Effects of propolis on blood biochemical and hematological parameters in nitric oxide synthase inhibited rats by Nω-Nitro-L-arginine methyl ester

Zeliha Selamoglu Talas[1]*, Ayse Gogebakan[1] and Ibrahim Orun[2]
[1]Department of Biology, Faculty of Arts and Sciences, Nigde University, Nigde, Turkey
[2]Department of Biology, Faculty of Arts and Sciences, Aksaray University, Aksaray, Turkey

Abstract: This study showed the effects of propolis on biochemical and hematological parameters in chronic nitric oxide synthase inhibited rats by N_{ω}-Nitro-L-arginine methyl ester (L-NAME). Rats are given L-NAME for 15 days and the propolis for the last 5 days with L-NAME together. The levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma glutamyltransferase in the L-NAME group compared to control group have increased (P<0.05). The levels of these parameters in L-NAME+propolis group compared to the L-NAME group have decreased (P<0.05). L-NAME caused increase (P<0.05) in levels of glucose, albumin, globulin, creatinine, urea, triglyceride and cholesterol. Erythrocyte number, total leukocyte, hemoglobin, hematocrit, neutrophil and monocyte decreased (P<0.05), platelets and lymphocyte increased (P<0.05) in L-NAME+propolis group compared to the L-NAME group. The study concluded that homeostasis is modulated in L-NAME administrated rats by adding propolis which causes increasing generation of vascular nitric oxide.

Keywords: Biochemical parameter, blood, hematological parameter, L-NAME, propolis, rat.

INTRODUCTION

Hypertension, which elevates endothelial disfunction and atherosclerosis, is major risk factor for cardiovascular disease leading death in industrialized societies (Ogita and Liao, 2004; Ryu *et al*., 2008). Nitric oxide (NO) plays a considerable role in the physiological control of blood pressure (BP) and causes vasodilation. Nitric oxide synthase (NOS) is responsible for production of NO in endothelial cells. Either genetic deletion or inhibition of this enzyme causes hypertension and profound alterations of vascular function. For NO produced by the endothelium to cause vasorelaxation of the underlying vascular smooth muscle, it must traverse the interstitial space between the endothelium and vascular smooth muscle (Harrison *et al*., 2007). NOS inhibitors such as N_{ω}-nitro-L-arginine methyl ester (L-NAME) are generally used in hypertensive animal models. L-NAME is the most used L-arginine analogues that inhibit NOS. This inhibition is competitive and completely reversible by excess of L-arginine At the same time, L-NAME reasons several functional and morphological conversions in the vascular endothelium and vascular smooth muscle cells. NO has been proved to have important role in the regulation of normal blood pressure and body fluid homeostasis (Kanematsu *et al*., 2006).

Recently, antihypertensive effects have been noted with various food and natural products (Maruyama *et al*., 2009; Talas *et al*., 2010; Gogebakan *et al*., 2012). Propolis is one of these natural products. It has been suggested that propolis is used as a sealant to block redundant openings in the beehive and to avoid invasion by fungi and bacteria. Propolis is used in folk medicines in many regions of the world and has been showed to have diverse biological properties, such as antibacterial, antiviral, anti-inflammatory, and anticancer, antihypertensive effect (Prytzyk *et al*., 2003; Bhadauria and Nirala, 2009; Maruyama *et al*., 2009). The major chemical substances found in propolis are flavonoids, phenolics, and various aromatic compounds. But its bioflavanoid ingredient is now recognition attention. Bioflavonoids are antioxidant molecules which play very big roles in scavenging of free radicals, which are generated in degenerative heart diseases, atherosclerosis and aging (Aisha *et al*., 2012). Some phenolic compounds like propolis have the antihypertensive and antioxidant ability in L-NAME-induced rats (Kumar *et al*., 2011).

To increase the production of NO and to activate NOS enzymes, there should be enough arginine to compete with L-NAME. It may be thought that this necessity can be provided with agents rich with arginine like propolis which contains enough flavonoids. The addition propolis to the environment is important for providing arginine to compete with L-NAME and for realizing of enzyme activation. The activated enzyme increases the formation of NO that shows vasodilator feature.

Biochemical and hematological parameters are the main homeostatic systems of humans and animals, sustaining normal viability, entirety and adaptive answers. Biochemical and hematological parameters of humans and animals are determined as an index of their health

**Corresponding author:* e-mail: ztalas@nigde.edu.tr

position as well. In this study, a regulatory effect of propolis in NOS inhibited rats has been observed by analyses of biochemical and hematological parameters. This work opens a new aspect on the search of primary therapeutic properties of propolis which is based on the biochemical and hematological parameters in blood of rats.

MATERIALS AND METHODS

Animals

Twenty-eight male Wistar rats weighing 200–250 g (all of animals are same age group) were placed in a temperature (21±2°C) and humidity (60±5%) controlled room in which a 12 h light–dark cycle was maintained. All tests were performed between 9:00 and 17:00 h. This work was performed in accordance with the guidelines for animal research from the National Institutes of Health and was confirmed by the Committee on Animal Research at Firat University, Elazig.

Experimental procedure

Rats were sectioned into four groups of seven rats each: (1) control, (2) propolis, (3) L-NAME and (4) L-NAME+propolis. L-NAME (Fluka Chemie, Switzerland) was dissolved in normal saline (0.09% NaCl w/v). The ethanolic extract of propolis was dissolved in distilled water. The rats in control group were injected normal saline intraperitoneally (i.p.) for 15 days. Rats in propolis group received propolis 200 mg/kg (Abo-salem *et al*., 2009; El-Sayed *et al*., 2009) with gavage. L-NAME group received non-specific NOS inhibitor L-NAME (40 mg/kg, i.p) for 15 days (Sahna *et al*., 2008). The L-NAME + propolis group received both L-NAME (40 mg/kg, i.p.) for 15 days and propolis (200 mg/kg, gavage) per day of the last 5 days.

Preparation of propolis extractive solution

The most general extracts used in biological experiments are ethanol, methanol and water. In the study, the propolis sample was collected from Balikesir in Turkey. Collected with hand that propolis was kept desiccated, in the dark, until the processing. Thirty grams of propolis was dissolved in 100 mL of 70% ethanol solution for a week at the room temperature. After a week, the ethanol extract was filtered and then evaporated by using a vacuum evaporator, safe from light and partially shaken for a day at room temperature. Then, the extracts were filtered twice, dried and stored in sealed bottles at 4°C until they are used (Bankova *et al*., 2002).

Biochemical assay

After these processes, rats were anaesthetised with 75 mg/kg sodium pentobarbital, chests were opened and 2 mL of blood was drawn from their heart. Blood samples were transferred to glass tubes, kept in cooled bath and analyzed. The blood was centrifuged at 3000g, at 4°C for 5 min. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT) were assayed; glucose, total protein, creatinine, triglyceride, total cholesterol, chloride, sodium, potassium, total calcium and phosphate levels were determined in plasma. All such analyses were done (Olympus Optical Corp., Shizuoka-ken, Japan) using commercially available kits (Roche).

Hematological analyses

Leukocyte counting was done in blood samples after proper dilution into Turck solution (De Wilde and Houston, 1961; Blaxhall and Daisley, 1973; Blaxhall, 1981). Hemoglobin (HGB) concentration was tested according to the cyano-methemoglobin procedure (Kit 525-A; Sigma Chemical, St. Louis, MO, USA; 14) (Azizoglu and Cengizler, 1996). The microhematocrit method was used in hematocrit (HCT) detection (Jewet *et al*., 1991).

Statistical analysis

Biochemical and hematological data were analyzed using the SPSS for Windows software, Version 10.0 (SPSS Inc.,Chicago, IL, USA). The differences between the groups were determined with the Kruskal-Wallis test and Mann-Whitney U test. A P value less than 0.05 was considered significant.

RESULTS

The effects on enzyme activities in blood of L-NAME, propolis and L-NAME+propolis treatments are shown in table 1. AST, ALT, ALP and GGT activities were increased ($P<0.05$) with L-NAME. But they were stable with the propolis administration ($P>0.05$) compared with control group (table 1). AST, ALT, ALP and GGT activities were reduced significantly with administration of L-NAME+propolis ($P<0.05$) as compared with the L-NAME group (table 1).

Results of biochemical analyses of control, propolis, L-Name and L-name+propolis groups are shown in table 2. Glucose, albumin, globulin, creatinine, urea, triglyceride, and cholesterol levels increased ($P<0.05$) significantly in L-NAME group compared to the control group (table 2). There weren't any differences ($P>0.05$) in the levels of glucose, albumin, globulin, creatinine, urea, cholesterol, total protein and triglyceride in propolis group compared to control group. Glucose, triglyceride, albumin, globulin, creatinine and urea levels reduced in L-NAME+propolis group ($P<0.05$) compared with L-NAME group (table 2). The serum levels of total protein and cholesterol unchanged in rats administrated L-NAME+propolis ($P>0.05$) compared with L-NAME group (table 2). Levels of electrolytes in control, L-NAME, propolis and L-NAME + propolis groups are demonstrated in table 3. There were not significant changes in electrolyte levels in control, L-NAME, propolis and L-NAME + propolis groups ($P>0.05$) (table 3).

The effects on hematological parameters of L-NAME, propolis and L-NAME+propolis are summarized in table 4. There were significant increases (P<0.05) in values of WBC, RBC, HGB, HCT, neutrophil and monocyte but PLT and lymphocyte accounts significantly decreased (P<0.05) in L-NAME group compared with control group (table 4). WBC, RBC, HGB, HCT, PLT, neutrophil, monocyte and lymphocyte levels in propolis group did not change (P>0.05) compared to control group (table 4). WBC, RBC, HGB, HCT, neutrophil, and monocyte levels reduced (P<0.05) in L-NAME+propolis group but PLT and lymphocyte counts increased compared with L-NAME group (table 4).

Table 1: Effects of propolis on the serum enzyme levels in blood of L-NAME treated rats.

Serum enzymes	Control	Propolis	L-NAME	L-NAME+Propolis
Alanine aminotransferase (ALT) (IU l^{-1})	83.49±18.4^{b}	89.14±16.5^{b}	201.23±18.7^{a}	130.25±25.5^{b}
Aspartate aminotransferase (AST) (IUl^{-1})	332.56±55.3^{b}	352.60±69.2^{b}	577.60±39.2^{a}	449.51±73.2^{b}
Alkaline phosphatase (ALP)(IU l^{-1})	140.90±1.1^{b}	138.85±2.4^{b}	178.0±4.93^{a}	142.90±3.07^{b}
Gammaglutamyltransferase (GGT) (IU l^{-1})	0.47±0.10^{b}	0.61±0.13^{b}	2.76±0.38^{a}	1.13±0.62^{b}

All data points are the average of n=7 with ± STDEVs. ^{a,b}The different letters in the same line are statistically significant (P< 0.05).

Table 2: Effects of propolis on the biochemical metabolites in blood of L-NAME treated rats.

Biochemical metabolites	Control	Propolis	L-NAME	L-NAME+Propolis
Glucose mg dL^{-1}	222.58±14.8^{b}	224.19±9.32^{b}	257.68±9.62^{a}	232.79±10.9^{b}
Total protein g dL^{-1}	5.77±0.05	6.18±0.1	5.67±0.47	5.91±0.06
Albumin mg dL^{-1}	2.90±0.31^{b}	2.99±0.19^{b}	3.73±0.27^{a}	3.24±0.1^{b}
Globulin mg dL^{-1}	2.70±0.14^{b}	2.78±0.19^{b}	3.94±0.42^{a}	3.14±0.2^{b}
Creatinine mg dL^{-1}	0.55±0.16^{b}	0.49±0.17^{b}	0.94±0.13^{a}	0.65±0.10^{b}
Urea mg dL^{-1}	60.19±2.35^{c}	62.12±4.39^{c}	79.94±1.24^{a}	72.12±3.97^{b}
Triglyceride mg dL^{-1}	101.03±2.52^{b}	107.90±6.72^{b}	212.64±3.12^{a}	121.9±5.39^{b}
Cholesterol mg dL^{-1}	57.61±6.1	60.14±5.83	72.24±3.37	66.45±4.96

All data points are the average of n=7 with ±STDEVs. ^{a,b}The different letters in the same line are statistically significant (P<0.05).

Table 3: Effects of propolis on the electrolytes in blood of L-NAME treated rats.

Electrolytes	Control	Propolis	L-NAME	L-NAME+Propolis
Chloride (mMol $L^{-1)}$	99.67±1.4	95.34±2.5	99.93±0.64	99.57±4.7
Sodium (mMol L^{-1})	102.23±3.37	109.02±6.8	126.68±10.2	121.46±11.6
Potassium (mMol L^{-1})	19.44±1.0	22.00±0.81	21.56±1.87	22.62±4.95
Calcium (mMol L^{-1})	8.46±0.46	8.17±0.85	8.93±0.15	8.73±0.7
Phosphate (mMol L^{-1})	8.00±0.65	9.13±1.65	8.14±0.6	12.52±5.05

All data points are the average of n=7 with ± STDEVs.

Table 4: Effects of propolis on the hematological parameters in blood of L-NAME treated rats.

Hematological parameters	Control	Propolis	L-NAME	L-NAME+Propolis
WBC ($m^3/10^3$)	6.12±0.2^{b}	6.34±0.24^{b}	8.62±0.4^{a}	6.41±0.28^{b}
RBC ($mm^3/10^6$)	8.30±0.12^{b}	8.45±0.14^{b}	9.40±0.24^{a}	8.85±0.18^{b}
HGB (g/dL)	14.20±0.47^{b}	14.70±0.4^{b}	16.40±0.42^{a}	15.20±0.4^{b}
HCT (%)	42.30±1.17^{b}	43.10±0.9^{b}	47.70±1.32^{a}	45.20±1.24^{b}
PLT (10^3/µL)	340.2±6.7^{a}	337.8±4.32^{a}	276.8±6.64^{b}	327.8±8.16^{a}
MCV(m^3)	50.96±2.2	51.00±1.92	50.74±1.8	54.36±1.62
MCH(mg)	17.10±0.82	17.39±0.91	17.44±0.84	17.17±0.79
MCHC(%)	33.56±1.18	34.10±1.07	34.38±1.14	33.62±1.2
Neutrophil	27.10±1.4^{b}	26.20±0.4^{b}	32.2±0.54^{a}	28.3±0.42^{b}
Eosinophil	0.2±0.01	0.2±0.01	0.4±0.01	0.3±0.01
Basophil	0.3±0.01	0.3±0.01	0.4±0.01	0.3±0.01
Monocyte	6.70±0.4^{b}	7.10±0.4^{b}	13.70±0.52^{a}	7.80±0.34^{b}
Lymphocyte	65.70±1.14^{a}	66.2±1.1^{a}	54.30±0.85^{b}	62.30±1.3^{a}

All data points are the average of n=7 with ± STDEVs. ^{a,b}The different letters in the same line are statistically significant (P<0.05).

DISCUSSION

We investigated the regulatory effects of propolis with analyses of blood biochemical and hematological parameters in rats given L-NAME. Pharmacologically long-term blockade of NO synthesis by the chronic administration of L-NAME, an inhibitor of NOS, generates systemic arterial hypertension, vascular structural alteration and renal disfunction. Because NO promotes vasodilatation and inhibits renal tubuler sodium reabsorption, reduced bioavailability of NO leads to peripheral vasoconstriction and sodium restraints (Nadaud *et al*., 2009).

The usage of plants and plant sections for therapeutic purposes has a long history. Recently, many studies have been performed for finding more suitable antihypertensive agents from natural sources (Prytzyk *et al*., 2003; Ryu *et al*., 2008; Maruyama *et al*., 2009; Gogebakan *et al*., 2012). In this study, levels of AST ALT, ALP and GGT increased in serum (P<0.05) indicated decomposition in the hepatic functions due to hypertensive effects of L-NAME. High levels of AST, ALT, ALP and GGT may be due to the escape of these enzymes from the liver cytosol into the blood stream and liver disfunction and defect in the biosynthesis of these enzymes with change in the permeability of liver membrane takes places. As a result, these enzymes are related with specific organ injuries, mainly cardiac and liver damages (Prytzyk *et al*., 2003; Mohammadzadeh *et al*., 2007). The levels of AST, ALT, ALP and GGT in propolis group unchanged (P>0.05) compared with the control group. Propolis caused decreases in AST, ALT, ALP and GGT activities compared with L-NAME group. Decreases in levels of AST, ALT, ALP and GGT in L-NAME+propolis group appeared compared with L-NAME group. Consequently, by concidering our results, propolis may prevent L-NAME-induced acute tissue damage and lipid accumulation, which is also indicated in the serum lipid profile.

There were significant increases in glucose, creatinine, urea, triglyceride, globulin, albumin and cholesterol levels of L-NAME group but decreases in propolis and L-NAME+propolis groups compared to control group. Increases in levels of these parameters in L-NAME group may be accepted as one of the markers of renal and liver disfunctions, and the failure in lipid metabolism. Propolis caused (P<0.05) decreases in most of levels of these parameters. Therefore propolis protected the status of cellular biomolecules towards normal by improving the cellular metabolism and reversed the hepatic necrosis and renal tubular damage (Bhadauria and Nirala, 2009).

Total leukocyte count, erythrocyte count, HGB, HCT, MCV, MCH and MCHC levels increased in L-NAME group. Increases in MCHC, HGB, MCV, MCH levels and erythrocyte count in L-NAME group may be an indicator of increasing blood pressure with inhibition of NOS in the circulation systems (Sforcin *et al*., 2002).

Our results suggested that blood viscosity as a hematological factor had a role in the regulation of arterial blood pressure. There is the relationship between arterial blood pressure and erythrocyte parameters. There are positive correlations between directly examined whole blood viscosity and a number of the components of the metabolic cardiovascular syndrome including systolic blood pressure, weight and blood lipids (Cinar *et al*., 2001). Wang *et al*., (2004) showed that increased erythrocyte count was associated with a variety of the metabolic syndrome.

Hypertension has been involved in defect in rheological properties. Metabolic abnormalities may affect rheological properties by means of both direct and indirect effects. The direct effects are mediated via alterartions in erythrocyte membrane composition and plasma factors, whereas indirect effects include oxidative stress. Whole blood viscosity results from the interaction of hematocrit, erythrocyte aggregation, erythrocyte deformability, and plasma viscosity. The whole blood viscosity at high share rates is analysed by erythrocyte deformability (Zhang *et al*., 2006).

The high leukocyte values depend on stimulatory effects of cytotoxic agents on the immune system. It has been recorded that the platelet count is decreased because of that (Sforcin *et al*., 2002). According to results of our study, propolis may act as an immunostimulant. Ample stimulation of the immune system by L-NAME can increase the total leukocyte values. The present work suggests that propolis may perform a big function in maintaining homeostasis as an antagonist substance to L-NAME. Phenolic compounds found in structure of propolis, due to the antioxidant properties acted an important role in the prevention of many diseases heart disease and cancer which threaten life of alive. At the same time, phenolic compounds have been revealed by investigated studies the blood pressure lowering effect by increasing the permeability of capillaries (Prytzyk *et al*., 2003; Ryu *et al*., 2008; Maruyama *et al*., 2009; Gogebakan *et al*., 2012).

Propolis caused a protective effect against L-NAME induced liver damage and improved lipid profile. We suggest that propolis may be used to protect against to hypertensive effects of L-NAME in the prevention of hematopoietic organ injuries, liver and on other degenerative diseases

ACKNOWLEDGEMENT

Nigde University Research Fund (FEB 2008/25) is

gratefully acknowledged for support of this work. Thanks for his help to Dr. Engin Sahna at Firat University in Turkey about animal research.

REFERENCES

Abo-salem OM, El-Edel RH, Harisa GEI, El-Halawany N and Ghonaim MM (2009). Experimental diabetic nephropathy can be prevented by Propolis: Effect On Metabolic Disturbances and Renal Oxidative Parameters. *Pak. J. Pharm. Sci*., **22**(2): 205-210.

Aisha AFA, Abu-Salah KM, Alrokayan SA, Ismail Z and Majid AMSA (2012). Evaluation of antiangiogenic and antoxidant properties of *Parkia speciosa* Hassk extracts. *Pak. J. Pharm. Sci.*, **25**(1): 7-14.

Azizoglu A and Cengizler I (1996). An investigation on determination of some hematologic parameters in healthy *Oreochromis niloticus* (L.). *Turk J. Vet. Anim. Sci*., **20**: 425-431.

Bankova V, Popova M, Bogdanov S and Sabatini AG (2002). Chemical composition of European propolis: expected and unexpected results. *Naturforsch (C)*., **57**: 530-533.

Bhadauria M and Nirala SK (2009). Reversal of acetaminophen induced subchronic hepatorenal injury by propolis extract in rats. *Environ. Toxicol. Phar*., **27**: 17-25.

Blaxhall PC and Daisley KW (1973). Routine hematological methods for use with fish blood. *J. Fish Biol.*, **5**: 771-781.

Blaxhall PC (1981). A comparison of methods used for the separation of fish lymphocytes. *J. Fish Biol*., **18**: 177-181.

Cinar Y, Enyol AM and Duman K (2001). Blood viscosity and blood pressure: Role of temperature and hyperglycemia. *Am. J. Hypertens*., **14**: 433-438.

De Wilde MA and Houston AH (1961). Hematological aspect of the therma acclamatory process in the rainbow trout, *Salmo gairdneri*. *J. Fish Res. Biol*., **24**: 2267-2281.

El-Sayed MS, Abo-Salem OM, Aly HA and Mansour AM (2009). Potential Antidiabetic and Hypolipidemic Effects of Propolis Extract in Streptozotocin-Induced Diabetic Rats. *Pak. J. Pharm. Sci*., **22**(2): 168-174.

Gogebakan A, Talas ZS, Ozdemir I and Sahna E (2012). Role of propolis on tyrosine hydroxylase activity and blood pressure in nitric oxide synthase-inhibited hypertensive rats. *Clin. Exp. Hypertens*., **34**(6): 424-428.

Harrison DG, Gongora MC, Guzik TJ and Widder J (2007). Oxidative stress and hypertension. *J. Am. Soc. Hypertens.,* **1**(1): 30-44.

Jewet MG, Behmer DJ and Johnson GH (1991). Effects of hyperoxic rearing water on blood hemoglobin and hematocrit levels of rainbow trout. *J. Aquat. Anim. Health.,* **3**: 153-160.

Kanematsu Y, Tsuchiya K, Ohnishi H, Motobayashi Y, Izawa Y, Ishihara M, Ishizawa K, Abe S, Kawazoe K and Tamaki T (2006). Effects of angiotensin II type 1 receptor blockade on the systemic blood nitric oxide dynamics in N_{ω}-nitro-L-arginine methyl ester-treated rats. *Hypertens. Res*., **29**: 369-374.

Kumar S, Prahalathan P and Raja B (2011). Antihypertensive and antioxidant potential of vanillic acid, a phenolic compound in l-NAME-induced hypertensive rats: A dose-dependence study. *Redox Rep*., **16**(5): 208-215.

Maruyama H, Sumitou Y, Sakamoto T, Araki Y and Hara H (2009). Antihypertensive effects of flavonoids isolated from brazilian green propolis in spontaneously hypertensive rats. *Biol. Pharm. Bull*., **32**(7): 1244-50.

Mohammadzadeh S, Shariatpanahi M, Hamedi M, Ahmadkhaniha R, Samadi N and Ostad SN (2007). Chemical composition, oral toxicity and antimicrobial activity of Iranian propolis. *Food Chem*., **103**: 1097-1103.

Nadaud S, Dupuis M, Brocheriou I, Haloui M, Louedec L, Capron F, Michel JB and Soubrier F (2009). Counter-regulation by atorvastatin of gene modulations induced by L-NAME hypertension is associated with vascular protection. *Vasc. Pharmacol.*, **51**: 253-261.

Ogita H and Liao J (2004). Endothelial function and oxidative stress. *Endothelium*., **11**: 123-132.

Prytzyk E, Dantas AP, Salomão K, Pereira AS, Bankova VS, De Castro SL and Aquino Neto FR (2003). Flavonoids and trypanocidal activity of Bulgarian propolis. *J. Ethnopharmacol*., **88**: 189-193.

Ryu SY, Oh KS, Kim YS and Lee BH (2008). Antihypertensive, vasorelaxant and inotropic effects of an ethanolic extract of the roots of *Saururus chinensis*. *J. Ethnopharmacol*., **118**: 284-289.

Talas ZS, Ozdemir I, Gok Y, Ates B and Yilmaz I (2010). Role of selenium compounds on tyrosine hydroxylase activity, adrenomedullin and total RNA levels in hearts of rats. *Regul. Peptides*., **159**: 137-141.

Sahna E, Deniz E, Karabulut AB and Burna O (2008). Melatonin Protects Myocardium from Ischemia-Reperfusion Injury in Hypertensive Rats: Role of Myeloperoxidase Activity. *Clin. Exp. Hypertens*., **30**: 673-681.

Sforcin JM, Novelli ELB and Funari RC (2002). Seasonal effect of Brazilian propolis on seric biochemical variables. *J. Venom. Anim. Toxins*., **8**(2): 244-54.

Wang YY, Lin SY, Liu PH, Cheung BM and Lai WA (2004). Association between hematological parameters and metabolic syndrome components in a Chinese population. *J. Diabetes Complicat*., **18**: 322-7.

Zhang L, Pu K, Zhang SY and Ren WQ (2006). Blood rheological properties are strongly related to the metabolic syndrome in middle-aged Chinese. *Int. J. Cardiol*., **112**: 229-233.