Radioprotective, anticarcinogenic and antioxidant properties of the Indian holy basil, *Ocimum sanctum* (Tulasi)

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*Ocimum sanctum* (tulasi), an Indian herb, well known for its medicinal properties, has been shown to have significant radioprotective, tumor preventive and antioxidant effects in animal models. The present paper reviews the recent literature on these aspects of this plant.

*Ocimum sanctum* (Sanskrit: Tulasi; English: holy basil; family: Labiatae), is found throughout the semi-tropical and tropical parts of India. It is grown in the gardens and is worshipped as a sacred plant by the Hindus. The medicinal value of this plant is known for millennia. The different parts of the plant are traditionally used in the Ayurveda and Siddha systems for the treatment of diverse ailments like infections, skin diseases, hepatic disorders, common cold and cough, malarial fever and as an antidiote for snake bite and scorpion sting. The Santal tribe uses the plant in fever, dropsy, vomiting, constipation, cough, postnatal complaints, etc. *O. sanctum* is also used as an ingredient of herbal preparations with other medicinal plants. Investigations on its pharmacological and therapeutic properties during the past decades have led to a number of publications. Literature on the ethnobotany, pharmacognosy and pharmacology of *Ocimum* sp. has been extensively reviewed by Satyavati and co-workers. Water extract of the leaves was reported to have a hypotensive action in dogs. A 50% ethanolic extract of *O. sanctum* leaves showed hypoglycemic effect in rats and antispasmodic activity in guinea pigs. The ether extract and essential oil of the leaves exhibited antibacterial activity against a number of bacterial species. The essential oil also exhibited antifungal activity. The extract and essential oil of the leaves exhibited antiviral activity against some common plant viruses like papaya leaf reduction virus, top necrosis virus of pea and bean mosaic virus. The acetone extract of the plant showed insecticidal activity against Spodoptera litura, while the water extract has nematocidal activity. The anti-stress effect of the plant has been reported in mice and rats. Hepatoprotective activity of the extract against carbon tetrachloride toxicity and paracetamol induced damage have been demonstrated. A methanol extract and an aqueous suspension of *O. sanctum* leaves were found to have anti-inflammatory and analgesic and immunostimulatory properties. Aqueous extract of the leaves and pure eugenol, which is a constituent of the extract, were reported to reduce the biochemical and membrane changes induced by restraint stress in rats. *Ocimum sanctum* extract also protected against experimental ulcers, which was related to its ability to reduce acid secretion and increase mucus secretion. The essential oil of *O. sanctum* was observed to have anti-inflammatory and anti-ulcer activities in rats. Oral feeding of *Ocimum* leaf powder for one month was found to reduce fasting blood sugar and cholesterol levels in blood, liver and heart of rats; the results indicated a hypoglycemic and hypolipidemic effect in diabetic rats.

A preliminary clinical trial showed that treatment with the aqueous extract of *O. sanctum* leaves gave a higher survival rate among patients suffering from viral encephalitis than in patients given steroid treatment. Oral administration of 250 g of dried leaf powder daily for 6 weeks reduced the blood pressure in hypertensive patients. There has been a high interest in the radioprotective, anticarcinogenic and antioxidant properties of this plant in the recent years. The present paper attempts to review the publications on these topics.

**Radioprotective effect**

Uma Devi and Ganasoundari first reported the protective effect of *Ocimum sanctum* against radiation injury. They compared survival of adult Swiss albino mice at 30 days after whole body lethal irradiation
with/without prior treatment with single graded doses of aqueous and ethanolic extracts from dried leaves of Krishna tulasi (dark-leafed variety of *O. sanctum*) and demonstrated that: (1) the aqueous extract was significantly more effective in increasing the 30-day mouse survival; (2) the optimum dose for protection of the aqueous extract by intraperitoneal (i.p.) administration was 50 mg/kg, while the acute LD₃₀ for the mice was more than 6 g/kg body weight; (3) administration of the optimum dose in fractions of 10 mg/kg /day on five consecutive days before irradiation gave better protection compared to a single administration of 50 mg/kg of the extract; (4) the extract was also effective orally in increasing mouse survival, although to a lesser extent than i.p. administration; and (5) the optimum dose of the extract, given i.p. before radiation, gave a dose modification factor (DMF, the ratio of the radiation dose needed to produce the same effect in the presence and in the absence of the protector) of 1.28 for 30 day mouse survival. These properties are similar to those reported for a synthetic drug, 2-mercaptopropionylglycine (MPG), which is a good antioxidant and a radioprotector at nontoxic doses.

Animal survival at 30 days after lethal whole body irradiation indicates recovery and regeneration of hemopoietic progenitor cells in the bone marrow. An i.p. injection of *Ocimum* leaf extract (OE) at the optimum dose (10 mg/kg daily for 5 days) before a sublethal (2 Gy) whole body gamma irradiation produced a significantly higher bone marrow stem cell survival (CFU-S) than a pretreatment with 300 mg/kg (-40 % of its LD₃₀) of the well known radioprotector WR-2721 suggesting that, in terms of the protective dose and toxicity, the extract may be a better protector than the synthetic compound. Analysis of chromosomal aberrations in the bone marrow of mice exposed to a range of sublethal whole body gamma doses showed that the extract significantly reduced the percent aberrant metaphases as well as the different aberrations, including dicentrics and rings, induced by radiation doses of 3-5 Gy. Decrease in percent aberrant metaphases by OE pretreatment was comparable to that given by 400 mg/kg of WR-2721; but the latter was more efficient in reducing the multiple aberrations (severely damaged cells, which contain more than 10 aberrations per cell) and polyploidy induced by 4.5 Gy of gamma radiation. While the OE pretreatment did not produce any toxic side effects, 300-400 mg/kg WR-2721 before exposure to 4.5 Gy resulted in delayed chromosome toxicity in the form of an increase in the percent aberrant cells at 14 days post-irradiation. Combination of OE treatment with WR-2721 before irradiation considerably enhanced the chromosome protection (giving a 2-fold increase in the protection factor from that obtained with either protector individually) and also eliminated the delayed chromosome toxicity of the latter. The extract was also found to protect mouse liver against radiation induced lipid peroxidation; this effect was not very different in animals treated with a single dose of 50 mg/kg OE or five doses of 10 mg/kg OE before irradiation. The anti-lipid peroxidative effect was associated with an increase in the levels of the cellular antioxidants like reduced glutathione (GSH), GSH-transferase, GSH-peroxidase and reductase as well as superoxide dismutase in the liver.

Nair et al. have reported the presence of ursolic acid, apigenin, luteolin, apigenin-7-O-glucuronide, luteolin-7-O-glucuronide, molludustin and orientin in *Ocimum* leaves. Norr and Wagner identified vicenin-2 (apigenin-6,8-C-diglucoside), gluteolin (luteolin-5-O-glucoside), cirsimelone (5,4'-dihydroxy-6,7,3'-trimethoxyflavone), eugenyl-β-D-glucoside (4-allyl-1-O-β-D-glucopyranosyl-2-methoxybenzene) and 4-allyl-1-O-β- D-glucopyranosyl-2-hydroxybenzene. In our laboratory attempts to isolate the chemical components from the aqueous extract yielded only two compounds in sufficient quantity for experimental studies. The compounds were identified as the C-glycoside flavonoids, orientin (8-C-β-D-glucopyranosyl-luteolin) and vicenin-1 (6-C-β-D-xylpyranosyl-8-C-β-D-glucopyranosyl apigenin). Both are readily soluble in water and did not show any systemic toxicity in mice even at a dose of 100mg/kg body-weight. Both compounds significantly increased mouse survival when administered i.p. 30 min before a lethal whole body gamma irradiation. The optimum dose for protection was found to be 50μg/kg body weight i.p., which was the most effective route; oral and intravenous routes also significantly increased survival, but to a lesser extent. At the optimum dose, vicenin gave a slightly higher protection (DMF = 1.37) than orientin (DMF = 3.0) and was better than 150 mg/kg WR-2721 in reducing chromosomal aberrations in bone marrow of mice exposed to 2 Gy of gamma radiation. Both the compounds were equally effective in protecting against radiation induced lipid peroxidation in mouse liver and also significantly...
inhibited the Fenton reaction induced OH radical activity in vitro.

These studies suggest that Ocimum leaf extract and its two flavonoids could be useful in radiation protection of healthy individuals engaged in radiation related work and for reducing the side effects of radiotherapy in cancer patients. For use in cancer patients, the protector should have a preferential effect on normal tissues, with little or no protection to tumors. Preliminary studies on two transplanted mouse tumors, B16F1 melanoma and fibrosarcoma, in the another's laboratory have shown that neither the extract nor the flavonoids protected solid tumors (unpublished). This, along with the finding that these protectors are effective orally and that the protective doses are much lower than the toxic level, strongly recommends low/non-toxic protectors from this plant for human application.

**Anticarcinogenic effect**

Aruna and Sivaramakrishnan, based on their findings that the leaves of Ocimum sanctum suppressed benzo(a)pyrene-induced chromosomal aberrations in bone marrow and elevated glutathione (GSH) and glutathione-S-transferase (GST) activities in the liver of mice, suggested a possible role of the plant in protecting against cancer. They also reported a suppressing effect of the plant on chemically induced hepatomas in rats and tumors in the fore-stomach of mice. The ethanolic extract of the leaves of this plant was shown to have an inhibitory effect on chemically induced skin papillomas in rats and tumors in the fore-stomach of mice. The alcoholic leaf extract increased the carcinogen metabolizing enzymes in vivo, while Prashat et al. reported that the extract prevented adduct formation between the carcinogen and DNA. More recently, Karthikeyan et al. showed an inhibitory effect of Ocimum leaf preparations on the induction of papillomas and carcinomas in the buccal pouch of hamsters. They found that the aqueous extract was more effective than the ethanolic extract or leaf paste and suggested that oral administration of the extract may be able to prevent or block the early events in carcinogenesis. However, more detailed and systematic studies are needed to establish the anticarcinogenic potential of O. sanctum.

**Antioxidant activity**

Earlier workers have reported the antioxidant properties of flavonoids from different sources. A relation between the antioxidant property and radiation protection by flavonoids has been suggested by Shimoi et al. Our studies have shown that the aqueous extract of Ocimum leaves have significant activity in inhibiting the OH radical-induced deoxyribose degeneration, which was better than that of DMSO. The effect was comparable to that of the synthetic radioprotector WR-2721; combination of the two protectors produced a significantly higher inhibition of the OH radical activity compared to either agent individually. Some of the compounds found in the Ocimum plant have been reported to possess strong antioxidant activity. Oleic acid and ursolic acid from O. sanctum were shown to protect against adriamycin induced lipid peroxidation in liver microsomes and against free radical damage induced by ascorbic acid. Rajkumar and Rao have demonstrated that isoeugenol, which has a double bond similar to that found in orientin and vicenin, is a good free radical scavenger. Tournare et al. studied 13 selected flavonoids and demonstrated their antioxidant activity in singlet oxygen quenching. Shimoi et al. based on a study of 12 plant flavonoids, concluded that plant flavonoids which show antioxidant activity in vitro also function as antioxidants in vivo and that their radioprotective effect may be attributed to their free radical scavenging activity. Antioxidant properties of flavonoids and their relation to membrane protection have been discussed by Saija et al. Orientin and vicenin, the radioprotective flavonoids from O. sanctum, have also exhibited strong inhibitory effect on the Fenton reaction-generated OH radical activity in vivo. The antioxidant activity of these flavonoids in vivo was expressed in a significant reduction in the radiation induced lipid peroxidation in mouse liver, as was also seen with the aqueous extract from which these flavonoids were isolated.

**Mechanisms of action**

Radiation and chemical toxins produce biological damage by forming reactive oxygen species like singlet oxygen and superoxides, hydroxyl and hydroperoxy radicals, hydrogen peroxide and organic peroxides. Several antioxidants, which are ingredients of our daily diet and/or cellular components, e.g. β-carotenes, vitamins A, C and E, flavonoids, have been reported to protect against
oxidative stress. The Ocimum leaf extract as well as their flavonoids orientin and vicenin have shown strong antioxidant activity in vitro and anti-lipid peroxidative effect in vivo which strongly suggest free radical scavenging as a major mechanism by which Ocimum products protect against cellular damage and tumor induction.

But the very low doses needed for radiation protection, 50 mg/kg body weight of the extract and 50 μg/kg body weight of orientin and vicenin, suggest that direct free radical scavenging alone may not be sufficient to explain the protection against radiation damage observed in vivo. Stimulation of cellular glutathione and antioxidant enzymes like glutathione transferase, reductase and peroxidase as well as superoxide dismutase has been demonstrated in mouse liver within 30-60 min after i.p. injection of the extract, which will significantly contribute to protection against radiation injury in vivo. This indicates that the protective activity of Ocimum sanctum may, to some extent, be mediated through the release of intracellular antioxidants which, in turn, will scavenge the free radicals and also may help in the repair of biochemical lesions. A similar mechanism, the non-protein sulphydryl (NPSH) release theory of protection against radiation injury, has been proposed for the thiol protectors earlier. In vitro studies further indicate that metal chelation may also have a role in the anti-lipid peroxidative effect of Ocimum leaves. In addition, immune stimulation was suggested as a mechanism contributing to the adaptogenic action of the plant. Based on studies in rats, Mediratta et al. concluded that O. sanctum may act at various levels in the immune mechanism, such as antibody production, release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs, in modulating the humoral immune responses.

Thus, it appears that different mechanisms like free radical scavenging, metal chelation, NPSH stimulation, as well as immune modulation may act at different levels individually or in combination to bring about the radioprotective and cancer preventive effects of this medicinal plant.

Clinical implications

The different areas where chemical radioprotectors can be employed include: protection against occupational exposures of persons working in nuclear industry; isotope production and medical radiology; protection of workers and public from radiation released from nuclear accidents; protection from exposures from military use of nuclear weapons; protection of embryos from maternal irradiation; normal tissue protection in cancer patients undergoing radiotherapy. For human application, ideally, the protectors should be nontoxic and orally effective. In addition, drugs contemplated for use in cancer patients for reducing side effects of radiotherapy should give preferential protection to normal tissues with little or no protection to the tumor. There is some experimental evidence to show that the Ocimum extract and the flavonoids have a preferential effect on normal tissues (unpublished) and thus fulfill the above conditions to a great extent. Another advantage of this plant is that it can be cultivated in all parts of India and, thus, is locally available.

Future prospects

Studies in mouse have indicated that the presence of flavonoids may be responsible for the antioxidant and radioprotective activities of the Ocimum sanctum leaf extract. Experiments in vitro have shown inhibitory effect of flavonoids on tumor cell growth and tumor invasion and metastasis. Flavonoid-enriched diet has been indicated to have a preventive effect on cancer, coronary heart disease and strokes. Therefore, the possibility of developing new radioprotective and cancer preventive drugs suitable for human application from Ocimum sanctum should be explored. In addition, its impressive antioxidant property and stimulatory effect on the cellular antioxidants and immune system can be exploited for prophylactic use against a number of human ailments such as cardiovascular diseases and stress-related disorders.

Acknowledgement

A major part of the study from the author’s group reported in this paper was supported by a grant from the Defence Research & Development Organisation, Govt. of India.

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