Effect of Egyptian Moringa Oleifera Lam. on blood hematology, serum biochemical parameters and lipid profile with special reference to kidney function in albino rats.

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Abstract: Moringa Oleifera is a well-known medical plant in Egypt and used extensively in medical research. To better understand the effect of Moringa Oleifera ethanolic extract on blood hematology, biochemical parameters, lipid profile and its role in modulation of renal function. Thirty male albino rats were divided into 3 groups gavaged 0.5 ml of distilled water, 250 mg/kg and 500 mg of Moringa Oleifera leaves ethanolic extract twice per weeks for 8 weeks. Rats were sacrificed and blood samples were tested for blood hematology, biochemical parameters and lipid profile. It was found that Moringa Oleifera at dose of 250 mg/kg increased significantly level of hemoglobin, WBCs and non-significantly increased RBCs and platelets counts while Moringa Oleiferaat dose of 500 mg/kg reduced significantly hemoglobin level. While at dose of 250 mg/kg of Moringa Oleifera showed significantly increased level of creatinine and total protein and non-significantly increased level of glucose and blood urea nitrogen while reduced significantly level of triglycerides, total cholesterol and high-density lipoprotein cholesterol when compared with control group. In contrast, Moringa Oleifera at dose of 500 mg/kg showing significantly increase of glucose, creatinine, blood urea nitrogen, total protein, albumin, triglycerides, total cholesterol and high density lipoprotein cholesterol levels when compared with control group. The histopathological examination determined that Moringa Oleifera at dose of 250 mg/kg showed interstitial inflammatory infiltrate and Moringa Oleifera at dose of 500 mg/kg showed focal tubular atrophy. On conclusion, ethanolic extract of Moringa Oleifera had effects on biochemical, hematology, lipid profile and kidney architecture in albino rats.

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Key words: Egyptian Moringa Oleifera ethanolic extract, blood hematology, biochemical parameters and lipid profile.

1. Introduction

Kidneys are one of the vital organs in the body with important role in the excretion of toxin that considered pathologically harmful to the human body. Therefore, nephrotoxic agents are of fatal consequences on human health **(Iwara et al. 2013).**

Moringa Oleifera is a well-known medical plant used in the treatment of hypercholesterolemia and hyperglycemia, alsoconsidered a nutritional additive. Additionally**,** there was no hepatotoxicity or nephrotoxicity of Moringa Oleifera and no hematological changes. Moringa Oleifera was genotoxic at level of 3,000 mg/kg body weight, **Asare et al. (2012).**

The extracts from the tropical tree Moringa Oleifera has anti-inflammatory, antidiabetic, antioxidant, and anticancer effects. Fermentation of these extract can further improve the nutritional and reduce its toxicity **(Joung et al., 2017).** Moreover, **Tang et al. (2017)** reported that Moringa Oleifera from Cambodia had anti-Oxidative Stress, anti-Hyperglycemia and improved renal Dysfunction in Type 2 Diabetic Mice.

The antioxidant activity of the Moringa Oleifera leaves extract was depending on their reducing power and radical scavenging properties. Moreover, anti-diabetic activity of Moringa Oleifera leaves extract and their role in the prevention of diabetic complications based on their digestive enzyme inhibitory activity and their potential in block the formation of early and advanced glycationend-products. The anti-glycation activity of the Moringa Oleifera leaves extract was based on the formation of amadori product (Fructosamine) and advanced glycationend-products **(Awasthi. 2013)**.

Adedapo et al. (2009) investigated the sub-acute oral toxicity of Moringa oleifera extract at dose of 400, 800 and 1600 mg/kg on male Wister rats through detection of hematological, biochemical and histological parameters. The extract at different doses caused significant changes in total RBCs, haemoglobin (Hb), total proteins, liver enzymes, and bilirubin.

Sub-lethal treatment of seed extract of Moringa Oleiferaat dose of12.40 mg/L gradually elevated WBC count, MCV, MCH, plasma glucose, AST, ALT and ALP activities at the end of 7, 14, 21, 28 and 35th days

in of exposed fish **Kavitha et al. (2012).** Additionally, **Awodele et al. (2012)** found that the LD50% of aqueous leaf extract of Moringa Oleifera was estimated to be 1585 mg/kg. The daily treatment of aqueous leaf extract of Moringa Oleifera at doses of 250, 500 and 1500 mg/kg orally for 60 days had no significant difference in biochemical and hematological parameters in the treated rats compared to the control. Moreover, there was no significant difference in weight gain of the control and treated animals.

Iwara et al. (2013) reported the effect of extracts of Vernoniaamygdalina (V.A) and Moringa Oleifera (M.O) on STZ induced kidney damage in Albino Wistar rats. Significant increase in K^+, Na^+, CL^- and urea concentration in groups treated with Moringa Oleifera extract compared to diabetic control, and closely related to normal control and insulin groups were seen. Serum glucose levels appeared a significant decrease compared with the diabetic control groups and closely related to normal control. Notably treatment of Ocimum Gratissimum and Moringa Oleifera in diabetic rats enhanced decrease the urea and creatinine concentrations for the treated groups and corrected the nephropathy complication, **Efiong and Ebong (2014**).

Aim of work

The aim of this study is to investigate the effect of ethanolic extraction of Moringa Oleifera on hematological parameters, lipogram and biochemical parameters especially kidney function parameters.

Material and methods

Experimental animals:

Thirty male albino rats weighting from (140±5 grams) obtained from medical experimental center, faculty of pharmacy, Mansoura University, Egypt. Animals were apparently clinical healthy and were housed in stainless steel cages with wood shavings as bedding. Animals were accommodating to laboratory condition for 2 weeks before being experimented. Animals were maintained on balanced ration prepared in the medical experimental research center. Water and feed were given ad-libitium throughout the experiment.

Plant identification and extraction

Two kilograms of leaves of Moringa Oleifera (Synonym: Moringa pterygosperma Geartner) belongs to a family of shrubs and tree, Moringaceae were collected from cultivated plants in Fayoum district and was authenticated by Dr. Zein Elaabdeen Metwaly, lecturer of Botany, P harmacognosy department, Faculty of Pharmacy, Mansoura University.

The leaves were air derided, powdered and sieved, the powder was allowed to stand in 10 liters of ethanol (5 times), 2 liters each respectively by slow percolation at room temperature. The filtrate was collected and evaporated with a rotatory evaporator then allowed to concentrate under reduced pressure till a greenish viscous paste was obtained which weighted 81.5 grams and stored in air tight dark glass bottle in the refrigerator at 4º Ctill using. A fresh solution of the extract (250 mg/ml and 500 mg/ml) dissolved in ethanol was prepared from this stock and used in the investigation, **Behery et al. (2012).**

Animals and treatments

The animals were maintained in accordance with the guidelines prescribed by the Faculty of Science and the study was approved by the Animal Ethics Committee of the University of Mansoura, Egypt. The experimental rats were divided into threegroups:

Group 1: Animals were fed on the standard diet and were served as control group.

Group 2: Animals were orally given250 mg/kg b.w. ethanolic extract of Moringa Oleifera, twice weekly for eight weeks.

Group 3: animals were orally given500 mg/kg b.w. ethanolic extract of Moringa Oleifera, twice weekly for eight weeks.

Hematological examination

Whole blood was collected on EDTA-vacuum tubes. Erythrocytescount and hemoglobin (Hb) was estimated and packed cell volume (PCV) was detected according of conventional method **(Duncan et al., 1997)**. Erythrocytes indices as mean corpuscular values (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscularhemoglobin concentration (MCHC) were calculated. Total leucocytes and platelets count were also determined as described by **Coles (1986)**.

Histological examinations

The treated animals and their controls were sacrificed by decapitation after 8 weeks of treatment. Their kidneys were removed and fixed in 10% neutral formalin. Fixed materials were embedded in paraffin wax and sections of 5 micrometer thickness were cut. Slides were stained with haematoxylin and eosin for histological examination.

Biochemical assays

For biochemical study serum were obtained by centrifugation of the blood samples at 4000 RPM for 5 minutes and stored at 4°C until assayed for the biochemical parameters.

a- Serum creatinine level was determined according to the method of **Bowers et al. (1980)**.

b- Serum urea level was determined according to the method of **Chaney et al. (1962)**.

c- Serum glucose level was determined according to the method of **Trinder et al. (1969)**.

d- Serum total cholesterol level was determined according to the method of **Tietz (1976**).

e- Serum triglyceride level was determined according to the principle of **Fossati et al. (1982)**.

f-Serum High density lipoprotein cholesterol (HDL-C) and Serum Low density lipoprotein cholesterol (LDL-C) levels were determined according to the method of **Tietz (1976)**.

g- Serum total protein level was determined according to the method which described by **Vassault et al. (1986)**.

h- Serum albumin level was determined according to the method of **Doumas et al. (1976)**.

Statistical analysis:

Data obtained in the current study were statistically analyzed for variance (ANOVA), and least significant difference (LSD) as described by **Snedecor and Cochran (1989)** by using computerized **SPSS** program (1996) version 13.0 (trial).

3. Results:

1-Effect of Moringa Oleiferaon body weight:

The ethanolic extract of Moringa Oleiferaa t doses of 250 and 500 mg/kg reduced body weight of treated male rats non-significantly when compared with control group. In the current study, kidney weight was reduced significantly in-group treated with Moringa Oleifera at doses of 250 or 500 mg/kg (table 1-fig 1).

Table (1) shows Effect of Moringa Oleifera on body weight, kidney weight and renal to body weight ratio.

Group	Body weight	Kidney weight	Renal to body weight ratio
Group 1	260±12.4	1.9±0.07	0.74±0.01
Group 2	237±19.0	1.7[a]±0.15	0.73±0.04
Group 3	242±8.0	1.7[a]±0.09	0.72±0.03

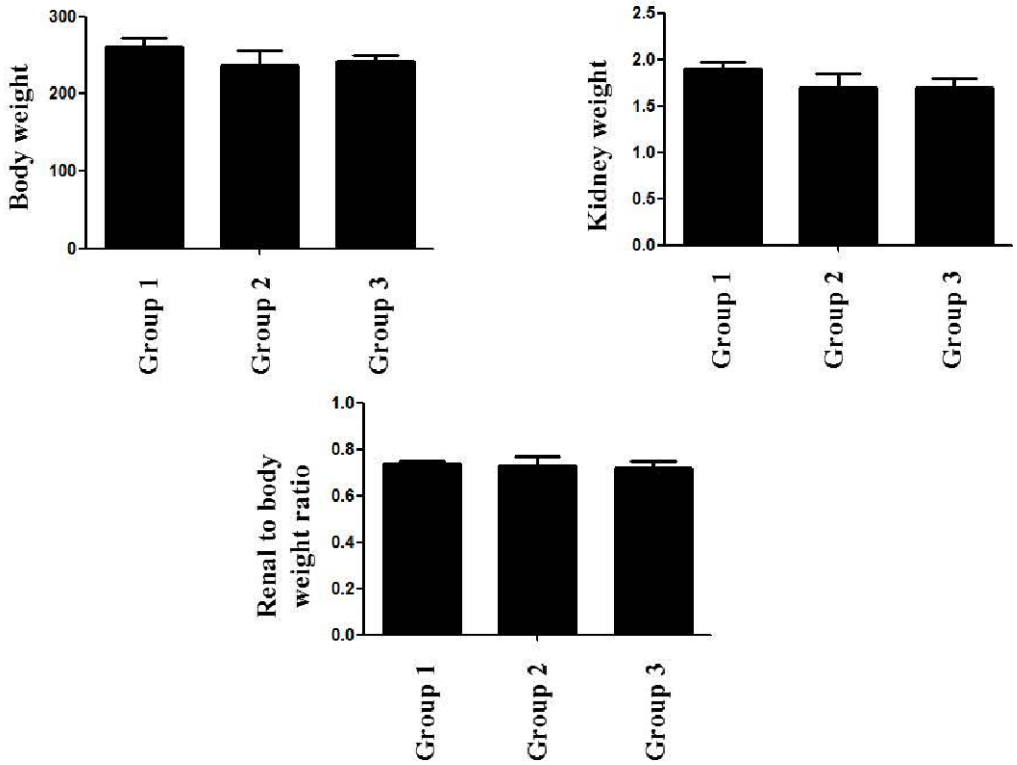

Figure (1) shows Effect of Moringa Oleifera on body weight, kidney weight and renal to body weight ratio.

2-Effect of Moringa Oleifera on blood hematology:

The ethanolic extract of Moringa Oleifera at dose of 250 mg/kg increased hemoglobin (HB), RBCs counts, WBCs counts, platelets and packed cell volume (PCV) significantly while Moringa Oleifera at dose of 500 mg/kg reduced non-significantly hemoglobin (Hb) level and increased WBCs counts, platelets significantly (table2-fig 2).

Table (2) shows effect of Moringa Oleifera on blood hematology

	Hb g/dL	**RBCs 10^6/µl**	**PCV %**	**MCV fL (µm^3)**	**MCH pg**	**MCHC g/dL**	**WBCs 10^3/ µl**	**Platelets 10^3/ µl**
Group 1	12.9±0.3	7.1±0.1	39.2±0.7	54.9±1.1	17.9±0.1	32.8±0.4	6.9±1.2	696±50
Group 2	14.6a±0.8	7.7±0.1	47.6a±3.2	61.4±4	18.8±1	30.6±0.2	12.7a±1.9	741±52
Group 3	12.4±0.7	6.9±0.5	41.4±1.5	61.2±3.8	18.1±0.56	29.9±0.9	10.7a±0.9	852a±75

A, b, c significant at ≤ 0.05

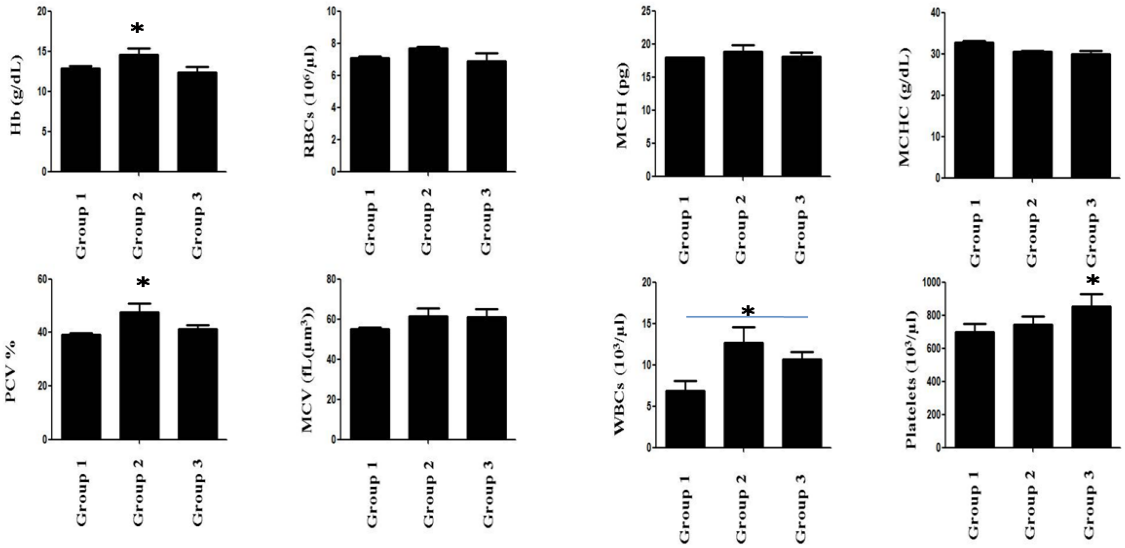

Figure (2) shows effect of Moringa oleifera on blood hematology

3- Effect of Moringa Oleifera on serum biochemical parameters:

In the current study, the ethanolic extract Moringa Oleifera at dose of 250 mg/kg increased significantly level of creatinine and total protein (T.P) and non-significantly increased level of glucose and blood urea while reduced significantly levels of triglycerides, total cholesterol and high density lipoprotein cholesterol (HDL-C) when compared with control group. In contrast, Moringa Oleiferaat dose of 500 mg/kg reduced significantly level of glucose, creatinine, blood urea nitrogen, total protein, albumin, triglycerides, total cholesterol and high density lipoprotein cholesterol (HDL-C) in comparison tocontrol group (table 3-figure 3).

4- Effect of Moringa Oleiferaon kidney tissue:

The ethanolic extract of Moringa Oleiferaat dose of 250 mg/kg showed an interstitial inflammatory infiltrate while at dose of 500 mg/kg showed a few focal tubular atrophy (figure 4).

Table (3) shows effect of Moringa Oleiferaon serum biochemical parameters.

	Glucose mg/dL	**creatinine mg/dL**	**Urea mg/dL**	**T.P g/dL**	**Albumin g/dL**	**Triglyceride mg/dL**	**T. chol. mg/dL**	**LDL-C mg/dL**	**HDL-C mg/dL**
Group 1	220±6.4	0.5±0.03	50.6±3.9	7±0.29	3.9±0.1	35.4±6.5	57.±7.6	29.9±6.3	23.2±3.1
Group 2	249± 1.9	0.7^a±0.3	56.4±4	9.7a±1.1	3.4±0.12	18.2^a±1.9	28.8^b±4.6	12.9^a±4.5	12.2^b±0.9
Group 3	111^a±1.1	0.44±0.04	28^a±2	3.8b±0.7	1.2^a±0.12	20.6^a±2.7	45^a±6.5	23.6±7.5	17.2^a±1.2

A, b, c significant at ≤ 0.05

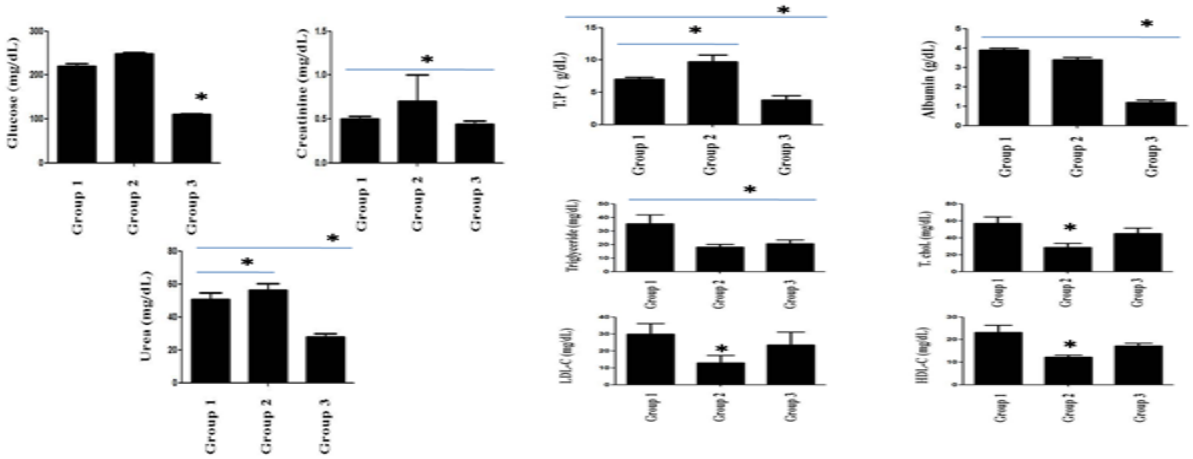

Figure (3) shows effect of Moringa oleifera on serum biochemical parameters.

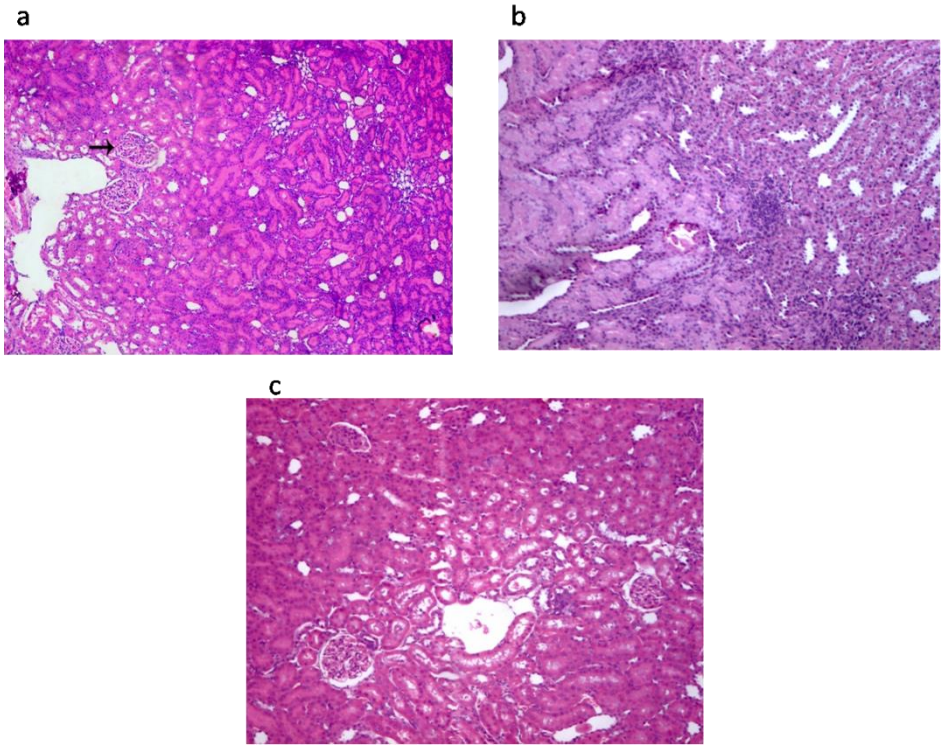

Figure (4) a-kidney is showing normal renal glomeruli (arrow) and normal renal tubules lined by normal renal tubular epithelium. **b**-Moringa Oleifera at dose of 250 mg/kg showed an interstitial inflammatory infiltrate. **c-** at dose of 500 mg/kg Moringa oleifera showed a few focal tubular atrophy. **(100x)**

4. Discussion:

In the current study, treatment with Oleifera at doses of 250 and 500 mg/kg reduced the body weight of treated animals. This result agree partially with **Oyagbemi et al., (2013)** who found that Wister rats received 400 mg/kg of methanolic extract

Moringa Oleifera decrease in body weight only on weeks 2,3,5 and 6 of the experiment.

Moringa Oleiferaat dose of 250 mg/kg increased significantly level of hemoglobin (Hb), WBCs and non-significantly increased of RBCs and platelets counts while Moringa Oleiferaat dose of 500 mg/kg reduced significantly hemoglobin (Hb) level. These results agree with **Osman et al. (2012)** who found that Moringa Oleifera increased significantly mean cell hemoglobin concentration (MCHC), platelets count, RBCs count and hemoglobin (Hb) in ratswhile RBCs and platelets count increased similarly in rabbits at dose of 300 mg/kg.

In the current study, Moringa Oleiferaat dose of 250 mg/kg increased significantly level of creatinine and total protein and non-significantly increased level of glucose and blood urea while reduced significantly level of triglycerides, total cholesterol and high density lipoprotein cholesterol (HDL-C) when compared with control group. In contrast, Moringa Olifera at dose of 500 mg/kg reduced significantly level of glucose, creatinine, blood urea, total protein, albumin, level of triglycerides, total cholesterol and high density lipoprotein cholesterol (HDL-C) when compared with control group. These result agree with **Oyagbemi et al. (2013)** found that prolonged consumption of Moringa Oleifera leaves as a beverage to Wister rat significantly increased serum total protein and globulin in a dose-dependent manner. Rats that received Moringa Oleifera at 200 and 400 mg/kg showed a significant increase in serum blood urea and creatinine which pointed to hepatic and kidney damage. Moreover, these results agree with **Omobowale et al., (2014)** who found that Wister rats received400mg/kg body of methanol extract of Moringa Oleifera for 5 weeks led to significant decrease in serum blood urea and creatinine when compared with control group.

Notably, these biochemical effects of Moringa Oleifera agree with **Tang et al., (2017)** who found that Moringa Oleiferaat dose of 150 mg /kg significantly ameliorated the altered fasting plasma glucose, triglyceride and low-density lipoprotein cholesterol in Type 2 diabetic mice and improved kidney dysfunction.

Moringa Oleiferaat dose of 250 mg/kg showed interstitial inflammatory infiltrate while kidney of rats treated with Moringa Oleiferaat dose 500 mg/kg showed few focal tubular atrophy. These results agree with **Osman et al. (2015)** who reported that kidneys treated with Moringa Oleifera showed hemorrhage, necrosis and degeneration of epithelial renal tubules. In contrast, **Asiedu-Gyekye et al., (2014)** found that acute or sub-acute of raw powder of Moringa Oleifera Leaves (40mg/kgto1000mg/kg) did not show any pathological alteration.

On conclusion, ethanolic extract of Moringa oleifera had effects on biochemical, hematology, lipid profile and kidney architecture in albino rats.

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