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Original Article

Effect of FPZ, a total flavonoids ointment topical application from *Pouzolzia zeylanica* var. *microphylla*, on mice skin infections

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ABSTRACT

The present study was designed to investigate the effect of FPZ, a total flavonoids ointment topical application from *Pouzolzia zeylanica* var. *microphylla* (Wedd.) Masam, Urticaceae, on skin infections in mice. FPZ ointment anti-infective effect was investigated on *Staphylococcus aureus*-induced skin abscess and skin ulcers in mice by evaluating the variation in abscess volume, histopathology of skin tissue and healing rate. Secondary, it is topical anti-inflammatory activities on carrageenan-induced hind paw edema in mice was estimated. Besides, FPZ ointment fingerprint was performed by using ultra-performance liquid chromatography and FPZ ointment chemical constituents were isolated and identified by repeated column chromatograph and spectroscopic methods. The results revealed that FPZ ointment topical application at the concentration of 2.5–10% could attenuate skin abscess and ulcers and accelerate wound healing, as compared with control group treated with vehicle ($p < 0.05$). The histological analysis indicated that FPZ ointment acted *via* inflammation inhibition, granulation promotion and epidermis formation. Moreover, FPZ ointment effectively inhibited carrageenan-induced paw edema in a dose-dependent manner, especially 10% FPZ which showed superior activities in comparison with dexamethasone used as reference drug. FPZ ointment topical application showed a significant anti-infective effect against pyogenic bacterial skin infection in mice.

Introduction

The plants of genus *Pouzolzia* (about 50 species) belong to the Urticaceae family, which are widely distributed in Japan, India, Malaysia, Indonesia, Australia and South China. Recent reports on the pharmacological activities indicated that the crude extracts from the plants of genus *Pouzolzia* had anti-inflammatory (Siriwatanametanon et al., 2010), antibacterial (Rabe and van Staden, 1997), antioxidant (Yadav et al., 2012), and anti-snake venom activities (Ahmed et al., 2010). *Pouzolzia zeylanica* var. *microphylla* (Wedd.) Masam is an important ethnomedicinal plant in the south of China. Its aerial parts have been used as a folk medicine for the treatment of skin and soft tissue infections, including skin abscesses, gangrenous ulcers, sores, boils, dysentery, syphilis, and gonorrhea, in Guangdong, Guangxi and Fujian Provinces for over 2000 years (Dangol and Gurung, 1991; Van Sam et al., 2008).

According to *P. zeylanica* traditional application, preliminary studies performed by our group showed that oral administration of *P. zeylanica* ethanol extract exerted a remarkable anti-inflammatory and analgesic effect in mice (Liu et al., 2012), while topical administration promotes wound healing of pyogenic bacterial skin infections (Li et al., 2012). Chemical constituents studies had shown that flavonoid glycosides are one of the main composition of *P. zeylanica* and their aglycones are mainly quercetin and kaempferol, most of such compounds possess anti-inflammatory activity (Chen, 2016). Therefore, we preliminary determined the curative effect of total flavonoids from *P. zeylanica* (FPZ) on SA-induced skin abscess and ulcers and evaluated *in vitro* anti-inflammatory activity of two flavonoid compounds from *P. zeylanica*. Our previous results showed that FPZ decreases the production of NO, TNF-α, IL-1 and PGE_2 on lipopolysaccharide-induced mice macrophage inflammation model (Li, 2014). On the other hand, ethanol extracts of *P. zeylanica* and FPZ did not show any significant antimicrobial effect *in vitro*, indicating that *P. zeylanica* extracts and FPZ may not directly inhibit or kill bacteria. Experimental evidences showed that FPZ functions exerting a topical anti-inflammatory action instead of the anti-bacterial one. Furthermore, we applied for the following patent "Application of *Pouzolzia* extract" granted for three flavonoid compounds from *P. zeylanica* (Guo, 2011). The total flavonoid content in the extract is not high and only three compounds were identified in this patent. On the basis of this patent, we optimized the purification process by AB-8

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macroporous resin to increase the flavonoids content from 28.81% to 68.18%. In order to meet the needs of practical application, we developed the FPZ into ointment.

Staphylococcus aureus is one of the most common bacteria causing skin and soft tissue infections, including impetigo and infected abrasions, and it is also one of the most important human pathogens. It can cause invasive and life-threatening infections, such as subcutaneous abscesses, cellulitis, folliculitis, infected ulcers and wounds. These *S. aureus* skin infections have become a major threat to public health as they resulted in 24 million outpatient all over the world (Martinez et al., 2009). Furthermore, *S. aureus* infections therapy has been an increasingly challenge for the widespread emergence of antibiotic-resistant strains such as methicillin-resistant *S. aureus* (MRSA). To date, no efficacious *S. aureus* infections treatment is available although intense research and multiple diverse therapeutic trials have been performed. Thus, there is an urgent need to discover new drugs which are efficient on treating *S. aureus* infections.

Therefore, the aim of the present study was to investigate the effect of FPZ ointment topical application on mice skin infections which were obtained by using *S. aureus*-induced skin abscess and ulcers.

Materials and methods

Phytochemical procedure

Plant material

Pouzolzia zeylanica var. *microphylla* (Wedd.) Masam, Urticaceae, was collected in December 2013 from Yulin, Guangxi province, China, and was identified by Professor Ji-Zhu Liu, Guangdong Pharmaceutical University. The voucher specimen (No. 20131210) has been deposited in the Laboratory of Traditional Chinese Medicine Chemistry, Guangdong Pharmaceutical University.

FPZ ointment preparation

Pouzolzia zeylanica was extracted three times with eightfold volume of 70% ethanol under reflux for 1 h. The extracted liquid were evaporated and diluted with 10 times distilled water. After separating the precipitate by centrifugation for 10 min at 3000×*g*, the supernatant was further separated by AB-8 macroporous resin under the conditions of sample mass concentration of 0.3 g/ml, pH 5, loaded amount of 5BV, and loading flow rate of 2 BV/h. Impurities were removed from the sample by 2BV water and by 2BV 60% ethanol elution. Ethanol eluent 60% was collected and enriched to obtain FPZ. Total flavonoids concentration increased to 68.18% (Chen, 2016).

Matrix formulation was shown in Table 1. FPZ 0.75 g, 1.5 g and 3 g were added in water phase respectively. Oil phase and water phase were dissolved and mixed at 85 °C to obtain 2.5% FPZ, 5% FPZ, and 10% FPZ ointment. Finally, a corresponding blank semisolid fraction was used as a vehicle. FPZ ointment quality standards were the following: brown, uniform fine, no rancidity, particle <180 μm in diameter, pH 6.36; no oil–water separation phenomenon after centrifugation or high and low temperature (–20~55 °C). The content of kaempferol-3, 7-*O*-α-L-dirhamnopyranoside and quercetin-3,7-*O*-α-L-dirhamnopyranoside in FPZ ointment was not less than 1000 μg/g and 110 μg/g, respectively.

Table 1
Vehicle formulation of FPZ, a total flavonoids ointment topical application from *Pouzolzia zeylanica* var. *microphylla*.

Oil phase	Weight/g	Water phase	Weight/g
Stearic acid	1.0	Propylene glycol	2.0
White vaseline	1.0	Trichloro-*tert*-butanol	0.1
Stearyl alcohol	2.0	Azone	1.0
Paraffin liquid	1.5	Triethanolamine	0.1
Glyceryl monostearate	0.8	Tween-80	1.0
Span-60	0.4	Water	19.1
Total weight/g		30	

FPZ ointment fingerprint

FPZ was prepared according to the method as described above. UPLC was performed using a Waters Acquity™ UPLC system (Waters, Milford, MA, USA) with ACQUITY BEH C_{18} column (150 mm × 2.1 mm, 1.7 μm). The mobile phase was 0.2% formic acid aqueous solution (A) and acetonitrile (B) using a linear gradient program of 15–19% (B) in 0–22 min, 19–24% (B) in 22–26 min, 24–24% (B) in 26–32 min, 24–38% (B) in 32–40 min. The flow rate was 0.3 ml/min and the injection volume was 0.2 μl. The UV absorbance was monitored at 330 nm using PDA.

Pharmacological experiment

Animals

Kun-Ming mice and Wistar rats, both SPF, weight 18–22 g and 180–220 g, respectively, were provided by the experimental animal center of Guangzhou University of Traditional Chinese Medicine (License No. SCXK (Guangdong) 2013-0020). The animals were kept under standard temperature (22 ± 1 °C), 12 h light/dark cycles, fed with standard diet and free access to water.

Bacterial culture

Staphylococcus aureus strains were separated and preserved in a basic microbiology and immunology laboratory of Guangdong Pharmaceutical University. *S. aureus* strains were grown overnight in 10 ml tryptic soy broth at 37 °C. Bacteria were harvested by centrifugation for 10 min at 3000×*g* and washed in 5 ml sterile saline. At last, bacteria were diluted to 1×10^9 CFU/ml with sterile saline.

FPZ ointment treatment on Staphylococcus aureus*-induced skin abscess*

Infectious skin abscess induced by *S. aureus* was obtained as previously described (Malachowa et al., 2013). Pearl ointment was used as reference drug and control group was treated with vehicle. Fifty female mice were randomly divided into five groups, such as vehicle, Pearl, 2.5% FPZ, 5% FPZ and 10% FPZ group. Firstly, mice back skin hair was shaved after anesthesia with pentobarbital (*i.p.*, 50 mg/kg weight), then 100 μl 1×10^9 CFU/ml *S. aureus* suspension was subcutaneously injected in their glabrous area for inducing skin abscess. Within the first 24 h after abscess was induced, the animals received a topical treatment with 0.1 g vehicle, Pearl, 2.5% FPZ, 5% FPZ or 10% FPZ once a day from day 0 to day 9 consecutively, starting immediately to evaluate the abscess-healing process after abscess induction. Skin abscess length (L) and width (W) were measured and recorded every other day with gauge calipers. Abscess volume was calculated according to the following formula (Malachowa et al., 2013):

$$\text{Abscess volume } (\text{cm}^3) = 4/3\pi(L/2)^2 \times W/2.$$

Mice skin abscess tissues were collected at specific intervals (*i.e.* day 1, day 5 and day 9) after inoculation with *S. aureus*. Tissues were dissected and fixed in 10% neutral buffered formalin, and embedded in paraffin. Microtome was used to cut 5–6 μm thick slices that were stained with hematoxylin and eosin. Finally, sections were analyzed at 100× magnification after selecting six random fields.

FPZ ointment treatment on Staphylococcus aureus*-induced skin ulcers*

Staphylococcus aureus skin ulcers were obtained as previously described with slight modification (Martinez et al., 2009; Yin et al.,

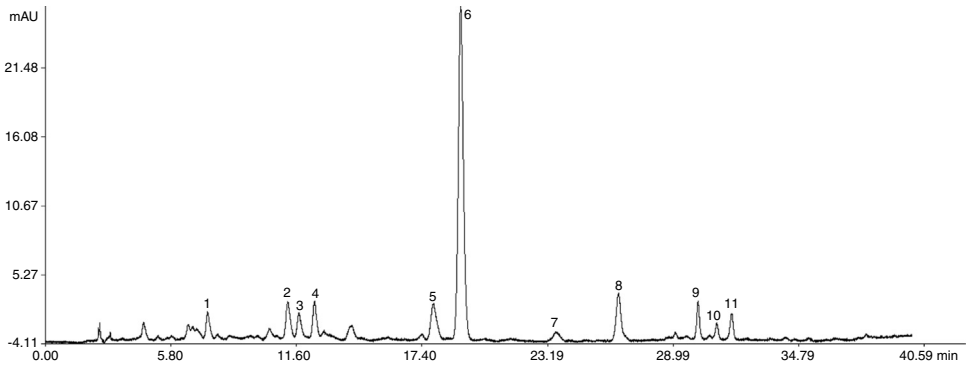

Fig. 1. FPZ ointment fingerprint. Compounds **1**–**7** corresponding to fingerprint peak **2**–**8.**

2013). Pearl ointment was used as reference drug and control group was treated with vehicle. Fifty male mice were randomly divided into five groups, such as vehicle, Pearl, 2.5% FPZ, 5% FPZ and 10% FPZ group. Mice back skin hair was shaved by a razor and removed using a depilatory cream after anesthesia with ethyl ether. After disinfection with ethanol, a circular 1 cm diameter mark was painted on the mice glabrous area and the marked area was immediately cut and removed. Subsequently, 100 μl 1×10^9 CFU/ml *S. aureus* suspensions were injected in the wound surface to induce skin ulcers. After 24 h the mice received a treatment with 0.1 g vehicle, Pearl, 2.5% FPZ, 5% FPZ and 10% FPZ once a day from day 0 to day 7 consecutively. Finally, skin ulcers length (L) and width (W) were measured and recorded every other day using gauge calipers. Ulcers area (A) and healing rate percentage were calculated according to the following formulas (VinodKumar et al., 2011):

$$(A)\ \ (\mathrm{cm}^2) = (\pi \times L/2) \times W/2,$$

$$\text{Healing rate}\ \ (\%) = (1 - A) \times 100\%.$$

FPZ ointment anti-inflammatory assay

Carrageenan-induced paw edema model in mice was used as previously described (Toker et al., 2004). Dexamethasone ointment was used as reference drug and control group was treated with vehicle. Fifty male mice were randomly divided into five groups, such as vehicle, dexamethasone, 2.5% FPZ, 5% FPZ and 10% FPZ group. 60 min after the topical application of the above mentioned drugs at the amount of 0.1 g, 30 μl (0.5 mg/25 μl) suspensions of carrageenan (Sigma, St. Louis, Missouri, USA) freshly prepared in physiological saline were injected into the sub-plantar tissue of the right hind paw. 30 μl physiological saline solutions were injected into the sub-plantar tissue of the left hind paw as a control. The difference in footpad thickness between the right and left foot was measured and calculated every 90 min during 360 min after inflammation induction.

Statistical analysis

Data were expressed as mean ± standard error of the means (S.E.M.). Differences between means were determined by analysis of variance (ANOVA) with Dunnett's t-test. Data were considered significant when $p < 0.05$.

Results

FPZ ointment fingerprint

FPZ ointment fingerprint and standard markers is shown in Figs. 1 and 2. There were eleven peaks obviously in the FPZ UPLC fingerprint, and seven peaks were identified as flavonoid compounds (Li et al., 2017; Liu et al., 2014; Luo et al., 2014). Compounds (corresponding to fingerprint peak **2**–**8**) were the following: apigenin-6-*C*-α-L-arabinoside-8-*C*-β-D-glucoside (**1**), apigenin-8-*C*-[α-L-arabinopyranosyl-(1→6)]-β-D-glucopyranoside (**2**), quercetin-3-*O*-[α-L-rhamnopyranosl-(1→6)-β-D-glucopyranosyl]-7-*O*-α-L-rhamnopyranoside (**3**), robinin (**4**), quercetin-3,7-*O*-α-L-dirhamnopyranoside (**5**), quercetin-3-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-7-*O*-α-L-glucopyranoside (**6**), kaempferol-3,7-*O*-α-L-dirhamnopyranoside (**7**).

Effect of FPZ ointment on Staphylococcus aureus*-induced skin abscess*

FPZ ointment could attenuate skin abscess compared to the abscess in the control group. During the time from day 3 to day 9, abscess volume decreased with the increase of FPZ concentration,

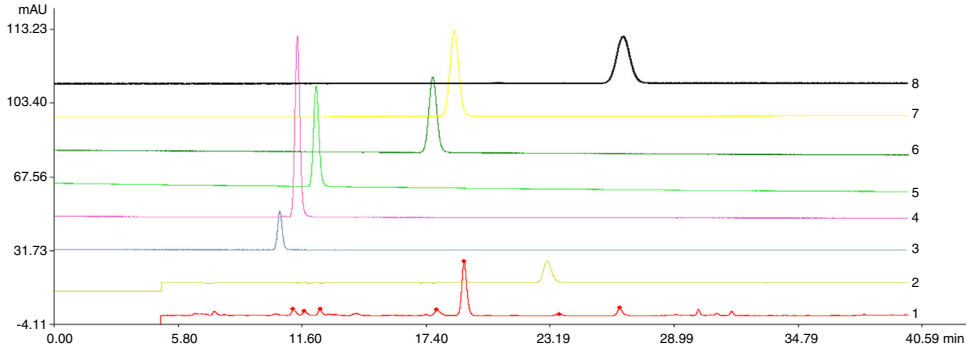

Fig. 2. FPZ ointment fingerprint and standard markers. 1, FPZ ointment fingerprint; 2, Compound **6**; 3, Compound **1**; 4, Compound **2**; 5, Compound **3**; 6, Compound **4**; 7, Compound **5**; 8, Compound **7**.

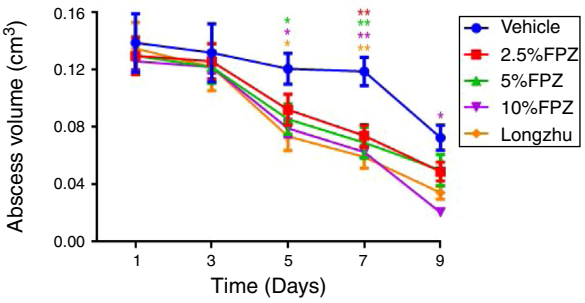

Fig. 3. Effect of FPZ ointment on *Staphylococcus aureus*-induced skin abscess. Graphs showing the changes in skin abscess volume of each group as time increased. Mice were treated with vehicle, Pearl and FPZ at different doses for 9 days. The results are expressed as mean ± S.E.M. ($n = 10$).

and its decrease in the FPZ ointment groups was significantly faster than in the control group. Analysis of variance results (Fig. 3) demonstrated that FPZ ointment exhibited a significant protective effect on *S. aureus*-induced skin abscess especially at the dose of 10% FPZ ($p < 0.05$). During the time from day 7 to day 9, 10% FPZ showed superior activities in comparison with Pearl ointment. FPZ ointment attenuated *S. aureus*-induced skin abscess and accelerated wound healing in a dose-dependent manner within the range of 2.5–10% from day 3 to day 9.

On the first day after induction of inflammation, the wound was in the early stages of inflammation. Skin abscess was clearly present and macroscopically visible in each group. *S. aureus* bacterial colony could be observed especially in the control group, while the epidermal cells were not visible. In addition, mice skin showed numerous infiltrations of inflammatory cells in the muscle and subcutaneous fat layer. Dermis showed many fusiform fibrocytes and little proliferating fibroblast (Fig. 4A–E). On day 5, the wound in mice skin showed a clear scab and was healing under it. The skin abscess was absent and the number of inflammatory cells was decreased in the Pearl group and FPZ ointment groups. Moreover, fibroblast proliferation and neovascularization were observed especially in the pearl group and FPZ ointment groups, explaining the granulation tissue formation and gradually filling the wound in the mice skin. In addition, epidermal cells were present except in the control group (Fig. 4F–J). On day 9, no congestion and necrosis on the skin abscess areas were observed in all groups, indicating that none of the treatments induced these effects during the study period. Granulation tissue was emerged, but epithelial cells were still not visible in the control group, which suggested that wounds healed slowly in the control group. The number of inflammatory cell decreased, while granulation tissue increased and filled the bottom of the wound in the therapy groups. Epidermis appeared and it was thin than before besides control group (Fig. 4K–O). The results showed that the wound healing stage of the mice skin in the treatment groups entered the remodeling phase while the control group remained in the proliferative phase.

Effect of FPZ on Staphylococcus aureus*-induced skin ulcers*

On day 1, mice wound were purulent and accompanied with swelling. However, mice wound were gradually healed as an effect of FPZ ointment treatment. One-way ANOVA revealed a significant difference among group effects ($p < 0.05$). Healing rate in FPZ groups was significantly higher than in control group, particularly from day 3 to day 7 (Fig. 5).

FPZ ointment anti-inflammatory effects

The topical carrageenan (0.5 mg/25 μl) application on the right hind paw resulted in the increase of footpad thickness. The

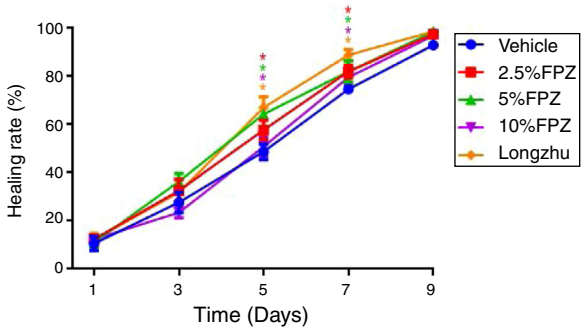

Fig. 5. Effect of FPZ ointment on *Staphylococcus aureus*-induced skin ulcers. Graphs showing healing rate change in each group as time increased. Mice were treated with vehicle, Pearl and FPZ at different doses for 9 days. The results are expressed as mean ± S.E.M. ($n = 10$).

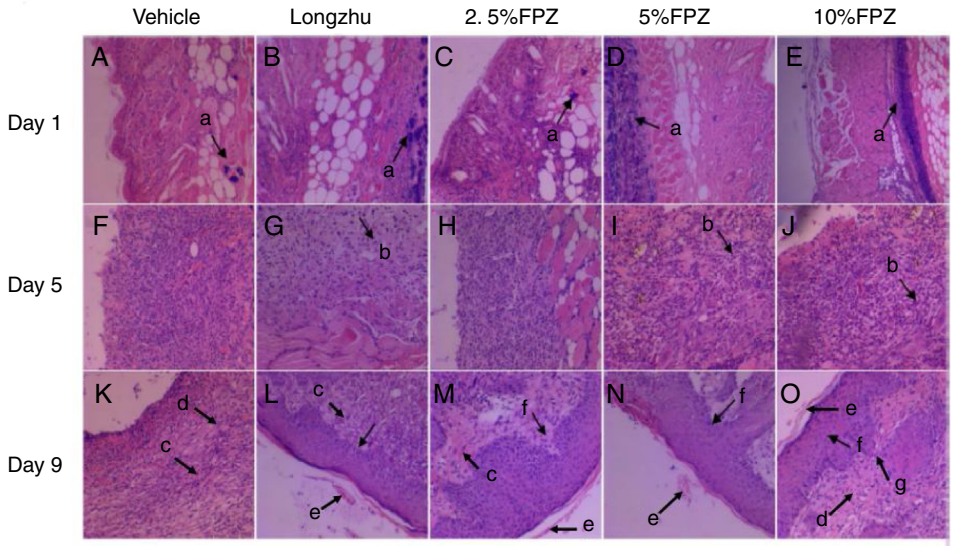

Fig. 4. Microscopic view of hematoxylin and eosin stained sections of wounds on day 1, 5 and 9. (A) Bacterial colony; (B) fibroblast and new capillary vessels; (C) fibroblast; (D) new capillary vessels; (E) re-epithelized epidermis; (F) epithelium; (G) hair follicle re-organization.

Table 2
Effects of FPZ ointment on carrageenan-induced paw edema.

Treatment groups	Swelling thickness ± S.E.M. (inhibitory %)			
	90 min	180 min	270 min	360 min
Vehicle	31.47 ± 1.53	35.82 ± 1.22	32.04 ± 2.61	26.30 ± 1.47
Dexamethasone	26.15 ± 1.96 (16.90%)	23.46 ± 1.07 (34.51%)	16.55 ± 1.35 (48.35%)[a]	11.29 ± 1.18 (57.07%)[a]
2.5% FPZ	30.39 ± 2.51 (3.43%)	29.06 ± 2.68 (18.87%)	28.17 ± 1.81 (12.08%)	25.32 ± 1.12 (3.73%)
5% FPZ	29.41 ± 1.74 (6.55%)	27.58 ± 1.46 (23.00%)	24.32 ± 2.11 (24.09%)	21.73 ± 1.75 (17.38%)
10% FPZ	24.03 ± 2.26 (23.64%)	19.64 ± 2.10 (45.17%)[a]	15.44 ± 1.34 (51.81%)[a]	10.63 ± 1.08 (59.58%)[a]

Results are expressed as mean ± S.E.M. ($n = 10$).
[a] $p < 0.05$ *vs.* control group.

difference in footpad thickness between right and left foot is shown in Table 2. The animals treated with FPZ ointment showed expressive activity and 10% FPZ was the most effective, inhibiting the edema formation.

Discussion and conclusions

The continuous search for new substances exerting skin abscess inhibition and wound healing activity, especially for protecting skin and wound associated with microbial infection, is still a challenging task for the pharmaceutical industry and academia (Martinez et al., 2009). Therefore, this study was performed to evaluate the potential role of FPZ ointment on *S. aureus*-induced skin abscess in a mice model. Our results showed that 10% FPZ topical administration profoundly inhibited *S. aureus* colonization, skin abscess and accelerated wound repair, which was confirmed by histological analysis. In our previous study, we investigated the effect of FPZ on cytokine and inflammatory mediator *in vitro* on mice peritoneal macrophages. Our previous results showed that FPZ inhibited TNF-α and IL-1 secretion level, NO and PGE_2 synthesis (Li, 2014). In the present study, FPZ topical application was able of reducing Carrageenan-induced hind paw edema in a dose-dependent manner. Our results suggested that FPZ had a significant anti-inflammatory effect, probably by acting on inflammatory factors involved in complex inflammatory pathways at one or more loci, leading to less NO, TNF-α, PGE_2 and IL-1 mediator release. According to a traditional explanation, hemostasis is firstly activated after trauma, and it is followed by chemotaxis of inflammatory cells to the wound site. However, some studies have revealed that reactive oxygen species played a critical role in initiating inflammatory response of wound healing (Yu et al., 2015). Reactive oxygen species, serving as a second messenger, can regulate numerous signal transductions and gene expressions. The second messenger molecule cyclic adenosine monophosphate (cAMP) in the cell plays an important role in regulating the development of inflammation. Intracellular elevated cAMP inhibits the release of free radicals from inflammatory cells and produces anti-inflammatory activity (Pan et al., 2009). Thus a further study is needed to evaluate FPZ ointment anti-inflammatory activity and its molecular mechanism by cellular second messenger and inflammatory signal transduction pathway.

Wound healing is a complex process involving integrated series of biochemical, cellular and physiological events. This process is divided into three stages: inflammation, proliferation and re-modeling. In the inflammatory phase, polymorph nuclear leukocytes and lymphocytes are attracted by soluble mediators that facilitate the adhesion to the endothelium and transmigration and play a key role by secreting cytokines and a great variety of growth factors. The proliferative phase is characterized by angiogenesis, collagen deposition, epithelialization and wound contraction, while the re-modeling phase is characterized by collagen and other extracellular matrix proteins re-modeling to form mature scar tissue (Wilgus, 2008). Therefore, the inflammatory response is an important step of the abscess inhibition and wound healing process since it creates a wound environment for the process of repairing. However, excessive inflammatory response may delay wound healing (Araujo et al., 2010). Our results showed that FPZ anti-inflammatory effect in mice was achieved through the promotion of proliferation and re-modeling phase, thus accelerating wound healing.

FPZ flavonoids composition indicated the presence of quercetin-3,7-*O*-α-L-dirhamnopyrnoside (57.42%), kaempferol-3,7-*O*-α-L-dirhamnopyrnoside (7.30%), quercetin-3-*O*-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranosyl]-7-*O*-α-L-glucopyranoside (6.79%) and robinin (5.09%). The potent anti-inflammatory activity of different types of flavonoids including quercetin and kaempferol derivatives were previously reported (Matsuda et al., 2002). The main flavonoid FPZ glycosides, quercetin-3,7-*O*-α-L-dirhamnopyrnoside and kaempferol-3,7-*O*-α-L-dirhamnopyranoside possess a potent anti-inflammatory activity without inducing any apparent acute toxicity or gastric damage (Toker et al., 2004). The flavonoid glycosides including quercitrin, hyperoside, tiliroside, astragalin and kaempferol 3-rutinoside exert inhibitory effects on NO production in LPS-stimulated macrophage RAW 264.7 cells (Zhang et al., 2015). Some quercetin derivatives hindered both the cyclooxygenase and lipoxygenase pathways of the arachidonate cascade at high concentrations, while at lower concentrations only the lipoxygenase pathway (Di Carlo et al., 1999) is hindered. Chemical studies suggest that FPZ contains mainly quercetin and kaempferol aglycones and this article confirms FPZ ointment anti-inflammatory effect, while the anti-inflammatory mechanisms need to be further analyzed.

In conclusion, FPZ ointment inhibits topical inflammation and accelerate cutaneous wound repair. These data validate its widespread use and suggest a potential therapeutic effect on skin immune system.

Ethical disclosures

Protection of human and animal subjects. The authors declare that all experimental procedures involving animals and their care were performed according to the guidelines of the Institutional Ethical Committee for Care and Use of Laboratory Animals, published by the US National Institute of Health (NIH publication, revised in 1985).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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Authors contributions

L-BG conceived and designed the experiments, performed the data analyses and performed the analysis with constructive discussions; X-MC performed the experiments and wrote the manuscript, drafted or revised the manuscript; Z-HL performed the experiments, included preparation of ointment and animal experiment; S-HT helped drafted or revised the manuscript and performed the analysis with constructive discussions; Y-FC helped performed the data analyses and instructed to complete animal experiments; Z-HC performed the experiments, included establishment of fingerprints and identification of compounds.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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References

Ahmed, A., Rajendaran, K., Jaiswal, D., Singh, H.P., Mishra, A., Chandra, D., Yadav, I.K., Jain, D.A., 2010. Anti-snake venom activity of different extracts of *Pouzolzia indica* against Russel viper venom. Int. J. ChemTech Res. 2, 744–751.

Araujo, L.U., Grabe-Guimaraes, A., Mosqueira, V.C., Carneiro, C.M., Silva-Barcellos, N.M., 2010. Profile of wound healing process induced by allantoin. Acta Cir. Bras. 25, 460–466.

Chen, Z.H., 2016. The preparation of *Pouzolzia zeylanica* (L.) Benn. var. *microphylla* (Wedd.) W.T. Wang. Ointment. MSc dissertation, Guangdong Pharmaceutical University.

Dangol, D.R., Gurung, S.B., 1991. Ethnobotany of the Tharu Tribe of Chitwan District, Nepal. Pharm. Biol. 29, 203–209.

Di Carlo, G., Mascolo, N., Izzo, A.A., Capasso, F., 1999. Flavonoids: old and new aspects of a class of natural therapeutic drugs. Life Sci. 65, 337–353.

Guo, L.B., 2011. Application of *Pouzolzia* extract. China Patent, 201110338498.

Li, K.Y., Xie, Y.F., Chen, Y.F., Liu, T.Z., Guo, L.B., 2012. Effect of *Pouzolzia zeylanica* extracts in mouse subcutaneous abscess. J. Guangdong Pharm. Univ. 28, 540–544.

Li, K.Y., 2014. Study on anti-inflammatory ingredients and mechanisms of *Pouzolzia zeylanica* var. *microphylla*. MSc dissertation, Guangdong Pharmaceutical University.

Li, Z.H., Chen, X.M., Tao, S.H., Zhong, C.Q., Guo, L.B., 2017. Study on chemical cosstituents from *Pouzolzia zeylanica* (III). J. Guangdong Pharm. Univ. 33, 177–180.

Liu, X.Y., Du, Q.T., Li, K.Y., Deng, Q., Guo, L.B., 2012. Anti-inflammatory and analgesic effects of different extract fractions from *Pouzolzia zeylanica* var. *microphylla*. Drugs Clinic 27, 356–369.

Liu, X.Y., Xie, Y.F., Zhang, H., Liu, T.Z., Wen, T., Guo, L.B., 2014. Chemical constituents from *Pouzolzia zeylanica* (L.) Benn. var. *microphylla* (Wedd.) W.T Wang. (I). J. Guangdong Pharm. Univ. 20, 43–47.

Luo, Z., Zhong, C.Q., Liu, X.Y., Yi, Z.B., Guo, L.B., 2014. Study on chemical constituents from *Pouzolzia zeylanica* (II). J. Guangdong Pharm. Univ. 30, 430–434.

Malachowa, N., Kobayashi, S., Braughton, K., DeLeo, F., 2013. Mouse model of *Staphylococcus aureus* skin infection. In: Allen, I.C. (Ed.), Mouse Models of Innate Immunity. Humana Press, pp. 109–116.

Martinez, L.R., Han, G., Chacko, M., Mihu, M.R., Jacobson, M., Gialanella, P., Friedman, A.J., Nosanchuk, J.D., Friedman, J.M., 2009. Antimicrobial and healing efficacy of sustained release nitric oxide nanoparticles against *Staphylococcus aureus* skin infection. J. Investig. Dermatol. 129, 2463–2469.

Matsuda, H., Ninomiya, K., Shimoda, H., Yoshikawa, M., 2002. Hepatoprotective principles from the flowers of *Tilia argentea* (Linden): structure requirements of tiliroside and mechanisms of action. Bioorg. Med. Chem. 10, 707–712.

Pan, J.S., Hong, M.Z., Ren, J.L., 2009. Reactive oxygen species: a double-edged sword in oncogenesis. World J. Gastroenterol. 15, 1702–1707.

Rabe, T., van Staden, J., 1997. Antibacterial activity of South African plants used for medicinal purposes. J. Ethnopharmacol. 56, 81–87.

Siriwatanametanon, N., Fiebich, B.L., Efferth, T., Prieto, J.M., Heinrich, M., 2010. Traditionally used Thai medicinal plants: *in vitro* anti-inflammatory, anticancer and antioxidant activities. J. Ethnopharmacol. 130, 196–207.

Toker, G., Kuepeli, E., Memisoglu, M., Yesilada, E., 2004. Flavonoids with antinociceptive and anti-inflammatory activities from the leaves of *Tilia argentea* (silver linden). J. Ethnopharmacol. 95, 393–397.

Van Sam, H., Baas, P., Keßler, P.J.A., 2008. Traditional medicinal plants in Ben En National Park Vietnam. Blumea 53, 569–601.

VinodKumar, C.S., Srinivasa, H., Basavarajappa, K.G., Patil, U., Bandekar, N., Patil, R., 2011. Abrogation of *Staphylococcus aureus* wound infection by bacteriophage in diabetic rats. Int. Pharm. Sci. Drug Res. 3, 202–207.

Wilgus, T.A., 2008. Immune cells in the healing skin wound: influential players at each stage of repair. Pharmacol. Res. 58, 112–116.

Yadav, I.K., Singh, H.P., Jain, D.A., Ahmed, A., Indranil, D., 2012. *In vitro* antioxidant and radical scavenging activity of different extracts of *Pouzolzia indica*. Elixir Int. J. 47, 9143–9148.

Yin, H., Li, X., Hu, S., 2013. IL-33 promotes *Staphylococcus aureus*-infected wound healing in mice. Int. Immunopharmacol. 17, 432–438.

Yu, J.K., Lu, Y.Y., Li, Y.P., Xiao, L.L., Xing, Y., Li, Y.S., Wu, L.M., 2015. Roleof S100A1 in hypoxia-induced inflammatory response in cardiomyocytes via TLR4/ROS/NF-κB pathway. J. Pharm. Pharmacol. 67, 1240–1250.

Zhang, T.-T., Wang, M., Yang, L., Jiang, J.-G., Zhao, J.-W., Zhu, W., 2015. Flavonoid glycosides from *Rubus chingii* Hu fruits display anti-inflammatory activity through suppressing MAPKs activation in macrophages. J. Funct. Food 18 (Part A), 235–243.