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Nephroprotective Evaluation of *Moringa oleifera* & *Beta vulgaris* Leaves in Murine Methotrexate Induced Toxicity

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ABSTRACT

Introduction: Methotrexate has been used for treatment of multiple diseases. Chronic use of this drug can result in oxidative damage resulting in nephrotoxicity. *Beta vulgaris* and *Moringa oleifera* are well known herbs for their antioxidant properties, so they can have potential nephroprotective effect against MTX induced renal damage. **Aims & Objectives:** The study was designed to evaluate the therapeutic role of *Moringa oleifera* and *Beta vulgaris* leaves against MTX induced nephrotoxicity in rats. **Place and duration of study:** Time duration of our research project was 6 months. It was carried out in PGMI Lahore and FPGMI, Shaikh Zayed Medical Complex, Lahore. **Material & Methods:** 5 groups of 9 rats each were made. Group1 were maintained as healthy controls while Group II as diseased control Groups III, IV and V were administered daily p.o for 24 days 200mg /kg of Ethanolic Extract of Beta Vulgaris(EEBV),and 800 mg/kg Ethanolic Extract of *Moringa oleifera* (EEMO) and combination of extracts (100mg/kg EEBV+400mg/kg EEMO respectively. Nephrotoxicity was produced on 21st day via single i/p route injection of 20mg/kg MTX in all groups except Group 1. RFTs were performed on days 1, 21 and 24th day. Nephroprotection was determined by measuring serum urea, creatinine along with histopathological study of kidneys at study end. **Results:** EEBV provided significant neproprotection in group 3 which had significantly lower levels of creatinine as compared to group 2 with p-value <0.001. The groups 4 and 5 had nonsignificant difference from group 2 with p-values 0.975 and 0.252. Further confirmation by histopathological examination of kidneys revealed group 3 to have improved status of kidney(normal kidney epithelium, normal tubules, mild congestion, mild necrosis) as compared to other groups with (distorted kidney epithelium ,dilated tubules ,moderate to severe congestion and necrosis). **Conclusion:** Nephroprotective effect was shown by *Beta vulgaris* plant while *Moringa oleifera* plant extracts remained unable to show their protection against MTX induced nephrotoxicity.

Key words: Ethanolic extract of *Beta vulgaris* (EEBV), Methotrexate, Ethanolic extract of *Moringa oleifera* (EEMO), Renal function tests (RFTs).

INTRODUCTION

The kidneys are important organs with many functions in the body including metabolism, body water and salts regulation, acid base regulation and waste products excretion along with excretion of hormones. Therefore, they are often at risk to damage caused by infections, harmful substances, prescribed and OTC drugs.[1]

Many drugs are responsible for acute nephrotoxicity. 20 % of all acute Renal Failure cases are caused by drugs.[2] Most drugs cause nephrotoxicity through different pathogenic mechanisms including, tubular cell damage, inflammation, crystal nephropathy. Commonly responsible drugs are NSAIDS, aminoglycosides, ACE inhibitors and amphotericin B.[3]

Methotrexate is a folic acid antagonist. It has multiple uses like blood dyscrasias, inflammatory diseases of joints, skin disorder and inflammatory bowel disease. Toxic dose and prolonged use of methotrexate can lead to nephrotoxicity. Despite all advantages with MTX, their extensive use is still reduced due to their adverse effects. Leucovorin, being antidote for methotrexate toxicities can save rapidly dividing cells only like gastrointestinal and hematopoietic cells. However, it is still unable to give protection against other adverse effects of MTX.[4]

Oxidative stress is responsible for MTX induced toxicities within kidney. The main reason behind this toxicity is low NADPH, which is essential for glutathione synthesis. Ultimately cells are more exposed to ROS (reactive oxygen species).[5]

Different plants have shown protective effect against different toxicities. Swiss *chard* (variety of BV) has shown nephroprotective effect in diabetic rats.[6] *Moringa oleifera* has proven to have nephroprotective activity against DMBA (7,12-dimethylbenz-anthracene) induced renal carcinogenesis.[7]

Taking into account the protecting effect of different herbs, *Moringa oleifera* and Beta vulgaris were chosen. *Beta vulgaris* leaves are useful as tonic, diuretic, anti-inflammatory.[8] *Moringa oleifera* is another herb, which has multiple uses like GIT diseases, diabetes mellitus and heart disorders.[9]

Initial phytochemical analysis has shown that leaf extracts of *Moringa oleifera* and *Beta vulgaris* contain various phytochemicals like tannins, flavonoids, alkaloids.[8,9] Being known antioxidants, these active principles may have protective role in management of MTX induced toxicities. So, the purpose of our study was to evaluate protective effect of these plants.

MATERIAL AND METHODS

Plant Materials: Authenticity of leaves of *Moringa oleifera* and *Beta vulgaris* was done by Botany Department of Punjab University Lahore. Ethanolic extracts of these herbs were made in PCSIR (Pakistan Council of Scientific & Industrial Research) Lahore.

The powdered form of *Beta vulgaris* leaves were extracted with 90% ethanol using Soxhlet apparatus. The material left after extraction was macerated with distilled water for 24 hours. Solvents were distilled off under reduced pressure and temperature to get greenish paste of EEBV.[8]

Absolute alcohol was mixed with powder form of *Moringa oleifera* leaves extract at room temperature. After evaporation of concentrated form, greenish paste was obtained.[10]

Animals: 45 rats weighing 150-200g were kept in iron cages under controlled room temperature (25±10 ◦C). Rats were provided with typical lab diet.

Settings: Animal House **&** Research laboratory of PGMI Lahore.

Grouping: Rats were randomly divided into groups of 9 animals. Extracts *of Moringa oleifera, Beta vulgaris* or their combination were administered daily orally throughout entire study period of 24 days as per group designation. On 21st day single injection of methotrexate was given through i/p route in all groups except group 1 to induce toxicity.
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Group 1 (negative, healthy control): The animals in this group were administered water only.

Group 2 (positive control): Single injection of MTX 20mg/kg i/p was administered in rats of this group on 21st day to induce nephrotoxicity.

Group 3 (Test): *Beta vulgari*s leaf ethanolic extract of 200 mg/kg/p/o

Group 4 (Test): Ethanolic extract of *Moringa oleifera*'s leaves in dose of 800mg/kg p/o.

Group 5 (Test): Combined EEBV 400 mg/kg and EEBV 100 mg/kg po.

Biochemical Parameters: On day 1 and 21, blood for analysis of biochemical parameters was collected from tail of rats.

3 cc syringes were used for collection of blood by intracardiac puncture on 24th day, RFTs were analyzed on chemistry analyzer in Biochemistry Department of Sheikh Zayed Hospital, Lahore.

Histopathology of kidneys: On 24th day animals were sacrificed and kidney samples was sent for histopathological examination Kidneys tissues samples were isolated after dissection, stained with haematoxylin and Eosin .Slides were prepared and examined under microscope.

Statistical analysis:

Data was evaluated by using SPSS (statistical package for social sciences) 20.0. Renal parameters like urea and creatinine were elaborated by using mean ±S.D. ANOVA was applied for comparison among groups. P value<0.001 was considered significant for all parameters.

RESULTS

The mean urea level for group 1 was 21.6±6.0 mg/dl, the group 2 had levels 91.6±14.9mg/dl and group 3, 4 and 5 had their mean urea levels at 98.1±13.0mg/dl, 81.1±18.6mg/dl and 91.3±15.3mg/dl respectively

Here the group, 2, 3, 4 and 5 all had significantly higher levels as compared to group1 with p-values <0.001. The group 2 had nonsignificant difference from group 3, 4 and 5 with p-values 0.863, 0.530 and 1.000 respectively. The group 3 and 4 had an nonsignificant difference with p-value 0.102 and other differences between experimental groups were also nonsignificant (Fig-A).

Group 1 had mean creatinine level of 0.39±0.11mg/dl which was lowest and group 5 had 0.87±0.05mg/dl which was highest. The group 2, 3 and 4 had creatinine levels of 0.79±.08mg/dl, 0.50±0.07mg/dl and 0.81±0.08 mg/dl.

Group 1 had significantly lower creatinine levels as compared to all other groups with p-values <0.001.

The group 4 and 5 had nonsignificant difference from group 2 with p-values 0.975 and 0.252 but group 3 had significantly lower levels as compared to group 2 with p-value <0.001. The group 3 also had significantly lower levels as compared to group 4 and 5 with p-values <0.001. The difference between group 4 and 5 was nonsignificant with p-value 0.581 (Fig-B).

Histopathological study of kidneys in group 2 showed detached epithelium, dilated tubules, congestion and necrosis ,showing damage of kidney by MTX (Fig 2),as compared to group 1(normal group) similar damage was observed in groups 4 and 5 (Fig-4,5) while group 3 showed improved kidney status as compared to other groups (Fig-3)

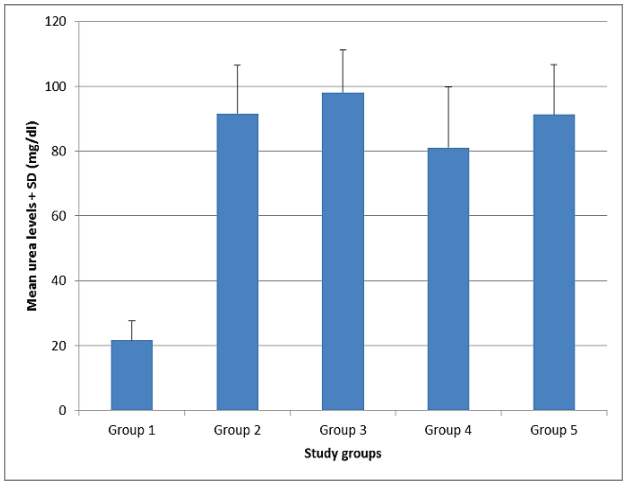

Fig- A: Mean urea levels of five study groups at 24th day

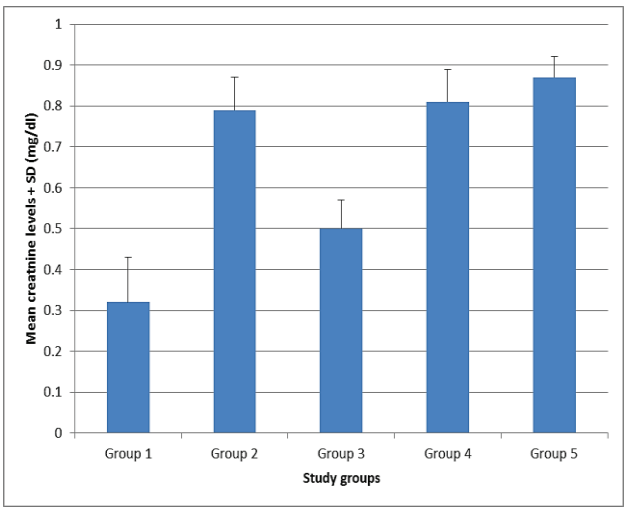

Fig-B: Mean creatinine level in five study groups at 24th day

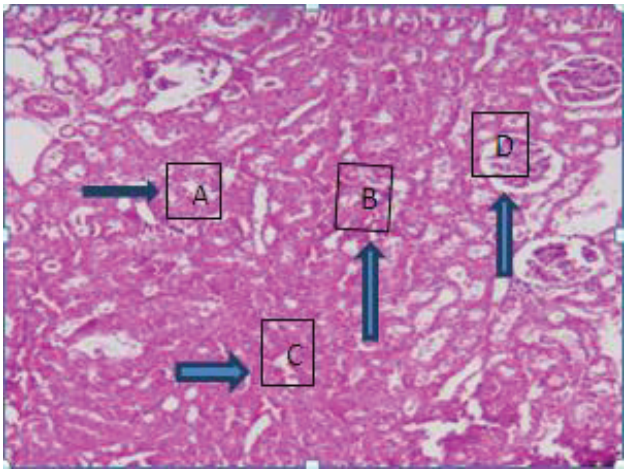

Fig-1: Showing normal histology of kidney in group 1 (negative control)

A. Normal kidney epithelium B. No congestion,
C. No tubular dilatation D. No necrosis

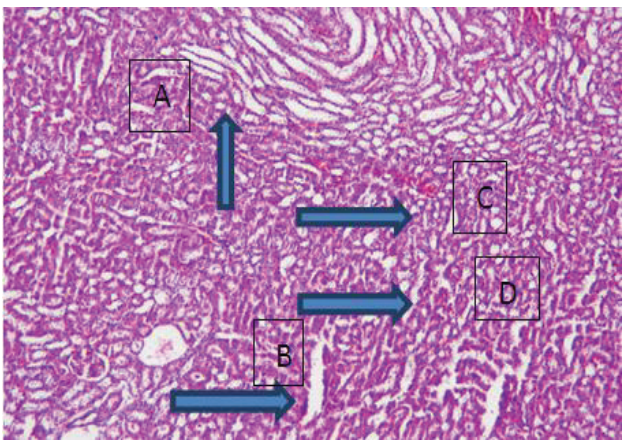

Fig-2: Showing Kidney damage by MTX in group 2

A. Distorted kidney epithelium B. Dilated tubules,
C. Congestion D. Necrosis

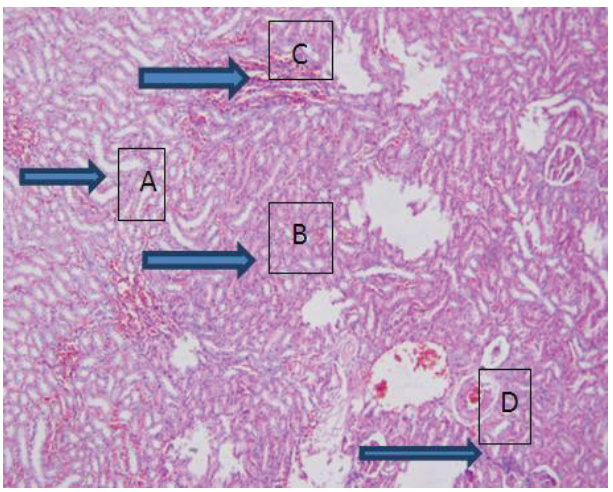

Fig-3: Showing protective effect on kidney tissue in group 3 received extract of *Beta vulgaris* showing

A. Normal kidney epithelium B. Normal tubules
C. Mild congestion D. Mild necrosis

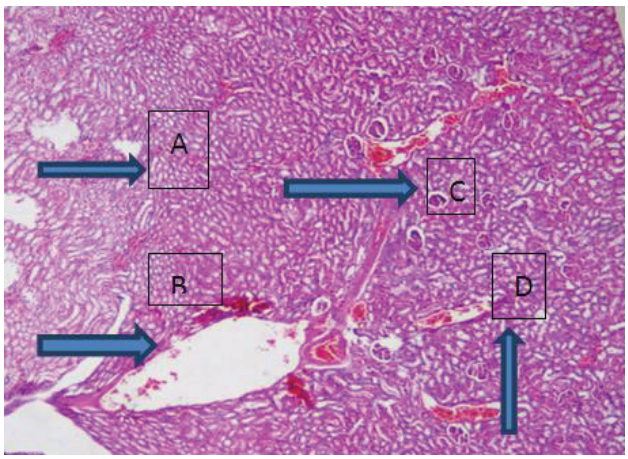

Fig-4: Showing damage of kidney in group 4 received extract of MO

A. Distorted kidney epithelium B. Dilated tubules,
C. Moderate to severe congestion D. Necrosis

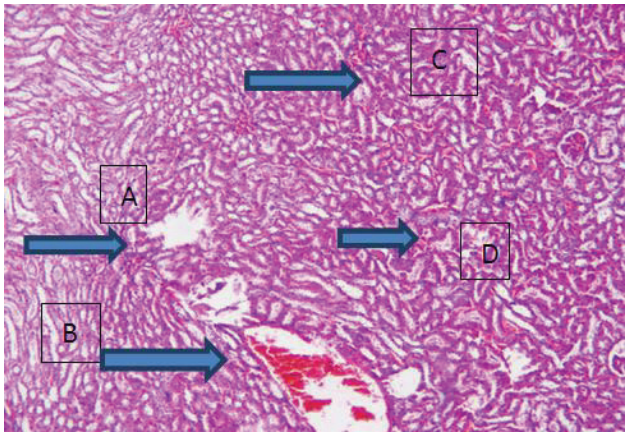

Fig-5: Showing damage of kidney in group 5 received combined extract of BV and MO

A. Distorted epithelium B. Dilated tubules
C. Moderate to severe congestion D. Necrosis

DISCUSSION

Drugs like MTX can affect multiple organs, it is employed for management of various diseases like inflammatory diseases of joints, GIT, blood cancers and skin diseases. Short and long-term use of drug has caused many adverse effects, which have been observed in many studies.[4]

MTX can affect multiple organs e.g bone marrow, GIT mucosal cells and hair follicles. Leucovorin being MTX antidote cannot rescue all adverse effects.[11] Phytotherapy is attaining increasing consideration. *Moringa oleifera* and *Beta vulgaris* herbs exhibited variety of active principles like flavonoids, alkaloids, saponins, tannins. These plants were used for the treatment of multiple diseases.[8,9] Main purpose of our research was to appraise these plants for their nephroprotective activity against MTX in rats.

At days 1 and 21 renal function test revealed values within normal range in comparison to group 1. This reflected safety of these herbal drugs if used as future potential medicines.

In our project, we have found that group 2 (MTX) has shown (405% & 133% increase of serum urea and creatinine in rats, reflecting lethal effects of MTX on kidney. Results were as expected.[12]

Histopathological examination of kidney samples from group 2 showed (kidney architecture damage, congestion, necrosis, tubular dilatation) in comparison to group 1 (Fig-2). Previous studies also demonstrated same results.[13]

At end of our project, we have observed that group 2 (MTX) showed (333% increase in urea level in comparison to group 1) and when we compared it with urea levels of groups 3, 4 and 5, we found out that there was no significance difference from urea levels (366%, 285%, 333%) observed in groups 3, 4 and 5, showing our plant extracts remained unsuccessful in reverting nephrotoxicity induced by MTX. However urea is a non-specific test renal functions.[15] Its level can also be raised because of other causes like dehydration[16], heart failure[17], GIT bleeding [18] so it is not a confirmatory indicator of failure of nephroprotection of our plant extracts.

At the end of study, mean creatinine level of group 1 was lowest 0.39±0.11 and group 5 had highest level 0.87±0.05. Nephrotoxicity was not reversed in groups 4 and 5 in terms of increase of creatinine levels (166% and 167%) as compared to group 2(MTX) which has shown 133% increase in creatinine level in comparison to group 1. However group 3 showed 66% elevation of creatinine level which was less as compared to group 2 (133%), reflecting nephroprotective effect of this extract. Nephroprotective effect of beet root extract against gentamicin induced nephrotoxicity seen in this study also favors our results[14].

As we detected in our project, nephrotoxicity was not reversed in two groups, 4 (EEMO) and 5 (EEBV+EEMO), rather it came out to be worst, studies in past exposed that when MTX is co-administered with certain drugs, their interactions might increase toxicity of MTX by competing with MTX for renal tubular secretion.[19] This mechanism might elucidate failure of nephrotoxicity reversal in our study.

Histological evaluation further supported our result, where status of histopathological changes (kidney epithelium, tubules, congestion, necrosis) was better in group 3 (EEBV) as compared to groups 4 and 5 (Fig-3,4,5) with (distorted kidney epithelium, dilated tubules,moderate to severe congestion and necrosis).

CONCLUSION

Nephroprotective effect was shown by *Beta vulgaris* plant while MO plant extracts remained unable to show their protection against MTX induced nephrotoxicity. This parameter should be further evaluated.

REFERENCES

1. Kumar V, Abbas A.K, Fausto N, Mitchell RN. Robbins basic pathology: 10thed. Delhi: Elsevier 2017.
2. Shahrbaf FG, Assadi F. Drug induced renal disorder.2015; 4(3):57-60.
3. Noilin TD, Himmelfarb J. Mechanism of drug Induced Nephrotoxicity. Handb Exp Pharmacol 2010; (196):111-30.
4. Harvey RA, Champe PC and William L. Lippincott illustrated Reviews Pharmacology. 6th ed Delhi: Wolters Kuwer 2015; 592-4.
5. Sener G, Demiralpe E, Cetiner M, Erean F, Yagen B. Beta glucan ameliorates MTX induced oxidative organ injury via its antioxidants and immunomodulatory effect. European J of Pharma 2006; 542(13):170-8.
6. Bolkent S, Yanardaq R, Saren O Bulan K. Effect of chard on kidney tissue, serum urea and creatinine levels of diabatic rat.2002;16(8):758-61
7. Paliwal R, Sharma V, Pracheta, Sharma S, Yadev S. Anti nephrotoxic effect of administration of DMBA induced renal carcinogenesis in swiss albino mice. Bio Medicine. 2011; 3(2):27-33.
8. Jain N, Singhai AK. Protective role of Beta vulgaris leaves extract and fractions on ethanol mediated hepatotoxicity. ACTA Poloniae Pharma Drug Res 2012; (69):945-50.
9. Mehta J, Shukla A, Bukhariya V, Charde R. The magic remedy of Moringa oleifera: an overview. International J Bio and AdvanR.2011;2(6):215-27
10. Yousaf FM, Khattab HR, Sindhi HA. Effectiveness of Moringa oleifera leaves Extract Against Methotrexate induced Acute Hepatotoxicity in Male Rats. International J Pharm. 2018; (14):1029-1037.
11. B G Ketzung, Susan B, Anthony J: 14th ed. New Dehli 2017; 957-58.
12. Kintzel PE, Anticancer drug induced kidney disorder 2010; 24(1):19-38.
13. Asci H, Ozer M.K, Calapoglu M, Savran M, Once M. Effect of misoprostol on methotrexate induced hepatic and renal damage. J Bio Life Sci 2011; 2(1):32-7.
14. ElGamal A, Alsaid M, Raish, Sohaibani MA, h. Beet root ameliorates gentamicin induced nephrotoxicity associated oxidative stress, inflammation and apoptosis in rodent model 2014:p12.
15. Higgins C. Urea and the clinical value of measuring blood urea concentration.2016:P1-6
16. Witting MD. ED predictors of upper gastrointestinal tract bleeding in patients without hematemesis. Am J Emerg Med 2006; 24(3):280-85.
17. Aronson D. Elevated blood urea nitrogen level as a predictor of mortality in patients admitted for decompensated heart failure. The Am J Med 2004; 116(7): 466-73.
18. Mehta AR. Why does the plasma urea concentration increase in acute dehydration? Advances in physiology education. 2008; 32(4):336.
19. Brigitte C, Widermann P, C Adamson. Understanding and managing methotrexate induced nephrotoxicities 2006; 11(6):694-703.

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