Effect of *Ocimum sanctum*, turmeric extract and vitamin E supplementation on the salivary gland and bone marrow of radioiodine exposed mice

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Significant increase in the salivary gland weight was observed after exposure to single therapeutic dose of 3.7 MBq of $^{131}$I in mice. Pre-supplementation of antioxidants, *O. sanctum* leaf extract, turmeric extract and vitamin E for 15 days before $^{131}$I exposure demonstrated significant reduction in the salivary gland weight. No major histopathological changes were observed in the salivary gland of experimental animals at 24 h of exposure. Micronuclei index in the bone marrow of polychromatic (PCEs) and normochromatic erythrocytes (NCEs) remained unchanged in all the experimental groups. However, PCE/NCE ratio in the bone marrow decreased significantly in all the $^{131}$I exposed animals irrespective of antioxidant supplementation status. The normalization of salivary gland weight by antioxidant pre-supplementation in radioiodine exposed mice is suggestive of the possible ameliorating effect of antioxidants on the salivary gland weight recommending further detailed studies regarding the functional aspect of the salivary gland in higher animals.

Keywords: Antioxidants, Bone marrow, Radioiodine, Salivary glands

Since decades, usage of various radionuclides *in vitro* or *in vivo* as a diagnostic or therapeutic modality has been practiced worldwide. It has been more than half a century since $^{131}$I was introduced in the medical practice and used widely for diagnosis and therapy of thyrotoxicosis and differentiated thyroid carcinoma. It has been postulated that around 90% radiation effects are due to beta radiation with track length of 0.4 mm in soft tissue. This relatively short range could indicate that extra thyroidal radiation is minimal and therefore side-effects as such should be few, making it a relatively safe isotope for internal administration, after following the standard radioprotection procedures.

However in practice, large dose of $^{131}$I therapy ranging from 18.5-37GBq has been found to be associated with certain acute side effects of radiation such as gastrointestinal discomfort, nausea, sialadenitis, xerostomia, transient hematological suppression, and radiation thyroiditis. Nevertheless many of these side effects are reversible in nature. Amongst all these, the salivary gland damage, as reflected by sialadenitis and xerostomia has been observed in around 30% of the patients with thyroid carcinoma receiving radioiodine therapy and may be of permanent nature. Majority of the patients receiving therapeutic ionizing radiation exposure are likely to be subjected to the risk of induction of genetic damage which in turn could be associated with secondary malignancy. Therefore it is essential to know the extent of genotoxicity associated with $^{131}$I exposure *in vivo*. Most of the literature available regarding the genotoxicity of $^{131}$I exposure is based either on the individuals who suffered from $^{131}$I fall out in Chernobyl accident or their offsprings. So far limited *in vivo* studies have been reported regarding genotoxicity of therapeutic dose of $^{131}$I exposure in patients.

The damaging effects of radioiodine exposure have been correlated with the *in vivo* generation of reactive oxygen species which can induce damage to critical macromolecules such as DNA. Antioxidants are known to scavenge the reactive oxygen species thereby protecting the *in vivo* milieu from the toxic and damaging effects of ionizing radiation. Currently, use of herbal formulations and natural compounds with antioxidant properties is being explored for the purpose of radioprotection. The beneficial effect
of *O. sanctum*, turmeric extract and vitamin E have been studied on lipid peroxidation and antioxidant defense enzymes in liver, kidney, stomach and salivary glands against *in vivo* therapeutic radioiodine exposure\textsuperscript{14,15}.

In the present experiment an animal model has been designed to study the damaging effect of the therapeutic radioiodine exposure and the effect of antioxidant supplementation on the bone marrow and salivary gland weight of the experimental mice. Salivary gland histopathology is also performed to see the changes in cellular architecture and its possible correlation with the salivary gland weight.

**Materials and Methods**

**Animals**—Male adult Swiss mice, 8-10 weeks age, weighing 25-30 g obtained from Animal House Facility, BARC, Trombay, Mumbai were used. They were maintained under controlled conditions of temperature and light in an animal house 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.

*O. sanctum*—Fresh tulsi (*Ocimum sanctum* L) leaves were collected locally, air dried, powdered and extracted with double distilled water by refluxing. The residue was concentrated and the extract was obtained from Saiba Chemicals Pvt Ltd, Mumbai, India. This extract was dissolved in double distilled water just before administration.

Turmeric extract—Fine powder of dry rhizomes of turmeric (*Curcuma longa* L) was subjected to cold ethanolic percolation. The ethanolic extract was then evaporated to obtain smooth paste of turmeric extract, which was obtained from Saiba Chemicals Pvt Ltd, Mumbai, India. The turmeric extract was dissolved in distilled water just before the administration.

**Experimental design**—The mice were divided in 8 groups of 6 each. All the animals were on normal diet and were treated as follows:

- **Group I**: Control mice.
- **Group II**: Oral administration of single dose of 3.7 MBq \textsuperscript{131}I (obtained from Board of Radiation and Isotope Technology, Vashi, India) 24 h preceding sacrifice.
- **Group III**: Oral supplementation of *O. sanctum* (40 mg/kg body weight) daily for 15 days preceding sacrifice.
- **Group IV**: Oral supplementation of *O. sanctum* (40 mg/kg body weight) daily for 15 days, followed by administration of single dose of 3.7 MBq \textsuperscript{131}I orally, 24 h preceding sacrifice.
- **Group V**: Oral supplementation of turmeric extract (40 mg/kg body weight) daily for 15 days preceding sacrifice.
- **Group VI**: Oral supplementation of turmeric extract (40 mg/kg body weight) daily for 15 days, followed by administration of single dose of 3.7 MBq of \textsuperscript{131}I orally, 24 h preceding sacrifice.
- **Group VII**: Oral supplementation of vitamin E (400 IU/kg body weight) daily for 15 days preceding sacrifice.
- **Group VIII**: Oral supplementation of vitamin E (400 IU/kg body weight) daily for 15 days, followed by administration of single dose of 3.7 MBq of \textsuperscript{131}I orally, 24 h preceding sacrifice.

The duration between the last dose of antioxidant supplementation and oral \textsuperscript{131}I administration was 24 h. The animals were sacrificed by cervical dislocation after 24 h of the oral dose of 3.7 MBq \textsuperscript{131}I. The salivary glands of the mice were dissected out, thoroughly washed in ice cold saline (0.9% NaCl) dried and weighed and then preserved in 10% formalin for histopathology.

Bone marrow samples of animals were collected in 5% bovine serum albumin in buffered saline for micronucleus test.

**Micronucleus test**—The micronucleus test in the bone marrow cells was performed according to Schmid\textsuperscript{16} and Heddle\textsuperscript{17}. Briefly, the animals were sacrificed by cervical dislocation and both femur bones were excised. Bone marrow was flushed with the help of syringe in tube containing 5% bovine serum albumin in buffered saline. The tube was centrifuged at 1000 rpm for 5 min. and the supernatant was removed. The sediment was mixed thoroughly and used for making bone marrow smears and allowed to dry overnight. The staining of the slides was done using 0.2% May-Grunwald in ethanol and 2% Giemsa stain as described by Schmid\textsuperscript{16} . For each animal minimum 2000 polychromatric erythrocytes (PCEs) and 2000 Normochromatic erythrocytes (NCEs) were analysed and number of micronucleated PCE and NCE was scored. The ratio of PCE/NCE was also assessed.

**Statistics**—Data were summarized as mean ± SE. It was tested for normal distribution and homogeneity of variance. The ANOVA with Post-Hoc correction was used for the comparison between the different groups. The *P* value of < 0.05 was considered to be significant.
Results
None of the experimental animals showed any noticeable toxic effects following supplementation of any of the three antioxidants for 15 days duration. In addition, single dose of 3.7 MBq oral dose of $^{131}$I did not give any morbidity or mortality in animals up to 24 h.

Weight of the salivary glands in animals supplemented with only *O. sanctum*, turmeric extract and vitamin E remained comparable to the controls (Gr III, Gr V, and Gr VII vs Gr I; Fig. 1). Animals from Gr II exposed to only single dose of 3.7 MBq $^{131}$I exhibited significant rise in salivary gland weight as compared to the control animals ($P<0.005$). Pre-supplementation of *O. sanctum*, turmeric extract as well as vitamin E for 15 days preceding $^{131}$I exposure resulted in significant reduction in salivary gland weights as compared to Gr II animals exposed to only $^{131}$I, thereby indicating the possible ameliorating effect of all the three antioxidants on the salivary gland weight (Gr IV, VI, VIII vs Gr II). However, amongst all three antioxidant treated groups, turmeric extract supplemented group demonstrated partial normalization of the weight of the salivary gland (Gr VI vs Gr I, $p=0.043$, Fig. 1).

The number of PCE and NCE were counted in each group and PCE/NCE ratio was calculated. No significant difference in PCE/NCE ratio was observed in animals supplemented with only *O. sanctum*, turmeric extract and vitamin E in comparison to controls (Gr III, Gr V and Gr VII vs Gr I). Significant reduction in PCE/NCE ratio was observed in animals from Group II exposed to only $^{131}$I as well as in antioxidant pre-supplemented and exposed to $^{131}$I in comparison to Group I (Group II, IV, VI and VIII vs Gr I, $P = 0.001$, Table 1). In addition, no significant change was observed in the frequency of micronucleated polychromatic erythrocytes (MPCE) and micronucleated normochromatic erythrocytes (MNCE) in the bone marrow of the experimental animals at 24 h of 3.7 MBq of $^{131}$I exposure (Table 1).

Salivary gland histopathology exhibited slight changes such as swelling of the cells along with lighter nucleus and sparingly vacuolated cytoplasm in the $^{131}$I exposed animals when compared with controls. However, no major appreciable changes were seen in the salivary gland architecture of any of the experimental animals as compared to controls.

Discussion
The effectiveness and utility of $^{131}$I therapy for thyroid cancer patients is unequivocal however, controversy still exists regarding the extent of health risk associated with the therapeutic radiation exposure$^{1,5,6,18}$. Presence of DNA damage in the peripheral blood lymphocytes of the thyroid carcinoma patients receiving high dose of radioiodine is widely reported$^{11}$. Large therapeutic dose of radioiodine is generally given to ablate the remnant thyroid tissue post thyroidectomy as well as metastasis in the differentiated thyroid carcinoma. Apart from thyroid, salivary gland also has the ability to concentrate iodine selectively due to the presence of sodium iodide symporter$^{19}$. Almost immediately after$^{131}$I therapy, transient swelling and pain with decreased salivary flow, usually bilateral involving parotid glands, have become a known problem$^{20}$. The swelling of salivary glands may subside after a fortnight of radiotherapy or may persist for a longer while, leading to partial or total xerostomia of transient or permanent nature affecting the quality of life of the patients$^{1,12,21}$. Konings *et al.*$^{22}$ in their extensive review on the mechanism of salivary gland damage in rodents have reported no major cellular loss in the early stages (0-10 days) of salivary gland radiation exposure of 15-50 Gy. However significant reduction in the water excretion and the salivary flow is observed at this stage. The conformational changes or the damage to the cellular membrane affecting the

![Fig. 1 — Effect of antioxidant supplementation on the salivary gland weight of the experimental mice. (Gr I = Control; Gr II= $^{131}$Iodine; Gr III= *O. sanctum*; Gr IV= *O. sanctum* + $^{131}$Iodine ; Gr V=turmeric extract; Gr VI = turmeric extract + $^{131}$Iodine; Gr VII = vitamin E; Gr VIII =vitamin E + $^{131}$Iodine $P$ values: $^a <0.005$ vs Gr I, $^b = 0.043$ vs Gr I; $^c <0.005$ vs Gr II; $^d =0.023$ vs Gr II)](image-url)
receptor mediated signal transduction is considered to be the key event which in turn could be responsible for the observed salivary gland damage after radiation exposure. A significant elevation in the levels of lipid peroxidation in the salivary gland of mice treated with only 3.7 MBq of $^{131}$I at 24 h was reduced significantly in antioxidant pre-supplemented group thereby indicating the possible ameliorating effect of antioxidant supplementation on the cellular damage. In the present study significant increase in the salivary gland weight of the mice exposed to $^{131}$I at 24 h may be the result of the fluid retention due to the radioiodine exposure. The decrease in water excretion in salivary glands at initial stages of the radiation exposure is well-documented. However, all the three antioxidant pre-supplemented and radioiodine exposed groups from the present study showed a significant normalization in weight of the salivary gland. These observations are suggestive of the possible beneficial effect of antioxidant supplementation on the salivary gland.

Amifostine, a known synthetic compound is currently used for the purpose of radioprotection, however the side effects associated with it limits its usage in patients. So far no other drug is available for effective radioprotection without side effects in the patients. In this context use of the antioxidants for radioprotection of salivary glands against radiation exposure is being explored. Vitolo et al. have observed the beneficial effect of Tempol administration on the salivary gland hypofunction after exposure to external irradiation from 5 to 20 Gy in mice. Recently pre-supplementation of antioxidant defence enzyme superoxide dismutase in lecithinized form to the UV-B irradiated human salivary gland cells, has exhibited improvement in the salivary secretion. The antioxidants used in the present experiments (O. sanctum, turmeric extract, and vitamin E) have been tested in past for the purpose of radioprotection against external $\gamma$ radiation and found to be quite effective. However limited data are available regarding the usage of antioxidant supplementation for radioprotection of salivary glands against the internal radioiodine exposure.

In the present study histopathological changes of salivary gland revealed a minor cytoplasmic vacuolization due to radioiodine exposure in experimental mice as compared to the control group. Severe hypofunction of salivary gland alongwith lipomatosis and inflammation at cellular level are documented as the late effects of the radiation exposure. However no major histopathological changes at 24 h of single dose of 3.7 MBq $^{131}$I exposure were observed. It may be due to the small dose as well as the short duration of the radioiodine exposure for the manifestation of the major cytological changes in salivary glands.

Bone marrow erythrocytes provide useful, convenient and reliable assay system for evaluation of the genotoxicity in animals. Increase in the micronuclei frequency in polychromatic and normochromatic erythrocytes serve as an index of

<table>
<thead>
<tr>
<th>Groups</th>
<th>MPCE/2000 cells</th>
<th>MNCE/2000 cells</th>
<th>PCE/NCE ratio</th>
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</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1.42 ± 0.200</td>
<td>0.42 ± 0.153</td>
<td>0.98 ± 0.030</td>
</tr>
<tr>
<td>Group II</td>
<td>1.42 ± 0.271</td>
<td>0.25 ± 0.112</td>
<td>0.74 ± 0.024</td>
</tr>
<tr>
<td>Group III</td>
<td>0.92 ± 0.374</td>
<td>0.67 ± 0.272</td>
<td>0.94 ± 0.024</td>
</tr>
<tr>
<td>Group IV</td>
<td>0.83 ± 0.167</td>
<td>0.83 ± 0.333</td>
<td>0.68 ± 0.030</td>
</tr>
<tr>
<td>Group V</td>
<td>1.33 ± 0.333</td>
<td>0.33 ± 0.167</td>
<td>1.03 ± 0.058</td>
</tr>
<tr>
<td>Group VI</td>
<td>1.00 ± 0.258</td>
<td>0.25 ± 0.112</td>
<td>0.74 ± 0.018</td>
</tr>
<tr>
<td>Group VII</td>
<td>0.92 ± 0.327</td>
<td>0.50 ± 0.183</td>
<td>1.03 ± 0.045</td>
</tr>
<tr>
<td>Group VIII</td>
<td>1.00 ± 0.258</td>
<td>0.58 ± 0.201</td>
<td>0.66 ± 0.040</td>
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Gr. I = Control; Gr. II= $^{131}$Iodine; Gr. III= O. Sanctum; Gr. IV= O. sanctum + $^{131}$Iodine; Gr. V= turmeric extract; Gr. VI= turmeric extract + $^{131}$Iodine; Gr. VII= vitamin E; Gr. VIII= vitamin E + $^{131}$Iodine

MPCE: Micronucleated Polychromatic erythrocytes and MNCE: Micronucleated Normochromatic erythrocytes.
P values: \( a < 0.001 \) vs Gr I; \( 0.001 < b < 0.005 \) vs Gr I; \( c < 0.005 \) vs Gr III; \( d < 0.005 \) vs Gr V; \( e < 0.005 \) vs Gr VII;
chromosomal damage\textsuperscript{35}. In the present study, no difference in bone marrow micronuclei frequency in polychromatic erythrocytes in any of the group indicates absence of genotoxicity due to the single dose of 3.7 MBq of radioiodine exposure. Transient suppression of cell proliferation is a known acute effect of radiation exposure\textsuperscript{35}. Decrease in the ratio of PCE/NCE demonstrates suppression in the generation of hematopoietic tissue\textsuperscript{35}. In the present study the observed decrease in PCE/NCE ratio at 24 h post radioiodine exposure may be due to the similar suppressive effect on the bone marrow which could be of transient nature. The antioxidant supplementation has not improved the observed decline in PCE/NCE ratio after radioiodine exposure. This may be due to the long time gap of 24 h between the last dose of the antioxidant supplementation and radiation exposure.

The present study is a modest attempt to address the two facets of therapeutic radioiodine exposure which includes association of genotoxicity due to the therapeutic single dose of radioiodine and ameliorative potential of the antioxidant pre-supplementation on the salivary gland damage. In conclusion, the single dose of 3.7 MBq of the radioiodine has not shown any marked signs of genotoxicity on the bone marrow of mice whereas the antioxidant pre-supplementation against the same dose has shown the possible beneficial effect on salivary gland weight. However, in order to justify usage of these antioxidants in patients for amelioration of the salivary gland damage, the dynamic study of the salivary gland function needs to be performed using imaging modalities in higher animals.

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References


