

Study on the Effect of Essence and Extract of *Thymus caramanicus Jalas* on the Process of Oral Wound Healing in Rats

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Research Article

Article Info

Received on: 29/03/2015
Accepted on: 15/04/2015
Published on: 29/05/2015



QR Code for mobile



ABSTRACT :

Oral ulcers are the most prevalent diseases of the oral cavity. Various factors may cause occurrence of such kinds of wounds, such as Physical and Chemical Trauma, Microbial infections, diseases or drug use, malignances and nonspecific causes. The aim of the present study is to determine the effect of *Thymus caramanicus Jalas* extract on healing Oral ulcers of white male rats. Present experimental clinical study was conducted on 48 adult male rats which were randomly divided into 3 groups of 16 rats, named Hydro-alcoholic extract, Essence and Control. On the first day of experiment a 2-millimeter wound was made by the application of a biopsy punch inside the mouth of each group. For the 1st group, the Hydro-alcoholic extract of *T. caramanicus Jalas* was used every 12 hours on a daily basis, for the 2nd group, the essence was used on the wound and, for the 3rd group, no drug was used. The progress of wounds healing was clinically examined on the 2nd, 4th, 6th and 8th days by the application of caliper, and histopathologic examination was conducted upon observation of the slides prepared. Data was analyzed by SPSS-21 software and ANOVA statistical test within the significance level of 0.05%. The results showed a significant decrease in the clinical size of the wound in extract group in comparison with control group on the 8th day. The average size of clinical ulcers on the 2nd day was similar in both groups. Furthermore, on the 6th and the 8th days, a significant decrease was observed in the size of the ulcers in extract group in comparison with the essence group ($P < 0.05$). Results of the present study showed that the extract of *T. caramanicus Jalas* causes improvement in the process of the oral ulcers healing in the rats.

Keywords: Oral, Ulcer, *Thymus caramanicus Jalas*, Healing.

INTRODUCTION:

Oral ulcers are the most prevalent diseases of the oral cavity, most of which, show similar symptoms, and may appear with damages of specified limits which are covered by Fibrinoleukocytic membrane [1, 2].

Generally, the local and systemic factors are the causes of appearance of the wounds. Oral ulcers may affect the quality of life due to interfere in nutrition, speaking and the social behavior. Sometimes Pemphigus and Pemphigoid, cause the patient to need to be hospitalized [3-5].

Oral ulcers destroy the epithelium which is a natural defensive barrier, and therefore, prepare the environ-

ment as a proper media for the growth of the microbes, and such a matter, may cause intensity of the pain [6]. Depending upon the kind of the wound, the intensity of pain, violence and the size of the lesion, the oral ulcers may heal under local and systemic treatments [7]. From the faraway times, benefiting from the traditional medicine for decreasing the pains in the human has been effective, and, the traditional medications are the most useful resource for treatment [8, 9]. Easy access, persons eagerness for treatment, and such a belief that the traditional treatments are more safe and reliable and chipper than the regular treat-

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doi: 10.15272/ajbps.v5i44.683

Conflict of interest: Authors reported none

ments, are the factors of leaning toward such kinds of treatments [10].

Kermanian thyme with the scientific name of *Thymus Caramanicus* Jalas is a local plant which may be found in Iran. Its main provenance is Kerman province, mainly at Hezar Mountain near Rayen city, Takht Sartashtak near Gishigan village, Sirch, Lalehzar Mountains and around Baft City. Such species may be considered as special kinds of Thyme in Iran and have the most of the properties of Thyme. The main compound of such a plant is Carvacrol. It is indicated that Carvacrol causes Prostaglandin (E1, E2 and F) to be controlled, and also may control inflammation and may decrease the level of destructive processes arising from the same. Furthermore, Carvacrol is of anti-inflammatory effect and causes control of Elastase Neutrophilia enzyme [11]. In the research of Safae and the colleagues, the antioxidant property of the methanol essence and extract of *T. caramanicus Jalas* and the main compound, i.e. Carvacrol, were analyzed, and they showed that the methanol extract of *T. caramanicus Jalas* is of high level of antioxidant effect [12]. In the year 2008, Nejad Ebrahimi and the colleagues showed the high antibacterial effect of *T. caramanicus Jalas* essence, facing with the gram negative bacteria and gram positive bacteria [13]. The research of Nilforushzadeh and colleagues and Hejazi and colleagues indicated that the extract of thyme is, significantly effective on decrease of the size of Leishmaniasis [14,15].

As, no similar study has been conducted regarding examination of anti-inflammatory effect of *T. caramanicus Jalas* up to now, therefore, the present study is conducted with the purpose of analysis of the effect of essence and the extract of *T. caramanicus Jalas* on the process of oral ulcers healing in Rats.

METHOD AND MATERIALS

Essence Preparation

In order to prepare the essence, 100 gr of the powder was carefully weighted which was prepared from the shoots of the plant, and the essence was extracted under the method of hydro-distillation by the application of Clevenger instrument for a period of 3 hours. The resulted essence was dehydrated with dry Sodium Sulfate, and its amount was reported at Volume/Weight percentage V/W. Resulted essence was kept in dark vials at the temperature of 4 degrees of centigrade [16].

Preparation of Hydro-alcoholic Extract

Twigs of the plant became dry in shadow. Next, they turned into powder in a mill with a mesh of 2 mm. In order to extract the essence, 250 gr of the powder

was dissolved in a two-liter flask including 1.5 liters of water and 96% ethanol in the ratio of 20-80, and then the essence was extracted during a period of 4 hours. Extracting was repeated for three times and the resulted extracts were mixed with each other and finally the mixture was condensed with Rotary Evaporator instrument at 30 degrees of centigrade [17].

Animals

In the present study, we used 48 white healthy rats of Vistar race with a weight of 250 to 300 gr. The animals were marked with numbers. They were placed in separate boxes and were kept under identical diet. Animals' room was ventilated under good conditions and the temperature and humidity were controlled, and the room was subject to the standard cycle of 12 hours light and 12 hours darkness. Animal was fastened to the anatomy table. In order to keep the mouth open, a hook-like clasp was attached to the inferior incisor and the string was fixed to the anatomy table. On the first day, all rats were anesthetized with ketamine at the dose of 60 mg/kg and zylazine as muscle relaxant at the dosage of 7.5 mg/kg through in-peritoneal method, and by the application of the punch No. 2, a 2×2 wound was made on the hard palate. In consideration the punch No. 2, the wound had a depth of 2 to 3 millimeters and such a depth included the epithelium and connective tissue [18, 19]. After hemostasis and formation of clotted blood, and drying the wound and the area around it, three groups were created. In the first group, the hydro-alcoholic extract was put on the wound by the application of sterilized swab for a duration of one hour, every 15 hours, and totally for 4 times, and such an action was repeated for the second group with essence and *T. caramanicus Jalas*. No medicinal interference was conducted in the third group. The clinical measurement of the wounds was made through application of a slide-calipers with an accuracy of 0.1 mm by a person who have no information about animal classification, from the first to the eighth days before the daily prescription.

From the group of 16, 8 animals were killed by ether, respectively, on the 2nd, 4th, 6th and 8th days. Then, by the application of the punch No. 5, sample was taken from the wounds of the animals. Such a sample comprised of the muscles around the wound and the healthy side. All samples subject of study were collected and were put inside the similar containers including the same concentration of 10% formalin (ten times as much as the sample volume).

Samples were coded and were sent to pathology lab to be tested. Identic sections were prepared from the samples at the laboratory and the processes of cell tissue passage were conducted by the application of tis-

sue processor, and the paraffin blocks were prepared. 5-micron sections were prepared from the mentioned blocks by microtome, and were stained under hematoxylin and eosin methods [20]. Then the following cases were examined for each sample by two pathologists who had no information about the condition of the samples.

1.Number of polymorphonuclear cells (number/10HPF) (table 2)

2.Number of Fibroblast cells (number/10HPF) (table 3)

3.Inflammation mean (table 4)

4.Size of re-epithelialization area (table 5)

5.epithelium thickness (μm) (table 6)

Groups	Days after oral injury				
	0	2	4	6	8
control	258 \pm 15	257 \pm 14	257 \pm 12	255 \pm 10	254 \pm 12
Extract	263 \pm 11	260 \pm 13	259 \pm 12	261 \pm 11	263 \pm 8
Essence	257 \pm 17	257 \pm 19	255 \pm 15	252 \pm 16	251 \pm 17

Table 1. Body weight of rats

Groups	Days after oral injury			
	2	4	6	8
control	156 \pm 10	105 \pm 13	58 \pm 13	33 \pm 8
Extract	114 \pm 7	56 \pm 12	36 \pm 13	17 \pm 5
Essence	185 \pm 15	100 \pm 18	65 \pm 9	40 \pm 8

Table 2. PMN cells count (number/10HPF)

Groups	Days after oral injury			
	2	4	6	8
control	105 \pm 21	119 \pm 29	135 \pm 31	176 \pm 27
Extract	157 \pm 18	185 \pm 36	327 \pm 26	386 \pm 42
Essence	112 \pm 36	126 \pm 38	143 \pm 56	165 \pm 31

Table 3. Fibroblast cells count (number/10HPF)

Groups	Days after oral injury			
	2	4	6	8
control	2.7 \pm 0.6	2.4 \pm 1	2 \pm 0.8	1.8 \pm 0.6
Extract	1.9 \pm 0.3	1.6 \pm 0.4	1.2 \pm 0.3	1 \pm 0.2
Essence	2.9 \pm 0.5	2.6 \pm 0.9	2.5 \pm 0.7	2.5 \pm 0.7

Table 4. Mean inflammation

Groups	Days after oral injury			
	2	4	6	8
control	2.6 \pm 0.3	2.7 \pm 0.4	2.9 \pm 0.3	3 \pm 0.4
Extract	4.5 \pm 0.5	4.7 \pm 0.7	5.2 \pm 0.8	5.6 \pm 0.8
Essence	2.5 \pm 0.4	2.6 \pm 0.3	2.8 \pm 0.8	2.9 \pm 0.5

Table 5. Mean re-epithelialization

Groups	Days after oral injury			
	2	4	6	8
control	117 \pm 25	128 \pm 16	147 \pm 18	169 \pm 13
Extract	126 \pm 26	169 \pm 35	198 \pm 26	243 \pm 19
Essence	123 \pm 16	129 \pm 18	138 \pm 36	156 \pm 19

Table 6. Mean epithelium thickness (μm)

All processes were performed under supervision of the protocol of Ethics Committee of Kerman University of Medical Science. Animals were killed under full anesthesia and were buried with mortar. Data was written in checklist and then it was entered into computer. In order to analyze, the ANOVA statistical test was applied in a significant level of 0.05%.

RESULTS AND DISCUSSION

Oral ulcers are the most prevalent diseases of the oral cavity and various medications may be used to heal them, most important of which are the anti-inflammatory medications, antimicrobial medications and anesthetic materials [21, 22]. In the present study, we analyzed the effect of essence and extract of Kermanian thyme with the scientific name of *Thymus Caramanicus Jalas* on the oral wound, and the results indicated than the extract of *T. caramanicus Jalas* had a meaningful effect on decrease of the clinical size of the wound from the 4th day in comparison with the control group (Fig 1). Results of the present study agree with the study of Hejazi and colleagues (2009), who examined the effect of Shirazi Thyme extract on healing the skin wounds arising from Leishmaniasis, and therefore, they stated that the thyme extract have a desirable effect on prevention of development of the wounds [15].

Thus, Nilforushzadeh and his colleagues (2008), demonstrated that, *Thymus Vulgaris* extract has a meaningful effect on decreasing the clinical size of the wound, which is created as a result of Leishmaniasis [14]. Motevasel *et.al* (2013) demonstrated that, alcoholic extract of Shirazi thyme leave has an antibacterial property [23]. Because of production of various enzymes by the microbes and the components of the immune system, infection may cause delay in wound healing.

Antibacterial factors may help the process of wound healing, through decrease in the quantity of the bacteria [24]. Because, in various studies, the antimicrobial effect of thyme and its main compounds (Carvacrol and thymol) are proved [18, 25], therefore, such a compound may improve the wound healing effect. In Didry study, it was demonstrated that Thymol and Carvacrol, which are the main components of *T. caramanicus Jalas*, may be effective in cure of the oral infections, because of their antibacterial effects [25]. Such a treatment process may be possible because of anti-inflammatory and antimicrobial properties of Thymol, which may improve the process of wound healing through control of the harmful inflammatory effects of immune system [26].



Fig 1: wound healing in treatment with extract of *T. caramanicus Jalas* (1st, 4th and 8th day, respectively)

Wound healing starts from the beginning moment of damage, and, therefore, the clinical size of the wound has been studied in different days. In the present study, it was demonstrated that, on the 4th (Fig 2), 6th and 8th (Fig 3) days, a meaningful decrease in the clinical size of the wound was observed in extract group in comparison with the essence group, but, in essence group, the clinical size of the wound was larger than that of the control group in all days (Fig 4). This may be because of the high concentration of essence and should be paid into attention in the further researches.

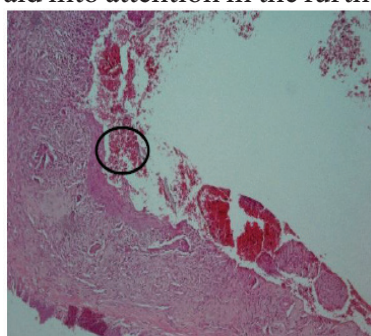


Fig 2: Low-power photomicrograph of rat palate. 4th day of treatment with extract of *T. caramanicus Jalas*.

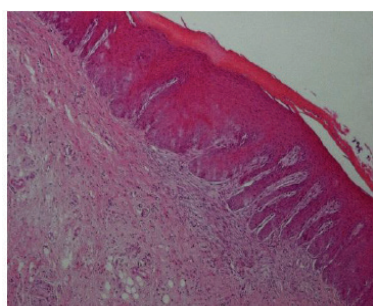


Fig 3: 8th day of treatment with extract of *T. caramanicus Jalas*.

Karkabounas demonstrated that the anti-accumulative property of the various antioxidants may be resulted because of neutralization of the free radicals produced in cyclooxygenase course, and may decrease the level of production of thromboxane A2 and the platelet receptors in fibrinogen molecules [27], and, as, *T. caramanicus Jalas* is of high level of antioxidant property, such a compound may improve the wound healing [12].

Furthermore, it has become distinct that Carvacrol may improve the wound healing and may moderate the inflammatory processes through control of Elastase neutrophil enzyme, control of platelet accumulation and decrease in production of the free radicals [27]. One of its most important properties is the antioxidant property of thyme. In various researches it has been demonstrated that, production of free radicals plays a significant role in inflammatory processes [28].

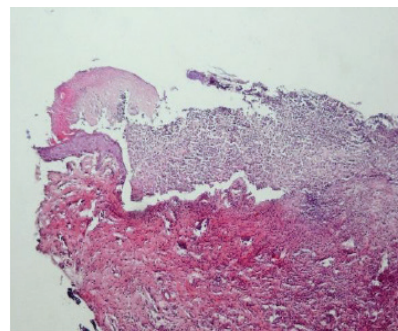


Fig 4: 8th day of treatment with essence of *T. caramanicus Jalas*

CONCLUSION

Results of the present research demonstrated that the extract of *T. caramanicus Jalas* may improve the process of wound healing in rats. In consideration of the effect of *T. caramanicus Jalas* in the process of oral wound healing, it is suggested that the effect of *T. caramanicus Jalas* to be studied and examined in the researches as oral mucus patch and mouthwash. Furthermore, as there is a rich level of essence component in a study of various concentrations, therefore, it is suggested to study on the effect of *T. caramanicus Jalas* Essence in wound healing.

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Cite this article as:

Molok Torabi, Maryam Aminizadeh, Hossein Karimiafshar, Majid Asadi, Mehrad Afzali. Study on the Effect of Essence and Extract of *Thymus caramanicus* Jalas on the Process of Oral Wound Healing in Rats. Asian Journal of Biomedical and Pharmaceutical Sciences, 5(44), 2015, 1-5.
