Tropical Journal of Pharmaceutical Research September 2019; 18 (9): 1909-1912
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v18i9.18

Original Research Article

Wound-healing effect of *Platycodon grandiflorus* (Jacq.) extract in rats

Liang Wang[1], Li Yang[2], Gang Yuan[1], Long-Jiao Hu[3]*
[1]Department of Pharmacy, [2]Department of Orthopedics, Traditional Chinese Medicine Hospital of Jiulongpo District, [3]Department of Pharmacy, People's Hospital of Jiulongpo District, Chongqing 400050, China

For correspondence: **Email: hulongjiao494@126.com; **Tel:** +86 18166572129*

Sent for review: 20 July 2018 *Revised accepted: 18 August 2019*

Abstract

Purpose: *To evaluate the healing effect of Platycodon grandiflorus (Jacq.). extract (PGE) on experimental burn wounds in rats.*
Methods: *Rats were randomly divided into four groups of eight rats each: control group, silver sulfadiazine (SSD)-treated group, moist exposed burn ointment (MEBO)-treated group and PGE-treated group. PGE, SSD and MEBO were applied topically twice daily for 7 days. SSD and MEBO were used as reference control. External observation of wound area contraction and histological analysis of wound tissues was performed respectively. The effect of PGE on matrix metalloproteases (MMPs), vascular endothelial growth factor (VEGF) and Type-III Collagen proteins of wound tissue in rats were analysed by Western blot.*
Results: *After 10 days of topical treatment with PGE, PGE-treated group showed faster reduction in wound area when compared with control groups ($p < 0.01$). Matrix metalloprotease-2 (MMP-2), matrix metalloprotease-9 (MMP-9), VEGF and type-III collagen expressions in the wound tissue increased significantly ($p < 0.05$) when compared with the control burn wounds. Histological results showed an overall early recovery and regeneration in PGE-treated group when compared with control group.*
Conclusion: *PGE possesses a significant wound-healing activity in full-thickness burn wounds in rats. Therefore, it can potentially be developed for the management of burns.*

Keywords: *Platycodon grandiflorus, Wound, Second-degree burns*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Burn injuries caused by heat, light, electricity, radiation and chemicals are one of the most common and devastating forms of trauma, particularly partial- and full-thickness burns [1]. According to reports, about 5000 - 10,000 in one million people suffer from burn in China every year [2]. Depending on severity, burn injuries are classified into three degrees, wherein the second degree is divided into shade and deep degrees [3]. Burn wound results in the increase of free radical-mediated damage and reactive oxygen species. It can delay granulation tissue formation, reduce angiogenesis and decrease collagen reorganization. Furthermore, due to its significantly prolonged period of rehabilitation, the healing of burn injuries is more difficult than

that of ordinary wounds and it is also associated with higher economic costs [4].

At present, the way to treat burn injuries include the use of antibiotics, antiphlogistics, and silver salts, which possess major drawbacks and unwanted side effects [5]. Nowadays, alternative and complementary medicines, such as traditional Chinese medicine and aromatherapy, are used because they are beneficial to effective with little toxicity and are less expensive than synthetic drugs. Many plants and plant-derived products have been found to possess potent wound-healing or burn-wound-healing activities [6].

Platycodon grandiflorus (Jacq.), is a Traditional Chinese Medicine preparation for treating burns. It has been used for treatment of inflammation, infection, jaundice, skin burns and hyperlipemia in China [7,8]. In the present study, the experiment was designed to investigate the effect of PGE on burn injuries in rats.

EXPERIMENTAL

Plant material

The plant material, *Platycodon grandiflorus* (Jacq.) A. DC. collected from Zhunyi City, Guizhou Province in China in October 2018. Taxonomic identification of the plant was performed by Professor Zhi Li of Chongqing University, in China. A voucher specimen (no. PGE 201805121) was deposited at College of Pharmacy, Chongqing University, China for future reference.

The herbal samples *Platycodon grandiflorus* (Jacq.) was dried in an oven. PGE was obtained by steeping the *Platycodon grandiflorus* (Jacq.) in water at 60 ℃ for three times, each for one hour. Then they were dried in an oven and freeze-dried to obtained. One gram powder was equivalent to 1.6 g crude samples.

Experimental animals and burn wound model preparation

Wistar rats weighing 200 – 220 g were obtained from Chongqing Center for Disease Control and Prevention, Chongqing. The animals had free access to food and water, and were allowed to acclimatize for at least one week before use. All animal experiments were approved by the Animal Care and Use Committee of People's Hospital of Jiulongpo District (approval no. 20120923) and were carried out in compliance with the Animal Welfare Act and the NIH guidelines (NIH publication No. 80-23, revised 1996) [9].

After the back hair of the rats were removed, and injecting 20 % of ethyl carbamate was injected into their abdominal cavities for anesthetic effects. The top of the electrical scald instrument (manufactured by Changhai Hospital of Second Military Medical University) was pressed onto the back skin with a certain force for 15 sec at the temperature of 75 ℃. A standard deep second-degree burn wound was prepared.

Experimental design

Rats were randomly divided into four groups of eight rats in each: control group, SSD-treated group, MEBO-treated group and PGE-treated group. PGE was applied topically and twice daily for 7 days in rats. Silver sulfadiazine (SSD) cream USP, 1.0 % w/w and moist exposed burn ointment (MEBO) were used for treating reference controls. The control group received the vehicle alone in an identical manner.

External observation of wound area contraction

The wound surface area of the rats was traced on a transparent paper and measured planimetrically [10].

Histological examination

The entire wound tissues of rats were fixed with 10 % formalin. After fixation, samples were embedded in paraffin, cut into 3 mm frozen sections with a cryostat microtome, and stained with hematoxylin and (H & E) eosin reagent. Collagen fiber, inflammatory cell, blood vessel, fibroblast and granulation tissue of burn skin of rats were observed by microscope.

Statistical analysis

Data are expressed as mean ± SE, and statistical significance between experimental and control values was analyzed by one way ANOVA followed by Dunnett's test using Graph Pad Prism 2.01 (Graph Pad Software Inc, La Jolla, CA, USA). A value of $p < 0.05$ was considered statistically significant.

RESULTS

Effect of PGE on wound area contraction in rats

Compare with control rats, the wound-healing activity of PEG was more effective. Table 1

shows the effect of topical application of PGE on wound area reduction at different time intervals. The rats treated with PGE showed significant faster reduction in wound area ($p < 0.05$, Table 1).

Histological features

On the day 10, the PGE group showed more advanced re-epithelialization and layering with continuous basement membrane in addition to a better organization of the collagen bundles. The PGE, SSD or MEB- treated animals showed more reduced congestion, edema and polymorphonuclear leukocytes infiltration. The control group showed a distinct space between old and new regenerating layers of epithelium. The histological studies showed an overall early recovery and regeneration in the PGE treated group, when compared with control group (Figure 1).

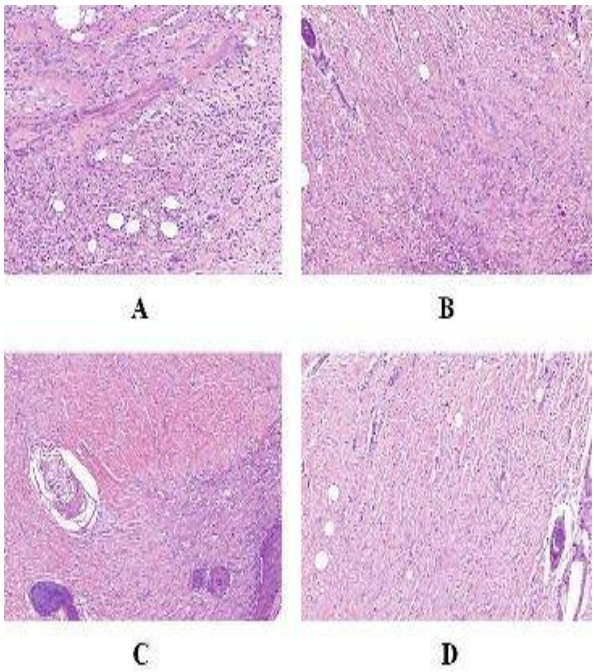

Figure 1: SEM of pathological sections of burns skin. A: Control group; B: SSD group; C: MEBO group; D: PGE group at day 10 (×200)

DISCUSSION

Thermal burn injury is still a major cause of death and disability in the world and its healing process is a challenge to modern medicine. Burn in human body may be treated by different methods depending on the extent and severity of the burn. Silver sulfadiazine (SSD) could kill a wide variety of bacteria, so it is commonly used to prevent and treat infections of the second and third degree burns.

Recent studies revealed that SSD ointment had positive effects on proliferation of fibroblasts which are the main source of collagen and fibronectin [12]. However, current reports suggest that silver-based drugs are better to be avoided due to their side effects and researchers are making efforts to seek better topical antimicrobial products.

The results of the present study indicate the PGE promotes wound healing in experimental burn wounds. The increased wound contraction in PGE treated rats might be due to enhanced activities of fibroblasts in regenerated wound tissue. Myofibroblasts are believed to play a key role in wound contraction by exerting tension on the surrounding extracellular matrix (ECM) and secreting ECM proteins such as collagen to stabilize the contraction. Collagen is a major protein of ECM and component that ultimately contributes to wound strength [13]. The enhanced expression of collagen type-III in SBT treated burn wounds when compared with the untreated control group was observed. Collagen type-III is a predominant form of collagen in the early stages of wound healing that helps in providing strength to the provisional ECM [13].

CONCLUSION

The findings of this study show that PGE possesses significant wound-healing activity in burn wounds in rats. Thus, the extract may be effective in the management of burns in humans but this requires further investigation.

Table 1: Effect of topical application of PGE on wound area contraction (mm^2)

Post-wound day	Burn control	SSD	MEBO	PGE
Day 0	193.27 ± 0.34	195.33 ± 0.28	190.37 ± 0.23	183.52 ± 0.69
Day 5	116.28 ± 3.43 (21)	123.27± 4.64 (20)	104.27 ± 4.03 (28)	102.33 ± 3.27* (25)
Day 10	85.16 ± 2.23 (40)	64.18 ± 3.29*(51)	61.38 ± 3.31* (59)	41.18 ± 2.87** (68)

Values are mean ± SE; $n = 8$; Numbers in parenthesis indicate percentage of wound contraction; $^{*}p < 0.05$, $^{**}p < 0.01$, compared with burn control

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Long-Jiao Hu designed all the experiments and revised the paper. Liang Wang and Li Yang performed the experiment, and Gang Yuan wrote the paper.

Open Access

REFERENCES

1. *Nacer KA, Mahlous M, Tahtat D. Evaluation of healing activity of PVA/chitosan hydrogels on deep second degree burn: pharmacological and toxicological tests. Burns 2013; 39: 98-104.*
2. *Kopp J, Wang GY, Horch RE. Ancient taditional Chinese medicine in burn treatment: a historical review. Burns 2003; 29: 473-478.*
3. *Edelman LS. Social and economic factors associated with the risk of burn injury. Burns 2007; 33: 958–965.*
4. *Shanmugasundaram N, Uma TS, Lakshmi TSR, Babu M. Efficiency of controlled topical delivery of silver sulfadiazine in infected burn wounds. J Biomed Mater Res A 2009; 89: 472-482.*
5. *Peng W, Qin R, Li X. Botany, phytochemistry, pharmacology, and potential application of Paris polyphylla: A review. J Ethnopharmacol 2013; 148: 729-745.*
6. *Dorsett-Martin, WA. Rat models of skin wound healing: A review. Wound Repair Regen 2004; 12: 591–599.*
7. *Hsu CY, Chan YP, Chang J. Antioxidant activity of Platycodon grandiflorus (Jacq.) A. DC. extract. Biol Res 2008; 43: 15-23.*
8. *Hsu CY, Chan YP, Chang J. Topical anti-inflammatory activity of Platycodon grandiflorus (Jacq.) A. DC. in the TPA model of mouse ear inflammation. J Inflamm 2009; 6: 11-14.*
9. *European Commission [homepage on the internet]. Directive 2010/63/EU on the protection of animals used for scientific purposes [cited 2013 Jan 16]. Available from:http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en..htm.*
10. *Gupta A, Upadhyay NK, Sawhney RC, Kumar R. A poly-herbal formulation accelerates normal and impaired diabetic wound healing. Wound Repair Regen 2008; 16: 784–790.*
11. *Upadhyay NK, Kumar R, Mandotra SK. Safety and healing efficacy of Sea buckthorn (Hippophae rhamnoides L.) seed oil on burn wounds in rats. Food Chem Toxicol 2009; 47: 1146–1153.*
12. *Coelho JM, Antoniolli AB, Nunese SD. Effects of silver sulfadiazine, ipêroxo (Tabebuia avellanedae) extract and barbatimão (Stryphnodendron adstringens) extract on cutaneous wound healing in rats. Rev Col Bras Cir 2010; 37: 45-51.*
13. *Singer AJ, Clark RAF. Cutaneous wound healing. N Engl J Med 1999; 341: 738–746.*
14. *Inkinen K, Turakainen H, Wolff H, Ravanti L, Ahonen J. Expression and activity of matrix metalloproteinase-2 and -9 in experimental granulation tissue. APMIS 2000; 108: 318–328.*