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The investigation of antioxidant and anti-inflammatory potentials of apitherapeutic agents on heart tissues in nitric oxide synthase inhibited rats via Nω-nitro-L-arginine methyl ester

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ABSTRACT

Background: High blood pressure effects heart and vessels. Development of pathogenesis is the result of oxidative stress. We aimed to investigate the antioxidant effects of propolis, caffeic acid phenethyl ester (CAPE), and pollen on the hearts of rats which chronic nitric oxide synthase (NOS) inhibited through Nω-nitro-L-arginine methyl ester (L-NAME). Paraoxonase 1 (PON1), total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), asymmetric dimethylarginine (ADMA), and nuclear factor-κB (NF-κB) were analyzed on the heart.

Material and Methods: Sprague-Dawley rats were divided five groups of seven rats in every group; Group I: Control, Group II: L-NAME, Group III: L-NAME+propolis, Group IV: L-NAME+CAPE and Group V: L-NAME +pollen. L-NAME become dissolved in regular saline (0.9% NaCl w/v). The ethanolic extract of propolis (200 mg/kg/days, gavage), pollen (100 mg/kg/days, by gavage), CAPE (50 μM/kg/days, intraperitoneally), and the NOS inhibitor L-NAME (40 mg/kg, intraperitoneally) had been administered.

Results: Blood pressure (BP) of rats treated with propolis, CAP,E and pollen statistically significant decreased. Decreasing in BP of the rats of pollen group was more than CAPE and propolis groups ($P < .05$). PON1 and TAS levels decreased in L-NAME-treated groups ($P < .05$), but ranges have been better in propolis, CAPE and pollen groups. TOS, ADMA and NF-κB levels increased ($P < .05$) in L-NAME group; however, these parameters were lower ($P < .05$) in propolis and CAPE groups ($P < .05$).

Conclusions: Vasorelaxant properties and free radical scavenging actions of propolis, CAPE, and pollen may reduce the oxidative stress and blood pressure in the rats chronic NOS inhibited through L-NAME.

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Introduction

Hypertension is one of the most important risk factors in the pathogenesis of cardiovascular diseases, causes mortality and morbidity due to complications. Hypertension has a detrimental effect on many organs such as heart, kidney, brain and vascular systems (1). The main point in the emergence and development of this pathogenesis is the occurrence of oxidative stress due to the formation of excess reactive oxygen species (ROS). Excessive ROS production also causes adverse effects of contractile and relaxation factors in the endothelium such as nitric oxide (NO), angiotensin-II and endothelin-I. NO is an endothelium-derived vasoactive mediator that plays a key role in regulating blood pressure in the human body. It has been determined that many cardiovascular diseases, including hypertension, are caused by reduction in nitric oxide synthesis or by decreasing in its bioavailability (2,3). Chronic inhibition of nitric oxide synthase (NOS) through Nω-nitro-L-arginine methyl ester (L-NAME) is one of the most popular in experimental hypertensive animal models (4). Increased ROS in hypertension cause the degradation of oxidant-antioxidant equilibrium in living organisms, which in turn affects its physiological functions such as intracellular signal transduction and gene regulation. Asymmetrical dimethylarginine (ADMA), an analogue of methylated L-arginine and effective in the production of nitric oxide in vascular tissues, is a competitive inhibitor of NO. In previous studies have shown that increased plasma ADMA concentration levels are directly related to hypertension pathogens. ROS production in vascular tissues has an important role in providing homeostasis. The decreases in ROS levels are perceived as a physiological warning. It stimulates signal transduction, apoptosis and gene expression and activates transcription factors such as nuclear factor-κB (NF-κB). The stimuli that activate NF-κB can be inhibited by enzymatic and nonenzymatic antioxidant molecules. Various functional dietary supplements are recommended for the treatment of hypertension and cardiovascular diseases. In the studies conducted so far, it is known that different apitherapeutic products have positive results in the treatment of cardiovascular diseases. Propolis has been used from ancient ages due to its useful features in the popular medicine for the treat of various diseases (5,6). Most investigators have supported the pharmacological mechanisms as antiseptic, anti-inflammatory, antioxidant features of propolis with their studies (7,8). In addition, some researchers have focused on the antihypertensive and cardioprotective effects of propolis (9,10). The chemical composition

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of propolis contains many functional compounds. One of the most effective components of propolis is caffeic acid phenethyl ester (CAPE) which a flavonoid-like compound. CAPE is an antioxidant component that is widely used in studies of free radical scavenging and oxidative damage removal (11). Its most important feature is that it is small and lipophilic so it can easily pass through the cell membrane and easily show its effect. Pollen, as another honey bee product, is a source of protein, amino acid, mineral and vitamin for human health. The differences in biochemical composition (flavonoids, anthocyanins, tannins, etc.) may be according to the source. In addition to exogenous antioxidant therapeutics in the treatment of cardiovascular diseases, the endogenous antioxidant molecules produced in the human body and the synergistic effect between them are important. Paraoxonase 1 (PON1) is synthesized endogenously from the liver and released into the blood and is closely related to high density lipoprotein (HDL). PON1 also plays a role in the prevention of atherosclerosis by hydrolyzing the proinflammatory platelet activating factor. Previous studies have shown the serum PON1 activities decreased in atherosclerotic diseases, myocardial infarction, slow coronary flow, cardiac syndrome, hypercholesterolemia, and diabetes. The antioxidants rich in flavonoids might be cause a 20% increasing in PON1 activity in the serum (12).

The oxidative stress and hypertension have processes related to eachother and also the heart is one of the most important organs in hypertension case. Considering that both conditions, hypertension and oxidative stress, might affect the heart tissue. In this regard, we aimed to test the effects of bee products such as propolis and pollen as therapeutic agents on the heart tissues of the hypertensive rats with PON1, ADMA, NF-κB, TAS, and TOS parameters.

Methods

Animals

Twenty-eight male Sprague -Dawley rats with a medium body weight of 250–300 g were brought from the experimental animal center (Central Lab. Animal Research at Firat University, Elazig, Turkey). The animal room was always regulated at 21 ± 2°C and at 60 ± 5% relative humidity with a 12-h light/dark cycle. Experimental rats were applied according to the regulations confirmed by the Committee on Animal Research Center at Firat University.

Experimental procedure

Sprague -Dawley rats were divided into five groups of seven rats in each group: Group I (control group), Group II (L-NAME group), Group III (L-NAME+propolis), Group IV (L-NAME+CAPE group), and Group V (L-NAME+pollen). L-NAME was dissolved in normal saline (0.9% NaCl w/v). Distilled water was used to dissolve the ethanolic extract of propolis. Normal saline was administered to the rats in the control group intraperitoneally (i.p.) for 28 days. The L-NAME group was given nonspecific NOS inhibitor L-NAME (40 mg/kg; i.p.) for 28 days (13). The L-NAME + propolis group was given both L-NAME (40 mg/kg; i.p.) for 28 days and propolis (200 mg/kg/d; by gavage) (14) CAPE (Sigma-Aldrich; 50 μM/kg/d; i.p.) (15) and pollen (100 mg/kg/d; by gavage) (16) were administered on the last 14 of 28 days.

Preparation of propolis extractive solution

Propolis (thirty grams of obtained from Balikesir, Turkey) were cute to pieces and extracted with 600 mL of 70% (v/v) ethanol at 60°C for 30 min. After this process, the obtained blend was centrifuged and the supernatant was evaporated to full dryness under vacuum at 40°C. The dry product was kept at 4°C for further use.

Preparation of pollen extractive solution

Bee pollen (30 g) was extracted three times with 100 mL of ethanol solvent at room temperature for 1 h. After sonication (15 min), maceration, and filtration, the filtrate was evaporated to dryness under vacuum. Throughout this process, dried extracts were dissolved in ethanol and stored at 4°C until samples were analyzed.

Preparation of tissues for biochemical analyzes

The rats were sacrificed after being anesthetized with 75 mg/kg of sodium pentobarbital well. After the treatments, 4 mL blood was taken from the anesthetized animals by entering into the right ventricle their hearts. Then, the chests of rats were opened the vena cava was cut and 30 mL of 0.9% NaCl was injected into the heart to rinse blood from body. The hearts of the rats were removed and frozen in liquid nitrogen. After the heart tissue carefully removing, they were stored at −80°C until used. Tissues were weighed and then homogenized in 100 mL of 2 mM phosphate buffer, pH 7.4 using homogenizer. Homogenized samples were then sonicated for 1.5 min (30 s sonications interrupted with 30 s pause on ice). Samples were then centrifuged at 12,000g for 10 min at 4°C and supernatants, if not used for enzyme assays immediately, were kept in the deep freeze at −80°C. Supernatants were used for determination of biochemical parameters.

Blood pressure measurement

Blood pressure (BP) was measured three times during the study; the zero-day, fourteenth day and twenty eighth days. Measurement was performed by using tail cuff method (MAY BPHR 9610-PC Tail-Cuff Indirect Blood Pressure Recorder. Commat Ltd., Ankara, Turkey). The animals were placed in a heated chamber at an ambient temperature of 34–37°C for 10 min and blood pressure values 1 to 9 groups were recorded for each animal. The minimum three readings were averaged to calculate the mean blood pressure.

Determination of PON1 activity

In the absence of basal activity, PON1 activity measurements were made. The paraoxon hydrolysis (diethyl-p nitrophenyl phosphate) rate was measured by monitoring the increase in

absorbency at 37°C at 412 nm. PON1 activity was expressed in terms of U/L serum (17).

Measurement of TAS

An automated measurement method using strong biological hydroxyl radical was used to determine TAS levels in serum. In the experiment, the iron ion solution in reagent-1 and hydrogen peroxide (H_2O_2) in reagent 2 were mixed. Sequentially produced radicals were also potent radicals, such as the dianisidine radical cation that was produced by the hydroxyl radical. The data were expressed in terms of mmol Trolox equivalents per liter (equiv/L) (18).

Measurement of TOS

Serum TOS levels were determined by a method developed by Erel, a new automatic measurement method (19). The ferrous ion–o-dianisidine complex is oxidized by sample oxidants to ferric ion. In addition, the oxidation reaction increases glycerol molecules that are abundant in the reaction medium. The assay was calibrated using H_2O_2. In study was expressed the results in units of μmol H_2O_2 equiv/L (micromolar H_2O_2 equiv/L).

Oxidative stress index

Percentage ratios of TOS levels to TAS levels were calculated as OSI. For calculation, the resulting unit of TAS was changed to mmol/L, and the OSI value was calculated according to the following formula (20): OSI (Arbitrary Unit) = TOS (μmol H_2O_2 Equiv./L)/TAC (mmol Trolox Equiv./L).

Determination of ADMA levels

The serum ADMA concentrations were assessed by the ADMA ELISA kit (Hangzhou EASTBIOPHARM CO., LTD). This assay is based on the methodology of competitive enzyme linked immunoassays. The color deviates from blue to yellow, and the photometer measures the absorbance at wavelength of 450 nm.

Determination of NF-κB levels

To observe the NF-κB activity, a colorimetric assay was applied using a 96-well-enzyme-linked immunosorbent assay (ELISA; Hangzhou EASTBIOPHARM CO, LTD). This assay has been shown to provide quantification of NF-κB activity in several studies. The absorbency of the samples was monitored using a spectrophotometric microplate reader set at 450 nm.

Statistical analysis

Biochemical data were tested with SPSS statistical software (SPSS for Windows, SPSS Inc., Chicago, IL, USA, version 20.0) for Windows using a one-way analysis of variance (ANOVA). Differences between means were determined using with Tukey test as post hoc test in which the significance level was defined as $P < .05$.

Results

Blood pressure

Blood pressure (BP) is measured by tail-cuff method. L-NAME (40 mg/kg/days, intraperitoneally) which is a NOS inhibitor was administrated to rats for 28 days to produce hypertension and also propolis (200 mg/kg/days, by gavage), CAPE (50 μM/kg/days, intraperitoneally) and pollen (100 mg/kg/days, by gavage) were administrated the last 14 of 28 days. In BP did not make any significant changes ($P > .05$) in all experimental groups at 0th day (Table 1). After application of L-NAME for 14 days, BP in all groups were determined statistically ($P < .05$) significant increases on BP in compared to control group (Table 1).

Abbreviations: CAPE, caffeic acid phenethyl ester; L-NAME, Nω-nitro-L-arginine methyl ester

0th day: Normal blood pressure of rats before application. **14th day**: Blood pressure measured at 14th day after L-NAME administration. **28th day**: Blood pressure measured at 28th days after antioxidant supplementation (propolis, CAPE, and pollen administration).

All data points are the average of n = 7 with ± STDEVs. ^{abc}Statistically significant of the data among all groups in the line ($P < .05$).

Results of BP at 28th day of control, L-NAME, propolis, L-NAME+propolis, L-NAME+CAPE, and L-NAME+pollen groups are shown in Table 1. This levels at 28th day increased ($P < .05$) significantly in L-NAME group compared to the control (Table 1). The BP levels of rats administered L-NAME+propolis, L-NAME+CAPE, and L-NAME+pollen decreased statistically ($P < .05$) compared to L-NAME group (Table 1). The most important decreasing of BP levels among therapeutic groups were statistically ($P < .05$) determined to in L-NAME+pollen group compared to L-NAME+propolis and L-NAME+CAPE groups (Table 1).

Table 1. Changes in blood pressure at 0th, 14th, and 28th days.

	Experimental Groups					
Days (mmHg)	Group I (Control)	Group II (L-NAME)	Group III L-NAME+Propolis	Group IV L-NAME+CAPE	Group V L-NAME+Pollen	P-value
0th day	103.71 ± 3.56	104.75 ± 1.69	106.02 ± 1.99	104.29 ± 1.82	104.29 ± 1.12	<0.003
14th day	104.75 ± 1.69^{b}	141 ± 1.21^{a}	141.11 ± 0.53^{a}	143.3 ± 0.56^{a}	143.97 ± 0.53^{a}	0.84
28th day	104.29 ± 1.82 ^{c}	154.56 ± 1.59^{a}	122.7 ± 1.67^{b}	129.29 ± 0.97^{b}	111.13 ± 1.68 ^{c}	0.57

Abbreviations: CAPE, caffeic acid phenethyl ester; L-NAME, Nω-nitro-L-arginine methyl ester

0th day: Normal blood pressure of rats before application. **14th day**: Blood pressure measured at 14th day after L-NAME administration. **28th day**: Blood pressure measured at 28th days after antioxidant supplementation (propolis, CAPE and pollen administration).

All data points are the average of n = 7 with ± STDEVs. ^{abc}Statistically significant of the data among all groups in the line ($P < 0.05$).

Biochemical parameters

The changes in PON1 activities and TAS, TOS, OSI ADMA, NF-κB levels in the heart tissues of control (Group I), L-NAME (Group II), L-NAME+Propolis (Group III), L-NAME+CAPE (Group IV), and L-NAME+Pollen (Group V) treated rats in the end of 28th day are shown in Table 2.

Abbreviations: ADMA, asymmetric dimethylarginine; CAPE, caffeic acid phenethyl ester; L-NAME, Nω-nitro -L-arginine methyl ester; NF-κB, nuclear factor-κB; OSI, oxidative stress index; PON1, Paraoxonase 1; TAS, total antioxidant status; TOS, total oxidant status

All data points are the average of n = 7 ± STDEVs. [abc]Statistically significant of the data among all groups in the line ($P < .05$).

Activities of PON1 in heart tissues in the end of 28th day were determined as 5.71 ± 1.25 U/L in the control group, 3.14 ± 1.06 U/L in L-NAME, 4.14 ± 1.06 U/L, in the L-NAME+propolis group, 3.57 ± 1.71 U/L in the L-NAME +CAPE group and 5.00 ± 0.81 U/L in the L-NAME+pollen group. According to the data obtained, there were statistically significant ($P < .05$) decreases in the PON1 levels of the L-NAME group compared to the control group. The increasing in the PON1 activities in L-NAME+propolis and L-NAME +pollen groups compared with L-NAME group were statistically significant ($P < .05$). It was determined that there was no significant change in the PON1 activity of the CAPE plus group ($P > .05$) (Table 2).

TAS values determined in the heart tissues of rats were determined as 2.16 ± 0.04 mmol/L in the control group, 1.99 ± 0.05 mmol/L in the L-NAME group, 2.60 ± 0.12 mmol/L in the L-NAME+propolis group, 2.20 ± 0.07 mmol/L in L-NAME +CAPE group, and 2.15 ± 0.05 mmol/L in the L-NAME+pollen group. According to the determined results; TAS data of L-NAME group compared to the control group values, statistically significant decreases were found ($P < .05$). In plus groups with propolis, CAPE and pollen, TAS values increased statistically significant compared to L-NAME group ($P < .05$) (Table 2). The highest TAS was observed in the propolis plus group ($P < .05$) (Table 2).

TOS data were determined as 2.26 ± 0.07 μmol/L in the control group, 7.28 ± 1.75 μmol/L in the L-NAME group, 2.50 ± 0.72 μmol/L in the L-NAME+propolis group, 4.92 ± 0.74 in the L-NAME+CAPE group, and 4.16 ± 0.94 μmol/L in L-NAME+pollen group. According to the obtained values, when the TOS value of the L-NAME group compared with the control group, it was determined that there was statistically significant increase ($P < .05$). TOS results of heart tissues in L-NAME+propolis, L-NAME+CAPE, and L-NAME+pollen groups compared to L-NAME group, there were statistically significant decreases ($P < .05$) (Table 2).

TOS/TAS ratio as OSI in the heart tissues were determined as 0.09 ± 0.02 in the control group, 0.36 ± 0.08 in the L-NAME group, 0.09 ± 0.02 in the L-NAME+propolis group, 0.22 ± 0.03 in the L-NAME+CAPE group, and 0.19 ± 0.04, in the L-NAME +pollen group. According to these data; when OSI value of the L-NAME group compared to the control group value, a statistically significant increase ($P < .05$) was detected. When the OSI values of L-NAME+propolis, L-NAME+CAPE, and L-NAME+pollen groups compared to L-NAME group, there were statistically significant decreases ($P < .05$) (Table 2).

ADMA levels of animals were examined as 3.11 ± 0.31 ng/mL in the control group, 4.67 ± 0.29 ng/mL in the L-NAME group, 3.28 ± 0.43 ng/mL in the L-NAME+propolis group, L-NAME+CAPE group 3.46 ± 0.30 ng/mL, and 2.91 ± 0.33 ng/mL in L-NAME+pollen group. According to the results of the analyzes, when the ADMA levels of the L-NAME group compared to the control group, it was found that there were statistically significant increases ($P < .05$). It was determined that the heart tissue ADMA levels in L-NAME +propolis, L-NAME+CAPE, and L-NAME+pollen groups compared to L-NAME group result were statistically significant ($P < .05$) and were close to the control group values (Table 2).

NF-κB values in heart tissues of rats were obtained as 4.03 ± 1.06 mmol/mL in the control group, 6.30 ± 0.62 mmol/mL in the L-NAME group, 3.80 ± 0.88 mmol/mL in the L-NAME +propolis group, 4.17 ± 0.73 mmol/mL in the L-NAME+CAPE group, and 2.79 ± 0.83 mmol/mL in L-NAME+pollen group. According to the data obtained; when NF-κB levels in the L-NAME group were compared to the control group values, it was found that statistically significant increase ($P < .05$) occurred. NF-κB levels in experimental groups with propolis, CAPE and pollen, showed statistically significant decreases ($P < .05$) compared to the data of L-NAME group. The lowest NF-κB levels were observed in the pollen supplemented group ($P < .05$) (Table 2).

Discussion

In the study, the effects on blood pressure of rats and PON activity, TAS, TOS, OSI, ADMA, and NF-κB levels in rat heart tissues of chronic NOS inhibition created by L-NAME and

Table 2. Changes in biochemical parameters in the heart tissues with propolis, CAPE, and pollen administration in L-NAME-induced rats.

Groups / Parameters	Group I (Control)	Group II (L-NAME)	Group III (L-NAME+Propolis)	Group IV (L-NAME+CAPE)	Group V (L-NAME+Pollen)
PON1 (U/L)	5.71 ± 1.25[a]	3.14 ± 1.06 [c]	4.14 ± 1.06[b]	3.57 ± 1.71 [c]	5.00 ± 0.81[a]
TAS (mmol/L)	2.16 ± 0.04[b]	1.99 ± 0.05 [c]	2.60 ± 0.12[a]	2.20 ± 0.07[b]	2.15 ± 0.05[b]
TOS (μmol/L)	2.26 ± 0.07 [c]	7.28 ± 1.75[a]	2.50 ± 0.72 [c]	4.92 ± 0.74[b]	4.16 ± 0.94[b]
OSI (ratio)	0.09 ± 0.02 [c]	0.36 ± 0.08[a]	0.09 ± 0.02 [c]	0.22 ± 0.03[b]	0.19 ± 0.04[b]
ADMA (ng/mL)	3.11 ± 0.31 [c]	4.67 ± 0.29[a]	3.28 ± 0.43[b]	3.46 ± 0.30[b]	2.91 ± 0.33 [c]
NF-κB(mmol/mL)	4.03 ± 1.06[b]	6.30 ± 0.62[a]	3.80 ± 0.88[b]	4.17 ± 0.73[b]	2.79 ± 0.83 [c]

Abbreviations: ADMA, asymmetric dimethylarginine; CAPE, caffeic acid phenethyl ester; L-NAME, Nω-nitro-L-arginine methyl ester; NF-κB, nuclear factor-κB; OSI, oxidative stress index; PON1, Paraoxonase 1; TAS, total antioxidant status; TOS, total oxidant status

All data points are the average of n = 7 ± STDEVs. [abc]Statistically significant of the data among all groups in the line ($P < 0.05$).

propolis, CAPE and pollen treatments were investigated. The most useful hypertensive animal model used in hypertension studies in recent years is the chronic NOS inhibition created with L-NAME. The increasing in blood pressure plays an important role in pathogenesis of hypertension by inhibiting the production of NO, which is one of the main endogenous relaxation factors, and permanent activation of the renin-angiotensin-aldosterone system (RAAS). RAAS system has been clinical object for antihypertensive therapy, myocardial infraction, stroke andrenal disfunctions (21). Also, RAAS is important for sustainable of hemodynamic equilibrium and pathophysiology of hypertension. Foods and natural products such as some flavonoids and polyphenols have antihypertensive properties due to their vasodilating and hypotensive effects. Flavonoids are free radical scavengers and metal chelators, showing antihypertensive, and antiarthritic actions. In recent years, the attentions have been focused on the preservative features of exogenous antioxidants in biological systems, and on the mechanisms of their effects. Most herbal and beneficial foods have been investigated on their the blood pressure decreasing roles like extract of the roots of *Saururus chinensis* (22). Saravanakumar and Raja studied by phenolic veratric acid which one of the major benzoic acid derivatives from vegetables and fruits (23). The meta-analysis by Ried *et al.* with garlic supplementation showed antihypertensive and antioxidant properties against to L-NAME induced hypertension (24). Our study showed lowering effects on the blood pressure by propolis, CAPE and pollen administrating, and thus utilizable as antihypertensive and antioxidant against to hypertension.

Natural honeybee products such as propolis and pollen have also been shown to neutralize reactive oxygen species (ROS), and due to its free radical scavenging activity, honeybee products have been used in medicine since ancient times [25-28]. Increase of ROS has been directly linked with atherosclerotic disorders, such as hypertension and hypercholesterolemia (29). In addition, experimental works have demonstrated that CAPE inhibits ROS (30). Increase of ROS has been directly correlated to atherosclerotic disorders, such as hypertension and hypercholesterolemia. Nowadays, there are many works on hypertension, in which the relevant receptors and their inhibitions were analyzed by experimental approaches.

The antioxidants rich in flavonoids might be cause a 20% increasing in PON1 activity in the serum (12). In our work, there were statistically significant decreases in the PON1 levels of the L-NAME group compared to the control group. The increasing in the PON1 activities in propolis and pollen treatment groups compared with L-NAME group were statistically significant. These results might be related to the flavonoid compounds of natural bee products such as propolis and pollen (31). PON1 plays a role in the prevention of atherosclerosis by hydrolyzing the proinflammatory platelet activating factor (32-34). Previous studies have shown the serum PON1 activities decreased in atherosclerotic diseases, myocardial infarction, slow coronary flow, cardiac syndrome, hypercholesterolemia, and diabetes (35-36).

These therapeutic agents may contribute to direct vasorelaxant properties and to reduce the elevated blood pressure. The antioxidant system is in equilibrium with reactive oxygen products that are constantly formed in living organisms under physiological conditions. Excessive ROS production or weakening of antioxidant defense causes oxidative stress by causing structural and functional modifications in biomolecules. Hypertension; it is one of the clinical aspects with cause and effect relationship of oxidative stress, which is not known exactly, and it causes excessive production of O^{-2}.

The harmful effects of oxidants can be eliminated with endogenous and exogenous antioxidants. Free radicals in the living organisms causing raised oxidative stress and tissue damage under pathological status. Oxidative stress may contribute to the generation and sustainable of hypertension via diminishing of the NO by ROS. Endothelium-derived nitric oxide is synthesized by the endothelial enzyme NO synthase (e-NOS) from the amino acid L-arginine. ADMA plasma levels may be linked with measure of NO bioavailability. However, researchers reported an inverse relationship between ADMA, though in the levels of the physiologic concentrations, and endothelium-dependent vasodilation. Increased serum ADMA levels have been determined on hypertension patients in past studies. Takiuchi et al. found similar deterioration in endothelial function in coronary and peripheral vascular territories in hypertensives (37). They hypothesized that increases in serum ADMA levels may be the underlying factor connecting the two pathologic alterations. Korandji et al. analyzed the time course of dimethylarginine compounds and oxidative stress levels and the relationship between these and cardiovascular function in fructose-hypertensive rats. The result of them disclosed increasing levels of ADMA associated with the development of oxidative stress and hypertension (38). Taner et al. showed that ADMA levels were increased in the hypertension group when compared with normotensive diabetics (39). Perticone et al. emphasized to association between ADMA and insulin resistance contributes to identify a possible novel mechanism by which ADMA promotes vascular damage, increasing individual cardiovascular risk in hypertensive patients (40). Achan et al. showed as acute infusion of ADMA increasing their blood pressure, vascular resistance via decreased cardiac output and cardiac dysfunction (41). Besides, Surdacki et al. and Wang et al. showed elevated plasma levels of ADMA and decreased systemic NO generation, indicated as reduced urinary NOx secretion (42,43). Boger et al. showed a important adverse relation between ADMA plasma levels and diastolic BP in big population (44). In our study ADMA levels were enhanced by L-NAME administrating. According to the result, the reason of increasing may be NOS inhibition. This situation may be responsible for endothelial dysfunction in subjects with vascular diseases and in those with cardiovascular risk factors. Several studies have demonstrated the ability of phenolic compounds to inhibit oxidative stress in rats through various mechanisms. Thus, the potent antioxidant effect was exerted by phenolic compounds might contribute to decreasing of ADMA levels in rats. Our present data indicated that propolis, CAPE, and pollen treatments suppress oxidative stress in heart tissue of rats. In this study, we found elevated serum ADMA levels in hypertensive rats compared to healthy controls. Impaired NO production in parallel NOS generation may be due to increased ADMA levels in hypertension cases. It may be simply explained by an increase in the generation of

oxidative stress which react with NO to form peroxynitrite. Therefore, blood pressure increased. Propolis, CAPE, and pollen applied to hypertensive rats occurred reductions in ADMA levels. The among these antioxidants, the pollen has been have the most positive results in levels ADMA. We can say that propolis, CAPE, and pollen showed healing properties on the ADMA levels in hypertensive rats. These findings match that of previous works (13). In line with this, reduction of serum oxLDL, LDL, and/or increase in antioxidant capacity may lead to a decrease of ADMA concentration. Therefore, supplementation of antioxidants may provide a further efficient strategy to reduce ADMA levels beside homocysteine lowering by B vitamins. In our study, supplementation of antioxidants resulted in an improved antioxidant status as shown by elevated serum Vitamin E and slightly increased TEAC values. The hypertension shows proinflammatory effects on different tissues, and RAAS activation can activate the NF-κB pathway in monocytes and cultured vascular smooth muscle cells (45–47). NF-κB is a family of transcription factors that modulate DNA transcription. It plays a key role in regulating immune response to infection and the inflammatory response. Activation of NF-κB can be induced by various molecules, such as cytokines and ROS, and dominates the transcription of several genes involved in the inflammatory response, cell growth and adhesion. Activation of NF-κB has been observed in association with many renal diseases (48,49), and inhibition of the NF-κB system could significantly attenuate renal injury (50).

Super oxide radical, which is one of the important radicals of oxidative stress, can directly affect endothelial NOS and decrease the production of NO in the vascular system. Therefore, hypertension associated with oxidative stress and decreased production of vascular NO is directly related. Therefore, we want to observe whether both oxidative stress and hypertension can be eliminated in the heart tissue by applying bee products, by reducing both oxidative stress and contributing to NO production by means of L-arginine, which is the substrate of the NOS. Therefore, inhibition of ROS generation has a useful potential for the prevention of vascular events.

Conclusions

The incidence of hypertension is increasing worldwide, and also hypertension is important because of its complications. The treatment of hypertension and its complications are vital in this context. The results obtained from this study; may be a pioneer in new treatment in cardiovascular diseases due to the anti-inflammatory effect of propolis and bee products as result of NF-κB neutralization. It has been obtained that ethanolic extracts of propolis and pollen, which are honeybee products in the modulation of increasing blood pressure. Propolis and pollen are thought to help in the regulation of cardiovascular system functions by inhibiting the functioning of inflammatory pathways leading to hypertension. It can contribute to the development of antioxidant system, and may contribute positively to lowering blood pressure, natural bee products in the prevention of the formation, and progression of diseases that develop due to hypertension. We can say that the application of specific antioxidants may be more effective and therapeutic in terms of cardiovascular diseases.

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Disclosure statement

There are no conflicts of interest.

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