



ORIGINAL ARTICLE

A Preclinical Study of Multiple Health Benefits of Tulsi, Holi Basil (*Ocimum sanctum* L.) on Different Rodents**Vishwakant**

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Email: gupta.vishwakant2@gmail.comReceived: 5th September 2019, Revised: 19th September 2019, Accepted: 26th September 2019**ABSTRACT**

India has always been a rich source of medicinal native plants since thousands of years in very beginning of civilization. Several medicinal plants across world and India have been used for curing different diseases. Even now in modern industrial and computerized era their importance is increasing day by day especially in the cases of microbial infections. In our traditional system of Ayurveda different parts of tulsi have been advised by ancient physicians to treat many ailments like many types of inflammation as sinusitis, pharyngitis, bronchitis, all types of viral infections, diarrhea, dysentery, arthritis, gastric troubles, healing wounds, skin, eye diseases etc. It has many properties like analgesic, antipyretic, anti-influenza, antifungal, antimicrobial, antiviral, anti-inflammatory, antidiabetic, anticancer, anti-fertility, cardio-hepato-pulmo-renal protective, antispasmodic and adaptogenic and so on. Active compound Eugenol (1-hydroxy-2-methoxy-4-allylbenzene) is found in *Ocimum sanctum* L. which is principally responsible for the therapeutic abilities. The present review focuses on therapeutic efficiencies of *Ocimum sanctum* L. on the basis of pharmacological studies under preclinical trials on rodents done by different researchers

INTRODUCTION

In accordance with Atharvaveda and Ayurveda around 1300 plant species have been used as phytochemical drugs across the globe. Many medicinal plants possess natural phytochemicals. Any part of plant from roots, stems, leaves, seeds, fruits, buds, barks may be beneficial in various way to mankind. In order to settle therapeutic uses of Tulsi (in Sanskrit means 'the incomparable one') in modern time drugs during couple of decades many Indian scientists, researchers have investigated pharmacological effects of distilled, ether petroleum and extracts of benzene from various parts of Tulsi [1] on CNS, immunology, biochemistry, hametology, hepatology, cardiology and likewise on trial animals like rats, rabbits and man. They elucidated therapeutic values of tulsi in curing and preventing several disorders.

Exploration of biochemical compounds of any plant and pharmacological screening provide the platform to develop new potential herbal drugs. Before this scientific examination of bioactive compounds have to pass through standardization and quality control for safety purposes.

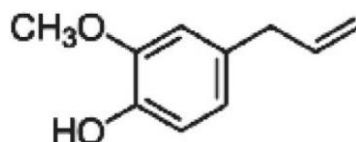
Tulsi is adorable in Hindu families almost every family plants the tulsi in home garden or any place in home in pots and it is worshipped daily. During winter tea is prepared from its leaves is a common practice in Hindu families. From mythological aspect tulsi-saligram wedding is celebrated as festival in many parts of our country as it symbolize health and wealth. Thus tulsi has its religious and spiritual sanctity since time immemorable as it had important medicinal values in traditional ayurvedic system of medicines [2].

Traditionally, *Tulsi* is taken in numerous forms like herbal tea, dried powder or fresh leaves. For hundred of years, the dried leaves of Tulsi have been assorting with stored grains to avoid insects [3]. Striking by its pleasant odor and acerbic taste, it is considered as a kind of 'elixir or amrit of life or magical potion' in Ayurveda and believed to uphold longevity [1].

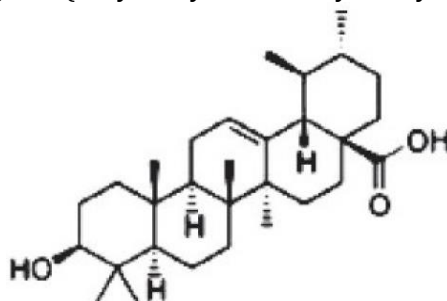
PHYTOCHEMICAL INGREDIENTS

1. The leaf volatile oil [4] –
 - a. eugenol (1-hydroxy-2-methoxy-4-allylbenzene [Figure 3]),
 - b. euginal (also called eugenolic acid),

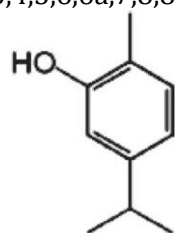
- c. urosolic acid [5] (2,3,4,5,6,6a,7,8,8a,,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid
 - d. carvacrol (5-isopropyl-2-methylphenol [Figure 5]),
 - e. linalool (3,7-dimethylocta-1,6-dien-3-ol [Figure 6]),
 - f. limatrol,
 - g. caryophyllene (4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene [Figure 7]),
 - h. methyl carvicol (also called Estragol: 1-allyl-4-methoxybenzene [Figure 8])
2. Seed volatile oil - fatty acids and sitosterol;
 3. Seed mucilage - some levels of sugars and the anthocyanins are present in green leaves. Sugars are comprised of xylose and polysaccharides.



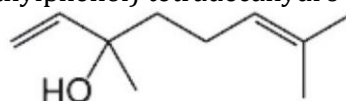
Eugenol (1-hydroxy-2-methoxy-4-allylbenzene)



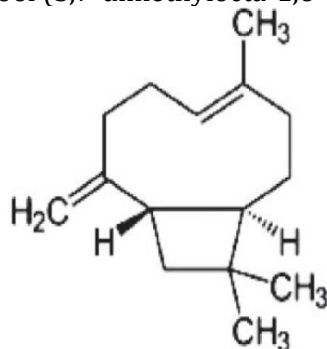
Urosolic acid (2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b



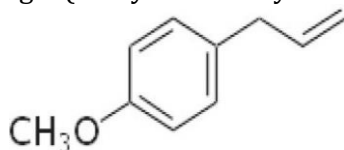
Carvacrol (5-isopropyl-2-methylphenol) tetradecahydro-1H-picene-4a-carboxylic acid)



Linalool (3,7-dimethylocta-1,6-dien-3-ol)



Estragol (1-allyl-4-methoxybenzene)



Caryophyllene (4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene)

The stem and leaves of holy basil contain many bioactive compounds like flavonoids, triterpenoids, saponins, and tannins [6].

Moreover other phenolic bioactive compounds have also been extracted loaded with antioxidant and anti-inflammatory power such as –

1. Rosmarinic acid ((2*R*)-2-[[[(2*E*)-3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]] oxy]-3-(3,4-dihydroxyphenyl) propanoic acid
2. Apigenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one
3. Cirsimaritin (5,4'-dihydroxy-6,7-dimethoxyflavone)
4. Isothymusin (6,7-dimethoxy-5,8,4'-trihydroxyflavone)
5. Isothymonin.

Two water-soluble flavonoids: [7]

6. Orientin (8-C-beta-glucopyranosyl-3',4',5,7-tetrahydroxyflav-2-en-3-one)
7. Vicenin (6-C-beta-D-xylopyranosyl-8-C-beta-D-glucopyranosyl apigenin), prevent radiation-induced chromosomal destruction in human lymphocyte.

THERAPEUTIC [HEALING] APPLICATIONS

These are based on pre-clinical studies on animal models, rodents during invitro testing. There are some remarkable research findings given one by one-

1. ANTI-DIABETIC ACTIONS:

O. sanctum L. Ethanolic extract pointedly decreases the blood glucose along with glycosylated hemoglobin, urea with a relative increase in glycogenesis, Hb formation in streptozotocin-dosed diabetic rats [8]. Such extracts also led to an increase in insulin, glucose tolerance and peptide chains.

O. sanctum L. has been used in treatment of diabetic-related metabolic ailment [9] and found to have action both in hypoglycemia and hyperglycemia and bring back glucose level [10].

Grovel *et al.* studied that *O. sanctum* L. extract given to fructose fed normal rats for 30 days and serum glucose level found lowered [11] comparable to control group. But he didn't find any significant effect on hyperinsulinemia.

Ghosap *et al.* [12] studied the possible pathway of glucose-dropping activity of *O. sanctum* L. in male mice. The study revealed that *O. sanctum* L. decreased the serum level of cortisol and glucose leaving antiperoxidative effect. He concluded that *O. sanctum* L. possibly regulate cortisol- induced diabetic mellitus.

In a study accomplished by Vats *et al.*, [13] in streptozotocin (STZ, 65 mg/kg)-induced model of diabetic rats for 30 days. Intrusion of *O. sanctum* L. extracts at a dose of 200 mg/kg for 30 days resulted in decreasing plasma glucose levels around 9.06 and 24.4% on 15th and 30th day respectively. Moreover *O. sanctum* L. moderately improved the activity of glucokinase, hexokinase and phosphofructokinase in the diabetic control.

Basil (*O. sanctum* L.) leaf powder [14] fed diabetic rats at the 1% level in normal and for a period of one month resulted a significant hypoglycemic effect at fasting.

2. CARDIO-PROTECTIVE ACTIONS:

Ocimum was found insightful effect on treatment and prevention of cardiovascular diseases as lowering blood lipid profile, subduing ischemia and strokes, alleviating hypertension. Moreover, it helps in antiplatelet aggregation and checks risk of pulmonary hypertension. These cardioprotective properties proven by many studies that *Ocimum* may be good remedy for prevention and treatment of cardiovascular diseases.

The AIIMS, Delhi researchers piloted blood tests and scrutinized the hearts of the rats and found that the rats those had got tulsi extracts earlier to the ISP injections revealed very less damage to hearts than rats who had not tulsi extracts. The tulsi had rats also showed better heart functions and less inflammation. The ISP injectables damage the heart by oxidative damage. In this experiment tulsi extracts provoked the synthesis of anti-oxidant enzymes in liver of rats. They further studied CK-MB enzyme, marker of injury of heart. The rats who had got tulsi extracts found to have lower values of CK-MB [15].

Tulsi ingredients like Eugenol and ursolic acid down the cortisol level and reduce stress and stress-related problems related with heart ailments. antioxidant property of *Ocimum* averts cardiac lipid peroxidation due to free radicals. This lowers the risk of cardiac diseases and keeps heart healthy [1, 16]. In another research Oral nurturing of hydroalcoholic extract of *Ocimum* (100 mg/kg) was done with male Wister rats exposed to chronic-resistant stress (6 h/day for 21 days). This significantly checked the chronic-resistant stress in plasma catalase activities, cAMP level and myocardial superoxide dismutase [17].

Wister rats feeding on fresh leaf homogenate of *Ocimum* as 50 and 100 mg/kg body weight, daily for 30 days obstruct isoproterenol-induced changes [18] in myocardial glutathione peroxidase and superoxide dismutase, and reduced glutathione level. In next study, the oil extract of Tulsi raised blood clotting time, compares with aspirin, might be attributed to tulsi power of inhibition towards platelet aggregation [19].

3. HYPOLIPIDEMIC ACTIONS:

In other experiment cholesterol administered lagomorphs were given tulsi seed oil (0.8 gm/kg body weight/day) for four weeks, result came as rabbits showed decreased serum triglycerides and LDL + VLDL and total cholesterol, in comparison to cholesterol fed rabbits without seed extract [20].

In next study afresh leaves of *Ocimum* (1 & 2 gm in 100 gm diet) administered in albino rabbits for 4 weeks showed significant alterations in the lipid profile [21] as dropping in TG,TC, LDL-cholesterol, phospholipids levels and noteworthy increased HDL-cholesterol. In other study to reconnoiter the effect of tulsi leaves powder on fasting blood sugar, uronic acid, total amino acids, and total cholesterol, triglyceride, phospholipids and total lipids the in serum and tissue lipids in normal and diabetic rats for one month The results revealed a significant fall in above parameters [22].

4. ANTIINFLAMMATORY ACTIONS:

Fresh tulsi leaves have been using since long as a potential adjuvant with conservative anti-inflammatory feature. The fresh tulsi leaf as paste shows considerable anti-inflammatory activity too as compared to Indomethacin when study done on rats animal models [23]. In a trial on albino rats showed significant ($P < 0.05$) anti-inflammatory outcome in Carrageenan induced edema of rat foot. The reduction of edema by tulsi found better than the standard anti-inflammatory medicine, indomethacin.

Ocimum sanctum has significant anti-inflammatory activity might be attributed to inhibition of cyclooxygenase and lipooxygenase bi-pathways for arachidonic acid metabolism [24]. Extracted Compounds from *Ocimum* like Eugenol, Apigenin, Isothymonin, Civsilineol, Civsimavatine, Rosavinic acid and were found to have anti-inflammatory potentials [25]. due to cyclooxygenase inhibitory activity. Eugenol proven 97% inhibitory actions of cyclooxygenase-1 when assayed with 1000 μM concentration.

Apigenin, Isothymonin, Civsilineol, Civsimavitin, and Rosavinic acid exhibited 65, 58, 37, 50, 37% cyclooxygenase-1 inhibitory doings respectively, when assayed with 1000 μM concentrations. The actions of such compounds were comparable to Naproxen, Ibuprofen and aspirin at 10, 10 and 1000 μM concentrations respectively. Singh doings on research [26] elucidated that linoleic acid available in different potency in oil extract of different species of *Ocimum* has the power to arrest cyclooxygenase and lipooxygenase bicyclic pathways of arachidonate metabolism which led to anti-inflammatory actions. In other trial methanolic extract [27] plus aqueous suspension of tulsi at 500 mg/kg succeed to inhibit acute and chronic inflammation in rats with pedal edema induced by carrageenan and granuloma induced by cratonoil and exudates, respectively. Outcome had compared to the response met with sodium salicylate at 300 mg/kg.

Moreover both extract and suspension showed analgesic effect in procedure of mouse hot plate, and the methanol extract produced prolong tail withdrawal reaction time of morphine drug in stand in analgesic dose. However both solutions have reduced typhoid plus paratyphoid a, b vaccine tempted pyrexia. The antipyretic action of both preparations was little and with shorter duration than dose of 300 mg/kg sodium salicylate. 60 rats in some other trial were taken and

looked the effect of seeds of *Ocimum basilicum* on histamine and prostaglandins induced inflammation. A mixture of Petroleum ether fraction at 400mg/kg, p.o and fraction of ethanol at 400mg/kg, p.o from seeds of same species was administered and found that mixture ominously inhibited the foot edema created by histamine along with PGF2- α . This concluded that seeds of *Ocimum basilicum* L. have persuasive anti-inflammatory efficiency [28].

5. IMMUNOMODULATORY ACTIONS:

Different experimental animal studies have clearly shown immunomodulatory properties in the extract of Tulsi leaves [29, 30, 31, 32]. In an experiment wistar rats, stressed and non stressed mice, and guinea pigs were taken, where the effects of Restraint stress, on humoral and cell-mediated immunity was observed. Animals were effectively tempered with OSSO (tulsi seed oil). It was concluded that OSSO proved modulating both types immune responses and these immune modulatory effects might be attributed to GABAergic pathways [31].

In other test to assess the immunomodulatory effect of *Ocimum* in wistar albino rats, aqueous extract of *Ocimum* were given with doses of 100 and 200 mg per kg per day for 45 days. This showed increased antibody production (immunomodulatory). It enhanced the erythropoiesis, WBC and haemoglobin production. It not affected the biochemical factors [33]. The results [32] to see Immunotherapeutic prospective of aqueous extract of tulsi in intramammary infused bovine sub-clinical mastitis revealed that such treatment diminished the total bacterial count and increased TLC and lymphocytes with higher phagocytic actions along phagocytic index.

Godhwani *et al.* [1987, 1988] examined the immune-regulatory contour of methanolic extract along with aqueous suspension of *Ocimum* leaves to antigenic encounter of Salmonella and sheep R.B.C. by computing agglutinating antibodies employed Widal agglutination vis-à-vis sheep erythrocyte agglutination experiments together with E-rosette development in albino rats. Outcome of study revealed an immune-stimulation of humoral immunogenic response and cellular immunologic response.

6. NEURO-PROTECTIVE ACTIONS:

Neuro-protection means the mechanisms and methodologies employed to protect the CNS against various injuries in cases of acute and chronic neurodegenerative diseases (NDs) such as stroke, ischaemia, nerve degeneration, Parkinson's, Epilepsy, Dementia, Alzheimer's, Huntington's disease, bipolar and motor disorder etc. The different forms of Tulsi aid to regulate neurotransmitter levels which play an inevitable role in nervous function [34].

Several done studies over *Ocimum* ethanolic extract showed positive results on the capability of the central nervous system from center of learning and memory point of view [35]. Tulsi extracts administration also encourages the density of pyramidal cells in the CA1 and CA3 regions facilitated by higher levels of ACh [36]. Through various in-vitro studies, OSE proven itself in maintaining the stability of ChAT expression at human cerebral microvascular endothelial cells imitate young age and restore ChAT expression at imitate old age [37]. Another evidence from experimental design told that OSE unveils neuroprotective effects against neuronal cell damage by H₂O₂ in SH-SY5Y neurons [38].

Ocimum EO expresses its anti-convulsing property by specific targeting GABAergic mechanism [39]. Moreover, observation also revealed the neuro-protective effects of rosmarinic acid (active compound in tulsi), which are almost marked by the up regulation of shielding genes, tyrosine hydroxylase levels, brain-derived neurotrophic factor and avert mitochondrial dysfunction [40]. Further in vitro employment of rosmarinic acid in N2A cells have also revealed the almost same neuro-protective properties [40].

The neuro-shielding effectiveness of rosmarinic acid at 100 mg/kg was clearly demonstrated temporal lobe epilepsy in rats induced by Kainic acid, an agonist of AMPA/KA receptor [41, 42].

Phytochemicals available in *Ocimum basilicum* preclude cerebral ischemia, re-perfusion as well as short-term memory loss and progress cognitivity and motor functions [43]. Singh *et al.* [44] demonstrated how to check neuron injury in ethyl acetate fed mice induced by reperfusion cerebral damage. Different extracts from stems, stem callus and leaves (induced with somewhat modified media of Murashige and Skoog along with supplementation of kinetin and 2,4-

dichlorophenonyacetic acid) were verified for anti-convulsant activity [6]. Tulsi was found to act as Nootropic agents which are a new class of explored drugs used in organic disorder in learning abilities. Joshi and Parle [45] evaluated the potential of *Ocimum* extracts as nootropic and anti-amensic agents in mice.

7. ANTI-MICROBIAL ACTIONS:

Singh *et al* [46] in his study suggested that higher content of linoleic acid in Tulsi oil could contribute towards its antibacterial activity. The oil show good antibacterial activity against *Staphylococcus aureus* (most sensitive), *Bacillus pumilus* and *Pseudomonas aeruginosa*. The combined extracts of aqueous seed oil and alcohol of holy basil exhibited antimicrobial properties against enteric pathogens [47, 48].

The Tulsi extract mixed with essential oil is effective against gram-positive and gram-negative bacteria [49]. Mahmood *et al.* [50] was also explored *Ocimum* having active compounds which have positive effect against strain of *S. aureus*. *Ocimum sanctum* extract also has the wide antibacterial effects against *S. aureus*, *P. aeruginosa* and *Bacillus pumilus*. These microbes render spoilage of food stuffs, fruits and vegetables leading to food poisoning and other serious ailments in humans. Devi *et al.* [51] also reconnoitered that the *Ocimum sanctum* leaves extraction is found excellent antimicrobial agent. In fact it is highly effective against both gram positive and gram negative bacteria such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *P. putida*, *B. subtilis* and *E. coli*.

Rao & Nigam [52] concluded in his research that the Tulsi leaves extract & essential oil were found effective against pathogenic fungi such as *Alternaria solani*, *Fusarium solani*, *Candida guilliermondii*, *Colletotricum capsici*, *Curvularia sp.* and *Helminthosporium oryzae*, which are responsible for rotting fresh produce. Extract of dried leaves and oil of basil is more effective against various fungal strains as compared to fresh leaves alone. The different types of holy basil extract (*O. sanctum*) contain many useful secondary metabolites as polyphenols described above which act as strong antiviral agents against various viruses. The aqueous extracts along with essential oil of basil were evaluated in viral encephalitis [53].

Moreover essential oil and extract of basil retain inhibitory effect on growth of viruses such as polio virus type-3 [54], hepatitis B virus and multiple RNA viruses viz. coxsackie virus B1 (CVB1), herpes viruses (HSV), adenoviruses (ADV) and enterovirus 71 (EV71) [55].

8. ANTI-CANCER ACTIONS:

The prime action of Holy Basil (Tulsi) in cancer control comes from its ability to inhibit an enzyme called COX-2 that stimulates an inflammatory response in the body and triggers cancerous activities. Holy basil, by blocking the action of this enzyme, prevents inflammation and reduces pain. Various animal and laboratory studies have indicated that Tulsi can repair and regenerate the cells damaged due to the exposure to radiation and oxidation demonstrating its immense potential at destroying the pre-cancerous lesions.

Studies have revealed that the phytonutrients in holy basil directly kill the cancer cells thereby limiting the growth and spread of the cancer mass. Prashar *et al.* in their study investigated that *Ocimum* leaves extract blocks or suppresses metabolic events associated with chemical carcinogenesis [56] by impeding metabolic activation of the carcinogen. In this study, rat hepatocytes cultures were infested with 0–500 µg of *Ocimum* extract for 24 hrs aftermath with 7,12-dimethylbenz[a] anthracene (10 or 50 µg) for 18 hrs. significant reduction was found in the levels of dimethylbenz [a] anthracene adducts was observed in all cultures treated with basil extract. This led to blocking of metabolic progression of carcinogen. The chemo-preventive activity [57] of seed oil of *Ocimum* was estimated against Swiss albino mice injected with 20-methyl cholanthrene provoked fibrosarcoma tumors in their thigh region.

The delaying in tumor possibility and longer survival rate was observed in seed oil fed mice. Liver enzymatic, non-enzymatic antioxidant malondialdehyde values and end products from lipid peroxidation were significantly moderated with oil feeding as compared to unfed 20- mice with methylcholanthrene injections. Infestation with aqueous and ethanolic extracts of *Ocimum sanctum* to mice having Sarcoma-180 solid tumors. Considerable reduction in tumorous volume and an longevity in lifespan was observed. These observations proven anticancer activity oil of tulsi [58].

Aruna and Sivaramakrishnan [59] assessed that administration of tulsi to mice led to meaningfully elevated glutathione level and more than 75% of glutathione S-transferase working and barred fore stomach tumors and hepatomas.

Ponugoti [60] observed that Tulsi has been bosom to have superb anticancer activity. They noted that detoxification of carcinogens and mutagens which is carried out by enzymes such as glutathione-S-transferase, cytochrome b5 and cytochrome P450, and aryl hydrocarbon hydroxylase is tempered by the alcoholic extract of leaves of *Ocimum*. Venkatachalam and Muthusamy [61] described in his review that management of Tulsi has been shown to decrease cell proliferation, invasion, angiogenesis, and apoptosis. They also noted that the leafy extract subdues the biochemical events associated with chemical carcinogenesis by checking metabolic activities, conversion of procarcinogen to active carcinogen.

Manikandan *et al.* [62] demonstrated that basil also reasons a downfall in the 7, 12-dimethylbenz[a] anthracene inductive genotoxicity, valued by micronuclei formation in bone marrow cells of mice. They found that tulsi keeps anti-genotoxic effects, and all these might aid to lessen the chemical carcinogenesis and declivity of cutaneous γ -glutamyl transpeptidase, a indicator of tumor progression, and glutathione-S-transferase-P, which is elevated in chemically induced hepato tumors. Moreover *Ocimum sanctum* has been evaluated for and demonstrated its anticancer properties against prostate cancer, breast cancer and gastric cancer [63].

CONCLUSION

In recent years there has been a renaissance for pharma interests in spreading the traditional health encouraging uses of Tulsi. The nutritional and pharmaceutical properties of the complete herb in its natural form, its derivative forms came from synergistic amalgamation of lots of different bioactive phytochemicals and compounds. Therefore, overall effects of Tulsi may not be fully repeated with isolated compounds or extracts. Since its inherent botanical and biophytochemical intricacy, Tulsi standardization has, until now, escaped modern science. Moreover Tulsi is known as a general vitalizer, immunity booster and physical endurance uplifter, it don't have caffeine or other stimulant.

Despite of the numerous landmark and history maker triumph of western medical science, of modern allopathic medicines has not been proven to deal with the growing range of chronic degenerative disorders, metabolic organs dysfunctions, multiple microbial & viral infections due environmental dismantling, rotten lifestyle and personal stress that surround modern society from all sides. When modern medicines fail to work then significant inevitable and complementary role has come out of traditional a variety of herbal medicines mostly native from India and all-inclusive methodologies towards better health in prevention and prophylaxis of the persistent illness of nowadays civilization. Be acquainted with the prominence of augmentation of western medical viewpoint, WHO has recommended that traditional health and folk medicine systems should be integral part of modern allopathic medical therapies to work more effectively discourse health issues globally.

Significant evidences has emerged from different researches tell us that besides basil's many wide range therapeutic as stated in this review article aforesaid applications, the herb's super natural adaptogenic properties give rise to substantial preventive and curative potential with respect to lot of disorders especially related with modern life style. Ongoing clinical trials of Tulsi's health encouraging qualities will put on four moon on crown of such holy plant.

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