

REVIEW ARTICLE

THE SCIENCE BEHIND SACREDNESS OF *TULSI*  
(*OCIMUM SANCTUM* LINN.)

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**Abstract :** Medicinal properties of *Tulsi* (*Ocimum sanctum* Linn) are known for thousand years to various civilizations of the world. This medicinal herb is considered as a sacred plant by the Hindus in the Indian subcontinent. Scientific explorations of traditional belief of medicinal properties of *Tulsi* have got momentum mostly after the middle of the 20<sup>th</sup> century.

In the present review, efforts have been made to sum up different aspects of scientific studies on this medicinal plant. Scientific evidences are available on various medicinal aspects i.e. antimicrobial, adaptogenic, anti-diabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, radio-protective, immunomodulatory, neuro-protective, cardio-protective, mosquito repellent *etc.* to name a few. Most of these evidences are based on *in-vitro*, experimental and a few human studies.

**Key words :** medicinal plant  
adaptogenic

antimicrobial properties  
immunomodulation

INTRODUCTION

*Tulsi* is described as sacred (1) and medicinal plant in ancient literature (2). The name *Tulsi* is derived from ‘Sanskrit’, which means “matchless one” (3). This plant belongs to the family *Labiatae*, characterized by square stem and specific aroma. Botanical name of *Tulsi* is *Ocimum sanctum* (Linn). In India, the plant is grown throughout the country from Andaman and Nicobar islands to the Himalayas up to 1800 meters above the sea level (1). It is also abundantly found in Malaysia, Australia, West Africa and some of the Arab countries. *Ocimum sanctum*

(Linn) is the most prominent species of the genera. The leaves of the plant are considered to be very holy and often form a consistent part of the Hindu spiritual rituals (*Tirtha* or *Prasada*) (3). *Ocimum sanctum* has two varieties i.e. black (*Krishna Tulsi*) and green (*Rama Tulsi*), their chemical constituents are similar (4). Both the varieties also have common medicinal properties (3).

Several medicinal properties have been attributed to the plant not only in Ayurveda and Siddha but also in Greek, Roman and Unani system of medicines (5). Traditionally,

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juice of the leaves of *Tulsi* plant was used as demulcent, stimulant, expectorant. *Tulsi* was also used in the cure of upper respiratory tract infections, bronchitis, skin infections (6) and earache. An infusion of leaf had been used as anti-spasmodic in gastric disorders of children. A concoction of root of *Tulsi* is still being used as a diaphoretic in malarial fevers in remote areas. The seeds are mucilaginous and demulcent and are given in different ailments of genito-urinary system (1). *Tulsi* is good for heart, stimulates digestion, reduces breathing difficulties and cough (3). It has also been used in the treatment of snake-bite and scorpion-sting as described in ancient texts by *Charaka* and *Sushruta* (2). Thus, every part of the plant has useful application. Even today people use different parts of this plant for treatment of various ailments based on traditional knowledge. However, in the modern scientific world such claims warrant scientific proof and validation. Although the ancient traditional claims about medicinal properties of *Tulsi* are being investigated scientifically, majority of these studies are only limited to *in-vitro* and experimental animal models only. Studies on human subjects are a very few. Therefore, an effort has been made to review various scientific studies that have considerably contributed on various aspects of the plant *Ocimum sanctum* (Linn) and described under specific headings.

#### Chemical properties

*Ocimum sanctum* has specific aromatic odour because of the presence of essential or volatile oil, mainly concentrated in the leaf. This aromatic volatile oil mainly contains phenols, terpenes and aldehydes. The oil extracted from seeds is called fixed

oil and mainly composed of fatty acids. Besides oil, the plant also contains alkaloids, glycosides, saponines and tannins. The leaves contain ascorbic acid and carotene as well (1). The present day information about the chemical properties is based on the various studies that have been done in different parts of the world (7–18) and it is likely that chemical constituents may be varying due to edaphic and geographic factors (19). The details of chemical constituents reported in various literatures are shown in Table I.

#### Toxicological properties

*Tulsi* is being used as medicinal herb for thousand years without any known adverse effects. There have been number of scientific studies conducted to evaluate the toxic effects of the plant. Bhargava and Singh (20) studied the toxicity to find out the lethal dose of ethanolic extract of *Tulsi* in adult mice. Approximate  $LD_{50}$  of *Ocimum sanctum* was found to be  $4505 \pm 80$  mg/kg body weight (bw) on administration by oral route and  $3241 \pm 71$  mg/kg, bw by intra-peritoneal (ip) routes. *Tulsi* leaves' aqueous and alcoholic extracts were injected ip in mice with graded doses (3500–6300 mg/kg, bw) and mortality was observed for a period of 72 hours. The administration of aqueous extract did not produce any acute toxic symptoms (100% survival) at doses up to 5 g/kg, bw and the alcoholic extract was well tolerated (80% survival) up to a dose of 4g/kg, bw. The acute  $LD_{50(30)}$  values for aqueous and alcoholic extracts were found to be 6200 mg/kg, bw and 4600 mg/kg, bw respectively (21). The toxicity of fixed oil (seed oil) of *Tulsi* has also been studied by intra-peritoneal administration in experimental rats. In acute toxicity study, fixed oil was given in a graded

TABLE I: Chemical constituents\* of *Ocimum sanctum* Linn.

<i>Essential oil from Leaves</i> (4, 7-18, 35, 100-110)	<i>Alcoholic extract of leaves/ Aerial parts</i> (9, 11-13, 111, 112)	<i>Fixed oil from Seeds</i> (113, 114)	<i>Mineral content</i> (per 100 gm) (1, 115)
$\alpha$ -Thujene	Ursolic acid	Palmitric acid	Vit. C (83 mg)
Octane	Apigenin	Stearic acid	Carotene (2.5 mg)
Nonane	Luteolin	Linolenic acid	Ca (3.15%)
Benzene	Apignin-7-O-glucuronide	Linoleic acid	P (0.34%)
(Z)-3-hexanol	Luteolin-7-O-glucuronide	Oleic acid	Cr (2.9 $\mu$ g)
Ethyl 2-methyl butyrate	Isorientin	Sitosterol	Cu (0.4 $\mu$ g)
$\alpha$ -pinene	Orientin	Dilinenoleno-linolins	Zn (0.15 $\mu$ g)
$\beta$ -pinene	Molludistin	Linolenodilinolin	V (0.54 $\mu$ g)
Toluene	Stigmsterol	Hexourenicacid	Fe (2.32 $\mu$ g)
Citronellal	Triacontanol ferulate		Ni (0.73 $\mu$ g)
Camphene	Vicenin-2		Insoluble oxalate
Sabinene	Vitexin		
Dimethyl benzene	Isovitexin		
Myrecene	Aesculectin		
Ethyl benzene	Aesculin		
Limocene	Chlorgenic acid		
1,8-cineole	Galuteolin		
Cis- $\beta$ -ocimene	Circineol		
Trans- $\beta$ -ocimene	Gallic acid		
p-cymene	Gallic acid methyl ester		
Terpinolene	Gallic acid ethyl ester		
Allo-ocimene	Procatechuic acid		
Butyl-benzene	Vllinin acid		
$\alpha$ -cubebene	4-hydroxybenzoic acid		
$\gamma$ -terpene	Vallinin		
trans-linalool oxide	4-hydroxybenzoic acid		
Geraniol	Caffiec acid		
$\alpha$ -copaene	Chlorogenic acid		
$\beta$ -bourbonene	Phenylpropane glucosides 1		
$\beta$ -cubebene	Phenylpropane glucosides 2		
Linalool	$\beta$ -Stigmsterol		
Eugenol	Urosolic acid		
Methyl eugenol			
$\beta$ -farnesene			
$\beta$ -elemene			
(E)-cinnamyl acetate			
Isocaryophyllene			
$\beta$ -caryophyllene			
Iso-eugenol			
$\alpha$ -guaiene			
$\alpha$ -amorphene			
$\alpha$ -humulene			
$\gamma$ -humulene			
4,11-selinadiene			
$\alpha$ -terpeneol			
Isoborneol			
Borneol			
Germacrene-D			
$\alpha$ -selinene			
$\beta$ -selinene			
Myrtenylformat			
$\alpha$ -muurolene			
$\delta$ -cadinene			
Cuparene			
Calamenene			
Geraneol			
Nerolidol			
Caryophyllene oxide			
Iedol			
Humulene oxide			
$\alpha$ -guaiol			
$\tau$ -cadinol			
$\alpha$ -bisbolol			
(EZ)-farnesol			
Cis-sesquisabinene hydrate			
Elemol			
Tetradecanal			
Selin-11-en-4- $\alpha$ -ol			
14-hydroxy- $\alpha$ -humulene			

\*References given in the brackets of respective columns.

manner up to 55 ml/kg, bw but a dose of 30 ml/kg, bw were well tolerated. There was no mortality at 30 ml/kg, bw while 100% mortality observed at 55 ml/kg, bw. Whereas, sub-acute toxicity study, a dose of 3 ml/kg, bw was administered and no behavioral as well as histological changes were seen in the brain, lungs, liver and kidneys. The LD<sub>(50)</sub> of fixed oil was calculated and found to be 42.5 ml/kg, bw (22).

#### Antimicrobial properties

Indian mythological book *Padmottara Purana* asserts that a house where a garden of *Tulsi* exists is itself a centre of pilgrimage; neither servants of *Yama* (The lord of death) nor disease can enter there and wherever fragrance of *Tulsi* goes, the air gets purified (3). This statement seems to have some relevance because the essential oil, which forms the specific fragrance, is volatile in nature and can kill various types of microbes. The essential oil is reported to possess antibacterial and insecticidal properties. The oil has been shown to have inhibitory effects on growth of *Mycobacterium tuberculosis* and *Micrococcus pyogenes* var. *aureus*. It has one-tenth anti-tubercular potency of streptomycin and one-fourth that of isoniazid (1). Aqueous and acetone extracts of *Ocimum sanctum* were also found to be sensitive to many plant fungi, *Alternaria tenuis*, *Helminthosporium spp*, and *Curvularia penniseli* (23). Essential oil of *Tulsi* was tested on plant pathogenic fungi as well e.g. *Alternaria solani*, *Candida guilliermondii*, *Colletotricum capsici*, *Curvularia spp*, *Fusarium solani*, *Helminthosporium oryzae* and the bacterial strains, *Anthrobacter globiformis*, *Bacillus megaterium*, *Escherichia coli*, *Pseudomonas spp*, *Staphylococcus aureus*, *Staphylococcus albus* and *Vibrio*

*cholerae* (24, 25). The essential oils of *Tulsi* have been effective against both Gram-positive and Gram-negative bacteria and the properties were comparable with the effectiveness of clove oil (26, 27). Antimicrobial activity of *Ocimum sanctum* was found to be higher as compared to commonly available other species of *Ocimum* (i.e. *O. canum*, *O. gratissimum*, *O. basilicum*) in India (28) more so, aqueous extract, alcoholic extract and seed oil of *Tulsi* shown antimicrobial properties against enteric pathogens (29, 30). It also exhibited significant antimicrobial activities against some of the clinical isolates and multi-drug resistant *Neisseria gonorrhoeae* (31, 32). The ethanolic extracts have ability to inhibit clinical isolates of  $\beta$ -lactamase producing methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* [MSSA] (33). Essential oil of *Ocimum sanctum* reported to have shown antimicrobial activity against *Propionibacterium acnes* in *in-vitro* study and minimum inhibitory concentration (MIC) value found to be 3.0% v/v (34). Fresh leaves essential oil had shown more antibacterial properties compared to dried leaves essential oil of *Tulsi* and in case of fungus the property is just the reverse (35).

#### Adaptogenic (Anti-stress) properties

Stress is very common problem in today's competitive life. The production of more free-radicals due to stress leads to adverse effects on various vital organs and tissues of the human body. The plant substance which increases the state of non-specific resistance against multiple stresses is called adaptogen. The ethanolic extract (dried whole plant) of *Tulsi*, given to mice and rats increased physical endurance and better healing effects

from experimentally induced ulcers were observed. Ethanolic extract were also prevented hepatotoxicity and leukocytosis when administered at a dose of 100 mg/kg, bw. A battery of stress tests which includes swimming endurance test, milk induced leukocytosis, aspirin induced ulcers and carbon tetrachloride induced hepatotoxicity have been tested in these animals (20). The treatment with ethanolic extract of *Tulsi* at a dose of 20 mg/kg, bw when given orally for seven days showed increased production of adrenaline, noradrenaline, monoamine oxidase and caused decrease in dopamine and 5-hydroxytryptamine (serotonin) levels in mice following swimming and gravitation induced stresses (36).

Restrained stress to the rat led to increase in levels of blood glucose, urea, lactate dehydrogenase (LDH) and alkaline phosphatase. Restrained stress also increased membrane protein clusterization, fluidity and reduced membrane thickness of red blood corpuscles (RBCs). Pretreatment with *Tulsi* essential oil significantly reduced the LDH and alkaline phosphatase levels. Enhanced aspartate transaminase and membrane dynamics of RBC were reversed near normalcy. This reversal gave reasonable ground to speculate that central neurotransmitters are involved in regulation process of stress responses (37). Experimental rats could prevent the elevation in plasma corticosterone levels following acute and chronic noise stress when pretreated with 100 mg/kg, bw ethanolic extracts of *Tulsi* leaves. Due to the experimental stress, plasma corticosterone level rose from 89.70 µg/dl in control group to 158.52 µg/dl whereas in pretreated group it remained near normal

*i.e.* 99.72 µg/dl. In chronic stress conditions, it also remained near normal level (38). In another study, treatment with the ethanolic extract of the roots of *Ocimum sanctum* (400 mg/kg, bw) increased the mean swimming time significantly when experimental mice were subjected to swimming stress test (39). Methanolic extract of fresh leaves of *Tulsi* has been effective to bring normal the altered values of acute noise induced neutrophil functions (40). Similarly, when the experimental rats were treated at dose of 200 or 500 mg/kg, bw petroleum ether extract of *Tulsi* 30 minutes before the pentobarbitone induced hypnotization, the treated group did less error to escape from water maze (41).

A polyherbal formulation containing *Tulsi* along with other plant extracts such as *Withania somnifera*, *Tribulus terrestris* and *Shilajeet* treated animals showed reduction in various induced stress related outcome results and was comparable with the proven adaptogen *Ginseng* (42). The methanolic extract of *Tulsi* when given at a dose of 50/100 mg/kg, bw could significantly reduce the various paradigms of oxidative stress caused by ischemia-reperfusion injury, cigarette smoke, foot shock and iron overload hepatotoxicity (43). *Tulsi* fresh leaves significantly reduced the effects of anemic hypoxia induced oxidative damage. In a study conducted on experimental animals by Sethi *et. al.* (44), it was observed that feeding of 2 g of fresh *Tulsi* leaves for 30 days, the hemoglobin, serum glucose and plasma malondialdehyde (MDA) levels significantly remained higher when anaemic hypoxia condition was induced. The alcoholic extract of *Tulsi* and its fraction is found to inhibit lipid peroxidation of erythrocytic membrane

in a dose dependant manner. The alcoholic extract produces greater inhibition ( $IC_{50}$  at 16  $\mu$ g) as compared to aqueous extract ( $IC_{50}$  at 80  $\mu$ g) (45). Administration of ethanolic extract of *Ocimum sanctum* (100 mg/kg, bw/day, i.p. for 15 days) had a normalizing action on discrete regions of brain and controlled the alteration in neurotransmitter levels due to noise stress (46).

#### Anti-diabetic properties

The anti-diabetic properties of *Ocimum sanctum* have been evaluated in experimental animal models and very few studies on human are available. Administration of fresh *Tulsi* leaves (1 and 2 g/day) for four weeks exerted significant hypoglycaemic and uricosuric effects on fasting glucose and 24-hour urine samples in experimental adult albino rabbits (47). Upon 15 days treatment with *Tulsi* leaf extract reduction in blood sugar level by 43% were noted in experimental rats with diabetes mellitus induced by alloxan. These diabetic rats lost control of sugar level when feeding of *Tulsi* leaves extract were stopped, giving a clear indication that the fresh leaves possess hypoglycaemic properties (48). Similarly, oral administration of ethanolic extract of *Tulsi* to the rats with diabetes, induced by glucose and streptozotocin, showed reduction in serum glucose level. This reduction was 91.55% and 70.43% in normal and diabetic rats respectively, when compared to hypoglycaemic agent tolbutamide treated groups (49). *Tulsi* also showed hypoglycaemic activity along with other herbal formulations. Dry *Tulsi* leaf powder when fed at 1% of total diet for 30 days to the rats with diabetes induced by alloxan, fasting blood sugar, uronic acid, total amino acids, total cholesterol, triglyceride,

phospholipids and total lipids reduced significantly (50). Similarly, methanolic extract of *Tulsi* when given to experimental animals at a dose of 200 mg/kg, bw for 30 days, the activities of glucokinase and hexokinase was increased significantly (51). The seed oil of *Tulsi* when given 800 mg/kg, bw/day to experimentally induced hyperglycaemic and hypercholesterolaemic rabbits for four weeks, cholesterol levels reduced significantly with no significant effects on blood sugar level (52). Five hundred mg/kg, body weight *Ocimum sanctum* extract found to reduce blood glucose and oxidative stress in rats with streptozotocin-induced diabetes (53). It was also found that feeding of 200 mg/kg, body weight aqueous extract of whole *Tulsi* plant for 60 days significantly delayed insulin resistance in fructose fed experimental mice (54). The alcoholic extract and other organic solvent fraction's extract has been found to stimulate insulin secretion from perfused rat pancreas, isolated islets and clonal pancreatic  $\beta$ -cells. The proposed mechanism of action for the secretion of insulin is that, *Tulsi* extract is able to stimulate adenylate cyclase/cAMP or the phosphatidylinositol or direct effect on exocytosis that induce mobilization of intracellular  $Ca^{++}$  as well as promoting  $Ca^{++}$  entry (55).

In one of the initial randomized controlled clinical trails, anti-diabetic properties have been studied in 40 non-insulin dependent diabetes mellitus (NIDDM) patients. It was observed that taking dried *Tulsi* leaf powder made from 2.5 g fresh leaves per day orally on empty stomach could reduce the fasting glucose level up to 21 mg/dl and postprandial blood glucose by 15.8 mg/dl (56). In another trial on 27

NIDDM patients, it was observed that supplementation of *Tulsi* powder along with hypoglycaemic drugs for one month could significantly decrease the blood glucose, glycosylated proteins, total amino acids, uronic acid, triglycerides, low density lipoprotein (LDL) and very low density lipoprotein (VLDL), compared to control group on similar hypoglycaemic drugs. However, there was no significant change in high density lipoprotein (HDL) level (57).

#### Hepatoprotective properties

*Tulsi* offered liver protection against various experimentally induced damages. *Tulsi* extract treated group showed no mortality while control group showed 60% mortality in carbon tetrachloride induced liver damage in rats (20). The ethanolic extract of *Tulsi* can protect the liver damage from anti-tubercular drugs in experimental rats (58). The ethanolic extract of *Tulsi* treatment prior to paracetamol induced liver damage, have shown to protect the liver. This has been evident by significantly enhanced levels of serum enzymes (aspartate, aminotransferase, alkaline and acid phosphatase) and liver glutathione in experimental rats (59). In a polyherbal formulation of four plants "Imu-21" including *Ocimum sanctum* tested for cytotoxicity by measuring splenic leukocyte natural killer (NK) cells activity against K-562 cell line, showed that pretreatment with Imu-21, for seven days, can increase NK cell activity in mice. The possible mechanism is probably due to activation of mature NK cells or precursor cells which were previously not active (60).

#### Anti-inflammatory properties

The aqueous and methanolic suspension

of *Tulsi* has shown to inhibit acute as well as chronic inflammation in rats. This test was conducted by carrageenan induced paw edema, croton oil induced granuloma and exudates, at a dose of 500 mg/kg, bw/day (61). The oils extracted from fresh leaves (essential oil) and seeds (fixed oil) of *Tulsi* have shown anti-inflammatory effects on experimental animal's hind paw edema induced by carrageenan, serotonin, histamine and prostaglandin-E-2. These experimental rats were administered with essential oil (200 mg/kg, bw), and fixed oil (0.1ml/kg, bw) before injection of phlogistic agents and was compared with standard drug flurbiprofen. It was noted that *Tulsi* extracts could significantly reduce the edema when compared with the saline treated control. However, its effect was less than the standard drug (62). The mechanism of action of the anti-inflammatory effects of *Tulsi* could be the cyclo-oxygenase and lipoxygenase pathways (63, 64). In order to compare the anti-inflammatory effects of fixed oils of various species of *Ocimum viz O. sanctum, O. basilicum, O. americanum*, which possess varying proportions of unsaturated fatty acids (particularly linolenic acid) showed different response against phlogistic agent induced paw edema. *Ocimum basilicum* possess highest percentage of linolenic acid (21.0%) and offered maximum inhibition of paw edema (72.42%), *O. sanctum* fixed oil containing 16.63% linolenic acid provided 68.97% inhibition while *O. americanum* offered least paw edema inhibition (65). Fixed oil of *Tulsi* can inhibit enhanced vascular permeability and leukocyte migration as evidenced by carrageenan induced inflammatory stimulus (63). Extract of seeds from three plants including *Ocimum sanctum* have been studied for anti-inflammatory effects of

carrageenan, leukotrine and arachidonic acid induced paw edema in rats. *Ocimum sanctum* seed oil showed maximum percentage inhibition of leukotrine induced paw edema (66).

#### Anti-carcinogenic properties

The anti-carcinogenic properties have been evaluated in the experimental animals induced by different types of carcinogens. *Tulsi* leaves when fed to experimental rats with 600 mg/g diet for ten weeks, significantly reduced the 3,4-benzo(a)pyrene [B(a)P] and 3'-methyl-4-dimethylaminoazobenzene (3'MeDAB) induced squamous cell carcinoma and hematoma incidences (67). The anti-cancer activity of *Tulsi* has also been reported from Philippines where juice of fresh leaves was applied on the skin of experimental mice thrice a week for 20-minutes along with tumor promoter agents (dimethylbenzanthracene as initiator and croton oil as promoter of cancer). No incidences of tumor were found in 20 weeks follow up period in *Tulsi* treated group (68). The ethanolic extract of *Tulsi* leaves at a dose of 400 and 800 mg/kg, bw have found to modulate carcinogen metabolizing enzymes such as cytochrome P-450, cytochrome-b5 and aryl hydrocarbon hydroxylase of mice liver (69).

#### Immunomodulatory effect

The fresh leaf of *O. sanctum* is consumed with the traditional belief that it enhances immunity. This claim has been investigated in experimental animals. Rats treated with methanolic extract of *O. sanctum* when challenged with typhoid H-antigen and sheep red blood cells (SRBCs) showed a significant rise in antibody titre in both groups as compared to saline treated controls. In the

Erythrocyte (E)-rosette formation test, it was observed that E-rosette formation in *O. sanctum* treated groups was significantly higher as compared to controls (70).

Steam distilled extract of fresh leaves of *O. sanctum* enhanced humoral immune responses in experimental rats. This was evident by enhanced count of anti-sheep red blood cell (anti-SRBC) haemagglutination titre and IgE antibody titre as measured by passive cutaneous anaphylaxis in rats. Antigen (egg albumin) induced histamine release from peritoneal mast cells of sensitized rats in *in-vitro* was significantly inhibited by fresh leaves extract of *O. sanctum* (71).

The *O. sanctum* seed oil (OSSO) has shown immunomodulatory potential. Humoral and cellular immunity were found to be increased in non-stressed and restrained experimental rats (72). To know the effect of OSSO in non-stressed group, rats were immunized with SRBCs on the beginning of day zero and divided into two groups. The control group received saline while experimental group received 3 ml/kg/day OSSO, i.p. for six days. Haemagglutination titre was estimated by using microtitre plates. The OSSO treated group produced a significant increase in anti sheep-RBC antibody titre. On the other hand, OSSO i.p. injection for 13 days with same dose significantly inhibited antigen induced histamine release from peritoneal mast cells of rats sensitized with egg albumin along with Freund's complete adjuvant and triple antigen. OSSO treated experimental mice showed significant reduction in footpad thickness test (paw volume) and percentage of leukocyte migration inhibition (LMI) also remained significantly low. In stressed



group, stress caused significant reduction in anti-SRBC antibody titre in saline control group while significantly increased in OSSO treated group. When diazepam and OSSO were given together, a synergistic effect was observed. Pre-treated animals with OSSO caused significant attenuation on the effects of restrained-strain on T-cell mediated response (72).

Bovine mastitis is a disease, usually caused to lactating bovine by bacterial infections, eventually damages the udder tissues. Aqueous extract of *O. sanctum* showed immunotherapeutic potential in bovine sub-clinical mastitis. Polymorphonuclear cells (PMNs) are the primary cellular defense cells of the mammary glands of the bovines and they are depressed during periparturient period. Use of antibiotics to treat mastitis further depresses the activity of PMNs. Use of 100 mg/teat/day aqueous extract infusion of *O. sanctum* for seven days reduced total bacterial count (TBC) in the milk and increased neutrophil and lymphocyte counts with enhanced phagocytic activities and phagocytic index. Similarly, lysozyme content of the milk PMNs were also enhanced significantly in animals pretreated with *O. sanctum*. It was suggested that the bioactive constituents could be urosolic acid, oleanolic acid and sarigenin, which may possess immunomodulatory potential indicated by percentage increase in lymphocyte, enhanced activity of the phagocytosis of PMN cells in the bovine mammary gland, and the reduction in TBC in the milk (73).

Therapeutic efficacy of *O. sanctum* seed oil was also studied in 23 confirmed cases of bovine mastitis in buffaloes. All animals were

divided into four groups and all were given different treatments through intra-mammary route for 3-5 days. Group I (n=5) received liquid paraffin 3 ml/teat/day. Group II (n=6) received *O. sanctum* seed oil 3 ml/teat/day. Group III (n=6) received antibiotics cloxacillin (200 mg) in addition to *O. sanctum* seed oil and group IV (n=6) received cloxacillin (200 mg) alone. It was observed that fixed oil treated group recovered completely within 5 days, antibiotics group within 4 days and combined group within 3 days. This result suggests that fixed oil also have the properties to cure the bovine mastitis (74).

#### Radio-protective properties

Umadevi and her group have established a domain in pioneering the research on radio-protective properties of *Tulsi* extracts on experimental animals. They established that water extract of *Tulsi* is more radio-protective than the alcoholic extract. Optimum dose for water extract was found to be 10 mg/kg, bw and optimum radioprotection observed when the route of administration was intra-peritoneal. Increase in the doses however did not increase the level of radio-protection. This was observed when experimental mice were given water extract at a dose of 10 mg/kg, bw for consecutive 5 days before whole body  $\gamma$ -radiation (11 Gy) and the survival of mice were observed for a period of 30 days (21). Among three plants extracts viz *Withania somnifera*, *Plumbago rosa* and *Ocimum sanctum*, tested on experimental mice bone marrow survival following 2 Gy  $\gamma$ -radiation, water extract of *Tulsi* provided highest radioprotection as measured by an exogenous spleen colony forming unit (CFU-S) assay. It was also observed that the *Tulsi* extract had

no toxic effects compared to synthetic radio-protector WR-2712 (75). Radio-protection efficacy of two flavonoids, orientin and vicenin, isolated from leaves of *Tulsi* (administered i.p. 10 mg/kg, bw/day to mice for five days) were compared with synthetic radio-protector aminothiols, 2-mercaptopropionyl-glycerine 'MPG' (20 mg/kg, bw), WR-2721 (150 mg/kg, bw). The experimental mice were subjected to whole body exposure to 2 Gy  $\gamma$ -radiations for 30 minutes and bone marrow chromosomal aberrations were studied. It was observed that vicenin provided maximum protection from radiation induced chromosomal aberrations and MPG the least, while orientin and WR-2721 provided almost similar effects (76). WR-2721 and aqueous extract of *Tulsi* showed synergistic effects when compared with the individual effects of these compounds (77). Umadevi and Ganasoundari (78) further explored the possible protection against radiations induced lipid peroxidation in liver of adult swiss mice. The experimental mice were given 10 mg/kg, bw water extract of *Tulsi* for 5 days and exposed to 4.5 Gy  $\gamma$ -radiation 30 minutes after the administration of last dose. They estimated levels of glutathione (GSH) and the antioxidant enzymes glutathione transferase (GST), glutathione reductase (GSRx), glutathione peroxidase (GSPx), super oxide dismutase (SOD) and lipid peroxide (LPx) in the liver at 15 min, 30 min, 1, 2, 4 and 8 hours post treatment. It was found that pretreatment with water extract of *Tulsi* significantly reduced the lipid peroxidation compared to the controls. It also accelerated the recovery of antioxidant enzymes to normal levels. The proposed mechanism of this protection is the free radical scavenging capacity of flavonoids of *Tulsi* plant (79).

Flavonoids of *Tulsi* (orientin and vicenin) also exhibited radio-protective effects on human lymphocyte chromosomes (80). The polysaccharides isolated from *Ocimum sanctum* also prevented  $\gamma$ -radiation mediated cell death in experimental mice (81).

#### **Neuro-protective and Cardio-protective properties**

Chronic oral administration of fresh *Tulsi* leaves augments cardiac endogenous antioxidants and prevents isoproterenol-induced myocardial necrosis in rats (82). The ethanolic extract of *Ocimum sanctum* found to have ameliorative effects in axotomy (experimental denervation) induced peripheral neuropathy in rats. It was observed that administration of *Tulsi* extract for ten days (post operative) attenuated axonal degeneration and nociceptive threshold. It also reduced thiobarbituric acid reactive species. (83).

#### **Mosquito-repellent/Larvicidal properties**

In search of safe natural products to repel/kill mosquitoes and plant pests studies have been conducted on various plants including *Tulsi*. Mosquitocidal activity of *Tulsi* was investigated using its eugenol and triglyceride (isolated from *Tulsi*'s hexane extract) on fourth instars *Aedes aegypti* larvae (84). When seeds of *Tulsi* was placed in water, it exude within one hour, a mucilaginous substance (polysaccharides) and larvae which came in contact with seeds became firmly attached to it and died due to drowning of larvae. When 100 larvae of *Culex fatigans* was spread over water containing 25, 50 and 75 *Tulsi* seeds/m<sup>2</sup> surface area of water for 48 h, 100% mortality was observed in 75 seeds/m<sup>2</sup> of water while 65% and 89% mortality observed in 25 and 50 seeds/m<sup>2</sup> of

water respectively (85). Mosquitocidal efficacy of essential oil of *Ocimum sanctum* against adult mosquitoes of different species viz. *Anopheles stephensi*, *Aedes aegypti*, *Culex quinquefasciatus* were investigated and 100% mortality observed in *A. stephensi*, *A. aegypti* at a dosage of 0.003 ml/43.0 cm<sup>2</sup>. However, mortality of *C. quinquefasciatus* was observed at a higher dose (0.01 ml/43.0 cm<sup>2</sup>) (86). Essential oil of *Tulsi* showed larvicidal efficacy against larvae of *A. stephensi*, *A. aegypti*, *C. quinquefasciatus* (87). Anees (88) has studied the mosquito larvicidal property of both leaf and flower extract of *Ocimum sanctum* against 4th instar larvae of *Aedes aegypti* and *Culex quinquefasciatus*. Compared to flower extract, leaf extracts were found to be more effective against both types of mosquitoes. In search of plant based insecticides, the anti-feedant and larvicidal properties of four plants including *Ocimum sanctum* against gram pod borer *Helicoverpa armigera*, cotton leaf roller *Sylepta derogata*, and mosquito *Anopheles stephensi* have been studied. Organic solvent extract of *Ocimum sanctum* were able to kill the larvae of tested pests and vector (89).

#### Contraceptive studies

In the search of a safe herbal contraceptive, *Tulsi* plant's properties have been studied systematically in experimental animals. However, some changes have been noted on different accounts but this plant can not be qualified as true contraceptive. The long term feeding of fresh *Tulsi* leaves (465 mg/kg, bw/day) have shown increase in body weight and decrease in weights of testes, prostate and adrenal gland. The fresh leaves intake led to changes like decrease in pH, hypotonic environment and chemical substances like mucoproteins,

alkaline and acid phosphatase in spermatogenic cells leading to the formation of non-viable spermatozoa. However, male mouse could mate normally but no pregnancy occurred (90). The benzene extract of *Ocimum sanctum* leaves at dosage of 100, 150 and 200 mg/kg, bw for 15 days altered the weight of testes significantly while it did not have any significant effect on epididymis, seminal vesicle, prostate and vas deferens. It was also effective in significantly reducing the sperm count and motility (91). Long term (three months) feeding of *Tulsi* leaves (at dose of 20, 200 and 400 mg/100 g, bw) could reduce sperm count, motility and weight of reproductive organs of male but weight remained unaffected in females. The mating behavior of experimental rats reduced after 2 months of treatment but those female rats that mated, few carried full term pregnancy with normal gestation and delivered normal weighted offspring without any congenital defects. It is possible that *Tulsi* powder decreases testosterone levels directly or by inhibiting LHRH (leutinizing hormone releasing hormone) and preventing LH (leutinizing hormone) release necessary for bringing about mating response (92). Significant decrease in reproductive behavior observed in male rats at a dose of 200 and 400 mg/kg, bw for 15 days. The sexual behavior in experimental rats were monitored by scoring different responses such as grooming, pursuit, mounting, intromission and ejaculation after giving graded doses (93).

#### Miscellaneous properties

It was found in a transdermal drug (Flurbiprofen) delivery study on abdominal skin of rat that a combination of natural

product, *Tulsi* oil and terpentine oil, demonstrated significantly higher drug delivery than the synthetic combination such as isopropylene and propylene glycol (94). Experimentally induced wound in rats recovered faster in *Tulsi* extract treated compared to the control group (95). *Ocimum sanctum* extract treated human lymphocyte culture could reduce experimentally induced genotoxic effects *i.e.* chromosomal aberrations, mitotic index, sister chromatid exchange and replication index in a dose dependent manner (96). In search of potential herbal remedy for catalepsy (behavioral state in which affected person/animal is not able to correct externally imposed posture), it was found that a polyherbal formulation, which also contained *Tulsi* extract, fed mice group improved catalepsy score and super oxide dismutase activity (97). Junctachote and Berghoter (98) have studied the antioxidant activities of *Ocimum sanctum* in order to preserve the packed food from rancidity (decomposition of fats, oils and other lipids by hydrolysis and/or oxidation). By taking battery of tests to assess the state of rancidity, it was found that *Tulsi* extract can be used as a preservative. To improve shelf life of a soybean product called 'Tofu', aqueous extract of *Tulsi* was added to it. The shelf-life of 'Tofu' increased from normal 3-4 days to 7-8 days (99).

#### Future direction of research

The future studies can be focused in the light of previous studies. It can be said that *Ocimum sanctum* showed very promising results in stress relating changes in experimental animals, antimicrobial properties in *in-vitro* studies against various microbes and immunomodulatory effects. The stress induced oxidative damage is one of the major causes of various diseases especially coronary artery diseases and diabetes. With emergence of increased resistance of microbes to different antibiotics it is imperative that antimicrobial properties of *Tulsi* may possess promising results in theory. *Ocimum sanctum* is present in almost every parts of the Indian subcontinent and its immunomodulatory properties may be explored to provide additional immunity to mankind at very low cost due to its easy availability. Besides these, other studies like neuro-protective and regenerative properties may be explored further to evaluate its use in neurological disorders like Parkinson's and Alzheimer's diseases.

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