compared to the significant increase ($p < 0.05$) in PCV and Hb concentration observed in the group treated with HLTO and MOTO. This finding is in tandem with the reports of Ujah *et al.*, (2013) who observed significant increments in PCV and Hb concentration. The presence of these bioactive agents in HLMO might have influenced a right shift in lymphocyte count in the treatment group; this finding is in agreement with the work of Gupta *et al.* (2010) and Ujah *et al.* (2013).

Furthermore, studies have shown that bioactive components of *Moringa oleifera* play vital roles in the immune system and immunomodulating action (Ravaglia *et al.*, 2000; Brisebe *et al.*, 2009). *Moringa oleifera* leaf is a rich source of essential amino acids and could be used to supplement protein requirement of animal and humans (Price, 2007). Except in group 4 that received combined extracts, the present study also showed moderation of total serum protein, albumin and globulin when compared with the control. This finding is in agreement with the previous reports of Ujah *et al.* (2013) and Eseyin *et al.* (2000b) who reported enhanced total protein and globulin production by *Moringa oleifera* and *Telfairia occidentalis* leaves extracts in animal model. However, in this study, the observed significant reduction in total serum protein and globulin might suggest antagonistic effect of combined extracts of *Telfairia occidentalis* and *Moringa oleifera* on globulin synthesis. We therefore recommend further studies on mode of action of leave extracts of *Telfairia occidentalis* and *Moringa oleifera* on protein synthesis in animal model.

5.0 CONCLUSION

A combined administration of leaves extracts of *Telfairia occidentalis* and *Moringa oleifera* has complementary effect that could better enhance erythropoietin and haemoglobin production than individual extract. The duo extracts modulated serum total protein exempting globulin synthesis in rats.

6.0 ACKNOWLEDGEMENT

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7.0 Conflict of Interests

The authors do not have a direct financial relationship with the commercial identity mentioned in this paper.

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