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Effects of $AlCl_3$ on the Liver Function of Wistar Rats Treated with *Moringa oleifera* Seed Extracts

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Abstract

In this study ALT and AST levels of experimental rats were significantly ($p<0.05$) increased from 11.91 ± 0.20 and 11.83 ± 0.40 to 57.23 ± 0.24 and 59.29 ± 0.50 respectively due to intoxication with aluminum chloride. When rats were treated with ethanol and aqueous extracts of *Moringa oleifera* at 100 mg/kg the levels of ALT dropped down to 11.62 ± 0.40 and 11.61 ± 0.17 respectively and the AST levels dropped to 11.39 ± 0.25 and 11.42 ± 0.15 respectively. A further increase in the concentrations of both extracts to 400 mg/kg resulted to a significant elevation of ALT 11.93 ± 0.17 (ethanol) and 11.94 ± 0.07 (aqueous) and AST 11.77 ± 0.25 (ethanol) and 11.78 ± 0.15 (aqueous). AST and ALT are common liver enzymes because of their higher concentrations in hepatocytes, but only ALT is remarkably specific for liver function. Therefore, an elevation in serum concentration of ALT is an indication of liver damage. The preliminary phytochemical screening for seed extracts of *Moringa oleifera* revealed that flavonoids, terpenoids, phenols, alkaloids, steroids and reducing sugars tannins were present in both the ethanol and aqueous extracts. Saponins and tannins were only found in the aqueous and ethanol extracts respectively. Experiments to observe for lethal conditions

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or changes in behavior showed no lethality or behavioral change at doses of 10, 100, 1000, 1600 and 2900 mg/kg bw. Weakness and drowsiness was exhibited at a dosage of 5000 mg/kg bw but no death occurred within 24 hrs. of administration.

Keywords: *Moringa oleifera,* AlCl3, Liver, Function, Wistar, Rats, Treated, Extracts

INTRODUCTION

Moringa oleifera contains sufficient amount of vital nutrients. The leaves are rich in calcium, potassium, zinc, magnesium, iron and copper. Beta-carotene of vitamin A, vitamin B such as folic acid, pyridoxine and nicotinic acid, vitamin C, D and E are also present in *Moringa oleifera* (Mbikay, 2012). Also present are phytochemicals like tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars along with anti-cancerous agents like glucosinolates, isothiocyanates, glycoside compounds and glycerol-1-9-octadecanoate (Berkovich, Earon, Ron, Rimmon, Vexler, and Lev-Ari, 2013). The leaves of moringa also has low calorific value and can be used in the diet of the obese. The pods contain fiber and are useful in the treatment digestive problems and thwart colon cancer (Oduro, Ellis, and Owusu, 2008). A research by Sánchez-Machado, Nú˜nez-Gastélum, Reyes-Moreno, Ramírez-Wong and López-Cervantes, (2010) shows that immature pods contain around 46.78% fiber and 20.66% protein content. Pods have 30% of amino acid content, the leaves have 44% and flowers have 31%. The immature pods and flowers showed similar amounts of palmitic, linolenic, linoleic and oleic acids.

A lot of minerals that are essential for growth and development are found in moringa. Calcium which is one of these minerals is considered an important mineral for human growth. While 8 ounces of milk can provide 300–400 mg, moringa leaves can provide 1000 mg and moringa powder can provide more than 4000 mg. Moringa powder can be used as a substitute for iron tablets, hence as a treatment for anemia. Beef has only 2 mg of iron while moringa leaf powder has 28 mg of iron. It has been reported that moringa contains more iron than spinach (Fuglie, 2005). A good dietary intake of zinc is essential for proper growth of sperm cells and is also necessary for the synthesis of DNA and RNA. *M. oleifera* leaves show around 25.5–31.03 mg of zinc/kg, which is the daily requirement of zinc in the diet (Gopalakrishnanb, Kruthi Doriyaa and Kumar, 2016).

Many chronic diseases have in their very core a functional and pathophysiological relationship with inflammation and oxidative stress and these effects can be measured in vitro (Fahey, Stephenson, Wade and Talalay, 2013).

In their studies Waterman, Cheng, Rojas-Silva, Poulev, Dreifus, Lila, and Raskin; (2014) showed the anti-inflammatory effects of isothiocyanate rich extracts of *M. oleifera* in vitro while Ndhlala, Mulaudzi, Ncube, Abdelgadir, du Plooy and Van Staden; (2014) compared the antioxidant variation between *M. oleifera* cultivars. However, it is much more challenging to associate anti-oxidative and anti-inflammatory measurements made in bodily fluids such as blood, urine, or sputum, with clinical symptoms of disease. Kushwaha, Chawla and Kochhar; (2014) conducted a trial on 30 post-menopausal women who were supplemented with 7g of *M. oleifera* leaf powder daily for 3 months. They reported that there were changes in the antioxidant profile and "oxidative status" upon monitoring blood/serum biomarkers in the women, and there was no report of symptom measurement.

Galuppo, Giacoppo, De Nicola, Iori, Navarra, Lombardo, Bramanti and Mazzon; (2014) have reported that the isothiocyanates of *M. oleifera* repress the inflammatory component of experimental autoimmune encephalomyelitis using the rodent models of disease.

Liver function tests are tests that are useful in the evaluation and treatment of patients with hepatic dysfunction. The liver carries out metabolism of carbohydrate, protein and fats. Biochemical markers for liver dysfunction are usually some of the enzymes and the end products of the metabolic pathway that are very sensitive for the abnormality (Gowda, Desai, Hull, Math, Vernekar and Kulkarni, 2009). Some of the such biochemical marker are serum bilirubin, alanine amino transferase, aspartate amino transferase, ratio of aminotransferases, alkaline phosphatase, gamma glutamyl transferase, 5' nucleotidase, ceruloplasmin, α-fetoprotein. An isolated or conjugated alteration of biochemical markers of liver damage in patients can challenge clinicians during the diagnosis of disease related to liver directly or with some other organs. This is because they reflect different functions of the liver that is, to excrete anions (bilirubin), hepatocellular integrity (transaminases), formation and the subsequent free flow of bile (bilirubin and ALP), and protein synthesis (albumin).

MATERIALS AND METHODS

Sample Collection and Preparation

Dried seeds of *Moringa oleifera* were collected from a healthy plant from its natural habitat around the Wukari area of Taraba State and was sent to the International Centre for Ethnomedicine and Drug Development in Nsukka, Enugu State where it was identified and authenticated by Ugwu Paschal Ifeanyichukwu (Herbarium Curator) and Alfred Ozioko (taxonomist).

The dried seeds of *Moringa oleifera* were pulverized to powdered specimen using a mortar and pestle.

Sample extraction

A 200g of the powdered seeds was macerated in ethanol and aqueous in the ratio 1:5 for exactly 48hrs. The extracts were filtered out first using a clean white sieving mesh and then using the Watman No. 1 filter paper. The filtrates were concentrated using a rotary evaporator. The concentrated extracts were then transferred to air-tight containers, corked and preserved in the refrigerator at 4 °C until required.

Animals Specimen

35 male albino rats of 100–150 g were purchased from the animal house of the Department of Zoology, University of Nigeria, Nsukka. They were kept in clean cages and maintained under standard laboratory conditions (Temperature 25 ± 5 °C, Relative humidity 50 – 60 %, and a 12/12h light/dark cycle) and allowed free access to standard diet and water ad libitum. Animals were allowed to acclimatized for 7 days before each of the experiments. All experiments were conducted in compliance with ethical guide for care and use of laboratory animals of the Department of Zoology, University of Nigeria, Nsukka.

Experimental Design

The rats were randomly divided into seven groups of five animals each (n = 5) as follows:

i. Group 1 Control received normal feed and water + 3 % tween 80 after 1 hour.
ii. Group 2 received 100 mg/kg bw Aluminium chloride + 3 % tween 80 after 1 hour.
iii. Group 3 received 100 mg/kg bw standard drug an hour after administering 100 mg/kg bw of Aluminium chloride.

iv. Group 4 received 100 mg/kg bw ethanol extract of *Moringa oleifera* seeds an hour after administering 100 mg/kg bw of Aluminium chloride.
v. Group 5 received 100 mg/kg bw aqueous extract of *Moringa oleifera* seeds an hour after administering 100 mg/kg bw of Aluminium chloride.
vi. Group 6 received 400 mg/kg bw ethanol extract of *Moringa oleifera* seeds an hour after administering 100 mg/kg bw of Aluminium chloride.
vii. Group 7 received 400 mg/kg bw aqueous extract of *Moringa oleifera* seeds an hour after administering 100 mg/kg bw of Aluminium chloride.

After the experimental period, animals were sacrificed and venous blood was collected by ocular puncture. Blood samples were collected into plain sample tubes containing no anticoagulant for the serum. The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 rpm for 5 min.

Biochemical Assay

Phytochemical Analysis

Phytochemical analysis of the organic extract was carried out according to the method of Harborne (1998). Basic phytochemical screening was carried out using simple chemical tests to detect the presence of secondary plant constituents such as alkaloids, tannins, flavonoids, saponins, triterpenes, sterols, phenols, glycosides, reducing sugars and soluble carbohydrates in the sample.

Toxicity study

The median lethal dose fifty (LD_{50}) for $AlCl_3$ was carried out according to the method described by Lorke (1983). Thirty-six experimental animals (mice with weight range of 15 – 30 g) were used for the test. In the investigation, six groups of mice containing three mice each were respectively administered with 10, 100 and 1000 g/kg of the aqueous and ethanol extract orally. They were observed closely for 24 hr. for lethality or any other behavioral response. Based on the result, further increased doses of 1600, 2900 and 5000 mg/kg of the aqueous and ethanol extracts were administered orally to six other mice groups respectively. They were also observed for 24 hours for any death or behavioral changes.

Determination of Liver Function States

Determination of the aspartate aminotransferase (AST) activity

The activity of aspartate aminotransferase was assayed by the method of Reitman and Frankel (1957) as outlined in the Randox kit used. Aspartate aminotransferase activity was measured by monitoring the formation of oxaloacetate hydrazine with 2,4-dinitrophenylhydrazine.

Determination of the alanine aminotransferase (ALT) activity

The activities of alanine aminotransferasewas assayed by the method of Reitman and Frankel Reitman and Frankel (1957) as outlined in Randox Kit.

Determination of the alkaline phosphatase (ALP) activity

The activities of alkaline phosphatase were assayed by the method of Babson (1966), as outlined in Randox kit, used. The alkaline phosphatase act upon the AMP-buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

Determination of total Bilirubin

Colorimetric method based on that described by Jendrassik and Grof (1938). Driect (conjugated) bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Determination of Albumin

Albumin react with bromocresol green (BCG) in a buffered solution to give a dye- binding reaction.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) using Graph Pad Prism version 5 and Tukey post hoc test. Values of $P< 0.05$ were considered to be significant. The data obtained were expressed in tables and charts.

RESULTS

Qualitative Phytochemical of Ethanol and Aqueous Extract Moringa oleifera seeds

Table 1 shows the presence of reducing sugars and tannins in mild, phenols, alkaloids and steroids in moderate amount while flavonoids and terpenoids were present in abundance in the ethanol extracts of *M. oleifera*. Whereas the aqueous extracts presence of terpenoids and steroids were mild, reducing sugars, phenols and flavonoids were moderate while saponins and alkaloids are in abundance.

Anthraquinones, carbohydrates and glycosides were absent in both the extracts while saponin was absent in the ethanol extract but abundant in the aqueous extract while tannin also absent in the ethanol extract and mild in the aqueous extract.

Table 1: Qualitative Phytochemical Composition of Ethanol and Aqueous Extract *Moringa oleifera* seeds

SN	Test	Ethanol	Aqueous
1	Saponin	-	+++/++
2	Anthraquinones	-	-
3	Carbohydrates	-	-
4	Reducing Sugar	**+**	**++**
5	Tannins	+	-
6	Glycosides	-	-
7	Phenols	++	++
8	Alkaloids	++	+++
9	Steroids	++	+
10	Flavonoids	**+++**	++
11	Terpenoids	**++++**	**+**

+mild, ++moderate, +++abundant

Acute Toxicity (LD_{50})

In the investigation, there was no lethality or behavioral change in the six groups of mice that received 10, 100 and 1000 mg/kg of both extract. Based on this result, further increased doses of 1600, 2900 and 5000 mg/kg of the extract were administered to six other groups respectively. Those that received 5000 mg/kg of the extract exhibited weakness and drowsiness. No death occurred within 24 hr. of administration.

Table 2 The median lethal dose of aqueous extract of the seed of *M. oleifera*

Phases	**Dosages mg/kg bw**	**Mortality**	**Behavioral Changes**
Phase I			
Group 1	10	0/3	Nil
Group 2	100	0/3	Nil
Group 3	1000	0/3	Nil
Phase II			
Group 1	1600	0/3	Nil
Group 2	2900	0/3	Nil
Group 3	5000	0/3	Weakness and drowsiness

Table 3 The median lethal dose of ethanol extract of the seed of *M. oleifera*

Phases	**Dosages mg/kg bw**	**Mortality**	**Behavioral Changes**
Phase I			
Group 1	10	0/3	Nil
Group 2	100	0/3	Nil
Group 3	1000	0/3	Nil
Phase II			
Group 1	1600	0/3	Nil
Group 2	2900	0/3	Nil
Group 3	5000	0/3	Weakness and drowsiness

Liver Function state

Presented in table 4 is the result of serum liver function of rats treated with $AlCl_3$, ethanol and aqueous extracts of *M. oleifera*. The result showed significant elevation in the concentrations of direct bilirubin, total bilirubin, albumin, AST, ALT and ALP levels in the group treated with $AlCl_3$. The result further revealed that admiration of $AlCl_3$ along with the extracts of *M. oleifera* seeds reduced such concentrations.

Direct Bilirubin levels increased from 37.17 ± 1.74 in the control group, 1 to 41.40 ± 1.44 in group 2 when treated with $AlCl_3$

Table 4 Effects of *M. oleifera* ethanolic and aqueous seed extract on the liver function of $AlCl_3$ treated rats.

	Bili (D) (mg/dL)	Bili (T) (mg/100L)	Alb (mg/dL)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Group 1	37.17 ± 1.74^{a}	0.47 ± 0.05^{b}	4.12 ± 0.34^{c}	11.83 ± 0.40^{b}	11.91 ± 0.20^{a}	88.63 ± 2.4^{b}
Group 2	41.40 ± 1.44^{b}	0.59 ± 0.03^{c}	4.73 ± 0.22^{d}	57.29 ± 0.50^{c}	59.23 ± 0.24^{b}	100.84 ± 12.34^{c}
Group 3	37.11 ± 0.87^{a}	0.47 ± 0.05^{b}	4.07 ± 0.21^{b}	11.70 ± 0.45^{b}	11.84 ± 0.27^{a}	88.63 ± 2.05^{b}
Group 4	36.34 ± 1.44^{a}	0.45 ± 0.05^{a}	4.23 ± 1.01^{c}	11.39 ± 0.25^{a}	11.62 ± 0.40^{a}	85.15 ± 4.11^{a}
Group 5	36.36 ± 0.57^{a}	0.42 ± 0.17^{a}	3.94 ± 0.13^{a}	11.42 ± 0.15^{a}	11.61 ± 0.17^{a}	84.51 ± 3.43^{a}
Group 6	37.22 ± 1.16^{a}	0.48 ± 0.03^{b}	4.11 ± 0.35^{c}	11.77 ± 0.25^{b}	11.93 ± 0.17^{a}	88.63 ± 1.37^{b}
Group 7	37.34 ± 1.16^{a}	0.48 ± 0.03^{b}	4.08 ± 0.27^{b}	11.78 ± 0.15^{b}	11.94 ± 0.07^{a}	88.75 ± 1.37^{b}

Each value represents the mean of 5 rats ± SD.

Bil (D) = Direct bilirubin, Bil (T) = Total bilirubin, Alb = Albumin, AST = Aspartaate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline Phosphatase

DISCUSSION

Phytochemicals of *Moringa oleifera*

A screening for phytochemicals or a qualitative analysis is used to reveal the chemical constituents or the secondary metabolites of plant extracts. In this study, preliminary phytochemical screening for seed extracts of *M. oleifera* revealed that flavonoids and terpenoids were abundantly present in the ethanol extracts as shown in Table 1 above. In the same ethanol extracts, phenols, alkaloids and steroids were moderately present whereas, reducing sugars and tannins were mild. Saponins which were not present in the ethanol extracts were now found to be abundant in the aqueous extracts. Alkaloids were also abundant. Reducing sugars and phenols were now seen to be present in moderate concentrations. Steroids and terpenoids were in mild concentrations. In their studies (Fahal, Rani, Aklakur, Chanu and Saharan, 2018) reported the presence of alkaloids, flavonoids, saponins, sterols and tannin in the ethanol and aqueous extracts of *M. oleifera* pods.

Several studies have shown that phytochemicals are supportive in the prevention and healing of infections and illnesses such as stimulating the body's immune system against various diseases causing agents like bacteria, fungi, viruses, etc.

Flavonoids have been said to help in treatment of inflammation and boosting the immune system while alkaloids, tannins, saponins and phenols have antimicrobial activity and as such essential in treatments of malaria. This suggest the use of this plant in the traditional treatment of several diseases (Khan, Suleman, Baqi, Ayub and Ayub, 2018).

Acute Toxicity Test

In the investigation, mice were treated with both the ethanol and aqueous extracts of *Moringa oleifera* seeds extracts at different concentrations in order to observe for lethal conditions or changes in behavior but there was no lethality or behavioral change in the six groups of mice that received 10, 100 and 1000 mg/kg of both extract. Based on the outcome further increase of dosage was administered to the mice. The new dosage was started on 1600 mg/kg bw, then it was increased to 2900 mg/kg bw and lastly to 5000 mg/kg bw. At the highest concentrations of 5000 mg/kg bw was when weakness and drowsiness were observed. Although, those that received 5000 mg/kg of the extract exhibited weakness and drowsiness no death occurred within 24 hr. of administration.

Liver function

Vital in various metabolic processes are the liver and kidneys, this exposes them to toxins and makes them primary targets of several exogenous compounds (Yakubu, Obot and Dawoye, 2016) such as $AlCl_3$. AST and ALT are common liver enzymes because of their higher concentrations in hepatocytes, but only ALT is remarkably specific for liver function (Crook, 2006). Therefore, an elevation in serum concentration of ALT is an indication of liver damage.

In this study (Table 4), ALT and AST levels were significantly ($p<0.05$) increased from 11.91 ± 0.20 and 11.83 ± 0.40 to 57.23 ± 0.24 and 59.29 ± 0.50 respectively when rats where induced with aluminum chloride toxicity. When rats were treated with ethanol and aqueous extracts of *M. oleifera* at 100 mg/kg the levels of ALT dropped down to 11.62 ± 0.40 and 11.61 ± 0.17 respectively and the AST levels dropped to 11.39 ± 0.25 and 11.42 ± 0.15 respectively. A further increase in the concentrations of both extracts to 400 mg/kg resulted to a significant ($P<0.05$) elevation of ALT 11.93 ± 0.17 (ethanol) and 11.94 ± 0.07 (aqueous) and AST 11.77 ± 0.25 (ethanol) and 11.78 ± 0.15 (aqueous).

Alkaline phosphatase, ALP, is a plasma and endoplasmic reticulum membrane-bound enzyme. Transient increase of this enzyme may be noticeable in all types of liver problems. $AlCl_3$ initiated an increase in the level of this enzyme. But treatment with the two extracts

of *M. oleidera* resulted in their decrease to 85.15 ± 4.11 (ethanol) and 84.51 ± 3.43 (aqueous) which were even lower than that of the control group (88.63 ± 2.4) but increasing their concentrations to 400 mg/kg lead to elevation to 88.63 ± 1.37 (ethanol) and 88.75 ± 1.37 (aqueous).

Serum bilirubin levels could be expressed as total bilirubin comprising of conjugated and non-conjugated or as direct bilirubin comprising only of the conjugated and an increase in bilirubin level could be attributed to three major causes such as haemolysis, biliary obstruction and liver cell necrosis (Tilkian, Conover and Tilkian, 1979). A decrease in both direct and total bilirubin levels were seen in animals that received treatment with both ethanol and aqueous extracts of *M. oleifera* after a noticeable increase in such levels upon inducing toxicity with aluminum chloride.

Albumin levels also increased when animals were administered with $AlCl_3$ but were significantly reduced when treatments of *M. oleifera* extracts were administered an hour after, although the group treated with 100 mg/kg showed the highest decrease.

CONCLUSION

This studies demonstrates the effectiveness of *Moringa oleifera* seeds against oxidative stress caused by $AlCl_3$ intoxication of Wistar rats. The low mortality rate observed during the experiment suggest the safety and efficacy of this plant extract in the system.

The hypotheses of this study is stated below

H1: $AlCl_3$ has effects on the liver function albino Wister rats treated with *Moringa oleifera* seed extracts.

Recommendation

Further studies in regards to the effects of *Moringa oleifera* seed extracts should be considered in novel drug development. The plant should be considered a traditional drug for health remedy. The extract is a promising source for therapeutic agent that can be used in combating infectious diseases caused by drug-resistant bacteria, since this study shows that the extract exhibited significant improvements in the activities of serum electrolytes as against albino Wister rats induced with $AlCl_3$ toxicity, further studies should be carried out for the isolation and identification of individual bioactive compounds which are

responsible for this therapeutic activity and the investigation of their mechanism(s) of action.

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Competing Interests

The authors declare that there are no competing interests.

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