POTENTIAL ANTIDIABETIC AND HYPOLIPIDEMIC EFFECTS OF PROPOLIS EXTRACT IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

EL-SAYED M EL-SAYED*, OSAMA M ABO-SALEM, HAMDY A ALY AND AHMED M MANSOUR

Pharmacology &Toxicology Department, Faculty of Pharmacy, Al-Azhar University, Nasr-City, Cairo, Egypt

ABSTRACT

Free radicals have been implicated in the pathogenesis of diabetes mellitus leading to various complications including atherosclerosis. Propolis was reported to have oxygen radical scavenging activity. The present study was designed to investigate the possible antidiabetic, hypolipidemic and antioxidant effects of ethanolic extract of propolis (EEP). Type I diabetes was induced in rats by injection of streptozotocin (STZ) in a dose of 60 mg/kg bwt, i.p. for 3 consecutive days. After 5 weeks of STZ injection, there were an apparent reduction in the animal body weight amounting to 21% and significant increases in serum glucose (184%), triglycerides (63%), total cholesterol (43%) and low density lipoprotein-cholesterol (LDL-C) (148%) with a concomitant decrease in serum high density lipoprotein-cholesterol (HDL-C) (51%) as compared to the control normal group. In addition, there was significant elevation in pancreatic lipid peroxides measured as malondialdehyde (MDA) and serum nitric oxide (NO) amounting to 185% and 224%, respectively with marked reduction in serum reduced glutathione (GSH) andcatalase (CAT) (66% and 31%, respectively) and pancreatic superoxide dismutase (SOD) (54%) in STZ-treated rats. On the other hand, oral daily treatment of animals with EEP in a dose of 200mg/kg bwt for a period of 5 weeks ameliorated STZ-induced alterations in the animal body weight as well as in serum glucose, lipids, lipoproteins, NO, GSH & CAT and pancreatic MDA & SOD. In conclusion, propolis extract offers promising antidiabetic and hypolipidemic effects that may be mainly attributed to its potent antioxidant potential. Further studies will be needed in future in order to determine which one(or more) of its active constituents has the main antidiabetic and hypolipidemic effects.

Keywords: Diabetes; streptozotocin; antioxidant; propolis extract.

INTRODUCTION

Free radicals have been implicated in the pathogenesis of many degenerative disease, including diabetes, and atherosclerosis and cancer (Baynes, 1991; Mercuri *et al*., 2000). Antioxidant defence systems are also disturbed in diabetes mellitus (Jones *et al*., 1988). Oxidative stress may cause oxidative damage of cellular membranes and changes in the structural and functional integrity of subcellular organelles and may produce effects that result in various complications in diabetic disease (Mercuri *et al*., 2000; West, 2000; Cam *et al*., 2003 ; Yavuz *et al*., 2003).

Streptozotocin (STZ) is an alkylating agent antibiotic that experimentally produces diabetes due to β-cell death by the mechanism of DNA damage in rodent islets (Yang and Wright, 2002). During STZ metabolism, various toxic intermediates are produced, including methyl cations, methyl radicals, reactive oxygen species (ROS) and nitric oxide (NO) (Peschke *et al.,* 2000, González *et al.,* 2002). Beta cells are very susceptible to oxidative changes since they possess a low antioxidative capacity (Hotta *et al.,* 1998, Kajimoto and Kaneto, 2004).

**Corresponding author:* elsayed200_1956@yahoo.com

Propolis is a complex resinous material collected by honeybees from buds and exudates of certain plant sources neighboring its hives. Propolis consisting of sap, bark and bee excreta, accumulates in bee hives. The chemical consistency of propolis is highly dependent on the flora of the region from where it is collected (Marcucci, 1995; Burdock, 1998; Banskota *et al*., 2001). Propolis contains at least 200 compounds have that been identified in different samples of propolis, with more than 100 being present in any given sample. These include fatty and phenolic acids and esters, substituted phenolic esters, flavonoids (flavones, flavanones, flavonols, dihydroflavonols, chalcones), terpenes, β-steroids, aromatic aldehydes and alcohols, and derivatives of sesquiterpenes, naphthalene and stilbenes (Greenaway *et al*., 1991; Aga *et al*., 1994; Bankova *et al*., 1995; Marcucci *et al*., 1996). The main types of flavonoids are rutin, quercetin, galangin (Isla *et al*., 2001) and caffeic acid phenethyl ester (Natarajan *et al*., 1996).

Propolis possesses a broad spectrum of biological activities and has a historical utilization in folk medicine. Thus, it is extensively being used in health food, pharmaceutical preparations (Havsteen, 1983; Wleklik *et al*., 1997; Khalil, 2006; Orsolic and Basic, 2006) and beverages with the aim of maintaining or improving

human health (Greenaway *et al*., 1991; Aga *et al*., 1994; Bankova *et al*., 1995; Marcucci *et al*., 1996). It was reported that propolis enhances immune system activities (Wleklik *et al*., 1997; Orsolic and Basic, 2003 & 2006), oxygen radical scavenging (Moreno *et al*., 2000; Chen *et al*., 2004), antimicrobial, anti-inflammatory (Mizoeva and Calder, 1996) and antitumor activities (Russo *et al*., 2004; Orsi *et al*., 2005; Silici and Kutluca, 2005; Duarte *et al*., 2006).

Based on the oxygen radical scavenging activity of propolis, the present study was designed to investigate the probable antidiabetic and hypolipidemic effects of ethanolic extract of propolis (EEP) in relation to its antioxidant effect in STZ-induced diabetic rats.

MATERIALS AND METHODS

Chemicals

Brazilian green propolis (Brazil, Minas Gerais State) was obtained from Sigma – Aldrich Chemical Company (St. Louis, MO, USA) as ethanolic extract that was stored at -20^{o}C prior to experimentation. It was suspended in a vehicle containing 2%Tween 80 and 2%sodium lauryl sulphate in a saline solution and administered orally at 9AM by a gastric tube in a dose of 200 mg/kg bwt (Bhadauria *et al*., 2007) for one week before induction of diabetes, 3 days during induction of diabetes and continued till the end of the experiment (i.e. total treatment period of 5 weeks).

Streptozotocin was purchased from Sigma-Aldrich Chemical Company. It was freshly dissolved in ice-cold 0.05 M citrate buffer (pH 4) and given i.p. in a dose of 60 mg/kg bwt for 3 consecutive days for induction of diabetes (Abdel-Wahab and Abd-Allah,2000; Yavuz *et al*., 2003).

Vitamin E (d-alpha tocopherol) was obtained from Kahira Pharmaceutical and Chemical Co., Cairo, Egypt and administered i.m. in a dose of 250 mg/kg bwt (Abdel-Naim *et al*., 1999). It was used as a reference standard antioxidant agent.

All the other used chemicals were of the highest analytical grades commercially available.

Animals

Adult male Wistar rats, weighing 225-235 g were purchased from the National Institute of Cancer, Cairo-Egypt. They were housed in the animal facility of the Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt for two weeks before starting the experiment. Animals were allowed to free access to standard diet, water and maintained under the optimum conditions of temperature (22 ± 2˚C), relative humidity (55%±5) and light period (12 h light/12h dark).

Experimental design

Forty rats were classified into 4 groups (8 animals/ group) and subjected to treatments as follows:

Group I: received 1ml distilled water per 100 g bwt per day by oral gavage for 5 weeks and served as a control group.

Group II: injected i.p. with streptozotocin (STZ) in a dose of 60 mg/kg bwt for 3 consecutive days and served as untreated diabetic group (UDG).

Group III: received ethanolic extract of propolis (EEP) alone,orally in a daily dose of 200mg/kg bwt for 5 weeks and served as propolis-treated group (PG).

Group IV: received EEP orally in a dose of 200 mg/kg bwt for 1week before induction of diabetes, 3 days during induction of diabetes and continued for 5 weeks and served as propolis treated diabetic group (PDG).

Group V: injected with vitamin E, i.m. in a dose of 250 mg/kg bwt as in the same schedule of EEP in group IV and served as vitamin E treated diabetic group (EDG)

Induction of experimental diabetes mellitus and propolis treatment

Type 1 diabetes mellitus was induced in rats by injection of STZ in citrate buffer (pH: 4.0) in a dose of 60 mg/kg i.p. for 3 consecutive days (Abdel-Wahab and Abd-Allah, 2000; Yavuz *et al*., 2003). On the fourth day, blood samples were taken from the tail veins to measure the blood glucose level. Ethanolic extract of propolis(EEP) was administered orally at 9.0 AM by gastric tube in a dose of 200 mg/kg bwt (Bhadauria *et al*., 2007) for 1 week before induction of diabetes,3 days during induction of diabetes and extended for a total duration period of 5 weeks. Vitamin E was administered i.m. daily in a dose of 250 mg/kg bwt (Abdel-Naim *et al*., 1999) in the same schedule as propolis and was considered as a standard antioxidant agent. At the end of the study, blood samples were collected from the tail veins, animals were killed by cervical dislocation and rat pancreas was taken out.

Tissue preparation

Each pancreas was quickly removed from the sacrificed rat, placed in ice cold saline solution and trimmed of adipose tissue. Each pancreas was finely cut, homogenized in 50 mM phosphate buffer, pH 7.4 and centrifuged at 10,000 × *g* for 15 min at 4 °C using Beckman cooling Ultracentrifuge. The supernatant was used for determination of MDA and SOD.

Determination of serum glucose, lipids and lipoproteins

Serum glucose, triglycerides (TG), total cholesterol, low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) were determined by enzymatic methods according to Diniz *et al*. (2006).

MDA determination

Malondialdehyde (MDA) contents of pancreatic tissues were measured as described by Draper and Hadley (1990). The method depends on spectrophotometric measurement of the purple color generated by the reaction of thiobarbituric acid (TBA) with MDA. In a test tube 0.5 ml supernatant was mixed with 2.5 ml of trichloroacetic acid solution (10%, w/v) and placed in a boiling water bath for 15 min. After cooling to room temperature, the tubes were centrifuged at 1000 × *g* for 10 min and 2 ml of each sample supernatant was transferred to a test tube containing 1 ml of TBA solution (0.67%, w/v). Tubes were then placed in a boiling water bath for 15 min and cooled to room temperature. The resultant color was measured spectophotometrically at 532 nm. The concentration of MDA was expressed as nmol/mg protein.

Determination of serum nitric oxide

Serum nitric oxide (NO) level can be estimated spectrophotometrically by determination of the NO stable end products, nitrite and nitrate. The serum nitrite level was determined using Griess reagent according to Hortelano *et al*. (1995). The Griess reagent, a mixture (1:1) of 1% sulfanilamide in 5% phosphoric acid and 0.1% 1-naphtylethylenediamine gives a red-violet diazo color in the presence of nitrite. The color intensity was measured at 540 nm. Results were expressed as µmol/l using a $NaNO_2$ calibration graph.

Antioxidant defense system assays

Serum reduced glutathione (GSH) was evaluated as described by Ellman (1959), where 0.5 ml of rat serum was treated with 0.5 ml Ellman's reagent (19.8 mg of 5,5′-dithiobisnitrobenzoic acid in 100 ml of 0.1% sodium nitrate) and 3 ml of phosphate buffer (0.2 M, pH 8). The absorbance was read at 412 nm. Data were expressed as nmol/ml.

Catalase (CAT) activity in the rat serum was measured as described by Aebi (1984). The change in the absorbance was monitored spectrophotometrically at 240 nm over a 5 min period and data were expressed as U/ml.

SOD activity in the rat pancreatic supernatant was determined by assessing the inhibition of pyrogallol auto-oxidation according to the method of Marklund (1985). Changes in the absorbance at 420 nm were recorded at 1 min interval for 5 min. SOD activity was determined from a standard curve of % inhibition of pyrogallol auto-oxidation with SOD activity and data were expressed as U/mg protein.

Protein determination

The protein concentration of pancreatic tissue samples were measured according to Lowry *et al*. (1951) using bovine serum albumin as a standard.

Statistical analysis of data

Data are presented as means ± SEM. Statistical comparison between groups were done by one-way analysis of variance (ANOVA) followed by Tukey Kramer test to analyze the differences. Statistical significance was achieved at $P < 0.05$.

RESULTS

Table 2 showed marked elevation in serum glucose (184%), triglycerides (63%), total cholesterol (43%), LDL-C (148%) and significant reduction in HDL-C (51%) after 5 weeks of streptozotocin (STZ) injection in rats. Moreover, there was an apparent reduction in the animal body weight amounting to 21%, as compared to the control group (table 1). Analysis of lipid peroxides revealed that pancreatic MDA contents were significantly increased by 185%.In addition, serum NO were noticeably elevated reaching 224%.,while there were marked decreases in serum GSH&CAT and pancreatic SOD, amounting to 66%, 31% & 54%, respectively (table 3). On the other hand, oral treatment of STZ-induced diabetic rats with ethanolic extract of propolis (EEP), daily for 5 weeks ameliorated alterations in the animal body weight as well as serum glucose, lipids, lipoproteins, NO and pancreatic MDA and antioxidant parameters (tables 1, 2 and 3). Based on the obtained results, EEP was nearly equal in efficacy in comparison with vitamin E on all mentioned parameters except on serum glucose and pancreatic SOD, where EEP was found to be more effective than vitamin E.

DISCUSSION

Oxidative stress is produced under diabetic conditions and it is likely involved in progression of pancreatic β-cell dysfunction (Kajimoto and Kaneto, 2004). Also, because of the relatively low expression of antioxidant enzymes such as catalase and superoxide dismutase, pancreatic β-cells may be vulnerable to ROS attack when the system is under oxidative stress situation (Lenzen *et al*., 1996; Tiedge *et al*., 1997). Similarly, elevated levels of free radicals, due to insufficiency of the antioxidant defense system, may lead to disruption of cellular function, oxidative damages to membranes and enhance their susceptibility to lipid peroxidation (Baynes, 1991). In recent years, it has been shown that dietary supplementation with natural antioxidants such as, vitamins C and E, melatonin and flavonoids attenuated the oxidative stress and diabetic state induced by STZ (Montilla *et al*., 1998; Kaneto *et al*., 1999; Coskun *et al*., 2005).

Lipid peroxidation products such as MDA are generated under high levels of un-scavenged free radicals (Levy *et al*., 1999). These products may be important in the pathogenesis of vascular complications in diabetes

mellitus (Halliwell, 2000). The increased MDA level may have an important role in pancreatic damage associated with diabetes. In addition, it has been suggested that overproduction of free radical NO under the influence of STZ may play a crucial role in destruction of the β-cells during the development of type 1 diabetes (Haluzik and Nedvidkova, 2000).

Our results revealed that there were marked increases in serum levels of glucose ,triglycerides, total cholesterol and LDL-C with a concomitant decrease in serum HDL-C in STZ-induced diabetic rats and this is in accordance with that demonstrated by Douillet *et al*. (1998) and Naziroglu *et al*. (1999). In addition, the observed decrease in the body weight of diabetic animals agree with Torres *et al*.(1999) who also noticed a marked reduction in the

Table 1: Effect of treatment of streptozotocin (STZ)-induced diabetic rats with ethanolic extract of propolis (EEP)on the animal body weight

Groups Body weight (g)	Control	UDG	PG	PDG	EDG
Initial	229.13 ± 1.06	229.00 ± 1.65	231.88 ± 0.91	226.50 ± 0.65	230.25 ± 1.39
Final	275.13 ± 2.12	218.63 ± 2.31[a]	272.50 ± 2.85[b]	267.50 ± 3.66[b]	277.50 ± 2.78[b]

Data are expressed as means ± SEM of 8 rats.
UPG:untreated STZ-induced diabetic group; PG: propolis-treated group; PDG: propolis-treated diabetic group;EDG:vitamin E-treated diabetic group.
a: Significantly different from control group; b: Significantly different from UDG using one-way ANOVA with Tukey-Kramer test at $P < 0.0$

Table 2: Effect of treatment of streptozotocin (STZ)-induced diabetic rats with ethanolic extract of propolis (EEP) on serum glucose, lipids and lipoproteins

Groups Serum levels (mg/dl)	Control	UDG	PG	PDG	EDG
Glucose	65.75 ± 1.53	187.00 ± 4.00[a]	69.50 ± 1.95[b]	93.88 ± 4.00[a,b,c]	124.25 ± 2.71[a,b,c,d]
TG	69.00 ± 2.70	112.63 ± 2.52[a]	66.75 ± 2.25[b]	60.00 ± 2.75[b]	66.88 ± 2.95[b]
Total cholesterol	83.50 ± 2.34	119.75 ± 2.48[a]	78.38 ± 3.23[b]	79.25 ± 2.46[b]	87.25 ± 3.74[b]
LDL-C	29.88 ± 1.08	74.13 ± 2.89[a]	27.75 ± 1.47[b]	31.75 ± 1.67[b]	34.75 ± 2.40[b]
HDL-C	40.50 ± 2.51	19.75 ± 3.04[a]	55.50 ± 1.86[a,b]	41.50 ± 2.99[b,c]	35.63 ± 2.56[b,c]

Data are expressed as means ± SEM of 8 rats.
UPG: untreated STZ-induced diabetic group; PG: propolis-treated group; PDG: propolis-treated diabetic group; EDG: vitamin E-treated diabetic group.
a: Significantly different from control group; b: Significantly different from UDG; c: Significantly different from PG; d: Significantly different from PDG using one -way ANOVA with Tukey-Kramer test at $P < 0.05$.

Table 3: Effect of treatment of streptozotocin(STZ)-induced diabetic rats with ethanolic extract of propolis(EEP) on oxidant and antioxidant parameters

Groups Parameters	Control	UDG	PG	PDG	EDG
Pancreatic MDA (nmol/mg protein)	0.33 ± 0.02	0.94 ± 0.03[a]	0.37 ± 0.03[b]	0.42 ± 0.02[b]	0.38 ± 0.03[b]
Serum NO (µmol/L)	5.27 ± 0.39	17.10 ± 0.55[a]	5.22 ± 0.47[b]	5.00 ± 0.42[b]	8.09 ± 0.30[a,b,c,d]
Serum GSH (U/ml)	12.16 ± 0.56	4.14 ± 0.38a	17.35 ± 0.44a,b	11.55 ± 0.53b,c	13.54 ± 0.61b,c
Pancreatic SOD (U/mg protein)	4.48 ± 0.24	2.07 ± 0.23a	4.07 ± 0.31b	4.97 ± 0.27b	3.66 ± 0.21b,d
Serum CAT (U/ml)	0.64 ± 0.02	0.44 ± 0.03a	0.58 ± 0.03	0.69 ± 0.04b	0.65 ± 0.05b

Data are expressed as means ± SEM of 8 rats.
UPG: untreated STZ-induced diabetic group; PG: propolis-treated group; PDG: propolis-treated diabetic group; EDG: vitamin E-treated diabetic group.
a: Significantly different from control group; b: Significantly different from UDG; c: Significantly different from PG; d: Significantly different from PDG using one- way ANOVA with Tukey-Kramer test at $P < 0.05$.

animal body weight with significant increase in serum triglycerides in STZ-induced diabetic rats. Moreover, our study showed that there were marked elevations in pancreatic tissue content of MDA as well as serum NO level with significant reduction in the antioxidant parameters (serum GSH & CAT and pancreatic SOD) in STZ-induced diabetic rats which were consistent with the observations obtained by Coskun *et al*. (2005).

On the other hand, our data showed that treatment of the diabetic rats with EEP resulted in marked decrease in serum glucose, lipids and lipoproteins as well as the animal body weight. In addition, it significantly decreased MDA pancreatic content as well as serum NO level to the normal level of control group. Furthermore, EEP had a potent increasing effect on serum GSH &CAT activities, along with, elevating pancreatic SOD activities compared to diabetic group. Our results agree with Matsui *et al*. (2004) who demonstrated that administration of propolis extract in rats had a potent antihyperglycemic effect, whereas in other studies, it was shown that long-term treatment of rats with propolis extract produced no alterations in the seric levels of cholesterol, HDL-C, total lipids and triglycerides (Mani *et al*., 2006). Also, our data of the antioxidant effects of propolis are parallel with Kwon *et al*.(2004) who reported that propolis attenuates kainate-induced neurotoxicity via decreasing lipid peroxidation in hippocampus. Similar results were demonstrated by Bhadauria *et al*. (2007) who revealed that propolis may play a hepatoprotective role through reducing oxidative stress in living system.

The antioxidative property of propolis extract certainly is due to its chemical constituents. Phytochemical investigations of propolis have demonstrated the presence of flavonoids and polyphenolic components as main active ingredients having potent antioxidant activities (Moreno *et al*., 2000; Hosnuter *et al*.,2004).

In conclusion, EEP offers a promising therapeutic value in prevention of diabetes and dyslipidemic profile. These effects could be mainly attributed to its antioxidant properties as shown by significant quenching impact on the extent of lipid peroxidation along with, enhancement of antioxidant defense systems in pancreatic tissue. Further studies will be needed in future to determine the main active ingredient having the beneficial antidiabetic, hypolipidemic and antioxidant effects.

REFERENCES

Abdel-Naim AB, Abdel-Wahab MH and Attia FF (1999). Protective effect of vitamin E and probucol against gentamycin-induced nephrotoxicity in rats. *Pharmacol. Res*., **40**(2): 183-187.

Abdel-Wahab MH and Abd-Allah AR (2000). Possible protective effect of melatonin and/or desferrioxamine against streptozotocin-induced hyperglycaemia in mice. *Pharmacol. Res.,* **41**(5): 533-537.

Aebi H (1984). Methods in Enzymology, Academic Press, New York , p.121.

Aga H, Shibuya T, Sugimoto T, Kurimoto M and Nakajima SH (1994). Isolation and identification of antimicrobial compounds in Brazilian propolis. *Biosci. Biotechnol. Biochem.,* **58**: 945-946.

Bankova V, Christov R, Kujumgiev A, Marcucci MC and Popov S (1995). Chemical composition and antibacterial activity of Brazilian propolis. *Z Naturforsch [C]*, **50**: 167-72.

Banskota AH, Tezuka Y and Kadota S (2001). Recent progress in pharmacological research of propolis. *Phytother. Res.*, **15**(7): 561-571.

Baynes JW (1991). Role of oxidative stress in the development of complications in diabetes. *Diabetes*, **40**: 405-412.

Bhadauria M, Nirala SK and Shukla S (2007). Propolis protects $CYP2E_1$enzymatic activity and oxidative stress induced by carbon tetrachloride. *Mol. Cell Biochem.*, **302**(1-2): 215-224.

Burdock GA (1998). Review of the biological properties and toxicity of bee propolis (propolis). *Food Chem. Toxicol.*, **36**(4): 347-363.

Cam M, Yavuz O, Guven A, Ercan F, Bukan N and Ustundag N (2003). Protective effects of chronic melatonin treatment against renal injury in streptozotocin-induced diabetic rats. *J. Pineal Res*., **35**: 212-220.

Chen CN, Weng MS, Wu CL and Lin JK (2004). Comparison of radical scavenging activity, cytotoxic effects and apoptosis induction in human melanoma cells by Taiwanese propolis from different sources. *Evid. Based Complement Alternat.Med*., **1**(2): 175-185.

Coskun O, Kanter M, Korkmaz A and Oter S (2005). Quercetin, a flavonoid antioxidant, prevents and protects streptozotocin-induced oxidative stress and β-cell damage in rat pancreas. *Pharmacol. Res.,* **51**: 117-123.

Diniz YS, Rocha KR, Souza GA, Galhardi GM, Ebaid GX, Rodrigues HG, Fihlo JN, Cicogna AC and Novelli EB (2006). Effects of N-acetylcysteine on sucrose-rich diet-induced hyperglycaemia, dyslipidemia and oxidative stress in rats. *Eur. J. Pharmacol.,* **543**: 151-157.

Douillet C, Bost M, Accominotti M, Borson-Chazot BF and Ciavatti M (1998). Effect of selenium and vitamin E supplements on tissue lipids,peroxides, and fatty acid distribution in experimental diabetes. Lipids, **33**: 393-399.

Draper H.H and Hadley M (1990). Methods in Enzymology, Academic Press, New York, p.421.

Duarte S, Rosalen P L, Hayacibara MF, Cury JA, Bowen WH, Marquis RE, Rehder VL, Sartoratto A, Ikeqaki M and Koo H (2006). The influence of a novel propolis on

mutans streptococci biofilms and caries development in rats. *Arch.Oral Biol.*, **51**(1): 15-22 .

Ellman M (1959). A spectrophotometric method for determination of reduced glutathione in tissues. *Analyt. Biochem*.,**74**: 214-226.

Gonzalez E, Jawerbaum A, Sinner D, Pustovrh, White V, Capobianco E, Xaus C, Peraka C and Rosello-Catafau J (2002). Streptozotocin-pancreatic damage in the rat: Moulatory effect of 15-deoxydelta12,14-prostaglandin j(2) on nitridergic and prostanoid pathway. *Nitric Oxide*, **6**: 214-220.

Greenaway W, May J, Scaysbrook T and Whatley FR (1991). Identification by gas chromatography-mass spectrometry of 150 compounds in propolis. *Z Naturforsch [C]*, **46**: 111-121.

Halliwell B (2000). Lipid peroxidation, antioxidants and cardiovascular disease: how should we more forward? *Cardiovasc. Res.* **47**: 410-448.

Haluzik M and Nedvidkova J (2000). The role of nitric oxide in the development of streptozotocin-induced diabetes mellitus: experimental and clinical implication, *Physiol. Res.*, **49**: 37-42.

Havsteen B (1983). Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol.*, **32**(7): 1141-1148.

Hortelano S, Dewer B Genaro AN, Diaz-Guerra MJ and Bosca L (1995). Nitric oxide is released resulting in regenerating liver after partial hepatectomy. *Hepatology*, **21**: 776-786.

Hosnuter M, Gurl A, Babuccu O, Armutcu F, Kargi E and Isikdemir A (2004).The effect of CAPE on lipid peroxidation and nitric acid levels in the pasma of rats of rats following thermal injury.Burns, **30**(2):121-125.

Hotta M, Tashiro F, Ikegami H, Niwa H, Ogihara T, Yodoi J and Miyazaki J (1998). Pancreatic beta cell-specific expression of thioredoxin, an antioxidative and antiapoptotic protein, prevents autoimmune and streptozotocin-induced diabetes. *J. Exp. Med*., **188**: 1445-1451.

Isla MI, Moreno MI, Sampietro AR and Vattuone MA (2001). Antioxidant activity of Argentine propolis extracts. *J. Ethnopharmacol.*, **76**: 165-170.

Jones AF, Winkles JW, Jennings PE, Florkowski CM, Lunec J and Barnett AH (1988). Serum antioxidant activity in diabetes mellitus. *Diabetes Res*., **7**: 89-92.

Kajimoto Y and Kaneto H (2004). Role of oxidative stress in pancreatic beta-cell dysfunction. *Ann. NY. Acad. Sci.*, **1011**: 168-176.

Kaneto H, Kajimoto Y, Miyagawa J, Matsuoka T , Fumigant Y, Umayahara Y, Hanafusa T, Matsuzawa Y, Yamasaki Y and Hori M (1999). Beneficial effects of antioxidants in diabetes, possible protection of pancreatic β-cells against glucose toxicity. *Diabetes*, **48**: 2398-2406.

Khalil ML (2006). Biological activity of bee propolis in health and disease. *Asian Pac. J. Cancer Prev.*, **7**(1): 22-31.

Kwon YS, Park DH, Shin EJ, Kwon MS, Ko KH, Kim WK, Jhoo JH, Jhoo WK, Wie MB, Jung BD and Kim HC (2004). Antioxidant propolis attenuates kainate-induced neurotoxicity via adenosine A_1-receptor modulation in the rat.Neuroscience, **355**(3): 231-235.

Lenzen S, Drinkgern J and Tiedge M (1996). Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues, *Free Radical Biol. Med.*, **20**: 463-466.

Levy U, Zalizber I , Ben-Amotz A, Kanter Y and Aviram M (1999). β-Carotene affects antioxidant status in non-insulin dependent diabetes mellitus. *Pathophysiology*, **6**: 157-161.

Lowry OH , Rosebrought NJ and Randell RJ(1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* **193**: 265-272.

Mani F, Damasceno HC, Novelli EL, Martins EA and Sforcin JM (2006). Propolis: Effect of different concentrations, extracts and intake period on seric biochemical variables. *J. Ethnopharmacol.,* **105**(1-2): 95-98.

Marcucci MC (1995). Propolis: Chemical composition, biological properties and therapeutic activity. *Apidologie*, **26**: 83-99.

Marcucci MC, DeCamargo FA and Lopes CMA (1996). Identification of amino acids in Brazilian propolis. *Z Naturforsch [C],* **51**: 11-14.

Marklund SL (1985). Pyrogallol autooxidation. *In*: Handbook of methods for oxygen radical research. Ed.: Greenwald RA, Boca Raton, FL, CRC Press, pp.243-247.

Matsui T, Ebuchi S, Fujise T,Abesundara KJ, Doi S, Yamada H and Matsumoto K (2004). Strong anti-hyperglycemic effects of water-soluble fraction of Brazilian propolis and its bioactive constituent,3,4,5,-tri-O-caffeoxylquinic acid. *Biol. Pharm. Bull.,* **27**(11): 1797-1803.

Mercuri F, Quagliaro L and Ceriello A (2000). Oxidative stress evaluation in diabetes. *Diabetes Technol. Ther*. **2**: 589-600.

Mizoeva OK and Calder PC (1996). The effect of propolis and its components on eicosanoid production during the inflammatory response. *Prostagland. Leuk. Essent. Fatty*, **55**: 441-449.

Montilla P, Vargas J, Tunez I, Munoz MC, Valdelvira ME and Cabrera E (1998). Oxidative stress in diabetic rats induced by streptozotocin: protective effects of melatonin. *J. Pineal Res*., **25**: 94-100.

Moreno M, Isla M, Sampietro A and Vattuone M (2000). Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. *J. Ethnopharmacol.*, **71**: 109-114.

Natarajan K, Singh S, Burker TR Jr, Grunberger D and Aggarwal BB (1996). Caffeic acid phenethyl ester is a potent and specific inhibitor of activation of nuclear transcription factor NF-kappa B. *Proc. Natl. Acad. Sci. USA*, **93**(17): 9090-9095.

Naziroglu M, Dilsiz N and Cay M (1999). Protective role of intraperitoneally administered vitamin C and E and selenium on the levels of lipid peroxidation in the lens of rats made diabetic with streptozotocin. *Biol. Trace Elem. Res.,***70**: 223-232.

Orsi RO, Sforcin J M, Funari S R and Bankova V (2005). Effects of Brazilian and Bulgarian propolis on bactericidal activity of macrophages against Salmonella Typhimurium. *Int. Immunopharmacol.*, **5**(2): 359-368.

Orsolic N and Basic I (2003). Immunomodulation by water-soluble derivative of propolis: a factor of antitumor reactivity. *J. Ethnopharmacol.*, **84**(2-3): 265-273.

Orsolic N and Basic I (2006).*Cancer chemoprevention by propolis and its polyphenolic compounds in experimental animals. Recent Prog. Med. Plants*, **17**: 55-113.

Peschke E, Ebelt H, Bromme HJ and Peschke D (2000).'Classical' and 'new' diabetogens-comparison of their effects on isolated rat pancreatic islets *in vitro*.*Cell. Mol. Life Sci*., **57**:158-164.

Russo A, Cardile V, Sanchez F, Troncoso N, Vanella A and Garbarino JA (2004). *Life Sci.*, **76**: 545-558.

Silici S and Kutluca S (2005).Chemical composition and antibacterial activity of propolis collected by three different races of honeybees in the same region. *J. Ethnopharmacol.*, **99**(1): 69-73.

Tiedge M, Lortz S , Drinkgern J and Lenzen S (1997). Relation between antioxidant enzyme gene expression and antioxidative defense system status of insulin producing cells. *Diabetes*, **46**: 1733-1742.

Torres MD,Canal JR and Perez C(1999).Oxidative stress in normal and diabetic rats. *Physiol. Res*.,**48**: 203-208.

West IC (2000). Radicals and oxidative stress in diabetes. *Diabetic Med*., **17**: 171-180.

Wleklik M, Zahorska R and Luczak V (1997). Interferon-inducing activity of flavonoids. *Acta Microbiol. Pol.*, **36**: 151-154.

Yang H and Wright JR (2002). Human beta cells are exceedingly resistant to streptozotocin *in vivo*. *Endocrinology*, **143**: 2491-2495.

Yavuz O, Cam M, Bukan N, Guven A and Silan F (2003). Protective effect of melatonin on beta-cell damage in streptozotocin-induced diabetes in rats. *Acta Histochem*., **105**: 261-266.