EXPERIMENTAL DIABETIC NEPHROPATHY CAN BE PREVENTED BY PROPOLIS: EFFECT ON METABOLIC DISTURBANCES AND RENAL OXIDATIVE PARAMETERS

OSAMA M ABO-SALEM[a]*, RAWHIA H EL-EDEL[b], GAMAL ELDIN I HARISA[c], NERMIN EL-HALAWANY[d] AND MABROUK M GHONAIM[e]

[a]Phamacology and Toxicology Departments & [c]Biochemistry Department, Faculty of Pharmacy, Al-Azahar University, Cairo, Egypt

[b]Clinical Pathology Department & [e]Microbiology and Immunology Department, Faculty of Medicine, Menoufiya University, Egypt

[d]Cell Biology Department, National Research Center, Giza, Egypt

ABSTRACT

Oxidative stress may play a key role in the pathogenesis of diabetic nephropathy. Propolis and its extract have antioxidant properties. The effect of ethanolic extract of propolis against experimental diabetes mellitus-associated changes was examined. Diabetes was induced experimentally in rats by i.p. injection of streptozotocin (STZ) in a dose of 60 mg/kg bwt for 3 successive days. Blood urea nitrogen (BNU), creatinine, glucose, lipid profile, malondialdehyde (MDA) and urinary albumin were measured. Superoxide dimutase (SOD), glutathione (GSH), catalase (CAT) and MDA were measured in the renal tissue. The results showed decreased body weight and increased kidney weight in diabetic animals. Compared to the control normal rats, diabetic rats had higher blood glucose, BNU, creatinine, total cholesterol, triglycerides, low-density lipoprotein-cholesterol (LDL-C), MDA and urinary albumin and lower high-density lipoprotein-cholesterol (HDL-C) levels. Moreover, renal tissue MDA was markedly increased while SOD, GSH and CAT were significantly decreased. Oral administration of propolis extract in doses of 100,200&300 mg/kg bwt improved the body and kidney weights, serum glucose, lipid profile, MDA and renal function tests. Renal GSH, SOD and CAT were significantly increased while MDA was markedly reduced. These results may suggest a strong antioxidant effect of propolis which can ameliorate oxidative stress and delay the occurrence of diabetic nephropathy in diabetes mellitus.

Keywords: Propolis; diabetes; nephropathy; oxidative stress; antioxidants.

INTRODUCTION

Diabetes mellitus is associated with microvascular, macrovascular and non-vascular complications (Gispen and Biessels, 2000; Montilla *et al*., 2005). Increased production of reactive oxygen species plays a role in pathogenesis and pathophysiological mechanisms that trigger diabetic complications (Auslander *et al*., 2002; Marjani**,** 2005; Nobecourt *et al*., 2005).

Diabetic Nephropathy is characterized by persistent albuminuria, a decline in the glomerular filtration rate and elevated arterial blood pressure (Cross *et al*., 2005) and is the leading cause of chronic renal failure (Nakai *et al*., 2005). Oxidative stress was reported to play a key role in pathogenesis of many diseases including diabetic nephropathy (Jee *et al*., 2005; Okutan *et al*., 2005).

Propolis is a resinous hive product collected by honeybees from many plant sources (Tan-No *et al*., 2006). It is a traditional medicine used as early as 300 BC and has been reported to exert a broad spectrum of biological functions including antioxidant activity (Burdock *et al*., 1998; Benguedouar *et al*., 2008). It has recently gained popularity as a healthy food in various parts of the world because it promotes health and prevents diseases (Inokuchi *et al*., 2006). The composition of propolis depends on local flora, types of plants and the vegetation at the site of collection. The major components of propolis in Europe and China are flavonoids and phenolic acid esters (Bankova *et al*., 2000). However, Brazilian propolis has terpenoids and prenylated derivatives of coumaric acids (Tazawa *et al*., 1999). Egyptian propolis was reported to contain 42 Polyphenolic compounds, 13 aromatic acids, esters and alcohols and 29 flavonoids (Abd El-Hady *et al*., 2007).

In the present study, the effect of propolis on diabete-associated metabolic disturbances, renal function and oxidative stress was examined STZ-induced diabetic rats.

MATERIAL AND METHODS

Chemicals

Chemicals and ethanolic extract of Brazilian green propolis were purchased from Sigma Chemical Company

**Corresponding author*: Ph: +20 127162100, +202 29885788, +966 560769519, +966 503540119, e-mail: oabosalem@yahoo.com

Table 1: Effect of propolis extract on the body and kidney weight of diabetic rats

Groups	BW (g)			KW/100 g BW
	1st day	40th day	Increase	
C	195.1 ± 1.13	259.3 ± 4.47	64.2 ± 4.39	0.37 ± 0.02
D	196.9 ± 1.03	219.0 ± 2.26[a]	22.1 ± 2.16[a]	0.83 ± 0.05[a]
P100	197.5 ± 1.02	236.2 ± 3.34[a,b]	38.7 ± 3.42[a,b]	0.58 ± 0.05[a,b]
P200	195.0 ± 0.94	237.7 ± 2.20[a,b]	42.7 ± 2.50[a,b]	0.57± 0.03[a,b]
P300	198.6 ± 0.60	248.2 ± 4.77[b]	49.6 ± 5.09[b]	0.47 ± 0.04[b]

Body weights were measured at the beginning and end of the experiment. Data are expressed as means ± SEM of 10 rats /group. BW: total body weight; KW: kidney weight; C: control normal group; D: STZ-induced diabetic group; P100, P200 & P300: diabetic groups treated with ethanolic extract of propolis, orally, in doses of 100, 200 & 300 mg/kg bwt, respectively , daily for 40 days after STZ injection.
[a]: significantly different from control group; [b]: significantly different from STZ-induced diabetic group, using one-way ANOVA with Tukey-Kramer test at P< 0.05.

Table 2: Serum biochemical parameters after 40 days of propolis administration in diabetic rats

Parameters	C	D	P100	P200	P300
Glucose (mg/dl)	61.60 ± 4.28	220.00 ± 5.77[a]	147.60 ± 4.71[a,b]	111.80 ± 5.27[a,b,c]	97.20 ± 6.57[a,b,c]
BUN (mg/dl)	18.70 ± 1.80	50.30 ± 2.75[a]	36.90 ± 1.93[a,b]	34.80 ± 1.65[a,b]	24.40 ± 2.03[b,c,d]
Creatinine (mg/dl)	0.46 ± 0.04	0.98 ± 0.07[a]	0.91 ± 0.06[a]	0.75 ± 0.05[a,b]	0.68 ± 0.02[a,b,c]
Total cholesterol (mg/dl)	79.20 ± 3.46	184.50 ± 8.18[a]	115.30 ± 5.57[a,b]	113.20 ± 7.92[a,b]	89.80 ± 3.71[b,c]
LDL-C (mg/dl)	32.80 ± 2.13	89.30 ± 3.73[a]	49.20 ± 4.64[a,b]	38.40 ± 3.53[b]	37.60 ± 2.83[b]
HDL-C (mg/dl)	38.90 ± 2.28	14.20 ± 1.29[a]	19.70 ± 1.86[a]	27.50 ± 1.65[a,b]	42.50 ± 3.48[b,c,d]
LDL-C/HDL-C	0.89 ± 0.10	6.76 ± 0.62 [a]	2.69 ± 0.34[a,b]	1.46 ± 0.18[b]	0.91 ± 0.05[b,c]
TG (mg/dl)	71.70 ± 2.81	129.50 ± 5.50[a]	99.40 ± 5.86[a,b]	97.20 ± 5.97[a,b]	90.50 ± 5.76 [b]
Total cholesterol /HDL-C	2.09 ± 0.14	14.21 ± 1.64[a]	6.39 ± 0.67 [a,b]	4.27 ± 0.43[b]	2.24 ± 0.20[b,c]
HDL-C/TG	0.55 ± 0.04	0.11 ± 0.01[a]	0.21 ± 0.03[a]	0.29 ± 0.03[a,b]	0.47 ± 0.03[b,c,d]
MDA (umol/L)	1.59 ± 0.12	7.80 ± 0.76[a]	4.70 ± 0.56[a,b]	3.60 ± 0.40[b]	4.10 ± 0.43[a,b]

Data are expressed as means ± SEM of 10 rats /group. C: control normal group; D: STZ-induced diabetic group; P100, P200 & P300: diabetic groups treated with ethanolic extract of propolis, orally, in doses of 100, 200 & 300 mg/kg bwt, respectively, daily for 40 days after STZ-injection.
[a]: significantly different from control group; [b]: significantly different from STZ-induced diabetic group; [c]: significantly different from P100 group; [d]: significantly different from P200 group, using one-way ANOVA with Tukey-Kramer test at P< 0.05.

(St. Louis, MO, USA). The Kits were supplied by Biodiagnostics Company, Egypt.

Animals

Adult male Albino Wistar rats, weighing 190-200 gm were purchased from the National Institute of Cancer**,** Cairo, Egypt. Rats were housed (5 per cage) in the animal facility of the Faculty of Pharmacy (boys), Al-Azhar University, Cairo, Egypt for two weeks for adaptation prior to starting the experiment. Free access was allowed to standard diet, temperature (22 ± 2˚C), relative humidity (55%), light period (12 h light/12 h dark) and water *ad libitum.*

Induction of diabetes

Type 1 diabetes was induced by i.p. injection of STZ (dissolved in citrate buffer 0.1 mol/L, pH 4.2) in a dose of 60 mg/kg bwt for 3 successive days (Abdel-Wahab *et al*., 1996). Rats were considered diabetic if blood glucose concentrations increased to 200 or more mg/dl.

Experimental protocol

Fifty rats were used in this study and classified into 5 groups (10 animals/ group) as follows:

Group I: Received the vehicle (2 % tween 80 + 2 % sodium lauryl sulfate in a saline solution) and served as a control group.

Group II: Injected with STZ, i.p. in a dose of 60 mg/kg bwt for 3 successive days and served as a diabetic group.

Groups III-V: Received ethanolic extract of propolis (EEP) at dose levels of 100, 200, 300 mg/kg bwt, respectively via oral gavage, daily for 40 days, starting after 3 days of STZ injection.

Biochemical parameters

On the 39th day of the study, individual rats were placed in metabolic cages to collect 24 h urine for measurements of urinary albumin. Assessment of micro-albumin (MA) was done on Beckman 360 ARRAY (Beckman Inst., USA) (Busby and Atkins, 2005).

Table 3: Effect of propolis extract on oxidant and antioxidant parameters in renal tissue

Parameters	C	D	P100	P200	P300
MDA (umol/g)	74.04 ± 4.98	167.96 ± 11.85[a]	124.87 ± 10.14[a,b]	118.84 ± 8.25[a,b,c]	93.86 ± 8.40[b,c]
GSH (umol/g)	35.53 ± 1.87	16.75 ± 1.27[a]	26.78 ± 1.29[a,b]	27.42 ± 1.48[a,b]	30.62 ± 1.73[b]
SOD (U/mg)	27.33 ± 1.19	11.89 ± 0.43[a]	19.01 ± 0.37[a,b]	24.70 ± 1.47[b,c]	26.26 ± 2.08[b,c]
CAT (U/mg)	20.83 ± 1.29	3.73 ± 0.40[a]	21.09 ± 1.86[b]	23.99 ± 1.73[b]	23.46 ± 2.25[b]

Data are expressed as means ± SEM of 10 rats /group. C: control normal group; D: STZ-induced diabetic group; P100, P200 & P300: diabetic groups treated with ethanolic extract of propolis, orally, in doses of 100, 200 & 300 mg/kg bwt, respectively, daily for 40 days after STZ-injection. [a]:significantly different from control group; [b]:significantly different from STZ-induced diabetic group; [c]:significantly different from P100 group, using one-way ANOVA with Tukey-Kramer test at P< 0.05.

On the 40th day of the study, venous blood samples were obtained from retro-orbital veins and used for determination of serum glucose, creatinine, BUN and lipid profile. These parameters were performed on Synchron CX5 autoanalyser (Bekman Inst., USA).

Rats were scarified by cervical dislocation under ether anesthesia. Kidneys were removed, washed with physiological saline, cleared of fatty tissue and weighed. They were homogenized in ice cold 20 mM Tris-HCl buffer (pH 7.4) and the homogenates were then centrifuged at 10,000 g for 10 min at 4ºC (Montilla *et al*., 2005). The supernatants were collected and used for assessment of protein, GSH (Beutler *et al*., 1963), SOD (Sun *et al*., 1998), CAT (Aebi, 1984) and MDA (Deniz *et al*., 1997).

Statistical analysis of data

The values are presented as means ± SEM. Quantitative data were analyzed by one-way ANOVA followed by Tukey-Kramer test for multiple comparisons using INSTAT soft ware.

RESULTS

Body and kidney weights

Table (1) showed that there was marked reduction in the body weight of diabetic animals reaching 17% compared to that of the control normal group. In addition, kidney weight in diabetic group was significantly increased amounting to 124 %. Oral administration of propolis extract in doses of 100, 200 and 300 mg/kg significantly increased the body weight recording 8%, 9% and 13%, respectively compared to the diabetic group. Moreover, propolis treatment led to significant reduction in the kidney enlargement in a dose-dependent manner, amounting to 30%, 31% and 43%, respectively.

Renal functions and lipid profile

Table 2 showed that serum BUN and creatinine were significantly elevated in the diabetic group compared to that of the normal rats. Compared to diabetic group, BUN declined significantly with all the three tested doses (27%, 31% and 51%, respectively) while creatinine decreased only at doses of 200 and 300 mg/kg bwt (29% and 31%, respectively). Administration of propolis extract at different doses significantly reduced blood glucose in a dose-dependent manner amounting to 33%, 49% and 56%, respectively compared to that of the diabetic rats. Serum cholesterol, LDL-C, triglycerides (TG) and MDA levels of the diabetic group were significantly higher while HDL-C was lower compared to the control normal rats. However, administration of propolis significantly improved these levels in a dose-dependent manner (table 2).

Urinary albumin excretion

At the end of the study, urinary albumin/24 h was significantly increased in the untreated diabetic group (301) compared to the normal rats (fig.).

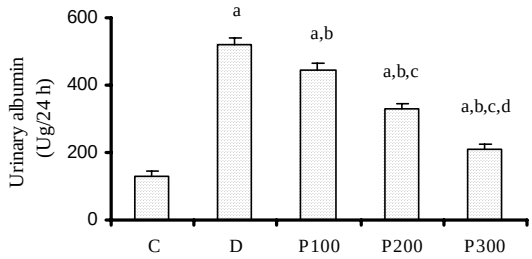

Fig. Effect of propolis extract on urinary albumin excretion in diabetic rats.

Urinary albumin was measured on the 40th day of the experiment. Data are expressed as means ± SEM of 10 rats /group. C: control normal group; D: STZ-induced diabetic group; P100, P200 and P300: diabetic groups treated with ethanolic extract of propolis, orally in doses of 100, 200 and 300 mg/kg bwt, respectively, daily for 40 days after STZ injection.

[a]:significantly different from control group; [b]:significantly different from STZ-induced diabetic group; [c]:significantly different from P100 group; [d]:significantly different from P200 group, using one-way ANOVA with Tukey-Kramer test at P< 0.05.

Administration of propolis extract in doses of 100, 200 and 300 mg/kg bwt produced marked reduction in the elevated urinary albumin excretion in the diabetic group in a dose dependent manner, recording 14%, 36% and 60%, respectively.

Lipid peroxidation and antioxidant defense systems in the renal tissue

Significant increase of MDA (127%) and decreases of GSH (53%), SOD (56%) and CAT (82%) were found in the diabetic group as compared to the normal control (table 3). Treatment of rats with propolis extract at different doses significantly reduced the kidney MDA and increased GSH, SOD and CAT activities compared to the diabetic group. Propolis normalized CAT activity at all tested doses. However, GSH returned to the normal value at the dose 300 mg/kg bwt only, while SOD at doses of 200 and 300 mg/kg bwt. These data may indicate that the protective effect of propolis against renal damage in diabetic rats is dose-dependent.

DISCUSSION

In diabetes mellitus, increased blood glucose, lipids, oxidized LDL and oxygen free radicals can induce glomerulosclerosis and chronic tubulointerstitial damage in the kidneys leading to DN (Yin *et al*., 2004; Masumi *et al*., 2005; Montilla *et al*., 2005; Okutan *et al*., 2005). A progressive decline in the glomerular filtration rate due to loss of functioning nephrons and histological renal damage are common characteristics in the development of diabetic nephropathy (Yamabe *et al*., 2006).

Our data revealed that there were marked reduction in the total body weight as well as elevation in the kidney weight of the diabetic group compared to that of the control normal group. Propolis treatment showed a significant amelioration in both body and kidney weights in a dose-dependent manner. Propolis has a strong antioxidant and free radical scavenging effect (Valadares *et al*., 2008).This finding suggests that propolis may improve the disturbed metabolism associated with diabetes. A similar observation was reported by Yamabe *et al*. (2006) after administration of (-)-epigallocatechin-3-*O*-gallate as an antioxidant.

Moreover,our results showed that serum BUN and creatinine were significantly elevated in diabetic rats compared to that of the control normal rats. BUN declined significantly in the propolis-treated groups, however, creatinine level decreased only with the intermediate and high doses of propolis. BUN reached the normal levels in the group treated with the highest dose of propolis (300 mg/kg). Moreover, urinary albumin excretion, a marker of early diabetic nephropathy, was improved after treatment with propolis in a dose-dependent manner. These results may indicate that propolis can attenuate renal damage in diabetic rats. In agreement with these findings, Yamabe *et al*. (2006) reported that antioxidants and good control of diabetes led to improved renal functions and urinary albumin excretion. Moreover, caffeic acid phenethyl ester (CAPE), a biological active component of propolis was found to improve renal function tests in a rat model with lithium-induced renal tubular damage and oxidative stress (Oktem *et al*., 2005).

In this study, serum levels of glucose, total cholesterol, LDL-C, TG and MDA were significantly elevated in diabetic rats compared to that of the normal control rats. Administration of propolis at different doses significantly improved these parameters in a dose-dependent manner. Moreover, the highest dose of propolis (300 mg/kg) was able to reduce blood glucose to the normal level. Moreover, the serum level of HDL-C, which was significantly decreased in diabetic rats, was also improved by propolis in a dose-dependent manner. These findings may indicate that propolis can improve the lipid profile of diabetic rats. In accordance with this observation, ethanol and water extracts of propolis were found to decrease glucose, fructosamine, MDA, nitric oxide, total cholesterol, TG, LDL-C and VLDL-C and increase HDL-C and SOD in rats (Fuliang *et al*., 2005). Improvement of lipid the profile, MDA and SOD activity in mice by propolis treatment was demonstrated by Luan *et al*. (2000) and Jasprica *et al*. (2007). Furthermore, propolis was found to modulate antioxidant enzymes and decrease lipid peroxidation processes in plasma, liver, lungs, and brain of mice in a dose- and tissue-dependent manner (Shinohara *et al*., 2002; Sobocanec *et al*., 2006). Therefore, propolis can control the blood glucose, modulate the metabolism of lipids leading to decreased outputs of lipid peroxidation and scavenge the free radicals in rats with diabetes (Fuliang *et al*., 2005).

In the present study, SOD, CAT and GSH activities were measured in renal tissue to evaluate the changes of antioxidant status in the kidney. Increased renal MDA content and decreased GSH, SOD and CAT activities were found in diabetic rats compared to the normal control group. However, treatment of diabetic rats with propolis significantly improved these parameters. Interestingly, CAT reached the normal values at all tested doses, SOD at doses of 200 and 300 mg/kg, and GSH at dose of 300 mg/kg only. These data may indicate that the protective effect of propolis against renal damage in diabetic rats is dose-dependent. Similar results were obtained illustrating the protective effect of CAPE against lithium carbonate-induced (Oktem *et al*., 2005) and electromagnetic radiation-induced (Ozguner *et al*., 2005) renal tubular damage in a rat model. In addition, Okutan *et al*. (2005) reported that the activities of SOD, CAT and GSH-peroxidase were markedly increased while MDA content was reduced in the cardiac tissues of diabetic rats after treatment with CAPE. These investigators concluded that diabetes increases oxidative stress in cardiac tissue and CAPE has an ameliorating effect on the oxidative stress via its antioxidant property. Moreover, Altug *et al*. (2008) showed that CAPE significantly had a protective role in permanent focal ischemia of brain by decreasing plasma MDA and increasing GSH and CAT.

Propolis was suggested to have potent antioxidant activity in vitro and in vivo (Ichikawa *et al*., 2002). Also, it was reported to save vitamin C (Sun *et al*., 2000), maintain cellular GSH, conserve the integrity of biomembranes and reduce leakage of cytosolic lactate dehydrogenase in the liver (El-Khatib *et al*., 2002). Moreover, it may diminish primary DNA damage of the cells (Benkovic *et al*., 2008).

In conclusion, propolis has an antioxidant effect which can decrease metabolic disturbances and oxidative stress that are associated with diabetes. Consumption of food and drink containing effective antioxidant agents as propolis may delay the onset and/or progression of diabetic nephropathy and delay the occurrence of diabetes-associated renal function impairment.

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