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Protective Effects of *Moringa oleifera* Ethanolic Seed Extract on Nutmeg induced Kidney Tumorigenesis in Wistar Rats

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ABSTRACT

Recent studies suggest that chronic nutmeg exposure to be toxic to the kidney inducing aberrant cellular proliferation due to the Myristicin component which could promote tumorigenesis [1]. This necessitates the search for protective agents that can mitigate nutmeg-induced renal tumorigenesis. Sixteen (16) Wistar rats weighing 150g, was randomly selected into four groups. Group A received no treatment while Group B was administered 500 mg/kg of nutmeg seed extract. Group C received 500 mg/kg of nutmeg seed extract and 200mg/kg of moringa ethanolic seed extract. Group D was administered 500 mg/kg of nutmeg seed extract and 400mg/kg of moringa ethanolic seed extract. From the immunohistochemical analysis, the photomicrographs of groups B and C showed positive immunoreactivity to both CD117 and vimentin, while groups A and D had negative immunoreactivity to both CD117 and vimentin. The results from this study confirmed Moringa to be a potent natural protective agent for nutmeg induced kidney tumorigenesis which however, should be explored more for its possible application in clinical practices.
Keywords: *Moringa oleifera*, Ethanolic Seed Extract, Kidney Tumorigenesis and Wistar Rats

INTRODUCTION

Nephrotoxic substances, including certain dietary components such as nutmeg, has been recorded as possible agents for tumorigenesis in the kidney [2]. Nutmeg has toxicological properties and chronic exposure has been linked to nephrotoxic and epithelial-mesenchymal transition (EMT), which is pivotal in renal fibrosis, tumor progression and carcinogenesis [3]. One of the major components of nutmeg, myristicin, has been documented to induce mitochondrial dysfunction, potential DNA damage, leading to apoptotic and necrotic cell death [4], and has been suggested to promote tumorigenesis [2]. Additionally, the carcinogenic potential of nutmeg is further supported by studies demonstrating upregulation of nephrotoxic markers such as vimentin and CD117 (c-Kit), which are associated with renal oncogenesis [5]. This necessitates the search for protective agents that can mitigate nutmeg-induced renal toxicity. The broad pharmacological properties of *Moringa oleifera* has been proven to counteract induced renal damages by neutralizing oxidative stress, modulating inflammatory pathways, and preventing tumor formation [6]. This study is targeted towards investigating this protective role of *Moringa oleifera* against nutmeg-induced kidney tumorigenesis.

MATERIALS AND METHODS

Ethical Approval

This study 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

Maceration method [7], was used for ethanolic extraction of moringa seed and Soxhlet method [8] for n-hexane extraction of moringa seed nutmeg ethanolic extraction was carried out using digestion method [8].

Animals and Managements

Sixteen Wistar rats weighing between 150g-200g, were acclimatized for two weeks and properly fed. Radom selection was used to select them into four groups. The experimental animals were housed in the animal house of the College of Medicine, Enugu State University of Science and Technology. The study was carried out following standard experimental procedures. Group A received no treatment while Group B was administered 500 mg/kg of nutmeg seed extract. Group C received 500 mg/kg of nutmeg seed extract and 200mg/kg of moringa ethanolic seed extract. Group D was administered 500 mg/kg of nutmeg seed extract and 400mg/kg of moringa ethanolic seed extract.

Collection of Blood Sample

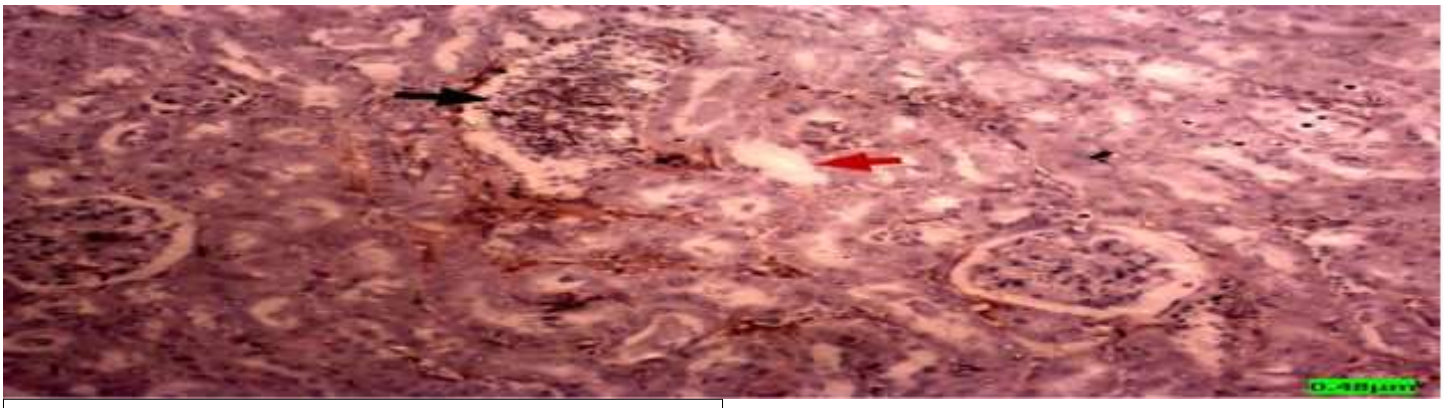
Experimental animals were anaesthetized using chloroform. Sacrificing was by cervical dislocation. The kidneys were carefully dissected out immediately and fixed in 10% neutral buffered formalin (NBF) and further prepared following normal histological procedures.

RESULTS

CD117 RESULTS

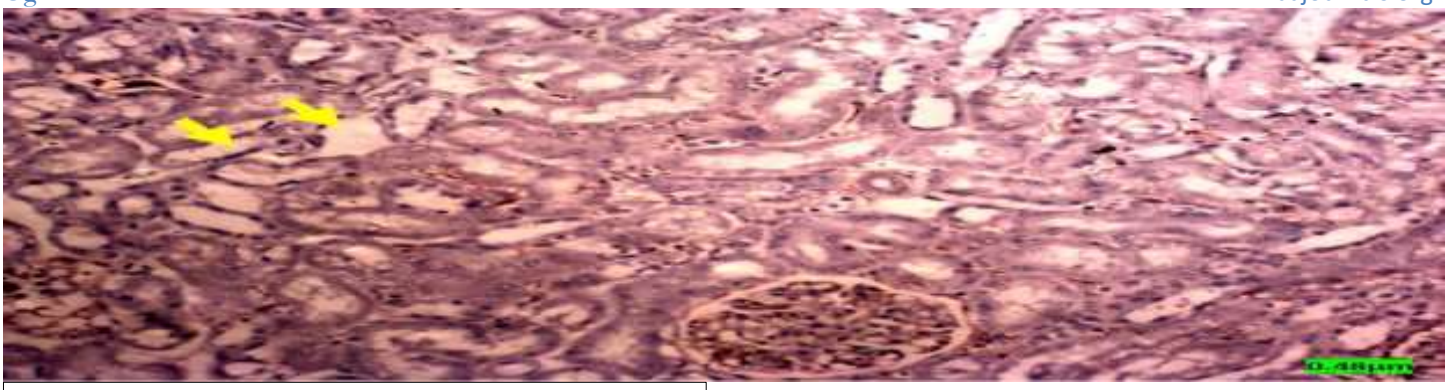
A: Photomicrograph of the kidney cortex area showing tubular negative immunoreactivity to chromophobe cell carcinoma.

CD 117. X300



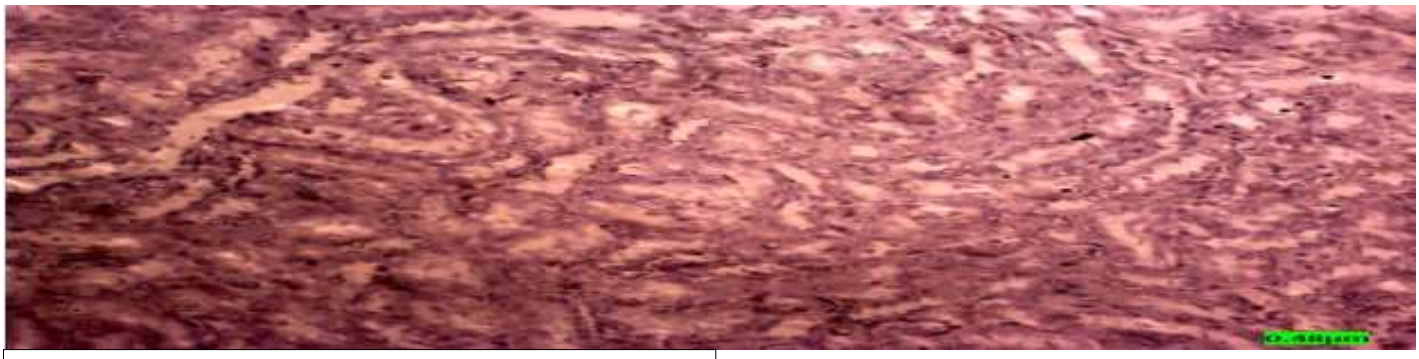
B: Photomicrograph of the renal cortex showing focal area of prominent vimentin expression with loss of brush borders (arrow)

CD117. X300



C: Photomicrograph of the renal cortex showing prominent immunoreactivity to renal cell carcinoma: eosinophilic cytoplasm (arrow)

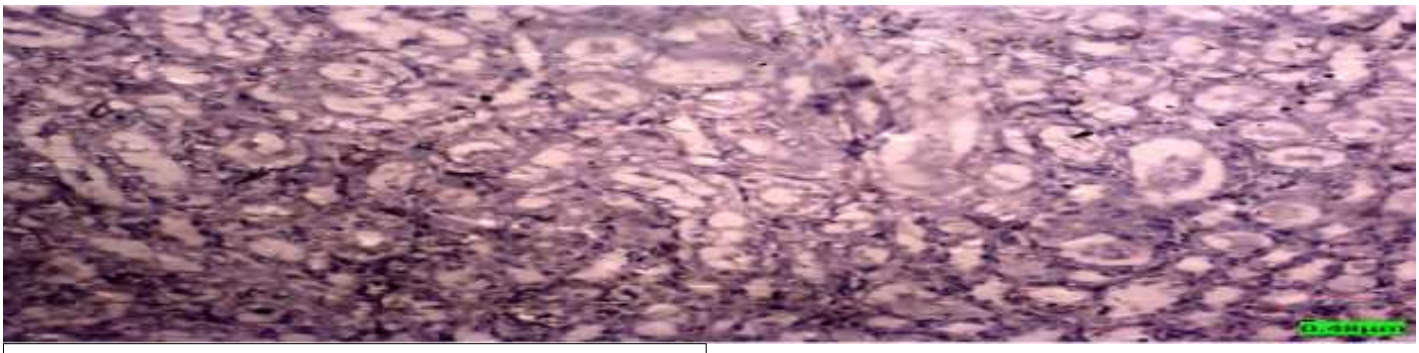
CD117. X300



D: Photomicrograph of the renal cortex showing negative immunoreactivity to renal cell carcinoma.

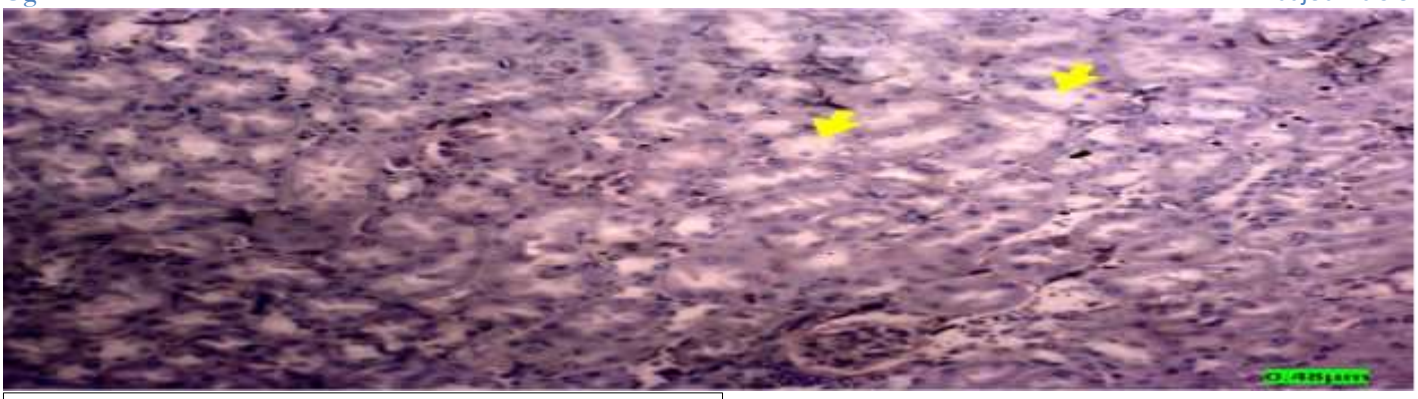
CD117. X300

VIMENTIN RESULTS



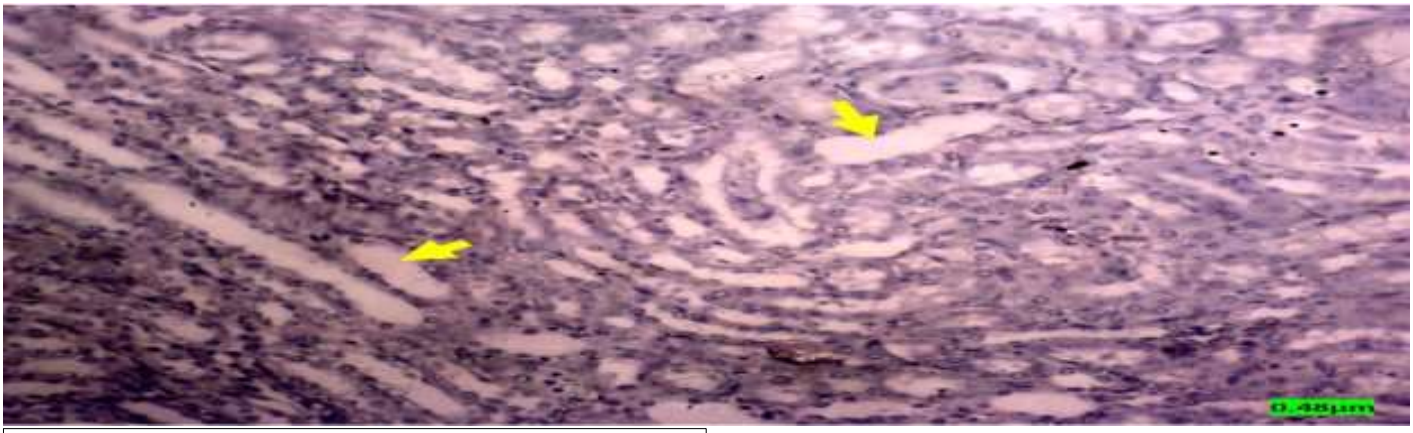
A: Photomicrograph of the renal cortex showing negative immunoreactivity to renal cell carcinoma.

Vimentin. X300



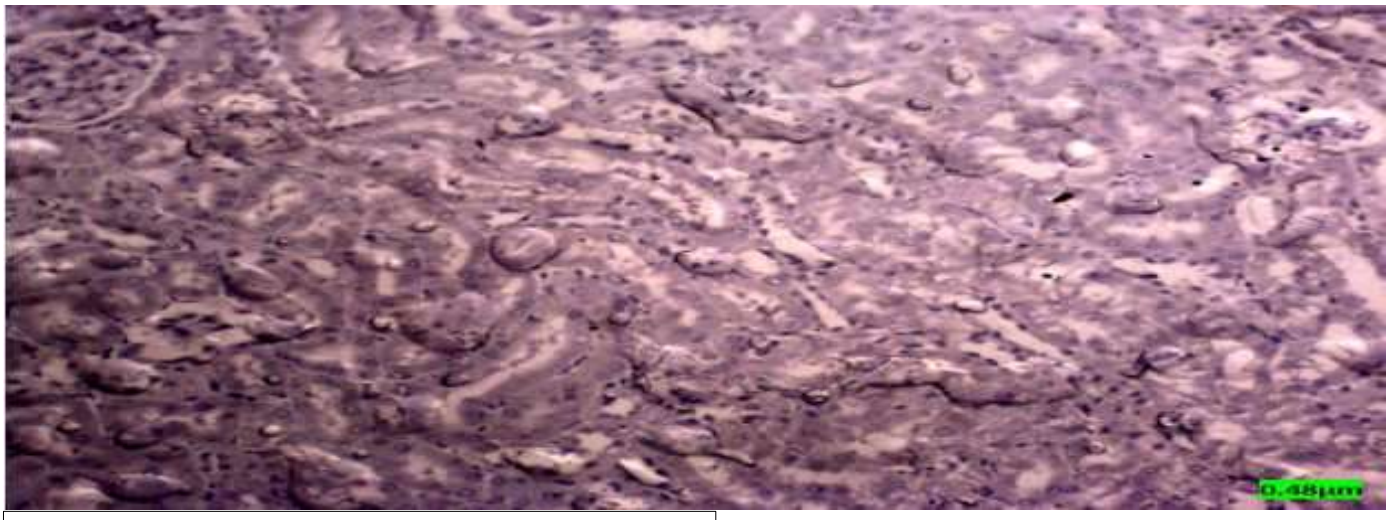
B: Photomicrograph of the renal cortex showing focal area of prominent vimentin expression with loss of brush borders (arrow)

Vimentin. X300



C: Photomicrograph of the renal cortex showing prominent immunoreactivity to renal cell carcinoma: eosinophilic cytoplasm (arrow)

Vimentin. X300



D: Photomicrograph of the renal cortex showing negative immunoreactivity to renal cell carcinoma.

Vimentin. X300

DISCUSSION

Immunohistochemical analysis results showed negative immunoreactivity for CD117 and Vimentin in the control group A and group treated with 400mg/kg moringa (group D). This indicates a normal renal architecture without evidence of any toxicity or pathological process, confirming the role of Vimentin and CD117 as markers for certain types of renal toxicity rather than normal renal cells. This is consistent with the result of [9] and [11]. It also points out the protective potency of Moringa on nephrotoxicity [12], [13], [14]. This further strengthens the attributes of moringa with potency of protecting the kidney from nephrotoxicity, attributed to the nutrient-rich profile of Moringa [15], [16], [17], [13]. The positive control group B showed positive immunoreactivity for Vimentin and CD117 confirming response to renal injury induced by nutmeg which is in alignment with the findings from other studies [18], [19], [18], [21],[22], [23], [24]. In treatment group C, positive immunoreactivity for CD117 and Vimentin implies that the protective effects of *Moringa oleifera* is dose dependent and at low dose, it could not completely mitigate the toxicity effect of the nutmeg.

CONCLUSION

Preservation of renal histoarchitecture in moringa treated groups have confirmed moringa to possess nephroprotective properties on nutmeg induced renal toxicity. More studies on the clinical application are advised.

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