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Research article

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

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Abstract

Chia, *Salvia hispanica* L., a plant containing lipid-antioxidant, has been shown to be beneficial for prevention of risk factors of type 2 diabetes. The objectives of this study were to evaluate effects oral chia seed extract on wound healing properties including wound contraction and histopathological examination in a diabetic wound model. C57BL/6J mice were fed with standard and high-fat diet for 27 weeks. The mice were divided into 4 groups (n=6) as follows: a non-diabetic group (normal control group) and 3 diabetic groups (4% chia seed extract group, glipizide group, diabetic group). The percentage of wound contraction, histopathological score and morphology were compared for evaluating wound healing properties. On day 12 post-wounding, there were statistically significant differences ($p < 0.05$) in the percentage of wound healing and histopathological score in the normal control, 4% chia seed extract, glipizide group, as compared to diabetic group (99.45 ± 0.865, 98.99 ± 1.948, 99.06 ± 0.779, 81.41 ± 10.759 and 10.50 ± 0.837, 10.00 ± 0.632, 9.92 ± 1.625, 3.83 ± 1.169, respectively). The results of histopathological morphology showed consistent results with histopathological scores, in which collagen, fibroblast, epithelialization and neovascularization were dominant in granulation tissue of better scores. It may be concluded that chia seed extract was beneficial for diabetic wound healing in mice

Keywords: Chia seed, Diabetic wound, Oral administration, *Salvia hispanica* L.

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INTRODUCTION

Diabetes mellitus which is an important chronic disease that is the cause of an abnormal metabolic rate. Type 2 diabetes affects many people worldwide. In 2011 it was reported that there were 366 million people with diabetes. It was considered that by 2030, the incidence would be likely to be as high as 552 million in the developing countries (Whiting et al., 2011). Diabetes is caused by lack of insulin and as a result, the cells in the body are unable to use sugar. Thus hyperglycemia is found in this disease as are abnormalities in various parts of the body such as kidney problems or neuropathy (Mogensen et al., 1983; Yagihashi et al., 2007). Additionally, much published research in humans and animals has reported delayed wound healing or chronic wounds correlated with diabetes patients. Diabetic patients are unable to respond with an adequate inflammation and they could be prone to sepsis and induction of limb amputation (Jeffcoate and Harding, 2003; Boulton et al., 2005).

The diabetic mice model, C57BL/6J is a genetically inbred mouse strain in which type 2 diabetes can be easily induced with a high-fat diet. The mechanism of diet-induced obesity is similar to type 2 diabetes in humans. Moreover, obese mice explicitly presented diabetes with severe hyperglycemia after feeding for 6 months (Surwit et al., 1988). The most common high-fat diet is prepared to 60 percent of food energy from fat; 60% high-fat diet (commonly using fat from lard) (Collins et al., 2004). Furthermore, an oral glucose tolerance test (OGTT) for testing blood glucose levels is usually done by feeding glucose directly into the stomach. Oral glucose tolerance tests show significant glucose levels compared to when glucose is injected into the abdomen (Intraperitoneal glucose tolerance test; IPGTT) (Andrikopoulos et al., 2008).

Salvia hispanica L. (Lamiaceae) or Chia seed is a plant from South America (Ayerza, 1995; Orona-Tamayo et al., 2019). Research studies have found that chia seeds contain oil which is the main component comprising approximately 25-40 % of oil yield (Mohd Ali et al., 2012). Moreover, chia seeds are also a significant source of linolenic acid (around 50-57 %), and α-Linolenic acid (approximately 17-26 %), as well as protein, dietary fiber, vitamins, important minerals such as calcium, phosphorus, magnesium and antioxidants (Bushway et al., 1981; Ayerza, 1995). Chia seed is also known as a functional food which contributes nutrition and bioactive compounds to humans and animals (Orona-Tamayo et al., 2019). Chia seed has been reported to possess other beneficial effects which are related to cardiovascular disease such as inflammatory factors (C-reactive protein), coagulation (fibrinogen, factor VIII, von Willebrand factor), fibrolytic factors (such as tPA), iron status and endothelial function (nitric oxide generation), and post-prandial glycemia (Vuksan et al., 2010). Other positive effects associated with diabetes include controlling blood pressure, hyperglycemia, triglycerides (Guevara-Cruz et al., 2011; Toscano et al., 2014) and some publications might also indicate that it helps promote weight loss in diabetes (Toscano et al., 2015). Although many positive effects of chia seed on diabetic patients have been revealed, some effects on diabetic wound and the accelerating effect of wound healing still have not been proved. Here we present a high-fat diet mouse model for inducing diabetes mellitus and an excisional wound model in order to study wound healing properties of oral chia seed extract. Wound healing properties were also evaluated by using wound contraction and histopathological examination (histopathological score and morphology).

MATERIALS and METHODS

Seeds and solvent extraction

Chia seed products imported from Bolivia (Nathary®) were available in Thailand. For solvent extraction, chia seeds were milled by grinder (Minimex® CG2) and a Soxhlet extractor was used to extract bioactive compounds with n-hexane at 80 ºC for at least 8 hours by using the standard method of IUPAC. Solvent was removed from the extract by using a rotary vacuum evaporator (Buchi, Flawil, Switzerland) at 40 ºC under nitrogen stream. This purified extract was prepared to analyze total phenolic content, antioxidant activity assays, and fatty acid compositions in order to test active ingredients as a quality control before mixing with diets.

Total phenolic content, antioxidant activity assay, and fatty acid compositions of chia seed extract

The mixture for testing total phenolic content by the method of Singleton et al. (1965) was prepared from 20 µl chia seed extract and 100 µl Folin-Ciocalteau reagent which were blended simultaneously and left for 1 min then 80 µl of 7.5% sodium carbonate (w/v) added, mixed well, and allowed to stand for 30 min at room temperature. The absorbance was read at 760 nm against a blank using a spectrophotometer and compared to a standard gallic acid concentration chart. The results were reported as gallic acid equivalent (mg GAE / mg oil).

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Prior et al., 2005) used 10 µl chia seed extract (1 mg/ml) and 190 µl of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) solution. The mixture was left in the dark for 30 minutes at room temperature. The absorbance was read at 515 nm against the blank using a spectrophotometer and compared to a standard trolox concentration graph. Finally, the results were reported in terms of trolox equivalent antioxidant capacity (mg TEAC / mg oil).

Ferric reducing antioxidant power (FRAP) assays (Thaipong et al., 2006) were prepared from 10 µl chia seed extract (1 mg/ml) and 190 µl FRAP reagent. The mixture was left in the dark for 15 minutes at room temperature. The absorbance was read at 593 nm against a blank using a spectrophotometer compared to a standard trolox concentration graph. The results were reported in terms of trolox equivalent antioxidant capacity (mg TEAC / mg oil).

ABTS [2,2′-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging assay (Thaipong et al., 2006) used 10 µl chia seed extract (1 mg/ml) and 190 µl of 7.0 mM ABTS cation radical reagent. The mixture was left in the dark for 15 minutes at room temperature. The absorbance was read at 734 nm against the blank using a spectrophotometer and compared to a standard trolox concentration graph. The results were reported in terms of trolox equivalent antioxidant capacity (mg TEAC / mg oil).

Fatty acid composition was determined using gas chromatography with a flame ionization detector (Ixtaina et al., 2011).

Animal preparation and experimental design

Twenty-four Male B57BL/6J mice (20-25 g), at the age of 8-10 weeks, were purchased from Nomura Siam International Co., Ltd, Bangkok, Thailand. Animals were housed in individual cage in a temperature-controlled room with a 12-hour light/dark cycle. They were acclimated and allowed ad libitum to feed water and standard pellet diet for 1 week. After adaptation, all mice (n=24) were randomly divided into the four groups (a non-diabetic group and three diabetic groups) and each group (6 mice/group) was fed with different diets as specified above (Singh et al., 2014). Afterward, mice were precisely diagnosed with diabetes by using fasting plasma glucose, 2-hour plasma glucose (OGGT), and area under curve (AUC). Eventually, diabetic and non-diabetic mice were inflicted with skin wounds at 27 weeks, and were observed for wound contraction and macroscopic lesions for 12 days' post-wounding (on day 0, 4, 6, 8, 10, and 12). On day 12, all wounds were surgically collected for preparation of the histopathological sections in order to evaluate histopathological score. Ethical approval was obtained from the Institutional Animal Care and Use Committee of Mahasarakham University (IACUC-MSU), Thailand (Ethics approval number: IACUC-MSU-028/2019) and complied with the guidelines of the National Research Council of Thailand.

Oral glucose tolerance test

At 27 weeks of age, all C57BL/6J mice were tested to confirm diabetes before wounding. Mice were refrained from water and food for 6 hours before being tested. Blood samples for OGGT were collected from the tip of the tail by using an Accu-Check Performa Blood Glucose Meter at different time points. First of all, fasting plasma glucose (0 hour) was collected from blood before orally administrated glucose. After that, glucose was fed to mice at a dose of 2 g/kg using a feeding tube (18-gauge, 38 mm) and measured glucose levels at 30, 60, 90, and 120 min respectively (Andrikopoulos et al., 2008). All mice also were diagnosed as clear-cut diabetes with fasting blood glucose levels of >180 mg/dl and 2-hour plasma glucose >240 mg/dl (OGGT) (Messier et al., 2007; Kim et al., 2009). Moreover, OGGT, graph of plasma glucose versus time (minutes), and area under curve of plasma glucose level (AUC-OGTT) calculated by trapezoidal rule were compared and there was a significant difference among groups by using ANOVA, followed by multiple comparison (Purves, 1992; Wang and Brubaker, 2002).

Diets

Each group of mice was fed one of four diets as follows:- (1) normal control group, mice were fed with standard diet (SD) until the end of the experiment (2) diabetes control group, mice were fed with high-fat diet (HFD) until the end of the experiment (3) chia seed extract group, mice were fed with high-fat diet for 27 weeks and changed to chia seed extract diet (CSED) after the 27 weeks of experiment (4) glipizide group, mice were fed with high-fat diet for 27 weeks and then glipizide (5 mg/kg) was fed together with high-fat diet for treatment of diabetes (Oza and Kulkarni, 2018). Commercial diet, CE-2 (CLEA Japan, Tokyo, Japan), was fed as the standard diet, and Quick fat diet (CLEA Japan, Tokyo, Japan) was a specific diet for inducing diabetes mixed together with lard for preparation of 60% high-fat diet (consisting of 60% calories from fat) (Collins et al., 2004; Li et al., 2016). The composition of diets is shown in Table 1.

Table 1 The compositions of different diets.

Ingredients (g/100 g diet)	SD	HFD	CSED
Protein	24.9	18.6	18.6
NFE (nitrogen-free extract)	51	35	35
Soybean oil	4.6	4	-
Lard	-	31.8	31.8
Chia seed extract	-	-	4
Fiber	4.1	1.83	1.83
Crude ash	6.6	3.75	3.75
Humidity	8.9	5.1	5.1
Nutrient composition (%)			
Energy from protein	28	14	14
Energy from carbohydrate	60	26	26
Energy from fat	12	60	60
Energy density (Kcal/g)	3.45	5.28	5.28

Wound contraction

The full-thickness excision wound model was performed to evaluate wound closure as percentage and to observe macroscopic lesions. All mice skins were shaved by mini shaver, were scrubbed with povidone iodine (Betadine®) and 70% alcohol with aseptic technique. Mouse anesthesia used 10 mg/kg xylazine and 50 mg/kg ketamine combination. Mice were unconscious at the surgical stage when circular wounds were created on the interscapular skin using a 6 mm biopsy punch, and then allowed to heal normally. All wounds were cleaned every day by using normal saline, a sterile cotton bud, Tegaderm® (a transparent polyurethane dressing). Wound area measurement was observed by a digital camera (Nikon, Japan) and calculated by the AutoCAD software on days 0, 4, 6, 8,10, and 12 (Koca et al., 2009). Wound contraction was calculated from the wound area which was healed as a percentage that had close using the modified formula (Greenhalgh et al., 1990) for this calculation as follows: contraction (%) = (wound area on day 0 - wound area on observed day)/wound area on day 0 x 100.

Histopathological score

6 mm of all wound skin was surgically collected from each group at 12 days post-wounding. Each wound specimen was fixed in 10% formalin. Wound blocks were embedded in paraffin, and were usually cut and mounted as 5 µm thin slides. Histological slides were stained with hematoxylin-eosin stain and Masson's trichrome stain. The histological scoring was evaluated by two blinded assessors according to the previous method by Greenhalgh et al. (1990) and ranged from 1 to 12 where 1-3, none to minimal cell accumulation, no granulation tissue or epithelial travel; 4-6, thin, immature granulation that is dominated by inflammatory cells but has few fibroblasts, capillaries, or collagen deposition, minimal epithelial migration; 7-9, moderately thick granulation tissue, can range from being dominated by inflammatory cells to more fibroblasts and collagen

deposition, extensive neovascularization, epithelium can range from minimal to moderate migration; 10-12, thick, vascular granulation tissue dominated by fibroblasts and extensive collagen deposition, epithelium partially to completely covering the wound. Conventional hematoxylin and eosin (H&E) stain was used to differentiate histopathological change of wound healing such as, leukocyte and macrophage infiltration, neovascularization, epithelialization while Masson's trichrome (MT) stain made visible the key cells for wound healing such as keratin, collagen fiber and arrangement, adipocytes, hemoglobin, and muscle fiber.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics Version 22 (IBM, Chicago, IL, USA). Data including wound contraction results and histopathological score results were presented as mean ± standard deviation. According to homogeneity of variance, groups of data were analyzed by one-way analysis of variance (ANOVA) or Welch's robust test of equality of means and then further multiple comparisons using Scheffe's or Tamhane's T2 for post hoc analysis. P-value with $p < 0.05$ considered as statistically significant.

RESULT

Total phenolic compound, antioxidant activity, and fatty acid compositions

The amount of total phenolic compounds in terms of gallic acid equivalent and antioxidant activity in terms of trolox equivalent are given in Table 2.

Oral glucose tolerance test and area under curve

Plasma glucose concentration during the oral glucose tolerance test (2 g/kg) was determined at age 26 weeks of C57BL/6J mice before testing wound contraction, in order to separate normal plasma glucose mice of the control group from diabetic mice of treatment groups (Andrikopoulos et al., 2008). We found that there was a significant difference at each time point of the oral glucose tolerance test between standard diet group and high-fat diet groups. All high fat-diet mice had diabetes mellitus, confirmed by measured blood glucose levels of >180 mg/dl and 2-hour plasma glucose of OGTT >240 mg/dl compared to standard diet feeding mice which had a normal fasting plasma glucose and 2-hour plasma glucose level as shown in figure 1 (Messier et al., 2007; Kim et al., 2009). The area under curve was also calculated from oral glucose tolerance tests by using the trapezoidal rule. For clarity, there was a significant difference of the area under the curve between standard diet group and high-fat diet groups, with standard diet feeding mice having a lower AUC than high-fat diet mice but AUC of high-fat diet feeding was not significantly different as shown in figure 2.

Table 2 Total phenolic compound, antioxidant activity assay, and fatty acid compositions of chia seed extract.

Chia seed extract compositions	Chia seed extract
Total phenolic compound (mg GAE/ mg oil)	0.16 ± 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
Unsaturated fatty acid (g/100 g)	88.7954
Alpha-Linolenic acid (C18:3n3)	62.0267
Cis9, 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 (g/100 g)	11.2046
Palmitic (C16:0)	7.2258
Stearic (C18:0)	3.4312
Arachidic (C20:0)	0.2898
Lignoceric acid (C24:0)	0.0934
Behenic acid (C22:0)	0.0767
Pentadecanoic acid (C15:0)	0.0456

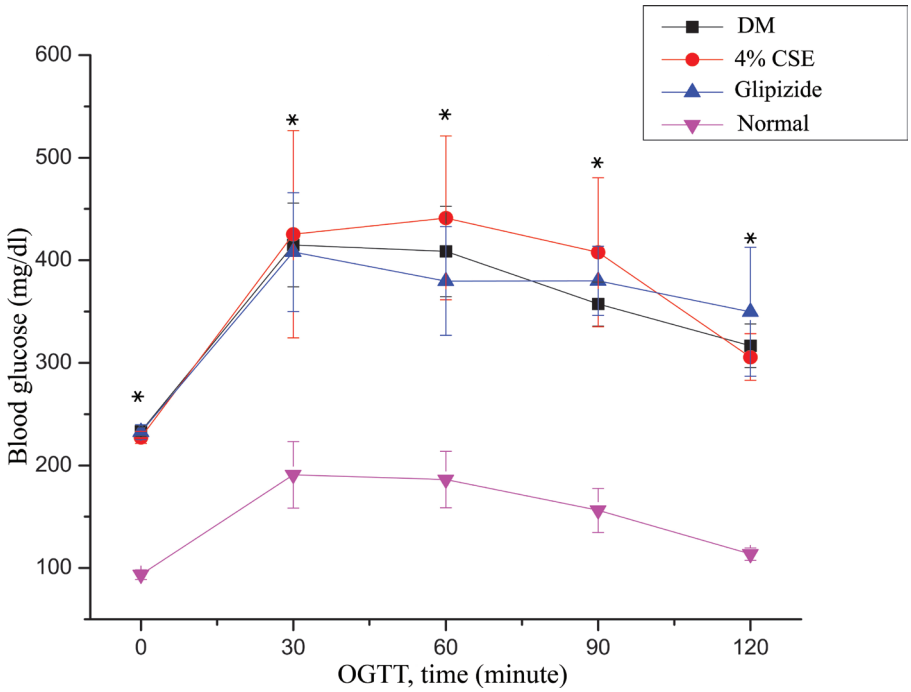

Figure 1 Oral glucose tolerance test (2 g/kg, 6-hour fasting) after 27 weeks of diet feeding in the four groups (* p <0.05).

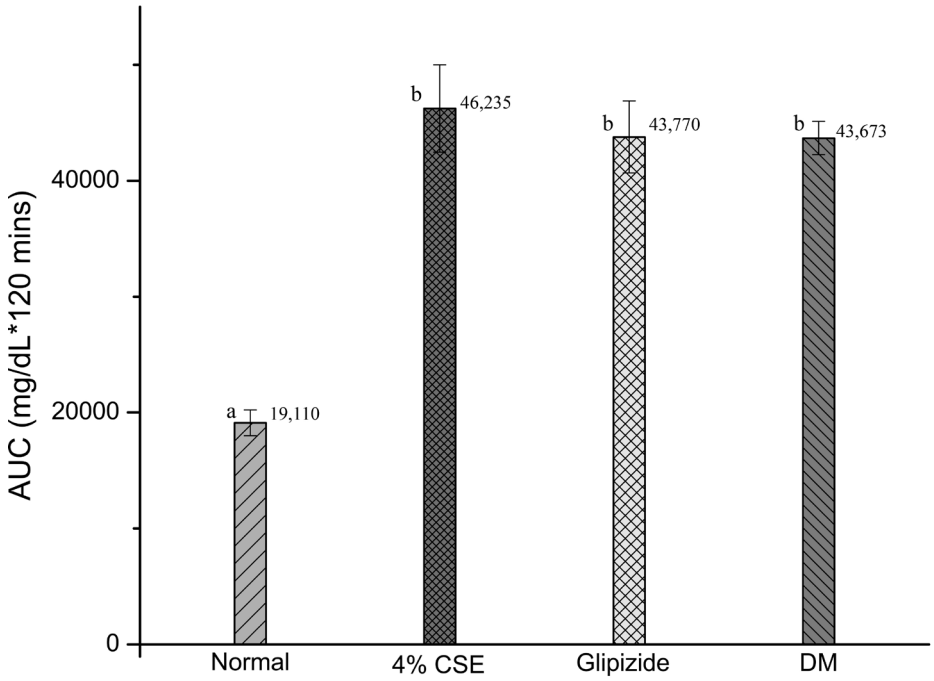

Figure 2 Area under curve of oral glucose tolerance test was significant difference (p <0.05) in the four groups after 27 weeks of diet feeding. Grouping after post hoc test shown as a and b.

Wound contraction

In the present study, wound contraction of full-thickness circular excisional wounds (6 mm in diameter) was compared in four different groups. In overall comparisons at the end of the experiment, it was seen that wounds healed more rapidly in the normal control group, non-diabetic mice fed only with standard diet; the 4% chia seed extract group, diabetic mice fed with high-fat diet and 4% chia seed extract; glipizide group, diabetic mice fed with high-fat-glipizide diet than in the diabetic group, diabetic mice fed with fed high-fat diet. The external appearances of wounds in normal control mice had a scab on the wound surface that fell off by day 8 compared to day 10 in both 4% chia seed extract group and glipizide-fed mice, while in diabetes mellitus mice (untreated groups), the wound was not closed on day 12 and was covered with a scab and crust as shown in figure 3. At 12 days post-wounding, the diabetes mellitus group, which was not given any medication, showed a significant delay ($p < 0.05$) in wound contraction over the period of observation as compared to other groups including the normal control group, 4% chia seed extract group, and glipizide group as shown in Table 3 and figure 4.

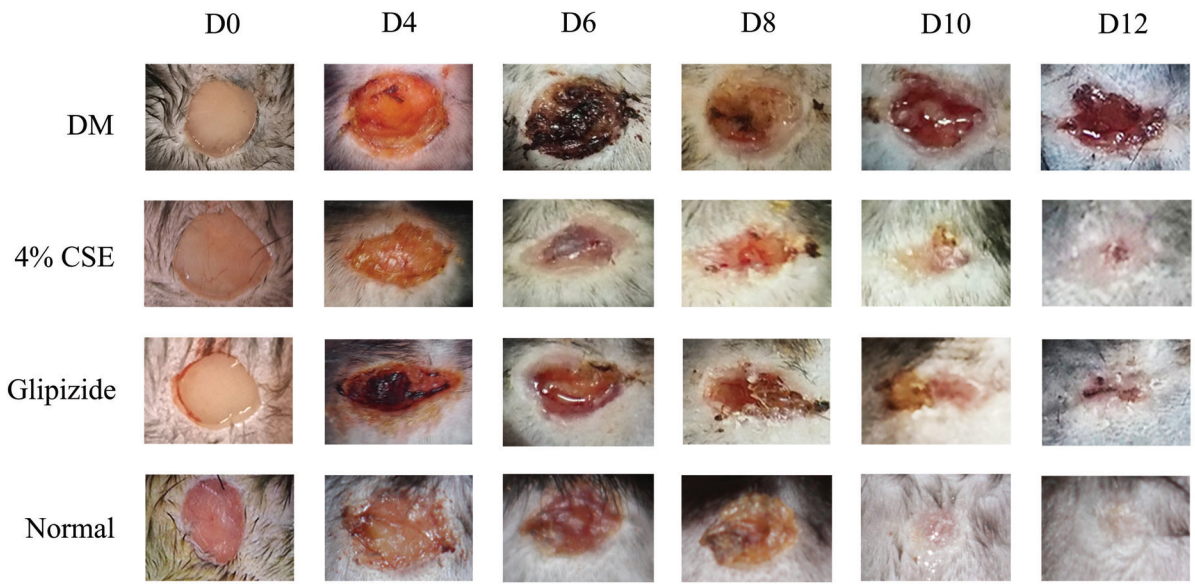

Figure 3 Macroscopic appearance of excision wound model in B57BL/6J mice of different groups from day 0 to 12 post-wounding.

Table 3 Effect of chia seed extract on wound contraction (%, mean ± standard deviation) on excisional wound model in mice.

Group	Wound contraction (%), mean ± SD					
	Day 0	Day 4*	Day 6*	Day 8*	Day 10*	Day 12*
Normal	0.00 ± 0.00	44.96^{c} ± 9.70	80.24^{a} ± 7.98	93.95^{a} ± 3.71	97.11^{a} ± 2.44	99.45^{a} ± 0.87
4% CSE	0.00 ± 0.00	36.54^{bc} ± 15.40	69.35^{b} ± 18.87	84.06^{b} ± 14.72	92.27^{b} ± 8.42	98.99^{b} ± 1.95
Glipizide	0.00 ± 0.00	19.83^{ab} ± 7.58	60.23^{b} ± 11.42	78.63^{b} ± 6.39	91.01^{b} ± 3.57	99.06^{b} ± 0.78
DM	0.00 ± 0.00	6.05^{a} ± 6.96	44.47^{b} ± 17.85	56.87^{b} ± 15.96	73.61^{b} ± 12.96	81.41^{b} ± 10.76

* p <0.05, grouping after post hoc test using a, b, or c

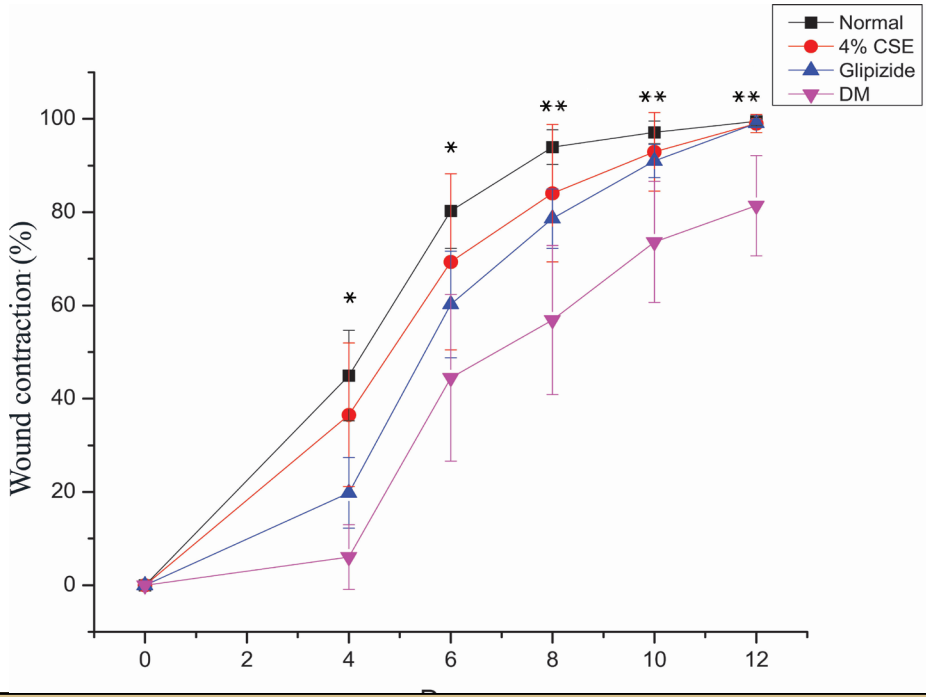

Figure 4 Contraction rate (%) of excisional diabetic wound model from 0 to 12 days post-wounding in normal control, 4% CSE, glipizide and DM group (* p <0.05).

Histopathological score

Microscopic appearance in the normal control group, 4% chia seed extract group, and glipizide group were relatively similar and achieved better results than in the diabetes mellitus group as follows: numerous fibroblasts, presenting neovascularization and granulations, extensive collagen deposition (green colour, Masson's trichrome stain), epithelium completely covering the wound; however, microscopic appearances in the diabetes mellitus group shown moderate inflammatory cells dominating between the dermal-epidermal junction and dermal area, a lack of re-epithelialization, less neovascularization, few fibroblasts with a small number of thin collagen fibers, a large number of adipose cells as shown in figure 5. Wound healing of biopsy wounds harvested on day 12 was assessed using histopathological score and microscopic morphology was evaluated for histopathological change. Histopathological scores showed that the normal control, 4% chia seed extract, and glipizide group had similar results and were able to improve wound healing as compared to diabetes mellitus group ($p < 0.05$, 10.50 ± 0.837, 10.00 ± 0.632, 9.92 ± 1.625 vs. 3.83 ± 1.169) as shown in figure 6.

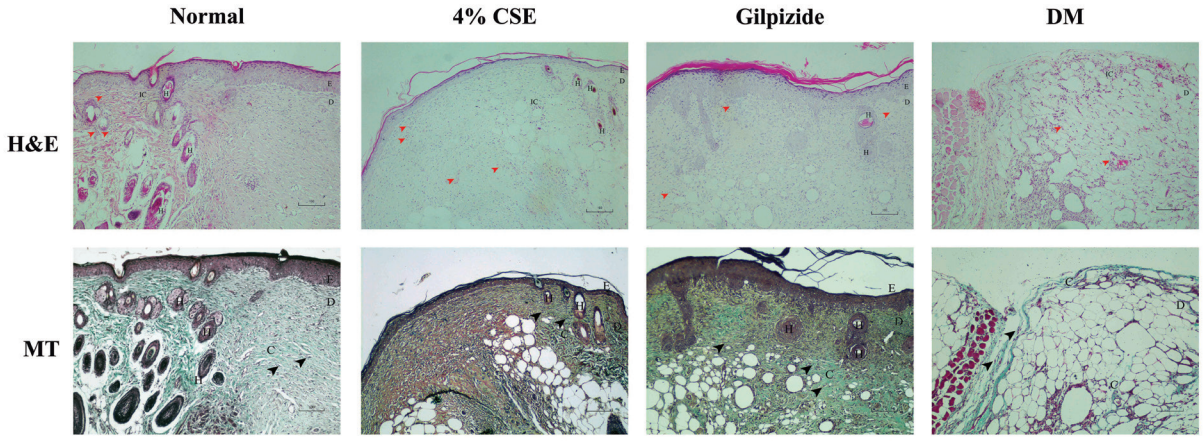

Figure 5 Macroscopic appearance of excision wound model in B57BL/6J mice of different groups froHistopathological examination of diabetic wound model on 12 days post-wounding. Each group stained with hematoxylin-eosin and Masson's trichrome stain shown as pink and green images respectively for evaluation of histopathological score and morphology (blood vessel, red arrow head; collagen, C; dermis, D; epidermis, E; fibroblast, black arrow head; hair follicle, H; inflammatory cell, IC;). All images shown as 100X with 100 µm scale bar.

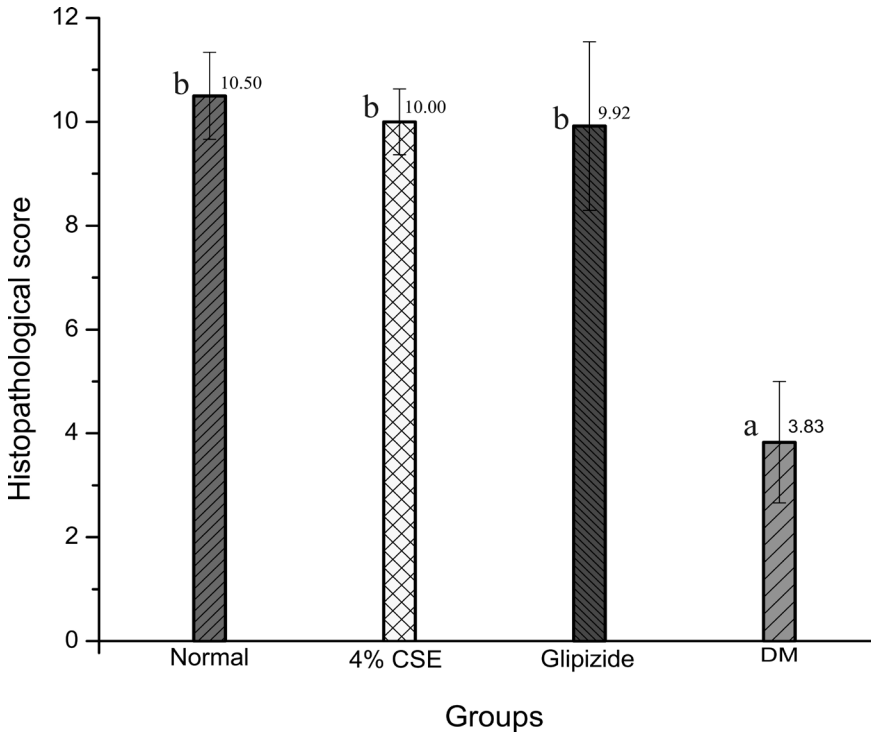

Figure 6 Histopathological score on 12 days post-wounding in normal control, 4% CSE, glipizide and DM group. Grouping after post hoc test shown as a and b.

DISCUSSSION

Wounds were cleaned, and mice were observed every day in the wound contraction study. After Anesthesia, mice were weak and had delayed recovery time because of metabolic diseases (obesity and diabetes). As a result, photographic images were not taken on day 2 post-wounding because restraining for taking photographs made them stressful or killed them. Mice also ate and drank normally after full recovery on first day and became normal physical status after day 2 post-wounding. We started taking photographs on day 4 post-wounding according to Thangavel et al. (2017).

Effective of dose (oral administration) of this plant was not published in a previous study of wound healing. However, in a previous metabolic study, there was a safe concentration (4% w/w) which was used for treatment of metabolic conditions in mice so 4 % w/w chia seed extract was prepared at this concentration for treatment in this study (Marineli et al., 2015). Hyperglycemia in diabetic patients may cause infection (leukocyte dysfunction and decreased phagocytosis), and atherosclerosis resulting in impaired wound healing (Chait and Bornfeldt, 2009; Pettersson et al., 2011). Moreover, endothelial dysfunction (damaged vessels) was seen in delayed wound healing, which were damaged by hyperglycemia-induced reactive oxygen species (ROS) and consequent oxidative stress (Koya et al., 2003; Patel et al., 2013). As mentioned above, hyperglycemia was a cause of many diabetic complications including wound healing. As a result, glipizide was used in this study for bringing blood glucose levels back to normal range, and helped wound healing return to normal. Antidiabetic drug (glipizide) was used as oral medication in the positive

control group, and also had beneficial effect on the glucose control by reducing blood glucose levels in diabetic mice according to the previous study of Singh et al. (2014). The optimum dosage of glipizide was recommended at 5 mg/kg, once daily for diabetic treatment (Oza and Kulkarni, 2018). The negative control group (DM group) was used to reveal diabetic wound healing which was delayed in wound contraction while better wound healing results were found in normal control group, 4% CSE group, and glipizide group. The normal control group in this study not only was used to compare wound healing between non-diabetic and diabetic mice, but also was used to check experimental error which directly affected wound healing results. Experimental design and grouping in this study were used according to previous study (Singh et al., 2014). Even though high-fat diet was used to induce hyperglycemia, insulin resistance and to increase body fat consumption in the short-period range, the 27-week high-fat diet of the long-term experiment was designed to test diabetic wounds which was similar to the cause of diabetes type 2 in humans. Impaired wound healing may be caused by obesity or hyperglycemia especially in inflammation phase. In inflammation phase, interfered pro-inflammatory cytokines can cause chronic wounds or diabetic wounds (Pence and Woods, 2014; Ganz et al., 2015). Although blood glucose levels were not analyzed during the wound contraction experiment, diets in 3 groups of diabetic mice were prepared from a base mixture of high-fat-diet formula in order to maintain diabetes.

Chia seed extract comprised fatty acids and antioxidants. Different locations, harvesting of different growth stages, and extraction methods of chia seed affected bioactive compound levels (Ayerza, 1995; Peiretti and Gai; 2009; Ixtaina et al., 2010). For confirming the presence of bioactive ingredients, extract of chia seed needs to be validated for its components or properties before testing in animals. The qualitative screening of antioxidants in chia seed extract was performed according to standard methods used in food and dietary supplements (Singleton and Rossi, 1965; Prior et al., 2005; Thaipong et al., 2006), while fatty acids in this study were analyzed by gas chromatography (Ixtaina et al., 2011).

Statistical analysis in this research compared the mean among groups using ANOVA or Welch test in the oral glucose tolerance test (OGTT), area under curve of oral glucose tolerance test (AUC-OGTT), rate of wound contraction (%), and histopathological score. ANOVA with Scheffe's test was used in the oral glucose tolerance test (0, 30 and 60 minutes), the percentages of wound contraction (day 4 and 6) while Welch test with Tamhane's T2 post hoc test was used in the area under curve of plasma glucose level, oral glucose tolerance tests (90 and 120 minutes), and the percentage of wound contraction (day 8, 10, and 12).

The effectiveness of wound healing was assessed by the degree of wound contraction and histopathological score. It was found that there was no difference in wound contraction and histopathological score of wound healing in mice among three different groups namely, the normal control group, 4% chia seed extract group, and glipizide group. Furthermore, microscopic appearances of wounds in these groups were examined in correlation with histopathological score. Nevertheless, diabetic mice showed impaired wound healing as well as chronic wounds. Apart from hyperglycemia-induced impaired wound healing,

there were many physiological factors in diabetes which interfered with many stages of wound healing. In the inflammation phase, diabetic ulcer patients experience delayed wound healing which were caused by the absence of growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), and insulin-like growth factor (IGF-I). These growth factors helped in the mitogenic response of fibroblasts in late inflammation and proliferative phase (Loots et al., 2002). Another common problem is that dysfunction of phagocytosis (macrophage dysfunction) was prone to infection (Zykova et al., 2000). In the normal proliferative phase, many cells collaborated with cytokines in many biological events of wound healing. However, the healing mechanism was interfered with diabetic patients. Several pathogenic abnormalities in this phase were as follows; decreasing collagen deposits; decreasing angiogenesis; decreasing epidermal nerves (Gibran et al., 2002; Falanga, 2005). In the remodeling phase, matrix metalloproteinases (MMPs) promoted physiological tissue turnover. Hyperglycemia-induced oxidative stress increased expression of MMPs, leading to microvascular complications (Koya et al., 2003; Stamenkovic, 2003; Signorelli et al., 2005). Chronic diabetic ulcers or wounds, which were caused by vasculopathy and neuropathy, are common problems in type 2 diabetes. Financial problems were more likely to increase because of cost of treatment (Boulton et al., 2005; Dinh and Veves, 2005). Wound healing occurred immediately after tissue injury, and involved many cells type and many growth factors collaborating in the four phases of wound healing (Brem and Tomic-Canic, 2007). As chia seed is functional food and contains many anti-inflammatory nutrients (alpha-linolenic acid, linoleic acid, and antioxidants), it is used orally in a murine model for treatment of metabolic disorders, resulting in decreasing plasma triglyceride and increasing high-density lipoprotein, and preventing insulin resistance (Ayerza and Coates, 2007; Chicco et al., 2008). In recent years, chia seed has also been reported to have promising antimicrobial activity on potential pathogenic and probiotic bacteria as a result of its antioxidants (Kobus-Cisowska et al., 2019). In addition, in oral administration, both alpha-linolenic acid and linoleic acid were able to accelerate wound healing by decreasing proinflammatory cytokines (interleukin-1, interleukin-6) in the inflammation phase of the wound healing process (Rodrigues et al., 2012). Another property is that antioxidants as well as flavonoids in chia seed play key roles in anti-free-radical properties and help in inflammatory skin disease including wound healing (Jeong et al., 2010; Scapin et al., 2016; Pintapagung and Asawapattanakul, 2020). These published properties and the results of the current study indicate that chia is a promising plant for helping to accelerate wound healing by reducing inflammatory cells and increasing fibroblast with intensive collagen. Furthermore, chia seed used orally has been reported to have some properties which were useful in treating diabetes mellitus such as weight loss, reducing hyperglycemia, helping insulin resistance, helping in many cardiovascular and inflammatory diseases (Guevara-Cruz et al., 2011; Vuksan et al., 2017). However, there were no significant results describing in weight loss in a previous study, which should further validate this property (Nieman et al., 2009). Apart from mentioned properties, this study was also the first attempt to using chia seed orally in wound healing of diabetic mice.

CONCLUSION

The obtained results showed that chia seed extract contained fatty acids and antioxidants had the beneficial properties on wound healing by increasing wound contraction and histopathological score in diabetic mice model.

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CONFLICT of INTEREST

There are no known conflicts of interest.

ABBREVIATIONS

4% CSE, 4% w/w chia seed extract; ABTS, 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid); ANOVA, one-way analysis of variance; AUC, area under curve; CSED, chia seed extract; DM, diabetes mellitus; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EGF, epidermal growth factor; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalent; H&E, hematoxylin and eosin; HFD, high-fat diet; IACUC-MSU, Institutional Animal Care and Use Committee of Mahasarakham University; IGF-I, insulin-like growth factor; IPGTT, intraperitoneal glucose tolerance test; IUPAC, international union of pure and applied chemistry; MMPs, matrix metalloproteinases; MT, Masson's trichrome; OGTT, oral glucose tolerance test; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; SD, standard diet; TEAC, trolox equivalent antioxidant capacity; tPA, tissue plasminogen activator.

REFERENCES

Andrikopoulos, S., Blair, A.R., Deluca, N., Fam, B.C., Proietto, J., 2008. Evaluating the glucose tolerance test in mice. Am. J. Physiol. Endocrinol. Metab. doi: 10.1152/ajpendo.90617.2008.

Ayerza, R., 1995. Oil content and fatty acid composition of chia (*Salvia hispanica* L.) from five northwestern locations in Argentina. J. Am. Oil Chem. Soc. 72, 1079-1081.

Ayerza, R., Coates, W., 2007. Effect of dietary α-linolenic fatty acid derived from chia when fed as ground seed, whole seed and oil on lipid content and fatty acid composition of rat plasma. Ann. Nutr. Metab. 51(1), 27-34.

Boulton, A.J., Vileikyte, L., Ragnarson-Tennvall, G., Apelqvist, J., 2005. The global burden of diabetic foot disease. Lancet. 366(9498), 1719-1724.

Brem, H., Tomic-Canic, M., 2007. Cellular and molecular basis of wound healing in diabetes. J. Clin. Invest. 117(5), 1219-1222.

Bushway, A.A., Belyea, P.R., Bushway, R.J., 1981. Chia seed as a source of oil, polysaccharide, and protein. J. Food Sci. 46(5), 1349-1350.

Chait, A., Bornfeldt, K.E., 2009. Diabetes and atherosclerosis: is there a role for hyperglycemia?. J. Lipid Res. 50(Supplement), 335-339.

Chicco, A.G., D'Alessandro, M.E., Hein, G.J., Oliva, M.E., Lombardo, Y.B., 2008. Dietary chia seed (*Salvia hispanica* L.) rich in α-linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. Br. J. Nutr. 101(1), 41-50.

Collins, S., Martin, T.L., Surwit, R.S., Robidoux, J., 2004. Genetic vulnerability to diet-induced obesity in the C57BL/6J mouse: physiological and molecular characteristics. Physiol. Behav. 81(2), 243-248.

Dinh, T., Veves, A., 2005. Microcirculation of the diabetic foot. Curr. Pharm. Des. 11(18), 2301-2309.

Falanga, V., 2005. Wound healing and its impairment in the diabetic foot. Lancet. 366(9498), 1736-1743.

Ganz, M., Bukong, T.N., Csak, T., Saha, B., Park, J.K., Ambade, A., Kodys, K., Szabo, G., 2015. Progression of non-alcoholic steatosis to steatohepatitis and fibrosis parallels cumulative accumulation of danger signals that promote inflammation and liver tumors in a high fat–cholesterol–sugar diet model in mice. J. Transl. Med. 13, 193.

Gibran, N.S., Jang, Y.C., Isik, F.F., Greenhalgh, D.G., Muffley, L.A., Underwood, R.A., Usui, M.L., Larsen, J., Smith, D.G., Bunnett, N., Ansel, J.C., Olerud, J.E., 2002. Diminished neuropeptide levels contribute to the impaired cutaneous healing response associated with diabetes mellitus. J. Surg. Res. 108(1), 122-128.

Greenhalgh, D.G., Sprugel, K.H., Murray, M.J., Ross, R., 1990. PDGF and FGF stimulate wound healing in the genetically diabetic mouse. Am. J. Pathol. 136(6), 1235-1246.

Guevara-Cruz, M., Tovar, A.R., Aguilar-Salinas, C.A., Medina-Vera, I., Gil-Zenteno, L., Hernández-Viveros, I., Lopez-Romero, P., Ordaz-Nava, G., Canizales-Quinteros, S., Guillen Pineda, L.E., Torres, N., 2011. A dietary pattern including nopal, chia seed, soy protein, and oat reduces serum triglycerides and glucose intolerance in patients with metabolic syndrome. J. Nutr. 142(1), 64-69.

Ixtaina, V.Y., Martínez, M.L., Spotorno, V., Mateo, C.M., Maestri, D.M., Diehl, B.W., Tomás, M. C., 2011. Characterization of chia seed oils obtained by pressing and solvent extraction. J. Food Compos. 24(2), 166-174.

Ixtaina, V.Y., Vega, A., Nolasco, S.M., Tomás, M.C., Gimeno, M., Bárzana, E., Tecante, A., 2010. Supercritical carbon dioxide extraction of oil from Mexican chia seed (*Salvia hispanica* L.): Characterization and process optimization. J. Supercrit. Fluids 55(1), 192-199.

Jeffcoate, W.J., Harding, K.G., 2003. Diabetic foot ulcers. Lancet 361(9368), 1545-1551.

Jeong, S.K., Park, H.J., Park, B.D., Kim, I.H., 2010. Effectiveness of topical chia seed oil on pruritus of end-stage renal disease (ESRD) patients and healthy volunteers. Ann. Dermatol. 22(2), 143-148.

Kim, K., Kim, H., Kwon, J., Lee, S., Kong, H., Im, S.A., Lee, Y.H., Lee, Y.R., Oh, S.T., Jo, T.H., Park, Y.I., Lee, C.K., Kim, K., 2009. Hypoglycemic and hypolipidemic effects of processed Aloe vera gel in a mouse model of non-insulin-dependent diabetes mellitus. Phytomedicine 16(9), 856-863.

Kobus-Cisowska, J., Szymanowska, D., Maciejewska, P., Kmiecik, D., Gramza-Michałowska, A., Kulczyński, B., Cielecka-Piontek, J., 2019. In vitro screening for acetylcholinesterase and butyrylcholinesterase inhibition and antimicrobial activity of chia seeds (*Salvia hispanica*). Electron. J. Biotechn. 37, 1-10.

Koca, U., Süntar, I.P., Keles, H., Yesilada, E., Akkol, E.K., 2009. In vivo anti-inflammatory and wound healing activities of *Centaurea iberica* Trev. ex Spreng. J. Ethnopharmacol. 126(3), 551-556.

Koya, D., Hayashi, K., Kitada, M., Kashiwagi, A., Kikkawa, R., Haneda, M., 2003. Effects of antioxidants in diabetes-induced oxidative stress in the glomeruli of diabetic rats. J. Am. Soc. Nephrol. 14 suppl. 3, 250-253.

Li, E., Nakata, M., Shinozaki, A., Yang, Y., Zhang, B., Yada, T., 2016. Betatrophin expression is promoted in obese hyperinsulinemic type 2 but not type 1 diabetic mice. Endocr. J. 63(7), 611-619.

Loots, M.A., Kenter, S.B., Au, F.L., Van Galen, W.J.M., Middelkoop, E., Bos, J.D., Mekkes, J.R., 2002. Fibroblasts derived from chronic diabetic ulcers differ in their response to stimulation with EGF, IGF-I, bFGF and PDGF-AB compared to controls. Eur. J. Cell Biol. 81(3), 153-160.

Marineli, R.D.S., Moraes, É.A., Lenquiste, S.A., Godoy, A.T., Eberlin, M.N., Maróstica Jr, M.R., 2015. Chemical characterization and antioxidant potential of Chilean chia seeds and oil (*Salvia hispanica* L.). LWT-Food Sci. Technol. 59, 1304-1310.

Messier, C., Whately, K., Liang, J., Du, L., Puissant, D., 2007. The effects of a high-fat, high-fructose, and combination diet on learning, weight, and glucose regulation in C57BL/6 mice. Behav. Brain Res. 178(1), 139-145.

Mogensen, C.E., Christensen, C.K., Vittinghus, E., 1983. The stages in diabetic renal disease: with emphasis on the stage of incipient diabetic nephropathy. Diabetes 32 Suppl. 2, 64-78.

Mohd Ali, N., Yeap, S.K., Ho, W.Y., Beh, B.K., Tan, S.W., Tan, S.G., 2012. The promising future of chia, *Salvia hispanica* L. J. Biomed. Biotechnol. 2012, 1-9. doi: 10.115/2012/171956.

Nieman, D.C., Cayea, E.J., Austin, M.D., Henson, D.A., McAnulty, S.R., Jin, F., 2009. Chia seed does not promote weight loss or alter disease risk factors in overweight adults. Nutr. Res. 29(6), 414-418.

Orona-Tamayo, D., Valverde, M.E., Paredes-López, O., 2019. Bioactive peptides from selected Latin American food crops–A nutraceutical and molecular approach. Crit. Rev. Food Sci. Nutr. 59(12), 1949-1975.

Oza, M.J., Kulkarni, Y.A., 2018. Formononetin treatment in type 2 diabetic rats reduces insulin resistance and hyperglycemia. Front. Pharmacol. 9(739), 1-11. 171956 doi: 10.3389/fphar.2018.00739.

Patel, H., Chen, J., Das, K.C., Kavdia, M., 2013. Hyperglycemia induces differential change in oxidative stress at gene expression and functional levels in HUVEC and HMVEC. Cardiovas. Diabetol. 12(1), 142.

Peiretti, P.G., Gai, F., 2009. Fatty acid and nutritive quality of chia (*Salvia hispanica* L.) seeds and plant during growth. Anim. Feed Sci. Technol. 148(2-4), 267-275.

Pence, B.D., Woods, J.A., 2014. Exercise, obesity, and cutaneous wound healing: evidence from rodent and human studies. Adv. Wound Care. 3(1), 71-79.

Pettersson, U.S., Christoffersson, G., Massena, S., Ahl, D., Jansson, L., Henriksnäs, J., Phillipson, M., 2011. Increased recruitment but impaired function of leukocytes during inflammation in mouse models of type 1 and type 2 diabetes. PLoS One 6(7), 1-9.

Pintapagung, T., Asawapattanakul, T., 2020. Effect of chia (*Salvia hispanica* L.) seed extract on wound healing in mice. Vet. Integr. Sci. 18(2): 103-117.

Prior, R.L., Wu, X., Schaich, K., 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agric. Food Chem. 53(10), 4290-4302.

Purves, R.D., 1992. Optimum numerical integration methods for estimation of area-under-the-curve (AUC) and area-under-the-momentcurve (AUMC). J. Pharmacokinet. Biopharm. 20, 211-227.

Rodrigues, H.G., Vinolo, M.A.R., Magdalon, J., Vitzel, K., Nachbar, R.T., Pessoa, A.F.M., dos Santos, M.F., Hatanaka, E., Calder, P.C., Curi, R., 2012. Oral administration of oleic or linoleic acid accelerates the inflammatory phase of wound healing. J. Investig. Dermatol. 132, 208-215.

Scapin, G., Schmidt, M.M., Prestes, R.C., Rosa, C.S., 2016. Phenolics compounds, flavonoids and antioxidant activity of chia seed extracts (*Salvia hispanica*) obtained by different extraction conditions. Int. Food Res. J. 23(6), 2341-2346.

Signorelli, S.S., Malaponte, G., Libra, M., Pino, L.D., Celotta, G., Bevelacqua, V., Petrina, M., Nicotra, G.S., Indelicato, M., Navolanic, P.M., Pennisi, G., Mazzarino, M.C., 2005. Plasma levels and zymographic activities of matrix metalloproteinases 2 and 9 in type II diabetics with peripheral arterial disease. Vasc. Med. 10(1), 1-6.

Singh, S., Ali, S., Singh, M., 2014. Biological screening of plants extract showing hypoglycaemic and wound healing properties: *Capparis zeylanica* and *Primula denticulata*. Am. J. Phytomed. Clin. Ther. 2(12), 1338-1345.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16(3), 144-158.

Stamenkovic, I., 2003. Extracellular matrix remodelling: the role of matrix metalloproteinases. J. Pathol. 200(4), 448-464.

Surwit, R.S., Kuhn, C.M., Cochrane, C., McCubbin, J.A., Feinglos, M.N., 1988. Diet-induced type II diabetes in C57BL/6J mice. Diabetes 37(9), 1163-1167.

Thangavel, P., Ramachandran, B., Chakraborty, S., Kannan, R., Lonchin, S., Muthuvijayan, V., 2017. Accelerated healing of diabetic wounds treated with l-glutamic acid loaded hydrogels through enhanced collagen deposition and angiogenesis: an in vivo study. Sci. Rep. 7(1), 1-15.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Byrne, D.H., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compost. Anal. 19(6-7), 669–675.

Toscano, L.T., da Silva, C.S.O, Toscano, L.T., de Almeida, A.E.M., da Cruz Santos, A., Silva, A.S., 2014. Chia flour supplementation reduces blood pressure in hypertensive subjects. Plant Foods Hum. Nutr. 69(4), 392-398.

Toscano, L.T., Toscano, L.T., Tavares, R.L., da Silva, C.S.O., Silva, A.S., 2015. Chia induces clinically discrete weight loss and improves lipid profile only in altered previous values. Nutr. Hosp. 31(3), 1176-1182.

Vuksan, V., Jenkins, A.L., Brissette, C., Choleva, L., Jovanovski, E., Gibbs, A.L., Bazinet, R.P., Au-Yeung, F., Zurbau, A., Ho, H.V.T., Duvnjak, L., Sievenpiper, J.L., Josse, R.G., Hanna A., 2017. Salba-chia (*Salvia hispanica* L.) in the treatment of overweight and obese patients with type 2 diabetes: A double-blind randomized controlled trial. Nutr. Metab. Cardiovasc. Dis. 27(2), 138-146.

Vuksan, V., Jenkins, A.L., Dias, A.G., Lee, A.S., Jovanoski, E., Rogovik, A.L., Hanna, A., 2010. Reduction in postprandial glucose excursion and prolongation of satiety: possible explanation of the long-term effects of whole grain Salba (*Salvia hispanica* L.). Eur. J. Clin. Nutr. 64, 436-438.

Wang, Q., Brubaker, P.L., 2002. Glucagon-like peptide-1 treatment delays the onset of diabetes in 8 week-old db/db mice. Diabetologia 45(9), 1263-1273.

Whiting, D.R., Guariguata, L., Weil, C., 2011. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes Res. Clin. Pract. 94(3), 311-321.

Yagihashi, S., Yamagishi, S., Wada, R., 2007. Pathology and pathogenetic mechanisms of diabetic neuropathy: correlation with clinical signs and symptoms. Diabetes Res. Clin. Pract. 77 Suppl. 1, 184-189.

Zykova, S.N., Jenssen, T.G., Berdal, M., Olsen, R., Myklebust, R., Seljelid, R., 2000. Altered cytokine and nitric oxide secretion in vitro by macrophages from diabetic type II-like db/db mice. Diabetes 49(9), 1451-1458.

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