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Beneficial Effect of Extracts of *Momordica charantica, Emblica officinalis, Tribulus terrestris* and *Trigonella foenum Graecium* in Combination against Streptozotocin Induced Diabetic Nephropathy

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Abstract
Present study evaluates the synergistic effect of *Momordica charantica, Emblica officinalis, Tribulus terrestris* and *Trigonella foenum graecium* in combination on the streptozotocin (STZ) induced diabetic nephropathy (DN) rats. Diabetes was induced in all the animals by single intraperitoneal (i.p.) injection of STZ at a dose of 55 mg/kg. Animals were treated STZ + MC + EO group which receives STZ + *Momordica charantia* (250mg/kg) + *Emblica officinalis* (250mg/kg); STZ + MC + EO+ TT group which receives STZ + *Momordica charantia* (250mg/kg) + *Emblica officinalis* (250mg/kg) + *Tribulus terrestris* (250mg/kg); STZ + MC + EO+ TT+TF group which receives *Momordica charantia* (250mg/kg) + *Emblica officinalis* (250mg/kg) + *Tribulus terrestris* (250mg/kg) + *Trigonella foenumgraceium* (250mg/kg); STD group which receives lisinopril 1 mg/kg for the duration of 28 days. Effect of MC+EO+TT+TFG was determine by estimating blood glucose level and body weight on 1st, 14th and 28th day of protocol. Biochemical parameters of renal function, level of oxidative stress and histopathology was also determined for the estimation of synergistic effect of MC+EO+TT+TFG treatment on the STZ induced DN rats. Result of the study suggests that treatment with MC+EO+TT+TFG ameliorates the altered level of blood glucose and body of STZ induced diabetic nephropathy. Moreover, level of parameters of oxidative stress and renal function was also found to be attenuated in MC+EO+TT+TFG treated STZ induced diabetic nephropathy rats. Histopathology study also suggests that TS of kidney tissues of MC+EO+TT+TFG treated rats show improvement compared of DN rats. In conclusion, data of the study reveals that treatment with MC+EO+TT+TFG in combination shows potential effect for the management of DN by reducing oxidative stress.

Keywords
Diabetic nephropathy; *Momordica charantica*; *Emblica officinalis*; *Tribulus terrestris*; *Trigonella foenum graecium*; oxidative stress

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INTRODUCTION

Diabetes is a metabolic disorder characterized by increased blood glucose level due to alteration in the secretion of insulin and its action. Literature suggests that approximately 422 million people in 2014 were suffering from diabetes throughout the world which may rise upto 629 million by 2045 [1]. Uncontrolled diabetes leads to development of many complications includes diabetic neuropathy, gastroparasis, retinopathy and nephropathy [2]. Diabetic nephropathy is a chronic complication of diabetes which leads to kidney failure. Nephropathy is reported to develop in 30–40% of patients with diabetes and has become a leading cause of end stage renal failure worldwide [3]. There are several markers of nephropathy may leads to altered in the patient suffering from DN such as urinary albumin excretion along with accumulation of extracellular matrix, thickening of basement membranes, mesangial expansion, hypertrophy and glomerular epithelial cell (podocyte) loss within the glomeruli [4]. There are several conventional treatments available for the management of DN or renal failure. These molecules (synthetic) has its own limitation and long-term use of these medicine may causes further decline in the renal function [5]. Thus, there is a need of development of alternative medicine for the management of nephropathy. Moreover, WHO also recommended that there is need of investigation on herbal medicine for the management of diabetes and its complication.

In the recent few decades herbal and alternative medicine has shown in the potent effect for the management chronic disorder. Amongst the different medicinal herbs; commonly used four herbs are Momordica charantica, Emblica officinalis, Tribulus terrestris and Trigonella foenum graecium. Momordica charantica improves the glucose metabolism and thus imparts a hypoglycemic effect in the diabetic patients [6-7]. Moreover, it has shown potent cholesterol and lipid lowering property with added anti-cancer and cytotoxic effect. Emblica officinalis which is popularly known as Alma reported for several therapeutic effects including those of usage in cardiovascular disorders, liver dysfunction, and inflammation [8]. Tribulus terrestris (Zygophyllaceae) is another herb known for its hypotensive effect and activity against angina pectoris and cardiac depressant [9]. It has been shown to increase the free serum testosterone and to be effective in the treatment of sexual and erectile dysfunction by conversion of its phytochemical derivative, protodioscin to De Hydro Epi Androsterone (DHEA). Fenugreek (Trigonella foenum graecum) is one of the oldest medicinal plants originating in India also reported to have strong antioxidant and antidiabetic property. The concept of polyherbal formulation is well documented in the ancient literature compared to the single herb. Thus, present investigation evaluates the synergistic effect of extract of Momordica charantia, Emblica officinalis, Tribulus terrestris and Trigonella foenum gracumalone for the management of STZ induced DN rats.

MATERIALS AND METHODS

Preparation of Extraction

The whole plant of *Emblica Officinalis, Tribulus terrstris, Trigonella foenum gracum and Momordica charantia* were obtained from local market and a sample of each was stored in the Museum of Department of Pharmacology, Dayanand Medical College and Hospital, Ludhiana. Coarse powdered drug (5 kg) was placed in closed vessel and macerated in hydro alcohol (1:1) for seven days with occasional shaking. Then filtration is done to get the clear solution. Remained solid residue was pressed again to get occluded solution. Filtered and pressed solution was then mixed and allowed to concentrate under reduced pressure so as to get the semisolid extract. All the standardized extracts were properly dried, reduced to fine powder and the powders were sieved through 80 mesh sieves separately. Percentage yield of *Emblica Officinalis, Tribulus terrstris, Trigonella foenum gracum and Momordica charantia* was found to be 12.4% w/w, 9.6% w/w, 13.2% w/w and 12.9 % w/w respectively.

Animals

Male Wistar rats (200-300gm) were used for the pharmacological screening. These rats were housed in polypropylene cages with wire mesh top and husk bedding and maintained under standard environmental conditions (25 ± 20° C, relative humidity 60 ± 5 %, light- dark cycle of 12 hours each) and fed with standard pellet diet and water ad libitum. The rats were housed and treated according to the rules and regulations of CPCSEA and IAEC. The protocols for all the animal studies were approved by the Institutional Animal Ethics Committee (IAEC). CPCSEA registration no: 816 / PO / a / 04 / CPCSEA / MS / 165**.**

Pilot Study

A pilot study was conducted in our lab to determine the anti diabetic potential of extracts of *Emblica officinalis* (100 and 250 mg/kg)*, Tribulus terrstris* (100 and 250 mg/kg)*, Trigonella foenum gracum* (100 and 250 mg/kg) *and Momordica charantia* (100 and 250 mg/kg). Study has shown that extract of all the herbs with 250 mg/kg p.o has shown the potent antidiabetic effect. Thus for further investigation all the plant extracts were used at a dose of 250 mg/kg.

Induction Diabetic Nephropathy

Diabetes was induced in all the animals by single intraperitoneal (i.p.) injection of Streptozotocin (STZ, 55 mg/kg) (Cam et al., 2003). STZ was dissolved in freshly prepared 0.1 M citrate buffer (pH 4.5). The age-matched control group of rats received citrate buffer. Diabetes was confirmed after 48 h of STZ injection. Blood samples were taken from retro-orbital sinus for various estimations and the animals having blood glucose level more than 200 mg/dl were selected for the further study.

All the animals were separated in to six different groups such as control group; diabetic control group which receives saline solution; STZ + MC + EO group which receives STZ + *Momordica charantia* (250mg/kg) + *Emblica officinalis* (250mg/kg); STZ + MC + EO+ TT group which receives STZ + *Momordica charantia* (250mg/kg) + *Emblica officinalis* (250mg/kg) + *Tribulus terrestris* (250mg/kg); STZ + MC + EO+ TT+TF group which receives *Momordica charantia* (250mg/kg) + *Emblica officinalis* (250mg/kg) + *Tribulus terrestris* (250mg/kg) + *Trigonella foenumgraceium* (250mg/kg); STD group which receives lisinopril 1 mg/kg for the duration of 28 days. Body weight and blood glucose level were observed after each week of drug treatment and percentage change in body weight was calculated for each group.

Estimation of Biochemical Parameter

Renal function was assessed by determining the concentration of serum creatinine, total protein and BUN in blood of all the animals. Level of serum creatinine, total protein and BUN was estimated using auto analyzed and the instruction given by the manufacturer for the kits. The urinary creatinine clearance was estimated by alkaline picrate method (Meyer et al., 1985, Toora and Rajagopal, 2002). The alkaline pirate reacts with creatinine to form orange colour complex. The absorbance of test and standard sample were noted against the blank at 520 nm spectrophotometerically.

Estimation of Parameters of Oxidative Stress

The amount of malondialdehyde (MDA), a measure of lipid peroxidation, was measured by reaction with thiobarbituric acid at 532 nm using a Perkin Elmer lambda 20 spectrophotometer. Reduced glutathione (GSH) in renal tissues was estimated according to the method described by (Ellman, 1959). Ellman's reaction was used for the estimation of plasma level of GSH and absorbance was determined at 412 nm. The accumulation of nitrite in the renal tissues supernatant, an indicator of the production of nitric oxide (NO), was determined by a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) ethylenediaminedihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by (Green et al., 1982). Equal volumes of supernatant and Greiss reagent were mixed, and this mixture was incubated for 10 min at room temperature in the dark. Absorbance at 540 nm was measured with a Shimazdu spectrophotometer.

Histological Studies

The kidney was excised and immediately immersed in 10% formalin. The kidney was dehydrated in graded concentrations of alcohol, immersed in xylene and then embedded in paraffin. One portion of kidney tissues was fixed in 10% buffered formalin and embedded in paraffin for a light microscopic study. The kidney tissue sections (4 μm) were stained with periodic acid-Schiff reagent (PAS) and examined under a light microscope. The degrees of mesangial matrix expansion in different groups of rats were determined as PAS-positive staining in the mesangial region excluding cellular elements. Morphological changes in glomeruli were assessed under microscopy.

Statistical Analysis

Results were expressed as Mean ± SD (n=6). The results were analyzed by using Graph pad prism software. Statistical analysis was carried out for One-way analysis of variance (ANOVA) followed by Dunnett's test. Minimum significant value was set as $P \leq 0.05$.

RESULTS

Effect of MC, EO, TT, TFG treatment on the serum glucose and body weight

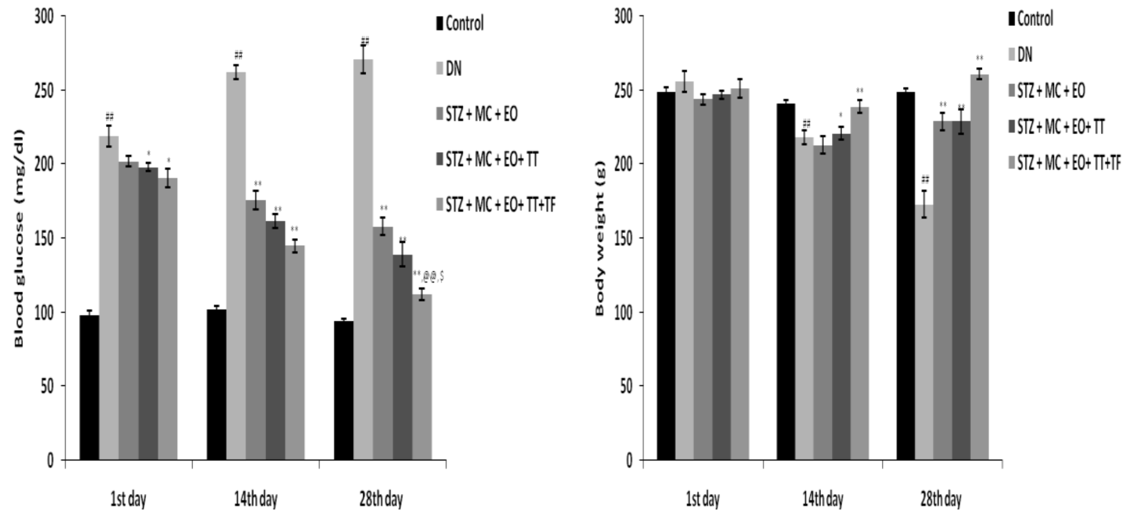
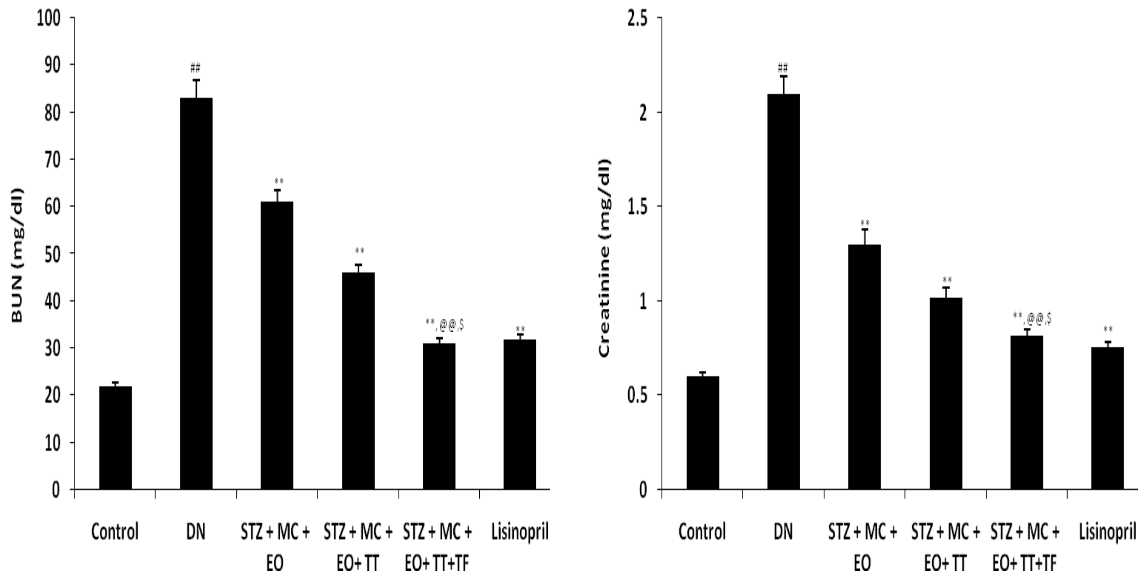


Fig 1. Effect of MC, EO, TT, TFG on serum glucose and body weight of STZ induced diabetic nephropathy rats. Data expressed as Mean ± SD (n=6)

$^{\#\#}$p<0.01 compared to control group; *p<0.05, **p<0.01 compared to DN group; $^{@@}$p<0.01 compared MC+EO treated group; $^{\$}$p<0.01 compared to MC+EO+TT

Effect of MC, EO, TT, TFG on the serum glucose level and body weight of STZ induced diabetic nephropathy after 1st, 14th and 28th day of treatment was shown in Fig. 1. There was significant increase in the (p<0.01) blood glucose level and decrease in the body weight of DN group compared to control group. However, treatment with MC+EO, MC+EO+TT and MC+EO+TT+TFG ameliorates the altered level of blood glucose and body of STZ induced diabetic nephropathy after 1st, 14th and 28th day of treatment. Moreover, level of blood glucose was found to be significantly reduced in MC+EO+TT+TFG treated group compared to MC+EO and MC+EO+TT treated group.

Effect of MC, EO, TT, TFG treatment on the parameters of renal function



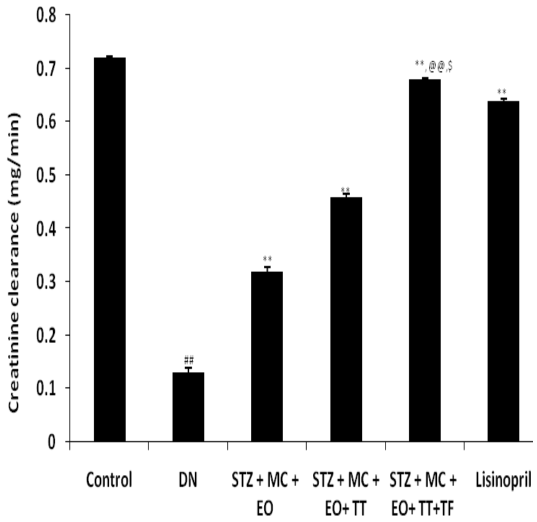


Fig 2. Effect of MC, EO, TT, TFG on the serum BUN and creatinine and creatinine clearance in urine of STZ induced diabetic nephropathy rats.
Data expressed as Mean ± SD (n=6)
$^{\#\#}$p<0.01 compared to control group; *p<0.05, **p<0.01 compared to DN group; $^{@@}$p<0.01 compared MC+EO treated group; $^{\$}$p<0.01 compared to MC+EO+TT

Effect of MC, EO, TT, TFG treatment on the serum BUN and creatinine and creatinine clearance in urine of STZ induced diabetic nephropathy rats was shown in Fig. 2. There was significant increase in the (p<0.01) serum BUN and creatinine and decrease in the creatinine clearance of DN group compared to control group of rats. However, treatment with MC+EO, MC+EO+TT and MC+EO+TT+TFG attenuates the altered serum level of BUN and creatinine and creatinine clearance in the urine of STZ induced diabetic nephropathy rats. Moreover, serum level of BUN and creatinine and creatinine clearance in the urine was found to be significantly reduced in MC+EO+TT+TFG treated group compared to MC+EO and MC+EO+TT treated group.

Effect of MC, EO, TT, TFG treatment on the oxidative stress parameters

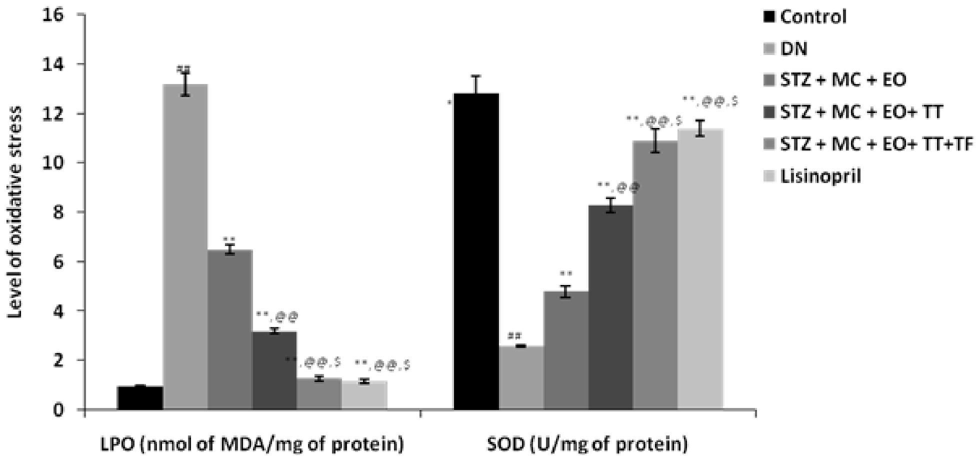


Fig 3. Effect of MC, EO, TT, TFG on the oxidative stress parameters in the renal tissue homogenate of STZ induced diabetic nephropathy rats.
Data expressed as Mean ± SD (n=6)
$^{\#\#}$p<0.01 compared to control group; *p<0.05, **p<0.01 compared to DN group; $^{@@}$p<0.01 compared MC+EO treated group; $^{\$}$p<0.01 compared to MC+EO+TT

Effect of MC, EO, TT, TFG on the oxidative stress parameters in the renal tissue homogenate of STZ induced diabetic nephropathy rats was shown in Fig. 3. There was significant increase in the ($p<0.01$) level of MDA and decrease in the activity of SOD in the renal tissue homogenate of DN group compared to control group of rats. However, treatment with MC+EO, MC+EO+TT and MC+EO+TT+TFG attenuates the altered level of MDA and activity of SOD in the renal tissue homogenate of STZ induced diabetic nephropathy rats. Moreover, level of MDA was found to be reduced and activity of SOD was found to be significantly enhanced in the renal tissues of MC+EO+TT+TFG treated group compared to MC+EO and MC+EO+TT treated group.

Effect of MC, EO, TT, TFG treatment on the histopathology of kidney

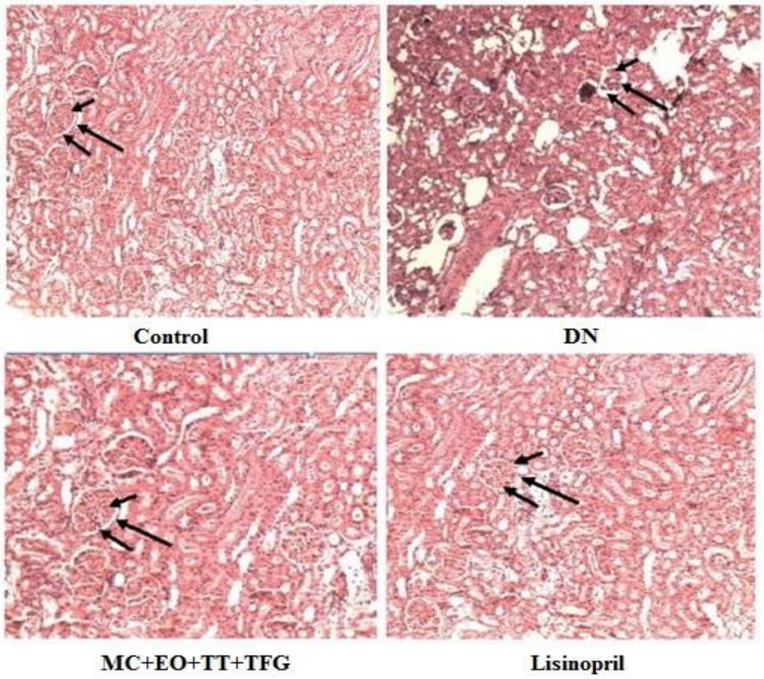


Fig 4. Histopathological photomicrographs of kidney slides by H7E staining (100×). A. Normal, B. STZ-treated Kidney, C. STZ + Lisinopril, E. STZ+MC+ EO+TT+TFG (250 mg/kg).

TS of kidney of DN rats shows pathological changes in the glomeruli such as glomerular capillary size reduction and extracellular mesangial expansion as compared to normal rats (Figure 4.). Pharmacological treatment with MC+EO+TT+TFG (250 mg/kg, p.o*,* 4 weeks) remarkably prevented the diabetes-induced pathological changes in the glomeruli by improving the glomerular capillary size and reducing the mesangial expansion in diabetic rats On the other hand, treatment with lisinopril (1 mg/kg *p.o.,* 4 weeks) markedly protected the diabetic kidney from pathological changes in the glomeruli.

DISCUSSION

Diabetes nephropathy is chronic complication associated with diabetes and management of DN is still a challenge with conventional treatment [10]. In the recent few decades alternative medicine gains the importance for the management of diabetic nephropathy. Present investigation evaluates the synergistic effect of MC+EO+TT+TFG treatment on the STZ induced DN rats. Effect of MC+EO+TT+TFG was determine by estimating blood glucose level and body weight on 1st, 14th and 28th day of protocol. Moreover, markers of renal function such as serum creatinine and BUN and creatinine clearance in urine were determined by using autoanalyzer kit. Level of oxidative stress and histopathology was also determined for the estimation of synergistic effect of MC+EO+TT+TFG treatment on the STZ induced DN rats.

Uncontrolled diabetes leads to development of several complication includes renal damage [11]. MC and TFG has reported for its strong anti diabetic and anti hyperglycaemic activity. Our report suggests that combination of MC+EO+TT+TFG shows significant reduction in the blood glucose and increase in the body weight compared to DN rats.

Literature reveals that level of creatinine and BUN in the serum is the markers of renal function and drugs used for the management of renal dysfunction attenuate the altered level of creatinine and Bun in the serum [12]. Data of the study reveals that treatment with MC+EO+TT+TFG ameliorates the altered level of BUN and creatinine in the serum and creatinine clearance in the urine of STZ induced DN rats. Oxidative stress plays an important role in the pathogenesis of DN as alteration in the glucose metabolism and lipid leads to increase in the production of cellular reactive oxygen species (ROS) [13]. ROS alters the cellular activity and due to which level of oxidative stress is enhanced in diabetic nephropathy. MDA and SOD are the markers of oxidative stress which found to be altered in the renal tissues of DN rats. Moreover MC, EO, TT and TFG reported to posse's strong antioxidant activity. Result of the study suggests that treatment with MC+EO+TT+TFG together shows the synergistic effect against the enhanced level of oxidative stress.

CONCLUSION
In conclusion, data of the study reveals that treatment with high dose of *Momrdica charantia, Emblica officinalis, Tribulus terrestris and Trigonella foenum graecium* in combination shows potential effect for the management of DN by reducing oxidative stress.

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Conflict of interest: No

REFERENCE
1. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, Malanda B. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018 Apr;138:271-281.
2. Edwards JL, Vincent AM, Cheng HT, Feldman EL. Diabetic neuropathy: mechanisms to management. Pharmacol Ther. 2008;120(1):1-34.
3. Lim AKh. Diabetic nephropathy - complications and treatment. Int J Nephrol Renovasc Dis. 2014; 7: 361-81.
4. Kolset SO, Reinholt FP, Jenssen T. Diabetic nephropathy and extracellular matrix. J Histochem Cytochem. 2012;60(12):976-86.
5. Pandey A, Tripathi P, Pandey R, Srivatava R, Goswami S. Alternative therapies useful in the management of diabetes: A systematic review. J Pharm Bioallied Sci. 2011;3(4):504-12.
6. Nain P, Saini V, Sharma S, Nain J. Antidiabetic and antioxidant potential of Emblica officinalis Gaertn. leaves extract in streptozotocin-induced type-2 diabetes mellitus (T2DM) rats. J Ethnopharmacol. 2012 Jun 26;142(1):65-71.
7. Deng R. A review of the hypoglycemic effects of five commonly used herbal food supplements. Recent Pat Food Nutr Agric. 2012;4(1):50-60.
8. Variya BC, Bakrania AK, Patel SS. Emblica officinalis (Amla): A review for its phytochemistry, ethnomedicinal uses and medicinal potentials with respect to molecular mechanisms. Pharmacol Res. 2016 Sep; 111:180-200.
9. Chhatre S, Nesari T, Somani G, Kanchan D, Sathaye S. Phytopharmacological overview of Tribulus terrestris. Pharmacogn Rev. 2014;8(15):45-51.
10. Dounousi E, Duni A, Leivaditis K, Vaios V, Eleftheriadis T, Liakopoulos V. Improvements in the Management of Diabetic Nephropathy. Rev Diabet Stud. 2015;12(1-2):119-33.
11. Nasri H, Rafieian-Kopaei M. Diabetes mellitus and renal failure: Prevention and management. J Res Med Sci. 2015;20(11):1112-20.
12. Lopez-Giacoman S, Madero M. Biomarkers in chronic kidney disease, from kidney function to kidney damage. World J Nephrol. 2015;4(1):57-73.
13. Mahjoub S, Masrour-Roudsari J. Role of oxidative stress in pathogenesis of metabolic syndrome. Caspian J Intern Med. 2012;3(1):386-96.
14. Kashihara N, Haruna Y, Kondeti VK, Kanwar YS. Oxidative stress in diabetic nephropathy. Curr Med Chem. 2010;17(34):4256-69.