IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 12 Ver. XI (Dec. 2017), PP 26-31
www.iosrjournals.org

Histological And Biochemical Evaluation of the Effect of Topical Application of Curcumin And Propolis on oral Ulcer in Albino Rats

Soha Basha[1]
[1](*Oral Medicine, Periodontology And Radiology Department, Faculty Of Dentistry/ Pharos University In Alexandria, Egypt.*
Corresponding Author: Soha Basha[1]

Abstract : *Pro-inflammatory cytokines such as tumor necrosis factor alpha and interlukin -8 played a major role in pathogenesis of oral ulceration. Recently, considerable attention has been focused on using natural herbs as an effective treatment modality for many types of oral ulcers. Curcumin has potent anti- inflammatory properties. Propolis extract is a bee-metabolized resinous substance that exerts antioxidant, anti-inflammatory and analgesic effects.*
Aim of study: *The present study was conducted to evaluate and compare the effect of topical application of curcumin and propolis on induced palatal ulcers in rats.*
Materials and methods: *A total of 30 adult male Albino rats were divided into three groups: Group I (control group), Group II (curcumin-treated group) and Group III (propolis-treated group). 5 rats from each group were sacrificed at day 2 and 8. Tissue samples were obtained and prepared for histological and biochemical evaluations. The data obtained were statistical analyzed and compared.*
Results: *Curcumin and propolis treated group showed a significant reduction in TNF- α after 8 days compared with control group. While no significant difference was detected between curcumin and propolis groups. Regarding IL-8 level significant difference was seen between control group and curcumin after 8 days while, no significant difference was detected between control and propolis or between curcumin and propolis. Histological examination revealed reduction in inflammation, epithelial regeneration and formation of thick keratin layer in group I and II after 8 days with minimal number of inflammatory cells in curcumin group.*
Conclusion: *we concluded that curcumin and propolis could be considered as a promising adjuvant treatment for oral ulcerations. However, curcumin is more effective than propolis.*
Keywords: *IL-8, curcumin, inflammatory mediators, propolis, TNF- α*

Date of Submission: 29 -12-2017 Date of acceptance: 29-12-2017

I. Introduction

Oral ulceration is one of the most common oral diseases. It is defined as a break in the skin or mucous membrane with a loss and degeneration of epithelial tissue. The etiology of oral ulcer is unclear in many cases; a common cause is accidental damage of the oral tissues.[1] Several studies[2,3]suggested that the crucial role in the pathogenesis of chronic ulcerations is inflammation caused by activated leukocytes. Throughout the healing process, inflammatory response is considered as a major trigger for this process. Persistent inflammation is an evidence of non-healing process; on the other hand the resolution of inflammation is associates with the healing process. The levels of the pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF-α) are the biochemical indicators of inflammation.

TNF- α (referred to as chaectin) is an important pro-inflammatory cytokine that control the response of the immune system and hasten the inflammatory process.[4] TNF- α is produced by many cells including stimulated monocytes, fibroblasts and endothelial cells. In addition, macrophages, T-cells, B-lymphocytes, granulocytes, smooth muscle cells, eosinophils, chondrocytes, osteoblasts, mast cells and keratinocytes are also produce TNF- α. Moreover TNF- α has the ability to activate multiple cells. It has been implicated in diverse range of inflammatory, infectious and malignant conditions. [5] The main role of TNF- α in inflammation has been demonstrated by the ability of agents that block the action of TNF- α to treat a wide range of inflammatory conditions, such as rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease and psoriasis. [6,7]

Another important pro-inflammatory cytokine that played a major role in host response to injury and inflammation is IL-8. It also referred to as neutrophil chemotactic factor (NCF) and neutrophil activating factor (NAF). It is a chemokine that has originally described as neutrophil chemo-attractant, it is now known to possess more diverse functions as activation of neutrophil and chemo-attraction of other cells including T cells and basophils.[8]

Therapeutic options for treating oral ulceration are varying according to the severity and frequency of ulceration and the main objectives of treatment are to relieve discomfort, reduce secondary infection, promote healing of existing ulceration and prevent occurrence of new ulcers. Topical analgesic can be used to reduce discomfort; Antiseptic mouthwashes containing chlorhexidine or povidone-iodine are widely used for preventing secondary infection.[9,10] Topical corticosteroids can be effective drugs in the treatment of severe cases of oral ulceration however; prolonged use of potent topical corticosteroids carries a risk of systemic absorption and associated with many adverse effects.[11]

In addition to traditional pharmaceutical treatments the implementations of anti-inflammatory interventions are very beneficial and they provide a more widely applicable treatment options. Appropriate dietary alterations including foods and supplements with established anti-inflammatory benefits have been shown to effectively reduce inflammation. [12]

Herbal medicine is one of the most commonly used complementary and alternative therapies that suppress the inflammatory process through its inhibitory effects on several pro-inflammatory cytokines. There have been a number of reports in the medical literature regarding these natural agents as ginseng, acai berry, omega-3, propolis and curcumin. [13,14,15,16,17]

Curcumin is herbal product that has anti-tumour, anti-oxidant, anti-viral and anti-inflammatory activities. [18,19,20] Turmeric (the common name for Curcuma longa) is an Indian spice that has a long history of use in treatment of many inflammatory conditions. Curcumin inhibits the production of the pro-inflammatory cytokines such as TNF-α, IL-1, IL-2, IL-6 and Il-8.[21,22] Systemic and topical use of curcumin has been advocated for treatment of several common diseases in India and China. It is nontoxic and has been consumed daily

for centuries in Asia. Recently extensive researches have performed to examine the use of curcumin for treatment of gingivitis and periodontitis. [23,24,25,26]

Another important natural remedy is propolis. It is known as bee glue, and it is created out of a mix of buds from some trees with the substance secreted from the bee's glands. Propolis is a complex resinous material that includes; fatty and phenolic acids and esters, substituted phenolic esters, flavonoids (flavones, flavanones, flavonols, dihydroflavonols, chalcones), terpenes, β- steroids, aromatic aldehydes and alcohols, and derivatives of sesquiterpenes, naphthalene and stilbenes.[27] Owing to its various chemical contents, several applications of propolis have been studied and described in detail for centuries. Propolis has antibacterial, antifungal, anti-infammatory, antiviral, anticancer and many other properties. [28,29,30,31] Propolis has a wide range of applications in various specialities of dentistry. [32,33] Many authors suggested that propolis modulates the non-specific immunity via macrophage activation and stimulation of cytokines production, such as IL-1 and TNF-α and Il-8. [30,34]

The purpose of this study was to evaluate and compare the effects of topical application of curcumin and propolis on oral ulceration through biochemical and histological examinations.

II. Materials And Methods

30 adult male Albino rats weighting (200-250 g) were used in the study. Animals were obtained from the animal house of Medical Research Institute, Alexandria University. During the study the animals were kept at the animal house of Faculty of Dentistry, Pharos University in polypropylene cages, 10 rats each with free access to water and normal diet. The room temperature was about 22-24°C and the animals were exposed to 12:12 hours light dark cycles. The present research protocol was approved by the Ethics Review Board of Faculty of Dentistry, Pharos University.

Ulcer induction: Prior to the creation of the ulcers, rats were fixed on their backs and all animals were anaesthetized with an intra-peritoneal injection of ketamine* and xylazine** (90 and 15 mg/kg, respectively). Round filter papers 5.5 mm in diameter were soaked in 15 ml of 50% acetic acid. In order to create round ulcer, an acid-soaked filter paper was pressed onto the palate for 60 seconds. The rat population were divided into 3 groups, 10 rats each

Group I: Control group: rats with oral ulcer and not receiving any treatment.
Group II: Curcumin group: Curcumin was obtained from El-hawag factory for raw oils Bader city -Cairo–Egypt.The concentration of 1% curcumin cream was prepared based on carboxyvinyl polymer and trolamine. [24] Curcumin was applied over the ulcer twice daily throughout the study period.
Group III: Propolis group: rats were treated by topical application of propolis (100 mg/kg b.wt) obtained from Sigma Pharmaceutical Industries (S.P.I.). For: International Business Establishment (IBE) Pharma. Propolis was applied over the ulcer twice daily throughout the study period.
5 rats from each group were sacrificed at day 2 and 8 by an overdose intra-peritoneal injection of 100 mg/kg Phenobarbital sodium (West Ward Pharm., USA). Each wound was excised, maintaining approximately 3 mm of mucosa around the incision.

Biochemical Evaluation:

Mucosal tissues from the rats were directly dissected and homogenized in appropriate buffer and then centrifuged, according to the instructions of the biochemical assay. The levels of TNF-α and IL-8 were measured using an enzyme-linked immunosorbent assay ELISA commercial kit (R&D Systems, EUA). Microplate reader measure absorbance at 450 nm was used in this study. This assay was a sandwich ELISA and was performed according to manufacturer's instructions.

Histological Evaluation:

The excised tissues were fixed with 10% formalin. Specimens of oral mucosa were obtained and fixed in 10% neutral formalin for 48 hours. Then they were dehydrated in ascending grades of alcohol and embedded in paraffin. Histological sections of 5µm thickness were obtained and stained with hematoxylin and eosin stain according to the conventional method. [35] Then the specimens were prepared for histological and biochemical examination.

III. Statistical Analysis Of The Data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. The Kolmogorov-Smirnov, Shapiro and D'agstino tests were used to verify the normality of distribution of variables, **Student t-test** was used to compare two groups for normally distributed quantitative variables while **ANOVA** was used for comparing the three studied groups and followed by **Post Hoc test (LSD)** for pairwise comparison. Significance of the obtained results was judged at the 5% level.

IV. Results Biochemical results

Table (1): Comparison between the three studied groups according to TNF alpha and IL-8

	Control	Curcumin	Propolis	p_1	p_2	p_3	p_4
TNF alpha							
After 2 days	32.7 ± 3.8	28.5 ± 5.5	30.0 ± 5.1	0.165	0.063	0.223	0.495
After 8 days	28.0 ± 3.4	20.2 ± 4.9	23.1 ± 4.5	0.001*	<0.001*	0.017*	0.144
% of change	↓14.4	↓29.1	↓23				
p_5	0.009*	0.002*	0.005*				
IL-8							
After 2 days	22.2 ± 3.0	20.5 ± 3.9	21.5 ± 4.0	0.583	0.306	0.671	0.545
After 8 days	18.1 ± 2.6	13.1 ± 3.7	15.2 ± 5.2	0.031	0.009*	0.116	0.249
% of change	↓18.5	↓36.1	↓29.3				
p_5	0.004*	<0.001*	0.007*				

p_1: p value for **ANOVA test** for comparing between the three studied groups
p_5: p value for **Student t-test** for comparing between the two studied periods
*: Statistically significant at $p \leq 0.05$

Regarding the TNF-α, no significant difference was observed between the three groups (control, curcumin and propolis) after 2 days. Though, the least value was detected in curcumin group (28.5 ± 5.5) followed by propolis (30.0 ± 5.1) and finally the control groups (32.7 ± 3.8). After 8 days significant difference was seen between control group and curcumin ($p<0.001$) and also between control and propolis ($p=0.017$). However, no significant difference was detected between curcumin and propolis ($p=0.144$). The percentage of decrease was greatest in curcumin group (29%) followed by propolis (23%) and the least were in the control group (14.4%).

Regarding the IL-8, no significant difference was observed between the three groups (control, curcumin and propolis) after 2 days. Though, the least value was detected in curcumin group (20.5 ± 3.9) followed by propolis (21.5 ± 4.0) and finally the control groups (22.2 ± 3.0). After 8 days significant difference was seen between control group and curcumin ($p=0.009$). No significant difference was detected between control and propolis or between curcumin and propolis ($p=0.116$ and 0.249 respectively). The percentage of decrease was greatest in curcumin group (36%) followed by propolis (29.3%) and the least was in the control group (18.5%).

Histological Findings:

Group I (Control group): Histological examination of control group after **2 days** showed sever epithelial and connective tissue degeneration with large number of inflammatory cells (**Figure 1)**. After **8 days** control group showed signs of ulcer healing in the form of very thin epithelial lining .Connective tissue showing area of degeneration with minimal inflammatory cells **(Figure 2).Group II (Curcumin group):** Histological examination after **2 days** revealed a signs of epithelial regeneration on one side of ulcer region and remnant of granulation tissue was observed. Connective tissue of lamina proporia was severely infiltrated with inflammatory cells **(Figure 3).** While after **8 days** a thick epithelial layer with long epithelial ridges and high
connective tissue papilla was observed. Regular layer of keratin can be seen. Lamia proporia showed dense connective tissue fibres with minimal degree of inflammatory cell infiltration (**Figure 4)**.

Group III (Propolis group): Histological examination of propolis group after **2 days** revealed epithelial degeneration with a deep ulcer formation and complete absence of the covering keratin layer .The lamina propria and submucous layers were infiltrated with inflammatory cells (**Figure 5)**, whereas after 8 days even thickness of epithelial layer with a thin keratin layer was observed. Lamina proporia moderately infiltrated with inflammatory cells. (**Figure 6)**

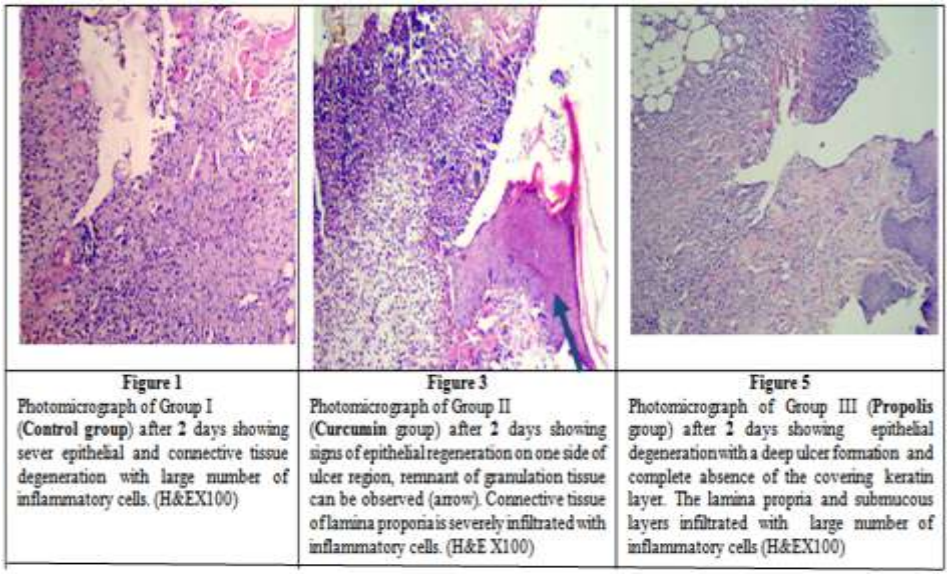

Figure 1
Photomicrograph of Group I (**Control group**) after **2** days showing sever epithelial and connective tissue degeneration with large number of inflammatory cells. (H&EX100)

Figure 3
Photomicrograph of Group II (**Curcumin** group) after **2** days showing signs of epithelial regeneration on one side of ulcer region, remnant of granulation tissue can be observed (arrow). Connective tissue of lamina proporia is severely infiltrated with inflammatory cells. (H&E X100)

Figure 5
Photomicrograph of Group III (**Propolis** group) after **2** days showing epithelial degeneration with a deep ulcer formation and complete absence of the covering keratin layer. The lamina propria and submucous layers infiltrated with large number of inflammatory cells (H&EX100)

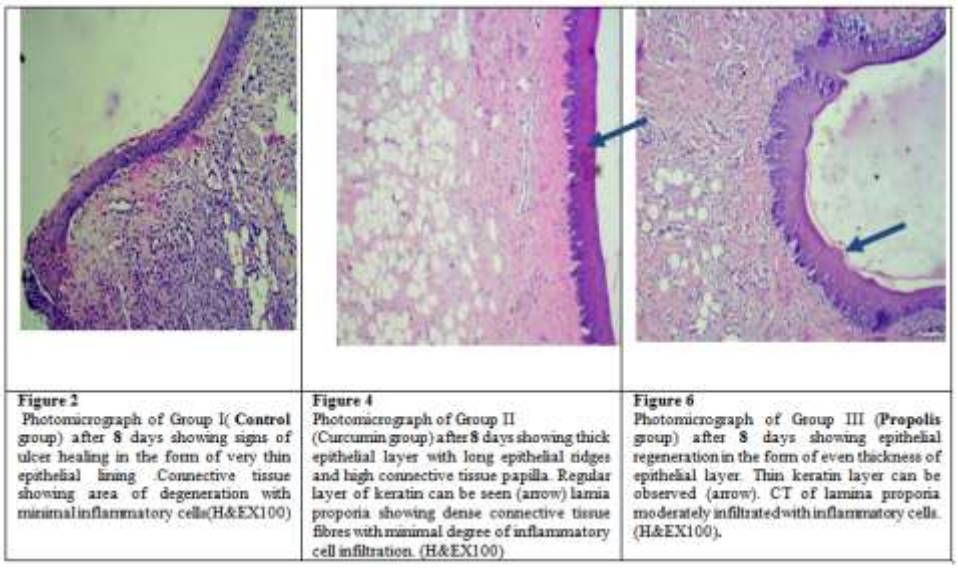

Figure 2
Photomicrograph of Group I(**Control** group) after **8** days showing signs of ulcer healing in the form of very thin epithelial lining .Connective tissue showing area of degeneration with minimal inflammatory cells(H&EX100)

Figure 4
Photomicrograph of Group II (Curcumin group) after **8** days showing thick epithelial layer with long epithelial ridges and high connective tissue papilla. Regular layer of keratin can be seen (arrow) lamia proporia showing dense connective tissue fibres with minimal degree of inflammatory cell infiltration. (H&EX100)

Figure 6
Photomicrograph of Group III (**Propolis** group) after **8** days showing epithelial regeneration in the form of even thickness of epithelial layer. Thin keratin layer can be observed (arrow). CT of lamina proporia moderately infiltrated with inflammatory cells. (H&EX100).

V. Discussion

Oral ulceration is a common complaint of patients attending dental clinics. The goal of treatment of oral ulcers is to relieve symptoms; consequently, finding suitable drugs with fewer side effects is the goal of many researchers. Clinical studies on the use of herbal remedies have reported favourable benefits to patients by reducing the discomfort and duration of ulcers with minimum or no side effects through inhibition of proinflammatory cytokines that incorporated in the pathogenesis of oral ulcers, like TNF-α, IL-8 and granulocyte macrophage colony stimulating factor (GM-CSF).[2]

The present study attempted to evaluate and compare the effect of curcumin and propolis on oral ulcers using histological and biochemical analysis through assessment of TNF-α and IL-8 levels. Regarding TNF-α level, significant difference was observed between curcumin group and control group after 8 days. Consequently we have concluded that curcumin is a potent inhibitor for TNF-α production. In agreement, different studies demonstrated that curcumin downregulate TNF- α production. [36,37,38] .This result could be attributed to the ability of curcumin to inhibit the production of nuclear factor-kappa beta (NF- κβ) which mediates the expression of many inflammatory mediators. NF- κβ is responsible for activation and regulation of TNF-α production. Accordingly, it plays a critical role in host defence and in chronic inflammatory diseases. [18,39]

Different studies[40,41,42] concluded that curcumin has an inhibitory effect on key signal pathways of mitogen activated protein kinases (MAPKs) which plays a key role in tumor necrosis factor production. Previous studies[43,44] established that curcumin is a potent inhibitor of phospholipid-dependent protein kinase C (PKC) production which mediates TNF-α release process. In addition, Chann[45]showed that curcumin inhibited TNF-α production in cell culture. Moreover, Gonzales et al[46] proved that curcumin blocked the expression of pro-inflammatory gene. Furthermore, the present study showed a significant difference between propolis group and control group after 8 days which emphasize the potent effect of propolis on reducing the level of TNF-α. These results were in accordance with Fatahinia[47] et al, who showed that oral administration of propolis resulted in suppression of TNF-α and IL-2 in the sera of mice when compared to controls. Propolis has been shown to inhibit the production of TNF- α by inhibiting NF-kB activity. [48,49] These results were also supported by Khayyal et al[50]who reported that administration of an aqueous extract of propolis decreased the levels of many pro-inflammatory cytokines including TNF-α, IL-6 and IL-8 .

Consequently, Al Ghamdi et al [51] proved that the anti-inflammatory properties of propolis arise from its direct inhibition of cytokine production by immune cells. Furthermore, the authors revealed that propolis suppresses TNF-α production in low dose. However, Zedan et al [52]found that increasing the dose of propolis causes significant increase in TNF- α level compared to other groups received low dose of systemic propolis. They concluded that effect of propolis on TNF- α production is dependent on the dose and duration of propolis administration. In the current study maximum decrease of TNF- α level was denoted in curcumin group (29%) followed by propolis group (23%) while the least decrease was in the control group(14.4%), which indicate the superiority of curcumin over propolis in TNF- α reduction. Our second biochemical parameter is IL-8, the result showed a significant reduction in IL-8 level of curcumin group comparing to control group after 8 days. This finding agreed with Cohen et al[53] who concluded that curcumin inhibits IL-8 production. This result was attributable to the ability of curcumin to inhibit NF-κβ which results in the downstream inhibition of the IL-6 and IL-8. Additionally, reduction of IL-8 level is explained by many studies which demonstrated that curcumin lower IL-8 secretion from monocytes. [54, 45]

In the present study, serum IL-8 was monitored in propolis group. There was no significant difference between propolis group and control group in day 2 or day 8. However, the amount of decrease was higher in propolis compared to control group (9.3% and 18.5). Our data are consistent with the results of a previous study by Skiba et al[55] who reported that propolis reduce secretion of IL-8. They concluded that propolis inhibits NF-kB, which in turn inhibits the production of many proinflammatory cytokines including IL-8. This was counteracted by Al Ghamdi et al [51] who evaluated the effect of propolis on mice by measuring the level of different pro-inflammatory cytokines including IL-8 and IL-10 and he reported that the IL-8 and IL-10 levels did not differ between control mice or propolis-treated diabetic mice. Our results showed that curcumin was more efficient in reducing the level of IL-8 compared to propolis (36% and 29.3%) which again confirms the superiority of curcumin in reducing the level of proinflammatory mediators. Our biochemical results were further confirmed by histological evaluation by means of light microscopic examination. Histological findings of our samples revealed faster healing process in curcumin group; Manifested by presence of a well formed parakeratinized epithelium and thick layer of keratin as well as presence of minimal number inflammatory cells in curcumin group after 8 days. On the other hand control group after 8 days shows a thin layer of epithelium and absence of keratin layer. Furthermore, connective tissue layer showing large number of inflammatory cells.

These results are in agreement with Zaher et al 2014 [56]who concluded that topical curcumin accelerates oral ulcer healing process. This study showed that topical administration of curcumin in rat with tongue ulcer result in more organized re-epithelialization, early fibroblast infiltration and well organized collagen fibres in curcumin group. Similar results were reported by Lim 2016[57]who reported that on applying curcumin to gingival ulcer; completely recovered epithelium was observed in curcumin group. Several studies revealed that curcumin is useful in conditions of impaired wound healing, as it increased synthesize of collagen and improved fibroblast densities, enhanced maturation and cross linking of collagen in curcumin treated group[58].The results of current study are consistent with a study by Panchatcharam et al[59], the authors observed faster wound closure, better maturation and cross linking of collagen in curcumin treated wounds.

The histological specimen of propolis group in day 8 showed gradual reduction in inflammation, epithelial regeneration and formation of thick keratin layer. In agreement, Hozzein et al [60]showed that topical application of propolis to wounds accelerated wound closure in rats. These improvements were evident during the entire two-week study period, indicating that propolis application impacts all stages of the healing process by enhancing the production of collagen. These data are consistent with previous studies in humans and animals, as well as with older reports describing the use of propolis to treat ulcers [61,62.] Samet et al studied the uses of propolis as an adjunctive treatment of recurrent aphthous stomatitis. This study showed that propolis is effective in decreasing the number of recurrences and improve the quality of life in patients who suffer from recurrent aphthous stomatitis. [63]

Furthermore, these observations have been confirmed in a study by Silva et al, where it was demonstrated that propolis is effective in reducing acute inflammatory processes.[64]However, different studies showed that high concentration of propolis might cause adverse effects. Cases of allergic reactions to the topical application of propolis have been reported[65,66] Another adverse effect of propolis is mucosal damage which is attributed to the high alcohol component of propolis.[67]Jacoub et al concluded that propolis shows potential to assist in wound healing process, depending on its concentration.[68]

VI. Conclusion

The findings of our study indicated that topical application of curcumin and propolis promotes healing process of oral ulcers in rats. However, curcumin is more effective than propolis. Moreover dose-limiting toxicity of curcumin and propolis should be evaluated; more researches were needed to determine the maximum safe dose of curcumin and propolis.

References

[1]. Femanio F, Lanz A, Buonaiuto A, et al. Guidelines for diagnosis and management of aphthous stomatitis. Pediatr Infect Dis J. 2007;26(8):728–732.
[2]. Koh TJ, DiPietro LA. Inflammation and wound healing: The role of the macrophage. Expert reviews in molecular medicine. 2011;13:11-24.
[3]. Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. J Clin Invest. 2007;117:514–521.
[4]. Gaspari AA: Innate and adaptive immunity and the pathophysiology of psoriasis. J Am Acad Dermatol 2006; 54: (3): 67-80.
[5]. Patil P, Patil L, Kadam V. TNF-α: A potential therapeutic target for inflammatory bowel disease. Asian Journal of Pharmaceutical and Clinical Research. 2011; 4(1): 158-166.
[6]. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448:427–434.
[7]. Andersen V, Hansen AK, Heitmann BL. Potential impact of diet on treatment effect from Anti-TNF drugs in inflammatory bowel disease. Nutrients. 2017;9(3):286-301
[8]. Durmazlar S U, lkar GB, Eskioglu F, Tatlican S, Mert A, Akgul A. Significance of serum interleukin-8 levels in patients with Behcet's disease: high levels may indicate vascular involvement. Int J Dermatol. 2009; 48(3):259-64.
[9]. Weinberg MA and Segelnick S L. Management of common oral sores. US Pharm. 2013; 38(6):43-48.
[10]. Kanagalingam J, Feliciano R, Hah J, Labib H, Le TA, Li J C. Practical use of povidone-iodine antiseptic in the maintenance of oral health and in the prevention and treatment of common oropharyngeal infections. International journal of clinical practice IJCP. 2015; 69, (11): 1247–1256.
[11]. Dhar S, Seth J, Parikh D. Systemic side-effects of topical corticosteroids. Indian Journal of Dermatology. 2014; 59(5):460-464.
[12]. Victor M, Aguirre B, Rosales C, Constantino L-Macías, and Gómez M .Control and resolution mechanisms of the inflammatory response. Mediators of Inflammation. 2016; (2016):1- 5.
[13]. Oyagi A, Ogawa K, Kakino M, Hara H. Protective effects of a gastrointestinal agent containing Korean red ginseng on gastric ulcer models in mice. Complement Altern Med. 2010; 10(45). 10-45.
[14]. Lee JY, Kim N , Choi YJ. Anti-inflammatory and Anti-tumorigenic Effects of Açai Berry in Helicobacter felis-infected mice. Journal of Cancer Prevention. 2016; 21(1):48-54.
[15]. Dawud FA, Mabrouk MA, Mohammed A, Omar IA. Effect of vitamins C & E on aspirin induced gastric mucosal damage and oxidative stress. Journal of Biological Sciences 2014;6(1): 36-41
[16]. Alexander JW, Supp DM. Role of Arginine and Omega-3 Fatty Acids in Wound Healing and Infection. Advances in Wound Care. 2014;3(11):682-690.
[17]. Shishodia S, Sethi G and Aggarwal BB. Curcumin: getting back to the roots. Ann NY Acad Sci. 2005; 11(1056): 206-217.
[18]. Duvoix A, Blasius R, Delhalle S, Schnekenburger M, Morceau F, Henry E, Dicato M , Diederich M. Chemopreventive and therapeutic effects of curcumin. Cancer Lett .2005; 223: 181-190.
[19]. Campbell FC, Collett GP. Chemopreventive properties of curcumin. Future Oncol 2005; 1: 405-414.
[20]. Vasudev S, Vakade C, Paramesh R Belgal P. Role of curcumin in management of potentially malignant disorders: A review of literature. Journal of Advanced Clinical & Research Insights. 2016; 3: 152–155.
[21]. Cho JW, Lee KS, Kim CW. Curcumin attenuates the expression of IL-1beta, IL-6, and TNF-alpha as well as cyclin E in TNF-alpha-treated HaCaT cells; NF kappa B and MAPKs as potential upstream. Int J Mol Med. 2007;19(3):469-74.
[22]. Aggarwal B, Gupta S C, a Sung B. Curcumin: an orally bioavailable blocker of TNF and other pro-inflammatory biomarkers. Br J Pharmacol .2013; 169(8): 1672–1692.
[23]. Lim YS, Kwon SK, Park JH, Cho CG, Park SW and Kim WK. Enhanced Mucosal Healing With Curcumin in Animal Oral Ulcer Model Laryngoscope. 2016; 126 (2): 68-73.
[24]. Nagpal M and Sood S. Role of curcumin in systemic and oral health: An overview. Journal of Natural Science Biology and Medicine. 2013; 4(1):3-7.
[25]. Hosadurga RR, Rao SN, Jose J, Rompicharla NC, Shakil M , Shashidhara R. Evaluation of the efficacy of 2% curcumin gel in the treatment of experimental periodontitis. Pharmacognosy Research. 2014; 6(4):326-333.
[26]. Farid RM. A Focus on Curcumin local application in oral diseases management: Mini review. Journal of Pharmacy: 2016; 6(1): 2250-3013.
[27]. Elshater AA, Salman MM, Abd-Elmegeed AA. Ameliorative effects of propolis extract on some biochemical and hematological parameters of burnt skin of male guinea pigs. Egypt. Acad. J. Biolog. Sci. 2017; 9(1):47-57
[28]. Bonvehi J S , Gutierrez AL. The antimicrobial effects of propolis collected in different regions in the Basque Country (Northern Spain).World Journal of Microbiology and Biotechnology. 2012; 28(4): 1351–1358.
[29]. Bruschi ML, Dota KF, Consolaro ME, Svidzinski TI. Antifungal activity of Brazilian propolis microparticles against yeasts isolated from vulvovaginal candidiasis. Evidence-based Complementary and Alternative Medicine.2011; l: 1-8.
[30]. Nassar S A, Mohamed A H, Soufy H, Nasr SM, and Mahran K M. Immunostimulant effect of Egyptian propolis in Rabbits. Science World Journal. 2012; 12:1-9.
[31]. Sawicka D , H Borawska M and Nikliński J. The anticancer activity of propolis. Folia Histochem Cytobiol. 2012; 50(1):25-37
[32]. Mazia RS, Pereira RR, Francisco LM, Natali MR, Filho BP, Nakamura CV, Bruschi ML, Nakamura TU. Formulation and Evaluation of a Mucoadhesive Thermo-responsive System Containing Brazilian Green Propolis for the Treatment of Lesions Caused by Herpes Simplex Type I, Journal of Pharmaceutical Sciences.2016;105 (1) 113-116.
[33]. Malhotra S, Gupta VK. Use of propolis in pediatric dentistry. J Dent Allied Sci 2014; 3:93-8.
[34]. Conti BJ, Santiago KB, Búfalo MC, Herrera YF, Alday E, Velazquez C, Hernandez J, Sforcin JM. Modulatory effects of propolis samples from Latin America (Brazil, Cuba and Mexico) on cytokine production by human monocytes. J Pharmp Pharmacol . 2015; 67(10):1431-8.
[35]. Bashkar, SN, 1990. Orban's Oral Histology and Embryology: 11th Ed. Baltimore, Boston, Chikago, London, Piladephia, Sydney, Toronto: Mosby st. Louis, pp: 339 – 41, 349 - 50, 365, 470-73.
[36]. Cho JW, Lee KS, Kim CW. Curcumin attenuates the expression of IL-1beta, IL-6, and TNF-alpha as well as cyclin E in TNF-alpha-treated HaCaT cells; NFkappa B and MAPKs as potential upstream. Int J Mol Med. 2007; 19(3):469-74.
[37]. Woo HM, Kang JH, Kawada T, Yoo H, Sung MK and Yu R. Active spice-derived components can inhibit inflammatory responses of adipose tissue in obesity by suppressing inflammatory actions of macrophages and release of monocyte chemoattractant protein-1 from adipocytes. Life Sci .2007;80: 926–931
[38]. Zhao C, Yang J, Wang Y, Liang D, Yang X and Li X. Synthesis of mono-carbonyl analogues of curcumin and their effects on inhibition of cytokine release in LPS-stimulated RAW 264.7 macrophages. Bioorg Med Chem .2010; 18: 2388–2393.

[39]. Klinger NV, Mittal S. Therapeutic Potential of Curcumin for the Treatment of Brain Tumors. Oxidative Medicine and Cellular Longevity. 2016; 2016: 9324085.

[40]. Campbell J, Ciesielski C, Hun A, Horwood N, Beech J, Hayes L, Denys A, Feldmann M, Brennan F and Foxwell B. A Novel Mechanism for TNF-α Regulation by p38 MAPK: Involvement of NF-κB with Implications for Therapy in Rheumatoid Arthritis. J Immunol . 2004; 173 (11): 6928-6937.

[41]. Zwerina J, Hayer S, Redlich K, Bobacz K, Kollias G, Smolen JS and Schett G. Activation of p38 MAPK is a key step in tumor necrosis factor–mediated inflammatory bone destruction. Arthritis Rheum. 2006;54(2):463-72.

[42]. Leclercq IA, Farrell GC, Sempoux C, Pena A and Horsmans Y. Curcumin inhibits NF-kappa B activation and reduces the severity of experimental steatohepatitis in mice. J Hepatol 2004; 41: 926-934.

[43]. Soetikno V, Watanabe K, Sari FR, Harima M, Thandavarayan RA, Veeraveedu PT, Arozal W, Sukumaran V, Lakshmanan AP, Arumugam S and Suzuki K. Curcumin attenuates diabetic nephropathy by inhibiting PKC-α and PKC-β1 activity in streptozotocin-induced type I diabetic rats.Mol Nutr Food Res. 2011;55(11):1655-65.

[44]. Pullakhandam R, Srinivas PN, Nair MK and Reddy GB. Binding and stabilization of transthyretin by curcumin. Arch Biochem Biophys . 2009 ;15; (2):115-124.

[45]. Noorafshan A and Esfahani S. A Review of Therapeutic Effects of Curcumin. Current Pharmaceutical Design, 2013; (19): 2032-2046

[46]. Gonzales MA and Orlando AR. Curcumin and resveratrol inhibit nuclear factor-kappaB-mediated cytokine expression in adipocytes. Nutrition & Metabolism 2008; 5(17):1-13.

[47]. Fatahinia M, Khosravi AR and Shokri H. Propolis efficacy on TNF-α, IFN-γ and IL2 cytokines production in old mice with and without systemic candidiasis. Journal of Medical Mycology.2012; 22, (3): 237-242.

[48]. Korish A and Arafa M. Propolis derivatives inhibit the systemic inflammatory response and protect hepatic and neuronal cells in acute septic shock Braz J Infect Dis .2011;15(4):332-338.

[49]. Buyukberber M, Savas C, Bagci C, Koruk M, Gulsen M , Tutar E, Bilgic T, Devec R and Kucuk C. The beneficial effect of propolis on cerulein-induced experimental acute pancreatitis in rats.Turk J Gastroenterol 2009; 20 (2): 122-128.

[50]. Khayyal MT, el-Ghazaly MA, el-Khatib AS, Hatem AM, de Vries PJ, el-Shafei S and Khattab MM.A clinical pharmacological study of the potential beneficial effects of a propolis food product as an adjuvant in asthmatic patients. Fundam Clin Pharmacol. 2003 Feb;17(1):93-102.

[51]. Al Ghamdi A and Gamal B Hozzein W, Allam A, Noori S, Al-Wadaan M and Garraud O. Oral supplementation of diabetic mice with propolis restores the proliferation capacity and chemotaxis of B and T lymphocytes towards CCL21 and CXCL12 by modulating the lipid profile, the pro-inflammatory cytokine levels and oxidative stress. BMC Immunology. 2015; **16(**54):1-14.

[52]. Zeedan, G S G, Allam, A M M, Nasr, S M, Aballhamed, A M. Evaluation the efficacy of Egyptian propolis against parapox viruses by production of IFN-γ, TNF-α and immunoglobulin in experimental rat. World Applied Sciences Journal, (2014). 31(2), 199-207.

[53]. Cohen N A, Veena SM, Srivatsan SE, and Wang B l. Suppression of Interleukin 6 and 8 Production in Head and Neck Cancer Cells With Curcumin via Inhibition of Iκβ Kinase. Arch Otolaryngol Head Neck Surg. 2009; 135(2):190-197

[54]. Jain SK, Rains J, Croad J, Larson Band Jones K . Curcumin supplementation lowers TNF-alpha, IL-6, IL-8, and MCP-1 secretion in high glucose-treated cultured monocytes and blood levels of TNF-alpha, IL-6, MCP-1, glucose, and glycosylated hemoglobin in diabetic rats. Antioxid Redox Signal 2009; 11: 241–249.

[55]. Skiba M, Szliszka E, Kunicka M and Król W. Effect of ethanol extract of propolis (EEP) on interleukin 8 release by human gastric adenocarcinoma cells (AGS) infected with Helicobacter pylori. Centr Eur J Immunol. 2011; 36 (2): 65-69.

[56]. Zaher A, Elsabaa H, Abou Elkhier M and Elhindawy M. Impact of Curcumin on Tongue Ulcer Healing in Albino Rats. Mansoura Journal of Dentistry 2014; 1(3):85-89.

[57]. Lim YS, Kwon SK, Park JH, Cho CG, Park SW and KimWK. Enhanced mucosal healing with curcumin in animal oral ulcer model laryngoscope. 2016 ;126: (2) 68-73.

[58]. Jagetia, GC. and Rajanikant, GK. Role of Curcumin, a Naturally Occuring Phenolic Compound of Tumeric in Accelerating the Repair of Exicision Wound in Mice Whole-Body Exposed to Various Doses of Gamma-Radiation. Journal of Surgical Research.2004; 120: 127-138.

[59]. Panchatcharam, M., Miriyala, S., Gayathri, V.S. and Suguna, L. Curcumin Improves Wound Healing by Modulating Collagen and Decreasing Reactive Oxygen Species. Molecular Cellular Biochemistry.2006; 290: 87-96.

[60]. Hozzein WN, Badr G, Al Ghamdi AA, Sayed A, Al-Waili NS, Garraud O. Topical application of propolis enhances cutaneous wound healing by promoting TGF-Beta/Smad-mediated collagen production in a streptozotocin-induced type i diabetic mouse model. Cell Physiol Biochem 2015; 37(3) :940-954.

[61]. Henshaw FR, Bolton T, Nube V, Hood A, Veldhoen D, frunder L, McKew GL, Macleod C, McLennan SVand Twigg SM. Topical application of the bee hive protectant propolis is well tolerated and improves human diabetic foot ulcer healing in a prospective feasibility study. J Diabetes Complications 2014; 28:850-857.

[62]. Pillai S, Palsamy P, Subramanian S and Kandaswamy M. Wound healing properties of Indian propolis studied on excision wound-induced rats. Pharm Biol 2010; 48:1198-1206.

[63]. Samet N, Laurent C, Susarla SM and Rubinsteen N. The effect of bee propolis on recurrent aphthous stomatitis: A pilot study. Clin.Oral Investig.2007; 11: 143–147.

[64]. Silva JC1, Rodrigues S, Feás X, Estevinho LM Antimicrobial activity, phenolic profile and role in the inflammation of propolis.food Chem Toxicol. 2012 May; 50(5):1790-5.

[65]. Fern´andez S G, Luaces E L, Madoz S E, Alem´an E A, Api˜n´aniz MA, Purroy AIT. "Allergic contact stomatitis due to therapeutic propolis," Contact Dermatitis, 2004; 50(5):321-327.

[66]. Basavaiah ND, Suryakanth DB. Propolis and allergic reactions. Journal of Pharmacy & Bioallied Sciences. 2012;4(4):345-348

[67]. Wimardhani YS, Soegyanto AI. Oral Mucosal Ulceration Caused by the Topical Application of a Concentrated Propolis Extract. Case Reports in Dentistry. 2014;2014: 307646.

[68]. Jacob A, Parolia A, Pau Allan and Amalraj F.The effects of Malaysian propolis and Brazilian red propolis on connective tissue fibroblasts in the wound healing process. BMC Complementary and Alternative Medicine.2015; 15:294-304.