Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant

S K Gupta*, Jai Prakash & Sushma Srivastava

Department of Pharmacology, All India Institute of Medical Sciences, New Delhi 110 029, India

In several ancient systems of medicine including Ayurveda, Greek, Roman, Siddha and Unani, *Ocimum sanctum* has vast number of therapeutic applications such as in cardiology, haemopathy, leucoderma, asthma, bronchitis, catarhal fever, otalgia, hepatopathy, vomiting, lumbago, hicups, ophthalmia, gastropathy, genitourinary disorders, ringworm, verminosis and skin diseases etc. The present review incorporates the description of *O. sanctum* plant, its chemical constituents, and various pharmacological activities.

*Ocimum sanctum* (OS), popularly known as 'Tulsi' in Hindi and 'Holy Basil' in English is one of the sacred herbs for Hindus in Indian sub-continent. It has a versatile role to play in traditional medicine. Several scientific studies are being conducted regarding the efficacy of whole plant or its parts for the treatment of different diseases. OS contains a number of chemical constituents that interact in a complex way to elicit their pharmacodynamic response. A number of active constituents responsible for the medicinal actions of OS have been isolated and are being characterized. All over the world scientific research is getting momentum to evaluate the effects, side effects and therapeutic uses of OS in various acute and chronic pathological conditions. Satyavati and co-workers in 1987 have reviewed the work done on various aspects of *Ocimum*. Since then OS has been extensively evaluated for its various phytochemical and pharmacological activities. Therefore, the present review summarizes the recent available literature on these aspects of the plant including our own findings.

**Plant description**

The plant is distributed and cultivated throughout India. It is an erect, much branched softly pubescent undershrub, 30-60 cm high with red or purple subquadrangular branches. Leaves are simple, opposite, elliptic, oblanceolate, obtuse or acute, with entire or sub serrate or dentate margins, pubescent on both sides, minutely gland dotted, with slender, hairy petioles.

*Correspondent author*

Phone: 91-11-6593633, 91-11-6593282
Fax: 91-11-6522663, 91-11-6521041
E-mail: skgup@hotmail.com

Flowers are purplish in elongate racemes in close whorls, stamens exerted, upper pair with a small bearded appendage at the base fruits nutlets, smooth, not mucilaginous when wetted. The plant is bitter and acrid. The whole plant of OS has medicinal value, few of them are aromatic, stomachic, demulcent, diaphoretic, digestive, diuretic, expectorant, febrifuge, vermifuge and alexiteric properties. Mostly leaves and sometimes the seeds are also used.

**Chemical constituents**

*O. sanctum* leaves contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpene hydrocarbon carvophyllene. Ursolic acid has been isolated from the OS leaves. Apart from ursolic acid, Nair *et al.* also isolated apigenin, luteolin, apigenin-7-O-glucuronide, luteolin-7-O-glucuronide, orientin, molludin. Isolation of two flavonoids, orientin and viccin from the aqueous leaf extract of OS is also reported. Kelm *et al.* have extracted, and purified the following phenolic compounds from the fresh leaves and stems of OS: cirsilineol, cirsimartinn, isothymusin, isothymonin, apigenin, rosmarinic acid and appreciable quantities of eugenol. The structures of these compounds have also been elucidated. Norr and Wagner identified viccin-2, rosmarinic acid, galuteolin, cirsilineol gallic acid, gallic acid methylester, gallic acid ethylester, protocatechic acid, vanilllic acid, 4-hydroxybenzoic acid, vanillin, 4-hydroxybenzaldehyde, caffeic acid, chlorogenic acid from the ehanolic extract of OS. They also detected 2-phenylpropaneglucoside 1 and 2. The leaves of OS are also known to contain traces of zinc, manganese and
blood sugar lowering activity than Catharanthus roseus, Gymnema sylvestre and Azadirachta indica\textsuperscript{23}.

To explore further evidence, effect of treatment with holy basil leaves on fasting and post-prandial blood glucose and serum cholesterol levels in humans were assessed through a randomized placebo controlled crossover single blind trial in patients of non-insulin dependent diabetes mellitus (NIDDM)\textsuperscript{24}. The results of the trial indicated a significant decrease in fasting and post-prandial blood glucose levels during treatment as compared to placebo. Fasting and post-prandial blood glucose levels fell by 21 mg/dl ($P<0.001$) and 15.8 mg/dl ($P<0.02$), respectively. The lower values of glucose represented reductions of 17.6 and 7.3% in fasting and post-prandial blood glucose levels, respectively. Urine glucose level showed similar trend. Mean total cholesterol level showed mild reduction during the treatment. The authors explored the possibility of basil leaves in improving β-cell function and enhancing insulin secretion. However, it was not clear which chemical constituent or a combination of constituents present in leaves was responsible for the hypoglycemic effect. The findings suggested that basil leaves might be prescribed as an adjunct to dietary therapy and as a drug treatment in mild to moderate NIDDM\textsuperscript{24}.

Tulsi leaf powder supplementation at 1% dose level showed significant hypoglycemic and hypolipidaemic effects in diabetic rats which could be associated with the essential oil, eugenol present in OS leaf powder\textsuperscript{25}. In addition there could be some other active insulinogenic ingredients present in OS leaf powder, bringing the blood sugar level down in the diabetic rats. Significant lowering in serum total cholesterol, triglycerides, phospholipid and LDL-cholesterol levels and a significant increase in HDL-cholesterol and total fecal sterol contents of rabbits was observed by oral administration of fresh leaves for 4 weeks at two dose levels of 1 and 2% w/w mixed in the diet\textsuperscript{26}.

Trasina, an Ayurvedic herbal formulation containing Ocimum sanctum as one of the ingredients showed little effect on blood sugar concentrations and islet superoxide dismutase activity (SOD) in euglycemic rats, in 100 and 200 mg/kg po doses administered once daily for 28 days. However, these doses of Trasina caused a dose related decrease in streptozotocin hyperglycemia and attenuation of streptozotocin induced decrease in islet SOD activity\textsuperscript{27}.

Adaptogetic activity—The ethanolic leaf extract of this plant at 100 mg/kg daily dose when fed orally
for 7 days was found to normalize the noise induced changes in total and differential leucocytes in rats. The mechanism of the extract as an adaptogen was not clear, however, it was claimed to induce the “state of non-specific increased resistance in animals and man”23. The ethanolic leaf extract of Ocimum sanctum was screened for antistress activity against acute and chronic noise induced changes in plasma corticosterone level in albino rats30. There was a significant elevation of the plasma corticosterone level in rats subjected to 30 min noise (100 dB) stress. Chronic exposure (4 hr daily for 30 days) to noise with same intensity reduced the hormonal level significantly. Treatment with the extract prevented the changes in plasma corticosterone level induced by exposure to both acute and chronic noise stress, indicating the antistress properties. The normalizing activity of OS leaf extract on noise induced changes in total and differential leucocyte counts in rats was also reported30,31.

Ethanolic extract (70%) of the whole plant increased the physical endurance (survival time) of swimming mice, prevented stress induced ulcers and milk induced leucocytosis in rats and mice, respectively, indicating induction of non-specifically increased resistance against a variety of stress induced biological changes by OS in animals32. Methanolic extract also increased the swimming time suggesting its antistress activity when given intraperitoneally at the dose of 400 mg/kg. The efficacy of the extract was comparable to that of desipramine, an antidepressant drug33. The immunostimulant capacity of OS may be responsible for the adaptogenic action of the plant34.

In a separate experiment, effects of restraint stress (RS) and its modulation by OS and eugenol were evaluated on some biochemical and biophysical parameters in rats. Neither OS nor eugenol affected the RS induced elevations in blood glucose and urea levels. However, both lowered RS induced cholesterol, lactate dehydrogenase and alkaline phosphatase levels. It seems possible that OS induced changes in biochemical parameters could partially be due to eugenol. RS induced changes in red blood cells (RBCs) membrane dynamics (increased membrane protein clusterisation, increased membrane fluidity, reduced RBC membrane thickening and effects on synaptosomal membrane) were reversed in a differential manner by OS and eugenol35. The authors speculate that neurotransmitter mediated changes during stress, influenced by change in lipid disposition in membrane, are reversed to near normalcy by OS and eugenol. Authors also speculate that CNS may be one of the sites for the antistress/the adaptogenic effects of OS. The subtle differences in biological effects of the whole extract and the pure active constituent, eugenol indicate that the other constituent of the plant, though present in minute quantities may play a major role in determining the overall biological activity of a medicinal plant.

Antifertility activity—Antifertility activity of OS leaves has been reported in rats, mice and rabbits. In one of the above studies benzene extract was more effective than petroleum ether extract or other extracts. Benzene extract of fresh OS leaves in male rats indicated significant reduction in sperm count, sperm motility and weight of testis36-39.

Long term feeding (up to 3 months) of Tulsi leaves (200 and 400 mg/kg) to adult male and female albino rats along with normal diet decreased sperm count, sperm motility and the weight of male reproductive organs. The mating behaviour of both male and female rats was inhibited severely. In some animals where mating took place only during the initial phase of the treatment, pregnancy was carried to term with birth of normal pups. However, short term oral administration of OS leaf extract to the rats in the graded doses of 100, 150, 200 and 400 mg/kg body weight along with normal diet for 15 days continuously, decreased the sexual behaviour. A significant decrease in sexual behaviour score was noticed when the dose was increased to 200 and 400 mg/kg. It suggested that tulsi plant could not be exploited for contraceptive use since it depresses mating behaviour and does not cause azoospermia40,41.

Hepatoprotective activity—The cold water extract at 3g/100g body weight dose when fed orally for 6 days was found to be effective against carbon tetrachloride (0.2 ml/100 g subcutaneously) induced liver injury (necrosis, fatty degeneration and hydropic degeneration) in albino rats42. Similar effect was observed with 70% ethanolic extract43.

Oral administration of the hydroethanolic leaf extract at 200 mg/kg dose to male Wistar albino rats provided protection against paracetamol induced liver injury. The studies were supported by significant reduction in the elevated serum enzyme levels and marked reduction in fatty degeneration in treated rats as compared to untreated control (paracetamol alone)43.

Immuno-modulatory activity—Steam distilled extract from the fresh leaves showed modification in the
The antiproliferative and chemopreventive activity of stress induced by a variety of techniques in different paradigms of oxidative stress was notably protected by oleanolic acid against adriamycin-induced convulsions. It also decreased apomorphine induced fighting time and ambulation in open field studies. Using a behavioural despair model involving forced swimming in rats and mice, the extract lowered immobility in a manner comparable to imipramine. This action was blocked by haloperidol and sulpiride, indicating a possible action involving dopaminergic neurons. In a similar study, there was a synergistic action has been reported when extract was combined with bromocriptine, a potent D2-receptor agonist.

Antioxidant activity—Uma Devi has recently reviewed the antioxidant activity of OS. Antioxidant properties of flavonoids from different sources have been reported. Shimoi et al. thought of a probable relationship between radioprotective and antioxidant activity which was also confirmed by Ganisoundari and co-workers. Ursolic acid isolated from OS offered remarkable protection against lipid peroxidation in isolated liver and heart microsomes in vitro. The compound did not induce lipid peroxidation by itself and thus improved therapeutic application. It also provided mild protection as compared to strong protection by oleanolic acid against adriamycin-induced lipid peroxidation.

Protective role of aqueous leaf extract against radiation induced lipid peroxidation, glutathione and allied antioxidant enzymes in liver of mice was observed. OS leaf extract exhibited significant antioxidant activity against several paradigms of oxidative stress induced by a variety of techniques in different rat tissues, which was comparable to that of vitamin E. Recently cyclooxygenase inhibitory properties of OS have also been reported along with its antioxidant properties.

Chemopreventive and anticarcinogenic activity—The antiproliferative and chemopreventive activity of OS aqueous leaf extract and seed oil was studied using 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay on HeLa cells. Significant antiproliferative activity was observed only with high concentrations (83.33 and 250 μg/ml) of seed oil. Leaf extract and low doses of seed oil did not affect the proliferation of the cells. Oral supplementation of maximal tolerated dose of 100 μl/kg body weight against 20-methylcholanthrene (MCA) induced fibrosarcoma tumours in Swiss albino mice reduced the cumulative tumour incidence and tumour volume. Increased survival rate and delay in tumour incidence was also observed. Liver enzymatic (superoxide dismutase, catalase, glutathione-S-transferase) and non enzymatic antioxidants (reduced glutathione) and lipid peroxidation end-product, malondialdehyde levels were significantly modulated with oil treatment suggesting that the potential chemopreventive activity of the oil is partly attributable to its antioxidant properties. The chemopreventive efficacy of 100 μl/kg seed oil was comparable to that of 80 mg/kg of vitamin E. The oil also showed significant chemopreventive activity against 7,12-dimethylbenz (a) anthracene (DMBA) induced papillomagenesis in Swiss albino mice. This was evidenced by significant reduction in incidence of papillomas, average number of papillomas/mouse, increased survival and modulation of level of reduced glutathione and lipid peroxidation, and activity of superoxide dismutase, catalase and glutathione peroxidase in papillomas by oil supplementation and histopathological parameters.

Anticarcinogenic potential of OS has also been reviewed earlier by Uma Devi. OS significantly decreased the incidence of benzo(a)pyrene induced neoplasia of stomach and 3-methyl-4-dimethylaminoazobenzene induced hepatomas in rats. Topical treatment of OS leaf extract in DMBA induced papillomagenesis significantly reduced the tumour incidence, average number of papillomas/mouse and cumulative number of papillomas in mice. Topical application of the extract significantly elevated reduced glutathione content and glutathione-S-transferase (GST) activity. The chemopreventive action of OS leaf extract is probably through the induction of hepatic/extrahepatic GST in mice. Elevated levels of reduced glutathione in liver, lung and stomach tissues in OS extract supplemented mice were also found as compared to untreated control mice.

Ethanolic leaf extract also had significant modulatory influence on carcinogen metabolizing enzymes (cytochrome P450, cytochrome b5 and aryl hydrocar-
bon hydroxylase, glutathione-S-transferase) and glutathione levels in mouse.

Incidence of papillomas and squamous cell carcinomas were significantly reduced when OS in the form of fresh leaves paste, aqueous and ethanolic extract was topically applied and the extracts were orally administered to buccal pouch mucosa of hamsters exposed to 0.5% of DMBA. The aqueous extract showed profound effect than the other two forms.

Analgesic, anti-inflammatory, antipyretic and antidiarrhoeal activity—In acute (carrageenan induced paw edema) and chronic inflammation (croton oil induced granuloma and exudate formation) models, the response observed with 500 mg/kg methanolic extract and aqueous suspension of OS was comparable to 300 mg/kg of sodium salicylate. Both the formulations showed analgesic activity, reduction in typhoid paratyphoid A/B vaccine induced pyrexia, but the antipyretic action was weaker and of shorter duration than 300 mg/kg sodium salicylate. They also delayed castor oil induced diarrhoea in rats when fed orally on prophylactic basis. Dose and time dependent antipyretic activity against Brewer’s yeast induced pyrexia in rats was also found in water soluble fraction of the ethanolic extract of OS. The mechanism of antipyretic action may be due to inhibition of prostaglandin synthesis.

The fixed oil from the seeds of OS demonstrated significant anti-inflammatory activity in both acute and chronic models of inflammation in rats. Antipyretic activity against typhoid-paratyphoid A/B vaccine induced pyrexia was also seen in albino rats without noticeable side effects. The oil was found to be devoid of analgesic activity in experimental pain models (tail flick, tail clip, tail immersion model). However, it was effective against acetic acid induced writhing method in mice in a dose dependent manner. The writhing inhibiting activity of the oil is suggested to be peripherally mediated (due to combined inhibitory effect of prostaglandins, histamine and acetylcholine). The OS fixed oil and linolenic acid possess significant anti-inflammatory activity against PGE2, leukotriene and arachidonic acid induced paw edema in rats by the virtue of their capacity to block both the cyclo-oxygenase and lipoxygenase pathways of arachidonic acid metabolism. In a subsequent study significantly lesser anti-inflammatory activity of OS fixed oil than O. basilicum and O. americanum fixed oil in experimental models of inflammation in rats was reported, which could be attributed to lesser percentage of linolenic acid present in O. sanctum.

Antiasthmatic activity—Ethanolic extract (50%) of fresh leaves and fixed oil from the seeds exerted significant antiasthmatic (against histamine and acetylcholine induced preconvulsive dyspnoea in guinea pigs) and anti-inflammatory activity (against carrageenan, serotonin, histamine and PGE2 induced inflammation in rats). The antiasthmatic activity of the ethanolic extract of the fresh leaves is suggested to be due to the presence of volatile oil consisting of several components. Ethanolic extract (50%) from the dried leaves, however, did not protect the guinea pigs against histamine induced preconvulsive dyspnoea which could be attributed to the loss of active constituents during the process of drying.

Antiaulcerogenic activity—OS was studied for antiaulcerogenic properties in pyloric ligated and aspirin treated rats. The extract of the leaves reduced the ulcer index, free and total acidity on acute and chronic administration. Seven days drug pretreatment increased the mucous secretion also. These results indicated antiaulcerogenic properties of the extract against experimental ulcers attributable to reduction in acid secretion and increased mucous secretion.

The fixed oil of OS was found to possess significant antiaulcer activity against aspirin, indomethacin, alcohol, histamine, reserpine, serotonin and stress induced ulceration in experimental animal models. Significant inhibition was also observed in gastric secretion and aspirin induced gastric ulceration in pylorus ligated rats. The oil had lipoxygenase inhibitory, histamine antagonistic and antisecretory effects, which could probably contribute to its antiaulcer activity.

Radioprotective activity—Uma Devi and co-workers have extensively worked on radioprotective activity of OS and Uma Devi has recently reviewed the same. Hydroalcoholic extract of OS provided radioprotective effect when given intraperitoneally before a whole body exposure to 11 Gy of Co. Intrapitoneal route offered best protection than other routes.

The aqueous extract exerted protective effect against radiation induced chromosome damage in mouse bone marrow and modified bone marrow radio sensitivity which could be attributed to its free radical scavenging activities.

The two isolated flavonoids from OS leaves, orientin and vicenin showed better radioprotective effect as compared with synthetic radioprotectors, WR-2721
and MPG (2-mercaptopyrrolglycine). Both the flavonoids showed a significant protection against chromosome aberration in mice, the activity of vicenin being significantly greater than orientin without systemic toxicity. Free radical scavenging appeared to be the likely mechanism of radiation protection by these flavonoids. In a separate in vitro system, orientin and vicenin provided almost equal protection against radiation induced lipid peroxidation (LPO) in mouse liver and a significantly greater free radical inhibiting activity than DMSO. They showed no pro-oxidant activity at the tested concentrations.

The combination of OS leaf extract with WR-2721 resulted in higher bone marrow cell protection and reduction in the toxicity of WR-2721 at higher doses, suggesting that the combination would have promise for radioprotection in humans.

At an optimum dose of 50 μg/kg, i.p. administration of orientin and vicenin, provided protection to mice against death from gastrointestinal and bone marrow syndrome before whole body exposure to 11 Gy γ-radiation. Survival and duration of protection were better with vicenin than at tested concentrations.

Anticataract activity — Anticataract effect of aqueous extract of OS leaves in selenite model of cataract both in vitro and in vivo was studied. Significant differences in the incidence of cataract and various biochemical parameters like antioxidant enzyme activity, glutathione and malondialdehyde levels were observed.

Aqueous extract from the fresh leaves of this plant delayed the process of cataractogenesis in experimental models of cataract (galactosemic cataract in rats by 30% galactose and naphthalene cataract in rabbits by 1 g/kg naphthalene). OS 1 and 2 g/kg delayed the onset as well as subsequent maturation of cataract significantly in both the models. In addition to delay in reaching the various stages of development of cataract, IV stage did not develop with high doses till the completion of 40 days of experimental period. Higher doses were found to be more effective and promising prophylactic rather than curative. This effect was more pronounced in galactosemic cataract. The anti-oxidant activity could be linked to the antioxidant, antistress and hypoglycemic activities of the extract.

Wound healing activity — The effect of aqueous extract of leaves of this plant on wound healing was assessed using excision and incision wound models in Wistar rats. Oral administration of 0.1 ml/100 g of the extract significantly increased the tensile strength in incision wound and promoted epithelialization in excision wound. The prohealing action of the extract seemed promising. The mechanism of prohealing action of the extract remains to be elucidated as yet.

Miscellaneous activities — In a four weeks trial, the seeds and leaves of OS exhibited significant hypouricemic and uricosuric effect in normal male albino rabbits. The leaf extract also inhibited the mitochondrial malate dehydrogenase and malic enzyme of a filarial worm Setaria digitata.

Data collected from the indigenous medical practices using local plants by interviewing 75 old members and 3 local doctors of Baiga tribe from three villages of Raigarh district of Madhya Pradesh showed that OS was used in cases of snakebite poisoning.

An OS seed based matrix has been developed for immobilization of the enzyme α-rhamnosidase. The α-rhamnosidase activity of naringinase was immobilized on this newly developed matrix. K_m, pH and temperature optima of the immobilised enzyme using p-nitro-α-L-rhamnopiranoside as the substrate were determined and found to be 0.17 mM, 4 and 60°C respectively.

The aqueous extract of the leaves of OS was evaluated for the regulation of thyroid function in male mice. The extract at the dose of 0.5 mg/kg body weight for 15 days significantly decreased serum T4 concentrations, hepatic LPO and glucose-6-phosphate activity. The activities of endogenous antioxidant enzymes, the SOD and catalase (CAT) were increased by the extract administration. However, no marked changes were observed in serum T3 level, T3/T4 ratio and in serum cholesterol level. Thus the antithyroidic and antioxidative nature of the extract was suggested.

Future prospects

Efforts should be directed towards the isolation and characterization of the active principles and elucidation of the structure activity relationship as in most of the studies crude extract has been used. Its potential antioxidant and immunomodulatory properties may help in fighting against a number of diseases, viz. cancer, AIDS, ageing, cataractogenesis etc. Incorporation of O. sanctum in various marketed formulations may be useful as adjunct therapy. However, its possible synergistic action with other drugs may be explored in a variety of diseases. As per regulatory requirements it may be put to clinical trials, after conducting preclinical pharmacological and toxicological
studies and its validity in various pathophysiological conditions may be proved. Future selective targeting/delivery may also be tried.

Conclusion
Keeping in view the tremendous pharmacological activities and a wealth of available literature, Ocimum sanctum may be utilized to alleviate the symptoms of a variety of diseases as evident from pre-clinical data. Clinical studies are only few with O. sanctum, however, it is one of the ingredients of several marketed formulations. The wide spread availability of this herb in India and other countries, makes it an attractive candidate for further pre-clinical and clinical research and hence studies are in progress throughout the globe.

References


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