Double Dose of Propolis Ameriolated Diabetic Nephropathy in Streptozotocin-Induced Diabetic Rats

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Abstract

The aim of the present study was to study the possibility that propolis can control blood glucose levels and prevent diabetic nephropathy in streptozotocin induced diabetic rats. The present study compared 60 streptozotocin (STZ)-induced diabetic rats, with ten nondiabetic rats used as a negative control. The experimental design comprised seven groups (n = 10 rats per group): (1) nondiabetic, used as a negative control; (2) nontreated, used as a positive control; (3) treated with insulin alone; (4) treated with a single dose of propolis alone; (5) treated with a double dose of propolis; (6) treated with insulin and a single dose of propolis; and (7) treated with insulin and a double dose of propolis. After 6 weeks of treatment, the rats were sacrificed and blood was collected from abdominal aorta. There was a significant reduction in FBG in all diabetic treated rats. Similarly, higher plasma insulin levels were observed in diabetic rats treated with propolis and insulin than in nontreated diabetic rats, although plasma insulin was not comparatively higher in diabetic rats treated with insulin alone. Furthermore, a significant decrease in the lipid peroxidation activity in propolis treated rats than non-treated diabetic (positive) group to approach near the mean value of the control group. The present study showed an increase in albuminuria, serum creatinine and BUN in the non-treated diabetic (positive control) group compared to the all treated groups with propolis alone in both doses or with insulin. However, insulin and double dose of propolis showed the best renal function results and the most significant improvement in albuminuria and creatinine levels. It could be concluded that, propolis administration especially the double dose (0.6 gm/kg) with the traditional treatment of type1 diabetes (insulin) is very beneficial in the control of diabetes and prevention of its most dangerous complications as nephropathy.

Keywords: Propolis-Nephropathy-Lipid Peroxidation-Insulin-Albuminuria-Creatinine-BUN.

Introduction

Diabetes has become a global health burden affecting 425 million people worldwide according to the International Diabetes Federation (IDF). IDF also estimated that this number will increase to 630 million in 2045 [1]. One of the most important medical complications in diabetic population is the diabetic nephropathy (DN). About one-third of all diabetic individuals are affected by this complication which is the leading cause of end- stage renal disease (ESRD) [2]. Streptozotocin induced diabetes in rats causes insulinopenia due to B-cell destruction in pancreas, which resembles type-1diabetes (T1DM) in humans and usually associated with decreased renal functions and finally renal failure [3]. Hyperglycemia is an important factor responsible for the intense oxidative stress in diabetes, and the toxicity induced by glucose autoxidation is likely to be one of the important sources of reactive oxygen species [4]. Additionally, lipid peroxidation plays an important role in the production of free radicals and

oxidative stress in diabetes [5]. Normally, there are several intra- and extracellular antioxidant defense mechanisms counteract the destructive effects of free radicals by attenuating or omitting their activities. However, in DM the oxidative stress exceeds the body's antioxidant defense mechanisms [6]. Oxidative stress and free radicals have been reported to play a significant role in diabetic nephropathy, so treatment with antioxidants has been reported to reduce these complications [7]. Several therapeutic interventions have been implemented to slow the progression of DN in cases of DM. However, these approaches have proved insufficient and new strategies are needed [8]. Propolis is a resinous substance collected by honeybees from various plant sources. It has been used as a folk medicine in many countries since ancient time because of its peculiar biological properties as an antioxidant, antimicrobial anti-inflammatory and anti-cancer material [9]. Recent articles on propolis credit it with curing diabetes mellitus and its complications, but detailed studies are few and no uniform standard methods

for the preparation of propolis in the treatment of diabetes mellitus and /or its complications [10]. Many studies have shown that propolis has a hypoglycemic, hypolipidemic, and strong antioxidant activity which can be used to prevent or delay the appearance of diabetic nephropathy [11]. Its hypoglycemic activity has been attributed to inhibition of intestinal maltase and/or alpha-glucosidase activity, preventing rise of blood glucose following carbohydrate intake [12]. Propolis has also been reported to enhance the antioxidant defense system to protect pancreatic tissues with restoration of insulin secretions from B-cells of pancreas [13]. In view of recent claims that propolis can cure streptozotocin (STZ)-induced DM, and prevent its complications, the aim of the present work was to investigate the effect of propolis on the control of diabetes and the prevention of its serious complication DN.

Material and methods

The present study was conducted at Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. A total of 70 male albino Wistar rats weighing 150–250 gm was obtained from the university's animal house for this study. All rats were maintained in a room at a constant temperature of 22 ± 1°C with 12-hour light/dark cycles and had free access to standard laboratory food pellets and water. Ethical approval of the protocol was taken from ethics committee of the university.

The study compared 60 streptozotocin (STZ)-induced diabetic rats, with ten nondiabetic rats used as a negative control. The experimental design comprised seven groups (n = 10 rats per group): (1) nondiabetic, used as a negative control (G-1),(2) nontreated, used as a positive control (G-2), (3) treated with insulin alone (G-3),(4) treated with normal dose of propolis (0.3 g/kg) alone (G-4) , (5) treated with a double dose of propolis (0.6 g/kg) (G-5); (6) treated with insulin and a normal dose of propolis (0.3 g/kg) (G-6), and (7) treated with insulin and a double dose of propolis (0.6 g/kg) (G-7).

T1DM was induced in the experimental rats by administering a single-dose intraperitoneal (IP) injection of STZ (60 mg/kg) (Sigma-Aldrich Co, St Louis, MO), dissolved in distilled water [14]. Three days after the STZ injection, urine strips (Medi-Test Combi -10 Macherey-Nagel GmbH & Co, Düren, Germany) were used to detect glycosuria in rats (a dark-green color indicated blood glucose ≥ 500 mg/dL [15]. These 60 STZ-induced diabetic rats were randomly divided into the six groups, G-2 to -7, while group 1 beeing the negative control was left intact.

Treatment of all rats included in groups 1-7 started daily at 8 am and continued for 6 weeks. Both the negative control and positive control groups (G-1 and G-2, respectively) received a daily IP injection with normal saline and 1 mL of water through a rat feeding needle (Kent Scientific Corporation, Torrington, CT) [15]. Groups G-3, G-6, and G-7 received an IP injection of insulin (5 IU/kg/day) (Humulin; Eli Lilly and Company, Indianapolis, IN) [16].

Groups G-4 and G-6 received propolis through oro-gastric metallic needle (in aqueous solution), 0.3 gm/kg [17], and

groups G-5 and G-7 received a double dose of propolis (in aqueous solution, 0.6 gm/kg), through an orogastric metallic needle [15].

At the end of the 6-week experimental period, the different treatment regimens were stopped, and food was stopped 12 hours before sacrificing the rats. Animals were anesthetized with an IP injection of ketamine (50 mg/kg) (Alfasan International BV, Woerden, Netherlands) [18]. Blood was collected directly from the abdominal aorta in two tubes by means of a vacutainer. One tube was heparinized for separation of plasma for hormonal studies, while the other tube was kept plain for fasting blood glucose (FBG) and separation of serum to determine the antioxidant activities, creatinine and BUN. Plasma and serum was separated from blood samples by centrifugation at 3000 rpm for 10 minutes at 4°C, collected, and stored at −80°C until the time of analysis.

Determination of Plasma insulin and glucagon

Plasma enzyme-linked immunosorbent assays (ELISAs) were used to estimate hormone levels of Insulin and Glucagon. The Insulin ELISA kit (ALPCO Diagnostics, Salem, NH) used for quantitative determination of plasma insulin concentration in rats is a one-step sandwich enzyme immunoassay using two monoclonal antibodies [19]. Pancreatic glucagon levels were determined by a highly specific ELISA kit (Wako Pure Chemical Industries, Ltd, Richmond, VA), based upon a competitive ELISA using a highly specific antibody to glucagon [20].

Serum FBG, Creatinine and BUN levels

FBG concentrations were determined by a glucometer (Accu-Chek Go, Roche Diagnostics GmbH, Indianapolis, IN) [21]. Serum creatinine and (BUN) were measured by colorimetric method using a spectrophotometer [22].

Oxidative status(TBARS) assay

The activity of thiobarbituric acid reactive substances (TBARS) assay kit (Cayman Chemical) was used to measure the product of the reaction between malondialdehyde, a product of lipid peroxidation, and TBARS by using ELISA reader [23].

Evaluation of Microalbuminuria

Rats, 24 hrs. before sacrificing, were housed in stainless steel metabolic cages individually prior to each collection. Collected 24 hr. urine sample of each cage was processed immediately for microalbuminuria by using Hemocue Instrument (Hemocue, Quest Diagnostics) [24].

Statistical analysis

All values reported are expressed as mean plus or minus standard error of the mean. Differences among means were analyzed for significance by analysis of variance using SPSS software (v 10; SPSS Inc, Chicago, IL). Groups were then compared by one-way ANOVA test, and $P < 0.05$ was considered statistically significant.

Results

Fasting Blood Glucose, Plasma insulin, Plasma glucagon and I/G ratio:

Table 1 illustrates the results of fasting blood glucose, plasma insulin, plasma glucagon and I/G ratios in the seven studied groups (G1-G7): It was found that, the mean fasting blood glucose for the non-treated diabetic (positive) group was highly significantly increased compared to all the other studied groups. Insulin treatment and supplementation of propolis with or without insulin (G3, G4, G5, G6 and G7) caused a significant decrease in fasting blood glucose, to approach near the mean value of negative control. The results of the present study showed that there was a significant decline in the means of plasma insulin concentration in all diabetic's groups(G2-G7) compared with negative control. However, the groups given propolis with insulin (G 6 and G7) has significantly higher insulin than non-treated (positive control) group. The mean of plasma glucagon concentration of the non-treated diabetic (positive control) group was found to be significantly higher than that of all other groups (G3, G4, G5, G6 and Group7). Moreover, with the double propolis dose and insulin treated group, the plasma glucagon concentration was found to be significantly lower than, insulin treated and propolis treated groups (G3 and G4), to approach near the mean value of negative control.

The means of insulin glucagon (I/G) ratio of all experimental groups were significantly lower compared to the negative control. Interestingly, the means of I/G ratios of the (G6 and G7) groups were found to be significantly higher than that of other groups (G2, G3, G4 and G5).

Groups	treatments	FBS mg/dl	Insulin ngm/ml	Glucagon	I/G ratio
G1(negative) N = 10 Mean ± SEM	none	143.9± 6.8	3.0±0.25	0.417± 0.029	7.182 ±0.09
G2(positive) N = 10 Mean ± SEM	none	509.3 ± 22.8 *G1	0.30± 0.04 *G1	1.2482 ± 0.16 *G1	0.207 ± 0.001 *G1
G3 N = 10 Mean ± SEM	Insulin	182.0 ± 8 *G1	1.10 ± 0.26 *G1	0.949 ± 0.078 *G1*, G2	1.158 ±0.03 G1*
G4 N = 10 Mean ± SEM	Propolis (n.dose)	153.7 ± 12.7 ♀G1*	0.84 ± 0.3 G1*	0.929 ±0.115 *G1*, G2	0.842 ±0.03 G1*
G5 N = 10 Mean ± SEM	Propolis(double)	127.6 ± 17.5 G2*	0.85 ± 0.4 G1*	0.755 ± 0.142 G2*	1.13 ±0.02 G1*
G6 N = 10 Mean ± SEM	Insulin+propolis (n.dose)	156.2 ± 12.5 G2*	1.50 ± 0.04 G1*G2*	0.669 ± 0.889 G2*	2.242 ±0.05 G1*G2*G3* G4*G5*
G7 N = 10 Mean ± SEM	Insulin+propolis (double)	127.1 ± 20.7 G2*	1.40 ± 0.06 G1*G2*	0.587 ± 0.591 G2*G3*G4*	2.382 ±0.05 G1*G2*G3* G4*G5*

Table 1: Means (± SEM) of Fasting Blood Glucose (FBG), Plasma Insulin, Glucagon and Insulin Glucagon (I/G) Ratio in the Control and Experimental Groups. Significancy* tested by using one-way ANOVA at P< 0.05.

Albumin in Urine and Serum Creatinine and Blood Urea Nitrogen (BUN)

Table 2 illustrates the data concerning analysis of albumin concentration in urine in the seven studied groups(G1-G7). The means of albumin concentration in urine in the non-treated diabetic (positive control) group were found to be significantly higher compared to the negative control and all the treated groups (II2, II3, II4, II5 and II6) with the lowest levels seen in in the group treated with double dose of propolis plus insulin.

Table 2 also illustrates the data concerning analysis of creatinine, and BUN in all the studied groups(G1-G7) The mean of serum creatinine for non-treated diabetic (positive) group was found to be significantly higher than the negative control and all the treated diabetic groups (G3, G4, G5, G6 and G7), while that of BUN is insignificantly higher, when compared to the control (negative) group and all treated groups (G3-G7).

Group	Albumin in Urine (mg/L)	Creatinine (mg/dl)	BUN (mg/dl)	Lipid peroxidation TBARS Umol/L
G 1: Control (negative) N = 10 Mean ± SEM	34.5 ± 10	0.79 ± 0.006	18.5 ± 1.5	49 ± 6.2
G2: Non-treated diabetic rats(positive) N = 10 Mean ± SEM	111 ± 40 G1*	0.87 ± 0.005 G1*	19 ± 2 insignificant	99.7 ± 12.1 G1*
G3: Insulin-treated rats N = 10 Mean ± SEM	60.1 ± 14.1 G2*	0.77 ± 0.008 G2*	18.3 ± 1.3 insignificant	64 ± 15.5 G2*
G4: Propolis-treated rats N = 10 Mean ± SEM	64 ± 29 G2*	0.78 ± 0.005 G2*G3*	18.2 ± 1.3 insignificant	56.5 ± 7.7 G2*
G5: Double propolis-treated rat N = 10 Mean ± SEM	48± 5 G2*	0.74 ± 0.007 G2*G3*G4*	18.4 ± 1.7 insignificant	37.5 ± 4.5 G2*
G6:Propolis+Insulin treated rat N = 10 Mean ± SEM	55 ± 12 G2*	0.77 ± 0.008 G2*G3*G5*	18.5 ± 1 insignificant	51.9 ± 10.3 G2*
G7: Double propolis+insulin treated rat N = 10 Mean ± SEM	48 ± 12 G2*	0.73 ± 0.006 G2*G3*G4*G6*	18.1 ± 1.2 insignificant	36 ± 12.1 G2*

Table 2: Means (± SEM) of Albumin Concentration in Urine (mg/L), Serum Creatinine (mg/dl), Blood Urea Nitrogen (BUN) (mg/dl) and lipid peroxidation(TBARS)Umol/L in all Groups Significancy*tested by using one-way ANOVA at P< 0.05.

Lipid Peroxidation (TBARS) Level

Table 2 illustrates the the level of lipid peroxidation (TBARS) in the seven studied groups(G1-G7) The results showed that the mean of TBARS level in the non-treated diabetic (positive) group(G2) was found to be statistically significantly higher than in the negative control group(G1). TBARS level in the group treated with insulin (G3) was not significantly lower than the non-treated diabetic (positive) group, while all other groups treated with propolis (G4, G5, G6 and G7) had significantly lower TBARS level compared to the non-treated diabetic (positive) group indicating high antioxidative effect of propolis.

Discussion

The cornerstone in reducing the risk of diabetic nephropathy is the control of blood glucose levels. The present study showed that propolis could almost control the hyperglycemia in the STZ-induced diabetic rat model.

The glycemic control achieved by propolis treatment could be multifactorial , some studies reported an insulin like action of propolis through increased stimulation of glucose uptake by peripheral tissues [25] ,inhibition of glucose release in circulation by decreased hepatic glucose production through the inhibition of Glucose -6- Phosphatase System by inhibiting the autophosphorylation of Y279 and Y216 of GSK3α and β, respectively, which are involved in the activation of GSK3 [26] and reduced glucose absorption in the gut through the inhibition of maltase and alpha-glucosidase enzymes [27]. The present study found that a decline in glucagon concentration in propolis-treated rats with elevation of the I/G ratio, again suggesting that decline in liver glucose output is an important mechanism in glycemic control by propolis. The study also found that a significant decline of glucagon concentrations in all propolis-treated diabetic rats compared with nontreated diabetic rats is due to insulin-induced inhibition of pancreatic α cells. This is because high insulin levels were found to be associated with significantly low glucagon

levels in diabetic rats treated with both propolis and insulin. Additionally, compared with single-dose propolis-treated rats, double-dose propolis-treated rats showed low glucagon levels that were nearing negative control group levels, despite the insulin dose being the same for both groups (propolis-treated diabetic and double-dose propolis-treated diabetic rats). However, a direct inhibitory effect of propolis on α cells cannot be excluded and requires further investigations. These findings suggest that propolis may be a potential antidiabetic agent for the treatment of insulin-dependent diabetes. However, the present study showed also that propolis treatment in STZ-induced diabetic rats was associated with significant elevation of plasma insulin and low glucagon levels with elevated I/G ratio. These findings suggest that propolis may stimulate and regenerate beta-cells of pancreas which showed more activation, however, the recovery of insulin secretion in diabetic rats is a partial restoration only, because insulin levels in all diabetic rats treated with propolis were significantly lower than in the negative control group. The source of insulin in propolis-treated diabetic rats could be the β cells of the pancreas and therefore two possibilities are suggested: either propolis induces partial regeneration or it prevents further deterioration of β -cells through its strong anti-oxidative and anti-inflammatory actions. Other investigators have reported similar findings on the ability of propolis to induce regenerative effects on β-cells [28-29]. As already noted, lipid peroxidation is the most potent oxidative defect that damages β- cells in T1DM [30]. All diabetic rats in the study treated with propolis alone or with insulin were observed showing significantly lowered lipid peroxidation levels. This strong anti-oxidant effect may be responsible for protection of the kidney from oxidative damage in these cases together with the glucose lowering effect. Diabetics are at increased risk for several types of kidney disease, and the predominant cause of end-stage renal disease, in this disorder is diabetic nephropathy [31]. However, clinical trials suggest that there is no effective treatment for diabetic nephropathy [32]. Therefore, prevention of occurance and progression of diabetic nephropathy has become a very important issue. Two processes play a role in the fully developed diabetic glomerular lesions, a metabolic defect linked to advanced glycosylation end products, that account for the thickened glomerular basement membrane and increased mesangial matrix and hemodynamic effects, associated with glomerular hypertrophy which contributes to the development of glomerularsclerosis [33]. Clinically, diabetic nephropathy starts early within few weeks from the diabetic state by microalbuminuria accompanied by increased glomerular filtration rate with increased glomerular capillary pressure, glomerular hypertrophy and increased glomerular filtration area [34]. Then, after longer time, persistant albuminuria, decline in glomerular filtration rate, elevation of BUN and creatinine, changes in serum electrolytes and hypertension occurs ending by chronic renal failure [35]. In the present study, our data showed a significant increase in microalbuminuria in non-treated diabetic (positive) group compared to the normal control group while treatment with propolis (in both doses) with or without insulin caused a significant decrease in the levels of microalbuminurea. The associated

significant increase in serum creatinine concentration may indicate some changes in glomerular filtration rate. However, treatment with propolis plus insulin caused an improvement in creatinine levels especially with the double dose of propolis plus insulin leading to return of creatinine to the levels of the control group.

Micoalbuminuria was found to be improved in all the treated groups especially by insulin and double dose of propolis and approached values near that recorded in normal controls. Simultaneously, treatment with insulin and propolis in both doses could prevent the increase in serum Hence control of blood glucose level might have improved renal functions and ameliorated the renal damage. Several studies have provided substantial evidence that multiple factors caused by hyperglycemia contribute to the development of diabetic kidney disease [36,37]. A suggested mechanism is hyperglycemic condition resulting in tissue damage via protein glycation reaction that leads to formation of glycosylated protein and AGEs [38].

Overproduction of glycation products and lipid peroxidation products plays a potential role in diabetic nephropathy [39], this would result in improvement of renal lesions caused by oxidative stress [40]. Control of hyperglycemia and consequently the oxidative stress were also reported by another study to improve renal functions and decrease in microalbuminuria occurence in diabetics [41]. Also, an improvemet in renal functions and decreased microalbuminuria in experimentally diabetic rats treated with propolis was reported in a previous study [42]. Indeed, the present results showed that, all treated rats had improved lipid peroxidation values, but propolis in double dose with or without insulin showed a significantly lowered lipid peroxidation to approach normal control (negative) group values with normal Albuminuria, so nephropathy was prevented. This suggests that, administration of propolis in double dose with the insulin would ameliorate oxidative stress under the diabetic condition and prevent diabetic nephropathy.

Conclusion

In conclusion, experimental treatment of STZ-induced diabetic rats with double dose propolis with insulin which is the traditional treatment of this type of diabetes was found to effectively control blood glucose level, improve function of the pancreatic islets, eliminate the oxidative stress, and protect the kidney from diabetic nephropathy. Clinical application of this combined therapy in humans requires further investigation and evaluation of its effectiveness and safety in T1DM patients.

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Conflicts of Interest: The author declares no conflict of interest regarding this study.

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