

**COMPARATIVE WOUND HEALING ACTIVITY OF DIFFERENT LEAF EXTRACTS OF *LANTANA CAMARA LINN.*****JAIN SHONU\* AND JOSHI AMIT**

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**ABSTRACT**

*Lantana camara Linn.* is a heavily branched shrub that can grow in compact clumps, dense thickets or as a climbing vine. and has covered large areas in India, Australia. and much of Africa. In order to evaluate the wound healing activity of *Lantana camara L.*, two different solvent extracts were prepared from the leaves of the plant. Ethyl acetate and Ethanol were used for the extraction of the active ingredients. Excision wound model on Wistar albino rats of 4 months, of both sexes, weighing between 100 to 150 gm was used to assess the wound healing activity of the leaves. Remarkable wound healing activity was observed with the ointment formulation of the ethanol extract at 2% concentration. Wound contraction was calculated as percentage of the reduction in wound area. A specimen sample of tissue was isolated from the healed skin of each group of mice for the histopathological examination, the results of which also supported the outcome of excision wound models. The present study demonstrated that the aerial parts of *Lantana camara L.* promote wound healing activity in mice as a preclinical study.



## KEYWORDS

Lantana camara Linn, Excision wound model, wound healing activity, histopathological examination.

## INTRODUCTION

Herbal medicine has been described as the oldest form of therapy practiced by humans today, with archaeological evidence of medicinal use of herbs dating back to 60,000 years. Herbal medicines often complement conventional treatments, providing safe, well tolerated remedies for chronic illness. World Health Organization (WHO) has also recognized potential of herbal medicines and according to WHO - "Any plant and its parts containing any substance which can be used therapeutically, or can be used as raw material for Chemical/Pharmaceutical synthesis is categorized as herbal medicine."<sup>1,2</sup>

*Lantana* is a heavily branched shrub that can grow in compact clumps, dense thickets or as a climbing vine. The stems are square in cross section, with small, recurved prickles. Most leaves are about 6 cm long and are covered in fine hairs. They are bright green above, paler beneath and have round-toothed edges. Leaves grow opposite to one another along the stem. When crushed, the leaves produce a distinctive odor. Flowers appear throughout most of the year in clustered, compact heads about 2.5 cm in diameter. Flower colors vary from pale cream to yellow, white, pink, orange and red. *Lantana* produces round, berry-like fruit that turn from glossy green to purplish-black when ripe<sup>3</sup>. The ethanolic extract of *Lantana camara* leaves heals gastric ulcers and also prevents development of duodenal ulcers in rats. Extracts of the fresh leaves are antibacterial and are traditionally used in Brazil as an antipyretic, carminative, in the treatment of respiratory system infections, Anti Diabetic, Anti spasmodic, Diaphoretic, Carminative, Tonic and are useful in the treatment of typhus<sup>4</sup>.

Wounds are usually caused by cuts or scrapes. Wounds may arise due to physical, chemical or microbial agents. Healing is survival mechanism and represents an attempt to maintain normal anatomical structure and function. Wound healing is a process by which tissue regeneration occurs. It is complex, dynamic process of restoring integrity and tissue layer, which involves an array of inter related and concomitant events. Wound healing involves a complex series of interactions between different cell types, cytokine mediators, and the extracellular matrix. The phases of normal wound healing include hemostasis, inflammation, proliferation, and remodeling. Each phase of wound healing is distinct, although the wound healing process is continuous, with each phase overlapping the next. Because successful wound healing requires adequate blood and nutrients to be supplied to the site of damage, the overall health and nutritional status of the patient influences the outcome of the damaged tissue<sup>5</sup>.

## MATERIALS AND METHODS

### *Plant Material and extract preparation*

The leaves of the plant *Lantana camara* Linn were procured from TIT College of Pharmacy campus Bhopal M.P. The collected, cleaned leaves of *Lantana camara* Linn were used for the extraction process. 200g of powder of Leaves were evenly packed in the soxhlet apparatus and extracted with methanol and ethyl acetate solvent respectively by hot continuous extraction process for about 26 hr. and porcelain bits were placed to avoid the bumping during extraction in round bottom flask.

The Soxhlet apparatus (with graduated) receiving the extract in round bottom flask. The extracts were filtered, while hot, through whatmann filter paper to remove any impurities, if present. The extracts were concentrated by vacuum distillation to reduce the volume 1/10. The concentrated extracts were transferred to 100 ml beaker and the solvent were removed by evaporating on the water bath. Then they were collected and placed in desiccator to remove the excessive moisture. The dried extracts were packed and labeled in air tight container for the further studies such as a phytochemical screening and pharmacological activities.<sup>6</sup>

### **Animal care and handling**

The experiment was carried out on Wistar albino rats of 4 months, of both sexes, weighing between 100 to 150 gm. They were provided by Sapience Bio-analytical Research Lab, Bhopal, (M.P.). The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature  $25\pm 2^{\circ}\text{C}$  relative humidity 44–56% and light and dark cycles of 12:12 hours, fed with standard pallet diet and water ad libitum during experiment. The experiment was approved by the institutional ethics committee and as per CPCSEA guidelines (approval no. 1413/a/11/CPCSEA).

### **Requirements**

Scale, Surgical blades (No.18), Xylocain (Local anesthetics), Annie French (Hair remover cream), Forceps, Cotton.

### **Formulation of the ointment**

2% w/w ointment of Ethanolic and Ethyl acetate extract of *Lantana camara* were prepared by using carbopol 934P by allowing it to swell in distilled water over night at room temperature. Then 0.5 gm of ethanolic or ethyl acetate extract of *Lantana camara* was added by mixing vigorously and sufficient amount of water was added for better cream like

preparation. Finally ointments were prepared and stored in cool place.

### **Experimental Design**

In the experiment, a total of 16 rats were used. The rats were divided into 4 groups comprising 4 animals in each group as follows:

**Group I:** Left untreated and considered as control.

**Group II:** Served as reference standard and treated with 5%w/w ointment Intadine.

**Group III:** Treated with 2% w/w Ointment of Ethanolic extract of *Lantana camara*.

**Group IV:** Treated with 2% w/w Ointment of Ethyl acetate extract of *Lantana camara*.

### **Excision wound model**

The wound was created using excision method. For this hair were removed from the posterior sides of rats using hair remover cream. An area of 314 mm<sup>2</sup> was measured with sterile scale and this area was marked with a marker pen. The marked area was anesthetized using a local anesthesia xylocain. After 2 minutes of applying xylocain the marked area of skin was excised with the help of surgical blade no. 18 and forceps. The skin was removed after creating the wound 314 mm<sup>2</sup>.

The Ointment of extracts and Intadine were topically applied once a day, starting from the day of the operation, till complete epithelialisation time. The parameters studied were wound closure and epithelialisation time. The wound was measured using transparency paper, a marker, scale and area was calculated. The period of epithelialisation was calculated as the number of days required for falling of the dead tissue remnants of the wound without any residual raw wound.<sup>7,8</sup>

### **Histopathological studies**

Normal skin removed from sacrificed mice was immediately fixed in 10% formalin fixative for 24h. The tissues were

then dehydrated in ascending series of alcohol, kept in 1:1 mixture of absolute alcohol and Xylene and then in Xylene for 1h each. Finally, tissue pieces were embedded in paraffin wax and 7 micron thick sections were cut and spread on glass slides, stained with hematoxylin and eosin, The sections were dehydrated with Canada balsam on the slides carefully with glass rod, covered with cover slip, and viewed under light microscope and photographed.<sup>9</sup>

**Statistical analysis:**

All the values are expressed as mean±standard error of mean (S.E.M.) and analyzed for ANOVA and posthoc Dennett’s test by employing statistical software, Graph Pad in Stat 3. Differences between groups were considered significant at  $P < 0.01$  levels.<sup>7, 8</sup>

**RESULTS AND DISCUSSIONS**

**1. Wound area**

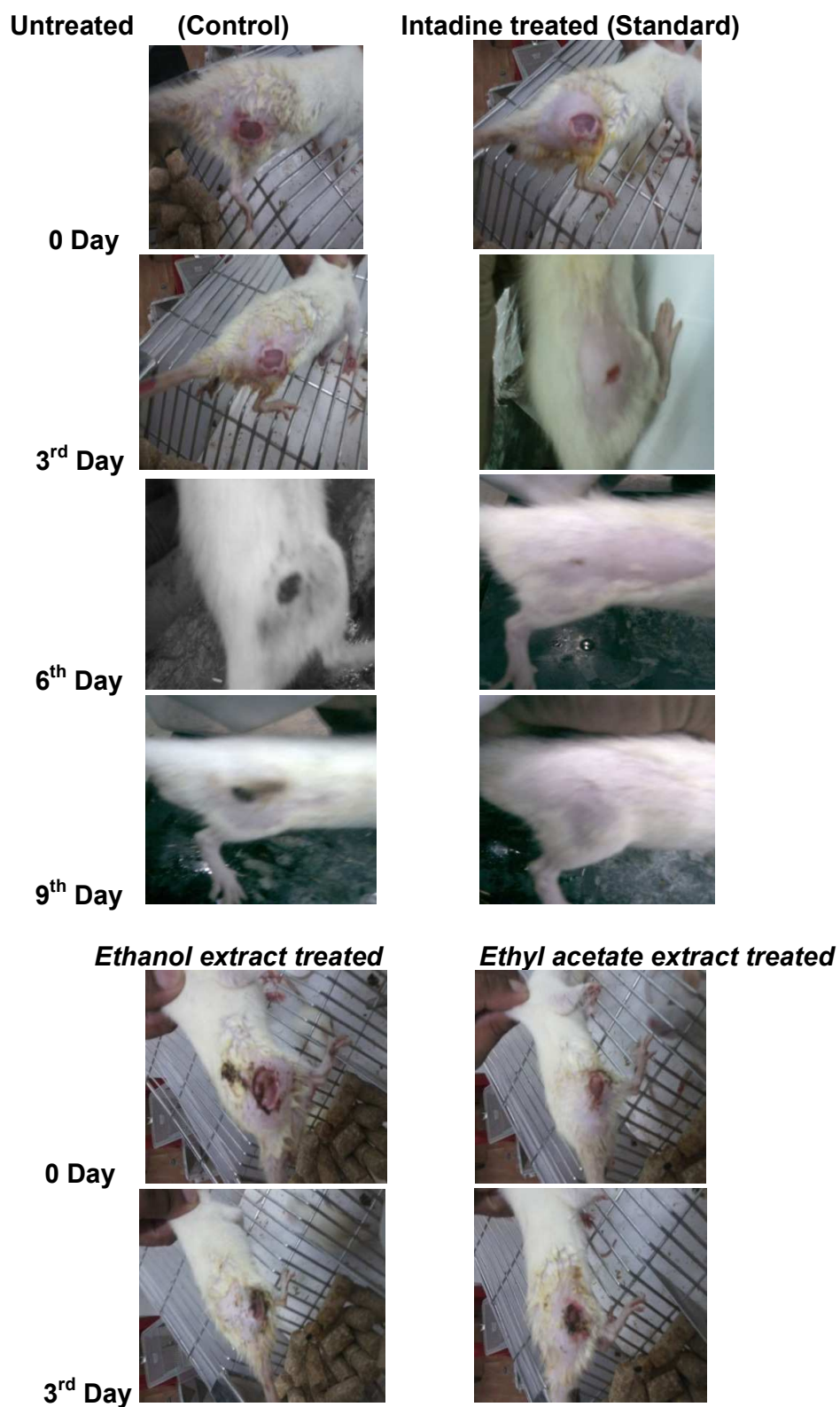
Significant wound healing activity was observed in animals treated with *Lantana camara* extracts compared with those who received the standard and control treatment. In excision wound model, *Lantana camara* extract treated animals showed a significant reduction in wound area and period of epithelisation. The mean percentage wound closure was calculated on the 3<sup>rd</sup>, 6<sup>th</sup> and 9<sup>th</sup> wounding days as shown in table No.1. The ethanolic and ethyl acetate extracts treated animals showed faster epithelisation of wound (10.34±0.11 and 11.86±0.17) than the control. The period of epithelisation was 9.14±0.21 in case of standard drug 5% Intadine ointment.

**Table 1**  
**Effect of topical application of 2%w/w Ointment of Ethanolic and Ethyl acetate extract of *Lantana camara* on Wound healing**

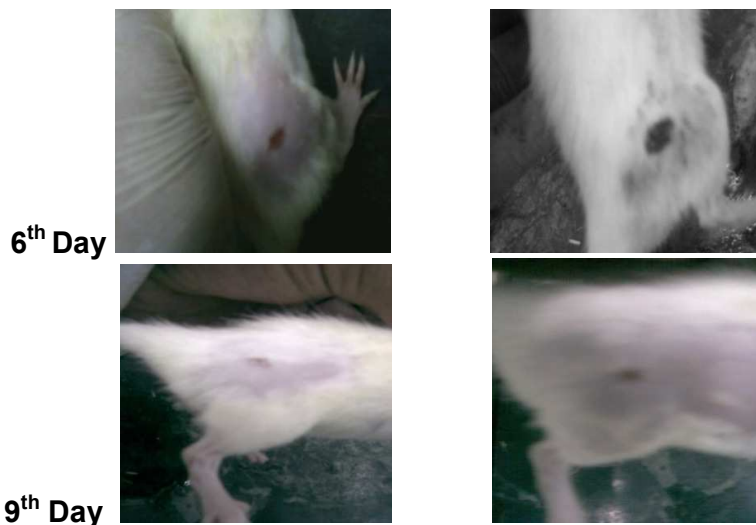
Groups	Wound Closure (mm) <sup>2</sup>			Epithelisation period (Days)
	3 <sup>rd</sup> day	6 <sup>th</sup> day	9 <sup>th</sup> day	
Control (Untreated)	103.93±6.80	173.67±5.00	217.58±5.64	13.23±0.57
Standard (Intadine treated)	193.99±6.96*	298.06±4.03*	314.46±0.31*	9.14±0.21*
Ethanolic extract of <i>Lantana camara</i>	166.53±3.34*	262.21±1.37*	312.74±0.20*	10.34±0.11*
Ethyl acetate extract of <i>Lantana camara</i>	146.53±3.34*	232.21±1.37*	310.74±0.20*	11.86±0.17*

Values are mean ± SEM from a group of four animals. \* $p < 0.01$  when all treatment groups compared to control group

**Figure 1**  
**Photographs of mice taken on Day 0, 3, 6 and 9.**







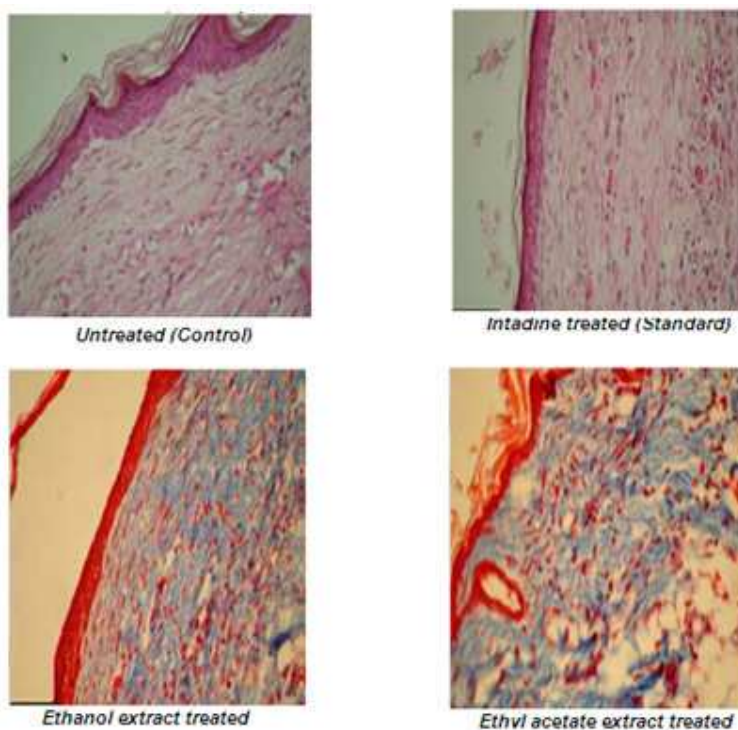
## 2. Histological studies

Histological evaluation was carried out for the treated and untreated samples. There was a marked infiltration of the inflammatory cells, increased blood vessel formation and enhanced proliferation of cells as a result of treatment with different Leaf extracts of *Lantana camara*. There was full thickness reepithelialisation, in which epidermis was thin and well organized, comparable to the normal adjacent skin which was not involved in the wound generation and healing process. The granular layer was well formed and one cell in thickness. All the animals when treated with standard (Intadine)

ointment and prepared ointment of ethanolic and ethyl acetate extract showed comparable results when compared with control. There was a full thickness epidermal regeneration which completely covered the wound area. The epidermis was thick and disorganized, especially when compared with the adjacent normal skin. In all, complete epithelialisation, vasculirisation and hair follicles formation were observed in treated mice.

Early dermal and epidermal regeneration in treated mice also confirmed that the extract had a positive effect towards cellular proliferation, granular tissue formation and epithelialisation.

**Figure 2**  
**Photographs of Histological studies.**



## CONCLUSION

In vivo studies were performed for wound healing potential by using excision model. In our study, 2%w/w ointment of the both ethanolic and ethyl acetate extract of *Lantana camara* significantly increased the rate of wound closure and rate of epithelisation. The wound was healed significantly as compared to standard Intadine. The drug has equally shown better and similar wound healing property as compared with Intadine. The constituents present in the *Lantana camara* may be responsible for promoting the wound healing activity. The Wound healing activity of Ethanol extract of *Lantana camara* L. Leaves is better in comparison to ethyl acetate extract.

Histological evaluation shows there was a marked infiltration of the inflammatory cells, increased blood vessel formation and enhanced proliferation of cells as a result of treatment with *Lantana camara* Leaf extract.

As infections being a major cause of morbidity and mortality in wound patients, these herbal extracts may prevent infection that leads to high risk of sepsis, and thereby prevents the prolongation of inflammatory phase. Further study on the fractionation of active components and the mutual effect of these plant extract machinery on infecting microbial species may provide a better understanding of the infection management in the process of wound healing.

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