Effect of “Nettle Oil” on Oxidative Damage to the Heart and Lungs Induced by Radiation

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We studied the effect of “Nettle Oil” on radiation-induced oxidative damage in the tissues of the heart and lungs. The experiment was performed on 40 Wistar male rats divided into 4 groups. Animals of the first and third groups received intragastric sunflower oil at a dose of 2.5 ml/kg body weight for 14 days. Animals of the second and fourth groups - the drug “Nettle Oil” in the same amount and mode. Then, rats of the third and fourth groups, an hour after the last injection of oil, were exposed to gamma radiation (6 Gr). Irradiation toxicity was manifested by an increase in blood serum activity of aspartate, alanine aminotransferase, creatinephosphokinase, lactate dehydrogenase, total cholesterol, triglycerides, low-density cholesterol lipoproteins, atherogenic indices against the background of a decrease in high-density cholesterol lipoproteins. In the tissues of the heart and lungs, the amount of malondialdehyde increased and the activity of superoxide dismutase, catalase, glutathione peroxidase and the level of reduced glutathione and non-protein thiol decreased. The introduction of “Nettle Oil” prior to irradiation provided significant antioxidant protection. Antioxidant enzymes increased along with the levels of reduced glutathione and non-protein thiol, which is associated with a significant decrease in the level of malondialdehyde in the tissues of the heart and lungs.

Keywords: ionizing radiation, “Nettle oil”, lung, heart, oxidative stress.
structures, including DNA, lipids, proteins and membrane formations\textsuperscript{3,19,29,56}, which correlates with many pathological conditions, including cancer, pulmonary, cardiac, immunosuppressive diseases\textsuperscript{39,53}. Therefore, the protection of a living system from radiation hazard is a priority in radiation biology. In addition, radiation protection is very important in radiation therapy of cancer cells. During this procedure, ionizing radiation can cause tissue damage, i.e. radiation can destroy tumor cells in these organs, and spread to neighboring healthy cells\textsuperscript{39}, which is considered a limiting factor in the use of radiotherapy\textsuperscript{36,37,44}. Thus, the search and development of radioprotective agents is especially relevant in radiotherapy. In addition to the natural endogenous antioxidant defense system of the body, humanity can consume external (dietary) antioxidants, which also help cells modulate the homeostasis of reactive oxygen metabolites\textsuperscript{30,46,49}. Indeed, people consume a wide variety of phytochemicals that can protect them from the harmful effects of radiation. This can be supported collectively by bioactive compositions of plant extracts, since each component has different properties, therefore, plant extracts can protect vital organs from many diseases\textsuperscript{25,60}. Thus, any molecules (preparations) that absorb or inhibit the formation of reactive oxygen species, i.e. possess the ability to remove free radicals and the property of an antioxidant, can act as a radioprotector, reducing damage to normal tissues undergoing radiotherapy\textsuperscript{42,52}.

Synthetic thiol compounds and nitroxides are very effective in reducing radiation mortality and are widely studied. However, at a clinically effective concentration, they are toxic and cause some side effects. And in most cases, toxicity appears in promising radioprotective agents, which limit their usefulness and applicability\textsuperscript{41}. Due to the low toxicity in an effective dose with minimal side effects\textsuperscript{29}, plant products with antioxidant properties and remove (inhibitory, binding) free radicals, “thereby” minimizing radiation damage to normal tissue, are considered radioprotectors\textsuperscript{54}.

Stinging nettle (\textit{Urticadioica L.}) is a perennial herbaceous plant of the \textit{Urticaceae} family, the many effects of which are associated with the content of a complex of valuable biologically active substances in it. Phytochemical studies have shown that its leaves are especially rich in potassium, calcium, iron, magnesium, manganese, sulfur, copper, zinc, selenium, vitamins K, PP, B1 B2, chlorophylls, proteins, terpenes, as well as such well-known antioxidants as  \textit{a}-carotene, ascorbic acid, and flavonoids\textsuperscript{43}. Due to this, nettle leaves have antioxidant, anti-inflammatory, antimicrobial, antihypertensive, antidiabetic, antitumor, hepatoprotective, antimutagenic, diuretic, anticholesterolemic, hypolipidemic, immunomodulating properties\textsuperscript{1,4,14,17,22,26}.

Based on the foregoing, the aim of this study is to study the possible protective effects of the drug “Nettle Oil” on oxidative damage in the tissues of the heart and lungs caused by gamma radiation in rats.

Materials and methods. The work was performed on 40 Wistar male rats weighing 170-190 g. The animals were in standard conditions in the vivarium of the Scientific and Practical Center of the Non-profit Joint-Stock Company “West Kazakhstan Medical University named after Marat Ospanov” (Aktobe, Republic of Kazakhstan) on a standard diet with free access to food and water. The experiments were carried out in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes (Strasbourg, 1986). The program of the experiment was discussed and approved by the regional ethical commission of the university.

10 days after acclimatization, the rats were randomly divided into 4 groups. Animals of groups 1 and 3 for 14 days received intragastric sunflower oil at a dose of 2.5 ml/kg body weight. Animals of groups 2 and 4 — the drug “Nettle oil” (RK–MD–5–#010970) in the same amount and mode. Then, rats of the 3rd and 4th groups, one hour after the last injection of the oils, were exposed to a single gamma irradiation of Cobalt 60 at a dose of 6 Gray (power 0.54 Gr/min) at the “Teragam” (Czech Republic) radiotherapy facility. The distance from the source to the skin was 70 cm.

The dose of “Nettle oil”, the route of administration and the duration of its use are justified according to the literature\textsuperscript{57,58}.

Euthanasia of animals in all groups was performed at the end of the experimental period by cervical instant decapitation under mild ether anesthesia to prevent stress. Blood was collected in test tubes and centrifuged at 2200 g for 10
min. Serum samples were taken and, if necessary, stored at -80 degrees Celsius. The heart and lungs obtained by dissecting the chest were placed in cold phosphate buffered saline to remove excess blood. Then it was ground, homogenized in Tris – HCL buffer (pH = 7.4) and centrifuged. The obtained supernatants were used for biochemical analyzes.

**Biochemical studies**

Using a standard set of reagents in serum, the activity of marker enzymes of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenesis (LDH), creatine phosphokinase (SC) and lipid profile — total cholesterol (TC), low-density triglycerides (TG), lipoprotein cholesterol was determined (LDL-C), high-density cholesterol lipoproteins (HDL-C) on a Architect 4000 biochemical analyzer (Abbott, USA). The ratios were also calculated: Atheroegenix Index (AI) = (TC - HDL-C)/(HDL-C).

The content of malondialdehyde (MDA) marker of lipid peroxidation intensity (LPO) in the tissues of the heart and lungs was determined spectrophotometrically by the method of Draper, Hadley. The essence of the method: at high temperature in an acidic medium, MDA reacts with 2-thiobarburic acid, forming a colored complex with an absorption maximum at 532 nm. The molar extinction coefficient is $1.56 \times 10^{-5}$ cm$^{-1}$. M. The MDA level was expressed as nmol/mg protein (nmol/mg Pt.)

Antioxidant status of the heart and lungs. Catalase activity (CAT) was measured in accordance with the method. The reaction was started by adding 2.0 ml of hydrogen peroxide to 10 il of the supernatant and after 10 minutes it was stopped by adding 1.0 ml of 4% ammonium molybdate. The absorption of the sample was measured at 410 nm. Activity was expressed in ìmol.

Superoxide dismutase (SOD) activity was evaluated by the Beauchamp and Fridovich method. The method is based on recording changes in the rate of reduction of nitro blue tetrazolium (NBT) in the presence of reduced nicotinamide adenine dinucleotide and phenazinemethosulfate. The optical density was measured at 560 nm. The unit of SOD activity was taken to be the amount of enzyme necessary to inhibit NBT recovery by 50% and the activity was expressed in units of mg protein (U/mg Pt).

Glutathione peroxidase (GPx) activity was measured by the method of Flohe and Gunzler. Enzyme activity is expressed as nmol of the oxidized amount of reduced glutathione (GSH) - nmol of GSH / min / mg protein.

Glutathione levels in the heart and lungs were determined by the Ellman method as modified by Jollow et al. The formation of yellow staining was determined when DTNB (5,5-dithiobis 2-nitrobenzoic acid) was added to the sample containing SH groups. Extinction was measured at 412 nm. The amount was expressed in mol/g of tissue. The non-protein thiol content (NPSH) was determined by the Ellman method, and the amount was expressed as nmol/mg protein.

Protein content was determined according to the method of Lowry et al using bovine serum albumin as a standard.

**RESULTS AND DISCUSSIONS**

**Biomarkers of cytotoxicity**

Gamma irradiation (6Gr) caused an increase in serum ALT, AST, LDH and CK activity by 31%, 60%, 102% and 121%, respectively, compared with the control group. The introduction of one “Nettle Oil” - led to a significant decrease in the activity of only LDH by 12% in comparison with the control. The use of “Nettle Oil” within 2 weeks before irradiation caused a significant decrease in the activity of ALT, AST, LDH and CK by 18%, 42%, 53% and 33%, respectively, compared with the data of the irradiated group (Table 1).
Blood lipid profile

Analysis of serum lipid profile data (table 2) showed that gamma irradiation causes a significant increase in TS levels (by 45%), TG (by 71%), and LDL cholesterol (by 190%) against a significant decrease in HDL–C (53%) compared with the control group. Atherogenic coefficients TC/(HDL–C), (LDL–C)/(HDL–C) and AI significantly increase by 208%, 510% and 58%, respectively. The introduction of “Nettle Oil” was accompanied by a significant decrease in LDL–C (by 15%), ratios (LDL–C)/(HDL–C) and AI by 21% and 22%, respectively, compared with the control. The use of “Nettle Oil” for 14 days before ionizing irradiation led to a significant decrease in the levels of TC, TG, HDL–C, LDL–C and all studied coefficients in comparison with the data of the exposed group. However, the lipid profile indices — TG, LDL–C, AI, TC/(HDL–C) and (LDL–C)/(HDL–C) remained significantly higher than the controls by 24, 65, 25, 21, and 89%, respectively, and HDL–C is lower than the value of the control group.

Table 1. The effect of “Nettle Oil” on the biomarkers of cytotoxicity in rats exposed to gamma radiation

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>LDH (U/L)</th>
<th>CK (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>42.0±1.4</td>
<td>70.0±2.6</td>
<td>340±15</td>
<td>156±13</td>
</tr>
<tr>
<td>“Nettle Oil”</td>
<td>40.0±1.1</td>
<td>66.0±2.3</td>
<td>300±12*</td>
<td>145±11</td>
</tr>
<tr>
<td>Gamma radiation</td>
<td>55.0±1.5*</td>
<td>112±5.3*</td>
<td>687±26*</td>
<td>345±15*</td>
</tr>
<tr>
<td>“Nettle Oil” and Gamma radiation</td>
<td>45.0±1.2*</td>
<td>65.0±3.3*</td>
<td>320±9.6*</td>
<td>230±12*</td>
</tr>
</tbody>
</table>

Note: x - p <0.05 compared with the control group, o - p <0.05 compared with the data of ionizing radiation

Table 2. The effect of “Nettle oil” on the lipid profile of blood in rats with radiation-induced damage to the heart and lungs

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>“Nettle Oil”</th>
<th>Gamma radiation</th>
<th>“Nettle Oil” and Gamma radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (TC)</td>
<td>1.16±0.071</td>
<td>1.09±0.063</td>
<td>1.68±0.09*</td>
<td>1.22±0.07*</td>
</tr>
<tr>
<td>Triglycerides (TG)</td>
<td>1.0±0.05</td>
<td>0.9±0.04</td>
<td>1.7±0.06*</td>
<td>1.24±0.06*</td>
</tr>
<tr>
<td>HDL–C (mmol/L)</td>
<td>0.53±0.03</td>
<td>0.56±0.04</td>
<td>0.25±0.02*</td>
<td>0.46±0.025*</td>
</tr>
<tr>
<td>LDL–C (mmol/L)</td>
<td>0.20±0.01</td>
<td>0.17±0.01*</td>
<td>0.58±0.04*</td>
<td>0.33±0.02*</td>
</tr>
<tr>
<td>Atherogenic Index (AI)</td>
<td>1.2±0.09</td>
<td>0.99±0.08*</td>
<td>1.9±0.15*</td>
<td>1.5±0.12*</td>
</tr>
<tr>
<td>TC/(HDL–C)</td>
<td>2.18±0.14</td>
<td>1.94±0.12</td>
<td>6.72±0.4*</td>
<td>2.65±0.15*</td>
</tr>
<tr>
<td>(LDL–C)/(HDL–C)</td>
<td>0.38±0.03</td>
<td>0.3±0.02*</td>
<td>2.32±0.16*</td>
<td>0.72±0.06*</td>
</tr>
</tbody>
</table>

Note: x - p <0.05 compared with the control group, o - p <0.05 compared with the data of ionizing radiation

Table 3 presents the effects of Nettle Oil, gamma irradiation and their combination on the levels of MDA, GSH, NPSH and the activity of SOD, CAT, GPx in cardiac tissue. Exposure to gamma radiation led to a significant decrease in the activity of SOD, CAT, GPx and the content of GSH, NPSH (by 34%, 25%, 57% and 50%, 31%) and a significant increase in MDA (by 66%), respectively, compared to the control group. Administration of CM alone for 2 weeks caused a significant (p<0.05) decrease in the MDA level by 15% and a significant increase in the content of GSH and NPSH (by 16% and 16%) in comparison with the control. The use of CM within 14 days prior to irradiation was accompanied by a significant decrease in MDA (by 29%) against a significant increase (p<0.001) in the activity of SOD, CAT, GPx (by 46%, 20%, 85%), respectively, and the levels of GSH and NPSH (by 55%, 31%) compared with the data of the irradiated group.

The data in table 4 show that gamma radiation (6 Gr) caused a significant increase in

MDA by 152% and a significant decrease in the activity of SOD, CAT, GPx and the content of GSH and NPSH in lung tissues by 36%, 41%, 46% and 42%, 32%, respectively, compared with the control group (p<0.001). The introduction of only “Nettle Oil” led to a decrease in the level of MDA (p<0.05), SOD activity (p<0.05) against the background of a significant increase in GSH (by 20%) and NPSH (by 13%) compared with control data. The two-week administration of Nettle Oil before irradiation caused a significant decrease in the content of MDA (by 45%) and an increase in the activity of SOD, CAT, GPx and levels of GSH and NPSH by 37.5%, 58%, 39% and 38%, 39%, respectively compared with the irradiated group.

Protecting normal cells from damage induced by radiation is a very important problem in radiation therapy. It is especially relevant for the heart and lungs. Since radiation-induced lung injuries and cardiovascular complications are disabling and potentially fatal toxicities, limiting the dose to the chest for lung cancer, breast cancer, lymphoma, cancer of the esophagus(10,11,35,55) and total radiation body. Temporary generation of reactive oxygen species that occurs within minutes after ionizing radiation to cells upsets the balance in the prooxidant - antioxidant status, activates lipid peroxidation, and leads to a constant increase in the generation of reactive oxygen metabolites(1), and ends in cell death(7). Therefore, the development of biologics based on antioxidants is necessary to prevent and/or treat radiation hazard(45).

In the present study, gamma radiation led to a significant increase in the level of malondialdehyde in the tissues of the heart and lungs, a marker of lipid peroxidation intensity,

Table 3. The effect of “Nettle oil” and gamma irradiation (6 Gr) on oxidative stress in rat heart tissues

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>“Nettle Oil”</th>
<th>Gamma radiation</th>
<th>“Nettle Oil” and Gamma radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA(à)</td>
<td>1.45±0.05</td>
<td>1.23±0.06*</td>
<td>2.41±0.1*</td>
<td>1.71±0.07*</td>
</tr>
<tr>
<td>SOD(b)</td>
<td>57.0±3.9</td>
<td>50.0±1.7</td>
<td>37.6±3.0*</td>
<td>54.8±2.6*</td>
</tr>
<tr>
<td>CAT(à)</td>
<td>58.0±3.4</td>
<td>60.0±4.0</td>
<td>43.5±2.0*</td>
<td>52.0±3.0*</td>
</tr>
<tr>
<td>GPx(à)</td>
<td>23.0±1.3</td>
<td>22.0±1.6</td>
<td>9.9±0.8*</td>
<td>18.3±1.5*</td>
</tr>
<tr>
<td>GSH(à)</td>
<td>6.2±0.12</td>
<td>7.2±0.15*</td>
<td>3.1±0.08*</td>
<td>4.8±0.08*</td>
</tr>
<tr>
<td>NPSH(f)</td>
<td>23.3±1.3</td>
<td>27.0±1.5*</td>
<td>16.0±0.8*</td>
<td>21.0±0.9*</td>
</tr>
</tbody>
</table>

Note: x - p <0.05 compared with the control group, o - p <0.05 compared with the data of ionizing radiation, à – nmol/mg protein; b – U/mg protein; c – µmoles H₂O₂ degraded/min/mg protein; d – nmol GSH/min/mg protein; e – µmol/gr tissue; f – nmol/mg protein

Table 4. The effect of “Nettle oil” and gamma irradiation on the state of lipid peroxidation and antioxidant protection in lung tissues

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>“Nettle Oil”</th>
<th>Gamma radiation</th>
<th>“Nettle Oil” and Gamma radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA(à)</td>
<td>2.58±0.11</td>
<td>2.2±0.08*</td>
<td>6.51±0.23*</td>
<td>3.6±0.13*</td>
</tr>
<tr>
<td>SOD(b)</td>
<td>75.0±1.6</td>
<td>66.0±1.5*</td>
<td>48.0±1.0*</td>
<td>66.0±1.3*</td>
</tr>
<tr>
<td>CAT(à)</td>
<td>73.0±1.5</td>
<td>76.0±1.8</td>
<td>43.0±1.1*</td>
<td>68.0±2.4*</td>
</tr>
<tr>
<td>GPx(à)</td>
<td>16.5±1.1</td>
<td>18.0±1.3</td>
<td>9.0±0.89*</td>
<td>12.5±0.9*</td>
</tr>
<tr>
<td>GSH(à)</td>
<td>5.0±0.11</td>
<td>6.0±0.1*</td>
<td>2.9±0.08*</td>
<td>4.0±0.12*</td>
</tr>
<tr>
<td>NPSH(f)</td>
<td>26.6±1.2</td>
<td>30.0±1.1*</td>
<td>18.0±1.3*</td>
<td>25.0±0.9*</td>
</tr>
</tbody>
</table>

Note: x - p <0.05 compared with the control group, o - p <0.05 compared with the data of ionizing radiation, à – nmol/mg protein; b – U/mg protein; c – µmoles H₂O₂ degraded/min/mg protein; d – nmol GSH/min/mg protein; e – µmol/gr tissue; f – nmol/mg protein
which determines the severity of oxidative stress in organ cells. These results are consistent with previous results\textsuperscript{8,32,48}. When applying “Nettle Oil” to ionizing radiation, this parameter decreased. Therefore, the introduction of “Nettle Oil” prior to irradiation significantly protected animals from radiation-induced lipid peroxidation, i.e. radiation-induced oxidative stress, which indicates its radical cleansing activity and the mechanism of chain destruction. With an increase in lipid peroxidation, biological membranes of internal organs (heart, lungs, kidneys, liver, and others) are affected, which leads to a loss of their fluidity, an increase in their permeability, and leakage of cytosolic enzymes.

The activity of transaminases, lactate dehydrogenase and creatine phosphokinase, which are markers of cytotoxicity, increases. In the present study, gamma radiation caused an increase in blood AST, ALT, LDH, and CK activity. These results are consistent with data from previous experiments in which researchers showed that ionizing radiation leads to an increase in serum LDH and CK and transaminases\textsuperscript{13,34,46}. The introduction of the drug “Nettle Oil” before ionizing irradiation led to a significant decrease in the activity of ALT, AST, LDH and CK compared with the ionizing irradiation group, which indicates a limitation of cell membrane damage.

Whereas, the use of “Nettle Oil” caused a decrease in LDH activity compared to control data. The antioxidant system of organs (the body), which is mainly involved in the neutralization and purification of ROS, prevents oxidative stress. In the present study, in addition to the MDA level, the OS intensity is characterized by a significant decrease in the activity of SOD, CAT, GPx and the content of GSH, NPSH in the lungs and cardiac tissue in irradiated rats, which is consistent with previous reports\textsuperscript{32,33}. Reduced glutathione (GSH) and non-protein thiol, the thiol constituents of the second line of cellular defense of AOS, function as a direct reactive free radical scavenger, removing hydroxyl radicals and singlet oxygen. Therefore, a decrease in the levels of GSH, NPSH reflects their increased need for cells, probably to combat ROS generated after irradiation\textsuperscript{50}. However, a decrease in GPx activity may be associated with a decrease in the availability of GSH, which is a substrate of GPx and is necessary for its catalysis\textsuperscript{47}.

Depletion of GSH (NPSH) causes an increase in hydroxyl radicals, which, in turn, attack lipid membranes and activate lipid peroxidation. While the decrease in SOD activity is due to inactivation of the enzyme, probably due to an increase in the production of superoxide radicals or inhibition by hydrogen peroxide ($H_2O_2$) as a result of a decrease in CAT activity.

The introduction of “Nettle Oil” within 2 weeks before irradiation increases the activity of SOD, CAT, GPx and levels of CSH, NPSH in the tissues of the heart and lungs compared with the data of the irradiated group, which indicates a decrease in oxidative stress, i.e. “Nettle oil” protects heart and lung tissue from the effects of superoxide ions, enhancing the activity of SOD, CAT and increasing levels of GSH, NPSH, which can be explained by the antioxidant properties of nettle leaves\textsuperscript{15,18,58,63}. The use of only one “Nettle Oil” led to a decrease in the level of malondialdehyde against a background of a significant increase in the levels of GSH, NPSH in the heart tissue and lungs, which once again proves the antioxidant activity of “Nettle Oil”\textsuperscript{57,62}, which is due to the content of a complex of valuable biologically active substances, including well-known antioxidants like $\alpha$-carotene, vitamin C and flavonoids.

The increase in the power of both the enzymatic and non-enzymatic AOS units in the tissues of the heart and lungs can be part of the cardioprotective, pulmonotropic effect of “Nettle oil”. Our results show that Nettle Oil reduces oxidative stress caused by ionizing radiation, i.e. exhibits antioxidant activity by removing free radicals and restoring the imbalance of prooxidant/antioxidant homeostasis with preventive administration (before irradiation). Probably, the explanation of the observed changes in antioxidant status during prophylactic use is that “Nettle Oil” contributes to an increase in the power of the antioxidant system in neutralizing and removing the oxidative stress radicals generated, which leads to a decrease in their levels.

In the present study, the lipid profile in rats irradiated with \(\alpha\)-rays showed significant changes: an increase in TS, TG, LDL-C and a decrease in HDL-C compared with the control\textsuperscript{8,32}. 


Obviously, “Nettle Oil”, when applied before irradiation, can successfully influence changes in the lipid profile due to gamma radiation and show an atherogenic (hypolipidemic, anticholesterolemic) property. Nettle Oil probably acts as an antioxidant (exhibits antioxidant activity) and inhibits the oxidation of lipids and lipoproteins (LDL-C) in cell membranes.

CONCLUSION

The results of the study show that the prophylactic use of “Nettle Oil” protects against radiation-induced oxidative damage by inhibiting lipid peroxidation and enhancing the antioxidant system in the tissues of the heart and lungs. We suggest that «Nettle Oil» has a high potential for the prevention of cardiovascular and pulmonary diseases during radiation therapy.

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