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Effectiveness of *Commiphora wightii* Gel in Treatment of Alveolar Osteitis

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ABSTRACT

Alveolar osteitis (AO) is a painful condition occurring in 0.5–68% of the dental patients following tooth extraction posing a challenge for clinicians due to lack of a definitive treatment. *Commiphora wightii* is a naturally occurring oleo-gum resin that has been shown to exhibit antibacterial and wound healing potential. This study was designed to formulate a 0.5%w/w *Commiphora wightii*-based mucoadhesive gel and investigate its role in bone healing in an animal model of AO. After obtaining ethical approval, *Commiphora wightii* based mucoadhesive gel was formulated. Eighteen male Wistar rats were randomly and equally divided into three groups comprising of nine subgroups (n = 2); C1, C2 and C3 (control group), A1, A2 and A3 (experimental group A) and B1, B2 and B3 (experimental group B). Tooth extraction in 1st maxillary right molar region was done in all groups followed by creation of AO in experimental group A and B only. *Commiphora wightii* based-gel was applied once only on 3rd day in extraction socket of experimental group B while no application of gel was performed in control group and experimental group A. All animals were sacrificed on 4th, 7th and 14th day after extraction of the tooth and creation of alveolar osteitis model. At the end of the experimental period, the animals were euthanized and the part of maxilla containing the extraction socket was surgically removed and all the samples processed for histological analysis. Initially, Control group had a higher healing score on 4th day as compared to both experimental groups. However, the experimental group B had the highest healing score on the 7th and 14th day as compared to both other groups, while experimental group A had the lowest score on all three days of sacrifice. Hence it was concluded that *Commiphora wightii*-based mucoadhesive gel improved healing in the extraction socket of experimentally induced model of alveolar osteitis rats belonging to group B.

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TZ did collection of experimental data and did manuscript writing. NN provided intellectual input through critical analysis of the data. SG conceived the study, did manuscript writing, provided intellectual input through critical analysis and did final approval of the manuscript.

Key words
Alveolar osteitis, *Commiphora wightii*, Mucoadhesive gel, Healing, Rat model

INTRODUCTION

Alveolar osteitis (AO), commonly known as dry socket is described as postoperative pain inside and around the extraction site, which increases in severity at any time between the first and third day after the extraction accompanied by a partial or total disintegration of blood clot within the alveolar socket with or without halitosis (Chow and Wang, 2020). The incidence of dry socket varies between 0.5 to 37.5 % in both surgical and non-surgical tooth extraction in humans (Jaafar and Nor, 2000). In Pakistan, the incidence of AO has been reported to be about 5.12% (Qureshi *et al*., 2018). Previous studies have shown that extraction of a tooth can have a significant effect on the quality of life in humans (Geissler and Bates, 1984; Ibikunle *et al*., 2016). The molar tooth region in both rats and humans consists of loose connective tissue containing blood and lymph vessels hence structural and functional changes are expected following extraction of the tooth which presents as pain, swelling and trismus. These sequalae have a significant effect on the quality of life in rats and humans alike in the immediate post operative period till the 3rd day (Kato *et al*., 1997; Ibikunle *et al*., 2016). Current management of AO requires multiple visits to the dentist requiring irrigation of the extraction socket followed by the placement of an obtundent dressing. This dressing contains eugenol and eucalyptus oil which provides a temporary relief in pain, however, its application causes delayed wound healing (Eshghpour *et al*., 2018). Another treatment involves copious irrigation of the extraction socket with normal saline followed by curettage which debrides all the extraction socket, removing the inflammatory cells as well as any cellular debris which may have accumulated withing the socket

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since the extraction of the tooth and allows the socket to fill up again with the fresh blood. This treatment improves the healing over time, however, both treatment modalities increases pain and discomfort and financial burden for the patient (Chow and Wang, 2020).

AO presents with pain and empty alveolus in almost all the patients (Mahmoud *et al*., 2019). In normal healing following extraction of the tooth a series of events take place beginning with the formation and maturation of blood clot. The blood clot is infiltrated by fibroblasts which replaces the blood clot with the granulation tissue that acts as a provisional matrix for bone tissue formation (Kuboki *et al*., 1988). On gross examination, the alveolar sockets of such rats present a similar picture as observed in human patients with an empty alveolar socket surrounded by necrotic bone (Rodrigues *et al*., 2011).

Commiphora is a woody shrub or a small tree which belongs to the Burseraceae family with 17 to 18 genera and about 540 species are distributed around the world. In Pakistan and in India, *Commiphora* species include *C myrrha*, *C. stocksiana, C. wightii*, *C. berryi* and *C. agallocha* (Bhardwaj and Alia, 2019). *C. wightii*, also known as Guggul in Urdu language, is an indigenous medicinal plant of South Asia distributed in the arid areas of Pakistan, India and Bangladesh. In Pakistan it is found in the arid hills of Jamshoro, Kirthar mountain range, Thar Desert and in some parts of Punjab and Balochistan (Kulloli and Kumar, 2013). Oleogum resin is the active component of *C. wightii* which is secreted by specialized cells or ducts from the bark of the stem. This oleogum is composed of volatile oils, steroids, guggltetrols, sugars, lignans and amino acids (Bhardwaj and Alia, 2019). Previous studies have suggested the role of *C. wightii's* oleogum resin as an effective antimicrobial and antifungal agent (Fraternale *et al*., 2011; Galehdari *et al*., 2016).

In humans, *C. wightii* has shown to improve wound healing in skin diseases as well as improved healing in bone fractures and symptoms of inflammatory condition such as arthritis (Kumar *et al*., 2018). *C. wightii* possesses significant antibacterial activity against gram-positive bacteria and moderate activity against gram-negative bacteria in invitro culture studies (Goyal *et al*., 2010; Singh and Singh, 2013). The antimicrobial property is attributed to the presence of compounds such as sesquiterpene and *T. cadinol*, which are the terpenoidal constituents of the volatile oils present in the gum resin (Bhardwaj and Alia, 2019). These compounds interact and disrupt the cell membrane of the bacteria leading to bacteriolysis (Goyal *et al*., 2010). Anti-inflammatory properties of *C. wightii* have been attributed to the inhibition of interferon gamma (IFNG), interleukin-2 (IL-2), tumor necrosis factor (TNF), interleukin-1β (IL1-β) and nitric oxide (NO) levels in cell culture of mouse macrophage cell line (Manjula *et al*., 2006). *Commiphora* species such as molmol and erythrea have shown a reduction of inflammatory markers at the site of injury indicating a healing process. This anti-inflammatory property was seen when the above-mentioned species of *Commiphora* were applied in crude or gel form on ear oedema in mice and superficial skin wounds in diabetic rats (Fraternale *et al*., 2011; Galehdari *et al*., 2016).

Bioadhesion is a term used to describe binding of two materials, one of which is biologic in nature, held together strongly at the interface. This bonding is non-toxic, biodegradable and is able to function under wet conditions (Wunderer *et al*., 2019). These properties of bioadhesive molecules, which are present in plant lectins becomes important in medical use as the biological surface of most interest for the administration of drugs is the mucosal surface, which is inherently wet (Woodley, 2001). The mucosal surface is covered by a layer of mucous, which is a complex hydrated gel-like material and any molecule binding to the mucosal surface binds to the mucous layer first. Hence the term mucoadhesion becomes more appropriate to describe binding of any material to the mucosal surface. *C. wightii* gum resin is not naturally a mucoadhesive material, however we aimed at formulating its mucoadhesive gel so it would remain at the site of tooth extraction for a longer duration and impart its antibacterial and anti-inflammatory effects towards healing of bone in AO model in rats. To our knowledge, this is the first study utilizing *C. wightii* based mucoadhesive gel for assessing its effectiveness through histological assessment of bone healing in experimental models of alveolar osteitis in rats.

MATERIALS AND METHODS

Preparation of mucoadhesive gel

Ethanol extract of 500 g air dried gum resin of *C. wightii* was obtained through rotary evaporator. Mucoadhesive gel at 0.5% w/w was prepared using Hydroxypropyl methylcellulose (HPMC) as a polymer. Propylene glycol was heated followed by addition of methyl paraben and propyl paraben, hand mixed, and cooled. Prepared extract was added in the cooled mixture until it was completely dissolved. HPMC was dissolved in distilled water. All the ingredients were mixed to obtain a homogenous gel (Mansour *et al*., 2014).

Preparation of animal and induction of alveolar osteitis

Eighteen adult healthy male Wistar rats weighing between 160–250 g were included in the study. Female rats and rats previously used in a study were excluded. All the rats were kept in clean and disinfected cages. Regular

light and dark cycles of 12/12 h were maintained. The humidity level was maintained at 35% with temperature in the range of 24 to 26 °C. Rats were given a solid chow diet prior to the experimental phase. They also receive sterile water *ad libitum* throughout the course of the study (Bano *et al*., 2019).

Eighteen Wistar rats were randomly and equally divided into nine groups (n=2). One control group having tooth extraction socket only divided into three sub groups as C1, C2 and C3, an experimental group A having tooth extraction socket with AO and without gel treatment divided into three subgroups as A1, A2 and A3 and experimental group B having tooth extraction socket with AO and with gel treatment divided into three subgroups as B1, B2 and B3. All rats were anesthetized by a combination of ketamine hydrochloride (25 mg/kg body weight) and xylazine hydrochloride (10 mg/kg body weight) injected intramuscularly followed by submucosal injection of 0.5 % lidocaine hydrochloride (7mg/kg body weight) at maxillary right 1st molar and extraction of this tooth was done (Kielty, 2011; Rodrigues *et al*., 2011). Once the tooth had been extracted in all the animals groups, the rats in the control group did not receive any intervention and were left to heal normally (Supplementary Fig. 1A). However, AO was created in the rats of experimental group A and experimental group B. This was done by placing epinephrine in the extraction socket followed by thioglycolate reduction medium to favor anaerobic growth on the day of tooth extraction. *C. wightii* based mucoadhesive gel was place on the third day of tooth extraction in experimental group B only. All animals were also given post-operative analgesic mixed in drinking water for 5 days (Fleischmann *et al*., 2017).

Rats of both the experimental groups were observed for signs of alveolar osteitis by daily monitoring of appearance of general malaise and necrotic appearance of the alveolar socket (Supplementary Fig. 1B). *C. wightii* based mucoadhesive gel was placed inside the alveolar sockets only once on the 3rd day of the extraction in experimental group B while no gel was placed in the experimental group A (Supplementary Fig. 1C,D). Body weights of all animals in all the groups were recorded once before the extraction of tooth and once on the day of sacrifice (Sharp and Villano, 2012). The rats in all the groups were sacrificed on 4th, 7th and 14th day respectively by an over dosage of chloroform.

Collection of histological samples

The maxilla was drilled to remove the bone containing the experimental section and placed in 10 % neutral buffered formalin overnight at 4°C. The soft tissue of the samples was removed and bony fragments were decalcified in 14% EDTA solution. After satisfactory softening of the bone, the tissues were embedded in paraffin wax to form blocks. Longitudinal sections of 7 µm in thickness were obtained and stained with hematoxylin and eosin for evaluation of histological healing score under the light microscope (Suvarna *et al*., 2012). The histological variables that were observed to assess the healing score included amount of osteoid deposition with an attempt to form bone, presence or absence of osteoblasts, osteocytes, osteoclasts, osteoid, inflammation, granulation tissue, presence or absence of red blood cells and presence or absence of small thin blood vessels. Variables such as, amount of bone formation, osteoblasts, osteocyte, osteoclast, osteoid and woven bone deposition were scored from 0-3, where 0 represents absence of the histological variable, 1 if variable is present peripherally, 2 if variable is present centrally and 3 if variable is present centrally and peripherally. Inflammation was scored from 0-3 in which 0 indicated severe inflammation, 1 for moderate inflammation, 2 for mild inflammation and 3 for absence of inflammation. Granulation tissue was also scored from 0-3. Where 0 indicated absence of granulation tissue, 1 if granulation tissue was scanty, 2 if granulation tissue was moderately present and 3 if granulation tissue appeared healed. The blood cells and vessels were scored 0-3. Where 0 represented absence of blood clot, 1 if blood clot was present, 2 if thin small blood vessels were present and 3 if large blood vessels were present. The higher cumulative score indicated improved bone healing (Lucaciu *et al*., 2015).

RESULTS

Change in body weight

Body weights of all the animals in each group was calculated, before the extraction of tooth and at the day of the sacrifice. The average weight of all the animals was 198.2 g. In controls, there was an initial decrease, at day 4, in the body weight of the animals after the extraction of the tooth recorded at -5.8 ±2.82 g. However, at days 7 and 14 there was a gradual increase in the body weight recorded at 9.15 ±3.5 g and 14.9 ±2.61 g, respectively. Increase in body weight over time indicates a normal healing process with overall wellbeing of the animal as evident by increase in body weight.

In experimental group A, there was a decrease in body weight across the three days of sacrifice which was recorded at -3.55±2.47 g, -5±11.4 g and -13.9±14.8 g at days 4, 7 and 14, respectively. In experimental group B, the mean change was recorded at -0.2±1.2 g, 10.45±8.83 g and 24.85±3.6 g at days 4, 7 and 14, respectively (Table I). The decrease in body weight in experimental group A,

Table I. Change in body weight of animal at the start of the experiment and then at the day of sacrifice.

Day of sacrifice	Control			Experimental A			Experimental B			P value
	Initial wt (g)	Final wt (g)	Mean change in wt (g) ± SEM	Initial wt (g)	Final wt (g)	Mean change in wt (g) ± SEM	Initial wt (g)	Final wt (g)	Mean change in wt (g) ± SEM	
4(n=2)	194.75	188.95	-5.8 ±2.82	191.75	188.2	-3.55 ±2.47	192.75	192.55	-0.2 ±1.2	< 0.001*
7(n=2)	193.95	203.1	9.15 ±3.2	200.1	195.1	-5 ±11.4	202.45	212.9	10.45 ±8.83	< 0.001*
14(n=2)	201.55	216.3	14.9 ±2.61	208.65	194.8	-13.9 ±14.8	201.65	226.5	24.85 ±3.6	< 0.001*

In the control group, body weight increased by day 14. In experimental Group A, weight decreased by day 7, and increased by day 14. In experimental Group B, weight increased by day 14. Initial wt: weight at time of intervention, Final wt: weight at the time of sacrifice, Change in wt: initial minus final weight, mean change in wt: mean change in body weight on each day, SEM: standard error of mean. *p-value ≤ 0.05 is statistically significant

which does not improve over time, indicated a delay in healing process due to the creation of AO model in this group. The initial decrease in body weight followed by a gradual increase in experimental group B, the results of which are comparable to control group. This increase in body weight over time in experimental group B indicated a normal healing process.

Healing in tooth extraction socket

The histological analysis of C 1 in Figure 1 showed that the extraction site was mostly occupied by blood clot which comprised of erythrocytes and platelets trapped in a network of fibrin and inflammatory cells containing neutrophils (Fig. 1A). Granulation tissue, osteoblasts, osteoclasts, osteocytes and osteoid deposition was not present in these samples. The total healing score of C1 was calculated to be 3. In C 2 there was a reduction in size of the blood clot which was replaced with moderate amount of granulation tissue (Fig. 1D). Granulation tissue comprised of a highly vascularized tissue with an inflammatory cell infiltrate comprising predominantly of macrophages and multinucleated cells. Osteoblasts, osteocytes and evidence of osteoid deposition was not present in these samples. The total healing score of C 2 was calculated to be 13. In C 3 the extraction site mostly contained healed granulation tissue which consisted of large blood vessels and mesenchymal cells. Few osteoclasts were also found dispersed within the granulation tissue (Fig. 1G). Control group showed a cumulative healing score of 33 across the three days of sacrifice.

In A1, there was no sign of blood clot within the extraction socket, however, areas of coagulative necrosis were present as well as large numbers of neutrophils (Fig. 1B). Only inflammation was prominent in this group of animals, all other variables were absent from these samples. The total healing score of A 1 was calculated to be 0. In A 2, scanty granulation tissue was seen which occupied a small area of the extraction site containing small thin newly forming blood vessels along with a few macrophages (Fig. 1E). Osteoid deposition was also observed in a few of the samples, though limited peripherally. These areas contained osteoblasts with peripheral rim of osteoclasts, indicating an attempt for bone formation. No osteocytes were seen in these samples. The total healing score of A 2 showed a little improvement and was calculated to be 13. In A 3 some samples showed large colonies of bacteria occupying the superficial parts of the sockets. No osteoid deposition was seen in these samples. Osteoblasts and osteocytes were also absent from theses samples. Most of the extraction site was occupied with inflammation containing macrophages and giant cells (Fig. 1H). Granulation tissue was present in half of the samples in which it appeared scanty mostly because of the extraction site being occupied by inflammatory cells. Blood vessels were also present, which appeared small and thin, containing mostly red blood cells. The total healing score of A3 was calculated to be 11. The experimental group A showed the lowest healing score of 22 across the three days of sacrifice.

On histological evaluation, the group B 1 exhibited the extraction socket which was filled with blood clot containing erythrocytes and platelets trapped in a network of fibrin (Fig. 1C). Inflammation was also seen in the superficial part of the extraction site containing neutrophils. No other variable was seen in samples of this group. The total healing score of B 1 was calculated to be 2. In B 2 the extraction site was mostly occupied by healed granulation tissue, which was richly vascular, containing large blood vessel with erythrocytes. Some residual inflammatory cells like giant cells were also present. B 2 was marked by the presence of osteoclasts which rimmed the extraction site. This rimming of osteoclasts was seen only in B 2 (Fig. 1I). Osteoblasts were also present along the regions of bone deposition. The total healing score of B 2 was calculated to be 21. In B 3 the extraction site was completely occupied by osteoid (Fig. 1F). Samples of this group showed marked osteoid deposition both centrally and peripherally. There was an evidence of woven bone formation which was accompanied by the presence of osteoblasts and osteocytes laying down the matrix. Osteocytes were exclusively seen

only in B 3 group. Healed granulation tissue was observed in these samples and inflammation was also noted to be only mildly present. The total healing score of B 3 was calculated to be 31. Experimental group B scored the highest with the cumulative score of 54 across all the three days of sacrifice. The individual score of each histological parameter is tabulated in Table II.

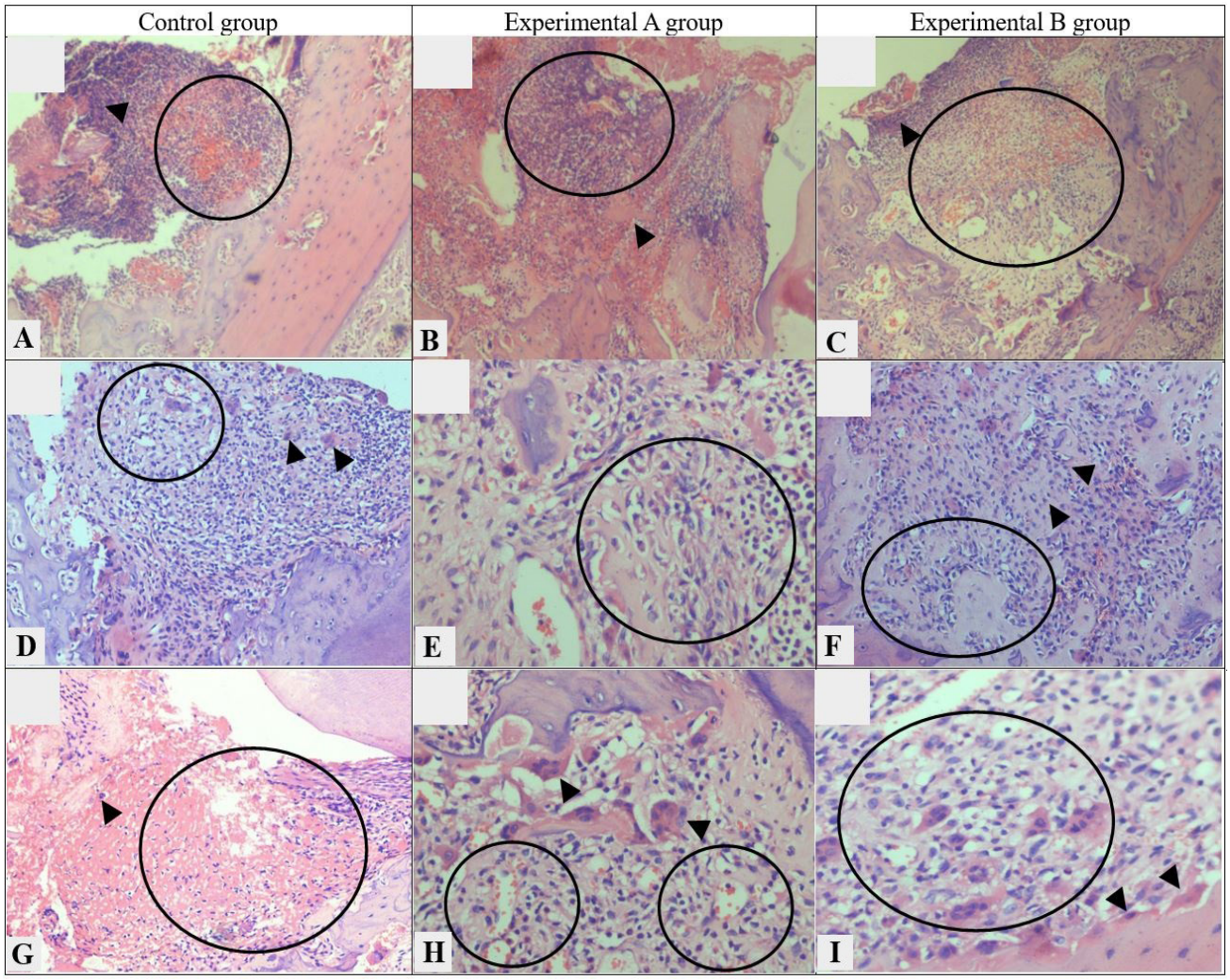

Fig. 1. Effect of *Commiphora wightii* gel on histological structure of tooth extraction socket in control, experimental A and experimental B group. **A,** Control group at day 4 (C1) with erythrocytes in the apical part of the socket (encircled) and the coronal part containing neutrophils (arrow heads) (10X magnification). **B,** Alveolar osteitis model in experimental A group at day 4 (A1) extraction site filled with mostly inflammatory infiltrate (encircled) no blood cells are visible, and areas of coagulative necrosis identified (arrow heads) (10X magnification). **C,** Experimental B group at day 4 (B1) after the placement of mucoadhesive gel in alveolar osteitis model, blood clot (encircled) and neutrophilic infiltrate (arrow head) are apparent (10X magnification). **D,** Control group at day 7 (C2) mostly filled with granulation tissue containing a few blood vessels (encircled) as well as a few osteoclasts (arrow head) (20X magnification). **E,** Experimental A group at day 7 (A2) containing scanty granulation tissue with blood vessels and neutrophilic infiltrate (encircled) (20X magnification). **F,** Experimental B group at day 14 (B3) group findings reveal osteoid at the periphery (encircled) and fragments of woven bone with osteocytes in the central region (arrow head) (20X magnification). **G,** Control group at day 14 (C3) mostly filled with granulation tissue (encircled) as well as a few osteoclast (arrow head) (20X magnification). **H,** Experimental A group at day 14 (A3) contains granulation tissue occupying the extraction site with thin blood vessels (encircled) macrophages and giant cells (arrow head) (40X magnification). **I,** Experimental B group at day 7 (B2) mostly filled with healing granulation tissue (encircled) containing a few osteoclast-like giant cells and rimming of osteoclasts at the periphery (arrow head) (40X magnification).

Table II. Histological evaluation record showing individual histological parameter's healing score for each animal. A higher score indicating a better healing as compared to a lower score.

Parameter	Group score for the individual histological parameter																	
	Control group						Experimental group A						Experimental group B					
	C 1		C 2		C 3		A 1		A 2		A 3		B 1		B 2		B 3	
Bone formation	1	0	0	0	0	1	0	0	0	2	0	0	0	0	0	1	3	1
Osteoblast	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	1	2	1
Osteocyte	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Osteoclast	0	0	0	2	0	1	0	0	0	2	1	0	0	0	1	2	2	1
Inflammation	0	0	0	1	2	2	0	0	0	0	2	1	0	0	2	1	2	2
Granulation tissue	0	0	2	2	2	3	0	0	1	1	1	0	0	0	3	3	3	2
Blood clot and vessels	1	1	3	3	3	2	0	0	2	2	3	2	1	1	3	3	3	2
Osteoid	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	1
Woven bone deposition	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
Total	2	1	5	8	7	10	0	0	3	10	8	3	1	1	9	12	19	12
	3		13		17		0		13		11		2		21		31	

DISCUSSION

Alveolar osteitis is an extremely distressing condition which can lead to increased intensity of pain at the site of extraction. This pain can be considerably debilitating which can lead to decreased intake of food and consequently decrease in the body weights as the symptoms can last up to a week if left untreated. Even after a week of tooth extraction if no therapeutic intervention is done there are chances of developing a superimposed infection in the tooth socket and necrosis of the underlying bone as the wound is exposed to the oral microbes that can easily colonize the site of the wound (Javed *et al*., 2017)

C. wightii (Francis *et al*., 2004) is an indigenous medicinal plant of Pakistan which has been shown to possess antibacterial and anti-inflammatory properties due to the presence of sesquiterpenes and *T. cadinol*. In this study 3 groups were made in the adult male Wistar rats. The first group was the control group in which only the 1st maxillary right molar was extracted. Second group was the experimental group A in which the alveolar osteitis model was created following extraction of the tooth and the third group was the experimental group B in which *C. wightii* based mucoadhesive gel was placed in the tooth extraction socket following the creation of the alveolar osteitis model. All the animals in these three groups were sacrificed at day 4, 7 and 14.

Changes in dietary intake

In the control group, the intake of food by the animals decreased initially following extraction of the tooth. However, this intake of food gradually improved as the extraction socket healed and found to be highest on the 14th day of sacrifice. Previous studies have shown that the healthy adult male Wistar rat consumes an average of 23 g of food per day (Pickering and Pickering, 1984). Although not directly related. however, a study on another rodent species i.e. rabbits also showed that the average daily intake of food reduces following painful condition such as extraction of the tooth (Cheng *et al*., 2015), which is in line with the results obtained in our study. Some previous studies have shown that extraction of a tooth can have a significant effect on the quality of life in humans (Geissler and Bates, 1984; Ibikunle *et al*., 2016). The molar tooth region in both rats and humans consists of loose connective tissue containing blood and lymph vessels hence structural and functional changes are expected following extraction of the tooth which presents as pain, swelling and trismus. These sequalae have a significant effect on the quality of life in rats and humans alike in the immediate post operative period till the 3rd day (Kato *et al*., 1997; Ibikunle *et al*., 2016).

Oral infections can cause a significant decrease in dietary intake due to pain and inflammation in the oropharyngeal mucosa. This inflammation can extend to involve the oropharyngeal musculature and can lead to trismus which further decreases the chewing ability in an individual and eventually lead to a decrease in food intake. Same is the sequalae which is apparent in patients with alveolar osteitis when not catered properly and in a timely manner. In experimental group A, inflammation of the orofacial area was evident on gross inspection as well

as microscopically where large bacterial colonies were present overlying the untreated alveolar osteitis socket. Although this group showed a reduction in dietary intake similar to the control group, however, unlike the control group the dietary intake did not improve after the 7th post operative day. We assume that the reduction in dietary intake was due to the experimentally created condition of alveolar osteitis as well as the superimposed infection in experimental group A rats.

According to previous studies, the pain associated with alveolar osteitis is severe throbbing which can last up to 3 weeks if left untreated (Sweet and Butler, 1979; Javed *et al*., 2017). Although, with topical treatment of obtundent dressings like alveogyl® the pain associated with AO improves but does not improve healing rather, it also extends the healing time. In our experimental group B, dietary intake improved as compared to those of the experimental group A and was found to be similar to that of control group. It is speculated that this was possible because of application of *C. wightii* based mucoadhesive gel, in experimental group B, within the extraction sockets after creation of the alveolar osteitis model. This may have led to reduced pain and improved healing leading towards normal dietary intake of the animals as compared to control group and experimental group A.

A study conducted by Francis *et al*. (2004) has shown that *C. wightii* has significant anti-inflammatory activity where it inhibits the cyclooxygenase 1 and 2 (COX-1 and COX-2) enzymes by 50% and lipid peroxidation by 60%. Thus, it may be possible that this anti-inflammatory property of *C. wightii* can significantly improve the intense inflammation of alveolar osteitis and reduce the painful sequalae observed in this condition. Another important inflammatory marker which has been indicated to play an important role in alveolar osteitis is tumor necrosis factor-α (TNF-α). Role of TNF-α in causing chronic inflammatory diseases like rheumatoid arthritis has been well documented, in which it acts as a pro inflammatory cytokine leading to the destruction of joint (Williams *et al*., 2016). Evidence from the studies related to the healing of post extraction sockets and induced alveolar osteitis reveal an increase in expression of TNF-α in cases where there is a delay in healing process. This increase in its expression has been correlated to the decrease in bone formation as well as causing the intense pain associated with the condition of alveolar osteitis in humans (Hall *et al*., 2016; Williams *et al*., 2016). As the *C. wightii* mucoadhesive gel contained guggulsterol as the active ingredient that has been documented to play an important role in inhibition of TNF-α, it can be deduced that guggulsterol may also have played a role in reduction of inflammation in the wound area, thereby, proving the dietary intake of the animals in experimental group B.

Histological assessment of wound healing

In this study, normal healing pattern was observed in the histological sections of the tooth extraction area of the control group rats. In normal healing following extraction of the tooth a series of events take place beginning with the formation and maturation of blood clot. The blood clot is infiltrated by fibroblasts which replaces the blood clot with the granulation tissue that acts as a provisional matrix for bone tissue formation (Kuboki *et al*., 1988). Similar histological events were demonstrated in the control group rats. The sequence of events in alveolar osteitis is similar to normal healing with a delay in healing. In this delayed healing of extraction socket there is a premature loss of the initial blood clot which normally persists for 24 h. This premature loss of blood clot is attributed to the process of fibrinolysis, which may be an inherent property of normal bone following the extraction of the tooth or may be a result of the microbiological activity (Bergmann and Hammerschmidt, 2007). Development of alveolar osteitis can be either due to the loss of the blood clot from the extraction socket, which is termed as dry type, or it can be due to loss of blood clot as well as superimposed infection, which is termed as the granulomatous type (de Melo *et al*., 2002).

Experimental group A showed histological findings that were similar to those observed in previous studies of experimental model of alveolar osteitis in rats and dogs (Cardaropoli *et al*., 2003; Rodrigues *et al*., 2011). The histological sections of the extraction site on the 4th day of sacrifice showed no red blood cells (loss of blood clot) in the samples of experimental group A as compared to the control group. Wound healing was consequently affected as evidenced by absence of proper granulation tissue in the samples of 7th and 14th days of experimental group A rats. Inflammatory cells like giant cells and macrophages were present in abundance within the extraction socket and bacterial colonies overlying it. These histological findings confirmed the creation of the alveolar osteitis model in accordance with the previous studies (de Melo *et al*., 2002; Cardaropoli *et al*., 2003; Cardoso *et al*., 2011; Rodrigues *et al*., 2011).

The histological sections of tooth extraction site in experimental group B were initially similar to those observed in experimental group A till day 4. On the 7th and 14th day samples there was a decrease in the inflammatory cells infiltrate in experimental group B. Osteoid was found to be mostly occupying the area of tooth extraction socket. Histological findings from the experimental group B showed improved healing which is assumed to be due to the application of *C. wightii* based mucoadhesive gel

on the 3rd day of creation of alveolar osteitis model. This was evident on the 14th day when the granulation tissue was completely replaced by osteoid. This oseoid was not present in either control group or the experimental group A.

Prolonged levels of TNF-α production, as has been shown in alveolar osteitis model which leads to a greater inflammatory infiltrate as compared to those undergoing normal healing following extraction of a tooth. Reduction in the inflammatory infiltrate has been shown to increase the bone deposition in alveolar osteitis model in dogs (Cardoso *et al*., 2011). The inflammation can be reduced either physically by curettage and irrigation of the extraction socket, which involves anaesthetizing the extraction area followed by physical debridement of the socket with the help of a bone curette and then finally irrigating the socket with normal saline. A previous study has shown that the animal models of alveolar osteitis, which underwent a treatment either of curettage and irrigation or placement of intra alveolar medicament, a reduction in the density of the inflammatory infiltrate occured (Bergmann and Hammerschmidt, 2007). Since physical debridement was not done in the experimental group B, inhibition of TNF-α protein can be considered as one of the possibilities which may aid in the improved healing and mature osteoid deposition in the experimental group B.

Previous studies on *C. wightii* reports its anti-inflammatory potential as it significantly inhibits Interferon gamma (IFN), interleukin 1 β (IL1 β) and up regulates nitric oxide (NO) (Manjula *et al*., 2006; Goyal *et al*., 2010). IL1 β facilitates synaptic activity and pain transmission at the site of injury, which leads to development of chronic pain. Recently it has been shown that at IL1 β is elevated both peripherally and centrally at multiple levels in painful and inflammatory conditions such as that of alveolar osteitis (Ren and Torres, 2009). Nitric oxide (NO) acts as a potent vasodilator contributing to improved angiogenesis. Angiogenesis is one of the essential events that occurs during the socket healing process and provides the inflammatory cells, growth factors, and progenitor cells that are required in the inflammatory and proliferative stages of socket healing. In addition, the regeneration and wound healing of alveolar bone is directly dependent on the angiogenesis process. *C. wightii* downregulates IL1 β and TNF-α markers by inhibiting the mitogen activated protein kinases (MAPK). Keeping in view the anti-inflammatory properties of *C. wightii,* it is possible that this property may also have led to the improved healing in the histological sections of alveolar osteitis model of experimental group B which received the *C. wightii* based mucoadhesive gel as compared to the experimental group A which did not receive any mucoadhesive gel.

Certain microorganisms of oro nasal origin have been demonstrated to possess the fibrinolytic property which causes dissolution of the blood clot and cause ease of invasion of the microorganisms into the origin site. These include the *Staphylococcus aureus* and the β hemolytic species of the *Streptococcus* that were isolated form the nose and throat region of the patients with epistaxis. *Bacteroides gingivalis* and *Treponema denticola* have also demonstrated fibrinolytic activity associated with periodontal disease and acute necrotizing ulcerative gingivitis (Fleetwood *et al*., 2015; Chi *et al*., 2017) *C. wightii* acts as an effective antimicrobial and antifungal agent. This antibacterial property is attributed to the presence of compounds such as sesquiterpene and *T. cadinol* which interacts and disrupts the cell membrane of the bacterial agents leading to bacteriolysis. Extracts of this locally available plant have been used extensively in the past for anti-fungal, anesthetic and anti-microbial properties without any reported side effect (Al-Daihan *et al*., 2013; Singh and Singh, 2013). These properties combined may have played a role in the overall improved wound healing of the rats in the experimental group B as compared to both the experimental group A and control group.

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IRB approval

The study was approved by the Institutional Review Board at University of health Sciences, Lahore (No: UHS/REG-18/ERC/917. Dated: 07 March 2018).

Ethical approval

The ethical approval for this experimental study was obtained from the institutional review committee of University of Health Sciences, Lahore. The study was carried out in the Department of Oral Biology and experimental research laboratory of the University. The sample size for the study was calculated by keeping the power of the study at 85% and the desired level of significance at 0.05 and it came out to be 18.

Supplementary material

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20220412070440

Statement of conflict of interest

The authors have declared no conflict of interest.

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