Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 1, 2012

Research Article

EVALUATION OF WOUND HEALING ACTIVITY OF CYPERUS ROTUNDUS ESSENTIAL OIL

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Received: 20 Aug 2011, Revised and Accepted: 28 Sep 2011

ABSTRACT

The 2009 National Burn Repository reports the most common cause of burns as direct fire/flame (43%) followed by scalds (30%).The aim of present study was to reveal the ameliorative effect of cyperus rotundus essential oil. The burn was induced in the rats by using method as described by Bairy and Somayaji[1]. The treatment was given with the cyperus rotundus essential oil (CREO) with dose 200 mg.kg and 500 mg/kg p.o. The animals were assessed for the percentage wound closure and histopathological studies like epithelization,inflammation,collagen,neovascularity and cellularity. It was found that CREO was improved the disease conditions. CREO was able to close the wound in significantly. It can be concluded that cyperus rotundus may be useful in amelioration of the burn injury.

Keywords: Cyperus rotundus essential oil, Burn wound, Silymarin

INTRODUCTION

A burn is an injury caused by transfer of energy into tissue with a resulting disruption in its functional integrity. The source of energy may be thermal, chemical, electrical or radiation.[2]

The pathophysiological reaction to a burn injury is complex and varies with cause. In thermal burn injury, changes in the burn wound are mainly caused by direct effect of heat, but superimposed on these are changes associated with an acute inflammatory process. A local response to a sudden increase in body surface temperature is the dilation of blood vessels as an attempt to dissipate heat. A further increase in body temperature results in inflammatory response. The key cells in the post burn inflammatory response are (polymorphonuclear neutrophils) endothelial cells. These cell types together with platelet represent the prime target sights responsible for the mediation, progression and resolution of inflammatory response. [3]

Burn wound healing is a complex process consisting of an early phase of abrupt energy depletion and necrosis, followed by a two stage inflammatory phase, delayed cell death, formation of granulation tissue, matrix formation and remodeling.[4,5,6]

Inflammation mediated delayed cell death occurs at the border of the wound and in the surrounding tissue. Increased accumulation of macrophages and fibroblast at the wound site. Surrounding epithelial cells proliferate and migrate to gradually cover the open wound surface. Increased extracellular matrix deposition, especially collagen and remodeling of the newly formed connective tissue persist for several months after injury.[4,5,6]

Free radical as well as histamine and prostaglandin released from the burn wound cause lipid peroxidation in the skin. In consequence they cause burn edema, intravascular heamolysis, hypovolemic shock and adult respiratory distress syndrome or multiple organ failure syndromes. Lipid peroxidation occurs after burn and is continue during the whole post burn period. This process can damage the lipid part of the biological membrane, which leads to the loss of its function and apoptosis. One of the major product of lipid peroxidation is MDA (malondialdehyde), which is an index of free radical activity.[7]

The dynamic process of healing takes place either by "Regeneration" i.e. the replacement of the lost tissue buy new structure known as granulation tissue which ultimately matures to form the scar tissue.

Burn wound healing and repair can occur accordingly whenever the whole body substrate is adequate for survival and functioning of the specialized cells of the organ parenchyma.[7]The stages of wound healing are inflammatory phase, proliferation phase, fibroblastic phase and maturation phase.[8]

Traditionally the extracts of cyperus rotundus is used for digestion and to treat constipation, flatulence, diarrhoea, astringent, bitter, acrid, cooling, carminative, anthelmintic, stimulant, febrifuge, colic, helminthiasis, diarrhoea, dysentery, agalactia, bilious and intermitant fever.It is also used as diuretic and has also been mentioned to heal wound and ulcers.[9] Cyperus rotundus extract has reported for amntimutagenic, bitter, acrid, astringent, cooling, anti-inflammatory, revulsive, galactogogue, depurative, intellect promoting, nervine tonic, digestive, carminative, anthelmintic, stomachic, constipating, diuretic, lithontriptic, expectorant, diaphoretic, emmenagogue, vulnerary, febrifuge, antiperiodic, tonic, antidiarrhoel and radical scavengers.[10]Some plats like *Aloe vera, Coriandrumsativum,Curcuma longa and Murrayakoenigii* having antioxidant potential are also used for their wound healing activity.[11]

Herbal drugs were found advantageous compared to synthetic and semisynthetic drugs due to their less adverse effects such as gastric irritation[12]. So the drugs which are traditionally used have to be reviewed for the activity in order to obtain a scientific confidence.

MATERIALS AND METHODS

Animals

Adult male wistar rats weighing 150-200g breed in the animal house, Shri. Venkateshwara Enterprises, Bangalore were used in the study. The ethical clearance was obtained from Institutional ethical committee local branch, Belgaum. Animals were divided into 5 groups containing 5 animals each.

Rats were housed in a group of five in clean galvanized iron cages in a thermostatically controlled room (28±2^{0}C) and maintained in 12hr natural dark/light cycle. The bedding material of the cages was changed every day. One-week time was provided to the animal acclimatization with our laboratory environment.

Grouping

Group I- (Control)- control with burn injury

Group II- Cyperus rotundus essential oil (CREO 250mg/kg),with burn injury (received250mg/kg,p.o.)

Group III- Cyperus rotundus essential oil (CREO 500 mg/kg), with burn injury (received 500 mg/kg, p.o.)

Group IV-Silymarin 250mg/kgwith burn injury,(received silymarin 250mg/kg)

Burn wound model

Adult albino rats of weight around 150-200gm were divided into 5 groups. On the zeroday, animals were anaesthetized with anesthetic

ether and secured to operation table in its natural position. An impression was made on the dorsal thoracic central region 5 mm away from the ears by using a round seal of 300 mm^2diameter as described by Bairy and somayaji[1]. During the experimental period the animals were housed individually and resuscitation was done with Ringer lactate (0.1 ml/100 mg) daily.Burn wound were created by pouring hot molten wax at 80ºC into a metal cylinder (300 mm area of circular openings,capacity to hold 4.6 g of wax) placed on the back of the rat.

On solidification of wax (8 min), the metal cylinder with wax adhered to skin was removed, which left distinctly demarked circular wounds of 300 mm^2. After this each animal was placed in a separate cage for full recovery from anesthesia before being returned to holding rooms. No local or systemic chemotherapeutic agents were given. Animal showing signs of infection were excluded from the study. Actual amount of heat delivered by molten wax to create burn wound was calculated by the following formula:

$$\frac{\Delta H/\ \Delta A = MS\ (T_1 — T_2)}{\text{Area of Skin exposed to molten wax (300 mm}^2\text{)}}$$

ΔH/ ΔA = Amount of heat delivered by molten wax to sq. mm. of exposed skin.

M = Mass of molten wax.

T_1 = Initial Temperature.

T_2 = Room Temperature.

S = Specific heat.

The animals were then placed back into individual cages. The physical attribute of healing viz. (wound closure) contraction which mainly contributes for wound closure was studied by tracing the raw wound area on the polythene paper on wounding day followed by 4,8,12 and 16th days after removing scab till complete epithelialization occurred, the criterion for complete epithelialization being fall of scab without any raw wound area. Wound area was measured by retracing the wound on a millimeter graph paper.

The degree of wound healing was calculated as percentage closure in wound area from original wound area using the formula,

Percentage closure= 1- AD/AO X 100

Where AO = wound area on day 0

AD= wound area on corresponding days

The mean and S.E.M. values of raw wound areas were calculated. The number of days for complete epithelialization was noted.

Histopathological studies

On various post wounding days a few animals were sacrificed by deep anesthesia .The wounds were excised, leaving a 5 mm margin of normal skin around the edges of the wound, and placed in 10% formalin for histopathological examination. After the tissue was processed, mid-wound vertical sections of each specimen were cut and stained with haematoxylin and eosin. The specimens are assessed under light microscopy for the progression of new epithelium, inflammation, vascular responses and the formation of collagen in the wound.

RESULTS

Wound contraction studies (percentage closure)

As shown in fig.1 the percentage of wound closure was estimated at day 0 before the treatment with CREO and after treatment with the same at day 4, 8, 12, 16. It was gradually increased due to chronic treatment of CREO. CREO-250 and CREO-500 increased percentage wound closure in dose dependent manner. Silymarin was found to be more effective than CREO.

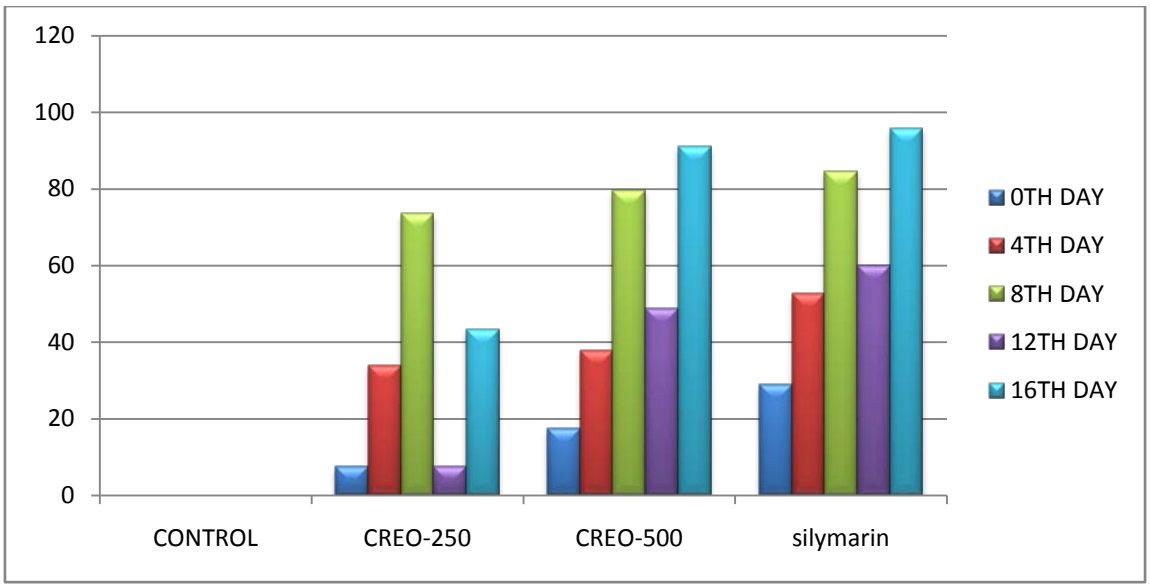

Fig. 1: Percentage closure

Histopathological results

As shown in table no. 1 From the result, it was observed that there was difference in epithelialization on the 10th post-wounding day between control and treated groups The Cyperus rotundus essential oil500 mg/kg treated groups showed significant (P<0.001) increase in epithelialization compared to control group.From the results obtained there was considerable difference in the inflammatory response between the test and control group for the significant (P<0.001) decrease in treated group.Collagen content was significantly (P<0.001) increased in treated group on compared group.Treated group showed significant (P<0.01) increase in neovascularization compared to control group.Treated group showed significant (P<0.01) change in the fibroblast content compared to control group on 10 th post wounding day.

The collagen content was estimated from regenerated tissues for control as well as treated groups there was a significant increase (P<0.001) in collagen content on 4th, 8th, 12th and 16th days in treated group compared to the control group.

Table 1: Histopathological results

Drug	Parameters	Day 4		Day 8		Day 12		Day 16	
		C	T	C	T	C	T	C	T
CREO-250	epithelization	0000	0000	0.852±0.01	1.25±0.021**	1.26±0.01	1.79±0.02*	2.51±0.08	3.77±0.04**
	Inflammation	4.37±0.13	3.48±0.15*	3.638±0.02	3.18±0.03**	2.27±0.07	1.61±0.03**	2.22±0.01	1.27±0.044**
	Collagen	1.15±0.037	2.55±0.10**	1.064±0.04	2.22±0.03**	1.77±0.06	3.16±0.01**	2.47±0.12	3.46±0.10**
	Neovascularity	3.39±0.057	4.30±0.021*	1.722±0.0	1.86±0.03*	2.59±0.05	2.76±0.02**	2.16±0.02	2.28±0.01**
	cellularity	3.62±0.01	3.74±0.02	3.366±0.01	3.63±0.03**	2.13±0.04	2.16±0.021**	1.63±0.01	1.89±0.02**
CREO500	epithelization	0000	0000	0.852±0.01	1.36±0.016**	1.26±0.01	2.19±0.02**	2.51±0.08	4.26±0.01**
	Inflammation	4.36±0.13	3.80±0.11**	3.638±0.02	2.93±0.092**	2.27±0.08	1.28±0.06**	2.22±0.01	1.11±0.01**
	Collagen	1.15±0.037	2.19±0.01**	1.064±0.04	2.81±0.08**	1.77±0.06	3.61±0.059**	2.47±0.11	4.30±0.09**
	Neovascularity	3.39±0.057	4.83±0.071**	1.722±0.06	2.01±0.045**	2.59±0.05	2.95±0.04**	2.16±0.02	2.56±0.01***
	cellularity	3.624±0.01	4.03±0.01**	3.366±0.01	3.91±0.026**	2.13±0.04	2.38±0.086**	1.63±0.06	2.04±0.01**

Values 5 for maximum similarity and 1 refer for least similarity of wound from the normal saline are expressed as mean ± SEM of 5 valuesFollowed by ANOVA and dunnett's test. P< 0.001 significant as compared to control.

DISCUSSION

A burn is an injury caused by transfer of energy into tissue with a resulting disruption in its functional integrity. The source of energy may be thermal, chemical, electrical or radiation.[13]

The burn wound healing studies of essential oils cyperus rotundus have shown dose dependently significant increase in wound contraction.The process of wound healing occurs in four phases: coagulation, which prevents blood loss, inflammation and debridement of wound and repair, including cellular proliferation and tissue remodeling and collagen depositions. Any agent that accelerates the above process is a promoter of wound healing. This may be effective in reducing tissue swelling and oozing of tissue fluids accompanying inflammation revealed a positive healing profile[6].

When a wound occurs and is exposed to external environment. It is more prone to attack by microbes, which invade through the skin and delay the natural wound healing process. The significant antibacterial effect of oils against eight pathogens confirmed that the compounds present in the oil are responsible for the effective antimicrobial activity[14].

The first step in collagen synthesis is the formation of precursor polypeptide which is proline and lysine residues. Hence, more the hydroxyproline, more collagen is formed. The remolding of scar tissue occurs by faster inter and intramolecular cross linking of collagen where as in control group healing is due to natural cell matrix interaction and further causes largest scar area. Hence it clearly means that on increase in the tensile strength, there is in inter and intramolecular cross linking of collagen fibers. The formation of granulation of tissue begins simultaneously with the fibroblast proliferation and the beginning of capillary formation [15].

Histopathological studies demonstrate a significant difference between the test and the control on all observed except for 4th day. The inflammatory response up to 8th day indicates the entire process. Inflammation results in the stimulation of fibroblast in synthesis of collagen.

The Cellularity and collagen content showed significant difference in the test and control groups. Hence, in test wounds the fibroblast content resulted in faster synthesis of collagen compared to control.

Report suggests that restoration of tissue continuity after injury and strengthening of repairing tissue depends primarily on the function of the fibroblast. More over, fibroblast can migrate during the healing process and become contractile as myofibroblasts. This contractile ability contributes to healing on wounds[6].

Cyprousrotundus at higher dose showed significant increase in epithelization, collagen fibroblast formation and neovascularization as compared to lower dose.

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