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Preparation and in vivo Evaluation of an Astragalus Gummifer Hydrogel-Based Dressing for Excisional Wound

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Abstract: This experimental study aimed to prepare a topical wound-healing gel based on natural compounds. For this purpose, the natural biopolymer of Astragalus gummifer (A.G.) was initially selected as a substrate. After pecan oil preparation, pecan oil emulsion's effect in concentrations of 0.3, 0.4, 0.5, and 0.8% was studied for wound healing in Wistar rats (200-250 gr). Animals were randomly divided into 2 groups: a control and an experimental group. An excisional Wound was applied on the dorsum of rats with scissors with the desired dimension. Antimicrobials, skin sensitivity, and macroscopic evaluation of animals were investigated on different days. In all samples, the diameter of the halos of non-growth of Staphylococcus aureus bacteria was 16 mm, and that of Gram-negative Escherichia coli was 12 mm. The samples also had very good antibacterial activity. No swelling or redness was observed. Also, the topical use of gel with a concentration of 0.8% was effective in the wound healing process, and the wound was healed after 7 days of treatment. Hence, this gel exhibits promising potential as an optimal wound dressing due to its antibacterial and anti-allergenic properties.

Keywords: Astragalus gummifer; hydrogel; wound healing; pecan oil; antibacterial; skin.

1. Introduction

The skin is very important to protect the body's internal organs against external factors and prevent dehydration, which must be protected against damage. This issue causes severe infections, hinders treatment procedures, and even causes life-threatening complications. For this purpose, many efforts have been made to develop new materials in order to protect damaged skin against infection and dehydration [1]. A wound refers to any loss of integrity or disintegration of the integrity of the skin layers (epidermis, dermis, and hypodermis) or the integrity of subcutaneous tissues. Wounds may be caused by physical factors (surgical incisions, trauma, pressure, and gunshot wounds) or chemical factors (acid burns) and may involve soft tissue, muscle, or bone [2]. Wounds are divided into two general categories: acute wounds that start suddenly, heal naturally, and usually leave no side effects. Wounds caused by accidents, surgical wounds, burns, and transplant sites are among the most common acute wounds [3]. Hydrocolloid dressings, hydrogel dressings, sponge dressings, alginate dressings, semi-permeable sticky film dressings, biological dressings, polysaccharide dressings, silver-containing dressings, nanofibrous dressings, Medicinal dressings, and smart dressings are types of new dressings [1,4,5]. Gels have been used in various biomedical fields due to their properties, such as ionic conductivity, thermal stability, and electrochemical stability [6]. Hydrogel dressings usually have the ability to hold water [7-9]. The ability to absorb exuded secretion, maintain moisture, and non-toxic, antibacterial properties are important characteristics of a desirable wound dressing [10].

In recent years, there has been a strong desire to learn more about how physiology affects how herbal medicines are used across the world, particularly in Iran [11,12]. Acidic anionic monosaccharides, calcium, magnesium, potassium salts, and a minor quantity of protein make up A.G. This gum is made up of an insoluble portion that swells in water and a soluble portion. Tragacanthin 5 or tragacanthic acid 6 is the soluble component of the gum and swells in water to create a gel [13,14]. Basorin is the gum's insoluble component. Alpha-di-galacturonic acid 7, alpha-di-galacturonic acid methyl ester 8, l-arabinose 9, beta-di-xylose 10, beta-di-galactose 11, and alpha-l-fucose 12 are the monosaccharide units of this gum [11,15]. Skin fibroblast cells' proliferation and survival are stimulated by arabinogalactan [16]. The perennial pecan plant, a member of the Zygophyllaceae family, is used medicinally in various ways [17]. The perennial plant P. harala may be found in Mexico, South America, Africa, Pakistan, and India, among other dry and semi-arid parts of the world. Antioxidant, anti-inflammatory, antibacterial, antifungal, antiviral, antioxidant, analgesic, heart-protective, antitumor, antidiabetic, tissue function, brain-protective, antiproliferative, and anti-cancer actions are only a few of the biological benefits of pecan plant extracts [18]. The majority of the pharmacologically active alkaloids in the seeds of this plant, which range in concentration from 2 to 6%, are beta-carboline compounds such as Harman, Harmine, Harmaline, and Harmalol [17,18]. Pecan extract has a significant anti-inflammatory effect by inhibiting some inflammatory mediators, including prostaglandin (1 microgram/100 mg) [19]. Also, during the research conducted on some other pecan compounds, such as Harman and Harmine, these compounds can stimulate opioid receptors. Since opioid substances have analgesic effects, the most well-known role of morphine-like substances in the spinal cord is that harmin and harmin act in the synapse between enkephalinergic interneurons (located in the posterior horn of the gray matter of the spinal cord) on the afferent terminals of peripheral nerves [20]. Khajovi *et al*. prepared scaffolding dressings from A.G. for wound care in wet conditions and investigated the drug's ability to release. Their results showed that many scaffolds containing gentamicin are able to be used for wound healing in wet environments by absorbing wound secretions and releasing the drug [21]. In another study, Qaympour and Montazer investigate the green synthesis of zinc oxide nanoparticles on cotton goods using A.G. biopolymer using ultrasound waves and in the presence of natural A.G. biopolymer [22]. Also, Fayazzadeh *et al*. used in vivo studies on laboratory mice to determine the therapeutic effect of A.G. on wound healing. A.G. increases the speed of wound healing and closure and also improves the formation of granulation tissue and mucosal regeneration [16]. Also, the study conducted by Moqbal *et al*. on the laboratory pig method confirms these results [23]. Forough Qashqaei *et al*. investigated the effect of the essential oils of Esfand, Thyme, and Choil plants on burn infections caused by Pseudomonas aeruginosa producing exotoxin A in laboratory mice [24]. Although the pharmacological effects of A.G. have been investigated in different diseases, its effect on wound healing has not been adequately studied.

2. Materials and Methods

2.1. Preparation of the plant.

First-grade band A.G. was obtained from the province of Fars; pecan plants were bought from the market in the province of Kerman; and oil was made. The Sepahan Nanogostar Company provided silver nanoparticles. German-made Triton X-100 Preparation of culture media (MHA) and (MHB) by Tisan Gene Biot Company German-made chloroform produced by Kimia Daru Company is betadine.

2.2. Animal model.

In order to carry out this research, 21 Wister male rats were obtained from the University of Science and Art and kept in the animal room of the University of Science and Art with appropriate laboratory conditions (temperature 22±2°C and 12 hours of light and darkness). A 30mm wound was created using sterile scissors on the back of each animal under aseptic conditions.

2.3. Astragalus gummifer dressing preparation.

A.G. (strip grade 1) was acquired from the region of Fars and ground into powder using a Chinese mortar to make it easier to employ in the testing procedure. The next step was to dissolve 1 gram of A.G. powder in 100 ml of double-distilled water using a magnetic stirrer for 3 hours to solute with a concentration of 1% by weight.

In order to prepare the effective wound-healing gel composition, 0.1 ml of Triton X-100 emulsifier was added to 1.5 ml of A.G. solution, and it was stirred using a magnetic stirrer until the sample became cream-colored. Then, specific amounts of pecan oil passed through a 0.22-micrometer filter were poured into the solution, and finally, stirring continued for 5 minutes.

After preparing the appropriate emulsion from A.G. and pecan oil, the intended wound dressing was prepared by impregnating cotton cloth with the obtained emulsion. In this regard, a cotton cloth with dimensions of 2 x 4 cm was prepared and coated with 2 ml of emulsion, and then, in order to determine the effectiveness of the wound dressings, tests such as the sensitivity test and the halo antibacterial test were performed, and the wound was repaired. Figure 1 shows a graphic of the use of Astragalus gummifer (A.G.) extract for an excisional wound healing model on rats.

Figure 1. Preparation of Astragalus gummifer (A.G) extract for excisional wound healing model on rat.

2.4. Antibacterial effect.

After transferring 5 colonies of the target microbe to Mueller Hinton Broth (MHB), the samples were placed in an incubator for 2 to 4 hours at 37°C until their turbidity reached the McFarland 0.5 standard. Reach After dilution, 500 microliters of 5.1x108 cfu/ml suspensions were transferred to Mueller Hinton Agar (MHA) medium and cultured in three directions by sterile swap. Then, wells with a diameter of 6 mm and a distance of 2.5 cm from each other were created on the agar surface, and 100 microliters of 30, 40, 50, and 80 g/ml solutions of the sample were injected into each well. Dimethyl sulfoxide 5%, which was used to dissolve the sample, was used as a negative control, and chloramphenicol antibiotic was used as a positive control. Then, the plates were incubated at 37°C, and the halos of non-growth were measured after 24 hours.

2.5. Sensitivity test.

In order to perform the skin sensitivity test, three rats with an average weight of 170 grams were prepared. Rats were anesthetized by injecting a mixture of 50 mg/kg ketamine and 5 mg/kg xylazine through a syringe. After that, the hair on the back of the rats was shaved and completely disinfected with alcohol. Animals were photographed before injection, and then 1 ml of the target sample was injected subcutaneously into the right side of each animal. 1 ml of normal saline is also injected into the left side of the rats as a control sample. Immediately after the injection of the sample and the control substance, pictures of the rats were taken (as time zero). Then at 0.5, 1, 24, 48, and 72 hours after the injection, photographs were also taken of the rats. The degree of sensitivity caused by the injection of the sample into rats is checked by comparing the degree of redness and swelling of the skin before and after the injection [25].

2.6. Wound healing test.

First, the animal was anesthetized with chloroform, and the hair on the back of the animal was cut to remove the hair completely. Then, the back area of the animal was disinfected with betadine, and by using a razor and following all surgical principles, a three-cm-the deep wound was created in the dermis and hypodermis, and the day of the operation was considered day zero. In this way, the samples were prepared, and the wounds were treated topically once a day. In addition, the area of the wound was also measured, and a photo was taken of each sample with a camera. For this, the animal was placed on its stomach in a lying position, and the wound length was calculated using a ruler. The measurement error was minimized by measuring 3 times and obtaining the average.

In order to measure the percentage of wound healing, the area of the wound was measured on the test days using a ruler with millimeter accuracy. The recovery percentage was calculated using the 1-2 relationship.

$$\text{Wound percentage} = \frac{\text{wound area on day x}}{\text{wound area on the first day}} \times 100 \quad (1)$$

3. Results and Discussion

The results of the evaluations carried out in order to prepare a hydrogel wound dressing with the property of accelerating the wound healing process have been investigated. In addition to A.G. being used as a substrate for wound dressing, its hydrogel properties and wound-healing ability have also been used. Investigating the effect of pecan oil as an herbal medicine has also been considered.

3.1. Antibacterial test.

In order to evaluate the antibacterial effect of the samples, a good diffusion method was used, and the results showed that the samples showed good antibacterial activity. As shown in Figure 1-3, the size of the non-growth halos of Staphylococcus aurous bacteria was the same for all 4 samples with 0.3, 0.4, 0.5, and 0.8 percent by weight, and the value was measured as 16 mm.

In investigating the effect of Escherichia coli gram-negative bacteria, it was observed that this bacterium showed resistance to the sample, and the halos of non-growth were fainter. The halo diameter for these bacteria with a 0.3% sample equals 5 mm, a 0.4% sample equals 8 mm, a 0.5% sample equals 10 mm, and a 0.8% sample equals 12 mm (Figure 2).

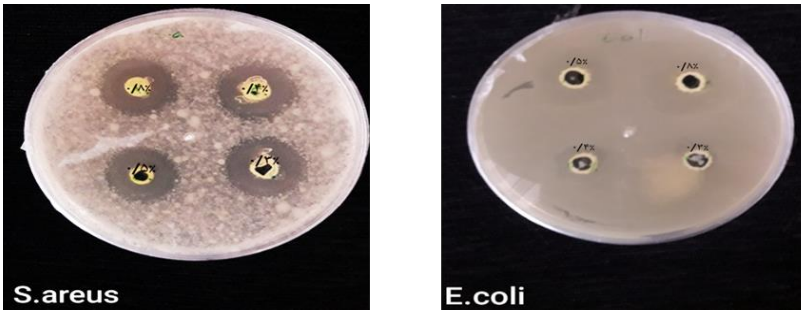


Figure 2. Area of bacterial growth inhibition.

Esfand plant seeds contain 2-6% alkaloids, most of which are beta-carboline compounds, including Harman, Harmine, Harmaline, and Harmalol. According to research, Harmaline has antifungal and antibacterial effects. The very good antibacterial results obtained from the samples in the present research also confirm this issue.

3.2. Sensitivity tests.

In order to investigate skin sensitivity, samples of the prepared emulsion, including A.G., Triton X-100, and pecan oil, were tested on 6 rats at 0.5, 1, 24, 48, and 72 hours. Photography was done. As the evaluation images of this test in Figure 3 show, no skin sensitivity such as swelling, redness, or inflammation was observed in the samples.

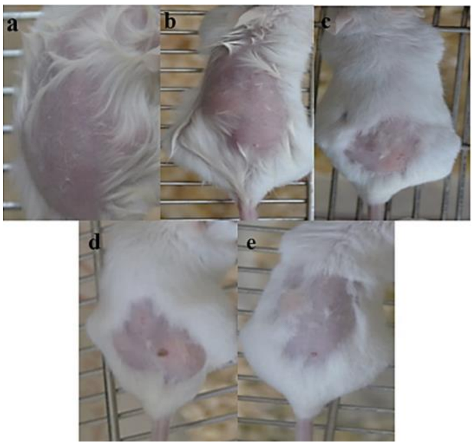


Figure 3. Sensitivity test after **(a)** 0.5; **(b)** 1; **(c)** 24; **(d)** 48; **(e)** 72 hours of hydrogel administration. No evidence of redness and swelling showed over time.

3.3. Wound healing test.

In order to evaluate the wound healing of 5 three groups of rats, including group (1), emulsion containing pecan oil 0.3%, Triton X-100, and A.G., group (2) emulsion containing pecan oil 0.8%, Triton X-100, and A.G., and group (3), were used as control samples. Wounds were created 3 cm deep in the dermis and hypodermis, and the wounds were treated topically once a day. Photographs were taken of the healing process of the wound every day. An emulsion containing 0.8% pecan oil showed better wound-healing properties than the other 3 samples. Pecan seed has long been considered one of Iran's most important medicinal plants in traditional medicine. The present research results showed that using this substance's oil on the wound increases the speed of healing and the wound's closure. In addition, the use of A.G.'s polymer substrate doubles its effect due to its favorable wound-healing properties. Figure 4 and Table 1 show the wound healing results in the control and experimental groups.

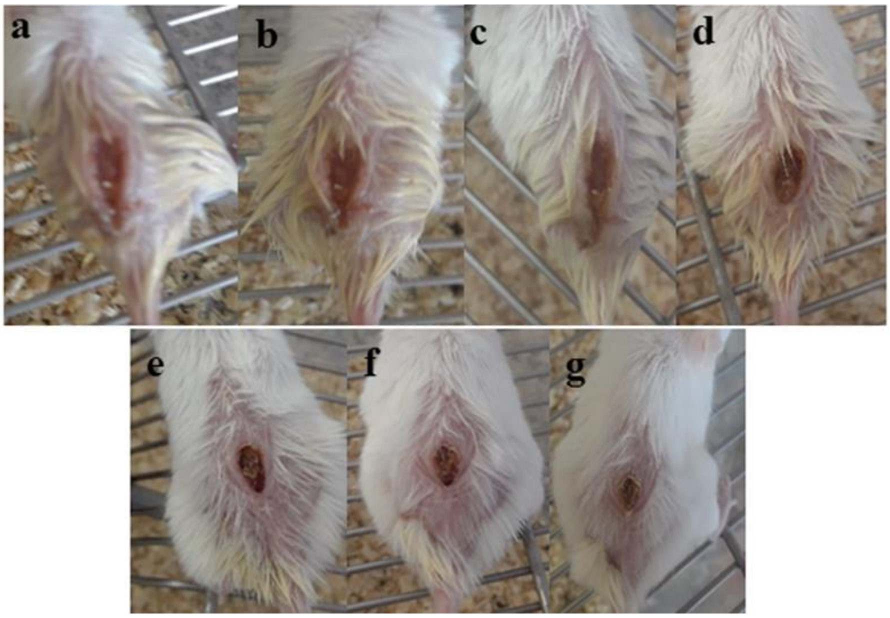
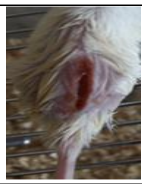


Figure 4. Macroscopic evaluation of animals in the control group with no treatment after excisional wound. **(a)** day 1st with no improvement percentage; **(b)** day 4th; **(c)** day 6th; **(d)** day 8th; **(e)** day 10th; **(f)** day 12th; **(g)** day 14th with 16.67, 20, 26, 26.6. 30 and 33.3 % of improvement percent, respectively.

Table 1. The animal treatment Group includes emulsion containing 0.8% pecan oil, Triton X-100, and A.G.

Day	Wound size (mm)	Wound (%)	Improvement (%)	Macroscopic observation
1	30	100	0	
2	25	83	17	
3	27	56	43	

Day	Wound size (mm)	Wound (%)	Improvement (%)	Macroscopic observation
4	12	40	60	
5	9	19	78	
6	5	16.6	83.34	
7	2.5	8	96	
8	0	0	100	

The rapid spread of bacterial infections has recently generated significant public health and economic concerns on a global scale. One of the many biological processes involved in wound healing is blood coagulation, inflammation, cell proliferation, and regeneration. During these dynamic and intricate responses, the body replaces the injured tissue. In light of this, one of humanity's aspirations is to discover substances that have the fewest adverse effects and that can affect how wounds heal. In this context, medicinal plants play a significant role, and since pecan plants have antibacterial and wound-healing properties, the impact of pecan plant oil on skin wound healing has been examined in this study. In order to perform additional research more effectively, it might be beneficial to compare the therapeutic effects of various sections of a medicinal plant [26,27]. Zinc oxide nanoparticles were added to the wound dressing to improve its efficacy once the target medicinal plant was located.

A successful dressing for the treatment of bedsores was created in a study by Ehsani *et al*. Using polyvinyl alcohol and A.G. hydrogels, a dressing was created for this purpose by layering fabric. The findings of this study demonstrated that the use of polyvinyl alcohol speeds up the healing of wounds because of its malleability and adequate mechanical qualities, in addition to the ointment's anti-inflammatory effects. The impact of pecan oil concentrations in order to treat superficial skin wounds in concentrations of 0.3, 0.4, 0.5, and 0.8 with A.G. was tested using the natural biopolymer of A.G. as a substrate. It has been demonstrated that plex gluco-arabinan polysaccharides derived from a similar Asian species (A. mongholicus) promote the development of T cells and plasma cells that produce antibodies [28].

In order to obtain the optimal sample, an antibacterial test was first taken, and in all the samples, the diameter of the staphylococcus aurous bacteria non-growth halos was reported to be 16 mm. In the investigation of the effect of Escherichia coli bacteria, it was observed that they showed resistance compared to the sample, and the halos of non-growth were fainter. The diameter of the halo for this bacterium with a 0.3% sample was equal to 5 mm; a 0.4% sample was equal to 8 mm; a 0.5% sample was equal to 10 mm; and finally, a 0.8% sample was equal to 12 mm. And the samples were antibacterial. This encapsulated bacterium is considered one of the most important causative agents of mortality in patients with bloodstream infections [29]. It can be concluded that after being placed on the wound, in addition to absorbing the wound secretions due to its high water absorption, the A.G. scaffold can prevent the possible infection of the wound due to the release of the drug and the worry caused by the infection in Reduce unopened dressings [30]. This, along with an acidic pH and preventing the wound from drying out, will accelerate healing. In order to evaluate skin sensitivity, an emulsion sample containing 0.3% of A.G. and pecan oil was injected into 6 heads of rats, and at 0.5, 1, 24, 48, and 72 hours after injection, the rats were photographed. In the evaluation of this test, no sensitivity was observed, including inflammation, swelling, or redness of the skin. According to various articles and recommendations of traditional medicine experts, studies were conducted on various medicinal plants such as pecan, yarrow, ghaziaghi, mountain bitter, henna, thyme, chamomile, and marshmallow, and evaluating their wound healing properties [31,32]. Further, according to the properties of the pecan plant and the few studies done in this field, the oil of this plant was used to investigate the wound healing properties.

Considering that this research was conducted on rats, it is suggested that it be conducted on a larger number of samples and, secondly, that this research be conducted on human samples.

4. Conclusions

In order to evaluate wound healing, 15 rats were used, and a 3 cm-deep wound was created in the dermis and hypodermis. The topical use of gel with a concentration of 0.8% was effective in the wound healing process, and the wound was healed after 7 days of treatment. Due to its antibacterial and anti-allergenic properties, this gel exhibits promising potential as an optimal wound dressing.

According to the said contents, it is possible that the scaffolds produced can be used to dress superficial wounds. Putatively, this relatively newly approached remedy with an ancient background is recommended.

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Conflicts of Interest

The authors declare no conflict of interest.

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