

Healing effects of *Pergularia tomentosa* L., a native medicinal plant in Bushehr province, Iran on burn, in animal model

Parviz Farzadinia¹, Gholamhossein Mohebbi^{2,3}, Afshar Bargahi², Samad Akbarzadeh⁴, Iraj Nabipour², Mohammadreza Abdi², Zeynab Hasanpour², Zahra Alipour² and Adel Daneshi^{2*}

¹Department of Anatomy, School of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran

²Department of Marine Toxinology, The Persian Gulf Marine Biotechnology Research Center, Bushehr University of Medical Sciences, Bushehr, Iran

³Food and Drug Department, Bushehr University of Medical Sciences, Bushehr, Iran

⁴Department of Biochemistry, School of Medicine, Bushehr University of Medical Sciences, Bushehr, Iran

Abstract: Burn injury is one of the most destructive events in the world. The *Pergularia tomentosa* L. is a medicinal plant that traditionally, applies for treatment of burning, in Bushehr province, Iran. Various bioactive compounds such as steroid glycosides, tannins, various vitamins, saponins, cardenolides and anthraquinones were identified into extract of the plant, which can be effective in burn wound healing. Twenty-one rats weighting every one 200±5 grams were divided equally into three groups. The second-degree burning induced on all groups. One of groups did not receive any treatment (The control group) and was treated locally with saline and eucerin. The Second group received the *P. tomentosa* L. as a topical ointment, and the third group received locally, a thin layer of silver sulfadiazine ointment 3% after washing the wound with saline. Afterward treatment period, the microscopic slides from histological sections were prepared. At that point, amounts of the fibroblast cells, blood vessels, wound area, necrotic tissues, and diameter of epidermis rate of wound healing were determined. Also the exterior status of wound in different days was considered. Results obtained from current study have revealed that the extract of *P. tomentosa* L. can significantly, cause qualitative and quantitative acceleration in healing of second degree burn wounds, due to their bioactive and vasoactive properties. In conclusion the *P. tomentosa* L. can be used as an overborne medicine with lower cost and side effect than the similar chemical medicines. Although, the further studies are needed on these plants, due to their some toxic effects.

Keywords: *Pergularia tomentosa* L., burn injury, therapeutic effect.

INTRODUCTION

Skin with a stratified squamous epithelial tissue is a defensive barrier against the chemicals and unpleasant microorganisms (Arturson, 1995; Ahmed & Amr, 1998; Ennis *et al.*, 2004; Naghavi *et al.*, 2009). Infection typically occurs when this layer is damaged. The injuries resulted from burning, can destroy the barrier and activate an inflammatory cycle, that ultimately lead to destruction both under and superficial tissues of the skin (Arturson, 1995).

Burn injury is one of the most critical events in the world (Lau, 2006; Senthil *et al.*, 2006). In 2000, there were expired about 238,000 people from burn and its complications (Peden *et al.*, 2002). Burn damages and their managing, are the greatest common health problems in African and Asian countries such as Nepal, Sri Lanka and Pakistan (Marsh *et al.* 1996; Mzezewa *et al.* 1999). The cost of treatment in burned patients is very expensive (Takayanagi *et al.* 1999).

Healing of wounds starts from the moment of damage and can last for varying periods of time depending on the

*Corresponding author: e-mail: adel_daneshi1@yahoo.com

degree of wounding and the process can be broadly categorized into three phases of inflammatory, proliferate, and finally the remodeling phase which eventually determines the strength and appearance of the healed tissue (Sumitra *et al.*, 2005).

Several therapeutic approaches and drugs were used for controlling and treatment of burn injuries, like nitrofurazone (Ron *et al.*, 2009), sulfadiazine (Roosterman *et al.*, 2006), tetracycline (Mashrekly *et al.*, 2008), gentamicin (Shila *et al.*, 2009), hydrocortisone (Takayanagi *et al.*, 1999), Alpha (Talalay, 2001), calendula cream (Trop *et al.*, 2006), rezhoderm cream (Visuthikosol *et al.*, 1995), phenytoin (Volgler & Ernst; 1999), and the other chemical agents. Chemical medicines may reason a number of complications such as allergies and drug resistance. Hence, scientists are obliged to look for the other remedies. Recently, scientific communities have noted the traditional herbal medicines in basis of their treatment experiences (Molan, 1992; Monafu & Freedman, 1987; Phan *et al.*, 2000; Kunwar *et al.*, 2010; Ghassemi *et al.*, 2012; Mosaddegh *et al.*, 2012). Herbal drugs such as Eucalyptus and Aloe Vera were practiced and used for treatment of burns. Eucalyptus was used in healing wounds as lotions and Ointments due to its high

tannin. Aloe Vera gel extracts, were applied for quick healing of skin, angiogenesis and perfusion in burned tissue, with a number of mechanisms like, increasing the collagen synthesis, rising the rate of epithelialization due to the acemanan effects on fibroblasts proliferation and their antimicrobial, anti-inflammatory and moisturizing effects (Volgler & Ernst, 1999). Kiwi fruit contains a large amount of protease enzyme that is effective in the treatment of burn wounds (Ryan, 2003). Roazinni and his colleagues in 2005, were evaluated macroscopically, the caring effect of honey on healing in burn wounds in Malaysia (Roazini *et al.*, 2005). Other herbal medicines include Canella asiatic, Elm powder, raw olive, Sesame oil, Leaves of hypercom, Fresh leaves of ivy, Marshmallow, mountain evergreen, Mountain Hindi chestnut (Phan *et al.*, 2000 and Poon & Burd, 2004; Sabitha *et al.*, 2012; Zolfaghari *et al.*, 2012).

P. tomentosa L. belongs to family of *Asclepiadaceae* that informally named “lábashir” and “shàtàr” in Bushehr. It is one of plants that have studied in recent times by investigators (Hammiche & Maiza, 2006; Zohra *et al.*, 2012). In addition to Bushehr, It grows in a number of areas of Iran like Khuzestan, Laar, Bandar Abbas, Lut Desert, Sefid-Abe (Rahimi-Golsefidi, 2008).

Several researches present the anti-microbial, anti-tumor and anti-fungal activities of *P. tomentosa* (Mahalel, 2012; Zohra, *et al.*, 2012). Also, the plant used as an expectorant and as a purgative (Sabitha *et al.*, 2012). In Indian traditional medicine, the *Pergularia* is commonly used to treat wound and related injuries (Jain, 1964; Ayyanar *et al.*, 2009). A number of studies have focused on identifying compounds in *P. tomentosa L.* by analytical methods and revealed the presence of bioactive substances such as alkaloids, cardiac glycosides, saponins, flavonoids and tannins in *P. tomentosa* (Morisaki *et al.*, 1995; Zohra, *et al.*, 2012).

The labashir plant traditionally, uses for treatment of burn and skin damages in Bushehr, a southern province of Iran. Nevertheless, no academic study was governed on therapeutic effects of *P. tomentosa L.* on second degree burn wounds. Therefore, their effects on burn injury on rats as our objective of study, was performed. Though, the further studies are needed on these plants. Because of their some deleterious effects like cardiac and toxicity effects obtained from the various researches and post mortem findings (Hamed *et al.*, 2006; Sonia *et al.*, 2009).

MATERIALS AND METHODS

Model selection and classification of groups

Twenty one mature male rats with weight of 200±5 grams were divided to three equal groups.

The study was approved by the Medical Ethics Committee of Bushehr University of Medical Sciences

and Health Services, Bushehr, Iran, and written informed consent was obtained from all subjects.

All animal study was permitted in accordance with the National Ethical Guidelines for Animal Research in Iran (2005) under a Project License, which was approved by the Animal Care and Use Committee of Bushehr University of Medical Sciences- Iran (Protocol #: DP/M13.24/72).

The Animals were kept under identical conditions (24°C in 12h light/12h dark cycles and humidity of 70-80 i). The Injuries were absterged with physiologic serum and were treated locally by Eucerin. The first group (negative control (I)) have received no treatment. Second group received the *P.tomentosa L.* as a topical serum after abstergent the wound (case group (II)). Third group (positive control (III)) received locally, a thin layer of silver sulfadiazine ointment (3%) after rinsing the wound, by physiologic serum.

Sample collection and preparation of extract

The *P. tomentosa L.* was collected from deserts around the Khormuj city in Bushehr province, Iran that were grown a large quantity of these Plants. Formerly, the Aerial portions of *P. tomentosa L.* were cleaned and rinsed several times to remove any soil and salts. Subsequently, the stems and leaves were kept into the oven at 40°C to contract and preventing the mildew. Then the dry parts were grinded by the mill and prepared them as powder. Following, 300 grams of the powder was added to 2 liters of distilled and healthy water and boiled for 30min. The subsequent stage, the obtained solution was incubated at 70°C for 24h and then was filtered with fabric (that washed previously with distilled water) and cloth filters, respectively. For separation of the minerals and impurities, the solution was centrifuged at 4000rpm for 15min. Extraction was done by rotary evaporator for eliminating the water. Dry extract was obtained from oven-drying of filtered extract at 45°C.

Preparation of animals and burning method

Before burn induction, behind of the rats, in top of left side section, were shaved by shaver and then, a small amount of depilated local cream was rubbed on shaved skin and remained there, for 5 minutes to get effective impression. Single day after shaving, rats were transferred to a surgery room. After anesthesia via 50mg/Kg Ketamine (I.M), the second-degree burning was carried out by a burning device with a plaque of 1cm² in the behind of rat. Intended for this act, the device with temperature of 318°C was located on the shaved area of skin for 15 seconds.

The treatment

According to classification of groups, the animals were treated by the rubbed ointment on the wound. Treatment was done, twice a day for every 12 hours, at the first week; once a day in the second week, and once each two

days for third and fourth weeks. The wounds were washed and cleaned by saline and sterile gauze thoroughly, and then a fresh ointment was applied to the wound. The room, cages and water containers, and the other conditions were controlled to prevent microbial growth. The Sawdust and drinking water was daily changed.

Biopsy and histological study

Samples were kept at days 0, 14 and 28, after anesthetizing the rats by Ketamine (50mg/Kg I.M). It was performed by sterilized surgical set from declared area of tissue. The specimens were fixed in 10% formalin and referred to the histopathology lab. For the optical microscopic study, samples were intake and molded by alcohol and Paraffin, respectively. There were formed the three microns pieces by a rotary microtome and stained normally. The microscopic slide photos were taken by microscope equipped with a Moticam camera model A352 (Netherlands) in a perform resolution (resolution *100). The measurable parameters such as the number of fibroblastic cells, blood vessels, wound zones; necrotic tissues and diameter of epidermis were evaluated using photomicrographs with software image tool (version 8). Also the qualitative studies (morphology) were recorded directly using observations in addition to a standard check list that able to monitor the wound during the healing process. The obtained results were involved the observed changes on wound such as type, color, amount of secretions and scar tissue in different days.

STATISTICAL ANALYSIS

Parameters obtained in different groups were analyzed by SPSS software and one-way ANOVA method with DUNCAN test. The data were shown as mean \pm standard deviation (SD) ($P \leq 0.05$).

RESULTS

Morphologic results (qualitative study)

According to the photomicrographs results, the histopathology alterations are followed as: The photomicrographs of skin cross section in different groups in 0, 14 and 28th days are shown in figs. 1, 2 and 3, respectively.

As can be identified of fig. 1, the epithelium and connective tissues of the dermis were regular, uniform and normal in all groups, at zero day (fig. 1).

After 14th day, they were relatively improved in control and treatment groups, while skin tissue without surface specific epithelium was found in negative control group. Also, the connective tissue has no particular changes in current group (fig. 2).

About the 28th day, not only the epithelium and connective tissues of the dermis were comparatively

improved in all groups, but also, the epithelium thickness and formidability of dermis were developed in positive control and treatment groups than the negative control (fig. 3).

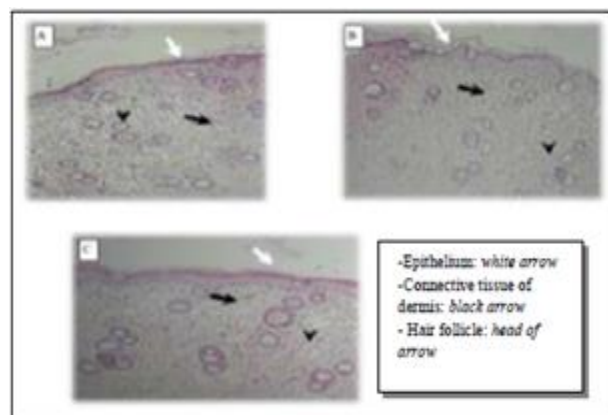


Fig. 1: The photo micrograph of skin cross sections in negative control (figure 1. A), positive control (fig. 1B), and treatment groups (fig. 1C) at 0th day (Normal staining and resolution *100). Epithelium (white arrow), Connective tissue of dermis (black arrow), Hair follicle (head of arrow).

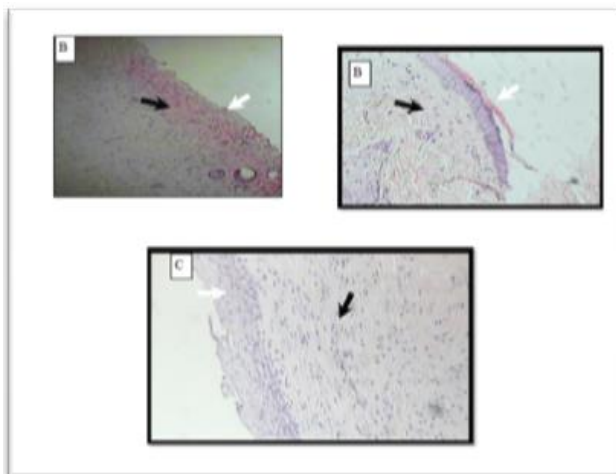


Fig. 2: The micrograph of skin cross section in different groups at 14th day. Epithelium (white arrow), Connective tissue of dermis (black arrow), Hair follicle (head of arrow), Negative control groups (fig. 2A), Positive control group (fig. 2B), treatment groups -with *Pergularia tomentosa* (fig. 2C). (Normal staining and resolution *100).

As can be seen in fig. 4, the connective tissue of dermis has thickened, uniform and dense collagen fibers. The dermis tissue has damaged and inflamed at 14th day, while granular and vascular tissue were seen in the other groups. The positive control and treatment groups had uniform, regular and soft filaments at the end of the treatment period (fig. 4).

Table 1: The qualitative changes of wounds such as types, amounts secretion colors and scars in different groups and days.

Rat Groups		I (Negative control)	II (Positive control)	III (Treatment)
Amount of secretions	5 th day	High	Moderate	Moderate
	10 th day	Moderate	No secretion	No secretion
	15 th day	Moderate	No secretion	No secretion
Type of secretions	5 th day	Purulent	Seropurulent	Sanguineous
	10 th day	Sanguineous	Serous	Serous
	15 th day	Seropurulent	No secretion	No secretion
Color of secretions	5 th day	Creamy	Creamy	Reddish
	10 th day	Creamy	Reddish	Reddish
	15 th day	Creamy	Bright Red	Bright Red
Scar tissue (Quality)	1 th day	Soft	Soft	Soft
	14 th day	Hard	Hard	Soft
	28 th day	Very Hard	Soft	Soft

Table 2: The mean± standard deviation (SD) for changes of wound such as epithelium thickness, number of blood vessels and fibroblast cells and also area of wound closure (%) in different groups and days

Rat		I (Negative control)	II (Positive control)	III (Treatment)
Epithelium thickness (micron)	Day 1	145.11±(5.17)	155.56±(5.21)	157.41±(1.45)
	Day 14	No epithelium	29.21±(1.28)*	33.31±(3.11)*
	Day 28	155.63±(2.49)	159.32± (2.23)	166.46±(2.44)
Number of blood vessels	Day 1	7.17±(1.23)	8.17±(1.35)	10.33±(2.43)
	Day 14	5.32±(3.57)	11.32±(2.65)*	15.12±(1.66)*
	Day 28	8.45±(6.15)	9.33±(2.48)	10.11±(3.24)
Number of fibroblast cells	Day 1	19.34±(2.34)	20.15±(1.12)	22.23±(4.15)
	Day 14	21.34±(2.05)	33.12±(3.15)*	38.10±(3.13)*
	Day 28	21.23±(1.46)	20.22±(1.46)	19.11±(2.12)
Area of closure wound (%)	Day 7	88. 12±(5.57)	83. 23±(3.21)	81. 21±(1.18)
	Day 14	85. 41±(1.56)	56. 13±(2.34)*	51. 67±2.46)*
	Day 21	66. 34±(1 .67)	33. 34±(2.11)*	31. 63±(2.24)*

*Sign shows significant difference with negative control (P≤0.05).

Furthermore, the properties of the wound areas related to 0 and 7th days (fig. 5), in addition to 14, 21 and 28th days (fig. 6), in different groups are presented in these figs, respectively. Images revealed that the consolidation and appearance of the wound are more regenerated in positive and treatment groups than negative control group.

The qualitative statuses of wounds were studied and results obtained from the morphological changes of wound include types, amounts secretion colors, and scars related to the 5, 10 and 15th days, were shown in table 1.

At this point, the amounts of secretions were more increased in negative control at 5th day, than the other groups. Also, the amounts of secretions were moderate in negative control group at 10 and 15th days; While, no secretions were seen in other groups. Moreover, type of secretions was relatively more purulent in negative control at 5th day than the other groups.

The obvious changes of wound from the viewpoint of secretions color, were studied at 5, 10 and 15th days in different groups and the color of secretions was creamy in negative control at 5th day, while their colors were red, in other groups. The colors of secretions were creamy in negative control at 10 and 15th while color was red in the other groups. Also, the seeming changes of wound as scar tissue (qualitative and quantitative) at 1, 14 and 28th days were shown. The scar tissue was contained the soft consolidation and thin connective filaments of collagen in all groups; while, scar tissue contains hard consolidation and thick connective filaments of collagen in groups of I, II at 14th day, However, its status in treatment group was soft in 14 and 28th days, as well as positive control in 28th day. Also the scar tissue in negative group at 28th day was very hard.

Morphometric aspects (quantitative studies)

The results obtained from wound grades, include the quantitative changes of wound such as epidermis

thickness, number of blood micro vessels and fibroblasts at 1, 14 and 28th days were shown in table 2. Also, the closure wounds were studied at 7, 14, 21 and 28th days. According to the table, the mean± SD of the epidermis thickness, number of blood vessels and fibroblast cells were significantly increased in positive control and treatment group at 14th day in compared with negative control. Whereas, the areas of closure wound were significantly reduced in positive control and treatment groups at 7, 14 and 21th days in compare with negative control ($P \leq 0.05$).

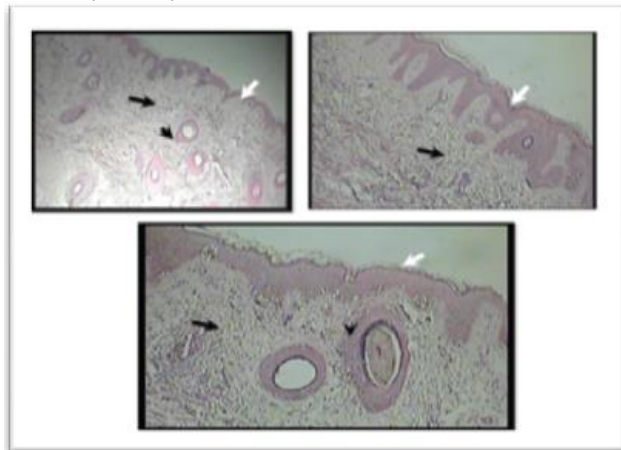


Fig. 3: Photomicrograph of skin cross section in different groups at 28th day. Epithelium (white arrow), Connective tissue of dermis (black arrow), Hair follicle (head of arrow), Negative control groups (fig. 3A), Positive control group (fig. 3B), and treatment groups with *Pergularia tomentosa* (fig. 3C). (Normal staining and resolution *100).

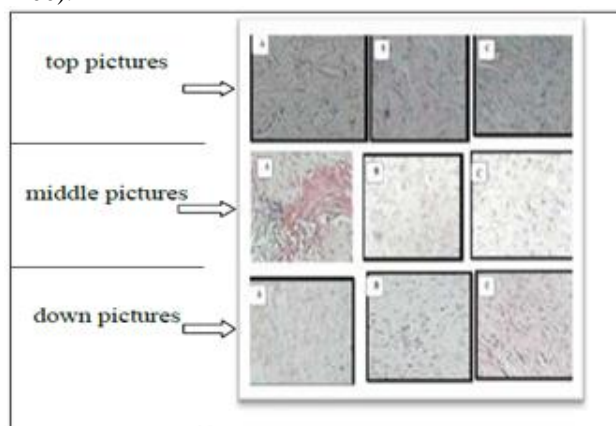


Fig. 4: Photomicrograph of skin connective tissue in different groups at 0 (top pictures), 14 (middle pictures) and 28th (down pictures) days. Negative control group (eucerin 4A), Positive control (sulphodiazine 4B), treatment group (4C).

DISCUSSION

Burn injuries are one of the most damaging events, can cause the physical, emotional- psychological, social and economic complications. Burn is the main cause of

morbidity and mortality in worldwide. Burn injuries, after car accident are the second reason of the mortality in United States (Mzezewa *et al.*, 1999). The burning creates destruction in tissue through the membrane instability, coagulation of proteins, depletion of energy resources, and cellular hypoxia, which, ultimately leads to extreme necrosis of tissue. Furthermore, burn-induced wound is a threat to the rest of the body because of invading the infectious microorganisms, antigen challenge and repeated trauma through wound cleaning (Marks *et al.*, 2006). The wound healing is a fundamental response to tissue injury that occurs through repairing of connective tissue and in conclusion, the epithelium tissue (Mendez *et al.*, 1999).

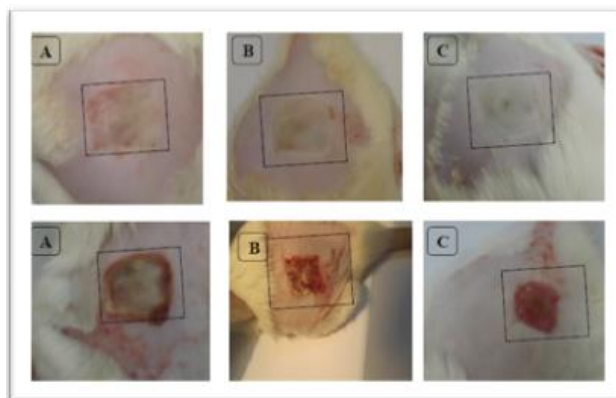


Fig. 5: photomicrograph of burn wound location (skin) in different groups. Negative control group (eucerin 5.A), Positive control (sulphodiazine 5.B), treatment group (5.C) at 0 (top) and 7th (below) days.

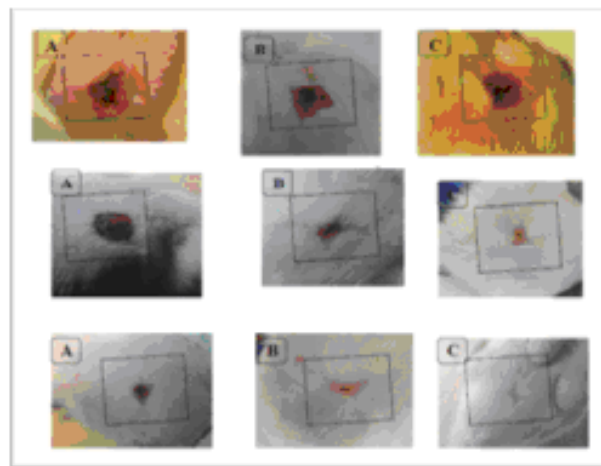


Fig. 6: Photomicrograph of burn wound location (skin) in Negative control group (eucerin 6.A), Positive control (sulphodiazine 6. B), treatment group (6.C) at 14 (top), 21 (middle) and 28th (down) days.

Since the high price, elevated consumption, several side effects such as scar, discoloration of the wound site after healing, allergy, and drug resistance of burn wound chemical medicines like, silver sulfadiazine; it have

increased the importance of medical herbs (Roazini *et al.*, 2005). Nowadays, the use of herbal remedies and traditional medicine is common among patients and many researchers and physician (Lee and Houghton, 2005; Ayyanar *et al.*, 2009). Since time immemorial man has used various parts of plants in the treatment and prevention of many diseases (Chah *et al.*, 2006).

Iran is one of the innovators in the use of herbal medicines (Ghassemi *et al.*, 2012; Mosaddegh *et al.*, 2012; Doozandeh *et al.*, 2015). The traditional medicine records in Iran contain the clinical experiences by great scientists such as Ibn Sina, Aghili Khorasani and Hakim Mohammad Momin (Blalr, 1997) especially, on wounds and burns, which unfortunately, are not used today. This subject has been emphasized In America. More or less, one billion dollars has spent for traditional therapies that more costs is related to herbal remedies (Monafo & Bessey, 1996).

According to the morphological studies of histological micrographs (figs. 1-5), the thickness and consolidation of dermal connective tissue is more improved in positive and treatment groups in comparison with negative control at 14 and 28th days. Also, the photo-micrographs from skin wound in different groups show that, the wound surface is wide and purulent in control group while wound surface is redder and smaller in positive control and treatment groups.

The results in table 1, indicated that inflammatory events were decreased in positive control (silver sulfadiazine) and treatment groups (*P. tomentosa*) in comparison with control group. Further, appearance and quality of wound (in terms of secretions type) were serous bloody in the treatment and positive control groups (sulfadiazine) than the control group on 10th day, whereas no secretion were seen in these groups on 15th day. Also in these groups, the apparent color of the wound was bright red, while apparent color of the wound was creamy and dark. It is revealed the improvement of inflammation in treatment and positive control groups, in comparison with the control group. Scar tissues had soft consolidations that have shown regulated growth process in connective tissues of dermis. Further, according to the table 2, the thickness of epithelium, numbers of fibroblasts and blood vessels were increased significantly in treatment group in compared with control group. A skin has collagen fibers and increasing of fibroblasts result in elevated production of basement connective tissue.

The improvement of wound surfaces and aggregation of wound edges were last parameters that were studied in current study. The wound surfaces were decreased significantly in treatment and silver sulfadiazine groups, in compared with negative control group on 7, 14, 21 and 28th days.

The latest researches have shown the antibacterial, antitumor and antifungal effects of *pergularia tomentosa* L., they were used for treatment of skin diseases in Iran (Hassan *et al.*, 2007). However, no researches were conducted to study of *P. tomentosa* effects on healing process in burn second-degree wounds. As, their usage in treatment of skin diseases and also, presence of active compounds in their root and leaves; we can expect *P. tomentosa* improves the healing process of burn wound in skin and be an effective gait in confirmation of traditional medicine and medical plants. Widespread researches on *P. tomentosa* by chromatography and analytical methods were found the bioactive materials such as alkaloids, cardiac glycosides, saponin, flavonoid, tannin and anthraquinones in their root and leaves (Morisaki *et al.*, 1995; Hassan *et al.*, 2007; Zohra, *et al.*, 2012).

The potential mechanism of healing effects for *P.tomentosa* on second degree burn wounds can totally as follow: Alkaloids, steroid glycosides presented in *P. tomentosa* extract inhibit potentially positive and negative gram bacteria and can sterilize the wounds, inhibit growth of bacteria in necrotic tissues (that are best cultural medium for bacteria) and accelerate wound healing process. Glycosides, tannins and different vitamins presented in *P.tomentosa* extract may accelerate the healing process of burn-induced wound by their antioxidant activity. Saponin existing in extract can improve the wound status and increase regeneration by enhancing of the angiogenesis. Cardenolides presented in extract can boost proliferation of fibroblasts result in enhancement in wound healing process and wound closure. Anthraquinones presented in different parts of plant can increase velocity of dermal wound healing by enhancing the synthesis of hydroxylproline and elevation inelasticity of skin (Hamed *et al.*, 2006; Sonia *et al.*, 2009).

CONCLUSION

In conclusion, the *P. tomentosa* is containing the bioactive compounds such as steroid glycosides, cardenolide, tannins, saponin, anthraquinone and different vitamins can improve potentially the healing process of skin wound in second-degree burns. It is hoped that results acquired from this study can create a manner to treat skin diseases. In future, *P. tomentosa* may be used as a novel burn medicine with lower cost and side effect than the similar chemical medicines. According to propose of the authors, the further studies are needed on these plants, because of their some toxic effects. Cardiac glycosides are responsible for cardiac effects of the plant.

REFERENCES

Ahmed EB and Amr RM (1998). Epidemiology of childhood burns in the burn unit of Ain Shams University in Cairo, Egypt. *Burns*, **24**: 728-732.

- Arturson G (1995). Pathophysiology of the burn wound and pharmacological treatment. *The Rudi Hermans Lecture. Burn*, **22**: 255-274.
- Ayyanar M and Ignacimuthu S (2009). Herbal medicines for wound healing among tribal people in southern India: Ethnobotanical and scientific evidences, *Internat. Jour. Appl. Resear. Nat. Prod.*, **2**(3): 29-42.
- Blarl B (1997). The medical manuscripts of Azerbaijan. *Azerbaij. inter.*, **5**: 51-52.
- Chah KF, Eze CA, Emuelosi CE and Esimone CO (2006). Antibacterial and wound healing properties of methanolic extracts of some Nigerian medicinal plants. *J. Ethnopharmacol.*, **104**: 164-167.
- Doozandeh K, Dejam M, Mohajeri F, Mohebbi GH, Abadi F and Talebi H (2015). Chemical composition of the essential oils of *Achillea eriophora* DC. growing wild in Iran. *J. Chem. Phar. Res.*, **7**(2): 748-754.
- Ennis WJ, Valdes W and Meneses P (2004). Specialty training. *Wound. Repair. Regent*, **21**: 724-732.
- Ghassemi DN, Norouzi M and Safaei A (2012). Collection and evaluation of the traditional applications of some plants of Jandagh. *J. Islam. Trad. Med.*, **3**: 105-112.
- Hamed AI, Plaza A, Balestrieri ML, Mahalel UA, Springuel IV, Oleszek W, Pizza C and Piacente S (2006). Cardenolide glycosides from *Pergularia tomentosa* and their pro apoptotic activity in Kaposi's sarcoma cells. *J. Nat. Prod.*, **69**: 1319-1322.
- Hammiche H and Maiza K (2006). Traditional medicine in central Sahara: Pharmacopoeia of Tassili Najjer. *J. Ethnopharmacol.*, **105**: 358-365.
- Hassan SW, Ladan MJ, Dogondaji RA, Umar RA, Bilbis LS, Hassan LG, Ebbo AA and Matazu IK (2007). Phytochemical and toxicological studies of aqueous leaves extracts of *Erythrophleum africanum*. *Pak. J. Biol. Sci.*, **10**(21): 3815-3821.
- Jain SK (1964). The role botanist in folklore research. *Folklore.*, **5**: 145-150.
- Kunwar RM, Shrestha KP and Bussmann RW (2010). Traditional herbal medicine in far-west Nepal, a pharmacological appraisal. *J. Ethnobiol. Ethnomed.*, **6**: 1-18.
- Lau YS (2006). An insight into burns in a developing country: A Sri Lankan experience. *Pub. Health.*, **120**: 65-95.
- Lee CC and Houghton P (2005). Cytotoxicity of plants from Malaysia and Thailand used traditionally to treat cancer. *Jour. Ethnopharmacol.*, **100**: 237-243.
- Mahalel UA (2012). Antibacterial sensitivity for some chemically diverse steroidal glycosides *in vitro*. *J. Agric So. Sci.*, **8**: 24-28.
- Marks JG and Miller J (2006). Looking bill and Marks' Principles of Dermatology (4th Ed.). Elsevier. Inc., p.6.
- Marsh D, Sheikh A, Khalil A, Kamil S, Zaman JU and Qureshi I (1996). Epidemiology of adults hospitalized with burns in Karachi, Pakistan. *Burns*, **22**: 225-229.
- Mashreky SR, Rahmana A, Chowdhury SM, Giashuddina S, Svanstromb L, Khand TF, Cox R and Rahmana F (2008). Burn injury: Economic and social impact on a family. *Pub. Heal.*, **122**: 1418-1424.
- Mendez MV, Park HY and Menzolan JO (1999). The proliferative capacity of neonatal Skin fibroblast in reduced after exposure to Venus ulcer wound fluid a potential mechanism for senescence in Venus ulcers. *J. Wasc. Surg.*, **30**: 734-743.
- Molan PC (1992). The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity. *Bee. World*, **73**: 59-76.
- Monafu WF and Fredman B (1987). Topical therapy for burns. *Surg. Clin. North. Am.*, **67**: 133-145
- Morisaki N, Watanabe S and Tezuka M (1995). Mechanism of angiogenic effects of saponin from Ginseng *Radix rubra* in human umbilical vein endothelial cells. *Brit. J. Phar.*, **115**(7): 1188-1193.
- Mosaddegh M, Naghibi F, Moazzeni H, Pirani A and Esmaeili S (2012). Ethno botanical survey of herbal remedies traditionally used in Kohgiluyehva Boyer Ahmad province of Iran. *J. Ethnopharmacol.*, **141**: 80-95.
- Mzezewa S, Jonsson K, Aberg M and Salemar L (1999). A prospective study on the epidemiology of burns in patients admitted to the Harare burn units. *Burns*, **25**: 499-504.
- Naghavi M, Abolhassani F, Pourmalek F, Moradi Lakeh M, Jafari N, Vaseghi S, Mahdavi Hezaveh N and Kazemeini H (2009). The burden of disease and injury in Iran 2003. *Popul Health Metr.*, **7**: 9.
- Peden M, McGee K and Sharma G (2002). The injury chart book: A graphical overview of the global burden of injuries. WHO (World Health Organization), Geneva, Switzerland, pp.6-19.
- Phan TT, Allen J and Hughes MA (2000). Up regulation of adhesion complex proteins and fibronectin by human keratinocytes treated with an aqueous extract from the leaves of *Chromolaena odorata* (Eupolin). *Eur. J. Dermatol.*, **10**: 522.
- Poon VK and Burd A (2004). *In vitro* cytotoxicity of silver: Implication for clinical wound care. *Burn*, **30**: 140-147.
- Rahimi-Golsefid M (2008). Zagros medicinal plants Bakhtiari (Iran). First Ed, Hafez Novin, p.250.
- Roazini MZ, Zouki ABZ and Noordin YMM (2005). Macroscopic evaluation of wound healing progress treated with different type of honey. *Pak. J. Boil. Sci.*, **200**: 721-733.
- Ron W, John JR and Robert IS (2009). Rosen's emergency medicine: Expert Consult Premium Edition Enhanced Online Features and Print (Rosen's Emergency Medicine: Concepts & Clinical Practice). St. Louis: Mosby. ISBN 0-323-05472-2.
- Roosterman D, Goerge T, Schneider SW, Bunnett NW and Steinhoff M (2006). Neuronal control of skin function: The skin as a neuroimmunoendocrine organ. *Physiol. Rev.*, **86**: 1309-1379.

- Ryan TJ (2003). Use of herbal medicines in wound healing: A perspective paper. *Int. J. Low. Extrem. Wounds*, **2**: 22-24.
- Sabitha S, Maher K and Mohamed M (2012). Medicinal plants diversity and their conservation status in the United Arab Emirates (UAE). *J. Med. Plant. Res.*, **6**(7): 1304-1322.
- Senthil KM, Sripriya R, Vijaya RH and Sehgal P (2006). Wound healing potential of *Cassia fistula* on Infected Albino rat model. *J. Surg. Res.*, **131**: 283-289.
- Shila G and Natasa S (2009). Wound healing properties of *Carica papaya* latex: *In vivo* evaluation in mice burn model. *J. Ethnophar.*, **121**: 338-341.
- Sonia P, Milena M, Nancy DN, Janique D, Arafa H, Robert K and Tatjana M (2009). Cardenolides from *Pergularia tomentosa* display cytotoxic activity resulting from their potent inhibition of Na⁺/K⁺-ATPase. *J. Nat. Prod.*, **72**: 1087-1091.
- Sumitra M, Manikandana P and Suguna L (2005). Efficacy of *Buteamono spermaon* dermal wound healing in rats. *Int. J. Biochem. Cell. Biol.*, **37**: 566-573.
- Takayanagi K, Kawai S and Aoki R (1999). Cost of burn care and implication for efficient care. *Clin. Perform. Qual. Health. Care*, **7**: 70-73.
- Talalay P (2001). The importance of using scientific principles in the development of medicinal agents from plants. *Acad. Med.*, **76**(3): 238-247.
- Trop M, Novak M, Rodl S, Hellbom B, Kroell W and Goessler W (2006). Silver-coated dressing acticoat caused raised liver enzymes and argyria like symptoms in burn patient. *J. Trauma*, **60**(3): 648-652.
- Visuthikosol V, Chowchuen B, Sukwanarat Y, Sriurairatana S and Boonpucknavig V (1995). Effect of aloe Vera gel to healing of burn wound a clinical and histologic study. *J. Med. Assoc. Thai.*, **78**(8): 403-409.
- Volgler BK and Ernst E (1999). Aloe Vera: A systematic review of its clinical effectiveness. *Br. J. Gen. Pract.*, **49**(447): 823-828.
- ZohraYB, Lakhdar S, Hala IA, Mahmoud AA and Musa HAZ (2012). Two new taraxasterol-type triterpenes from *Pergularia tomentosa* growing wild in Algeria. *J. Asia. natur. Prod. Res.*, **14**: 1137-1143.
- Zolfaghari B, Sadeghi M, Tiri I and Yousefali TM (2012). Collection, identification and evaluation of the traditional applications of some plants of Babol. *J. Islam. Iran. Trad. Med.*, **3**: 113-123.