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Cavernous Lymphangioma of Unusual Location: A Case Report

By Dr. Rajesh B. Dhirawani, Dr. Ankit Sharma, Dr. Sanyog Pathak, Dr. Sumit Asrani,
Dr. Garvita Sahu & Dr. Ankit Gupta

Abstract- Lymphangioma is a benign tumor leading to hyperplasia of lymphatic vessels. The gold standard for treating lymphangiomas is surgical resection; alternative options being, sclerotherapy reduces the impact and complications of surgery.

Keywords: lymphangioma, cavernous lymphangioma, lymph.

GJMR-J Classification: NLMC Code: WU 290



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Cavernous Lymphangioma of Unusual Location: A Case Report

Dr. Rajesh B. Dhirawani ^α, Dr. Ankit Sharma ^σ, Dr. Sanyog Pathak ^ρ, Dr. Sumit Asrani ^ω, Dr. Garvita Sahu [¥]
& Dr. Ankit Gupta [§]

Abstract- Lymphangioma is a benign tumor leading to hyperplasia of lymphatic vessels. The gold standard for treating lymphangiomas is surgical resection; alternative options being, sclerotherapy reduces the impact and complications of surgery.

Keywords: lymphangioma, cavernous lymphangioma, lymph.

I. INTRODUCTION

Lymphangioma is a benign tumor involving the proliferation of lymphatic vessels. The commonly affected regions are head and neck, presenting two-thirds of cases at birth and 90% by the second year of life, and some victims may not manifest lifelong. [1,2] Lymphangioma formed along the tissue planes or penetrate in adjacent tissues, become canalized and accumulates fluid in them. These are classified into three types, namely Capillary lymphangioma, Cavernous lymphangioma, and Cystic hygroma. [2] Another variety being Hemolymphangioma shows vascular as well as lymphatic component.

These hamartomatous lesions contain clear lymph fluid, but some may present clinically as transparent vesicles containing RBCs because of extravasation. Mostly superficial, presenting as a swelling or a mass, but some may extend deeply involving the connective tissue. Amongst these, Cavernous lymphangioma contains dilated sinusoidal endothelium-lined vascular channels devoid of erythrocytes and may appear as subcutaneous nodules, with a rubbery consistency. [3] These are certainly demarcated from capillary lymphangioma. The overlying skin does not show any lesions or changes.

II. MATERIALS AND METHODS

Cavernous lymphangioma of cheek region is discussed in the present article. The lesion presented with vestibular obliteration on the ipsilateral side and extending to the left maxilla. Diagnosis was made based

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on radio imaging and histopathology. Both clinical and histological features for proper management concerning this hamartomatous entity are incorporated in the present article.

III. CASE REPORT

A 30-year-old male reported to our department with complaint of slow-growing mass in the right cheek region for ten years giving an unaesthetic appearance. There were no associated symptoms like pain and discharge. He noticed a gradual increase in size for three years. There was no history of trauma or any past illnesses. He complained of mild restriction during mouth opening.

Extra-oral examination revealed facial asymmetry concerning the right middle third of the face, round to oval solitary diffuse swelling extending from the right lateral ala of the nose to preauricular region anteroposteriorly and from right inferior orbital rim to the right commissure area supero-inferiorly. The lesion was measured about 3 × 3 cms in diameter with no change in the color of overlying skin and evident obliteration of the right nasolabial groove [Figure 1].



Figure 1: Pre OP Profile View

On palpation, the swelling was diffuse, soft in consistency; mobile, not fixed to skin, non-tender on palpation, no localized rise in temperature, non-reducible, and compressible, non-pulsatile. Intra-oral examination revealed vestibular obliteration in the right upper region posteriorly.

Fine-needle aspiration cytology (FNAC) revealed the presence of fat cells having eccentrically placed nuclei and empty looking cytoplasm. Fatty material with plenty of polymorphs and few lymphocytes were present [Figure 2].

Figure 2: FNAC Report

Under general anesthesia, intraorally 2.5-3 cms maxillary vestibular incision taken. The Lesion was exposed, followed by dissection in the subcutaneous plane [Figure 3]. The facial vein was seen passing through the tumor and hemostasis achieved by ligating the parent vessel and circumscribed 3 X 3 X 3 cms tumor excision was done [Figure 4].



Figure 3: Exposed Lesion



Figure 4: Lesion excised

Histopathologic examination of the excised specimen showed numerous dilated, sinusoidal spaces of varying sizes within the deeper connective stroma. The vascular areas consist of walls of variable thickness and lined by a single layer of endothelial cells. Some areas show eosinophilic material with lymphocytes and few RBCs [Figure 5].

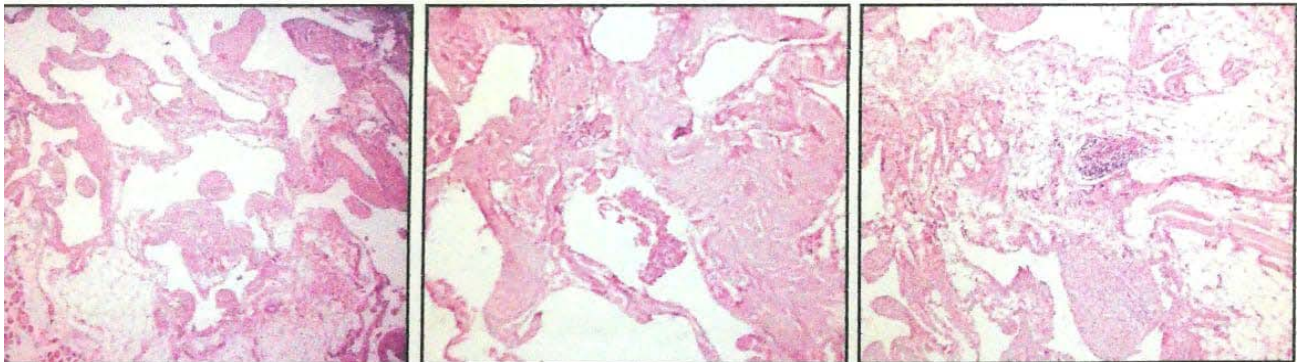


Figure 5: H & E stained section shows numerous dilated, sinusoidal spaces of varying sizes within the deeper connective stroma. The vascular spaces consist of walls of variable thickness and lined by single layer of endothelial cells. Some spaces show eosinophilic material with lymphocytes and few RBCs. Vascular channels are infiltrating into adjacent muscle.

IV. DISCUSSION

Oral lymphangiomas usually involve anterior tongue, causing macroglossia, lips, and buccal mucosa. Clinically they appear as nodular or elevated masses and may resemble surrounding mucosa. Histopathologically multiple intervening lymphatic channels are contained within a loose fibrovascular

stroma. Lymphangioma is usually confused with hemangiomas very often. The absence of valves and the presence of numerous erythrocytes in hemangiomas is a characteristic feature for differentiation.

The origin of this abnormality explained on the basis of three theories. The first theory entails about any blockage or disruption in the growth of primitive lymph channels during embryogenesis; the second states that

the primary lymphatic sac does not communicate the venous system, while the third hypothesized, that lymphatic tissue was laid down in the faulty region during embryogenesis.

Poor aesthetics becomes the primary concern in patients with cavernous lymphangioma of the cheek. In this case, the lesion was present on the right cheek region with unaltered speech or breathing. Differential diagnoses include hemangioma, lipoma, amyloidosis, neurofibroma. Lymphangioma accounts for about 6% of all tumors, is benign vascular malformation. Lymphangioma of the cheek is a rare entity that often shows slow progressive enlargement of the lesion resulting in swelling over the affected region. Whereas, it can be associated with syndromes like Turner's syndrome, Noonan's syndrome, trisomies, cardiac anomalies, fetal hydrops. Treatment depends on the size and location of the lesion, proximity to anatomic structures, and infiltrating into the surrounding tissues, further complicating treatment. Macrocystic lesions are localized and can be excised easily, while microcystic lesions are diffuse and are difficult to remove into Completely excised mass with two cms of safe margin, involving complete depth removed from the adjacent unaffected mucosa. No after-surgery complications like wound dehiscence and scarring were noticed figure [6]. Regular follow up at definite time intervals are being done for assessing the recurrence.



Figure 6: Post OP Profile View

There are ample of techniques for management of this benign lesion like surgical resection, radiotherapy, cryotherapy, electrocautery, sclerotherapy, administration of steroids, embolization, ligation, laser surgery, and radiofrequency tissue ablation technique.^[4] A definitive diagnosis and intervention help to reduce the functional, psychological disturbances, and cosmesis.

Surgery is considered to be the gold standard and is the treatment of choice for every surgeon, but the absence of capsule and infiltrating nature of the tumor makes complete removal next to impossible, and therefore chances of recurrence are more often.

Lymphangiomas did not response to sclerosing agents like hemangiomas.

V. CONCLUSION

The Differential diagnosis in cases of soft tissue swelling of the buccal mucosa should involve the lymphangioma. As earlier diagnosis will help in better treatment options for the patient. In the present study, surgical excision seems to be the safe and effective treatment of lymphangioma, preventing further recurrence in the future.

Conflicts of Interest: Jabalpur Hospital and Research Center, Jabalpur (M.P.).

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Mouthguard and Orofacial Traumatismo in Young Roller Hockey Practicers

By Lopes, L & Santos, M

Abstract- Objective: This study aimed to investigate the prevalence of mouthguard used and orofacial trauma in a roller hockey population and verify the several associated factors.

Materials and Methods: An observational cross-sectional study based on clinical observation and questionnaire application to the athletes under search. The sample consisted of 117 roller hockey athletes between 13 and 19 years old, in Portugal, in several clubs which were, Sporting Clube de Portugal, Sport Lisboa e Benfica, Clube Desportivo de Paço de Arcos and Parede Futebol Clube.

Results: The prevalence of mouthguard used was 16.2%, and 56.2% of them used Type II, and 47.4% Type III.

The prevalence of orofacial trauma was 38.5%, in which 71.1% of these dental injuries involved the upper incisors, which were the most affected teeth, both the deciduous and definitive dentition.

At the time of injury, only four athletes wore mouthguards, which 1 type I, 2 of type II and 1 of model III.

Conclusion: Roller hockey is a sport in which the prevalence of mouthguard use is low, and that of orofacial trauma is high. Thus, the mouthguard is a device that should be more used by athletes, since it is useful in preventing these injuries.

Keywords: sports, roller hockey, mouthguard, orofacial injuries.

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Lopes, L^α & Santos, M^σ

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Keywords: sports, roller hockey, mouthguard, orofacial injuries.

I. INTRODUCTION

The cause of traumatic dental injuries are usually the result of an external impact on a tooth and its surrounding tissues. (Soares et al., 2016) Dental trauma is thus a public health problem that affects a large number of people, leading in certain cases to irreversible tooth loss, not only during the accident but also during or after treatment. In athletes, trauma often occurs in youth and contact sports, representing an important group in the etiology of dental trauma. (Ferrari & Medeiros, 2002)

Contact sports are those in which practitioners are in direct physical contact with other players or objects, which leads to a higher likelihood of trauma and injury. (Fernandes et al., 2019)

Despite efforts to reduce the number of dental injuries, the incidence is relatively high in children and young people. (Sigurdsson, A., 2013) Mouthguards are a viable option for the prevention of traumatic injuries of

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the oral cavity during sports practice, and their use is more relevant in contact sports, despite their low frequency. (Collins et al., 2016)

The practitioners of Hockey should use mouthguards to prevent the occurrence of oral trauma, since Hockey is a high-risk collective sport in which physical contact is marked (Galic et al., 2018)

Understand the importance of use the mouthguard by young people who practice a collective contact sport and its relationship with the orofacial trauma suffered by them is the aim of this study.

II. MATERIALS AND METHODS

This is an observational cross-sectional study with a sample of 117 athletes between 13 and 19 years old practicing a collective contact sport.

It was conducted a study at the Sporting Clube de Portugal, Sport Lisboa e Benfica, Clube Desportivo de Paço de Arcos e Parede Futebol Clube, in their respective sports halls.

Observation and personal and direct interview with the athletes and father/mother/legal guardian were used to collect the following data: gender; age; occurrence of orofacial trauma; circumstances of the fact of orofacial lesion (outside sports, during sports or both); injury location (dental and/or soft tissue); in case of dental trauma, what type of wound occurred in concrete; if a dentist was report after the harm occurred; if you went to a dentist, how long did it take to do so; in case of avulsion of a tooth, is it aware of the possibility of its reimplantation and, if so, how long do you think is proper to do that intervention; and in which transport medium do you think is suitable for; what kind of impact caused the trauma; if it was during sports practice, was mouthguard used at the time of injury; if you used a mouthguard at the time of injury, what type did you use; type of occlusion; presence of risk factors (increased overjet and high caries index); knowledge about what is and what is the function of a mouthguard; frequency of mouthguard use; type of mouthguard; frequency of mouthguard replacement; existence in the club concerned of any advice regarding the use of mouthguards; opinion regarding the relevance of the mouthguard; what are the reasons for not using it.

The observation was made with the individual sitting, facing the observer, with the parent/legal guardian next to him/her and in the place/sports hall/room where the training took place, with the aid of

artificial light. Occlusion and risk factors were evaluated, as well as the presence of traumatic injuries and orthodontic appliances not previously mentioned by the guardians, as well as the DMFT index.

Descriptive statistics were performed for the final sample (absolute and relative frequencies), analyzing universal and independent variables. The binomial test was used to compare two proportions.

The data collected were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 22.0 software program for Windows and synthesized in figures in the Microsoft Office Excel 2019 software program.

III. RESULTS

For this research, we considered a final sample of 117 young people between 13- and 19-years old

roller hockey practitioners, who're mean, and mode age was 15.3 years and 13 years, respectively. Regarding gender, only 0,9% (n=1) was female. As for sports clubs, 23,1% (n=27) were athletes from Sporting Clube de Portugal, 22,2% (n=26) from Sport Lisboa e Benfica, 32,5% (n=38) from Clube Desportivo de Paço de Arcos and 22,2% (n=26) of Parede Futebol Clube.

Overall, 38,5% (n=45) of the athletes suffered orofacial trauma at least once (Figure 1), which specifically affected the teeth in 71,1% (n=32) of the cases (Figure 2).

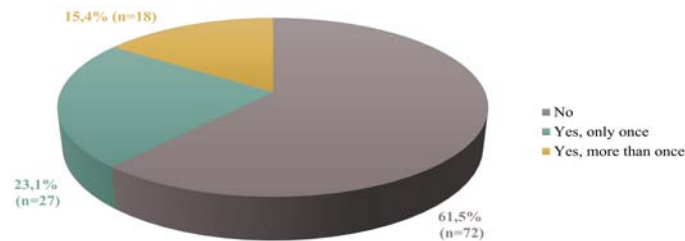


Figure 1: Sample distribution regarding the occurrence of orofacial trauma

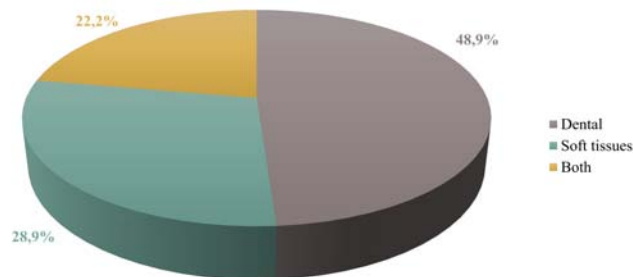


Figure 2: Distribution of the orofacial trauma sample by location

Of these, 68,8% (n=22), the affected dentition was the definitive one (most often the definitive maxillary incisors), 25% (n=8) affected the deciduous dentition (most often the deciduous maxillary incisors) and 6,3% (n=2) both dentitions.

Regarding the type of injuries suffered, 62,5% (n=20) of them were coronary fractures, 9,4% (n=3) subluxations, 6,3% (n=2) intrusions, 12,5% (n=4) avulsions and 9,4% (n=3) of another type unknown.

It was observed, regarding the type of impact that led to trauma, that 71,9% (n=23) occurred due to impact with some equipment and 28,1% (n=9) due to impact with another athlete (Figure 3).

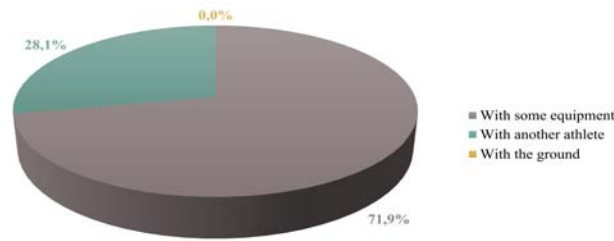


Figure 3: Distribution of the sample that suffered orofacial trauma during sports related to the type of impact

Facing the knowledge about the function of the mouthguard, 90,6% (n=106) of the athletes reported knowing their role, and 9,4% (n=11) reported not knowing his paper (Figure 4).

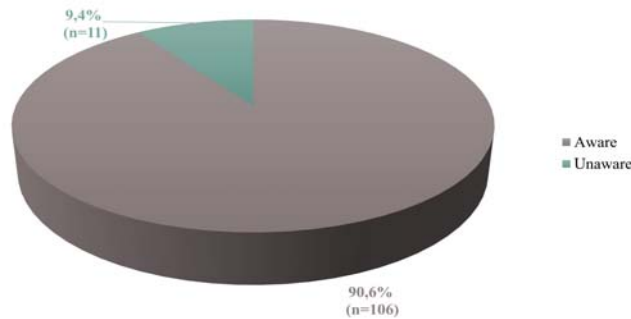


Figure 4: Total sample distribution regarding knowledge of mouthguard function

Considering the counseling on the use a mouthguard in the respective sports club, 90,6% (n=106) reported no recommendation, 6,0% (n=7) related that it exists and 3,4% (n=4) describes not knowing/not responding.

The opinion about the use of a mouthguard is variable, 60,7% (n=71) indicated that it could be used, but it is their choice, 37,6% (n=44) that should be used and 1,7% (n=2) indicated not knowing/not answering (Figure 5).

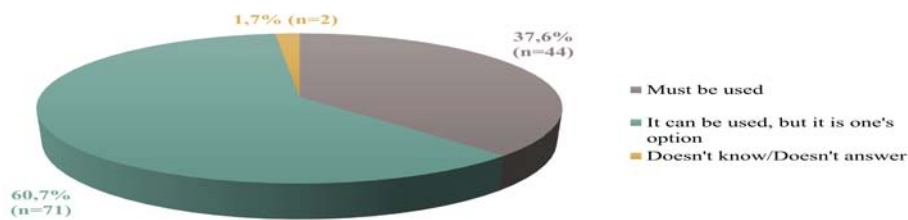


Figure 5: Sample distribution regarding opinion about the use of a mouthguard

Of all athletes, only 16,2% (n=19) used mouthguard, 52,6% (n=10) Type II and 47,4% (n=9) Type III (Figure 6 and Figure 7).

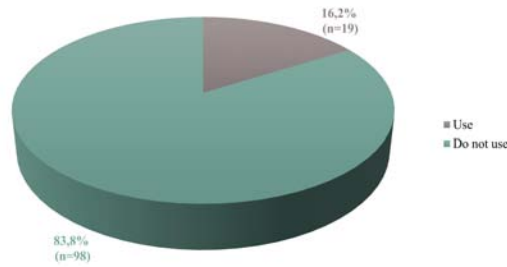


Figure 6: Total sample distribution regarding the use of a mouthguard

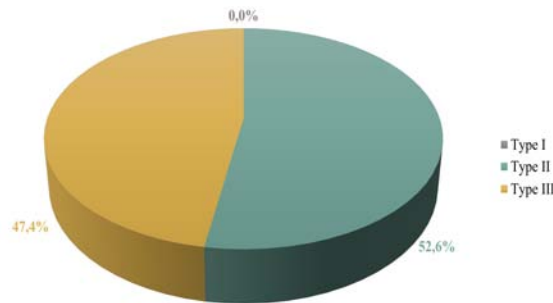


Figure 7: Distribution of the sample that uses a mouthguard about to the type used

Of these, 10,5% (n=2) of the individuals had orofacial injury in the past, but already used mouthguard, and 21,1% (n=4) of the individuals had orofacial injury in the past, but not since they started using mouthguard (Figure 8).

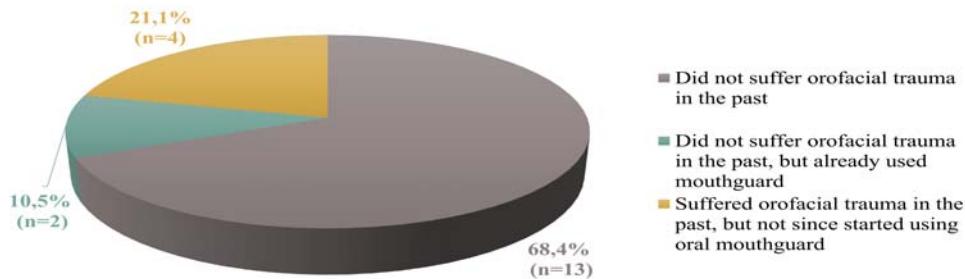


Figure 8: Distribution of the sample that currently uses mouthguard regarding the occurrence of orofacial trauma during sports practice in the past

At the time of the injury, only four athletes used a mouthguard, which 25% (n=1) used Type I, 50% (n=2) Type II and 25% (n=1) Type III (Figure 9 and Figure 10)

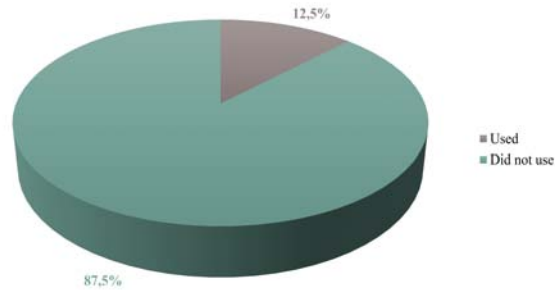


Figure 9: Distribution of the sample that suffered orofacial trauma during sports practice regarding the use of a mouthguard

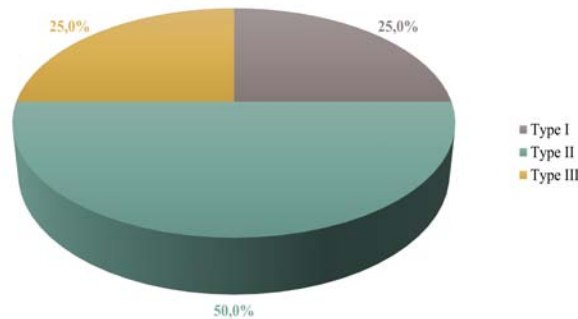


Figure 10: Distribution of the sample that used mouthguard during orofacial trauma about the type of a mouthguard used

Regarding the 52,6% (n=10) of individuals currently using Type II mouthguard, 31,6% (n=6) had no orofacial lesion in the past, 5,3% (n=1) had orofacial wound in the past, but already used mouthguard at the time and 15,8% (n=3) suffered orofacial trauma in the past, but has not suffered any more injuries since they began using mouthguard.

Relatively to the 47,3% (n=9) of individuals currently using Type III mouthguard, 36,8% (n=7) had no orofacial trauma in the past, 5,3% (n=1) had damage in the past, but already used mouthguard and 5,3% (n=1) suffered orofacial harm in the past, but not since they began using oral mouthguard.

IV. DISCUSSION

Regarding the athletes' knowledge about the mouthguard, in the study sample, it was concluded that 106 athletes (90,6%) knew the mouthguard and its function. In Portugal, the values range between 41,8% and 100%, similar to those in other countries where the values range between 51,6% and 97,3%. (Antunes et al., 2016; Collins et al., 2016; Galic et al., 2018; Goswami et al., 2017; Lopes, 2014; Matos, 2011; Pacheco, 2012; Sethi et al., 2016; Tiwari et al., 2014)

Thus, it was essential to understand the prevalence of mouthguard use. It was found in the sample of 117 young roller hockey practitioners under study, that 19 (16,2%) used a mouthguard in their sports practice. Of these 19, 10 (52,6%) used Type II mouthguard, and 9 (47,4%) used Type III mouthguard. There are several studies on this subject, both national and foreign. In Portugal, the values range between 0,8% and 18%, and abroad the values range between 4,25% and 91,3%. However, differences between studies should be taken in consideration because of the different age groups and modalities. (Al-arfaj et al., 2016; Antunes et al., 2016; Çağlar et al., 2005; Collins et al., 2015; Ferrari & Ferreira De Medeiros, 2002; Figueroa, 2018; Galic et al., 2018; Goswami et al., 2017; Hersberger et al., 2012; Kroon et al., 2016; Lahti et al., 2002; Lieger & Von Arx, 2006; Lopes, 2014; Matos, 2011; Pacheco, 2012; Sethi et al., 2016; Silva, 2013; Tiwari et al., 2014; Vucic, Drost, Wijk et al., 2016)

It was also recorded the prevalence of orofacial injuries. In the study sample of 117 athletes, 45 of them (38,5%) reported having suffered orofacial trauma at least once, and 18 of them (15,4%) suffered trauma for more than once. Of the 45 (38,5%), 32 (71,1%) suffered

a dental trauma, 4 of them (12,5%) reported using a mouthguard during the incident, 1 of which Type I (25%), 2 Type II (50%) and 1 Type III (25%). In Portugal, the values range between 3,9% and 90%, while abroad, the oscillation between 10,7% and 57,9% is smaller. (Al-arfaj et al., 2016; Çağlar et al., 2005; Collins et al., 2016; Ferrari & Ferreira De Medeiros, 2002; Figueroa, 2018; Galic et al., 2018; Goswami et al., 2017; Hersberger et al., 2012; Juneja et al., 2018; Kroon et al., 2016; Lieger & Von Arx, 2006; Lopes, 2014; Martins et al., 2013; Matos, 2011; Pacheco, 2012; Rattai & Levin, 2018; Silva, 2013; Tiwari et al., 2014; Traebert et al., 2004; Vucic et al., 2016; Zamora-Olave et al., 2018)

It was also under analysis which type of dental injury was the most prevalent, verifying that it was the dental fracture, because of the 32 athletes who suffered a dental trauma, 20 (62,5%) suffered a coronary fracture. In similar studies conducted both in Portugal and abroad, the results were in line with ours with dental fracture as the most prevalent dental injury. (Figueroa, 2018; Galic et al., 2018; Goswami et al., 2017; Lopes, 2014; Rozi et al., 2017; Santos, 2013; Spinass, Mameli, & Giannetti, 2018)

Regarding the type of tooth most affected, it was found that the maxillary incisors were the most injured tooth type in both dentitions, both deciduous with seven individuals (6%) reporting trauma to these teeth, and the definitive one in which 18 individuals (15,4%) stated the same. In studies carried out in Portugal, the results were identical to ours, with the upper incisors being the target of trauma more frequently, as in outside studies. (Juneja et al., 2018; Lahti et al., 2002; Martins et al., 2013; Rozi et al., 2017; Santos, 2013; Spinass et al., 2018)

It was found several kinds of impacts during sports that may be responsible for orofacial trauma, of the 32 of injuries sustained during sports, 23 (71,9%) occurred due to impact with some equipment, and 9 (28,1%) due to impact with another athlete, with no injury due to impact with the ground. As for other studies conducted abroad the results vary: (Lahti et al., 2002) reported that 48,9% of athletes suffered impact-related orofacial trauma with sports equipment, specifically with the stick, (Collins et al., 2016) reported that 61,3% of dental injuries were due to contact with another athlete, 31,5% to impact with some equipment, 4,1% to impact with the playing surface and 3,2% for another or unknown and (Vucic et al., 2016) reported that the main cause of orofacial trauma was impacted with the ball (57%), followed by impact with the stick (39%)

One of the objectives of this study was to verify whether there is a correlation between increased overjet and orofacial trauma. We then observed that of the 32 athletes who suffered a dental trauma, 6 (18,7%) had an overjet greater than 3mm, unlike the remaining 26 (81,3%) who had an overjet less than 3mm. Comparing with similar studies, the results obtained were quite

different. We have (Traebert et al., 2004) who reported that children with overjet larger than 5mm suffered more dental trauma (15,3%) than children with overjet up to 5mm (9,9%), (Martins et al., 2013) who mentioned that the prevalence of overjet children over 3mm was 21,6%, not being statistically significant in relation to dental trauma and also (Juneja et al., 2018) who found that among children with overjet than 3mm 13,4% suffered traumatic dental injury. Among children with an overjet smaller than 3mm, 9,6% suffered traumatic dental injury.

Finally, our main objective was to understand if the mouthguard use decreased the prevalence of orofacial trauma. Orofacial injuries occurred to only four athletes using mouthguard, which 2 (6,3%) only once, and 2 (6,3%) more than once. So, the distribution was as follows: 1 athlete (25,0%) used Type I, 2 (50,0%) used Type II, and 1 (25,0%) used Type III mouthguard. We also observed that of the 19 athletes currently using mouthguard, 6 (31,6%) had already suffered orofacial trauma during past roller hockey practice, and 2 (10,5%) had orofacial trauma in the past, but already used mouthguard and 4 (21,1%) suffered orofacial trauma in the past, but not since they began using mouthguard. Thus, the remaining 13 (68,4%) had never suffered orofacial trauma in the past. Regarding the type of mouthguard currently used by these 19 athletes, there was the following distribution: 10 athletes currently use Type II mouthguard, and 6 (31,6%) did not suffer orofacial trauma in the past, 1 (5,3%) had orofacial trauma in the past, but already used mouthguard and 3 (15,8%) had orofacial trauma in the past, but not since they started using mouthguard. Regarding the Type III mouthguard, 9 athletes currently use it, and 7 (36,8%) did not suffer orofacial trauma in the past, 1 (5,3%) had orofacial trauma in the past, but already used mouthguard and 1 (5,3%) has suffered orofacial trauma in the past, but not since they started using mouthguard. In Portugal, Silva (2013) concluded mouthguard would be effective. In studies abroad, (Lieger & Von Arx, 2006) and (Tiwari et al., 2014) had satisfactory results relatively the reduction of trauma by the mouthguard, unlike (Kroon et al., 2016) who found that 135 athletes used mouthguard at the time of injury and 131 did not, thus registering a higher occurrence of injuries among athletes using mouthguard.

V. CONCLUSION

The mouthguard should be increasingly used by all roller hockey athletes, and they must be sensitized and advised to use it to avoid orofacial injuries and whose consequences may be manifestly negative.

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Application of Aloe Vera in Dentistry

By Aniket U. Vaidya & Manisha M. Khorate

Abstract- Research since ancient times have claimed the therapeutic benefits of Aloe Vera in medical field. The role of Aloe Vera in reducing inflammation is already established. The recent research has thrown light on the immunomodulatory properties of Aloe Vera gel. The immunomodulatory property of Aloe Vera is attributed to the various polysaccharides present in the gel, primarily Acemannans. In this review the possible use and applications of Aloe Vera in dentistry is elaborated.

Keywords: aloe vera, herbal, alternative medicine.

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Application of Aloe Vera in Dentistry

Aniket U. Vaidya ^α & Manisha M. Khorate ^ο

Abstract- Research since ancient times have claimed the therapeutic benefits of Aloe Vera in medical field. The role of Aloe Vera in reducing inflammation is already established. The recent research has thrown light on the immunomodulatory properties of Aloe Vera gel. The immunomodulatory property of Aloe Vera is attributed to the various polysaccharides present in the gel, primarily Acemannans. In this review the possible use and applications of Aloe Vera in dentistry is elaborated.

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I. INTRODUCTION

Alternative medicine is described as 'Any of various systems of healing or treating disease (such as chiropractic, homeopathy or faith healing) not included in the traditional medical curricula of the United States and Britain' (Mandala, 2017). This term is loosely used for almost all the forms of medicine except Allopathy. Alternative medicine practice exists in all the cultures worldwide and terms such as traditional medicine, indigenous medicine or folk medicine etc. are used to describe such practices. Every country or region has its own traditional system of health and medical care such as the Chinese created acupuncture, the French practice magnetic healing, the Germans widely use Heilpraxis, the English use Herbalism, the Indians believe and practice Ayurveda with Siddha, the Japanese are known for Shiatsu etc (Newcomer, 2017).

In India, Ayurveda is being practiced for more than 5000 years. Ayurvedic philosophy is such that illness is a state of imbalance among the body's systems that can be detected through such diagnostic procedures as reading the pulse and observing the tongue. Nutrition counselling, massage, natural medications like various herbs, meditation and many other modalities are used to address a broad spectrum of ailments (Newcomer, 2017; Indian Board of Alternative Medicines, 2014). Presently, herbal medicines are gaining more attention due to their lesser side effects. Recently the popular interest and use of Aloe Vera (AV) gel has increased dramatically. It is one of the most widely used ingredients in healthcare and cosmetic products and is readily available all over the country. This review throws light upon the various uses and applications of AV in dentistry.

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II. ALOE VERA (AV)

AV is perennial succulent xerophyte, several species of which have been identified namely Aloe Vera, Aloe barbadensis, Aloe ferox, Aloe chinensis, Aloe indica, Aloe peyrii, etc. Amongst these *Aloe barbadensis Miller* is accepted unanimously as the correct botanical source of aloe (IARC Monographs, 2015). This plant is commercially cultivated in India, Haiti, Aruba, the United States of America, Bonaire, South Africa and Venezuela (IARC Monographs, 2015). In India, it is seen plenty in the coastal areas of Maharashtra, Gujarat and South India (Reddy et al., 2012).

Historical Perspective: In Mesopotamia, clay tablets dated 1750 B.C. showed that AV was being used in a pharmaceutical manner. In 74 A. D., a Greek physician, Discordes wrote a book titled, '*De Materia Medica*' in which he stated that AV can treat wounds, heal infections of the skin, cure chapping, decrease hair loss and eliminate haemorrhoids (Shelton R M 1991). Now a days it is used very much in cosmetic industry whereas the original commercial use of the AV plant was in the production of a latex substance called Aloin which is a yellow sap used for many years as a laxative ingredient (IARC Monographs, 2015). *The Ayurvedic Pharmacopoeia of India* recommends the use of dried juice of leaves in dysmenorrhoea and diseases of the liver (Rajeswari R et al., 2012).

AV extracts shows antimicrobial activity and hence it is used the treatment of pimples, acne and mouth ulcers. It has also been used to control bleeding, itching of piles and relief from arthritic pain. Historically the Chinese used the aloe vera juice as a mild laxative wash for piles, abscesses and scabies. In the Philippines, it was used to treat dysentery and pain in the kidneys. It was also used as aperients, anthelmintic, carminative, deobstruent, stomachic and diuretic (Kumbhar PS et al., 2015). Juice is also used in skin care medicine, amenorrhoea, dyspepsia, burns, colic, hepatopathy, splenopathy, constipation, span menorrhoea, abdominal tumors, carbuncles, sciatica, lumbago and flatulence (Kumbhar P S et al., 2015).

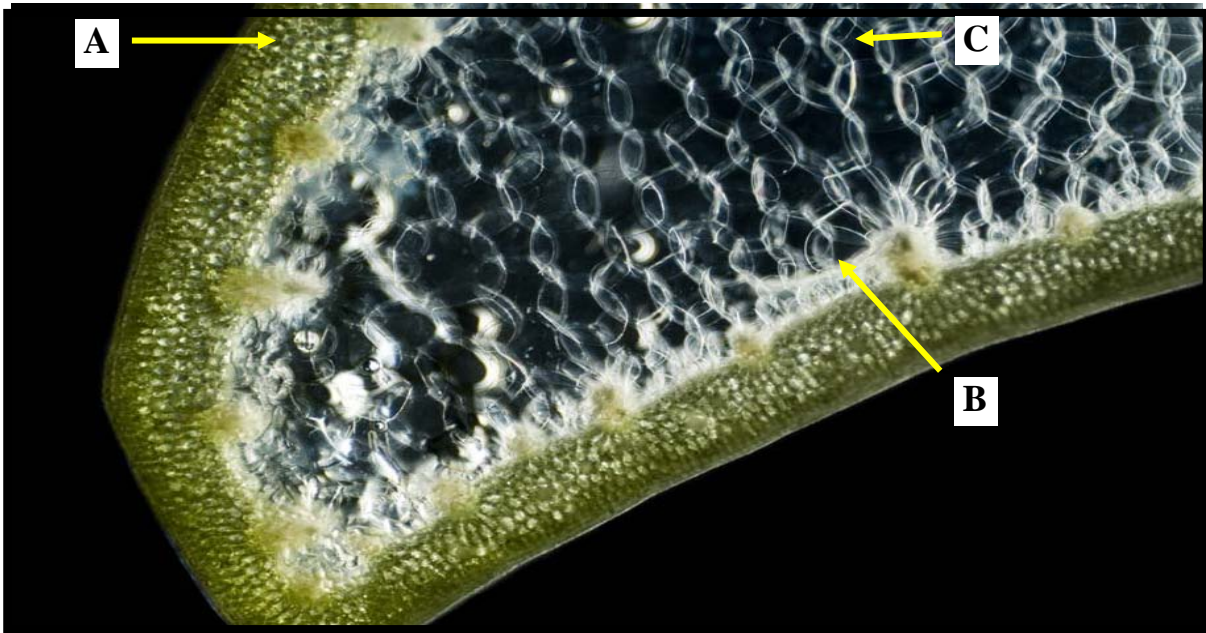


Fig. 1: The Transverse Section of the Leaf Exhibiting Three Cells Layers; A) The Protective Layer, B) Middle Layer and C) Colourless Inner Layer.

III. PARTS OF THE ALOE VERA LEAF: (FIG. 1)

a) Outer Protective Layer of the Leaf

The bitter yellow latex of pericyclic tubules in the outer layer of the leaves contain derivatives of hydroxyanthracene, anthraquinone and glycosides aloin A and B in the percentage of 15– 40%. The other active principles of Aloe include hydroxyanthrone, aloe – emodin-anthrone 10-C-glucoside and chrones (Sahu PK et al., 2013).

b) Middle Layer of the Leaf

The middle layer of the leaf also contains bitter yellow latex containing anthraquinones and glycosides. The parenchymatous tissue or pulp contains proteins, lipids, amino acids, vitamins, enzymes, inorganic compounds, small organic compounds and different polysaccharides (Sahu PK et al., 2013).

c) Inner Layer of the Leaf

The innermost layer of leaf gel contains water upto 99%, with glucomannans, amino acids, lipids,

sterols and vitamins. The other potentially active ingredients include enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids. It has numerous monosaccharide’s and polysaccharides; vitamins B1, B2, B6, and C and several inorganic ingredients, enzymes (acid and alkaline phosphatase, amylase, lactate dehydrogenase, lipase) and organic compounds (aloin, barbaloin, and emodin). The main functional component of AV is a long chain of acetylated mannose (Sahu PK et al., 2013).

IV. CHEMICAL COMPOSITION OF ALOE VERA

The AV plant contains 98 - 98.5 % water, with an average pH of 4.5, refractive index of 1.3340 - 1.3355 and specific gravity of 1.0030 - 1.0070. The remaining solid material contains over 75 different components (Kumbhar PS et al., 2015). (Table 1)

Table 1: Composition of Aloe Vera Gel (IARC Monographs, 2015; Kumbhar PS et al., 2015; Mangaiyarkarasi SP, 2015; Nair GR et al. 2016; Naroor N et al., 2014; Pol JS et al., 2016)

1. The principal ingredient	Water 98.5%
2. Other ingredients	Aloin, Barbaloin, Etheral oil, Cinnamic acid, Isobarbaloin, Emodin, Emoding lucosides of d – Arabinose, Ester of cinnamic acid, Resitannol
3. Inorganic ingredient	Calcium (30mg/dl), Potassium (13.4meq/l), Chlorine (3meq/l), Sodium (8.7meq/l), Manganese, Zinc (31mg/dl), Copper, Iron (15mg/dl), Magnesium (3.9mg/dl)
4. Amino acids	Lysine (5-6ppm), Histidine (2.8-3.3ppm), Arginine (4.5-5.5ppm), Hydroxyproline, Aspartic Acid (13-15ppm), Threonine(5-6ppm), Serine (6-7ppm), Glutamic acid (13.5-15.5ppm), Proline (8-9ppm),

	Glycine (7-8ppm), Alanine (1-1.3ppm), Valine (6.5-7ppm), Methione (1.5-2ppm), Isoleucine (3.5-4ppm), Leucine (8.5-9ppm), Tyrosine (2.8-3.3ppm), Phenylalanine (4.3-4.7ppm)
5. Monosaccharides & polysaccharides	Glucose (77.8mg/dl), Mannose, Acemannan, Uronic acid
6. Enzymes	Amylase, Carboxypeptidase, Catalase, Cycloxygenase
7. Vitamins	Vitamin A, Vitamin E, Vitamin B ₁ (6-7mg/100ml), Niacinamide(30-37mg/100ml), Vitamin B ₂ (6-7mg/100ml), Vitamin B ₆ (3-3.7mg/100ml), Vitamin C(47-61mg/100ml), Choline (9.5-11.2mg/100ml), Folic Acid (13.2ng/ml), Alpha-Tocopherol, Beta Carotene.
8. Miscellaneous	Cholesterol (8mg/dl), Triglycerides (2.4mg/dl), Steroids, beta-Sitosterol, Lignins, Uric Acid (1mg/dl), Gibberellin, Salicylic acid, Arachidonic acid

V. MEDICINAL PROPERTIES AND THERAPEUTIC POTENTIAL OF ALOE VERA

a) Burns and Wounds

AV gel has been tested for its efficacy on inflammation many times. The earliest experiments were carried out in 1930 which involved use of AV gel on skin burns. It was believed that the aloe or its components are useful for wound healing and in burns (Kumbhar PS et al., 2015).

b) Tissue Oedema

In an experiment, a swelling was produced caused by fluid accumulation in a tissue (Oedema) initiated by irritating compounds and it was used as an inflammatory model in the mouse ear or rat hind paw as subjects. Croton oil, a powerful irritant, was applied to the right ear with the left remaining as control. Inflammation was measured by weighing a tissue punch sample and it was shown to decrease after topical application of aloe gel. A subsequent trial demonstrated an even greater decrease when the gel was combined with a corticosteroid (Kumbhar P S et al., 2015).

c) Digestion

The Anthroquinones present in the outer leaf acts as a laxative and stimulates the bowels to move and helps with elimination if a person is constipated (Kumbhar P S et al., 2015; Reddy L et al., 2012).

d) Rheumatoid Arthritis

AV helps to strengthen joint flexibility and helps in the regeneration of body cells. It strengthens joint muscles which is beneficial to reduce pain and inflammation in weakened or aged joints (Kumbhar P S et al., 2015).

e) Anti-Cancer Activity

Acemannan is a molecule in AV gel. It was seen that growth of a murine sarcoma implanted in mice, showed regression after Acemannan treatment, probably due to an immune attack. Injection of mice with Acemannan inhibited the growth of murine sarcoma cells implanted subsequently and decreased mortality by about 40%. Aloe polysaccharides show evidence of anticancer effects by modifying and accentuating the

immune response during activation of macrophages. Aloe-emodin induces apoptosis in T-24 human bladder cancer cells (Joeng H Y et al., 1994; Kumbhar P S et al., 2015; Merriam E A et al., 1996).

f) Anti-Diabetic Activity

Historically, dried aloe exudates have been used in Arabia in diabetes treatment. In an experimental study, in normal mice, both Glibenclamide (10 mg/kg twice daily) and aloe (500 mg/kg twice daily) induced hypoglycaemia after 5 days. In the diabetic mice, fasting plasma glucose was significantly reduced by Glibenclamide and AV after 3 days (Ghannam N et al., 1986). Thus it can be concluded that AV contains a hypoglycaemic agent which lowers the blood glucose, the mechanism of which is yet known.

g) Antimicrobial Activity

The comparative antimicrobial activities of the gel and leaf of AV were tested against Staphylococcus Aureus, Pseudomonas Aeruginosa, Trichophyton Mentagra-phytes, Trichophyton Schoeleinii, Microsporium Canis and Candida Albicans. Ethanol was used for the extraction of the leaf after obtaining the gel from it. Antimicrobial effect was measured by the appearance of zones of inhibition. It was found that Anthraquinone inactivates various viruses such as herpes simplex, varicella zoster and influenza (Kumbhar PS et al., 2015).

In an in vitro experiment, the antimicrobial activity of an AV tooth gel (forever bright tooth gel) and commercially popular tooth pastes (Colgate Palmolive) was evaluated. It was concluded that AV tooth gel was effective than the commercially popular toothpastes in controlling all the oral organisms like Streptococcus Mutans, Candida Albicans, Lactobacillus Acidophilus, Streptococcus Mitis, Enterococcus Faecalis, Prevotella Intermedia, and Peptostreptococcus Anaerobius (George D et al., 2009).

h) Immunomodulatory Property

The immunomodulatory activity of Processed Aloe Vera gel (PAG) was evaluated *in vivo* in mice. Oral administration of PAG significantly reduced the growth of *C. Albicans* in the spleen and kidney following intravenous injection of *C. Albicans* in normal mice. PAG

administration also reduced the growth of *C. Albicans* instreptozotocin-induced diabetic mice. PAG administration did not increase ovalbumin (OVA)-specific cytotoxic T lymphocyte (CTL) generation in normal mice, but did increase it in high fat-diet induced diabetic mice. These findings provide the first clear evidence for the immunomodulatory activity of orally administered AV gel (Im S A et al., 2010).

i) *Periodontitis*

Bhat G et al. (2011) evaluated the clinical effects of subgingival application of AV gel in periodontal pockets of adult periodontitis patients after mechanical debridement. In this study, 15 subjects were evaluated for clinical parameters such as plaque index, gingival index, probing pocket depth at baseline, followed by scaling and root planning. Test site comprised of scaling procedures followed by intra-pocket placement of AV gel which was compared with the control site in which only scaling and root planning was done and clinical parameters were compared between the two site sat 1month and 3 months from baseline. Results of this study exhibited encouraging findings in clinical parameters of the role of AV gel as a drug for local delivery and it was concluded that subgingival administration of AV gel results in improvement of periodontal condition and hence AV gel can be used as a local drugdelivery system in periodontal pockets.

j) *Alveolar Osteitis (Dry Socket)*

Acemannan hydro gel is used in the treatment of dry socket. Poor MR et al. (2002) carried out a comparative study of treatment of Alveolar Osteitis with Clindamycin soaked gel foam and freeze dried pledget that contains Acemannan Hydrogel obtained from inner gel of AV. A retrospective evaluation was done of records of 587 patients (1031 sockets) treated with Clindamycin soaked gel foam and a prospective trial was performed by treating 607 patients (1064 sockets) by placing Acemannan hydrogel into the sockets immediately after extraction. The results of the study showed that 78 of 975 sites (8.0%) in the Clindamycin Gelfoam group developed Alveolar Osteitis whereas only 11 of 958 sites (1.1%) in the Acemannan Hydrogel group developed Alveolar Osteitis ($P < 0.0001$).

k) *Oral Submucous Fibrosis*

Sudarshan R et al. (2012) carried out a preliminary study to compare the efficacy of AV with antioxidants in the treatment of oral submucous fibrosis (OSMF). In this study, 20 subjects with OSMF were included. Patients are divided into two groups, Group A received 5 mg of AV gel 3 times daily for 3 months and Group B received antioxidant capsules twice daily for 3 months. At the end of the study the authors concluded that AV group showed a better treatment response in reducing the burning sensation and enhanced mouth

opening as compared to the antioxidants group. Hence it can be applied topically and effective in the treatment of OSMF.

l) *Oral Lichen Planus (OLP)*

Hayes S M (1999) was first to report the medical of use of AV for treating lichen planus. The patient was asked to drink 2 oz. of stabilized AV juice daily in addition to application of AV lip balm for lesions on the lip and 75% cutaneous AV cream. After one month there was complete healing of the oral lesions and the cutaneous lesions decreased gradually over a period of 4-5 months without any recurrence.

In a study done by Reynolds T and Dweck A C (1999), on various components of AV leaf gel they reported that polysaccharides of the inner gel of AV have a varied immunomodulatory activity. The polysaccharides from the AV gel contains multiple factors and components in the form of interleukin monotypes which directly acts on the immune system to reduce symptomatic inflammatory features which can prove effective in treatment of OLP.

A randomized controlled study was performed to compare the efficacy of AV and placebo in the topical management of OLP. The AV gel which they formulated consisted of 70% AV mucilage, sorbitol, potassium sorbate, sodium metabisulphite and hydroxyethyl-cellulose and the placebo gel contained the same ingredients except AV mucilage. The AV gel used in their studyproved to be statistically significantly more effective than placebo in inducing clinical and symptomatological improvement of OLP (Choonhakarn C et al., 2007)

Salazar Sanchez N et al. (2010) conducted a study on 64 patients to check the efficacy of topical AV in patients with OLP. In this study, patients were asked to apply 0.4 ml AV gel [70% with water, sorbitol, E-202 (potassium sorbate) and E-223 (sodium metabisulfite)] and 0.4 ml of placebo gel [water, sorbitol, E-202 (potassium sorbate) E-223 (sodium metabisulfite)] in the mouth three times a day, keeping it into the oral cavity for one minute for 12 weeks. The patients treated with AV showed a greater reduction in pain than the placebo group. After 12 weeks, 19patients in the AV group showed complete remission, whereas 9 patients presented partial remission and 3 patients failed to respond to the treatment. In ananother recent randomized controlled trial on 40 patients to test the efficacy of AV versus Triamcinolone Acetonide ointment in the treatment of OLP. The authors concluded that AV gel was safe and effective in reducing both clinical signs and burning sensations of the lesion when compared to triamcinolone acetamide (Reddy L et al., 2016).

m) *Recurrent Aphthous Stomatitis*

Bhalang K et al., 2013conducted a study to evaluate the effectiveness of Acemannan (polysaccharide) found in AV, in the treatment of oral

aphthous ulcers. 180 subjects with recurrent aphthous ulceration randomly received one of three treatments; 0.1% Triamcinolone Acetonide, 0.5% Acemannan in Carbopol 934 and just pure Carbopol 934 (control). The authors observed that the effectiveness of Acemannan in reducing ulcer size and pain was superior to that of control but inferior to that of 0.1% triamcinolone acetonide.

n) Denture Cleanser and Adhesive

Acemannan was formulated into a denture adhesive and evaluated for adhesive strength in both wet and dry conditions; the adhesive also was used to evaluate cytotoxicity to human gingival fibroblasts. An optimal formula with a high and relatively stable adhesive bond strength and minimum cytotoxicity was observed (Mangaiyarkarasi SP et al., 2015).

VI. ROUTES OF ADMINISTRATION

It is currently available in the market as an external applicator like gel, oil, face powder, face wash and toothpaste (Kumbhar PS et al., 2015; Naroor N et al., 2014).

VII. ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION OF ALOE VERA

There are no reports of studies to determine the absorption, distribution, metabolism or excretion of topically applied AV gel or whole leaf extract in experimental animals or humans (IARC Monographs, 2015). AV gel contains non-starch polysaccharides of high molecular weight (Mostly Acemannan) that are composed of sugar moieties linked by β -1, 4-glycosyl bonds. In certain ex vivo experiments, it was observed that Acemannan labelled with Fluoresceinyl Isothiocyanate (FITC) in a suspension of fresh human faeces produced metabolites for 5 days which means that Acemannan is catabolized by human intestinal bacteria (IARC Monographs, 2015; Yagi A et al., 1999).

AV latex contains the Anthrone C-Glycosides Aloin A (Barbaloin) and Aloin B (Isobarbaloin) that are linked by β -Glycosyl bonds to D-Glucopyranose. Orally ingested Anthrone C-Glycosides (i.e. aloin A and aloin B) pass unchanged through the upper gastrointestinal tract and only in the lower gastrointestinal tract these molecules are cleaved to Aloe-Emodin-9-Anthrone by human Eubacterium sp. BAR, these results were obtained in germ-free rats (IARC Monographs, 2015; Akao T et al., 1996).

VIII. ADVERSE EFFECTS OF ALOE VERA

a) Topical Use

It may cause redness, burning, stinging sensation and rarely generalized dermatitis in sensitive individuals. Allergic reactions are mostly due to Anthraquinones (Aloin and Barbaloin). It is best to apply

it to a small area first to test for possible allergic reaction (Rajeswari R et al., 2012).

b) Systemic Use

Diarrhea, red urine, abdominal cramps, hepatitis and dependency can lead to worsening of constipation. Laxative effect may sometimes cause electrolyte imbalances (Kavyashree G and Rosamma G, 2015). Verma A et al. in 2011 conducted a study with an aim to evaluate the extent of cytogenetic toxicity of the crude leaf extract of AV using the onion root tip and murine bone marrow cells. The authors stated that when onion root tip cells were exposed to the AV extract, a highly significant increase in mitotic index (MI) was observed resulting from an increase in the number of cells in prophase. However there was no increase in chromosomal abnormalities.

The mice treated with AV extract also exhibited a marked increase in the number of dividing cells (metaphases) and thus the MI of bone marrow cells increased significantly. No significant increase was observed in structural abnormalities (break and break-related abnormalities) in chromosomes but the cells with variations in chromosome number were found to increase significantly.

IX. CONTRAINDICATIONS OF ALOE VERA

1. Contraindicated in cases of known allergy to plants in the Liliaceae family (Rajeswari R et al., 2012).
2. Oral aloe is not recommended during pregnancy due to theoretical stimulation of uterine contractions and in breastfeeding mothers, it may sometime causes gastrointestinal distress in the nursing infant (Rajeswari R et al., 2012).
3. AV should not be used in patients with intestinal obstruction or stenosis, severe dehydration with electrolyte depletion (Sahu PK et al., 2013).
4. AV should not be administered to patients with inflammatory intestinal diseases, such as appendicitis, Crohn's disease, ulcerative colitis, irritable bowel syndrome or to children less than 10 years of age (Sahu PK et al., 2013).

X. CONCLUSION

The use of Aloe Vera looks very promising and must be explored further with long term studies to gain better insights. Advanced studies can be carried out to isolate individual compounds present in Aloe Vera using advanced techniques like High Pressure Liquid Chromatography. The emphasis is to find out the beneficial active constituents in Aloe Vera and their pharmacological actions in order to use them accurately in medical formulations.

Source of funding or financial interest

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Oral Health Status in India- Where do We Stand?

By Dr. L S Sreela, Dr. Anita Balan & Dr. Admaja K Nair

Abstract- Oral health is an imperative part of general health. Moreover, dental diseases are easily preventable to a huge extent. In India, dental diseases, particularly caries and periodontal disease as well as oral cancer, still continues to be widespread in the population. Only two large scale Oral Health Surveys were conducted in the country till now: (i) National Oral Health Survey & Fluoride Mapping by Dental Council of India in 2003 and (ii) Oral Health in India: Report of multi-centric oral health survey by Ministry of Health and Family Welfare in collaboration with Dental Department AIIMS in 2005. This article highlights the desperate necessity of a nationwide oral health survey for proper identification and correction of real barriers in achieving good oral health for the population.

Keywords: oral health status, national health survey, india..

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Oral Health Status in India- Where do We Stand?

Dr. L S Sreela ^α, Dr. Anita Balan ^ο & Dr. Admaja K Nair ^ρ

Abstract- Oral health is an imperative part of general health. Moreover, dental diseases are easily preventable to a huge extent. In India, dental diseases, particularly caries and periodontal disease as well as oral cancer, still continues to be widespread in the population. Only two large scale Oral Health Surveys were conducted in the country till now: (i) National Oral Health Survey & Fluoride Mapping by Dental Council of India in 2003 and (ii) Oral Health in India: Report of multi-centric oral health survey by Ministry of Health and Family Welfare in collaboration with Dental Department AIIMS in 2005. This article highlights the desperate necessity of a nationwide oral health survey for proper identification and correction of real barriers in achieving good oral health for the population.

Keywords: oral health status, national health survey, india.

Key message: In India, there is a crucial need for conducting a proper national oral health survey so that there will be evaluation of present dental needs of the whole population which will enable in framing an apt National Oral Health policy.

I. INTRODUCTION

India is one in all the world's oldest civilizations and one of the most inhabited countries in the world. According to the census reports of Indian Census 2011, the population of India is 1,210,854,977 with 623,724,248 males and 586,469,174 females. The density of population is 382 persons/sq.km. In regards to sex ratio, at present, there are 940 females on average on per 1000 males, and the child sex ratio is 914 females per 1000 males.^[1] In the Health sector, India has created monumental strides over the past decades. The average life expectancy is around 67.9 years, infant and under-five mortality rates are declining as is the rate of disease incidence.^[2] Several diseases, such as polio, guinea worm illness and tetanus, have been efficaciously eradicated. In spite of all the progress within the field of health, still, there are several communicable and noncommunicable diseases posing a constant threat to our manpower and economy.

Oral health is a vital component of general health. According to the World Health Organization fact

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sheet on oral health, "Oral health is essential to general health and quality of life. It is a state of being free from mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal (gum) disease, tooth decay, tooth loss, and other diseases and disorders that limit an individual's capacity in biting, chewing, smiling, speaking, and psychosocial wellbeing."^[3] Oral diseases are also associated with non-communicable diseases (NCD) like diabetes, cardiovascular diseases, cancers, and respiratory diseases.^[4] Majority of the population is even unaware regarding the preventive aspects of dental problems like dental caries, periodontal diseases or oral cancer.

II. GLOBAL STATUS OF ORAL HEALTH

The WHO has established a Global Oral Health Data Bank in 1967 and is continuing persistent efforts to maintain surveillance in oral health. It is essential to have regional epidemiological data based on standard protocol for facilitating international comparison. WHO encourages to conduct clinical oral health surveys in every 5-6 years in the same community or setting, and for comparison between countries, certain indicator age groups are suggested: 5-6 years, 12 years, 15 years, 35-44 years and 65 years or more (or 65-74 years). To ensure data of high validity and reliability, standard criteria including use of basic instruments and record forms for recording clinical conditions are recommended. The survey protocol focuses on dentition status, prosthetic status and needs, dental caries and dental treatment needs, developmental anomalies of teeth, periodontal disease and treatment needs, oral mucosal lesions and oral precancer or cancer.^[5]

There has been minimal progress regarding country wide oral health surveys in poorly developed or developing countries like that of Africa. In a couple of nations in Asia, customary overviews for the assessment of public health initiatives have been embraced. In Thailand, the Ministry of Health has led regular surveys every 5 years since 1984. Regardless of the numerous dental awareness programs, improper life style as well as die try habits continues to depreciate oral health, particularly in young children.^[6] The Global Burden of Disease (GBD) Study 2016 estimated that oral diseases affected half of the world's population (3.58 billion people) with dental caries in permanent teeth being the most prevalent condition assessed whereas periodontal disease, was estimated to be the 11th most prevalent

disease globally. In some Asian-Pacific countries, the incidence of oral cancer is within the top 3 of all cancers.^[7]

III. ORAL HEALTH STATUS IN INDIA

a) *The burden of oral diseases*

There is a lack of evidence regarding recent epidemiology of dental diseases affecting the whole of Indian population and the available studies are mostly loco-regional or hospital-based. The prevalence and incidence rates of major dental diseases affecting the population is mostly based on the two large scale Oral Health Surveys conducted in the past: (i) National Oral Health Survey & Fluoride Mapping by Dental Council of India in 2003^[8] and (ii) Report of multi-centric oral health survey by Ministry of Health and Family Welfare in collaboration with Dental Department AIIMS in 2005.^[9]

In India dental caries (with a prevalence rate of 50-84.7% among index age groups), appear to be the major dental problem affecting the population followed by periodontal diseases (prevalence rate 55.4- 79.4%).^[8] Balaji et al. reviewed the 2016 level India-specific dental disease data using the GBD approach and compared the same with the South Asian-level data. For every 100,000 Indians males 31,489 have prevalence of dental caries where as among females, it is 34,426 for every 100000. Nearly one third of the population suffers from dental caries that require treatment.^[7,10] The unmet need for dental caries or periodontal pathologies is not still clearly defined. The age standardized the incidence of oral cancer in India is 12.6 per 100,000 population.^[6] In spite of significant awareness campaigns and cessation programs, more than 28% of the population still uses any forms of tobacco, which are strongly associated with oral mucosal premalignant lesions and neoplasms.^[11]

b) *Barriers to improvement of oral health status in India*

In India, the dental manpower to population proportion is low. WHO estimates the ideal dentist: population ratio as about 1:7500. In India, it was 1:30000 in 2004 which improved to 1:10000 in 2014. Even though there is short a lack of dentists based on this ratio, the main reason for lack of adequate dental care to the population is the inequality in the distribution of dentists across the country.^[12] As per recent data from Dental Council of India, there are about 1.5 lakh registered dentists for a population of about 1.3 billion, out of which 72% live in villages which remain deprived of dental care.^[13] Majority of the public (government) dental health-care setups are poorly equipped, understaffed, and oral health is not a priority in budgetary allocations. Not even 20% of the rural primary healthcare centers (PHCs) around the country have a dentist or a DPH professional.^[14] The National Oral Health Survey 2003 was able to cover only 19 states/ union territories even though it was originally planned to

cover all states/ union territories in India. However, this was not achieved as some of the state authorities, such as the North Eastern States, had expressed their inability to participate due to lack of dental colleges and related resource deficiencies in their states. The general public often does not appreciate the importance of oral health, perceives it as independent from and secondary to general health. Many public health policy makers do not understand or value oral health as part of general health care, thus sidelining oral health to a policy of lower priority. Because of their economic status, under privileged or those with compromised socio economic status are unable to pay for oral health care services. Dental health insurance is of much importance in most of the developed nations. However, in India, it is still in its nascent stage with very few insurance companies providing the service.^[15]

IV. STRATEGIES TO IMPROVE ORAL HEALTH STATUS

It is clear from the above discussion that India strongly needs an oral health policy that is formulated on basis of the discussed aspects. Like in developed and few developing countries, oral health must be included in family health policies. The Government should implement new programs focusing on preventive, curative, and educational oral health, integrated into the existing system utilizing government and private health and educational infrastructure in both rural as well as urban areas with equal importance. Moreover, parental approaches toward the importance of oral hygiene, plays a key role in the preservation of children's dental health. Provision of oral health education to parents through health workers or teachers is essential to raise their awareness regarding the importance of oral health. More systematic studies focusing on public attitude toward oral health care is desirable. The identification of geographical areas or population with high requirement or inaccessibility of oral health status and dental treatment needs will be possible only by conducting a nationwide oral health survey which is the utmost need of the hour. Then only a population beneficial national oral health policy be able to be implemented successfully.

V. CONCLUSION

Oral diseases are highly prevalent in our population, and there is an urgent need to combat them. The rapid growth of dental education institutions has not helped the Indian public health system as a whole. Moreover, in a diverse country like India, there is wide variation in the distribution of dental diseases as well as treatment needs across the different states. Estimation of dental diseases and their demographic characterization would help the administration to frame successful policies for oral health. Optimizing the

provision of general and specialty dental services both under private and public sectors, is essential in the present scenario. A rough statistics point out the dentist population ratio is something like 1:5000, which is much above WHO recommendations. The system fails to provide specialty care to the needy. To conclude, there is a crucial need for conducting a systematic national oral health survey to evaluate present dental needs of population, which will facilitate in drafting a proper National Oral Health policy to extend good professional care to all.

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Copaiba Oral Hydrogel for Oral Mucositis Control - Search Note

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Abstract- Oral mucositis is a common and debilitating consequence of cancer patients undergoing antineoplastic treatment. There is no consensus on the most appropriate treatment for this condition. The best protocol to use should consider each patient's specific needs and cost-effectiveness of treatment. Copaiba oil is a low-cost herbal medicine with scientifically proven anti-inflammatory, bactericidal, antitumor, and healing action.

Keywords: *copaiba, dentistry, oncology, oral care, oral mucositis.*

GJMR-J Classification: *NLMC Code: WU 600*



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Copaiba Oral Hydrogel for Oral Mucositis Control - Search Note

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consider each patient's specific needs and cost-effectiveness of treatment. Copaiba oil is a low-cost herbal medicine with scientifically proven anti-inflammatory, bactericidal, antitumor, and healing action. So, from this reality, this unpublished study set out to create and verify the efficacy of a Copaiba-based Oral Hydrogel in the control of chemo and radio induced oral mucositis, compared with the use of Low Power Laser and the Standard Operating Protocol of Oral Care. The study was developed at a referral center for cancer treatment, where it was possible to identify the good acceptance of the three methods used. The data obtained are under analysis. This previous note intends to report a clinical case in which the positive relationship between good oral hygiene and reduction of acute complications of head and neck radiotherapy in the stomatognathic system was verified when the patient is under specialized dental care.

Keywords: copaiba, dentistry, oncology, oral care, oral mucositis.

I. INTRODUCTION

The use of medicinal plants and their extracts has been studied and used for medicinal purposes and can be said to have been born with humanity. Evidence of this use has been found in older civilizations and is considered one of the most remote practices used by humans to cure, prevent and treat diseases, serving as an important source of biologically active compounds^{1,2}.

A increased interest in the use of medicinal plants and their extracts in therapy has been observed and, under certain circumstances, constitutes an aid in primary health care and a therapeutic complement, compatible with conventional medicine². Among the medicinal plants, we can highlight the Copaiba that has been arousing the interest of the scientific community because of its therapeutic possibilities.

The copaiba is a common tree in Latin America and West Africa, which can be found in Brazil in the Southeast, Midwest, and Amazon. Such plants come to live about 400 years, reach a height between 25 and 40 meters. From the tree of copaiba, an oil-resin is extracted, of color that varies from golden yellow to brown, depending on the species. This oil-resin is used since the arrival of the Portuguese to Brazil in traditional folk and forestry medicine for various purposes, and

today is one of the most relevant Amazonian natural products marketed and exported to the United States, France, Germany and England³.

The chemical structure of the oil-resin constituents, as well as the oil obtained from the seeds, is formed by various diterpenes, sesquiterpenes and polylactic acid responsible for the analgesic, anti-inflammatory, antiseptic and healing effect⁴. According to the literature, a variety of therapeutic properties associated with the use of copaiba oil-resin are related in addition to those already mentioned, such as antiseptic, antibacterial, germicidal, antitumor, analgesic, diuretic and expectorant action³.

Oral mucositis (OM), characterized by acute inflammation of the oral mucosa seen in immune compromised patients, is a common and debilitating consequence of cancer patients undergoing high doses of chemotherapy or radiotherapy in the head and neck region. Radiation is a very effective therapy and can be an alternative to surgical treatment or a valuable adjunctive modality for surgery and chemotherapy in loco regional treatment and control of head and neck cancer. However, besides acting on tumor cells, radiation-induced tissue ionization is capable of causing very harmful damage to the stomatognathic system^{5,6}.

These patients may have decreased oral functions such as swallowing, speaking, and chewing, which impairs their quality of life. Also, oral mucositis is a gateway to opportunistic microorganisms, increasing the risk of morbidity and mortality in these patients⁵. Several disadvantages may result in increased length of

stay or hospitalization, as well as burdening treatment costs⁶.

Although therapy in the treatment of OM has shown to be supportive and palliative, there is still no consensus on the most appropriate treatment⁷⁻⁹. The use of low power laser (LPL) has a preventive and curative nature to treat injuries caused by OM, and several studies show its benefits for the treatment of these injuries⁶. Its effect is related to tissue biomodulation that results in decreased damage, repair, and analgesic effect⁵.

Despite the various therapeutic possibilities available, the best protocol to be used should consider the specific needs of each patient and the cost-benefit ratio⁸⁻¹⁰. So, from this reality, this unpublished study proposed to create and test the Copaiba-based Oral Hydrogel, after approval of the project by the Research Ethics Committee (Approval Protocol: 2.143.119) whose partial result is exemplified below in this case report.

II. CASE DESCRIPTION

The Copaiba Oral Hydrogel with bacterial cellulose membranes (MCB) ground with a magnetic stirrer in aqueous medium is a innovative product. The prepare of formulations included mixing the reagents by a mechanical stirrer. We cannot describe the reagents as the formula is under patent. The final product has the consistency of hydrogel for oral use with a production of 50g and 100g. (Figure 1)



Figure 1: Presentation of Copaiba-based Oral Hydrogel.

The study is a randomized clinical trial¹¹. The sample consisted of 60 individuals (male and female), aged at 18 years or older, diagnosed with solid or hematological malignant neoplasia and under chemotherapy and/or radiotherapy treatment, the latter in the head and neck region. The study took place at a referral center for cancer treatment. All participants signed the Informed Consent Form, thus allowing the

use, after random drawing, of one of the three methods used in this study: a) Standard Operating Protocol of Oral Care (POP -Oral); b) POP-Oral + LPL; c) POP-Oral + Copaiba Oral Hydrogel for prevention and treatment of OM. The data obtained are under analysis.

Since there are no objective, practical methods of pain measurement, this information was obtained through scales¹². Thus, the Numerical Pain Scale (0-10)

consisting of a ruler divided into eleven equal parts, successively numbered from 0 to 10, was applied. The patient had to identify the correlation between the intensity of his pain and a numerical score, where 0 corresponds to "no pain" and 10 to "maximum pain"¹³.

The World Health Organization (WHO) Classification was used to record OM, where grade 0 means no mucositis; grade I presence of erythema; grade II presence of erythema, edema, and painful ulcer; grade III severe oral ulceration; and grade IV patients require enteral or parenteral nutritional support^{6,14-16}.

Aiming to anticipate and share the findings of this unpublished study, we described in the following case report the use of POP-Oral and the topical application of Copaiba-based Oral Hydrogel used to control radio-induced grade II MO presented by the patient. The participant signed the Informed Consent Form, consenting to the disclosure of her case for academic purposes.

BMJ, female, 77 years old, illiterate, stable union, mother of 8 children, farmer. She smoked for about 70 years, ten cigarettes a day, and quit smoking in March 2018 after the diagnosis of oral cancer. She sought the Head and Neck service of the Oncology Center, presenting ulcerated lesion on the left border of the tongue for about one year. After the biopsy and histopathological analysis (AP 191438), the diagnosis concluded for moderately differentiated invasive squamous cell carcinoma (histological grade 2), pathological staging pT3, T2N0M0.

The treatment proposed by the medical team for the case was oncologic surgery, with partial glossectomy performed in April 2018, plus association with exclusive radiotherapy. BMJ protocol consisted of

6MV nominal energy photons generated with MEVATRON linear particle accelerator. Tumor Dose/Day: 200cGy and Total Tumor Dose: 7.000cGy, from 01/11/2018 to 28/12/2018. Before the beginning of radiotherapy, BMJ went to the dentistry service for oral adequacy.

The intraoral clinical examination showed bad oral hygiene, the presence of dental caries, and chronic periodontitis with marked mobility in all teeth present. Panoramic radiography of the jaws helped to complete the analysis, planning, and correct adequacy of the oral cavity. Multiple dental extractions were necessary to remove all compromised dental elements and root remnants, to minimize the side effects of radiotherapy.

It was instituted The Standard Oral Care Protocol (POP-Oral) for edentulous individuals, using a toothbrush with a small head and soft bristles, non-abrasive toothpaste, mouthwash with sodium bicarbonate solution (8/8h), mouthwash with oral nystatin solution (100,000 IU) four times a day and topical application of vitamin E (oily) 12/12h. Also, after signing the Consent and Authorization Form for the use of Copaiba Oral Hydrogel, it was used through mouthwash twice a week, once a day, throughout the radiotherapy treatment (Figure 2B).

At each consultation, the researchers applied the Numerical Pain Scale before topical application and mouthwash with the copaiba hydrogel. In the 18th session of radiotherapy, BMJ reported pain corresponding to grade 5 on the pain scale (Figure 2A) and managed to reach the absence of pain symptoms, identified as grade 0, after the third session of topical application in the ulcer and mouthwash with the Copaiba-based hydrogel (Figure 2C).



Figure 2: 2A: Clinical aspect of grade II OM presented at the 18th session of radiotherapy with pain complaint corresponding to grade 5, on the numerical pain scale. 2B: Topical application of copaiba-based oral hydrogel for later mouthwash. 2C: Clinical aspect of grade II OM in regression and control phase after the third session of topical application and mouth wash with Copaiba-based Hydrogel, with no pain symptoms, identified as grade 0, on numerical pain scale.

BMJ underwent weekly follow-up, and as can be seen in the last week of radiotherapy treatment, BMJ presented asymptomatic integral oral mucosa (Figure 3). BMJ is lucid, conscious, oriented, and very collaborative.

BMJ presented good tolerance to side effects of radiotherapy, developing throughout the treatment

episodes of xerostomia and MO grade II (WHO scale)^{6,14,15,16}. BMJ is currently undergoing an oral rehabilitation phase (removable total denture) and is being followed up by a multidisciplinary team.



Figure 3 (A and B): Clinical aspect of the oral mucosa in the last week of radiotherapy treatment, showing recovered mucosa and absence of painful symptoms.

III. DISCUSSION

Innovation in cancer treatment resulted in increased survival, as well as changes in the incidence, nature, and severity of oral complications, affecting the quality of life of these individuals. Thus, the need for dental follow-up of these patients has become increasingly recognized, in addition to establishing specific oral care for them, aiming to ensure their oral health and general well-being¹⁷.

Since a lack of a balanced diet, poor oral hygiene, the presence of fractured teeth or defective restorations, root remnants, periodontal disease or poorly adjusted prostheses can all contribute to the development of local or systemic infections, it is consensus in the literature that dental treatment before the beginning of radiotherapy, as reported in this clinical case, optimizes antineoplastic protocol avoiding interruptions during its application and improving patients' quality of life^{16,17,18}.

Corroborating the clinical case described in this article, the study developed by Brennan¹⁸, evaluated the level dental disease prior to radiotherapy. Based on dental examination, 163 (49.5%) of the participants had recommended dental treatment before radiotherapy, with dental cleaning in 137 (41.5%); recommended extractions in 116 (35.3%); a new dental restoration or replacement of a restoration in 82 (25%); pre-prosthetic surgery in 21 (6.4%); and endodontic therapy in 8 (2%).

Therefore, teeth with a poor prognosis, as in the case of advanced periodontal disease, high rate of dental caries, poor oral hygiene, need for endodontic treatment, or extensive restorations, have an indication of extraction, as described and performed in the clinical case of this article. The necessary extractions should be performed at least 14 to 21 days before the beginning of radiotherapy, in an atraumatic manner, eliminating bone spicules, with primary closure of the surgical wound and without soft tissue tension to promote rapid and complete healing¹⁶.

Oral mucositis (MO) is an acutely painful inflammation that has age, oral hygiene, renal function, genetic factors, previous antineoplastic treatment, among others, as risk factors linked to the patient; whereas, the dose of chemotherapy and radiotherapy, the chemotherapy used, and the mode of application are factors related to therapy⁵.

The routine eating behavior that was a habit in the life of these patients undergoes radical changes. Painful symptoms and difficulty in swallowing food cause extreme discomfort in patients with mucositis lesions, which makes eating difficult. Besides, the taste of food is altered as a result of head and neck radiotherapy and the use of chemotherapeutic agents and discourage eating. As the lesions evolve, they increase the sensation of pain and discomfort within the oral cavity and, as a result of these clinical manifestations, the patient opts to use pasty or liquid food. This complication may result in increased length of stay or hospitalization, as well as burdening treatment costs⁶.

The severity of mucositis lesions interferes with the systemic treatment of the individual since the degree of morbidity is high and may lead to the suspension of antineoplastic therapy. In this context, mucositis classifications are of fundamental importance, as they are instruments for assessing the success or failure of a given therapy^{11,19,20}.

The most commonly used scale to evaluate and measure oral mucositis is recommended by WHO, which classifies the OM in four degrees^{6,14,15} as described in this article, in the methodology item. This case report demonstrates an OM grade II, which is characterized by the presence of ulcers, pain, but the patient still maintains oral feeding.

As demonstrated in the report of this clinical case, it is essential to establish before the cancer treatment a clinical and surgical dental treatment plan, when these needs are diagnosed in the initial dental evaluation, aiming to promote oral health as a

fundamental role, enabling to minimize the risk of sequelae, as is the case with MO^{9,16-18}.

Also, for control of OM and other complications such as dehydration, cachexia, and infections^{7,15}, prophylactic approaches are indicated, as awareness for the improvement of oral hygiene, and adoption of POP-Oral as demonstrated in this case report²¹. With regular oral care, it is possible to reduce the influence of oral bacterial flora, pain symptoms, and bleeding related to anticancer therapy and prevent soft tissue infections, which may promote systemic sequelae. Also, maintaining oral hygiene reduces the risk of dental complications, including caries and gingivitis^{7,9,16,17}.

Still, the management of OM often recommends the use of chamomile, betamethasone, benzydamine, acetylsalicylic acid, lidocaine, polymyxin E, lozenges, tobramycin, LBP and cryotherapy, among others, as therapeutic resources¹⁵. In this study, Copaiba-based Hydrogel developed and used as a preventive and therapeutic form for OM was able to reduce the severity of mucositis degree and also oral pain, providing rapid relief, inflammation control, maintenance of mucosal integrity, better tissue repair and greater patient comfort.

In other words, POP-Oral²¹ associated with topical application and mouthwash with Copaiba-based Hydrogel showed a positive clinical and functional response, promoting excellent comfort and quality of life for patients under head and neck radiotherapy. Soon we will release data from the randomized controlled trial conducted.

Coupled with specific dental care, Copaiba Oral Hydrogel can represent a low cost and affordable possibility, and with anti-inflammatory and antimicrobial properties, corroborated in the literature, to be used as an alternative and effective method to control oral mucositis, considering the efficacy observed in this case report against radio-induced OM.

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Conflict of interest

The authors declare no conflicts of interests.

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Keywords: *Enterococcus faecalis*, root canal irrigants, doxycycline, sodium hypochlorite, *camellia sinensis* and *azadirachta indica*, and *aloe barbadensis miller*.

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Dr. Tanzeem Qureshi^α, Dr. Mayuri Singh^σ, Dr. Vartika Vashistha^ρ, Dr. Amit Singh^ω, Dr. Garvita Sahu[¥] & Dr. Rajiv Prajapati[§]

Abstract- Aims and objectives: Successful root canal treatment involves the complete elimination of pathogenic microorganisms from the root canal system. *Enterococcus faecalis* is one of them which is most commonly detected in asymptomatic persistent endodontic infections. Chemical irrigation of the root canals along with biomechanical preparation helps in the elimination of such microorganisms. Thus, the present study was done to evaluate and compare the antibacterial efficacy of Doxycycline, *Camellia Sinensis*, *Aloe barbadensis* Miller, *Azadirachta indica* and saline with Sodium hypochlorite as an intracanal irrigant against *E. faecalis*.

Material and Method: Five different intracanal irrigants were selected for this study (*ie.* Doxycycline, *Camellia Sinensis*, *Aloe barbadensis* Miller, *Azadirachta indica*, and normal saline) and their comparison was done with Sodium hypochlorite against *E. faecalis* to check their antimicrobial efficacy. The extracts were prepared, and MIC was determined. A total of 6 wells per plate were made on the agar plate with the help of cork borer. Once wells on plates are made, the testing irrigating solutions were pipetted into each well, and then the zone of inhibition was determined after 72hrs by Agar well diffusion method.

Results: The average size of zones of inhibition after 72 hours were: Doxycycline has the highest zone of inhibition (34.00 ± 0.71) followed by *Azadirachta indica* (16.00 ± 0.63), *Camellia Sinensis* (14.00 ± 0.71), Sodium hypochlorite (10.17 ± 0.98) (positive control), and least zone with *Aloe barbadensis* Miller (8.17 ± 0.75) and no zone of inhibition was observed with normal saline (negative control). Results were subjected to statistical analysis by using *Mann-Whitney U test* and *Kruskal-Wallis test*.

Conclusion: Tested herbal irrigants like *Aloe barbadensis* Miller, *Camellia Sinensis* and *Azadirachta indica* showed inhibitory zone against *E. faecalis*. Hence, these can be used as root canal irrigating solutions.

Keywords: *Enterococcus faecalis*, root canal irrigants, doxycycline, sodium hypochlorite, *camellia sinensis* and *azadirachta indica*, and *aloe barbadensis miller*.

Running Title: Qureshi et al. Antibacterial Efficacy of root canal Irrigants against *E. faecalis*.

I. INTRODUCTION

The long term success of endodontic treatment completely depends upon the debridement of non-commensal microorganisms from the root canal system.¹ However, due to complex root canal configuration, complete debridement through mechanical instrumentation alone cannot remove the entire bacterial load. Since it is a difficult task to completely eradicate microorganisms from the infected root canal system, intracanal irrigant seems necessary for eradication of infected tissues and microorganisms in addition to mechanical debridement. Moreover, low oxygen tension, less nutrient availability and enormous bacterial interactions lead to predominant colonization of facultative anaerobic species prevailing in the root canals.²

Persistence of microorganisms in apical third of the root canals leads to failure of endodontic treatment. *E. faecalis* microorganism is one of them which is the most prevalent (24-77%) gram positive and facultative anaerobe persistently found in root canal failures. Thus intracanal irrigant play's an important role in eliminating residual bacteria after biomechanical preparation and providing a favourable environment for obturation and periapical healing.³ Nowadays, various irrigants are used for root canal disinfection, which includes sodium hypochlorite, gly-oxide, EDTA (ethyl diamine tetra acetic acid), and citric acid but none of them fulfil the requirement of ideal root canal irrigant.⁴

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An ideal irrigation should be:-

- Nonirritating to periapical tissues.
- Remain stable in solution
- Be active in the presence of blood, serum and protein derivatives of tissue
- Low surface tension
- Prolonged antimicrobial effect
- Not stain tooth structure
- Do not interface with repair of perapical tissues
- Completely remove smear layer
- Disinfect the underlying dentin & its tubules
- Inexpensive
- Dissolve pulp tissue and organic debris
- Should be a good lubricant
- Be systemically non toxic, non caustic to periodontal tissue.

Therefore in the present study various plants extracts were used such as Green tea Aloe-vera, and Neem which has anti-inflammatory, anti-bacterial, anti-fungal, antimicrobial property.⁵

Green tea is harvested from leaves of the young *Camellia sinensis* tree. Its antibacterial activity is due to inhibition of the DNA gyrase bacterial enzymes, which act by binding to the ATP binding sites of the ATP subunit. Ramezani *et al.* found that green tea has antibacterial activity against *E. faecalis*.⁶

Aloe Vera (*Aloe barbadensis* Miller) is a kind of plant that is well known for its numerous biologic as well as therapeutic functions such as wound healing, hypoglycemic effects, anti inflammatory, immunomodulation features and also antimicrobial properties. It has been proven in several studies that Aloe Vera shows considerable antimicrobial activity against various species such as *Enterococcus faecalis*, *Candida albicans* and *Staphylococcus aureus*.⁵

It is well known that neem leaves (*A. indica* or AI) have antibacterial, antifungal, antiviral, antioxidant, anti-inflammatory, antipyretic, and analgesic effects without any side effect. AI has several active constituents like nimbidin, nimbin, nimbolide, gedunin, azadirachtin, mahmoodin, margolone, and cyclictrisulfide which are responsible for its antibacterial action. AI causes maximum reduction in adherence of *E. faecalis* to dentin.⁷

Although many irrigating solutions are used routinely, the search for biocompatible, non-cytotoxic and therapeutic solution is still on. Thus, the present study was done to evaluate and compare the antibacterial efficacy of Doxycycline, *Camellia Sinensis*, *Aloe barbadensis* Miller, *Azadirachta indica* and saline with Sodium hypochlorite as an intracanal irrigant against *E. faecalis*

II. MATERIALS AND METHOD

The present *in-vitro* study was done in the Department of Paedodontics and Preventive Dentistry,

Hitkarini Dental College and Hospital, Jabalpur, Madhya Pradesh, in collaboration with Daksh Laboratories and Research Center, Jabalpur which was aimed to evaluate the antibacterial efficacy of Green tea (Tetley Green Tea Regular, Tata Global Beverages Ltd., Bengaluru, India), Aloe vera (Fresh Leaves), Neem (Fresh Leaves), Doxycycline HCL (100mg) (Doxycept Cap, Concept Biosciences (P) Ltd. Baddi Distt., Solan (H.P), India.) and their comparison was done with Sodium hypochlorite (3%) (Vishal Dentocare Pvt. Ltd., Ahmedabad, Gujarat, India) against *E. faecalis* (ATCC-11700) (Himedia Laboratories Pvt. Ltd., Mumbai, India).

a) Preparation of test organism and Agar plates

The organism *E. faecalis* strain (ATCC 29212) is obtained from Himedia Laboratories. The vial containing the lyophilized bacteria was opened under complete aseptic conditions, then the test tube containing 5 - 6 ml of Brain Heart Infusion broth, 0.5 ml to 1 ml was pipette out using a sterile pipette and incorporated into vial to rehydrate the pellet. After mixing it well the suspension was transferred to the test tube containing the broth, again it is mixed properly and then several drops of this suspension was used to inoculate the Brain Heart Infusion agar plate and kept for incubation at a temperature of 37° C for 24 hours. Pure cultures were initially identified according to their staining characteristics and ability to produce catalase. By using sterile cotton buds bacteria from broth is spread over the Muller-Hinton agar plate.

b) Preparation of intracanal irrigant's and determination of MIC for each irrigating solution

i. Preparation of Green Tea Extract

Green tea extract was prepared by a Soxhlet extractor by mixing 10gm of the green tea bag in 250ml ethanol at a temperature of 40°C for 6-7 hrs under reduced pressure in a rotary evaporator. Once concentrated to 10gm volume, the extract was transferred to a pre-weighed beaker and allowed to dry completely in a bench-top oven (30°C) overnight. A final concentration of dried powder of 10gm was added to 500ml of distilled water to obtain a concentration of 0.05mg/ml.⁸

ii. Preparation of Doxycycline solution

The enteric-coated hard gelatin capsule shell seal is broken into two halves, and the powdered ingredients were mixed with 100ml of distilled water to make a homogenous aqueous solution of concentrated 1mg/ml.

iii. Preparation of Neem leaf extract

100 g of neem leaves were tied in a muslin cloth and soaked in 800 ml of distilled water in a beaker. This beaker was boiled under low flame until the extract reduced to 400 ml to obtain a 25% concentration of aqueous neem extract. After the extract cooled, it was filtered using a filter paper and stored for usage.⁹

iv. Preparation of Aloe vera extract

Aloe Vera gel is extracted from the plant's leaves. The leaves of the plants are washed with distilled water, and the surfaces of the leaves are disinfected with 70% ethyl alcohol. After cutting, the fresh pulp was collected and homogenized. 80 gram of the gel was dissolved in 20 milliliters distilled water to prepare 80% Aloe Vera solution.¹⁰

c) Determination of Minimum Inhibitory Concentration of the Prepared intracanal irrigants

100 μ l of each dilution is added to 100 μ l of Mueller Hinton broth. 5 μ l of bacterial suspension, which is adjusted to 0.5 McFarland, was added, and this mixture is then incubated at 37°C for 24 hrs. After 24 hours, the tubes were visually checked for turbidity. The lowest dilution inhibiting the growth is taken as MIC.

After determination of MIC, the following irrigants were randomly divided into six groups:

Group A= NaOCl (positive group)

Group B=Saline(negative group)

Group C=Green Tea

Group D= Doxycycline HCL

Group E=Aloe vera

Group F=Neem.

d) Placement of intracanal irrigants in Agar plates

A total of 6 wells per plate with a diameter of 6mm and depth 4mm were made equidistant from each other on a prepared agar plate with the help of cork borer. Once wells on plates were made, spreading of bacteria from the nutrient broth was done by using sterile cotton buds, and then 50 μ l of the test irrigant solution was pipetted into each well, and then zone of inhibition is determined after 72hrs for the experimental groups. The size of the resulting zones of inhibition were measured by an independent observer with sliding calipers and calculated as follows:

Size of growth inhibition zone = (diameter halo - diameter specimen) / 2.

The results were recorded in terms of average diameter of growth inhibition zone. Each specimen was tested 15 times. (Fig. 1)



Fig. 1: Zone of inhibition of different irrigating solutions after 72hrs

III. RESULTS

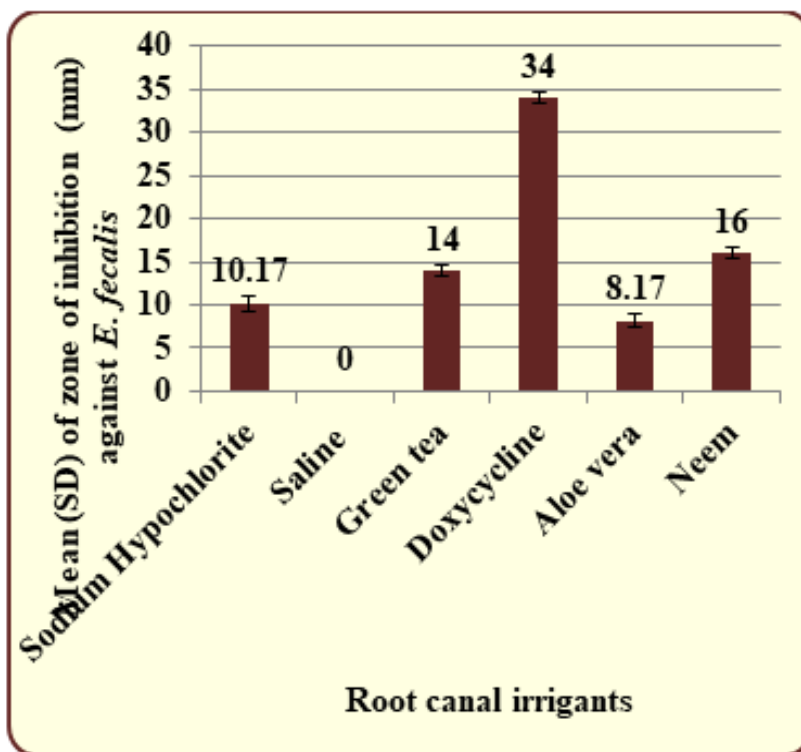
Mean, and standard deviation are calculated for all the root canal irrigants, i.e., Sodium hypochlorite (positive control), saline (negative control), Aloe vera, Neem, Green tea and Doxycycline (Experimental group). Table-1 shows Means of the Zones of inhibition showed by all the root canal irrigants against *E. faecalis*.

In Table-1 and Graph-1, there was a significant difference among the groups as the p-value was <0.001 (Kruskal-Wallis test). Doxycycline had the highest zone of inhibition (34.00 \pm 0.71). In herbal groups, Neem had

the higher zone of inhibition (16.00 \pm 0.63) followed by Green tea (14.00 \pm 0.71) and least in Aloe vera (8.17 \pm 0.75). Whereas, there was no significant difference between Aloe vera and Sodium hypochlorite with p-value = 1.000 against *E. faecalis*.

Table 1: Comparison of antibacterial efficiency of root canal irrigants against *E. faecalis*

Root canal irrigants	n	Zone of inhibition (mm) against <i>E. faecalis</i>		
		Mean ± SD	Median	Min-Max
Sodium Hypochlorite (Positive Control)	06	10.17 ± 0.98	10.50	9.00-11.00
Saline (Negative Control)	06	0.00 ± 0.00	0.00	0.00-0.00
Green tea	06	14.00 ± 0.71	14.00	13.00-15.00
Doxycycline	06	34.00 ± 0.71	34.00	33.00-35.00
Aloe vera	06	8.17 ± 0.75	8.00	7.00-9.00
Neem	06	16.00 ± 0.63	16.00	15.00-17.00
Kruskal wallis test	$\chi^2 = 34.086$, $df = 5$, $P = 0.000$ (<0.001), Sig. diff.			
Mann Whitney U test	Doxycycline > Neem > Green tea > Sodium Hypochlorite > Aloe vera > Saline			



Graph 1: Mean (SD) Zones of inhibition in mm between different irrigating agents against *E. faecalis*.

IV. DISCUSSION

A successful endodontic treatment completely depends on proper diagnosis, thorough biomechanical preparation and elimination of the infective

microorganism from the root canal system, which favours the periapical healing. Regardless of these treatment protocols, pathogenic microorganism still persists in the root canal system because of the complex root canal configurations such as ramifications,

webs and fins.⁵ The most common species is *Enterococcus faecalis* which is a gram positive, facultative anaerobic and coccoid bacterium which is persistently found in root canal failure. Thus, in order to reduce such bacteria from root canal, intracanal irrigant seems necessary.⁶

Various studies have been done to evaluate intracanal irrigant such as sodium hypochlorite, glyoxime, EDTA (ethyl diamine tetra acetic acid), and citric acid against *Enterococcus faecalis*. Despite of number of studies, none of the intracanal irrigant shows promising result in eradication of *E. faecalis*. Henceforth, the search for a potent substance with high antimicrobial potential and low cytotoxicity continues to be a relevant issue for endodontics.⁷

Doxycycline is broad-spectrum antibiotics effective against a wide range of microorganisms. It inhibits collagenase and matrix metalloproteinases of bacteria and consequently releases antigenic by products such as endotoxins. It also increases the level of interleukin-10, an anti-inflammatory cytokine. Jaju *et al.* stated that Doxycycline is the most potent anticollagenase antibiotic among commercially available tetracyclines. This is in confirmity with the present study as a significant difference in zone of inhibition was observed (34.00 ± 0.71), which suggests that doxycycline has substantial, the therapeutic effect on *E. faecalis* but it has demerits, the calcium chelates are formed within the dentinal tubules which may later cause discoloration of tooth.¹¹

Sodium hypochlorite is another most ideal irrigant, as it covers most of the requirements for endodontic irrigant than any other known compound, which has a unique capacity such as antimicrobial activity, tissue dissolving ability, lack of tooth discoloration and availability. The present study revealed that the zone of inhibition (10.17 ± 0.98) against *E. faecalis* obtained with 3% of NaOCl can also be used as an endodontic irrigating solution. However, toxicity and potential for the severe inflammatory response in the periradicular area, making its concern for the search of other alternating solutions.¹² The results of the present study were similar to the research conducted by Kini *et al.* where sodium hypochlorite and doxycycline shows effectivity in eliminating *E. faecalis*.¹³

Recently, plant extracts are introduced in dentistry. Green tea, Aloe vera and Neem has not only antimicrobial, antifungal, anti-immunomodulatory but also stimulates dental pulp proliferation, differentiation, and extracellular matrix mineralization.⁵

Rosaline *et al.* used NaOCl, EDTA, saline, *Moriandacitrifolia*, *Azadiractaindica*, and *Camellia sinensis* as a final irrigant. Significantly, less bacterial adhesion is noticed in samples treated with Neem, NaOCl, Green tea and *Moriandacitrifolia*, respectively.¹⁴

Neem (*Azadiracta indica*) consists of isoprenoid (diterpenoids, triterpenoids, and steroids) and in-

isoprenoid compounds containing proteins, amino acids, and flavonoids, etc. The antibacterial effect of neem marks it as a good root canal irrigant with the highest inhibition zone of (16.00 ± 0.63) which concur with current studies of Ghonmode *et al.*, Hegde *et al.*, Damre *et al.* between neem and NaOCl.⁹

Green tea extracts have shown to completely eradicate *E. faecalis* in 6 min. In the present study, green tea showed good antibacterial activity against *E. faecalis* compared to the control group with the minimum inhibitory zone of 14.00 ± 0.71 . Its healing properties such as antioxidant activity, anti-inflammatory, and radical scavenging properties because of catechins present in it make it appropriate for intra-canal irrigation.⁶ No report of microbial resistance of green tea is documented yet.

The antibacterial activity of Aloe Vera is related to anthraquinones and also contains a derivative of 2-ethylanthraquinone, used in the production of H_2O_2 . According to Sureshchandra *et al.* observed that chloroform extract of Aloe vera had a significant antimicrobial effect against *E. faecalis* (9mm). The results of the current study shows that Aloe vera does have an inhibitory effect on *E. faecalis*, but it is less than the antimicrobial effect of doxycycline and NaOCl and also with both herbal irrigants *i.e.*, green tea and neem with the zone of inhibition (8.17 ± 0.75). Karkare *et al.* concluded that aloe vera showed the highest zone of inhibition against *E. faecalis* similar to NaOCl.¹⁰ The low inhibitory zone of aloe vera could be because of pH of substrate and incubation period of drug which may have neutralize the effect of aloe vera.

Although Doxycycline has high inhibition zone and is considered as one of the best root canal irrigant, but research is continuing for the option of biocompatible, non-cytotoxic, therapeutic solutions.

V. CONCLUSION

Based on the findings of the present study *Aloe barbadensis Miller*, *Camellia Sinensis*, and *Azadirachta indica* showed inhibitory zone against *E. faecalis*. Hence, these can be used as an alternative root canal irrigating solutions.

Limitation of the study

The present study was an *in-vitro* study a large sample size with a wider spectrum of bacteria needs to be tested to validate its use as an intracanal irrigant.

Further research

Herbal irrigants are proven to be effective antimicrobial agents against *E. faecalis*. Results in this study proved herbs as antimicrobial, anti-inflammatory, cost-effective but further research is needed to evaluate the role of herbs against root canal microbes also.

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Conflicts of interest

There are no conflicts of interest.

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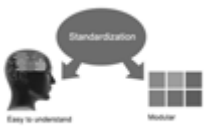
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After nomination of your institution as “Institutional Fellow” and constantly functioning successfully for one year, we can consider giving recognition to your institute to function as Regional/Zonal office on our behalf. The board can also take up the additional allied activities for betterment after our consultation.

The following entitlements are applicable to individual Fellows:

Open Association of Research Society, U.S.A (OARS) By-laws states that an individual Fellow may use the designations as applicable, or the corresponding initials. The Credentials of individual Fellow and Associate designations signify that the individual has gained knowledge of the fundamental concepts. One is magnanimous and proficient in an expertise course covering the professional code of conduct, and follows recognized standards of practice.



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- In addition to above, if one is single author, then entitled to 40% discount on publishing research paper and can get 10% discount if one is co-author or main author among group of authors.
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- • This individual has learned the basic methods of applying those concepts and techniques to common challenging situations. This individual has further demonstrated an in-depth understanding of the application of suitable techniques to a particular area of research practice.

Note :

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- In future, if the board feels the necessity to change any board member, the same can be done with the consent of the chairperson along with anyone board member without our approval.
- In case, the chairperson needs to be replaced then consent of 2/3rd board members are required and they are also required to jointly pass the resolution copy of which should be sent to us. In such case, it will be compulsory to obtain our approval before replacement.
- In case of “Difference of Opinion [if any]” among the Board members, our decision will be final and binding to everyone.

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We accept the manuscript submissions in any standard (generic) format.

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Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.



Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
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- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

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The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

- a) A title which should be relevant to the theme of the paper.
- b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
- c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
- d) An introduction, giving fundamental background objectives.
- e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
- f) Results which should be presented concisely by well-designed tables and figures.
- g) Suitable statistical data should also be given.
- h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

- i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
- j) There should be brief acknowledgments.
- k) There ought to be references in the conventional format. Global Journals recommends APA format.

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The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

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The full postal address of any related author(s) must be specified.

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The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

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A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

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Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

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Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

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Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

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Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.



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1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

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Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

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15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

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19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.



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INFORMAL GUIDELINES OF RESEARCH PAPER WRITING

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- Please note the criteria peer reviewers will use for grading the final paper.

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One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

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This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

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To make a paper clear: Adhere to recommended page limits.



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- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
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- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
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An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.



The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

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This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.



Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
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- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
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- Never confuse figures with tables—there is a difference.

Approach:

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Put figures and tables, appropriately numbered, in order at the end of the report.

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Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."



Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

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- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

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<i>References</i>	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



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