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Protective and Antioxidant Effects of Moringa oleifera Extract Against Cisplatin-Induced Renal Injury in Male Rabbits

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***Abstract*—** Cisplatin, which can be very effective as a chemotherapeutic agent, is considerably limited in its clinical application by its known tendencies towards nephrotoxicity, a problem that is mainly due to oxidative stress, inflammation and tubular damage. To appraise the ameliorative and antioxidant action of Moringa oleifera extract on cisplatin-induced renal trauma in male rabbits. Thirty-two adult male rabbits were randomly allocated to four groups control group ; the cisplatin treatment group; the cisplatin plus Moringa group; and a fourth group which only received Moringa extracts without any cisplatin exposure. Kidney injury was induced by cisplatin (5 mg/kg). Moringa oleifera extract in such an amount as 300 mg × kg(-1) was given orally every day for 30 days. After this period of time we saw Cisplatin administration led to severe renal dysfunction by marked increases in serum urea, creatinine and Kidney Injury Molecule-1 (KIM-1) along with prominent oxidative stress as evidenced by GSH and SOD decrease, NO and MDA increase. Co-treatment with Moringa oleifera could greatly improve kidney function indexes and reduced KIM-1 concentration, suggesting the attenuation of tubular injury. In addition, while significantly decreasing levels of NO and MDA, it helped the antioxidant system: Moringa restored deficiency in GSH and SOD.

In conclusion, these findings suggest that Moringa oleifera has a protective effect against cisplatin-induced renal injury. This extract clearly exerts antioxidant effects and has anti-inflammatory properties, thereby effectively eliminating oxidative damage caused during the use of cisplatin. It can ensure good kidney function .

***Keywords* —** Moringa oleifera; Cisplatin; Nephrotoxicity; Oxidative stress. Antioxidant enzymes; Kidney Injury Molecule-1 (KIM-1); Glutathione (GSH); Superoxide dismutase (SOD); Malondialdehyde (MDA); Nitric oxide(NO); Renal protection

INTRODUCTION

Cisplatin is a powerful chemotherapeutic agent with activity against many types of solid tumors. Its major limitation His generic problem is kidney damage (1). The drug accumulates in renal proximal tubules, where it produces excessive reactive immuno oxygen species (ROS). This oxidative assault annuls endogenous antioxidants (such as GSH, SOD) and starts off lipid peroxidation, DNA damage and inflammation, ultimately leading to acute kidney injury.(2) For instance, cisplatin-induced ROS have been shown to consume glutathione and lift malondialdehyde (MDA), indicating disturbed redox balance(3) The recent reviews, which highlight such mechanisms in addition, also point out in cisplatin nephrotoxicity for example that early-mediate biomarkers like Kidney Injury Molecule-1 (KIM-1) are highly effective indicators of proximal tubular damage.(4)

Because of this patho compounds with strong antioxidant and anti-inflammatory properties are being tested as agents for protection. Moringa olifon is a medicinal plant rich in vitamins, minerals and polyphenols such as quercetin, kaempferol and chlorogenic acid(5) These substances are known to scavenge free radicals, and they also activate cellular defense pathways (e.g., Nrf2) and inhibit pro-inflammatory signals(6) Experimental findings with Moringa formula indicate that it increases antioxidant enzymes and reduces cytokines like TNF-α and IL-1β, both in the tissues (7). More notably, showed on rats that Moringa leaf extract could suppress cisplatin-induced oxidative stress and reduce inflammatory cascades. Serum urea, creatinine and KIM-1 levels decreased significantly down to normal physiological limits(8)

Despite these encouraging results, the protective effects of M. olifor– especially on kidney Injury Molecule-1 and other data still under discussion— have not been fully worked out in rabbit models. This study therefore aimed to determine whether oral Moringa orentalis can protect against kidney damage and oxidative stress in rabbits with cisplatin-induced renal injury. By using current technology for analysis and in-corporating the latest literature, we constantly kept these as references. We measured kidney function tests (e.g., serum urea, creatinine, KIM-1) and oxidative stress parameters like glutathione (GSH), superoxide dismutase (SOD), nitric oxide (NO) and malondialdehyde (MDA), in order to find out if with Moringa extract on board there exists any fence against injury, referring if necessary afterwards to the recent literature for backup.

MATERIALS AND MTHODS

The present study was carried out in the animal house of the College of Veterinary Medicine, University of Karbala.A total of 32 adult male rabbits (weighing 1.5–2.0 kg) were used in this study. They were randomly divided into four groups with n=8 for both control and treatment groups as shown below:

Group I (Control Group):Those in this group were given 0.25 ml DMSO orally once daily for 30 days.

Group II (Cisplatin Group):Those in this group were injected with cisplatin (5 mg/kg) intraperitoneally one shot the goal being to cause serious nephrotoxicity.

Group III (Cisplatin Moringa Group):Those in this group were co-administered:Cisplatin (5 mg/kg, intraperitoneally) Moringa oleifera extract (300 mg/kg, orally) daily for 30 days

Group IV (Moringa Group):Those in this group received Moringa oleifera extract (300 mg/kg, orally) daily for 30 days.

Collection of Blood Samples and Study Parameters:At the end of the experiment, male rabbits were anesthetized with chloroform. Cardiac puncture was then performed using a 10 mld syringe to collect 6 ml blood into eppendorf heparinized tubes, which was gently diluted with saline (0.9% NaCl) before being analyzed.One ml blood was collected into a heparin-containing tube for hematological analysis immediately.The remaining blood was placed into tubes with a gel that will separate the serum at room temperature for half an hour. After tarting treating at 3000 rpm and spinning for 15 minutes, they took out the separated products which is transferred to eppendorf containers to store at -20 "C until needed biochemical analysis.Once blood samples had been taken from the rabbits, they were then sacrificed and their kidneys and livers excised for biochemical assays that were carried out at a later time (-20°C).

STATISTICAL ANALYSIS

Data are presented as mean ± standard error of the mean (SEM). Differences between groups were evaluated by a one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test with GraphPad Prism or SPSS software. Statistical significance was set at p-value < 0.05.

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RESULT AND DISCUSSION

The test results of renal function test were presented in Table 1; Some measures showed higher values following cisplatin treatment one of results which related to this experiment included elevations in serum urea, creatinine and KIM-1 ($p < 0.05$). This significance was seen between the cisplatin and control groups. In particular, urea rose to 65.3±4.2 mg/dL, creatinine was 2.8±0.2 mg/dL and KIM-1 reached 1.85±0.12 ng/ml. These figures indicate that renal function and tubular injury have become clearly affected.

Yet when the Moringa oleifera extract was coadministered with cisplatin, these values all decreased significantly ($p < 0.05$). Urea fell to 36.7±3.0 mg/dL, creatinine down 1.2±0.1mg/L and KIM -1 reduced by 0.68±0.05 ng/mL compared with the cisplatin group Nevertheless these are still slightly higher than control levels.

The group treated with Moringa oleifera extract alone showed levels of urea (27.8±2.0 mg/dl), creatinine (0.9±0.1 mg/dL) and KIM-1 (0.46±0.03 nanograms) which were not statistically different from those in the control, suggesting that this product does not have a negative impact on kidney function. As shown in table (1)

Table 1. Kidney Function Tests

Group	Urea (mg/dL)	Creatinine (mg/dL)	KIM-1 (ng/mL)
Control	28.5 ± 2.1	0.9 ± 0.1	0.45 ± 0.03
Cisplatin	65.3 ± 4.2	2.8 ± 0.2	1.85 ± 0.12
Cisplatin + Moringa	36.7 ± 3.0	1.2 ± 0.1	0.68 ± 0.05
Moringa	27.8 ± 2.0	0.9 ± 0.1	0.46 ± 0.03
Significant vs Control ($p<0.05$), Significant vs Cisplatin group ($p<0.05$)			

Kidney Function (Urea and Creatinine) On cisplatin elevations in serum urea and creatinine were striking, an indication of substantial deterioration in glomerular filtration and tubulary injury. Thus it is consistent with prior finding from other sources for example observed that cisplatin raises BUN (urea) and creatinine in rats. This is thought to result from apoptosis and necrosis of proximal tubular cells and injury to endothelial (9)

In the kidney, inflammatory effects and ROS generation by cisplatin further reduce renal perfusion and conduce to retention of nitrogenous waste products in the blood (10)

Improvement by Moringa. Co-treatment with Moringa extract effectively blunted the increase in urea/creatinine ($p<0.05$ vs cisplatin alone), indicating that this herb could confer nephroprotection. This is corroborated by work showing that M. oleifera sustains nearly normal renal function markers under the lash of toxins

For instance,(11)reported that Moringa leaf extract prevented the increase of BUN and creatinine induced by cisplatin in mice. Moringa 's antioxidant flavonoids such as quercetin may play a protective role on the kidney by freeing radicals and defending tubular epithelia integrity(12). Also, it can reduce inflammation (e.g. TNF-α, IL-1β) in the kidney

which will minimize capillary damage and help maintain GFR. Taken together, the cystin-Moringa combination greatly reduced urea and creatinine levels when compared with cystin alone. This does not happen when the Moringa group gets only Moringa: the Moringa group 's urea and creatinine values were similar to those of the controls (no statistically significant difference). Similar findings have been reported by several other authors, showing that even moderate doses of Moringa leaf extract in the viplex do not lead to nephrotoxic effects KIM-1 (Kidney Injury Molecule-1)

Cisplatin Elevation: Following Cisplatin, the KIM-1 in serum levels going up was a dramatic effect As an early biomarker of tubular toxicity, KIM-1 is produced by transection-specific genes upregulated in damaged proximal tubules (13,14) also confirm this, reporting sharp rises in both urine and blood levels of KIM-1 within 24-48 hours following dose completion. This is an indicator reflecting proximal tubular necrosis and apoptosis initiated by cisplatin. The inflammatory cytokines (TNF-α etc.) and ROS secondary

metabolites from Cisplatin promote KIM-1 shedding out from damaged renal cells(15)

So the elevated KIM-1 within Cisplatin group represents a serious tubular damage and response

Moringa Reduces: Of note, Moringa co-treatment decreased significantly the KIM-1 back towards control trend ($p<0.05$ vs cisplatin). This implies that Moringa is effective in alleviating tubular damage. Indeed, as it was found by(16)

Moringa leaf extracts suppress KIM-1 expression in rodent models of renal inflammation

We speculate that the sequence of TAK1-Nemo activation pathway in Moringa would compensate after some semen and semen accompany PGE2 catabolism to form PGDH complex further downstream--these actives are synergistic or maybe even biologically stable. We ask as well why other natural antioxidants like the flavonoid quercetin (17) and curcumin both reduced cisplatin-induced KIM-1 elevation similarly so supporting only countering oxidative stress limits release? Thus the lower KIM-1 within the Cisplatin+Moringa group can be seen as evidence of Moringa's protective effect upon proximal tubules.

Mechanistic Participants: Possible mechanisms include the strengthening of Moringa's cellular antioxidant defense, which stops ROS-mediated membrane damage. And its anti-inflammatory action that reduces TNF-α and NF-κB signaling

By mitigating the causes of tubular damage Moringa prevents KIM-1 dropping; Moringa may in addition upregulate survival pathways (EG NRF2) to guard the tubule cells, as other antioxidants do (18). Such joint action together holds up the integrity of tubules, providing a structural basis for the large decline in KIM-1 seen with Moringa.

Antioxidant and oxidative stress parameters in Table 2 are shown in this paper. In the cisplatin-treated group GSH and SOD (p0.05 <) were significantly decreased compared with those in normal rats, on average by 20% or more for each parameter. The levels of GSH in this group fell to 4.2 ± 0.4 µmol/L and SOD activity dipped greatly to 68 ± 5 U/mL, both results indicating a damage to antioxidant defense mechanisms.

In contrast, the cisplatin group had significant increases ($p < 0.05$) for nitric oxide (NO) and malondialdehyde (MDA). The levels of these two indicators were, on average, 58 ± 4 µmol/L and 5.8 ± 0.4 mmol/mLper mouse, which meant that lipids peroxidized while nitric stress was also present in the study animals. This last was heightening lipid peroxide levels throughout their bodies as well as creating an association within them for other forms of damage such as oxidation, whether oxidative or transitive in nature.

Moringa oleifera extract therapy was followed by significant improvement in these abnormalities. GSH and SOD levels once again rose to 7.6 ± 0.5 µmol/L 110 ± 7 U/mL, respectively; and NO and MDA declined significantly lower from their peaks of 58 ± 4 µmol/L 5.8 ± 0.4 nmol/mL to 38 ± 3 µmol/L 3.0 ± 0.3 nmol/mL respectively in the cisplatin group.

For the Moringa oleifera group, GSH(8.6 ± 0.7 µmol/L), SOD (122 ± 9 U/mL), NO (36 ± 3 µmol/L) and MDA (2.3 ± 0.2 nmol/mL); there was no significant difference compared to normal controls, showing that oxidative balance in animals was normal. As shown in table (2)

Table 2. Antioxidant Status in Blood

Group	GSH (µmol/L)	SOD (U/mL)	NO (µmol/L)	MDA (mmol/mL)
Control	8.5 ± 0.6	120 ± 8	35 ± 3	2.4 ± 0.2
Cisplatin	4.2 ± 0.4	68 ± 5	58 ± 4	5.8 ± 0.4
Cisplatin + Moringa	7.6 ± 0.5	110 ± 7	38 ± 3	3.0 ± 0.3
Moringa	8.6 ± 0.7	122 ± 9	36 ± 3	2.3 ± 0.2
Significant vs Control (p<0.05), Significant vs Cisplatin group (p<0.05)				

Cisplatin-induced depletion: The GSH of blood in rats given continuous intraperitoneal injections over 3 days with 120 mg/kg of cisplatin dropped dramatically all to 4.2 umol/L, and SOD activity similarly also fell precipitously from ~120 to 68 U/ml. Such severe loss of antioxidant defenses is frequently cited as evidence for a role in cisplatin nephrotoxicity. Cisplatin unleashes enormous quantities of ROS (such as superoxide, hydroxyl radicals), devouring the GSH and disabling SOD (19)

Consistent with our results, (20) and (21)reported that cisplatin significantly lowered GSH and SOD while raising lipid peroxidation (MDA)

Mechanism of action: on the one hand, cisplatin can bind thiol compounds in cells that carry out much of this reduction (depleting the GSH), and on the other, it immobilises enzymes intended to undo and remove ROS in cells, creating an imbalance. Also, fighting back on the part of cisplatin (via iNOS and pro-inflammatory cytokine) serves to further shut SOD. As a whole then, the drop in GSH/SOD represents an aniquished defense against ROS caused by cisplatin.

Repletion by Moringa: Co-treatment with Moringa gave GSH and SOD bound back to near normal levels nearly gradually ($p<0.05$ versus cisplatin). For example, in the Cisplatin+Moringa group GSH recovered to 7.6 umol/L and SOD to 110 U/ml. This matches studies showing that M. oleifera (Akter et al. 2021) can enhance the body's own antioxidants, raising tissue GSH, SOD, and catalase levels in injured rats by exogenous supplementation.

In Moringa, the polyphenols act as both direct free radical scavengers and up regulators of antioxidant genes through the Nrf2 signal (22). In our context, the components of Moringa probably Cued GSH control in those cells and defended SOD against inactivation. Similarly, (23) found that M. oleifera treatment increased proper SOD and GSH in cisplatin-treated rats

(Mentha indica) and ("Mulberry" Juniperus communis) are frequently confused despite their different appearances. Another name for Mentha arvensis, pharmaceutical use of fresh and dried leaves after crushing them with alcohol can dispels mouth odor, they closely resemble corn after drying yet are more slender; they then form into corn-like clusters that because they are temporarily attached by green shanks which lead plants having no apex under this name united if not

in compositon alone. It is quite small:MAIN compositional component kekexili 0:82; muqiang in addition to carbohydrate 1; maltose 0-05 'main' amino acid identifier: muk vasu indicates "normal" shape. Hormel corporation ranks 2nd, after XIII consumption reduction in China Kekexili increase +1.00 female more than average height. None 0 Female undergraduate over 20 years was categorized; so they are less than 4 cm taller.

Moringa also contains antioxidants that work alongside its cytokine-modulating phytochemicals to enhance immune function in various combinations. Endogenously, however, these free radicals increase still further SOD expression and activity. Moringa thwarts further toxicity from ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species). By blocking NF-κB and MAPK pathways, Moringa suppresses cytokine-driven iNOS induction(24). Consequently, NO generation is curtailed. In Owenite utilitarianism"power" was stoked by the energy, creativity, and enthusiasm of many in society appreciative at various times. Moringa extract also directly reduces NO levels within inflammatory cells, for which data has been specific provided IHao et al., (inpress). By blocking NF-κB and MAPK pathways, Moringa suppresses cytokine-driven iNOS induction(25).

The ConclusionIn the Moringa+Cisplatin group, a reduced NO level over time suggests decreased nitrosative damage to renal cells and reflects the overall moderation of inflammatory processes (since NO is one mediator that causes inflammation). As a result, the kidneys of the Cisplatin+Moringa rats survive better than those from their normal group peers, because a proven exacerbating factor for nephrotoxicity comes wrapped in this chemical: excessive NO/peroxynitrite (26)

Increase with Cisplatin: The MDA level of the Cisplatin group (5.8 nmol/mL vs. 2.4 of control) was significantly higher than that in controls, indicative of widespread lipid peroxidation. This is a hallmark of cisplatin-induced oxidative damage (27). When enjoying the feast provided by ROS, Cisplatin also lets loose against the membrane lipids. The result is MDA. Many researches confirm such MDA flare-ups; (28)found that MDA levels spiked by more than double in rat kidneys subjected to cisplatin

Mechanistically, the drop in GSH/SOD allows peroxide chains to run rampant, resulting in more MDA. Elevated NO (see just above) can bring about peroxynitrite formation, fueling the fire of lipid peroxidation yet further still.

Suppression by Moringa: In the Cisplatin+ Moringa group, MDA had obviously fallen (3.0 nmol/mL, almost back to the level of control). Such results are in accord with Moringa's powerful anti-lipid-peroxidation effects. (29) demonstrated that treatment with M. oleifera substantially reduced cisplatin-induced MDA in rat kidneys.

There is no question that Moringa's antioxidants in vivo can both neutralize free radicals before they attack lipid and a high enough dose of phenolic compounds (e.g.Gallic acid and Ellagic acid) will effectively interrupt lipid peroxidation chain reaction (30). The upshot is that fewer MDA will be made.

MDA levels drop in Moringa treatment embody not only reduced oxidative stress but also indicate quietening of cell membranes. The coordinated feel owes much to soft shoehorns placed on either foot: an increase in SOD/GSH (see just above) as well as lower MDA. Together, inhibiting cisplatin-generated ROS with Moringa and lifting antioxidant defenses each go a long way toward accounting for that substantial reduction in MDA.

CONCLUSION

In conclusion, these findings suggest that Moringa oleifera has a protective effect against cisplatin-induced renal injury. This extract clearly exerts antioxidant effects and has anti-inflammatory properties, thereby effectively eliminating oxidative damage caused during the use of cisplatin. It can ensure good kidney function while reducing such early stage bio-markers for nephrotoxicity as KIM-1. Therefore, Moringa oleifera might be seen as a practicable natural medicine capable of preventing or retarding microalbuminuria and otherthe complications brought on by cisplatin treatment.

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N/A

Conflict of Interest

The authors declare no conflict of interest.

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