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Effects of *Moringa oleifera* seed Extracts on Nutmeg Induced Renal Dysfunction in Wistar Rats

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ABSTRACT

Nutmeg is a common household spices because of its rich nutrient composition, that has been found to have very serious toxic effects on the kidney function at high doses. This study investigated the ameliorative potency of *Moringa oleifera* seed extracts on nutmeg induced renal dysfunction. Twenty-four (24) adult Wistar rats weighing 80-150g were randomly separated into six groups of four rats each. Group A was only fed with food and water. Group B received oral doses of 500mg/kg/day of nutmeg extract for 21 days. Group C received oral doses of 500mg/kg of nutmeg extract and 200mg/kg of *Moringa oleifera* ethanolic seed extract simultaneously for 21 days. Group D received oral doses of 500mg/kg of nutmeg extract and 400mg/kg of ethanolic moringa seed extract simultaneously for 21 days. Group E received oral doses of 500mg/kg of nutmeg extract and 200mg/kg of *Moringa oleifera* n-hexane seed extract simultaneously for 21 days. Group F received oral doses of 500mg/kg of nutmeg extract and 400mg/kg of n-hexane moringa seed extract simultaneously for 21 days. Blood sample was collected by ocular puncture and kidney function was assessed through serum electrolytes, urea and creatinine levels. Data was analyzed using the statistical program SPSS version 27.1. The positive control group B showed significant decrease in the electrolyte levels when compared to the negative control group A. Also, there was a significant increase in the mean serum urea level in group B when compared with group A which was founded to be ameliorated in the treatment groups. There was no significant difference in the creatinine level across the groups. However, when the urea levels across the groups were compared with the positive control group (B), there were significant decrease in the mean urea levels ($P<0.05$) in treatment groups D, E and F. The findings from this study demonstrated that *Moringa* had an ameliorative effect on the serum urea imbalance but showed no ameliorative potency on the electrolyte imbalance.

Keywords: Nutmeg Toxicity, Renal Dysfunction, *Moringa oleifera*, Kidney Function, Ameliorative Effects, Serum Urea And Electrolyte Imbalance

INTRODUCTION

Nutmeg (*Myristica fragrans* Houtt) is a popular household spice that has rich nutrient composition [1]; [2]; [3], at high doses, it has been found to have serious toxic effects on the body organs leading to organ kidney dysfunction and cellular apoptosis amongst others [4]; [5]; [6]. While, *Moringa Oleifera*, a perennial tree enriched with numerous nutritional and vital phytochemicals such as glucosinolates, vitamins, flavonoids, carotenoids, phenolic acids, alkaloids, isothiocyanates, polyphenols, tannins, and saponins [7]; [8], has been a popular potent herbal medicine used for the treatment of many health diseases including renal injuries [9]; [10]. The kidney is bilateral bean-shaped organs located in the posterior abdominal wall that plays an important role in chemical metabolism

and elimination making it highly vulnerable to toxicities such as that of myristicin from nutmeg. Recently, there is growing dissatisfaction of people on orthodox medicine which could be as a result of the high cost or their adverse effects. This has increased the shift of people interest to the use and abuse of some medicinal plants such as nutmeg for either its aphrodisiac, nutritional or medicinal effects with the naive believe that every herbal plant or medicine is healthy at any dosage [11]. Therefore, this works tends to find an alternative protective remedy for nutmeg induced kidney toxicities that can be readily available as well as affordable.

MATERIALS AND METHODS

Ethical Approval

Ethical approval was obtained from the research ethics committee of the Faculty of Basic Medical Sciences, College of Medicine, Enugu State University of Science and Technology, Enugu, Nigeria.

Preparation of Plant Extract

The moringa pods was broken and the seeds removed and properly grounded followed by ethanolic extraction using maceration methods [12] and n-hexane extraction using Soxhlet method [13]. The nutmeg seeds were also properly grounded followed by alcoholic extraction using digestion method [13].

Animals and Managements

Twenty-four (24) Wistar rats weighing between 80g - 150g was purchased and housed in the animal house of the College of Medicine, Enugu State University of Science and Technology. The animals were acclimatized for two weeks in a well-ventilated cage and properly fed and allowed water *ad libitum*. The animals were handled according to the guidelines for animal research in the National Institute of Health (NIH) guidelines for the care and use of laboratory animals. After acclimatization, the animals were randomly grouped into six (6) groups; each group consisting of four (4) rats. Group A was only fed with food and water. Group B received oral doses of 500mg/kg/day of nutmeg extract for 21 days. Group C received oral doses of 500mg/kg of nutmeg extract and 200mg/kg of *Moringa oleifera* ethanolic seed extract simultaneously for 21 days. Group D received oral doses of 500mg/kg of nutmeg extract and 400mg/kg of ethanolic moringa seed extract simultaneously for 21 days. Group E received oral doses of 500mg/kg of nutmeg extract and 200mg/kg of *Moringa oleifera* n-hexane seed extract simultaneously for 21 days. Group F received oral doses of 500mg/kg of nutmeg extract and 400mg/kg of n-hexane moringa seed extract simultaneously for 21 days.

Collection of Blood Sample

Experimental animals were anaesthetized using chloroform. 4ml blood sample was collected from the retro-orbital venous plexus for all the rats using capillary tubes and collected into the plain bottle and allowed to clot. Kidney function was assessed through serum urea, creatinine and electrolytes (Na⁺, K, Ca⁺, Cl⁻, Mg⁺, PO₄³⁻ and HCO₃) levels.

Determination of Urea and Creatinine Levels

Measurement of serum creatinine was carried out using Jaffe reaction method [14]; [15]; [16], While serum urea was carried out using Urease method [17]; [18].

Statistical Analysis

Data analysis was carried out using the IBM SPSS package (IBM Corp., IBM SPSS Statistics for Windows, Version 27.1) and evaluated using ANOVA followed by Post hoc test.

RESULTS

Table 4.1 Result of the Kidney Function Test

GRO UP	Na (mmol/L)	Ca (mg/dl)	K (mmol/L)	mg (ng/dL)	Phosphate (mg/dl)	CL- (mEq/L)	HCO ₃ (mEq/L)	Creatinine (mg/dl)	Urea (mg/dl)
1A	135.50±2.12 ^a	6.05±0.07 ^a	6.53±0.24 ^a	56.14±5.76 ^a	5.01±0.01 ^a	95.50±2.12 ^a	41.20±1.27 ^a	1.03±0.13	1.84±0.82 ^a
2B	114.00±2.83 [*]	2.60±0.28 [*]	2.50±0.28 [*]	12.22±1.68 [*]	0.85±0.13 [*]	60.50±6.36 [*]	22.50±2.12 [*]	4.12±2.67	4.95±1.23 [*]
3C	112.00±2.83 [*]	2.40±0.99 [*]	2.23±0.23 [*]	20.50±3.54 [*]	2.00±0.28 ^{*a}	63.00±5.66 [*]	11.50±2.12 ^{*a}	2.52±0.21	3.00±0.22
4D	115.50±2.12 [*]	4.60±0.42 ^a	4.10±0.71 ^{*a}	30.61±9.18 [*]	4.50±0.42 ^a	95.50±3.54 ^a	24.00±4.24 [*]	1.74±0.27	2.36±0.36 ^a
5E	116.30±2.55 [*]	2.70±0.28 [*]	2.50±0.28 [*]	30.00±4.24 [*]	2.90±0.14 ^{*a}	64.50±4.24 [*]	14.00±1.41 [*]	2.44±0.44	1.33±0.16 ^a
6F	115.50±6.36 [*]	2.85±0.21 [*]	2.95±0.35 [*]	30.90±0.25 [*]	2.35±0.21 ^{*a}	82.50±10.61	19.50±2.12 [*]	1.14±0.13	1.48±0.40 ^a

Table 1 shows the result of the kidney function test. Values were expressed as mean ± standard deviation. Where **P*<0.05 showed a significant difference when compared to group 1, *αP*<0.05 showed a significance difference when compared to group 2.

DISCUSSION

The kidney function test result from this study shows that the positive control group B, which received 500 mg/kg of nutmeg, had a significant decrease in serum electrolyte concentration compared to the negative control group A. This finding aligns with previous

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studies that have documented nutmeg induced nephrotoxicity to cause electrolyte imbalance [19]; [20]; [21]. [22] and [23], reported the opposite but this is probably due to the lower nutmeg dosage used in their study. The electrolyte levels of the treatment groups (C, D, E, and F), was also decreased significantly when compared to the negative control group A, but had no significant difference when compared with each other and with the positive control group B. Though some study findings have documented moringa to stabilize electrolyte levels in nephrotoxicity [24]; [25], [26]; [27]; [28], the result of this study aligns with studies where *Moringa oleifera* extracts did not ameliorate serum electrolyte imbalance [29]; [30]; [31]. There was a significant increase in the mean serum urea levels in the positive control group B when compared to the negative control group A. This finding is consistent with the documentation of urea as a key marker of renal function, where elevated levels indicate impaired glomerular filtration [32]. Also, the urea levels in treatment groups D, E, and F showed significant decreases when compared to the positive control. This is in tandem with the documentations that Moringa extracts can improve renal function [9]; [11]; [24]; [26]; [27] [32]. There was no significant difference in serum creatinine levels across all the groups suggesting that while nephrotoxic effects were present, they did not progress to the point of significantly affecting creatinine levels which may be due to the short duration of this study. Similar results have been recorded by other authors, where renal toxicity markers like urea were elevated, but creatinine levels remained stable [33];[34].

CONCLUSION

In conclusion, this study demonstrates that despite the rich nutrient profile of nutmeg that it has very strong nephrotoxic effects when abused. Also, this study demonstrated that Moringa had an ameliorative effect on the serum urea imbalance but showed no ameliorative potency on the electrolyte imbalance. More studies with higher doses of moringa are encouraged for future studies.

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