World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

Centella Asiatica L enhance wound healing potential in dead space wound in diabetic rats

Kalpana Krishnaraju[1], Saravanan VS[1], Krishnaraju Venkatesan[2*], Manimekalai Pichaivel[3], Premalatha Paulsamy[4], Divya Kuppan[5]

[1]Department of Pharmacy, Erode College of Pharmacy, Veppampalayam, Erode, India
[*2]Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, KSA
[3]Professor & Head Department of Pharmacology, SVCP, Tiruchengode, Tamil Nadu, India
[4]King Khalid University, Khamis Mushayit, Asir Province, Saudi Arabia
[5]JKK Nataraja College of arts and science, Kumarapalayam, Namakkal District, India

Received: 06-06-2021 / Revised Accepted: 24-07-2021 / Published: 27-07-2021

ABSTRACT

Centella asiatica (*C.asiatica)* is a well-known medicinal herb used in Indian medicine to treat a variety of skin problems. The purpose of the research reported in this article was to assess the wound-healing capacity of the plant's ethanolic extract in diabetic rats. Wistar albino rats were used in the investigation, and dead space wounds were used as a model. When compared to controls, the extract of *C.asiatica* considerably enhanced wound breaking strength in wound models. In a dead space wound model, wet and dry granulation tissue weights, granulation tissue breaking strength, and hydroxyproline, hexuronic acid, tissue protein, and lysyl oxidase content all increased statistically significantly. In a rat model, the results showed that the leaf extract accelerates wound healing substantially.

Key words: *Centella asiatica*, Wound healing; Diabetic; Dead space wound; Granulation tissue; Streptozotocin.

INTRODUCTION

Wounds are inherent and unavoidable in our lives. Various sorts of wounds may necessitate different treatments. Thankfully, the human body has a sophisticated self-healing system. Nonetheless, the care of wounds becomes difficult and often costly when diseases such as microbial infection, diabetes, and poor blood circulation exist (Thakur et al., 2011).[1] To address these issues, a variety of treatments have been introduced to the market that promise to cure wounds in the quickest period feasible while also reducing pain, discomfort, and scarring.

The goal of this study is to offer *Centella asiatica (C.asiatica)* extract as a traditional herb with wound healing characteristics that can help the body recover itself. *C.asiatica (L)* Urban is also known as Asiatic pennywort and pegaga in its native region. This plant has been used as medicine from ancient times, particularly in India's

Address for Correspondence: Dr. Krishnaraju Venkatesan, Department of Pharmacology, College of Pharmacy, King Khalid University, Abha, KSA; Email: kvenkatesan@kku.edu.sa

How to Cite this Article: Kalpana Krishnaraju, Saravanan VS, Krishnaraju Venkatesan, Manimekalai Pichaivel, Premalatha Paulsamy, Divya Kuppan. *Centella Asiatica L* enhance wound healing potential in dead space wound in diabetic rats. World J Pharm Sci 2021; 9(8): 125-128.

Ayurvedic system and in Chinese and Madagascar's traditional medicine. Although traditional healers in Malaysia employ *C. asiatica* in herbal treatments, its appeal is limited to that of a vegetable rather than a medicinal plant.

C. asiatica is one of the most essential medicinal plants to be protected and grown, according to the World Health Organization (WHO) (Jabatan Perhutanan Semenanjung Malaysia, n.d.).[2] Previous research on *C.asiatica* extracts has shown that it can serve as an antioxidant, antibacterial, collagen synthesis agent, and even a wound healer (Taemchuay et al.2009, Hashim et al., 2011, Idrus et al., 2012).[3,4] According to most research, asiaticoside is the active ingredient responsible for the action. Asiaticoside is a triterpene chemical that forms a saponin glycoside when sugar molecules are attached to a triterpene unit. Glucose–glucose–rhamnose are the sugar molecules. The wound healing ability of *C. asiatica* extract was investigated in diabetic rats utilising a dead space wound model.

Wound healing is frequently hindered in people with diabetes mellitus (DM), resulting in non-healing, delayed healing, or persistent skin ulcers.[5] In diabetes, delayed wound healing can be caused by an imbalance in the inflammatory response, changed cytokine production, altered collagen synthesis, insufficient angiogenesis, extracellular matrix differentiation, lower tensile strength, or diminished growth factors.[6,7] Incision and excision wound models were used to investigate the effects of *C. asiatica* on wound healing activities. The tannin impact on a diabetic wound model, on the other hand, is unknown. As a result, the goal of this study is to see how *C.asiatica* affects diabetic wounds.

MATERIALS AND METHODS

Preparation of Ethanol Extract: *C.asiatica* leaves were pulverised after being dried in the shade. The powder (75 g) was extracted in a soxhlet device at 60°C to 75°C with 700 mL of 95 percent ethanol and concentrated. The yield ranged between 10% and 15%. The extract was kept cool in the fridge.

Animals: Healthy Wistar rats of either sex (150–200 g) were utilised in this study with no prior pharmacological treatment. The animals were fed a commercial pellet diet and given unlimited access to water. The animals were acclimatised to laboratory hygienic conditions for 10 days prior to the start of the trial. The therapy was carried out with the approval of the animal ethics committee of King Khalid University, as well as the National Institute of Health's standards for the care and use of laboratory animals in the United States (NIH Publication No. 85-23, revised 1996). For the dead space wound model, animals of either sex were divided into three groups, each with six animals: Group I- Normal control; group II-diabetic control; and group III received *C.asiatica* (1000 mg/kg/day). The extracts were administered orally to the individual animal groups once a day.

Wound healing activity:

Dead Space wound model: Rathi et al. described a technique for creating dead space wounds.[8] Eighteen rats were divided into three groups of six individuals each. Subcutaneous dead space wounds were produced in the area of the axilla by creating a pouch through a tiny nip in the skin under general anaesthesia (achieved with 10 mg/kg body weight of xylazine hydrochloride and 50 mg/kg ketamine hydrochloride). The development of granulomas was induced by implanting sterile cotton pellets (30 mg) in each axilla. Sutures were used to close the wounds, which were then cleaned with an alcoholic swab. After being grouped together, the animals were placed individually in a metal cage to prevent them from biting each other's wounds.

For 8 days, the treatment groups were given extract or normal saline (1 ml/kg). Rats were euthanized on day 10 and the cotton pellets and granuloma tissues were carefully removed, dried in a 60°C oven, weighed, and compared to the control. The hydroxyproline, hexosamine concentration, and hexuronic acid were determined using the neutralised acid hydrolyzate of the dry tissue. For the measurement of lysyl oxidase and tissue protein, a piece of the moist granulation tissue was utilised.[9]

Induction of diabetes: The rats were given a newly produced solution of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) dissolved in citrate buffer pH 4.5 at a dosage of 65 mg/kg intraperitoneally (i.p.) 15 minutes after receiving 110 mg/kg body weight nicotinamide (HiMedia labs Pvt. Ltd.). The rats were given a 10% glucose solution after 6 hours of STZ treatment for additional 24 hours to prevent hypoglycemia owing to large pancreatic insulin secretion. Blood was collected from the tail veins of the rats 72 hours after the STZ injection, and rats with a fasting blood glucose level of more than 200 mg/dl were deemed diabetic and used in this investigation.[10]

Statistical analysis: The information is presented as a mean with a Standard Error Mean (SEM). The differences between means were investigated using one-way analysis of variance (ANOVA), with p values less than 0.05 deemed significant. The data was analysed using one-way analysis of variance (ANOVA) with a post hoc Scheffe's test in Graph Pad, and the mean and standard deviation were calculated. P values less than 0.05 were deemed statistically significant.

RESULTS

The animals treated with *C.asiatica* showed a substantial increase in wound healing activity when compared to those that received placebo control treatments. The effects of *C.asiatica* given orally for 8 days on wound healing activity in rats with a dead space wound are shown in Table 1. Tannin treatment rats had considerably higher granulation tissue breaking strength and wet and dry granulation tissue weight than diabetic and control rats (table 1).

Table1: Physical and biochemical analysis of granulation tissue in streptozotocin induced diabetic rats

Groups	Blood glucose (mg/dl)	Wet tissue weight (mg/ 100g rat)	Dry tissue weight (mg/ 100g rat)	Tissue breaking strength (g)
Wounded Control	81.4 ± 8.4	246.2 ± 12.19	30.18 ± 4.81	270.39±15.43
Diabetic Control	275.48 ± 15.8[a]	172.4 ± 11.02[a]	21.5 ± 4.40[a]	177.41±1.60a
C.asiatica	272.30 ± 15.2[a]	278.5 ± 12.19[a]	33.4 ± 4.40[a]	316.19±13.27a

Values are mean ± SD of 6 replications. P values: [a]:<0.01vs control.

In streptozotocin induced diabetic rats, the concentration of hydroxyl proline in granulation tissue was dramatically reduced. The experimental group's glycosamino glycan contents, such as hexuronic acid and hexosamine concentration, were considerably lower. When diabetic rats were compared to control rats, tissue protein content was quite low. The level of lysyl oxidase in the experimental group was considerably lower. When compared to diabetic and control rats (group II), all of the following metrics increased considerably in the *C.asiatica* treatment group (table 2).

Table 2: Biochemical analysis of granulation tissue in streptozotocin induced diabetic rats

Groups	Hydroxyproline (mg/g tissue)	Hexosamines (mg/g tissue)	Hexuronic acid (mg/g tissue)	Tissue protein (mg/g tissue)	Lysyl oxidase (SFU)
Wounded control	13.12 ± 4.12	10.39 ± 2.17	12.11 ± 3.09	40.18 ± 3.70	1707± 59
Diabetic Induced	12.38 ± 2.20[a]	8.2 ± 1.40[a]	9.4 ± 1.22[a]	24.5 ± 2.60[a]	1123 ± 57[a]
C.asiatica	14.62 ± 5.22[a]	14.47 ± 2.67[a]	12.01 ±3.19[a]	45.38 ± 3.60[a]	1910 ± 67[a]

Values are mean ± SD of 6 replications. (SFU- Spctroflourimetric units), P values: [a]:<0.01 vs control.

Discussion

Granulation, collagen maturation, and scar formation are only a few of the numerous wound healing processes that occur simultaneously yet separately. The findings show that an ethanolic extract of *Centella asiatica* improved wound healing in diabetic lesions. An increase in collagen concentration as well as fibre stability may be responsible for the observed improvements in wound breaking strength and hydroxyl proline content in treated wounds.[11] Higher protein content and collagen bundle production are generally associated with increases in wet and dry granulation tissue weight.

Fibroblasts are primarily responsible for the increased granulation mass. The scientists are aware that these characteristics were discovered in a rat model and that further research is needed before any potential advantages of the plant extract to improve diabetic wound healing in humans can be shown. Triterpenes from *Centella asiatica* promote extracellular matrix build up and glycosamino glycan production in rat experimental wounds, according to research. [12]

The increase of collagen production was attributed only to asiatic acid.[13] Impaired wound healing has been linked to oxidative stress.[14] Free radicals play a key part in the failure of ischemic wound healing, and antioxidants have been shown to help repair ischemic skin wounds.[15] Vitamin E, vitamin C, superoxide dismutase, catalase, and glutathione peroxidase have all been found to be considerably increased by the active component of *C.asiatica* in other research.[16] The antioxidant properties of *C.asiatica* leaf extract are likely to have contributed to the quicker wound healing seen in this diabetic rat wound model.

Conclusion: The findings were seen in a rat model of acute wound healing, which the authors are aware of. Before such findings can be applied to the human model, where the majority of wound healing issues are chronic, there are many stages that must be completed. However, these promising results point to the need for more research and clinical trials to determine the extract's safety and efficacy in humans.

Acknowledgments: The authors are grateful to Deanship of Scientific Research, King Khalid University for sponsoring this study through the Large Research Group Project under grant number RGP 2/186/42.

Conflicts of Interest: "The authors state that they have no competing interests. The funders had no involvement in the study's design, data collection, analysis, or interpretation, manuscript preparation, or the decision to publish the findings."

REFERENCES

1. R. Thakur, N. Jain, R. Pathak, S.S. Sandhu. Practices in wound healing studies of plants. *Evidence-Based Complementary and Alternative Medicine*. 2011:1-17,
2. Jabatan Perhutanan Semenanjung Malaysia, n.d. Pegaga [WWW Document]. URL http://www.forestry.gov.my/herba/pegaga_en.pdf
3. D. Taemchuay, T. Rukkwamsuk, T. Sakpuaram, N. Ruangwises. Antibacterial activity of crude extracts of *Centella asiatica* against *Staphylococcus aureus* in bovine mastitis. *Kasetsart Veterinarians*. 2009;19:119-128.
4. P. Hashim, H. Sidek, M.H.M. Helan, S. Aidawati, U.D. Palanisamy, M. Ilham. Triterpene composition and bioactivities of *Centella asiatica*. *Molecules*.2011;16:1310-1322,
5. Beckmann KH et al. Low level laser therapy for the treatment of diabetic foot ulcers: a critical survey. *Evid Based Complement Alternat Med*. 2014;4(1): 1–9.
6. Rosado P et al. Influence of diabetes mellitus on postoperative complications and failure in head and neck free flap reconstruction: a systematic review and meta analysis. *Head Neck*. 2014;37:615–648.
7. Bagdas D et al. In vivo systemic chlorogenic acid therapy under diabetic conditions: wound healing effects and cytotoxicity/genotoxicity profile. *Food Chem Toxicol*. 2015;81:54–61.
8. AB Gokhale, AS Damre, KR Kulkami, and MN Saraf. Preliminary evaluation of anti-inflammatory and anti-arthritic activity of S. lappa, Achyranthes speciosa and Achyranthes aspera. *Phytomedicine*.2002;9:433–437.
9. Rathi B, Patil PA, Baheti AM: Evaluation of aqueous extract and seeds of Moringa oleifera for wound healing in albino rats. *J Nat Remedies*. 2004;4(2):145–149.
10. Komal M Parmar, Priyanka R Shende, Nitin Katare, Mahaveer Dhobi, Satyendra K Prasad. Wound healing potential of *Solanum xanthocarpum* in streptozotocin-induced diabetic rats. *Journal of Pharmacy and Pharmacology*. 2018;70(10):1389–1400.
11. Udupa SL, Udupa AL, Kulkarni DR. Influence of Tridax procumbens on lysyl oxidase activity and wound healing. *Planta Med*. 1991;57(4):325-7.
12. Maquart FX, Chastang F, Simeon A, Birembaut P, Gillery P, Wegrowski Y. Triterpenes from *Centella asiatica* stimulate extracellular matrix accumulation in rat experimental wounds. *Eur J Dermatol*. 1999;9(4):289-96.
13. Maquart FX, Bellon G, Gillery P, Wegrowski Y, Borel JP. Stimulation of collagen synthesis in fibroblast cultures by triterpene extracted from *Centella asiatica*. *Connect Tissue Res*.1990;24(2):107-20.
14. Maiere CM, Chan PH. Role of superoxide dismutase in oxidative damage and neurodegenerative disorders. *Neuroscientist.* 2002; 8:323-34.
15. Senel O, Cetinkale O, Ozbay G, Ahcioglu F, Bulan R. Oxygen free radicals impair healing in ischemic rat skin. *Ann Plast Surg*. 1997; 39:516-23.
16. Shukla A, Rasik AM, Dhawan BN. Asiaticoside induced elevation of antioxidant levels in healing wounds. *Phytother Res*.1999;13(1):50-4