

Haematological Profile of Rats Administered With Aqueous Leaf Extract of *Moringa Oleifera* Following Thermoxidized Palm Oil Diet Induced Toxicity

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Abstract: In assessing the effect of haematological parameters of rats administered with aqueous leaf extract of *Moringa oleifera* following thermoxidized palm oil induced toxicity. Twenty-five (25) Female rats weighing 150-170g body weight were assigned randomly into five groups: A-E of 5 rats, after permission was granted by the Collage Ethical Committee on Experimental Animals. Groups A serves as control received rat chow and water, Group B was fed with fresh palm oil diet (Diet I), Group C was fed with thermoxidized palm oil diet (diet II), Group D was fed with fresh palm oil diet and 600mg/kg body weight aqueous *Moringa oleifera* leaf orally for 28days, Group E received thermoxidized palm oil diet and 600mg/kg body weight orally for 28days. They were allowed free access to water and standard feeds ad libitum throughout the experimental periods. The animals were housed in stainless cages at temperature of $28\pm 20^{\circ}\text{C}$ and 12hrs dark cycle and humidity of 60%. After 28th day of feeding, the rats were allowed an overnight fast. They were anaesthetized with chloroform/diethylether mixture, their thorax was opened and blood was withdrawn by cardiac puncture with sterile syringe into EDTA sample bottle for haematological studies. Sequel to this, the bioactive component of *Moringa oleifera* was determined using standard methods. Results shows the presence of alkaloids, glycosides, saponins, tannins, flavonoids, reducing compounds, polyphenol, terpenes, steroid with highest concentration of flavonoids and polyphenols while anthraquinones, hydroxyl methyl and phlobatannins are not detected. Hematological result from RBCs showed significant increase ($P < 0.001$) in FPO, FPO+MO and TPO+MO; WBC were significantly increased in TPO, FPO+MO and TPO+MO. Platelets, HB and HCT were significantly reduced in TPO but were significantly ($P < 0.001$) higher in FPO+MO and TPO+MO. MCV and MCHC were significantly ($P < 0.001$, $P < 0.05$) increase in test groups compared with the control. The granulocyte % were significantly reduced ($P < 0.001$) in TPO and FPO+MO but were increased in FPO while monocyte were significantly reduced in test groups compared with the control. Lymphocytes were significantly ($P < 0.05$) increased in test groups compared with control. The results are indications of its haematopoietic and erythropoietic effect which suggests that the bioactive component of *Moringa oleifera* might possess a chelative or scavenging effect on the TPO diet.

Keywords: Bioactive components, EDTA, Haematological parameters, *Moringa oleifera*, Thermoxidized palm oil.

I. Introduction

Formation of free radicals result from the by-product of many normal cellular reactions including energy generation, breakdown of lipids and proteins and inflammatory processes [1] as well as intracellular accumulation resulting from exogenous products such as environmental pollutants that cannot be broken down by cells [2]. They may accumulate transiently causing reversible damage or permanently resulting in irreversible damage following their cytotoxic effects. It has been theorized that they are involved in degenerative disorders such as *Alzheimer dementia* and *Parkinsonism* plaque formation in atherosclerosis and cancer [3]. Free radical injury has been known to be the final pathway to tissue damage by injurious agents. Uncontrolled free radical formation damage cell membranes, cross-link proteins, damage the nucleic acid that make up DNA. An imbalance between free radicals generation and free radicals scavenging system results in oxidative stress. Free radical mediated damage contributes to such varied process as chemical injury, ischemia-reperfusion injury [4,5,6,7] mitochondrial dysfunction [8,9], loss of membrane phospholipid, cytoskeletal abnormalities. [8]. Haemoglobin alteration in auto mechanics exposure to motor spirit fumes [10] has been reported.

The list of chemicals/substances that may cause cell injury defies compilation [11]. Simple chemicals such as glucose and salt in hypertonic concentration may cause cell injury directly or by deranging electrolyte homeostasis of cells. Nutritional excesses and nutritional deficiencies [12,13] such as obtain in urbanised or rural

areas have become important causes of ill health. Whether over nutrition or under nutrition the common position of the diet makes a significant contribution to a number of diseases.

Thermally oxidised palm oil (TPO) remains the one form of palm oil used by road-side food, fish sellers. Normally palm oil is consumed either freshly or as thermally oxidized. It is thermally oxidized when the fresh form is subjected to several rounds of heating at high temperature. This way the exogenous fumes are inhaled and/or taken into the body by those who prepare as well as when taken in diets. Its cytotoxic and destructive by-products are known to be injurious to cells, tissues and organs [11]. Long term consumption of oxidised oils and fats causes devastating physiological and biochemical effects [14]. Free radicals generated are said to be involved in the aetiology of disease state [15,16,17].

Moringa oleifera, family *moringaceae* is a native to sub-Himalayan region India, Pakistan, Asia Minor, Africa and Arabia [18,19]. *Moringa oleifera* is widely cultivated and has become naturalized in many parts of the tropics [20]. All parts of the plant: root, bark, fruit, seed, leaf, pods) have been reported to have medicinal, social, nutritional, industrial value [21,22]. The plant contains protein, vitamins, B-carotene, amino acids and various phenolics [23] which confer its use in man's health challenges. *Moringa oleifera* leaves are used for preparation of soup and additionally as antipyretic, antidiarrhea cases but how it affect blood cells remain to be researched. It is believed that inhaling oil fumes and consumption of thermally oxidized oil and fat induced free radicals which are injurious to the tissues and cause haematological disorders. Therefore, in this study it is necessary to investigate the effect of *Moringa oleifera* on blood cells of rats fed with thermoxidized palm oil diet.

II. Materials and Methods

Collection of plant material

The leaves of *Moringa oleifera* were collected in March, 2013 from Okuku, Yala, Local Government Area, Cross River State, Nigeria. The plant material was authenticated in the Department of Botany, University of Calabar, Nigeria. The leaves were washed with distilled water and sun dried, pulverised by electrical homogenizer into snuff-like powder and macerated 1:10 w/v solvent (water) and left for 12hrs, filtered using Whatman 1 filter paper. The filter was dried in an aerated oven at 45°C to constancy. The resulting extract was stored in screw-capped bottle until use.

Evaluation of the crude phytoconstituent of *Moringa oleifera* leaf

The screening of the leaf extract for the presence of phytochemicals was carried as described using standard methods: alkaloids with Mayer and Dragendoffs reagent; flavonoid, with Mg and HCl, ; tannin ferric chloride test;steroids;glycoside; polyphenol potassium ferrocyanate test ;saponin heated and cooled;phlobatinin test ;Anthraquinones Benzene and 10% ammonia test ; hydroxy-antranquinone 5% ammonia test; reducing Sugars Fehling's test[2021,22,23] .

Animal

Female rats (25) weighing 150-170g body weight were obtained from Animal House of the Department of Human Physiology, Faculty of Basic Medical Science, Cross River University of Technology were assigned randomly into five groups: A-D of 5 rats, after permission was granted by the Collage Ethical Committee on Experimental Animals. Groups A serves as control received rat chow and water, Group B was fed with fresh palm oil diet (Diet I), Group C was fed with thermoxidized palm oil diet (diet II), Group D was fed with fresh palm oil diet and 600mg/kg *Moringa oleifera* leaf orally for 28days, Group E received thermoxidized palm oil diet and 600mg/kg MOLE orally for 28days. They were allowed free access to water. The animals were housed in stainless cages at temperature of 28±20Cand 12hrs dark cycle and humidity of 60%.

Preparation of thermoxidized palm oil and formulation of diets.

Five (5) litres of fresh palm oil was purchased from Okuku market, Yala Local Government Area, Cross River State, Nigeria was divided into 2 equal portions: one portion was subjected to thermal oxidation and the other was used as fresh. These forms are the two states palm oil is used for cooking. Palm oil was heated at 150 °C in stainless pots for 20mins and allowed to cool for 5hrs. This was done five times. In most West African diet the level of palm oil is 15%[24], 15g of the cooled thermoxidized palm oil was mixed with 85g of rat feed. This is (diet II). On the other hand 15g of fresh palm oil was mixed with 85g of rat feed form (diet I). All diets were stored in dark container to prevent further oxidation of oil content.

Collection of blood

After 28th day of feeding, the rats were allowed an overnight fast. They were anaesthetized with chloroform/diethyl ether mixture, their thorax was opened and blood was withdrawn from the heart with sterile syringe into EDTA sample bottle for haematological studies.

Haematological Analysis of blood samples

Analysis of blood samples was done using automated machine VITROST DT System: Vitros DTSC II module. Johnson Johnson, UK, 2004

Statistical analysis

Data was expressed as mean \pm SEM. Student t-test was used to compare means. P values of 0.05 were accepted significant.

III. Results

The result reveals that aqueous extract of *Moringa oleifera* contain bio active phytonutrients like Alkaloids, Glycosides, Saponins, Tannins, Flavonoids, Reducing compound, Polyphenol, Phlobatannins, Triterpenes, Steriod, though, polyphenols and flavonoids appears in higher concentration while Anthraquinones, Hydroxymethyl are not detected (Table 1). RBCs showed significant increase ($P < 0.001$) in FPO, FPO+MO and TPO+MO; WBC were significantly increased in TPO, FPO+MO and TPO+MO. Platelets, HB and HCT were significantly reduced in TPO but were significantly ($P < 0.001$) higher in FPO+MO and TPO+MO. MCV and MCHC were significantly ($P < 0.001$, $P < 0.05$) increase in test groups compared with the control. The granulocyte % were significantly reduced ($P < 0.001$) in TPO and FPO+MO but were increased in FPO while monocyte were significantly reduced in test groups compared with the control. Lymphocytes were significantly ($P < 0.05$) increased in test groups compared with control (Table 2).

Table 1: Phytochemical contents of *Moringa oleifera* leaf

Chemical constituent	Aqueous extract
Alkaloids	+
Glycosides	+
Saponins	++
Tannins	+
Flavonoids	+++
Reducing compound	+
Polyphenol	+++
Phlobatannins	-
Triterpenes	+
Steroid	+
Anthraquinones	-
Hydroxymethyl	

Key:

- + Present
- ++ Present in excess
- +++ Present in much excess
- Absent

Table 2: Result of Haematological Analysis of Thermo oxidized Palm Oil Diet

Parameter	Ctrl	FPO	TPO	FPO+MO	TPO+MO
RBC($\times 10^9/L$)	6.27 \pm 0.21	6.84 \pm 0.26**	5.74 \pm 0.17**	8.00 \pm 0.10**	7.66 \pm 0.07*
WBC($\times 10^6/L$)	6.17 \pm 0.33	5.13 \pm 0.24	7.60 \pm 0.20*	8.44 \pm 0.24**	9.02 \pm 0.03**
PLT($\times 10^6/L$)	469 \pm 3.80	439.17 \pm 1.19**	426.33 \pm 6.80*	539.36 \pm 6.80*	536.36 \pm 15.8**
HB(g/dl)	13.02 \pm 0.25	13.05 \pm 0.25	10.77 \pm 0.25**	13.16 \pm 0.24*	13.18 \pm 0.10*
HCT(%)	40.4 \pm 0.69	41.18 \pm 0.49	34.71 \pm 0.25**	42.04 \pm 0.06*	42.62 \pm 0.04*
MCV(fl)	45.50 \pm 0.24	54.67 \pm 0.33**	52.89 \pm 0.20**	51.67 \pm 0.73*	54 \pm 0.45**
MCH (pg)	17.76 \pm 0.61	17.73 \pm 0.20	16.77 \pm 0.15	16.63 \pm 0.30	17.27 \pm 0.29
MCHC(g/dl)	30.63 \pm 0.01	31.75 \pm 0.57*	30.60 \pm 0.25	30.68 \pm 0.08	31.73 \pm 0.17*
GRAM(%)	20.28 \pm 0.04	21.45 \pm 0.14*	15.42 \pm 0.13**	14.07 \pm 0.05*	20.20 \pm 0.08
MONO(%)	16.28 \pm 0.08	10.67 \pm 0.09*	9.37 \pm 0.10*	12.37 \pm 0.15*	10.67 \pm 0.08**
LYM(%)	6.03 \pm 0.03	6.35 \pm 0.12*	17.45 \pm 0.18**	7.13 \pm 0.58*	9.88 \pm 0.31**

Data expressed as mean \pm SEM, n=6, *= $P < 0.05$, **= $P < 0.01$, ***= $P < 0.001$, ns= not statistically significant.

IV. Discussion

Drugs and or chemicals induced anaemia [25] as well as free radical induced injury[1,26] on cells and tissue has been known. We fed rats with thermally oxidized palm oil diets for 28 days with daily administration of *Moringa oleifera* 600mg/kg body weight oral dose to study the effect on haematological parameters most especially on the TPO+MO groups.

The results showed that red blood cell counts were significantly higher in the *Moringa oleifera* feed groups compared with FPO, TPO and control. An increased red blood cell in male rats has been reported by [27,28]. This suggests that the leaf extract contain agents that induced the production of red blood cells or enhances erythropoiesis by the bone marrow. Although, the mechanisms involved were not studied. Red blood cells carry oxygen to various parts of the body by haemoglobin [10]. Haemoglobin content in the *Moringa oleifera* fed groups was significantly higher. This increase may arise as a result of increase red blood cell count and possibly the mineral content of the leaf extract especially iron content, an increase in iron supply is necessary to elevate the haemoglobin content. This suggests that the leaf may be useful in management of anaemic conditions, supporting the acclaimed folkloric usage that *Moringa oleifera* leaf relieves conditions of malaria and typhoid.

The haematocrit of the extract fed rats were significantly higher compared with TPO and Control. The measure of haematocrit is the number and size of red blood cells [26]. Similar increase was noted by [29]. The result suggests an increase in volume of cells which is confirmed by the increase in mean cell volume (MCV). A high MCV is an indication of macrocytic anaemia suggesting vitamin B₁₂ and folate deficiency but it is not clear whether the biochemical consequence of cobalamin is one of inability to synthesise new DNA bases or defect in cell division seen in cytotoxic drugs or haematological disease in the marrow such as myelodysplasia[29]. The mean cell haemoglobin concentration (MCHC) was seen to be increased in TPO+MO group. This suggests hyperchromasia although blood films were not studied. The presence of macrocytes, abnormally rounded cells with increased cell thickness greater than normal to increased MCHC [25] as seen in TPO+MO. The *Moringa oleifera* fed groups showed increased platelet count this result agree with that of [30]. [31], observed that Epo and Tpo are closely related in the production of red blood cells and platelets. Epo and Tpo are identical at the N-terminal region and therefore have some effect on erythropoiesis, although the exact role is not clear. It is recognised that several types of anaemia are associated with arise in platelets. Increase in total white blood cell count may be beneficial as they are vital in the body's defence mechanism [32].

Taken separately, the TPO+MO group exhibited mark changes in the haematological parameters indicating that the insult on the thermoxide palm oil diet fed rats has been ameliorated. Scientists continue to investigate the use of free radical scavengers to protect against cell injury during periods when protective cellular mechanisms are impaired [33]. Antioxidants are involved in the prevention of free radical cellular damage, the common pathway of a variety of diseases. The subdued effect of free radicals in the TPO+MO group must be due to the presence of such radical scavengers as zeatin, quercetin, beta sitosterol, caffeoylquinic acid and kaempferol, important for *Moringa oleifera* medicinal values. These antioxidants in the *Moringa oleifera* act as defence system against free radical damage.

This report is in accordance with the report of [33,34].

In conclusion, *Moringa oleifer* aqueous leaf extract given to rats orally at the dose of 600mg/kg body weight improved full blood count in female rats. MCV, MCHC were be increased indicating the presence of macrocytes and hyperchromasia respectively. The extract should be taken be with caution despite of its antioxidant and haematopoietic effect.

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