

## Wound healing activity of *Sesamum indicum* L seed and oil in rats

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The seeds of *S. indicum* L (Pedaliaceae) are used traditionally in the folklore for the treatment of various kinds of wounds. The present study was undertaken to verify the effect of *S. indicum* seeds and its oil on experimentally induced excision wound, incision wound, burn wound and dead space wound models in rats. *Aloe vera* was used as standard wound healing agent. A formulation of seeds and oil was prepared in carbopol at 2.5% and 5% concentrations and applied to the wounds. In the excision and burn wound models, the so treated animals showed significant reduction in period of epithelization and wound contraction (50%). In the incision wound model a significant increase in the breaking strength was observed. Seeds and oil treatment (250 mg and 500 mg/kg; po) in dead space wound model, produced a significant increase in the breaking strength, dry weight and hydroxyproline content of the granulation tissue. The results suggest that *S. indicum* seeds and oil applied topically or administered orally possesses wound healing activity.

**Keywords:** Burn wound, Dead space wound, Excision wound, Incision wound, *Sesamum indicum* L.

Wounds are inescapable events of life, which arise due to physical injury, 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. Though some of these drugs have been screened scientifically for evaluation of their wound healing activity in different pharmacological models and patients, the potential of many of the traditionally used herbal agents remains unexplored. In few cases, active chemical constituents were identified<sup>1</sup>.

Sesame (*Sesamum indicum* L) is one of the oldest cultivated plants in the world that is mainly grown for its oil rich edible seeds. The seeds are widely used as spice globally. Sesame seed may be the oldest condiment known to man and probably was the first crop grown for its edible oil<sup>2</sup>. The seeds possess potent antioxidant effect due to the presence of sesamol<sup>3-6</sup>. Traditionally, sesame seeds are used in the treatment of wounds, especially burn wounds. In

Ahmednagar and Buldhana districts of Maharashtra state (India), a paste of seeds is applied over the wounds and it is believed that the paste possesses soothing effect and can cure burn wounds and cracks. There is no scientific report on the effect of sesame seeds on the wounds. The present study has been undertaken to ascertain the effect of sesame seeds and sesame oil (the main constituent of the seeds) on experimentally induced wounds in rats.

### Materials and Methods

**Experimental animals**—Male albino Wistar rats weighing between 250-275 g were used. The animals were caged individually after wounding for treatment till completion of wound healing. In each group of different models six animals were used. The experimental protocol was approved by Institutional Animal Ethics Committee and animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

**Chemicals**—Ketamine injection was procured from Prem Pharmaceuticals Pvt. Ltd. (Indore, India) and xylazine was from Indian Immunological Ltd. (Guntur, India). Hydroxyproline and paradimethyl-amino benzaldehyde were procured from SD Fine Chemicals Pvt. Ltd. (Mumbai, India), sodium hydroxide (NaOH), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and

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copper sulphate ( $\text{CuSO}_4$ ) were purchased from Nice Chemicals Pvt. Ltd. (Mumbai, India), hydrochloric acid (HCl) was obtained from Ranbaxy Fine Chemicals Pvt. Ltd. (Mumbai, India). Carbopol 940 was from Loba Chemie (Mumbai, India). Sesame seeds packet (100 g; date of packing: April 2007; date of expiry: March 2010) and double refined sesame oil (500 ml; date of packing: June 2007; date of expiry: June 2009) were purchased from local market (The Food World, Bangalore). The seeds were observed carefully for presence of contaminants and the manufacturing date printed on the oil was not more than six months from the date of start of study. Voucher specimens of seeds and oil are kept in the Department.

*Selection of dose, gel base and treatment period*—No acute toxicity study was carried out as both the seeds and oil are used as food and are considered to be very safe. The doses for topical administration was used based on the information provided by the traditional healers and the oral dose was selected assuming that seeds are very safe at a dose of 5 g/kg, po as per limit tests of OECD guidelines<sup>7</sup>,  $1/10^{\text{th}}$  and  $1/20^{\text{th}}$  of the safe dose was used for oral administration.

The sesame seeds contain around 60-70% oil<sup>8</sup>. Hence, a water soluble base like carbopol containing methyl paraben (0.01%) and propyl paraben (0.1%) was selected as base for both seeds and oil for local application as gel<sup>9</sup>.

Sesame seed (SSLD 2.5%), sesame oil (SOLD 2.5%) and sesame seed (SSHHD 5%), sesame oil (SOHD 5%) were selected as low and high dose respectively in carbopol base. Since, modern medicine uses mainly antibacterial agents for treatment of wound, a herbal drug, *Aloe vera* (10%) in carbopol was used as standard for topical application in excision, incision and burn wound models<sup>10</sup>. The methanolic extract of aloe contains all the chemical constituents responsible for wound healing activity. For oral administration, suspension of crushed sesame seed (250 and 500 mg/kg) and emulsion of sesame oil (250 and 500 mg/kg) was prepared using acacia (5%) as suspending/emulsifying agent. *Aloe vera* extract (300 mg/kg, po)<sup>11</sup> in the form of suspension (acacia 5%) was used as standard drug. The treatment period was 10 days for incision and dead space wound models and in excision and burn wound models, the treatment was continued till the day of scab falling.

*Excision wound*<sup>12,13</sup>—The animals were anesthetized using ketamine (100 mg/kg, im) and xylazine (16 mg/kg, im). An impression was made on the dorsal thoracic region 1 cm away from vertebral column and 5 cm away from ear on the anaesthetized rat. The particular skin area was shaved one day prior to the experiment. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm<sup>2</sup>. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. The animals were then grouped and different formulations were applied to cover the entire wounded area as follows: Group I: Carbopol (1%) gel (control), Group II: *Aloe vera* extract (10%) gel formulation, Group III: SOLD (2.5%), Group IV: SOHD (5%), Group V: SSLD (2.5%), Group VI: SSHHD (5%). Wound area was measured by tracing the wound on a millimeter scale graph paper on predetermined days i.e., 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 22 days post-wounding. The wound contraction-50% (days) was determined by plotting the wound area vs days on a graph paper. Falling of scab leaving no raw wound behind was taken as end point of complete epithelization and the days required for this was taken as period of epithelization.

*Incision wound*<sup>14-16</sup>—Para vertebral straight incision of 6 cm length was made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel. After complete haemostasis, the wounds were closed by means of interrupted sutures placed at approximately 1 cm apart. Animals were treated daily with drugs, as mentioned above under excision wound model from 0<sup>th</sup> day to 9<sup>th</sup> post-wounding day. The wound breaking strength was estimated on 10<sup>th</sup> day by continuous, constant water flow technique.

*Burn wound*<sup>17</sup>—Partial thickness burn wounds were inflicted on overnight-starved animals under ketamine (100 mg/kg, im) and xylazine (16 mg/kg, im) anesthesia by pouring hot molten wax (2 g) at 80°C. The wax was poured on the shaven back of the animal through a cylinder of 300 mm<sup>2</sup> circular opening. The wax was allowed to remain on the skin till it gets solidified. Immediately after the injury and on subsequent days, the drugs or base was applied topically as mentioned above.

*Dead space wound model*<sup>18</sup>—This type of wound was created by implanting subcutaneously a 2.5×0.5 cm polypropylene tube in the lumber region in

anesthetized rats. Animals received one of the following treatments from 0<sup>th</sup> day to 9<sup>th</sup> post wounding day. Group I: 5% acacia solution (control), Group II: *Aloe vera* extract (300 mg/kg, po), Group III: SOLD (250 mg/kg, po), Group IV: SOHD (500 mg/kg, po), Group V: SSLD (250 mg/kg, po), Group VI: SSHD (500 mg/kg, po). On the 10<sup>th</sup> post wounding day, the animals were sacrificed and the granulation tissue harvested on the implanted tube was carefully dissected out along with the tube. The tubular granulation tissue was cut lengthwise to obtain a sheet of granulation tissue. The breaking strength was measured as described under incision wound model. The pieces of granulation tissue were collected, dried at 60° C for 24 hr to get a constant weight and weighed. The tissue was then used for the determination of hydroxyproline content<sup>19</sup>.

**Statistical analysis**—Results are expressed as mean  $\pm$  SE. The differences between experimental groups were compared using one-way Analysis of Variance (ANOVA) followed by Bonferroni's test. The results were considered statistically significant at  $P < 0.05$ .

## Results

**Effect on excision and incision wound**—All the prepared formulations; SOLD (2.5%), SOHD (5%), SSLD (2.5%) and SSHD (5%) produced a significant decrease in period of epithelization when compared to control ( $P < 0.001$ ). Treatment with *Aloe vera* extract also produced significant reduction in the period of epithelization ( $P < 0.001$ ). All the treatments also showed significant decrease in wound contraction (50%) as compared to control ( $P < 0.001$ ). Comparative analysis revealed that the high dose of both oil and seeds (5%) were significantly less

effective in reducing the epithelization period compared to the low dose (2.5%). The low dose of the oil was significantly more effective compared to the low dose of the seeds. The low dose of seeds was also more effective than high dose in reducing wound contraction-50% (Table 1).

The breaking strength of 10 days old incision wound was increased by all treatments. The high dose of the seeds was more effective than high dose of the oil in increasing the breaking strength of the incision wound (Table 1).

**Effect on burn wound**—Like the excision wound model, application of SOLD (2.5%), SOHD (5%), SSLD (2.5%), SSHD (5%) and *Aloe vera* extract (10%) gel topically shortened the period of epithelization significantly ( $P < 0.001$ ) and also produced a significant decrease ( $P < 0.001$ ) in wound contraction-50% (days) when compared to control. Comparative analysis of different groups indicate that high dose of seeds and oil were less effective in reducing the epithelization period compared to the low dose of the seeds and oil. The high dose of the seeds was less effective in reducing wound contraction compared to low dose of seeds. Similarly, like the excision wound, the low dose of the seed is less effective compared to the low dose of the oil (Table 2).

**Effect on dead space wound**—The breaking strength of 10 days old granulation tissue was significantly promoted by all the treatments; SOLD (250 mg/kg, po), SOHD (500 mg/kg, po), SSLD (250 mg/kg, po), SSHD (500 mg/kg, po) and *Aloe vera* extract (300 mg/kg, po). The dry tissue weight and hydroxyproline content were significantly increased ( $P < 0.001$ ) by all the treatments when compared to

Table 1—Effect of *S. indicum* seeds and oil on period of epithelization and wound contraction in excision wound and breaking strength in incision wound model

[Values are mean  $\pm$  SE from 6 rats in each group]

Treatment	Excision wound		Incision wound Breaking strength (g)
	Epithelization period (days)	Wound contraction -50% (days)	
Control (1% carbopol gel)	22.16 $\pm$ 0.3073	11.70 $\pm$ 0.3578	289.16 $\pm$ 3.005
<i>Aloe vera</i> extract (10%)	15.83 $\pm$ 0.3073 <sup>a</sup>	9.26 $\pm$ 0.2692 <sup>a</sup>	399.16 $\pm$ 3.005 <sup>a</sup>
SOLD (2.5%)	14.50 $\pm$ 0.2236 <sup>a,d</sup>	8.33 $\pm$ 0.2917 <sup>a</sup>	443.33 $\pm$ 3.073 <sup>a,e</sup>
SOHD (5%)	16.66 $\pm$ 0.2108 <sup>a,b</sup>	9.46 $\pm$ 0.4072 <sup>a</sup>	380.00 $\pm$ 2.887 <sup>a,b,e</sup>
SSLD (2.5%)	15.66 $\pm$ 0.2108 <sup>a,c</sup>	8.91 $\pm$ 0.2414 <sup>a</sup>	437.50 $\pm$ 3.354 <sup>a,e</sup>
SSHD (5%)	17.50 $\pm$ 0.2236 <sup>a,b,e</sup>	9.20 $\pm$ 0.3670 <sup>a</sup>	417.50 $\pm$ 2.141 <sup>a,b,c,e</sup>

control. There was no significant difference in any of the parameters between various treatment groups (Table 3).

### Discussion

The present study was undertaken to evaluate whether *S. indicum* seeds and oil promote wound healing in experimentally induced wounds in rats. The results of the present study substantiate the use of *S. indicum* seeds and oil in folklore medicine for the treatment of wounds. The gel containing seeds or oil

applied topically or administration of seeds or oil orally promoted the breaking strength, wound contraction and period of epithelization in different models of experimental wounds.

Collagenation, wound contraction and epithelization are crucial phases of wound healing. The phases of inflammation, macrophasia, fibroplasia and collagenation are intimately interlinked. Thus an intervention into any one of these phases by drugs could eventually either promote or depress one, other or all phases of healing. Growth hormone is known to

Table 3—Effect of *S. indicum* seeds and oil on breaking strength, dry tissue weight and hydroxyproline content in dead space wound model

[Values are mean  $\pm$  SE from 6 rats in each group]

Treatment	Breaking strength (g)	Dry tissue weight (g)	Concentration of hydroxyproline ( $\mu\text{g/g}$ of tissue)
Vehicle (1 ml/kg)	284.16 $\pm$ 8.983	69.16 $\pm$ 4.729	2533.33 $\pm$ 245.85
<i>Aloe vera</i> extract (300 mg/kg, po)	535.83 $\pm$ 12.01 <sup>a</sup>	175.83 $\pm$ 7.574 <sup>a</sup>	6266.66 $\pm$ 245.85 <sup>a</sup>
SOLD (250 mg/kg, po)	530.83 $\pm$ 10.678 <sup>a</sup>	165.00 $\pm$ 5.859 <sup>a</sup>	5466.66 $\pm$ 321.11 <sup>a</sup>
SOHD (500 mg/kg, po)	540.00 $\pm$ 12.845 <sup>a</sup>	163.5 $\pm$ 4.624 <sup>a</sup>	6266.66 $\pm$ 245.85 <sup>a</sup>
SSLD (500 mg/kg, po)	497.50 $\pm$ 8.732 <sup>a</sup>	164.00 $\pm$ 4.676 <sup>a</sup>	5600.00 $\pm$ 292.12 <sup>a</sup>
SSHHD (250 mg/kg, po)	522.50 $\pm$ 6.922 <sup>a</sup>	157.33 $\pm$ 5.232 <sup>a</sup>	6133.33 $\pm$ 168.65 <sup>a</sup>

<sup>a</sup> $P < 0.001$  vs. control

Table 2—Effect of *S. indicum* seeds and oil on period of epithelization and wound contraction in burn wound model

[Values are mean  $\pm$  SE from 6 rats in each group]

Treatment	Epithelization period (days)	Wound contraction 50% (days)
Control (1% carbopol gel)	21.83 $\pm$ 0.3073	8.56 $\pm$ 0.3621
<i>Aloe vera</i> extract (10%)	16.00 $\pm$ 0.2582 <sup>a</sup>	5.76 $\pm$ 0.2076 <sup>a</sup>
SOLD (2.5%)	13.33 $\pm$ 0.2108 <sup>a,c</sup>	4.76 $\pm$ 0.1406 <sup>a</sup>
SOHD (5%)	16.50 $\pm$ 0.2236 <sup>a,c</sup>	5.66 $\pm$ 0.1430 <sup>a</sup>
SSLD (2.5%)	16.66 $\pm$ 0.3073 <sup>a,d</sup>	5.10 $\pm$ 0.1238 <sup>a</sup>
SSHHD (5%)	17.00 $\pm$ 0.2582 <sup>a,c</sup>	6.30 $\pm$ 0.3183 <sup>a,b</sup>

Other details are same as in Table 1

promote the healing process by enhancing epithelial cell proliferation and cell collagen formation. Collagen is the family of protein, which provide structural support and it is the main component of tissue such as fibrous tissue and cartilage. The collagen synthesis is stimulated by various growth factors<sup>20</sup>. Growth hormone is also known to promote the proliferation of fibroblasts<sup>21</sup> and fibroblast proliferation form the granulation tissue. In the dead space wound model, *S. indicum* treatment increased granuloma tissue weight and breaking strength. The exact mechanism(s) by sesame increased the granuloma tissue weight and breaking strength of granulation tissue can not be explained with the present data.

Lipid peroxidation is an important process of several types of injuries like burn, inflicted wound and skin ulcers. A drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils, increasing the strength of collagen fibers by an increase in circulation, thereby preventing the cell damage and promoting the DNA synthesis<sup>22</sup>. Antioxidants such as vitamin C, metronidazole and vitamin E are reported to increase the wound healing<sup>23</sup>. Sesame seeds and its constituents possess very potent antioxidant activity<sup>36</sup>. Sesamol (3,4-methylenedioxyphenol), a coumarin derivative present in sesame seeds is known to efficiently scavenge hydroxyl, one-electron oxidizing, organo-haloperoxyl, lipid peroxy, and tryptophanyl radicals *in vitro* and *in vivo*, it was found to inhibit lipid peroxidation, hydroxyl radical-induced deoxyribose degradation and DNA cleavage<sup>6</sup>. The other constituent of sesame, sesaminol, sesamol and sesamol (lignans) reduce lipid oxidation in liver and kidney<sup>24</sup>. Since, both coumarins and lignans reach systemic circulation after oral administration<sup>25-27</sup>, it can be suggested that the wound healing activity of sesame seeds and oils after both local and systemic administration may at least be in part due to its potent antioxidant activity.

The oil of sesame was more effective in healing of excision and burn wounds than incision wound. From the results, it can be speculated that constituents such as sesamol, sesaminol, sesamol and sesamol present in both seeds and oil may be responsible for the wound healing activity. However, further studies are needed to determine the reason for less effectiveness of oil in healing of incision wound.

To conclude, seeds and oil of *Sesamum indicum*

possess good wound healing activity when applied locally or administered orally. The low dose of both seeds and oil are more effective when applied locally and the high dose of seeds and oil showed greater effect in dead space wound when administered orally.

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