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THE ACTION OF *COLEUS AROMATICUS* AS A POTENTIAL WOUND HEALING AGENT IN EXPERIMENTALLY INDUCED DIABETIC MICE

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Abstract

The ethanolic and aqueous extract of *Coleus aromaticus* leaves and roots were evaluated for its wound healing activity in diabetic mice induced experimentally with Monosodium Glutamate (MSG) via excision and dead space wound models with the application of 100 mg kg-1 of body weight of extract. Excision wound was created on five different groups of mice and the wound contraction was measured for ten uninterrupted days. Dead space wound model was performed on four different groups of mice for the estimation of the weight of both wet and dry granulation tissue followed by the histological investigation of the wet granulation tissue after ten days. Ethanolic extract treated wounds showed 76.6% of wound area reduction compared to the controls that only showed 55.9 % of wound area reduction. The rate of epithelialization in ethanolic extract treated mice was interestingly higher compared to the controls. Histological investigation shows an increase in granulation tissue, good proliferation of collagen tissue and satisfactory angiogenesis in the leaves and root extract treated mice compared to the controls. *Coleus aromaticus* stimulates significant wound healing in experimentally induced diabetic mice and evaluation of the effectiveness of *Coleus aromaticus* as wound healing agent on human organism is highly suggested.

Keywords: wound healing; *Coleus aromaticus*; excision wound model; dead space wound model; epithelialization

Abstrak

Ekstrak *Coleus aromaticus* yang disediakan dengan menggunakan etanol dan air telah dikaji untuk mengenalpasti aktiviti penyembuhan luka ke atas tikus diabetik yang dirangsang dengan kaedah eksperimen melalui penyuntikan Monosodium Glutamat (MSG). Aktiviti pemyembuhan luka telah disiasat dengan menggunakan 2 model luka iaitu "*Excision Wound Model*" dan "*Dead Space Wound Model*" dan dos sebanyak 100mg/kg ekstrak kepada berat badan telah digunakan. "*Excision Wound Model*" telah dijalankan ke atas 5 kumpulan tikus yang berlainan dan keluasan pengecutan luka telah diukur untuk 10 hari yang berterusan. "*Dead Space Wound Model*" pula telah dijalankan ke atas 4 kumpulan tikus untuk mendapatkan anggaran berat tisu *granulation* serta dikuti dengan penyediaan slaid histologi daripada tisu basah *granulation* selepas 10 hari. Rawatan ekstrak beretanol ke atas luka telah menyebabkan pengecutan luka sebanyak 76.6% manakala luka yang tidak menerima sebarang rawatan hanya menunjukkan pengecutan luka sebanyak 55.9%. Kadar pembentukan tisu epitelium adalah memberangsangkan dengan adanya ekstrak etanol tumbuhan *Coleus aromaticus*. Siasatan histologi telah membuktikan bahawa kadar pembentukan tisu kolagen dan salur darah baru agak memuaskan dengan adanya ekstrak etanol *Coleus aromaticus*. Dengan ini, boleh dikatakan bahawa *Coleus aromaticus* memainkan peranan yang penting ke atas penyembukan luka diabetik dan penilaian aktivitinya adalah disyorkan untuk dijalankan ke atas luka manusia.

Katakunci: penyembuhan luka; *Coleus aromaticus*; excision wound model; dead space wound model; pembentukan epitelium

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1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder which is associated with high blood sugar level and it is usually accompanied with low level of insulin production, insulin secretion, insulin action, or both. Lack of insulin level in the blood usually leads to chronic hyperglycemia and diabetic patients sometimes show deterioration of metabolic processes overall including those of carbohydrates, fats and proteins [1]. The steady growth of population, aging, urbanization, obesity and physical inactiveness have high influences on the number of diabetic patients that increases steadily with the estimated number of patients with diabetes would increase from 171,228 number of cases in 2000 to 366,212 number of cases in 2030 comprising all the countries over the world [2]. These drastic changes on the number of diabetic incidence could always trigger the need of eradicating this disorder as effective as possible and to minimize the risks of its complications.

Diabetes mellitus has been documented to demonstrate lots of complications that are usually chronic in nature and these chronic complications target the organs of different systems that form the entire human organism which either becomes the direct or indirect factor that increases the rate of mortality and morbidity among the diabetic patients. Chronic complications can be classified into vascular and nonvascular complications in which the vascular complications are further subdivided into microvascular and macrovascular complications. Microvascular complications affect the eye causing retinopathy, affect the nerves causing neuropathy, and affect the kidneys causing nephropathy where as the macrovascular complications affect the heart causing Coronary Artery Disease (CAD), affect the peripheral vessels causing peripheral vascular disease, and affect the brain stimulating the cerebrovascular diseases. The nonvascular complications on the other hand include sexual abnormalities, skin changes and others [3].

Although the features of wound look quite simple, but the mechanism underlying it are usually complicated and it is quite hard to be understood clearly. A wound is a breach of epidermis that is caused by either a trauma or pathological changes which stimulate the wound repair processes. There are basically two types of wound in terms of onset and occurrence. Acute wounds are the wounds that appear suddenly and it persists only for a short period of time.

Surgical wounds and burns are the examples of acute wounds that heal quickly with few complications established. Chronic wounds on the other hand are the wounds that remain unhealed for more than 6 weeks and it is usually associated with complications that are caused by various factors that slow down the healing process. In chronic wound the entire biochemical and physiological processes of wound healing face some sort of instability that decreases the wound closure capabilities. Sometimes it is quite unfortunate to say that these chronic wounds tend not to respond well to the treatments that are designed especially for them. Chronic wounds also contribute to the changes of a person's lifestyle especially eating habit and it can lead to imbalanced diet or imperfect food digestion and absorption thus leading to unsatisfactory wound healing and closure. Pressure ulcers, fungating ulcers and malignant wounds are the common examples of chronic wounds [4].

Wound healing is a process to restore skin to a state of soundness of any injury those results in an interruption in the continuity of external surfaces of the body [5]. There are four main phases of wound healing which are the hemostasis, inflammation, proliferation and remodeling. Wound healing is a dynamic process with certain level of continuity which by right does not rest in between the series of repairing mechanisms .However all the stages of wound healing overlaps each other and that could be a reason why someone could not really distinguish the phases of wound healing independently [6]. Non healing wounds become everybody's interest in the area of medicine and ways to treat non healing wounds are constantly treasured and it involves developed, developing or even under developed countries worldwide. While majority of health care areas comprising different disciplines experience some sort of benefits when it comes to the treatment procedures as well as the outcome of their management and their medical care but unfortunately wound management team of health care industry has not faced any evidence based promising prognosis with the current trend of medications, therapeutic measures and referral [7].

According to World Health Organization (WHO), majority of the population in developed countries for about 70-80% of them somehow rather have used some forms of traditional or alternative medicines that were derived from plants for their primary health care needs [8]. Developed and even developing countries are dependent on traditional medicines at some point of their life because it has several advantages like the cost of treatment that is quite affordable

and some of our social life and structure has been already incorporated perfectly with the alternative form of medicine which began to serve as our cultural landmark [9].Many such medicinal plants have hepatoprotective, neuroprotective, anti-inflammatory and also antioxidant or radical scavenging properties that create an opportunity for its usage [10].

Coleus aromaticus Benth (Lamiaceae) also known as Indian or country Oregano. *C. aromaticus* Benth syn. *Coleus amboinicus* (Lour) Spreng or *Plectranthus amboinicus* is often used as a remedy for curing wounds in some countries [11]. It is a type of large succulent herb that has aromatic leaves and it is found abundantly in tropical countries even without extensive and serious cultivation [12]. The leaves are thick, succulent, juicy and it emanates pleasant mint smell upon crushing or squeezing [13].

C. aromaticus has several capabilities to aid in the process of wound healing in which one of it is the immunostimulant properties as well as the effective antimicrobial properties which aids in the process of eradicating pathogenic microorganism that slows down the normal wound healing route of progression [14]. It has high content of zinc and the fact is zinc is a wonderful mineral that serves important functions to promote and facilitate wound healing. *C. aromaticus* is also credited high for its anti-inflammatory characteristics that prevent the prolongation of inflammatory phase of a wound healing. This is quite important because the longer is the inflammatory phase the slower will be the rate of wound healing [8].

C. aromaticus also noted to have good antibacterial activity and obviously antibacterial agents can enhance wound healing activity so *C. aromaticus* can be considered as to be one of the important antibacterial agents that assures optimal wound healing by preventing infections in most cases [13]. There are lots of phytochemicals found in all the parts of the plant including its roots, stems, buds, leaves, flowers and fruits. Some of the phytochemicals found in *C. aromaticus* has been proven to play an important role in contributing towards blood sugar level lowering mechanisms. Since *C. aromaticus* has the capability of lowering the blood sugar level so it could be beneficial in terms of treating diabetic wounds to heal faster because of its ability to prevent or decrease the risk of infection and its complications [8].

C. aromaticus as one of the famous herbal plants are commonly used in cephalgia, otalgia, anorexia, dyspepsia, flatulence, colic, diarrhoea, cholera, halitosis, convulsions, epilepsy, cough, chronic asthma, hiccough, bronchitis, renal calculi, vesical calculi, strangury, hepotopathy, malarial fever and spasm. In Malaysia, bruished leaves of C. *aromaticus* are used to apply on burns and their poultice are applied on centipede and scorpion bites. In Jawa, it is used to treat cracks at the corner of the mouth [15]. *C. aromaticus* also has been reported to have good antihelminthic activity and because of this, it is extensively cultivated to accommodate the increasing trend of herbal medicine requirements [16]. On the other hand, researches and analyses had been conducted thoroughly to show the antioxidant activity of *C. aromaticus* as a tool to assist prevention of diseases that are triggered by biochemical oxidants [8]. It also has been accounted for exhibiting potent anticarcinogenic property and it defends tumour promoting activities [17].

In the sense that this golden herb has been used for ages as an important traditional medicine effectively, it is pretty impressive to suggest these herbs as an alternative tool for wound treatment due to its minimal side effects that are not that significant to be considered. Research has been done to investigate the lethal toxic dose of this particular herbs on the laboratory mice and the outcome of that research shows that *C. aromaticus* has minimal side effects or it can be concluded that this amazing herb has no side effects [18].

There are quite number of research done to evaluate the wound healing activity of *C. aromaticus* and one of the researches showed augmentation of wound healing by means of immunostimulation on the diseased giant murrels with lesions and deformed fins at the dorsal side and with reddish spot on the fifth day of application [14]. On the other hand, good wound healing activity was observed on excision, incision and dead space wound models with the help of polyherbal suspension of *C. aromaticus* together with *Punica granatum* in which the investigation was done on laboratory mice [11].

Research was also done to evaluate the wound healing activity of *C. aromaticus* on mice by performing excision wound model in albino mice with the application of ten percent ointment that was prepared from the aqueous extracts of leaves and roots of this herb. The result was

quite surprising because the experiment revealed exciting results of wound healing by establishing complete epithelialization on the 12th day post treatment [19].

The present study has been undertaken to conceal the wound healing activity of the leaves and roots extract of *C.aromaticus* in experimentally induced diabetic mice with the assistance of excision and dead space wound model.

2. METHODS

The technique of Chandrappa [20] was adopted for the preparation of ethanolic extract. 50g of dried leaves and 50g of dried roots of *C. aromaticus* were homogenized with 500ml of pure ethanol with the help of mortar and pestle. Homogenized mixture was centrifuged at 7000 rpm for 10 minutes. Clear supernatant was concentrated using rotary evaporator from 38° to 40°C. The extract was dissolved in ethanol and kept at 4°C until its future use.

Dried extract was prepared according to the method described by Nayak [21]. 200 g of leaves and roots were cleaned with distilled water and then it was homogenized using mortar and pestles with 50 ml of distilled water. Once the fine texture is obtained, the mixture was then filtered using muslin cloth. The filtrate was left aside for 48 hours at room temperature. Finally the filtrate was oven dried at 40°C to obtain dried extract. The dried extract was stored in a clean container until its future use.

Mice husbandry was performed by using the guidelines obtained from Babu [22]. Sprague Dawley with approximate weight of 150-200 g was fed under normal ratio. The mice were placed under optimum temperature (26-34°C), good ventilation and illumination with low light density. The mice was fed palatable (natural ingredients diets which is composed primarily of cereal grains and they are supplemented with additional proteins, vitamins and minerals), uncontaminated diets that meet their nutritional and physical needs at least daily. Foods and water were protected from feces and urine contamination. Bedding was used to absorb moisture and to curtail the growth of microorganisms and to limit animal contacts with their body waste. The mice were protected from any source of infection. The animals were randomly distributed into five groups in which each group contains 5 mice.

Induction of diabetes was done by applying the techniques utilised by by Singh [23]. Animals of groups 3, 4 and 5 were weighed and their fasting blood sugar levels were determined prior to experimental diabetic induction. Then the animals were injected subcutaneously (under the skin) with 1 ml saline water containing Monosodium Glutamate (MSG) with the dose of 4 mg/g body weight for 6 uninterrupted days and the blood sugar level was determined on the 28th day to confirm the presence of *Diabetes mellitus*.

Method from Nayak [24] was adopted to execute excision wound model. Both sides of the back were depilated and sterilized with alcohol swab. The area of wound was outlined at the back (circular area 300mm² with 2 mm thickness) using sterile stainless steel stencil. Excision wound was created along the outlined margin after anesthesia. Animals were divided into five groups. The normal controls (Group 1) were applied with Vaseline , experimental controls (Group 2) were applied with the ethanolic extract of *C. aromaticus*, diabetic controls (Group 3) were applied with Vaseline, diabetic experimental mice (Group 4) were applied with the ethanolic extract and the positive controls (Group 5) were applied with mupirocin ointment. The treatment was done with the topical administration of the substances investigated in all the mice tested. The ethanolic extract was applied at a dose 100mg/kg/day twice a day for 10 days. Feeding and husbandry were continued as usual. Wound areas were measured on daily basis using transparency sheet and permanent marker. Recordings of the wound areas were measured accurately using graph paper. The day in which complete wound closure occurs were observed and recorded. Comparison was done among the groups.

Dead space wounds were created by implanting sterile cotton pellets (10mg each), on both axilla and groin on the ventral surface of each and every mouse by using the technique adopted from Khan [25]. Mice were divided into four groups of 5 each. Group 1 animals (non diabetic mice) were provided plain water orally, Group 2 animals (non diabetic mice) were provided with 100 mg kg-1 day-1 dried extract for 10 days, Group 3 animals (diabetic mice) were provided with plain water orally and Group 4 animals (diabetic mice) were provided with dried extract 100 mg kg-1 day-1 for 10 days. Since an average mouse drinks approximately 110 ml of water/kg/day, 100 mg of dried extract was dissolved into 100 ml of drinking water. At the 10th day of wound, the sterile cotton pellets were removed carefully under anesthesia. Wet weight of granulation tissue was measured before it was dried at 60°C for 12 hours to measure

the dry weight of the granulation tissue. Additional piece of wet granulation tissue was preserved in 10% formalin solution for histological studies.

The means of wound areas were compared between the groups using one way ANOVA analysis followed by Post Hoc Tukey-Kramer pairwise comparison test. ANOVA analysis was used to examine the mean differences in excision and dead space wound model with P value less than 0.05 for all the analyses.

3. RESULTS

3.1.Excision Wound Model

The percentage of wound contraction increased steadily in the experimental control group (non diabetic mice treated with ethanolic extract of *C.aromaticus*) with the highest rate of wound progression. It was followed by the diabetic mice treated with ethanolic extract of *C.aromaticus* as well as the diabetic mice treated with modern antiseptic cream Mupirocin. The progression of wound healing in non diabetic mice which were applied with Vaseline is quite moderate and the least and not satisfying rate of wound healing progression was noted in diabetic mice that were applied with only Vaseline (Figure 1).

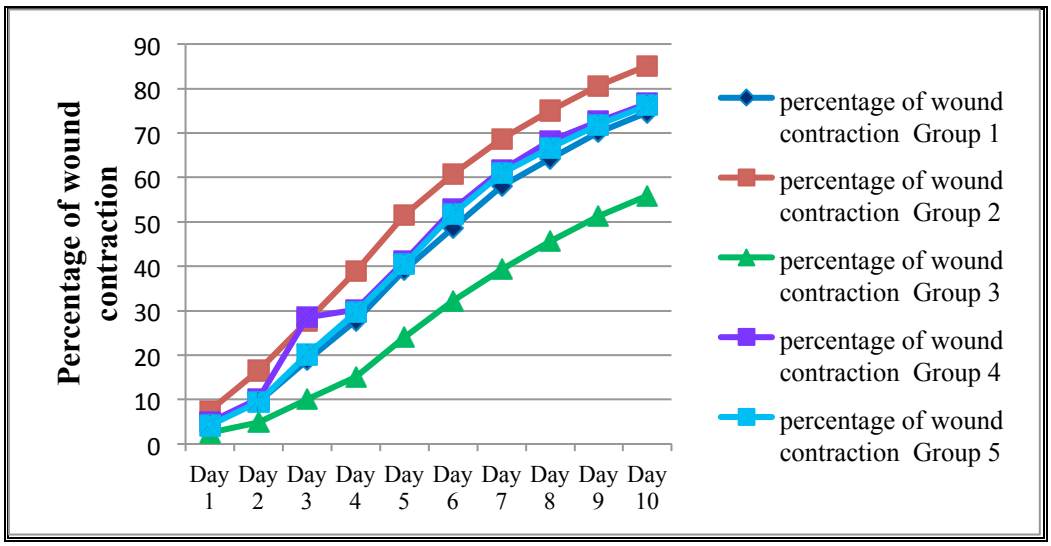

Figure 1: Wound healing activity of *C.aromaticus* in Monosodium Glutamate induced Diabetic mice

The wound area contraction was noted to be highest in non diabetic mice treated with ethanolic extract of *C.aromaticus* with about 85.1% of wound area reduction at day 10 (Table 1). When comparison is done between the diabetic mice that were treated with ethanolic extract of *C.aromaticus* and the diabetic mice that were treated with Mupirocin, it is quite interesting to say that they both achieved good rate of wound contraction for about 76.6% and 76.2% respectively on the tenth day. Non diabetic mice with topical administration of Vaseline showed only 74.6% of wound area contraction and its value seemed to be inferior to the non diabetic mice treated with ethanolic extract of *C.aromaticus*. Unfortunately, the least wound area reduction was observed in diabetic mice in which their wounds were treated with nothing but just Vaseline and their wound area reduction was about 55.9% only.

Table 1: Wound healing activity of *C.aromaticus (Excision Wound Model)* in Monosodium Glutamate induced Diabetic mice

Values are the means from 5 animals in each group					
Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Wound area (mm^2)					
Day 1	286.2 (4.6%)	277.4 (7.5%)	292.4 (2.5%)	285.2 (4.9%)	287.4 (4.2%)
Day 2	271.4 (9.5%)	250.2 (16.6%)	285.4 (4.9%)	269.4 (10.2%)	271.6 (9.5%)
Day 3	243.4 (18.9%)	216.8 (27.7%)	269.8 (10.1%)	214.4 (28.5%)	239.6 (20.1%)
Day 4	216.6 (27.8%)	183.4 (38.9%)	254.8 (15.1%)	209.4 (30.2%)	210.8 (29.7%)
Day 5	182.8 (39.1%)	145.4 (51.5%)	227.6 (24.1%)	176.4 (41.2%)	178.8 (40.4%)
Day 6	154.2 (48.6%)	117.6 (60.8%)	203.2 (32.2%)	141.4 (52.9%)	144.2 (51.9%)
Day 7	125.8 (58.1%)	93.8 (68.7%)	182.2 (39.3%)	114.8 (61.7%)	117.4 (60.9%)
Day 8	107.2 (64.3%)	74.6 (75.1%)	162.8 (45.7%)	95.4 (68.2%)	99.8 (66.7%)
Day 9	89.8 (70.1%)	57.8 (80.7%)	146.4 (51.2%)	82.2 (72.6%)	84.4 (71.9%)
Day 10	76.2 (74.6%)	44.8 (85.1%)	132.2 (55.9%)	70.2 (76.6%)	71.2 (76.2%)

The rate of wound contraction in diabetic mice in which their wounds were applied with Vaseline is quite slow and its initial wound contraction was only for about 2.5% after the first day of Vaseline application. Unfortunately, the speed of wound closure in diabetic wounds is

considerably low with not more than 10% of increase between each day. Diabetic wounds that were applied with ethanolic extract of *C.aromaticus* on the other hand showed more than 10% of wound area reduction between some of the days. Inter comparison between the groups were made using ANOVA analysis from the data collected from Day 10 followed by Tukey-Kramer Post Hoc tests to compare each and every group with each other. ANOVA confirms the presence of difference between the groups ($P < 0.05$ and $F > F$ critical).

Post Hoc Tukey-Kramer (Table 2) shows significant differences between Group 1 animals (Non diabetic mice applied with Vaseline) with all the other groups. There are differences between Group 2 animals (Non diabetic mice applied with ethanolic extract of *C.aromaticus*) with the rest of the groups as well. Group 3 animals (Diabetic mice applied with Vaseline) also showed significant differences with all the other groups of mice. However, it was noted that there is no significant difference between Group 4 (Diabetic mice applied with ethanolic extract of *C.aromaticus*) and Group 5 (Diabetic mice applied with Mupirocin antiseptic cream).

Table 2: Wound healing activity of *C.aromaticus* in Monosodium Glutamate induced Diabetic mice at Day 10 (Post Hoc Tukey-Kramer Pair wise Comparison) – Excision Wound Model

Group	**Group 1** (Non diabetic mice applied with Vaseline)	**Group 2** (Non diabetic mice applied with ethanolic extract of *C.aromaticus*)	**Group 3** (Diabetic mice applied with Vaseline)	**Group 4** (Diabetic mice applied with ethanolic extract of *C.aromaticus*)
Group 1 Non diabetic mice applied with Vaseline				
Group 2 Non diabetic mice applied with ethanolic extract of *C.aromaticus*	significant difference noted			
Group 3 Diabetic mice applied with Vaseline	significant difference noted	significant difference noted		
Group 4 Diabetic mice applied with ethanolic extract of *C.aromaticus*	significant difference noted	significant difference noted	significant difference noted	
Group 5 Diabetic mice applied with Mupirocin antiseptic cream	significant difference noted	significant difference noted	significant difference noted	no significant difference noted

The range of epithelialization rate for non diabetic mice with topical Vaseline administration is from 16 to 18 days with the average of 16.8 days (Table 3). The epithelialization period for non diabetic mice treated with extract is considerably faster with the range between 12 to 13 days

that has an average of 12.4 days. Diabetic mice showed slower rate of epithelialization without any treatment (applied with Vaseline basically) which is about 18 to 19 days with the average of 18.4 days. It is quite amazing to say that ethanolic extract application on the wound area increases the rate of epithelialization to approximately 4 days earlier in diabetic mice compared to the non treated diabetic wounds. The extract treated diabetic wounds reepithelialised from 13 to 15 days with the average of 14 days. The ethanolic extract of *C.aromaticus* has almost equal strength with the modern antiseptic cream because both remedies caused wound epithelialization with almost similar period of days in average.

Table 3: Wound healing activity of *C.aromaticus* in *Monosodium Glutamate* induced diabetic mice at day 10 (Epithelialization Day)

Epithelialization Day					
	Group 1	Group 2	Group 3	Group 4	Group 5
Mouse 1	16	12	18	14	13
Mouse 2	17	13	18	14	15
Mouse 3	16	12	19	13	14
Mouse 4	18	13	18	14	14
Mouse 5	17	12	19	15	15
Average	16.8	12.4	18.4	14	14.2

ANOVA showed that there are differences between the groups ($P < 0.05$ and $F > F$ critical). Post Hoc Tukey-Kramer (Table 4) shows significant differences between Group 1 animals (Non diabetic mice applied with Vaseline) with all the other groups. There are differences between Group 2 animals (Non diabetic mice applied with ethanolic extract of *C.aromaticus*) with the rest of the groups as well. Group 3 animals (Diabetic mice applied with Vaseline) also show significant differences with all the other groups of mice. However, it was noted that there is no significant difference between Group 4 (Diabetic mice applied with ethanolic extract of *C.aromaticus*) and Group 5 (Diabetic mice applied with Mupirocin antiseptic cream).

Table 4: Wound healing activity of *C.aromaticus* in *Monosodium Glutamate* induced Diabetic mice at day 10 (Epithelialization Day) – Post Hoc Tukey Kramer Test

Group	**Group 1** (Non diabetic mice applied with Vaseline)	**Group 2** (Non diabetic mice applied with ethanolic extract of *C.aromaticus*)	**Group 3** (Diabetic mice applied with Vaseline)	**Group 4** (Diabetic mice applied with ethanolic extract of *C.aromaticus*)
Group 1 Non diabetic mice applied with Vaseline				
Group 2 Non diabetic mice applied with ethanolic extract of *C.aromaticus*	significant difference noted			
Group 3 Diabetic mice applied with Vaseline	significant difference noted	significant difference noted		
Group 4 Diabetic mice applied with ethanolic extract of *C.aromaticus*	significant difference noted	significant difference noted	significant difference noted	
Group 5 Diabetic mice applied with Mupirocin antiseptic cream	significant difference noted	significant difference noted	significant difference noted	no significant difference noted

3.2 Dead space wound model

The weight of wet granulation tissue in diabetic mice that were treated with 100mg/kg/day of dried extract of *C.aromaticus* orally was found to be highest which ranged from 163.8 to 116.2 mg/100g of mouse where as the lowest weight of wet granulation tissue was obtained in diabetic mice without any treatment (Table 5). The range of wet granulation tissue weight for

diabetic mice without treatment was from 90.3 to 92.2 mg/100g of mouse. The second highest weight of wet granulation was noted in non diabetic mice treated with 100mg/kg/day of dried extract orally for ten days which ranged between 151.9 till 154.1 mg/100 g of mouse. However, in non diabetic mice that were given plain water orally with the absence of extract, the range of wet granulation tissue weight was from 100.5 till 102.3 mg/100 g of mouse.

Table 5: Wound healing activity of *C.aromaticus* in *Monosodium Glutamate* induced Diabetic mice at day 10 (Dry and Wet Granulation Tissue)

	Weight of Wet granulation tissue (mg/100 g mouse)			
	Group 1	**Group 2**	**Group 3**	**Group 4**
Member 1	100.5	153.4	90.3	166.2
Member 2	101.5	152.4	90.8	165.8
Member 3	100.9	151.9	91.2	164.9
Member 4	101.6	153.1	92.2	164.8
Member 5	102.3	154.1	90.7	163.8
Average	101.36	152.98	91.04	165.1
	Weight of Dry granulation tissue (mg/100 g mouse)			
	Group 1	**Group 2**	**Group 3**	**Group 4**
Member 1	30.5	44.2	28.4	41.5
Member 2	31.1	45.3	29.1	40.9
Member 3	30.6	44.6	28.6	40.5
Member 4	29.9	45.1	29.2	41.2
Member 5	30.3	44.9	28.6	41.5
Average	30.48	44.82	28.78	41.12

The administration of dried extract of *C.aromaticus* increases the granulation tissue in both diabetic and non diabetic mice. The weight of dry granulation tissue was noted to be the highest on the non diabetic mice treated with dried extract of *C.aromaticus* which ranged from 44.2 till 45.3 mg/100 g of mouse. The weight of dry granulation tissue ranged between 40.5 till 41.5

mg/100 g of mouse in diabetic mice treated with 100mg/kg/day of dried extract and it was slightly lower than the non diabetic mice treated with extract. The range of the dry granulation weight was found to be least in diabetic mice given only plain water orally to drink which was from 28.4 to 29.2 mg/100 g of mice. Finally the weight of the dry granulation tissue was about 29.9 to 31.1 mg/100 g of mouse in non diabetic mice that were given plain water orally. So, if the mass of dry granulation tissue is used as a criteria for arranging the strength of granulation tissue, it can be arranged in such a way in which diabetic mice with plain water oral administration be placed on the bottom of ascending hierarchy, followed by the non diabetic mice with plain water oral administration, then with diabetic mice treated with dried extract orally and finally, the non diabetic mice treated with 100mg/kg/day extract for ten days can be placed at the highest rank in the hierarchy. ANOVA analysis showed significant differences between all the 4 groups investigated and it confirms the presence of difference between the groups ($P < 0.05$ and $F > F$ critical) (Table 6).

Table 6: Wound healing activity of *C.aromaticus* in *Monosodium Glutamate* induced Diabetic mice at day 10 (Wet and Dry Granulation Tissue) – Post Hoc Tukey-Kramer test

Group	Group 1 - Non diabetic mice with oral plain water administration	Group 2 - Non diabetic mice with oral extract administration	Group 3 - Diabetic mice with oral plain water administration
Group 1 - Non diabetic mice with oral plain water administration			
Group 2 - Non diabetic mice with oral extract administration	significant difference noted		
Group 3 - Diabetic mice with oral plain water administration	significant difference noted	significant difference noted	
Group 4 - Diabetic mice with oral extract administration	significant difference noted	significant difference noted	significant difference noted

Histopathological investigation of the wet granulation tissue of the non diabetic mice without any treatment found to show high level of fibroblast cells with lesser amount of collagen fibres. The wet granulation tissue of non diabetic mice treated with 100mg/kg/day of dried extract for ten days revealed lesser fibroblast and abundant amount of collagen fibres which are arranged uniformly and the formation of new blood vessels were also noted accompanied with lesser amount of inflammatory cells. Histopathological investigation of the wet granulation tissue obtained from the diabetic mice without any treatment showed greater number of fibroblast cells but less collagen fibres. However, condition was quite better in diabetic mice that were

treated with dried extract orally that showed moderate collagen fibres and fibroblast with the formation of new blood vessels. (Figure 2)

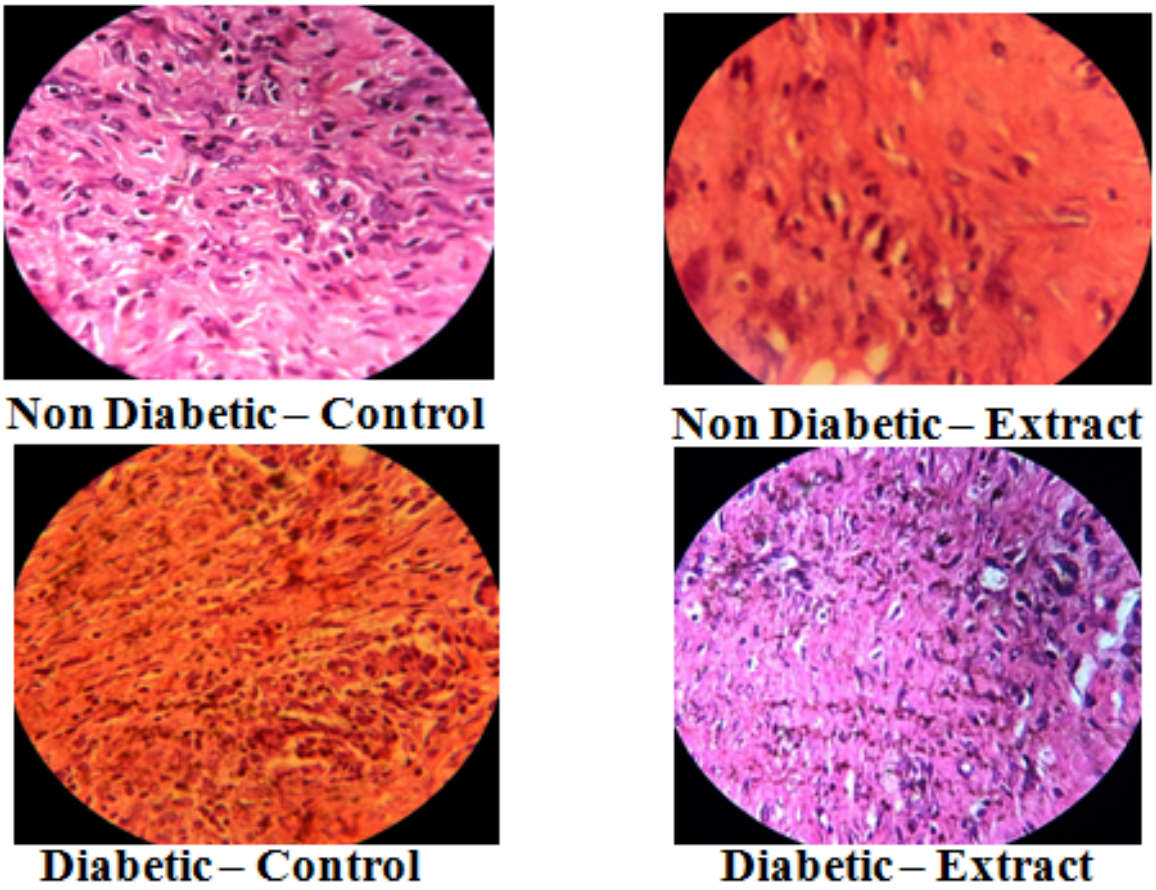

Figure 2: Histopathological slides of the wet granulation tissue to investigate the wound healing activity of *C.aromaticus* in *Monosodium Glutamate* induced Diabetic mice

4. DISCUSSION

Diabetes mellitus is a chronic hyperglycaemic disorder that has the highest rate of epidemiological distribution involving all the countries of the world with the fact that it is quite common to the awareness of society. Everybody knows about this disorder and intensive way of preventing the disorder as well at its complications always takes place with the provision of mutual benefit to all as the prime concern. This disorder affects more than one human body system and it even has a significant role in the alteration and modification of normal wound

healing process. *Diabetes mellitus* Type 2 commonly affects the elder aged group of people and since this age group of people usually demonstrates greatest risk of wound healing in which the process itself can be delayed and apart from that a wound infection may always accompany the wound which may even contribute to slower rate of wound healing and it seemed that the rate of diabetic cases among older generation is increasing steadily [5].

Wound healing process can be subdivided into few major stages which initiate with the stage of inflammation, then after some time it undergoes proliferation and epithelialization and finally the wound will undergo the stage of remodelling or maturation [26].The inflammatory phase is characterised by the migration of neutrophils from the blood to the site of wound and slowly it will be replaced by agranulocytes which are the macrophages. The proliferative phase is then customised with the formation of new blood vessels or it also termed as the process of angiogenesis in the medical concept. Angiogenesis is associated with the development extracellular matrix with the help of collagen synthesis, granulation tissue formation, and re-epithelialization. The extracellular matrix that is formed here eventually undergoes the remodelling stage that is accomplished with the final touch that is concerned with the formation of scar tissue and with the absence of blood vessels [27].

Infection and wound healing always show some sense of relationship in an inverse pattern in which the greater the incident of wound infection the lesser will be the wound healing capabilities. In other words, it can be said that infection could slow down the mechanics of normal progression of wound healing. Infections therefore are capable of causing wound breakdown, herniation of the wound and complete wound dehiscence that are actually serve as the unwanted ending episode of wound healing. The effort to control wound infection as a whole has became very complicated and tangled in today's day situation because most of the bacteria have achieved mutation in which they are capable of resisting common modern antibiotics and thus these is something that has to be worried by all since it is going to cause major problem in the area of bacterial eradication. Such problem has already be encountered with *Staphylococcus aureus* that has demonstrated greater level of resistance against methicillin and this species is definitely spreading and reproducing more similar like progeny [28].

C. aromaticus as a unique herb has been investigated by various researchers from all over the world and recognition has been given to this particular plant due to its important contribution in the area of microbiology, reproductive medicine, wound healing and many others. None other than the phytochemical components of *C.aromaticus* could rather have contributed towards its achievements in the field of herbal medicine. Phytochemical screening and qualitative estimation of the crude yields of the chemical constituent of the plants showed that the leaves and the stems of *C. aromaticus* (Benth) consist of medically essential alkaloids, tannins and saponins. Each of these phytochemicals exhibits their own impressive therapeutic values in various medical disciplines. Tannins demonstrate antimicrobial activity by preventing the development of pathogenic microorganisms. The mechanism of action of the tannins is actually related to the action of them to cause the precipitation of microbial proteins while inhibiting the presence of nutritious proteins to microbes [29].

C. aromaticus is used for centuries as a medicinal agent because it has several interesting properties like antilithiotic, chemopreventive, anti-oxidant and antiepileptic potential. Some people use it to treat ulcers, boils, swellings and other forms of wounds. In India, it is almost used by all the local civilians for the purpose of treating common cold, cough, fever, headache and indigestion effectively. The leaves are even said to have specific actions on the urinary bladder and in regards with that it can be used to treat urinary and vaginal diseases [14].

Topical administration of *C.aromaticus* extract in the current study has somehow rather improved the wound healing process even as early as on the fifth day of wound treatment and it has improved the range of wound contraction because it reduces the epithelialization period up to 12 days. Obviously, for any sort of wounds, the most important outcome is to achieve epithelialization quicker. Since the ethanolic extract of *C.aromaticus* acts as a potent wound healing remedy, it is not wrong to use it as an alternative choice of wound healing agent. The restoration of the epidermal layer on the wounded area is performed through epithelialization and the faster the rate of epithelialisation the greater will be the wound healing progression and this always gives a guideline to us on the mode of action of certain wound healing remedy [19]. While considering into the point mentioned above, *C. aromaticus* has evidently proved that it promotes quicker epithelialization process by reducing the timeframe for the wound epithelialization.

Collagen is the major protein molecule that composes the extracellular matrix and without this collagen the granulation tissue could not be formed. So in that sense, a fact can be derived to say that the amount of collagen in a wound directly indicates the strength of a wound [30]. The condition of granulation tissue is often used as a tool to estimate the condition of the wound as a whole and it also gives a rough idea on how the wound healing progresses [31]. The granulation tissue that is present on the area of the wound is usually composed with fibroblast cells, collagen, and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin transform themselves into fibroblast, which then start migrating into the wound gap along with the fibrin strands [24]. The study of dead space wound model shows increased level of wet and dry weight of granulation tissue in the ethanolic extract treated animals when it is compared with the control groups and this indicates that the extract improves the strength of wound by stimulating the generation of healthy granulation tissue.

Non healing wound is biologically characterised by prolonged inflammation, defective re-epithelialization and impaired matrix remodelling [15]. Since the histopathological investigation of the wet granulation tissue obtained from the extract treated animals demonstrated high level of collagen deposition with reduced inflammatory cells, it can be suggested that the extract prevents the prolongation of inflammatory phase but promotes the granulation and proliferation phase the wound healing. That is the reason, why the wound heals faster in the presence of extract.

5. CONCLUSIONS

This study demonstrates that *C.aromaticus* extract promotes wound healing, increases wound contraction, enhance collagen deposition and reduces wound epithelialization period in monosodium glutamate induced diabetic mice.

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