

## The Role of *Moringaoleifera* Seed Extract in Amelioration of Kidney Injury Induced by Sodium Nitrite in Male Rats

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### Abstract

The present study aimed to reveal the biological vitality of *Moringaoleifera* seeds aqueous extract in the protection of the kidney tissues from the oxidative stress induced by Sodium Nitrite (NaNO<sub>2</sub>). 36 Male rats have been divided at random to 6 groups every one of which includes 6 rats dosed for 30 days, The first group a control, group 2 treated by NaNO<sub>2</sub> in a concentration of 40mg/kg orally by gavage, Group 3 normal rat dosed aqueous extracts of *MoringaOleifera* seeds 350 mg/kg, The Group 4 normal rats dosed aqueous extracts of *MoringaOleifera* seeds 450mg/kg, while Group 5,6 normal rats received aqueous extracts of *MoringaOleifera* seeds concentration 350 and 450mg/kg and before 4 hour of taking (Sodium Nitrite) NaNO<sub>2</sub> with 40mg/kg of body weight. The results of this study pointed to that a Histologically the activity of extracts of *MoringaOleifera* seeds has been as well capable of protecting the kidney from the damage that has been induced by sodium nitrite, represented by shrinkage of glomerular, and degeneration of epithelial lining cell of renal tubular, congestion, and expanded Bowman Space, hemorrhage, The aqueous extract *MoringaOleifera* seeds at doses of 350mg/kg and 450mg/kg orally considerably protected the Sodium nitrite induced kidney toxicity in the rats by the increase in the Catalase and Glutathione enzymes and reduces the MDA. In conclusions, *Moringaoleifera* seeds extract results in the enhancement of the defense status of the oxidative stress against the renal toxicity.

### Introduction

*Moringaoleifera* is a part of the Moringaceae family species. Its leaves are rich of macro and micro-nutrients including, vitamins, phenolic acids, carotenoids, flavonoids, and alkaloids, polyphenols, minerals (Liang *et al.*, 2020) Therefore, *Moringaoleifera* plant is used in nurturing of human as an excellent nutritive supplement (Sanjay & Dwivedi, 2015), the moringa tree nutritional and medical importance have been used for ages in treating many different diseases in conventional medicine, such as anti-hypertension, heat, diabetes, fats, cardiac stimulant, circulatory, immunology, antioxidant, tumors, infections, ulcers, depression, bacteria, fungi, cramping, aging, diuretic, and liver diseases (Jahan, *et al.*, 2018). The seeds of *M. Oleifera* are nutritionally important as they are freshly eaten largely green or as ground seed in the northern part of Nigeria (Zade *et al.*, 2013). The percentage of protein in the seeds is high, as the dried

seeds contain 18-25% of protein, which is almost twice the amount found in the grains .., and effective plant components were also observed in the seed extract of *MoringaOlivera*, such as alkaloids, flavonoids, steroids, and phenols. Phenolics, tannins, saponines(Ogbunugaf,*et al.*,2011 :Ma *et al.*2020). Examination of these components helps to reassess the chemical components of the plant and which one prevails over the other. It also helps to search for biologically active agents such as its launch of a product that is partially used in some useful medicines (Harbone, 1998) and thus it is considered the most important seed Legumes for human nutrition, as there are many saturated fatty acids, including arachidic acid, stearic acid, palmitic acid and benic acid, in addition to that it contains the most important unsaturated fatty acids Oliec acid, as it reaches a high percentage (67.9 - 70.0%) (2011) . The *moringa* plant contains many active substances, the most important of which are flavonoids, that play a role In curbing oxidative stress that results from the generation of free radicals as well as protecting the body from cancer and heart diseases (Gopalakrishnane*et al.*, 2016) ). And flavonoids also improve the human protective enzyme system and protect it from diseases associated with aging.) (It provides protection for the human body from oxidative stress resulting from the use of preservatives(Anwar *et al.*,2007; Dubey *et al.*, 2013) . Preservatives are very important in the food, cosmetics and pharmaceutical industries to extend their shelf life, inhibit the growth of microorganisms, to preserve taste and texture and to improve the nutritional value (Smith, 2011 ; Caroch, *et al.* , 2014 ) . Sodium Nitrite is an inorganic salt compound with the chemical formula  $\text{NaNO}_2$ , its crystals have a white to yellowish white color, used as a food preservative (Abdel-Reheim*et al.*, 2014) .Its international code is E250 (Sindelar&Milkowski, 2012; Aldaamy&AlZubiady, 2020). Nitrite salts are added to meat, poultry and fish in minute quantities as a method of preservation and this practice has been common for several years (Sherif&Al-Gayyar,2013;Hussan*et al.*, 2020 ).Adding sodium nitrite as a food additive may interact with food amines in the stomach and produce nitrosamine or large numbers of free radicals. These free radicals cause oxidative stress, which can be harmful to various organs including the kidneys(Aboulgasem*et al.*, 2015 ; Abdulshahed,2020).This study, therefore, is designed for evaluating the protective effects of the aqueous extract of*the M.Oleifera* seeds against Sodium Nitrite–induced kidney damage .

## Materials and Methods

### *M. oleifera* seeds collection and extraction

Dry seeds of *MoringaOleifera* were obtained from the local market of kerbala , Iraq . The seeds were cleaned and dried for 3 days at room temperature, the dried seed were milled to fine powder using a mechanical grinder . A 20 g. of dry powder was taken and blending with 400 ml of distilled water for 24 hound at room temperature, and filtered . the extract was dried in the oven after placing it in sterile glass dishes at 30oC for 24 (Hernandez *et al.*, 1994). . The concentrated extract was stored in the refrigerator until use for this study .

## Experimental animals

Thirty six male rats from the ages of approximately 2-3 months old were used in this experiment. weight between 280 – 400 g , have been obtained from the animal house . college of pharmacy , Univ. of kerbala, The animals were placed in special plastic cages with mesh wire covers, under standard condition with 12hrs. Light and 12hrs. dark cycle throughout the entire experimental period. and were given food and water ad libitum. The 36 rats have been randomly divided to 6 groups (6/group ).Group1: Considered as a control group, Group2: rats administration of sodium Nitrite orally at a dose of 40mg/kg per day , Group 3: has been dosed orally of *Moringaoleifera* seeds extract at a concentration of 350mg/kg , Group 4: was oral administered *MoringaOleifera* seeds extract (450 mg /kg b. wt), Group 5: was oral administered *MoringaOleifera* seeds extract at a 350mg/kg dose before 4 hours of receive Sodium Nitrite orally at a 40mg/kg per daydose, Group 6 : was oral administered *MoringaOleiferaseeds* extract at a dose 350 mg/kg before 4 hours of receive Sodium Nitrite orally at a 40mg/kg dose per day, All treatment were carried out for 30 days.

## Blood Samples

blood samples were collected from heart puncture, blood was kept into eppendorf tubes without EDTA, the serum was separated by centrifugation at 3000 rpm for 14 minutes. and frozen at 20C0 for later biochemical analyses. The Serum used for measurement of ( Reduced Glutathione (GSH), Malondialdehyde (MDA) , Catalase ) concentrations. Histopathological studies The tissue samples collected from the kidney of all the rats , from all groups were anaesthetized with chloroform , Immediately after death the kidney was excised and fixed in 10 % formalin for 48 hours. till the preparation of histological sections according to(Suvarnaet *al.*,2013; ; Obeid *et al.*, 2020 ).

## Statistical analysis:

Data has been estimated by one-way analysis of variance (ANOVA) and have been analyzed with the SPSS v.22 software and presented in forms of mean  $\pm$  standard deviation, Statistical significance, was set at ( $p < 0.05$ ). the standard studied for different groups using the(LSD)

## Results

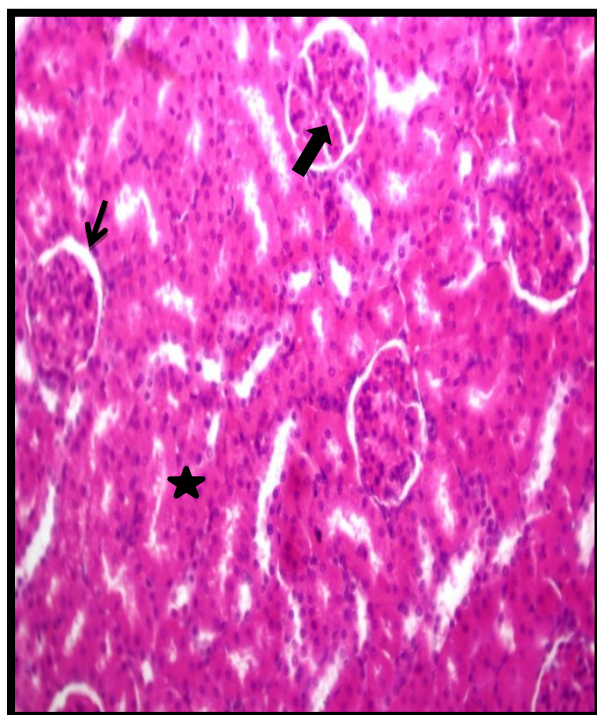
### Histopathological study

It was observed that, in this study, Fig (1), the kidney of control rats, showing section for normal kidney structure, including normal appearance of Bowman Capsule and glomerulus , Renal tubules , Fig (2) also sodium nitrite rat group showed histopathological changes including shrinkage of glomerular with expanded Bowman space ,degeneration renal tubular and congestion . Fig (3), (4) groups , Administration of *Moringaoleifera* seed extract showeing

normal appearance glomerulus ,normal Renal tubules and intact Bowman capsule. Fig (5) section of rat kidney received 350 mg /kg of *MoringaOleifera* seed extract with sodium nitrite 40 mg/kg showing nearly normal renal corpuscles ,expanded Bowman Space and some degeneration of epithelial lining cell of renal tubule and hemorrhage . In the other hand using *MoringaOleitera* seed extract and sodium nitrite 40 mg/kg, showed normal glomerulus and renal tubules no inflammation no significant congestion.

### Biochemical Results

Table (1) has shown a significant decrease ( $P \leq 0.05$ ) in level of Catalase(CAT) enzyme activities and Glutathione (GSH) in groups G2 treated with Sodium nitrite for 30 day and significant increase ( $P \leq 0.05$ ) in MDA level compared to the control group G1. On the other hand, rats received *Moringaoleifera* seed extract in groups G3 , G4 with a concentration of ( 350 , 450) mg /kg has shown a significant increase ( $P \leq 0.05$ ) in GSH and CAT enzyme level compared with those of control group . A significant increase in the CAT enzyme , GSH concentration in the groups( G5 ,G6 ) compared with those of rats received Nitrite sodium(G2).



Figure(1): Kidney section of rat (control) Showing normal appearance of BowmanCapsule (thin arrow), glomerulus (thick arrow), Renal tubules (star).(H&E 200X)

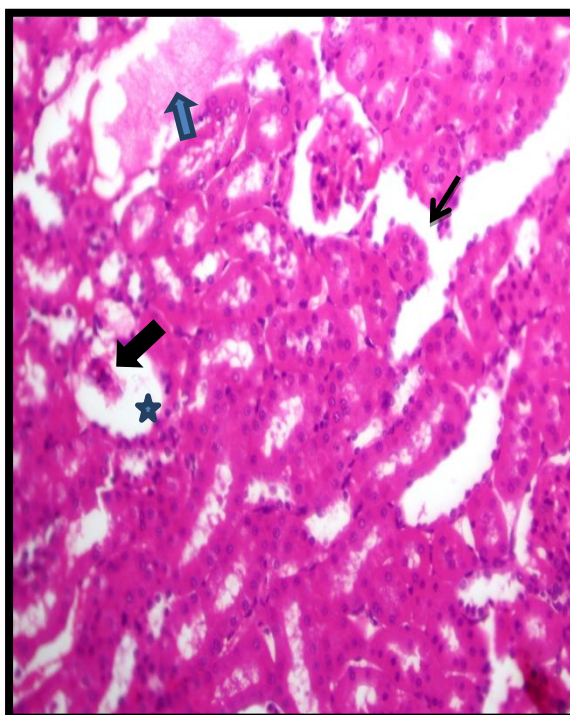
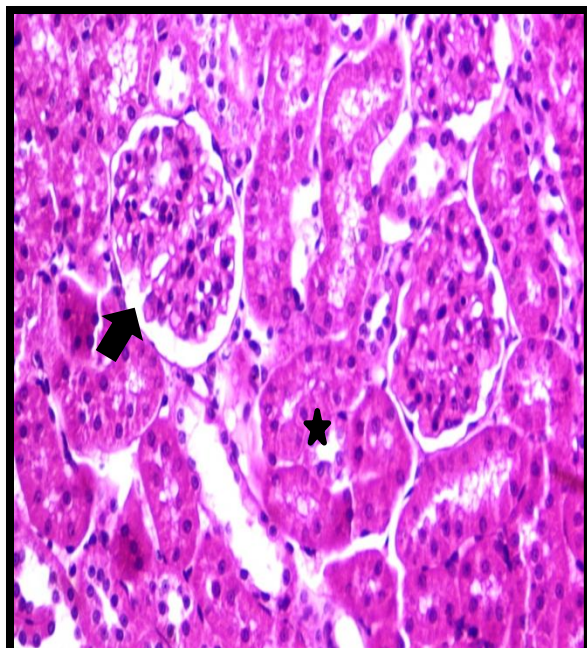
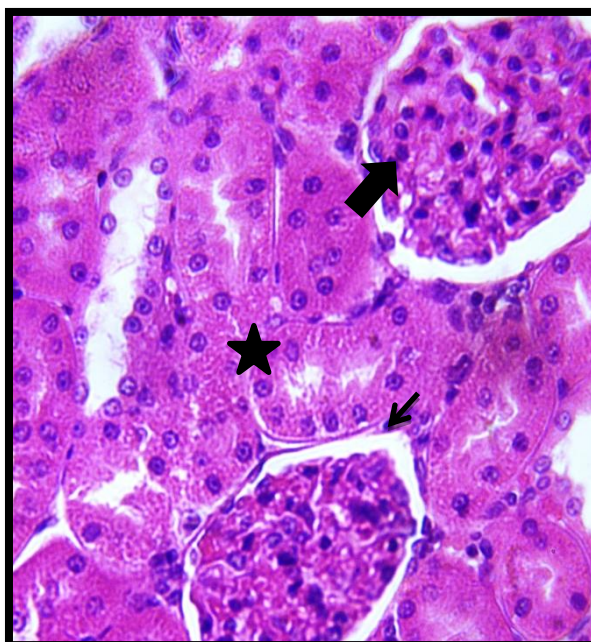


Figure (2) : Kidney section of rat, treated with sodium nitrite 40 mg/ kg showing shrinkage of glomerular (thick arrow), expanded Bowman Space (star), degeneration renal tubular (thin arrow), congestion ( blue arrow ).(H&E 200X)

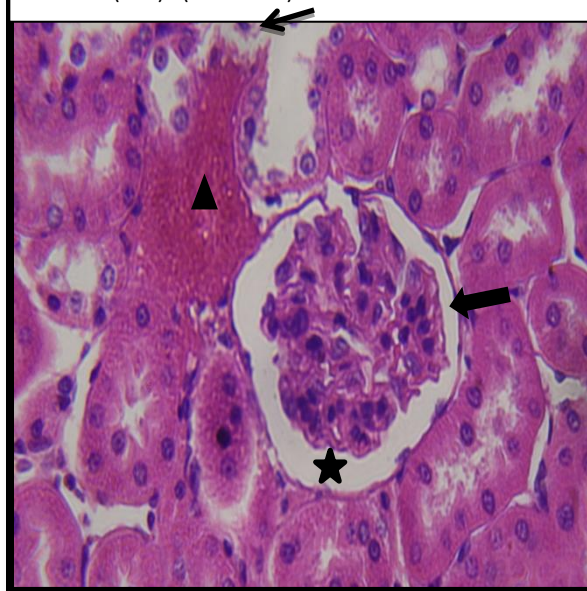




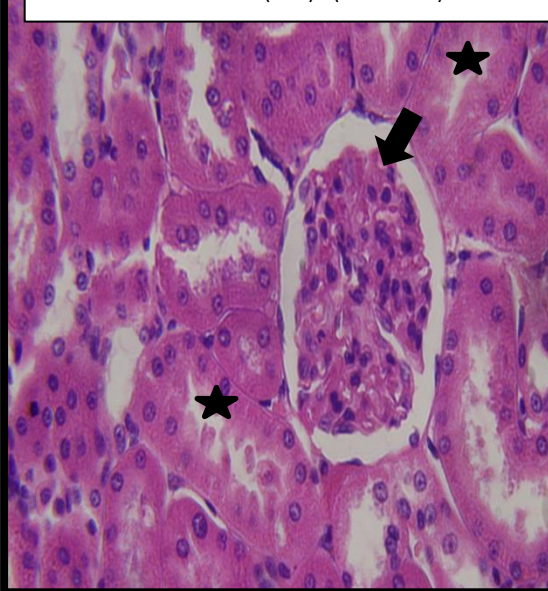
Figure(3): Kidney section of rat dosed 350 mg/kg of MoringaOleifera seed extractshowing normal appearance glomerulus (thick arrow), normal Renal tubules (star) .(H&E 200X)



Figure(4): Kidney section of rat dosed 450 mg/kg of MoringaOleifera seed extractshowintact glomerulus (thick arrow), and BowmanCapsule(thin arrow) normal Renal tubules (star) .(H&E 400X)



Figure(5): Kidney section of rat received 350 mg/kg of MoringaOleifera seed extractand sodiumnitrite 40 mg/kgshowing nearly normal renal corpuscles (thick arrow ), expandedBowman Space(star), and some degeneration of epithelial lining cell of renal tubule ( thin arrow), and hemorrhage( ) (H&E 400X) .



Figure(6): Kidney section of rat received 450 mg/kg of MoringaOleifera seed extract and sodiumnitrite 40 mg/kg showing nearly normal glomerulus (thick arrow), and normal renal tubulesno inflammation, No significant congestion (star) .(H & E 400X).

**Table 1: Effect of *MoringaOleifera* seed extract on Antioxidant (Glutathione , Catalase ) and Malondialdehyde(MDA)concentration in male Rats Treated with Sodium Nitrite**

Mean $\pm$ S.D Treatment	Glutathione GSH $\mu\text{mol/L}$	Malondialdehyde MDA $\mu\text{mol/L}$	Catalase CATKU/L
G1 contral	A 12.20 $\pm$ 0.47	A 13.54 $\pm$ 0.36	A 3.45 $\pm$ 0.04
G2 Sod .Nitrite 40 mg	B 6.04 $\pm$ 0.23	B 16.23 $\pm$ 0.67	B 3.05 $\pm$ 0.03
G3 MoringaOl. Se. 350mg/kg	C 13.10 $\pm$ 0.27	C 11.32 $\pm$ 0.25	A 3.32 $\pm$ 0.04
G4 MoringaOl. Se. 450mg/kg	D 14.50 $\pm$ 0.13	C 10.20 $\pm$ 0.14	C 3.52 $\pm$ 0.03
G5 MoringaOl. Se. 350mg/kg +Sod. Nitrite	E 7.56 $\pm$ 0.62	D 14.40 $\pm$ 0.54	D 3.13 $\pm$ 0.03
G6 MoringaOl. Se. 450mg/kg +Sod. Nitrite	F 9.40 $\pm$ 0.23	E 13.67 $\pm$ 0.32	E 3.23 $\pm$ 0.03

Data are showed as mean  $\pm$  SD , N=6 ,Capital letters in vertical direction mean significant verities ( $p \leq 0.05$ ).

### Discussion:

The increase in MDA concentration in the sodium nitrite group was related to the level of oxidative stress, which is quite clearly associated with damage to the lipid membrane (Atialtet *al.*, 2019) and deterioration of its health, which increases the generation of MDA (Dellavallet *al.*, 2013; Akhzari *et al.* 2018).

The decrease in MDA concentration in groups treated with only the extract as well as both the extract and sodium nitrite together is caused by the presence of active antioxidants that work to scavenge free radicals and prevent peroxidation fats in the membranes (Liang *etal.* 2012; Ahmed 2020), which prevent or reduce the release of MDA (Gonzalez *et al.* 2012). The

reason for the decrease in the concentration of GSH and CAT in the blood serum of rats treated with sodium nitrite is the failure of the antioxidant defense system to overcome the reactive oxygen species resulting from exposure to NaNO<sub>2</sub>, and this was agreed with (Ansari *et al.* 2019).

As for the reason for the high concentration of GSH AND CAT in the dosed groups, Moringa seed extract is due to the reduction of oxidative stress that occurred in the cells of the kidney tissues and the scavenging activity of free radicals that this extract possesses, which may be due to the presence of polyphenols in the extract of Moringa seeds (Sancedo-pompa *et al.* 2018).

This is in agreement with (Ahmed ,2021).The reason for the high concentration of GSH and CAT in the dosed groups, *Moringa* seed extract with sodium nitrite, is due to the protective role of this extract due to its possession of many phenolic compounds, vitamins and tocopherols that act as a scavenger for free radicals, being hydrogen donors and preventing membrane lipid peroxidation (Hamza,2010; ;Jaiswal *et al.* 2013;Sancedo-pompa *et al.* 2018).

Histological study,The cause of glomerular atrophy, congestion, and hemorrhage in the kidney tissue of groups dosed with sodium nitrite is due to exposure to nitrogen oxides resulting from nitrite metabolism (El-Sheikh& Khalil,2011;Ansari *et al.*2018), which has toxic effects on tissues due to free radicals that ultimately cause lipid peroxidation and breakdown of membranes in the tissues of the urinary tubules. (Mohammed *et al.*2016) As for the lack of influence of the kidney tissue in our current study in the groups dosed with the extract of Moringa seeds, it is due to the anti-inflammatory property of the plant with its effective compounds (Ravindra *et al.* 2006 )such as flavonoids that work to renew cells, break down free radicals and enhance antioxidants (Hamza,2010; ;Jaiswal *et al.* 2013; Sancedo-pompa *et al.* 2018). As for the groups that dosed the extract with sodium nitrite, the reason for the improvement of the kidney tissue was due to the effect of this antioxidant extract in stopping the damage to this tissue due to Oxidative stress stops the lipid peroxidation process and reinforces its internal repair system (Rafaela da *et al.*2020; Liang *et al* 2020).

**Conclusions**, *Moringa oleifera* seeds extract improves the defense status of the oxidative stress against renal toxicity, high antioxidant effectiveness, and reduces oxidative stress. It has protective significance in Sodium nitrite-induced kidney damage

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