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Effect of Ethanolic Leaf Extract of *Moringa oleifera* on Aluminum-induced Anemia in White Albino Rats

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Abstract

The study aimed to investigate the effect of ethanolic leaf extract of *Moringa oleifera* on $AlCl_3$-induced anemia in albino rats. Twenty-four rats (average body weight 284 g) were divided into four groups each of six: group (A) was provided with 0.5 mg/kg body weight of $AlCl_3$, group (AM) with $AlCl_3$ and 10 minutes later with *M. oleifera* extract (300 mg/kg body weight), group (M) with *M. oleifera* extract (300 mg/kg) alone and the control group (C) with normal saline solution only. The treatments were given orally by gavage and continued daily for 21 days. Then blood sample was collected from each rat and measured for blood parameters and electrolytes. The results showed that $AlCl_3$ had led to a significant decrease ($P< 0.05$) in red blood cells count (RBCs), hemoglobin (Hb), mean corpuscular hemoglobin concentration (MCHC), hematocrit (PCV) and iron level, and a significant increase in the mean cell volume (MCV) and no significant change in the white blood cells (WBC), platelets (PLT) and lymphocytes (LMY). The results also showed ethanolic extract of *M. oleifera* co-administered with $AlCl_3$ caused significant increase ($P< 0.01$) in RBC, Hb, PCV and MCHC when compared with the group provided with $AlCl_3$ alone. Moreover, administration of *M. oleifera* extracts alone lead to significant increase in RBCs, Hb, and MCHC ($P< 0.01$) as compared with the control group. However, the results revealed that ethanolic extract of *M. oleifera*, whether provided with $AlCl_3$ or alone, mitigated $AlCl_3$ – induced anemia in Albino rats.

Keywords*: Moringa oleifera,* aluminum chloride, albino rats, anemia, hemoglobin, iron deficiency.

1. Introduction

Anemia is usually caused by deficiency of hemoglobin, the oxygen carrying molecule in the red blood cell. While many minerals including iron and copper are important in the body's manufacture of hemoglobin, heavy metals intoxication such as that of arsenic and its derivative arsine, copper, gold, lead, and zinc are associated with anemia (Ringenberg *et al*., 1988). Aluminum toxicity is another factor that contributes to anemia both in humans and animals (Short *et al*., 1980; Zaman *et al*., 1993; Mahieu *et al*., 2000).

Aluminum (Al) is a trivalent cation found in its ionic form in most kinds of animal and plant tissues and in natural waters everywhere (Domingo *et al*., 1991). It has no known biological role, nevertheless, when accumulates in the body it can induce several clinical disorders such as neurotoxicity (Campbell, 2002), hepatotoxicity (Klein *et al*., 1984), bone diseases and anemia (Ward *et al*., 1978). Aluminum has also a direct effect on hematopoiesis (Wills and Savory, 1983) and its high levels in serum of hemodialysis patients were associated with impaired erythropoiesis and iron-deficiency anemia (Wills and Savory, 1983; Mahieu *et al*., 2000).

Moringa oleifera (Moringaceae) is a small fast-growing ornamental tree and was originally brought from India. For centuries, *M. oleifera* has been cultivated for its nutritional (Thurber and Fahey, 2009; Verma *et al*., 2009) and nutraceutical values (Sasikala *et al*., 2010; Forsch, 2010). Root, bark, pods, flowers, seeds, gum and leaves of this tree are used in traditional medicine for the treatment of various human diseases (Lowell, 2002). Leaves of *M. oleifera* are also known as a great source of vitamins and minerals including calcium, copper, sulphur, vitamin A and B-vitamins (Lowell, 2002). In addition, *M. oleifera* is

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very rich in iron and it was estimated that, the dried 100 g leaf powder to contain about 28.29 mg (Oduro *et al*., 2008).

Although, *M. oleifera* was reported to be used against various metal intoxications including arsenic (Gupta *et al*., 2005) and lead (Sirimongkolvorakul *et al*., 2012), there is no information regarding the effect of *M. oleifera* against aluminum toxicity. Therefore, this study was conducted to examine the effect of ethanolic leaf extract of *M. oleifera* on the treatment of aluminum chloride-induced anemia in white albino rats.

2. Materials and Methods

2.1. Study animals

White albino rats (male sex, n=24, average body weight = 284 g) maintained under standard environmental conditions and fed with normal diet concentrate (dried meat, milk powder, oil and flour in some water) were used in this study. The rats were divided into four groups each of six. The 1st group denoted (A) was provided with $AlCl_3$ (0.5 mg/kg body weight). The 2nd group denoted (AM) was provided with $AlCl_3$ and 10 minutes later with *M. oleifera* extract (300 mg/kg body weight). The 3rd group denoted (M) was provided with *M. oleifera* extract (300 mg/kg body weight) alone and the 4th group denoted (C) was provided with physiological normal saline and used to act as a control group. All experimental doses were administered orally by Gavage and were given daily for 21 days. Rats in all groups, before and after this period, were measured for body weight using Mettler sensitive balance (number 202845).

2.2. Extract preparation

The leaves of *M. oleifera* were harvested from different trees cultivated in central Sudan. The leaves were first rinsed with distilled water, dried in shade and were completely extracted with ethanol (70%) using Soxhlet apparatus for 3 days. The percolated extract was then dried in Rotary Evaporator apparatus, weighed and dissolved in distilled water to give the final concentration of 300 mg extract /kg body weight.

2.3. Hematological and electrolyte measurements

About 4 ml of blood sample was collected by hematocrit capillary tube from retro-orbital of each rat. Then 2 ml of this sample was placed in EDTA tube and used immediately for measurement of blood parameters using automated coagulating Sysmex apparatus of the type 8999. The blood parameters included: hemoglobin (Hb), mean cell volume (MCV), red blood cells count (RBCs), white blood cells count (WBC), mean cell hemoglobin concentration (MCHC), platelets (PLT), lymphocytes (LYM) and packed cell volume (PCV). However, MCV value was calculated from RBCs count and PCV, used the following formula, MCV = PCV/RBCs X 10 (Androw, 1972; Merghani, 2010).

The remaining 2 ml of blood sample was taken in a plane tube, left to coagulate, centrifuged at 1000 rpm and the supernatant (serum) was used for measurement of Na, K, Ca, P and Fe. For Na and K, aliquot for each element was placed in a cuvette and measured using flame photometer apparatus (Hald,1946), and the same was done for measuring Ca, P and Fe using Spectrophotometers apparatus of the type (30122) (Sanchez *et al*.,1997). Both instruments were adjusted to zero by blanks brought with the kit for each element.

2.4. Statistical analysis

Student T – test was used for comparison between (mean ± standard deviation) values of $AlCl_3$ - treated group (A) and the control group (C) and each of them with the mean values of the group treated with $AlCl_3$ plus *M. oleifera* (AM) and the group provided with *M. oleifera* alone (M) . The significance is taken at $P<0.05$ and $P<0.01$.

3. Results

3.1. Blood parameters

The results of blood parameters in group (A), group (AM), group (M) and group (C) are shown in Table 1. The results showed that, WBC count, PLT and Lym of group (A) were not significantly affected ($P>0.05$) when each compared with that in group (C), group (AM) and group (M). On the other hand, RBCs, Hb and PCV of group (A) were each significantly low ($P<0.01$) when compared with that in group (C), group (AM) and group (M) where in these groups each pair was not significantly different ($P>0.05$).

For MCHC, its mean value in group (A) was significantly lower than each one of those in group (C) ($P<0.01$), group (AM) ($P<0.05$) and group (M) ($P<0.01$) whereas its value was significantly higher ($P<0.01$) in group (M) but significantly lower in group (AM) when each compared with that of the control group (C). However, for the mean value of MCV in group (A), it was significantly higher ($P<0.01$) than any one in the other groups while in group (AM) it was significantly higher than that in group (C) as well as in group (M).

Table 1. Mean values of blood parameters in the four groups: the control group (C), the group treated with $AlCl_3$ (A), with $AlCl_3$ plus *M. oleifera* (AM) and with *M. oleifera* alone (M).

Parameters	C	A	AM	M
WBCs x $10^3/mm^3$	9.3± 1.4	8.6± 1.1	9.5± 1.1	9.8± 1.1
RBCs x $10^6/mm^3$	7.0± 0.5	5.0± 0.8**	6.7± 0.3##	7.2± 0.5**##
Hb level (g/dl)	12.2± 1.2	8.8± 0.7**	11.9± 0.5##	13.3± 1.2*##
MCHC (%)	33.5± 1.3	25.5± 0.1**	32.5± 0.1*##	36.1± 1.5**##
PLT x $10^3/mm^3$	986.2± 150.2	1011.2± 114.9	979± 177.8	1018.2± 94.9
MCV fl	52± 1.1	68.8± 0.8**	54.6± 0.9**##	51.1± 1.5 ##•
PCV (%)	36.4± 0.4	34.4± 0.5**	36.6± 0.9##	36.8± 0.7##
LYM (%)	78.2± 3.4	75.7± 6.2	78.8± 2.1	76.6± 6.4

Values are means ± SD, n= 6, * = $P \leq 0.05$, ** = $P \leq 0.01$ versus group C, # = $P \leq 0.05$, ## = $P \leq 0.01$versus group A, and • = $P \leq 0.05$ versus group AM.

3.2. Blood electrolytes:

The results of the mean values of electrolytes; namely: Na, K, Ca, P and Fe are shown in Table 2. The results revealed that, with the exception to Fe concentration, no significant changes occurred in the levels of Na, K, Ca and P in all experimental groups (P>0.05) when each compared with that in group (C). For Fe concentration it was significantly reduced (P<0.01) in group (A) when compared with its levels in group (C) and group (AM).

Table 2. Mean values of blood electrolytes in the four groups: the control group (C), the group treated with $AlCl_3$ (A), with $AlCl_3$ plus *M. oleifera* (AM) and with *M. oleifera* alone (M).

Parameters	C	A	AM	M
Na (mm/l)	137.8± 6.9	136.5± 5.7	138.5± 7.5	140.7± 4.7
K (mm/l)	3.8± 0.3	3.8± 0.2	3.9± 0.2	3.8± 0.3
Ca (mg/dl)	8.5± 1.6	8.2± 1.3	8.8± 1.3	9.2± 1.0
P (mg/dl)	0.8± 0.2	0.7± 0.1	0.9± 0.1	0.9± 0.1
Fe (µmol/l)	3.6± 3.7	3.0±1.8**	3.3± 4.5	3.7± 3.3##

Values are means ± SD, n= 6, * = $P \leq 0.05$, ** = $P \leq 0.01$ versus group C, # = $P \leq 0.05$, ## = $P \leq 0.01$versus group A.

3.3. Body weight:

The results showing the mean values of the body weight of experimental animals in all groups are shown in Table 3. The results showed that the rats in group (A) had a significant reduction (P<0.01) in their final body weight when compared to their initial body weight, while in group (M) a significant increase (P<0.05) in the final body weight was obtained. The average difference of reduced body weight was 0.21 for group (A) and 1.7 for average increase body weight in group (M).

Table 3. Mean values of Initial and final in the four groups: the control group (C), the group treated with $AlCl_3$ (A), with $AlCl_3$ plus *M. oleifera* (AM) and with *M. oleifera* alone (M).

Parameters	C	A	AM	M
Initial weight	292.2± 25.4	295± 24.4	273.7± 33.6	288.8± 25.8
Final weight	296.3± 22.7	290.6± 24.3*	277.3± 33.9	325.0 ±39.9*
Difference in weight per day (g)	0.2	0.21↓	0.2	1.7
Deference in weight (%)	1%	1.5%↓	1.3%	12%

Values are means ± SD, n= 6, * = $P \leq 0.05$, versus initial weight, (↓) reduction.

4. Discussion

The effect of the ethanolic extract of *M. oleifera* on aluminum-induced anemia was investigated in albino rats of the present study. It was evident that deficiency of blood indices namely: RBCs, Hb, PCV and iron level which are routinely checked for clinical diagnosis of anemia was indicated in $AlCl_3$ – treated animals of this study. This was coupled with alterations of the mean values of MCHC and MCV. The lack of consistency of the mean value of MCV compared to the other anemia indices, was probably, due to the reduced number of RBC by which the PCV was divided to estimate this parameter and that had led to its higher significant increase in $AlCl_3$ – treated animals as well as in the animal group treated with both $AlCl_3$ and *M. oleifera* (Spender, 2010). Moreover, the increased level of MCV of this study would agree with the report saying that more reticulocytes were produced than mature RBCs due to Al toxicity (Lewis *et al*., 2006) and that it had led to iron deficiency.

However, aluminum-induced haematolotgical alterations leading to microcytic hypochromic anemia in albino rats of this study are similar to previous reports on the same blood parameters in rats (Zaman *et al*., 1993; Chmielnicka *et al*., 1994; Savage *et al*., 2000). Moreover, patients with anemia caused by aluminum toxicity often have increased reticulocytes counts, decreased mean corpuscular volume, and mean corpuscular hemoglobin concentration and the latter agreed with results of this index obtained in this study.

Several mechanisms have been proposed for the aluminum-induced anemia, but the exact mechanism of aluminum-induced anemia is unknown. The proposed mechanisms appear to involve inhibition of heme synthesis, either by inhibition of enzyme activity or interference with iron incorporation or utilization (Kaiser and Schwartz, 1985; Ganchev *et al*., 1998; Han *et al*.,

2000). In a different study, it was confirmed that Al overload accumulation in all tissue to be the cause of anemia and it had led to Alzheimer's disease when accumulated in the tissue of the brain (Florence *et al*., 1994). Furthermore, disturbances in the distribution pattern of trace elements: Zinc, cooper, and iron together with lipid peroxidation in plasma and erythrocytes were also suggested as a mechanism of aluminum-induced anemia in rats (Guo *et al*., 2004).

For the role of *M. oleifera*, it was mentioned to treat Alzheimer's disease that was caused by Al accumulation (Obulesu and Dowlathabad, 2011). The leaves of this plant which contain vitamins and Fe in significant amount were mentioned to improve iron and blood status of rats (Verma *et al*. , 1976; Dhar and Gupta ,1982).

In the present study, uptake of ethanolic extract of *M. oleifera* alone or co-administered with $AlCl_3$ had mitigated aluminum chloride – induced anemia and raised the values of blood indices of anemia, almost, to their normal levels. As inferred from other reports, two mechanisms were suggested for the prevention of aluminum toxicity by *M. oleifera*: first the plant extract inhibited or reduced Al absorption from intestine and the second: aluminium overload might modulate gastrointestinal iron absorption and hinder the cellular uptake and use of iron for hemoglobin synthsis (Cannata *et al*., 1991 Ganchev T *et al*., 1998), and *M. oleifera* corrected this toxic effect of Al by enhancing iron absorption, cellular uptake or use.

The findings of this study seem to support this claim and to suggest that the accumulation of Al reaches also the tissue involved in the maintenance of normal blood parameters and disrupts its machinery, and restoration of function of this machinery entails the presence of *M. oleifera* extract. The principal role of *M. oleifera* extract seems to facilitate iron absorption, as adequate amount of this element is necessary for Hb synthesis and for the animal tissues such as the kidneys and bones to take part in manufacture of RBCs. The normal levels of WBC, PLT and LYM could furtherly confirm this claim as their manufacture does not necessitate iron absorption.

On the other hand, insignificant changed levels of electrolytes, particularly, Na, K, Ca and P in this study, would indicate that these elements were not affected; neither by $AlCl_3$ alone nor by *M. oleifera* whether provided singly or co-administered with $AlCl_3$. However, the need for these trace elements was probably secured as they can be transported actively or in passive form, co-transported with a molecule or facilitated by a carrier and either through the luminal or baso-lateral membranes of the gastrointestinal tract (Mergani, 2010).

However, the increase in the body weight of the group treated with $AlCl_3$ plus *M. oleifera* as well as in the group provided with *M. oleifera* alone indicates that the plant contains high nutritional values and this agreed with other report showing its nutritional ingredients (Goyal *et al*., 2007).

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