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The Effects of *Moringa oleifera* Leaves on Complete Blood Count, Renal and Liver Functions as Potential Therapy for Malnutrition

Gamar Musa Kodi¹, Howeida Abdullah Mustafa², Alkhair Abd Almahmoud Idris³¹School of Health Sciences, Ahfad University for Women, Omdurman, Sudan²Faculty of Veterinary Medicine, University of Khartoum, Khartoum, Sudan³Department of Human Biology and Histology, Ahfad University for Women, Omdurman, Sudan

Background: *Moringa oleifera* which is available in many areas all over the world including Sudan is low-cost and traditionally used in the treatment of many disorders, including malnutrition. This study aimed to determine the effect of aqueous extract of *M. oleifera* leaves in renal, liver functions and complete blood count (CBC) parameters, and its potential as therapy for malnutrition.

Materials and methods: This was an experimental case control study using twenty-five Wistar albino rats. Rats were divided into three groups: normal protein diet group, low protein diet with or without *M. oleifera* extract groups. We determined rats' weight, CBC parameters, blood mineral concentrations, as well as liver and renal functions at day 0, 7, and 14.

Results: Our findings showed that rats' weight were significantly different between the three groups at day 0, 7, and 14. Rats' weight, blood sodium, potassium, calcium, and urea concentration, as well as Hb concentration, TWBCs count, total platelets count, and %lymphocyte showed significant differences between three groups at day 0, 7, and 14.

Conclusion: *M. oleifera* leaves can be used as potential therapy for malnutrition because they have some effects on weight, blood mineral concentrations, renal and liver function, as well as CBC parameters.

Keywords: ALP, AST, ALT, creatinine, *Moringa oleifera*

Introduction

Malnutrition can lead to mental or physical disability, illness, and possible death.¹ The malnutrition prevalence rate in Sudan increased from 18% in 1995 to 23% in 1999, both in the North and the government-controlled areas in the South.²

Ready To-Use Therapeutic Foods (RUTFs) supplied by UNICEF for helping severe malnourishment are commonly used all over the world. However, it is expensive and it takes a long time to reach affected areas.^{3,4} In folklore medicine, many herbs are used to prevent and treat malnutrition and many other conditions e.g., snakebite and tumor. Among

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Corresponding Author:

Alkhair Abd Almahmoud Idris

Department of Human Biology and Histology

Ahfad University for Women, Omdurman, Sudan

e-mail: alkhair20@hotmail.com

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these herbs, *Moringa oleifera* leaves are commonly used to treat malnutrition.⁵

These problems motivated us to search for an available, cheap, affordable, and effective malnutrition treatment. *M. oleifera* which is available in many areas all over the world including Sudan is low-cost and traditionally used in the treatment of many disorders, including malnutrition. This study aimed to determine the effect of aqueous extract of *M. oleifera* leaves in the treatment of malnutrition-induced Wistar albino rats.

Materials and methods

Study Design and Animal Treatment

This was an experimental case control study using twenty-five Wistar albino rats. The samples were taken under anesthesia, animals were kept in well-ventilated area and fed properly.

Rats were divided into three groups. Group I (control) consisted of 5 rats that were fed a well-balanced diet containing 50% flour, 20% meat, 20% milk, 5% salt, and 5% oil.^{6,7} Group II consisted of 10 rats that were fed with low protein diet only. Group III consisted of 10 rats that were fed low protein diet added with aqueous extract of *M. oleifera*. All treatments were given for 7 days. The research was approved by the research committee of the Faculty of Health Sciences-Ahfad University for Women and Ministry of Health Ethical Research Committee (No. MH-RES/07-021-09, year 2021).

Plant Materials

M. oleifera fresh leaves were collected from Faculty of Agriculture, University of Khartoum. It was identified and authenticated by Department of Taxonomy, University of Khartoum. Twenty g of *M. oleifera* leaves were dried in oven at 40°C overnight for 8 h.

Hematological and Biochemical Analysis

All rats were weighed and the blood samples were collected at day 0, 7 and 14 from all rats for the determination of CBC parameters, magnesium, calcium, and phosphorus concentrations, as well as liver and renal functions to ensure the induction of malnutrition. Blood samples were collected from the retro-orbital plexus of all rats, using non heparinized and heparinized capillary tubes according to previous reports.⁶⁻⁸

The levels of total protein, albumin, urea, creatinine, sodium, potassium, calcium, phosphorus as well as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) enzymes were determined using Plasmatec kits.⁹

Biochemical and hematological blood analysis was conducted at the Khartoum Hospital Laboratory Research Unit. CBC was estimated using Sysmex machine (Model KX-21N).^{10,11}

Statistical Analysis

Mean values of whole blood and plasma parameters were compared using the student's t-test to detect the differences ($p \leq 0.05$).^{12,13}

Results

Weights of Rats

Table 1 showed the mean values of rats' weight after administration of three different diets. Results showed significant differences between 3 groups at day 0, 7, and 14. In all groups, rats' weight were increased in a time dependent manner.

Mineral Concentrations of Rats' Blood

Table 2 showed the mean values of sodium, potassium, magnesium concentrations measured after feeding the

Table 1. Body weight of rats on day 0, 7, and 14.

Group	Weight (g)		
	Day 0	Day 7	Day 14
Normal protein diet	72.68±11.23	95.36±20.00	99.22±18.04
Low protein diet without <i>M. oleifera</i>	54.72±30.12	76.16±38.63	110.48±28.40
Low protein diet with <i>M. oleifera</i>	116.32±28.07	128.60±28.77	150.56±34.72
<i>p</i> -value	0.001	0.001	0.002

Table 2. Mineral concentrations of rats' blood on day 0, 7, and 14.

Group	Day 0	Day 7	Day 14
Sodium (mEq/L)			
Normal protein diet	69.20±23.37	70.10±17.22	55.30±22.57
Low protein diet without <i>M. oleifera</i>	127.60±12.98	105.90±12.50	83.30±18.44
Low protein diet with <i>M. oleifera</i>	141.90±14.04	115.90±14.50	106.10±18.18
<i>p</i> -value	0.001	0.010	0.002
Potassium (mEq/L)			
Normal protein diet	2.20±0.74	2.12±0.72	1.66±0.68
Low protein diet without <i>M. oleifera</i>	3.92±1.47	4.90±2.02	4.13±0.96
Low protein diet with <i>M. oleifera</i>	4.26±0.54	4.47±1.82	3.36±0.57
<i>p</i> -value	0.002	0.002	0.002
Magnesium (mg/dL)			
Normal protein diet	1.02±0.34	0.82±0.29	0.65±0.26
Low protein diet without <i>M. oleifera</i>	1.73±0.23	3.16±0.57	3.67±1.10
Low protein diet with <i>M. oleifera</i>	1.79±0.13	2.65±0.61	1.76±0.33
<i>p</i> -value	0.100	0.010	0.010

rats. The mean values of blood sodium concentration showed significant differences between three groups at day 0, 7, and 14. In Group II and III, sodium concentrations were decreased in a time dependent manner. The mean values of blood potassium concentration also showed significant differences between three groups at day 0, 7, and 14. Meanwhile, the mean values of blood magnesium concentration were not significantly different at day 0, but significantly different at 7 and 14.

Table 3 showed the mean values of calcium and phosphorus concentration. The mean values of blood calcium concentration showed significant differences between three groups at day 0, 7, and 14. While the mean values of blood phosphorus concentration were not significantly different at day 0 and 7, but significantly different at day 14.

Blood Urea and Creatinine

Table 4 shows that the mean values of blood urea and creatinine concentration measured after feeding the rats. The mean values of blood urea concentration showed significant differences between three groups at day 0, 7, and

14. Meanwhile, the mean values of creatinine concentration were significantly different at day 0, but not significantly different at day 7 and 14.

Liver Function Test

Table 5 shows the mean values of blood total protein concentration, albumin concentration, as well as ALT, AST and ALP concentrations that were measured after feeding the rats. The mean values of total protein concentration were significantly different at day 0 and 14, but not significantly different at day 7. The mean values of serum albumin concentration were significantly different at day 0, but not significantly different at day 7 and 14. The mean values of blood ALT concentration were significantly different at day 0, but not significantly different at day 7 and 14. The mean values of blood AST concentration showed no significant differences between three groups at day 0, 7, and 14. Lastly, the mean values of blood ALP concentration were significantly different at day 0 and 7, but not significantly different at day 14.

Table 3. Mineral concentrations of rats' blood on day 0, 7, and 14.

Group	Day 0	Day 7	Day 14
Calcium (mg/dL)			
Normal protein diet	4.68±1.56	4.63±1.54	3.34±1.36
Low protein diet without <i>M. oleifera</i>	8.57±0.96	11.35±1.40	9.26±2.10
Low protein diet with <i>M. oleifera</i>	9.93±0.14	9.71±1.26	7.53±1.26
<i>p</i> -value	0.004	0.006	0.040
Phosphorus (mEq/L)			
Normal protein diet	1.77±0.62	1.60±0.60	1.37±0.57
Low protein diet without <i>M. oleifera</i>	3.07±0.41	1.45±0.23	1.03±0.26
Low protein diet with <i>M. oleifera</i>	3.19±0.25	1.62±0.21	3.04±0.53
<i>p</i> -value	0.060	0.900	0.010

CBC Parameters

Table 6 showed the mean values of hemoglobin (Hb) concentration, total white blood cells (TWBCs) count, red blood cells (RBCs) count, total platelets count, %neutrophil, %lymphocyte, %monocyte, %basophil, and %eosinophil that were measured after feeding the rats.

Hb concentration, TWBCs count, total platelets count, and %lymphocyte showed significant differences between

three groups at day 0, 7, and 14. The mean values of RBCs count were significantly different at day 0 and 7, but not significantly different at day 14.

The mean values of %neutrophil were significantly different at day 0, but not significantly different at day 7 and 14. The mean values of %monocyte, %eosinophil and %basophil were not significantly different at day 0, 7, and 14.

Table 4. Blood urea and creatinine levels on day 0, 7, and 14.

Group	Day 0	Day 7	Day 14
Urea (mg/dL)			
Normal protein diet	17.90±6.18	19.00±6.56	16.20±6.73
Low protein diet without <i>M. oleifera</i>	37.00±4.41	61.40±7.54	61.90±11.02
Low protein diet with <i>M. oleifera</i>	35.90±3.30	68.30± 8.91	52.30±9.74
<i>p</i> -value	0.0100	0.0002	0.0050
Creatinine (mg/dL)			
Normal protein diet	0.52±0.18	0.52±0.18	0.46±0.19
Low protein diet without <i>M. oleifera</i>	0.94±0.12	0.93±0.39	0.92±0.60
Low protein diet with <i>M. oleifera</i>	1.12±0.13	0.98± 0.41	0.92±0.60
<i>p</i> -value	0.0400	0.0700	0.2000

Table 5. Mineral concentrations of rats' blood on day 0, 7, and 14.

Group	Day 0	Day 7	Day 14
Total protein (mg/dL)			
Normal protein diet	3.77±1.23	3.60±1.20	2.29±1.20
Low protein diet without <i>M. oleifera</i>	6.90±0.78	4.24±0.57	3.22±0.77
Low protein diet with <i>M. oleifera</i>	7.55±0.18	4.16±0.60	5.73±0.97
<i>p</i> -value	0.020	0.800	0.001
Serum albumin (U/L)			
Normal protein diet	2.11±0.71	2.10±0.70	1.45±0.31
Low protein diet without <i>M. oleifera</i>	3.88±0.45	1.88±0.29	1.51±0.38
Low protein diet with <i>M. oleifera</i>	4.12±0.20	1.71±0.35	2.46±0.49
<i>p</i> -value	0.010	0.300	0.400
ALT (U/L)			
Normal protein diet	19.80±7.36	14.50±5.8	11.90±5.44
Low protein diet without <i>M. oleifera</i>	31.00±5.07	24.80±4.6	23.70±5.92
Low protein diet with <i>M. oleifera</i>	40.30±4.09	30.20±5.6	28.30±5.36
<i>p</i> -value	0.050	0.100	0.200
AST (U/L)			
Normal protein diet	12.10±4.35	13.70±4.77	10.30±4.44
Low protein diet without <i>M. oleifera</i>	22.10±3.95	25.20±5.01	23.30±7.26
Low protein diet with <i>M. oleifera</i>	23.80±2.55	23.20±4.01	20.30±4.62
<i>p</i> -value	0.070	0.100	0.100
ALP (U/L)			
Normal protein diet	30.20±10.86	30.70±11.02	21.40±12.20
Low protein diet without <i>M. oleifera</i>	66.00±11.70	71.90±12.31	48.40±19.22
Low protein diet with <i>M. oleifera</i>	82.20±8.68	64.80±8.63	46.30±18.34
<i>p</i> -value	0.005	0.030	0.200

Discussion

The mean weight of rats that were fed a low protein diet added with aqueous extract of *M. oleifera* leaves (Group

III) was increased in a time dependent manner, indicating the nutritive role of *M. oleifera* leaves. This result was aligned with previous studies.¹⁴⁻¹⁷ In addition, young post-

Table 6. CBC parameters on day 0, 7, and 14.

Group	Day 0	Day 7	Day 14
Hb concentration (mEq/L)			
Normal protein diet	28.00±9.72	34.50±7.94	27.00±7.80
Low protein diet without <i>M. oleifera</i>	65.10±8.39	63.90±7.82	40.30±8.50
Low protein diet with <i>M. oleifera</i>	70.54±4.67	50.00±11.70	59.70±7.56
<i>p</i> -value	0.0030	0.0500	0.0050
TWBCs (mEq/L)			
Normal protein diet	2,715.00±950.67	3,500.00±121.11	2,190.00±918.51
Low protein diet without <i>M. oleifera</i>	4,300.00±594.47	5,560.00±447.45	5,000.00±178.70
Low protein diet with <i>M. oleifera</i>	5,675.00±518.18	7,045.00±765.90	4,050.00±704.00
<i>p</i> -value	0.0004	0.0020	0.0400
RBCs (mg/dL)			
Normal protein diet	2,237,000±4,948	2,570,000±5,226	2,307,000±3,142
Low protein diet without <i>M. oleifera</i>	4,792,800±6,229	5,945,700±6,200	3,616,500±7,070
Low protein diet with <i>M. oleifera</i>	4,902,000±4,109	6,613,800±6,268	4,343,000±6,351
<i>p</i> -value	0.0100	0.0040	0.2000
Total platelets (mg/dL)			
Normal protein diet	121,000±45,276	104,000±44,076	99,000±49,068
Low protein diet without <i>M. oleifera</i>	287,000±42,165	356,000±54,123	299,000±67,831
Low protein diet with <i>M. oleifera</i>	330,000±36,788	432,000±61,583	525,000±63,495
<i>p</i> -value	0.0030	0.0040	0.0300
Neutrophil (%)			
Normal protein diet	28.40±9.53	27.90±5.70	22.60±7.70
Low protein diet without <i>M. oleifera</i>	49.70±6.05	45.40±13.09	32.20±12.87
Low protein diet with <i>M. oleifera</i>	53.50±2.67	42.90±11.87	45.40±17.78
<i>p</i> -value	0.0200	0.1000	0.2000
Lymphocyte (%)			
Normal protein diet	19.30±6.46	19.40±7.94	15.10±3.00
Low protein diet without <i>M. oleifera</i>	36.80±4.66	30.10±6.80	31.90±17.99
Low protein diet with <i>M. oleifera</i>	42.70±2.73	41.40±6.09	32.07±16.65
<i>p</i> -value	0.0050	0.0200	0.0100
Monocyte (%)			
Normal protein diet	0.80±0.67	1.10±0.90	1.10±0.78
Low protein diet without <i>M. oleifera</i>	1.41±1.00	1.70±0.97	2.20±0.98
Low protein diet with <i>M. oleifera</i>	1.42±1.05	2.12±1.02	1.70±0.88
<i>p</i> -value	0.4000	0.0600	0.2000
Eosinophil (%)			
Normal protein diet	1.30±0.12	1.1±0.20	1.0±0.90
Low protein diet without <i>M. oleifera</i>	1.40±0.33	1.6±0.50	2.3±1.89
Low protein diet with <i>M. oleifera</i>	1.30±0.88	1.9±0.89	1.1±0.67
<i>p</i> -value	0.9000	0.3000	0.1000
Basophil (%)			
Normal protein diet	0.20±0.15	0.50±0.03	0.20±0.15
Low protein diet without <i>M. oleifera</i>	0.80±0.71	0.90±0.61	0.90±0.61
Low protein diet with <i>M. oleifera</i>	0.70±0.54	0.90±0.61	0.50±0.03
<i>p</i> -value	0.0700	0.4000	0.1000

weaning rabbits that were given *M. oleifera* supplement feed formulation had the highest weight average compared to mixed supplement and standard feed formulation.¹⁸

The average of blood sodium, potassium and calcium concentrations showed significant differences between Group I, II and III at day 0, 7, and 14. Sodium, potassium and calcium concentrations of Group III at day 14 were 106.10 ± 18.18 , 3.36 ± 0.57 and 7.53 ± 1.26 , respectively. These values fall within the normal ranges of well-nourished rats. Thus, *M. oleifera* leaves have good nutritive values in terms of balanced blood mineral concentration.

Average blood urea concentration of Group III was increased from day 0 to 7, then decreased at day 14, while the mean values of blood creatinine concentration was decreased in a time dependent manner in Group II and III. Creatinine concentrations of Group III at day 0, 7, and 14 were 1.12 ± 0.13 , 0.98 ± 0.41 and 0.92 ± 0.6 , respectively. Low blood urea and creatinine concentrations are closely associated with good nutritional status; hence indicate that *M. oleifera* leaves are potential for malnutrition treatment.

Average total protein and serum albumin concentrations of Group III were slightly decreased from day 0 to 7, then increased at day 14. The mean concentration of blood ALT was increased in a time dependent manner. Inversely, the mean concentration of blood AST and ALP were decreased in a time dependent manner. However, all liver function parameters fall within the normal range of ideal liver function.

Hb concentration, TWBCs count and total platelets count showed significant differences between three groups at day 0, 7, and 14. Moreover, the mean values of RBCs count were significantly different only at day 0 and 7. In Group III, the mean value of total platelets count was increased in a time dependent manner. Similar results were revealed by Idohou and his colleagues.¹⁹

The mean values of %lymphocyte showed significant differences between three groups at day 0, 7, and 14. The mean values of %neutrophil were significantly different only at day 0. The mean values of %monocyte, %eosinophil and %basophil were not significantly different at day 0, 7, and 14. In Group III, the mean value of %lymphocyte was decreased in a time dependent manner. All blood parameters fall within the normal range. There are no available studies regarding the effects of *M. oleifera* leaves on blood parameters.

M. oleifera leaves are mostly used for food as well as medicinal purposes. They have a distinctive strong, mustard-

like taste and are rich in antioxidants and other nutrients, such as calcium and iron, which are commonly deficient in people living in developing countries.²⁰⁻²² *M. oleifera* leaves have been used for the treatment of various diseases from malaria and typhoid fever to hypertension and diabetes.²³

There are some limitations in this study, including small sample size and short duration of experimental analysis. This study could not be applied as a nutritional therapy in human subjects, too. Further studies should be conducted using larger sample sizes and different parts of the *M. oleifera* tree should be used. Additional studies in humans, including clinical trials are needed to confirm the effects of *M. oleifera* leaves on malnutrition.

Conclusion

We suggest that *M. oleifera* leaves can be used as potential therapy for malnutrition. *M. oleifera* leaves have some effects on weight, blood mineral concentrations, renal and liver function, as well as CBC parameters.

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