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Original article

Effects of *Nigella sativa* oil and thymoquinone on radiation-induced oxidative stress in kidney tissue of rats

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ABSTRACT

Ionizing radiation leads to release of free radicals into the systemic circulation from irradiated tissues. These free radicals cause oxidative stress in distant organs. Oxidative status may be reversed by naturally occurring antioxidant agents. The aim of this study was to investigate protective and antioxidant effects of *Nigella sativa* oil (NSO) and thymoquinone (TQ) in kidney tissue of rats exposed to cranial irradiation. Forty-eight Sprague-Dawley rats were divided into six groups: *IR group* received irradiation (IR) to total cranium plus saline; *IR plus NSO group* received IR and NSO; *IR plus TQ group* received IR and TQ; *sham group* did not receive NSO, TQ or IR; *control group of TQ* received dimethyl sulfoxide; *control group of NSO* received saline. Total oxidant status (TOS), oxidative stress index (OSI) and lipid hydroperoxide (LOOH) levels were studied as oxidative parameters, and total antioxidant status (TAS), total sulfhydryl levels, paraoxonase (PON), ceruloplasmin (Cp) and arylesterase activities were determined as antioxidative parameters in the kidney tissue of rats. Kidney TOS, OSI and LOOH levels were significantly lower in *IR plus TQ*, *IR plus NSO* and *sham groups* compared to *IR group* ($p < 0.001$). TAS, PON and Cp activities in *IR group* were significantly lower compared to the control group ($p < 0.001$). PON and Cp activities were significantly higher in *IR plus NSO* and *IR plus TQ groups* compared to *IR group* ($p < 0.001$). In conclusion, free radicals generated by cranial ionizing radiation exposure cause oxidative stress in kidney. NSO and TQ exhibit protective and antioxidant effects against oxidative damage in rats.

1. Introduction

Ionizing radiation is widely used in management of malignant diseases. Effects of ionizing radiation occur either directly or indirectly after penetration into tissues. Ionizing radiation may affect directly with breaks in DNA and/or indirectly with generation of free radicals via radiolysis of water which is the major component of human body. Radiolysis of water results in generation of reactive oxygen species (ROS) [1] which interact with DNA, RNA, proteins and membranes, and finally cellular damage takes place [2]. Furthermore, ionizing radiation exposure leads to release of free radicals into the systemic circulation from the irradiated tissues which may generate oxidative stress in distant organs [3].

Ionizing radiation injury may be seen in hematopoietic, gastrointestinal, and central nervous systems, therefore, use of protective and antioxidant compounds became important. In this respect, effects of various agents in prevention of normal tissues against radiation induced oxidative stress have been investigated [1,4,5]. Radioprotective agents are capable of decreasing free radical formation and improving antioxidant status, and as a result may ameliorate DNA damage and reduce

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radiation induced inflammation [1].

Nigella sativa oil (NSO) and its essential oil component, thymoquinone (TQ) are reported to exhibit important antioxidant properties against generation of free radicals which are responsible in cellular oxidative damage. Antioxidant, hepatoprotective, neuroprotective, antidiabetic, antiinflammatory, nephroprotective, and anticarcinogenic properties of NSO and TQ are reported in many studies [6–12]. NSO and TQ establish antioxidant effects by acting as natural free radical scavengers, by improving antioxidant status parameters [13–16], and by inhibiting lipid peroxidation related cellular membrane damage which results in cell death [17,18].

Although several recent studies investigated the efficiency of NSO and TQ, to our knowledge, there is no study that investigates the effects of both of these agents on oxidative stress in kidney of rats **exposed** to total cranial irradiation. The aim of this study was to investigate protective effects of NSO and TQ on oxidative stress in the kidney tissue of rats generated by transferred free radicals via systemic circulation.

2. Materials and methods

2.1. Chemicals

Dimethyl sulfoxide (DMSO) and thymoquinone were purchased from Sigma Chemical Co. (St Louis, MO, USA). All other chemicals and reagents were obtained from the store of the Department of Medical Biochemistry, Gaziantep University, School of Medicine.

2.2. Animals and experiments

Forty-eight male albino Sprague-Dawley rats **(12–16 weeks old, weighing 220 ± 25 g at the time of irradiation)** bred at department of animal laboratory were used for the experiment. The rats were quarantined for at least seven days before irradiation, housed ten to a cage in a windowless laboratory room with automatic temperature (22 ± 1 °C) and lighting controls (12 h light/12 h dark), and fed with standard laboratory chow and water. Animal experimentations were carried out in an ethically proper way.

The rats were randomly divided into six groups. Groups were organized as follows:

Irradiation (IR) group: A single dose of 5 Gray (Gy) gamma IR to the total cranium and 1-ml saline through an orogastric tube were administered to the rats.

IR plus NSO group: A single dose of 5 Gy gamma IR to the total cranium and NSO (1 g $kg^{-1}day^{-1}$) were administered to the rats in this group. NSO administration was made one hour before the IR through an orogastric tube and continued for 10 days after IR.

IR plus TQ group: A single dose of 5 Gy gamma IR to the total cranium and TQ (50 mg $kg^{-1}day^{-1}$) were administered to the rats. TQ administration was made by intraperitoneal (IP) injections 30 min before IR and continued for 10 days after IR. TQ was dissolved in dimethyl sulfoxide (DMSO) with a final concentration of 0.1% and immediately injected to the rats before IR.

Control group of IR plus NSO group: Only saline (1 ml) through an orogastric tube was administered to the rats in this group. Saline administration continued for 10 days. IR, NSO or TQ were not added.

Control group of IR plus TQ group: Only DMSO with IP injections was administered to the rats. The volume of the DMSO was same as TQ used in *IR plus TQ group*. DMSO injections continued for 10 days. IR, NSO or TQ were not administered.

Sham group: Rats in this group received no IR, no NSO, or no TQ.

All animal experimentation was carried out in an ethically proper way according to the guidelines of Ethical Committee of Gaziantep University. Concentrations of NSO and TQ administered to the rats were determined according to the previous studies [3,17,19,20]**.** All rats were anesthetized with 80 mg/kg ketamine hydrochloride (Pfizer Ilac, Istanbul, Turkey) prior to total cranium IR and placed on a tray in the prone position. A Cobalt-60 teletherapy unit (Theratron Equinox, MDS Nordion, Kanata, Ontario, Canada) from a source-to-surface distance of 80 cm by 5 × 5 cm anterior fields with a dose rate of 0.49 Gy/min was used in IR. The central axis dose was calculated at a depth of 0.5 cm. Irradiation dose was arranged according to the previous studies [3,20, 21]**.**

2.3. Biochemical analysis

Ten days after total cranial irradiation, all rats were anesthetized with 80 mg/kg ketamine hydrochloride (Pfizer Ilac, Istanbul, Turkey) and sacrified, and kidneys of the rats were removed. Homogenization of kidneys was made in isotonic saline (IKANERKE, GmBH & CO. KB D-79219, Staufen, Germany) and centrifugation of homogenate was made at 10,000 *g* for 1 h to remove debris. Clear upper supernatant was collected and this fraction was used for all of the evaluations. Procedures were performed at 4 °C.

Oxidative status parameters measured in the study were total oxidant status (TOS), lipid hydroperoxide (LOOH) and oxidative stress index (OSI). Antioxidant status parameters were evaluated with changes in activities of arylesterase (ARYL), paroxonase (PON) and ceruloplasmin (Cp), in total sulfhydryl (-SH) groups and total antioxidant status (TAS).

A colorimetric method defined by Erel [22] was used in the measurement of TAS and TOS levels. Expressions of the findings were made as millimolar Trolox equivalent per liter (mmol Trolox equivalent/gr protein) for TAS and micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/gr protein) for TOS. The ratio of TOS to TAS was defined to be OSI. The resulting unit of TAS was converted to μmol/gr protein. Calculation of OSI value was made using the formula [23];

$$\text{OSI (arbitrary unit)} = [\text{TOS } (\mu\text{mol } H_2O_2 \text{ equivalent/gr protein}) / \text{ TAS } (\mu\text{mol Trolox equivalent/gr protein})] \times 100$$

Paroxonase activity was measured in the basal activity. Measurement of paraoxon hydrolysis rate was made by monitoring the increase in absorbance at 412 nm at 37 °C. Generated p-nitrophenol amount was calculated from the molar absorptivity coefficient at pH 8, which was 17,000 M/cm [24]. PON activity was expressed as U/gr protein.

In measuring ARYL activity, phenylacetate was used as a substrate and increase in absorbance at 270 nm at 37 °C was monitored. Calculation of activity was done from the molar absorptivity coefficient of the produced phenol, 1310 M/cm [25]. One unit of ARYL activity was defined as 1 μmol phenol generated/min under the above conditions and expressed as U/gr protein.

In the measurement of Cp enzymatic activity, Erel's method [26] was used. Ferrous ion was oxidized to ferric ion via ceruloplasmin ferroxidase activity in this assessment and results were expressed as U/gr protein.

Ellman's method (modified by Hu et al.) was used in the assessment of total -SH levels and results are expressed as mmol/gr protein [27].

In the measurement of LOOH levels, ferrous ion oxidation-xylenol orange method was used [28] and results are expressed as μmol/gr protein.

2.4. Statistical analysis

Data analysis was performed using statistical analysis with the Statistical Package for the Social Sciences for Windows (SPSS, version 21.0, Chicago, IL). Kolmogorov Smirnov test was used to determine whether the continuous variables were normally distributed. Data were expressed as mean ± SD. Differences between groups in normally distributed variables were analyzed using the independent sample *t*-test. The Mann Whitney *U* test was used to analyze data that were not normally distributed. The results were accepted statistically significant when *p* value is less than 0.05.

3. Results

Oxidative and antioxidant parameters were measured in all of the groups. *IR group*, *IR plus NSO group* and *IR plus TQ group* were compared with *sham group, control group of NSO* and *control group of TQ*, respectively. To determine the protective and ameliorating effects of NSO and TQ against radiation induced oxidative stress, *IR group* was compared with *IR plus NSO* group and *IR plus TQ group*. In addition, *IR plus NSO group* was compared with *IR plus TQ group* to assess the superiority of the two supplements against each other.

3.1. Antioxidant parameters

There was a statistically significant difference between *IR group* and each of the other groups (Table 1). When IR group was compared to sham group, TAS, PON, and Cp activities were found statistically lower ($p < 0.001$). PON and Cp activities were significantly lower in *IR group* was compared to *IR plus NSO group*. Also PON and Cp activities were lower in *IR group* compared to *IR plus TQ group* ($p < 0.001$). Cp activity in *IR plus NSO group* was significantly higher compared to *control group* ($p < 0.001$). In addition, Cp activity in *IR plus NSO group* was significantly higher compared to *IR plus TQ group*.

ARYL activity and total -SH levels were compared across all of the groups and statistically significant difference was not found ($p > 0.1$).

3.2. Oxidative parameters

When *IR group* was compared to each of other groups, TOS, OSI, and LOOH levels were found to be significantly different (Table 2). All of the oxidative parameters were significantly higher in *IR group* compared to *sham group* ($p < 0.001$). When compared to *IR group*, TOS, OSI, and LOOH levels were found to be decreased in *IR plus TQ group* ($p < 0.001$). Additionally, all of the oxidative parameters were found decreased in *IR plus NSO group* compared to *IR group* ($p < 0.001$). When *IR plus NSO group* was compared to *IR plus TQ group,* LOOH levels were found significantly lower ($p < 0.001$).

4. Discussion

Ionizing radiation interacts with tissues, generates free radicals by radiolysis of water and causes cell death by damaging DNA. Furthermore, free radicals may be transferred to distant organs through systemic circulation. High dose of ionizing radiation may damage hematologic, gastrointestinal, and central nervous systems. In response to free radical formation after ionizing radiation exposure, expression of antioxidants increases in cells to minimize or to eliminate free-radical induced cellular damage. Hence, improvement of effective protectors became important, especially natural products functioning as antioxidants and immune-stimulants have been investigated in several studies [1,3,20].

Table 2
Oxidative parameters.

	LOOH (μmol/gr protein) (mean±SD)	TOS (μmol H_2O_2 equivalent/gr protein) (mean±SD)	OSI (arbitrary unit) (mean±SD)
Sham Group	0.86 ± 0.06[a]	4.16 ± 0.44[a]	6.74 ± 1.15[a]
Control group of IR plus TQ group	0.86 ± 0.05	4.01 ± 0.34	7.18 ± 0.79
Control group of IR plus NSO group	0.89 ± 0.06	4.26 ± 0.28	8.01 ± 1.16
IR only group	1.13 ± 0.10	5.30 ± 0.65	12.80 ± 4.15
IR plus TQ group	0.90 ± 0.05[a]	4.22 ± 0.43[a]	7.57 ± 1.11[a]
IR plus NSO group	0.84 ± 0.03[a,b]	4.12 ± 0.17[a]	7.09 ± 0.80[a]

IR: irradiation; NSO: *Nigella sativa* oil; TQ: thymoquinone; LOOH: lipid hydroperoxide; TOS: total oxidant status; SD: standard deviation.
[a] $p < 0.001$ vs. IR only group,
[b] $p < 0.001$ vs. IR plus TQ group.

4.1. Effects of ionizing radiation on antioxidant and oxidative parameters in kidney

In our study, TOS, OSI and LOOH were assessed as oxidative parameters to determine ionizing radiation induced oxidative stress in the kidney of rats exposed to cranial irradiation and statistically significant increase in these parameters was found compared to the control groups. Furthermore, TAS, PON, and Cp activities were assessed as antioxidant markers in these rats, and found significantly lower when compared to the control groups. These findings show that ionizing radiation may generate oxidative stress in distant organs beside local irradiated tissues.

4.2. Effects of Nigella sativa oil and thymoquinone on antioxidant and oxidative parameters in kidney

Oxidative damage associated with free radical generation has been researched in pathogenesis of many diseases in many studies. As ROS generation takes place in vivo, several enzymatic or non-enzymatic antioxidants may prevent body from certain diseases [29]. Natural occurring antioxidant agents have been investigated in several experimental studies in different tissues and organs for their protective effects from radiation induced oxidative/nitrosative stress [3,17].

Nigella sativa belongs to the Ranunculaceae family and known as black seed in traditional use. For centuries *Nigella sativa* and its oil have been used for treatment and prevention of various diseases in several

Table 1
Antioxidant parameters.

	ARYL (U/g protein) (mean±SD)	PON (U/g protein) (mean±SD)	-SH (mmol/g protein) (mean±SD)	TAS (mmol Trolox equivalent/g protein) (mean±SD)	Cp (U/g protein) (mean±SD)
Sham Group	10.43 ± 0.7	1.26 ± 0.2[a]	0.07 ± 0.008	0.06 ± 0.01[a]	44.18 ± 1.7[a]
Control group of IR plus TQ group	10.15 ± 0.9	1.28 ± 0.3	0.07 ± 0.007	0.06 ± 0.01	44.34 ± 1.6
Control group of IR plus NSO group	10.63 ± 0.6	1.29 ± 0.2	0.07 ± 0.004	0.06 ± 0.01	43.84 ± 2.4
IR only group	10.38 ± 0.8	1.04 ± 0.2	0.06 ± 0.007	0.05 ± 0.02	42.50 ± 0.8
IR plus TQ group	10.04 ± 1.2	1.31 ± 0.1[a]	0.06 ± 0.002	0.06 ± 0.01	47.12 ± 5.5[a]
IR plus NSO group	10.12 ± 0.8	1.27 ± 0.1[a]	0.07 ± 0.003	0.06 ± 0.01	62.43 ± 14.7[a,b,c]

IR: irradiation; NSO: *Nigella sativa* oil; TQ: thymoquinone; ARYL: arylesterase; PON: paraoxonase; -SH: sulfhydryl group; TAS: total antioxidant status; Cp: ceruloplasmin; SD: standard deviation.
[a] $p < 0.001$ vs. IR only group.
[b] $p < 0.001$ vs control group of IR plus NSO.
[c] $p < 0.001$ vs. IR plus TQ group.

countries. TQ is the volatile component of *Nigella sativa* seed and composed of approximately 27–57% of the quinone constituents [17,30,31]. NSO and TQ have been investigated (both in vitro and in vivo) in cancer, sepsis, atherosclerosis and asthma, and their antiinflammatory, antioxidant and anticarcinogenic effects have been reported. Antioxidant properties of NSO and TQ have been shown to exhibit by scavenging ROS or reactive nitrogen species (RNS) [18] and by reducing oxidative stress induced by ionizing radiation or other oxidant agents [14,16]. NSO and TQ have also been shown to protect cellular membranes from lipid peroxidation [17,18,20,32]. In our study, TOS, LOOH, and OSI levels in kidney tissue were lower in the rats received NSO and TQ with IR compared to those did not receive these agents. Supplementation with both agents, NSO and TQ, was shown to reduce oxidative damage in the kidney tissue of the irradiated rats and these results are in consistent with our research hypothesis.

Ceruloplasmin, ARYL, PON and total –SH groups are the components of defense system against oxidative stress and have important role in protecting cells against ROS/RNS [33]. In the present study, there were statistically significant reductions in TAS, PON and Cp activities in the kidney tissue of irradiated rats which indicate the radiation induced oxidative stress.

Ceruloplasmin is an extracellular antioxidant and has a role in Fe^{+2} oxidation [34]. We found that Cp activity was statistically increased in *IR plus NSO*, *IR plus TQ* and *sham groups* compared to IR only group. Our results showed that Cp activity was reduced only in the irradiated rats, and elevation in the Cp activity with supplementation of NSO and TQ exhibits the ameliorating effects of these products. In addition, Cp activity in *IR plus NSO group* was significantly higher when compared to *IR plus TQ group* which may suggest superiority of NSO against TQ.

Paroxonase is an important antioxidant enzyme and has a role in hydrolyzing lipid peroxides in oxidized lipoproteins [35–37]. In our study, PON activity was statistically higher in *IR plus NSO*, *IR plus TQ* and *sham groups* compared to *IR group*. Our results showed that PON activity was significantly decreased in the rats received irradiation alone, and supplementation of NSO and TQ improved this decrease.

Free radical formation may also result in lipid peroxidation related damage in the cells. LOOH is one of oxidative stress parameters and formed through peroxidative reactions from unsaturated phospholipids, glycolipids and cholesterol [38]. The current study showed that LOOH levels were significantly higher in *IR group* compared to *IR plus TQ*, *IR plus NSO* and *control groups*. Supplementation of NSO and TQ improved the increase in LOOH levels in the kidney tissue of the irradiated rats. Lipid hydroperoxides have been shown to inhibit PON activity during lipoprotein oxidation [39]. In our study, lower PON activity and higher LOOH levels in the irradiated rats may explain the ionizing radiation related oxidative stress. Administration of NSO and TQ prevented kidney tissue against radiation injury by increasing PON activity and decreasing LOOH levels. Moreover, supplementation with NSO and TQ significantly decreased TOS and OSI besides LOOH levels in the kidney tissue of the irradiated rats. The results of this study are similar to the previous studies investigated protective effects of NSO and/or TQ against oxidative stress induced by ionizing radiation or other oxidant agents [3,7,20]. When the groups treated with NSO and TQ were compared, LOOH levels were found lower and Cp activity was found higher in *NSO plus IR group* than in *TQ plus IR group*. These results may suggest that NSO is more effective than TQ, however detailed further investigations are needed.

Significant differences in terms of ARYL activity and total -SH levels were not able to be detected in none of the groups.

In conclusion, free radicals generated by cranial exposure of ionizing radiation and released into systemic circulation cause oxidative stress in kidney. Supplementation with NSO and TQ clearly protect kidney tissue of the rats against radiation induced oxidative stress by improving antioxidant status and/or by inhibiting generation of oxidative stress parameters.

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None of the authors declares a conflict of interest regarding the data presented in this publication.

CRediT authorship contribution statement

Hilal Alkis, Elif Demir, Seyithan Taysi: Wrote the main manuscript text. **Hilal Alkis, ST**: Analyzed the data. **Hilal Alkis:** Prepared the tables and figures. **Hilal Alkis, Elif Demir, MRT, Suleyman Sagir, Seyithan Taysi**: Contributed to the design and implementation of the experiment. All authors reviewed and revised the manuscript. All authors approved of the manuscript text.

Conflict of interest statement

The authors declare no competing financial and non-financial interests.

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