Veterinary Integrative Sciences 2020; 18(2): 103-117

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Effect of chia (*Salvia hispanica* L.) seed extract on wound healing in mice

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Abstract

Salvia hispanica L. either commonly known as chia seed, can help in inflammatory skin disease including wound healing. However, the pharmacological activity and benefits of chia seed for wound healing need to be validated. The current study is designed to evaluate the effect of different concentrations of chia seed extract used as a topical drug for secondary wound healing in mice. Chia seed extract was analysed some active ingredients and properties, such as fatty acid compositions, total phenolic content, antioxidant activity, and antimicrobial activity. Male BALB/cAJcl mice were divided into 4 groups (n = 24), given 6 mm circular area, treated for 9 consecutive days with different applications: propylene glycol, 4% chia seed extract, 8% chia seed extract, and 1% silver sulfadiazine. The investigated parameters were the percentage of wound contraction and histopathological examination. Chia seed extract consists of major fatty acids, alpha-linolenic and linoleic acid; total phenolic compound, also has ability to eliminate free radicals by using DPPH, FRAP, and ABTS assay, and did not show the antibacterial property. Wound contraction increased significantly on day 9 in groups treated with 8%, 4% v/v chia seed extract, and 1% silver-sulfadiazine, rising to 97.98, 97.14, 89.17 percent respectively, compared to propylene glycol group, 76.49 ($P<0.001$). Histopathological studies showed infiltration of a number of fibroblasts, collagen, and vessels occurring in the treated groups. Chia seed extract is a good natural plant which could be used as topical treatment for activating wound healing.

Keywords: Antioxidant, Chia seed, *Salvia hispanica* L., Wound healing

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Article history; *received manuscript: 19 March 2020, revised manuscript: 27 March 2020, accepted manuscript: 7 April 2020, published online: 14 April 2020*
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Wound healing is a dynamic complex process, which comprises four phases: hemostasis, inflammation, proliferation, and tissue remodeling. In the hemostasis phase, platelet aggregation is an immediately active process after tissue injury, forming a platelet plug, and then originating fibrin production which reinforces the platelet plug (Li et al., 2007). Activated platelets in this phase also secrete a number of growth factors and cytokines which significantly influence the later stages of wound healing (Werner and Grose, 2003). In the inflammation phase, inflammatory cells such as mast cells, lymphocytes, monocytes, and macrophages occupy the wound tissue. These cells are not only involved in the phagocytosis of bacteria, wound debris, and foreign particles, but also are an important source of mediators, which induce the proliferative phase (Koh and DiPietro, 2011). In the proliferative phase, there are many processes: fibroblast proliferation, collagen synthesis, angiogenesis, re-epithelialization, and wound contraction. The dominant cells helping skin re-growth in this phase are keratinocytes, fibroblasts, and myofibroblasts (Werner et al., 2007). Finally, in the remodeling phase, the wound increases its strength by the arrangement of scar collagen (Rohani and Parks, 2015).

People using herbal medicine as primary health care in many general diseases around the world comprise approximately 60-90%, and are especially in developing countries. There is a tendency to use more in the future due to the affordability of herbal medicine. Additionally, people are concerned about chemical drugs (WHO, 2002). As herbal medicine becoming more important to treat skin diseases or wound, plants have been reported in the treatment of wound healing or skin problems in approximately 34% of all traditional medicines. In contrast, just only 1-3% of modern drugs were used for wound or skin treatment. (Mantle et al., 2001). On the other hand, Phytochemicals, known as bioactive substances, can be extracted from various plants. Their properties stimulate wound process in several different mechanisms such as inhibition of bacteria, scavenging free radicals, and stimulating mitogenic activity e.g. boosting cell proliferation, stimulating vascular production, activating collagen production, and promoting DNA synthesis (Ghosh and Gaba, 2013).

Salvia hispanica L. is generally known as either chia or chia seed. It is endemic in Central America and South America, especially in Mexico (Ayerza et al., 2002). Chia seed, which is a natural source of lipid antioxidants, comprises omega 3 (alpha-linolenic acid; 18: 3), omega 6 (linoleic acid; 18: 2), and antioxidant phytochemicals such as myricetin, quercetin, kaempferol, and chlorogenic acid (Marineli et al., 2015). Previous reports have showed that alpha-linolenic acid and linoleic acid had beneficial effects of anti-inflammation, and decreased the risk factors in cardiovascular diseases as well as chronic diseases (Simopoulos, 2008). Moreover, topical applications of these fatty acids have more advantages in many stages of wound healing (Pérez-Recalde et al., 2018). The antioxidants of chia seed play an important role in anti-inflammatory activities. As previously mentioned, chia seed properties likely improved wound regeneration and repair, so topical chia seed oil has received a great deal of attention. Consistent with the study of Jeong et al. (2010), chia

seed was reported to have the effect of a successful topical anti-pruritic agent on skin disorder. However, there is limited knowledge of the pharmacological activities of chia seed and require validation.

The aim of the present study was to investigate the effectiveness of different concentrations of topical chia seed extract in the full-thickness excision wound in mice. In addition, data on wound contraction and histopathological tissues were collected for evaluating the wound healing process.

MATERIALS and METHODS

Seeds

Commercial chia seed products (Nathary®, Thailand) were imported from Bolivia. Seeds were packed, sealed with plastic, and stored at 4°C until use.

Plant extraction

Chia seeds were ground and extracted with n-hexane in a Soxhlet extractor under thermal cycles at 80 ºC for 8 hours, following the IUPAC Standard Method (Paquot and Hautffene, 1992). The solvent was removed using a rotary vacuum evaporator (Buchi, Flawil, Switzerland) at 40 ºC under nitrogen stream (Capitani et al., 2012). This extract was reserved for all tests described below. For this study, 4 and 8% v/v chia seed extract were prepared with a base mixture of propylene glycol for the excision wound model.

In vitro antimicrobial activity

The antimicrobial activity was evaluated using disc diffusion method (Wayne, 2015). The standard bacteria were both gram-negative and gram-positive bacteria comprising *Staphylococcus aureus* (TISTR2329), *Staphylococcus intermedius* (TISTR668), *Escherichia coli* (TISTR527), and *Pseudomonas aeruginosa* (TISTR1287). These strains were obtained from the Thailand Institute of Scientific and Technological Research, and were cultured on tryptic soy broth (TSB) at 37 °C for 24 hours. The turbidity of the test suspension was adjusted by sterile saline (0.9%) and equivalent to a 0.5 McFarland's standard (approximately 1.5 x 108 CFU/ml). Standardized TSB was spread on Mueller-Hinton agar and then paper disks of various concentrations of chia seed extract (4%, 8%, 50%, and 100% v/v); propylene glycol, 0% v/v of chia seed extract (negative control), and antibiotic (positive control; amikacin) were placed on a plate and incubated at 37 ºC for 16-24 hours. Finally, all specific zones of inhibition were interpreted in diameters as a susceptible based on CCLSI (2015) recommendation.

Fatty acid composition of chia seed extract

Fatty acid compositions of chia seed extract were tested by GC-FID based on AOAC method 996.06 (AOAC, 2012).

Total phenolic content and antioxidant activity

Determination of total phenolic content. The total phenolic compound was measured by using the modified Folin-Ciocalteau method as described by Singleton and Rossi (1965). The 20 µl of chia seed extract (1 mg/ml) was mixed with 100 µl of Folin-Ciocalteau reagent and left for 1 minute. Then added 80 µl of 7.5% Na_2CO_3 (w/v) was added and left at ambient temperature for 30 minutes. The extracted total phenolic compound was measured a wavelength at 765 nm and compared with the standard gallic acid concentration and reported as gallic acid equivalent (mg GAE/mg oil).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Prior et al., 2005) was performed as follows. The 10 µl of chia seed extract (1 mg/ml) was mixed with 190 µl of 0.1 mM DPPH solution. The mixture was incubated for 30 minutes in a dark chamber. Measurement of absorbance used a microplate reader at 515 nm. The results were expressed as the concentration of chia seed extract, which inhibited DPPH compared with the standard Trolox concentration and reported as Trolox equivalent (mg TEAC / mg oil).

Ferric reducing antioxidant power (FRAP) assay (Thaipong et al., 2006). The 10 µl of chia seed extract (1 mg/ml) was mixed with 190 µl of FRAP reagent. Measurement of absorbance using a microplate reader at a wavelength of 593 nm was compared with a standardized graph of Trolox concentration and reported as Trolox equivalent (mg TEAC / mg oil).

2,2'-Azino-bis (3-ethylbenzothialine-6-sulfonic acid) (ABTS) scavenging activity (Thaipong et al., 2006) was assayed as follows. The 10 µl of chia seed extract (1 mg/ml) was mixed with 190 µl of ABTS•+ solution (7.0 mM ABTS solution was prepared by reaction of 2.45 mM $K_2S_2O_8$ and incubated for 15h in the darkroom). The mixture was incubated for 15 min in a dark chamber. Measurement of absorbance using a microplate reader at a wavelength of 734 nm was compared with the standard graph of Trolox concentration and reported as Trolox equivalent (mg TEAC/mg oil).

Animal preparation

Male BALB/cAJcl mice (25-30 g), 8-10 weeks of age, were imported from Nomura Siam International Co., Ltd, Bangkok. The animal experiments were permitted by the Northeast Laboratory Animal Center, Khon Kaen University, Thailand (Ethics approval number: ACUC-KKU-60/60). This study was performed according to the ethical principles and guidelines of the National Research Council of Thailand. All mice were housed in an individual cage in an air-conditioned room at 25±3 °C with a 12/12-hour light/dark cycle and acclimated to the new environment for 2 weeks. They were fed water and standard pellet diet ad libitum. To study the effects of topical chia seed extract, mice (n=24) were inflicted with wounds and divided into four groups (six mice per group). These groups of animals (treated groups; 4%v/v chia seed extract, 8%v/v chia seed extract and 1% w/w silver sulfadiazine; and untreated group, propylene glycol) were treated topically once a day for 9 days (Zhu et al., 2018).

Wound contraction

The wound model was designed to measure wound contraction and observed macroscopic lesion. In each group of animals, interscapular hair was shaved off and the skin prepared aseptically with 70% ethyl alcohol (Saratha et al., 2010). The mice were anesthetized by 10 mg/kg xylazine® and 60 mg/kg ketamine® (Cavalcanti et al., 2012). A circular full-thickness wound was cut off from prepared skin (Interscapular area) of the mice with a 6 mm biopsy punch (Akkol et al., 2011). For daily wound management, the wound areas were cleaned, removed debridement with normal saline and sterile cotton bud , treated with different applications according to topical medication in each group, covered with Tegaderm® transparent film dressing, and allowed to heal naturally. Wound areas were monitored by a digital camera (Nikon Corporation, Tokyo, Japan) on days 0, 3, 5, 7, and 9. The wound area was measured in images by using the AutoCAD software (Koca et al., 2009). Wound contraction was measured from the wound area as a percentage of the initial wound area (day 0) (Rashed et al., 2003).

Histopathological examination

The full thickness wounds were surgically removed from each group at the end of the experiment (day 9). The tissues were fixed in 10% neutral buffered formalin, processed, embedded in paraffin, and after that were serially sectioned into 5 µm thick sections. The sections were stained with hematoxylin and eosin stain (H&E), and Masson's trichrome stain (MT) (Cavalcanti et al., 2012). The stained sections were imaged by Nikon light microscope (Nikon Corporation, Tokyo, Japan).

Statistical analysis

Obtained results are reported as mean ± SD. The percentage of wound contraction was compared using one-way analysis of variance (ANOVA) followed by the Duncan test. The results were considered a significant difference at $p < 0.05$.

RESULTS

In vitro antimicrobial activity

The antimicrobial activity of chia seed extract was determined using in vitro disc diffusion method and also showed in Table 1.

Fatty acid composition of chia seed extract

The fatty acid composition of *Salvia hispanica* as shown as Table 2.

Table 1 The antimicrobial activity of chia seed extract using in vitro disc diffusion method (mean ± SD)

Sample	Zone of inhibition (mm)			
	Staphylococcus aureus	***Staphylococcus intermedius***	***Escherichia Coli***	***Pseudomonas aeruginosa***
Propylene glycol (0 % v/v CSE)	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
4 % v/v CSE	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
8 % v/v CSE	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
50 % v/v CSE	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
100 % v/v CSE	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000	0.00 ± 0.000
Amikacin	25.25 ± 0.354	24.88 ± 0.177	19.50 ± 0.707	18.63 ± 1.591

Table 2 Fatty acid composition of chia seed extract (*Salvia hispanica* L.).

Fatty acids	chia oil (g/100 g)
Unsaturated fatty acid	**88.7954**
alpha-Linolenic acid (C18:3n3)	62.0267
cis-9,12-Linoleic acid (C18:2n6)	19.8989
cis-9-Oleic acid (C18:1n9c)	6.5175
Palmitoleic acid (C16:1n7)	0.2378
Erucic acid (C22:1n9)	0.0617
cis-11,14-Eicosadienoic acid (C20:2)	0.0529
Saturated fatty acid	**11.2046**
Palmitic (C16:0)	7.2258
Stearic (C18:0)	3.4312
Arachidic acid (C20:0)	0.2898
Lignoceric acid (C24:0)	0.0934
Behenic acid (C22:0)	0.0767
Pentadecanoic acid (C15:0)	0.0456
Myristic acid (C14:0)	0.0421

Total phenolic content and antioxidant activity

The total phenolic content and antioxidant activity (DPPH, FRAP, and ABTS) were showed in Table 3.

Wound contraction

Full-thickness excision wounds were created on back skin in mice and also allowed to heal as secondary intention. Between day 1 and 3, there were no differences in macroscopic lesions, all wound became inflamed, and were covered with a dry scab. By day 5, remained scab was found in all wounds and gradually fell off until by day 7, in the 8% v/v chia seed extract group, the scab disappeared and wound contraction was obviously evident a difference (Figure 1). The percentage of wound contraction revealed a significant difference on day 7 ($P<0.05$) and day 9 ($P<0.001$). The wound areas of chia seed extract group (4% and 8% v/v) healed much more than 50% between day 5 and 7. On the other hand, both the propylene glycol group and 1% silver sulfadiazine showed contraction rate and healed more than 50% between day 8 and 9 (Figure 2). By day 9, comparison of treated groups and propylene glycol group revealed significant differences in wound contraction rate. Treated groups with more than 80% of the wound were almost completely heal, while wound contraction in the propylene glycol group was only 76% (Table 4).

Table 3 Phenolic compound and antioxidant activity of chia seed oil (*Salvia hispanica* L.).

Methods	Unit	antioxidant activity (mean ± SD)
total phenolic	mg GAE/ mg oil	0.160 ± 0.007
DPPH assay	mg TEAC/ mg oil	0.563 ± 0.045
ABTS assay	mg TEAC/ mg oil	1.621 ± 0.013
FRAP assay	mg TEAC/ mg oil	0.977 ± 0.035

Table 4 Percentage of wound contraction on day 3, 5, and 9 post-wounding (Mean ± SD).

Group	Day 3	Day 5	Day 7*	Day 9**
PG	9.76 ± 3.89	31.35 ± 13.35	49.20 ± 16.82[a]	76.49 ± 8.30 [a]
4% CSE	19.42 ± 13.67	46.24 ± 26.36	67.10 ± 28.13[a]	97.14 ± 6.42 [b]
8% CSE	19.05 ± 13.67	44.09 ± 27.43	77.15 ± 14.44[a, b]	97.98 ± 2.27 [b]
1% SSD	9.43 ± 5.76	19.96 ± 12.66	43.97 ± 19.22[b]	89.17 ± 10.15[b]

* Significant difference $P<0.05$, ** Significant difference $P<0.001$

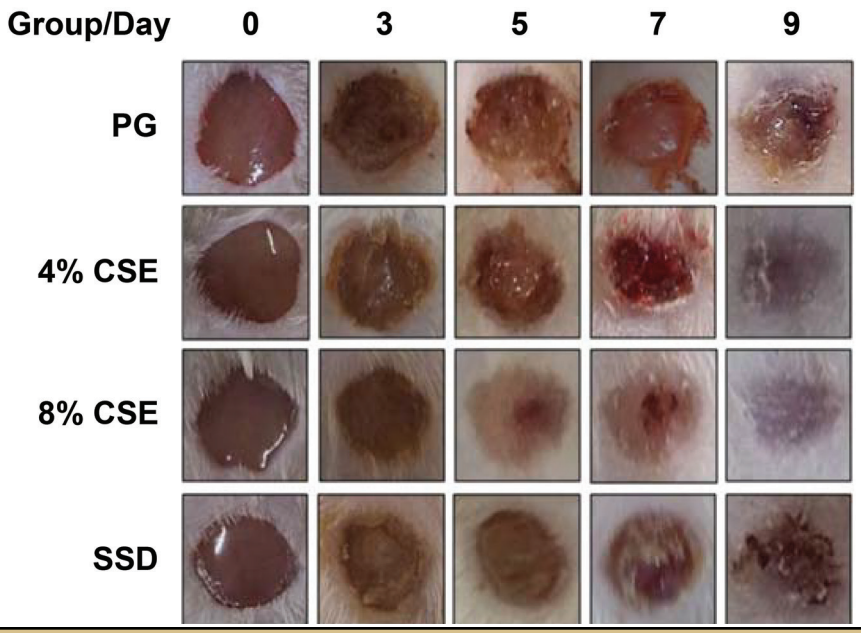

Figure 1 Macroscopic wound healing treated with 4%, 8% chia seed extract, and silver sulfadiazine, compared to untreated propylene glycol in mice on the different days post-wounding.

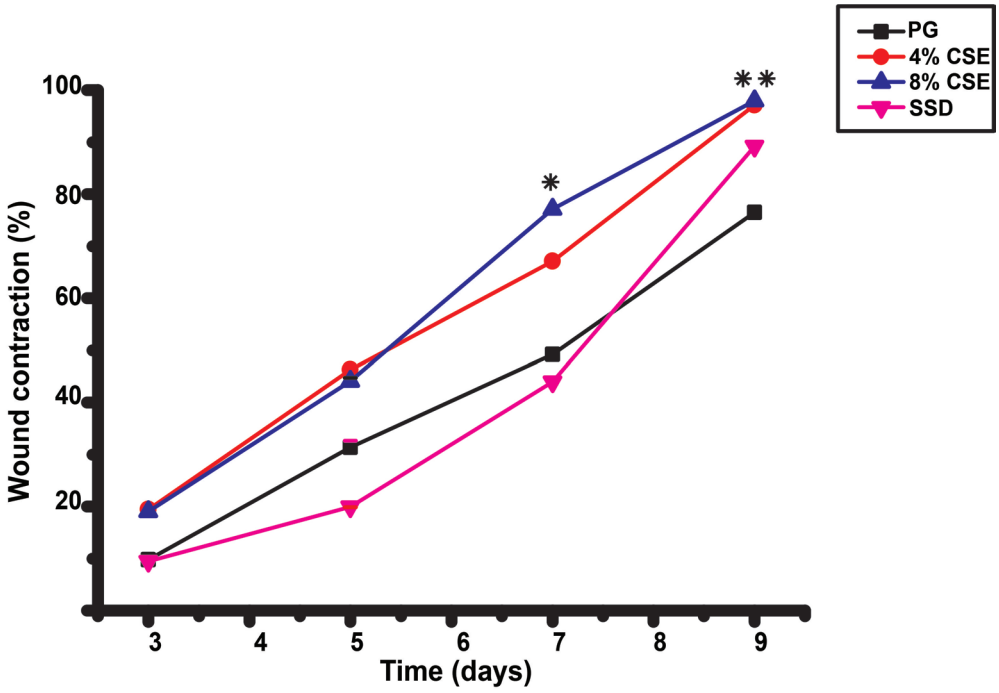

Figure 2 The graph illustrates the different percentage of contracted areas at prepared date post-wounding.

Histological examination

Histological sections stained with H&E and MT were executed in both treated groups and untreated group on day 9. The propylene glycol group and 1% silver sulfadiazine group with H&E staining had unbridged epithelium, some PMNs, slight neovascularization, and mild hemorrhage. MT staining found reviewed negligible fibroblasts and little collagen formation, but the 1% silver sulfadiazine group found had more collagen than the propylene glycol group. In contrast, in both the 4% v/v chia seed extract group and 8% v/v chia seed extract groups, there was complete dermal epithelialization, and the majority of cells were fibroblasts, there was collagen formation, marked neovascularization and keratinization. While PMNs cells were found less in the 4% v/v chia seed extract group and 8% v/v chia seed extract groups than in the propylene glycol group and 1% silver sulfadiazine group (Figure 3).

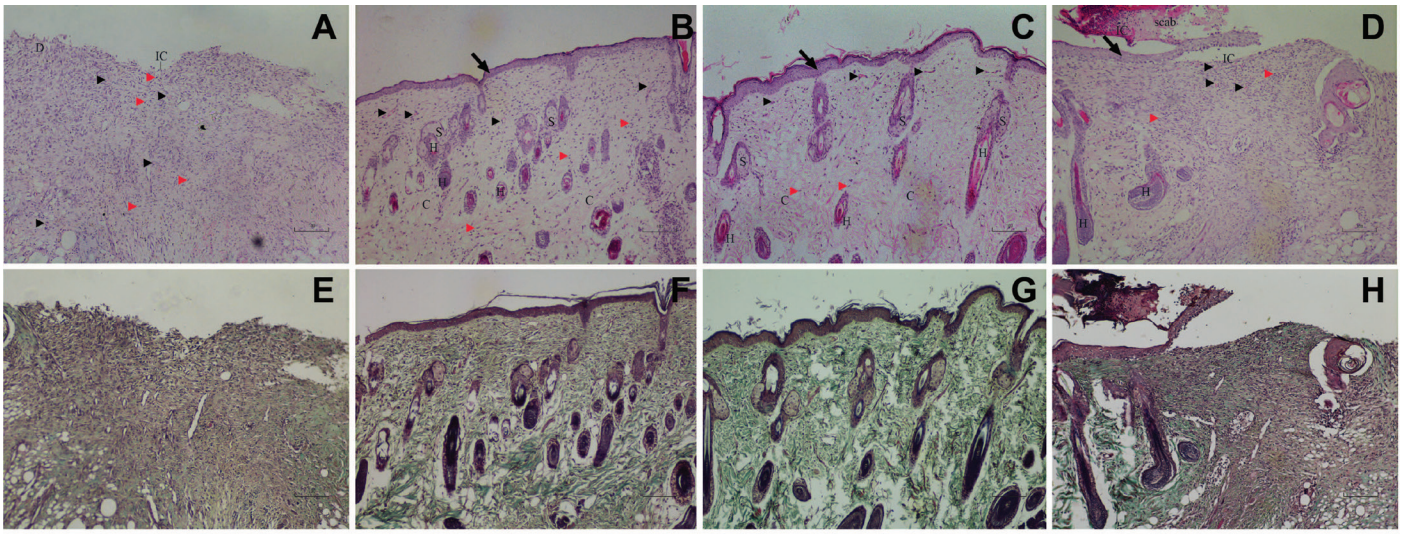

Figure 3 Histological examination of full-thickness excisional murine wound model on day 9 post-wounding. Images of wound sections stained with Hematoxylin and Eosin; 100 X (A, B, C, & D) and Masson's trichrome; 100 X (E, F, G, & H). A & E mice treated with propylene glycol, B & F mice treated with 4% chia seed extract, C & G mice treated with 8% chia seed extract, and D& H treated with 1% silver sulfadiazine. Wound scab and inflammatory cell (IC) were obviously observed in mice treated with 1% silver sulfadiazine. The black arrows indicate epidermis. The red arrow heads and black arrow heads indicate fibroblasts and blood vessels. Collagen stained with Masson's trichrome was showed in green color. Scale bar 100 µm.

DISCUSSSION

In vitro antimicrobial activity: all concentrations of chia seed extract and propylene glycol failed to inhibit all bacterial strains and did not show specific zones of inhibition. In contrast, amikacin clearly shown specific zones of inhibition had effective activity against *Staphylococcus aureus* (TISTR2329), *Staphylococcus intermedius* (TISTR668), *Escherichia coli* (TISTR527), and *Pseudomonas aeruginosa* (TISTR1287). These tested strains were the cause of many common skin diseases in animal and human (Stulberg et al., 2002; Talan et al., 1999). Obtained results of antimicrobial activity were inconsistent

with those previous studies. Chia seed has been previously reported antimicrobial activity against some pathogenic microorganisms: *Clostridium difficile* ATCC 9689, *Clostridium butyricum* ATTC 860, *Clostridium butyricum* ATTC 860, *Pseudomonas aereuginosa* ATCC 2785, *Salmonella typhimurium* ATCC 140, *Salmonella enteritidis* ATCC 13076, *Proteus mirabilis* ATCC 12453, *Escherichia coli* ATCC 25922, ; probiotic microorganisms: *Lactococcus lactis*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus paracasei*, *Streptococcus thermophilus*, *Lactobacillus reuteri* DSM 12246; periodontal microorganisms, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans* (Divyapriya et al., 2016; Kobus-Cisowska et al., 2019). In many experiments which were reported effective of inhibiting bacteria tended to be caused by the active ingredients, including proteins (bioactive peptides), antioxidants in chia seed (Divyapriya et al., 2016; Kobus-Cisowska et al., 2019; Segura-Campos et al., 2013). In present study, chia seed extract did not find any effect on inhibiting bacterias as mentioned above. In addition, we found that some studies provided consistent results which did not show antimicrobial activity in some microorganisms such as *Staphylococcus aureus* NCTC 8530, *Escherichia coli* BL21, *Bacillus subtilis* NRRL-B209, and *Listeria monocytogenes* ATCC 7644 (Tuncil and Celik, 2019). In our sample, a lack of antimicrobial activity of chia seed extract may be caused by high temperature of Soxhlet method using n-Hexane which are likely to destroy some active proteins and antioxidants (Capitani et al., 2012). Different locations can also cause different levels of antioxidants, whichaffect antimicrobial activity (da Silva et al., 2017). A previous study showed that antimicrobial activity of chia seed extract was species-specific or strain-specific resulting in no positive results found in the test (Tuncil and Celik, 2019). These factors were likely to be the cause of antimicrobial inactivity in this.

Fatty acid composition of chia seed extract: the fatty acid composition of *Salvia hispanica* (Table 2) was similar to previous reports previously (Ayerza et al., 2002; Coates, 2011). Alpha-linolenic acid (C18:3n3), which is an unsaturated fatty acid, was the major fatty acid of *Salvia hispanica* comprising 62.0267% of total fatty acids. The second largest fatty acid component was cis-9, 12-linoleic acid (C18:2n6), which comprised 19.8989% of total fatty acids. The other constituents were unsaturated and saturated fatty acids such as palmitic, oleic acid and stearic. Total phenolic content and antioxidant activity.

The result of the present study showed that chia seed extract contained total phenolic compounds (measured in terms of Gallic acid equivalent), and exhibited different antioxidant activity in terms of Trolox equivalent. Chia seed extract from Soxhlet method in this study was solvent extraction by using n-Hexane. Colour of extract was yellow. Although this conventional method provided more oil than other method, Less preservation of antioxidant content was found because of high temperature of Soxhlet method (Capitani et al., 2012). Furthermore, the developmental stage and location of chia seed affected chemical compositions, especially antioxidants and fatty acids (da Silva et al., 2017; Marineli et al., 2015; Coelho and Salas-Mellado, 2014).

In the present paper, the mixture that we used with chia seed extract was propylene glycol because of its properties as follows: It could produce moisture environment of wound by mixing this solvent with fatty acid used

as topical ointment, helped the wound heal normally without interfering with healing mechanism, was non-irritating skin agent . For this reasons, propylene glycol was criticized as advantageous as a base mixture of topical application (Eyarefe et al., 2019; Gupta et al., 2006). Concentration of chia seed extract we used in this study as 4% v/v according to previous study of Jeong et al.(2010). We tried to double concentration of chia seed extract at 8% v/v. This extract or chia seed oil on skin wound could be safe although pure extract was dropped into wound (Salih and Fadhil, 2018). 1% Silver sulfadiazine, topical ointment, was important to treat wound especially open wound which could be infected from normal flora bacteria on skin, commonly used to control healing in murine wound model (Afshar et al., 2015).

It was found that both concentrations of topical chia seed extract (*Salvia hispanica* L.) significantly stimulated the rate of wound healing compared to the propylene glycol control group. Healing effectiveness has been enhanced by increasing fibroblast, increasing collagen synthesis, and neovascularization. These were also shown in the histological examination. The mechanism for accelerating wound healing remains unclarified. However, the efficiency of wound healing by various plants is enhanced by inhibiting bacteria (antibacterial activity), antioxidant activity (free radical scavengers), and stimulating mitogenic activity. As chia seed extract did not show antibacterial properties in the screening disc diffusion method, the lack of antibacterial activity of chia seed extract was not relevant to promoting wound healing. Nevertheless, the major components of chia seed extract, which are probably useful in wound healing, are alpha-linolenic acid (ALA; 18: 3) and linoleic acid (LA; 18: 2). At the same results of Jeong et al. (2010) found that chia seed extract played a crucial role in inflammation phase. ALA, LA, and antioxidants in chia seed extract had the benefit of exhibiting anti-inflammatory agent on pruritic skin. Topical ALA can also regulate inflammation on wound healing, declined some pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α; moreover, it still enhanced fibroblast proliferation, collagen synthesis, and angiogenesis in the late inflammation or proliferative phase of wound healing (Li et al., 2017). Furthermore, topical linoleic acid can accelerate wound closure with the result of increased moisture, wound flexibility, angiogenesis, and enhancing fibroblast function in the proliferative phase (Bardaa et al., 2016). In this study, phytochemical analysis of chia seed extract (*Salvia hispanica* L.) has been clearly shown total phenolic content exhibited antioxidant activities. So chia seed extract is a good source of antioxidants in agreement with similar studies which have reported that some antioxidants play a significant role in accelerating the wound healing process in inflammation phase by protecting tissues from oxidative damage (Kant et al., 2014).

CONCLUSION

The current study revealed that the phytochemicals of chia possibly reduced of PMNs cells, increased the number of fibroblast and collagen cells, increased the percentage of wound contraction. Chia seed extract was a good natural plant which might used topically for wound healing.

ACKNOWLEDGEMENTS

This research was supported grant by Mahasarakham University.

CONFLICT of INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

ABBRIVATIONS

DNA; deoxyribonucleic acid; IUPAC, International Union of Pure and Applied Chemistry; TSB, tryptic soy broth; NCCLS, National Committee for Clinical Laboratory Standards; GC-FID, gas chromatography-flame ionization detector; AOAC, Association of Official Analytical Chemists; GAE; gallic acid equivalent; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TEAC, trolox equivalent antioxidant capacity; FRAP, ferric reducing antioxidant power; ABTS, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid); K2S2O8, potassium persulfate; H&E, hematoxylin and eosin stain; MT, Masson's trichrome stain; PG, propylene glycol group; 4% CSE, 4% chia seed extract group; 8% CSE, 8% chia seed extract group; SSD, silver sulfadiazine group; IC, inflammatory cell; PMNs, polymorphonuclear leukocytes; ALA, alpha-linolenic acid; LA, linoleic acid; IL-1β, interleukin-1 beta; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha

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How to cite this article;

Tanagorn Pintapagung and Thanaporn Asawapattanakul. Effect of chia (*Salvia hispanica* L.) seed extract on wound healing in mice. Veterinary Integrative Sciences. 2020; 18(2): 103-117.