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Stachytarpheta jamaicensis ethanolic leaf extract as wound healer on alloxan-induced diabetic sprague dawley rats

M.H.Wan Rozianoor*, K.Nurul Nadia, S.Nurdiana

Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, (MALAYSIA)

E-mail : rozianoor@salam.utim.edu.my

ABSTRACT

The present study investigates the wound healing activity of *Stachytarpheta jamaicensis* leaf extract in normal and alloxan-induced male diabetic rats. Wound healing activities involved six groups of four rats for a period of 20 days. The groups consisted of Group I (normal control), Group II (diabetic control), Group III (diabetic treated with metformin), Group IV (diabetic treated with 0.2% extracts), Group V (diabetic treated with 2% extracts) and Group VI (diabetic treated with 20% extracts). The most effective dose to speed up the wound closure was the 0.2% of *S. jamaicensis* extract. The topical application of plant extract cream on diabetic excision wound significantly improved ($p < 0.05$) the percentage of wound contraction for 88% when compared to untreated diabetic rats. However, the most well organized structure of new cells was the treatment group with 20% of *S. jamaicensis*. Histological analysis had confirmed that *S. jamaicensis* altered the arrangement of granulation on the wound site as compared to the other treatments. These findings suggest that *S. jamaicensis* has excellent potential as a plant based wound healing agent towards improving the quality of life for diabetic. In line with the GC-MS analysis, the most probably bioactive compounds contributed to the wound healing activity were phytol, linolenic acid and palmitic acid.

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KEYWORDS

Stachytarpheta jamaicensis;
Wound healing;
Diabetes;
Ethanolic extract;
Sprague dawley rats.

INTRODUCTION

Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion or insulin action, which results in hyperglycemia with disturbances of carbohydrate, fat and protein metabolism^[1,2]. There are two types of diabetes which are type I and type II. Type I diabetes or also known as insulin dependent diabetes mellitus is caused by lack of insulin secretion, while type II

diabetes is caused by whether resistance toward insulin or due to the abnormalities in β -cell function in pancreas^[3]. Patients with diabetes are commonly known to experience impairment in the wound healing process where diabetic wound exhibits significant delays in healing due to the impairment of the cellular infiltration and granulation tissues formation^[4] that can result in the wound infection and vascular complications^[5].

The use of medicinal plants and herbs has recently

increasing throughout the world for the maintenance and improvement of health and for the treatment of various human conditions and disease^[6]. *Stachytarpheta jamaicensis* is one of the medicinal plants used to treat various ailments such as inflammation, fever, diabetes, malaria and also being used externally to treat ulcer, sores, cuts and wound^[7]. It also provides suitable environment in natural healing process^[6,8]. *S. jamaicensis* is a weedy plant that grows annually. This plant can grow about 60 to 120 cm tall. The flower of *S. jamaicensis* can be reddish purple to deep blue in colour.

Recent study has reported the use of *S. jamaicensis* as an analgesic, anthelmintic, antacid, and anti-inflammatory^[9]. This plant is traditionally used and is believed to lower the blood glucose level. However, little is known on their effectiveness as having wound healing activity especially those related to type I diabetes mellitus. Therefore, the present study was designed to evaluate the effectiveness of *S. jamaicensis* leaf extract related to wound healing activity in alloxan-induced male diabetic rats.

EXPERIMENTAL

Collection of plant materials and preparation of extracts

The leaves of *Stachytarpheta jamaicensis* were collected from Kota Bharu, Kelantan, Malaysia and the plant material was authenticated by Assoc. Prof. Dr. Norrizah Jaafar Sidek, a plant taxonomist from Universiti Teknologi MARA, Malaysia. The plant leaves were washed and oven dried at temperature between 40°C to 60°C for one week. The dried leaves were coarsely powdered with blender. 10g of the leaf powder was homogenized in 100ml of 70% ethanol and was then stored in an oven for 48 hours^[10] with initial stirring. The extract was filtered using Whatman No.1 filter paper and concentrated in vacuum at temperature below 40°C using rotary evaporator. The final dark semi-solid extract was then transferred into a plastic vial and kept at 4°C prior to use.

Determination of bioactive compounds

GC-MS analysis was performed using an Agilent-5973 network system. A mass spectrometer with an

ion trap detector in full scan mode under electron impact ionization (70 eV) was used. The chromatographic column used for the analysis was an HP-5 capillary column (30 m × 0.32 mm i.d., film thickness of 0.25 µm). Helium was used as a carrier gas at a flow rate of 1 mL/min. The injections were performed in splitless mode at 230 °C. With the column held initially at 60 °C for 2 min, 1 µL of essential oil solution in hexane (HPLC grade) was injected and analyzed, and then the temperature was increased to 240 °C with a 3 °C/min heating ramp.

Experimental design

Twenty-four of male Sprague dawley rats weighing between 150g to 250g were divided into six groups, which were: Group I (normal control), Group II (diabetic control), Group III (diabetic treated with metformin), Group IV (diabetic treated with 0.2% extracts), Group V (diabetic treated with 2% extracts) and Group VI (diabetic treated with 20% extracts). The animals were kept in the Animal Room, Faculty of Applied Sciences under standard laboratory conditions of humidity, temperature and light. The rats were fed on a standard pellet diet and water *ad libitum*.

Induction of diabetes animals

The rats were administered intraperitoneally with alloxan monohydrate in physiological saline by using syringe^[11] after the 24-hours of fasting. After 72 hours, the blood of the rats were withdrawn and tested for the blood glucose level by using glucometer strips. Diabetic rats with the blood glucose levels higher than 250mg/dL^[12] were selected for the study.

Preparation of the topical formulation

The topical formulation was prepared by using petroleum jelly from Vaseline^[13] to give the ointment effect and mixed with different concentration of *S. jamaicensis* leaves extract i.e. 0.2%, 2% and 20%. These treatments were then been tested on the diabetic group which consisted of four rats in each groups. Positive control group was treated with a commercialize drug, Solcoceryl ointment. It is a type of aminoglycoside antibiotic, which is used as a topical treatment for various types of infections and injuries, such as boils and ulcers.

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Introduction of excision wound model

The rats were anesthetized by using low concentration of chloroform before being excised by using a sharp scalpel. The furs on the mid-dorsal part of the rats were removed and the skin was disinfected by alcohol. The area for the excision wound model was made as 1cm², 1cm by 1cm. The entire wounds were kept left opened.

Determination of the rate of wound contraction

The effects of topical formulation on wound healing were assessed by the rate of wound closure or contraction. The rates were measured as percentage reduction of the wound size from its initial size^[14]. Measurement was done for 20 days of treatment by using the following formula:

$$\text{Rate of closure} = \frac{\text{Initial size of wound at day}^2 - \text{size of wound at day}^n}{\text{Initial size of wound at day}^2} \times 100\%$$

n = number of days

Histological analysis

Rats in each group were sacrificed for the wound healing comparison. The samples from the wound were fixed in 4% buffered formaldehyde and embedded in paraffin wax. The paraffin blocks were cut in 4µm and stained with hematoxylin and eosin for evolution of histological changes. The prepared samples were then observed under the inverted microscope with 40X magnification.

Statistical analysis

The quantitative measurement was made on four animals in each group. The mean and variance of each analysis was calculated and the standard deviation from the mean was determined. Data obtained were sub-

jected to ANOVA via SPSS software 18th edition followed by paired T-test with the level of significance at p<0.05.

RESULTS AND DISCUSSION

The GC-MS analysis

The GC-MS analysis was in line with a phytochemical screening conducted by^[15] where it revealed the content of *S. jamaicensis* to include of saponin^[15], tannins^[15] and flavonoids^[15,16]. Docking performed on plants extract has revealed three flavonoids, which were phytol, linolenic acid and n-hexadecanoic acid or also known as the palmitic acid^[17]. In addition, *S. jamaicensis* also has been documented to contain another flavonoid called scutellarein, which have the properties as antitumorous, antibacterial and also antiviral^[18].

Percentage of wound contraction

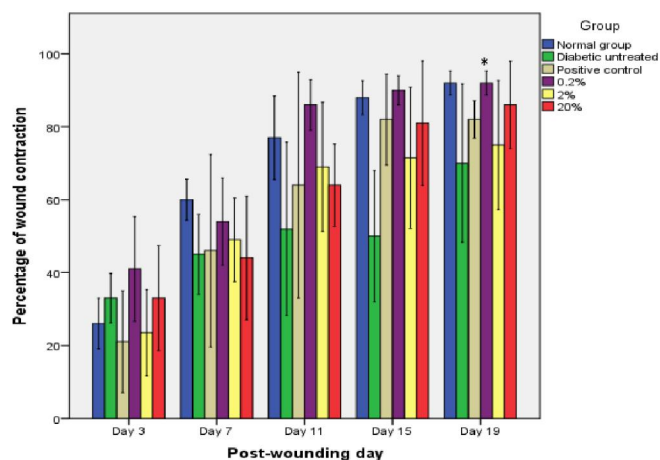


Figure 1 : Graph of percentage of wound contraction against day. * Paired T-test shows p<0.05 when compared to untreated diabetic rats (n=4).

TABLE 1 : Bioactive compounds present in *S. jamaicensis* ethanolic leaf extract from GC-MS analysis

| No | Retention time | Area | Library/ ID | Quality | Common name |
|----|----------------|-------|--|---------|------------------|
| 1 | 11.985 | 5.93 | Cyclopenta[c] pyran-4-carboxylic acid, 7 methyl-, methyl ester | 94 | Genipin |
| 2 | 17.386 | 11.25 | n-Hexadecanoic | 96 | Palmitic acid |
| | | | Tridecanoic acid | 91 | Tridecanoic acid |
| 3 | 18.800 | 13.18 | Phytol | 93 | Phytol |
| | | | 9,12,15-Octadecatrienoic acid, methyl ester, (z,z,z)- | 91 | α-linolenic acid |
| 4 | 19.097 | 15.68 | Cis,cis,cis-7,10,13-hexadecatriena | 91 | α-linolenic acid |
| | | | 9,12,15- Octadecatrien-1-ol,(z,z,z)- | 90 | Linolenic acid |

Typical process of healing under normal condition was faster compared to the diabetic condition^[19]. This probably due to healthy immunity system organizes collagen fiber formation and also better blood circulation around the wound site. The diabetic untreated group exhibits significant delay and prolonged in healing process, which probably due to the impairment of the cellular infiltration and granulation tissue formation^[4]. Starting from day 3, the rate of the wound closure for the diabetic untreated group was slower when compared with the treated diabetic groups. There are many factors which result in the delay and the impairment of the wound healing process. Factors could be either local for example poor oxygenation, prior irradiation and recurrent trauma, or systemic such hypoxia, collagen disorder, diabetes, malnutrition or autoimmune disorder^[20]. Therefore, one of these factors may leads to the impairment of the wound healing; thus, prolonged the healing process.

The delay in wound healing process for the diabetic untreated group in this research may also be due to the disrupted at one or more points in the healing process phases, either haemostasis, inflammation, proliferation or remodelling^[21]. In addition, under hyperglycemic condition, production of the reactive oxygen species (ROS) and free radicals via oxidation of glucose can also affect the healing process as it leads to a state of oxidation stress^[22], consequently prolonged and delayed the healing process. Besides the production of the ROS, hyperglycemia is also well known for the formation of the advance glycation end-products (AGEs), that is associated with impaired wound healing in diabetic mice^[22]. AGEs are protein or lipids that become glycated after exposure to sugars and can contribute to a variety of microvascular and macrovascular compli-

cations^[23].

Among the treated diabetic groups, it shows that, the wounds treated with 0.2% concentration of *S. jamaicensis* gave the most effective effects for the closure of the wounds, as it jumped from 50% to about 88% rate of closure. This was then followed by the positive control group, which was treated with Solcoceryl ointment, the 20% and 2% concentration of *S. jamaicensis*.

Flavonoid can aid in healing process as it is a water soluble antioxidants and free radical scavengers which helped in prevention of oxidative damage and have strong anticancer activity^[24,25]. It can prevent injury caused by the free radicals, by allowing its own compound to be oxidized by the radicals, resulting in more stable, less-reactive radical. Flavonoids also have been recognized as agents that can be used to antagonize lipid peroxidation that usually occurs in case of wound injury^[26]. Similar to other types of antioxidants like vitamin C and E, flavonoids are also able to increase blood circulation which results in increased of collagen viability and reducing cell damage^[27]. As a conclusion, it shows that *S. jamaicensis* able to become a wound healer as it contains phytochemical constituents that can help in wound healing activities (Figure 2).

Histological analysis

Figure 3 shows the histology overview of wound healing in normal control, diabetic untreated and diabetic treated groups with different treatments after 20 days. From the figure, it shows that the formation of the granulation tissues and organize collagen formation was abundant in the normal control group indicated that wound was recovering well as compared to the other groups. This may be due to the endlessness supply of

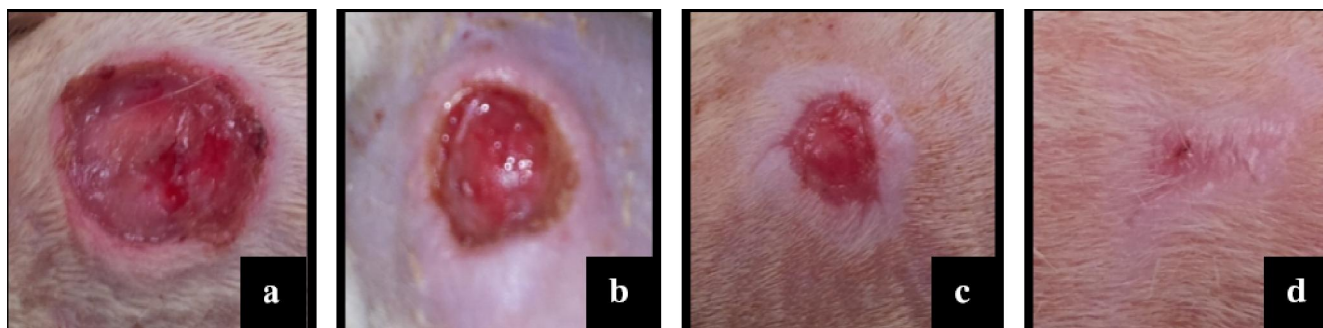


Figure 2 : The wound closure along the post-wounding days. a= wound at day 3, b= wound at day 7, c= wound at day 11, d= wound at day 19

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oxygen and red blood cells to promote the healing process. There was lacked of collagen formation for the diabetic untreated group resulted from the lack of formation of fibroblasts. The loss of collagen observed in the diabetic untreated group also may be due to the decreased levels of synthesis or enhanced catabolism of newly synthesized collagen or both^[28].

accelerate the conversion of soluble collagen to insoluble collagen. Altogether, flavonoids also have shown to inhibit the catabolism of soluble collagen^[29]. Therefore, *S. jamaicensis* plant extract can enhance the wound healing process, by speeding up the wound contraction and also managed to form organize tissues structure.

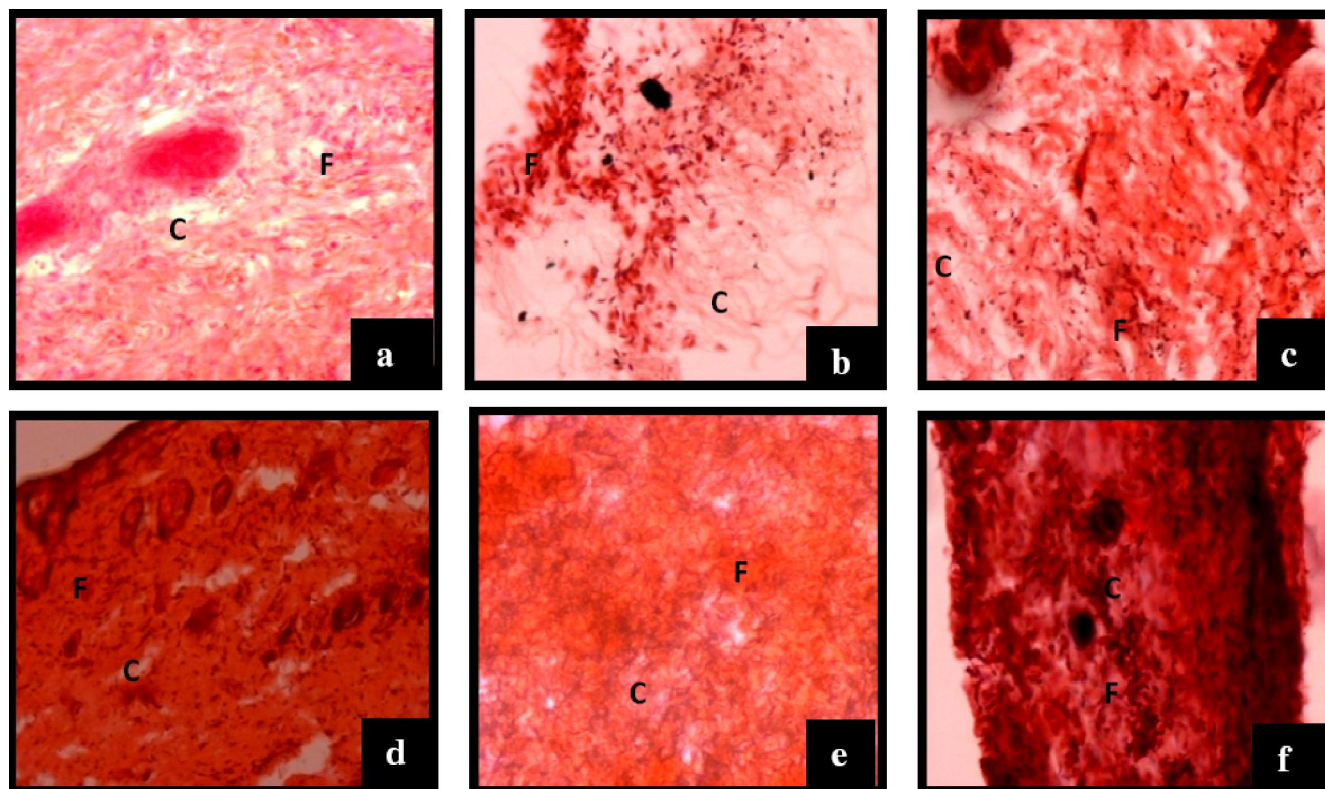


Figure 3 : Histological overview on the formation of new tissues. Magnification power of 40X by using haematoxylin-eosin staining. a=Normal control, b=Diabetic untreated, c= Positive control, d=0.2% *S. jamaicensis*, e=2% *S. jamaicensis*, f=20% *S. jamaicensis*, F= fibroblast, C= collagen fiber.

Among treated diabetic groups, the most well organized structure of new cells was the treatment group with 20% of *S. jamaicensis*. From the result on the wound contraction earlier, the most effective dose to speed up the wound closure was the 0.2% treatment group. Even though 0.2% treatment speeds up the contraction, the new cells formations were not really well organized. Fibroblast and also collagen formation were more abundant and organized at the 20% concentration of *S. jamaicensis*, followed by the positive control group, 2% and 0.2% of *S. jamaicensis*. This probably due to the presence of flavonoid in the 20% extracts concentration. Flavonoids have shown to increase the collagen synthesis, promote the cross-linkage of collagen, decrease the degradation of soluble collagen, and

CONCLUSION

This study indicates that *Stachytarpheta jamaicensis* has beneficial effect as wound healer related to diabetes mellitus type I. The most effective dose to speed up the wound closure was the 0.2% of *S. jamaicensis* extract. However, the most well organized structure of new cells was the treatment group with 20% of *S. jamaicensis*. Phytol, linolenic acid and palmitic acid which group under flavonoid were the most probably bioactive compounds responsible for the wound healing activity. These findings may form a basis of *S. jamaicensis* indication as a plant based wound healer. But, the possible mechanism involved in the short term

of wound healing effects of the leaf extract cannot be established by the current study and remain speculative. This suggests further investigation.

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