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## EVALUATION OF WOUND HEALING ACTIVITY OF B-GLUCAN+*HIBISCUS ROSA SINENSIS LINN* IN ALBINO RATS

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### ABSTRACT

In Indian medicine, *Hibiscus rosa sinensis* (*HRS*) is a well-known medicinal plant that is used to cure a variety of skin ailments. The purpose of the research presented in this article was to assess the wound-healing capacity of the *Hibiscus rosa sinensis* ethanolic extract alone and in combination with  $\beta$ -glucans. The experiment was divided into two parts, excision wound model and incision wound model and betadine was used to compare the wound healing properties of the *Hibiscus rosa sinensis* flower extract. Along with a control group of rats, the groups were given their medications. The current research concludes that *Hibiscus rosa sinensis* flower extract +  $\beta$ -glucan (BG) improved wound breaking strength more effectively than the control but not as efficiently as the betadine ointment. *Hibiscus rosa sinensis* flower extract + BG aids wound healing by promoting early epithelization and reducing wound size. Betadine is linked to a variety of negative effects, including life-threatening allergic responses, water retention and more.

### KEYWORDS

$\beta$ -glucan, Betadine, *Hibiscus* and Wound healing.

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### INTRODUCTION

Wounds and their healing are a major source of concern. Pharmaceutical companies are taking advantage of these difficulties to introduce a slew of new treatments, many with significant commercial stakes. There are numerous therapies available today to combat the many elements that impede the normal wound healing process<sup>1</sup>. Given these considerations,

a simple, inexpensive, readily available and effective wound healing technique that may be performed without medical supervision is required<sup>2</sup>.

*Hibiscus rosa sinensis* extract (*Hibiscus rosa sinensis*) was shown to be efficacious for better wound care because it is inexpensive, readily available, has no side effects and can be applied without help<sup>3</sup>. There are only a few trials comparing the efficacy of *Hibiscus rosa sinensis* extracts to current pharmaceuticals in the literature. Due to the stimulation of immunological and cutaneous cells,  $\beta$ -glucan (BG) molecules enhance moist wound healing and repair<sup>4</sup>. As a result, the current study aimed to compare the efficacy of *Hibiscus rosa sinensis* flower extract + BG as a wound healing agent to betadine ointment.

## MATERIAL AND METHODS

### Collection and preparation of alcoholic extract of *Hibiscus rosa sinensis* flower

*Hibiscus rosa sinensis* flowers were collected and shade dried before being crushed and powdered. The powder was placed into a Soxhlet extractor in 8 batches of 250g each and extracted for 30-40 hours with 95 percent ethanol. The extract was concentrated under decreased pressure on a water bath at a temperature below 50°C to a syrupy consistency after the solvent was distilled out. It was then dried in a desiccator.

### Animal care and Handling

Rats were only employed following a 7-day acclimation period to the laboratory setting (12:12 hours, light: dark cycle). They were kept in typical nutrient and environmental conditions, including light, temperature and humidity. They were fed conventional laboratory chow and given unlimited water. The rats were rehabilitated and returned to the animal house following the experiment. Animals were carefully monitored for signs of infection and those that showed signs of infection were removed from the trial and replaced. The treatment was carried out in accordance with the consent of King Khalid University's animal ethics committee and the National Institute of Health's guidelines for the care and use of laboratory animals in the United States (NIH Publication No. 85-23, revised 1996).

### Ointment formulations

Raduan S *et al* and Meena A K *et al*<sup>5,6</sup> determined that a dose of about 5% (w/w) of HRS extract was the best amount for wound healing. The extract of 5 percent (w/w) was used to make ointment formulations, with 5 grams of extract put into 100 grams of petroleum jelly. A standard medication, betadine topical ointment (10 percent w/w), was utilized to compare the extract's wound healing ability.

### Induction of anesthesia

The injections of ketamine (50mg) and xylazine (2 percent) were given intra-peritoneally<sup>7</sup>. Because it is a dissociative anesthetic, it disrupts neuronal communication between the cortex and the thalamus. Sustained reflex movements, tense muscles, unconsciousness and wide eyes define this effect. The absence of a response to painful stimuli suggests that anesthesia has been fully induced.

### Excision wound<sup>8</sup>

Under anesthesia, excision wound was made by cutting away a 500mm<sup>2</sup> complete thickness of skin on the back, as described by Morton J J *et al*<sup>9</sup>. To expose the wound to the open environment, it was left naked. The rats were separated into groups and given the following treatments:

Starting on the day of the operation and continuing until complete epithelization, the ointment was used topically once a day. The wound contraction and wound closure time were tracked using this model. The % reduction in wound area was used to calculate wound contraction. Every other day, the wound margin was traced on graph paper to track the progression of the wound. Wound closure percentage = (Wound area on Day 0 - Wound area on Day 'n') / (Wound area on Day 0) \* 100.

### Incision wound<sup>10,11</sup>

A 2cm long paravertebral incision was made through the complete thickness of the skin on the left side (flank) of the vertebral column of the rats back after inducing anesthesia, as described by Ehrlich H P *et al*<sup>12</sup>. Interrupted sutures (ethilon/nylon) were used to seal the wound. The rats were separated into groups and given the following treatments:

Once a day, the ointment was applied topically. On the seventh day, the sutures were removed. A

tensiometer was used to test wound-breaking strength (wound healing) in sedated rats on the 10th day following wounding.

## RESULTS AND DISCUSSION

The animals treated with the *Hibiscus rosa sinensis* extract and combination showed a considerable increase in wound-healing activity as compared to those that received placebo control treatments. In the incision wound model, the effects of the ethanolic extract of *Hibiscus rosa sinensis* flower alone and in combination were shown in Table No.3, where a considerable increase in wound breaking strength was found when compared to the controls. *Hibiscus rosa sinensis* treated rats alone and in combination demonstrated a substantial reduction in wound area ( $p < 0.001$ ) and epithelization duration in the excision wound model (Table No.4).

### Discussion

The goal of this study was to compare the wound healing activity of *Hibiscus rosa sinensis* as well as combination with BG in albino rats to standard treatment. This study's findings and outcomes are comparable to those of several other research that used *Hibiscus rosa sinensis* flower extracts<sup>9,11,13</sup>. Betadine is a povidone-iodine-based product. Povidone iodine is a bactericidal antiseptic that does not obstruct wound healing and is effective against both gram positive and gram-negative bacteria<sup>14-16,13</sup>. However, adverse effects such as life-threatening allergic responses, apparent water retention and temperature swings in boys have been recorded in various trials<sup>17</sup>. *Hibiscus rosa sinensis* flower-treated rats had greater collagen content, according to Shivananda N B *et al.* They discovered a rise in hydroxyproline, an amino acid that is found in large amounts in collagen<sup>9</sup>. Although *Hibiscus rosa sinensis* flower does not have any antibacterial properties of its itself, its wound-healing properties can be linked to its many hormonal actions, which include hypoglycemic, androgen-like, antioxidant and anticonvulsant properties<sup>6</sup>. The higher tensile strength, improved wound contraction and reduced epithelization duration all point to *Hibiscus rosa sinensis* flower extract having a wound healing-promoting activity.

$\beta$ -glucans have a wide range of biological activities that improve human immunity. The use of  $\beta$ -glucans for topical treatments is on the rise, thanks to their pluripotent qualities. During wound healing, the main target cells of  $\beta$ -glucans are macrophages, keratinocytes and fibroblasts.  $\beta$ -glucans aid wound healing by promoting macrophage infiltration, which promotes tissue granulation, collagen deposition and re-epithelialization.  $\beta$ -glucan wound dressings are a good wound healing agent because they are stable and resistant to wound proteases. Based on a fore said independent properties of *Hibiscus rosa sinensis* flower extract and  $\beta$ -glucan, combining the two in a multi-modal treatment method considerably improved wound healing. *Hibiscus rosa sinensis* flower extract improved wound contraction impact and epithelization could be used clinically to aid in the healing of open wounds. However, a well-designed clinical assessment will be required to confirm this suggestion.

**Table No.1: Grouping for excision wound model**

S.No	Group	Total no. of rats	Treatment
1	I (Control)	6	Untreated, saline
2	II (Standard)	6	10% w/w betadine topical ointment
3	III (Test 1)	6	50mg of ointment prepared from 5% of ethanolic extract of <i>HRS</i>
4	III (Test 2)	6	5% ointment of <i>HRS</i> +BG 80mg/kg, po

**Table No.2: Grouping for incision wound model**

S.No	Group	Total no. of rats	Treatment
1	I (Control)	6	Untreated, saline
2	II (Standard)	6	10% w/w betadine topical ointment
3	III (Test)	6	50mg of ointment prepared from 5% of ethanolic extract of <i>HRS</i>
4	III (Test 2)	6	5% ointment of <i>HRS</i> +BG 80mg/kg, po

**Table No.3: Wound healing effect of *HRS*+  $\beta$ -glucans in Incision wound model**

S.No	Parameter	Placebo control	Standard	<i>HRS</i>	<i>HRS</i> + $\beta$ -glucans
1	Skin breaking strength (g)	319.14±3.23	465.0 ±4.63**	421.0 ± 4.43**	471.0 ± 4.41**

N = 6, Values are expressed as mean ± SD

\*p < 0.05 and \*\*p < 0.001 vs. control. Independent t-test.

**Table No.4: Wound healing effect of *HRS*+  $\beta$ -glucans in Excision wound model**

S.No	Parameter	Placebo control	Standard	<i>HRS</i>	<i>HRS</i> + $\beta$ -glucans
Wound area (mm <sup>2</sup> ):					
1	Day 1	226.3±23.80	235.50 ± 12.7	232.50 ± 14.7	235.50 ± 12.7
2	Day 5	183.6 ± 22.8	174.17±32.58	183.17 ± 31.58	172.16 ± 32.58
3	Day 15	128.8±25.90	67.41±24.8**	65.41 ± 23.8 **	65.40 ± 24.8 **
4	Period of epithelization (day)	14.7 ± 0.10	9.33 ± 0.14**	10.21 ± 0.14**	9.20 ± 0.14**

N = 6, Values are expressed as mean ± SD

\*\*P < 0.001 vs. control. Independent t-test.

## CONCLUSION

*Hibiscus rosa sinensis* flower extract +  $\beta$ -glucan has characteristics that make it capable of boosting rapid wound healing activity as compared to placebo controls, according to the current investigation. Wound contraction and enhanced tensile strength necessitate additional research into the topical therapy and management of wounds using *Hibiscus rosa sinensis* flower extract +  $\beta$ -glucan.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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