

# Research Paper: Systematic Evaluation of Effect of Myrtle and Fenugreek on Initial Inflammatory Response of Lingual Muscles to Different Types of Suturing Material in Diabetic Rats



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## ABSTRACT

**Introduction:** Several studies have been conducted to examine the effect of suture material on the skin during healing of the wound while a few articles reviewed the musculoskeletal response. In this study, the effects of the two plants, myrtle and fenugreek, on the primary inflammatory response of the tongue muscles to the types of suture in diabetic rats are examined.

**Materials and Methods:** For conducting this study, 96 Wistar healthy male white rats weighing between 250 and 300 g were selected. The animals were divided into 3 groups, 32 each, consisting of the diabetic group, the diabetic-myrtle group, and the diabetic-fenugreek group. All the animals became diabetic two weeks before the study using Streptozotocin (STZ). For carrying out the task, all animals were anesthetized on the first day using ketamine with a dosage of 60 mg/kg and xylazine (Rampon) with a dosage of 5.7 mg/kg. Four wounds were made using No. 15 blade, and each wound was sutured using one sample of suture string (simple interrupted suture). Then, in the myrtle and fenugreek groups, these materials were placed on the sutures using swabs. After this, from each group, 8 animals were sacrificed using ether. On days 2, 4, 7 and 14, samples were taken from the suture sites with intact margins and were sent to the pathological lab, and the degree of inflammation was examined using a light microscope with magnifications of 40 and 100. At last, the data were analyzed using Wilcoxon, post hoc ( by SPSS version 13.5).

**Results:** This study showed that the least amount of inflammation on all days was associated with silk string followed by plain catgut in the diabetic group, and there was a significant relationship ( $P=0.001$ ) in that regard with the two other suture strings used; chromic catgut and monofilament polyamide. In the myrtle group, chromic catgut and monofilament polyamide inflammation were not significantly different as compared with the other two suture strings in the control group on all days of the study. The findings showed that in the fenugreek group and the control silk and monofilament polyamide, the inflammation severity was not significantly different from the two suture strings in the control group on all days of the study.

**Conclusion:** The findings of this study showed that the two plants myrtle and fenugreek reduce the inflammatory response in plain catgut though the effect of myrtle was more significant. Myrtle is also effective in reducing inflammation in silk while fenugreek is effective in reducing the inflammation caused by chromic catgut.

## Keywords:

Fenugreek, Myrtle, Diabetes, Suture string

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## 1. Introduction

**H**ealing of muscular tissue after surgery depends on the internal and external factors that induce an inflammatory response. The innate response is the normal immune response to the injury while external factors include inflammatory response to the presence of a foreign body such as suture in place of tissue repair. Therefore, one of the important factors that should be considered in surgery is the biocompatibility of suturing materials. Choosing the best suturing material for surgery is substantial and significant [1].

Studies have shown that most surgical procedures are done in the oral and maxillofacial region vitiate and create defects in the oral and facial muscle tissue because of a variety of surgical errors. Similarly, defects such as jaw pressure on masticatory muscles make the surgeons restore the situation to almost normal in terms of anatomy and function. Reconstruction surgeries of orofacial clefts are the most special surgeries where a high priority is given to repairing muscles, because it dictates the beauty and function of the mouth and face, and has a big impact on speaking, mastication, swallowing, as well as the quality of life [2, 3].

Muscular tissue regeneration is different from other tissues because of the presence of satellite cells, that are precursor cells for myosis, and it is located between the basal layer and plasma membrane. These cells proliferate and differentiate into multi-nucleated myotubes, and eventually transform into myofibers. The interaction between inflammatory cells and skeletal muscle cells could have an impact on proliferation, differentiation, and damage of muscle cells [4]. Tidball et al. [1] studied the effect of macrophages in skeletal muscle recovery. They concluded that macrophages have a drastic role in muscular tissue repair and regeneration by using growth factors and cytokines.

Inflammatory response caused by suturing material is one of the most important factors that affect the healing process. Yaltirik et al. suggested in a study that an indirect method of evaluation of healing process could be done by studying the inflammatory response of tissue specimens [5]. In this study, they used nylon suture, polydioxanone, catgut, and polygalactic because they are all used for amending tissue [5].

Today, there are many herbal combinations rendered for the treatment of many diseases, including skin diseases. It is customary to use herbal medications for skin

injuries such as wounds and burns after conducting various studies and proving the effectiveness of those medications. Modern medicine has approved the beneficial effects of plants such as aloe, calendula, and lavender in healing wounds and burns [6-9].

Several studies have been conducted to examine the effect of suturing material on the skin during closure and healing of wounds [4-6] while a few studies investigated the response of skeletal muscles [7]. Thus, the experts suggest more exploration in the field of oral tissue reactions. The oral environment is different from other parts of the body in term of special flora and biological conditions. Based on the literature, no research has been conducted on the muscle response in diabetic patients. In this study, we will evaluate the effect of fenugreek and myrtle extract on the initial inflammatory response of lingual muscles of diabetic rats to different types of suturing materials.

## 2. Materials and Methods

### Supplying and preparing herbal extracts

The plants were collected from their habitat and fenugreek seeds were purchased from the market. After verification of the scientific name of each plant, fenugreek was mixed with water and myrtle was mixed with methanol 80%, and the extract was prepared by maceration for 72 hours. The obtained extracts were concentrated by vacuum distillation, and stored at 20°C until the start of the test [10]. Two subsequent rounds of filtration were used as an effective method of sterilization.

### Animal sample preparation

For this purpose, 96 Wistar rats weighing between 250 to 300 g were selected. Animals were divided into 3 groups, and each group contained 32 members (groups are as follow: diabetic, diabetic and myrtle, diabetic and fenugreek). Groups with 8 members each were kept in a separate glass cage; we fed the animals with the same soft diet. We made animals diabetic two weeks prior to the conduction of the study by intraperitoneal injection of streptozotocin (Chemical Co., St. Louis, Mo, USA Sigma) (60 mg/kg in 0.2 mL of 10 mM). The blood sugar of each animal was checked daily by using a digital device (Easy Gluco made by Infopia in South Korea), and when the blood sugar level was above 250 mg on two consecutive days, the animals were diabetic and ready for the research [11]. The type of diabetic rats used in the current study is the same as those used in the study conducted by Romana et al. where streptozotocin induced

diabetes, and it would bring about the pathological characteristics of long-term diabetes in human beings. The rates also exhibit the same physiological signs as human beings like increase in blood sugar level, weight loss, and body malaise [6].

The limbs of animals were tied to the dissecting table, and a hook-like clamp was inserted around the upper incisor teeth of the animals, and the thread attached to the clamp was fixed on the table so that the mouth would be kept open. For conducting the research, all 96 animals were injected with ketamine (dosage: 60 mg/kg) for general anesthesia and xylazine as a muscle relaxant [3]. Simple incisions were made on the tongue of each animal (with a depth of two millimeters), and each incision was sutured (simple interrupted suture) using different suturing materials. Sutures used chromic catgut (SUPA company c63026b3), plain catgut (Huaiyin Medical Instruments Co. Ltd. Made in China-030805), monofilament polyamide (SUPA) and silk (SUPA company S630300B3) (Figure 1).

In the fenugreek and myrtle groups, we used cotton swabs to apply the extracts on the sutured lesions. These procedures were continuously performed until the end of the study. Then, from each group, 8 animals were sacrificed, on days 2, 4, 7, 14 by ether, and a sample of the sutured section with healthy margins was removed and fixed in formalin 10%.

The specimens were taken to a pathology laboratory and after applying paraffin, they were colored using hematoxylin & eosin. After this, the specimens were evaluated by a pathologist who was not aware of the groups of the specimens, using a light microscope



**Figure 1.** Sutures with chromic catgut, plain catgut, monofilament polyamide and silk

(magnification 40 and 100) [12]. The degree of inflammation in this study was based on 0=no acute inflammation, 1=perivascular scattered acute inflammatory cells, 2=perivascular and submucosal scattered acute inflammatory cells, 3=submucosal band-like inflammatory infiltrate, less than 1/4 of one power field, 4=submucosal band-like inflammatory infiltrate, between 1/4 and 1/2 of one power field without tissue necrosis and 5=submucosal band-like inflammatory infiltrate, more than 1/2 of one power field with tissue necrosis [12]. This research is based on the international law of animal ethics (Helsinki), and was performed under the supervision of the Ethics Committee of Kerman University of Medical Sciences. The data were then analyzed using Wilcoxon, post hoc (by SPSS13.5). The statistical significance used in this study was  $P < 0.05$ .

### 3. Results

The study examined 3 groups that are as follows: Group 1: diabetic animals that did not receive any medication; Group 2: diabetic animals that received fenugreek extract; Group 3: diabetic animals that received myrtle extract. The study showed that the least severity of inflammation was seen in lesion sutured by a silk suturing material and after that in the lesion sutured by plain catgut, and there is a significant relationship between two other suturing materials in this field (monofilament polyamide and chromic catgut) ( $P=0.001$ ).

Table 1 shows the severity of inflammation in the lingual muscle of rats that was sutured with silk suturing material in diabetic rats and diabetic rats that received myrtle extracts. The severity of inflammation was the same in both groups on the 4<sup>th</sup> day, but on the 7<sup>th</sup> and 14<sup>th</sup> day, it is less in the group that received myrtle extract compared to the control group, and there was a significant relationship found in this field.

This study shows the severity of inflammation in the lingual muscle of rats that were sutured with plain catgut in groups of diabetic rats and diabetic rates that received myrtle extract. The severity of inflammation decreased in the group that received the myrtle extract compared to the control group, and there is a significant relationship in this field. This study shows no significant difference in the severity of inflammation in rats that were sutured with monofilament polyamide and chromic catgut in the group of diabetic rats that received myrtle extract and the control group. In other words, myrtle extract could not decrease the inflammatory response when we used the abovementioned types of suturing materials (Table 1).

**Table 1.** Severity of inflammation of the tongue muscles of diabetic rats in myrtle plant with respect to the control group

	Days	Silk Suture						P
		Decrease		Increase		Equal		
		No <sup>£</sup>	%	No <sup>£</sup>	%	No <sup>£</sup>	%	
	Second	1	12.5	1	12.5	6	75	0.41
	Fourth	4	50	0	0	4	50	0.12
	Seventh	5	62.5	0	0	3	37.5	0.04*
	Fourteenth	5	62.5	0	0	3	37.5	0.04*
Plain catgut suture	Second	5	62.5	0	0	3	37.5	0.001*
	Fourth	6	75	0	0	2	25	0.001*
	Seventh	8	100	0	0	0	0	0.001*
	Fourteenth	8	100	0	0	0	0	0.001*
Chromic catgut suture	Second	0	0	0	0	8	100	0.12
	Fourth	1	12.5	0	0	7	87.5	0.41
	Seventh	1	12.5	0	0	7	87.5	0.41
	Fourteenth	2	25	0	0	6	75	0.41
Monofilament polyamide	Second	0	0	0	0	8	100	0.12
	Fourth	1	12.5	0	0	7	87.5	0.41
	Seventh	1	12.5	0	0	7	87.5	0.41
	Fourteenth	2	25	0	0	6	75	0.41

£: Number of animals, \* P<0.05 is significant.



The result of this study shows no significant difference in the severity of inflammation in rats that were sutured with monofilament polyamide and silk in the group of diabetic rats that receive fenugreek extract and the control group. In other words, myrtle extract could not decrease the inflammatory response when we used the mentioned types of suturing materials. The severity of inflammation decreased on the 7<sup>th</sup> and 14<sup>th</sup> day in the group of diabetic rats that received fenugreek extract compared to the control group, and there was a significant relation found in this field (Table 2). Finally, the results obtained in this study show that fenugreek extract and myrtle extract could decrease the inflammatory response in the group of rats sutured with plain catgut although the impact of myrtle is more than that of fenugreek, but there is no significant relationship in this field (P=0.31) (Figure 2).

Fenugreek extract and myrtle extract did not have any impact on the inflammatory reaction in the group of rats that were sutured using monofilament polyamide suturing materials. In other words, they did not decrease the inflammation. Myrtle extract decreased inflammatory response in the group of rats sutured by silk material and fenugreek extract decreased the inflammatory response in the group of rats sutured using chromic catgut material.

#### 4. Discussion

Sutures have an important role in the wound healing process after operation; therefore, it is important to choose the best suturing material. This matter is quite important in the oral cavity as the oral cavity is different from other parts of body because of the constant presence of saliva, specific microbial flora, and its vascularization [13].

Several factors like diabetes, malignancy, infection, malnutrition, obesity, previous surgery, use of corticosteroids and type of surgical technique are effective in wound healing [14, 15], of which the best known is diabetes [16]. Although diabetes is a chronic progressive disease, it can lead to failure in tissue repair, and might induce defects in immune cells [15].

Inflammatory response could prevent the wound healing process and make the wound susceptible to infection. Therefore, the ideal suturing material should have enough tensile strength and cause an inflammatory response with limited intensity and duration [15]. Several studies were conducted to evaluate the effect of suturing materials on wound closure and the wound healing process [12, 15,

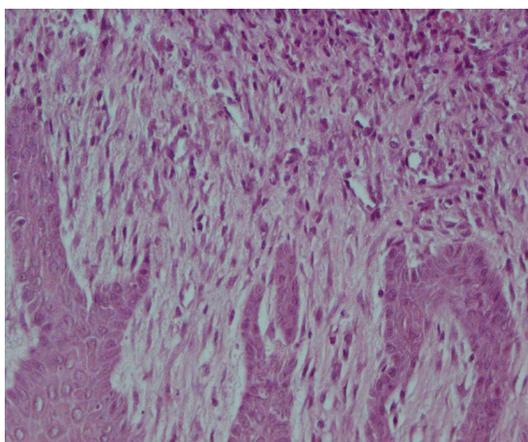
**Table 2.** Severity of inflammation of the tongue muscles of diabetic rats in fenugreek plant with respect to the control group

	Days	Silk Suture						P
		Decrease		Increase		Equal		
		No <sup>£</sup>	%	No <sup>£</sup>	%	No <sup>£</sup>	%	
	Second	1	12.5	1	12.5	6	75	0.21
	Fourth	0	0	0	0	8	100	0.12
	Seventh	1	12.5	0	0	7	87.5	0.41
	Fourteenth	1	12.5	0	0	7	87.5	0.41
Plain catgut suture	Second	1	12.5	1	12.5	6	75	0.21
	Fourth	2	75	2	25	4	50	0.28
	Seventh	5	62.5	0	0	3	37.5	0.03*
	Fourteenth	7	87.5	0	0	1	12.5	0.001*
Chromic catgut suture	Second	1	12.5	1	12.5	6	75	0.12
	Fourth	2	25	1	12.5	5	62.5	0.41
	Seventh	3	37.5	2	25	3	37.5	0.41
	Fourteenth	6	75	0	0	2	25	0.41
Monofilament polyamide	Second	0	0	0	0	8	100	0.12
	Fourth	1	12.5	0	0	7	87.5	0.41
	Seventh	1	12.5	0	0	7	87.5	0.41
	Fourteenth	1	12.5	0	0	7	87.5	0.41

£ Number of animals, \*P<0.05 is significant.

[16] while very few ones examined the response of muscles of oral cavity to suturing material [12].

This study showed that the minimum inflammatory response was observed in samples that were sutured using silk and plain catgut suturing material. Silk is naturally made up of a braided non-absorbable protein called fibroin, which is produced by silkworm larvae. The silk suturing material does not result in considerable inflammatory response of the body tissue; therefore, it is



**Figure 2.** The inflammatory response in the group using plain catgut

considered as the gold standard [1, 17]. The presence of protein in silk texture would cause a minimal body reaction and collagen production would be better [18, 19]. Although, some studies showed that silk suturing material caused the formation of keloid [20, 21], and this suturing material is susceptible to bacterial invasion and induces severe tissue inflammatory response [21].

This study showed that the maximum inflammation was observed using monofilament polyamide and chromic catgut which was in agreement with the Bhargava et al. study results [12]. On the other hand, other studies done by Silva et al. [22] and Hochborg et al. [6] showed that fibrosis and healing process was maximum in groups sutured using a nylon suturing material, which could be a trial for fibrous encapsulation.

Among all suturing materials, chromic catgut is mostly used for closing wounds in veterinary medicine. Chromic catgut is a poly-filament, bio-absorbable suturing material that is made by twisting collagen fibers around each other and coated with chromium salts in order to increase the tensile strength, accelerating the absorption rate, and increasing biocompatibility [17].

Studies have shown that chromic catgut is a biological suturing material that can induce a serious inflammatory

response [15, 19, 23, 5]. A study conducted by Lily et al. [24] showed that increased inflammatory response in sutured cases using polyfilament suturing material is due to the presence of bacteria in between the strands of the suturing material. They considered capillary phenomenon as the main reason of movement of saliva and bacteria along the polyfilament suturing materials. The current study showed that plain catgut suturing material caused negligible inflammation after silk, which is in agreement with the other studies [12], and that inflammation decreases after using monofilament catgut suturing material. Although Bhargava et al. [12] showed the second suturing material in terms of severity of inflammation is plain catgut, which is not in agreement with the current study. Plain catgut suture induces a less inflammatory response in oral environments compared to different parts of the body, and the response of muscle tissue to the foreign body could be a reason for the mismatch.

The tensile strength of plain catgut suturing material would be expunged in a short time, and muscle tissue needs more time of closure for healing. For some patients, the appearance of healed tissue is more important than the original disease. Therefore, choosing the suturing material should be commensurate with the type and location of the wound and the status of the patient. All suturing materials are not appropriate for different uses, and we need to determine the most appropriate suturing material based its use. Nowadays, using suturing materials that release anti-inflammatory and anti-bacterial drugs in the wounds could be a solution for reducing the risk of inflammation and infection and speed up the healing procedure.

Finally, the results obtained in this study show that fenugreek extract and myrtle extract decrease the inflammatory response in a group of rats that were sutured with plain catgut although the impact of myrtle is more than that of fenugreek, but there is no significant relationship here. Myrtle extract decreased the inflammatory response in a group of rats that were sutured using silk suturing material and fenugreek extract decreased the inflammatory response in a group of rats that were sutured using chromic catgut suturing material.

Myrtle (*Myrtus communis* Linn. from Myrtaceae family) is a traditional medicine used as a disinfectant, anti-septic and for lowering blood sugar. This plant is highly regarded because of its anti-inflammatory, anti-viral, decontamination, and disinfection property. The fruits of this plant are also used for diarrhea, dyspnea, internal ulcers, rheumatism, foot ulcers, funky wounds, vesicles, sinusitis, hair loss, bleeding, and bronchitis [25].

Fenugreek (*Trigonella foenum-graecum* L.) is a plant whose seeds and leaves are used in traditional medicine. Fenugreek has many pharmacologic effects such as hypoglycemic, hypocholesterolemic, anti-oxidant, and appetite stimulation effects. In addition, the aqueous extracts of fenugreek seeds have a protective effect against ethanol toxicity. This plant is used in traditional Persian medicine for its hypoglycemic and anti-rheumatic effects [7]. The presence of saponins and flavonoids as the main component in fenugreek almost accounts for its anti-inflammatory effect. Flavonoids are potential inhibitor of cyclooxygenase and lipoxygenase synthase, and anti-pyretic and anti-inflammatory activity of fenugreek leaves has also been reported [17].

Thakur et al. [7] reported significant anti-inflammatory activity of the ethanol extract of this plant. A recent report showed that the ethanol extract of fenugreek seeds causes an increase in the number of macrophages, which indicates its action through activation of macrophages. Studies done by Sharififar et al. [7, 8] showed that these plants have strong anti-inflammatory, anti-oxidant and anti-bacterial effects. In some cases, it would have an analgesic effect and it could have a drastic effect on wound healing because it contains diverse compounds. Ghassemi et al. and Hashemipour et al. showed that fenugreek and myrtle has a dramatic effect on wound healing in the oral cavity of rats [25, 26].

## 5. Conclusion

The results of this study showed that myrtle and fenugreek reduce the inflammatory response in plain catgut groups, and in this case, the effect of myrtle is more considerable than that of fenugreek. Myrtle and fenugreek had no significant effect on monofilament polyamide suturing material. Myrtle has a significant effect on the reduction of the inflammatory response induced by silk suturing material and fenugreek has a significant effect on the reduction of the inflammatory response induced by chromic catgut.

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## Conflict of Interest

The authors declared no conflicts of interest.

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