Cellular and Molecular Biology

Journal homepage: www.cellmolbiol.org

Nephroprotective effects of polyherbal extract via attenuation of the severity of kidney dysfunction and oxidative damage in the diabetic experimental model

Mohd. Adnan Kausar[1,#*], Kehkashan Parveen[#2], Waseem A. Siddiqui[2], Sadaf Anwar[1], Aqeela Zahra[3], Abrar Ali[4], Riadh Badraoui[5], Azfar Jamal[6,7], Naseem Akhter[8], Neha Bhardwaj[9], Mohd Saeed[5*]

[1]*Department of Biochemistry, College of Medicine, University of Hail, Hail, Kingdom of Saudi Arabia*
[2]*Interdisciplinary Biotechnology Unit, Aligarh Muslim University, Aligarh, Uttar Pradesh, 202002, India*
[3]*Department of Family and Community Medicine., College of Medicine, University of Hail, Hail, Kingdom of Saudi Arabia.*
[4]*Department of Ophthalmology, College of Medicine, University of Hail, Hail, Kingdom of Saudi Arabia*
[5]*Department of Biology, College of Sciences, University of Hail, Hail, Kingdom of Saudi Arabia*
[6]*Health and Basic Science Research Centre, Majmaah University, Majmaah 11952, Kingdom of Saudi Arabia*
7*Department of Biology, College of Science, Al-Zulfi-, Majmaah University, Majmaah 11952, Saudi Arabia*
[8]*Department of Laboratory Medicine Faculty of Applied Medical Sciences Al- Baha University, Al- Baha, Kingdom of Saudi Arabia*
[9]*Department of Medical Elementology and Toxicology, School of Chemical and Life Sciences, Jamia Hamdard, New Delhi 110062, India*
[#]*These authors share equal contribution*

ARTICLE INFO

Original paper

Article history:
Received: July 23, 2021
Accepted: October 31, 2021
Published: December 01, 2021

Keywords:
Diabetes; Inflammation, Oxidative stress; Polyherbal extract; Nephroprotective

ABSTRACT

In view of many complications of diabetes, kidney failure is considered as one of the main complications. The oxidative stress-induced due to persistent hyperglycemic conditions is the major cause of kidney disease. The present study was designed to explore the nephroprotective efficacy of polyherbal (PH) extract in a diabetic model induced by streptozotocin (STZ). STZ (55 mg/kg body weight, intraperitoneal) was injected in overnight fasting rats to develop the diabetic experimental model. Effect on kidney injury was evaluated by investigating biochemical and histological evidences in renal tissue after 56 days of treatment of PH extract. Results showed the high glucose level in STZ treated rats that suggested hyperglycemia persistence along with the successful establishment of nephropathy in diabetic rats with altered renal function, inflammatory cytokines level as well as oxidative and nitrosative stress. Administration of PH extract significantly improved the glycemic condition, glomerular function and proximal reabsorptive markers. Further, elevated pro-inflammatory cytokines levels and disturbed redox status were restored. Moreover, findings were fostered and substantiated by histopathological examinations. Our work strongly proposes that the nephroprotective effect of the PH extract on renal damage could be attributed due to its anti-inflammatory and antioxidant properties. Thus, PH extract could have potential as a pharmaceutical drug for diabetes mellitus (DM). Additional long-term study or clinical trial is required for further investigations.

DOI: http://dx.doi.org/10.14715/cmb/2021.67.4.6

Introduction

Diabetes mellitus (DM) is described as a metabolic syndrome that causes morbidity and mortality leading to tissue damage with consequent microvascular and macrovascular manifestations (1, 2). Diabetic nephropathy (DN) is one of the foremost severe manifestations of DM, which is described as the dysfunction of the nephrotic system due to persistent hyperglycemic conditions. It became the most important explanation of the end-stage renal disease, compelling dialysis or kidney replacement for survival (3). Studies in patients with type 2 diabetes mellitus indicate that while poor metabolic control is the most important determinant of the development of nephropathy, hyperlipidemia and hypertension are equally responsible for this condition (4). DN is characterized by a change in functional as well as structural integrity, which causes intraglomerular hypertension leading to further deterioration of the kidney and consequently resulting in end-stage renal

*Corresponding author. E-mail: ma.kausar@uoh.edu.sa; mo.saeed@uoh.edu.sa

disease (5-7). Structural and functional changes occur at glomeruli and tubular level and are marked as a glomerular epithelial cell (podocyte) loss, renal hypertrophy, mesangial expansion, expansion of tubular basement membranes, tubular atrophy, and glomerular basement membrane thickening and fibrosis due to excessive accumulation of extracellular matrix (ECM) proteins (8).

Accumulating evidence showed hyperglycemia-induced oxidative stress leads to overproduction of reactive oxygen species (ROS) and depletion of antioxidants, which alters the metabolic pathways in the kidneys and causes progression of DN (9-11). Excessive ROS generation stimulates renal fibrosis and inflammation and leads to significant tissue damage by increasing lipid peroxidation, DNA fragmentation, and protein modification, as well as mitochondrial dysfunction (12, 13). It is reported that the induction of inflammation and oxidative stress by the mechanism of hyperglycemia may play a crucial role in the pathogenesis of DN (14-16). The development of DN is linked with significant inflammatory molecules infiltration such as tumour necrosis factor-α (TNF-α), transforming growth factor-β (TGF- β) and interleukin -1β (IL-1β) (17, 18). Although several mechanisms have been proposed in the progression of DN, the exact mechanism remains unexplored, and it seems that these mechanisms act synergistically in rendering renal disease.

Synergism is a versatile phenomenon that has been preferred by nature for decades. Pharmaceutical preparation using different formulations is preferred for the treatment of diabetes in the modern system of medicine. It has been assumed that combining different properties of herbs like blood glucose control, antioxidants, and anti-inflammatory helps to make a golden remedy provided by herbal formulations (19, 20). Polyherbal formulations are plant-based preparation that may work collaboratively by their diverse, active constituents within themselves (21). These preparations provide maximum therapeutic efficacy without causing any side effects.

Co-administration of different medicinal plants has been shown to prevent DN (18). In the present study four plants (*Cichorium intybus* Linn., *Cucumis melo* Linn, *Cynomorium coccineum*, *Cynomorium songaricum*) were selected for polyherbal preparation. In the traditional system of medicine different preparations of *Cichorium intybus* L., commonly known as Chicory or Kasni, have been practiced for digestive and hepatic problems and lower glucose levels and inflammation (22, 23). Several studies have reported the antioxidant, inflammation-reducing, and blood-glucose-lowering activities of Cichorium and, to some extent investigating the mechanistic approach also (24-27). The effectiveness of chicory is attributed owing to the consistency of medically important phytochemicals, including alkaloids, inulin, sesquiterpene lactones, coumarins, minerals, vitamins, unsaturated sterols, monomeric flavonoids, saponins, tannins and polyphenols (23).

Cucumis melo has been shown to possess an array of medicinally important beneficial effects in traditional systems of medicine, including anti-inflammatory, antioxidant and anti-diabetic and also managing the severity of renal ailments such as renal and urinary bladder stones, ulcers in the urinary tract and burning micturition (28-31). Phytoconstituents present in the seeds of *Cucumis mel*o contain chromone derivatives, phenolic glycoside, arginine, aspartic acid, glutamic acid, alpha-galactosidases, dihydroxy triterpenes, sitosterol 2, and beta-sitosterol, etc.(31).

Cynomorium sps. has been used in the traditional Chinese medicine system and other parts of the world due to their various health benefits and has been regarded as a "treasure of drugs". Many research studies have shown that two sps of Cynomorium family namely *C. songaricum* and *C. coccineum*, displayed a huge array of pharmacological activity, including antihypoxia, antioxidant, antidiabetic, immunity improving and neuroprotective (32-35). The major phytoconstituents present in *Cynomorium* plants were phenolic compounds, steroids, triterpenes, organic acids, saccharides and glycosides (36).

Keeping on the view of the above effects of the herbals for possible nephrotic disorders and other biological activities, the present study was focused on experimentally elucidating the combining beneficial outcomes of polyherbal preparation in the management of DN.

Materials and methods

Chemicals and reagents

Streptozotocin (STZ), ethylene diamine tetraacetic acid (EDTA), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), pyrogallol,

Glutathione (GSH), 5, 5'-dithiobis-2-nitrobenzoic acid) (DTNB), 1,1,3,3-tetramethoxypropane (TMB), p-nitrophenyl-N-acetyl-β-d-glucosaminide, sodium nitrite, thiobarbituric acid (TBA), sodium nitrate, were obtained from Sigma–Aldrich Chemicals Pvt. Ltd. (India). Bovine serum albumin (BSA) was purchased from Srl and Merck Chemical Pvt. Ltd. (India). All the other chemicals were of analytical reagent grade. Plant materials were purchased from the local market of Delhi, INDIA.

Preparation of Polyherbal (PH) extract

Four single herbal materials, namely *Cichorium intybus* (Root and seeds), *Cucumis melo* (Seeds), *Cynomorium coccineum* (Stem) and *Cynomorium songaricum* (Stem) were mixed equally to make PH extract. The respective part of plant material was dried in the shade and made into coarse powder for extraction. Extraction was done with distilled water using soxhlet apparatus for 3 days. After cooling the extraction was filtered to remove the residue. The extraction was concentrated using a rotary evaporator under reduced pressure and then dried to get a fine powder. The yield of crude extract, called PH extract, was stored in an air-tight bottle for further analysis. The dried powder was dissolved in normal saline with proper proportion for the study.

Acute Toxicity Study

According to OECD guidelines, the acute toxicity study of the PH extract was assessed using male Wistar rats weighing 130–140 g to find out the dose. The animals were fasted 6-8 hours prior to carrying out the experimental plan. Different doses of PH formulation were given intraperitoneally (i.p.). The LD50 was calculated according to Miller and Tainter. Polyherbal formulation did not show any mortality even at the higher dose (2000mg/kg, p.o); on this basis, the dose (200mg/kg) of formulation (36) was selected for further studies in animal models.

Experimental animals

Male Wistar rats (150±10 g), 7–8 weeks old, were obtained from the Animal House facility of Jamia Hamdard, New Delhi. Animals were freely permitted to acclimatize with animal house conditions for one week before the experiment and were provided free access to standard laboratory rodent feed and water ad libitum prior to the experiment. All procedures for using animals were reviewed and approved by Institutional Animal Ethics Committee registered under the Committee for the Purpose of Control and Supervision of Experimental Animals (173/CPCSEA).

Experimental Design

Animals were divided into four groups of six rats each. Diabetes was induced in rats by giving STZ (55 mg/kg body weight) intraperitoneally (i.p.). After three days of STZ administration, blood was taken from 12 h overnight fasted rats via the tail vein. Fasting blood glucose (FBG) level was assessed using a glucometer. The animals with fasting blood glucose levels of more than 250 mg/dl were considered diabetic and used in the study. The experimental design and treatment protocols were as follows:

Control (C): served as normal control and given single i.p. injection of normal saline only.

Diabetic (D): served as diabetic and given a single dose of STZ (55mg/kg body weight; i.p.) and then kept untreated for 56 days to develop DN.

D+PH: animals were given PH extract orally (200 mg/kg body weight) for 56 days after diabetes induction.

C+ PH: served as treatment control and given PH extract orally (200 mg/kg body weight) for 56 days.

FBG concentration using a strip-operated glucometer was determined after 56 days in all groups and 24 hours urine was also collected for estimation of urinary protein, 8-Oxo-7,8-dihydro-2-deoxyguanosine (8-OHdG), N-acetyl-β-D-glucosaminidase (NAG) and creatinine estimation in order to mark the progression of renal damage.

Collection of sample and tissue preparation

During a 24-hour cycle, the urine samples were collected and stored under oil (mineral) to prevent vaporization. After collection, the measurement of the volume of the urine was done. Blood was taken retro-orbitally from the eye and serum was separated and stored at −80 °C for further analysis. The rats were sacrificed, one kidney was quickly extracted, and the cortex was separated from the medulla for homogenate preparation. Subsequently, the other kidney was detached and stored for histopathological tests in a 10% buffered formalin solution. Homogenization of Cortical slices was carried out in ice-cold phosphate

buffer. The centrifugation of homogenate was done at 800×g for 5 min at 4◦C to remove the nuclear debris and a small quantity of supernatant was stored to measure malondialdehyde (MDA). The remaining supernatant was further centrifuged to obtain the post-mitochondrial supernatant (PMS), which served to estimate other biochemical assays.

Evaluation of kidney function

Creatinine level in urine and serum was estimated using a commercial assay kit using the sarcosine oxidase method. Clearance was calculated by using the standard equation of Arreola-Mendosa et al. (37). Glomerular filtration rate (GFR) was measured from creatinine clearance. Blood urea nitrogen (BUN) level was measured in serum by the urease method using commercial assay kits and expressed as mmol/l. Estimation of protein excreted in urine had been used as a sensitive indicator of renal disease. Proteinuria was measured as total protein (mg/24 hr) described by the Bradford method (38). NAG level in urine was measured to detect damage at the tubular level. It was estimated using the method previously reported by Price et al. (39), with slight modification by Pedraza-Chaverri and co-workers (40). One unit of NAG was expressed as the amount of enzyme releases per 1µmol of p-nitrophenol in the assay medium.

Urinary 8-OHdG level

Urine samples were centrifuged at 2,000g for 20 min and after that dilution was done to assess the 8-OHdG level. Urine level of 8-OHdG was determined by ELISA assay (Elabscience, Texas, USA) and expressed as total amounts excreted in 24 h.

Assessment of Inflammatory cytokines in the Serum

ELISA assay kits determined serum levels of inflammatory molecules (TGF-β1, IL-1β and TNF-α) according to the manufacture's protocols (Elabscience, Texas, USA).

Assay for Nitric oxide (NO) as (NO2- + NO3-)

As nitric oxide is an unstable product, it was determined as a combined nitrite/nitrate level (the stable degradation by-products of nitric oxide). Reducing agent copperized cadmium was utilized to reduce nitrate into nitrite and then color development reaction was done by adding Griess reagent in acidic condition (41).

Assessment of oxidative stress in renal tissue

Renal lipid peroxidation was evaluated as MDA and is expressed as nmol MDA formed/h/ mg protein using Ohkawa et al. (42) method. The total GSH level was assessed using DTNB as reported earlier by Gherghel and co-workers (43). Glutathione peroxidase (GPx) activity was estimated using H_2O_2 as the substrate (44). The glutathione reductase (GR) activity was measured by the protocol of Carlberg and Mannervik (45), with some modifications. The assay mixture comprised of phosphate buffer, GSSG, NADPH, EDTA, and PMS (10%) in a final volume of 2.0 ml. The enzyme activity was estimated at 25°C by determining the vanishing of NADPH at 340 nm. Superoxide dismutase (SOD) enzyme was assessed using the Marklund and Marklund (46) method and expressed as protein units/mg. Claiborne (47) method was used for catalase activity. The assay system comprised phosphate buffer, hydrogen peroxide, and PMS. A deviation in absorbance was noted down at 240 nm.

Histopathological analysis of tissues

In histopathological tests, the kidney retained in 10 % buffered formalin solution was used. A small piece of renal tissue with medulla & cortex and the whole kidney tissue was dehydrated and immersed in paraffin after fixation in buffered formalin solution. At least three cross-sections of each kidney were taken as slices of 3-4 µm thickness and then stained with hematoxylin and eosin (H and E), respectively. The tissue sections were mounted with DPX mountant after two changes of xylene washes of 2 min each. The slides were then observed for microscopic evaluation (bright field). A microscope system was used for microphotographs. Kidney stains PAS were used to determine the severity of glomerular lesions, mesangial growth, absence or existence of any protein casts in the tubular atrophy, tubules, and lymphocytic infiltration. The kidney tissue's H & E staining procedure was done to evaluate the degree of damage to the renal architecture.

Protein assay

Total protein estimation was done in the homogenate and PMS as described previously (Bradford, 1976) (38) utilizing BSA as a standard.

Statistical analysis

Analysis was done employing the statistical software SPSS 23. ANOVA test was used for comparison between the groups followed by Tukey–Kramer test post-analysis test for multiple comparisons with P <0.05 being considered statistically significant. The data were expressed as mean±S.E.M.

Results and discussion

Effect of PH extract on FBG level

The blood glucose level (Figure1) measured after 56 days showed significant (P<0.05) persistent elevation in the diabetic group compared to the control group. Supplementation with PH extract significantly (P<0.05) reduced the blood glucose level in the D+PH group after 56 days compared to the diabetic group. No significant change was noted in the normal rat treated with PH extract C+PH group.

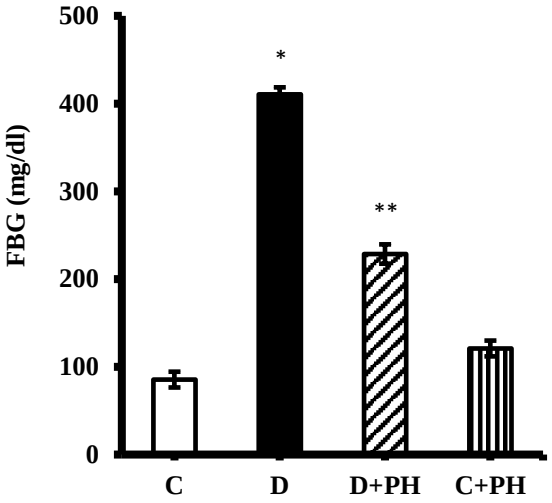

Figure 1. Effect of PH extract treatment on the blood glucose level. Values represented as mean ± S.E.M. (*P<0.05 diabetic (D) group vs. control (C)/C+PH group; **P<0.05 D+PH group vs. D group).

Effect of PH extract on kidney function markers

Serum creatinine and creatinine clearance

The level of serum creatinine was significantly (P<0.05) elevated. In contrast, creatinine clearance was significantly (P<0.05) decreased in the diabetic group as compared to the control group at the end of 56 days study period (Figures 2 A and 2 B). High serum creatinine levels and decline in creatinine clearance were taken as an index of altered GFR in DN. Supplementation with PH extract significantly (P< 0.05) ameliorated the alterations in GFR at the end of the study by decreasing serum creatinine level while improving the creatinine clearance compared to diabetic rats.

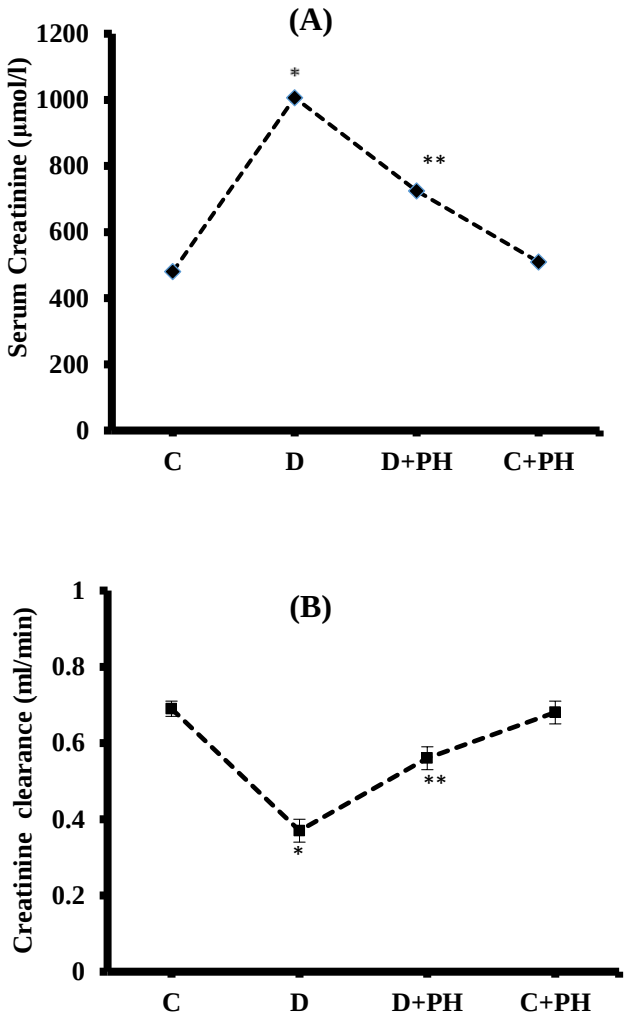

Figure 2. (A) Effect of PH extract treatment on serum creatinine level in experimental groups. Values represented as mean ± S.E.M. (*P<0.05 diabetic (D) group vs. control (C)/C+PH group; **P<0.05 D+PH group vs. D group). (B) Effect of PH extract on creatinine clearance in experimental groups. Values represented as mean ± S.E.M. (*P<0.05 diabetic (D) group vs. control (C)/C+PH group; **P<0.05 D+PH group vs. D group).

Blood Urea Nitrogen (BUN)

Estimation of BUN level is a sensitive index to evaluate kidney function. After 56 days of diabetes induction, diabetic rats had a significant (P0.05) rise in BUN compared to control rats. (Figure 3). Treatment with PH extract lowered the BUN level in D+PH,

wherein there was no significant change in BUN level in the C+PH extract-treated group compared to the control group.

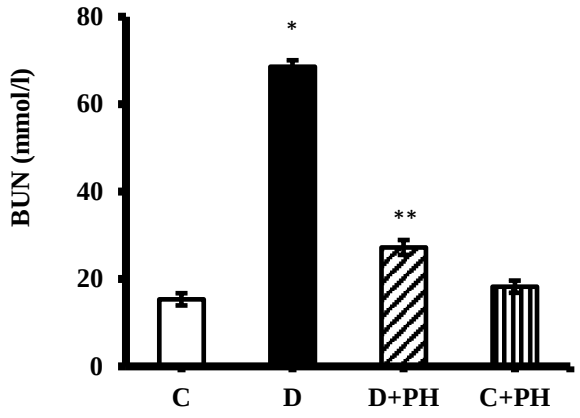

Figure 3. Effect of PH extract treatment on BUN level in experimental groups. Values represented as mean ± S.E.M. (*P<0.05 diabetic (D) group vs. control (C)/C+PH group; **P<0.05 D+PH group vs. D group).

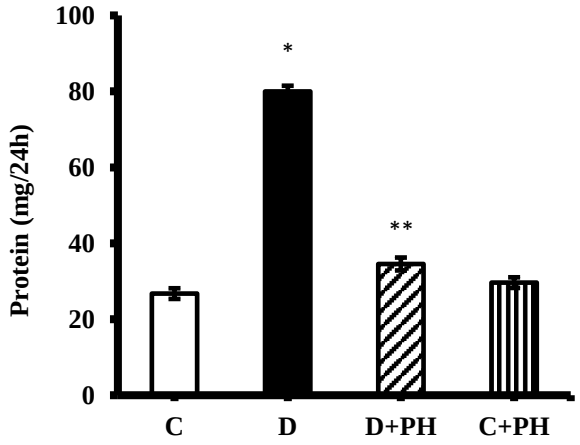

Figure 4. Effect of PH extract treatment on excretion of urinary protein in experimental groups. Values represented as mean ± S.E.M. (*P<0.05 diabetic (D) group vs. control (C)/C+PH group; **P<0.05 D+PH group vs. D group).

Proteinuria

The urinary excretion of protein is a more sensitive indicator of renal damage that occurs during DN. The total protein level in urine (Figure 4) was significantly (P<0.05) increased in the diabetic group compared to the control group. Supplementation with PH extract significantly (P<0.05) decreased the proteinuria in the D+PH group at the end of the study, no significant reduction was found in the C+PH group. Proteinuria levels in the PH extract supplemented group were similar to those in the control group.

Effect of PH extract on NAG level

The effect of PH extract on NAG level in urine was also determined in this study. The urine concentration of NAG (Figure 5) in the diabetic group was significantly higher than those in the control groups (P <0.05). Treatment with PH extract 200 mg/kg in the diabetic group caused a significant decrease in the NAG urine level in comparison with the diabetic control group (P <0.05). The NAG concentration in the C+ PH extract was not significantly different compared to the control group (P >0.05). Thus, 56 days of treatment of PH extract in diabetic rats significantly reduced NAG level as compared to the control.

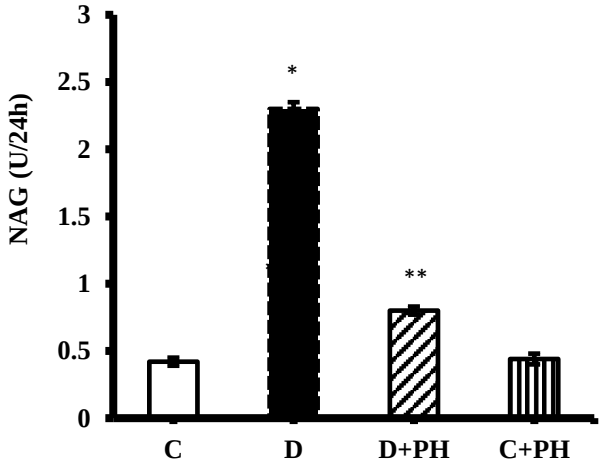

Figure 5. Effect of PH extract treatment on NAG level in experimental groups. A significant increase in NAG level was observed in the D group in serum. PH extract treatment preserved the level significantly. Values represented as mean ± S.E.M. (*P<0.05 diabetic (D) group vs. control (C)/C+PH group; **P<0.05 D+PH group vs. D group).

Effect of PH extract on urinary 8-OHdG excretion

8-OHdG is suggested as one of the overriding patterns of ROS-induced oxidative DNA modification, and it has been indicated index of oxidative stress. Therefore, measuring 8-OHdG excretion in the urine functioned as a valid biomarker for monitoring diabetes-related oxidative damage. A dramatic increase in urinary 8-OHdG level was observed in diabetic rats versus the control group (Figure 6). PH extract

administration for 56 days produced a significant decline in the 8-OHdG level in the D+PH group as compared to the untreated diabetic group. The synergistic therapy reverted 8-OHdG back to the normal control level.

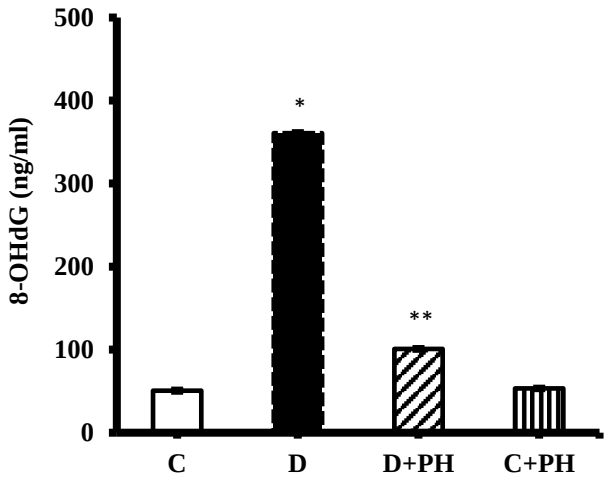

Figure 6. Effect of PH extract on urinary 8-OHdG excretion in experimental groups. A significant increase in the 8-OHdG level was noted in the D group in the urine sample. PH extract treatment significantly decreased its level in the D+PH group. Values represented as mean ± S.E.M. (*P<0.05 diabetic (D) group vs. control (C)/C+PH group; **P<0.05 D+PH group vs. D group).

Effect of PH extract on inflammatory cytokines production

To investigate the anti-inflammatory effect of PH extract, we measured the level of TGF-β1, IL-1β and TNF-α in serum. The levels of TGF-β1, IL-1β, and TNF-α found in the serum of diabetic rats were all considerably (P >0.05) higher than the levels found in the serum of control rats. (Table 1). PH extract administration significantly (P >0.05) diminished inflammatory molecule levels in diabetic rats compared to untreated diabetic rats. Therefore, our data indicate that diabetes resulted in significantly (P <0.05) elevated levels of TGF-β1, IL-1β and TNF-α of control values. Treatment of diabetic rats in the D+PH group, significantly (P <0.05) improved the levels of TGF-β1, IL-1β and TNF-α in comparison to that of diabetic untreated rats.

Table 1. Effect of PH extract on inflammatory cytokines in the serum

	Control (C)	D	D+PH	C+PH
TGF-β1(ng/ml)	1.24±0.53	3.12±0.94* (+151.61%)	2.12±0.66** (-32.05%)	1.27±0.58 (+2.41%)
IL-1β (pg/ml)	80.39±2.1	239.85±8.4* (+198.35%)	136.74±5.2** (-42.99%)	83.31±3.9 (+ 3.63%)
TNF-α (pg/ml)	305.48±7.9	689.99±10.5* (+125.87%)	387.45±6.1** (-43.85%)	310.22±5.9 (+1.55%)

Values represented as mean ± S.E.M. Diabetic group indicated a significant elevation in TGF-β1, IL-1β and TNF-α in kidney tissue. PH extract treatment preserved the marker levels significantly. *P<0.05 diabetic (D) group vs. control (C) / C+PH group; **P<0.05 D+PH group vs. D group.

Effect of PH extract on cortex NO

Total nitric oxide level in the renal cortex was raised (P< 0.05) significantly in the diabetic rats while compared to control ones (Figure 7) showing the nitrosative stress condition. Administration of PH extract significantly modulated this increase in the D+PH group when compared to the diabetic group.

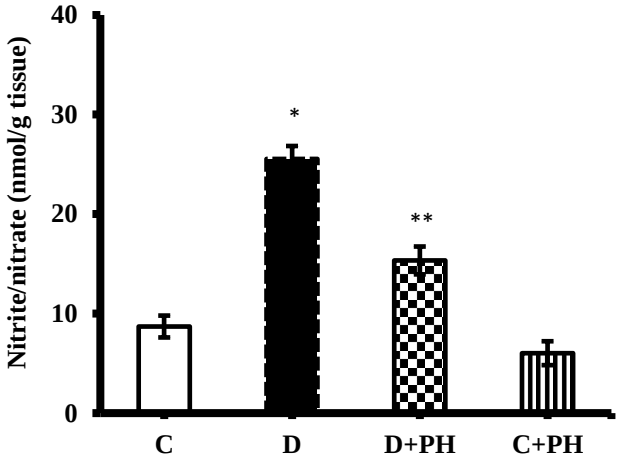

Figure 7. Effect of PH extract on renal cortex NO level in experimental groups. A significant increase in NO level was observed in the D group in the renal cortex. While administration with PH extract significantly augmented its level in the D+PH group. Values represented as mean ± S.E.M. (*P<0.05 diabetic (D) group vs. control (C)/C+PH group; **P<0.05 D+PH group vs. D group).

Effect of PH extract on oxidative stress in the kidney

Renal MDA was evaluated as an indicator of renal lipid peroxidation. GSH, GPx, GR, SOD and Catalase levels were determined as an index of antioxidant status during diabetes-induced oxidative stress. GSH, GPx, GR, SOD and Catalase levels were significantly ($P < 0.05$) reduced, whereas the levels of MDA were significantly ($P<0.05$) increased in the kidney of the diabetic group compared to the control group (Table 2). Administration of PH extracts significantly ($P<0.05$) restored the level of GSH and antioxidant enzymes and diminished the lipid peroxidation in kidney tissues as compared to the diabetic group.

Table 2. Effect of PH extract on oxidative stress markers in the kidney

	Control (C)	D	D+PH	C+PH
MDA (nmol MDA formed/h/mg protein)	0.38±0.23	1.11±0.34* (+192.10%)	0.64±0.46** (-42.34%)	0.35±0.41 (-7.89%)
GSH (nmol/mg protein)	7.12±1.6	2.47±0.91* (-65.31%)	3.32±1.2** (+34.41%)	7.89±1.4 (+10.81%)
GPx (nmol NADPH/min/mg protein)	25.04±3.9	11.89±4.5* (-52.51%)	18.35±5.1** (+54.33%)	26.10±4.9 (+4.23%)
GR (nmol NADPH/min/mg protein)	11.87±1.9	5.21±3.5* (-56.11%)	8.32±4.1** (+59.69%)	10.47±3.9 (-11.79%)
SOD (U/mg protein)	6.74±0.86	2.69±0.89* (-60.09%)	4.13±1.1** (+53.53%)	6.12±1.3 (-9.20%)
CAT (nmol H_2O_2 consumed/min/mg protein)	45.55±4.6	19.67±3.4* (-56.82%)	36.88±4.8** (+87.49%)	44.88±3.8 (-1.47%)

Values represented as mean ± S.E.M. Diabetic (D) rats exhibited a significant increase in MDA level with a decreased GSH, GPx, GR, SOD and CAT activity in kidney tissue. PH extract treatment restored all these changes.*P<0.05 diabetic (D) group vs. control (C)/ C+PH group; **P<0.05 D+PH group vs. diabetic (D) group.

Histopathological findings

Histologically control renal tissue shows well preserved renal parenchyma. Normal glomeruli were seen surrounded by the Bowman's capsule and renal tubules. There were no inflammatory or degenerative lesions were seen. While in the diabetic group, degenerative changes were seen in renal tubular epithelial cells characterized by necrotic changes, edema and vacuolation. Atrophy of the glomeruli was also seen. The focally hyaline deposit was seen in renal tubules. Findings showed that treatment with PH extract restored most of the renal tissue with well-preserved renal parenchyma. Mild degenerative changes were seen focally. There were no inflammatory lesions seen (Figure 8).

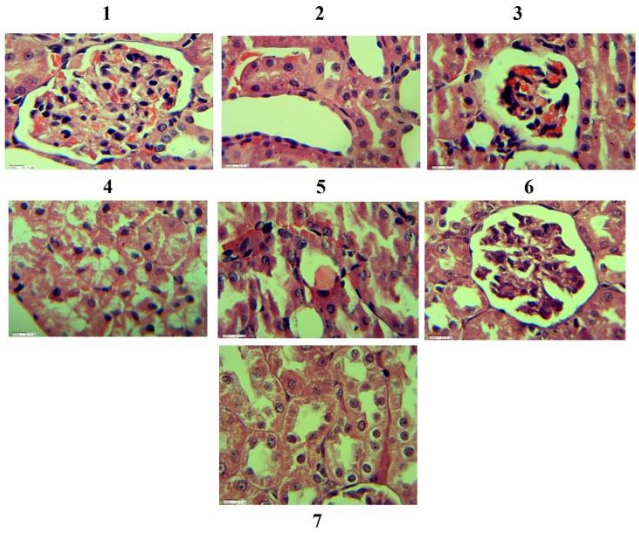

Figure 8. H&E stained section of Kidney showing 1) Control Kidney– Apparently normal glomerulus and renal tubules 40X. 2) Control Kidney– Normal renal tubules 40X. 3) Diabetic Kidney – Atrophied glomerulus and mild degenerative changes in the surrounding renal tubular epithelial cells 40X. 4) Diabetic Kidney- Oedematous changes in tubules, vacuolation in tubular epithelial cells and necrotic changes 40X. 5) Diabetic kidney – Hyaline deposit in renal tubule 40X.6) Treated Kidney – Normal glomerulus and renal tubules 40X. 7) Treated Kidney– Normal renal tubules 40X.

Polyherbal formulations have been known to show potential response in the treatment of a vast number of diseases. The therapeutic effect of the polyherbal formulation was employed due to the presence of various phytoconstituents acting synergistically (48). The potentiality of these formulations was found high even at low doses and safe at high doses. Considering the therapeutic efficacy of polyherbal formulation, our study was focused to evaluate the hypoglycemic and nephroprotective action of PH extract composed of four plant's extracts *Cichorium intybus*, *Cucumis melo*, *Cynomorium coccineum* and *Cynomorium songaricum* in STZ-induced DN in rats. Treatment with the PH extract had significantly ameliorated the severity of renal dysfunction and improved hyperglycemia-induced oxidative damage during DN. Our data revealed that the administration of PH extract at a dose of 200 mg/kg body weight significantly reduced blood

glucose levels in STZ-induced diabetic rats. This may be because the PH extract used in our study involves four individual medicinal plants, which were already reported for their antidiabetic activity (23, 34, 49) as well as for reducing hepatic glucose-6-phosphatase activity (50) in diabetic rats. Another possible cause for the noted response could be the remarkably enormous concentration of phytoconstituents present in PH extract, which may enhance glucose uptake, increase insulin-sensitizing and secreting properties (51-54).

Earlier reports suggested that DN progression leads to a decline in GFR due to expansion of mesangial matrix and increased glomerular matrix proteins level caused by persistent hyperglycemia (55, 56). In diabetic rats, measurement of BUN and serum creatinine was done to evaluate the altered GFR during DN progression (57). In the present study, serum creatinine and BUN level were found to be increased, whereas a reduction in creatinine clearance was found in the diabetic group coinciding with a previous study (57). We demonstrated that treatment with PH extract for 56 days ameliorated the GFR in diabetic rats significantly, indicating the nephroprotective role of herbal formulation. The study suggested the antihyperglycemic action of PH extract to improve GFR, subsequently reducing mesangial expansion and accumulated matrix proteins.

The proteinuria in DN is mainly due to diminished reabsorption of filtered plasma proteins by tubules, resulting in an elevated loss of low molecular weight proteins in urine (58)(56). Many studies have also reported that altered GFR resulted in the development of proteinuria by changing blood flow and vascular permeability, resulting in protein penetration into bowman's space (6, 59). The present study showed a significantly elevated level of total protein excreted in the urine in the diabetic group. While supplementation with PH extract in diabetic rats decreased the degree of proteinuria, improvement in proteinuria in diabetic rats receiving PH extract may be due to hypoglycemic and inhibiting TGF-β induced tubular injury and NO synthesis that could contribute to protein infusion into bowman's space (57).

N-acetyl-β-d-glucosaminidase (NAG) is a somewhat high molecular weight enzyme found in the proximal tubules' epithelial cells. As DN progression occurs, it results in secretion of NAG in urine following the renal tubular damage (60). Actually, urinary excretion of NAG has been suggested as a foremost tubular susceptible marker for the evaluation of tubule-interstitial damage during the advancement and progression of renal injury (60, 61). The present study showed that with the progression of DN, the NAG level was found to be increased in diabetic rats. It is suggested that the antioxidant property of PH extract is the main cause of the reduction of NAG level in the treatment group, further showing nephroprotective action through a subsequent decline in tubule-interstitial damage and kidney inflammation elicited due to hyperglycemic condition consistent with previous studies (4, 62).

According to recent studies, 8-OHdG is produced following DNA oxidation and is the most common quantifiable marker for oxidative stress to indicate DNA damage (63, 64). Research has reported the increased levels of 8-OHdG in the urine of diabetic patients (63). Hence, urinary excretion of 8-OHdG may be considered as an index of oxidative damage to DNA in the whole body. Our study found that the amount of urinary 8-OHdG excretion was markedly elevated in the diabetic group, while this alteration was modulated after PH extract administration. This response may be due to the antioxidant property of the higher phytoconstituent present in PH extract in line with the earlier studies (4, 65).

Considerable studies have suggested a strong association of chronic inflammation and hyperglycemia with the severity of kidney dysfunction (66, 67). Accumulation of pro-inflammatory molecules has been connected to insulin resistance and renal fibrosis progression in a clinical study during diabetes (68, 69). In general, accumulated inflammatory cytokines evoked TGF-β1, resulting in fragmentation of the epithelial-mesenchymal transition (EMT), leading to renal fibrosis (70). Furthermore, TGF-β1 leads to the progression of DN, contributing to extracellular matrix (ECM) accumulation, ultimately leading to glomeruli scarring or hardening in diabetes (71). Increased levels of inflammatory cytokines such as TNF-α and IL-1β were found in the development of nephropathy, thus playing a role in DN's pathogenesis (72). In the present study, elevated levels of TGF-β1, IL-1β and TNF-α were found in diabetic rats consistent with the findings of the earlier studies. This study evaluated that PH extract significantly lowered TGF-

β1, IL-1β, and TNF-α levels in the serum of diabetic rats due to their anti-inflammatory property (73). Also, *Circhorium intybus* was found to inhibit NF-kB and TNF-α expression through modulation of NLRP3 inflammasome activation, leading to an anti-diabetic effect by improving insulin resistance and inhibiting inflammation (74).

According to reports, NO has a function in maintaining GFR and renal perfusion, whereas excessive NO generation induces afferent arteriolar expansion, glomerular thickening, and hyperfiltration (75)(76). $NO2^{-}$ +$NO3^{-}$ together can be taken as a basis of NO generation due to its instability. This study demonstrated that cortex NO level was higher in diabetic rats compared to control in line with the previous studies in animals and humans (76, 77). Additionally, NO carried out renal damage through the production of cytotoxic agent peroxynitrite by binding to superoxide radicals agent (76). The supplementation of PH extract for 56 days significantly diminished the NO level in the diabetic-treated group. This beneficial effect of PH extract can be correlated with the antioxidant and anti-inflammatory potential by downregulating the expression of inducible NO synthase (77).

Considerable evidences have suggested that high blood glucose level leads to ROS formation, which is closely associated with renal dysfunction (78, 79). ROS accumulation decreased the antioxidant system that resulted in oxidative damage to DNA, lipids, and proteins during DN. Along with this, persistent hyperglycemia impaired scavenging mechanisms via the glycosylation of antioxidant enzymes, such as SOD and catalase (80). Similar to these findings, the present study showed that the level of MDA, a lipid peroxidation product increased as well as levels of GSH, GR, SOD, GPx and catalase were decreased significantly in diabetic rats while treatment with PH extract restored all these ailments. Our results showed that the PH extract counteracts oxidative stress and increases antioxidant defense status in experimental animals due to antioxidant properties (81). Also, the PH extract may contain an enormous level of phytoconstituents that could synergistically preserve renal damage during oxidative stress, probably via attenuation of NFκB and the JNK/MAPK pathway (82). Thus, our findings are consistent with prior research that has shown that inhibiting the NF-kB and MAPK signal transduction pathways decreases hyperglycemia-induced DN. (82). Another possible mechanism of action of PH extract may be due to the occurrence of its active constituents: quercetin upregulated glucose transporter 4 (GLUT 4) and insulin receptor resulted in raised glucose uptake, and gallic acid, a quencher of free radicals reducing oxidative damage associated with the progression of diabetes (83).

Our results were also consistent with histopathological examinations. Significant abnormal changes were observed in renal glomerulus and tubules in diabetic rats. All severe changes were restored with PH extract treatment, suggesting the nephroprotective potential of PH extract in DN.

Conclusions

In summary, the present work evaluated that the PH extract exhibits synergistic nephroprotective activity revealed by lowering blood glucose, improving the renal function, inflammatory and oxidative stress markers, thus providing a multifactorial approach for prevention of DN. PH extract has a significant therapeutic beneficial effect on the protection of the kidney against STZ-induced DN, possibly through attenuating kidney function markers and inhibiting oxidative stress and cytokine levels. Hence, it might help in the preservation of renal integrity as well as the hyperglycemia-induced oxidative stress condition. This study showed the beneficial effects of PH extract on DN might play a vital role in managing complications of diabetes and also attain a therapeutic value; further exploration is needed.

Acknowledgment

This research has been funded by Research Deanship at the University of Ha'il – Saudi Arabia through project number RG-20 042.

References

1. Parveen K, Siddiqui WA, Kausar MA, Kuddus M, Shahid SM, Arif JM. Diabetic nephropathy-a major macrovascular complication. International journal of pharmaceutical research & allied sciences. 2016;5(4).

2. Nabi R, Alvi SS, Saeed M, Ahmad S, Khan MS. Glycation and HMG-CoA Reductase Inhibitors: Implication in Diabetes and Associated Complications. Curr Diabetes Rev. 2019;15(3):213-23.

3. Al-Trad B, Ashankyty IM, Alaraj M. Progesterone ameliorates diabetic nephropathy in streptozotocin-induced diabetic Rats. Diabetol Metab Syndr. 2015;7:97.

4. Parveen K, Siddiqui WA, Arif JM, Kuddus M, Shahid SMA, Kausar MA. Evaluation of vegetables and fish oils for the attenuation of diabetes complications. Cell Mol Biol (Noisy-le-grand). 2019;65(7):38-45.

5. Cooper ME. Pathogenesis, prevention, and treatment of diabetic nephropathy. Lancet. 1998;352(9123):213-9.

6. Chiarelli F, Verrotti A, Morgese G. Glomerular hyperfiltration increases the risk of developing microalbuminuria in diabetic children. Pediatr Nephrol. 1995;9(2):154-8.

7. Saeed M, Kausar MA, Singh R, Siddiqui AJ, Akhter A. The Role of Glyoxalase in Glycation and Carbonyl Stress Induced Metabolic Disorders. Curr Protein Pept Sci. 2020;21(9):846-59.

8. Kanwar YS, Wada J, Sun L, Xie P, Wallner EI, Chen S, et al. Diabetic nephropathy: mechanisms of renal disease progression. Exp Biol Med (Maywood). 2008;233(1):4-11.

9. Coimbra TM, Janssen U, Grone HJ, Ostendorf T, Kunter U, Schmidt H, et al. Early events leading to renal injury in obese Zucker (fatty) rats with type II diabetes. Kidney Int. 2000;57(1):167-82.

10. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature. 2000;404(6779):787-90.

11. Nangaku M, Izuhara Y, Usuda N, Inagi R, Shibata T, Sugiyama S, et al. In a type 2 diabetic nephropathy rat model, the improvement of obesity by a low calorie diet reduces oxidative/carbonyl stress and prevents diabetic nephropathy. Nephrol Dial Transplant. 2005;20(12):2661-9.

12. Allen DA, Harwood S, Varagunam M, Raftery MJ, Yaqoob MM. High glucose-induced oxidative stress causes apoptosis in proximal tubular epithelial cells and is mediated by multiple caspases. FASEB J. 2003;17(8):908-10.

13. Ozbek E. Induction of oxidative stress in kidney. Int J Nephrol. 2012;2012:465897.

14. Banba N, Nakamura T, Matsumura M, Kuroda H, Hattori Y, Kasai K. Possible relationship of monocyte chemoattractant protein-1 with diabetic nephropathy. Kidney Int. 2000;58(2):684-90.

15. Sassy-Prigent C, Heudes D, Mandet C, Belair MF, Michel O, Perdereau B, et al. Early glomerular macrophage recruitment in streptozotocin-induced diabetic rats. Diabetes. 2000;49(3):466-75.

16. Chow F, Ozols E, Nikolic-Paterson DJ, Atkins RC, Tesch GH. Macrophages in mouse type 2 diabetic nephropathy: correlation with diabetic state and progressive renal injury. Kidney Int. 2004;65(1):116-28.

17. Rivero A, Mora C, Muros M, Garcia J, Herrera H, Navarro-Gonzalez JF. Pathogenic perspectives for the role of inflammation in diabetic nephropathy. Clin Sci (Lond). 2009;116(6):479-92.

18. Navarro-Gonzalez JF, Mora-Fernandez C, Muros de Fuentes M, Garcia-Perez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. Nat Rev Nephrol. 2011;7(6):327-40.

19. Ahmad I, Aqil F, Owais M. Modern phytomedicine: Turning medicinal plants into drugs: John Wiley & Sons; 2006.

20. Reddy KS, Sudheer A, Pradeepkumar B, Reddy CS. Effect of a polyherbal formulation in streptozotocin-induced diabetic nephropathy in wistar rats. Indian J Pharmacol. 2019;51(5):330-6.

21. Suvarna R, Shenoy RP, Hadapad BS, Nayak AV. Effectiveness of polyherbal formulations for the treatment of type 2 Diabetes mellitus - A systematic review and meta-analysis. J Ayurveda Integr Med. 2021;12(1):213-222.

22. Soliman HA, El-Desouky MA, Hozayen WG, Ahmed RR, Khaliefa AK. Hepatoprotective effects of parsley, basil, and chicory aqueous extracts against dexamethasone-induced in experimental rats. J Intercult Ethnopharmacol. 2016;5(1):65-71.

23. Saeed M, Abd El-Hack ME, Alagawany M, Arain MA, Arif M, Mirza MA, et al. Chicory (*Cichorium intybus*) herb: Chemical composition, pharmacology, nutritional and healthical applications. International Journal of Pharmacology. 2017;13(4):351-60.

24. Samarghandian S, Borji A, Tabasi SH. Effects of *Cichorium intybus* linn on blood glucose, lipid constituents and selected oxidative stress parameters in streptozotocin-induced diabetic rats. Cardiovasc Hematol Disord Drug Targets. 2013;13(3):231-6.

25. Abdel-Rahim EA, Rashed MM, El-Hawary ZM, Abdelkader MM, Kassem SS, Mohamed RS. Anti-diabetic Effect of *Cichorium intybus* Leaves and Plantago ovate Seeds in High Fat Diet-streptozotocin Induced Diabetic Rats. Journal of Food and Nutrition Research. 2016;4(5):276-81.

26. Ferrare K, Bidel LPR, Awwad A, Poucheret P, Cazals G, Lazennec F, et al. Increase in insulin sensitivity by the association of chicoric acid and chlorogenic acid contained in a natural chicoric acid extract (NCRAE) of chicory (*Cichorium intybus* L.) for an antidiabetic effect. J Ethnopharmacol. 2018;215:241-8.

27. Lightowler H, Thondre S, Holz A, Theis S. Replacement of glycaemic carbohydrates by inulin-type fructans from chicory (oligofructose, inulin) reduces the postprandial blood glucose and insulin response to foods: report of two double-blind, randomized, controlled trials. Eur J Nutr. 2018;57(3):1259-68.

28. Ismail HI, Chan KW, Mariod AA, Ismail M. Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts. Food chemistry. 2010;119(2):643-7.

29. Fahamiya N, Aslam M, Javid K, Siddiqui A, Shiffa M, Yaqub S, et al. Nephroprotective activity of methanolic extract of *Cucumis melo* Linn. in gentamicin induced nephrotoxicity. International Journal of Drug Formulation and Research. 2012;3(2):40-53.

30. Ibrahim DS. Neuroprotective effect of *Cucumis melo* Var. flexuosus leaf extract on the brains of rats with streptozotocin-induced diabetes. Metab Brain Dis. 2017;32(1):69-75.

31. Waseem M, Rauf A, Rehman S, Ahmed R. Pharmacognostical and pharmacological review of *Cucumis Melo* L. including Unani medicine perspective. International Journal of Pharmacognosy & Chinese Medicine. 2018;2(3).

32. Ma C-M, Sato N, Li X-Y, Nakamura N, Hattori M. Flavan-3-ol contents, anti-oxidative and α-glucosidase inhibitory activities of *Cynomorium songaricum*. Food chemistry. 2010;118(1):116-9.

33. Wang J, Zhang J, Zhao B, Wu Y, Wang C, Wang Y. Structural features and hypoglycaemic effects of *Cynomorium songaricum* polysaccharides on STZ-induced rats. Food chemistry. 2010;120(2):443-51.

34. Cui Z, Guo Z, Miao J, Wang Z, Li Q, Chai X, et al. The genus *Cynomorium* in China: an ethnopharmacological and phytochemical review. J Ethnopharmacol. 2013;147(1):1-15.

35. Cui J-L, Gong Y, Xue X-Z, Zhang Y-Y, Wang M-L, Wang J-H. A phytochemical and pharmacological review on *Cynomorium songaricum* as functional and medicinal food. Natural Product Communications. 2018;13(4):1934578X1801300428.

36. Ghosh M. Fundamentals of experimental pharmacology. Indian Journal of Pharmacology. 2007;39(4):216-.

37. Arreola-Mendoza L, Reyes JL, Melendez E, Martin D, Namorado MC, Sanchez E, et al. Alpha-tocopherol protects against the renal damage caused by potassium dichromate. Toxicology. 2006;218(2-3):237-46.

38. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-54.

39. Price RG, Dance N, Richards B, Cattell WR. The excretion of N-acetyl-beta-glucosaminidase and beta-galactosidase following surgery to the kidney. Clin Chim Acta. 1970;27(1):65-72.

40. Pedraza-Chaverri J, Barrera D, Medina-Campos ON, Carvajal RC, Hernandez-Pando R, Macias-Ruvalcaba NA, et al. Time course study of oxidative and nitrosative stress and antioxidant enzymes in K2Cr2O7-induced nephrotoxicity. BMC Nephrol. 2005;6:4.

41. Sastry KV, Moudgal RP, Mohan J, Tyagi JS, Rao GS. Spectrophotometric determination of serum nitrite and nitrate by copper-cadmium alloy. Anal Biochem. 2002;306(1):79-82.

42. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8.

43. Gherghel D, Griffiths HR, Hilton EJ, Cunliffe IA, Hosking SL. Systemic reduction in glutathione levels occurs in patients with primary open-angle glaucoma. Invest Ophthalmol Vis Sci. 2005;46(3):877-83.

44. Lawrence RA, Burk RF. Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun. 1976;71(4):952-8.

45. Carlberg I, Mannervik B. Purification and characterization of the flavoenzyme glutathione

reductase from rat liver. J Biol Chem. 1975;250(14):5475-80.

46. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469-74.

47. Claiborne A. Handbook of methods for oxygen radical research. Florida: CRC Press, Boca Raton. 1985:283-4.

48. Nayak B, Roy S, Roy M, Mitra A, Karak K. Phytochemical, antioxidant and antimicrobial screening of *Suaeda maritima* L (Dumort) against human pathogens and multiple drug resistant bacteria. Indian Journal of Pharmaceutical Sciences. 2018;80(1):26-35.

49. Babulreddy N, Sahoo SP, Ramachandran S, Dhanaraju M. Anti-hyperglycemic activity of *Cucumis Melo* Leaf extracts in Streptozotocin induced Hyperglycemia in Rats. Inter J. 2013;2(4):22-7.

50. Srivastava AK, Mukerjee A, Tripathi A. Antidiabetic and antihyperlipidemic activities of *Cucumis melo* var. momordica fruit extract on experimental animals. Future Journal of Pharmaceutical Sciences. 2020;6(1):1-9.

51. Pushparaj PN, Low HK, Manikandan J, Tan BK, Tan CH. Anti-diabetic effects of *Cichorium intybus* in streptozotocin-induced diabetic rats. J Ethnopharmacol. 2007;111(2):430-4.

52. Muthusamy VS, Anand S, Sangeetha KN, Sujatha S, Arun B, Lakshmi BS. Tannins present in *Cichorium intybus* enhance glucose uptake and inhibit adipogenesis in 3T3-L1 adipocytes through PTP1B inhibition. Chem Biol Interact. 2008;174(1):69-78.

53. Tousch D, Lajoix AD, Hosy E, Azay-Milhau J, Ferrare K, Jahannault C, et al. Chicoric acid, a new compound able to enhance insulin release and glucose uptake. Biochem Biophys Res Commun. 2008;377(1):131-5.

54. Ghamarian A, Abdollahi M, Su X, Amiri A, Ahadi A, Nowrouzi A. Effect of chicory seed extract on glucose tolerance test (GTT) and metabolic profile in early and late stage diabetic rats. Daru. 2012;20(1):56.

55. Falk RJ, Scheinman JI, Mauer SM, Michael AF. Polyantigenic expansion of basement membrane constituents in diabetic nephropathy. Diabetes. 1983;32 Suppl 2:34-9.

56. Nerlich A, Schleicher E. Immunohistochemical localization of extracellular matrix components in human diabetic glomerular lesions. Am J Pathol. 1991;139(4):889-99.

57. Sugimoto H, Shikata K, Wada J, Horiuchi S, Makino H. Advanced glycation end products-cytokine-nitric oxide sequence pathway in the development of diabetic nephropathy: aminoguanidine ameliorates the overexpression of tumour necrosis factor-alpha and inducible nitric oxide synthase in diabetic rat glomeruli. Diabetologia. 1999;42(7):878-86.

58. Pennell JP, Meinking TL. Pattern of urinary proteins in experimental diabetes. Kidney Int. 1982;21(5):709-13.

59. Vedel P, Obel J, Nielsen FS, Bang LE, Svendsen TL, Pedersen OB, et al. Glomerular hyperfiltration in micro-albuminuric NIDDM patients. Diabetologia. 1996;39(12):1584-9.

60. Fu WJ, Xiong SL, Fang YG, Wen S, Chen ML, Deng RT, et al. Urinary tubular biomarkers in short-term type 2 diabetes mellitus patients: a cross-sectional study. Endocrine. 2012;41(1):82-8.

61. Gluhovschi C, Gluhovschi G, Petrica L, Timar R, Velciov S, Ionita I, et al. Urinary Biomarkers in the Assessment of Early Diabetic Nephropathy. J Diabetes Res. 2016;2016:4626125.

62. Takao T, Horino T, Kagawa T, Matsumoto R, Shimamura Y, Ogata K, et al. Possible involvement of intracellular angiotensin II receptor in high-glucose-induced damage in renal proximal tubular cells. J Nephrol. 2011;24(2):218-24.

63. Valavanidis A, Vlachogianni T, Fiotakis C. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2009;27(2):120-39.

64. Serdar M, Sertoglu E, Uyanik M, Tapan S, Akin K, Bilgi C, et al. Comparison of 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels using mass spectrometer and urine albumin creatinine ratio as a predictor of development of diabetic nephropathy. Free Radic Res. 2012;46(10):1291-5.

65. Naito Y, Akagiri S, Uchiyama K, Kokura S, Yoshida N, Hasegawa G, et al. Reduction of diabetes-induced renal oxidative stress by a cantaloupe melon extract/gliadin biopolymers, oxykine, in mice. Biofactors. 2005;23(2):85-95.

66. Wellen KE, Hotamisligil GS. Inflammation, stress, and diabetes. J Clin Invest. 2005;115(5):1111-9.

67. Navarro JF, Mora C. Diabetes, inflammation, proinflammatory cytokines, and diabetic nephropathy. ScientificWorldJournal. 2006;6:908-17.

68. Tilg H, Moschen AR. Inflammatory mechanisms in the regulation of insulin resistance. Mol Med. 2008;14(3-4):222-31.

69. Meng XM, Nikolic-Paterson DJ, Lan HY. Inflammatory processes in renal fibrosis. Nat Rev Nephrol. 2014;10(9):493-503.

70. Sato M, Muragaki Y, Saika S, Roberts AB, Ooshima A. Targeted disruption of TGF-beta1/Smad3 signaling protects against renal tubulointerstitial fibrosis induced by unilateral ureteral obstruction. J Clin Invest. 2003;112(10):1486-94.

71. Reeves WB, Andreoli TE. Transforming growth factor beta contributes to progressive diabetic nephropathy. Proc Natl Acad Sci U S A. 2000;97(14):7667-9.

72. Donate-Correa J, Martin-Nunez E, Muros-de-Fuentes M, Mora-Fernandez C, Navarro-Gonzalez JF. Inflammatory cytokines in diabetic nephropathy. J Diabetes Res. 2015;2015:948417.

73. Lee D, Yoo JH, Lee BC. Therapeutic Effect of *Cucumis melo* L. Extract on Insulin Resistance and the Gut Microbiome in Lep(^{ob})/Lep(^{ob}) Mice. Evid Based Complement Alternat Med. 2018;2018:8159261.

74. Rezagholizadeh L, Pourfarjam Y, Nowrouzi A, Nakhjavani M, Meysamie A, Ziamajidi N, et al. Effect of *Cichorium intybus* L. on the expression of hepatic NF-kappaB and IKKbeta and serum TNF-alpha in STZ- and STZ+ niacinamide-induced diabetes in rats. Diabetol Metab Syndr. 2016;8:11.

75. Komers R, Allen TJ, Cooper ME. Role of endothelium-derived nitric oxide in the pathogenesis of the renal hemodynamic changes of experimental diabetes. Diabetes. 1994;43(10):1190-7.

76. Manikandan R, Beulaja M, Thiagarajan R, Priyadarsini A, Saravanan R, Arumugam M. Ameliorative effects of curcumin against renal injuries mediated by inducible nitric oxide synthase and nuclear factor kappa B during gentamicin-induced toxicity in Wistar rats. Eur J Pharmacol. 2011;670(2-3):578-85.

77. Walker LM, Walker PD, Imam SZ, Ali SF, Mayeux PR. Evidence for peroxynitrite formation in renal ischemia-reperfusion injury: studies with the inducible nitric oxide synthase inhibitor L-N(6)-(1-Iminoethyl)lysine. J Pharmacol Exp Ther. 2000;295(1):417-22.

78. King GL, Loeken MR. Hyperglycemia-induced oxidative stress in diabetic complications. Histochem Cell Biol. 2004;122(4):333-8.

79. Ha H, Hwang IA, Park JH, Lee HB. Role of reactive oxygen species in the pathogenesis of diabetic nephropathy. Diabetes Res Clin Pract. 2008;82 Suppl 1:S42-5.

80. Giacco F, Brownlee M. Oxidative stress and diabetic complications. Circ Res. 2010;107(9):1058-70.

81. Ning C, Wang X, Gao S, Mu J, Wang Y, Liu S, et al. Chicory inulin ameliorates type 2 diabetes mellitus and suppresses JNK and MAPK pathways in vivo and in vitro. Mol Nutr Food Res. 2017;61(8).

82. Chen MF, Liou SS, Hong TY, Kao ST, Liu IM. Gigantol has Protective Effects against High Glucose-Evoked Nephrotoxicity in Mouse Glomerulus Mesangial Cells by Suppressing ROS/MAPK/NF-kappaB Signaling Pathways. Molecules. 2018;24(1).

83. Elavarasi S, Saravanan K, Renuka C. A systematic review on medicinal plants used to treat diabetes mellitus. International Journal of Pharmaceutical, Chemical & Biological Sciences. 2013;3(3).