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Radiation-modifying abilities of *Nigella sativa* and Thymoquinone on radiation-induced nitrosative stress in the brain tissue

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ABSTRACT

To investigate *Nigella sativa* oil (NSO) and Thymoquinone (TQ) for their antioxidant effects on the brain tissue of rats exposed to ionizing radiation.

Fifty-four male albino Wistar rats, divided into six groups, were designed as group I (normal control group) did not receive NSO, TQ or irradiation; group II (control group of TQ) received dimethyl sulfoxide and sham irradiation; group III (control group of NSO) received saline and sham irradiation; group IV (irradiation plus NSO group) received both 5 Gray of gamma irradiation to total cranium and NSO; group V (irradiation plus TQ group) received both irradiation and TQ; group VI (irradiation alone group) received irradiation plus saline. Alterations in nitric oxide ($NO^{\bullet}$) and peroxynitrite ($ONOO^{-}$) levels, and nitric oxide synthase (NOS) enzyme activity were measured by biochemical methods in homogenized brain tissue of rats.

Levels of $NO^{\bullet}$ and $ONOO^{-}$, and enzyme activity of NOS in brain tissue of the rats treated with NSO or TQ were found to be lower than in received IR alone ($p < 0.002$)

Nigella sativa oil (NSO) and its active component, TQ, clearly protect brain tissue from radiation-induced nitrosative stress.

Introduction

Oxygen-free radicals including reactive oxygen species (ROS) such as superoxide radical ($O_2^{\bullet -}$) and hydroxyl radical ($OH^{\bullet -}$), and reactive nitrogen species (RNS) such as nitric oxide ($NO^{\bullet}$), peroxynitrite ($ONOO^{-}$) are produced in humans as a consequence of intracellular metabolic processes and after exposure to genotoxic agents, i.e. ionizing radiation (IR) (Cadenas 1989). IR is an important source in generation of ROS/RNS among many physical/chemical agents and killing action of IR is mainly mediated through oxygen-free radicals (Hall and Giaccia 2006). ROS or RNS mediated oxidative or nitrosative injury plays a crucial role in manifestation of health effects of radiation exposure (Hannig et al. 2000).

Nitric oxide ($NO^{\bullet}$), a double-edged molecule, is an important biological messenger that plays an important role in the physiology of the central nervous system (CNS) (Yun et al. 1996). $NO^{\bullet}$ is produced from L-arginine by enzyme activity of nitric oxide synthase (NOS) and acts as an important physiological signaling molecule mediating a large variety of cellular functions. In other respects, its overproduction induces cytotoxic and mutagenic effects (Kaynar et al. 2005). When present in excess, $NO^{\bullet}$ can react with $O_2^{\bullet -}$ and produce $ONOO^{-}$ anion which is a powerful oxidant that can cause lipid peroxidation (LPO), inhibit mitochondrial electron transport, oxidize thiol compounds and oxidize and nitrate DNA (Powell et al. 2005; Valko et al. 2006). There is a balance between production and scavenging of ROS/RNS. If the balance changes in support of production of ROS/RNS, oxidative or nitrosative stress takes place and may result in a variety of diseases; cancer, cardiovascular, and neurological diseases (Reuter et al. 2010).

Brain is an important tissue of CNS and IR is a mandatory part in the treatment of brain malignancies. Brain is particularly sensitive to the oxidative and nitrosative injury due to its high content of polyunsaturated fatty acids, relatively low antioxidant

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capacity, low repair mechanism activity, non-replicating nature of its neuronal cells, and high rate of oxidative metabolic activity and overproduction of ROS/RNS metabolites compared to other organs (Evans 1993). In CNS, oxidative or nitrosative stress results in acute and chronic injury and plays an important role in the pathogenesis of neuronal damage (Facchinetti et al. 1998). Therefore, agents which can protect cellular membranes against IR and ROS/RNS will have potential benefits as radioprotectors, antioxidant and antimutagens (Odin 1997; Stavric 1994).

Nigella sativa (NS), commonly known as black seed, belongs to the botanical family of Ranunculaceae and generally grows in Eastern Europe, Middle East and Western Asia. The seeds of NS and their oil have been widely used throughout the world in the treatment of several diseases for centuries. NS contains amino acids, proteins, carbohydrates, both fixed oils (84% fatty acids, including linolenic and oleic acids) and volatile oils, alkaloids, crude fiber, saponins and minerals, such as calcium, iron, sodium and potassium. The volatile component of NS seed, TQ, constitutes about 27–57% of the quinone constituents and has been attributed to be the most important active present in the whole seeds or their extracts among the other active ingredients (Ali and Blunden 2003; Salem 2005). Antihistaminic, antihypertensive, analgesic, anti-inflammatory, hypoglycemic, antibacterial, antifungal, antitumour, hepatoprotective, renal protective and antioxidant effects of NS oil (NSO) and TQ have been shown in a large number studies (Ali and Blunden 2003; Burits and Bucar 2000; Padhye et al. 2008).

Besides the known antioxidant properties of NSO and TQ, data on the radiation-protective ability of these agents are limited (Cemek et al. 2006; Rastogi et al. 2010). We hypothesized that NSO and TQ whose antioxidant effects are proven by many studies could protect brain tissue from radiation-induced nitrosative damage. For this reason, we measured the nitrosative biomarkers, $NO^{\bullet}$, NOS and $OONO^{-}$, in the brain tissue of rats with or without exposing of gamma radiation to total cranium with a single dose of 5 Gray (Gy).

Materials and methods

Rats and experiments

Fifty-four male Wistar albino rats, 12–16 weeks old, weighting 220 ± 25 g at the time of irradiation and bred at Gaziantep University Medical School, department of animal laboratory were used for the experiment. All procedures involving the Wistar albino rats adhered to the ARVO Resolution on the Use of Animals in Research. Animal experimentations were carried out in an ethically proper way by following guidelines as set by the Ethical Committee of the Gaziantep University. 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. The rats were randomly divided into six groups. Control groups included 8 rats and the other groups included 10 rats for each. Group I (normal control group) did not receive NSO, TQ or irradiation. Group II (control group of TQ) did not receive NSO, TQ or irradiation, but received sham irradiation and intraperitoneally (i.p.) injections of dimethyl sulfoxide (DMSO) at an equal volume to that of TQ used in group V (the first dose of DMSO was started 30 min before the irradiation and continued during ten days). Group III (control group of NSO) received 1-ml saline through an orogastric tube and sham irradiation. Group IV (irradiation plus NSO group) received both 5 Gy of gamma irradiation as a single dose to total cranium and NSO (1 g/kg/day) starting 1 h before irradiation and continuing for 10 days through an orogastric tube. Group V (irradiation plus TQ group) received both 5 Gy of gamma irradiation as a single dose to total cranium and TQ (30 mg/kg/day, i.p.) injection starting 30 min before the radiation dose and subsequently daily for 10 days after irradiation. TQ was dissolved in DMSO just before giving to the rats. Group VI (irradiation alone group) received 5 Gy of gamma irradiation as a single dose to total cranium plus 1-ml saline through an orogastric tube. Prior to total cranium irradiation, the rats were anesthetized with 50 mg/kg ketamine HCL (Pfizer Inc, Istanbul, Turkey) and placed on a plexiglas tray in the prone position. While the rats in the control groups of II and III received sham irradiation, the rats in the groups of IV, V, VI were irradiated using cobalt 60 teletherapy unit (Theratron Equinox, MDS Nordion, Kanata, Ontario, Canada) from a source-to-surface distance of 100 cm by 20 cm × 20 cm anterior fields with 5 Gy to the total cranium as a single fraction. The central axis was calculated at a depth of 1 cm. The dose rate was 0.49 Gy/min.

Fractination of brain samples

At the end of the study, the rats were anesthetized with 50 mg/kg ketamine i.p. Then an intracardiac withdrawal of blood was performed. Following this process, the rats were sacrificed and their brains were removed. Brain tissues were homogenized by a homogenizer (IKA-NERKE, GmBH & CO. KB D-79219, Staufen, Germany) in isotonic saline (1/10 weight/volume) on ice for 1 min. The supernatant was stored at −80 °C in aliquots for biochemical measurements. $NO^{\bullet}$ and $ONOO^{-}$ levels and enzyme activity of NOS were determined from these supernatants spectrophotometrically.

Determination of NOS activity and $NO^{\bullet}$ and $ONOO^{-}$ levels

NOS activity assay is based on the diazotization of sulfanilic acid by $NO^{\bullet}$ at acid pH and subsequent coupling to N-(1-naphthyl) ethylenediamine. To 0.1 ml of sample, 0.2 ml of 0.2 M arginine was added and incubated at 37 °C for 1 h. Then, the combination, 0.2 ml of 10 mM HCl, 100 mM sulfanilic acid, and 60 mM N-(1-naphthyl) ethylenediamine was added. After 30 min, the absorbance of the sample tube was measured against a blank tube at 540 nm (Durak et al. 2001). Results are expressed as U/mg protein. $NO^{\bullet}$ levels in brain tissue were measured using the Griess reagent as previously described (Bories and Bories 1995; Moshage et al. 1995). Griess reagent, the mixture (1:1) of 0.2% N-(1-naphthyl) ethylenediamine and 2% sulphanilamide in 5% phosphoric acid, gives a red-violet diazo dye with nitrite, and the resultant color was measured at 540 nm. First nitrate was converted to nitrite using nitrate reductase. The second step was the addition of Griess reagent, which converts nitrite to a deep purple azocompound; photometric measurement the absorbance of 540 nm determines the nitrite concentration. Results were expressed as μmol/g wet weight. Peroxynitrite assay was determined as described (Al-Nimer et al. 2012; Vanuffelen et al. 1998). Ten microliters of samples was added to 5 mM phenol in 50 mM sodium phosphate buffer (pH 7.4) to get a final volume of 2 ml. After 2 h incubation in a dark place at 37 °C, 15 μl of 0.1 M NaOH was added and the absorbance, at wavelength of 412 nm, of the samples were immediately recorded. The yield of nitrophenol was calculated from $\varepsilon = 4400\ M^{-1}\ cm^{-1}$. Results were expressed as μmol/g wet weight. The protein content was determined as described (Bradford 1976). Biochemical measurements were carried out using a spectrophotometer (Shimadu U 1601, Japan).

Statistical analyses

Analyses were conducted using Statistical Package for the Social Sciences (SPSS, version 18) software. Data were analyzed with one-way analysis of variance (ANOVA) followed by a post hoc test (LSD alpha) for multiple comparisons. Data were expressed as