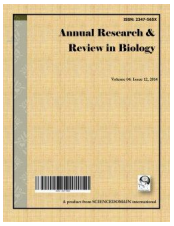

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Nerium oleander Distillate Can Reduce Oxidative Deoxyribonucleic Acid Damage in Rats Fed with High Cholesterol Diet

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EM and BD designed the study, performed the statistical analysis, and wrote the first draft of the manuscript. Authors AS and ALB wrote the protocol and managed the analyses of the study. Authors EM, BD and MT managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the effect of *Nerium oleander* distillate on the high cholesterol diet(HCD) induced oxidative deoxyribonucleic acid damage via assessing blood 8-hydroxy-2'-deoxyguanosine(8-OHdG) and superoxide dismutase(SOD) levels.
Methodology: Twenty-one male Sprague-Dawley rats were divided equally into three groups. The first group (control group) was fed a normal diet and administered 0.5 ml distilled water via gavage for 90 days. The second and third groups were fed with HCD. The second group was administered 0.5 ml distilled water and the third group was administered 0.5 ml *Nerium oleander* distillate(0.375 mg/rat) via gavage for 90 days, after being fed the HCD for two weeks. Blood samples were collected, and 8-OHdG and SOD levels were measured.

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Results: 8-OHdG levels were statistically significantly different in all groups. Highest 8-OHdG levels were determined in the second group whereas *Nerium oleander* treatment reduced the level of 8-OHdG. In addition, decreased SOD levels were detected in the rats fed with HCD(Groups 2 and 3) when compared to the control group.
Conclusion: It may be stated that HCD may cause oxidative damage in deoxyribonucleic acid and *Nerium oleander* distillate may reduce this damage. Hence, *Nerium oleander* distillate may show beneficial effects in the treatments of diabetes mellitus, hypercholesterolemia, and metabolic syndrome. In the future, it should investigate the effect of *Nerium oleander* distillate on different antioxidant pathways.

Keywords: Nerium oleander; 8-hyroxy-2'-deoxyguanosine; superoxide dismutase; hypercholesterolemia.

1. INTRODUCTION

Nerium oleander of the dogbane (Apocynaceae) family grows along the whole Mediterranean coast starting in southern Portugal, Syria, and streambeds of the Western and Southern Anatolia. In the literatures, cardiotonic, antibacterial, anticancer, antidiabetic, anti-hyperlipidemic and anti-platelet aggregation activities of various extracts of *Nerium oleander* have been reported [1-5]. In addition to these effects of *Nerium oleander*, *Nerium oleander* may show antioxidant activity, as well [5-7]. It has been determined that *Nerium oleander* distillate (NOD) has no toxic effect when acute and subchronic administration to rats [8,9], and in vitro in cell line [10].

Oxidation and reduction of molecules, occur in cells, and these reactions can lead to the productions of free radicals [11]. Free radicals generate some reaction with organic substrates such as proteins, lipids and deoxyribonucleic acid(DNA), and they damage these substrates. The status disrupts their normal function and may contribute variety of diseases. Thus, reactive oxygen species(ROS) and antioxidant capacity are commonly analyzed for determining the oxidative damage in various diseases. The defense system includes enzymes such as superoxide dismutase(SOD), glutathione peroxidase(GPx) and catalase that decrease ROS concentrations [11,12]. SOD converts superoxide radicals into oxygen and hydroperoxide, and it has an important role in removal of excess of superoxide radicals in aerobic organisms [13]. These enzymes counteract oxidative damage and target damaged molecules for destruction and replacement [11].

8-hyroxy-2'-deoxyguanosine (8-OHdG) was identified by Kasai and Nishimura [14] as a marker of oxidative damage due to its sensitivity and mutagenic potential [15]. Guanine has the lowest ionization potential consisting of the DNA [16,17]. Ionizing radiation, chemicals, endogenous cell metabolism products, drugs, solar light, cigarette smoking, and air pollution can induce oxidation of DNA components [15]. Besides, biomarkers of oxidative DNA damage are a great of interest and can potentially be used for the early detection of disease, monitoring the progression of the disease and determining the efficacy of therapy [18]. Polyunsaturated fatty acid and hypercholesterolemic diets induce DNA damage [19,20].

To our best knowledge, there is no investigation that the effect of NOD on oxidative DNA damage in rats fed high cholesterol diet (HCD). The aim of the present study is to evaluate the effect of NOD on oxidative DNA damage and SOD levels in rats fed HCD.

2. MATERIALS AND METHODS

2.1 Preparation of *Nerium oleander* Distillation and Dosage

Nerium oleander plant was collected among new shoots in March-September period from the Mediterranean region of Turkey. The plant was identified by the Biology Department, University of Selcuk, Konya, Turkey. Initially, the collected plant was washed, fresh shoots were chopped, and adequate distilled water was added. The mixture was heated in a heat resistant container. After the liquid started to evaporate, the container lid was covered, and vapor was separated to other clean glass containers by causing it to come in contact with a surface cooled with cold water. *Nerium oleander* distillate (NOD) was lyophilized by using lyophilizator. Lyophilized NOD was dissolved at concentrations of 750 µg/ml in distilled water.

2.2 Experimental Design

The experimental protocol was approved by the Ethics Committee in Animal Experimentation of Selcuk University, Turkey. (No: 2011/059). Twenty one male Sprague Dawley rats (6–8 weeks) were allocated to metabolic cages individually in an automatic ambient humidity (50 ± 5%), temperature (22 ±2ºC), and light-dark (12 : 12) controlled room.

The rats were divided into equal three groups; group 1 (n=7) was a control group which was fed with commercially normal diet during the experiment, and 0.5 ml distillated water was administrated via gavage for 90 days. Group 2 and group 3 were fed 1% cholesterol diet for 2 weeks. After hypercholesterolemia (HC) was induced, the animals were maintained with cholesterol diet (Table 1) during the experiment [21]. In addition, distillated water (0.5 ml) was administrated via gavage rats in group 2 (n=7) for 90 days after two weeks following HC. Group 3 (n=7) was another HC group, and the group was treated 0.5 ml distillate NOD (0,375 mg/rat) via gavage for 90 days two weeks after inducing the HC. NOD administrations were carried out once a day in all groups.

Table 1. High cholesterol diet composition

Ingredients	%
Cholesterol	1
Lard	5
Soybean oil	5
Casein	20
Corn starch	35.1
Sucrose	20
AIN-76 minerals	3.5
AIN-76 vitamins	1
Choline	0.4
Methyl-cellulose	9

2.3 Oxidative and Antioxidant Evaluations of the Animal Groups

Blood samples were collected from cardiac puncture into non-heparinized tubes under general anesthesia and centrifugated at 1600 g for 10 minutes. The serum samples were analyzed to determine the levels of 8-hydrox-2-deoxyguanosine (BIOXYTECH 8-OHdG-EIA Kit, OXIS Int, CA, USA, cat no: 21026) and superoxide dismutase (Cayman Chemical Superoxide Dismutase Assay Kit USA, cat no: 706002) by using Enzyme-Linked ImmunoSorbent Assay (ELISA) method according to the manufacturer's protocol with Rayto RT 2100 C microplate reader (Rayto Electronics, China).

2.4 Statistical Analysis

All experimental values are presented as mean ± standard error (SE). The data were analyzed by ANOVA, and the Duncan test was used as a *post-hoc* test to analyze the differences of the groups (SPSS 22.0). $P < 0.05$ was considered statistically significant.

3. RESULTS

8-hydroxy-2'-deoxyguanosine and SOD levels are presented in Figs. 1 and 2. The levels of control and group 3 were significantly lower than levels of group 2 ($p<0.05$). The highest level of 8-OHdG was found in group 2, whereas the levels were statistically different from the levels of group 3 ($p<0.05$).

Higher SOD levels were detected in control group when compared to group 2 and 3 ($p<0.05$). However, between levels of group 2 and 3 were not statistically significant different ($p>0.05$).

4. DISCUSSION

Nerium oleander, a small tree of all Mediterranean region [1,2], may show antioxidant [5,7], antihyperglycemic and antihyperlipidemic [1,2]. Although the researchers have focused on the antioxidants effects of *Nerium oleander* [5,7], it has not been fully understood the effect of NOD on the oxidative stress induced DNA damage.

As it is well known that hydroxyl radicals attack bases and leads to mutations. The most affected bases are guanine and cytosine in oxidative stress. Guanine has the lower ionization potential compared to another component of DNA, therefore it is powerfully attacked by free radicals. 8-OHdG, is a modified base and is formed because of attacks to its C8 by hydroxyl radicals. It is a sensitive marker of oxidative DNA [14]. In the present study, 8-OHdG levels of group 2 were statistically higher than the control group and group 2, whereas NOD reduced 8 OHdG levels in the group 3 ($p<0,05$, Fig 1). HCD increases the oxidative DNA damage [20]. Haegele et al. [22] determined important positive correlation of 8-OHdG concentration with diet lipid iodine number, which was a function of the degree of unsaturated fatty acid. In the result;

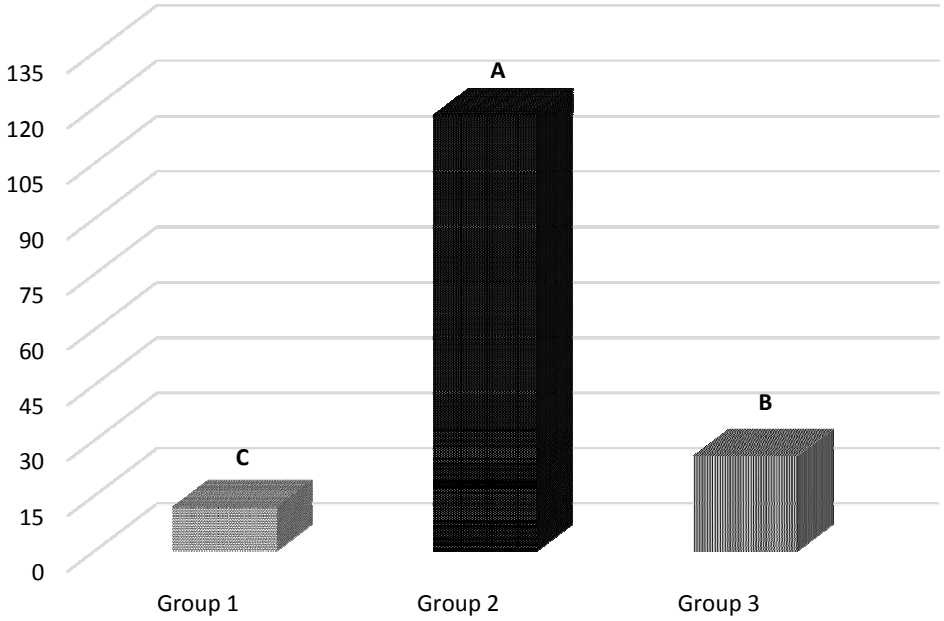

Fig. 1. 8-OHDG levels after NOD treatment (0.375 Mg/Rat, P.O.) in rats fed high cholesterol diet

^{A,B,C} : Different letters are statistically significant (p<0,05).

Group 1: Healthy Control, Group 2: High Cholesterol Diet, Group 3: NOD Treatment (0.375 Mg/Rat, P.O.) and High Cholesterol Diet

8-OHdG : 8-hydroxy-2' -deoxyguanosine, NOD : Nerium oleander distillate

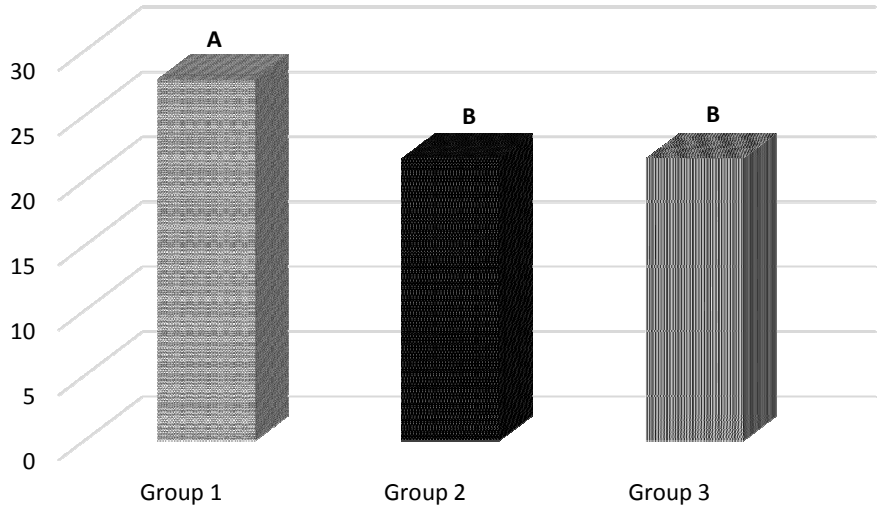

Fig. 2. SOD levels after NOD treatment (0.375 Mg/Rat, P.O.) in rats fed high cholesterol diet

^{A,B} : Different letters are statistically significant (p<0,05).

Group 1: Healthy Control, Group 2: High Cholesterol Diet, Group 3: NOD Treatment (0.375 Mg/Rat, P.O.) and High Cholesterol Diet

SOD : Superoxide Dismutase, NOD : Nerium oleander distillate

NOD decreases the oxidative DNA damage in hypercholesterolemia rats. Therefore, NOD has a lipid lowering effect and may rearrange the oxidative DNA damage as positive by

intercepting oxidation of DNA. High fat diet causes a significant increase in cardiac lipids and lipoproteins. Extract of *Nerium oleander* treated to increase in lipid and lipoprotein levels. They highlight the possible mechanism of *Nerium oleander* as a hypolipidemic agent in its use in medicine [23]. NOD has different anti-diabetic and anti-cholesterolemic effects as a dose dependent manner [2]. In the present study, although NOD has significantly decreased 8-OHdG levels in hypercholesterolemia, it is not as much as control ($p<0,05$, Fig. 1). These results can be attributed to the dose manner or oxidative DNA damage could be prevented by the catalase enzyme in the oleander plant [7,24,25]. If the administration doses of *Nerium oleander,* increases these levels may reach to the control levels.

Chemical reactions, including oxidation and reduction of molecules, occur in every cell. These reactions can lead to the productions of free radicals [11]. Free radicals interact giving a reaction with proteins, lipids and DNA [11,12]. Antioxidant enzymes neutralize the exposition of reactive oxygen species (ROS) [14]. The defense system includes enzymes such as SOD, GPx and catalase that decrease ROS concentrations [11,12]. SOD converts superoxide radicals into oxygen and hydroperoxide, thus removes excess of superoxide radicals in aerobic organisms [13]. These enzymes counteract oxidative damage and target damaged molecules for destruction and replacement [11]. The cytotoxic effects of free radicals are the initiation of peroxidation of polyunsaturated fatty acids in the membrane or plasma lipoproteins, the direct inhibition of mitochondrial respiratory chain enzymes, the activation of membrane sodium channels and other oxidative modifications of proteins [26]. High fat diet prompts metabolic pattern inducing ROS production in mitochondria. Antioxidants from plant sources may be an imperative remedy against the chronic disorders. Hyperlipidemia could destroy the antioxidant defense system by decreasing the activity of SOD and elevating the lipid peroxide levels [27]. Furthermore, hypercholesterolemia increases the cholesterol content of platelets, polymorph nuclear leukocytes and endothelial cells; which leads to the formation of free radicals; and accelerate the process of lipid peroxidation [28]. Wang et al. [29] defined SOD levels significantly decreased in the rats fed with high fat diets and increased administration of aqueous enzymatic extract from rice bran. Methanol and aqueous methanol of *Nerium oleander* extracts effect antioxidant capacity. The extracts have different levels of efficacy in a dose-dependent manner [7]. In addition, methanolic flower extract of *Nerium oleander* reduces lipid peroxide, hydroxyl radical, superoxide anion, 2,2-diphenyl-1-picrylhydrazyl and 2,2′-azinobis [30]. In the current research, HCD decreased antioxidant defense in metabolism and SOD levels are not different in group 2 and 3 and statistically significant from the control group ($p<0,05$, Fig. 2). There were no important changes in, to point out the effects of NOD on the antioxidant system, it may be sample different efficient solvents for extraction or different parts of *Nerium oleander*.

5. CONCLUSION

In conclusion, NOD reduced the level of 8-OHdG, which is accepted as damaged DNA with oxidation, but it has no effect the SOD level in hypercholesterolemic rats. It may be stated that the antioxidant effect of NOD on the DNA may not be related to the SOD activity. However, NOD may have other antioxidant enzymes or may induce them. Further studies are needed to elucidate the effect of NOD on antioxidant-oxidant system enzymes in hypercholesterolemia.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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