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WOUND HEALING ACTIVITY OF *COLEUS AROMATICUS* IN EXPERIMENTALLY INDUCED DIABETIC MICE

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Abstract–Background. The ethanolic and aqueous extract of *C.aromaticus* leaves and roots (100 mg kg^{-1} day^{-1} for 10 days) were evaluated for its wound healing activity in Monosodium Glutamate induced diabetic mice using excision and dead space wound models. Excision wound model was performed on five different groups of mice to measure the progress of wound area for ten consecutive days. Dead space wound model was performed on four different groups of mice to estimate wet and dry granulation tissue as well as to examine the histological appearances of granulation tissue after ten days. Ethanolic extract treated wounds was found to show 76.6% of wound area reduction when it is compared with controls that exhibited 55.9 % of wound area reduction. The ethanolic extract treated wound had epithelised faster compared to controls. The wet and dry granulation tissue weight has increased significantly in extract treated mice when it is compared with the controls. Extract treated mice exhibited good proliferation of collagen tissue with satisfactory angiogenesis. Coleus aromaticus promotes significant wound healing in induced diabetic mice and evaluation of this activity on human organism is highly suggested.

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and relative insulin deficiency, resistance or both. Ten to fifteen percent of diabetic patients develop foot ulcers at some stage in their lives. Diabetic foot problems are responsible for nearly 50% of all diabetes related hospital admissions (Kumar and Clark, 2012). Wounds can heal in patients with diabetes but healing may take longer because the healing process is impaired and it is prone to complications such as infection and trauma which can eventually delay the healing process. (Sharp and Clark, 2011)

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 (Mosby's medical dictionary, 8th edition, 2008). The phases of normal wound healing include hemostasis, inflammation, proliferation and remodeling. Each phase of wound healing is distinct although the wound healing process is continuous, with each phase overlapping the next (Mackay and Miller, 2003). Non healing wounds are one of the significant problems in health care system all over the world. Unlike other areas of health care, wound management has not had the benefits of evidence-based, standardized treatment and referral plans with the current trend of medical technology (Gotrup, 2003).

Earlier studies on traditional medicinal plants revealed that the use of medicinal plants is predominantly due to its low cost and sometimes it is a part of their social life and culture (Muthu *et al*., 2006). Many such medicinal plants have hepatoprotective, neuroprotective, anti-inflammatory and also antioxidant or radical scavenging properties (Perry *et al*., 1999).

Coleus aromaticus Benth (Lamiaceae) also known as Indian or country Oregano. (Pritima *et al*., 2010). *C. aromaticus* Benth syn. *Coleus amboinicus* (Lour) Spreng or *Plectranthus amboinicus* is traditionally used to cure wounds. (Soni *et al*., 2011). It is large succulent herb with aromatic leaves, found abundantly in tropical countries (Revathi *et al*., 2011). A dense shrub with a foetidscent, the flowers white with the throat barred with red or yellow (Kalaiarasan & John, 2011). The leaves are thick,

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succulent and juicy (Pritima *et al*., 2008) and it emanates pleasant smell upon crushing or squeezing.

C. aromaticus can improve wound healing because it has immunostimulant property and it has antimicrobial capability to fight against infection that eventually increases the wound healing process (Sunitha *et al*., 2010). It has high content of zinc (mineral) which can facilitate wound healing. Its anti-inflammatory effect will slow down the process of inflammation and decreases the timeframe for the inflammatory phase of wound healing and eventually speeds up the process of wound healing (Khare *et al*., 2011).

On the other hand, *C. aromaticus* has antibacterial activity naturally to rally wound healing externally which we can consider as to be the most essential factor that can promote optimal wound healing. (Pritima & Pandian, 2008) *C. aromaticus* also contains various kinds of phytochemicals that are be found in the roots, stems, buds, leaves, flowers and fruits which can lower the blood sugar level and it aids in the process of diabetic wound healing (Malathi *et al*., 2011).

They are commonly used in cephalgia, otalgia, anorexia, dyspepsia, flatulence, colic, diarrhea, cholera, halitosis, convulsions, epilepsy, cough, chronic asthma, hiccough, bronchitis, renal calculi, vesical calculi, strangury, hepotopathy, malarial fever and spasm. In Malaysia, bruished leaves are applied to burns and their poultice on centipede and scorpion bites. In Jawa, it is used to treat cracks at the corner of the mouth (Patel *et al*., 2010). *C. Aromaticus*, a Malay species has been cultivated widely because of its antihelminthic activity (Prameela & Oommen, 2011). Some researchers reported that *C. aromaticus* has antioxidant activity which is capable of preventing diseases. (Khare *et al*., 2011). It exhibits its anticarcinogenic property and it tends to defend tumour promoting activities. (Rasineni *et al*., 2008).

Since this herb has been used for ages traditionally and effectively, it is presumed that side effects should be less. Research has been done to check the lethal toxic dose on the laboratory mice and that credited greatest value for *C. aromaticus* since minimal or no side effects were documented. (Preeja *et al*., 2011)

C. aromaticus showed enhancement of wound healing by the 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 (Sunitha *et al*., 2010). Improved wound healing activity was observed on excision, incision and dead space wound models with the use of polyherbal suspension of *C. aromaticus* and *Punica granatum* (Soni *et al*., 2011).

Anand K. Jain from G.R Medical College, India has evaluated the wound healing activity of aqueous extract of leaves and roots of *C.aromaticus* using excisional wound model in albino rats in 2012 and it was proven that ten percent ointment of aqueous extract of root showed complete epithelialization after 12 days (Jain *et al*., 2012)

The present study has been undertaken to demonstrate wound healing activity of the leaf and root extract of *C.aromaticus* in experimentally induced diabetic mice by utilizing excision and dead space wound models.

METHODS

The method of Chandrappa *et al*., (2010) was adopted for the purpose of ethanolic extract preparation. 50g of dried leaves and 50g of dried roots of *C. aromaticus* were homogenized with 500mL of pure ethanol using mortar and pestle. Homogenized mixture was centrifuged at 7000 rpm for 10 minutes. Clear supernatant was concentrated using rotary evaporator at 38° to 40°C. The extract was dissolved in ethanol and kept at 4°C.

Dried extract was prepared according to the method described by Nayak *et al*., 2007. 200 g of leaves and roots were cleaned with distilled water and it was homogenized using mortar and pestles with 50 ml of distilled water until 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 method of Babu *et al*., (2012) was adopted for mice husbandry. 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 which are supplemented with additional proteins, vitamins and minerals), uncontaminated diets that meet their nutritional and physical needs at least daily. Foods and water was protected from feces and urine contamination. Bedding was used to absorb moisture, minimize the growth of microorganisms and dilute and limit

animal contacts with excreta. The mice were protected from any source of infection. The animals were randomly distributed into five groups of 5 each.

The method by Singh *et al*., (2011) was adopted to induce diabetes in mice. Animals of groups 3, 4 and 5 were weighed and their fasting blood sugar levels were determined prior to diabetic induction. Then the animals were injected subcutaneously with 1 ml water containing Monosodium Glutamate (MSG) with dose level of 4 mg/g body weight for 6 consecutive days and the blood sugar level was determined on the 28th day to confirm the diabetic status of mice.

Method from Nayak *et al*, (2008) was adopted to conduct 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 300mm^2 with 2 mm thick) 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 topically in all the cases. 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 every day 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. Results were documented.

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 *et al*., (2007). 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 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 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.

RESULTS

Excision Wound Model

Wound Area

Significant increase in wound healing activity is noted in extract treated mice (Table 1). In comparison between Vaseline treated non diabetic mice with *C.aromaticus* ethanolic extract treated non diabetic mice, it is evidently shown that the percentage of wound contraction is greater in animals treated with extract (85.1%) on day 10. On the other hand, comparison between the groups of diabetic mice on day 10, it is proven that the percentage of wound contraction is greater in diabetic mice that were treated with ethanolic extract (76.6%) and in diabetic mice that were treated with modern antiseptic cream (76.2%).

When the percentage of wound contraction is compared between diabetic and non diabetic mice, it was noted that the percentage of wound contraction in diabetic mice was found to be lesser than the non diabetic mice. The highest percentage of wound contraction was noted in non diabetic mice that were treated with ethanolic extract of *C.aromaticus* (85.1%). On the other hand, the lowest percentage of wound contraction was noted in diabetic mice that were applied with Vaseline (55.9%).

When diabetic mice that were treated with ethanolic extract is compared with the diabetic mice that were treated with modern antiseptic cream, it is quite visible that both ethanolic and mupirocin has approximately equal effect on wound healing. However, ethanolic extract treated diabetic mice show slight increase of wound contraction

Table 1. Wound healing activity of *C.aromaticus* (excision wound model) in Monosodium Glutamate induced Diabetic mice

	Values are means from 5 animals in each group				
Parameter	Group 1	Group 2	Group 3	Group 4	Group 5
Wound area (mm²)					
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%)

compared to the modern antiseptic cream. (Fig. 1)

Inter comparison between the groups were made using ANOVA (Table 2) from 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).

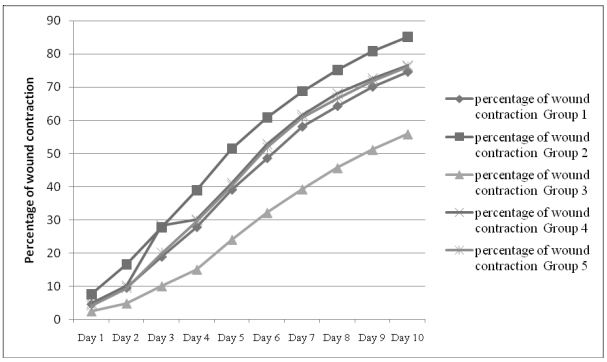

Fig. 1. Wound healing activity of *C. aromaticus* in Monosodium Glutamate induced Diabetic mice

Post Hoc Tukey Kramer (table 3) 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)

Epithelialization

Animals of Group 1 (Non diabetic mice applied with Vaseline) showed complete epithelialization after 17 days. Animals of Group 2 (Non diabetic mice applied with ethanolic extract of *C.aromaticus*) showed complete epithelialization approximately after 12 days. Animals of Group 3 (Diabetic mice applied with Vaseline) showed complete epithelialization only after 18 days. Group 4 (Diabetic mice applied with ethanolic extract of *C.aromaticus*) and Group 5 (Diabetic mice applied with Mupirocin antiseptic cream) animals showed epithelialization after 14 days. (Table 4)

ANOVA (Table 5) showed that there are differences between the groups ($P < 0.05$ and $F > F$ critical). Post Hoc Tukey Kramer (Table 6) 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)

Dead space wound model

Dry and wet granulation tissue

A significant increase of wet and dry weight of granulation tissue was noted in the animals that were administered with dried extract orally (Table 7).

Table 2. Wound healing activity of *C.aromaticus* in Monosodium Glutamate induced Diabetic mice (excision wound model) at Day 10

ANOVA Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
77	4	304	76	3.333333
46	4	178	44.5	3
133	4	528	132	6.666667
71	4	280	70	0.666667
74	4	282	70.5	3

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	16642.8	4	4160.7	1248.21	1E-18	3.055568
Within Groups	50	15	3.333333333			
Total	16692.8	19				

Table 3. 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

Histology

Histopathological investigation shows an increased collagen formation and satisfactory angiogenesis with less fibroblast cells in extract treated animals compared to its control.

DISCUSSION

Diabetes mellitus is a hyperglycaemic multisystem disorder that affects the wound healing process. Diabetes mellitus Type 2 (non insulin dependent) can be considered as to be increasing in incidence worldwide. Diabetes mellitus Type 2 commonly affects the older generation who exhibits highest risk of slow wound healing and wound infection (Mousley, 2003).

Wound healing could be divided into four different stages which is haemostasis, inflammation, proliferation and maturation. Haemostasis is characterized by haemorrhage, platelet activation,

Table 4. 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

Table 5. Wound healing activity of *C.aromaticus in Monosodium Glutamate induced Diabetic mice* at Day 10 (epithelialization day)

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
16	4	68	17	0.666667
12	4	50	12.5	0.333333
18	4	74	18.5	0.333333
14	4	56	14	0.666667
13	4	58	14.5	0.333333

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	93.2	4	23.3	49.92857	1.71E-08	3.055568
Within Groups	7	15	0.466667			
Total	100.2	19				

Table 6. 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	

Table 6. *Continued ...*

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 5 Diabetic mice applied with Mupirocin antiseptic cream	significant difference noted	significant difference noted	significant difference noted	no significant difference noted

Table 7. Wound healing activity of *C.aromaticus in Monosodium Glutamate induced Diabetic mice* at Day 10 (dry and wet granulation tissue)

	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

	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

Table 8. Wound healing activity of C.aromaticus in Monosodium Glutamate induced Diabetic mice at Day 10 (wet granulation tissue)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Group 1	5	506.8	101.36	0.478
Group 2	5	764.9	152.98	0.737
Group 3	5	455.2	91.04	0.523
Group 4	5	825.5	165.1	0.88

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	20377.82	3	6792.606667	10378.31423	1.62E-26	3.238872
Within Groups	10.472	16	0.6545			
Total	20388.292	19				

Table 9. Wound healing activity of *C.aromaticus in Monosodium Glutamate induced Diabetic mice* at Day 10 (dry granulation tissue)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Group 1	5	152.4	30.48	0.192
Group 2	5	224.1	44.82	0.187
Group 3	5	143.9	28.78	0.122
Group 4	5	205.6	41.12	0.182

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	931.228	3	310.4093333	1817.917033	1.79E-20	3.238872
Within Groups	2.732	16	0.17075			
Total	933.96	19				

Table 10. Wound healing activity of *C.aromaticus in Monosodium Glutamate induced Diabetic mice* at Day 10 (wet and dry granulation tissue) – 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

complement cascade activation and blood clotting. Inflammation can be characterized by cell recruitment, phagocytises and debridement. Proliferation can be characterized with the release of cytokines, cell growth and activation, neovascularisation and granulation tissue formation. Maturation can be characterized by wound contraction, wound closure, dissolution of granulation tissue and remodelling. (Sharp & Clark J, 2011)

Infection in a wound delays healing process and they may cause wound breakdown, herniation of the wound and complete wound dehiscence. The control of wound infection has be more challenging because bacteria have developed antibiotic resistance that causes bacterial eradication becomes unsuccessful and to a greater incidence of infections caused methicillin resistant *Staphylococcus aureus*, polymicrobial flora and fungi (Shittu *et al*.,2002)

C. aromaticus has been investigated by various researchers to identify its medicinal importance in the area of microbiology, reproductive medicine,

wound healing and others. Phytochemical constituents of *C.aromaticus* may have contributed towards its achievements in the area of traditional medicine. Phytochemical screening and qualitative estimation of the crude yields of chemical constituent of the plants showed that leaves and stems of *C. aromaticus* (Benth) consist of alkaloids, tannins and saponins. These phytochemicals exhibits impressive medicinal values. Tannins demonstrates antimicrobial activity preventing the development of microorganisms and it causes the precipitation of microbial proteins and inhibiting the availability of nutritious proteins to microbes (Ramya *et al*., 2012)

C. aromaticus has been reported to contain vitamin C and flavonoids that has free radical scavenging activity which helps to reduce lipid peroxidation by preventing cell necrosis or cell death by improving vascularity. *C. aromaticus is* capable of increasing viability of collagen fibrils and the strength of collagen fibres by improving circulation and promoting DNA synthesis. (Jain *et al*., 2012). Evaluating the chemical patterns of *C.aromaticus* in the above mentioned studies could be useful to justify the undebatable strength of this unique plant in the process of wound healing.

Topical administration of *C.aromaticus* improved wound healing as earlier as at the 5th post wounding day. Ethanolic extract had improved wound contraction and it helps in reducing the epithelialization period up to 12 days and its activity is incomparable with mupirocin the modern antiseptic cream. Restoration of the epidermis layer can be done via epithelialization and obviously this could be used as one the best parameter to indicate quality of wound healing. In regards with that, *C. aromaticus* possesses significant wound healing activity as evidenced by the significant increase in the rate of wound contraction and decreased period of epithelialization (Jain *et al*., 2012).

Due to its intrinsic and unique quality as well as its wide approach of use the traditional medicine becomes tolerable and affordable and it serves an important function to be the best alternative medicine continuously and it is being broadly used to solve medical related issues and problems especially in remote or rural areas. Modern health care system alone could not meet the health care need of the entire population of the world. In conjunction with this, World Health Organization (WHO) had launched the policy of urging its member's states to promote and integrate traditional medicine into their modern health care system (Tolera *et al*., 2011). In this study, *C. aromaticus* has demonstrated almost equal activity of wound healing in comparison with the modern antiseptic cream as proven by the results documented thus it helps the progress of herbal treatment in the area of wound healing and this eventually fulfils the expectation of WHO to incorporate traditional medicine into current trend of modern medicine.

Collagen is the major protein of extracellular matrix that forms granulation tissue and it is a component that ultimately contributes to wound strength (Manjunatha *et al*., 2005). Granulation tissue is a term used to describe the new wound matrix which is composed with collagen fibres and an extracellular substance which is called as ground substance. The condition of granulation tissue is often used as a tool to estimate the condition of the wound and how wound healing progresses (Flanagan, 2000). Collagen constitutes more than fifty percent of the protein in scar tissue and its production is quite important for the healing process to accomplish. Fibroblast is responsible for the production of collagen fibres thus it promotes protein regeneration. (Monaco & Lawrence, 2003). In the reparative dermis, fibroblasts and endothelial cells are the most prominent cell types present and support capillary growth, collagen formation, and the formation of granulation tissue at the site of injury (Guo & DiPietro, 2010). The granulation tissue of the wound is basically composed of fibroblast, collagen, and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands (Nayak *et al*., 2006). Since the study of dead space wound model shows increased proliferative level of wet and dry granulation tissue in extract treated animals in comparison with the control groups, we could suggest the presence of wound strength in extract treated animals due to increased level collagen synthesis and deposition that evidently proves the efficiency of C.*aromaticus* to promote wound healing and to accomplish wound repair within the short period of time.

Non healing wound is also biologically characterised by prolonged inflammation, defective re-epithelialization and impaired matrix remodelling (Edwards & Harding, 2004). Healing abnormalities are often associated with collagen deposition aberrations. (Monaco & Lawrence, 2003). Histological investigation of granulation tissue from

the extract treated animals demonstrated increased collagen deposition with reduced inflammatory cells. Delayed wound healing is often related to imbalanced state of wound healing stages that prevents progression from one phase to another phase. Chronic leg ulcers are stuck in the phase known as early inflammation (Collier, 2003). Reduced inflammatory cells that have been noted in the histological studies could suggest that inflammatory phase is not prolonged in extract treated animals. Since histological study shows less inflammatory cells with increased collagen cells, this can be used to suggest that extract decreases inflammatory cells and increases the rate of progression from one phase of wound healing to another. That is to say that the wound healing has progressed from the inflammatory phase to the proliferative phase and thus it can progress quite quickly into the stage of maturation, the final stage of wound healing.

CONCLUSIONS

The present study demonstrates that *C.aromaticus* extract that were applied topically promotes wound healing, increases wound contraction, enhance collagen deposition and reduces wound epithelialization period in monosodium glutamate induced diabetic mice where wound healing is delayed.

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REFERENCES

Adam, J., Singer Richard and Clerk, A.F. 1999. Cutaneous wound healing. *The New England Journal of Medicine*. 1 (1) : 738-746.

Ahmad Oryan, Aboutorab T. Naeini and Behrooz Nikahval, 2010. Effect of aqueous extract of *Aloe vera* on experimental cutaneous wound healing in rats. V*eterinarski Arhiv*. 80 (4) : 509-522.

Ailsa Sharp and Jane Clark, 2011. Diabetes and its effects on wound healing. *Nursing Standard*. 25 (45) : 41-47.

Anand K. Jain, Ashish Dixit and Swaroop C. Mehta, 2012. Wound healing activity of aqueous extract of leaves and roots of Coleus aromaticus in rats. *Acta Poloniae Pharmaceutica -Drug Research*. 69 (6) : 1119-1123.

Ashoka Babu, V.L., Goravanakolla, A., Murali, A., Madhavan, V. and Yoganarasimhan, S.N. 2012. Wound healing activity of the leaves of *Wattakaka volubilis* (L.f.) Stapf (Asclepiadaceae). *International Journal of Applied Research in Natural Products*. 5 (3) : 23-29.

Ayyanar, M. and Ignacimuthu, S. 2009. Herbal medicines for wound healing among tribal. *International Journal of Applied Research in Natural Products*. 2 (3) : 29-42.

Annie Pritima, R. and Selvaraj Pandian, R. 2008. Antimicrobial Activity of Coleus aromaticus (Benth) against Microbes. *Afr. J. Infect. Diseases*. 1 (1) : 18-24

Benjamin Wedro. 2012. *Wound signs and symptoms.* Available: http://www.emedicinehealth.com/wound_care/page4_em.htm. Last accessed 4th Nov 2012.

Bhattacharjee Prasenjit, Hullah, K.K. and Vijay Kumar, 2011. Anthelminthic and Antioxidant Activity of Alcoholic Extracts of Different Parts of Coleus amboinicus Lour. *International Journal of Research in Ayurveda and Pharmacy*. 2 (1) : 181-185.

Chellaiah Muthu, Muniappan Ayyanar, Nagappan Raja and Savarimuthu Ignacimuthu. 2006. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. *Journal of Ethnobiology and Ethnomedicine*. 2 (43) : 1-10.

Dan L. Longo, Anthony S. Fauci, Dennis L. Kasper, Stephen L. Hauser, J. Larry Jameson and Joseph Loscalzo, 2005. *Endocrinology and Metabolism*. In: Dan L. Longo, Anthony S. Fauci, Dennis L. Kasper, Stephen L. Hauser, J. Larry Jameson, Joseph Loscalzo *Harrison's Principle of Internal Medicine.*16th ed. United States of America: McGraw Hill. 2152-2179.

Douglas MacKay and Alan Miller, L. 2003. Nutritional Support for Wound Healing. *Alternative Medicine Review*. 8 (4) : 359-377.

Esimone, C.O, Ibezim, E.C. and Chah, K.F. 2005. The wound healing effect of herbal ointments formulated with Napoleona imperalis. *Journal of Pharmaceutical and Allied Sciences*. 3 (1) : 294-299.

Finn Gotrup. 2003. Multidisciplinary wound healing concepts. *European Wound Management Association Journal*. 3 (1) : 5-11.

Flanagan, M. 2000. The physiology of wound healing. *Journal of Wound Care*. 9 (6) : 299-300.

Gemechu Feyera Tolera, Nasir Tajure Wabe, Mulugeta Tarekagn Angamo, SultanSuleman Wega, Nezif Hussein Dekama, Sadikalmahdi Hussen Abdella, Mohammed Adem Mohammed, Alima Hassen Ali 2011. Attitude of modern medical practitioners towards theintegration of modern and traditional medical practices in Ethiopia . *Spatula DD*. 1 (4) : 199-205.

Girish, K. Rasineni, Dayananda Siddavattam and Attipalli, R. Reddy, 2008. Free radical quenching activity and polyphenols in three species of Coleus. *Journal of Medicinal Plants Research*. 2 (10) : 285-291.

Guo, S. and Di Pietro, L.A. 2010. Factors Affecting Wound

Healing. *J Dent Res*. 89 (3) : 219-229.

Harold Brem, Marjana Tomic-Canic, 2007. Cellular and molecular basis of wound healing in diabetes. *The Journal of Clinical Investigation*. 117 (5) : 1219-1222.

Himesh Soni, Govind Nayak, Sita Sharan Patel, Mishra, K. and Singhai, A.K. 2011. Synergistic effect of polyherbal suspension of Punica granatum and Coleus aromaticus in evaluation of wound healing activity. *Journal of Herbal Medicine and Toxicology*. 5 (1) : 111-115.

Jackson, C.J. and Xue, M. 2006. Activated protein C (APC) as a novel agent to promote wound healing. *Primary Intention*. 14 (1) : 31-34.

Janet, C., Garber, R., Wayne, Joseph and Bielitzki, T. 2011. *Guide for the Care and Use of Laboratory Animals*. 8th ed. United States of America: National Academy if Sciences. 1-207

Joan, L., Monaco, W. and Thomas Lawrence, 2003. Acute wound healing An overview. *Clinics in Plastic Surgery*. 30 (1) : 1-12

Jon, C. Tilburt A., Ted, J. and Kaptchuk, J 2008. Herbal medicine research and global health: an ethical analysis. *Bulletin of the World Health Organization*. 86 (8) : 577-656.

Joshi, R.K, Badakar, V. and Kholkute, S.D. 2011. Carvacrol rich essential oils of Coleus aromaticus (Benth) from Western Ghats. *Advances in Environmental Biology*. 5 (6) : 1307-1310.

Kalaiarasan, A. and Ahmed John, S. 2011. *In vivo* studies on the stem cuttings of coleus aromaticus. Benth, following growth hormones. *Journal of Pharmacy Research*. 4 (4) : 970-972.

Karodi, R., Jadhav, M., Rub, R. and Bafna, A. 2009. Evaluation of the wound healing activity of a crude extract of *Rubia Cordifolia* L in mice. *International Journal of Applied Research in Natural Products*. 2 (2) : 12-18.

Kathryn, A. Vickers, Kate B., Jolly and Sheila, M. Greenfield, 2006. Herbal medicine: women's views, knowledge and interaction with doctors: a qualitative study. *BMC Complementary and Alternative Medicine*. 6 (40) : 170-178.

Keith, F. Cutting, 2003. Wound healing, bacteria and topical therapies. *European Wound Management Association Journal*. 3 (1) : 17-19.

Kerstin Cunningham, 2011. *What are the causes of wounds that slow to heal?*. Available: http://www.livestrong.com/article/185601-what-are-the-causes-of-wounds-that-are-slow-to-heal/. Last accessed 15th Sept 2012.

Kotade Kiran, Mohammad Asad. (2008). Wound healing activity of *Sesamum indicum* L seed and oil in rats. *Indian Journal of Experimental Biology*. 46 (1), 777-782.

Kuldip Singh, Arvindpreet Kaur and Ahluwalia P. (2011). Effect of Monosodium Glutamate on lipid peroxidation and various lipid fractions in plasma of hypercholestremic adult male mice. *Indian Journal of Fundamental and Applied Life Sciences*. 1(4): 316-323

Kumar, A., Elango, K., Markanday, S., Undhad, C.V., Kotadiya, A.V., Savaliya, B.M., Vyas, D.N. and Datta, D. 2007. Mast cell stabilization property of *Coleus aromaticus* leaf extract in rat peritoneal mast cells. *Indian Journal of Pharmacology*. 39 (2) : 119-120.

Khan, M., Patil, P.A. and Shobha, J.C. 2004. Influence of *Bryophyllum pinnatum* (Lam.) leafextract on wound healing in albino rats. *Journal of Natural Remedies*. 4 (1), 41-46.

Lars Tue Sorenson, 2003. Smoking and wound healing. *European Wound Management Association Journal*. 3 (1): 13-15.

Lorimer,M., Kanku, J.P., Signorini, M.A. and Pardini, A. 2009. *Plectranthus amboinicus*: an ornamental plant of medical and food interest. *Science Association of Tropical Agriculture Journal*. 1 (1) : 1-2.

Manjunatha, B.K., Vidya, S.M., Rashmi, K.V., Mankani, K.L., Shilpa, H.J. and Jagadeesh Singh, S.D. 2005. Evaulation of wound-healing potency of *Vernonia arborea* Hk. *Indian J. Pharmacol*. 37 (4) : 223-226.

Mark Collier, 2003. *Understanding wound inflammation.* Available: http://www. nursingtimes.net/home/clinical-zones/wound-care/understanding-wound-inflammation/ 205361.article. Last accessed 30th June 2013.

Monica Cheesbrough, 2006. *District Laboratory Practice in Tropical Countries*. 2*nd* ed. India: Cambridge University Press. 1-407.

Mosby's Medical Dictionary (8th ed.). (2008). St. Louis, MO: Mosby.

Mousley. Maria , 2003. Diabetes and its effect on wound healing and patient care. *NursingTimes.net*, [Online]. 99, 70. Available at: http://www.nursingtimes.net/diabetes-and-its-effect-on-wound-healing-and-patient-care/205050.article [Accessed 30 June 2013].

Malathi, R., Cholarajan, A., Karpagam, K., Jaya, K.R. and Muthukumaran, P. 2011. Antimicrobial studies on selected medicinal plants (*Coleus amboinicus*). *Asian J. Pharm. Tech*. 1 (2) : 53-55.

Nirmala Devi Kaliappan, Periyanayagam Kasi Wiswanathan, 2008. Pharmacognostical studies on the leaves of *Plectranthus amboinicus* (Lour) Spreng. *International Journal of Green Pharmacy*. 2 (3) : 182-184.

Nirmala Devi, K. and Periyanayagam, K. 2010. *In vitro* anti-inflammatory activity of Coleus aromaticus. *International Journal of Pharmaceutical Studies and Research*. 1 (1) : 26-29.

Om Prakash Rout, Rabinarayan Acharya and Sagar Kumar Mishra. 2011. *In vitro* antioxidant potentials in leaves of Coleus. *Journal of Applied Pharmaceutical Science*. 1 (8) : 194-198.

Om Prakash Rout, Kedar Kumar Rout, Rabinarayan Acharya and Sagar Kumar Mishra, 2010. Preliminary pharmacognostical and phytochemical evaluation of Coleus aromaticus leaf. *International Journal of Pharma World Research*. 1 (4) : 1-19

Onyema Oscar Okwudiri, Alisi Chinwe Sylvanus and Ihetuge Adaeze Peace, 2012. Monosodium Glutamate Induces OxidativeStress and Affects Glucose Metabolism in the Kidney of Rats. *International*

Journal of Biochemistry Research & Review. 2 (1) : 1-11.

Parveen Kumar and Michael Clark, 2012. Diabetes mellitus and other disorders of metabolism. In: Parveen Kumar, Michael Clark *Clinical Medicine*. 8th ed. United Kingdom: Elsevier Science Limited. 1069-1100.

Perry, E.K., Pickering, A.T., Wang, W.W., Houghton, P.J. and Perru, N.S. 1999. Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. *Journal of Pharmacy and Pharmacology.* 51 : 527–534.

Prameela, T.S. and Oommen, P. Saj 2011. Phytochemical screening, antimicrobial and antihelminthic studies on Coleus aromaticus Benth. *International Journal of Pharmaceutical Research and Development*. 3 (4): 93-103

Preeja, G., Pillai, P. Suresh, Gayatri Aggarwal, Gaurav Doshi and Vidhi Bhatia, 2011. Pharmacognostical standardization and toxicity profile of the methanolic leaf extract of *Plectranthus amboinicus (Lour) Spreng*. *Journal of Applied Pharmaceutical Science*. 1 (2) : 75-81.

Pritima, R.A. and Pandian, R.S. 2008. Antimicrobial Activity Of Coleus aromaticus (Benth) Against Microbes Of Reproductive Tract Infections Among Women. *African Journal of Infectious Diseases.* 1 (1) : 18-24.

Damanik, R., Damanik, N., Daulay, Z. Saragih, S., Premier, R., Wattanapenpoiboon, N. and Wahlqvist, M.L. 2001. Consumption of bangun-bangun leaves (*Coleus amboinicus Lour*) to increase breast milk production among Bataknese women in North Sumatera Island, Indonesia. *Asia Pacific Journal of Clinical Nutrition*. 10 (4) : 1-2.

Revathi, A., Thangabalan, B., Vengal Rao, P. and Vadivel, K. 2011. Microbiological activity of essential oil extracted from *Coleus aromaticus* Linn. leaves. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2 (1) : 12-14.

Rajinder Raina, Shahid Prawez, P. K. Verma and Pankaj, N. K. 2008. Medicinal Plants and their Role in Wound Healing. *VetScan*. 3 (1) : 1-24

Rashmi Sahay Khare, Shanta Banerjee and Kanika Kundu, 2011. *Coleus aromaticus*: A nutritive medicinal plant of potential therapeutic value. *International Journal of Pharma and Bio Sciences*. 2 (3) : 488-500.

Robert Warriner and Robert Burrell, 2005. Infection and the chronic wound: A focus on Silver. *Advances in Skin and Wound Care*. 18 (1) : 2-12.

Roshan Patel, 2011. Hepatoprotective effects of *Plectranthus amboinicus (Lour) Spreng* against carbon tetrachloride-induced hepatotoxicity. *Journal of Natural Pharmaceuticals*. 2 (1) : 28-35

Roshan, P., Naveen, M., Manjul, P.S., Gulzar, A., Anita, S. and Sudarshan, S. 2010. *Plectranthus amboinicus (Lour) Spreng*: An Overview. *The Pharma Research, A Journal.* 4 (1) : 1-15.

Ruth Edwards and Keith G. Harding, 2004. Bacteria and wound healing. *Current Opinion in Infectious Disease*. 1 (17) : 91-96.

Srinivasa Rao, D., Prasada Rao, V. and Sambisiva Rao, K.R.S. 2010. Pharmacological effects on forskolin isolated from *Coleus aromaticus* on the lung damage rats. *An International Journal of Advances In Pharmaceutical Sciences*. 1 (1) : 17-21.

Shiney Ramya, B., Ganesh, P. and Suresh Kumar, R. 2012. Phytochemical Screening of Coleus aromaticus and Leucas aspera and their Antibacterial Activity against Enteric Pathogens. *International Journal of Pharmaceutical & Biological Archives*. 3 (1) : 162-166.

Shivananda Nayak, B., Lexley Pereira and Dale Maharaj, 2007. Wound healing activity of *Carica papaya* L. in experimentally induced diabetic rats. *Indian Journal of Experimental Biology*. 45 (8) : 739-743.

Sunitha, K.S. , Haniffa, M.A., James Milton, M. and Arthi Manju, 2010. *Coleus aromaticus* Benth act as an immunostimulant in *Channa marulius*. *International Journal of Biological Technology*. 1 (2) : 55-59.

Sasa Vukelic, Olivera Stojadinovic, Irena Pastar, Constantinos Vouthounis, Agata Krzyzanowska, Sharmistha Das, Herbert H. Samuels and Marjana Tomic-Canic. 2010. Farnesyl Pyrophosphate Inhibits Epithelialization and Wound Healing through the Glucocorticoid Receptor. *Journal of Biological Chemistry*. 285 (3) : 1980-1988.

Sharma, 2009. Wound healing activity of the Ether-Chloroform Benzene-95% extract of Momordica charantia fruits in albino rats. *Journal of Optoelectronics and Biomedical Materials*. 1 (4) : 389-392.

Shivananda Nayak , Poorna Nalabothu , Steve Sandiford, Vidyasagar Bhogadi and Andrew Adogwa, 2006. Evaluation of wound healing activity of Allamanda cathartica. L. and Laurus nobilis. L. extracts on rats. *BMC Complementary and Alternative Medicine*. 6(12) : 1-6.

Sharp, A. and Clark, J. 2011. Diabetes and its effects on wound healing. *Nursing Standard*. 25 (45) : 41-47

Shittu, A.O., Kolawole, D.O. and Oyedepo, E.A.R. 2002. A study of wound infections in two health institutions in Ile-Ife, Nigeria. *African Journal of Biomedical Research*. 5 (1) : 97-102.

Thamizhiniyan, P., Panneerselvam, M. and Lenin, M. 2009. Studies on the growth and biochemical activity of *Coleus aromaticus* Benth as influenced by AM Fungi and Azospirilum. *Recent Research in Science and Technology*. 1 (6) : 259–263.

Thenmozhi, S., Bhuvana, M. and Ahmed John, S. 2011. Screening of antimicrobial and phytochemical investigation of *Coleus aromaticus* leaf against five respiratory pathogens. *Journal of Pharmacy Research*. 4 (7) : 2261-2262.

Subhas Chandrappa M, Harsha R, Dinesha R. and Thammana Gowda, S.S. 2010. Antibacterial activity of *Coleus aromaticus*. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2 (3) : 63-66.

Venkatesh, G., Baburao, K., Rajesh Babu, M., Dhanalakshmi, S. and Indira Priya Darshini, G. 2010. Anti Urolithiatic and anti hyperlipidemic activity of Coleus aromaticus : An explanation of the underlying mechanisms. *International Journal of Phytomedicine*. 2 (1) : 284-291.