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**Evaluation of wound healing activity of herbal drug combination**  
**of *Rubia cordifolia*, *Centella asiatica*, *Terminalia***  
***belerica*, *Plumbago Zeylanica* and *Withania somnifera***

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**Abstract**

A herbal drug combination of *Rubia cordifolia*, *Centella asiatica*, *Terminalia belerica*, *Plumbago zeylanica* and *Withania somnifera* was evaluated for wound healing activity, on excision wound of albino rats. Wound healing activity of the plant was evaluated by formulating the drug in ointment dosage form and then compared with a marketed formulation (Soframycin cream) as reference drug. The parameters studied includes the percentage closure of excision wound, period of epithelization. The herbal drug combination has been observed to promote healing of wounds in animals. The results of analysis were validated statistically and by recovery studies.

**Key-Words:** Wound healing, Herbal drug, Evaluation

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**Introduction**

Wound healing involves a complex process including induction of acute inflammation by the initial injury, followed by parenchymal and mesenchymal cell proliferation, migration, and activation with production and deposition of extracellular matrix. Out of the different types of wounds, excision wound is considered to be important for research. Plant kingdom consists of number of medicinal plants claimed to be useful in wound healing. A herbal drug combination of *Rubia cordifolia*, *Centella asiatica*, *Terminalia belerica*, *Plumbago zeylanica* and *Withania somnifera* was investigated on albino rats for its effect on healing of excision wound.

Normally on wound site various mechanisms of body participate in wound healing i.e. white blood cell<sup>1</sup>, fibroblasts, keratinocytes Cytokines, etc. while carbohydrates<sup>2</sup> lipids<sup>3</sup> and protein<sup>4</sup> metabolism increases with the increase in the resting energy expenditure {RES}. Wound healing is also affected by other diseases such as diabetes etc, antineoplastic drug and antibiotics may also interfere with the wound healing.

The herbal drug combination of *R.cordifolia*, *C.asiatica*, *T.belerica*, *P.zeylanica*, and *W.somnifera* was formulated in the form of ointment and evaluated for wound healing activity by the evaluation of histology, the period of epithelization, and wound-contraction parameters. Two types of creams was prepared as medicated cream (drug) and non-medicated cream (only base cream) by using all purpose type of cream formula represent in Table I.

**Material and Methods**

**Plant materials:**

The plants were collected and were identified by Taxonomist.

**Animals:** Healthy male albino rats weighing between 150-250 gm were used in studies. They were individually housed and maintained on normal diet, water, and vitamin B tablets.

**Wound Model:** Partial thickness excision wound of 1.5cm (150mm<sup>2</sup>) diameter were inflicted on starved animals under light chloroform anesthesia with a sterile rod. After complete wounding, wound was washed and cleaned out with normal saline solution and the medicated cream was immediately applied.

**Experimental Protocol:** Animals bearing partial thickness wound were distributed into various groups such as control, standard and drug treated means A, B & C group. Each group had six animals.

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**Assessment of Wound Healing:** Animals were inspected daily up to 20<sup>th</sup> days and healing was assessed based on physical parameter namely, wound contraction, period of epithelization and histological study.

**Wound Contraction:** It was studied by tracing the raw wound area on transparent polythelene paper on a day 5<sup>th</sup>, 10<sup>th</sup> & 15<sup>th</sup> post-wounding day. Later the area was assessed using a graph paper. The wound contraction was measured as the percentage decrease of original wound size for each animal.

**Re-epithelization:** The end point of complete epithelization and the days required for this process was taken as period of epithelization.

**Histopathology:** On 10<sup>th</sup> day some of animals under each group were sacrificed and wounds were excised together with surrounding skin. The 5  $\mu$  thin paraffin section of wounds bed material were fixed in 10% neutral buffer formalin and histological evaluation was performed on heamatoxylin and eosin {HE} stain.

After complete staining the slides, microscopic photographs of collagen tissue were taken as were shown in the figure for control, nonmedicated and medicated cream formulations.

**Statistical Analysis:** Results are reported as means  $\pm$  SEM. The data was analyzed with Unpaired Student's 't' test in case of control, medicated & nonmedicated cream A, B & C animal groups.

Percentage closure of the original excision wound area i.e. 200mm<sup>2</sup>. At different time interval {Mean + S.R} in control, base and drug formulation treated is represented in Table II.

Percentage closure of the original excision wound area 200 mm<sup>2</sup> for control was 17%, 64% & 84% and 28%, 82% & 95% for drug formulation on 5<sup>th</sup>, 10<sup>th</sup> & 15<sup>th</sup> post wounding day which represents significant healing of wound with drug the herbal drug combination of *R.cordifolia*, *C.asiatica*, *T.belerica*, *P.zeylanica*, and *W.somnifera*. However, wound treated animals showed signs of advanced healing such as complete restoration of epidermis, well-organized high amounts of collagen bundles in dermis.

### Results and Conclusion

The results of present study shows that cream formulation of the herbal drug combination of *R.cordifolia*, *C.asiatica*, *T.belerica*, *P.zeylanica*, and *W.somnifera* was formulated promotes contraction and epithelization of excision wound.

The pharmacological action of *R.cordifolia* are antimicrobial, anti-inflammatory, antioxidant and antifungal activity<sup>5</sup>, *C.asiatica* are anti-inflammatory, wound healing, immunomodulatory activity<sup>6</sup>,

*T.belerica* are antimicrobial, antioxidant and antidiabetic activity<sup>7,8</sup>, *P.zeylanica* are antiplasmodial, antimicrobial, antifungal and antihyperglycemic activity<sup>9</sup>, *W.somnifera* are antitumor, antibacterial and anti-fungal activity<sup>10</sup>.

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Table I: Formula for All Purpose Type of Cream

Ingredients	Non medicated cream formulation	Medicated cream formulation
<b>PART A</b>		
Cetyl alcohol	2.0 gm	2.0 gm
Glyceryl monostearate	7.5 gm	7.5 gm
Mineral oil	7.5 gm	7.5 gm
Isopropyl palmitate	1.0 gm	1.0 gm
Polyethylene glycol	12.5 gm	12.5 gm
<b>PART B</b>		
Sodium lauryl sulphate	2.0 gm	2.0 gm
Glycerin	5.0 gm	5.0 gm
Propyl glycol	3.0 gm	3.0 gm
Dist. Water	61.3 gm	Herbal drug combination of <i>R.cordifolia</i> , <i>C.asiatica</i> , <i>T.belerica</i> , <i>P.zeylanica</i> , and <i>W.somnifera</i>
Methyl paraben	0.15 gm	0.15 gm
Propyl paraben	0.05 gm	0.05 gm

Table II: Base and Drug Formulation Treated

Drug{n}	Period of epithelization {days}	Wound contraction {%} on a days		
		5 <sup>th</sup>	10 <sup>th</sup>	15 <sup>th</sup>
Control (6)	42.66 ± 2.10	17.8 ± 1.2	64.0 ± 3.0	84.66 ± 1.96
Base {nonmedicated} (6)	39.83 ± 0.30	22.06 ± 0.72	69.55 ± 0.4	90.6 ± 0.74
Herbal drug combination {medicated} (6)	38.66 ± 1.54	28.16 ± 1.87	82.43 ± 3.0	95.00 ± 2.54