Ameliorative effect of septilin, an ayurvedic preparation against γ-irradiation-induced oxidative stress and tissue injury in rats

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Ionizing radiation is known to induce multiple organ dysfunctions directly related to an increase of cellular oxidative stress, due to overproduction of reactive oxygen species (ROS). This study was aimed to investigate the effect of septilin (an ayurvedic poly-herbal formulation containing the principal herbs, namely Commiphora wightii, Trinospora cordifolia, Rubia cardifolia, Emblica officinalis, Saussurea lappa and Glycyrrhiza glabra) against whole body γ-irradiation-induced oxidative damage in hepatic and brain tissues in rats. Administration of septilin for 5 days (100 mg/kg) prior to radiation resulted in a significant increase in both superoxide dismutase (SOD) activity and total glutathione (GSH) level in hepatic and brain tissues, while serum high-density lipoprotein-cholesterol (HDL) was reduced by γ-irradiation. Also, septilin resulted in a significant decrease in NO(x), nitric oxide and malondialdehyde (MDA) levels in hepatic and brain tissues and a significant decrease in serum triglycerides, low-density lipoprotein-cholesterol (LDL) and total cholesterol levels and serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) levels and alkaline phosphatase (ALP), γ-glutamyl transferase (GGT) activities, as well as serum tumor necrosis factor-alpha (TNF-α), compared to irradiated group. In conclusion, data obtained from this study indicated that septilin exhibited potential antioxidant activity and showed radioprotective effect against γ-radiation by preventing oxidative stress and scavenging free radicals.

Keywords: Septilin, γ-Radiation, Antioxidant, Tissue injury.

Radiation therapy is used commonly for solid cancers. More than 50% of patients with cancers receive radiation as a component of their treatment. It has been known that therapeutic radiation affects not only malignant tumors, but also the surrounding normal tissues and injuries to central nervous system, gastrointestinal tract and kidney occur commonly in patients undergoing cancer therapy. The risk of injury depends on the size, number and frequency of radiation fractions, volume of irradiated tissue, duration of treatment and method of radiation delivery.

Whole body exposure to ionizing radiation in humans and animals may trigger multiple organ dysfunctions directly related to an increase of cellular oxidative stress due to overproduction of reactive oxygen species (ROS) from molecular ionization. When cells or tissues are exposed to ionizing radiation, the water molecules undergo dissociation (radiolysis) and produce free radicals and related species in the form of ROS. These, in turn, can act on biomolecules, such as DNA, lipids and proteins and cause oxidative damage and subsequently elicit cellular defense mechanisms, such as cell cycle arrest, DNA repair, inflammation and activation of transcription factors like nuclear factor-κB (NF-κB). Septilin is an ayurvedic poly-herbal formulation containing the principal herbs, namely Commiphora wightii, Trinospora cordifolia, Rubia cardifolia, Emblica officinalis, Saussurea lappa and Glycyrrhiza glabra. It is extensively used in India for the treatment of acute and chronic infections. Various experimental and clinical studies suggest that septilin possesses immunomodulatory activity and radioprotective effect. Some of the plants like G. glabra, E. officinalis, R. cordifolia and Aegle marmelos have been found to possess antioxidant properties. It is reported that septilin's components having antioxidant principles show cytotoxicity towards tumor cells.
Septilin is reported to potentiate the non-specific defense mechanism of the body and thus helps overcome infections and inflammatory processes. It is also found to counteract cyclophosphamide-induced humoral suppression (both IgG and IgM suppression) in rats and myelo suppression and subsequent leucopenia in mice. Septilin is reported to enhance natural killer cell-mediated cytotoxicity and antibody-dependent cellular toxicity in normal mice, as well as tumor bearing mice. Thus, immune-stimulation by septilin may be responsible for enhanced anti-inflammatory effect of Septilin in chronic model. Therefore, in this study, we have evaluated the radioprotective effect of septilin against γ-irradiation-induced oxidative stress and tissue injury in rats.

Materials and Methods

Animals

Adult male Sprague-Dawley rats, weighing 120-150 g were obtained from the experimental animal house of the National Cancer Institute (NCI), Cairo University. Animals were kept under standard conditions and allowed free access to a standard requirement diet and water ad libitum. Animals were kept under a controlled lighting condition (light: dark, 13 h–11 h). The animals' treatment protocol was approved by the Animal Care Committee of the National Cancer Institute, Cairo, Egypt, following the guidelines of the National Institutes of Health (NIH).

Chemicals

EDTA, nitroblue tetrazolium (NBT), Greiss reagent, trichloro-acetic acid (TCA), thiobarbituric acid (TBA), 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) were procured from Sigma Chemical Co., St. Louis, USA. All other chemicals and solvents used were of the highest purity grade available. Septilin liquid preparation was obtained from the distribution unit of Himalaya Drug Company, Bangalore, Karnataka, India.

Irradiation

Wholebody γ-irradiation was performed at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt, using an AECL (137cesium) Gamma Cell-40 biological irradiator. Animals were irradiated at an acutesingle dose level of 4 and 6 Gy delivered at a dose rate of 0.012 Gy/s.

Experimental design

Sixty adult male albino rats were randomly divided into 6 groups, having 10 animals each. Group I was injected intraperitoneally with saline solution for 5 consecutive days and served as a control group. Group II was injected i.p. with septilin (100 mg/kg b.wt.) for five consecutive days. Group III was irradiated with a single dose of 4 Gy. Group IV was irradiated with a single dose of 6 Gy. Group V was injected with septilin (100 mg/kg, i.p.) for five consecutive days and 30 min after last injection rats were irradiated with 4 Gy. Group VI injected with septilin (100 mg/kg, i.p.) for five consecutive days and 30 min after last injection rats were irradiated with 6 Gy. Animals were anesthetized with ether 24 h after the last dose of the specific treatment and then sacrificed by decapitation. Blood samples were obtained by heart puncture and serum samples were separated for the measurement of indices of hepatotoxicity, lipid profiles and tumor necrosis factor-α (TNF-α).

Biochemical assays

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, serum alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), serum triacylglycerides, low-density lipoprotein-cholesterol (LDL), high-density lipoprotein-cholesterol (HDL) and total cholesterol were estimated. Serum TNF-α was determined by a sandwich ELISA method according to the manufacturer’s instructions (Becton Dickinson, USA).

Brains and livers were quickly excised, washed with saline, blotted with a piece of filter paper. Brain was homogenized using a Branson sonifier (250, VWR Scientific, Danbury, CT, USA). The homogenates were centrifuged at 800 g for 5 min at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 g for 20 min at 4°C to obtain the post-mitochondrial supernatant which was used to assay superoxide dismutase (SOD) activity, malondialdehyde (MDA) level, total glutathione (GSH) content and total nitrate/nitrite [NO(x)] level.

MDA, GSH levels and SOD activity in liver and brain tissue homogenates were determined spectrophotometrically. Total nitrate/nitrite [NO(x)] was measured as the stable end product, nitrite.

Statistical analysis

Differences between obtaining values (mean ± SE, n = 10) was carried out by one-way analysis of variance (ANOVA), followed by the Tukey-Kramer multiple comparison test. A p-value of 0.05 or less
was considered as a criterion for a statistically significant difference.

Results

Septilin injection daily for 5 consecutive days produced no significant changes in the measured biochemical parameters in serum, liver and brain tissue.

\(\gamma\)-Radiation (4 and 6 Gy) induced a significant increase in serum levels of cholesterol, triglycerides and LDL-C and significant decrease in HDL-C level, compared to control group \((P < 0.001\), Table 1); these effects were maximum when exposed to 6 Gy. Administration of septilin prior to IRR resulted in a significant decrease in cholesterol, triglyceride and LDL-C levels and significant increase in HDL-C levels, compared to the irradiated groups.

\(\gamma\)-Irradiation of 4 and 6 Gy induced a significant increase in the activities of serum ALT, AST, ALP and GGT \((P < 0.001)\) compared to the control group. Administration of septilin for five consecutive days prior to irradiation restored the ALT, AST, ALP and GGT activities to the normal control value. Serum TNF-\(\alpha\) level was markedly increased in IRR groups, as compared to control group. Administration of septilin prior to radiation significantly decreased serum TNF-\(\alpha\) levels, as compared to the IRR groups \((P < 0.001\), Table 2).

\(\gamma\)-Irradiation (4 and 6 Gy) resulted in a significant decrease in SOD activity and GSH content in brain and hepatic tissues compared to the control group \((P < 0.001)\) and significant increase in MDA and NO(x) levels in the brain and hepatic tissues compared to each control group. However, septilin induced non-significant change, compared to the control group. Administration of septilin for 5 consecutive days prior to irradiation resulted in a significant increase in SOD activity and GSH content and significant decrease \((P < 0.01)\) in MDA and NO(x) levels in brain and hepatic tissues, compared to irradiated groups (Fig. 1A-D).

### Table 1—Effect of septilin, radiation (IRR) and their combination on serum levels of triglycerides (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), and low density lipoproteins-cholesterol (LDL-C)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>LDL-C (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>28.40 ± 1.6</td>
<td>39.83 ± 0.8</td>
<td>54.32 ± 2.1</td>
<td>57.38 ± 0.6</td>
</tr>
<tr>
<td>IRR 4 Gy</td>
<td>40.89 ± 2.3闻言</td>
<td>24.91 ± 0.9闻言</td>
<td>97.92 ± 0.2闻言</td>
<td>77.12 ± 0.4闻言</td>
</tr>
<tr>
<td>IRR 6 Gy</td>
<td>45.44 ± 1.6闻言</td>
<td>20.38 ± 1.5闻言</td>
<td>109.36 ± 0.3闻言</td>
<td>89.98 ± 1.1闻言</td>
</tr>
<tr>
<td>Septilin</td>
<td>29.54 ± 1.5闻言</td>
<td>38.62 ± 1.0闻言</td>
<td>49.68 ± 0.4闻言</td>
<td>55.87 ± 0.5闻言</td>
</tr>
<tr>
<td>Septilin + IRR 4 Gy</td>
<td>31.70 ± 0.9闻言</td>
<td>36.70 ± 1.8闻言</td>
<td>57.11 ± 2.4闻言</td>
<td>62.48 ± 4.4闻言</td>
</tr>
<tr>
<td>Septilin + IRR 6 Gy</td>
<td>32.69 ± 0.9闻言</td>
<td>35.94 ± 2.4闻言</td>
<td>58.69 ± 2.5闻言</td>
<td>64.15 ± 3.7闻言</td>
</tr>
</tbody>
</table>

\(a\), \(b\), and \(c\) indicate significant change from control, irradiation (4 Gy) and (6 Gy), respectively at \(p \leq 0.05\) using ANOVA, followed by Tukey-Kramer as a post ANOVA test.

### Table 2—Effect of septilin, radiation (IRR) and their combination on serum tumor necrosis factor-alpha (TNF-\(\alpha\)), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and \(\gamma\)-glutamyl transferase (GGT) activities

<table>
<thead>
<tr>
<th>Parameters</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>GGT (U/L)</th>
<th>ALP (U/L)</th>
<th>TNF-(\alpha) (Pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.1 ± 0.62</td>
<td>68.6 ± 0.22</td>
<td>2.5 ± 0.25</td>
<td>403.7 ± 7.60</td>
<td>55.08 ± 0.8</td>
</tr>
<tr>
<td>IRR 4 Gy</td>
<td>40.2 ± 0.23闻言</td>
<td>76.1 ± 0.26闻言</td>
<td>6.6 ± 0.18闻言</td>
<td>446.8 ± 5.24闻言</td>
<td>225.0 ± 5.5闻言</td>
</tr>
<tr>
<td>IRR 6 Gy</td>
<td>54.3 ± 0.21闻言</td>
<td>84.6 ± 0.33闻言</td>
<td>10.3 ± 0.26闻言</td>
<td>460.2 ± 6.14闻言</td>
<td>277.0 ± 2.6闻言</td>
</tr>
<tr>
<td>Septilin</td>
<td>26.2 ± 0.21闻言</td>
<td>69.3 ± 0.20闻言</td>
<td>2.5 ± 0.11闻言</td>
<td>404.4 ± 8.2闻言</td>
<td>55.99 ± 1.3闻言</td>
</tr>
<tr>
<td>Septilin + IRR4 Gy</td>
<td>29.8 ± 1.66闻言</td>
<td>69.3 ± 1.6闻言</td>
<td>2.9 ± 0.11闻言</td>
<td>418.0 ± 1.3闻言</td>
<td>66.10 ± 2.9闻言</td>
</tr>
<tr>
<td>Septilin + IRR6 Gy</td>
<td>30.4 ± 1.57闻言</td>
<td>70.8 ± 3.26闻言</td>
<td>2.9 ± 0.15闻言</td>
<td>421.6 ± 2.1闻言</td>
<td>67.36 ± 3.9闻言</td>
</tr>
</tbody>
</table>

\(a\), \(b\), and \(c\) indicate significant change from control, irradiation (4 Gy) and (6 Gy), respectively at \(p \leq 0.05\) using ANOVA, followed by Tukey-Kramer as a post ANOVA test.
Fig. 1—Effect of septilin, radiation (IRR) and their combination on malondialdehyde (MDA) level (A), total nitrate/nitrite level [NO(x)] level (B), superoxide dismutase (SOD) activity (C) and reduced glutathione (GSH) content (D) in brain and hepatic tissues [Data presented as mean ± S.E., n = 10. a, b and c indicate significant change from control, irradiation (4 Gy) and (6 Gy), respectively at p ≤ 0.05 using ANOVA, followed by Tukey-Kramer as a post ANOVA test]

Discussion

Ionizing radiations are known to induce oxidative stress through the generation of ROS, resulting in an imbalance in the pro-oxidant, antioxidant status in the cells. Radiotherapy is an important modality for cancer treatment and it is estimated that approx. 50% of cancer patients are benefit by it. Although it is a common and important tool for cancer treatment, the radio-sensitivity of normal tissues adjacent to the tumor limits its therapeutic gain. Tissue injury from ionizing radiation ultimately begins with oxidative stress from radiolytic hydrolysis and the formation of ROS.

In the present study, exposure of rats to γ-irradiation induced a marked elevation in the activities of serum ALT, AST, ALP and γ-GGT and TNF-α level. This might be due to the release of these enzymes from the cytoplasm into the blood circulation rapidly after rupture of the plasma membrane and cellular damage due to oxidative damage generated as a result of increased free radical generation caused by irradiation. Treatment with septilin significantly reduced the activities of these marker enzymes in the serum of γ-irradiated rats. This suggested that septilin tends to prevent liver damage, preserved the integrity of the plasma membranes and hence restored these enzymes levels. In agreement with our study, previous studies have reported that S. lappa and glycyrrhizic acid found in G glabra (septilin’s components) show a transaminase-lowering and hepatoprotective effect and used for the treatment and control of liver disorders and chronic viral hepatitis.

The present study showed that exposure of rats to γ-irradiation induced a significant increase in cholesterol, triglycerides and LDL levels with a significant decrease in HDL compared to control group. In agreement with our results, previous studies have reported that whole body exposure to γ-radiation induces hyperlipidemia. Increased levels of serum cholesterol fractions was probably due to its release from tissues, destruction of cell membranes
and increase rate of cholesterol biosynthesis in the liver and other tissues. Lipoprotein modifications that appeared following radiation exposure might result from an induced inflammatory state and might further contribute to vascular damage. The increase in triglyceride levels might be related to the decrease in lipoproteinlipase activity in adipose tissue, leading to a reduction in the uptake of triglycerides.

The present data showed that γ-irradiation induced a significant increase in TNF-α level. This was in agreement with an earlier study which has reported that irradiation produces more NO in response to interferon-γ and/or lipopolysaccharide in murine macrophage cell lines and this increase is mediated by induction of TNF-α.

Previous reports have indicated that the radiation-induced injury might be caused, in part, by chronic oxidative stress and inflammatory responses, as well as increased macrophage activation. Another study has reported that irradiating macrophage cells in vitro causes to an increase in a variety of pro-inflammatory mediators, including the cytokines, TNF-α and IL-1β and the chemokine. Moreover, an in vivo study has reported an increase in pro-inflammatory mediators within hours of irradiating the rodent brain.

Pre-administration of septilin to irradiated rats significantly ameliorated the changes in cholesterol, triglycerides, HDL and LDL levels, compared to irradiated group. These results were in accordance with the previous study which has reported that E. officinalis (one of septilin’s components) decreases serum cholesterol, triglycerides, phospholipid and LDL levels. Also, E. officinalis shows anti-hypercholesterolemia, hypolipidemic, anti-atherosclerotic, hepatoprotective, nephroprotective and neuroprotective properties.

Moreover, a reduction of blood cholesterol levels in both normal and hypercholesterolemic men is reported on treatment with P. emblica (one of septilin’s components)9. Another study with alloxan-induced diabetic rats given an aqueous P. emblica extract has shown a significant decrease of the blood glucose, as well as triglyceridemic levels and an improvement of the liver function caused by a normalization of the liver-specific ALT activity.

On the other hand, C. mukul (one of septilin’s components) is known to normalize lipid metabolism, lower cholesterol and triglycerides, while maintaining or improving the HDL to LDL ratio. On the other hand, the methanolic extract of T. cordifolia stem (one of septilin’s components) is found to exhibit significant hypoglycemic and antioxidant activities in alloxan-induced diabetic rats. Moreover, septilin is reported to significantly block the lipopolysaccharide mediated nitric oxide (NO) production and expression of inducible NO synthase (iNOS) gene and inhibits TNF-α production in lipopolysaccharide activated monocytes and macrophage. C. mukul and S. lappa have shown strong anti-inflammatory potential. The anti-inflammatory activity of S. lappa is found to be due to the stabilization of lysosomal membranes, anti-proliferative effects and the inhibition of iNOS. The dehydrocostus lactone from S. lappa is found to decrease the TNF-α levels.

In the present study, we observed a significant increase in levels of MDA and NO(x) in concomitant with a significant decrease in the activity of SOD and the level of GSH in hepatic and brain tissues in γ-irradiated rats, indicative of oxidative stress in the liver and the brain. In agreement with our results, earlier studies have reported a significant depletion in the antioxidant system, accompanied by enhancement of lipid peroxides after whole body γ-irradiation. The increase in MDA level might be attributed to the high level of oxidative stress and the overproduction of ROS which interact with the polyunsaturated fatty acids in the lipid portion of biological membranes, initiating the lipid peroxidation and finally damaging the cell membranes.

The present study demonstrated a significant reduction in hepatic and brain GSH content and SOD activity, following radiation exposure. This could be due to the enhanced utilization of the antioxidant system, as an attempt to detoxify the free radicals generated by radiation. Depletion of GSH is known to cause an inhibition of the glutathione peroxidase activity and is shown to increase lipid peroxidation.

Septilin administration for 5 consecutive days prior to γ-irradiation effectively restored the depleted levels of the cellular antioxidants and lowered the radiation-induced lipid peroxidation and NO in the hepatic and brain tissues. This indicated the protective role of septilin by quenching the free radicals formed, thereby restoring the levels of the antioxidants. Earlier study has shown that E. officinalis (one of septilin’s component) pretreated irradiated animals exhibits a significant increase in GSH and decrease in lipid peroxidation level. E. officinalis, G. glabra,
R. cordifolia and P. emblica (septilin’s components) have also been reported to restore GSH levels and possess antioxidant and free radical scavenging activities. These findings suggested that the protective action of septilin was mediated via its antioxidant activity. Moreover, it is reported that oral administration of T. cordifolia roots extract (one of septilin’s component) exhibits antioxidant effects against alloxan-induced diabetes in rats. T. cordifolia is also found to increase GSH content and the activities of SOD, catalase and glutathione peroxidase in the heart and brain of diabetic rats.

Our results showed that whole body γ-irradiation of rats enhanced the formation of NO(x). Previous studies have reported that γ-irradiation might enhance endogenous NO biosynthesis in liver, intestine, lung, kidney, brain, spleen or heart of the animals, presumably by facilitating the entry of Ca ions into the membrane, as well as the cytosol of NO-producing cells though irradiation-induce membrane lesions. Administration of septilin ameliorated the increase in NO(x) level in hepatic and brain tissues. This result was in agreement with earlier study which reported that R. cordifolia (one of septilin’s components) inhibits the nitric oxide production in macrophages and exhibits free radical scavenging effect. T. cordifolia (one of septilin’s components) has shown a potent immunostimulatory activity which increases levels of antibodies and activates macrophages. S. lappa (one of septilin’s components) inhibits NO production. E. officinalis (one of septilin’s components) decreases free radical production and increases antioxidant enzyme levels. The high scavenging activity might help to arrest the chain of reactions initiated by the excess generation of NO that is detrimental to the human health. Septilin could also significantly block the lipopolysaccharide-mediated NO production and expression of inducible NO synthase (iNOS) gene.

P. emblica Linn., commonly known as amla in Ayurveda is shown to possess antipyretic, analgesic, anti-atherogenic, cardioprotective, gastroprotective, hepatoprotective, nephroprotective, neuroprotective, radiomodulatory, chemomodulatory, chemopreventive, free radical scavenging, anti-oxidant, anti-inflammatory, anti-mutagenic, anti-diabetic, hypolipidemic, chemopreventive and immunomodulatory activities that are efficacious in the treatment and prevention of cancer.

In conclusion, the present study demonstrated that septilin decreased lipid peroxidation and NO and thereby augmented the endogenous antioxidant enzymes in the hepatic and brain tissues of γ-irradiated rats. All these parameters suggested that septilin exhibited potential antioxidant activity and showed radioprotective effect against γ-radiation by preventing oxidative stress and scavenging free radicals.

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