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Wound healing effect of alcoholic extract of *Ocimum sanctum linn.* on rats

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Abstract
Wound healing is the process of repair that follows injury to the skin and other soft tissues. It can result from injurious process ranging from acute disruption of tissue by surgeon's knife to wide spread trauma, such as burns. It is well known that traditional herbal medicines existed before the application of the modern scientific methods to health care and even today most of the rural Indian population depend on herbal care practices. Since time immemorial indigenous plant material are being used for healing of wounds. This research work focus to find out healing effect of *Ocimum Sanctum* (Alcoholic extract) on incisional wound and its effects were compared on the 10th day by wound breaking strength. The wound breaking strength of control group (275gm), standard group (474.4gm) and alcoholic extract 400mg/kg (449.4gm), 800mg/kg (474.3gm). It is concluded that *Ocimum Sanctum* leaf extract i.e. alcoholic (400 & 800 mg) has significant wound healing effect.
Keywords: Wound healing, wound breaking strength, *Ocimum Sanctum*, Alcoholic Extract

1. Introduction
Wound healing is the process of repair that follows injury to the skin and other soft tissues. It is fundamentally a connective tissue response. Initial stages of wound healing involve an acute inflammatory phase followed by synthesis of collagen and other extracellular matrix which are later remodelled to form scar.

It is well known that traditional herbal medicines existed before the application of the modern scientific methods to health care and even today most of the rural Indian population depend on herbal care practices. Therefore standardization of herbal preparation and investigations about their clinical use and efficacy is recommended.

Since time immemorial indigenous plant material are being used for healing of wounds. *Ocimum sanctum* (family: Labiaceae), is found throughout the semitropical and tropical parts of India. Different parts of the plant are traditionally used in Ayurveda and Siddha systems for the treatment of diverse ailments like infections, skin diseases, hepatic disorders and as an antidote for snake bite and scorpion sting [1]. A methanol extract and an aqueous suspension of *O. sanctum* leaves have anti-inflammatory, analgesic and immunostimulatory properties [2]. Flavonoids isolated from *O. sanctum* scavenged free radicals *in vitro* and showed antilipoperoxidant activity *in vivo* at very low concentration [3]. The free radical scavenging activity of plant flavonoids help in the healing of wounds [4]. Low levels of antioxidants accompanied by raised levels of markers of free radical damage play a significant role in wound healing in rats [5].

Free radical scavenging activity is a major mechanism by which *O. sanctum* products protect against cellular damage [6]. Based on studies in rats, *O. sanctum* may act at various levels in the immune mechanism, such as antibody production, release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs in modulating the humoral immune responses [7]. Significant inhibition of growth of pathogenic microorganisms was observed *in vitro* by traditional drugs like *O. sanctum, Azardicta indica* and *Annona squamosa.* Healing of the wound by indigenous ointment formulation was comparable with that of Nitrofurazone and Propamidine cream in mice infected by the organisms [8].

Keeping in view the tremendous pharmacological activities and a wealth of available literature, *O. sanctum* may be utilized to alleviate the symptoms of a variety of diseases as evident from pre-clinical data [9]. With these background of wide spread availability, regular use of these plant products by common people and lack of information of any adverse side effects even on chronic use, the present work is undertaken to assess the wound healing actions, of ethanolic extracts of *Ocimum sanctum* leaf in a scientific manner in rats.

2. Material and Methods

2.1 Collection and Preparation of Leaf Extracts

The fresh leaves of *Ocimum sanctum* collected from young matured plant. Before collecting the plant materials in bulk the fresh leaves was identified for its genuinity by the taxonomists of G.M. College, Sambalpur. After comparing with the voucher specimen present in the herbarium after authentification. The leaves collected fresh in bulk were washed with running tap water to remove adhering dust and derbis followed by rinsing with the distilled water.

The collected leaves were spread on paper and dried under shade which took about some days (30days) for complete drying. Then the dried leaves were grinded and then used for extraction process in the Departmental Lab.

The powders were weighted (72gm) and extraction was done in 95% ethanol (600ml) as solvent by soxhlet apparatus. Then the extracts were collected (300ml) and evaporated to dryness in water bath at 40-50°C. The extract was stored at 4°C for further use.

75 g of leaf powder was extracted with 700 ml of 95% ethanol in a soxhlet apparatus at 60–75°C. Extract was concentrated by evaporation [12]. The yield was about 10–15%. The semisolid extract was dissolved in saline by using gum acacia as a vehicle during the study.

2.2 Experimental Animals

Healthy albino rays of either sex, inbred in the departmental animal house, weighing between 100 to 200mg. The animals under experiment were isolated and were kept in a separate room. They were fed on standard diet, i.e. soaked grams, green leafy vegetables; milk and water *ad libitum*. Every experimental animal was clinically examined preoperatively for any disease like infection. Female rats if showing signs of pregnancy were discarded from the study. All the animals were starved overnight with water *ad libitium* prior to the day of operation to avoid any post operative complication due to anaesthesia. All the animals were kept under observation for 1 week before operation. The animals were divided into different groups of ten each and kept individually in separate spacious clean cages under standard laboratory conditions.

2.3 Method

2.3.1 Resutured incisional wound

Under light anaesthesia the dorsal surface of each animal was shaved with a sterile blade under all aseptic measures.

One linear incisional wound of whole skin thickness of length 6cm was made on back of each animal. Parallel and 1cm lateral from the vertebral column on either side. After complete hemostasis the wounds were closed by means of interrupted stitches at 1cm gap with 3-0 silk (sterile).

All the above surgical measures were done with full aseptic measure. Immediately after wounding the rats were kept in separate spacious clean cages to avoid damage to wound. As the rats took 10- 20 minutes to come out of the effect of anaestesia, food and water was given *ad libitium* after 2 to 3 hrs of the day of operation. The sutures were removed on the 7^{th} day. Wound braking strength (WBS) was measured on the 10^{th} post- wounding day.

No local or systemic antibiotics were given in the post operative period. The animals were inspected daily for any evidence of infection and the animals showing infection were excluded from the study and replaced with fresh animals. The day wounding was referred as day-1.

The animals were divided into five groups of six animals each and were daily administered the extract of *O. sanctum* via intragastric tube. The extracts were dissolved by using the vehicle gum acacia in normal saline during the study. Two grams of gum acacia was dissolved in 100 ml of normal saline. From this, 10 ml of solution, which contains 200 mg of gum acacia, was used for dissolving 1 g of aqueous and alcoholic extracts. So each ml of solution contains 100 mg of extracts.

Group I: Control group with wound and treated with gum acacia in normal saline
Group II: Test group with wound and treated with alcoholic extract (400 mg/kg body weight)
Group III: Test group with wound and treated with alcoholic extract (800 mg/kg body weight)

2.3.2 Drug administration

After suturing the ointments were applied locally to all rats according to the experimental protocol for 10days.

Table 1: Experimental protocol

Group	No. of rats	Drug & dose	Nature of drugs
Gr. I	10	Normal saline	Control
Gr. II	10	Povidone Iodine	Standard
Gr. III	10	Alc. Extract (400mg/kg)	Test
Gr. IV	10	Alc. Extract (800mg/kg)	Test

2.3.3 Determination of Wound Breaking Strength

On 10th post wounding day each animal was anaesthetized with ketamine and the suture were removed under all aseptic measures.

Rats were secured to the operation table and a line was drawn on either side of the wound 3 mm away from the wound. Two allice forceps were firmly applied on to the line facing each other. One of the forceps was fixed, while the other was connected to a freely suspended lightweight polypropylene graduated container through a string run over to a pulley. Water was allowed to flow from the reservoir slowly and steadily into the container. A gradual increase in weight was transmitted to the wound site pulling apart the wound edges. As and when the wound just opened up, the water flow was arrested and the volume of water collected in the container (approximately equal to its weight) was noted. Three readings were recorded for a given incision wound and the procedure was repeated on the wound on the contralateral side.

The average reading of the group was taken as an individual value of breaking strength. Mean value gives the breaking strength for a given group. The data obtained were analyzed statistically.

2.4 **Statistical analysis of the results**

The mean, the standard deviation (SD) and the standard error of the mean (SEM) were calculated for each group of observations. To find out any difference between the means among the treatment groups, one–way ANOVA was applied on each set of observations. To find out any difference among the means for the paired data, Paired 't' test was applied. P value < 0.05 was taken as statistically significant.

In the present study one-way ANOVA followed by post ANOVA and Paired 't' test were applied by using MS-excel and Graph Pad Instat software in a personal computer. P value <0.05 was considered statistically significant.

3. Results

The present study was undertaken to assess the potential of alcoholic extracts in wound healing in Wistar albino rats. The rats were divided into three groups of ten animals each. Group 1 is normal wounded control and the other two groups were treated with two different doses each of alcoholic extract of *O. sanctum*. The wound healing parameters were evaluated by using incision, wounds in extract-treated rats and controls. The alcoholic extract significantly increased wound breaking strength, when compared with the control group. The results suggest that *O. sanctum* has antioxidant properties, which may be responsible and favorable for faster wound healing and this plant extract may be useful in the management of abnormal healing and hypertropic scars.

In the present work, the wound healing effect of alcoholic and aqueous extract of *Ocimum Sanctum* leaf were studied. Their effects on breaking strength of healing wound on 10th post-operative day were compared with rats given normal saline.

Table 2: Effect of *Ocimum sanctum* extract on breaking strength of post operative healing wounds (Comparative study)

Rat No.	Normal saline (Control) In gm	Povidone iodine (Standard) in gm	Alc. Extract (400mg/kg) In gm	Alc. Extract (800 mg/kg) In gm
1	**275**	**475**	**450**	475
2	**280**	**472**	**445**	479
3	**285**	**474**	**455**	475
4	**270**	**470**	**450**	470
5	**279**	**476**	**440**	468
6	**274**	**478**	**455**	477
7	**270**	**475**	**453**	479
8	**272**	**474**	**447**	470
9	**275**	**472**	**451**	475
10	**270**	**478**	**448**	475
Mean	**275**	**474.4**	**449.4**	474.3
SD	**5.011**	**2.59**	**4.647**	3.80
SEM	**1.58**	**0.82**	**1.47**	1.20
P			>0.05	<0.01

* denotes significant

4. Discussion

In our study on the effect of alcoholic and aqueous extracts of *O. sanctum* on wound healing (400 and 800 mg/kg body weight), we found that aqueous extract possesses a better effect than alcoholic extract at a dose of 800 mg/kg body weight. Since *O. sanctum* is ubiquitous and abundantly grown, it could be a fairly economical therapeutic agent for wound management as a prohealer, as well as to control abnormal healing.

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