WOUND HEALING AND ANTI-BACTERIAL EFFECTS OF *CENTELLA ASIATICA* EXTRACT

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ABSTRACT

Objective of the study was to find out the wound healing and antibacterial effects of *Centella asiatica* extract (CA Et) in rats. For wound healing studies Hot water burn wounds and Wax burn wound methods used. For anti-bacterial studies cup-plate method used.Indicated that the extracts of *Centella asiatica* possesed wound healing and antibacterial activities.

Key words: Wound healing, Anti-bacterial activity, Centella asiatica extract ,burn,Cup-plate.

1. INTRODUCTION:

Renewed interest on biological activities of medicinal plants emerged in early 1980's as the Council of Scientific and Industrial research have published the information on the screening of biological activities of many medicinal plants using experimental models (Somchic MR, 2004). Medicinal plants are an important therapeutic aid for various ailments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th Century (Jones FA, 1996). Recently the use of herbal preparations in remedies for various medical conditions have been rapidly increasing especially in India. In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects.

A pathogen that can cause life – threatening infections in patients with burns and wounds. The extracts obtained from plants are usually made in to different formulation, ether as ointment or as lotion applied to the skin for wound (Milhau G, 1997). During the past decade anaerobic bacteria especially non – poring ones, have found as important causative organisms of wound infection. A chemotherapeutic agent may act by destroying the organism (bactericidal) or by inhibiting its growth (bacteriostatic).

Centella asiatica (family- Umbelliferae.) is used as anti-oxidant and analgesic drug . Natural antimicrobials can be derived from plants, animal tissues, or microorganisms. In this present study, *Centella asiatica* extract were screened for wound healing and antibacterial activity in rats and micro organism respectively.

Even though earlier studies showed that alcoholic extract of centella asiatica possessed wound healing effect, in our present study we have focused only on aqueous extract of *Centella asiatica* by administering orally. But invitro study of anti bacterial effect by using both aqueous & alcoholic extracts of *Centella asiatica*. Thus, water soluble component of centella asiatica extract may be responsible for wound healing effect. Now day's attempts were made to capture biological activities by using the preparation sighted in the folkloric claim instead of chemically processed material after capturing the activities attempts can be made to isolate active principles.

2. MATERIALS & METHODS

Animals (For wound healing activity)

Healthy male wistar rats weighing between 150-200 g were used in the studies. They were individually housed and maintained on normal diet and water *ad libitum*. Animals were randomly distributed into various groups each containing around 10 animals.Burn wounds were inflicted on overnight starved animals under pentobarbitone (25 mg/kg) anaesthesia. Test extract given orally at a

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dose of 300 mg /kg .Apart from the drugs under investigation, no local/systemic chemotherapeutic covers were provided to animals. Animal showing signs of infection was excluded from the study and replaced with a fresh animal (Malik Rao and Mathew G, 2000).

Organisms (For anti-bacterial activity)

The antibaitecial activity of centella asiatica extract were studied by cup-plate method with ciprofloxacin as reference standard using two gram +ve organisms- *Staphylococcus aureus* and *Bacillus subtilis* and two gram –ve organisms namely *Escherichia coli* and *Pseudomonas auruginosa* (Ghosh MN, 1984).

Plant material

Centella asiatica plant was collected freshly from in and around Erode district of Tamilnadu, India. Plant dried under shade, made into coarse powder by grinding. Plant was identified and authenticated at the herbarium Tamilnadu Agricultural University, Coimbatore. Ref: ID 08/NCP/ 2006.

Preparation of plant extract

Aqueous Extract

To 20 g of each dried plant powder form , 500 ml water were added and contents of flask were mixed thoroughly by gentle shaking . Flasks were kept for four days with frequent shaking. After the completion of maceration process the filtrates were obtained and water evaporated to get the dried extract.(evaporation by keeping flasks in electric mantle at 80^0C).The residual extract was dissolved in water and used in the studies.

Alcoholic (ethanol) extract

To 20 g of each dried plant powder form , 500 ml ethanol were added and contents of flask were mixed thoroughly by gentle shaking . Flasks were kept for four days with frequent shaking. After the completion of maceration process the filtrates were obtained and solvent evaporated to get the dried extract.(evaporation by keeping flasks in electric mantle at 80 ^{0} C.

Group and Treatment

For Wound healing

Burn wound models: Method of infliction of Burn

a) Hot water burn wounds

The methods of Farrial et al (1994) was modified to produce the hot water burn wound. On day '0' the animals were anaesthetized and given a fur clipping on dorsal side. A 2 X 3 c.m glass cylinder was placed on the shaven back of a rat. Hot water (2 ml) at 98 0 C was placed in the glass cylinder. Thirty seconds later the water was quickly drained off and the exposed area was wiped off the water to see the whitish marked area (Farrial M, 1994).

b) Wax burn wound

On zero day, under anaesthesia, the dorsum of each rat was shaved . A 2 X 2cm metal cylinder was placed on the shaven back of the animals. To this was poured melted wax at 80 ^{0} C and the wax was allowed to solidify into the metal cylinder. Eight minutes after this (during this time wax solidified completely), the metal cylinder containing solidified wax adhering to the layers of skin was gently removed to inflict a distinctly demarked burn wound (Malik Rao and Mathew G, 2000).

Assesment of burn wound healing:

The animals were inspected daily and the healing was assessed based on physical parameters, namely, wound contraction and epithelization, as well as histologically.

a. Wound contraction: was studied by tracing the raw wound area on a transparent polythene paper on every alternate day upto 14 th post wounding day. These wound tracing were retraced on a graph paper to assess the area. The wound concentration was calculated as percentage of original wound size(300 mm2) for each animal of a group. From this group mean on predetermined days, viz., 2^{nd}, 6^{th},10^{th} and 14^{th} day was calculated for final analysis of the results.

b. Epethelization: Falling of eschar leaving no raw area was considered as end point of complete reepithelization and the days required for this was taken as a period of epithelization.

c. Histopathology: on day 0,2 and 10 some of the animals under each model were sacrificed and the wounds excised together with the surrounding skin. They were fixed in formalin and embedded in

paraffin. Histological evaluation was performed on the haematoxylin and eosin(HE) stained paraffin section (Inoue T, 2002).

For Antibacterial activity:

The antibacterial activity of extract was studied by cup – plate method (Jansen AM, 1987).

1. Medium have been prepared as described in Indian pharmacopoeia.
2. Sterilized Petri dishes, pipettes, boiling tubes and beakers.
3. 8 to 24hrs. old growth cultures in nutrient broth
4. Sterilized test tubes
5. Sterile 6mm cork borer.
6. Sterile inoculation loops
7. Sterilized fine pointed forceps
8. Nutrient agar
9. Tuberculin syringes.

Preparation of media

Media mentioned in Indian pharmacopoeia was prepared by dissolving bacteriological peptone (6g), pancreatic digest of casein (4g), yeast extract (3g), beef extract (1.5g), dextrose (1.0g) and agar (15.0g) in distilled water to produce one liter of medium. The pH of the solution was adjusted to 6.5-6.6 by using 1M sodium hydroxide and 1m hydrochloric acid. Then it was sterilized for 30 minutes at 15lbs pressure.

The organisms used in the present study for evaluating antibacterial activity of test compounds were obtained from laboratory stock. On the day of testing, the organisms were sub-cultured into sterile nutrient broth. After incubating the same for three hours, the growth thus obtained was used as inoculums for the test.

Sterilization of media & Glass wares

The media used in present study, nutrient agar and nutrient broth, were sterilized in conical flasks of suitable capacity by autoclaving at 15 lbs pressure for about 20 minutes. The cork borer, Petri dishes, test tubes and pipettes were sterilized in hot air oven at 160°c for an hour.

Preparation of solutions of test compounds

10mg of each test compound was dissolved in 10ml of DMF (dimethyl formamide) in serially and suitably labeled sterile test tubes, thus giving a final concentration of 100μg/0.1ml

Method of testing

Cup-plate method

This method depends on the diffusion of an antibiotic from a cavity through the solidified agar layer in a Petri dish to an extent such that growth of the added micro-organism is prevented entirely in a circular area or zone around the cavity containing a solution of antibiotic.

A previously liquefied medium was inoculated appropriate to the assay with the requisite quantity of the suspension of micro-organisms between 40-50°c and the inoculated medium was poured in to Petri dishes to give a depth of 3 to 4m.m .Care had been taken to see that the layers of the medium were uniform in thickness by placing the Petri dishes on a leveled surface.

The dishes thus prepared were stored in a manner so as to ensure that no significant growth or death of the test organism occurs before the dishes were used and the surface of the agar layer was dry at the time of use. With the help of sterile cork borer, three cups of diameter, each 6m.m were punched and the set agar in each Petri dish was scooped out. Using sterile pipettes the standard and the sample solutions (0.1ml) of known concentrations were fed into the bored cups. The order of the solutions was as follows;

Cup-1: Standard (ciprofloxacin)

Cup-2: solvent control (DMF)

Cup3-: Test compound

The Petri dishes were left standing for one to four hours at room temperature as a period of pre-incubation diffusion to minimize the effects of variation in time among the applications of different solutions. These were then incubated for 24 hrs at 37°C.

The zone of inhibition developed, if any was then accurately measured and recorded. Each zone of inhibition recorded were average of six measurements. Solvent control (DMF) was also tested for zone of inhibition.

Index

Concentration of ciprofloxacin -10μg/0.1ml in DMF.

Concentration of test compound-100μg/0.1ml in DMF.

Diameter of cup-6m.m

Quantity in each cup-0.1ml

Table 1: Epithelization period in partial thickness wounds

Groupno:	Wound	No.of animals	Period of epithelization: mean ±S.E
1	Hot water	9	16.48±0.8 (aqueous extract)
2	Melted wax	10	17.49±0.58 (aqueous extract)

Table 2: Wound contraction chronology in partial thickness wounds

Group no	Wound	No.of animal	Wound contraction:% of original wound size (300 mm2)mean ±S.E			
			On day 2	On day 6	On day 10	On day 14
1	Hot water	9	36±1.5	55±2.1	74±2.4	92±1.4
2	Melted wax	10	34±2.6	58±3.4	68±3.6	89±1.8

Table 3: Effect of extracts on epithelization of partial thickness wounds

Group no.	Drugs(n)	Dose (mg/kg)	Period of epithelization mean ±S.E (days)
1	Control 10 (0.05% cmc)	2.5 ml	14.25 ± 0.45
2	Aqueous extract(10)	100	11.32±0.55*

*= P<0.01 significantly lesser than control
n= number of animals

Table 4: Anti- bacterial activity of *C.asiatica* extracts

Treatment	**Zone of inhibition in m.m**			
	E. coli	**Pseudomonas.a.**	**S. aureus**	**B-subtilis**
Standard	26	24	26	32
Control dimethyl formamide(D.M.F.)	-	-	-	-
Alcoholic extract	17	19	21	24
Aqueous extract	11	14	16	17

3. RESULTS

In wound healing studies

The results of the study imply that extract *C.asiatica* accelerates significant healing process. In control animals wound contraction was to the extent of 33%, 56%, 66%, and 87% by day 2, 6, 10 and 14 respectively. These animals took 14.25 ± 0.45 days for reepithelization. Cassia asiatica, administered orally shortened the period of epithelization significantly ($p<0.01$) by 3 days. Besides, it also promoted the wound contraction throughout (Table 1).

Histological examination performed on the ten-day old wounds showed a steady and progressive wound healing in control animals (Table 2 & 3). The dermis proliferated almost to reach normal level. Eschar was getting separated off leaving space for epidermis to grow and complete reepithelzation. Moderate amount of collagen and numerous inflammatory cells could be seen in corium. However, wounds in *Centella asiatica* extract treated animals showed signs of advanced healing such as complete restoration of epidermis, well organized high amount of collagen in dermis, and absence of inflammatory cells in fully grown dermis. A reduction of lipid peroxidase of wounds may reduce the further loss of tissue in wound area and may thus promote healing.

In anti-bacterial studies

Antibacterial activities of *C.asiatica* extracts possess significant antibacterial activity was compared with 10 mcg of standard drugs, ciprofloxacin against the organisms like *E.coli, P.aeruginosa , S.aureus* and *B.subtilis*(Table 4).

4. DISCUSSIONS

Wound healing effect

A pathogen that can cause life – threatening infections in patients with burns and wounds (Ikram M and Inamul H, 1984). The extracts obtained from plants are usually made in to different formulation. Various biological and metabolic alterations occur in wound infections (Izzo AA, 1995). These include the degradation of adenosine triphosphate, significant of polyunsaturated fatty acid in the red cell membrane, elevation of the activity of serum enzyme and fall in the level of vitamin. These changes have been associated with the formation of the lipld peroxidation product namely malondialdehyde (MDA) as a consequence of the wound infection. More over, there is experimental evidence documenting super oxide radical (O_2) involvement in the pathogenesis of wound (Kubo L, 1993).

The selective toxic action on the infecting organism is the key to beneficial actions of antibiotic. These drugs can hit at least is targets in bacteria.

- The cell wall
- The cytoplasmic membrane
- The Ribosome
- The RNA molecules involved in transcription of genetic information

Antimicrobial can bind to ribosome and may interfere with peptide chain formation in bacteria or with the transcription mechanisms (Zaika LL, 1975).The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient .The use of plant extracts and phyto chemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. These products are known by their active substances, for example, the glycosides, terpenes (Velickovic DT, 2003). Saponins present in CA Et beneficially affect collagen (the material that makes up connective tissue), for example, inhibiting its production in hyperactive scar tissue.

Anti bacterial effect

No obvious difference in susceptibility was found between gram-negative and gram-positive bacteria. There was no inhibition of growth with the vehicle control (10% DMF). Our data express:

Plant extracts have great potential as antimicrobial / anti bacterial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes.

These plant extracts were also compared with standard antibiotic. Aqueous extracts showed less activity than ethanol extracts possibly because i) the same active substances were present in water extracts, but in low concentrations ii) active substances were soluble in organic solvents and, therefore, not present in water extracts as also suggested by de Boer et al (De Boer HJ, 2005). The antibacterial action of the extracts is more pronounced on Gram positive than on Gram negative bacteria, and these findings correlate to the observations of previous screenings (Nair R, 2005; Rabe T, 1997) of medicinal plants for antibacterial activity.

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