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## Effects of the aqueous leaf solution of *Moringa oleifera* on some hematological and biochemical parameters in male Wistar rats

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### Abstract

*Moringa oleifera* is a widely used medicinal herb but often in crude forms and arbitrarily. Thus, this study aimed to investigate the effects of the crude aqueous leaf solution of *Moringa oleifera* (ALSMO), on some haematological and biochemical parameters in male Wistar rats. Twenty (20) male Wistar rats were randomly separated into 5 groups of 5 rats each. While Group 1 served as control with no treatments, groups 2, 3 and 4 were treated with 40, 60 and 80mg/kg respectively of the ALSMO. After 28 days of treatment, analysed blood samples from the rats revealed dose-dependent significant ( $p < 0.05$ ) increases in blood cells in the treated animals compared to the control group. And significantly ( $p < 0.05$ ) reduced triglyceride and high density lipoprotein cholesterol levels in treated rats. While ALSMO may boost some haematological parameters and reduce risk of atherosclerosis, higher doses of ALSMO may result in hepato-renal toxicities in the study animals.

**Keywords:** *Moringa oleifera*, hematological parameters, ethnobotanical applications, hypo triglyceridaemic

### Introduction

Of recent, researches into the beneficial activities of various medicinal plants for the purpose of management of many disorders, following their perceived beneficial advantages over the orthodox medications, has being on the increase<sup>[1,2]</sup>.

Unarguably, plants remain the major reservoir for production of novel medicines and other therapeutic agents, hence the huge dependence of the populace on medicinal plants or herbs<sup>[2,3]</sup>. This is due to the fact that plants, as natural products, are readily available, less costly and with less side effects on the biological system<sup>[1]</sup> of which *M. oleifera* is a leading example<sup>[4]</sup>.

*M. oleifera* is of the Moringaceae family and a perennial tree that is vastly cultivated in many tropical countries<sup>[4]</sup>. It has the ability to grow even in adverse conditions. *M. oleifera* is also referred to as the miracle tree, this is due to its multiple beneficial indications in many ethnomedicinal applications. Many claims have revealed that there is no record of any side effects following the use of different parts of *M. oleifera* plant.<sup>[4]</sup> *M. oleifera* is reported to possess great therapeutic potentials and helps in improving conditions like diabetes, malnutrition, anemia, stress, arthritis, amongst other disorders.<sup>[4]</sup> It has also been shown to possess the capacity to stabilize many cardiovascular disorders, blood sugar concentration and has anti-oxidant effect, anti-inflammatory and anti-carcinogenic potentials<sup>[5]</sup>

Thus considering the increasing utilization of herbal medicines and products, the issue of efficacy and safety of herbal medicines must be taken seriously as to ensure a safe public health.<sup>[6]</sup> This is very important as adverse health effects associated with the use of herbal products have been linked to both inherent toxicity of herbal medications and their contaminants. Hence the need to ensure the efficacies and safety profile of these medicinal herbs through scientific studies<sup>[7]</sup>. Therefore, this study is an example of such efforts to investigate the effect of aqueous leaf solution of *M. oleifera* on some haematological and biochemical parameters in male Wistar rats using the crude leaf solution as is being used locally<sup>[7]</sup>.

Owing to the popular claim of the benefits of the *M. oleifera* plant, there is an increasing ethnobotanical applications of the plant and more often than not, it is without minding whether using such crude form of the various parts of the plant is actually efficacious<sup>[8]</sup>.

Hence the need to investigate the actual effects of aqueous leaf solution of *M. oleifera*

(ALSMO) on some hematological and biochemical parameters in male Wistar rats using the crude aqueous leaf solution as in local setting.

### Materials and Methods

This experimental/laboratory-based study used an animal model, male Wistar rats. Twenty (20) male Wistar rats weighing between 170 and 200g were procured from and housed in the Animal House of Faculty of Basic Medical Sciences (FBMS), University of Port Harcourt, and were kept under the natural light/dark cycle. Institutional Ethical approval was sought and obtained from the University of Port Harcourt Central Ethics Committee and the guidelines for handling and use of laboratory animals were observed.

The rats had free access to standard rat feed and tap water *ad libitum*. The animals were acclimatized to their new housing and handling for two weeks after which administration of the ALSMO decoction commenced which lasted for 28 consecutive days.

### Collection and Identification of *M. oleifera* plant and Preparation of ALSMO Decoction

Fresh leaves of *M. oleifera* were procured from the Fruit Garden Market in Port Harcourt Metropolis, Rivers State of Nigeria and were authenticated at the University of Port Harcourt Herbarium and voucher number (UPP/P-062) obtained.

The methods of Ojeka *et al.*,<sup>[9]</sup> and Yi and Chang,<sup>[10]</sup> were adopted for the preparation of the aqueous leaf solution of the *M. oleifera* plant. The fresh leaves of *M. oleifera* were collected, washed and shade dried before intermittent boiling in a pot of water for 24 hours. The decoction was then sieved into clean sample containers and stored in the refrigerator at 4 °C. The stock for the administration was prepared from this filtrate and thereafter, the different doses of 40, 60 and 80mg/kg of the *M. Oleifera* leaf solution were determined according to the average body weights of the rats in the respective test groups.

It is important to note that, the decision to use the specified doses (of 40, 60 and 80mg/kg) in the present study was based on the report of an earlier study by Ojeka,<sup>[9]</sup> which reported

an LD<sub>50</sub> of over 1000mg/kg bw for the aqueous leaf solution of *M. Oleifera*.

### Experimental Protocol and Treatment of Study Animals

The 20 male Wistar rats were divided into 4 groups of 5 rats each after the two weeks of acclimatization. The experimental protocol and 28 days administrations with the ALSMO decoction followed thus:

1. Group 1: Consisted of 5 rats, served as control and received 1ml of distilled water.  
Groups 2, 3 and 4 had 5 rats each, and served as test groups and received 1ml of different doses of *M. oleifera* leaf as follows:
2. Group 2 received 40mg/kg of ALSMO.
3. Group 3 received 60mg/kg of ALSMO.
4. Group 4 received 80mg/kg of ALSMO.

The administration of the ALSMO decoction commenced and lasted for 28 consecutive days after which, laboratory samples were collected from the study rats and were analysed.

Quantitative data from the laboratory analysis were then subjected to statistical analyses using analysis of variance (ANOVA) and LSD Post HOC test tools of the IBM's SPSS version 20.0. A p-value of less than 0.05 was considered statistically significant.

### Results

Table 1 show the effect of ALSMO on some haematological parameters. The ALSMO shows the tendency to significantly ( $p < 0.05$ ) increase RBC, control group PCV and Hb levels in the treated rats when compared to the untreated rats. Notably, these increases were in a dose-dependent manner. The levels of WBC and platelet count in all ALSMO treated groups showed graded significant ( $p < 0.05$ ) increase when compared to that of the control group.

Values are presented as mean $\pm$ SEM. N = 5.  $p < 0.05$ . \* Values are statistically significant when compared to the control; <sup>a</sup>Values are statistically significant when compared to 40mg/kg ALSMO; <sup>b</sup> Values are statistically significant when compared to 60mg/kg ALSMO.

**Table 1:** Effects of Aqueous Leaf Solution of *M. oleifera* (ALSMO) on some hematological parameters

Groups	Red Blood Cells (RBC) (X10 <sup>12</sup> /L)	PCV (%)	Hb (g/dL)	White blood cells(WBC) (x 10 <sup>9</sup> /L)	Platelets (x 10 <sup>9</sup> /L)
Control	4.84 $\pm$ 0.02	30.20 $\pm$ 2.99	10.02 $\pm$ 1.0	5.60 $\pm$ 0.15	205.20 $\pm$ 0.26
40mg/kg ALSMO	5.27 $\pm$ 0.03*	34.00 $\pm$ 2.76*	11.32 $\pm$ 0.93*	7.18 $\pm$ 0.04*	208.00 $\pm$ 1.04*
60mg/kg ALSMO	6.02 $\pm$ 0.01*, <sup>a</sup>	40.80 $\pm$ 2.08*, <sup>a</sup>	13.56 $\pm$ 0.71*, <sup>a</sup>	10.32 $\pm$ 0.04*, <sup>a</sup>	210.8 $\pm$ 0.71*, <sup>a</sup>
80mg/kg ALSMO	6.58 $\pm$ 0.06*, <sup>a,b</sup>	45.80 $\pm$ 3.48*, <sup>a,b</sup>	15.24 $\pm$ 1.16*, <sup>a,b</sup>	11.34 $\pm$ 0.05*, <sup>a,b</sup>	259.20 $\pm$ 0.51*, <sup>a,b</sup>

Values are presented as mean $\pm$ SEM. N = 5.  $p < 0.05$ . \* Values are statistically significant when compared to the control; <sup>a</sup> Values are statistically significant when compared to 40mg/kg ALSMO; <sup>b</sup> Values are statistically significant when compared to 60mg/kg ALSMO.

**Table 2:** Effects of Aqueous Leaf Solution of *M. oleifera* (ALSMO) on some Liver Enzymes

Groups	Aspartate aminotransferase (AST) (U/L)	Alanine aminotransferase (ALT) (U/L)	Alkaline phosphate (ALP) (U/L)
Control	4.80 $\pm$ 0.04	25.00 $\pm$ 7.11	27.00 $\pm$ 10.73
40 mg/kg ALSMO	6.40 $\pm$ 0.05*	20.20 $\pm$ 2.78	28.80 $\pm$ 3.44
60 mg/kg ALSMO	7.20 $\pm$ 0.04*, <sup>a</sup>	16.60 $\pm$ 1.12*	34.20 $\pm$ 14.79*
80 mg/kg ALSMO	10.40 $\pm$ 0.04*, <sup>a,b</sup>	41.40 $\pm$ 3.75*	34.80 $\pm$ 10.75*

**Table 3:** Effects of Aqueous Leaf Solution of *M. oleifera* (ALSMO) on Lipid Profile

Groups	Total Cholesterol (TC) (mmol/L)	Triglycerides (TG) (mmol/L)	High density Lipoprotein (HDL-C) (mmol)	Low density Lipoprotein (LDL-C) (mmol)
Control	2.92 $\pm$ 0.17	1.89 $\pm$ 0.05	0.84 $\pm$ 0.21	1.53 $\pm$ 0.13
40 mg/kg ALSMO	3.26 $\pm$ 0.29	0.72 $\pm$ 0.05	0.54 $\pm$ 0.19	2.09 $\pm$ 0.41

60 mg/kg ALSMO	3.24±0.39	0.57±0.04*	0.50±0.04*. <sup>a</sup>	3.04±0.40*
80 mg/kg ALSMO	2.40±0.09	0.63±0.16*	0.25±0.03*. <sup>a</sup>	1.80±0.13

Values are presented as mean±SEM. N = 5.  $p < 0.05$ . \* Values are statistically significant when compared to the control; <sup>a</sup> Values are statistically significant when compared to 40mg/kg ALSMO; <sup>b</sup> Values are statistically significant when compared to 60mg/kg ALSMO.

**Table 4:** Effects of Aqueous Leaf Solution of *M. oleifera* (ALSMO) on some Serum Electrolytes

Groups	Sodium ions (Na <sup>+</sup> ) (Meq/L)	Potassium ions (K <sup>+</sup> ) (Meq/L)	Bicarbonate ions (HCO <sub>3</sub> <sup>-</sup> ) (Meq/L)	Chloride ions (Meq/L) (Cl <sup>-</sup> )
Control	135.60±0.19	4.82±1.04	22.40±2.77	55.80±0.23
40 mg/kg ALSMO	128.80±0.11*	4.04±0.41	23.40±2.69	68.40±0.27*
60 mg/kg ALSMO	134.0±0.16*. <sup>a</sup>	3.58±0.28	26.20±0.91	78.40±0.30*. <sup>a</sup>
80 mg/kg ALSMO	114.80±0.32*. <sup>a, b</sup>	3.62±0.26	24.80±1.43	68.80±0.42*

Values are presented as mean ± SEM. Values are statistically significant (i.e.,  $p \leq 0.05$ ) when compared to the control.

**Table 5:** Effects of Aqueous Leaf Solution of *M. oleifera* (ALSMO) on Urea and Creatinine

Groups	Urea (mmol/L)	Creatinine (μmol/L)
Control	2.60±0.03	116.80±0.18
40 mg/kg ALSMO	3.04±0.02*	125.0±0.29*
60 mg/kg ALSMO	3.10±0.07*	125.40±0.29*
80 mg/kg ALSMO	2.84±0.03*. <sup>a, b</sup>	131.20±0.28*. <sup>a, b</sup>

Values are presented as mean±SEM. Values are statistically significant (i.e.,  $p \leq 0.05$ ) when compared to the control.

The result in Table 2 displays the effect of ALSMO on some serum liver enzymes. Considering the outcome on the effects of aqueous leaf solution of *M. oleifera* (ALSMO) on liver enzymes, increasing doses of the ALSMO possibly elicited corresponding significant ( $p < 0.05$ ) elevations in the serum level of AST. While the ALT level at 60mg/kg ALSMO was observed to be significantly ( $p < 0.05$ ) reduced, at 80mg/kg of the ALSMO, it significantly ( $p < 0.05$ ) increased. Also, the ALP levels at 60 and 80mg/kg of the ALSMO, it was observed to be significantly ( $p < 0.05$ ) increased compared to that of the control. These may imply that, the higher doses of ALSMO may have the potential of increasing serum liver enzyme levels.

Table 3 above shows the effect of ALSMO treatment on lipid profile. The results show that only at 80mg/kg of ALSMO treated group shows a non-significant ( $p > 0.05$ ) decrease in total cholesterol (TC) level when compared to that of control group. There was a progressive decrease in the triglyceride (TG) levels of all ALSMO treated rats but at 60 and 80mg/kg of ALSMO treated rats there were significant ( $p < 0.05$ ) reductions. The effect of ALSMO on the TG level appears to be a hypotriglyceridaemic effect. The HDL-C levels decreased in all ALSMO treated rats and these decreases were significant ( $p < 0.05$ ) in the higher doses (60 and 80mg/kg) of the ALSMO treated groups. Only the LDL-C level of the 60mg/kg ALSMO treated group had a significant ( $p < 0.05$ ) increase when compared to that of the control group.

In Table 4 above, the result of ALSMO treatment on some electrolytes is shown. It was observed that all ALSMO treated groups had significantly ( $p < 0.05$ ) reduced Na<sup>+</sup> level when compared to that of the control group. On the other hand, all ALSMO treated groups indicated remarkable ( $p < 0.05$ ) increase in Cl<sup>-</sup> levels when compared to that of the control group. No significant differences were observed in K<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> when compared to that of the control.

Table 5 above shows the result of ALSMO treatment on urea and creatinine levels in male Wistar rats. The different doses of ALSMO treated animals showed significantly ( $p < 0.05$ ) raised levels of urea and creatinine.

## Discussion

*Moringa oleifera* (*M. oleifera*), is said to possess numerous

nutritional and therapeutic benefits hence has been the focus of most biomedical researches [11]. The most widely studied part of *M. oleifera* is the leaves and has many reported beneficial uses in several chronic conditions such as hypercholesterolemia, high blood pressure, diabetes, insulin resistance, non-alcoholic liver disease, cancer and inflammation [5]. Owing to the much acclaimed therapeutic benefits of *M. oleifera*, most ethnomedicinal applications of the plant is in the crude forms, which is in utter disregard to the possible adverse effect of the plant.

Hence, the present study which investigated the effects of the crude aqueous leaf solution of *Moringa oleifera* (ALSMO) on some hematological and biochemical parameters in male Wistar rats had some useful findings as discussed below.

In the present study, the administration of ALSMO was found to have significantly raised the levels of RBC, PCV and Hb in the study animals, while the levels of WBC and platelet count in all ALSMO treated groups showed graded significant increases when compared to that of the control group.

The above observation is consistent with the finding of Ufelle *et al.*, [12] but disagreed with that of Ajibade *et al.*, [13] however, these other works were on crude extracts and not decoction, as the case in this study. The inferences here may be that the ALSMO decoction may have therapeutic relevance in anaemic conditions and could be helpful in potentiating WBC functions in infectious states. Thus indiscriminate consumption may be avoided in normal health conditions so as to prevent possible polycythaemic or leukocytosis conditions and their associated disorders [14]. While the actual phytochemicals in *M. oleifera* responsible for the possible RBC, Hb, PCV and WBC boosting effects are yet to be known, the report of Gopalakrishnan *et al.*, [15] mentioned that the leaves of *M. oleifera* have abundance of minerals, vitamins and more phytochemicals with antioxidant properties and that extracts of the leaves are consumed for nutritive purposes which can boost the production of the blood cells. Thus, considering the findings of Wambi *et al.*, [16] that said, maintenance of antioxidant diet was traceable to the improved recovery of bone marrow subsequent to a potentially lethal irradiation and a consequent boosting of RBC, it may be suggestive of rich antioxidant constituents of *M. oleifera* which may be responsible for the enhancement of the levels of these blood cells.

The outcome of ALSMO on Liver enzyme level indicated a possible hepatotoxic potential at the higher doses of the decoction. This finding agrees with the earlier report of Ajibade *et al.*, [13] which reported increased AST and ALT values in higher doses of *M. Oleifera* extract treated rats.

The finding of the current study validates the report of Ekor [6] which stated that adverse reaction and safety levels should be evaluated alongside therapeutic benefits of herbs.

Increased serum enzyme levels are indicators of tissue

damage especially in liver and bone marrow diseases [17, 18]. Caution should therefore be taken in the indiscriminate consumption of ALSMO especially at higher doses which may have potential toxic effects on the liver and other tissues. Considering the effect of ALSMO on lipid profile, only at 60mg/kg ALSMO treated group shows significantly raised LDC-C levels.

The TG level was observed to be decreased progressively at different doses of ALSMO. This result has shown that ALSMO may possess the ability to reduce serum level of TG in the rats and this is in line with the earlier submission of Yuan *et al.*, [19] which stated that the totality of evidence suggests that elevated triglyceride levels contributes independently to increased risk of cardiovascular disease. The finding of the present study validates Islam *et al.*'s [11] position that which stated that *M. oleifera* is a plant that possess both nutritional and medicinal properties that may be important for normal functioning of the body and prevention of certain diseases.

The progressive decrease of HDL-C may be an indication of higher tendency for plaque formation in vascular lumen—atherosclerosis. This line of thought is supported by the report of Morton *et al.*, [20] which stated that without an adequate HDL level, there is a higher risk for plaques formation which may in turn occlude arteries. This would indeed mean a very high risk for several cardiovascular diseases. Thus, considering the finding of the present study, it is instructive to state that, the consumption of higher doses of crude ALSMO may not be helpful in overall lipid profile regulations in a mammalian model.

The significant decreases in Na<sup>+</sup> level and the corresponding significant increases in Cl<sup>-</sup> levels in the study animals, has been observed. Of course as it is known, the plasma levels of electrolytes, creatinine and urea are among the major biochemical indices renal functions. And the stability of their normal levels indicates optimal performance of the renal system [21, 22].

Apart from the possible consequential imbalance in the ECF electrical equilibrium, it may result in metabolic acidosis. This submission is buttressed by the arguments of Fouche and Tubben [23] and Sharma *et al.*, [24] which explained that hyperchlorhydria forces bicarbonate to move intracellularly to maintain ionic equilibrium, thus reducing the available bicarbonate for the pH buffering system leading to net acidosis. Chloride is also known to be involved in the exchange of oxygen and carbon dioxide in the red blood cells [25]. This is thus, a further indicator of the fact that the consumption of increasing doses of ALSMO in a mammalian model, may result in some undesirable health effects which may outweigh its intended benefits. Thus, while a mild to moderate consumption of ALSMO decoction may be somewhat beneficial, chronic consumption of the ALSMO may have some adverse effects on the mammalian kidneys and general metabolism.

Of course, like the submission of Pandya *et al.*, [26], urea and creatinine are good indicators of a normal functioning kidney, but their increase in the serum are likely indications of kidney dysfunction. Therefore, the habitual consumption of crude ALSMO in a mammalian model especially in higher dose may result in adverse effect on the mammalian kidneys. Further, creatinine is a by-product of muscle metabolism and under normal physiological condition; the amount excreted per day is constant and correlates with body mass. Therefore, considering the outcome of the present study, it may be stated that while moderate and periodic consumption of ALSMO

may have biological benefits, the arbitrary and chronic consumption of the ALSMO may elicit some negative effect on the renal physiology. More so, this finding of the present study is contrary to an earlier report that *M. oleifera* crude extract consumption offers protection against lead-induced nephrotoxicity [27]. This is also a pointer to the fact that proper extraction of the leaf *M. oleifera* may have better therapeutic benefits than the mere consumption of the crude solution as evident with some ethnobotanical application of the plant [7, 8]. Further, whether or not creatinine is accumulated in the plasma, is useful for evaluating glomerular filtration rate [22]. Urea, on the other hand, is a nitrogenous waste product from the degradation of protein, and it must be excreted by the kidneys. Elevated plasma urea level has been linked to reduced renal function [21, 22]. This re-emphasizes the earlier submission of the present study that frequent consumption of particularly higher doses of ALSMO may have disordered biological effects.

## Conclusion

The overall findings of the present study have shown that the ALSMO may be a potent booster of RBC, PCV, Hb, WBC and platelet count in male Wistar rats. The ALSMO administration in the study animals also specifically indicated a possible hypotriglyceridaemic effect. It could therefore have a possible anti-anaemic/haematopoiesis stimulatory and hypotriglyceridaemic effect. Also, the administration of higher doses of ALSMO in Wistar rats may have hepatorenal toxicities and increased risk of atherosclerosis.

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