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EFFECT OF THE METHANOLIC EXTRACT OF *POROPHYLLUM TAGETOIDES* ON DIABETIC NEPHROPATHY

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Abstract

Diabetes mellitus is a global health problem. Persistent hyperglycemia is associated with several functional and structural complications, including kidney damage. Hyperglycemia-induced oxidative stress plays a critical role in diabetic nephropathy pathogenesis. Antioxidant and anti-inflammatory properties have been reported for *Porophyllum tagetoides*, Kunth DC (Asteraceae).
The aim of this study was to investigate the antioxidant effect of a methanolic extract of *P. tagetoides* (PT) leaves on kidney damage in diabetic rats.
Diabetes was induced to male Wistar rats by streptozotocin injection. The animals were divided in six groups. After six weeks of treatment diabetic rats developed hyperglycemia, decreased body weight, increased renal weight, proteinuria, and decreased activity of antioxidant enzymes. Treatment with PT at a dose of 200 mg/kg, but not at a dose of 100 mg/kg, prevented the increased expression of angiotensin II receptor type 1 (AT1R), and t*ransforming growth factor* β1 (TGFβ1). The treatment also partially reduced the proteinuria developed by the diabetic animals, but did not restore the activity of the renal antioxidant enzymes. Hyperglycemia and kidney weight/total body weight ratio were similar to those of untreated diabetic rats. The DPPH radical scavenging capacity showed by PT may be due to the presence of flavonoids. Captopril and vitamin E used as controls, decreased the proteinuria and restored the activity of antioxidant enzymes. These data show that the methanolic extract of *P. tagetoides* (PT) did not restore the antioxidant enzymatic activity but inhibited TGF-β and AT1 receptor expression; both TGF-β and angiotensin II are considered key factors for the development of diabetic nephropathy.

Keywords: *Porophyllum tagetoides, diabetic nephropathy, antioxidants, streptozotocin, diabetes mellitus*

Introduction

Diabetes mellitus incidence and prevalence have increased worldwide in developed and developing countries. Hyperglycemia initiates the development of systemic complications including diabetic nephropathy (DN), which is one of the main causes of end-stage renal disease (1-4). At the onset of diabetes, there is an elevation of glomerular filtration rate (GFR), and renal blood flow, followed by renal hypertrophy, glomerular damage, and progressive renal failure (5). Several lines of evidence suggest that glomerular hyperfiltration during the early phase of diabetes is due to vasodilatation of the afferent arterioles, in excess to that of the efferent arterioles. Moreover, the increased blood flow through the glomerular capillaries causes glomerular hypertension; high intraglomerular pressure has been considered an important factor in the pathogenesis of DN (2-3). Sustained hyperglycemia may enhance the production of reactive oxygen species (ROS) and impair the activity of cellular antioxidant enzymes in the kidneys (6, 7). An imbalance of ROS and endogenous antioxidants production may be a common end pathway of oxidative kidney damage, considered an important factor for DN development (8, 9).

Preclinical studies have explored the effectiveness of whole herbs, plants, or seeds, and their active ingredients in established DN (10). Some of these compounds have been reported to ameliorate oxidative stress-induced kidney damage, enhance the antioxidant system, and decrease the inflammatory process and fibrosis (11).

Porophyllum tagetoides Kunth DC (Asteraceae) is a plant with antioxidant properties (12). It is an annual warm-weather herb with a typical intense odor. It is found in the Mexican States of Queretaro, Durango, Guanajuato, Hidalgo, Jalisco, Michoacan, Mexico, Morelos, Puebla, Veracruz and Oaxaca. Its leaves are used in traditional medicine for the treatment of inflammatory and degenerative conditions (12, 13). It has volatile compounds, vitamin C, aldehydes, flavonoids, terpenes and polyphenols. The aim of the study was to investigate the antioxidant effect of the methanolic extract of *P. tagetoides* on the kidney of rats with streptozotocin-induced diabetes, using captopril and vitamin E as positive control drugs.

Methods

Fresh leaves of *P. tagetoides* were acquired in a market in Tlalnepantla de Baz, State of Mexico, Mexico, and authenticated by Edith López-Villafranco, biologist in charge of the Herbarium of the Department of Botany of the Facultad de Estudios Superiores Iztacala, Universidad Nacional Autónoma of México (UNAM). A voucher specimen was deposited at the herbarium (IZTA 2891).

Preparation of the extract of P. tagetoides

P. tagetoides dried leaves (500 g) were powdered and extracted with 2 L of methanol for 24 h at room temperature. The extract was filtered through a number 4 Whatman paper and concentrated under reduced pressure at 50° C in a rotary evaporator. The dried extract was dissolved in propylene glycol to be administered to the rats.

Phytochemical screening

Phytochemical screening of the methanol extract of *P. tagetoides* (PT) was carried out by using chromogenic reagents –NaOH, Braemer, Borntrager, Dragendorff's, Mayer, Baljet, Liebermann Burchard, ferric chloride, Salkowski, hydrochloric acid, and sulfuric acid– to detect saponins, coumarins, tannins, anthraquinones, alkaloids, sesquiterpene lactones, steroids, flavonoids, and terpenoids, by standard protocols (14). The analytical response to these qualitative tests was the precipitate color intensity, using 10% (w/v) solution of the extract in methanol.

Antioxidant activity

PT antioxidant capacity was determined by measuring the percentage of discoloration of the DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical in methanol solution (15). When DPPH reacts to an antioxidant compound undergoes purple to yellow discoloration. Samples at various concentrations (9, 18, 27, 36, 45, 54, 63, 72 and 90 µg/mL) of PT were plated out in triplicate in a 96 wells plate. 50 µL of methanol were added. DPPH (150 µL, 100 µM in methanol) was added to the test samples, 150 µL of methanol was used as control. The plate was incubated in dark at 37° C for 30 min. Absorbance was measured after incubation using a SLT Spectra ELISA reader, at a wavelength of 517 nm. Discoloration percentage was determined (equation 1) and the efficient concentration value (EC_{50}) was calculated.

Quercetin was used as reference standard. Discoloration % = $[(Abs_{control}-Abs_{test})/Abs_{control}] \times 100$. (1) where Abs = Absorbance at 517 nm, $Abs_{control}$ = average absorbance of DPPH/average absorbance of methanol, Abs_{test} = average absorbance obtained in the test sample.

Total phenolic content

The total polyphenol content of the extracts was determined using the Folin-Ciocalteu reagent technique (16). Gallic acid was used as reference standard. A standard curve was prepared using solutions of gallic acid in water (0.00625 to 0.2 mg/mL). A 1 mL aliquot of PT stock solution (0.05 mg/mL) was transferred to a tube containing distilled water (7 mL), and Folin-Ciocalteu reagent (0.5 mL). After incubation (5 min), 1.5 mL of Na_2CO_3 (20%) was added. The mixture was kept at room temperature for 20 min. Total phenols were determined by absorption at 760 nm in a spectrophotometer (COLEMAN Junior II, UV-VIS). The result was expressed as milligrams of gallic acid equivalents per gram of dry material.

Animals and treatment

Male Wistar rats aged 10 weeks (initial body weight 230-250 g) were obtained from the animal facility of the Facultad de Estudios Superiores Iztacala, UNAM. Rats had free access to standard rat chow (rodent Laboratory Chow 5001, Ralston Purina, Richmond Indiana, USA) and tap water, with 12/12 light-dark cycles throughout the experiment. Diabetes mellitus was induced by a single streptozotocin (STZ; Sigma-Aldrich Co., St. Louis, MO, USA) intraperitoneal (i.p.) injection (65 mg/kg of body weight) in 10 mM sodium citrate buffer, pH 4.5 (17). Control (C) rats received vehicle (10 mM citrate buffer, pH 4.5) alone. Forty-eight hours after STZ injection, blood glucose concentration was determined in tail vein blood samples using a reflectance meter (Accu-Check Active glucometer, Roche USA). Only animals with blood glucose levels > 300 mg/dL were included in the study. Diabetic rats were randomized into five groups, each group consisted of five animals: 1) untreated diabetic rats (DM) receiving vehicle (propylene glycol 1 mL/kg po), 2) diabetic rats treated with PT 100 mg/kg po (DM+PT100), 3) diabetic rats treated with PT 200 mg/kg po (DM+PT200), 4) diabetic rats treated with vitamin E 500 mg/ kg po (DM+VE 500), and 5) diabetic rats treated with captopril 25 mg/kg po (DM+CAP). Once hyperglycemia with serum glucose levels ≥ 300 mg/dL was confirmed, the treatments were administered daily during 6 weeks of the experiment. Two days before STZ injection and two days before the end experiment, the animals were placed in metabolic cages to measure food and water consumption, urinary volume, and to obtain urine samples to measure protein and creatinine. At the end of the study, the rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p.). Blood samples were obtained to measure blood glucose and creatinine levels. Both kidneys were quickly removed, decapsulated, weighed and dissected into cortex and medulla for total DNA and protein extraction, and immunoblot analysis and enzyme activity measurements.

Animal care and all procedures were performed in compliance with the Mexican Federal Regulations for Animals Investigation and Care (NOM-062-ZOO-2001). The protocol was approved by the institutional ethics review board.

Measurement of renal hypertrophy

Kidney weight/body weight ratio was used as a kidney hypertrophy index. Total DNA and protein from cortex tissue was extracted and quantified by Trizol reagent method (Invitrogen, Grand Island, New York, USA), and the protein/DNA ratio was calculated as an index of relative hypertrophy.

Analytical methods

A 24 h urine sample was collected placing the animals in metabolic cages. Samples were immediately frozen and stored at -80° C to measure proteins and creatinine. Protein concentration in urine was measured by the Bradford method (Bio-Rad), Bovine serum albumin (BSA) (Sigma-Chemical Co.) was used as standard, and creatinine was measured with Cayman reagents (Cayman Chemical, Ann Arbor, Michigan, USA) (18).

Evaluation of oxidative stress

To evaluate oxidative stress, the activity of 3 intracellular antioxidant enzymes was measured in renal cortex homogenates in 50 mM phosphate buffer, pH 7.0, with protease inhibitors (Complete Mini, Roche).

Catalase (CAT), was analyzed at 25° C by Aebi method (19) based on the disappearance of H_2O_2 from a solution of 30 mM H_2O_2 in 10 mM potassium phosphate buffer (pH 7.0), read at a wavelength of 240 nm in the presence of the tissue homogenate, with the results expressed as UI/mg protein.

Glutathione peroxidase (GPx) was analyzed by the method of Paglia and Valentine (20). GPx consumes glutathione and NADPH as cofactor. This reaction is coupled with the glutathione reductase reaction, which regenerates the cofactor. The reaction becomes cyclic and the difference in absorbance at a wavelength of 340 nm between the 2 and 4 min reaction is proportional to the glutathione peroxidase activity present; the results are expressed as UI/mg protein.

Superoxide dismutase (SOD) was measured by a competitive inhibition assay using the xanthine oxidase/xanthine system to reduce nitroblue tetrazolium (21); the results are expressed as UI/mg protein.

Western blot analysis

Western blot technique was performed with homogenates of renal cortex prepared by RIPA lysis buffer, consisting of: 1% sodium dodecylsulfate (SDS) (Amersham Biosciences, Great Britain), 1% Triton X-100, 0.5% sodium deoxycholate in PBS, and a complete protease inhibitor cocktail Mini (Roche Diagnostics, Germany). The suspension was centrifuged at 13,000 g for 10 min at 4° C and the protein concentration was determined using the Bradford method. 50 µg of proteins were diluted in 12 µL of a dissociation solution consisting of: 5% P/V SDS, 10% v/v β-mercaptoethanol, 0.5 M Tris-HCl, 20% v/v glycerol and bromophenol blue 0.5% w/v. Samples were heated to 95° C for 10 min. The proteins were then separated by electrophoresis in denaturing polyacrylamide mini-gels with SDS (10%) according to the Laemmli technique, applying a constant voltage of 88 V for about 2 h. Standard proteins of known molecular weight (Biorad) distributed in a molecular weight range of 26 to 180 kDa were run on the same gel. Subsequently, membranes of polyvinylidene difluoride (PVDF) (Bio-Rad, Hercules, CA, USA) were electroblotted for 1 h by applying a voltage of 19 V (22). The transfer buffer consisted of 25 mM Tris-HCl, 192 mM glycine, 0.05% w/v SDS and 20% v/v methanol. For blocking non-specific sites, the PVDF membrane was incubated with a blocking agent consisting of 5% fat-free milk in 0.5 M Tris-saline solution, pH 7.5 for 2 h. Immunodetection of the proteins was performed by incubating the membrane overnight at 4° C with the following antibodies: a) mouse anti-TGFβ1 monoclonal antibody (Santa Cruz Biotechnology), and b) rabbit anti-AT1R polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). After three washes, the membranes were incubated with the secondary antibody at room temperature for 2 h. In all cases, detection was performed by revealing the peroxidase activity in the presence of hydrogen peroxide with an enhanced chemiluminescence immunoblot (ECL) kit (Luminol, Santa Cruz Biotechnology Inc., Santa Cruz, California, USA) as chromogen, until the appearance of brown bands (5-10 min); the reaction was quenched with distilled water. The monoclonal anti-β-actin goat antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used to detect the β-actin protein used as a marker to normalize the relative expression of other proteins. Relative optical densities of the immunoreactive bands were obtained with MultiGauge Version 2.2 (Fuji Photo Film Co., LTD.) Program on digital images of PVDF membranes

Statistical analysis

Data are expressed as the mean ± standard error of the mean. Data were analyzed with ANOVA followed by Bonferroni adjustment for multiple comparisons of means. Differences were considered statistically significant with $p < 0.05$.

Results

Phytochemical screening of the PT revealed the presence of coumarins, tannins, sesquiterpene lactones, flavonoids, and sterols. The concentration of flavonoids and tannins was highest (Table 1). PT antioxidant activity or scavenging capacity (IC_{50}) was 319.76 µg /mL according to the DPPH assay, and that of quercetin was 4.78 µg/mL phenolic content in the PT methanolic extract was 50.08 mg gallic acid equivalents/g extract.

Six weeks after STZ injection, the control group of diabetic rats had polyuria/polydipsia and delayed weight gain as compared to the normoglycemic control group. Kidney weight/body weight ratio (Kw/Bw) was higher in all diabetic rats groups as compared to the control group. Treatment with

vitamin E decreased ratio as compared to the group of diabetic rats. The animals treated with captopril also decreased the Kw/Bw ratio, but it did not reach a statistically significant difference with that of the DM group (Table 2).

The blood glucose levels in diabetic rats were significantly higher as compared to the control group. Food and water consumption, and urinary volume were markedly increased in the 4 groups of diabetic animals (Table 3).

The protein/DNA ratio was significantly higher in diabetic rats than in control group. Treatment with captopril or vitamin E reversed this increment but treatment with PT did not (Fig. 1). Creatinine clearance diminished in diabetic rats as compared to the control. PT did not restore creatinine clearance. However, captopril and vitamin E treatment increased creatinine clearance (Fig. 2). creatinine clearance of diabetic rats (DM); control group (C), diabetics treated with vitamin E 500 mg/kg (VE500) and captopril (CAP) 10 mg/kg. Data are expressed as the mean ± SEM (n = 5); * p ≤ 0.05 vs control rats, # p ≤ 0.05 vs diabetic rats.

Urinary protein excretion increased in diabetic rats as compared to the control group. The treatment with captopril, and vitamin E significantly reversed this increment, but PT treatment did not (Fig. 3).

The activity of the antioxidant enzymes catalase (Fig. 4), superoxide dismutase (Fig. 5) and glutathione peroxidase (Fig. 6) of the renal cortex was reduced in diabetic rats as compared to the control group; this effect was reversed by captopril or vitamin E. PT treatment did not restore the activity of these enzymes. The expression of AT1 receptor protein was increased in diabetic rats as compared to the control group. Administration of captopril, vitamin E or PT (200 mg/kg) reversed this effect (Fig. 7).

Expression of TGFβ1 was increased in diabetic rats as compared to the control group. Treatment with captopril, vitamin E or PT (200 mg/kg) significantly decreased it (Fig. 8).

Discussion

These results indicate that PT contains flavonoids and DPPH radical scavenging, which correlated with its *in vitro* antioxidant activity. This is in accordance with the results by Jiménez et al., (2012); however, they did not report in vivo results (12). STZ-induced diabetes is characterized by hyperglycemia and body weight loss (23). STZ produces a selective destruction of pancreatic β cells and reduction of insulin secretory capacity; its mechanism as a diabetogenic agent is mediated by reactive oxygen species (ROS) and many other reactive intermediates production, activating the renin-angiotensin system (RAS) and the expression of proinflammatory cytokines such as TGFβ1 (3, 24). ROS deplete the cells antioxidant defenses rendering them susceptible to oxidative damage. ROS increase results in lipids, DNA and proteins oxidation, changing their structure and function (7, 25). Glutathione plays a crucial role as a free radical scavenger and maintains the antioxidant status of plasma. SOD converts superoxide anion into hydrogen peroxide, a less reactive ROS, which is further reduced to water by CAT (26). Diabetes mellitus increases oxidative stress in most tissues, manifested by decreased activity of antioxidant enzymes CAT, SOD, and GPx, which may increase the detrimental effect of free radicals (27). SOD, CAT, and GPx catalyze important reactions responsible for free radicals elimination (28); intracellular glucose loading decreases the enzymatic activity by protein glycation and lipid peroxidation (29).

PT administration did not reverted body weight loss, and did not restore CAT, SOD, and GPx antioxidant enzymatic activity. It also was associated with AT1 receptor and TGFβ1 expression increments, explained by RAS activation and oxidative damage. PT, at a dose of 200 mg/kg, but not of 100 mg/kg, attenuated these abnormalities. PT treatment at a dose of 200 mg/kg, significantly decreased hyperglycemia as compared with the untreated diabetic rats. Diabetic nephropathy is associated with renal hypertrophy (30). PT treatment decreased proteinuria, but did not prevent renal hypertrophy and did not improve creatinine clearance.

Vitamin E and captopril treatment was associated to antihypertrophic effect as demonstrated by decreased Kw/Bw ratio, and proteins/DNA ratio. Both drugs also significantly increased creatinine clearance and restored the activity of the antioxidant enzymes, as it has been previously reported (31, 32). Nuclear factor-erythroid 2-related factor 2 (Nrf2) induces the expression of antioxidant enzymes; vitamin E increases Nrf2 target protein expression (31). On the other hand, because angiotensin II, via

AT1 receptor, stimulates NADPH oxidase expression and activation, captopril can effectively suppress NADPH oxidase, superoxide anion production, and proteinuria (33)

In the present study, PT treatment did not significant improve in renal cortical SOD, CAT, and GPx activity, in spite of its flavonoids content. Flavonoids have been reported to have nephroprotective activity via attenuation of nicotinamide adenine dinucleotide phosphate oxidase-induced oxidative stress and inhibition of advanced glycation end-product generation (34). These results suggest that PT at the tested doses has a low antioxidant capacity, possibly due to its low antioxidant phenolic compounds content.

However, PT treatment decreased AT1 receptor and TGFβ1 expression. TGFβ1 is considered to be a crucial factor in the development of renal disease (35). Angiotensin II can increase glomerular capillary pressure and permeability. Angiotensin II non-hemodynamic effects include activation of TGF-β1 and other cytokines, activation of ROS production in mesangial cells, stimulation of extracellular matrix (ECM) production, and inhibition of ECM degradation (36). Thereby, PT treatment may exert a mild protective effect against renal damage development in diabetes.

These results show that a methanolic extract of *P. tagetoides* leaves prevents diabetes-induced AT1R and TGFβ1 expression increment and partially reduces proteinuria in DN. These effects may retard the development of renal damage in STZ-induced diabetes.

Conflicts of interest

The authors state that there are no conflicts of interest.

Acknowledgments

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Table 1: Phytochemistry of the methanolic extract of *P. tagetoides*

Components	Methanolic extract
Saponins	-
Coumarins	+
Tannins	+++
Anthraquinones	-
Alkaloids	-
Sesquiterpene lactones	++
Sterols	++
Flavonoids	+++
Terpenoids	-

Table 2: Final body weights, renal weight and renal weight/body weight ratio of all groups

Groups	Body weight (g)	Kidney weight (g)	Kidney weight / Body weight ratio (g/100 g)
Control	316 ± 5	0.990 ± 0.022	0.313 ± 0.005
STZ	220 ± 10*	1.140 ± 0.085*	0.526 ± 0.006*
STZ+Vit E	258 ± 13*#	1.193 ± 0.027*	0.466 ± 0.025*#
STZ+Cap	230 ± 12*	1.148 ± 0.079*	0.497 ± 0.011*
STZ+PT100	230 ± 9*	1.130 ± 0.088*	0.541 ± 0.024*
STZ+PT200	233 ± 9*	1.170 ± 0.022*	0.505 ± 0.015*

Data are expressed as the mean ± standard error of the mean (SEM) (n = 5) * p ≤ 0.05 vs control rats, # p ≤ 0.05 vs diabetic rats

Table 3: Glycemia and consumption of food and water per day; urinary volume/day of all groups.

Groups	Glycemia (mg/dL)	Food intake /24 h (g)	Water intake /24 h (g)	Urinary volume /24 h (mL)
Control	104 ± 3	11 ± 0.5	45 ± 5	22 ± 1.6
STZ	533 ± 16*	34 ± 2.2*	128 ± 15*	123.7 ± 11.5*
STZ+Vit E	515 ± 36*	32 ± 4*	105 ± 22*	72.2 ± 8.9*#
STZ+Cap	518 ± 27*	33 ± 4*	83 ± 14*	59.2 ± 9.5*#
STZ+PT100	526 ± 23*	31 ± 4*	147 ± 16*	97 ± 19*
STZ+PT200	446 ± 27*#	29 ± 2*	150 ± 12*	101.6 ± 4.5*#

Data are expressed as the mean ± SEM (n = 5); * p ≤ 0.05 vs control rats, # p ≤ 0.05 vs diabetic rats

Figure 1. Effect of methanolic extract of *P. tagetoides* 100 mg/kg (PT100) and 200 mg/kg (PT200) on protein/DNA ratio in renal cortex of diabetic rats (DM); control group (C), diabetics treated with vitamin E 500 mg/kg (VE500) and captopril (CAP) 10 mg/kg. Data are expressed as the mean ± SEM an (n = 5); ***** p ≤ 0.05 vs control rats, # p ≤ 0.05 vs diabetic rats.

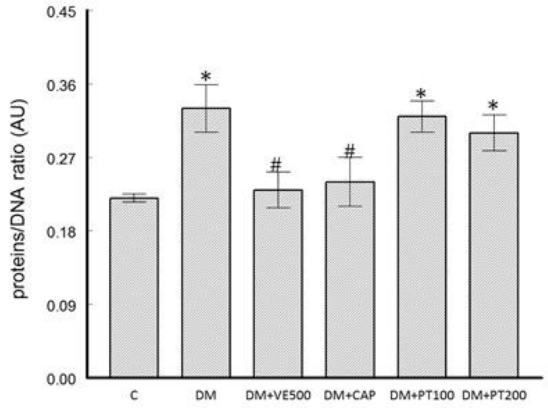

Figure 2: Effect of the methanolic extract of *P. tagetoides* 100 mg/kg (PT100) and 200 mg/kg (PT200) on the creatinine clearance of diabetic rats (DM); control group (C), diabetics treated with vitamin E 500 mg/kg (VE500) and captopril (CAP) 10 mg/kg. Data are expressed as the mean ± SEM (n = 5); ***** p ≤ 0.05 vs control rats, # p ≤ 0.05 vs diabetic rats.

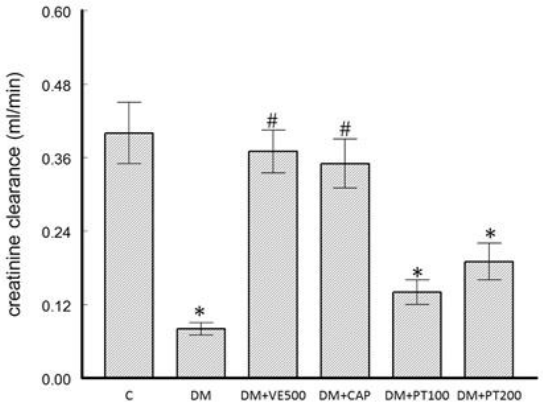

Figure 3: Effect of the methanolic extract of *P. tagetoides* 100 mg/kg (PT100) and 200 mg/kg (PT200) on the protein excretion in 24-h urine of diabetic rats (DM); control group (C), diabetics treated with vitamin E 500 mg/kg (VE500) and captopril (CAP) 10 mg/kg. Data are expressed as the mean ± SEM (n = 5); ***** p ≤ 0.05 vs control rats, # p ≤ 0.05 vs diabetic rats.

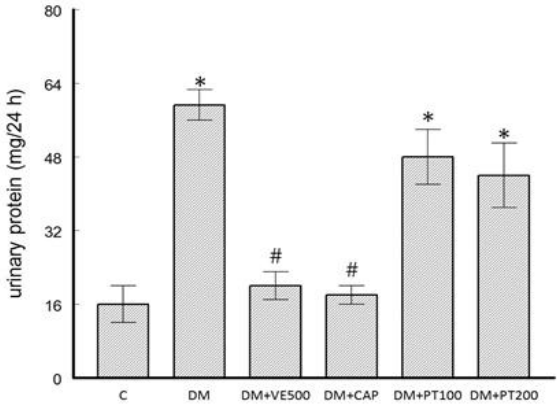

Figure 4: Effect of methanolic extract of *P. tagetoides* 100 mg/kg (PT100) and 200 mg/kg (PT200) on the activity of renal cortex catalase in diabetic rats (DM); control group (C), diabetics treated with vitamin E 500 mg/kg (VE500) and captopril (CAP) 10 mg/kg. Data are expressed as the mean ± SEM (n = 5); ***** p ≤ 0.05 vs control rats, # p ≤ 0.05 vs diabetic rats.

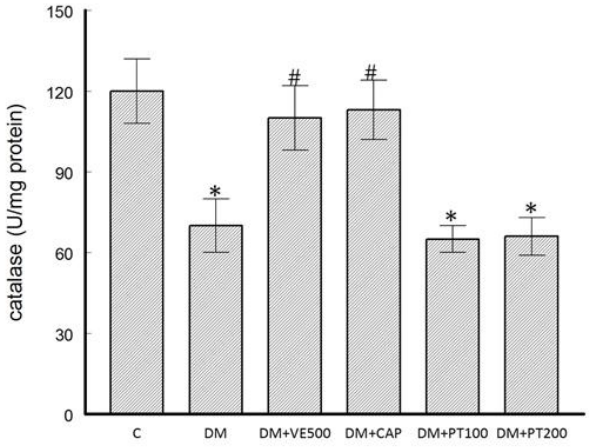

Figure 5: Effect of the methanolic extract of *P. tagetoides* 100 mg/kg (PT100) and 200 mg/kg (PT200) on the renal cortex superoxide dismutase (SOD) activity of diabetic rats (DM); control group (C), diabetics treated with vitamin E 500 mg/kg (VE500) and captopril (CAP) 10 mg/kg. Data are expressed as the mean ± SEM (n = 5); ***** p ≤ 0.05 vs control rats, # p ≤ 0.05 vs diabetic rats.

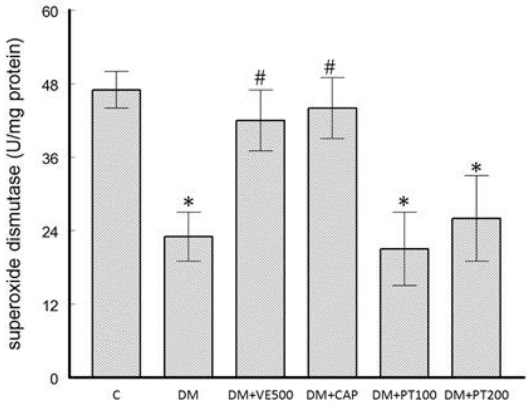

Figure 6: Effect of the methanolic extract of *P. tagetoides* 100 mg/kg (PT100) and 200 mg/kg (PT200) on the activity of glutathione peroxidase (GPx) of renal cortex of diabetic rats (DM); control group (C), diabetics treated with vitamin E 500 mg/kg (VE500) and captopril (CAP) 10 mg/kg. Data are expressed as the mean ± SEM (n = 5); ***** p ≤ 0.05 vs control rats, # p ≤ 0.05 vs diabetic rats.

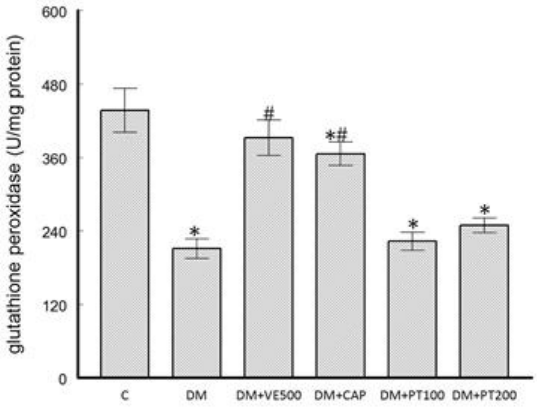

Figure 7: Effect of methanolic extract of *P. tagetoides* 100 mg/kg (PT100) and 200 mg/kg (PT200) on the protein expression of AT1 receptor in renal cortex of diabetic rats (DM); control group (C), diabetics treated with vitamin E 500 mg/kg (VE500) and captopril (CAP) 10 mg/kg. Data are expressed as the mean ± SEM (n = 5); *p ≤ 0.05 vs control rats, #p ≤ 0.05 vs diabetic rats.

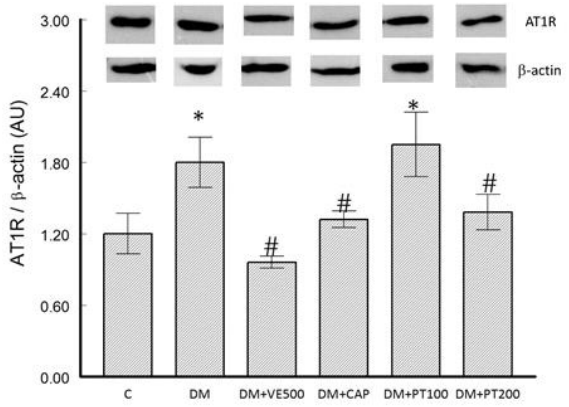

Figure 8: Effect of methanolic extract of *P. tagetoides* 100 mg/kg (PT100) and 200 mg/kg (PT200) on the protein expression of TGFβ1 in renal cortex of control (C) and diabetic (DM) rats; treated with vitamin E 500 mg/kg (VE500) and captopril (CAP) 10 mg/kg. Data are expressed as the mean ± SEM (n = 5); * p ≤ 0.05 vs control rats, # p ≤ 0.05 vs diabetic rat