Protective effect of *Ocimum sanctum* L after high-dose $^{131}$iodine exposure in mice: An *in vivo* study

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Radioprotective effect of aqueous extract of *Ocimum sanctum* (40 mg/kg body weight, for 15 days) in mice exposed to high-doses (3.7 MBq) of oral $^{131}$iodine was investigated by studying the organ weights, lipid peroxidation and antioxidant defense enzymes in various target organs like liver, kidneys, salivary glands and stomach at 24 hr after exposure in adult Swiss mice. The mean weight of the salivary glands showed significant increase after $^{131}$iodine administration. $^{131}$Iodine exposure significantly increased lipid peroxidation in kidneys and salivary glands in comparison to control animals. Pretreatment with *O. sanctum* in radioiodine exposed group showed significant reduction in lipid peroxidation in both kidneys and salivary glands. In liver, reduced glutathione (GSH) levels showed significant reduction after radioiodine exposure while pretreatment with *O. sanctum* exhibited less depletion in GSH level even after $^{131}$iodine exposure. However, no such changes were observed in stomach. The results indicate the possibility of using aqueous extract of *O. sanctum* for ameliorating $^{131}$Iodine induced damage to the salivary glands.

**Keywords**: Antioxidant enzymes, $^{131}$Iodine, Mice, *Ocimum sanctum*, Radioprotection, Salivary gland

Ionizing radiation is known to cause cellular damage mainly by formation of reactive oxygen species. These reactive oxygen species induce peroxidation of lipid as well as strand breaks and base alterations which ultimately leads to DNA damage. Following total or subtotal thyroidectomy in patients with well differentiated thyroid cancer high dose of radioiodine is often given for ablation of the thyroid remnant or metastasis. The other possible target organs likely to get substantial radiation exposure are salivary glands, liver, kidneys and stomach. Due to the presence of sodium iodide symporter, salivary glands concentrate almost 30 to 40 times of $^{131}$iodine in comparison to that found in plasma, which in turn is sufficient to cause irreversible damage to the glands in patients receiving large doses of radioiodine. The complications observed after radioiodine therapy impairs the quality of life of the patients during entire lifetime.

Recently, interest has been generated to develop effective drugs of plant origin for ameliorating radiation damage. Plant products appear to have advantage over the synthetic compounds in terms of low or no toxicity. Recently various plant products have been reported for their radioprotective potential in experimental animals as well as patients. Amongst them, *Ocimum sanctum* L (Tulasi) a medicinal plant in India is known to have antibacterial, immunomodulatory, anti-inflammatory, antifungal, antiviral and hepatoprotective properties. Uma Devi et al. have demonstrated radioprotection by using aqueous extract of *O. sanctum* and two flavonoids, orientin and vicenin, which were isolated from it against $^{60}$Co gamma radiation.

Thyroid cancer patients are administered with large doses of oral $^{131}$iodine at our Centre. However, there are no publications specifically studying the radioprotective effect of *O. sanctum* on $^{131}$iodine induced damage to various organs. Therefore, the aim of the present work is to study the damaging effects of large doses of oral $^{131}$iodine on the target organs like liver, kidneys, salivary glands and stomach and to
explore whether *O. sanctum* offers any protection against damaging effects in terms of lipid peroxidation and various antioxidant enzymes in mice.

**Materials and Methods**

**Animals**—Male adult Swiss mice of 8 to 10 week old, weighing 25 to 30 g, obtained from Animal House facility, BARC, Trombay, Mumbai, were used. They were maintained in an air conditioned room and were fed on standard mouse diet and water *ad libitum*. Animal studies were performed in compliance with BARC Institutional animal ethics committee’s guidelines.

**Experimental Design**

The mice (48) were divided into following 4 groups of 12 each. Group I mice were on normal diet and served as controls. Group II were given daily 40 mg/kg body weight aqueous extract of *O. sanctum* (Saiba Industries Pvt. Ltd. Mumbai, India) orally for 15 days. Group III mice were on normal diet and were given 3.7 MBq (100 µCi) 131Iodine (obtained from Board of Radiation and Isotope Technology, Vashi, India.) orally 24 hr prior to the sacrifice. Group IV mice were given 40 mg/kg body weight. *O. sanctum* orally for 15 days and 3.7 MBq of 131iodine was administered 24 hr after the last dose of *O. sanctum*.

The mice were sacrificed by cervical dislocation after 24 hr of oral administration of 131iodine. The liver, kidneys, salivary glands and stomach were dissected out, thoroughly washed in ice cold saline (0.9%), dried and weighed. The tissues were homogenized with 9 volume of cold phosphate buffer (pH 8) with 5 mM EDTA and 25 mM sucrose. The homogenate was used to measure malondialdehyde (MDA) content as an index of lipid peroxidation16, reduced glutathione (GSH)17 and protein18. The remaining homogenate was treated with Triton X-100 (0.05%) and centrifuged at 10,000 g for 30 min at 4°C. The supernatant obtained was used to estimate glutathione peroxidase (GPx)19, catalase (CAT)20 and superoxide dismutase (SOD)21.

All values were expressed as mean±SD and the data analysed using Student’s *t*-test for statistical significance.

**Results**

None of the experimental mice showed any noticeable toxic effect following administration of aqueous extract of *O. sanctum*. In addition, 3.7 MBq of oral dose of 131iodine did not give any morbidity or mortality in mice up to 24 hr.

In all the four groups, the weight of the organs like liver, kidneys, salivary glands and stomach was compared. Except for the weight of the salivary glands, no other organ showed any significant change (Fig. 1). The mean weight of the salivary gland shows significant reduction in group IV animals, pretreated with *O. sanctum* for 15 days prior to 131iodine administration.

Levels of lipid peroxidation, various defense enzymes and reduced glutathione levels in liver, kidney, salivary gland and stomach of animals from all the groups are given in Table 1.

Lipid peroxidation levels remained unaltered in liver while significant increase was observed in kidneys and salivary glands in animals exposed to 131iodine (Gr III). Pre supplementation with *O. sanctum* showed significant reduction in lipid peroxidation levels in kidneys and salivary glands in group IV animals. Liver GSH levels showed significant reduction in group III and IV as compared to group I. Kidney tissue demonstrated significant reduction in catalase levels in group III, while GPx levels remained raised in all the three groups in comparison to controls.

![Fig. 1 — Effect of *O. sanctum* supplementation on the weight of the salivary glands in experimental mice. (Group I = Control, Group II = *O. sanctum*, Group III = 131iodine, Group IV = *O. sanctum* + 131iodine. P values : * < 0.001 vs Group I; ** < 0.001 vs Group III)](image-url)
Discussion

Oral administration of $^{131}$Iodine is extensively used in diagnosis and therapy in patients suffering from thyroid diseases. Due to the effects of ionizing radiation, tissue destruction at the uptake site is observed\(^{22}\). In this process apart from the thyroid other organs like liver, kidney, salivary glands and stomach may also receive considerable radiation exposure. The present results clearly demonstrate that the administration of high doses of $^{131}$Iodine in mice show membrane damage at 24 hr especially in kidneys and salivary glands by increasing the levels of lipid peroxidation \textit{in vivo}. Radiation induced free radicals are known to produce oxidation of lipids, which leads to structural and functional damage to cellular membranes\(^{23}\). Further, the supplementation of \textit{O. sanctum} 15 days prior to radioiodine administration brings down the levels of lipid peroxidation significantly thereby showing membrane protection in these two organs against radiation induced oxidative damage.

The beneficial effect of antioxidants to reduce the deleterious effects of various mutagens has been studied in animal models for more than fifty years.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Organs</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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<tr>
<td>Lipid peroxidation</td>
<td>A</td>
<td>30.9 ± 4.3</td>
<td>28.0 ± 6.7</td>
<td>30.8 ± 3.8</td>
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<td>(nm MDA/gT)</td>
<td>B</td>
<td>27.5 ± 7.3</td>
<td>24.0 ± 3.9</td>
<td>38.6 ± 4.7(^b)</td>
<td>20.5 ± 1.9(^c)</td>
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<td>C</td>
<td>48.8 ± 5.1</td>
<td>40.6 ± 6.3</td>
<td>55.0 ± 3.5(^a)</td>
<td>41.3 ± 9.6(^c)</td>
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<td></td>
<td>D</td>
<td>36.4 ± 3.5</td>
<td>39.7 ± 2.4</td>
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<td>39.1 ± 3.8</td>
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<tr>
<td>GPx (10^4 × U/gT)</td>
<td>A</td>
<td>14.9 ± 2.8</td>
<td>20.6 ± 6.7(^h)</td>
<td>25.3 ± 2.2(^e)</td>
<td>25.0 ± 2(^c)</td>
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<td></td>
<td>B</td>
<td>18.6 ± 3.5</td>
<td>25.6 ± 3.3(^b)</td>
<td>31.9 ± 2.8(^e)</td>
<td>30.4 ± 2.1(^c)</td>
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<td></td>
<td>C</td>
<td>91 ± 3.0</td>
<td>93 ± 11.7</td>
<td>105 ± 15.0</td>
<td>83 ± 2.5(^d)</td>
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<td></td>
<td>D</td>
<td>30.3 ± 11.7</td>
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<td>Catalase</td>
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<td>1483 ± 220</td>
<td>1246 ± 355</td>
<td>1227 ± 235</td>
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<td>(U/gT)</td>
<td>B</td>
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<td>500 ± 79(^b)</td>
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<td>SOD</td>
<td>A</td>
<td>354 ± 10</td>
<td>324 ± 26</td>
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<td>B</td>
<td>252 ± 26</td>
<td>247 ± 6</td>
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<td></td>
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<td>48.3 ± 12.5</td>
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<td>GSH</td>
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<td>1.9 ± 0.3(^e)</td>
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<tr>
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<td>D</td>
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<td>Protein</td>
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<td>228 ± 36</td>
<td>238 ± 18</td>
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<td>246 ± 22</td>
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<tr>
<td>(mg/gT)</td>
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<td>243 ± 28</td>
<td>250 ± 18</td>
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<td>C</td>
<td>161 ± 44</td>
<td>135 ± 17</td>
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<td>154 ± 64</td>
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<tr>
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<td>D</td>
<td>115 ± 30</td>
<td>122 ± 48</td>
<td>115 ± 37</td>
<td>125 ± 43</td>
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</tbody>
</table>

\(P\) values, \(^a\)<0.05, \(^b\)<0.01, \(^c\)<0.001 vs Group I, \(^d\)<0.02, \(^e\)<0.01, \(^f\)<0.001 vs Group III

Group I = Control, Group II = \textit{O. sanctum}, Group III = $^{131}$iodine, Group IV = \textit{O. sanctum} + $^{131}$iodine
Uma Devi and co-workers have extensively studied the radioprotective effect of aqueous extract of *O. sanctum*\(^{11-13,24,25}\), as well as its two flavonoids, orientin and vicenin isolated from its leaves\(^{14,15}\). The present study showed a significant reduction in liver GSH in radioiodine treated group. Similar findings were reported by Uma Devi et al. after \(^{60}\)Co whole body irradiation\(^{25}\). This could be due to the enhanced utilization of the antioxidant system in an attempt to detoxify the free radicals generated by radiation. In the intact and healthy cells, the enzymes are restored immediately after each interaction\(^{26}\), which is also seen in the present experiment, where animals supplemented with *O. sanctum* before radiation exposure show significant increase in liver GSH level. However, the levels of liver GSH did not reach absolute normal level. It could be due to the fact that, in the irradiated animals the normal synthesis/repair may be disrupted due to damage to DNA and membranes. As a result, restoration will be delayed till the cells recover. However GPx levels remained raised in radioiodine treated groups when compared with control up to 24 hr. This may be required to combat the oxidative stress due to radioiodine exposure as indicated by depleted GSH levels in group III and IV.

In contrast to liver, kidneys showed increased lipid peroxidation thereby indicating the possibility of cellular damage at 24 hr after radiation exposure. Kidneys are known to eliminate the major portion of \(^{131}\)Iodine within 24 hr after radioiodine therapy thereby receiving extensive exposure\(^{4}\). However, permanent kidney damage was not seen after long duration of \(^{131}\)Iodine therapy in thyroid cancer patients\(^{27}\). It is possible that the increase in lipid peroxidation seen in kidneys in the present animals could be a transient phenomenon of cellular damage.

In the present study the \(^{131}\)Iodine was administered orally to simulate the mode by which thyroid cancer patients are given \(^{131}\)Iodine therapy. The oral route may expose the stomach to considerable amount of \(^{131}\)Iodine for longer time period. However, no significant changes suggestive of radiation damage were observed in the antioxidant defense system in the stomach at 24 hr.

Radiation damage to the salivary glands is a known complication of radioactive iodine therapy ranging from sialadenitis, xerostomia, to neoplasia in patients with differentiated thyroid cancer\(^{5,7}\). Moreover, patients with differentiated thyroid cancer has a very good prognosis when treated with \(^{131}\)I, hence it is essential to minimize the morbidity of the radioiodine treatment. Our major interest was to study the damaging effect of high dose of radioiodine in various organs such as liver, kidneys, stomach and salivary glands in particular, and the radioprotection possible by pretreatment with *O. sanctum*. In the present study, the increase in weight of the salivary glands after \(^{131}\)Iodine administration showed inflammation to the gland, which was not observed when the animals were pretreated with *O. sanctum*, thereby showing protection against the inflammation. Similar was the finding with salivary gland lipid peroxidation, where increased level was brought down to normal by pretreatment with *O. sanctum*. Konings et al. have proposed two different mechanisms causing salivary gland damage after irradiation\(^{28}\). During the initial phase, the defects in cellular function are observed due to selective membrane damage while late effects are observed due to shortage of properly functioning secretory cells. The present observation in this regard is for 24 hr of the \(^{131}\)Iodine exposure where increase in lipid peroxidation may reflect the early membrane damage. It is possible that more significant changes may develop at longer duration of \(^{131}\)Iodine exposure which may be expressed in succeeding cell generations of the salivary glands\(^{6,28}\).

So far only drug available for reducing the side-effects of ionizing radiation in salivary glands is amifostine, an organic thiophosphonate, which undergoes dephosphorylation within the tissues to its active metabolite WR-1065. It acts as a scavenger of oxygen free radicals which are the cause of radiation induced tissue damage\(^{5}\). This treatment, though proved beneficial, against radiation damage, is known to give the side-effects such as lowering of blood pressure\(^{6,29}\). Present practice is the use of sour candy or lemon juice to increase the salivation during \(^{131}\)Iodine administration to prevent accumulation of \(^{131}\)Iodine in the gland thereby reducing the salivary gland damage\(^{30-32}\). Nonetheless, there has been no established evidence that such a treatment actually reduces injury to the salivary gland after \(^{131}\)Iodine therapy. Recently Nakada et al. have done the study to determine whether an early start of sucking lemon candy decreases the side-effects of radioiodine therapy on the salivary glands\(^{33}\). Surprisingly they have found that it actually induces a significant increase in salivary gland damage.

The mechanism of action of *O. sanctum* seems to be via free radical scavenging ability as is also
observed with amifostine. However, scavenging of free radicals may not be the only pathway by which *O. sanctum* acts as a radioprotectant *in vivo*. Talalay *et al.* have suggested that protection against chemical carcinogenesis by phenolic antioxidants could be due to the bonding of the compounds to receptors which results in increase in activity of detoxifying enzymes in cytosol and microsomes and enzymes that increase glutathione synthesis. Moreover, orientin and vicenin, up to the concentration of 100 mg/kg body weight did not show any toxicity. Hence it makes *O. sanctum* a potential candidate which can be used for radioprotection against 131Iodine induced salivary gland damage.

All these studies on radioprotection have been performed *in vivo* as well as *in vitro* by using 60Co gamma radiation. In the present study, the animals were exposed to 131Iodine *in vivo* which also emit β radiation in addition to gamma rays. To our knowledge, so far no such study has been done by using high doses of 131Iodine and the protective effect offered by *O. sanctum* in various target organs. The present experiments have shown encouraging results. In conclusion, the study in higher animals on the action of *O. sanctum* and on the mechanism of protection against 131Iodine damage in salivary glands for longer duration should be performed before coming to clinical trials in patients with thyroid cancer.

References


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