

Original Article

PROTECTIVE EFFECT OF *MORINGA OLEIFERA* SEED EXTRACT ON CISPLATIN INDUCED NEPHROTOXICITY IN RATS

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Received: 27 Feb 2021, Revised and Accepted: 02 Apr 2021

ABSTRACT

Objective: The main objective of this study is to investigate the antioxidant and nephroprotective efficacy of *moringa oleifera* seed extract (MOSE) against cisplatin which induced acute renal injury.

Methods: Forty male Wister rats were equally segregated into 4 groups (10 rats per group): group I (0.5 ml of sterile saline orally), group II (200 mg MOSE/kg b. wt orally for 10 consecutive days), group III (7.5 mg cisplatin/kg b. wt/intraperitoneally as a single dose on the 5th day of the experiment) and group IV (200 mg *moringa oleifera* seed extract (MOSE)/kg orally for 10 d followed by 7.5 mg cisplatin/kg body weight/intraperitoneally once as a single dose on the 5th day of the experiment. Serum biochemical analysis of renal biomarkers (urea, uric acid, and creatinine), oxidative stress markers (malondialdehyde [MDA]), a crucial antioxidant enzyme (catalase) and the expression of renal activity interleukin (IL)-6, (IL)-10 and Tumor necrotic factor (TNF- α) mRNA were determined. Histopathological examination of renal tissue was done.

Results: Cisplatin induced renal damage, increased renal biomarkers (urea, creatinine and uric acid)(375.87 \pm 1.65, 5.238 \pm 0.25, 4.47 \pm 0.25). Tissue concentrations of malondialdehyde, IL-6 and TNF- α (387.56 \pm 0.97, 2.188 \pm 0.20, 3.06 \pm 0.27) compared to control group(140.58 \pm 1.25, 0.938 \pm 0.017, 1.24 \pm 0.17), (163.99 \pm 1.34, 1.008 \pm 0.05, 0.982 \pm 0.026) Moreover, cisplatin induced significantly down-regulation of anti-inflammatory (IL-10) and catalase (0.780 \pm 0.47, 1.62 \pm 0.06) compared to control one (1.010 \pm 0.02, 3.12 \pm 0.11). The histopathological examination showed renal tissue damage and degeneration of tubules in the cortical portion in cisplatin group. However, interestingly concurrent administration of the MOSE with cisplatin can alleviate the renal damage, oxidative stress and renal toxicity caused by cisplatin.

Conclusion: These results suggest that the antioxidant and the anti-inflammatory effects of MOSE alleviate the cisplatin-induced nephrotoxicity.

Keywords: Cisplatin, *Moringa Oleifera*, Oxidative stress, Nephrotoxicity, Cytokines

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DOI: <https://dx.doi.org/10.22159/ijpps.2021v13i5.41125>. Journal homepage: <https://innovareacademics.in/journals/index.php/ijpps>.

INTRODUCTION

Cisplatin is an important anticancer drug. It kills cancer cells via various mode of actions varying from oxidative stress, reactive oxygen species production, lipid peroxidation and activation of pro-inflammatory cytokines. Knowing these mechanisms of action may be an effective way in the strategy of prevention of its side effects on normal cells [1]. Despite considering it as a useful and effective drug in the treatment of many solid tumors, it has many hazard effects including hepatotoxicity, sever kidney injury, ototoxicity and cardiac toxicity [2]. The cisplatin-induced kidney injury remains a serious complication that still lacks effective ways of prevention.

Nutraceuticals from medicinal plants are considered the most desired adjunctive therapy with chemotherapeutic drugs in the treatment of most cancers. MOSE, is one of these effective plants. *Moringa oleifera* belongs to family *Moringaceae* is an effective remedy for malnutrition. *Moringa* is rich in nutrition owing to the presence of essential phytochemicals present in its leaves, ponds and seeds [3]. *Moringa* leaf extract is rich in antioxidants, polyphenols, oxidase and catalase. Accordingly, it exhibited a great pharmacological and nutritional benefits including protection against hepatorenal, cardiovascular and neurological disorders. It also has anti-inflammatory, antipyretic, anticancer and antioxidant activities [4, 5].

The pharmacological adjunctive usage of *moringa oleifera* to alleviate the cisplatin induced nephrotoxicity was the main objective of this study.

MATERIALS AND METHODS

Plant and chemicals

Moringa oleifera

The seeds of *Moringa oleifera* were purchased from Haraz Company of agricultural seeds, spices and medicinal plants, Cairo, Governorate, Egypt. The seeds were collected in March 2019.

Preparation of extract of *Moringa oleifera* seeds

Moringa oleifera seeds were flowed back in tap water and then with bi-distilled water, dried at room temperature and coarsely minced using a pestle and mortar. Extracts were prepared by macerating a weighed amount of the minced parts (200 g) in volume (2 Liters) of water/organic solvent (bi-distilled water: absolute ethanol, 70:30, and v/v). Maceration continued for 72 h in freezing with intermittent shaking. The extract was then strained through muslin mesh, filtered through what man paper (1). The obtained filtrate was then concentrated using a shaking water bath at 70 °C in a wide-mouthed containers and brown semisolid extract was obtained. It was then weighed (25 g). It re-dissolving in measured amount of iso saline (0.85%, w/v). The extract was stored in airtight container in freezing below 10 °C. a stock solution was diluted which were used for the antioxidant activity testing. For *in vivo* studies [6]. It was used as a dose of (200 mg/kg b. wt MOSE orally for 10 consecutive days) [7].

Chemicals, reagents and kits

Cisplatin was used under the trade name of (Cytoplatin 10 aquas) imported by star INT company and manufacture by CIPLA LTD. Each

20 ml vial contain 10 mg cisplatin. It was used at dose of 7.5 mg/kg b. wt at the 5th day of the experiment [8].

Kits

Kits for estimating Urea, creatinine and uric acid were supplied by Centronic GmbH Company Germany by chem7, Kits for estimating of oxidative stress marker Malondialdehyde (MDA), catalase (CAT) in kidney homogenate were purchased from BioDiagnostic Company, Cairo, Egypt by spectro nanodrop. Genes expression was made using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA), and SYBR green master (Applied Biosystems, CA, USA)

Animals

About forty healthy Wistar male rats weighting 200-250 gm were obtained from the animal house of faculty of veterinary medicine, Benha University. They were fed on standard rodent diet and clean water ad libitum. Rats were left for two weeks for acclimatization before the beginning of the experiment.

Experimental design and treatment protocol

Forty male Wistar rats were divided randomly into 4 groups (10 animals per group). Group I (control group) they were administered 0.5 ml of saline orally for 10 consecutive days. Group II (*moringa* group) they were administered 200 mg/kg b. wt *Moringa oleifera* seed extract (MOSE) orally for 10 consecutive days. Group III (cisplatin group) they were administered cisplatin at dose of 7.5 mg/kg body weight/intraperitoneally as single dose on the 5th day of experiment. Group IV (Cis+*Moringa*), they were administered *Moringa oleifera* seed extract MOSE at dose of 200 mg/kg orally for 10 d and single dose of cisplatin at dose of 7.5 mg/kg body weight/intraperitoneally on the 5th day of experiment one hour prior to dose of MOSE.

Blood sampling

At the end of the experiment blood samples were taken from each rat for biochemical analysis from the median canthus of the eye. Blood sample was collected without anticoagulant for separation of clear serum for biochemical analysis. These serum samples were used for biochemical analysis of (creatinine, urea and Uric acid).

Tissue samples

At the end of the experiment all rats were scarified, and tissue samples were taken from kidney for both biochemical study of oxidative stress markers (MDA, CAT, IL-10, IL-6 and TNF- α) and for histopathological examination.

Assay methods

Serum biochemical analysis

Serum creatinine was measured spectrophotometrically according to [9] using specific kits from Diamond Company. Serum urea concentration was determined according to [10] by kits from Diamond Company.

Evaluation of renal oxidative stress markers

Both kidneys were taken immediately after scarification, washed in physiological saline. One kidney was preserved at -80°C until preparation of tissue homogenate which used for assessment of (MDA and CAT) levels calorimetrically. According to [11, 12] respectively.

Gene's expression by real time PCR

Total RNA was isolated from kidney tissue using the Total RNA Isolation System (Promega Co., Madison, WI, USA) according to the manufacturer's protocol. Briefly, RNA was extracted and re-suspended in 50 μ L RNase free water, then stored at -80°C. The total RNA concentration was determined spectrophotometrically (SPECTRO star Nano, BMG Labtech Co., Ortenberg, Germany) and then 1 μ g of RNA was used for cDNA synthesis using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA). The condition was achieved in line with the kit of cDNA reverse transcription as follow; step I (10 min/25°C), step II (120 min/37°C), step III (5 min/85°C) and step IV (∞ at 4°C), then, 1

μ g of the obtained cDNA with the SYBR green master mix were used in a total of 20 μ l reaction volume 7 μ l of nuclease-free water, 10 μ l of master mix, 1 μ l of forward primer, 1 μ l of reverse primer, and 1 μ l of DNA template) for HSP70 and β -actin gene amplification using the following primers:

IL 6 Forward GACTTCCAGCCAGTTGCCTTCTTG, IL 6 Reverse TGGTCTGTTGTGGGTGGTATCCTC, IL 10 Forward TGCCAAGCCTTGTCAGAAATGATCAAG, IL 10 Reverse TGCCAAGCCTTGTCAGAAATGATCAAG, TNF- α Forward AAATGGGCTCCCTCTCATCAGTTTC, TNF- α Reverse TCCGCTTGGTGGTTGCTACGAC, GAPDH 5'-AACTCCCA TTCCTCCACCTT-3' forward, 5'-GAGGGCTCTCTTGTCTCT-3' reverse.

The genes were amplified then the expression levels were analyzed using a real-time PCR (7500 Fast Real-Time PCR System, Applied Biosystems, CA). The cycling condition was justified first the initial activation (3 min/95°C), denaturation (3 s/95°C), annealing/extension (30 s/60°C), and the number of cycles were 40 according to [13]. All gene expression level was normalized against the GAPDH gene.

Histopathology examination

Autopsy from the kidney of rats in different groups were taken and fixed in 10% formal saline for twenty-four hours. Washing by tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty-four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The tissue sections were taken on glass slides, deparaffinized, stained by hematoxylin and eosin stain for examination through the light electric microscope [14].

Statistical analysis

The results were done as mean \pm SE of the experimental groups using (one-way ANOVA) followed by Duncan's multiple range test. All analysis was performed by Statistical Package for Social science Software (SPSS (16) software (SPSS Inc., Chicago, USA).

RESULTS

There was no mortality observed in any group of the experiment during the period of the experiment. Regarding to the biochemical renal parameters (urea, creatinine and uric acid) there was significant increase ($p < 0.001$) in the urea (375.87 \pm 1.65), creatinine (5.23 \pm 0.25) and uric acid (4.47 \pm 0.25) levels in the cisplatin group when compared to control group, *moringa* group and Cis+*Moringa* group table 1.

Analysis of the lipid peroxidation parameter MDA, there was significant elevation in MDA level ($p < 0.001$) in the cisplatin group (387.5 \pm 1.49) when compared to control group (163.99 \pm 1.3), *moringa* group (213.34 \pm 1.3) and Cis+*Moringa* group (180.68 \pm 0.97) table 2.

The catalase level showed significant decrease ($p < 0.001$) in cisplatin group (1.62 \pm 0.06) when compared to control group (3.12 \pm 0.11), *moringa* group (3.56 \pm 0.17) and Cis+*Moringa* group (2.15 \pm 0.08).

The IL-6 gene expression level was significantly higher ($p < 0.001$) in the cisplatin group (2.19 \pm 0.20) than in the control, Cis+*Moringa* and *Moringa* groups (1.01 \pm 0.05, 1.40 \pm 0.02, and 1.34 \pm 0.05 respectively). The gene expression level of IL-10 was down regulated in the kidney tissue of the Cisplatin group rats (0.78 \pm 0.473) compared with other groups and TNF- α upregulated in cisplatin groups (3.0620 \pm 0.277) compared with other groups. All these data illustrated in table 3.

Histopathological findings

Regarding to the histopathological examination of the renal tissue, the cisplatin group showed focal infiltration of the inflammatory cells in between the degenerated tubule in the cortical portion fig. 1(C) with congestion in the blood vessels and glomeruli as well as degeneration and desquamation in the tubular lining epithelium fig. 1(D and E). There was no histopathological alteration and the glomeruli and tubules at the cortex appeared with normal histological structure in control group fig. 1(A) and in *moringa* group

fig. 1(B). The cortex showed normal histological structure in cisplatin+moringa group fig. 1(F), while the corticomedullary

portion showed degeneration and desquamation in the lining epithelium in fig. 1(G).

Table 1: Effect of oral administration of MOSE at 200 mg/kg body weight for successive 10 d on renal biomarker (Urea, creatinine, uric acid) on cisplatin (7.5 mg/kg b. wt.) intoxicated rats.(n=10)

Groups/parameters	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	140.58±1.25 ^c	0.938±0.017 ^c	1.246±0.17 ^b
Moringa	139.6±1.58 ^c	0.946±0.022 ^c	1.540±0.22 ^b
Cisplatin	375.87±1.65 ^a	5.238±0.25 ^a	4.47±0.25 ^b
Cis.+Moringa	192.30±1.51 ^b	2.276±0.22 ^b	2.377±0.22 ^b

Data are represented as (mean of 10rats±S. E). Mean values with different superscripted letters in the same column are significantly different

Histopathological findings

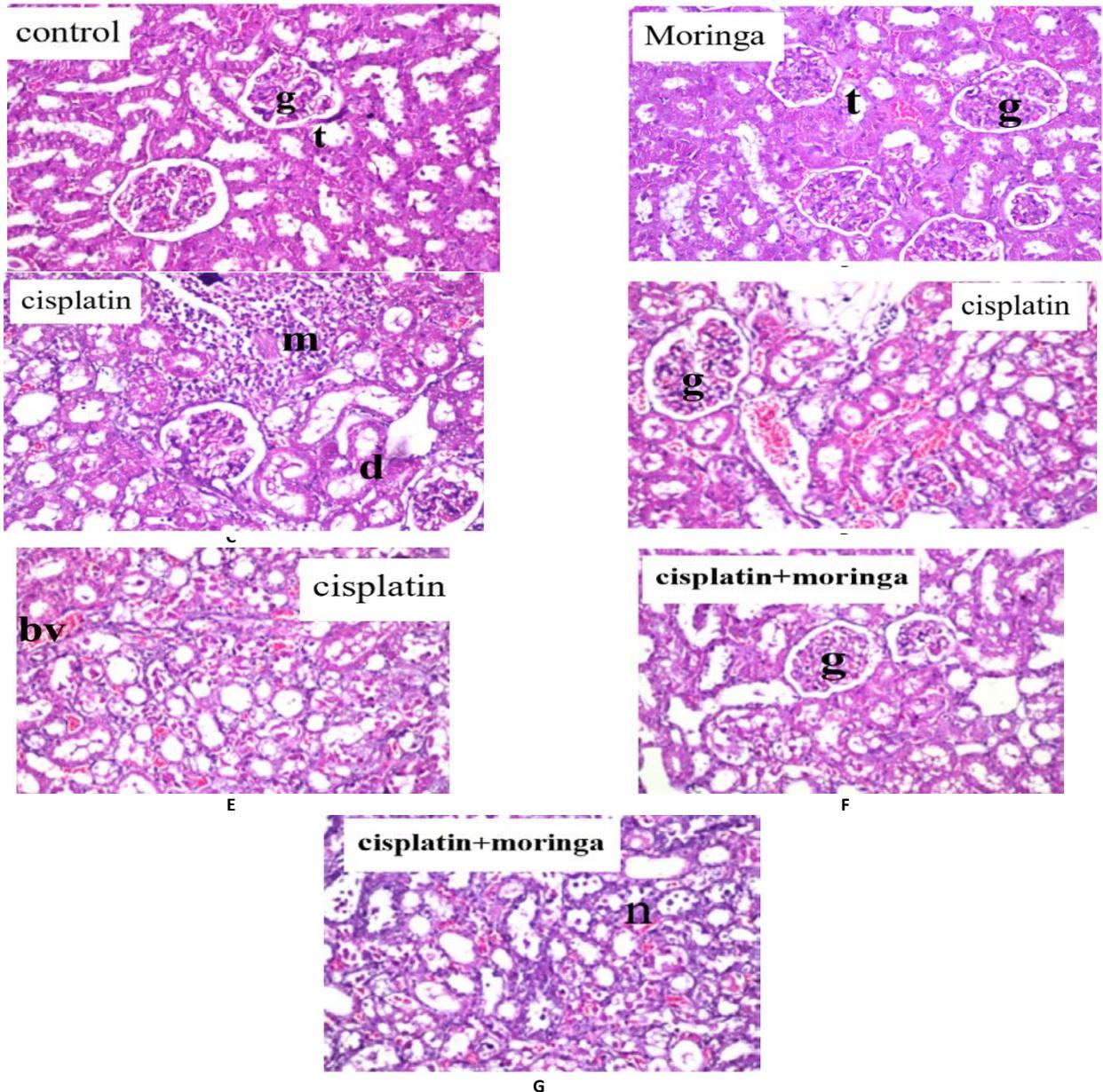


Fig. 1: Histopathological examination of rat kidney sections of different experimental groups stained with (HandE X40). There was no histopathological alteration and the normal histological structure of the glomeruli and tubules at the cortex in control (A) and moringa group (B). The cortical portion showed focal inflammatory cells infiltration in between the degenerated tubule. m: Focal inflammatory cell infiltration g: glomeruli d: degenerated tubules at cisplatin group (C). Congestion in the blood vessels and glomeruli as well as degeneration and desquamation in the tubular lining epithelium in cisplatin group (Dand E). bv: congested blood vessels, g: glomeruli. Cortex showed normal histological structure in cisplatin+moringa group (F), while the corticomedullary portion showed degeneration and desquamation in the lining epithelium in cisplatin+moringa group (G)

Table 2: Effect of oral administration of MOSE at 200 mg/kg body weight for successive 10 d on renal oxidative stress biomarker (MDA, CAT) on cisplatin (7.5 mg/kg b. wt.) intoxicated rats.(n=10)

Groups/parameters	MDA (nmol/gm)	CAT (U/gm)
Control	163.99±1.34 ^d	3.12±0.11 ^b
<i>Moringa</i>	213.34±1.33 ^b	3.56±0.17 ^a
Cisplatin	387.50±1.49 ^a	1.62±0.06 ^d
Cis.+ <i>Moringa</i>	180.68±0.97 ^c	2.15±0.08 ^c

Data are represented as (mean of 10rats±SE). Mean values with different superscripted letters in the same column are significantly different

Table 3: Effect of oral administration of MOSE at 200 mg/kg body weight for successive 10 d on renal IL-6, IL-10 and TNF-α on cisplatin (7.5 mg/kg b. wt.) intoxicated rats.(n=10)

Groups/parameters	IL-6	IL-10	TNF-α
Control	1.0080±0.05 ^c	1.010±0.02 ^b	0.9820±0.026 ^b
<i>Moringa</i>	1.3420±0.05 ^b	1.1520±0.04 ^b	1.0180±0.27 ^b
Cisplatin	2.1886±0.20 ^a	0.780±0.47 ^c	3.0620±0.277 ^a
Cis.+ <i>Moringa</i>	1.4020±0.02 ^b	1.832±0.13 ^a	1.5880±0.19 ^b

Data are represented as (mean of 10rats±S. E). Mean values with different superscripted letters in the same column are significantly different

DISCUSSION

Cisplatin is an important anti-neoplastic agent. It is a useful drug in the treating of many types of solid tumors. Nephrotoxicity is the main adverse side effect of cisplatin. This experiment was done to determine the antioxidant and nephroprotective efficacy of MOSE against cisplatin induced acute renal damage. *Moringa oleifera* seed extract MOSE has antioxidant properties.

Our results showed a state of oxidative stress-mediated renal injury after treatment of rats with a single i. p. dose of cisplatin as it is indicated by significant elevation in serum urea, uric acid, creatinine and renal tissue levels of MDA with significant reduction in renal tissue level of catalase. This state of renal injury was confirmed with histopathological examination of kidney tissue that revealed focal inflammatory cells infiltration in between the degenerated tubules in the cortical portion with degeneration and desquamation in the lining epithelium fig. 1(c). This state of renal injury may be because of the oxidative stress and inflammatory effects of cisplatin. This injury may be because of the liberated free radicals cause membrane lipid peroxidation and denaturation of both DNA and proteins. This damage leads to enzymatic inactivation and mitochondrial dysfunction that enhance ROS production via the disruption of the respiratory chain [15]. Catalase enzyme is a thiol-containing enzyme. It is an important enzyme for the neutralization of ROS [16, 17].

The increased serum levels of renal biomarkers urea, creatinine, and uric acid after cisplatin administration in a single i. p. dose coincided with [18] who recorded that cisplatin enhances glomerular filtration membrane damage through oxidative stress, inflammation, and apoptosis. This damage resulting in low glomerular filtration rate and loss of normal membrane permeability.

In our study MOSE administration reduced the oxidative stress state through significant reduction in serum creatinine, urea and renal MDA level with significant elevation in the renal catalase level. Our results matched with [19] who said that MOSE protected the renal tissue by restoring the kidney integrity and regenerative capacity and also by decreasing the elevated urea and creatinine in response to exposure to cisplatin. The possible protective effect of *Moringa oleifera* seed extract (MOSE) on these markers may be a result of the antioxidant action of oleic and linoleic acid, which are both constituents of the *moringa* plant. In this regard [20]. Reported that any natural compound with antioxidant effects may help in preserving a healthy state when continuously taken naturally or therapeutically. As agreed with [21] who reported that *moringa* extract effectively inhibit CCL4-induced tissue damage due to presence of various antioxidant bioactive compound.

Moringa oleifera seed extract (MOSE) is a highly enriched compound with flavonoids and phenolic constituents, which have a cytoprotective and antioxidant effects on the renal tissue, [22-24]. Antioxidant compounds of plant are able to perform their safety

roles in living organisms by stopping free radicals production or by counterbalancing them [25, 26].

The antioxidant activity of *Moringa oleifera* seed extract (MOSE) is thought to be the key factor as a renal protective agent.

In the present study the cisplatin increased the expression levels of IL-6 and TNF-α that is in agreement with [27] were found Many cytokines (e. g. TNF-α, IL-1 and IL-6) are upregulated in the renal tissues inflammatory conditions caused by cisplatin. While the levels of IL6 and TNFα restored to normal levels in the *Moringa* with cisplatin group because of *Moringa* has antibiotic, antitrypanosomal, hypotensive, antispasmodic, antiulcer, anti-inflammatory, hypocholesterolemic and hypoglycemic activities [28]. Cisplatin induced TNF-α is highly dependent upon the Reactive oxygen species (ROS) production, NF-κB and p38 MAPK activation. However, the activation of TNF-α and IL are involved in many signal transduction mechanisms, involving the NF-κB and AP-1 pathways. Exactly, the stress-activated group of MAPKs (JNK and p38) is strongly activated by TNF-α and IL [29].

Antioxidant property of *Moringa* which could be possibly the primary mechanism of protection against cisplatin toxicity due to *Moringa* contains antioxidant compounds that was confirmed in this study, such as vitamin A, which quench ROS, combined with metal ions and regenerate membrane-bound antioxidants. These findings are matched with previous reports of [30].

Several studies have expressed that cytokines such as IL-10 counterbalance or inhibit the increase in other pro-inflammatory cytokine levels in order to protect tissue against injury in various pathophysiological conditions. [31]. IL-10 exerts anti-inflammatory and anticytotoxic effect in kidney injury accompanied with lupus nephritis, immune complex glomerulonephritis and ischemic or cisplatin-induced nephrotoxicity [32]. The findings of the other study stated the animals receiving cisplatin the levels of IL-10 decreased [33]. While IL-10 gene expression regained to normal level with administration of *Moringa* with cisplatin to normal level due to *moringa* decrease inflammation and oxidative stress caused by cisplatin corresponding to the findings have revealed that *Moringa* prevent oxidative stress-mediated toxicity in the brain tissue, testis, liver, and kidney of rats [34-36]. *Moringa* leaves extracts have a powerful antioxidant action against free radicals, prevent oxidative damage to major biomolecules and provide substantial protection against oxidative damage [37-38].

CONCLUSION

The results of this study show a possible explanation for the protective effects of *Moringa oleifera* seed extract (MOSE) against cisplatin induced nephrotoxicity. Concurrent administration leads to significant decreased renal biomarkers (urea, creatinine and uric acid) (192.30±1.51, 2.276±0.22, 2.377±0.22), tissue concentrations of

malondialdehyde, IL-6 and TNF- α . (180.68 \pm 0.97, 1.40 \pm 0.02, 1.58 \pm 0.19) compared to cisplatin group (375.87 \pm 1.65, 5.238 \pm 0.25, 4.47 \pm 0.25), (387.50 \pm 1.49, 2.188 \pm 0.20, 3.06 \pm 0.27) Moreover, induced significantly up-regulation of anti-inflammatory (IL-10) and catalase (1.832 \pm 0.13, 2.15 \pm 0.08) compared to cisplatin group (0.780 \pm 0.47, 1.62 \pm 0.06). All this enhanced with a great protective effect of MOSE on renal tissue represented in no histopathological alteration. It may be due to its antioxidant scavenging properties and down regulation of the proinflammatory cytokine like (IL-6). These ensures the possible applied values of MOSE in preventing nephrotoxicity.

FUNDING

Nil

CONFLICT OF INTERESTS

No potential conflict of interest was reported by the authors.

AUTHORS CONTRIBUTIONS

All Authors have contributed equally.

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