Effects of Extracts Obtained from *Nepeta italica* L. and *Nepeta cilicia* Boiss. Apud Bentham on Antioxidant Enzymes

Nepeta italica L. ve *Nepeta cilicia* Boiss. Apud Bentham Türlerinden Elde Edilen Ekstraktların Antioksidant Enzimler Üzerine Etkileri

Research Article

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ABSTRACT

In the study, the effects of methanol and hexane extracts obtained from *Nepeta italica* L. and *Nepeta cilicia* Boiss. apud Bentham on antioxidant enzymes in healthy rat erythrocyte were studied. The ethanol extract of N. cilicia caused a decrease in glucose-6-phosphate dehydrogenase (G6PD) activity and an increase in catalase (CAT) activity. There wasn't significant enzyme value in the group treated with the hexane extract of *N. cilicia*. However, the ethanol extract of *N. italica* caused a decrease in superoxide dismutase (SOD) and CAT activities. In group treated with hexane extract of *N. italica*, a significant decrease in SOD and CAT activities was observed, whereas a significant increase in G6PD activity was observed. Also, a statistically significant difference in MDA wasn't found in all groups. In conclusion, it may suggest that the extracts are devoid of pro-oxidant properties, because lipid peroxidation hasn't been observed, although the extracts have caused some changes in antioxidant enzymes.

Key Words

Nepeta species, Antioxidant enzymes, Lipid peroxidation

ÖZET

Bu çalışmada, *Nepeta italica* L. and *Nepeta cilicia* Boiss. apud Bentham türlerinden elde edilen hekzan ve metanol ekstraktlarının sağlıklı rat eritrosit antioksidan enzimleri üzerine etkileri araştırılmıştır. *N. cilicia* etanol ekstraktı, glukoz-6-fosfat dehidrogenez (G6PD) aktivitesinde azalmaya, katalaz (CAT) aktivitesinde artışa neden olmuştur. *N. cilicia* hekzan ekstraktı uygulanan grupta, enzim değerlerinde anlamlı bir fark bulunmamıştır. Bununla birlikte, *N. italica* etanol ekstraktı, süperoksit dismutaz (SOD) ve CAT aktivitelerinde azalmaya neden olmuştur. *N. italica* hekzan ekstraktının uygulandığı grupta ise G6PD aktivitesinde artış gözlenirken, SOD ve CAT aktivitelerinde azalma gözlenmiştir. Tüm gruplarda, malondialdehid (MDA) seviyelerinde istatistiksel olarak anlamlı bir fark bulunmamıştır. Sonuç olarak, ekstraktların antioksidan enzimlerde bazı değişimlere neden olmasına karşın lipid peroksidasyonu gözlenmemiştir, bu nedenle bitki ekstraktlarının pro-oksidan bir özellik göstermediği söylenebilir.

Anahtar Kelimeler

Nepeta türleri, Antioksidan enzimler, Lipid peroksidasyonu.

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INTRODUCTION

Reactive oxygen species (ROS) are free radicals which responsible of oxidative stress in living organism. ROS are appeared as a factor in many illnesses and/or result of many illnesses. Many defense mechanisms, which are used to prevent the damage and formation of ROS, are called as antioxidant defense system or shortly antioxidant [1].

Antioxidant defense system is divided to groups which are in enzymatic and non-enzymatic structure. Some non-enzymatic antioxidants are vitamin E, vitamin C, vitamin A (β-carotene), flavonoids, selenium, transferrin and laktoferrin [2]. Catalase (CAT), superoxide dismutase (SOD), and glucose-6-phosphate dehydrogenase (G6PD) are the most important antioxidant enzymes in cells [1]. SOD accelerates the dismutation of superoxide (O_2^-) to hydrogen peroxide (H_2O_2), which is considered a primary primary defense, as it prevents further generation of free radicals. Catalase catalyzes the removal of H_2O_2 formed during the reaction catalyzed by SOD. G6PD is the first and regulator key enzyme of the pentose phosphate pathway. NADPH (nicotinamide adenine dinucleotide phosphate) formed by G6PD play a role in the protection of sulfhydryl groups, in the reaction of free radicals and peroxide [3].

In recent years, it has been demonstrated that medicine and mixture including antioxidant are protecting and curative in arteriosclerosis, diabetes, alzheimer and cancer [4]. However, the antioxidant properties of many herbs and spices have been reported [5], and this has raised a growing interest in phytochemicals of plants worldwide.

Many plant extracts and their productions have antioxidant properties. Moreover, it has been reported biological effectiveness of the antioxidants, such as antibacterial, antiviral, antiallergic [6, 7]. Nevertheless there are studies showing that plants have pro-oxidant features [8, 9].

The genus *Nepeta* (Lamiaceae) is represented by approximately 280 species, that are distributed over Asia, and also Europe and Africa and 33 species are present, 17 of which are endemic in Turkey [10].

Nepeta species are widely used by the reason of their antiseptic, diuretic antitussive, and antiasthmatic activities in folk medicine. [11]. There are many studies on biological activities of *Nepeta* genus in literature. It has been reported that some *Nepeta* species have insect repellant [12], analgesic [13], anti-inflammatory [14], anti-fungal and antimicrobial [15] and sedative activities [16].

Most conducted studies on Nepeta species were to determine their essential oil composition and the biological activities (17, 18, 19, 20). In a previous study, a major compound of the essential oil of Nepeta flavida Hub-Mor. was found to be 1,8-cineole, and it has been reported that 1,8-cineole has remarkable antioxidant activity (18). Nevertheless, Alim et al. (2009) found that the antioxidant capacity of essential oil of Nepeta nuda L. subsp. albiflora (Boiss.) Gams. is weak, but it has a strong antibacterial activity (5). In another study conducted with methanol extract of Nepeta sibthorpii Bentham, a direct correlation was found between the anti-inflammatory activity and antioxidant capacity of the plant (14). In addition, the extract of Nepeta cataria L. and its essential oil have shown not only antioxidative activity but also antimicrobial, bacteriostatic and fungistatic activities (19, 21).

Nepeta species contain monoterpenes, sesquiterpenes and cyclopentanoid iridoid derivatives, as well as nepetalactone (22). The essential oil of *N. italica* has been characterized by a high percentage of monoterpenes (95%), mainly 1,8-cineole (20). However, the major component of essential oil of *N. cilicia* has been found to be β-caryophyllene oxide (% 40.7), a sesquiterpene (23). In another study, limonene was the major component, and chemical differences between the plant contents are explained via difference in collecting area (24). According to our literature survey, in vitro antioxidant activity of *Nepeta* species has been studied (5, 14, 17, 18, 19), but we could not find report dealing with in vivo antioxidant activity of the *Nepeta* species.

The aim of this study was to evaluate the effects of ethanol and hexane extracts obtained from *N. italica* and *N. cilicia* species in rat erythrocyte antioxidant enzymes, SOD, CAT and G6PD, and malondialdehyde (MDA) levels.

MATERIALS AND METHOD

Animals

Three-month male Spraque-Dawley rats weighing 200–250 g were used. The current work was carried out after approval by our institutional animal ethical committee.

Collection of plant materials and preparation of the extracts

Wild growing *N. italica* and *cilicia* was collected at the stage of full blooming. The voucher specimen was identified by Dr. Hasan Özçelik[1] and Dr. Hasan Genç[2] at the Department of Biology, [1]Suleyman Demirel University and [2]Mehmet Akif Ersoy University and has been deposited at the Herbarium of the Department of Biology, Suleyman Demirel University, Isparta-Turkey.

The ground plants were dried in the shade at room temperature and then pulverized. Extracts of plant materials were prepared by using solvents of varying polarity. The extraction protocol of each is given below:

50 g of dried plant materials was extracted with hexane (HE), followed by ethanol in soxhlet apparatus (3h for each solvent). All extracts obtained were kept in the dark at +4°C prior to use.

Experimental diet and design of animals

In this experiment, a total of 40 rats were used. The rats were divided into 5 groups (control and plant extracts treated) of 8 animals each, and were kept separately in cages with temperature at 25°C with controlled lighting. The animals fasted for 6 hours without water restriction before the experiment. The extract treated animals received 1 g/kg body weight orally a single dose by gastric tube. Control rats were treated with the same volume of distilled water. Animals were killed by cervical dislocation 3 hours after the dose and blood was collected from the heart into heparinized eppendorf tubes. Fresh blood collected from the rats under the study was centrifuged for 5 min; 2500x g: Preparation of the haemolysate was done as described elsewhere [25]. The haemolysates were kept at -80°C until analysis.

Biochemical measurements

G6PD activity determination

G6PD activity was measured spectrophotometrically as described by Beutler [26]. The activity measurement was done by monitoring the increase in absorption at 340 nm due to the reduction of $NADP^+$ at 37 °C. One enzyme unit represents a reduction of 1 mmol of $NADP^+$ min^{-1} at pH 7.0.

SOD activity determination

SOD activity was assayed using the nitroblue tetrazolium (NBT) method of Sun et al. [27]. The principle of SOD activity assay was based on the inhibition of NBT reduction. The reduction of NBT by superoxide radicals to blue colored formazan was followed at 560 nm.

CAT activity determination

CAT activity was determined according to Aebi's method [28]. The principle of the method was based on the determination of the rate constant (s^{-1}, k) for the H_2O_2 decomposition rate at 240 nm.

Lipid peroxidation (LP) determination

The erythrocyte MDA concentration was determined using the method described by Jain et al. [29], based on thiobarbituric acid (TBA) reactivity.

Statistical analysis

Values were represented as means±SD. Differences between the means were estimated by a one-way analysis of variance, p values greater than 0.05 were considered insignificant. All statistical analysis was performed using SPPS 15.0 packet program.

RESULTS AND DISCUSSION

In the study, G6PD, CAT, SOD enzyme activities and MDA levels were examined in rats treated with the extracts obtained from N. italica and N.cilicia species. According to statistical significance, results are shown in Table 1.

In the group treated with the hexane extract of N. cilicia, the increased G6PD activity was statistically insignificant and there was no significance for other enzyme values when compared to the control group. In a previous study, ethanol extract of Terminalia arjuna has been treated to healthy rat group; it was

found that there is no statistical difference in the antioxidant enzyme activities and lipid peroxidation level. In the same study, the extract has exhibited the antioxidant properties when some dose has been given to diabetic rats [30].

In the group treated with the ethanol extract of *N. cilicia*, the decreased G6PD activity was found, whereas there was a significant increase in CAT activity compared to the control group ($p<0.05$). G6PD catalyzes the conversion reaction of glucose to glucose-6-phosphate producing one of the main reducing equivalents of the cell NADPH. NADPH plays a role in protecting oxidant/antioxidant balance in the cell and reducing the oxidative stress. Hence, the decrease in G6PD activity means less NADPH formation [31, 32]. Karuna et al. (2009) [33] found that extract of *Phyllanthus amarus* given to healthy rats resulted in a significant decrease in lipid peroxidation and a significant increase in SOD, CAT and non-enzymatic antioxidant activities. They have suggested that the non-toxic nature of the extract, and consumption of the extract, can be linked to improved antioxidant status and reduction in the risk of oxidative stress.

The hexane extract of *N. italica* caused a significant increase in G6PD activity compared to the control group. The increased G6PD activity probab1y reflects an adaptation response to oxidative stress which is in ion effect [34]. Moreover, it is known that the use of NADPH for different biological functions also causes an increase in G6PD activity. Also, treatment of ethanol and hexane extracts of N.italica resulted in decreased SOD and CAT enzyme activities ($p<0.05$). It leads us to believe that the decrease in enzyme activities may be related to other mechanisms, or different doses of the plant should be examined.

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The distribution of SOD enzyme in living organisms should be examined with CAT. SOD and CAT are the most important defense enzymes against toxic effects of oxygen metabolism. It is suggested that excessive superoxide causes differences in SOD activity [35]. However, increased H_2O_2 leads to lipid peroxidation on membrane lipid, inactivation of SOD activity, and damage of DNA [36, 37]. Husain et al. (2001) [38]has reported that inhibition of SOD activity may be a consequence of decreased *de novo* synthesis of SOD proteins or irreversible inactivation of enzyme proteins from increased free radical production. Another explanation regarding the decrease in the enzyme

Table 1. G6PD, CAT, SOD activities ve MDA levels

		Nepeta cilicia		Nepeta italica	
	Control (X ± SD)	Hexane (X ± SD)	Ethanol (X ± SD)	Hexane (X ± SD)	Ethanol (X ± SD)
G6PD (EU/mL)	0.635± 0,05	0.751±0.01	0.401±0.09*	2.35±0.27*	0.634±0.19
CAT (EU/mL)	453±66	427±69	657±60*	139±18*	140±16*
SOD (EU/mL)	0.021±0.001	0.020±0.002	0.022±0.002	0.008±0.002*	0.007±0.001*
MDA (mmol/L)	1.64±0.26	1.2±0.649	1.17±0.53	1.26±0.44	1.18±0.42

Significant (*P < .05) compared with control (means±SD, n:8)

activities is that the free radicals are low, so there is no need for SOD activities.

Antioxidant activity is related to both urosilic acid and phenols in plants [14], but some phenol antioxidants can accelerate oxidative damage of DNA, protein and carbohydrates [39]. Ajiboye et al. (2010) [40] found that antioxidant enzymes significantly decreased administration of the extract in a dose-dependent manner, while MDA levels increased.

MDA is the last product of lipid peroxidation and a carcinogen compound [41]. When SOD and CAT enzymes are inadequate in oxidative stress, free radicals can induce lipid peroxidation reaction. Therefore, MDA is one of the most sensitive indicators of oxidative stress [42, 43]. According to our findings, there was an insignificant decrease in MDA levels in all groups ($p > 0.05$). The decreased MDA levels might indicate that negative effects of free radical are removed.

In conclusion, we found that the extracts didn't cause lipid peroxidation, which has destructive effects on the body defense system, although antioxidant enzymes altered. Therefore, it suggests that the extracts are devoid of pro-oxidant properties and plants have different effects on antioxidant enzymes.

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