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Efficacy of Ethanolic Extracts for Two Plants on Wound Healing in Diabetic Albino Rats

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Research Article

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ABSTRACT

Background: Wounds in diabetic patients are critical to treat. The use of allopathic drugs has various side effects. New natural sources for therapies are so required.

Materials and methods: This research aim to determine the possibility for wound healing for the ethanolic extract of *Eucalyptus globulus* leaves and *Curcuma longa* rhizomes. To find out how plant extracts affected diabetic rats' wound healing, albino rats were given oral administration of various plant extracts.

Results: These plant extracts greatly accelerated the wound's ability to close in rats associated with diabetes or non-diabetes ($P<0.05$) this increases the proliferation and migration of the fibroblast result with a faster healing process. These plant extracts do not cause cytotoxicity. After treatment with extracts the proliferative assay that showed the least amount of cell death. Results found positive.

Conclusion: This research may indicate that the combination of ethanolic extracts of both plants have potential as novel therapies for diabetic patients wound healing.

Keywords: Diabetes; Healing; Extraction; Contraction; Epithelialization

INTRODUCTION

Natural ingredients have demonstrated the ability to create new leads for pharmacological, nutraceutical, and agrochemical products. The most frequent sources of biologically active molecules include plants, bacteria, fungi, and marine natural products. One of the richest plant based traditions in the world is found in India. Examining medical indications according to the compounds they contain revealed that natural products and closely similar medications are utilised to treat 87% of all classed human disorders, including their use as anticancer, anticoagulant, antiparasitic, and immunosuppressive medicines. Herbs are being used from centuries by the peoples all over the world. They may be the oldest "evidence based medicine". Herbal medicines have been recorded to cure all kinds of diseases of humans. Traditional healers have a broad knowledge on medicinal plants and its healing properties. Medicinal plant usage formed the backbone in many rural communities for treating ailments with varying severity. Most of the people use herbal preparations with modern medicines, WHO noticed that 80% of people in the world depend on Complementary and Alternative systems of Medicines (CAM); herbal medicines have major contributions for some aspect of their primary health care needs. According to WHO, twenty one thousand plant species have been identified to have therapeutic value [1].

Diabetes is a highly widespread and common disease. Globally, it is thought to impact 381 million adult populations, moreover, it is estimated that about 0.175 billion cases are still undiagnosed. The disease is becoming increasingly prevalent; for example, in the United States, 0.011 billion people were diagnosed in 2001, accounting for about 4 persons out of hundreds of the whole population, number is expected to increase to 0.0 29 billion during 2050. IDF which is well known explained as international diabetes federation estimates that these numbers will continue to grow up in the future. Type two diabetes is the most public, secretarial for 85–90% of diabetic patients, whereas type 1 diabetes accounts for about 10%–15% only [2].

Various drugs from synthetic class are available for the treatment of Diabetes Mellitus (DM). Despite this, about 80% of diabetic patients die due to vascular complication. If such herbs selected to develop Polyherbal Formulations (PHF) in palatable or oral dose forms, it will benefit to the people on multidrug therapy such as antidiabetic, antihyperlipidemic, wound healers etc [3].

The goal of the current research is to offer a research based perspective on efficiency of several herbal plants in the medication and treatment of diabetic wounds. The usage of these herbs in traditional medicine served as the main factor for their selection. Most importantly, the ancestral communities used a vast array of plants to treat wounds, cuts, and skin conditions. Two plant species were chosen as a result based on their ability to both *in vivo* and *in vitro* repair wounds. The selected plants are shown in the following Figure 1.

Figure 1. Plant investigated for this study. *Eucalyptus globulous, Curcuma longa*.

Large genus *Eucalyptus* comes under Myrtaceae family. This genus contains more than or equals to 900 different

species and subspecies. Leaves, fruits, buds, and bark of *eucalyptus* can be used for the extraction of oil and has been shown to have lethal effects on bacteria, can stop growth of bacteria, contains oxidative properties, can work on inflammation, and neoplastic activities. It is also used for the medication of upper respiratory infection condition like the common cold, influenza, and sinus congestion [4].

Curcuma longa is an herb which is available throughout the year and member of the genus *Curcuma*, and Zingiberaceae is the family. The rhizomes part of the plant has got most of its pharmacological activities and is widely used as an anti-diabetic, hypolipidemic, anti-inflammatory, anti-diarrhoea, hepatoprotective and anti-asthmatic. It is also widely used in cosmetic products. The fresh extract of turmeric is believed to have anthelmintic property [5].
This research was designed for the purpose of wound healing in diabetic albino rats. This was designed for the potential evaluation of ethanolic extracts of *eucalyptus* leaves and *curcuma longa* rhizomes in diabetic albino rats. In this study cytotoxic and lethal effects of selected plant extracts on human fibroblast cell line were also examined.

MATERIALS AND METHODS

Identification and collection of plant

In July 2021 and October 2021, leaves of *eucalyptus* globulus and rhizomes of *Curcuma longa* were collected respectively from nearby locations in Newai. Principal, school of pharmaceutical studies, faculty of health sciences of Dr. K. N. Modi university, confirmed the selected plants. Herbarium department of Rajasthan university, authenticated by receiving voucher specimens (RUBL21218 and RUBL21219).

Preparation of the ethanolic extract

Leaves of *Eucalyptus globulus* and rhizomes of *curcuma longa* before being submerged in 100% ethanol, they were cleaned, dried, and blended. For 3 days at 25°C-28°C temperature, dried leaves were submerged in ethanol (1:10 weight/volume ratio) with constant shaking. To eliminate the solid contaminants, whatman filter paper was used and it was dried with the help of rotary evaporator which was set to 50 degrees centigrade at less pressure. The unprocessed extracts were kept at -20°C in sealed containers [6].

Diabetes generation and collection of samples

Diabetes was brought on by injection which contains streptozotocin (strength 50 mg/kg according to body weight). It was given by a single intraperitoneal route. As it takes time at least 3 days. After mentioned days, diabetes was determined by using glucometer strips to measure the level of blood glucose from vein present in tail (measured by freestyle freedom lite device by Abbott pharmaceuticals). Diabetic rats were defined as animals having level of glucose in blood excessive than 200 micrograms/ml (Figure 2) [7].

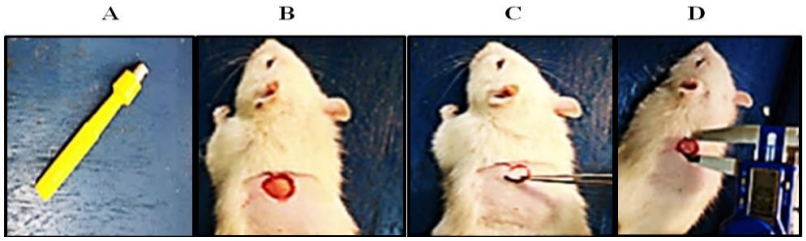
Figure 2. Places for collection of blood. A: Caudal vein; B: Femoral trigone; C: Sinus retro orbital blood collection.

Animals and dose selection for wound healing activity

The anti-inflammatory and healing effects for wound by ethanolic extracts of *curcuma longa* rhizomes and *Eucalyptus globulus* leaves were examined in rats (180-200 g). Researchers historically chose male wistar albino rats because they thought female rats responded differently to the same stimuli due to hormonal changes during the female reproductive cycle. The outcomes of the scientists several experiments with female rats varied. In the animal house at BNIPS (B.N. institute of pharmaceutical sciences) Bhupal Noble's University, Udaipur each animal was housed in a typical plastic and stainless steel cage [8].

The 24 degree centigrade temperature was set by maintaining day and night from light to dark 12 hours for each according to biological clock for the animal. Natural environment was maintained for better easy result finding. BNIPS (Bhupal Nobles institute of pharmaceutical sciences), Bhupal Noble's university, committee of animal ethics granted the study after taking the presentation and *viva*. Time requires anyone get set in new environment. Rats were given specific time to become used to new place before studies began. The rats kept apart during the studies using different cages. Blood samples were obtained using a variety of techniques (Figure 3). The serum was subjected to additional biochemical assays. The doses of plant extracts given according to the LD_{50} in rats (*eucalyptus* leaves extract can be used 5000 mg/kg body weight and *curcuma longa* rhizomes 5000 mg/kg weight [9].

Figure 3. Generation of wound. (A) 10 mm with punch; (B–C) Removal of skin; (D) Measurement of created wound.



Wound creation

Rats were sedated by inhaling diethyl ether. An electrical hair trimmer (Phillips hair trimmer) was used to shave the dorsal portion, and a 10 millimetres biopsy punch (disposable biopsy punch, BNIPS, Udaipur) was used to remove the skin. Image 2. The closer of wound was measured until the complete wound get close or epithelized. The main factor was considered that is contraction. The contraction % was calculated using following formula:

Contraction%=(Initial wound diameter day by day wound diameter)/(Initial wound diameter) X 100

Initial wound diameter is the diameter of wound which was initiated for the study at day 1st.

The duration of complete contraction was determined by counting days until the scab disappeared, there would not be

any presence of open naked wound. Sampling of blood done as per depicted in Figure 3.

Experiment design

Rats divided into two primary categories: diabetic (group 1-6) and non-diabetic (group 1-3) (Table 1). Three subgroups of five animals each make up each main group (n=5) as follows:

Vehicle (suspension of CMC in concentration of 0.05% in normal saline) was given to Group 1 which was known as control group) [10].

Vitamin E (100 mg/kg body weight) was given to group 2 (the positive control).

Group 3 received a vehicle borne dose of extract of plant (200 mg/kg body weight).

For continuous 15 days, equal doses of both plant extracts (5 ml/kg body weight) were given. The wounds were made in the manner that Mieczkowski, et al., described. On days 0 through 15, wound areas were gauged with a calliper. Digital photography was used to capture the images of the injuries. The variation in closing of the wounds was observed form day 0 to day 15th (Table 1).

Table 1. Groups given oral extract for treatment for all 1-6 categories.

Group No	Category	Treatment given
1	Non-diabetic rats	Normal saline
2	Non-diabetic rats	Vitamin E (100 mg/kg)
3	Non-diabetic rats	200 mg/kg plant extract
4	Diabetic rats	Normal saline
5	Diabetic rats	Tocopherol (100 mg/kg)
6	Diabetic rats	Plant extract (200 mg/kg)

Fibroblast migration and proliferation in an *in vitro* study of the effects of ethanolic plant extract on wound healing

- ***In vitro* toxicity test:** Various extracts obtained from plants were tested in a biochemistry lab for toxicity against a fibroblast cell line at Dr. K.N. Modi university. The cells were grown in a medium known as Dulbecco's MEM. It grown at 37 degrees Celsius in 5% CO_2. Cells were fractionated after three to four days by withdrawing the liquid culture, separating the cells with 2 millilitres of trypsin, and then addition of fresh media. Different quantities of the plant extract (10-100 g/mL) were examined. Triplicates of each experiment were run. The IC_{50} was determined by using the dose response curve. A 24 well plate was used to test the cytotoxicity of various plant extract concentrations (10, 25, 50, and 100 g/mL). Transform in cell morphology and shape were considered as toxic signals. Cell lines lacking extract were utilised as a negative control.
- **Dulbecco's modified eagle medium:** It is also known as DMEM. It contains high glucose without L-GLT with HEPES. Also contains sodium bicarbonate, sodium pyruvate. Which is sterilized and filtered with 0.2 micron. It used to store at 2-8 degree centigrade [11].
- **Antiproliferative assay:** After the investigated substances were given to cell lines, the hindrance of cell replication was measured using the G-banding stain method. The wounds were cleaned with 0.5 mL SPB (Saline Phosphate Buffer) after the medium was evacuated from them and fixed by ethanol at 36 degree

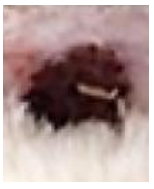
centigrade for nine minutes. Before washing and fixing plates were dried for 2 minutes. Giemsa stain (1:10 in SPB) was used to stain each well for 9 minutes. Aspirated the dye and followed it with a deionized water rinse. The antitumor activity was assessed using an ELISA reader at 630 nm by microplate after the bound dye was removed with 0.1 N HCl (Instrument ELX 800). The proportion of live cells in the tested sample which related with control was used to represent cell mortality. The formula listed below was used to compute the fatality percentage (Tables 2-5) [12].

Mortality (%)=100-(Controlled absorbance-Treated cells absorbance)/(Controlled absorbance) X 100

Table 2. *Eucalyptus globulus* ethanolic extract effect on wound on all groups.

Eucalyptus globulus ethanolic extract effect on wound on all groups							
Day	Case	Control negative		Tocopherol (100 mg/kg)		*Eucalyptus* extract (200 mg/kg body weight)	
		Diameter of wound (mm)	Wound contraction (%)	Diameter of wound (mm)	Wound contraction (%)	Diameter of wound (mm)	Wound contraction (%)
0	Non-diabetic	10.60 ± 0.50	0	10.80 ± 0.40	0	10.70 ± 0.60	0
	Diabetic	10.60 ± 0.50	0	11.00 ± 0.40	0	11.20 ± 0.50	0
3	Non-diabetic	10.40 ± 0.50	1.8	10.20 ± 0.20	5.9	10.00 ± 0.50	6.2
	Diabetic	10.30 ± 0.40	2.6	10.40 ± 0.40	5.6	10.00 ± 0.30	10.7
6	Non-diabetic	9.60 ± 0.40	10	8.70 ± 0.50	19.3	8.70 ± 0.40	18.8
	Diabetic	9.40 ± 0.30	11.5	8.60 ± 0.40	21.8	8.20 ± 0.10	27.1
9	Non-diabetic	8.60 ± 0.60	19.5	6.30 ± 0.70	42	6.20 ± 0.20	42
	Diabetic	8.20 ± 0.10	22.7	6.30 ± 0.30	42.6	7.40 ± 0.30	33.9
12	Non-diabetic	7.50 ± 0.40	29	4.00 ± 0.80	63	5.20 ± 0.10	51.8
	Diabetic	7.30 ± 0.20	31.4	4.50 ± 0.40	59.7	5.30 ± 0.20	52.6
15	Non-diabetic	5.80 ± 0.30	45	1.50 ± 0.30*	86	2.60 ± 0.60*	75.2
	Diabetic	6.00 ± 0.60	43.7	2.10 ± 0.60*	81.1	3.20 ± 0.70*	71.6

Table 3. Wound contraction by *eucalyptus* globulus leaves ethanolic extract for both non-diabetic albino (A) Diabetic albino; (B) Rats.

Days							
Groups	Treatment	0	3	6	9	12	15
Non-Diabetic A	Normal saline						
	Vit. E						

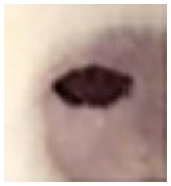
	Eucalyptus extract 200 mg/kg						
Diabetic-B	Normal saline						
	Vit. E						
	Eucalyptus extract 200 mg/kg						

Table 4. *Curcuma longa* ethanolic extracts extract effect on wound on all groups.

***Curcuma longa* ethanolic extracts extract effect on wound on all groups**							
Day	**Case**	**Control negative**		**Tocopherol (100 mg/kg)**		***Eucalyptus* extract (200 mg/kg body weight)**	
		Diameter of wound (mm)	**Wound contraction (%)**	**Diameter of wound (mm)**	**Wound contraction (%)**	**Diameter of wound (mm)**	**Wound contraction (%)**
0	Non-diabetic	10.60 ± 0.30	0	10.40 ± 0.20	0	10.50 ± 0.30	0
	Diabetic	10.30 ± 0.20	0	10.30 ± 0.10	0	10.40 ± 0.10	0
3	Non-diabetic	10.30 ± 0.30	2.4	10.00 ± 0.30	8	9.50 ± 0.30	9.3
	Diabetic	10.20 ± 0.20	2	9.70 ± 0.20	6.5	9.60 ± 0.20	7.6
6	Non-diabetic	9.70 ± 0.40	8.18	8.80 ± 0.33	15.4	8.20 ± 0.30	21.9
	Diabetic	9.30 ± 0.20	107	8.80 ± 0.32	14.2	8.60 ± 0.30	16.7
9	Non-diabetic	9.10 ± 0.70	14.4	7.00 ± 0.60	33.3	6.80 ± 0.50	35
	Diabetic	8.30 ± 0.20	20.5	7.00 ± 0.40	32.2	6.70 ± 0.60	35.1
12	Non-diabetic	7.50 ± .0.70	29.8	5.10 ± 0.70	50.5	4.80 ± 0.60	53.7

	Diabetic	7.40 ± 0.20	29.3	5.20 ± 0.70	48.9	4.90 ± 0.60	52.4
15	Non-diabetic	6.00 ± 0.80	43.9	1.80 ± 0.20*	83	1.90 ± 0.30*	81.9
	Diabetic	6.00 ± 0.40	42.5	2.10 ± 0.35*	79.1	2.50 ± 0.40*	75.4

Table 5. Wound contraction by *curcuma longa* ethanolic extract for both non-diabetic albino. (A) Diabetic albino; (B) rats.

Days							
Groups	**Treatment**	**0**	**3**	**6**	**9**	**12**	**15**
Non-Diabetic A	Normal saline						
	Vit. E						
	Curcuma extract 200 mg/kg						
Diabetic-B	Normal saline						
	Vit. E						
	Curcuma extract 200 mg/kg						

- **Migration assay:** As fibroblasts were developing in wounds with the density of 35000 cells per wound incubated until it got closed. Juszczak, et al., description in a paper they referenced of each well was scratched using a 200 L pipette tip as. The media was removed, and cells were washed with SPB prior

addition of medium which was in suitable condition with various doses of every extract of plant in concentration of 10 and 20 g/ml (saline phosphate buffer). Optical microscope used to analyse various places along each well's scratches at 0, 12, 24, and 48 hours after the damage was generated. In comparison to untreated control cells. It was possible to measure and quantify the distance between the scratch's edges as a percentage of area closure [13-16].

Statistical analysis

The information was displayed as multiple results division plus SD. The statistical significance of differences between groups was calculated using graph pad prism version 7 with a significance level of P 0.05. The standard deviation abbreviation is SD.

RESULTS AND DISCUSSION

Toxicity study: Fatal concentrations of multiple extract from *Eucalyptus globulus* and *curcuma longa* was confirmed from multiple research articles and that was found to be safe till 5000 mg/kg body weight for both extracts.

Effect of orally administrated plants extracts on cutaneous wound in diabetic rats: The *Eucalyptus globulus* leaves ethanolic solution (200 mg/kg body weight) gave expressive ($P<0.05$) effects on healing of wounds. This healing was compared with the vitamin E treated group. On separate section, ethanolic solution of *curcuma longa* rhizomes (200 mg/kg body weight) showed much better activity in respect to healing as compared to *Eucalyptus globulus*. The potential of healing both extracts are expressed in different charts and healing process of wound is also elaborated in tables having figures [17].

In therapeutic treatment, wounds are typically viewed as a severe concern since they represent a clinical problem. Standard wounds heal in some days, but wounds which not closes easily are far more problematic due to societal and financial considerations. Finding novel natural products that perform well and are cheaper is crucial as a result. Diabetes can also delay recovery or raise the risk of infection in the area that was injured, necessitating a lengthy hospital stay. In comparison to the majority of past wound care medications, which are applied externally as ointments, in this study, extracts of plants were taken orally. Improvement of new therapeutic agents employing extracts from medicinal plants was inspired by the current investigation. According to the findings, the untreated group heals wounds to some extent, which may be the result of auto-immunity. Although, ethanolic extracts of plants were used on selected groups for experiment resulted good and fast healing process. In this process between the time periods of observation no object was found as any irritation, comfortless and grooving at site of wound. An activity for healing of wounds was reported by PK Sen, et al., they stated that extracts are also responsible for the contraction and collagen formation. The current findings, which showed that the plant extracts promotes a quick closing of wound, which accelerates mitigation, support this interpretation. Very good results were found from *curcuma longa* rhizomes ethanolic extract, this can be preferred as an alternative treatment for diabetic wounds. Some studies were reported as with a fruit extract with aqua for wound management. That showed the same response like an increase in concentration of hydroxyproline deposition of collagen protein, reduce collagenase-II, enzyme myeloperoxidase which results in good and smoother wound healing [18].

It was reported with leaves of G. alypum's extract with the help of mixture of ethanol and water. This extract was

found rich with flavonoids and phenyl ethanoid, so antioxidative activity was found in G. alypum's extract. These kind of antioxidative constituents helps into healing of wound. Ethanolic mixture of constituents of *eucalyptus* leaflet showed less healing comparing with *Curcuma* rhizomes.

Fibroblast proliferation in test tube and resettling in healing of wound: The table displays the findings of the IC_{50} analysis and the toxicity of extracts of plant at strength of 100 µgm/ml. *Eucalyptus* globulus had the lowest IC_{50}, while *curcuma longa* had the lowest cytotoxicity. The score method, that demonstrated the resettling of the cell line of fibroblast after the score assay method and at the time of culturing at 10 micro gram/mL and 20 micro gram/ml of extract of plants, was used to study the capacity of extract of plants to promote proliferation of fibroblast in test tube and resettling. After 48 hours of treatment, 20 micro gram/mL of *curcuma longa* extract produced the strongest stimulatory effect, followed by *Eucalyptus globulus* [19].

The growth and migration of cells are crucial to the healing process. In test tube investigations on healing of wounds, the scratch method is frequently employed. As, protein is necessary to restore the stability, power, and build up required to restore the architecture and role of injured matter, the fibroblasts are crucial for protein settling is necessary in the reformation of skin after getting injured or damaged. Cellular replication and migration are the fibroblasts' main activities in the early proliferative phase. However, the large number of fibroblasts start to make and increase the amount of collagen in the wounded tissues on the third day following the injury, which characterises the wound healing activity. The findings of this study demonstrated that the ethanolic extracts of *curcuma longa* and *eucalyptus* globulus significantly induced fibroblast migration. These findings corroborate and reinforce the current in vivo investigations, which demonstrated that ethanolic extracts of *Eucalyptus* and *Curcuma longa* strongly stimulated fibroblast migration. The medicinal effectiveness of the herbal plants for injured cells discovered by the current study [20].

CONCLUSION

Medicinal advantages of the herapy using phytoconstituents advantage of the widely distributed herbs. *Eucalyptus globulus* leaves and *curcuma longa* rhizomes were the focus of this investigation. This study is significant since it established and reported for the first time the advantages of plants for wound healing is an ideal situation for diabetes and non-diabetic objects. Ethanolic extract of *Curcuma* and *Eucalyptus* both displayed strong wound healing abilities. Furthermore, the extracts were secure, and the rats showed no signs of systemic toxicity.

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