International Journal of Pharmacognosy and Clinical Research

ISSN Print: 2664-763X
ISSN Online: 2664-7648
Impact Factor: RJIF 8.00
IJPCR 2024; 6(1): 25-30
www.pharmacognosyjournal.in
Received: 15-11-2023
Accepted: 22-12-2023

Amandeep Swami
Associate Professor, Department of Pharmacology, Sciences, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Dr. Rajesh Asija
Principal, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Ajeet Singh
Research Interns, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Corresponding Author:
Amandeep Swami
Associate Professor, Department of Pharmacology, Sciences, Maharishi Arvind Institute of Pharmacy, Jaipur, Rajasthan, India

Protective effect of ethanolic extract of *Moringa oleifera* root in gentamicin induced nephrotoxicity in mice

Amandeep Swami, Dr. Rajesh Asija and Ajeet Singh

DOI: https://doi.org/10.33545/2664763X.2024.v6.i1a.46

Abstract
Moringa oleifera Lam. is referred as "Miracle tree" in tropics and sub-tropics regions of world. *Moringa oleifera* Lam. is not a big size tree with approximately 4 to 8 m height. It is cultivated all over the world due to its multiple food & pharmaceutical utilities. Every part of Moringa is used for certain nutritional and/or medicinal purpose. Besides being a good source of protein, vitamins, oils, fatty acids, micro-macro minerals elements and various phenolics, it is also reported as anti-inflammatory, antimicrobial, antioxidant, anticancer, cardiovascular, Hepatoprotective, anti-ulcer, diuretic, antiurolithiatic and antihelmintic. This research is oriented to design nephroprotective effect of ethanolic extract of *Moringa oleifera* root in gentamicin induced experimental model. The ethanol extract of roots of *Moringa oleifera* (ERMO) selected for further studies in the present work. The *Moringa oleifera* phenolic compounds like flavonoids, saponins etc., are a major group of components that acts as primary antioxidant or free radical scavengers. The antioxidant activity of n-hexane, ethanol and aqueous extracts of the *Moringa olieifera* was measured by the scavenging activity of DPPH radical, hydroxyl radical (OH.), hydrogen peroxide radical and reactive oxygen species. The acute toxicity studies of moringa extracts were performed by up and down method (OECD 425). No mortality and morbidly were found up to 2000mg/kg body weight in ethanol extracts of *Moringa oleifera*.

Keywords: *Root extract, Moringa oleifera, antioxidant, Gentamicine, Albino mice*

Introduction
The medicinal plant *Moringa oleifera*, which has been used since ancient times for the treatment and cure of several diseases, has been reported to be potent against hematin. Biologists have revealed the valuable medicinal properties and essential nutrients of the *Moringa olieifera* plant. Standardized extraction methods have revolutionized the process, allowing the extraction of bioactive compounds without changing the original composition and structure of the plant. However, there is little literature on the leaf type of *Moringa olieifera* leaves with hematine effect [1, 2]. The aim of this study was to find out the phytochemical diversity of young and old *Moringa olieifera* leaves, effect on body weight, food intake, biochemical nephrological & hematological parameters. Nephrotoxicity is defined as the rapid deterioration of kidney function due to the toxic effects of drugs and chemicals. There are many forms, and some drugs can affect kidney function in more than one way. Various mechanisms cause nephrotoxicity, including renal tubular toxicity, inflammation, glomerular damage, and crystalline nephropathy. Traditional markers of nephrotoxicity and renal failure are blood urea and serum creatinine, which are considered to have low sensitivity for detecting early kidney damage. Thus, new biomarkers that are more sensitive and highly specific, providing insight into the location of the underlying kidney damage, were needed to detect early kidney damage. The Symptoms & signs of damage done to the kidney due to the drugs include: Shortness of breath, excessive fatigue nausea. Gentamicin (GM) is a widely used aminoglycoside antibiotic against serious and life-threatening infections. However, the usefulness of gentamicin is limited by the development of nephrotoxicity. Nephrotoxicity induced by gentamicin is a complex phenomenon leading to the development of an array of morphological and functional alterations, including glomerular lesions that interfere with glomerular hemodynamics, and proximal tubule injury ranging from alterations in tubular transport to tubular epithelium necrosis followed by deterioration to acute renal failure.

Drug-induced nephrotoxicity tends to be more common among certain patients and in specific clinical situations. Therefore, successful prevention requires knowledge of pathogenic mechanisms of renal injury, patient-related risk factors, drug-related risk factors, and preemptive measures, coupled with vigilance and early intervention [3]. *Moringa olieifera* is a valued medicinal plant in traditional folk medicine. *Moringa olieifera*, native to India, grows in the tropical and subtropical regions of the world. It is commonly known as 'drumstick tree' or 'horseradish tree'. *Moringa olieifera* can withstand both severe drought and mild frost conditions and hence widely cultivated across the world. With its high nutritive values, every part of the tree is suitable for either nutritional or commercial purposes. Many pharmacological studies have shown the ability of this plant to exhibit analgesic, anti-inflammatory, antipyretic, anticancer, antioxidant, nootropic, Hepatoprotective, gastro protective, anti-ulcer, cardiovascular, anti-obesity, antiepileptic, antiasthmatic, antidiabetic, anti-urolithiatic, diuretic, local anesthetic, anti-allergic, anthelmintic, wound healing, antimicrobial, immunomodulatory and antidiarrheal properties [4-6]. This review is a comprehensive summary of the phytochemical and pharmacological activities as well as the traditional and therapeutic uses of this plant. *Moringa olieifera* has wide traditional and pharmacological uses in various pathophysiological conditions [7].

Mechanism underlying the anti-inflammatory activity may be attributed to the regulation of neutrophils and c-Jun N-terminal kinase pathway. Active ingredients contributing to anti-inflammatory property are tannins, phenols, alkaloids, flavonoids, carotenoids, β-sitosterol, vanillin, hydroxymellein, moringine, moringinine, β-sitostenone, and 9-octadecenoic acid [8-10]. Mahajan SG *et al*., 2007 investigated the anti-inflammatory activity of isolated compounds with the lipopolysaccharide (LPS)-induced murine macrophage RAW 264.7 cell line. *Moringa olieifera* may also possess some beneficial properties that act against chemically stimulated immune-mediated inflammatory responses that are characteristic of asthma in the rat [11]. Nandave *et al*., evaluated cardio protective effect of lyophilized hydroalcoholic extract of *M. oleifera* in the isoproterenol (ISP)-induced model of myocardial infarction. Chronic treatment with *M. oleifera* demonstrated mitigating effects on ISP-induced hemodynamic [HR, (+) LV dP/dt, (-) LV dP/dt, and LVEDP] perturbations. Chronic *M. olieifera* treatment resulted in significant favorable modulation of the biochemical enzymes (Superoxide dismutase, catalase, glutathione peroxidase, lactate dehydrogenase, and creatine kinase-MB) but failed to express any significant effect on reduced glutathione compared to the ISP control group. *Moringa* treatment significantly prevented the rise in lipid peroxidation in myocardial tissue. Furthermore, *M. oleifera* also prevented the deleterious histopathological and ultrastructural perturbations caused by ISP. Based on the results of the present study, it can be concluded that *Moringa olieifera* extract possesses significant cardio protective effect, which may be attributed to its antioxidant, antiperoxidative, furthermore myocardial preservative properties [11-12]. The present article is taken up to evaluate the ethanolic extract of root of *Moringa olieifera* for nephroprotective activity. The present study has been designed to protective effect of ethanolic extract of *Moringa olieifera* root in gentamicin induced nephrotoxicity in Swiss albino mice according OECD guidelines.

Materials and Methods

Materials

Root of *Moringa olieifera* was collected in central park Jaipur Rajasthan. The samples were authenticated in Department of Botany, Janta PG collage, APS University Rewa MP. The Root of *Moringa oleifera* were cleaned dried and course powdered for ethanolic extracts.The authenticated root of *Moringa oleifera* was extracted using soxlet apparatus at different concentrations ERMO.

Equipments: Equipment used in studies like-digital balance (Shimadzu), UV/Visible Spectrophotometer (UV-PC 180 software), Tissue Homogenizer laboratory, cooling centrifuge (REMI R-8C), Serum analyzer (Inkarp, ES-100), Trinocular Microscope (olmpus), B.O.D incubator (Barath biotech).The selected ethanolic extract of roots of *Moringa olieifera* (ERMO) were subjected to preliminary phytochemical investigation for the presence of secondary metabolites such as alkaloids, glycosides, saponins, tannins, flavonoids, phytosterols, fats/oils, phenolic compounds, by using standard methods of analysis.

Selection of animals: Albino mice either sex weighing 30-35 gm were procured form authenticated breeder in IIRT New Delhi. Around 36 animals was kept at a temperature of 20±2◦C with a 12 hour light/dark cycle and a relative humidity of 50-60%. Free access to food and water will be allowed at all the time. Experiment approval will be taken from IAEC (Institutional Animal Ethical Committee) for investigate nephron protective effect in gentamycin induced nephrotoxicity in Albino mice. The animal ethical committee approval number is IAEC/2024/22/027.

Results

Phytochemical screening of selected plant

The ethanolic extracts of *Moringa oleifera* roots was subjected to preliminary phytochemical investigation for the presence of secondary metabolites such as alkaloids, glycosides, saponins, tannins, flavonoids, phytosterols, fats/oils, phenolic compounds, gums and mucilages by utilizing standard methods of analysis. The phytochemical constituents existing in all the extracts are given the following Table 2.

Table 1: Phytochemical constituents studies of ethanolics plant extracts

S. No	Phytoconstituents	Moringa extract	
		ERMO1	ERMO2
1.	Carbohydrates	-	+
2.	Amino acids	-	+
3.	Glycosides	-	+
4.	Alkaloids	+	+
5.	Tannins	+	+
6.	Triterpenoids	+	+
7.	Saponins	+	+
8.	Steroids	-	-
9.	Flavonoids	+	+
10.	Polyphenols	+	+
11.	Proteins	-	+
12.	Resins	+	-

(+) =Presence of the constituent, (-) =Absence of the constitute.

Acute toxicity studies

Acute toxicity study was carried out as per OECD guidelines for testing of chemicals. The OECD guidelines

425 suggests two types of acute oral toxicity tests, i.e. the limit test and the main test. The limit test is used in situations where the experimenter has information indicating that the test material is likely to be non-toxic. Acute toxicity study is by up and down method adopted by OECD (425, 2008a) to assess the safety of plant extracts selected for the present study. Animals were observed for 14 days (post administration) with special attention for first 4 hours, later all animals were carefully observed for signs and symptoms of toxicity continuously up to 24 hrs. and later up to 14 days. It was observed that all the animals were normal and active within two hours of post treatment. No other sign of toxicity or no mortality was observed and all the animals were survived for 14 days post administration of test drugs. Thus, the dose (2000 mg/kg, P.O) was considered as safe.

Dose selection

The plant extracts were found to be safe at 2gms/kg, the present study was carried out by selecting $1/10^{th}$ of maximum tolerated dose, that is 200mg/kg as low dose, 400mg/kg as moderate dose and 600mg/kg as high dose for ethanolic extracts of *Moringa oleifera* for prophylactic studies and 600mg/kg as a single dose for curative studies.

Effect of ERMO on body weight in gentamicin induced nephrotoxicity

When compared to the disease control, all the prophylactic groups of rats treated with ERMO (group IV,V,VI) and also animals treated with plant extract alone (Group II), showed significant increase in body weight ($p<0.05$). Gentamicin treatment caused a significant reduction in bodyweight in disease control (group III), When compared with the normal animals (group I), which is an indication of renal impairment ($p<0.05$). After inducing nephrotoxicity by administration of gentamicin, treatment with ERMP, curative treatment (group VII), has shown significant increase in the body weight when compared with control group, revealing conservation of renal function. The results are shown in Table 2 and Figure 1.

Table 2: Effect of ERMO on gentamicin induced nephrotoxicity on body weights

S. No	Group	Initial body weights (gms)	Final body weights (gms)	Difference in body weights (gms)
1	Normal	178.2±9.254	198.2±6.535	19.5±2.65
2	Plant control (200 g/kg, P.O)	198.5±6.569[a]	218.5±9.561[a]	18.2±1.365[a]
3	Control (Gentamicin 40mg/kg, I.P)	145.73±2.342[b]	152.0±4.721[b]	6.27±2.379[b]
4	Prophylactic (200 mg/kg, P.O)	174.8±4.241[c]	185.5±5.050[c]	10.6±0.809[c]
5	Prophylactic (400 mg/kg, P.O)	170.0±3.858[c]	182.8±4.001[c]	12.8±0.143[c]
6	Prophylactic (600mg/kg, P.O)	175.2±3.400[c]	189.0±3.832[c]	15.8±0.432[c]

All values are expressed as Mean ± SEM. B indicates $p<0.05$when compared gentamicin control with normal group. A and C indicates $p<0.05$when compared plant control test groups with disease control respectively.

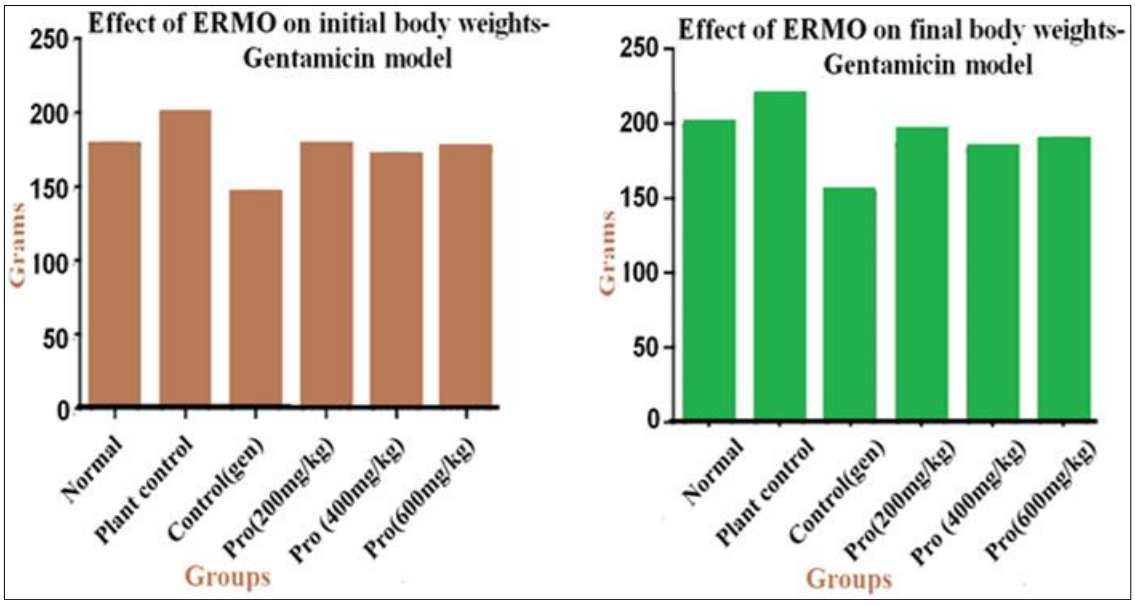

Fig 1: Effect of ERMO on Gentamicine induced nephrotoxicity on body weights

A significant increase in blood urea nitrogen (BUN) levels was observed in control (group II) mice after induce in gnephro toxicity with gentamicin, comparison to the normal group (I) showing that nitrogenous wastes has been accumulated in the disease control groups ($p<0.05$). In prophylactic treatment groups (IV, V, VI) treated with ERMO, there was a significant decrease in BUN levels when compared to the disease control group (III). In the same manner, significant reduction in BUN levels was observed in plant extract treated group (II) when compared to the disease control group ($p<0.05$). A significant inhibition in BUN levels was observed when compared to the control, group (IV) betraying that ERMO is potent enough to decrease the accumulation of nitrogenous wastes ($p<0.05$). The results are shown in table3 and Figure 2.

Table 3: Effect of ERMO on BUN in Gentamicin induced nephrotoxicity

S. No	Groups	ERMO BUN (mg/dl)
1	Normal	13.25±0.214
2	Plant control	12.55±0.345[a]
3	Control(Gentamicin40mg/kg, I.P)	45.18±0.417[b]
4	Prophylactic (Low dose, P.O)	19.64±0.458[c]
5	Prophylactic (Medium dose, P.O)	18.10±0.743[c]
6	Prophylactic (High dose, P.O)	16.32±0.897[c]

All values are expressed as Mean ± SEM. B indicates $p<0.05$when compared gentamicin control with normal group. A and C indicates $p<0.05$when compared plant control test groups with disease control respectively.

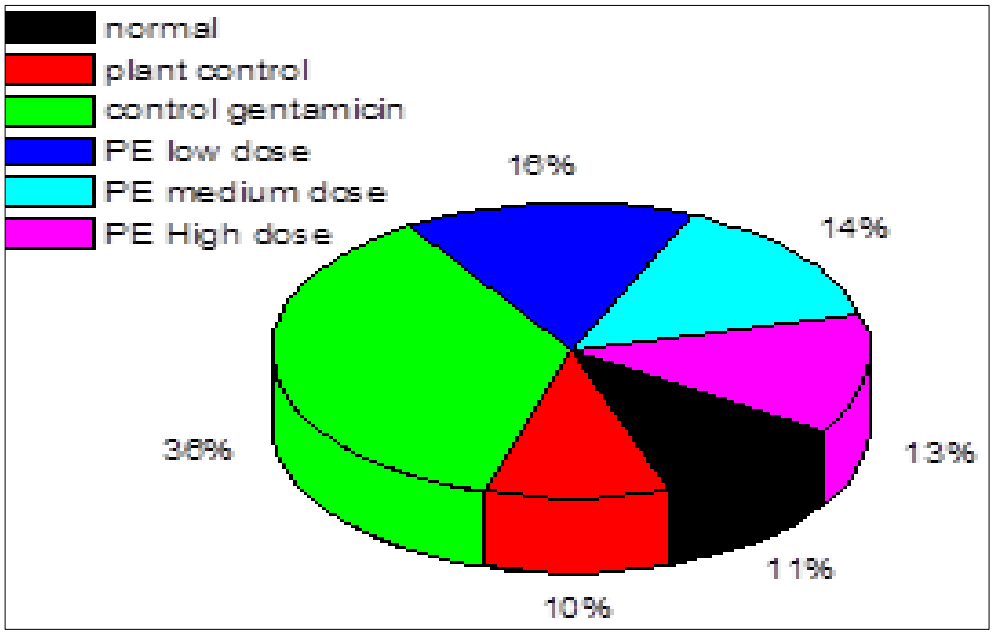

Fig 2: Effect of ERMO on BUN in gentamicin induced nephrotoxicity

Effect of plant extracts on serum creatinine

In prophylactic groups (IV, V, VI), treatment with ERMO for a period of 14 days has shown a significant inhibition in serum creatinine levels when compared to the control group ($p<0.05$). Similarly treatment with plant extract alone (group II) has also shown a significant decrease in serum creatinine levels when compared to the control group ($p<0.05$). Gentamicin induced nephrotoxicity in control (Group II) has shown a significant enhancementinserum creatinine levels when compared to the normal group (I), showing that there is a significant fall in renal physiology. A significant reduction in serum creatinine levels was also shown in curative group (VIII), when compared to control group ($p<0.05$). The results are shown in table 4 and Figure 3.

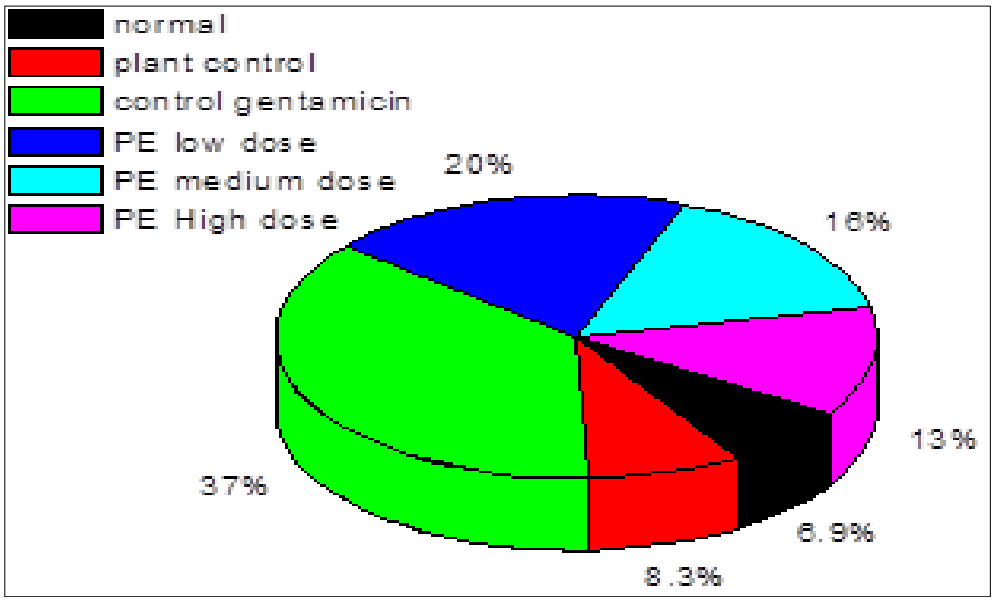

Fig 3: Effect of ERMO on serum creatinine in gentamicin induced nephrotoxicity

Table 4: Effect of ERMO on serum creatinine in gentamicin induced nephrotoxicity

S. No	Groups	ERMO Serum Creatinine (mg/dl)
1	Normal	0.195±0.002
2	Plant control (250mg/kg, P.O)	0.234±0.003[a]
3	Control (Gentamicin40mg/kg, I.P)	1.043±0.038[b]
4	Prophylactic (Low dose, P.O)	0.554±0.009[c]
5	Prophylactic (Medium dose, P.O)	0.4481±0.007[c]
6	Prophylactic (High dose, P.O)	0.354±0.009[c]

Effect of plant extracts on total proteins

Kidney injury induced by gentamicin significantly decreased the serum protein concentrations in the control (Group III) on comparison with the normal group (I) suggesting that it is due to impairment in the kidneys (p<0.05). Pre-treatment with ERMO significantly increased the serum total proteins in prophylactic treatment groups (IV, V, VI) when compared to the control group (III), (p<0.05). Similarly, upon the treatment with ERMO alone (Group II), serum total protein levels also increased significantly when compared to the control (III) reflecting preservation of glomerular function (p<0.05). The results are shown in table 4 and Figure 3.

Table 5: Effect of ERMO on total proteins in gentamicin induced nephrotoxicity

S. No	Groups	ERMO Total proteins (gm/dl)
1	Normal	6.240± 0.02
2	Plant control (250mg/kg, P.O)	6.329 ± 0.05[a]
3	Control(Gentamicin40mg/kg, I.P)	10.71 ± 0.004[b]
4	Prophylactic (Low dose, P.O)	9.26 ± 0.008[c]
5	Prophylactic (Medium dose, P.O)	8.93 ± 0.04[c]
6	Prophylactic (High dose, P.O)	7.013 ± 0.05[c]

All values are expressed as Mean± SEM. B indicates p<0.05when compared gentamicin control with normal group. A and C indicates p<0.05 when compared plant control test groups with disease control respectively.

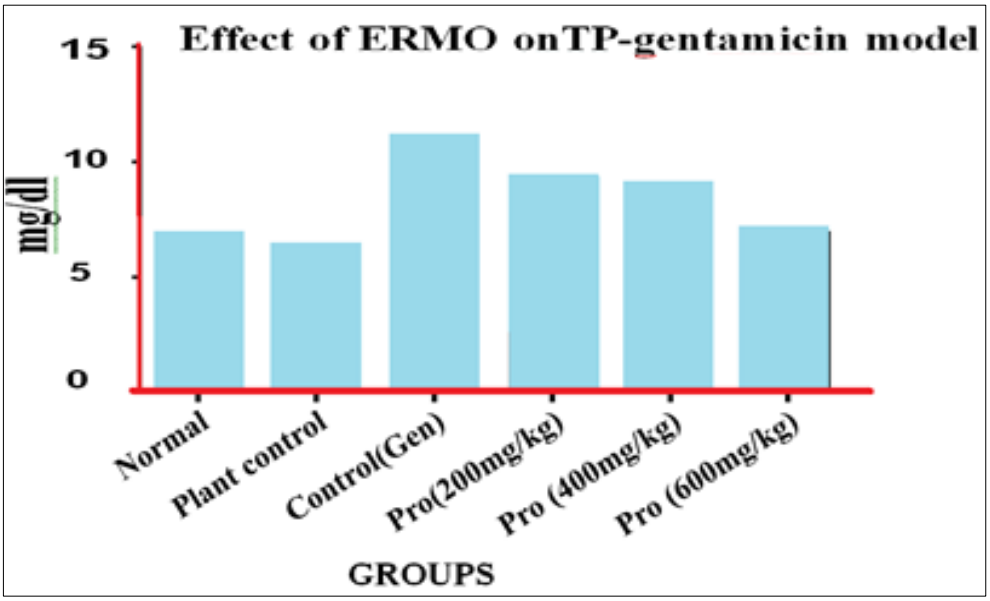

Fig 4: Effect of ERMO on total proteins in gentamicin induced nephrotoxicity

Discussion

The crude extracts of the plants in the present study were tested for the presence of various above mentioned constituents. The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

Phytochemical screening of the plants revealed the availability of some secondary metabolites such as glycosides, alkaloids, triterpenoids, saponins, flavonoids. The phytoconstituents detected are known to have medicinal importance. The antioxidant ability and free radical scavenging properties of plants are associated with their medicinal values. The plant phenolic compounds like flavonoids, saponins etc. are a major group of components that acts as primary antioxidant or free radical scavengers [10]. Many indigenous plants have been reported to have nephroprotective property among which *M. Oelifera* are widely used as nephroprotective agents in acute kidney failure by the traditional practitioners. The renal toxicity induced by gentamicin (40mg/kg, I.P.) in rats was evidenced by the alteration in the serum and urine biomarkers of glomerular and tubular damage. The changes in the biochemical parameters were well correlated with the renal histological score of gentamicin treated mice. From the results obtained in the present study, the ethanolic extracts of *Moringa olieifera* reemployed body weight, exhibited significant decrease in serum creatinine, BUN and

significant increase in serum total proteins, urine volume and clearance of creatinine in urine in both prophylactic and curative groups. This is an indication that extract of roots of *Moringa olieifera* (ERMO) can produce both prophylactic and curative nephroprotection.

Conclusion

This research is oriented to design nephroprotective effect of nuts ethanolic extract of *Moringa oleifera* root in gentamicin induced experimental model. Gentamicin has been suggested to cause renal damage to the kidneys and alter all the functioning of kidneys. It results in vascular resistance and constriction of vascular smooth muscle cells (VSMC) leading to reduced renal blood flow, decreased GFR, and hypoxia of renal tubular cells, leading to kidney damage. This research focuses on therapeutic effects of ethanolic extract of *Moringa olieifera* root on the kidney diseases. Since, it presents herbal drug and natural resources favorable to adjuvant treatment in addition to the conventional treatment process. Acute oral toxicity test was performed to find out the safe dose of test extract before going to *in-vivo* evaluation. Acute toxicity studies of test extracts carried out in rats and were found safe up to 2000mg/kg. The maximum tolerated dose (MTD) of the test extracts was found to be >2000mg/kg in rats. The nephroprotective activity of moringa ethanolic extract was designed to carry out for 21days in gentamicin nephrotoxicant model. The dose of 40mg/kg Gentamicin was used as the nephrotoxicant for 7 days. Its nephrotoxicity was evident by the significant increase in the levels of serum creatinine, BUN, total proteins, whereas the levels of GSH, SOD, and catalase and urine volume was significantly decreased and the histopathology findings confirmed the tissue damage.

Acknowledgement

I wish to express my deep gratitude and respects Principal Sir Dr Rajesh Asija Maharishi Arvind Institute of Pharmacy, Jaipur Rajasthan, India for his keen interest and valuable guidance, strong motivation and constant encouragement during the course of the work. I thank him for his great patience and extensive discussions around my work.

References

1. Sattar S, Hasan MJ, Ray NC, Quddus ASMR. Non-steroidal anti-inflammatory drugs induced acute kidney injury: Incidence, clinical presentation, management and outcomes of patients at Tertiary Hospital based longitudinal study in Mymensingh, Bangladesh. Community Based Med J. 2018;7(2):9-16.
2. Stojiljkovic N, Veljkovic S, Mihailovic D, Stoiljkovic M, Radenkovic M, Rankovic G, *et al*. Protective effects of pentoxifylline treatment on gentamicin-induced nephrotoxicity in rats. Ren Fail. 2009;31(1):54-61.
3. Naughton CA. Drug-induced nephrotoxicity. Am Fam Physician.2008;78(6):743-50.
4. Bhattacharya A, Naik MR, Agrawal D, Rath K, Kumar S, Mishra SS. Anti-pyretic, anti-inflammatory, and analgesic effects of leaf extract of drumstick tree. J Young Pharm. 2014;6:1-5.
5. Dilard CJ, German JB. Phytochemicals: Nutraceuticals and human health: A Review. J Sci Food Agric. 2000;80:1744-56.
6. Awanish P, Rishabh DP, Poonam TP, Gupta JH, Saumya BA. *Moringa oleifera* Lam. (Sahijan)-a plant with a plethora of diverse. Therapeutic benefits: An updated retrospection. Med Arom Plants. 2012;1:1-8.
7. Amrutia JN, Lala M, Srinivasa U, Shabaraya AR, Semuel MR. Anticonvulsant activity of *Moringa olieifera* Leaf. Int Res J Pharm. 2011;2:160-2.
8. Mehta J, Shukla A, Bukhariya V, Charde R. The magic remedy of *Moringa oleifera*: An overview. Int J Biomed Adv Res. 2011;2:215-27.
9. McKnight M, Allen J, Waterman JD, Hurley S, Idassi J, Minor RC. Moringa tea blocks acute lung inflammation induced by swine confinement dust through a mechanism involving TNF-α expression, c-Jun N-terminal kinase activation and neutrophil regulation. Am J Immunol. 2014;10:73-87.
10. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J Agric Food Chem. 2003 Apr 9;51(8):2144-55. DOI: 10.1021/jf020444
11. Mahajan SG, Mali RG, Mehta AA. Effect of *Moringa oleifera* Lam. Seed extract on toluene Diisocyanate-Induced immune-mediated inflammatory responses in rats. Journal of Immunotoxicology. 20074(2):85-96.
12. Nandave M, Ojha SK, Joshi S, Kumari S, Arya DS. *Moringa oleifera* leaf extract prevents isoproterenol-induced myocardial damage in rats Evidence for an antioxidant, antiperoxidative, and cardio protective intervention. J Med Food. 2009;12(1):47-55.
13. Effect of ethanolic and aqueous extracts of *Bauhinia variegata* Linn on gentamicin-induced nephrotoxicity in rats. Ind J Pharm Edu Res. 2011;45(2):192-197.