The Nephroprotective Effects of *Moringa Oleifera* Extract against Contrast Induced Nephrotoxicity

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Authors’ contributions

This work was carried out in collaboration between both authors. Author RAA wrote the protocol and wrote the first draft of the manuscript. Author QJF designed the study, performed the statistical analysis and managed the analysis of the study and literature searches. Both authors read and approved the final manuscript.

ABSTRACT

**Objectives:** The present research has been conducted to assess the Nephroprotective effects of the *Moringa Oleifera* Extract against contrast-induced nephrotoxicity in male rabbits.

**Study Design:** Experimental design

Place and Duration of Study: it was carried out in the Faculty of Pharmacy/University of Kufa, Iraq/AI-Najaf Town. The study began at October 2020 and end at March 2021.

**Methodology:** Twenty one adult (male rabbits) weighing range(1.2-1.5kg). Rabbits are divided in a random manner into three equal groups each group including seven rabbits. Group1: control group administered DW, Group2: has been treated with iodide contrast(2.5mg/kg) of (370mg/ml), group 3: dosed alcoholic extracts of *Moringa* 250 mg/kg and contrast (2.5mg/kg). By accounting data on the MDA levels, Glutathione level and features of kidney histopathology. All efforts have been made to reduce the suffering of animals prior to and throughout the experiment and sampling.

**Results:** The alcoholic *Moringa* extract at 250mg/kg body weight doses orally considerably protected contrast induced toxicity of the kidneys in the rabbits through the increment of the level of GSH and reduces the level of MDA. In the Histological data, the extract activities also protect the damage of the kidney, which is induced by the contrast that has been represented by the degeneration of some tubules and atrophy of glomerular, glomerular tuft congestion and expanded Bowman’s Space.

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1. INTRODUCTION

Medicinal plants are one of the oldest plants known and used by human beings for food and medicine throughout the ages and until the present time, in which the greatest inspiration of these plants has been manifested, and medicinal plants have always been considered an essential source of human health, herbal medicine has always been the most widespread by human beings. Many traditional cultures emphasize the importance of preventive and curative plant prescriptions and their other benefits, they are low cost, easy to obtain and are safer and more successful than manufactured medicines [1]. The kidney consists of numerous cell types organized into the nephron, which is the basic functional unit of the kidney. Any stimuli that induce loss of these cells can induce kidney damage and renal failure. The cause of renal failure can be intrinsic or extrinsic. Extrinsic causes include cardiovascular disease, obesity, diabetes, sepsis, and lung and liver failure. Intrinsic causes include glomerular nephritis, polycystic kidney disease, renal fibrosis, tubular cell death, and stones. The kidney plays a prominent role in mediating the toxicity of numerous drugs, environmental pollutants and normal substances. Drugs known to be nephrotoxic include several cancer therapeutics, drugs of abuse, antibiotics, and radiocontrast agents [2]. Nephrotoxicity is a poisonous effect of some substances (both toxic, Chemicals and drugs) on the kidney function [1]. Most drugs found to cause nephrotoxicity exert toxic effects by one or more common pathogenic mechanisms [3]. Intraglomerular, hemodynamics, tubular cell toxicity, inflammation, Crystal nephropathy, rhabdomyolysis. The proposed pathophysiology mechanism of contrast-media-mediated nephrotoxicity. Contrast media mediates renal toxicity via the interplay of (1) direct cytotoxicity to the renal endothelial and tubular cells, leading to a cycle of oxidative stress, hypoxia, and further tubular damage, and (2) viscos properties of contrast-triggering vasoconstriction, reduced urinary flow rate, and medullary hypoperfusion [4]. *Moringa Oleifera* which has been referred to as the “Miracle tree” as well, because if its multiple uses and adaptability. Due to the pharmacological and nutritive values, the leaves of *Moringa Oleifera* have been found to be rich source of vitamins, minerals and numerous important for the healthy [5]. The wild type *Moringa Oleifera* is different in the phyto-chemistry from the domestic *Moringa Oleifera* [6]. The *Moringa Oleifera* Lam plant is known locally as “Drum-stick tree” in English and “Sohanjana” in Punjabi and it is part of the Moringaceae family. The Moringaceae, can be defined as a monogenic family, comprising approximately 33 species. It is cultivated mainly in Africa, Pakistan, India, Mexico, Sri Lanka and South America. Presently, the species of the *Moringa* acquired an elevated significance, due to their various utilization [7]. Various parts of the plant were found to have a set of the medicinal characteristics, like the treatment of ascites, rheumatism anti-hypertensive (seed and flower), anti-inflammatory (flower and root), hypolipidemic (flower), and anti-ulcer [8], liver diseases, inflammation [9], venous bites [10], hematological, cancer, renal and hepatic functions [11]. The genus of *Moringa* has high anti-oxidant activity, mostly as a result of its high contents of the bio-active polyphenols [12]. The leaves of *Moringa Oleifera* have been considered as one of the most significant sources of the flavonoid compounds [13] such as Myricetin, Quercetin and kaempferol [14]. Moreover, it contains alkaloids, tannins, reducing sugars, saponins, eugenol, glycosides and carbohydrates. [14,15,16].

2. MATERIALS AND METHODS

2.1 Experimental Animals

Twenty one local domestic male rabbits were used in the current study. The weight of the rabbits ranged between 1-1.5 kg. All rabbits were accommodated in the animal house of Faculty of Pharmacy/University of Kufa. The rabbits were retained in cage under the influence of room climate of a twelve hour light, twelve hour dark and about 25°C. 60–65% was the range of humidity under which the rabbits should be retained. Food and water have been provided ad libitum in the current study. They have been adapted for a one week; during this week, all efforts have been made to reduce the suffering of...
a animals prior to and throughout the experiment and sampling.

2.2 Study Groups

After one week of adaptation, the rabbits have been divided in a random manner into three groups (7 rabbits in each group) as follows:

Control group: (negative control administered D.W)

Iodide contrast group: (positive control ). Iodide contrast (2,5mg/kg) of (370mg/ml) was injected

Moringa group: which received 250mg/kg body weight of Moringa Oleifera orally once daily for 27 sequential days by oral gavage and on the 27day iodide contrast (2,5mg/kg) of (370 mg/ml) was injected,and then the animals were sacrificed. About 3ml of blood has been obtained from every one of the rabbits through the cardiac puncture using a disposable syringe, centrifugally at 3000 cycles /minute for 15 minutes. Then, the abdomen has been opened via a mid-line incision and kidneys have been removed quickly. One of the kidneys has been isolated, maintained at a temperature of −70°C and then homogenized, whereas the second one has been fixed in a10% formalin for the histopathological examinations.

2.3 Sampling

Twenty one adult (male rabbits) of 1.2-1.5kg of body weight. Rabbits are divided in a random manner into three equal groups each group including seven rabbits.

2.4 Plant Collection and Extraction

After purchasing the Moringa plant from the nurseries in Karbala, the leaves were collected, washed , dried in shade , After in-blender pulverization, a fine powder has been made and extracted by ethanol alcohol 70% , which was mixed with ethanol alcohol and left for (24) hours at room temperature. Later, this mixture has been filtered by multi-layer medical gauze to remove impurities, and then prepare Moringa Oleifera extract for dosing (250 mg/kg) Orally by gavage.

2.5 Parameters Measurement

2.5.1 Malondialdehyde (MDA)

Malondialdehyde was measured by using the interaction between thiobarbituric acid (TBA) and malondialdehyde, according to Al-Zamely, Al-Nimer and Al-Muslih, [17].

2.5.2 Glutathione (GSH)

The concentration of glutathione was measured by the Ellman's reagent detector, which is a di- acid (Dithio-bis-(2-nitrobenzoic acid)) DTNB, according to.Eyer and Podhradský, [18] method.

2.6 Preparation of Histological Sections

By a merciful killing, the sampled animals were slaughtered, dismembered for examination, and solved in a saline 10% formalin solution. Fixed tissue has been dehydrated by lowering ethanol series concentrations. Blocks and sections were put in a rotary microtome at 5μum and Hematoxylin-eosin-stained parts. The slides were examined under light microscopy. To illustrate tissue changes, by an ocular micrometer, [19].

2.7 Statistical Analysis

The statistical analysis have been performed via the SPSS software (v. 24). Data were represented as mean ± Standard error mean (SEM). Independent-Samples T-test was used for 2 groups' comparisons. The importance of the differences between average values has been determined by the Analysis of Variance (ANOVA). Data from each group have been simultaneously combined and the nephrotoxicity, oxidative stress, markers have been compared with the scores of the nephrotoxicity severity with the use of a Pearson correlation analysis. In all tests, P < 0.001 has been judged as statistically significant.

3. RESULTS AND DISCUSSION

In this study, administered contrast resulted in significantly (P<0.001) greater MDA level in kidney tissue of the rabbits. The increased MDA level indicates the occurrence of the oxidative stress in the renal tissue. As the lipid peroxidation end product, its responsibility is exaggerating the damaging effects of the reactive species on the cellular membrane and the DNA, Table 1 showed that Moringa caused significantly decreased in the serum level of MDA in treated group, while the serum level of GSH has been considerably increased in the treated group as compared with the contrast group. The histological study showed that the histological sections of the kidney of the laboratory rabbits in the control group showing normal appearance of
Bowman Capsule, glomerulus, distal tubules and proximal convoluted tubules as shown in Fig. 1. The second group in which contrast was intravenously administered, recorded significant tissue changes represented Kidney section of rabbits, treated with contrast showing glomerular atrophy, glomerular tuft congestion, expanded Bowman Space, degeneration renal tubular and dissolution in the cortex as shown in Fig. 2. Group 3 sections of kidneys of rabbits showed alcoholic extract of Moringa concentrations of 250 mg/kg and 2.5 mg/kg of contrast showing normal appearance Bowman Capsule, and glomerulus, distal tubules and proximal convolute tubules under a microscopic enlargement of stain Hematoxylin -Eosin. And these have not differences with control group. Contrast induced nephropathy is the 3rd most common one of the causes of the hospital-acquired acute renal failures following the nephrotoxic treatments and impaired renal perfusion. A number of the definitions on the contrast induced nephropathy in the individuals that underwent the cardiac interventions was characterized [20]. The MDA (i.e. Malondialdehyde) can be defined as a final product of the peroxidation of the polyunsaturated fatty acids within cells. The increase in the free radicals results in causing the overproduction of the MDA. The levels of the MDA are mainly known as one of the oxidative stress and antioxidant status markers. The present study Result significant elevation of MDA Level in rabbits treated with contrast in compare with control group, those results are similar to a research that has been conducted by (Yoshioka et al. 1992) Which said that pathologic mechanisms through which the radio-contrast media induced those changes. This result is in similarity with [21] Which said that the intoxications of the rats of the G2 with Thioacetamide resulted in the significant decrease of the anti-oxidant biomarker(GSH) concentration, whereas it results in significantly increasing the level of the lipid peroxidation bio-marker MDA, and the concentration of the nitric oxide, in comparison with the ones of control group. The TBARS and the MDA are the lipid peroxidation end products of the poly-unsaturated fatty acids membrane with the free radicals and have been considered as oxidative damage indicators. Those results coincide with (Naziroğlu et al. 2013) which explained the clinical radio-contrast media-induced acute renal failures. The radio-contrast media-mediated cytotoxicity molecular basis is still difficult to understand at that time. None-the-less, the release of the inorganic iodide, with the successive toxicity of the iodide does not seem involved; and the radio-contrast media may results in the destabilization of plasma and possibly, mitochondrial membranes. Which might result in the loss of the critical plasma membrane and the mitochondrial (cytochrome c) proteins and increased susceptibility of the plasma membrane to Ca2, Contrast media induced over-production of the free oxygen radicals via activating the phagocytic cells. The current study results have shown dosed of the contrast induces increased levels of the serum MDA and renal tissue. Such finding was coinciding with results that have been found by Çetinet al. [22,23], Which stated that the administrations of the contrast agent results in the induction of increased levels of serum MDA and renal tissue, and levels of the renal tissue TBARS on the experimental contrast result in the induction of the models of nephropathy. The study in pathophysiology has suggested the fact that such condition is most same to be the renal ischemia result, oxidative injury and direct toxicity to the tubular epithelial cells [24] post administering the contrast media, ROS result in the enhancement of the contrast induced nephropathy and result in the cytotoxic damage and lipid peroxidation [25], suggesting that the oxidative injury has been defined as one of the main factors in pathogenesis of the contrast induced nephropathy. glutathione as a cytoprotective agent, earlier researches showed that the exogenous glutathione treatment of the renal cortical slices has result in the increase of the cellular glutathione concentration levels (R Christopher Harmon et al., 2005). The present study The Contrast cause significantly decreases the renal concentration of GSH (P < 0.001) of the treated rabbits in comparison with the control group. This finding coincided with the study that has been conducted by (Abd Eldaim et al. 2017) Which stated that Moringa Oleifera aqueous extract resulted in the prevention of changes in histo-architecture of the hepatic tissues and the normalized kidney levels of the glutathione, MDA, and caspasein hepatic tissue; gene expression. Moringa Oleifera resulted in the reduction of the necrosis of the hepatic cells because it includes the coumarins, phenols, essential oil, lignans, carotinoids, monoterpenes, flavonoids, glycosides, lipids, organic acids, ascorbic acid, alkaloids and xanthenes, phenolics (quercetin, kaempferol, apigenin, ellagic acid, catechin, cyanidin, ferulic acid, epicatechin, myricetin and ellagic acid). The results of this study in consistency with a study that has been conducted by Wongmekiat,
Leelarugrayub and Thamprasert, [26] which said that the Polyphenolic compounds were shown to be attenuating renal dysfunction, increasing the anti-oxidant enzyme activity, improving renal architecture, decreasing the lipid peroxidation and ROS in the nephrotoxicity and agreement with the study [27] which said that *Moringa Oleifera* maintained the anti-oxidant defense mechanism through the suppression of the NO and the MDA, and enhancement of the non enzymatic as well as the enzymatic anti-oxidant molecules. Moreover, the polyphenols and the flavonoids have been taken under consideration as a donor of hydrogen atom, which has the ability of neutralizing the free radicals and protecting the mammalian cells against the reactive oxygen species. The *Moringa Oleifera* restored renal function following the daily administrations, which suggests that the *Moringa Oleifera* contents protected the kidney integrity as well as increasing its reparative and regenerative capacities. The normalization or alterations to the bio-chemical parameters have been related to renal histological results. On the histological observations, the kidneys of the groups of intoxicated rabbits that has gotten the MO exhibited reparative behaviors. The changes above may be taken under consideration as an expression of functional enhancement of the renal tubules that can result from accelerated regenerations of the tubular cells. The protective effects of the *Moringa Oleifera* on contrast -induced nephrotoxicity in the present research have been observed as well from the histological examinations. Those findings have coincided with a research that has been conducted by Ouédraogo et al. [28], their research has stated that the kidneys exhibited reparative behaviors in the case of increasing *Moringa Oleifera* doses. The light microscope examination of the kidneys in the control group has shown normal renal parenchyma morphology with the well-defined tubules and glomeruli (Treatments using the gentamicin on its own resulted in a considerable proximal tubular necrosis, desquamation and parenchyma degeneration of tubular epithelial cells and the interstitial nephritis .The histopathological result in this study in consistency with study [29] which said that *Moringa Oleifera* reduced the histopathological changes significantly (P<0.05) by observing paracetamol treated group. The treatment with various *Moringa Oleifera* doses along with the paracetamol produced only a slight degeneration degree and absences of the necrosis in comparison with the group that has been treated with the paracetamol.

Fig. 1.Kidney section of rabbit (control) Showing normal appearance of Bowman Capsule (thick arrow), glomerulus (thin arrow) and proximal convoluted tubules (green arrow), distal tubules (blue arrow), (H&E 200X)
Fig. 2. Kidney section of rabbit, treated group with 2.5mg/kg contrast Showing that atrophy of glomerular(thin arrow), congestion ( star), expanded Bowman Space( blue arrow) inflammatory cells infiltration (thick arrow), Destruction renal tubules(red arrow),necrosis (orange arrow) (H & E 200X)

Fig. 3. Kidney section of rabbit dosed 250mg/kg of *Moringa Oleifera* L. alcoholic extract and2.5mg/kg of contrast show intact Bowman Capsule (thick arrow ) and glomerulus (thin arrow), distal tubules (blue arrow), and proximal convoluted tubules ( green arrow ) .(H&E 200X)

Table 1. Comparison between MDA &GSH among different group

| Group                  | MDA nmol/mg | GSH μmole/mg |
|------------------------|-------------|--------------|
| Group1 (negative control) | 4.94±0.10  | 65.28±1.74   |
| Group2 (positive control)  | 7.11±0.124  | 33.42±1.32   |
| Group3 (Treatment Group)   | 3.67±0.155  | 63.85±1.85   |
4. CONCLUSIONS

This study proposed that Maringa's alcoholic extract results in the enhancement of the defense status of the oxidative stress against the renal toxicity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All procedures in this study were conducted in strict compliance with the ethical standards of the National Institutes of Health (NIH) guidelines on the care and use of laboratory animals, prior approval was provided from the Institutional Animal Care and use Committee of University of Kufa.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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