Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [5]April 2023 : 46-58

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Eriodictyol Ameliorates the Diabetic Nephropathy in Experimental Rats via WNT/B-Catenin Signalling Pathway

Renu Malik*, Balvindner Singh
Faculty of Pharmaceutical Sciences, Baba Mastnath University, Asthal Bohar, Rohtak
Corresponding Author's Email: balvindersinghpharmaco@gmail.com

ABSTRACT

Diabetic nephropathy is a significant cause of chronic kidney disease and end-stage renal failure globally. This research will examine the current concepts of diabetic nephropathy management in the context of some of the basic science and pathophysiology aspects relevant to the approaches taken in novel, investigative treatment strategies. In recent years, the Wnt/β-catenin signalling has gained tremendous attention due to its ability to modulate a number of diseases including diabetic nephropathy. In current study, Eriodictyol is found effective to downregulate Wnt/β catenin signalling and restores its various effects on cells. It also ameliorates AGEs induced renal cells deterioration and dysfunctioning. It reduces various kind of cast formations, lymphocytes infiltrations and deposition in a tissue, podocytes lost, thickening of basement membrane. It also maintains few cellular events like epithelium integrity by prohibiting epithelial-masenchymal transition. The down-regulation of Wnt/β-catenin signalling leads to detrimental effects on kidney. Eriodictyol also regulates the anti-inflammatory and anti-oxidantscascades which were deteriorated by activation of Wnt/β-catenin signalling pathways. In conclusion, Eriodictyol is providing a significant improvement in blood and tissue parameters and renal pathology by downregulating the Wnt/β-catenin signalling pathway.
Keywords: Diabetic nephropathy, GFR, Eriodictyol, Wnt/B-Catenin Signalling

Received 08.032.2023 Revised 22.03.2023 Accepted 20.04.2023

INTRODUCTION

Diabetic nephropathy is the leading cause of kidney disease in patients starting renal replacement therapy and affects ~40% of type 1 and type 2 diabetic patients (1). Diabetic nephropathy (DN) or diabetic kidney disease is a syndrome characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions, and loss of glomerular filtration rate (GFR) in diabetics. Diabetes may be classified as type 1 (autoimmune β-cell destruction and absolute insulin deficiency), type 2 (relative insulin deficiency and resistance), and other types (eg, pancreatic disease) (2). Besides, diabetic nephropathy is associated with cardiovascular disease (3), and increases mortality of diabetic patients. Several factors are involved in the pathophysiology of DN, including metabolic and hemodynamic alterations, oxidative stress, and activation of the renin-angiotensin system. In recent years, new pathways involved in the development and progression of diabetic kidney disease have been elucidated; accumulated data have emphasized the critical role of inflammation in the pathogenesis of diabetic nephropathy. Expression of cell adhesion molecules, growth factors, chemokines and pro-inflammatory cytokines are increased in the renal tissues of diabetic patients, and serum and urinary levels of cytokines and cell adhesion molecules, correlated with albuminuria (4).

Wnt signalling is extremely complex, and there are more than 50 proteins that participate in Wnt signalling at various stages, which include 19 Wnt ligands, 10 Frizzled receptors, and 2 co-receptors, a dozen inhibitors, multiple intracellular mediators, transcription factors, and co-activators (5). Wnt/β-catenin is an evolutionary conserved signal transduction pathway that plays an essential role in organ development, tissue homeostasis and repair injuries nephron formation and renal development (6,7,8). In the canonical Wnt/β-catenin signalling pathway, Wnt protein binds to cell surface receptors to inactivate glycogen synthase kinase-3 (GSK-3), thereby allowing β-catenin translocation to the nucleus to promote Wnt gene expression.β-catenin is activated in early age but remains silent in normal adult but found activated in chronic kidney disease patients(9,10,11).In diabetic nephropathy, podocyte injury and glomerulosclerosis are common by upregulating Wnt ligands and β catenin activation (11,12). Recent studies have also demonstrated that high glucose (HG)-induced production of the extra cellular matrix by mesangial cells was associated with impairment of Wnt/β-catenin signalling (13). Studies have shown

that Wnt/β-catenin signalling in mesangial cells is involved in the development of diabetic glomerulosclerosis.Thus, the Wnt/β-catenin pathway in mesangial cells may be a therapeutic target for the treatment of DN (14).

Eriodictyol is a flavanone,which is found in various vegetables, citrus fruits and medicinal plants.it is extracted from yerba santa (Eriodictyon californicum) plant, a plant from north America(15,16).It has a promising antioxidant and anti-inflammatory activity(17,18).Eriodictyol efficiently improve the various diseases conditions via restoring different signalling pathyways.Eriodictyol improves the myocardial ischemic condition by regulating the JAK2 pathway, it also reduces the cardiac muscles injury by modulating Bcl-2 and BAX pro apoptotic signalling pathways (19,20). Eriodictyol also shows anti-cancer activity by inducing apoptosis through redirecting the mTOR/PI3/Akt pathway(21).Eriodictyol found to stimulate insulin production and secretion by cAMP/PKA signalling cascade (22). It also found effective in glucose metabolism in hepatocellular carcinoma patients by controlling PI3K/Akt signalling cascade (23).It protects from ensuing lipopolysaccharides induced oxidative stress, neuroinflammation and synaptic brokenness by MAPKs, NF-κB/Sirt1, and Nrf2/Keap1 pathways (24) activations.Eriodictyol repairs the retinal ganglial cells in rodents in hyperglycaemic induced oxidative stress and cell apoptosis by stimulating Nrf2/HO-1 cascades (25).

In current study, we evaluated the pathway by which Eriodictyol acts upon on diabetic nephropathy. This study revealed the role of eriodictyol in diabetic nephropathy treatment whether it is Wnt/β -catenin signalling pathway agonist or antagonist.

MATERIAL AND METHODS

Drug and chemicals-Eriodictyol (purity>98%, CAS-552-58-9) was procured from Biosynth Carbosynth Limited, UK. Streptozotocin was procured from Sigma Aldrich (USA), metformin fromAbott India Limited, Methyl Vanillate from TCI Chemicals. Creatinine (Cr), triglyceride (TG), totalcholesterol (TC), high -density lipoprotein (HDL), low -density lipoprotein (LDL), very low -density lipoprotein (VLDL-C)and blood urea nitrogen (BUN) colorimetric detection kit procured from Elabsciences. AGEs (Advanced Glycation End Products) ELISA kit from Cusabio. HBA1C from creative diagnostic kit. Tumor necrosis factor - α (TNF - α, EK0526), interleukin - 6 (I L -6, EK0412), rat albumin ELISA kit (E111 -125 Bethyl Laboratories),rat TG F - β 1 (transforming growth factor beta 1) ELISA kit (Elabscience) were procured. Malondialdehyde (TBARS), glutathione (GSH), superoxide dismutase (SOD), and catalase colorimetric detection kit from Bio – diagnostic. All other reagents used in study, were of analytical grade and purchased from Himedia Labs, Mumbai (India).

Drug preparations- Eriodictyol dissolved in 5% tween 80, 20% PEG and 75% Saline (26)**.**STZ was freshly prepared in 0.1 M cold Citrate buffer 4.5 Ph (27). Metformin solution was prepared with distilled water. Methyl Vanillate was prepared by dissolving in corn oil [28].

Experimental Animals-Adult male Wistar rats (6 weeks of age) weighing 200-250 gm were purchased from CPCSEA approved registered breeder Central Animal Facility, AIIMS, Delhi. The protocol for antidiabetic activity was approved by Institution Animal Ethics Committee (IAEC) of Swift College of Pharmacy, Ghaggar Sarai, Rajpura, Patiala, Punjab (Reg No. 724/PO/a/02/CPCSEA) with protocol No SSP/IAEC/2022/06 on dated 9/06/2022. The animals were housed under standard laboratory conditions with temperature (23 ± 1°C), relative humidity (55 ± 10%), 12/12 h light /dark cycles and fed with standard pellet diet from Ashirwad Industry and purified water *ad libitum.* All rats were free to drink clean water and normal pallet diet during the experimental period.

Induction of Diabetic Nephropathy- After acclimatization of two weeks, adult, albino wistar rats kept fasted overnight. Next morning, freshly prepared STZ 55mg/kg was injected intraperitoneally to animals (27,29). After 72 hours, blood glucose level was checked with glucometer by collecting the blood sample from tail vein and diabetes was confirmed (30). The rats with more than 250mg/dl were considered diabetic. The sustained level of glucose for 8 weeks will induce diabetic nephropathy (27,29).

Experimental Design- Fifty-six rats were randomly divided in to non-diabetic animals and diabetic animals' group. Non diabetic animals' group was treated with cold citrate buffer during whole experimental period and considered as control group. Then diabetic rats were divided into six groups and each group contains 8 rats. The sustained level of blood glucose, induced diabetic nephropathy in rats. Diabetic nephropathy was found developed at the end of 8th week. DNP (Diabetic nephropathy) group received only STZ (55 mg/kg) in cold citrate buffer. ED1 group received eriodictyol 1 mg/kg *p.o.* and ED10 group animals received 10 mg/kg *p.o*. for 28 days after 8 weeks of diabetes. Met group animals received metformin 200 mg/kg, orally (31) for 28 days after 8 weeks of diabetes. MV group received methyl vanillate (100 mg/kg orally) and for 28 days after 8 weeks of diabetes induction. MV+ED10 group was treated with eriodictyol (10 mg/kg orally) with methyl vanillate 100 mg/kg orally (28) after 8 weeks

for 28 days. Plasma glucose, feed/water intake and body weight were recorded every week during study period. Rats were supplied with normal food and water during experimental period.

Sacrification and specimen collection- At the end of study, rats were housed in metabolic cages for 24 hrs for urine collection to assess the various urine parameters. All rats were sacrificed by overdosing of thiopental sodium (40 mg/kg).Blood samples were collected by retroorbital route with the help of pointed capillary dipped in heparin solution to avoid the blood clotting. Blood samples of rats were centrifuged to separate serum which was used to assess various biochemical parameters or stored at -80 °C for later analysis.

The animals' body weights were recorded and both kidneys were harvested, rinsed in cold saline and weighed them for calculation of kidney index= (kidney wt/body wt) x 1000 to check the kidney impairment. Kidney specimens were frozen at −80 °C for preparation of homogenate, estimation of kidney content of oxidative stress and inflammatory biomarkers and advanced glycation end products (AGEs).

Estimation of serum parameters- Blood sample was kept for 2 hours at room temperature or at 2-8 °C for overnight. Then the sample was centrifuged for 20 min. at 1000rpm. Supernatant fluid was collected and different procedures for serum albumin, serum creatinine, HbA1C and insulin before and after treatment were carried out. These all were calculated, according to the as per given manufacturer instructions on kits. Blood urea nitrogen (BUN) and glycated hemoglobin (HbA1C) was assayed by ELISA test kit and optical density (OD) of sample was noted at 550 nm and 450 nm respectively. Serum creatinine (Scr) content in sample was checked by Sarcosine Oxidase Method. OD of sample for serum creatinine was measured by biochemical analyzer at 515 nm.

Lipid profile was estimatedby use of remaining serum with colorimetric method with help of biochemical analyser and spectrophotometer. Assessment of Total-cholesterol (TC), triglycerides (TGs), LDL cholesterol (LDL-C), VLDL cholesterol (VLDL-C), and HDL cholesterol (HDL-C) was doneas per instructions given by manufacturer kits by colorimetry method. HOMA-IR is measured by (Fasting serum glucose x fasting serum insulin)/22.5and HOMA -β can be calculated as 20x insulin (IU/ml)/(Glycemia-3.5).

Urine albumin (UAlb) was estimated by using ELISA kit and creatinine level (UCr) in urine was assessed by colorimetry analysis. Urine albumin excretion (UAER) was estimated by using (UAER) = UAlb (ng/mL) × 24 h urine volume (mL)} and creatinine clearance (CrCl) = UC r (mg/ dL) × urine volume (mL) /SC r (mg/dL) × 1000 /body weight (g) × 1/ 1440 (min)}.

Advanced Glycation End products estimation- AGE estimation was done with ELISA test kit. Kidney was isolated from rat and 100mg tissue was rinsed with 1X phosphate buffer saline (PBS), homogenized in 1 ml of 1X PBS and stored overnight at -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 rpm, 2 - 8°C. The supernatant was removed and assayed immediately. Alternatively, aliquot and store samples at -20°C or -80°C. Centrifuge the sample again after thawing before the assay. Incubate the sample at 37 °C. Protect it from light. Check the reading at 450 nm within 5 minutes.

Estimation of antioxidants and lipid peroxidation- The excised kidney, rinsed in ice cold normal saline followed by 0.15 M Tris-HCl (pH 7.4) blotted dry and weighed. A 10% w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation (TBARS) by the method of Ohkawa H *et al* (32).

A part of homogenate after precipitating proteins with Trichloric acetic acid (TCA) was used for estimation of glutathione (GSH) by the method of Ellman GL *et al* [34]. The rest of the homogenate was centrifuged at 15000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of Catalase activities was measured by the method of Claiborne *et al* [34]. Nitrites assessed by using copper cadmium alloy to reduce nitrate to nitrite in an hour followed by Griess reagent by colour development in acidic medium [35]. 100µL supernatant from the kidney homogenate is thoroughly mixed 100µL Griess reagent. Check the absorbance at 540 nm and compared from the standard curve of sodium nitrite (0-100 µL).

Super Oxide Dismutase assessment (SOD) was based on its ability to inhibit the reduction of nitro blue tetrazolium (NBT) by using 0.067M potassium phosphate buffer Ph 7.8,0.1 M EDTA contain 0.3 Mm sodium cyanide, 0.12 mM Riboflavin and 1.5 Mm NBT(36). Glutathione peroxidase (GPx) by using selenium as a cofactor and colour developed was read at 412nm (37).

Estimation of inflammatory cytokines- Harvested kidney tissues were homogenised by homogeniser to estimate the inflammation assessment.IL -6 was estimated by using the Sandwich-ELISA principle. The reaction was terminated by showing the yellow colour after adding stop solution. The optical density (OD) was measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is

proportional to the concentration of Rat IL-6. IL-6 concentration was calculated by comparing the OD of the samples to the standard curve.
TGF-β1 was estimate by Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit. Inactive TGF-β1 in sample was first turned to activate. The reaction was terminated by showing yellow colour.The O.D. absorbance read with a microplate reader at 450nm, within 30 minutes of stopping the reaction.TNF-α was estimated by using ELISA kit, by noted the absorbance at 450 nm within 30 minutes of stopping reaction.

Urine analysis- 5 ml of collected urine from 24 hours housed rats in metabolic cages, was centrifuged for 5 minutes at 3000 rpm. Supernatant fluid was separated out without any sediment's traces. Acetic acid Papanicolaou staining was used as given by Raju (38). After giving 1 %ethanoic acid treatment, to fix the stain 95%ethanol was used. Harris haematoxylin stain was used and slide was rinsed under running water followed by 1% ethanoic acid. OG-6 and EA -50 dye were also used followed by ethanoic acid and methanol washing respectively. After that xylenefor cleaning processedwas involved for mounting of slides. Grading was done on the basis of granular casts, immune cells and renal tubular epithelial cells to check the renal permeability, severeness of injury. Urinary microscopic analysis was executed and graded according to established scoring norms.
Histopathology- A paraffin enterenced,5µm sliced kidney tissue was fixed with sodium azide by natural buffered formalin. Haematoxylin and eosin stain was used to check the tissue assessment by polarized optical microscope. The renal tissuedysfunctioning was assessed by bowman's capsule expansion,cast development in medullary and cortical area, renal tubular damage and vascular congestion recognition and categorized as per injury grading: 0%-0,20%-1,20 to 40 %3, 40-60 %-3,60 to 80%-4 and >80%-5. The overall histopathological score was determined by summing up all individual score in cohort. Leukocyte intrusion and nuclei differentiation were accomplished in the renal interstitium along with their enumeration in 20 regions of 0.14 mm 2 (magnification ×100), and data was stated as (in) per mm 2. Brush border mutilation and basement membrane thickness were observed using Periodic acid - Schiff base (PAS) stain at ×200 magnification.
Statistical analysis- Statistical analysis was done by using Graph Pad 6. Means of normally dispersed variables were examined and followed by one or repeated measures two -way analysis of variance (ANOVA). If ANOVA conclusions were significant ($p < 0.05$) in F -statistics, multiple comparison tests viz. then Tukey' s HSD (Honest Significant Difference) test or Bonferroni test were used. Statistical significance was estimated at $p<0.05$ and results were stated as mean standard deviation.

RESULTS

Effect of Eriodictyol on body weight, water/food intake, blood glucose level,kidney weight and kidney index – During the initial phase of experimental period, body weight, water and feed intake was almost equal of every rat. There was no significant difference in these parameters. when rats were injected with single dose of STZ, except body weight, all parameters showed a significant hike when comparison was done with control group animals. Body weight of animals was decreased significantly on comparison to control group animals. Eriodictyol (1mg/kg, 10 mg/kg) showed a significant restoration of body weight, water/feed intake and plasma glucose. ED 10 revealed a highly significant decreased in water/ feed intake (Fig. 1 B, 1 C) and plasma glucose level (Fig. 1 D) whereas body weight (Fig. 1 A) increased significantly when comparison was done with DNP (Diabetic Nephropathy) ED10+ MV group showed the antagonist side of ED 10. It reduces the agonist effect of MV and restore the body weight, water and feed intake and plasma glucose level, when compared with DNP groups animals.
Kidney weight and kidney index are important parameters to show the progression of nephropathy. On STZ administration, both parameters were increased. Met and Eriodictyol treated animals showed a significant decrease in kidney weight and index. Among them, ED10 showed a highly significant results and restore the kidney weight and index. MV treated group showed the synergistic effect with STZ and enhanced the degree of severity of nephropathy whereas the ED10 antagonized the effect of MV and restore the animal's kidney weight and index.

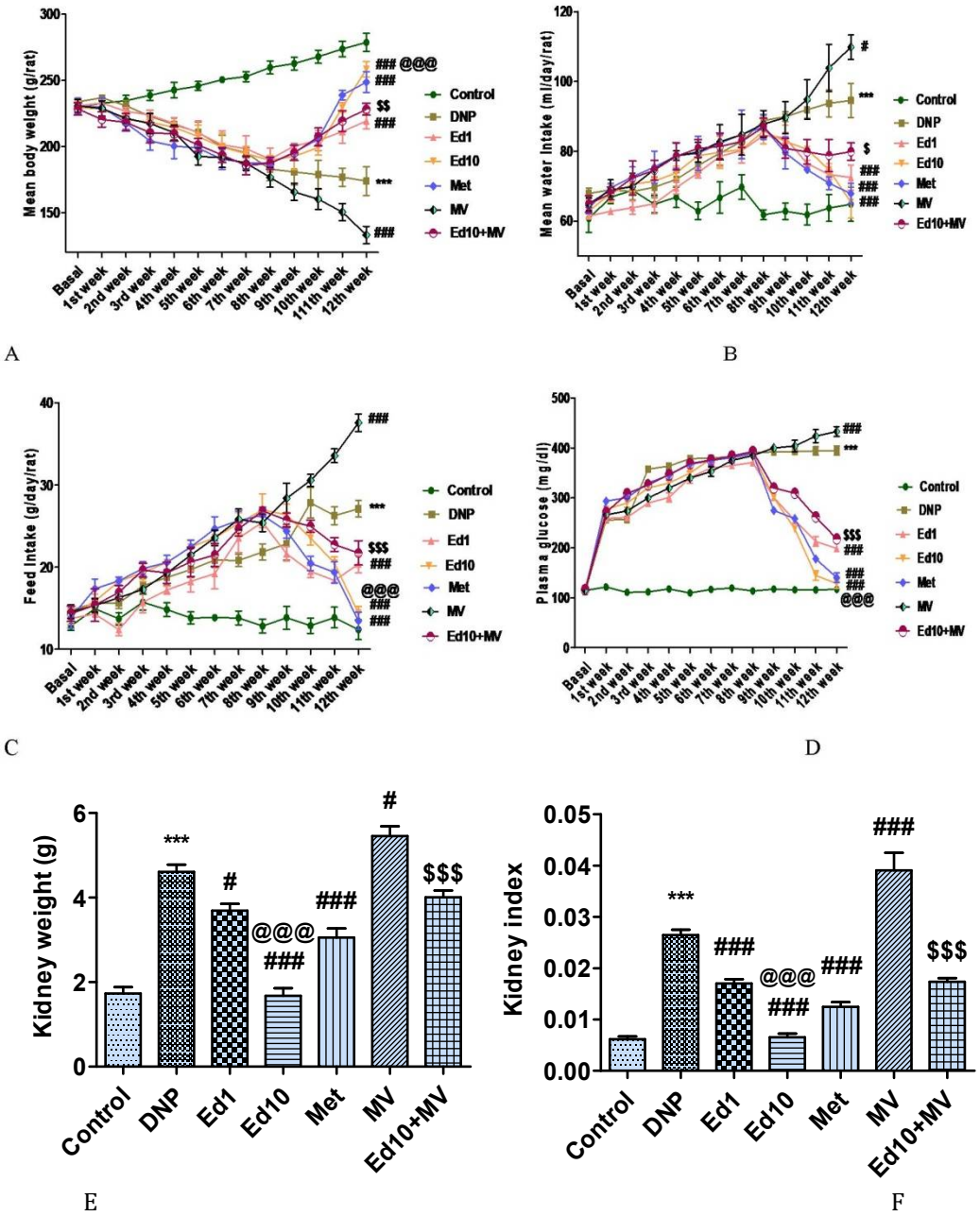


Fig-1. Effect of ED on (A) mean body weight, (B) mean water intake, (C)mean feed intake, (D) Plasma Glucose level, (E) Kidney weight, (F)Kidney index. Data of water/feed intake, Plasma Glucose level and body weight were analyzed using a repeated measure two-way ANOVA and Bonferroni posthoc test. One way ANOVA followed by Tukey's test HSD was used to analyze kidney weight and kidney index. Data explored as mean ± S.E.M, (n=8) symbols represent statistical significance.

*** Vs Control,### Vs DNP, @@@ Vs ED1, $$$Vs MV, p^{***}= <0.001, p ###=<0.001, p ##=<0.01, $p^{\#}$=<0.05, p @@@=<0.001, $p^{\$\$\$}$=<0.001. DNP- Diabetic Nephropathy, ED1- Eriodictyol 1 mg/kg, ED10- Eriodictyol 10 mg/kg, Met- Metformin 200mg/kg, MV-Methyl Vanillate.

Effect of Eriodictyol on serum parameters and urine analysis- Diabetic Nephropathy is characterized by enhanced level of lipid metabolism, serum creatinine, insulin resistance (HOMA IR) and significant decrease in serum albumin, insulin, pancreatic activity (HOMA β)was found in diabetic nephropathy group relative to control group. These serum parameters were ameliorated by Eriodictyol and Met treatment in rats (Table-1). BUN, SCr,Hb1AC, HOMA IRwere significantly increased in diabetic nephropathy group. Orally treatment with ED or Met attenuated the level of these parameters.

An impairment also observed in lipid profile of STZ injected animals relative to control group rats. On STZ administration, a hike in VLDL-C and LDL-C level whereas a decline was noted in HDL-C level on blood analysis.ED10 and Met treatment showed a highly significant result when data was compared with DNP group animals (Table-1). MV treated animals did not show any improvement in lipid profile, but also worst the animal condition relative to DNP animals. A significant increase in urine albumin (UAlb) and urine albumin excretion rate (UAER) was observed in DNP group when comparison was done with control group animals whereas urine creatine (Ucr) and creatinine clearance (CrCl) level got attenuated. ED and Met administration showed a significant upsurge of UAlb, UAER and diminution of Ucr, CrCl level. MV treatment to DNP animals, significantly enhanced the urine albumin and its excretion and decline the Ccr and Ucr level (Table-1).

Table-1. Evaluation of serum and urine parameters

Parameters	Controlgroup	DNP	ED1	ED10	MET	MV	MV+ ED10
SAlb (ng/mL)	295.2±20.73	89.2±32.9***	142.2±25.12##	283.5±16.77###@@	260.7±27.75##	44.34±24.55##	179.8±14.51$$$
HbA1C (ng/mL)	14.62±3.345	69.5±9.564***	52.38±4.673###	26.55±7.207###@@	34.2±6.207###	80.96±4.953#	46.39±5.67$$$
Insulin BT (µIU/mL)	18.89±1.432	9.954±1.822***	10.28±1.697***	9.955±1.244***	9.369±1.196***	8.985±1.646***	8.997±1.518***
Insulin AT (µIU/mL)	19.2±1.164	9.614±2.049***	13.46±2.524##	17.3±1.335###@@	17.56±1.17###	6.197±2.952#	13.25±2.348$$
BUN (mmol/L)	5.299±2.272	21.17±2.756***	16.17±4.423#	9.312±2.895###@@	10.09±3.464###	26.65±3.191#	15.82±2.836$$$
SCr (µmol/L)	97.61±8.298	354±47.97***	290±36.55#	160.7±58.59###@@	158.8±20.87###	418.6±26.1#	229.1±45.15$
HOMAβ BT	211.1±14.68	10±1.669***	10.4±1.987***	11.32±3.512***	10.01±1.229***	9.752±1.698***	9.398±1.219***
HOMAβ AT	207.3±19.26	11.92±4.38***	18.53±5.856#	19.8±4.804##	19.15±2.919#	5.222±1.093#	12.14±3.218$$
HOMA IR BT	2.727±0.8552	9.117±1.464***	9.517±1.173***	9.65±1.081***	9.641±1.229***	8.935±1.501***	9.067±1.214***
HOMA IR AT	2.915±0.6777	8.948±0.9569***	7.103±0.7401##	4.939±0.7007###@@@	6.14±0.6577###	10.78±1.606##	7.516±0.7891$$$
HDL±C (mg/dL)	2.782±0.643	0.8355±0.1313***	1.564±0.4093#	2.127±0.6823###	1.838±0.4267###	0.07716±0.0183##	1.295±0.4177$$
LDL±C (mg/dL)	1.565±0.4052	9.415±0.7848***	7.941±1.006#	2.407±0.6409###@@@	5.739±1.024###	11.11±0.77##	7.576±1.051$$$
VLDL±C (mg/dL)	292.3±60.75	914.1±162.8***	745.1±140.6#	335.6±51.79###@@	507.3±83.49###	1088±120.1#	658.8±60.28$$$
TC (mg/dL)	5.359±2.45	18.42±2.239***	14.71±2.769#	5.892±1.667###@@	7.574±1.675###	22.2±1.86##	13.02±1.153$$$
TGs (mg/dL)	2.499±1.273	9.847±1.082***	7.852±1.087#	3.287±1.197###@@	6.57±0.6867###	12.27±1.357##	7.57±1.199$$$
UAlb(ng/ml)	29.08±9.82	298.7±34.64***	244.1±36.08#	101.8±39.8###@@@	151.8±41.13###	354±37.08#	200.6±38.33$$$
UCr(µmol/l)	725.2±44.31	152.3±56.11***	275.1±47.78###	675.9±55.59###@@	627.6±31.23###	64.79±25.45##	429.9±49.83$$$
CrCl (ml/min)	3.969±0.671	1.656±0.417***	2.539±0.534#	3.836±0.594###@@	3.244±0.542###	0.5584±0.1816##	2.429±0.406$$$
UAER (µg/min)	0.8916±0.328	56.18±7.177***	36.51±6.693###	7.32±3.713###@@@	14.95±3.76###	85.29±15.5###	31.62±6.804$$$

Value Mean ± S.E.M, (n=8) symbols represent statistical significance.*** Vs Control,### Vs DNP, @@@ Vs ED1, $$$Vs MV, *p****= <0.001, *p* ###=<0.001, *p* ##=<0.01, *p*#=<0.05, *p* @@@=<0.001, *p*$$$=<0.001. One way ANOVA followed by Tukey's HSD Multiple Comparison test. DNP- Diabetic Nephropathy, ED1- Eriodictyol 1 mg/kg, ED10- Eriodictyol 10 mg/kg, Met- Metformin 200mg/kg, MV-Methyl Vanillate, SAlb- Serum Albumin, SCr- Serum Creatinine, BUN- Blood Urea Nitrogen, BT= Before Treatment,AT=After Treatment, HbA1C= Glycated Haemoglobin, HOMA-β=Homeostasis Assessment Model for β cells, HOMA-IR=Homeostasis Assessment Modelfor Insulin Resistance, HDL-C=High Density Lipoprotein Cholesterol, LDL-C= Low Density Lipoprotein Cholesterol, VLDL-C=Very Low Density Lipoprotein Cholesterol, TC= Total Cholesterol, TGs-Triglycerides,UAlb- Urine Albumin, CrCl= Creatinine Clearance, UCr- Urine Creatinine , UAER- Urine Albumin Excretion Rate.

Effect of Eriodictyol on oxidative stress, inflammatory parameters- Diabetic nephropathy group showed a significant decrease in GSH,GPx,catalase and SOD activity and increase in TBARS and nitrites level in kidney relative to control group. ED and Met treatment to DNP animals, exhibit a significant decrease in nitrites level, reduce lipid peroxidation by decreasing TBARS level and restore the level of antioxidant enzymes to reduce oxidative stress (Table-2).MV is the drug which enhanced the effect of STZ on animals and severe the level of renal toxicity by decline the antioxidant enzymes level and enhancing lipid peroxidation relative to DNP group. ED 10 decreased the lipid peroxidation and restore the activity of antioxidant enzymes.

DNP group showed a significant increase in inflammatory biomarkers**(**IL-6, TNF-α and TGF-β1) in comparison tocontrol group. When ED and Met treatments was given to DNP animals, the showed a significant decrease in inflammatory biomarkers relative to DNP group (Table -2).MV administration to DNP animals, enhanced the blood level of inflammatory biomarkers by act as agonist to Wnt receptors. ED10 treatment to MV and STZ injected animals significantly upsurge the inflammatory biomarkers level.

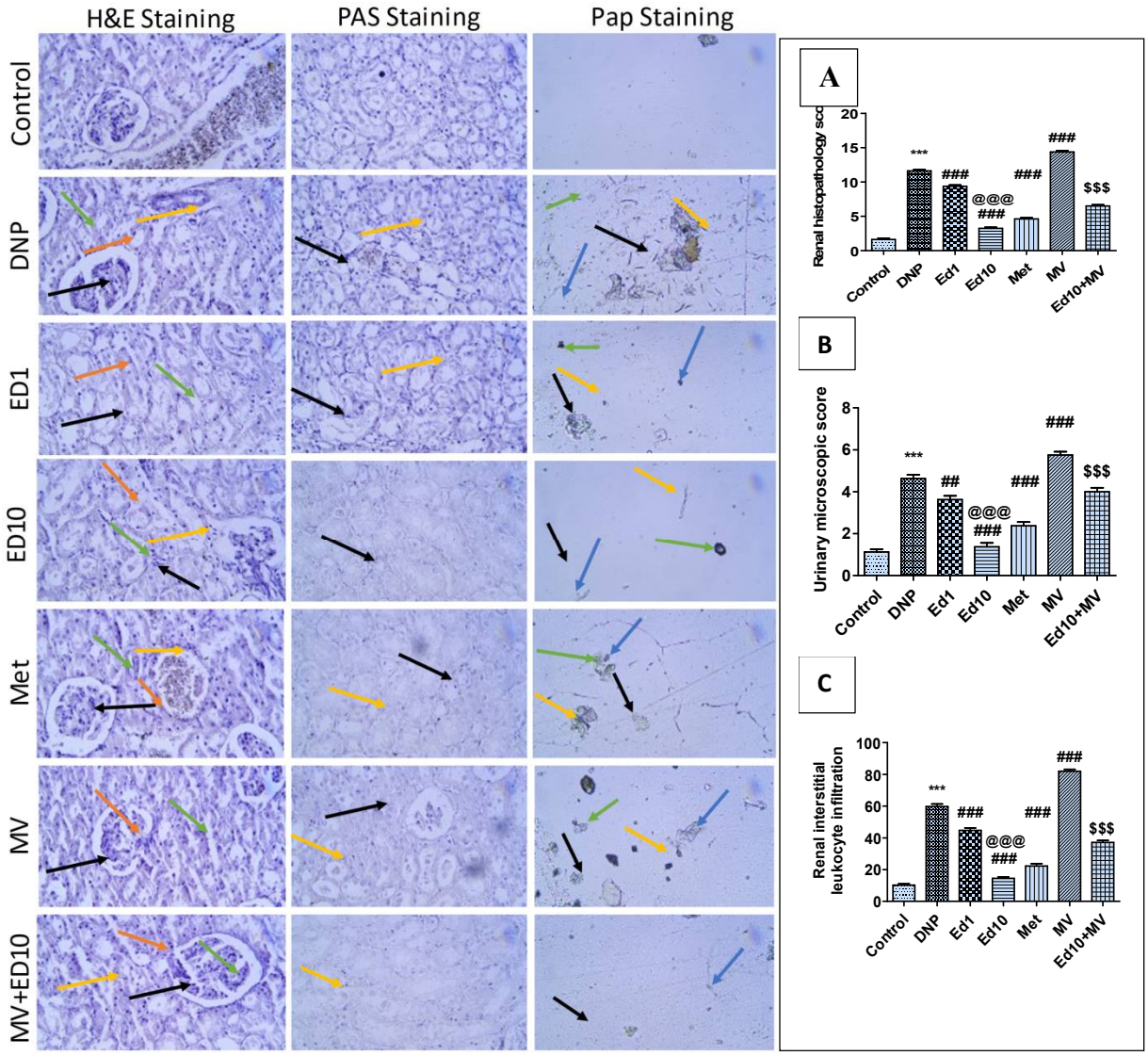
Table-2. Evaluation of oxidative stress, inflammatory parameters and AGEs.

Parameters	Control group	DNP	ED1	ED10	MET	MV	MV+ ED10
GPx (nmol/mg)	41.7±9.57	12.99±3.39***	21.65±5.01#	37.88±5.5###@@@	31.84±5.24###	4.425±2.826#	23.17±4.19$$$
GSH (µmol/mg)	102.3±9.79	30.92±9.63***	45.78±9.24#	95.65±8.26###@@@	85.57±11.34###	16.31±7.25#	67.42±5.62$$$
SOD (U/mg)	37.04±5.91	17.01±4.17***	25.08±3.39#	37.6±7.50###@@@	31.85±6.72###	7.918±3.31#	27.11±3.01$$
TBARS (nmol/mg)	14.94±5.44	60.54±9.93***	48.24±7.64#	21.74±6.15###@@@	29.03±5.31###	80.89±9.16#	42.93±7.93$$$
CAT (units /mg tissue)	66.12±11.6	22.14±3.25***	33.31±9.12#	65.54±4.60###@@@	56.72±3.97###	10.19±2.27##	44.74±3.66$$$
NITRITES(µmol/mg)	16.04±5.28	75.46±6.79***	65.1±9.18#	27.42±4.19###@@@	30.25±5.76###	85.05±4.19#	51.68±6.66$$$
IL±6 (pg/ml)	58.3±14.01	275.5±35.77***	226.7±33.38#	85.81±16.37###@@@	134.3±27.01###	337±34.78##	166.4±26.95$$$
TNF±α(pg/ml)	67.95±10.68	188.2±35.63***	147.9±21.45##	86.87±19.04###@@@	97.11±11.18###	231.5±23.54###	127.9±11.33$$$
TGF±β1 (pg/ml)	61.03±27.61	281.7±25.95***	220±23.38##	90.52±29.68###@@@	120.6±20.71###	358.4±33.58###	178.2±37.42$$$
AGEs (µg/ml)	28.14±6.83	147.8±31.4***	110.4±28.71##	53.4±16.91###@@@	80.76±15.87###	184±13.92#	111.9±17.15$$$

Value Mean ± S.E.M, (n=8) symbols represent statistical significance.*** Vs Control,### Vs DNP, @@@ Vs ED1, $$$Vs MV, *p****= <0.001, *p* ###=<0.001, *p* ##=<0.01, *p*#=<0.05, *p* @@@=<0.001, *p*$$$=<0.001. One way ANOVA followed by Tukey's HSD Multiple Comparison test. DNP= Diabetic Nephropathy, ED1= Eriodictyol 1 mg/kg, ED10= Eriodictyol 10 mg/kg, Met=Metformin 200mg/kg, MV-Methyl Vanillate, GSH= Glutathione, GPx= Glutathione Peroxidase, SOD= Superoxide Dismutase, TBARS/LPO= Thiobarbituric acid reactive substances/Lipid Peroxidation, IL-6= Interlukin-6, TNF-α= Tumor Necrosis Factor- alpha, TGF-β1=**Transforming Growth Factor-beta,** AGEs= Advanced Glycation End Products**.**

Effect of Eriodictyol on Adavnced Glycation End products- Advanced Glycation End Products level (Table-2) was found high in DNP and MV group animals. ED treatment showed highly significant restoration of AGEs on comparison to DNP by decreasing its level in tissue. ED 10 also ameliorated the effect of STZ with MV.

Histopathological evaluation of Eriodictyol–Tubular cast and tubular injury in medulla and cortex, vascular congestion was observed in histopathology of isolated kidney of DNP rats and Methyl Vanillate treated rats (Fig. 2D). Bowman's capsule enlargement, mesangial expansion, hypercellularity were also the characteristics of diabetic kidney and observed by various staining of kidney tissue (Fig. 2D). ED treatment showed a remarkable improvement in histopathological score (Fig. 2A) and different form of tissue damage relative to DNP rats (Fig. 2D). ED treatment reduces the infiltration of interstitial leucocyte in tubular area and lymphocytes clusters as compared to DNP rats (Fig. 2C). Urinary microscopic score reveals the renal tubular epithelial cells, granular cast, leukocyte cells and crystals in urine of DNP rats which was attenuated by ED treatment (Fig. 2B).



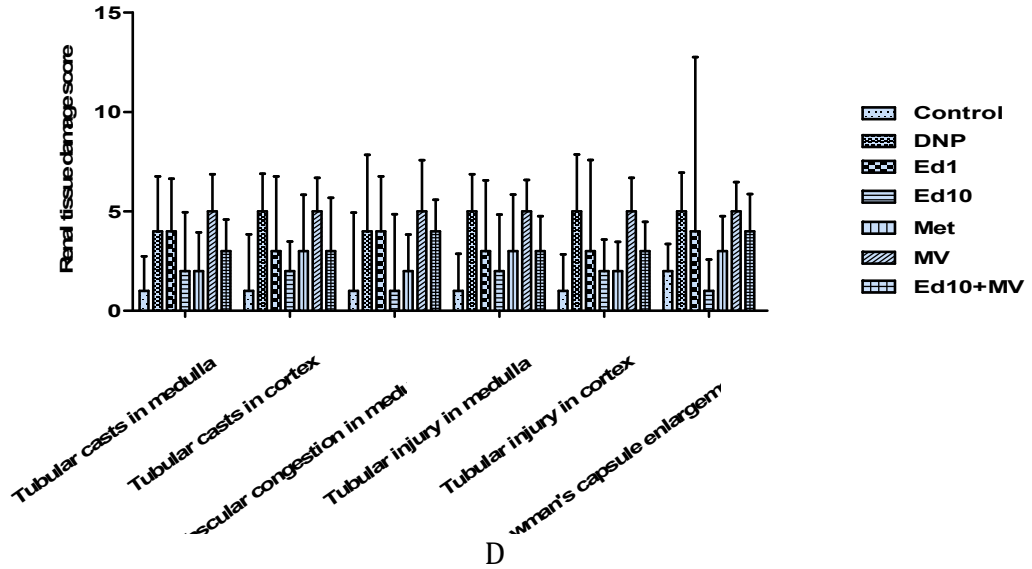


Fig-2. Renal histopathology H&E stain (× 100, 100 μm). Vascular congestion (orange arrow), interstitial leukocyte (monocytes, eosinophils, basophils, neutrophils) infiltration in tubular regions (black arrow), the cluster of lymphocytes (yellow arrow), and tubular cast formation (green arrow). PAS stain (× 100, 50 μm).Bowman's capsule enlargement, tubular dilation, hypercellularity (black arrow), and mesangial expansion (yellow arrow). Papanicolaou staining (Pap stain) of urinary sediments- Leakage of leukocytic cells in the urine (black arrow), granular cast (GC) (green arrow), renal tubular epithelial cells (RTECs) (blue arrow), and crystals (green arrow). Fig A- renal histopathology score, B- Urinary microscopic score, C-Renal interstitial leukocyte infiltration, D- Renal Tissue Damage Score.

Value Mean ± S.E.M, (n=8) symbols represent statistical significance.*** Vs Control,### Vs DNP, @@@ Vs ED1, $$$Vs MV, p***= <0.001, p ###=<0.001, p ##=<0.01, p#=<0.05, p @@@=<0.001, $p^{\$\$\$}$=<0.001. One way ANOVA followed by Tukey's HSD Multiple Comparison test,DNP= Diabetic Nephropathy, ED1= Eriodictyol 1 mg/kg, ED10= Eriodictyol 10 mg/kg, Met=Metformin 200mg/kg, MV-Methyl Vanillate.

DISCUSSION

Diabetic nephropathy is an end stage renal disease and clinically it can be identified by the level of albuminuria. Mismanagement of glucose level in blood for prolonged time mediates the podocyte dysfunctioning, deposition of various kind of cell proteins like actin, collagen fibers in tissue causes fibrosis and glomerulosclerosis (39). It alters the glomerular filtration rate by initiating podocytes loss. It leads to thickening of basement membrane, mesangial expansion, epithelial mesangial transmission (40). These all changes in renal tissue, are the results of impairment of various signalling pathways. Among them Wnt/β catenin signalling pathway is one of the important pathway, by targeting this pathway can find some new treatment alternatives of DN.

In Wnt/β catenin signalling pathway, Wnt ligands or Wnt proteins binds to frizzled receptor and dephosphorylates the β catenin from destruction complex composed of APC, CK1 GSK in cytosol and increase the cytoplasmic availability of β catenin (41). This β catenin is a transcription regulator in cell, responsible for kidney growth in early stage by regulating the podocyte formation (42). Podocytes cells are act as a filtration membrane in glomerulus for protein in a nephron. In adult age, β catenin remains silent in body (43,44). Wnt/ β catenin reactivation is found activated in many other diseases pathogenesis (45). When it gets activated in podocytes, it enhances the albumin secretion in urine (46). High glucose level in blood mediates the apoptosis of podocytes by activating Wnt/β catenin pathway (47). Various studies revealed that, this cascade also promotes the renal fibrosis on activation. Methyl vanillate is a Wnt/β catenin signalling pathway agonist which activates the Wnt/β catenin cascade and stimulates podocytes apoptosis and renal fibrosis (48). It enhances the severity of nephropathy in presence of STZ and hyperglycaemic conditions in body.

Flavonoids are well known for their anti-oxidant activity. They are the terminator of free radical chain and enable to catalyse the lipid peroxidation of cell membrane (49). They have electron donating capability to chelates redox active metal ions and scavenges other reactive oxygen species also. Eriodictyol possess many unsaturated bonds and hydroxyl groups in its structure to donate the free electron to neutralize various reactive oxygen species and stabilize them. It intract hydrophobically to the membrane and protect it from leakage followed by damage.lipid peroxidation (50). Flavonoids contain

more OH groups possess more potent antioxidant and anti-inflammatory activity (50,51). Eriodictyol expressed better antioxidant activity in comparison to other flavonoids like luteolin, quercetin and taxifolin in renal cells 51. Eriodictyol is a phenolic compound and contains two sites for chelation: 5,7-dihydroxy-4-chromane and 3',4',-dihydroxyl groups. The structure of eriodictyol is efficient to free radical scavenging properties, by which it can easily improve oxidative stress of a cell (52).

In this study, STZ treatment to rats, damages their pancreatic β cells and decreases the insulin formation and its secretion. It initiates the β cells necrosis by stimulating DNA fragmentation via PARP pathway. Insulin deficiency leads to alteration in glucose, lipid, fat and protein metabolism and create a hyperglycaemic condition in body. STZ also alters various biological reactions in pancreas. In this study a single dose of STZ 55 mg/kg is given to the rats to generate hyperglycaemic condition in body. This hyper glycaemic condition remains untreated for 8 weeks to develop diabetic nephropathy (53,54). It stimulates catabolism of protein and fats, contributes to increase body weight. Eriodictyol is found highly significantly effective in restoring the body/kidney weight and decrease polydipsia and polyphagia, increased plasma glucose level. Kidney weight was also controlled by protecting renal cells from hypertrophy and hyperplasia (55).

HOMA is a homeostatic Assessment Model known to check the progression of diabetes and its complications. HOMA-IR is stands for homeostatic Assessment Model for Insulin Resistance whereas HOMA-β stands for β-cells activity of pancreas. High values of HOMA-IR indicate the high chances of cardiovascular diseases and diabetes development (56). Presence of IR in blood of diabetes's patient or end stage renal disease anticipate the cardiovascular mortality which is the result of genetic factors and environment. This event in blood leads to dyslipidaemia (57). Impairment in lipid and protein metabolism can leads to atherogenic dyslipidaemia. Triglycerides and LDL level increases due to insulin resistance whereas HDL level goes to downfall in diabetes malletus and end stage kidney disease (58). Insulin resistance and triglyceride interaction are bidirectional. Hypertriglyceridemia and increased fats metabolism can contribute to insulin resistance (59). It has been reported that elevated HOMA-IR level also correlated with increased prevalence of microalbuminuria (60,61). In current study STZ treatment to rats, elevate their HOMA-IR and decline HOMA-β. Eriodictyol and metformin treatment restored their levels by decreasing HOMA-IR and increasing HOMA-β level in blood.

In diabetic nephropathy, decreased insulin secretion results enhanced lipolysis and decreased lipogenesis and decreased HDL level and increased LDL level. Eriodictyol improve lipid metabolism, leads to increased HDL level and decreased TGS, TC, LDL level in blood.

To maintain the oxidative stress of a cell, there is a lot of antioxidant enzymes are present in a cell. In DN, excessive formation of free radical ions which leads to DNA damage and other pathological changes in tissue. These reactive oxygen species leads to renal tissue damage by lipid peroxidation of cell mambrane1 and generate oxidative stress in cell (62). In normal condition, oxidative stress is maintained by antioxidant enzymes, which was responsible for removal of reactive oxygen species. Eriodictyol treatment enhances the decreased level of SOD, GSH, Gpx and Catalase enzymes.

AGEs are the results of glycation of reduced sugar in hyperglycemic condition and mediates blood vessel damage, in irreversible condition (63). It is remarkably high in nephropathy as compared to other complication (64). In DN, its increased level plays an important role in pathogenesis of renal tissue damage. Eriodictyol treatment declines its level and prevent renal tissue from damage.

Anti-oxidants and anti-inflammatory are the two main defense systems of cell which protect it from the various diseases. These two activities help in maintain the renal structure and functions(65). In current study proinflammatory markers (IL-6, TNF-α and TGF-β1) are measured and found increased in blood stream. Increase in these values leads to stimulates inflammatory reactions. Over production of IL-6 leads to glomerulosclerosis (66) while TNF-α mediates decreased glomerulus filtration rate by damaging the epithelial cells in kidney tubule and glomerulus (67).TGF-β enhances the extracellular matrix protein in renal cells and causes glomerular basement membrane thickening (68). Eriodictyol treatment decreased their elevated levels and improves the renal tissue condition.

In histopathology, STZ provoked DN characteristic features such as Bowman's capsule enlargement, mesangial expansion, hypercellularity, vascular congestion, leukocytes infiltration in tubules, tubular cast formation. Microscopic analysis of urine also explore leakage of leukocytic cells, granular cast, crystals and renal tubular epithelial cells. Met and eriodictyol preserve the integrity of renal cells and leukocytes infiltration relative to diabetic nephropathy. Natural products are getting attention these days in diabetes and its complication managment. Metformin is already used for diabetic nephropathy treatment but it also generates a lot of side effects like anaemia, hypoglycaemia, anorexia and lactic acidosis. Eriodictyol might be an effective treatment against DN in long term use or may be a complement drug with reduced clinical dose of metformin, by which side effect of metformin can be managed.

CONCLUSION

Eriodyctiol found effective to treat DN and prevent its progression via Wnt/β- catenin pathway. It also showed improvement in pathological changes against diabetic nephropathy. Its anti-inflammatory and antioxidant activity and restoring of disease symptoms, make it a good alternative against existing drugs of nephropathy.

REFERENCES

1. Gross JL, De Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. (2005). Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes care. ;28(1):164-76.
2. Lim AK. (2014). Diabetic nephropathy–complications and treatment. International journal of nephrology and renovascular disease. :361-81.
3. Hovind, P., Rossing, P., Tarnow, L., Smidt, U. M., & Parving, H.-H. (2001). Progression of diabetic nephropathy. Kidney International, 59(2), 702–709. doi:10.1046/j.1523-1755.2001.059002702.
4. Duran-Salgado MB, Rubio-Guerra AF. (2014). Diabetic nephropathy and inflammation. World journal of diabetes. ;5(3):393.
5. Zhou D, Tan RJ, Fu H, Liu Y. (2016). Wnt/β-catenin signaling in kidney injury and repair: a double-edged sword. Laboratory investigation.;96(2):156-67.
6. Reeves R. (2015). High mobility group (HMG) proteins: Modulators of chromatin structure and DNA repair in mammalian cells. DNA repair. ;36:122-36.
7. Angers S, Moon RT. (2009). Proximal events in Wnt signal transduction. Nat Rev Mol Cell Biol;10:468–477. [PubMed: 19536106]
8. Clevers H, Nusse R.(2012). Wnt/beta-catenin signaling and disease. Cell;149:1192–1205. [PubMed: 22682243]
9. Zhou L, Li Y, Hao S, et al. (2015). Multiple genes of the renin-angiotensin system are novel targets of Wnt/ beta-catenin signaling. J Am Soc Nephrol ;26:107–120. [PubMed: 25012166]
10. Zhang H, Luo W, Sun Y et al: Wnt/beta-catenin signaling mediated-UCH-L1 expression in podocytes of diabetic nephropathy. Int J Mol Sci, 2016; 17(9): pii: E1404 (5,9,10)
11. Liu BL, Chen YP, Cheng H et al: The protective effects of curcumin on obesity-related glomerulopathy are associated with inhibition of Wnt/betacatenin signaling activation in podocytes. Evid Based Complement Alternat Med, 2015; 2015: 827472 23.
12. Dai C, Stolz DB, Kiss LP, et al. (2009). Wnt/beta-catenin signalling promotes podocyte dysfunction and albuminuria. J Am Soc Nephrol ; 20:1997–2008. [PubMed: 19628668]
13. Huang L, Lin T, Shi M, Chen X, Wu P. (2020). Liraglutide suppresses production of extracellular matrix proteins and ameliorates renal injury of diabetic nephropathy by enhancing Wnt/β-catenin signaling. American Journal of Physiology-Renal Physiology. ;319(3):F458-68.
14. Xie L, Zhai R, Chen T, Gao C, Xue R, Wang N, Wang J, Xu Y, Gui D. (2020). Panax notoginseng ameliorates podocyte EMT by targeting the wnt/β-catenin signaling pathway in STZ-induced diabetic rats. Drug Design, Development and Therapy.:527-38.
15. L. Marin, I. Gutierrez-del-R ´ ´ıo, P. Yag¨ue, A. Manteca, ´ C. J. Villar, and F. Lomb´o,(2017). "De novo biosynthesis of apigenin, luteolin, and eriodictyol in the actinomycete Streptomyces albus and production improvement by feeding and spore conditioning," Frontiers in Microbiology, vol. 8, p. 921.
16. Ley J P, Krammer G, Reinders G, Gatfield I L, and H J Bertram (2005) Evaluation of bitter masking flavanones from Herba Santa (Eriodictyon californicum (H. & A.) Torr . , Hydrophyllaceae) . J AGRI FOOD CHEM 53: 6061-66
17. Park SJ, Lee YH, Lee KH, Kim TJ (2013). Effect of eriodictyol on the development of atopic dermatitis-like lesions in ICR mice. Biol Pharm Bull 36:1375–1379. https://doi.org/10.1248/bpb. b13-00296
18. Lee ER, Kim JH, Kang YJ, Cho SG (2007) .The anti-apoptotic and anti-oxidant effect of eriodictyol on UV-induced apoptosis in keratinocytes. Biol Pharm Bull 30:32–37. https://doi.org/10.1248/ bpb.30.32
19. D. Li, N. Lu, J. Han et al., "Eriodictyol attenuates myocardial ischemia-reperfusion injury through the activation of JAK2," Frontiers in Pharmacology, vol. 9, p. 33, 2018
20. Y. Xie, R. Ji, and M. Han,(2019). "Eriodictyol protects H9c2 cardiomyocytes against the injury induced by hypoxia/reoxygenation by improving the dysfunction of mitochondria," Experimental and therapeutic Medicine, vol. 17, no. 1, pp. 551–557.
21. Y. Zhang, R. Zhang, and H. Ni,(2020). "Eriodictyol exerts potent anticancer activity against A549 human lung cancer cell line by inducing mitochondrial-mediated apoptosis, G2/M cell cycle arrest and inhibition of m-TOR/PI3K/Akt signalling pathway," Archives of Medical Science, vol. 16, no. 2, p. 446.
22. A. Hameed, R. M. Hafizur, N. Hussain et al.,(2018). "Eriodictyol stimulates insulin secretion through cAMP/PKA signalling pathway in mice islets," European Journal of Pharmacology, vol. 820, pp. 245–255.
23. W.-Y. Zhang, J.-J. Lee, Y. Kim et al.,(2012). "Effect of eriodictyol on glucose uptake and insulin resistance in vitro," Journal of Agricultural and Food Chemistry, vol. 60, no. 31, pp. 7652– 7658.
24. P. He, S. Yan, X. Wen et al., "Eriodictyol alleviates lipopolysaccharide-triggered oxidative stress and synaptic dysfunctions in BV-2 microglial cells and of Cellular Biochemistry, vol. 120, no. 9, pp. 14756–14770, 2019.
25. P. Lv, J. Yu, X. Xu, T. Lu, and F. Xu, "Eriodictyol inhibits high glucose-induced oxidative stress and inflammation in retinal ganglial cells," Journal of Cellular Biochemistry, vol. 120, no. 4, pp. 5644–5651, 2019

26. Bucolo C, Leggio GM, Drago F, Salomone S. (2012). Eriodictyol prevents early retinal and plasma abnormalities in streptozotocin-induced diabetic rats. Biochem Pharmacol. ; 84(1):88-92.
27. Kuhad A, Chopra K. (2009). Attenuation of diabetic nephropathy by tocotrienol: involvement of NFkB signalling pathway. Life Sci.; 84(9-10):296-301.
28. Cha PH, Shin W, Zahoor M, Kim HY, Min do S, Choi KY. (2014). Hovenia dulcis Thunb extract and its ingredient methyl vanillate activate Wnt/β-catenin pathway and increase bone mass in growing or ovariectomized mice. PLoS One, 22(1): 85546
29. Kadian S, Mahadevan N, Bala Kumar P. (2013). Differential effects of low-dose fenofibrate treatment in diabetic rats with early onset nephropathy and established nephropathy. Eur J Pharmacol.; 698(1-3):388-96.
30. Soetikno V, Sari FR, Veeraveedu PT, Thandavarayan RA, Harima M, Sukumaran V, Lakshmanan AP, Suzuki K, Kawachi H, Watanabe K. (2011). Curcumin ameliorates macrophage infiltration by inhibiting NF-κB activation and proinflammatory cytokines in streptozotocin induced-diabetic nephropathy. Nutr Metab (Lond). 10;8(1):35
31. Kundu A, Dey P, Sarkar P, Karmakar S, Tae IH, Kim KS, Park JH, Lee SH, Lee BM, Renthlei L, Puia Z, Kim HS. (2020). Protective effects of Croton hookeri on streptozotocin-induced diabetic nephropathy. Food Chem Toxicol., 135: 110873.
32. Ohkawa H, Onishi N, Yagi K. (1979). Assay for lipid per oxidation in animal tissue by thiobarbituric acid reaction. Anal Biochem, 95: 351-358.
33. Ellman GL. (1959). Tissue sulfhydryl groups. Arch Biochem Biophysics, 82:70-77
34. Claiborne, A., 1985. Catalase activity. In: Greenwald, R.A (Ed.), Handbook of Methods for Oxygen Radical Research. CRC Press Inc., pp. 283–284
35. Sastry KV, Moudgal RP, Mohan J, Tyagi JS, Rao GS. (2002). Spectrophotometric determination of serum nitrite and nitrate by copper-cadmium alloy. Anal Biochem.;306(1):79-82. doi: 10.1006/abio.2002.5676. PMID: 12069417.
36. Winterbourn CC, Hawking RE, Brain M, Carrel RW (1975). Determination of Superoxide Dismutase. J. Lab. Clin. Med. 2:337-341
37. Rotruck, J.T., et al. (1973) Selenium: Biochemical Role as a Component of Glutathione Peroxidase. Science, 179, 588-590
38. K. Raju, Evolution of pap stain, Biomed. Res. Ther. 3 (2016) 490 –500.
39. Samsu N. (2021). Diabetic Nephropathy: Challenges in Pathogenesis, Diagnosis, and Treatment. Biomed Res Int. ;2021:1497449. doi: 10.1155/2021/1497449. PMID: 34307650; PMCID: PMC8285185.
40. Qi C, Mao X, Zhang Z, Wu H. (2017). Classification and Differential Diagnosis of Diabetic Nephropathy. J Diabetes Res.:8637138. doi: 10.1155/2017/8637138. Epub 2017 Feb 20. PMID: 28316995; PMCID: PMC5337846
41. Latres E, Chiaur DS, Pagano M. (1999). The human F box protein beta-Trcp associates with the Cul1/Skp1 complex and regulates the stability of beta-catenin. Oncogene. 18(4):849-854. https://doi. org/10.1038/sj.onc.1202653
42. Zhang H, Luo W, Sun Y et al: (2016). Wnt/beta-catenin signaling mediated-UCH-L1 expression in podocytes of diabetic nephropathy. Int J Mol Sci, 17(9): pii: E1404
43. Liu BL, Chen YP, Cheng H et al: (2015). The protective effects of curcumin on obesity-related glomerulopathy are associated with inhibition of Wnt/betacatenin signaling activation in podocytes. Evid Based Complement Alternat Med,: 827472
44. Yang X, Wang X, Nie F et al: (2015). miR-135 family members mediate podocyte injury through the activation of Wnt/beta-catenin signaling. Int J Mol Med, 36: 669–77
45. Clevers H, Nusse R. (2012). Wnt/beta-catenin signaling and disease. Cell. 149(6):1192–1205. doi:10.1016/j.cell.2012.05.012
46. C, Stolz DB, Kiss LP, Monga SP, Holzman LB, Liu Y. (2009). Wnt/betacatenin signaling promotes podocyte dysfunction and albuminuria. J Am Soc Nephrol. 20(9):1997–2008. doi:10.1681/ASN.200 9010019
47. . Guo J, Xia N, Yang L, et al. (2014). GSK-3beta and vitamin D receptor are involved in beta-catenin and snail signaling in high glucose-induced epithelial-mesenchymal transition of mouse podocytes. Cell Physiol Biochem. 33(4):1087–1096. doi:10.1159/000358678
48. Cha PH, Shin W, Zahoor M, Kim HY, Min do S, Choi KY. (2014). Hovenia dulcis Thunb extract and its ingredient methyl vanillate activate Wnt/β-catenin pathway and increase bone mass in growing or ovariectomized mice. PLoS One. 22;9(1):e85546. doi: 10.1371/journal.pone.0085546. PMID: 24465596; PMCID: PMC3899039.
49. Lee ER, Kim JH, Kang YJ, Cho SG. (2007). The anti-apoptotic and anti-oxidant effect of eriodictyol on UV-induced apoptosis in keratinocytes. Biol Pharm Bull;30:32–7.
50. Areias FM, Rego AC, Oliveira CR, Seabra RM. (2001). Antioxidant effect of flavonoids after ascorbate/Fe(2+)-induced oxidative stress in cultured retinal cells. Biochem Pharmacol;62:111–8.
51. Hendriks JJ, de Vries HE, van der Pol SM, van den Berg TK, van Tol EA, Dijkstra CD. (2003). Flavonoids inhibit myelin phagocytosis by macrophages; a structure– activity relationship study. Biochem Pharmacol ;65:877–85.
52. Johnson J, Maher P, Hanneken A. (2009). The flavonoid, eriodictyol, induces long-term protection in ARPE-19 cells through its effects on Nrf2 activation and phase II gene expression. Invest Ophthalmol Vis Sci ;50:2398–406
53. A. Kaikini, D. Dhodi, S. Muke, V. Peshattiwar, S. Bagle, A. Korde, J. Sarnai k, V. Kadwad, S. Sachdev, S. Sathaye, (2020). Standardization of type 1 and type 2 diabetic nephropathy models in rats: assessment and characterization of metabolic features and renal injury, J. Pharm. Bioallied Sci. 12; 295 –307, https://doi.org/ 10.4103/jpbs.JPBS 239_19 .

54. O. Palygin, D. Spires, V. Levchenko, R. Bohovyk, M. Fedoriuk, C. A. Klemens, O. Syke s, J. D. Bukowy, A. W. Jr, J. Cowley, D. V. Lazar, A. (2019). S t a r u s c h e n k o Ilatovskaya, Progression of diabetic kidney disease in T2DN rats, Am. J. Physiol. Rena l Physiol. 31:7, F145 0 –F146 1, https://doi.org/10.1152/ajprenal.00246.201 9.
55. Amin A, Bajbouj K, Koch A, Gandesiri M, Schneider-Stock R (2015). Defective Autophagosome Formation in p53-Null Colorectal Cancer Reinforces Crocin Induced Apoptosis. International Journal of Molecular Sciences 16: 1544-1561
56. Schrauben, S.J., Jepson, C., Hsu, J.Y., Wilson, F.P., Zhang, X., Lash, J.P., Robinson, B.M., Townsend, R.R., Chen, J., Fogelfeld, L. & Kao, P. (2019). Insulin Resistance And Chronic Kidney Disease Progression, Cardiovascular Events, And Death: Findings From The Chronic Renal Insufficiency Cohort Study. Bmc Nephrology. 20(1), Pp.1-11.
57. Bianchi, S., Baronti, A., Cominotto, R. & Bigazzi, R. (2016). Lipid Metabolism Abnormalities In Chronic Kidney Disease. Giornale Italiano Di Nefrologia. Organo Ufficiale Della Societa Italiana Di Nefrologia. 33(68).40-47
58. Mikolasevic, I., Zutelija, M., Mavrinac, V. & Orlic, L. (2017). Dyslipidemia In Patients With Chronic Kidney Disease: Etiology And Management. International Journal Of Nephrology And Renovascular Disease. 10 (3): 35-46.
59. Guthoff, M., Wagner, R., Vosseler, D., Peter, A., Nadalin, S., Harimg, H.U., Fritsche, A. & Heyne, N. (2017). Impact Of End-Stage Renal Disease On Glucose Metabolism-A Matched Cohort Analysis. Nephrology Dialysis Transplantation.32 (4): 670-676.
60. Sun X, Xiao Y, Wang B, et al. (2015). Associations of pancreatic β-cell function and insulin resistance with microalbuminuria in type 2 diabetes. Diabetes Res Clin Pract.;110(3):e22–26. doi:10.1016/j.diabres.2015.09.012
61. Sarafidis PA, Ruilope LM. (2008). Insulin resistance, microalbuminuria, and chronic kidney disease. ***Curr Hypertens Rep***. 10(4):249–251. doi:10.1007/s11906-008-0046-6
62. Fernandes, S. M., Cordeiro, P. M., Watanabe, M., Fonseca, C. D. D., & Vattimo, M. D. F. F. (2016). The role of oxidative stress in streptozotocin-induced diabetic nephropathy in rats. Archives of Endocrinology and Metabolism, 60(5), 443–449.
63. Sohn E, Kim J, Kim C, et al. (2013). The extract of Litsea japonica reduced the development of diabetic nephropathy via the inhibition of advanced glycation end products accumulation in db/db mice. Evid Based Complement Altern Med. 2013:1-9.
64. N. Kaur, L. Kishor, R. Singh, (2017). Dillenia indica L. attenuates diabetic nephropathy via inhibition of advanced glycation end products accumulation in STZ-nicotinamide induced diabetic rats Journal of Traditional and Complementary Medicine xxx 1-13
65. S. Sifuentes -Franco, D. E. Padilla -Tejeda, S. Carrillo -Ibarra, A. G. Miranda -Díaz, (2018). Oxidative stress, apoptosis, and mitochondrial function in diabetic nephropathy, Int. J. Endocrinol. 187587 0, https://doi.org/10.1155/2018 /187587 0.
66. F. F. Kreine r, J. M. Kraaijenho f, M. von Herrat h, G. K. K. Hoving h, B. J. von Scholten, (2022). Interleukin 6 in diabetes, chronic kidney disease, and cardiovascular disease: mechanisms and therapeutic perspectives, expert rev, Clin. Immunol. 18; 377 –389, https://doi.org/10.1080/1744666X.2022.204595 2.
67. Fernández-Real, J. M., Vendrell, J., García, I., Ricart, W., & Vallès, M. (2012). Structural damage in diabetic nephropathy is associated with TNF-α system activity. Acta Diabetologica, 49(4), 301–305.
68. L. Zhao, Y. Zou, F. Liu, (2020). Transforming growth factor -beta 1 in diabetic kidney disease, Front. Cell Dev. Biol. 8 187, https://doi.org/10.3389/ fcell.2020.00187

CITATION OF THIS ARTICLE

Renu Malik, Balvindner Singh. Eriodictyol Ameliorates the Diabetic Nephropathy in Experimental Rats via WNT/B-Catenin Signalling Pathway. Bull. Env. Pharmacol. Life Sci., Vol 12[5] April 2023: 46-58.