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WOUND HEALING ACTIVITY OF LEAF EXTRACTS OF *SALVADORAOLEOIDES*DECNE (SALVADORACEAE)

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ABSTRACT

The leaves of SalvadoraoleoidesDecne (Salvadoraceae) are usedtraditionally in the folklore for thetreatment of various kinds of wounds. The present study was undertaken to verifythe effect of Salvadoraoleoides leaves on experimentally induced wounds in rats inexcision wound model. The chloroform, ethyl acetate, alcohol and water extracts of Salvadoraoleoides leaf were suspended in simple ointment base and used for the topical application to the animals for wound healing activity. A significant increase in the wound-healingactivity was observedin the animals treated with the alcohol and water extracts of Salvadoraoleoides leaves at a dose of 200 and 400 mg/kg of body weight, when compared with the control treatments. The results suggest that Salvadoraoleoides leaf extracts applied topically possesses wound healing activity.

KEY WORDS

Excision wound model, Leaf extracts,Salvadoraoleoides and Wound healing activity.

INTRODUCTION

Only two species of *Salvadora* is found in Gujarat i.e. *Salvadoraoleoides*and *Salvadoraphlomoides*. It is a small herb, grows particularly along rocks and in the dry mountainous areas of Gujarat. The plant generally grows during rainy season (June – September). The whole plant is traditionally used in the treatment of various uterine and skin disorders by the local people of Kachchh region[1-2].

Leaves of *Salvadora* used to relieve cough while the root bark is used as vesicant. The leaves are said to possess anti-inflammatory, analgesic and antiulcer activity[3]. Fruits are employed in the treatment of enlarged spleen, rheumatism and fever[3-4]. The seed fat is used in the treatment of rheumatic pains, in preparation of suppositories and as a base for ointments. Leaves paste is used to treat throat swelling and wounds of domestic animals in India[5]. However, there is no scientific evidence for confirmation of their wound healing activity. Thus, the present study was carried out to ascertain the effect of various extract of *Salvadoraoleoides* leaves on experimentally induced wounds in rats.

MATERIAL AND METHODS

Preparation of the plant extracts:

Fresh leaves of *Salvadoraoleoides* were collected and dried under shade and powdered by a mechanical grinder. 1 Kg of the pulverized plant material was extracted with different solvents like petroleum ether, chloroform, ethyl acetate, ethanol and water using a soxhlet

apparatus.After concentration and drying of each extract, chloroform, ethyl acetate, alcohol and water extracts were selected for the biological screening in various animal models.All the plant extracts and standard drug Povidone iodine were suspended in simple ointment base and applied topically to animals.

Experimental animals:

Albino rats (Wistar strain) of either sex weighing between 150-200 g were used for experimental purpose. The animals were kept in polypropylene cages at room temperature and under 12:12 hours light/ dark cycle. The animals had free access to standard rat pellet and water under strict hygienic conditions. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize, if any non-specific stress. The animals were divided into groups of six animals each and fasted for 12 hours before the experiment. The study was approved by Institutional Animal Ethical Committee.

Excision wound model: [6]

The animals were randomly allocated into groups comprising six animals each (n=6) for the study as described in **Table 1.** Animals were anesthetized prior to and during creation of the wounds, with light ether anesthesia. The rats were inflicted with excision wounds. The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue. A full thickness of the excision wound of 1.5 cm in width was created along the markings using toothed forceps, scalpel and pointed scissors. The negative control group was applied with simple iodine ointment. The positive control group was applied with 3% Povidone iodine ointment. The remaining twelve groups applied with the chloroform, ethyl acetate, alcoholic and water extracts of *Salvadoraoleoides* at the two different dose levels of 200 and 400 mg/kg body weight. All the test extracts at different dose level and standard were administered topically once daily from day 0 to the day of complete healing or the 21st postoperative day, whichever occurred earlier. The parameters were evaluated such as wound area measurement, percentage wound contraction, period of epithelialization and scar area. The rate of wound closure was assessed by tracing the wound on days 0, 4, 8, 12, 16 and 21 post-wounding using transparency papers and a permanent marker. The wound areas recorded were measured using graph paper. The day ofescharfalling, after wounding, without any residual raw woundwas considered as the period of epithelialization.

Statistical analysis:

The data for each experiment were expressed as standard error of mean (± SEM). Unless otherwise specified the significance difference between the rats treated with test extracts, controls and standards groups were tested using analysis of variance (ANOVA) followed by suitable multiple comparision of either Dunnett's, Dunn's or Fisher LSD test. A value of $P < 0.05$ were considered statistically significant.

Table 1 Experimental design for Wound healing studies in rat

Group	Treatment	Dose
I-Control	Simple ointment base	100 mg/kg; topically
II-Standard	3 % w/w Povidone iodine ointment	100 mg/kg; topically
III-Test Extract CESO-200	Chloroform extract of *Salvadora oleoides* (prepared with simple ointment base)	200 mg/kg; topically
IV-Test Extract CESO-400	Chloroform extract of *Salvadora oleoides* (prepared with simple ointment base)	400 mg/kg; topically
V-Test Extract EESO-200	Ethyl acetate extract of *Salvadora oleoides* (prepared with simple ointment base)	200 mg/kg; topically
VI-Test Extract EESO-400	Ethyl acetate extract of *Salvadora oleoides* (prepared with simple ointment base)	400 mg/kg; topically
VII-Test Extract AESO-200	Alcohol extract of *Salvadora oleoides* (prepared with simple ointment base)	200 mg/kg; topically
VIII-Test Extract AESO-400	Alcohol extract of *Salvadora oleoides* (prepared with simple ointment base)	400 mg/kg; topically
IX-Test Extract WESO-200	Water extract of *Salvadora oleoides* (prepared with simple ointment base)	200 mg/kg; topically
X-Test Extract WESO-400	Water extract of *Salvadora oleoides* (prepared with simple ointment base)	400 mg/kg; topically

Table 2 Percentage wound contraction in excision wound model

Group/ Treatments	Wound Area in mm^2 (% Wound Contraction)						
	Dose	Day 0	Day 4	Day 8	Day 12	Day 16	Day 21
Control (Distilled water)	100 mg/kg	275.17±0.014	232.33 ±0.067 (15.56)	221.17 ±0.027 (19.61)	209.07±0.012 (24.01)	188.54±0.039 (31.47)	174.39±0.054 (36.61)
Standard	100 mg/kg	271.37±0.048	166.29 ±0.037 (38.72)	103.83 ±0.019 (61.74)	47.83 ±0.041 (82.37)	00.00 (100)	0.00 (100)
CESO 200	200 mg/kg	289.34±0.012	250.97 ±0.030 (13.26)	238.12 ±0.052 (17.70)	218.84 ±0.031 (24.37)	178.22 ±0.073 (38.40)	143.16 ±0.016 (50.52)
CESO 400	400 mg/kg	292.17±0.050	240.75 ±0.041 (17.60)	229.65 ±0.033 (21.40)	202.47 ±0.018 (30.70)	161.28 ±0.024 (44.80)	110.44 ±0.019 (62.20)
EESO 200	200 mg/kg	274.87±0.015	243.70 ±0.063 (11.34)	235.11 ±0.049 (14.46)	214.34 ±0.059 (22.02)	184.99 ±0.030 (32.70)	148.16 ±0.034 (46.10)
EESO 400	400 mg/kg	276.93±0.029	232.62 ±0.045 (16.00)	225.14 ±0.040 (18.70)	204.65 ±0.011 (26.10)	164.22 ±0.026 (40.70)	128.77 ±0.040 (53.50)
AESO 200	200 mg/kg	290.49±0.015	239.51 ±0.063 (17.55)	207.11 ±0.049 (28.70)	163.14 ±0.059 (43.84)	100.95 ±0.030 (65.25)	55.25 ±0.034 (80.98)
AESO 400	400 mg/kg	289.26±0.029	229.96 ±0.045 (20.50)	200.46 ±0.040 (30.70)	145.79 ±0.011 (49.60)	69.13 ±0.026 (76.10)	17.93 ±0.040 (93.80)
WESO 200	200 mg/kg	277.15±0.015	225.58 ±0.063 (18.61)	174.43 ±0.049 (37.06)	121.88 ±0.059 (56.03)	60.38 ±0.030 (78.21)	30.19 ±0.034 (89.11)
WESO 400	400 mg/kg	279.53±0.029	193.71 ±0.045 (30.70)	120.76 ±0.040 (56.80)	74.91 ±0.011 (73.20)	26.00 ±0.026 (90.70)	0.00 ±0.040 (100)

Table 3 Effect of various extract of *Salvadora oleoides* on percentage wound

Day of obs.	Control	Std.	CESO 400	EESO 400	AESO 400	WESO 400
	% Wound Contraction					
4	15.56	38.72	17.60	16.00	20.50	30.70
8	19.61	61.74	21.40	18.70	30.70	56.80
12	24.01	82.37	30.70	26.10	49.60	73.20
16	31.47	100	44.80	40.70	76.10	90.70
21	36.61	100	62.20	53.50	93.80	100
Conclusion	-	-	Poor	Poor	Excellent	Excellent

Table 4 Period of epithelialization in extract treated excision wound model of rat

Group/Treatments	Dose (mg/kg)	Period of Epithelialization (days)	Scar Area (mm^2)
Control (Distilled water)	100	32.20±0.10	25.64±1.5
Standard (3% Povidone Iodine Ointment)	100	15.66±0.11	14.32±1.2
CESO 200	200	30.43±0.14	24.05±2.2
CESO 400	400	27.78±0.11	22.13±1.7
EESO 200	200	31.52±0.16	24.67±2.5
EESO 400	400	26.36±0.03	23.38±1.8
AESO 200	200	25.81±0.25	20.16±4.2
AESO 400	400	22.94±0.15	17.29±3.1
WESO 200	200	24.26±0.10	21.34±2.3
WESO 400	400	18.17±0.21	15.47±1.6

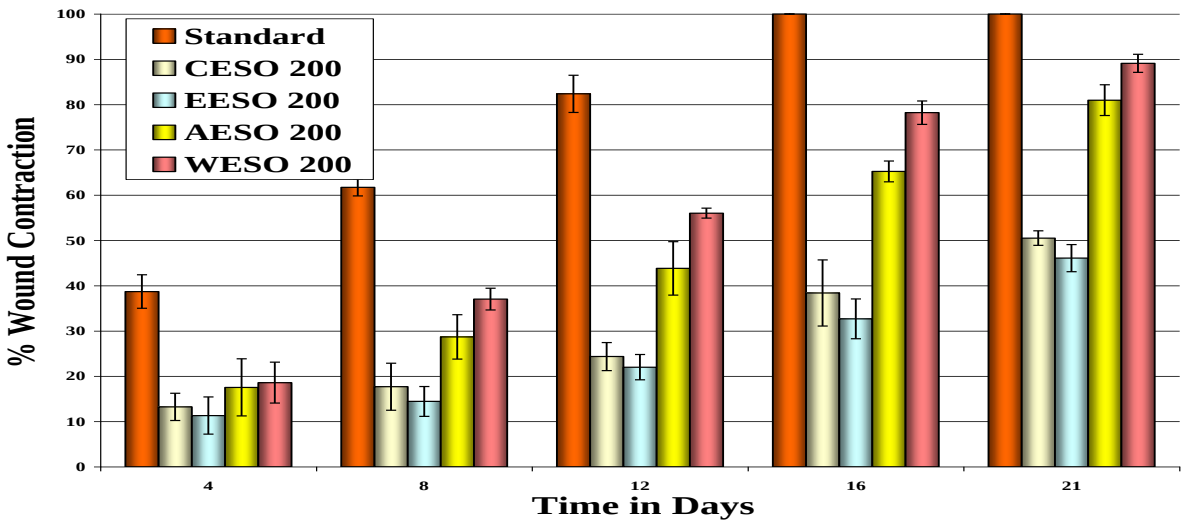

Figure 1 Percentage wound contraction by various extracts of *Salvadora oleoides* at the dose level of 200 mg/kg of b.w., in comparison with standard

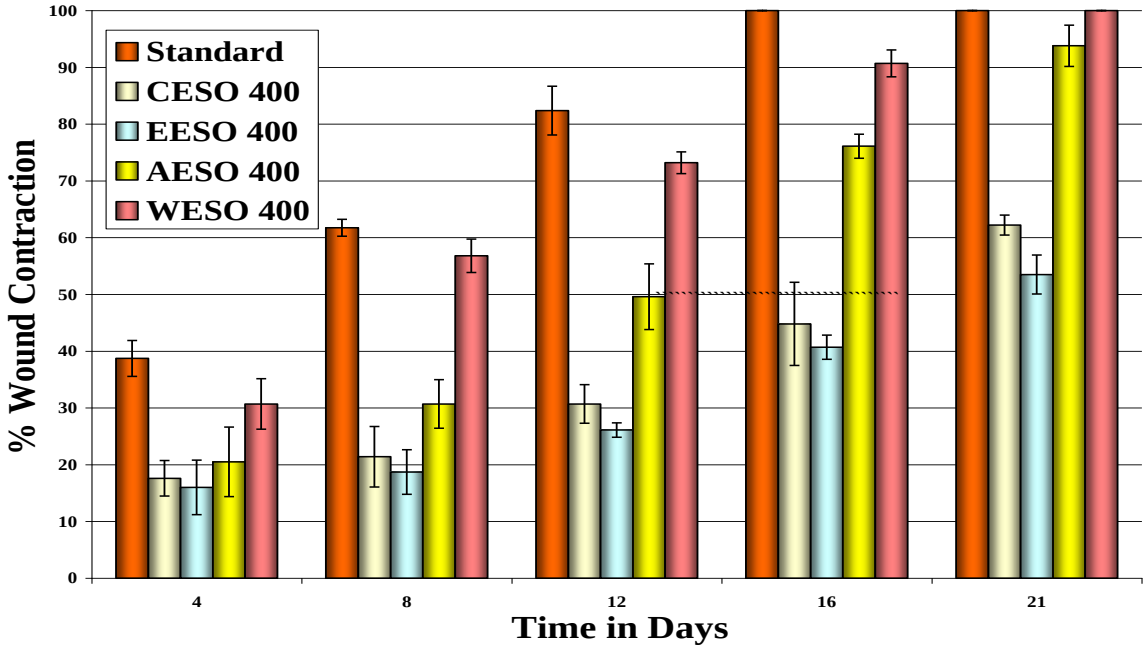

Figure 2 Percentage wound contraction by various extracts of *Salvadora oleoides* at the dose level of 400 mg/kg of b.w., in comparison with standard

RESULTS AND DISCUSSION

Wound healing activity:

A significant increase in the wound-healingactivity was observedin the animals treated with the alcohol and water extracts of *Salvadoraoleoides* leaves at a dose of 200 and 400 mg/kg of body weight, when compared with the control treatments.

Table 2, 3and **4**shows the effect of the alcohol and water extracts of*Salvadoraoleoides* on wound-healingactivity in rats inflicted with excision wound. In this model,the extract-treated animals showed a more rapid decrease inwound size and a decreased time to epithelializationcompared with the control rats which received distilledwater. The rate of wound contraction was less in control group of animals, whereas the percentage of wound closure was high in water extract treated group followed by alcohol extract treated animal group, indicating the effect of the plant on promoting healing of excision wound. Results were shown in **Figure 1 and 2.**

Control rats showed a time dependent increase in percent wound contraction from 15.56 % to 36.61 % from day 4th to day 21st, while complete wound closure and epitheliazation was observed on 32nd day of wound induction compared with day 0 which was taken as 0%. The mean epitheliazation period and scar area were 32.20 days and 25.64 mm^2 respectively in control rats. Standard drug (Povidone iodine ointment) treated rats shown increase in percent wound contraction from 38.72 % to 100 % from day 4th to 16th. The mean epitheliazation period and scar area were 15.66 days and 14.32 mm^2 respectively.

Both the alcoholic and water extracts of *Salvadoraoleoides* (200 and 400 mg/kg body weight) showed dose dependent increase in percent wound contraction. The alcoholic extract of *Salvadoraoleoides* at a dose of 400 mg/kg (AESO400) shown increase in percent wound contraction from 20.50 % to 93.80 % from day 4th to 16th, while epitheliazation period and scar area were 22.94 days and 17.29 mm^2 respectively. The

water extract of *Salvadoraoleoides* at a dose of 400 mg/kg (WESO 400) shown increase in percent wound contraction from 30.70 % to 90.70 % from day 4th to 16th, while epitheliazation period and scar area were 18.17 days and 15.47 mm^2 respectively.

Wound represents a major health problem, both in terms of morbidity and mortality. Wound healing is a fundamental response to tissue integrity. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue[7]. A therapeutic agent selected for the treatment of wounds should ideally improve one or more phases of healing without producing deleterious side effects[8].

Traditional Indian system of medicine has many plants with versatile medicinal properties, which require detailed investigation for effective drug development. Plant products are potential agents for wound healing and largely preferred because of their widespread availability, non-toxicity, absence of unwanted side effects and their effectiveness as crude preparations. In continuation of the development of drugs from plants to medicine, the extracts of *Salvadoraoleoides* leaves were selected for the wound healing effect.

The preliminary phytochemical analysis of the *Salvadoraoleoides* revealed the presence of carbohydrates, glycosides, triterpenes, proteins, amino acids and mucilages. Several studies, including our earlier work with other plantmaterials demonstrated the presence of similar phytochemicalconstituents which were responsible for promoting woundhealingactivity in rats[9-10]. Thus wound healing property of *Salvadoraoleoides* may be attributed to the phytoconstituents present in it, which may be either due to their individual or additive effect that fastens the process of wound healing. Since *Salvadoraoleoides* are abundantly grown, it could be a fairly economical therapeutic agent for wound management as a pro-healer, as well as to control abnormal healing.

Thus, the results of present study do indicate an important healing effect of both alcoholic and water extracts of dried leaves of *Salvadoraoleoides* and their effects were comparable to standard drug povidone iodine ointment on various parameters of wound healing. However further phytochemical studies are needed to find outthe active compound(s) responsible for promoting the woundhealingactivity.

Wounds are a major cause of physical disabilities. Wound healing consists of orderly progression of a series of events that establish the integrity of the damaged tissue. Normal wound healing can be adversely affected by many factors. If the healing fails to progress in the usual stepwise manner then it may lead to development of chronic wound. Many medicinal plants are found useful in treating wounds. Wound healing is a biological process that begins with trauma and ends with scar formation. The goals of wound care include reducing risk factors that inhibit wound healing, enhancing the healing process and lowering the incidence of wound infections. Many medicinal plants have been found useful in wound healing. Medicinal plants provide leads to find therapeutically useful compounds, thus more efforts should be made towards isolation and characterization of the active principles and elucidation of the relationship between structure and activity. The combination of traditional and modern knowledge can produce better drugs for wound healing with fewer side effects.

Wounds are inescapable events of life and they arise due to physical trauma, chemical injury or microbial infections. Healing of wounds usually takes place in a direction away from its normal course and under-healing, over-healing or no healing of wounds is common. Management of

under healing of wounds is a complicated and expensive program and research on drugs that increase wound healing is a developing area in modern biomedical sciences. Several drugs obtained from plant sources are known to increase the healing of different types of wounds. Some of these drugs have been screened scientifically for evaluation of their wound healing activity in different pharmacological models and patients, but the potential of many of the traditionally used herbal agents remains unexplored. In few cases, active chemical constituents were identified[11].

For the management and treatment of wounds, the phytomedicines for wound healing are not only cheap and affordable but are also purportedly safe as hyper sensitive reactions are rarely encountered with the use of these agents. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. However, there is a need for scientific validation, standardization and safety evaluation of plants of the traditional medicine before these could be recommended for healing of the wounds.

CONCLUSION

The present study proves scientifically the wound healing activity of *Salvadoraoleoides* leaves and thus provides pharmacological support for the traditional use. However further studies may reveal the exact mechanisms of action responsible for activity of leaves of *Salvadoraoleoides*.

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