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RESEARCH ARTICLE

Influence of propolis on oxidative stress, inflammation and apoptosis in streptozotocin-induced diabetic rats

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Abstract

Propolis is a complex mixture of several resinous substances which are collected from plants by honeybees. In the present study, an attempt has been made to evaluate the possible antioxidant effects of propolis on diabetes-induced oxidative stress, inflammation and apoptosis in rats. Diabetes was induced in male rats by intraperitoneal injection of a single dose (45mg/kg body weight) of streptozotocin (STZ). After induction of diabetes, rats were orally administered propolis in two separate doses, 50 or 100 mg/kg body weight daily for 6 weeks. Obtained results showed that administration of propolis remarkably decreased the oxidative stress resulting from diabetes induction, as reflected by lowered hepatic and pancreatic malondialdehyde (MDA) and protein carbonyl (PC) levels, and enhanced the antioxidant defense system capability by increasing hepatic and pancreatic contents of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and total antioxidants (TAC), compared to the diabetic group. Also, treatment of diabetic rats with propolis significantly ameliorated the inflammatory disorders as indicated by markedly decreased serum inflammatory markers such as C-reactive protein (CRP), interleukin 10 (IL-10) and transformation growth factor β (TGF-β), compared to the untreated diabetic rats. In addition, propolis was found to induce apoptosis and cell cycle arrest in diabetic rats, which was confirmed by increased pancreatic p53, caspase-3 and G0/G1; and decreased Bcl-2, compared to the diabetic group. Current findings provided additional evidence that propolis has antioxidant, anti-inflammatory and apoptotic properties which may be of health benefits in ameliorating various diabetes side effects.

INTRODUCTION

Diabetes mellitus (DM) disease acute or chronic is the most common endocrine disorder and it remains danger to human health. It is basically characterized by homeostatic disorder of glucose metabolism which includes hyperglycemia and defects in insulin secretion and/or action **(Gupta and Mukherjee, 2014).** DM is the fourth cause that leads to death in most low-income countries, due to increased risk of health complications which associated with the reduction in quality of life.

Type I DM is induced by pancreatic β-cells destruction. The disease is mediated by an autoimmune mechanism in which several inflammatory cytokines and oxidative stress are produced, that play a major role in causing β-cell destruction. Experimentally, STZ has been found to produce a selective toxic effect on β-cells and induces type I DM in most laboratory animal species **(Oche *et al.*, 2014).** Cytotoxic effect of STZ is mostly due to

DNA alkylation and nitric oxide generation which lead to destruction of pancreatic β-cells resulting in insulin deficiency and eventually hyperglycemia **(Harb *et al*., 2007).** Overproduction of free radicals inducing oxidative stress and lipid peroxidation which associated with decreased antioxidant defense system capabilities might also be implicated in the pathogenesis of pancreatic injury induced by injection of high doses of STZ **(Szkudelski, 2001 and El-Sayed *et al*., 2009).**

Exposure to the cytotoxic STZ was found to increase proinflammatory cytokines level including IL-6, IL-1β, IL-8, IL-12, IL-2, IL-3, IL-10, tumor necrosis factor (TNF-α) and interferon (IFN-γ) in serum of diabetic animals **(Yu *et al*., 2015 and Rashid and Sil, 2015).** It has been reported that hyperglycemia can cause apoptosis. In rat liver tissue, diabetes induced by STZ led to production of a significant increase in hydroxyl radical associated with increased levels of lipid peroxidation. This was accompanied with overproduction of apoptotic markers which in turn led to cellular apoptosis **(Francés *et al*., 2010 and Ghosh *et al*., 2015).**

Recently, much attention has been focused on the alternative use of natural products in medicinal purposes. It has been reported that some natural products contain a bioactive components that has the potential to prevent or overcome diabetes **(Kashkooli *et al*., 2011).** Propolis (bee glue) is one of these natural products, which has long history of medicinal use. The propolis composition varies according to its botanical origin; generally it contains more than 250 individual compounds. Phenolic acids, esters, and flavonoids have been shown to account for most important of propolis composition **(Najafi *et al*., 2007).** Because of its potential therapeutic use, propolis is greatly utilized by several cultures as a folk medicine **(Funakoshi-Tago *et al*., 2015).**

Several studies reported that propolis can decrease levels of blood glucose, modulate blood lipid and thus reduces atherosclerosis in patients with DM **(Fuliang *et al*., 2005 and El-Sayed *et al*., 2009).** Propolis has been shown to be an effective antioxidant since it mopped up free radicals caused by various toxic agents like radiation. Moreover, it can protect many body's healing substances such as GSH, SOD and CAT **(Frozza *et al*., 2014 and Ghosh *et al*., 2015).** It is also effective in inflammation-related diseases such as rheumatoid arthritis and diabetes as it inhibits the increase of inflammatory medium and decreases the inducing effects of cytokines **(Hu *et al*., 2005 and Oršolić *et al*., 2013).** Furthermore, propolis effectively induces inhibition of growth in several human cancer cell types **(Ahn *et al*., 2013).** In view of available literatures, present study was designed to investigate the probable antioxidative effect of propolis against production of oxidative stress, inflammation and apoptosis in STZ-diabetic rats.

MATERIALS AND METHODS

Chemicals:

STZ was purchased from Sigma Chemical Co. (St. Louis, MO 6, USA). Bio propolis capsules were purchased from Egyptian Sigma Pharmaceutical Industries Company, Egypt.

Animals

Adult male albino rats (*Rattus rattus*), weighing 140-160 g, were kept under a photoperiod of 12 h light: 12 h darkness. They were housed in stainless cages in a good-ventilated animal room and fed on adequate stable diet. Water was allowed *ad libitum*. After a week of acclimatization, used rats were divided into six groups each of six animals. Care and use of the animals were conducted under supervision of the Animal Ethics Committee of Mansoura University, Egypt.

Induction of diabetes

Overnight fasting rats were injected intraperitoneally with a single dose of freshly prepared STZ solution (45 mg/kg body weight) dissolved in citrate buffer, pH 4.6. Two days after induction, diabetes was confirmed by examining blood glucose level using Glukotest of diagnosis glucose level by ACCU–CHEKGo apparatus obtained from Roche Company, Germany **(Rajasekaran *et al*., 2005).** Rats with fasting blood glucose level over 200 mg/dl were considered as diabetic rats **(Peres *et al*., 2013 and Koroglu *et al*., 2015).**

Preparation of propolis

Known weight of propolis powder was mixed well with known amount of distilled water. A suspension was formed and the selective doses were calculated and given orally to rats through the stomach tube. The preparation was thoroughly mixed before each use.

Experimental design

Used rats were divided into six groups of 6 rats each: the first group served as control and received citrate buffer solution (pH 4.6); the second group received propolis in a dose of 50 mg/kg body weight; the third group received propolis in a dose of 100 mg/kg body weight; the fourth group injected intraperitoneally with a single dose of STZ solution, 45 mg/kg body weight, dissolved in citrate buffer, pH 4.6 (diabetic group); the fifth group was diabetic and received propolis in a dose of 50 mg/kg body weight; the sixth group was diabetic and received propolis in a dose of 100 mg/kg body weight. All treatments were continued for 6 weeks.

Samples collection

At the end of the experimental period, overnight fasted rats were sacrificed by cervical decapitation. Blood samples were collected in clean centrifuge tubes, and let stand for 15 min, after which they were centrifuged at 3000 rpm for 15 min. Blood sera were carefully separated, labeled and kept at - 20°C for selective biochemical analysis. On the other hand, the animals were quickly dissected, liver and pancreas specimens were removed, weighed, homogenized in distilled water, labeled and kept at - 20°C until further assays.

Biochemical determinations

Hepatic and pancreatic levels of MDA, GSH and TAC as well as the activities of SOD and CAT were estimated by the methods of **Ohkawa *et al*. (1982), Prins and Loose (1969), Koracevic *et al*. (2001), Nishikimi *et al*. (1972)** and **Bock *et al*. (1980)** respectively using kits from Bio diagnostic Company (Egypt) according to the instructions of the supplier. PC content was measured by the method of **Smith *et al*. (1991).** Serum level of CRP was determined using CRP-SPINREACT diagnostics kit (Spain) according to the instructions of the supplier **(Vaishnavi, 1996).**

Flow cytometric analysis of serum IL-10 and TGF-β and pancreatic p53, caspase-3, BcL-2 and G0/G1were determined according to the method of **Tribukait *et al*. (1975),** using FACS caliber flow cytometer (Becton Dickinson, Sunnyvale, CA, USA), equipped with a compact air cooked low power 15 m watt argon iron laser beam (488nm). Average evaluated nuclei per specimen are 20.000 (120 nuclei/second). Dean and Jett computer program for mathematical analysis was used to obtain the DNA histograms **(Dean and Jett, 1974).**

Statistical analysis

Obtained data were statistically evaluated with SPSS 17.5 software. Differences among groups were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests. *P* values equal or less than 0.05 was considered the minimal level of significance. All values were expressed as the mean ± SE for six animals per group. Percent of changes in the treated groups were calculated.

RESULTS

Table 1 shows hepatic and pancreatic MDA and PC concentrations. The results showed that daily administration of low or high doses of propolis for 6 weeks produced no significant changes in hepatic and pancreatic MDA and PC concentrations when compared to the control group. In STZ-diabetic group, significant increases in mentioned parameters were obtained, comparing with control. Diabetic rats treated with propolis showed significant decreases in hepatic and pancreatic contents of MDA and PC, when compared to the diabetic group. The results of these groups, however, appeared significantly high in comparison with control. There were no remarkable changes between the results of low and high doses of propolis in STZ-diabetic rats.

The data of hepatic and pancreatic GSH, SOD, CAT and TAC were summarized in table 2. Daily administration of propolis in low and high doses for 6 weeks induced no marked alterations in all mentioned antioxidant parameters, when compared to control group. However, diabetic groups displayed significantly decreased these variables, comparing with control. Administration of both low and high doses of propolis partially ameliorated this adverse effect of STZ-induced diabetes since obtained data showed significant increases in all tested antioxidant parameters when compared to the diabetic group. In comparison with control group, levels of hepatic GSH and SOD in diabetic rats treated with low and high doses of propolis and pancreatic SOD and CAT activities in

diabetic rats treated with high dose of propolis appeared unchanged, while levels of remaining data were still significantly decreased. Obtained results displayed non-significant changes between the beneficial effects of low and high doses of propolis in STZ-diabetic rats.

Serum levels of the inflammatory markers CRP, IL-10 and TGF-β were shown in table 3 and figure 1 (a and b). Obtained results exhibited that daily administration of low or high dose of propolis for 6 weeks resulted in non significant changes in these parameters, when compared to the control group. Remarkable increases in tested inflammatory markers were seen in diabetic group, compared to the control group. The results also revealed significant decreases in serum levels of these inflammatory markers in STZ-diabetic rats treated with low and high doses of propolis, comparing with diabetic group. However, serum levels of CRP, IL-10 and TGF-β in diabetic groups treated with low and high doses of propolis were still markedly high, when compared to control group. No detectable changes were recorded between low and high doses of propolis in STZ-diabetic rats.

Table 4 and figure 2 (a,b,c and d) illustrates pancreatic p53, caspase-3, Bcl-2 and G0/G1. Following daily administration of low and high doses of propolis for 6 weeks, obtained results showed no significant changes in all tested apoptotic markers, when compared to control group. In STZ-diabetic group, obtained results showed significant decreases in pancreatic p53, caspase-3 and G0/G1, along with markedly increased Bcl-2, when compared to control group. Following treatment with propolis in STZ-diabetic rats, significant rise in pancreatic p53, caspase-3 and G0/G1, accompanied with marked decline in Bcl-2 were detected, comparing with untreated diabetic rats. In comparison with control group, significantly elevated pancreatic p53, caspase-3 and G0/G1 were observed after treatment with high dose of propolis, and after low dose treatment in case of G0/G1. In contrary, marked decrease in Bcl-2 was observed following administration low or high doses of propolis in STZ-diabetic groups, compared to control results. Diabetic rats treated with high dose of propolis showed no significant variations in the mentioned parameters, except in case of G0/G1 where obtained results were significantly increased, compared to diabetic rats treated with low dose of propolis.

Table 1. Hepatic and pancreatic MDA and PC contents in different animal groups.

Parameters	Control	Propolis (P)		Diabetic (D)	D + P	
		Low	High		Low	High
Hepatic MDA (nmol/g)	69.47 ±1.25	72.70 ±2.01	71.03 ±2.54	182.55 ±11.03 [a]	102.61 ±5.80 [ab]	96.52 ±4.62 [ab]
		+ 4.64*	+ 2.24*	+ 162.77*	+ 47.70*	+ 38.93*
					- 43.79**	- 47.12**
pancreatic MDA (nmol/g)	10.39 ±0.86	10.25 ±0.97	11.33 ±0.67	31.54 ±1.47 [a]	15.21 ±0.83 [ab]	16.74 ±1.07 [ab]
		- 1.34*	+ 9.04*	+ 203.56*	+ 46.39*	+ 61.11*
					- 51.77**	- 46.92**
Hepatic PC (µmol/mg)	49.61 ±1.36	49.30 ±0.82	49.38 ±0.76	87.83 ±1.76 [a]	60.58 ±0.83 [ab]	59.36 ±0.50 [ab]
		- 0.62*	- 0.46*	+ 77.04*	+ 22.11*	+ 19.65*
					- 31.02**	- 32.41**
pancreatic PC (µmol/mg)	5.05 ±0.10	5.11 ±0.15	5.01 ±0.11	9.25 ±0.21[a]	6.97 ±0.18 [ab]	6.54 ±0.15 [ab]
		+ 1.18*	- 0.79*	+ 83.16*	+ 38.01*	+ 29.50*
					- 24.64**	- 29.29**

Values expressed as mean ± SE (n = 6); **[a, b]** Significant difference ($P \leq 0.05$) comparing to control and diabetic groups respectively;* ، ** are % of changes comparing to control and diabetic groups respectively.

Table 2. Hepatic and pancreatic levels of GSH, TAC, SOD and CAT in different animal groups.

Parameters	Control	Propolis (P)		Diabetic (D)	D + P	
		Low	High		Low	High
Hepatic GSH (mg/gm)	19.83 ±1.64	19.99 ±0.76	21.32 ±0.76	6.43 ±0.80 [a]	17.99 ±0.82 [b]	20.95 ±0.57 [b]
		+ 0.80*	+ 7.51*	- 67.57*	- 9.27*	+ 5.64*
					+ 179.78**	+ 225.81**
pancreatic GSH (mg/g)	14.22 ±0.88	13.55 ±0.63	14.88 ±0.63	6.16 ±0.64 [a]	10.66 ±0.54 [ab]	10.93 ±0.55 [ab]
		- 4.71*	+ 4.64*	- 56.68*	- 25.03*	- 23.13*
					+ 73.05**	+ 77.43**
Hepatic SOD (U/gm)	86.76 ±3.60	78.81 ±3.85	86.56 ±1.71	37.57 ±2.92 [a]	79.22 ±2.08 [b]	78.99 ±2.02 [b]
		- 9.16*	- 0.23*	- 56.69*	- 8.69*	- 8.95*
					+ 110.85**	+ 110.24**
pancreatic SOD (U/gm)	42.99 ±0.87	39.05 ±0.82	40.68 ±0.47	20.30 ±1.00 [a]	37.62 ±0.97 [ab]	41.43 ±1.54 [b]
		- 9.16*	- 5.37*	- 52.77*	- 12.49*	- 3.62*
					+ 173.99**	+ 104.08**
Hepatic CAT (U/gm)	2.12 ±0.09	2.02 ±0.11	2.03 ±0.05	0.64 ±0.05 [a]	1.56 ±0.04 [ab]	1.60 ±0.06 [ab]
		- 4.71*	- 4.24*	- 69.81*	- 26.41*	- 24.52*
					+ 143.75**	+ 150.00**
pancreatic CAT (U/gm)	0.79 ±0.04	0.76 ±0.04	0.85 ±0.04	0.39 ±0.04 [a]	0.67 ±0.03 [ab]	0.75 ±0.02 [b]
		- 3.79*	+ 7.59*	- 50.63*	- 15.18*	- 5.06*
					+ 71.79**	+ 92.30**
Hepatic TAC (mg/gm)	2.32 ±0.01	2.30 ±0.01	2.32 ±0.01	0.96 ±0.06 [a]	2.00 ±0.03 [ab]	2.02 ±0.05 [ab]
		- 13.79*	0.00*	- 58.62*	- 13.79*	- 12.93*
					+ 108.33**	+ 110.41**
pancreatic TAC (mg/g)	1.72 ±0.05	1.60 ±0.09	1.49 ±0.05	0.90 ±0.05 [a]	1.40 ±0.02 [ab]	1.46 ±0.04 [ab]
		- 6.97*	- 13.37*	- 47.67*	- 18.60*	- 15.11*
					+ 55.55**	+ 62.22**

Values expressed as mean ± SE (n = 6); **[a, b]** Significant difference ($P \leq 0.05$) comparing to control and diabetic groups respectively;* ' ** are % of changes comparing to control and diabetic groups respectively.

Table 3. Serum levels of CRP, IL-10 and TGF-β in different animal groups.

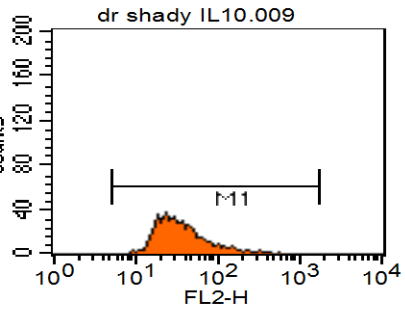
Parameters	Control	Propolis (P)		Diabetic (D)	D + P	
		Low	High		Low	High
CRP (g/dl)	7.30 ±0.22	7.13 ±0.30	7.82 ±0.31	12.84 ±0.31 [a]	9.66 ±0.17 [ab]	9.76 ±0.18 [ab]
		- 2.32*	+ 7.12*	+ 75.89*	+ 32.32*	+ 33.69*
					- 24.76**	- 23.98**
IL-10 %	38.22 ±1.73	40.75 ±2.25	41.11 ±1.34	72.17 ±1.78 [a]	51.24 ±3.65 [ab]	54.56 ±3.96 [ab]
		+ 6.61*	+ 7.56*	+ 88.82*	+ 34.06*	+ 42.75*
					- 29.00**	- 24.40**
TGF-β %	34.68 ±1.48	36.64 ±1.12	35.74 ±1.33	68.72 ±4.02 [a]	53.06 ±3.02 [ab]	52.57 ±3.40 [ab]
		+ 5.65*	+ 3.05*	+ 98.15*	+ 52.99*	+ 51.58*
					- 22.78**	- 23.50**

Values expressed as mean ± SE (n = 6); [a, b] Significant difference ($P \leq 0.05$) comparing to control and diabetic groups respectively;* ' ** are % of changes comparing to control and diabetic groups respectively.

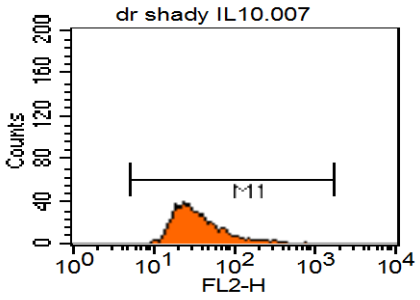
Table 4. Pancreatic p53%, caspase-3%, Bcl-2% and G0/G1% in different animal groups.

Parameters	Control	Propolis (P)		Diabetic (D)	D + P	
		Low	High		Low	High
P53%	21.33 ±0.43	21.72 ±0.43	20.83 ±0.15	14.53 ±0.22 [a]	23.69 ±1.97 [b]	26.08 ±0.22 [ab]
		+ 1.82*	- 2.34*	- 31.87*	+ 11.06*	+ 22.26*
					+ 63.04**	+ 79.49**
Caspase-3%	14.08 ±0.75	12.97 ±0.50	13.26 ±0.23	10.98 ±0.16 [a]	15.58 ±0.43 [b]	16.36 ±0.42 [ab]
		- 7.88*	- 5.82*	- 22.01*	+ 10.65*	+ 16.19*
					+ 41.89**	+ 49.00**
BCL2%	17.45 ±0.12	16.08 ±0.22	16.40 ±0.17	21.78 ±0.42 [a]	11.57 ±0.19 [ab]	11.92 ±0.30 [ab]
		- 7.85*	- 6.01*	+ 24.81*	- 33.69*	- 31.69*
					- 46.87**	- 45.27**
G0/G1%	70.62 ±1.35	71.69 ±0.46	73.94 ±0.92	65.22 ±0.45 [a]	83.58 ±0.79 [ab]	92.99 ±0.77 [abc]
		+ 1.51*	+ 4.70*	- 7.64*	+ 18.35*	+ 31.67*
					+ 28.15	+ 42.57

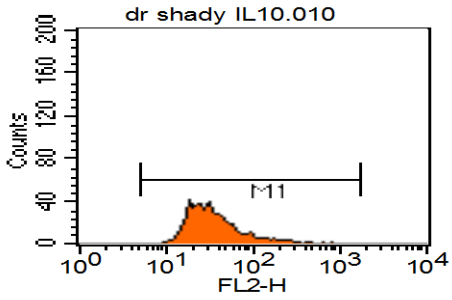
Values expressed as mean ± SE (n = 6); [a, b, c] Significant difference ($P \leq 0.05$) comparing to control, diabetic and D+P low dose groups respectively; * ' ** are % of changes comparing to control and diabetic groups respectively.



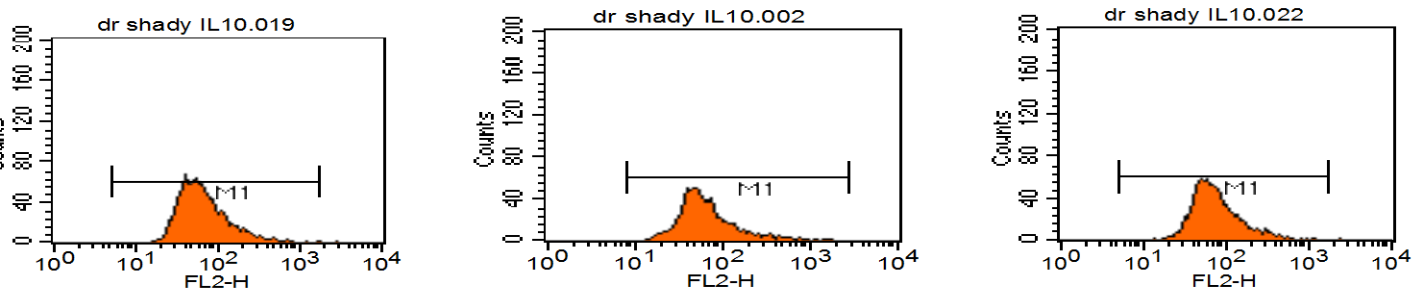
Control group



P (low dose) group

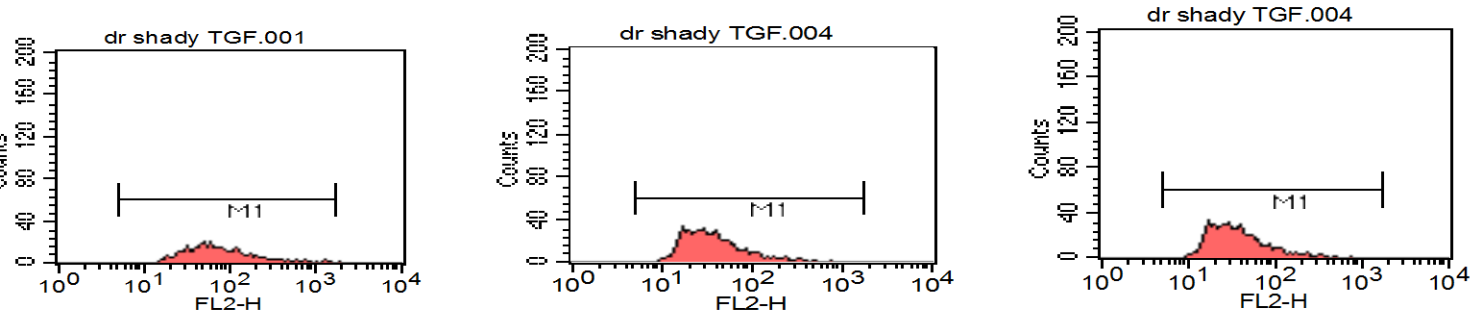


P (high dose) group

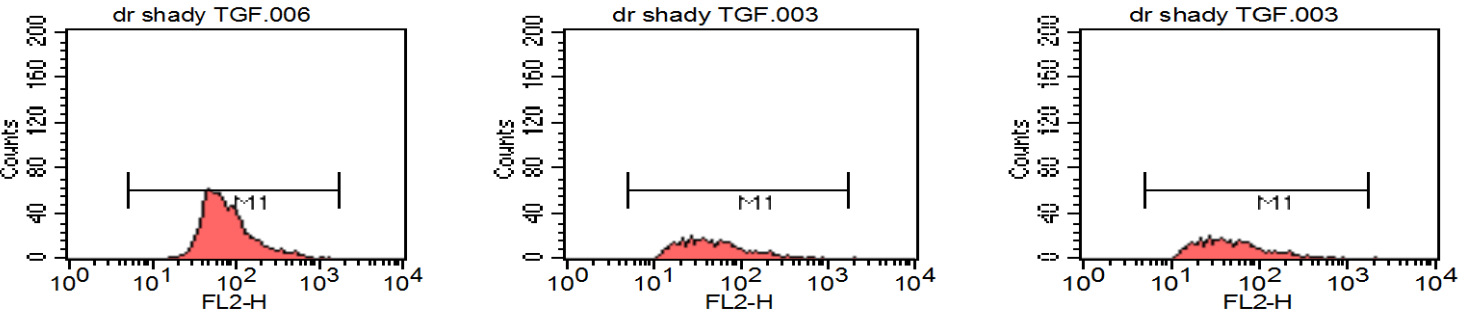


Diabetic group D + P (low dose) group D + P (high dose) group

Figure (1a): Serum IL-10 levels in different animal groups.

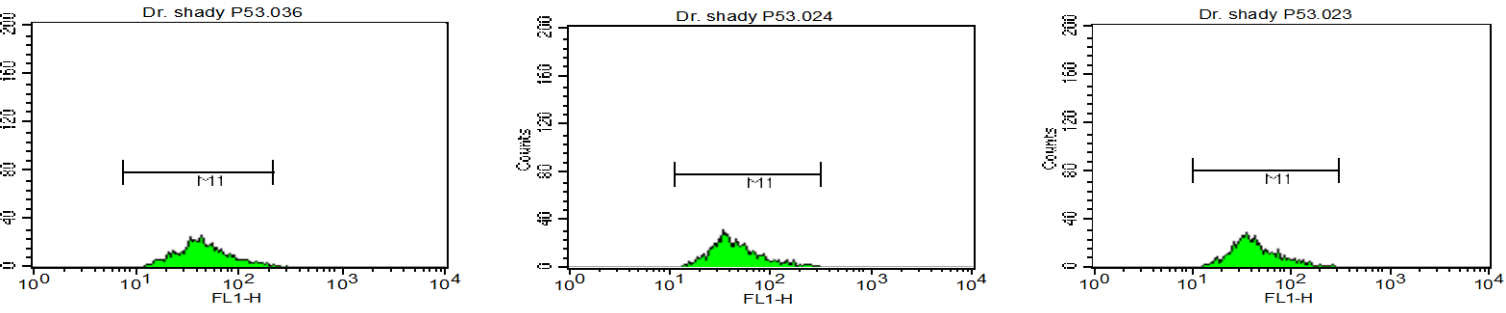


Control group P (low dose) group P (high dose) group

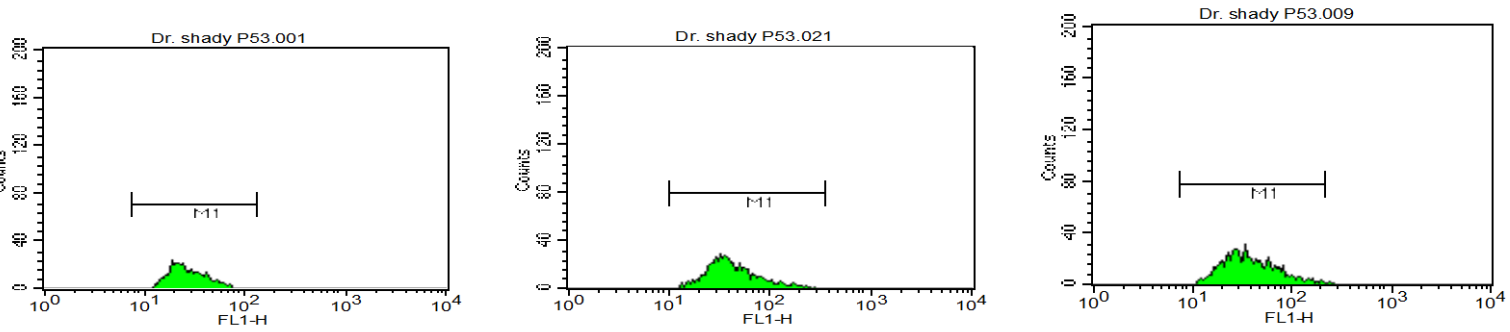


Diabetic group D + P (low dose) group D + P (high dose) group

Figure (1b): Serum TGF-β levels in different animal groups.

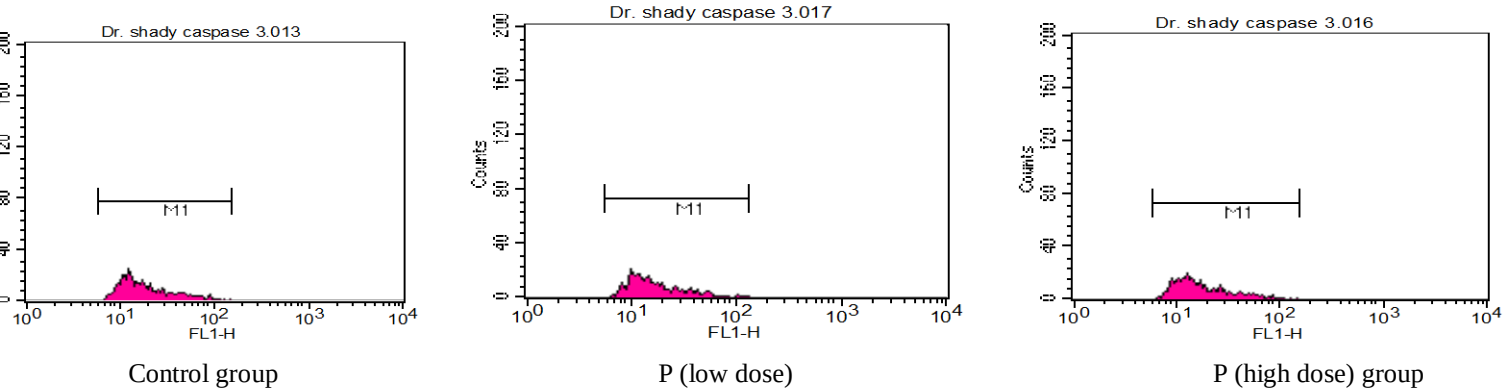


Control group P (low dose) group P (high dose) group

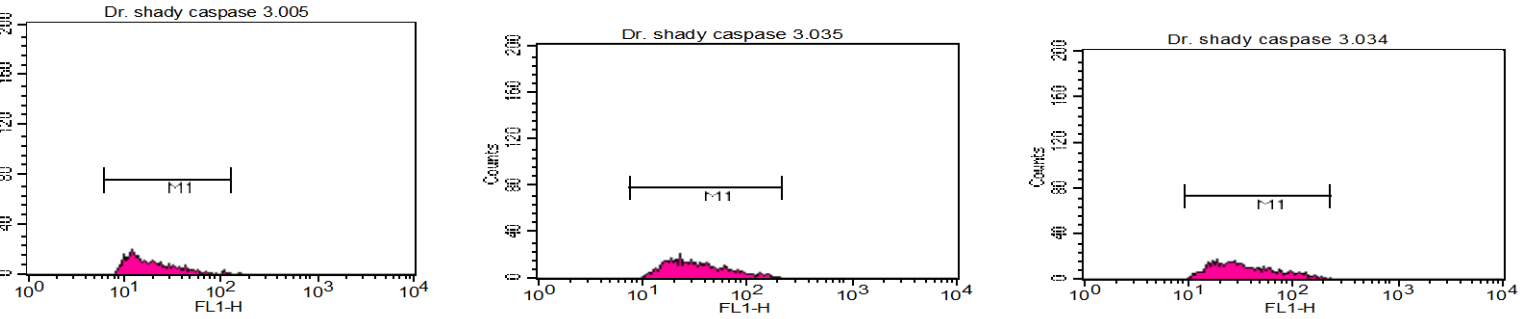


Diabetic group D + P (low dose) group D + P (high dose) group

Figure (2a): Pancreatic p53% in different animal groups.

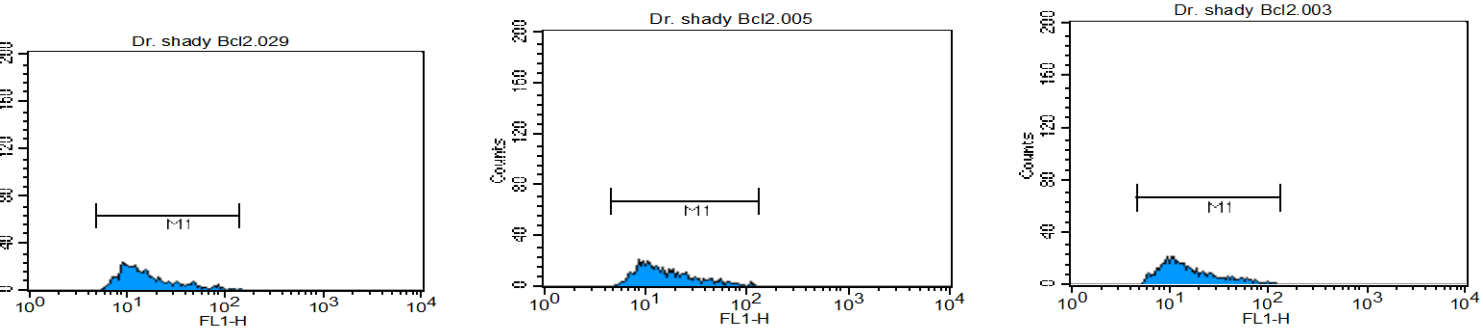


Control group P (low dose) P (high dose) group

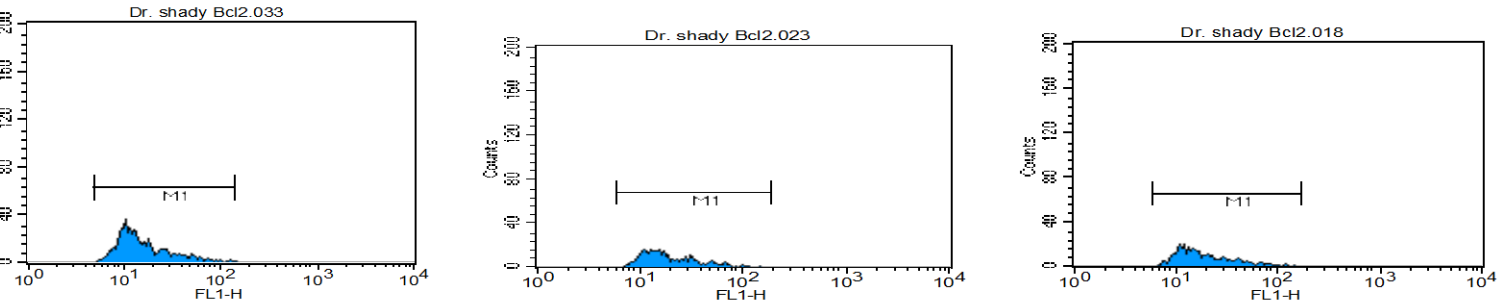


Diabetic group D + P (low dose) group D + P (high dose) group

Figure (2b): Pancreatic caspase-3% in different animal groups.

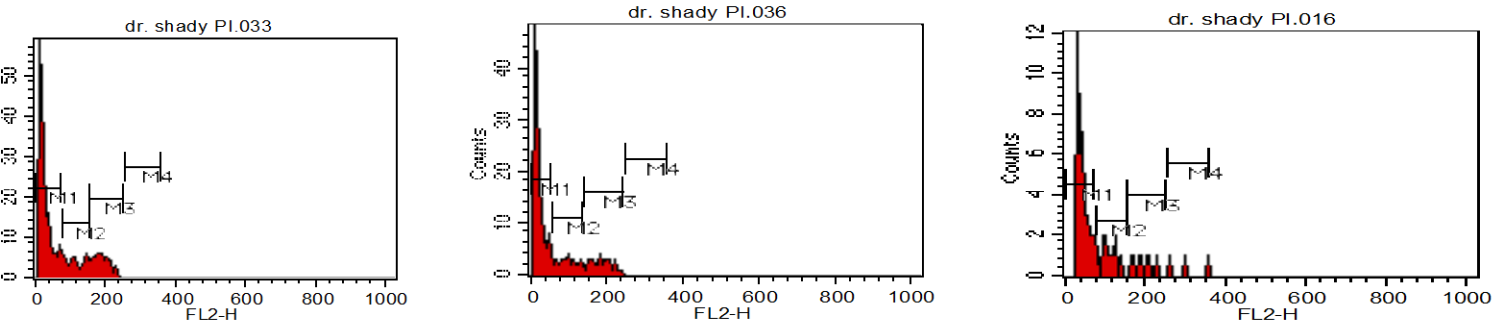


Control group P (low dose) group P (high dose) group

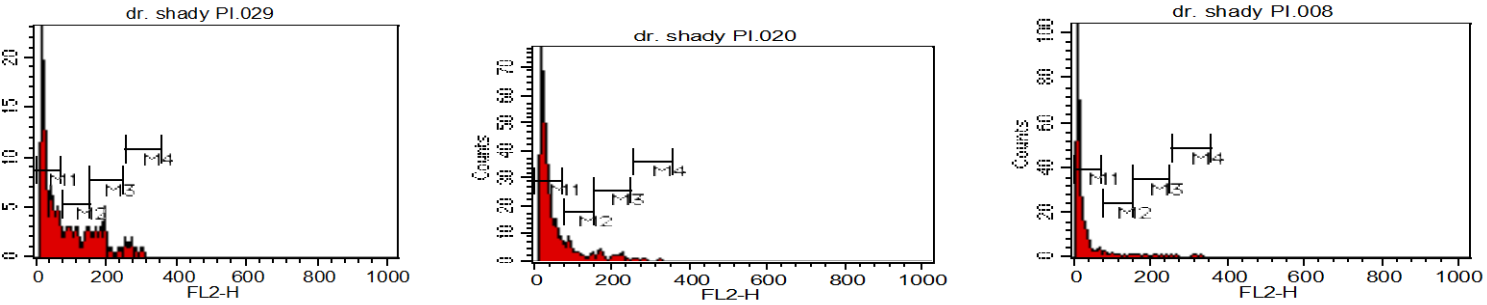


Diabetic group D + P (low dose) group D + P (high dose) group

Figure (2c): Pancreatic Bcl-2% in different animal groups.



Control group P (low dose) group P (high dose) group



Diabetic group D + P (low dose) group D + P (high dose) group

Figure (2d): Pancreatic G0/G1% in different animal groups.

DISCUSSION

Increased oxidative stress and insufficient antioxidant defense mechanism are widely accepted factors in the development and progression of DM and its complications **(Kedziora-Kornatowska *et al*., 2009).** In the present study, STZ-induced diabetes in rats clearly resulted in an increased oxidative stress in both liver and pancreas. Obtained results displayed production of lipid peroxidation and protein oxidation, as indicated by enhanced concentration of hepatic and pancreatic contents of MDA and PC respectively. Concomitantly, decreased hepatic and pancreatic contents of antioxidant agents including TAC, GSH as well as the activities of SOD, and CAT were observed. These results are in accordance with several published data. In recent studies, **Arcaro *et al*. (2014)** and **Zhou *et al*. (2015)** demonstrated significant increases in serum and liver MDA level in STZ-diabetic rats. Other studies displayed increased pancreatic PC and advanced oxidation protein products in STZ-induced diabetic rats **(Ardestani, 2008 and Kurt *et al*., 2011).** Decreased antioxidant parameters in various tissues in STZ-diabetic rats have also been reported **(Ardestani *et al*., 2008 and Kurt *et al*., 2011).** In liver tissue, STZ-diabetic rats showed

decreased activities of antioxidant parameters SOD, CAT, glutathione peroxidase (GSH-Px) and GSH levels **(Arcaro *et al*., 2014)**.

Several mechanisms have been introduced to explain the potent destructive action of STZ on pancreatic β-cells. Present findings in combination with previous studies confirmed that production of free radicals, lipid peroxidation and depletion of antioxidant parameters are implicated in the pathogenesis of STZ-induced destruction of pancreatic β-cells **(Szkudelski, 2001).** Moreover, elevated blood sugar in diabetes can trigger direct protein glycation and glucose auto-oxidation which results in molecular and functional changes in proteins and production of free radicals that contribute to development of oxidative stress in STZ-diabetic rats **(Ghosh *et al*., 2015 and Koroglu *et al*., 2015).** Furthermore, increased level of serum glucose, as a reducing agent, can enolize and thereby reduce molecular oxygen (O_2) under physiological conditions, yielding aketoaldehydes, hydrogen peroxide (H_2O_2) and free radical intermediates **(Thornalley and Stern., 1984).** Adding to this, increased intracellular glucose levels, when glucose uptake occurs in an insulin-independent manner; results in an overproduction of reactive oxygen species-induced oxidative stress mostly via mitochondrial electron transport chain **(Brownlee, 2005).**

Although there are different types of anti-hypoglycemic agents are available for the treatment of diabetes mellitus, there is increasing demand by patients to use antidiabetic from natural products to avoid the undesirable side effects of the existing drugs **(Zhou *et al*., 2012).** In this regard, propolis was taken into account as a natural product for its emerging antioxidant **(Talas *et al*., 2014)**, anti-inflammatory **(Wang *et al*., 2013)** and apoptotic efficacy **(Frozza *et al*., 2014).** In agreement, present study provided additional evidence that propolis can exert antioxidant effect against oxidative stress in STZ-diabetic rats. The results showed decreases in hepatic and pancreatic contents of MDA and PC together with significantly increased levels of SOD, CAT, GSH and TAC in STZ-diabetic rats treated with used two doses of propolis, compared to the untreated diabetic group. Interestingly, some of these changes (hepatic GSH and SOD in diabetic groups treated with low and high doses of propolis, and pancreatic SOD and CAT in diabetic group treated with high dose of propolis) appeared not significant when they compared to the control results. In this line, published data showed that propolis administration to STZ-diabetic rats resulted in a significant decrease in MDA and PC levels, probably by increasing the level of hepatic antioxidants including GSH, SOD and CAT **(Talas *et al*., 2014)**, which participate in free radicals scavenging and lowering lipid peroxidation **(El-Mazoudy *et al*., 2011).** On the other hand, daily treatment of animals with high dose of ethanolic extract of propolis for long period ameliorated STZ-induced dramatic alterations in serum glucose, lipids, lipoproteins, nitric oxide, GSH and CAT and pancreatic MDA and SOD **(El-Sayed *et al*., 2009).** In non-diabetic rats, propolis treatment afforded a hepatoprotective effect against lipid peroxidation by improving activity of CAT and level of nitric oxide in case of hepatotoxicity **(Selamoglu *et al*., 2015).** Also, prior administration with propolis extract significantly eliminated the effect of doxorubicin on hepatic lipid peroxidation, while propolis co-treatment led to improve GSH status in treated rats **(Singla *et al*., 2014).**

Inflammation is a vital physiological process generated in response to tissue injury and infection, and it is necessary for the body when facing invading microbes or the diseases. Number of proinflammatory mediators and cytokines are characterized along with their important roles in inflammatory responses, such as nitric oxide, IL-1β and IL-6 **(Rankin, 2004).** Current study showed significant elevations in the serum levels of inflammatory markers CRP, IL-10 and TGF-β in STZ-diabetic rat, compared to the control group. These results are consistent with findings of **Yu *et al*. (2015)** who reported increase in serum levels of TNFα, interferon-γ, IL-6, IL-1β, IL-8, IL-12, IL-2, IL-3 and IL-10 in STZ-diabetic rats. Also, the inflammatory markers TNF-α, IL-6 and IL1-β were up-regulated in the retina of STZ- diabetic rats **(Mima *et al*., 2012).** In addition, rats receiving STZ showed marked increase in the TNF-α, IL-1β and TGF-β contents in myocardial tissue **(Rizk, *et al*., 2014).** Studies on cardiovascular complications associated with STZ-diabetic rats showed significantly increased CRP levels **(Goyal *et al*., 2008 and 2009).**

Agents which able to suppress or inhibit the activation of the inflammation-linked factors may have therapeutic potential. Propolis, a resinous substance collected by honey bees, can exert anti-inflammatory effects **(Wang *et al*., 2013).** In the present study, administration of propolis to STZ-diabetic rats produced a marked decline in serum levels of CRP, IL-10 and TGF-β, compared to the untreated diabetic group. This result added support that propolis has anti-inflammatory activity and increased its potential therapeutic efficacy in various inflammatory diseases such as diabetes. Published data exhibited that propolis has been reported to prevent the destruction of β-cells in rats by inhibiting the activation of IL-1β and nitric oxide synthase activity **(Matsushige *et al*., 1996).** In addition, propolis derivatives (caffeic acid phenethyl ester) decreased the systemic inflammatory cytokines and increased the anti-inflammatory cytokines levels and protect hepatic and neuronal cells in acute septic shock

(Korish and **Arafa, 2011).** Also, **Bachiega *et al*. (2012)** found *in vitro* that propolis and its isolated compounds significantly inhibited the cytokines IL-6 and IL-10 production by murine macrophages. In another *in vitro* study, Chinese propolis was found to exert anti-inflammatory effects since it significantly inhibited the production of nitric oxide, IL-1β and IL-6 **(Wang *et al*., 2013).** Moreover, the numbers of IL-17-producing cells in mice fed propolis were significantly decreased **(Tanaka *et al*., 2012).**

Present study displayed down-regulation of cell cycle and apoptotic markers expression in pancreatic cells of STZ-diabetic rats. Obtained results showed marked decreases in pancreatic p53 (tumor suppressor protein), caspase-3 (a protein which activates deoxyribonuclease, the enzyme required for nuclear apoptosis) and G0/G1 in STZ-diabetic rats along with significantly increased Bcl-2 (anti-apoptotic marker), when compared to the control group. In contrast to the current findings on pancreatic tissue, previous studies showed that STZ can induce apoptosis and cell cycle arrest in most tissues such as liver **(Kapoor and Kakkar, 2014; and Ghosh *et al*., 2015)**, heart **(Latha *et al*., 2012 and Amin *et al*., 2015)** and testes **(Zhao *et al*., 2010 and Jiang *et al*., 2013)** as well as lymphocytes **(Arya *et al*., 2011).** In the liver, production of reactive species in STZ-induced diabetic rats resulted in loss of integrity of mitochondrial inner membrane and formation of pore through which several apoptogenic proteins like cytochrome c and apoptosis inducing factor (AIF) released into to the nucleus. As a consequence, the expression of caspase-3 and caspase-9 up regulated while BcL-2 expression down regulated in the liver of diabetic rats **(Kapoor and Kakkar, 2014).** In the heart of rats treated with STZ for 21 days, **Amin *et al*. (2015)** found increases in number of apoptotic cells and apoptotic markers such as p53, CD95 and caspases (3, 8 and 9) along with downregulation of Bcl-2.

However, studies on the mode of cell death caused by STZ in a murine pancreatic β- cell line demonstrated that higher doses of STZ caused the cells to undergo necrosis (22%) as well as apoptosis (17%). The results of such study suggested that STZ causes beta cell death at low doses by activation of apoptotic pathway, whereas, at high doses, it causes beta cell death, predominantly, by necrosis **(Saini *et al*., 1996).** In another study, **Fehsel *et al*., (2003)** did not find apoptosis-specific features (DNA stand breaks) during islet cell death using diabetogenic STZ. Instead they observed massive necrosis as evidenced by disrupted plasma membranes and spilled-out cellular constituents *in vitro* as well as during disease manifestation in rats. Furthermore, poly ADP-ribose polymerase 1 (PARP-1), an enzyme used for DNA repair, was not up-regulated during CD95-mediated apoptosis, resulting in restoration of high ATP levels in the diabetic liver. In contrast, CD95-dependent apoptosis was not observed in the diabetic pancreas due to up-regulation of PARP-1 and ATP depletion, resulting in necrosis **(Aseer *et al*., 2015).** In view of these published data, present finding of decreased pancreatic apoptotic markers in STZ-diabetic rats is suggested to be due to STZ-induced necrosis rather than apoptosis.

Generally, studies on the mechanisms of action of STZ-induced diabetes suggested that STZ may exhibit different strategies in causing pancreatic cell death and this may explain the ability of STZ to induce destruction of pancreatic β-cells by necrosis rather than apoptosis. It has been reported that injection of high dose of cytotoxic STZ can cause: (1) severe DNA alkylation leading to DNA damage **(Sajad *et al*., 2008),** (2) stimulation of PARP-1 leading to extremely fall in cellular NAD+ (the substrate of mentioned enzyme) and thus to a progressive depletion in ATP concentrations (due to inhibition of glucose oxidation), loss of membrane integrity, and eventually necrotic β-cell death **(Okamoto, 1985 and Heller *et al*. 1994),** (3) generation of free radicals via stimulation of xanthine oxidase that can also induce DNA damage **(Nukatsuka *et al*. 1990)** and (4) liberation of toxic amounts of nitric oxide that can induce further damage to DNA **(Kröncke *et al*., 1995*).*** As the result of all of these cytotoxic effects of high dose of the diabetogenic STZ, pancreatic β-cells undergo acute cellular injury that causes the death of cells by unregulated digestion of cell components (necrosis) rather than by programmed cell death (apoptosis). However, this subject needs further clarification.

On the other hand, treatments of STZ-diabetic rats with propolis extract, in the present study, modulated some apoptotic factors providing evidence that propolis can induce apoptosis. Obtained results showed up-regulation of p53 and caspase-3, and down-regulation of Bcl-2 following administration of propolis in STZ-diabetic rats, compared to the untreated STZ-diabetic rats, and to control group when used high dose of propolis. Treatment with propolis, dose-dependently, also caused G0/G1 cell cycle arrest in pancreas of diabetic groups treated with propolis, which further confirms the apoptotic activity of propolis. Available literatures demonstrated that artepillin C, an active component in Brazilian propolis, inhibited cell growth by inducing G0/G1 arrest in human colon cancer cells in a dose dependent manner **(Shimizu *et al*., 2005).** Also, caffeic acid phenyl ester**,** another active component of propolis, induced S- and G2/M-phase cell-cycle arrests and initiated apoptosis in human cervical cancer lines **(Hsu *et al*., 2013).** In addition, propolis has been shown to induce apoptosis and cell cycle arrest in the G2/M phase,

in leukemic U937 cells in a dose-dependent manner, and increased apoptosis level was associated with down-regulation of Bcl-2 and activation of caspase-3 **(Motomura *et al*., 2008).** Based on these published data and current findings, propolis-induced apoptosis in STZ-diabetic rats could be suggested as a further protective mechanism against STZ and diabetes cytotoxicity, by avoiding the cell to go wrong, i.e. to be a cancer cell, or to exposing to the process of unprogrammed death (necrosis).

In conclusion, present study provided additional evidence that STZ-induced diabetes in rats possibly via direct damage to DNA, and generation of oxidative stress and inflammatory effect. However, mechanism of STZ high dose-induced pancreatic cell death seemed to be mediated by necrosis rather than apoptosis since some major apoptotic markers were inhibited in pancreatic tissue. On the other hand, current findings gave an additional reference that propolis has obvious antioxidant potency, anti-inflammatory effect and apoptotic activity by which it reduced STZ-diabetes side effects, leading eventually to restore some of β-cell functions.

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