

The Effects of Asphodel (*Asphodelus aestivus* Brot.) Rhizome Extract Administration in the Treatment of Thermally Induced Wound in Rats

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ABSTRACT

Background: Burn wound is an important health problem worldwide. Serious outcomes related with burn occur and treatments focus on healing of the wounded area with dermal preparations containing chemicals avoiding growth of pathogenic organisms. Plants are important sources for both modern pharmacy to find new molecules used in modern medication as well as in traditional medicinal practices. Many plants are used for treatment of burn wounds. Asphodel (*Asphodelus aestivus* Brot.) is a naturally found plant in the Mediterranean flora. It is also used in traditional medicinal practices in Turkey.

Aim: In this study it was aimed to test wound healing effect of Asphodel. **Materials and Methods:** An ointment prepared with a 5% methanolic extract of Asphodel was used. 4 groups were formed as control, wound, wound + asphodel and wound + standard drug (oxytetracyclin hydrochloride) ($n=8$ in each group). Burn wound was established with a thermal induction. Biochemical, microbiological and histopathological evaluations were performed. **Results:** Biochemical results (Alt, Ast, Glucose, Ldh) obtained from blood samples showed no adverse effect of this administration. Microbiological results showed a protective effect of Asphodel similar with standard drug. Histopathological evaluation also presented an ameliorating effect as standard drug. **Conclusion:** Results of this study states that traditional use of this plant for the treatment of burn/wound/ulcer in traditional medicine applications is valid.

Key words: Burn, Asphodel, *Asphodelus*, Rat, Skin, Ethnopharmacology.

INTRODUCTION

Plants provide both important chemicals for modern chemical therapeutic methods and for traditional folk medicine treatments. According to fossil records humans have been using plants as medicines at least for 60,000 years.¹ Modern medicine brought effective treatments for diseases which devastated various ancient populations, however, almost more than 80% of the World's population still depends on traditional medicinal practices for certain skin disorders.^{2,3} Number of plant species used in the world for medical purposes are estimated in a range between 50,000

to 70,000.⁴ *Asphodelus aestivus* Brot. (Xanthorrhoeaceae) which is a species of Mediterranean element is defined from Portugal and are seen in northwest, east and west parts of Turkey.⁵ Relevant information about the healing effect of wounds with Asphodel (*Asphodelus aestivus* Brot.) among these species can be found in the records of Ibn-i Sina (Avicenna). There are records for Asphodel for treatment of wounds and eczema in ethnobotanical studies carried out in various regions of Turkey.⁶ Tuzlaci and Aymaz recorded that it is also used against burn and hemorrhoids as well as for wound healing in Balikesir-Gonen, Northwestern-

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Turkey.⁷ In another study, Asphodel was reported for wound healing around Manisa-Yunt Mountain, Western Turkey.⁸ Asphodel was used against skin diseases in Cyprus and Spain⁹ in a similar manner. However, no scientific study has been carried out in an animal model to test the effect of this species on wound healing. In another study, it has been shown that the Asphodel has an anti-ulcerogenic effect.¹⁰ When the ethnobotanical data and the anti-ulcerogenic activity are evaluated together, it can be thought that the wound healing of the Asphodel requires scientific investigation. In addition, the antimicrobial effect of another species of Asphodel (*Asphodelus tenuifolius*) was studied and ethanol, methanol and aqueous extracts were observed to exert similar activity.¹¹

For wound healing, in general the skin is shaved in rats or rabbits and a wound is formed as a mechanical wound by means of a biopsy instrument or as a thermal wound with a hot object or setup.^{12,13} In wound treatment, it is aimed to prevent all or part of the damage in the tissue to be ameliorated and to prevent further deterioration of the damaged skin by pathogens.¹⁴ In addition avoiding bacterial growth is another target of wound healing treatment. Studies on pathogenic micro-organisms causing wound infections have been performed in the World.¹⁵ Aim of this study is to examine therapeutic effect of *Asphodelus aestivus* Brot. following a thermal wound in rats.

MATERIALS AND METHODS

Following approval from Animal Experiments Ethics committee a total of 32 female Wistar albino rats were used. Rats in Van Yuzuncu Yil University Experimental Medicine Research Facility were divided into 4 groups, each consisting of 8 individuals. After shaving of the hair in the dorsal part of rat, wound was formed with heat under anesthesia. For this application, a cylindrical metal with a diameter of 2.5 cm was placed in the water at 100°C for 30 min and then contacted for 10 sec to the dorsal skin without pressure.¹² Subcutaneous (SC) 10 ml/kg of saline was injected to compensate the loss of fluid in the rat after the burn. This SC administration was applied away from injury site.

Asphodel specimen was collected in 2017 from Southern Turkey (C5 Adana): Sarıçam, Çukurova University Campus, surroundings of dam lake, road side, *Pinus* openings, 37° 03' 54" N, 35° 21' 31" E, 70 m, 08.03.20016, H. Eroğlu 1218 and a voucher specimen was formed in Van Yuzuncu Yil University Herbarium (number 164102). Dried underground parts of plant were extracted with methanol in a soxhlet extractor for

48 h. Methanol at the end of the extraction method was purged by rotary evaporator. Then, concentrated under reduced pressure and finally freeze-dried at -40°C. The yield of this process was 16.6 %.

Group 1 (Control Group, $n = 8$): No wound was formed. The tissues were collected and compared with the other groups. Group 2 ($n = 8$): No treatment was performed after wound formation. Group 3 (Wound+Asphodel Group, $n = 8$). After the wound was formed, the ointment prepared with *Asphodelus aestivus* extract (5% in an inert wax) was applied topically to the wound area twice a day. Group 4 (Wound+Standard Drug Group, $n = 8$). After the wound was formed, the standard wound cream (antibiotic ointment containing oxytetracyclin hydrochloride) was applied topically to the wound area twice a day. Preparation of 5% extract ointment was performed according to our previous preliminary experiences on the subject.

After the wound formation, this administration was continued for 12 days and skin samples were incised from rats under anesthesia for histopathological and biochemical analysis at the end of 12 days. Macroscopic and microscopic findings were compared between the groups. In addition, swab samples were taken from the wound area on the 5th and 10th days for the examination of the antibacterial properties of the ointment prepared with extract and evaluated in the microbiology laboratory. In addition to these assays, blood samples were taken for biochemical analysis.

Histopathological Method

Skin tissue samples taken for histopathological evaluation of necropsy were fixed in 10% formalin solution for 48 hr. Following routine tissue procedures, they were embedded in paraffin blocks. 4µm thick sections were taken from each block. Preparations for histopathological examination were stained with hematoxylin-eosin (HE) and examined with light microscopy (Leica DM 1000, Germany). Sections were evaluated semi-quantitatively as non-positive (-), mild (+), moderate (++) and severe (+++).

Microbiological Method

The experimental animals were divided into three groups (2, 3 and 4) for microbiological evaluation. Swab specimens were taken from wound sites twice on 5th and 10th days. The swab sampling was obtained from the area up to the site joining with the intact tissue region of the wound site and directly sown into the Shadler Broth. Shadler Broth was incubated at 37°C for 24-48-72 hr. From tubes in which bacterial growth is observed inoculations were performed into 5% sheep blood agar,

McConkey agar, Eosin Methylene Blue agar (EMB) and incubated at 37°C for 24-48-72 hr. Colony morphology, hemolysis properties, gram staining, catalase test, oxidase test, lactose activity, PYR test (L-pyrrolidonyl- β -naphthylamide hydrolysis activity), coagulate test and color rendering properties in EMB agar were taken into consideration. Identifications were performed in Vitek II device (bio Merieux) following incubation in 5% sheep blood agar at 37°C for 24-48-72 hr.

Biochemical Analysis

Following anesthesia blood samples were obtained from venous blood into special biochemical sampling tubes. Alanine transaminase (ALT), aspartate amino transferase (AST), glucose and lactate dehydrogenase (LDH) were measured with automatic sampling device for biochemical tests. Animals were not fasting during blood sampling.

Statistical Analysis

In the evaluation of data, Kruskal-Wallis and Mann Whitney U tests for evaluation of continuous variables and other relevant tests for semi-quantitative results were used. SPSS 20 software program was used for analysis.

RESULTS

Histopathology results

When the skin tissues of the rats in Control Group were examined, the epidermis and dermis layers were found to have normal histological structure (Figure 1a). In the skin tissues of rats in Wound Group, there was an intense necrotic mass in the wound area and a mononuclear cell infiltration composed of neutrophils. Epithelial regeneration was started and the presence of a loose collagen was determined. The granulation tissue was found to develop slightly (Figure 1b). Most of the necrotic mass in the skin tissues of Wound+Asphodel Group was cleared. It was determined that granulation tissue and angiogenesis was developed intensely, a tight collagen tissue was developed and epithelial regeneration progressed considerably (Figure 1c).

When compared with wound group, it was determined that epithelial regeneration, angiogenesis, granulation tissue, inflammation and collagenization were significantly increased ($p < 0.05$). The necrotic mass was completely cleared in Wound + standard drug Group. It was determined that a chronic granulation tissue and angiogenesis was developed and a tight collagen structure was formed. Epithelial regeneration was well established (Figure 1d). When compared with the wound group, there was a statistically significant increase in epithelial regeneration, angiogenesis, granulation tissue, inflammation and collagenization in Wound+Asphodel group ($p < 0.05$). Histopathological findings are summarized in Table 1.

Microbiological Findings

Staphylococcus epidermidis and *Escherichia coli* were observed in all 3 burn groups. However in lone burn wound group which had no treatment (2nd group) *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium* were

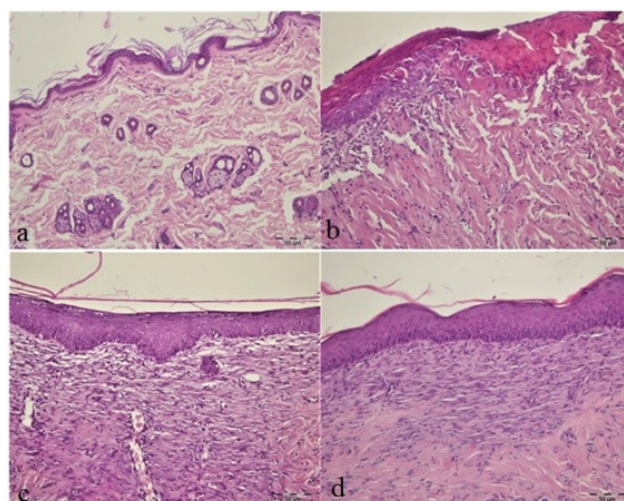


Figure 1: Histopathological findings of four groups. H&E, Bar: 50µm.

a) Control group, Normal histopathological structure. b) Wound group, Necrotic mass, mononuclear cell infiltration, a recent epithelial regeneration, a loose connective tissue in dermis in wound area were observed. c) Wound+Asphodel Group, well developed epithelial regeneration, tight connective tissue in dermis, light mononuclear cell infiltration, strong angiogenesis was observed. d) Wound+standard drug Group, well developed epithelial regeneration, a collagenization that began to mature in the dermis, a slight infiltration of mononuclear cells were observed.

Table 1: Histopathology evaluation table. Sections were evaluated semi-quantitatively as non-positive (-), mild (+), moderate (++) and severe (+++).

	Control Group	Wound group	Wound + Asphodel Group	Wound + Standard drug Group
Connective tissue proliferation	-	++	+++	+++
Mononuclear cell infiltration	-	++	+++	++
Angiogenesis and Collagenization	-	++	+++	+++
Epithelial proliferation	-	++	+++	+++

Table 2: Results of isolated and identified bacteria from wound samples. Results of isolated and identified bacteria species from wound samples are listed according to each individual rat. A.N. (Animal Number in each group).

A. N.	Wound Group		Wound + Asphodel Group		Wound + standard Drug Group	
	5 th day	10 th day	5 th day	10 th day	5 th day	10 th day
1	<i>S. epidermidis</i>	<i>E. coli</i>	<i>E. coli</i>	<i>S. epidermidis</i>	<i>E. coli</i> + <i>S. epidermidis</i>	<i>S. epidermidis</i>
2	<i>S. epidermidis</i>	<i>E. coli</i>	<i>E. coli</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>S. epidermidis</i>
3	<i>S. epidermidis</i>	<i>E. coli</i>	<i>E. coli</i>	<i>S. epidermidis</i>	<i>E. coli</i> + <i>S. epidermidis</i>	<i>S. epidermidis</i>
4	<i>S. aureus</i>	<i>S. aureus</i>	<i>E. coli</i> + <i>S. epidermidis</i>	<i>E. coli</i>	<i>E. coli</i>	<i>S. epidermidis</i>
5	<i>E. faecalis</i>	<i>E. coli</i>	<i>E. coli</i> + <i>S. epidermidis</i>	<i>E. coli</i>	<i>S. epidermidis</i>	<i>S. epidermidis</i>
6	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>E. coli</i>
7	<i>E. faecium</i>	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>E. coli</i>	<i>S. epidermidis</i>
8	<i>E. coli</i>	<i>E. coli</i>	-	-	-	-

Table 3: Biochemical results of groups. ALT: Alanine aminotransferase U/L, AST: Aspartate aminotransferase U/L, GLU: Glucose mg/dL, LDH: Lactate dehydrogenase U/L. No statistically significant difference was found between groups in listed parameters. $p > 0.05$.

	ALT	AST	GLU	LDH
Control	37.2	87.3	158.8	571.8
Wound	30.8	77.4	143.4	555.2
Wound + Asphodel	37.5	93.3	166.3	897.8
Wound + Standard drug	47.2	104.8	172.3	877.0

also observed in addition to *Staphylococcus epidermidis* and *Escherichia coli*. The results of the bacteria isolated from the wound area are given in Table 2.

Biochemical Results

There was no significant difference between the groups in terms of biochemical results. Although statistically insignificant, highest ALT and AST results were obtained in standard drug group. With the administration of Wound + Asphodel or Wound + Standard drug Glucose and LDH levels rise compared to control and wound groups ($p > 0.05$) (Table 3).

DISCUSSION

This study was aimed to evaluate wound healing property of Asphodel which is used in traditional medicinal practices for burn wounds.

Histopathological evaluation shows that the thermally generated model has been successfully applied.

Decrease in necrotic mass, enhancement of granulation tissue and angiogenesis in plant extract and standard drug treated groups showed that the improvement in both groups was significantly increased compared to the untreated burn group. This finding shows that its ethnopharmacological use is relevant. Ethnopharmacological use of *A. aestivus*, *A. luteus*, *A. microcarpus* and *A. tenuifolius* in skin disorders is in parallel with our histopathological findings.^{16,17} Although rat skin shows some certain differences in skin structure such as its loose skin and difference in Vitamin C metabolism which is important for collagen formation, it is still a widely used model for wound healing.¹⁸ Therefore our results should be evaluated as an animal model and not as direct testing of ethnopharmacological knowledge. In similar studies focusing on burn wound healing, ethnopharmacological applications guides researchers to test their effectiveness in modern scientific methodology.

Microbiological results of our study show that number of bacteria present on wound was attenuated with administration of standard drug as well as Asphodel ointment. This situation states a possible antimicrobial activity of Asphodel in this burn model. Microbiological evaluation following a burn was investigated by researchers in both experimental models as well as in field studies. In a study conducted by Bayram Y *et al.*¹⁹ between 2009 and 2011 on 179 patients, 250 bacteria were isolated from the wound swab samples. In burn units over a 6-month period in Iran *P. aeruginosa* (72.7%), *S. aureus* (14.4%) and *Staphylococcus epidermidis* (7.8%) were isolated from 82 biopsy specimens obtained from 82

patients who were hospitalized.¹⁵ In a study by Agnihotri N *et al.* between 1997 and 2002, they isolated 665 bacteria from burn wounds. The most isolated bacteria were *P. aeruginosa* (392) and *S. aureus* (119).²⁰ In a study in India, 336 burn wound specimens were evaluated. 54.16% *P. aeruginosa*, 20.83% *S. aureus*, 3.57% *Klebsiella* spp. and 2.67% *E. coli* isolation were performed. In our study, burn wounds were formed by a thermal induction in an animal model. 44 swab samples from the wounds were taken from the experimental animals (2nd, 3rd and 4th group) on the 5th and 10th days. Single isolation from 40 samples and mix isolation from 4 samples were conducted. 26 isolates were *E. coli*, 18 isolates were *S. epidermidis*, 2 isolates were *S. aureus* and 2 isolates were *Enterococcus* spp. The presence of different pathogenic bacteria in those listed studies was determined as a result of isolation in burn wounds. The differences in bacteria isolated from burn wounds in humans and animals are thought to vary depending on the flora of the experimental animal facility environment and their body flora. Nevertheless, absence of *Staphylococcus aureus*, *Enterococcus faecalis* and *Enterococcus faecium* in Asphodel as well as standard drug which was present in lone burn group suggests a protective activity of Asphodel. A study by Salhi *et al.*²¹ shows an antifungal activity of *Asphodelus tenuifolius*. Although we did not test antifungal activity in this study, literature on *Asphodelus tenuifolius* may support our observed antimicrobial activity. Although burn wounds are not infected at the beginning bacterial growth delays the healing and posing a threat for the patient. Thus antibacterial property is an important counterpart of burn therapy with certain ointments. This property is valued in relevant research studies concerning substances of natural origin used for this purpose.²² Current study with the prepared 5% ointment of methanolic extract of *Asphodelus aestivus* plant shows possible protective nature of this plant species in treatment of burn wounds. Arylcoumarins from *Asphodelus microcarpus* was shown to exert antimicrobial activity in *in-vitro* conditions.²³ Another study on *Asphodelus microcarpus* also shows antimicrobial activity and also a potential anti-viral activity.²⁴ Future studies on *Asphodelus aestivus* may present active substituents which are responsible from our observed antibacterial activity. Results of biochemical findings state that administration of asphodel or standard drug did not cause a significant alteration in evaluated biochemical parameters. Although there were differences in AST and LDH values between plant and standard drug groups with control in biochemical parameters, no statistically significant difference was observed in these differences. Differences can be detected if they will be repeated with a higher

number of samples. Present results state no apparent toxic impact of dermal administration of plant extract on liver. There are reports presenting certain toxicities due to consumption of *Asphodelus* plant. Two studies by Birincioglu *et al.*^{25,26} report neurological symptoms, pigmentation in various organs of the body including liver due to *Asphodelus aestivus* ingestion. However no toxicity was visible on liver in our study according to biochemical results. This situation may be due to route of administration (dermal) compared to oral ingestion in aforementioned studies.

The present findings suggest that traditional use of this plant for the treatment of burn/wound/ulcer in traditional medicine applications is valid to some extent. Similar ointment preparation techniques used in literature²⁷ also shows that ethnopharmacological information on wound healing with plant based treatments may reveal some scientifically sound results. In further studies, the investigation of the molecules responsible for the effect in this plant has the potential to present new molecules in this area.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

Alt: Alanine transaminase; **Ast:** Aspartate transaminase; **Ldh:** Lactate dehydrogenase; **SC:** Subcutaneous; **A.N:** Animal number.

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PICTORIAL ABSTRACT



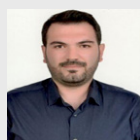
SUMMARY

- Asphodelus* species are used in traditional medicinal practices for wound healing
- Asphodelus aestivus* plant was collected and a methanolic extract was prepared. Methanol was evaporated.
- A thermal wound was formed on the back of the rats with a metal rod.
- An ointment containing 5% of the extract was administered for 12 days on the back of rats.
- Results show an ameliorative activity of *Asphodelus* in microbiological and histopathological aspects without negatively altering liver parameters in blood such as ALT and AST in this animal model.

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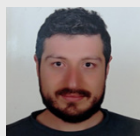
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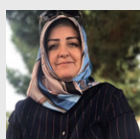
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