

Anti-inflammatory, anti-oxidant effect and cytotoxicity of *Ocimum sanctum* intra oral gel for combating periodontal diseases

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Abstract:

It is of interest to evaluate the anti-inflammatory, anti-oxidant effect and cytotoxicity of *Ocimum sanctum* (an Indian herb, Thulasi) intra oral gel in combating periodontal diseases. Hence, 2% of *O. sanctum* gel was prepared with Carbopol940 soaked in purified water containing 0.2% w/v sodium benzoate overnight. Hydroxy propyl methyl cellulose (HPMC) solution was mixed with propylene glycol using tissue homogenizer. Anti-oxidant effect was analyzed using DPPH radical assay and anti-inflammatory effect was assessed using the inhibition of albumin denaturation assay. *Ocimum sanctum* gel with various dilutions from 10 micro litres to 50 micro litres showed exponential increase in percentage of inhibition from 60.9 to 72.2 exhibiting antioxidant activity. The anti-inflammatory effect of *Ocimum sanctum* gel showed comparatively equivalent effect with standard diclofenac gel with values ranging from 76.6 for 50 micro liters of *Ocimum sanctum* gel and 89.6 for standard gel at 50 micro liters. *Ocimum sanctum* showed less toxicity towards brine shrimp nauplii. Thus we show that *Ocimum sanctum* gel showed potent anti-oxidant and anti-inflammatory effect and less toxic to brine shrimp nauplii as a promising agent for the treatment of periodontal diseases.

Key words: *Ocimum sanctum*, periodontal diseases, anti-oxidant effect, anti-inflammatory effect, cytotoxic effect.

Background:

Periodontal diseases are chronic inflammatory diseases in which host, microbes, environment and genetics plays an important role in causing the disease or alter the rate of progression of the disease. Dental plaque, which exists in a state of biofilm, is the etiological agent for periodontal disease [1]. The organisms, which are commonly detected in Periodontitis, are *Porphyromonas gingivalis*, *Aggregatibacter actinomycetem comitans*, *Tannerella forsythia*, *Prevotella intermedia*. These microbes are present in sub gingival dental plaque and exist in a state of biofilm. In the presence of biofilm, antimicrobial agents won't be effective in killing the organisms. Hence it's very important to destroy the biofilm environment for controlling the organisms [2]. Treatment of periodontal disease aims at removal of etiological factors like plaque and calculus by scaling and root planing there by removing the biofilm loaded with microorganisms. But certain microorganisms like A.a, P.g reside inside the tissues and also in echniches and repopulate the diseased site [3]. These microorganisms trigger the host inflammatory process and make it as hyper inflammatory trait, leading to release of enzymes like Matrix Metalo Proteinases (MMP) to host tissues. The destruction from the hyperresponsive host inflammation is more than the microorganism itself. Hence modulating the host immune response holds a paramount importance. Anti-inflammatory agents, anti-oxidants to control reactive oxygen species, anti-cytokine therapy have been tried for controlling the host inflammatory response. All these therapies has unwanted side effects when used in long term, hence search for natural extract which has anti-microbial, anti-inflammatory, and anti-oxidant property would be beneficial for the treatment of periodontal disease [4,5]. Ocimum sanctum (O.sanctum) is known as the "Queen of Herbs". It is described as sacred and medicinal plant in ancient literature and has been used in different formulations for the treatment of a wide range of disorders, including those of the mouth and throat, lungs, heart, blood, liver, kidney and other systems. It is also called Holy Basil and in Sanskrit Tulsi means 'the incomparable one' has got two varieties, Krishna Tulsi (black) and Rama Tulsi (green). *O. sanctum* has demonstrated anti-microbial, anti-oxidant and anti-inflammatory effects in various pharmacological studies [6]. Therefore, the aim of the study was to estimate the anti-inflammatory and anti-oxidant effect of Ocimum sanctum intra oral gel for the treatment of periodontal disease.

Materials and Methods:

The study was presented to institutional ethical committee and got approved. The study was in accordance with Helsinki's declaration. There are three parts in materials and methods. First is preparation of Ocimum sanctum gel followed by evaluation of anti-

inflammatory activity and evaluation of anti-oxidant activity of the prepared Ocimum sanctum insitu gel.

Preparation of Ocimum sanctum gel:

Ethanolic extract of *O. sanctum* was prepared by maceration method. 250gm of Tulsi powder is soaked in 1000 ml of ethyl alcohol for 48hrs and filtered with whatman filter paper. Filtered liquid was evaporated to get supercritical fluid and stored in suitable container with appropriate temperature until further use. Two percentage of *O. sanctum* gel was prepared with following method: Carbopol940 was soaked in purified water containing 0.2% w/v sodium benzoate overnight. Using tissue homogenizer hydroxypropyl methyl cellulose (HPMC) solution was mixed with propylene glycol. 2ml of tulsi extract (supercritical fluid extract) was transferred into HPMC solution and homogenized (see Figure 1). This drug solution were later transferred to carbapol solution and homogenized. Triethanolamine was added to neutralize the pH. (Figure 1)

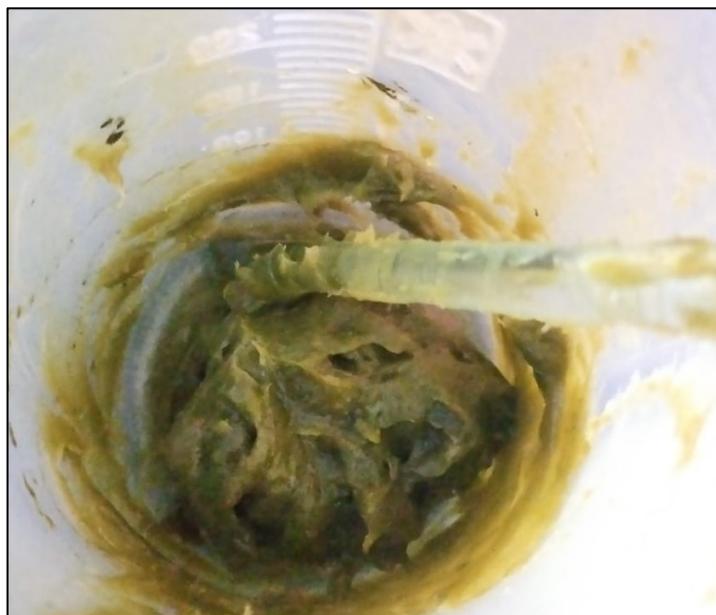


Figure 1: Ocimum sanctum *in situ* gel prepared in the lab.

Antioxidant Activity:

DPPH radical assay: The DPPH free radical scavenging activity of Ocimum sanctum gel was determined according to the methodology followed in this study [7]. Typically, different concentration (2-10 µg/ml) of plant extract was mixed with 1 ml

of 0.1 mM DPPH in methanol solution and 450 μ l of 50 mM Tris-HCl buffer (pH 7.4) and incubated for 30 min. After incubation, the reduction in the number of DPPH free radicals was measured based on the absorbance at 517 nm. BHT was used as controls. (Figure 2 and Figure 3) The percent inhibition was calculated from the following equation: % Inhibition = [Absorbance of control - Absorbance of test sample / Absorbance of control] \times 100 results are shown in (Table 1 & Figure 5)

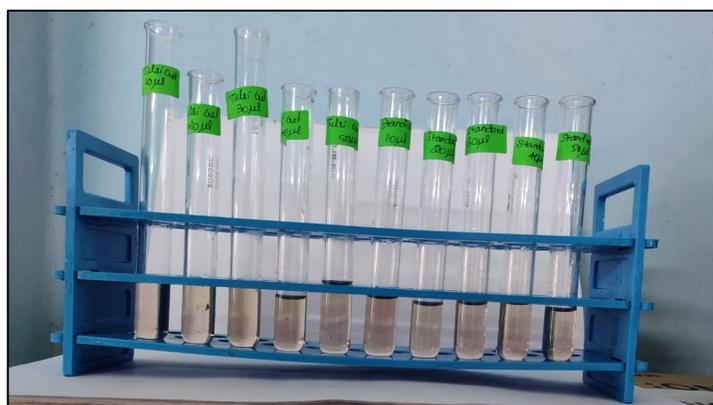


Figure 2: Different concentrations of Ocimum sanctum gel for estimation of anti-oxidant effect.



Figure 3: Double beam Spectrophotometer used for evaluation

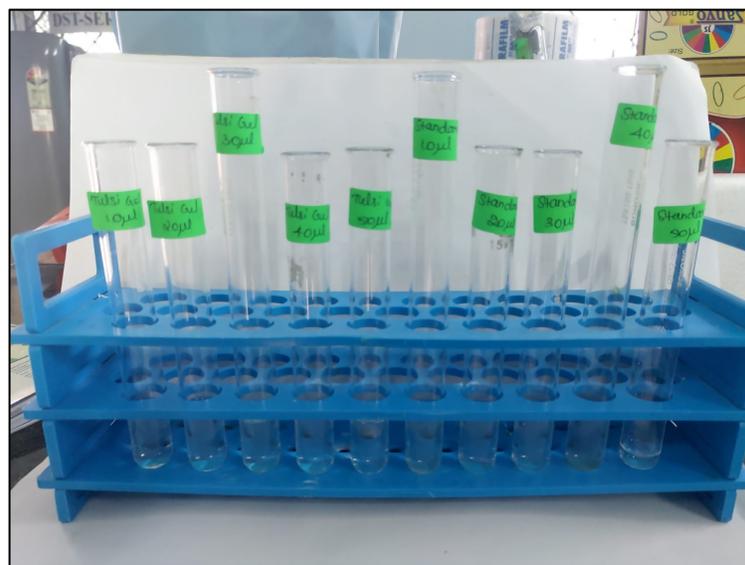


Figure 4: Different concentrations of Standard and Ocimum sanctum gel for estimation of anti-inflammatory effect.

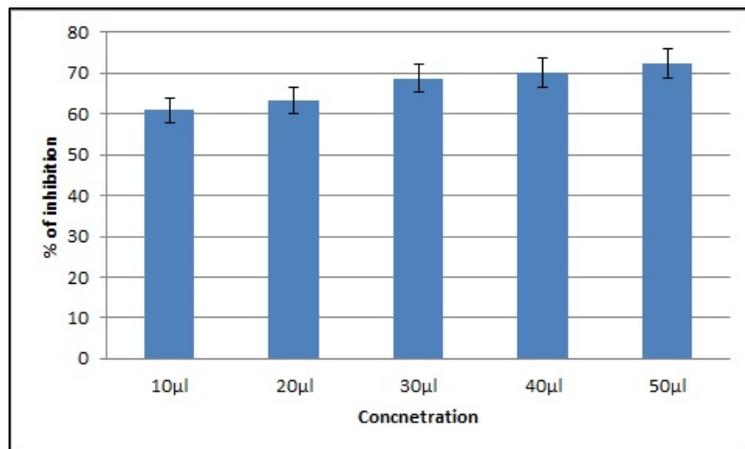


Figure 5: shows Anti-Oxidant effect of Ocimum sanctum gel in different concentrations Ocimum sanctum gel has excellent anti-oxidant effect, at the concentration of 50microliters it has demonstrated peak anti-oxidant effect.

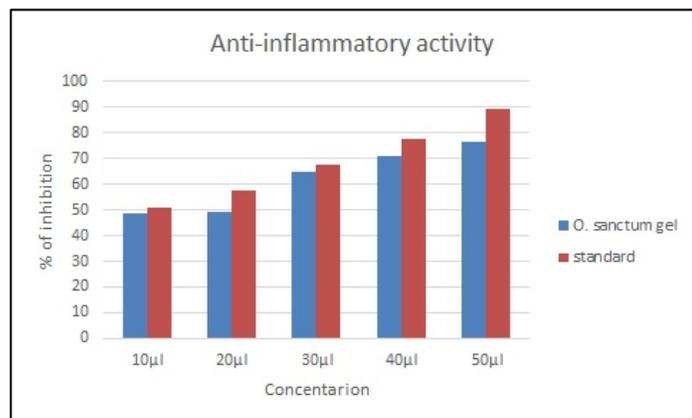


Figure 6: shows Anti-Inflammatory effect of *Ocimum sanctum* gel in different concentrations against the Standard. There is a significant increase in anti-inflammatory effect with varying concentrations of *O. sanctum* gel and it is compared to standard drug diclofenac.

Anti-inflammatory activity:

Inhibition of albumin denaturation assay: BSA (Bovine serum albumin) was used as a reagent for the assay. Bovine serum albumin (BSA) makes up approximately 60% of all proteins in animal serum. It is commonly used in cell culture, particularly when protein supplementation is necessary and the other components of serum are unwanted. BSA undergoes denaturation upon heating and starts expressing antigens associated with Type III hypersensitive reaction which are related to diseases such as rheumatoid arthritis, glomerulonephritis, serum sickness and systemic lupus erythematosus. 2 mL of 1% Bovine albumin fraction

Table 1: shows Anti-Oxidant effect of *Ocimum sanctum* gel in different concentrations measure in a single beam UV spectrophotometer at absorbance value of 660nm.

Different Concentrations of <i>O. sanctum</i> Gel	Absorbance at 517nm	Percentage of Inhibition
10µl	0.391	60.9
20µl	0.368	63.2
30µl	0.313	68.7
40µl	0.3	70
50µl	0.278	72.2

Table 2: shows anti-Inflammatory effect of *O. sanctum* gel in different concentrations measured in a single beam UV spectrophotometer at absorbance value of 660nm.

Different Concentrations of <i>O. sanctum</i> Gel and Standard	Percentage of Inhibition for <i>O. sanctum</i> gel	Percentage of Inhibition for Standard (Diclofenac Gel)
10µl	48.8	58.8
20µl	49.1	57.8
30µl	64.7	67.6
40µl	70.9	77.9
50µl	76.6	89.6

was mixed with 400 µL of plant crude extract in different concentrations (500-100 µg/mL) and the pH of reaction mixture was adjusted to 6.8 using 1N HCl. The reaction mixture was incubated at room temperature for 20 minutes and then heated at 55°C for 20 min in a water bath. The mixture was cooled to room temperature and the absorbance value was recorded at 660 nm. An equal amount of plant extract was replaced with DMSO for control. Diclofenac sodium in different concentrations was used as standard [8]. The experiment was performed in triplicate. (Table 2 & Figure 6)

% Inhibition was calculated using the following formulae:

$$\% \text{ Inhibition} = \frac{\text{Control O.D} - \text{sample O.D}}{\text{Control O.D}} * 100$$

Cytotoxicity Test:

Cytotoxic Assessment was done by brine shrimp egg. In this experiment, brine shrimp eggs are kept in the fish tank and iodide free salt is added in the beaker along with water. The solution is then stirred for 10 minutes till the crystallized salt gets dissolved. Sodium bicarbonate was then added in the fish tank in small amount of 0.5mg. The brine shrimp eggs were kept in the tank for 24 hours. After 24 hours nauplii were hatched and they were identified and separated. Then 10 naupliis are added in each Elisa well and *O. sanctum* insitu gel was added in the range of 10µl, 20µl, 30µl, 40µl, 50µl in all wells and control level also added. To determine the mortality rate of prepared *O. sanctum* insitu gel movement of nauplii was noted and stable ones were checked under microscope to assess it micromovement (Table 3).

Table 3: shows the cytotoxicity test results at Day 0,1 and 2 days after incubation. Nauplii survived even at higher concentration. At less concentration 80% of nauplii survived which shows *Ocimum sanctum* gel is less toxic.

Ocimum sanctum Insitu gel	Nauplii at Day 0	Nauplii at Day 1(after 24hrs)	Nauplii at Day 2 (after 48 hrs)
10 µl	100%	90%	80%
20 µl	100%	80%	70%
30 µl	100%	80%	60%
40 µl	100%	70%	60%
50 µl	100%	70%	50%

Results and Discussion:

Treatment of periodontal disease starts at removing the etiological factors and disturbing the biofilm. Anti-microbial therapy is given to reduce the microbes penetrated inside the tissues and eradicate the organisms from econiche. It can be done systemically as well as locally. Systemic medications has side effects hence locally delivered drugs into gingival crevice has the advantage. Locally delivered drugs should be retained in the sulcus for required period of time and it should be above the minimum inhibitory concentration for periodontal pathogens. Pharmacokinetics of the locally delivered drugs should follow zero order kinetics for effective drug delivery. Drugs like tetracycline, doxycycline, minocycline, metronidazole, chlorhexidine has been tried in the treatment of periodontal disease with potential effects. But these drugs don't have anti-inflammatory, anti-oxidant property to modulate the host. Hence search of drug or natural extract, which has antimicrobial, antioxidant and anti-inflammatory property, is in place [9]. *O. sanctum* (Tulsi) belongs to plant family Lamiaceae. It has made important contribution to the field of science from ancient times as also to modern research due to its large number of medicinal properties. Phytoconstituents of Tulsi: The leaves of *O. sanctum* contain 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. The oil also contains carvacrol and sesquiterpine hydrocarbon caryophyllene₄. Fresh leaves and stem of *O. sanctum* extract yielded some phenolic compounds (antioxidants) such as cirsilineol, circimaritin, isothymusin, apigenin and rosameric acid, and appreciable quantities of eugenol [10]. Two flavonoids, viz., orientin and vicenin from aqueous leaf extract of *O. sanctum* have been isolated. Ursolic acid, apigenin, luteolin, apigenin-7-O-glucuronide, luteolin-7-O glucuronide, orientin and molludistin have also been isolated from the leaf extract₆. *O. sanctum* also contains a number of sesquiterpenes and monoterpenes viz., bornyl acetate, elemene, neral, and pinenes, camphene, campesterol, cholesterol, stigmasterol and sitosterol [11]. A study done by Vishwabhan *et al.* proposed the antimicrobial activity of Tulsi by virtue of its essential oil content [12]. Various other studies also project the idea that Tulsi yields various essential oils, which are responsible for the medicinal uses including antimicrobial, antioxidant, antifungal, and anti-inflammatory activities that can probably explain its activity against the microbes

discussed so far. It has also been postulated that Tulsi has an immunomodulatory effect and acts by increasing the levels of interferon, interleukin-4 and T helper cells that can strengthen host response to infections [13,14]. The methanol extract and the aqueous extract of *O. sanctum* have shown to inhibit acute as well as chronic inflammation in rats. Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *O. sanctum*, has been found to be largely responsible for the anti-inflammatory property of tulsi. It demonstrated 97% cyclooxygenase-1 inhibitory activity when assayed at 1000 µM concentration [15-18]. In an animal study done by Hosadurga *et al.* 2% *O. sanctum* was used in the treatment of experimental periodontitis in Wistar albino rats. Anti-Inflammatory effect and duration of action was assessed for edema reduction. Results of the study showed *O. sanctum* gel showed anti-inflammatory effect by reducing edema to 33.66% compared to control group and action was sustained till 24 hrs. However antioxidant effect and toxicity levels were not assessed in that study [19]. In our present study *O. sanctum* showed excellent anti-oxidant effect of 72.2 percentage of inhibition in 50 micro liters, which is equivalent to any standard anti-oxidant agent. Also anti-inflammatory effect of *O. sanctum* in our study showed 76.6 percentage of inhibition in comparison with the standard diclofenac gel, which showed 89.6 percentage of inhibition at 50 microliters. Though the anti-inflammatory effect is lesser than standard drug it is sufficient for producing anti-inflammatory effect when delivered in local delivery form. Cytotoxicity of *O. sanctum* gel was assessed by Brine shrimp eggs assessment method. Nauplii was hatched from brine shrimp eggs and was tested with *O. sanctum* gel. Results showed survival rate of nauplii at variable concentrations of *O. sanctum* gel. Nauplii survived better after 24 hrs and 48 hrs in lesser concentrations of the gel when compared to higher concentration of the gel. Survival rate of nauplii is within the acceptable limits.

Conclusion:

Ocimum sanctum has shown promising actions towards the treatment of periodontal diseases by having anti-inflammatory, anti-oxidant action with less toxicity. Cytotoxicity test showed *O. sanctum* insitu gel is less toxic. *O. sanctum* gel showed potent anti-oxidant and anti-inflammatory effect comparable to standard drug. *O. sanctum* intra oral gel is a potential local drug delivery agent in the

treatment of periodontal disease. However, adequate clinical trials are required to validate its therapeutic potential.

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Conflict of Interest: Both the authors have declared no conflict of interest for the study.

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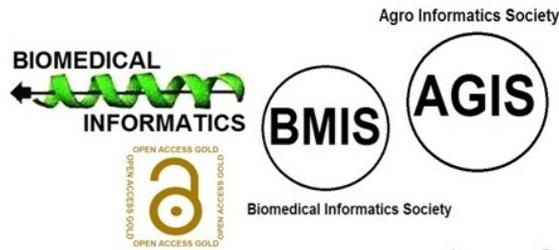
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