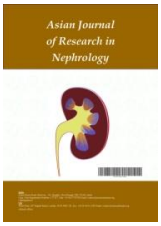

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Protective Effect of Aqueous Extract of *Moringa oleifera* on Nephrotoxicity and Liver Injury Induced by Sodium Chloride in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NC, EA and NCI designed the study. Authors NC, OAN and DNJ performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AC, NC and EA managed the analyses of the study. Authors NC and NCI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Protective effect of aqueous extract of *Moringa oleifera* on nephrotoxicity and liver injury induced by sodium chloride in Wistar albino rats was studied. *M. oleifera* collected from Imo State University School farm was prepared into an extract, and given orally to groups (III, IV and V) of test rats against experimental control rats (group II) that were only induced, alongside with normal control rats (group I) that were neither induced with sodium chloride nor received aqueous extract as treatment. Result of weight changes in rats revealed significant increase ($p<0.05$) in % weight

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change of test rats (groups III, IV and V) against the experimental control rats (group II). % weight change in test rats increased insignificantly ($p>0.05$) when compared to normal control. Urea and creatinine levels significantly reduced ($p<0.05$) in test rats against experimental control but increased significantly ($p>0.05$) when compared to those of the normal control. The liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) significantly reduced ($p<0.05$) when compared to those of experimental control. The ameliorating effect as observed in test rats against the experimental control rats in this study, could be as a result of *M.oleifera* extract. The extract may have offered a protective effect against the severity of the induced sodium chloride in the test rats. This study has revealed the protective effect of aqueous extract of *M.oleifera* on nephrotoxicity and liver injury induced by sodium chloride in Wistar albino rats.

Keywords: Aqueous extract; liver injury; Moringa oleifera; nephrotoxicity; sodium chloride.

1. INTRODUCTION

Research studies in recent years have revealed the enormous benefits of plants found within the environs [1-5]. Different authors have evaluated and tried to harvest these benefits using analytical procedures [6-13]. The biologically active constituents of plants are behind these benefits and these constituents and harnessed by man [14-20]. The benefits being derived from plants by man are numerous and cover so many areas of life [1-20]. Aside from serving as food [21-32], plants are used as raw materials in industrial productions, in building, for constructions and decorations amongst others. The ability of plants to act as colouring agents, remediation agents against pollution [33], coagulating agents, water purifier [34,35] and as raw materials to folkloric medicine [22-32,36-37] are recognised.

Moringa oleifera commonly called “horseradish” tree or “drumstick tree”, is one of such plants whose ability to act in different capacities to benefit man has been reported [38]. Ibok Oduro and Deborah [38] noted that *M.oleifera* is one of the underutilised tropical crops. It is an Indian crop but grows in both subtropical and tropical regions of the world. Every part of *M.oleifera* is suitably positioned to benefits man and his environs [36,39-40]. The plant is rich in vitamins, minerals, and other phytochemicals that are very essential to man [38]. Extract from *M.oleifera* plant is used by lactating mothers to augment breast milk. It has been implicated against cancer, inflammation, diabetes, and so many diseases causing pathogens [36,38-39]. The plant *M.oleifera* has often been associated to remedy over three hundred diseases cases [37-38], and has been in use as an indispensable raw material for folkloric medicine in India and African countries for ages [37-38]. Recent studies have also promulgated the coagulating ability of *M.oleifera* seeds in water purification [33-35,40].

Sodium chloride (NaCl) is regarded as the common salt or the Table salt used at home. It is an electrovalent compound of sodium metal and chloride ion and shares electrovalent properties. It is a cognisant found in the body fluid and helps majorly to facilitate nervous impulse through electrode potential permittivity of the nerve surface. Vincent et al. [41], noted that 0.9% of sodium chloride has been used as physiological saline incorrectly. The same authors further noted that such concentration contains higher chloride against the plasma and can cause hyperchloremic metabolic acidosis, and as well, affect renal perfusion. The study done by Wang et al. [42] linked liver fibrosis through excess reactive oxygen species (ROS) production to induced high salt intake. The kidneys are the top of the renal system of the body while the liver is the chief processing unit of the body [3], and both are amongst the visceral organs [3]. Generally, visceral organs deserve maximum protection for optimal functioning of the body system.

Since there is a paucity of data in the study of *M. oleifera* and protection of visceral organs, this study looked into this area and evaluated the protective effect of aqueous extract of *M. oleifera* on nephrotoxicity and liver injury induced by sodium chloride in Wistar albino rats.

2. MATERIALS AND METHODS

2.1 Sample Collection, Identification and Preparation

M. oleifera sample used in the present study was collected in July 2019, from Imo State University School farm in Owerri, Nigeria. The sample was identified in the Department of Plant

Science and Biotechnology, Imo State University and the voucher specimen was deposited at the Herbarium unit of Imo State University, Owerri. The leafy parts of the freshly harvested and identified sample were cut, rinsed, and air-dried for seven days before they were milled and packaged in sterile well labelled polyethene bags for storage and further use.

2.2 Preparation of Aqueous Extracts

The extract was prepared using the method as described by Ikewuchi and Ikewuchi [43]. The milled *M. oleifera* sample was soaked in distilled H_2O for 12 hours, after which the resultant mixture was filtered and the filtrate was stored for subsequent use in the refrigerator at very low temperature. A known volume of this extract was evaporated to dryness and the weight of the residue used to calculate the concentration of the filtrate, which was in turn used to determine the dose of administration of the extract to the test animals. Lorke [44], was used to determine LD_{50} for *M. oleifera* and was found to be well above 2,950 mg/kg.

2.3 Sodium Chloride Procurement and Induction

The sodium chloride used in this study was commercially purchased from a dealer, and was formulated into concentration capable of inducing nephrotoxicity and liver injury using the lethal dose Table [45].

2.4 Experimental Design and Experimental Animals

Forty male Wistar albino rats weighing between 90 to 170 g were procured from the animal colony of Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria. The rats were placed on drinking water, food and room temperature of 25 ºC for the initial acclimatization of four days before they were separated into five groups of 8 rats each and induced with the NaCl. The rats were randomly distributed into groups and the group weights were equalised as nearly as possible. The treatments of the rat groups were designated as follows:

Group I: (Normal control): Water and feed.
Group II: (Experimental control): Rats induced with NaCl, water, and feed.
Group III: Rats induced with NaCl and treated with 150 mg/kg body weight of *M.oleifera.*
Group IV: Rats induced with NaCl and treated with 250 mg/kg body weight of *M.oleifera..*
Group V: Rats induced with NaCl and treated with 350 mg/kg body weight of *M.oleifera..*

Groups III, IV and V were test rats. The extracts were given to the rats orally on daily basis as the experiment lasted for twenty-one days. The feed for the rats was a commercially purchased pelletized feed. The floors of the cages were constantly cleaned on daily basis, their feed consumption and body weights were taken on daily basis.

Experimental handling of animals was in accordance with international guidelines on animal care and uses of National Institute of Health [46].

2.5 Blood Samples Collection for Analysis

The rats in each group were sacrificed at end of treated period after subjecting them to overnight fast. The rats were subsequently anaesthetized with chloroform and blood samples were collected by cardiac puncture into clean tubes for nephrotoxicity and liver injury studies. The tubes were properly labelled and used for analysis.

2.6 Nephrotoxicity and Liver Injury Studies

Nephrotoxicity was evaluated by ascertaining the levels of urea, creatinine and electrolyte ions; while liver injury assessment was done by with the evaluation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total bilirubin of the liver. Urea was analysed using the Bethlot Searcy's method [47]; creatinine was determined by the method described by Larsen [48]. Sodium ion (Na^+) and chloride (Cl^-) ion levels were determined according to the instructions on their diagnostic kits purchased from Randox laboratories (UK). Potassium ion (K^+) was determined by direct spectrophotometric method. Bicarbonate (HCO_3^-) was determined using Forrester et al. [49] method. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using Reitman and Frankel [50] methods; alkaline phosphatase (ALP) was carried out using the phenolphthalein monophosphate method [51]; total bilirubin was measured using Doumas et al. [52] method.

2.7 Statistical Analysis

Results are presented as mean and standard deviations of triplicate determinations. Group comparisons were done using least significant difference (LSD). Significant difference was established at 5% level as described by Onu and Igwemma [53].

3. RESULTS AND DISCUSSION

Weight changes of rats given an aqueous extract of *M.oleifera* as presented in Fig. 1, shows Initial weight of rats ranged from 88.30 g in group V to 89.67 g in normal control (group I). Final weight ranged from 114.80 g in experiment control rats (group II) to 148.10 g in normal control (group I) rats. Weight change in rats after 21 days ranged from 25.90 g in experimental control rats to 58.43 g in normal control rats. The percentage of weight gained ranged from 10.91% in experimental control rats to 24.61% in. Percentage weighed gained increased significantly ($p<0.05$) test groups (III, IV and V) against the experimental control but percentage weight gained in test groups IV and V increased insignificantly ($p>0.05$) when compared to that of normal control (group I).

Results are mean and standard deviation of triplicate determinations. Values with different letters of alphabets along the same row are statistically significant at 5% significant levels.

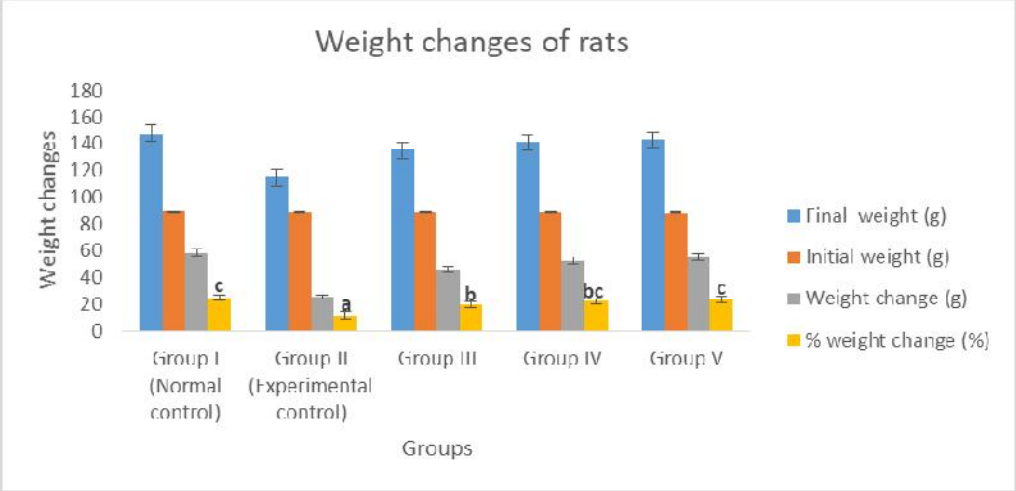

Fig. 1. Weight changes of rats given aqueous extract of *M. oleifera*

Table 1. Nephrotoxicity indices of rats given aqueous extract of *M. oleifera*

Parameters	Groups				
	I	II	III	IV	V
Urea (mg/dl)	30.52 ± 1.90^{a}	58.44 ± 1.07^{d}	46.01 ± 1.51^{c}	41.87 ± 1.00^{b}	31.34 ± 1.00^{a}
Creatinine (mg/dl)	1.10 ± 0.08^{a}	3.04 ± -0.36^{b}	1.99 ± 0.10^{a}	1.57 ± 0.11^{a}	1.15 ± 0.14^{a}
Na^{+} (mEq/L)	129.02 ± 1.37^{a}	146.38 ± 2.41^{d}	138.38 ± 1.26^{c}	135.01 ± 2.98^{b}	131.00 ± 1.04^{b}
K^{+} (mEq/L)	3.10 ± 0.30^{a}	3.54 ± 0.50^{b}	2.98 ± 0.13^{a}	3.27 ± 0.28^{ab}	3.14 ± 0.50^{a}
Cl^{-}(mEq/L)	93.04 ± 1.56^{a}	102.70 ± 1.04^{e}	99.15 ± 1.60^{d}	95.93 ± 1.07^{c}	94.22 ± 2.87^{b}
HCO_3^{-}(mmol/L)	23.50 ± 0.99^{a}	34.08 ± 1.52^{b}	31.97 ± 2.11^{b}	24.02 ± 1.03^{a}	22.56 ± 1.21^{a}

Table 2. Liver enzyme of rats given aqueous extract of *M. oleifera*

Enzyme	Groups				
	I	II	III	IV	V
AST (U/L)	35.21 ± 2.09^{a}	47.84 ± 1.05^{d}	43.90 ± 1.13^{c}	43.08 ± 1.74^{c}	37.75 ± 1.02^{b}
ALT (U/L)	43.98 ± 1.18^{a}	59.34 ± 1.25^{d}	55.13 ± 1.80^{c}	50.46 ± 1.93^{b}	44.02 ± 1.94^{a}
ALP (U/L)	56.13 ± 2.44^{a}	69.04 ± 1.11^{e}	64.20 ± 1.90^{d}	61.85 ± 1.80^{c}	57.74 ± 1.10^{b}
Bilirubin (mg/dl)	0.86 ± 0.04^{a}	0.98 ± 0.08^{b}	0.92 ± 0.01^{ab}	0.89 ± 0.01^{a}	0.88 ± 0.01^{a}

High salt intake has long linked to increased blood pressure and excessive water retention in the body, which could result in diuresis. The renal system with the kidney as the chief organ, regulates the balance of salt in the body. Nephrotoxicity indices of rats given an aqueous extract of *M.oleifera* as presented in Table 1 revealed that urea ranged from 30.52 to 58.44 mg/dl. Creatinine ranged from 1.10 to 3.04 mg/dl. Na^+ was between 129.02 to 146.38 mEq/L. K^+ ranged from 2.98 to 3.54 mEq/L. Cl^- ranged from 93.04 to 102.70 mEq/L while HCO_3^- ranged from 22.56 to 34.08 mmol/L. Levels of urea in test groups (III, IV and V) reduced significantly ($p<0.05$) when compared to the experimental control (group II). Urea levels in test groups III and IV increased significantly ($p<0.05$) against normal control (group I) while test group V had urea levels that insignificantly ($p>0.05$) increased when compared to normal control (group I). Creatinine levels in test groups III, IV and V reduced significantly ($p<0.05$) against the experimental control (group II). Urea in test groups insignificant ($p>0.05$) increase when compared to normal control (group I). Na^+ and Cl^- levels of test groups (III, IV and V) reduced significantly ($p<0.05$) when compared to experimental control but increased significantly ($p<0.05$) against normal control. K^+ levels of test groups III and V but not test group IV significantly ($p<0.05$) reduced against experimental control. K^+ levels of all the test groups increased insignificantly ($p>0.05$) against the normal control. The induced sodium chloride may have altered the filtration integrity of the renal system as observed in experimental control rats (group II), while the *M.oleifera* extract offered as a treatment to the rats may have ameliorated the severity as observed in test group rats.

The observed levels of the enzymes as shown in above Table 2 may have indicated a compromise of the liver integrity by the induced sodium chloride in rats. Levels of AST and ALP reduced significantly ($p<0.05$) in test rats (groups III, IV, V), when compared to the experimental control (group II), but increased significantly ($p<0.05$) in test groups against the normal control (group I). ALT levels reduced significantly ($p<0.05$) in test rats (groups III, IV and V) when compared to experimental control but increased insignificantly ($p>0.05$) in test group V against normal control. Different authors [2,4,6,9,11,16-17,20] have implicated increased levels of AST, ALT and ALP enzymes to compromise of hepatocellular integrity by hepatocellular injury or diseases.

Bilirubin levels increased significantly ($p<0.05$) in test groups IV and V against experimental control. However, test groups (III, IV and V) increased insignificantly ($p>0.05$) when compared to normal control. The protective effect of *M. oleifera* extract used in the present study was observed against the liver enzyme increase, which could be as a result of the compromise of hepatocellular integrity by the induced sodium chloride in rats. The protective effect of *M.oleifera* against liver injury has been reported by Islam and Alam [54] on paracetamol induced hepatotoxicity in rats. Das et al. [55] also reported that *M.oleifera* Lam. leaf extract prevents early liver injury and restores antioxidant status in mice fed with high-fat diet.

4. CONCLUSION

Reduced severity in nephrotoxicity and liver injury of rats induced with sodium chloride and orally treated with *M. oleifera* extract were observed in this study. The extract may have improved the affected cells of the renal system and improved the integrity of the liver cells. This study has evaluated the protective effect of aqueous extract of *M. oleifera* on nephrotoxicity and liver injury induced by sodium chloride in Wistar albino rats.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was approved by Ethical Committee of the Department of Medical Laboratory Science, Imo State University, Owerri, Nigeria**.**

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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