



## RESEARCH ARTICLE

### EX- VIVO HISTOPATHOLOGY OF TEETH WITH IRREVERSIBLE PULPITIS IN CONTACT WITH ESSENTIAL OIL EXTRACT OF OCIMUM SANCTUM (TULSI) FOR ITS ANTI-INFLAMMATORY PROPERTIES. A PILOT CLINICAL STUDY

1,\*Navin Mishra and 2Isha Narang

<sup>1</sup>Endodontist, IIT, New Delhi, India

<sup>2</sup>Endodontist HCMS, Haryana, India

#### ARTICLE INFO

##### Article History:

Received 28<sup>th</sup> December, 2015

Received in revised form

20<sup>th</sup> January, 2016

Accepted 15<sup>th</sup> February, 2016

Published online 31<sup>st</sup> March, 2016

##### Key words:

Ocimum Sanctum,  
Herbal,  
Irreversible Pulpitis.

#### ABSTRACT

**Introduction:** Intracanal medicament has been widely advocated to help eliminate pulpal or periapical tissue microorganism either as local dressing after excavation of carious lesions or as a root canal medicament. Herbal medicine, also called “botanical medicine” or “phytomedicine”, refers to the use of plant seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Ocimum sanctum (Holy basil, Tulsi) is a plant native to India and has been known to have antimicrobial and anti-inflammatory properties. The present pilot clinical study used Ocimum sanctum’s essential oil extract to test its anti-inflammatory properties in deep carious teeth with irreversible pulpitis slated for extraction.

**Materials and methods:** Six patients with irreversibly inflamed pulp in carious third molar slated for extraction were randomly divided into two groups. Gross carious excavation was done in all the selected teeth. IRM restoration was placed in teeth of control Group A while cotton pellet dipped in Ocimum sanctum’s essential oil extract was placed in group B before restoration. Both groups were called after 48 hrs for extraction and teeth were longitudinally grooved with a fissure bur and split in half. The dental pulps were extracted carefully from all the teeth using a spoon excavator which was fixed in 10% formalin solution. It was then subjected to dehydration with graded alcohol 70%, 80%, and 90% successively. The tissue embedding was done in blocks of paraffin wax after clearing with Xylene. The specimens were examined under compound microscope after staining with haematoxylin and eosin stain.

**Results: Group A (Control):** Cellular collagenous pulp tissue showing interstitial edema with moderate diffuse infiltrate of neutrophilic polymorphonuclear cells with evidence of acute inflammation.

**Group B (Ocimum Sanctum (Tulsi) essential oil extract):** Cellular collagenous pulp tissue with no evidence of acute inflammation was seen.

**Conclusion:** After 48 hours deep carious third molars in contact with Tulsi oil the mediators of acute inflammation was totally eliminated. Thus extract showed its efficacy against acute inflammation.

Copyright © 2016, Navin Mishra and Isha Narang. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Citation:** Navin Mishra and Isha Narang, 2016. “Ex- vivo histopathology of teeth with irreversible pulpitis in contact with essential oil extract of ocimum sanctum (tulsi) for its anti-inflammatory properties. A pilot clinical study”, *International Journal of Current Research*, 8, (03), 28677-28680.

## INTRODUCTION

Role of microorganisms in the development and maintenance of pulpal and periapical inflammation have been well documented. Primary root canal infections are polymicrobial, typically dominated by obligate anaerobic bacteria (Nair, 2004). The success of endodontic therapy largely depends on the elimination of these microbial contaminations from the root canal system.

\*Corresponding author: Navin Mishra,  
Endodontist, IIT, New Delhi, India.

The objectives of root canal instrumentation are thorough debridement, creation of an optimum space for delivery of antimicrobial substances and to facilitate 3D obturation of the root canal system to prevent recolonization by oral microbiota (Sundqvist and Taxonomy, 1994). Mechanical instrumentation in combination with a chemically inert irrigating solution cannot adequately reduce viable microorganisms in the infected root canal system (Chavez *et al.*, 2003). With the current nickel titanium and traditional stainless-steel instruments, almost half of the root canal walls were shown to be left unprepared (Engstrom, 1964).

Hence the use of intracanal medicaments has been widely advocated to help eliminate these remaining bacteria. Intra canal medicaments can be classified on the basis of their chemical composition into phenolic compounds (e.g. eugenol and camphorated monochlorophenol), aldehydes (Formocresol), halides (e.g. iodine potassium iodide), calcium hydroxide, antibiotics, and various combinations (Haapasalo *et al.*, 1983). The majority of these preparations are not used in contemporary endodontic practice due to reported toxicity, development of resistant strains, and suppression of the immune system (Waltimo *et al.*, 1997). Intracanal medicaments that exert their antibacterial action in a vapour form are formocresol, camphorated mono-chlorophenol, merthiolate, metacresylacetate, beechwood creosote, and glutaraldehyde (Waltimo *et al.*, 1997; Schilder, 1974). Although, they are effective against certain microorganism implicated in periradicular disease, alpha hemolytic streptococci and enterococci have been found to be resistant to the vapors of these medicaments. Moreover they have a limited role because they are extremely toxic, antigenic and its effect is lost after a few days.

The medicaments reduce periapical inflammation, pain and induce healing (Schilder, 1974). It also aids in controlling inflammatory root resorption and prevent contamination between appointments by acting as a physicochemical barrier, precluding the proliferation of residual intracanal microorganisms and preventing reinfection of the root canal by bacteria from the oral cavity (Grahnén and Krasse, 1962). Ideal requirements for an intra canal medicament are that they should be biocompatible, easily retrievable, non-staining and have no effect on obturating materials. Change is the law of nature. Long practiced outside of conventional medicine, herbal therapy is getting importance as research shows their value in the treatment and prevention of diseases. Herbal medicine, also called “botanical medicine” or “phytomedicine”, refers to the use of plant seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Recently, the World Health Organization estimated that 80% of people worldwide relied on herbal medicines for some aspect of their primary healthcare. The benefits of herbal medicine are its abundance, biocompatibility with minimum side effects and no resistance development on its prolonged and repeated use (Neelakantan *et al.*, 2007).

*Ocimum sanctum* (Holy basil, Tulsi) is a plant native to India with known medicinal properties since the Vedic period. It is classified as a “rasayana”, - a herb that nourishes a person’s growth to perfect health and promotes long life. It has known antibacterial (Bystrom and Sundqvist, 1981), antifungal (Mayer *et al.*, 2002) and antiviral properties (Peters, 2004). Extracts of *Ocimum sanctum* inhibits acute as well as chronic inflammation and is used in the treatment of arthritis (Bystrom and Sundqvist, 1983). In addition, this oil has a strong analgesic effect (Ikhlas and John, 2007).

The dried leaves of the plant can be powdered, mixed with mustard oil to make a dentrifice. It can prevent dental caries and aphthous ulcers in the mouth (Harrison, 1979). There are few studies documenting the use of herbal extracts as intracanal medicaments (Santos *et al.*, 2006; Pulok *et al.*, 2006), however no study has been reported on essential oil extract of *Ocimum*

*sanctum* as an intracanal medicament The aim of the present preliminary study was to evaluate the essential oil extract of *Ocimum sanctum* for its anti inflammatory property for its proposed use as an intracanal medicament.

## MATERIALS AND METHODS

In the present study after taking informed consent, six patients with irreversibly inflamed pulp in carious third molar slated for extraction were randomly divided into two groups A control and Group B test respectively. Gross carious excavation was done in all the selected teeth. IRM restoration was placed in teeth of control Group A while cotton pellet dipped in *Ocimum sanctum*’s essential oil extract was placed in group B before restoration. Both groups were called after 48 hrs for extraction and teeth were longitudinally grooved with a fissure bur and split in half. The dental pulps were extracted carefully from all the teeth using a spoon excavator which was fixed in 10% formalin solution. It was then subjected to dehydration with graded alcohol 70%, 80%, and 90% successively. The tissue was embed in blocks of paraffin wax after clearing with Xylene. The embedded pulp tissue was cut in 5 micron meter section through microtome and then section fixation was done on albumin coated slide by heating around 60-70 degree. The specimens were examined under compound microscope after staining with haemotoxylin and eosin stain.

Both groups received antibiotics and analgesics prior to extraction which were continued for 5 days after extraction.

## RESULTS

### Group A (Control)

Cellular collagenous pulp tissue showing interstitial edema with moderate diffuse infiltrate of neutrophilic polymorphonuclear cells with evidence of acute inflammation. (Fig 1.)

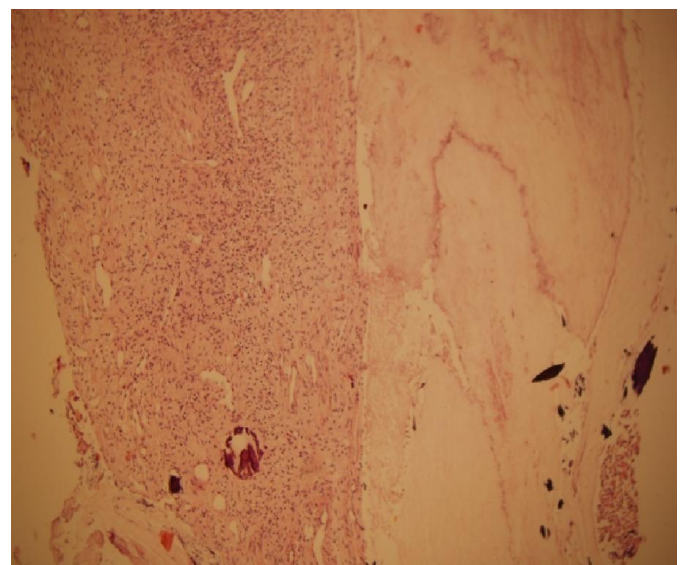
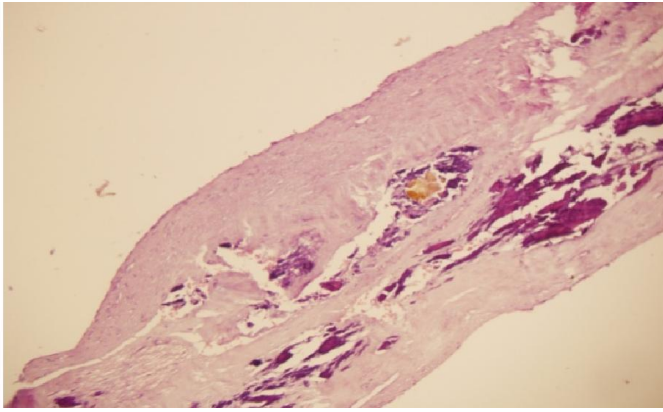


Figure 1. Histopathology of dental pulp (Group 1. Control)



**Figure 2. Histopathology of dental pulp Group 2. Essential oil extract of *Ocimum sanctum*)**

#### **Group B (*Ocimum Sanctum* (Tulsi) essential oil extract)**

Cellular collagenous pulp tissue with no evidence of acute inflammation was seen. (Fig. 2) After 48 hours deep carious third molars in contact with Tulsi oil the mediators of acute inflammation was totally eliminated. Thus extract showed its efficacy against acute inflammation.

#### **DISCUSSION**

Irreversible pulpitis are based on subjective and objective findings that the vital inflamed pulp is incapable of healing and that root canal treatment is indicated. Signs and symptoms of symptomatic pulpitis which is irreversible may include sharp pain upon thermal stimulus, lingering pain (often 30 seconds or longer after stimulus removal), spontaneity (unprovoked pain) and referred pain. Sometimes the pain may be accentuated by postural changes such as lying down or bending over and over-the-counter analgesics are typically ineffective. Common etiologies may include deep caries, extensive restorations, or fractures exposing the pulpal tissues. Such teeth may be difficult to diagnose because the inflammation has not yet reached the periapical tissues, thus resulting in no pain or discomfort to percussion. In such cases, dental history and thermal testing are the primary tools for assessing pulpal status while asymptomatic irreversible pulpitis is a clinical diagnosis based on subjective and objective findings indicating that the vital inflamed pulp is incapable of healing and that root canal treatment is indicated.

These cases have no clinical symptoms and usually respond normally to thermal testing but may have had trauma or deep caries that would likely result in exposure following removal. In the present pilot clinical study 6 human third molars having deep caries showing signs and symptoms of symptomatic irreversible pulpitis slated for extraction were taken. The anti-inflammatory action of essential oil extract of *Ocimum sanctum* is attributed to the presence of alpha linolenic acid. It is an omega-3 fatty acid which inhibits both lipooxygenase and cyclooxygenase pathways i.e. it has a dual inhibitory action. The alpha linolenic acid is progressively metabolized to 6, 9, 12, 15 octadecatetraenoic acids, stearadonic acid and eicosapentaenoic acid, which are the end products. Eicosapentaenoic has the capacity to competitively inhibit the formation of prostaglandins and leukotrienes.

It also acts on histamine, serotonin and kinins. Another proposed mechanism for its antigranulomatous efficacy is, its ability to regulate the AP-1 transactivation (Neelakantan *et al.*, 2007). This results in the inhibition of epidermal and fibroblast growth factors, which gives the oil; its antiproliferative property. Anti-inflammatory effect of dexamethasone was tested on a similar animal model. Following endodontic overinstrumentation the periapical tissues were examined histologically (Pallota and Ribeiro, 2007). They concluded that the local infiltration of dexamethasone produced a significant anti-inflammatory effect on the periapical tissues of teeth with vital or partially necrotic pulp tissue. However, steroidal preparations have local side effects such as immunosuppression and impairment of periapical healing (Paquette *et al.*, 2007). Hence the use of essential oil extract of *Ocimum sanctum* having a good anti-inflammatory property is advantageous over the other steroidal/non steroidal intracanal medicament (Wang *et al.*, 2007).

#### **Conclusion**

This study is first of its kind and hence based on the encouraging results of this *ex-vivo* histopathological study, which documented the beneficial anti-inflammatory properties of the essential oil extract of *Ocimum sanctum*, further research is needed to extrapolate these and the other potential beneficial properties for its potential use in endodontics as an medicament in pulpal and periapical diseases.

#### **Acknowledgement:**

The Authors deny's any conflict of interest.

#### **REFERENCES**

- Bystrom, A. and Sundqvist, G. 1981. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. *Scand J Dent Res.*, 89:321- 8
- Bystrom, A. and Sundqvist, G. 1983. Bacteriologic evaluation of the effect of 0.5 percent sodium hypochlorite in endodontic therapy. *Oral Surg Oral med Oral Pathol*, 55:307-12
- Chavez, L.E., Dahlén, G., Molander, A., Moller, A. and Bergenholtz, G. 2003. Bacteria recovered from teeth with apical periodontitis after antimicrobial endodontic treatment. *Int Endod J.*, 36:500-8
- Engstrom, B. 1964. The significance of Enterococci in root canal treatment. *Odontol Revy.*, 15:87-106
- Grahnén, H. and Krasse, B. 1962. The effect of instrumentation and flushing of non-vital teeth in endodontic therapy. *Odontol Revy.*, 13:167-77
- Haapasalo, M., Ranta, K., Ranta, H. 1983. Facultative Gram-negative enteric rods in persistent periapical infections. *Acta Odontol Scand.*, 91:458-63
- Harrison, W. 1979. The clinical toxicity of endodontic medicaments. *J.Endod.*, 5:2-6
- Ikhlas, E.L. and John, K. 2007. The antimicrobial effect of root canal irrigation and medication. *Oral Surg Oral Med Oral Pathol.*, 103:560-69
- Mayer, B.E., Peters, O.A. and Barbakow, F. 2002. Effects of rotary instruments and ultrasonic irrigation on debris and

- smear layer scores: a scanning electron microscopic study. *Int Endod J.*, 35:582–9
- Nair, P.N. 2004. Pathogenesis of apical periodontitis and the causes of endodontic failures. *Crit Rev Oral Biol Med.*, 15:348–81
- Neelakantan, P., Sanjeev, K. and Subbarao, V. Duration – dependent susceptibility of endodontic pathogens to calcium hydroxide and chlorhexidine gel used as intracanal medicaments: an in vitro evaluation. *Oral Surg Oral Med oral Pathol Oral Radiol Endod*; 104:138-41
- Pallota, C. and Ribeiro, S. 2007. Determination of minimum inhibitory concentration of four medicaments used as intracanal medication. *Aust Endod J.*, 33:107-11
- Paquette, L., Legner, M., Fillery, E.D. 2007. Friedman S. Antibacterial efficacy of chlorhexidine gluconate intracanal medication in vivo. *J Endod.*, 33(7):788-95
- Peters, O.A. 2004. Current challenges and concepts in the preparation of root canal systems: a review. *J Endod.*, 30:559–67
- Pulok, K. Mukherjee, A., Wahile, L. 2006. Integrated approaches towards drug development from Ayurveda and other Indian system of medicines. *Jour Ethnopharmacol*, 103: 25–35
- Santos, L.L., Vilhena, D. E., Toledo, Medeiros S. and Souza, P. 2006. The use of herbal medicine in Alzheimer's disease-a systematic review. *Evid Based Complement Alternat Med.*, 3(4):441-5
- Schilder, H. 1974. Cleaning and shaping the root canal. *Dent Clin North Am.*, 18:269-96
- Sundqvist, G. 1994. Taxonomy, ecology, and pathogenicity of the root canal flora. *Oral Surg Oral Med Oral Pathol.*, 78:522–30
- Waltimo, T.M., Sirén, E.K., Torkko, H.L., Olsen, I. and Haapasalo, M.P. 1997. Fungi in therapy-resistant apical periodontitis. *Int Endod J.*, 30:96 –101
- Wang, C.S., Arnold, R.R., Trope, M., Teixeira, F.B. 2007. Clinical efficiency of 2% chlorhexidine gel in reducing intracanal bacteria. *J Endod.*, 33(11):1283-9

\*\*\*\*\*