

EFFECT OF THE TOPICAL APPLICATION OF THE EXTRACT OF *GLYCOSMIS PENTAPHYLLA* ON EXCISIONAL WOUND MODEL IN MICE.**Megha Jha^{1*}, Versha Sharma², Nitin Nema³, Tahziba Hussain⁴**¹Department of Research, Jawaharlal Nehru Cancer Hospital and Research Centre, Idgah hills, Bhopal (M.P.), 462001 India²Department of Zoology, Dr. H. S. Gour Vishwavidyalaya, Sagar-470 003, Madhya Pradesh, India³Department of Pharmaceutical Sciences, Dr. H. S. Gour Vishwavidyalaya, Sagar-470 003, Madhya Pradesh, India⁴Central JALMA Institute for Leprosy & Other Mycobacterial Diseases (ICMR), Agra, IndiaAddress for correspondence-* Email: meghajhabtbpl@gmail.com, megha_jha_bt_bpl@yahoo.com**Summary**

Glycosmis Pentaphylla is an aromatic shrub found in southern part of India. On the basis of its traditional use and literature references, this plant was selected for the screening of wound healing activity. The methanolic extract of leaves of *Glycosmis Pentaphylla*, in the form of an ointment with two different concentrations (10% and 15% w/w ointment of leaf extract in simple ointment base) was evaluated for wound healing potential in an excision wound model in rats. It is observed that the wound contraction ability of the ointment containing *Glycosmis Pentaphylla*, 10% and 15% was significantly greater than that of the control (simple ointment). The 15%w/w extract containing ointment group showed significant wound healing which was comparable to that of the nitrofurazone ointment treated animals. The wound closure time was less and the percentage of wound contraction was much more with the 15%w/w extract ointment treated group. On 18th day 100% contraction was observed which was almost similar to that of the nitrofurazone ointment group. 10%w/w extract ointment group of animals showed significant wound healing activity and achieved 98.51% wound contraction. Both concentrations of the methanolic extract of *Glycosmis Pentaphylla*, ointment showed significant responses when compared with the control group. Thus, it was concluded that *Glycosmis Pentaphylla* leaf extract applied topically (10 % and 15 % extract ointment) possesses wound healing activity.

Key words: *Glycosmis Pentaphylla*, nitrofurazone, ointment, wound healing.**Introduction**

Natural products are a source of synthetic and traditional herbal medicine and are still the primary health care system¹. An important aspect of the use of traditional medicinal remedies and plants in the treatment of burns and wounds is the potential to improve healing and the same time to reduce the financial burden². Wound occurs when the continuity of the skin or mucous membrane is broken³. Wound healing is a dynamic process that begins at the moment of wounding. It is essentially a survival mechanism and represents an attempt to maintain normal anatomical structure and function⁴. Wound healing occurs in three stages: inflammation, proliferation, and remodeling. The proliferative phase is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction⁵. Normal wound healing response begins the moment the tissue is injured. The healing cascade begins immediately following injury when the platelets come into contact with exposed collagen.

As platelet aggregation proceeds, clotting factors are released resulting in the deposition of a fibrin clot at the site of injury. The fibrin clot serves as a provisional matrix and sets the stage for the subsequent events of healing. The inflammatory cells also arrive along with the platelets at the site of injury and they provide key signals are known as cytokines or growth factors⁶. In normal tissues collagen provides strength, integrity and structure. Collagen, the major component which strengthens and supports extracellular tissue, contains substantial amounts of hydroxyproline, which has been used as a biochemical marker for tissue collagen⁷.

Glycosmis Pentaphylla is an aromatic shrub with few branches, grows from 0.5 to 2meter in height. The leaves are compound, imparipinnate⁸. The whole plant *Glycosmis Pentaphylla* is used in anemia, jaundice and rheumatism⁹. *Glycosmis Pentaphylla* plant is most commonly used in Ayurveda and homoeopathic system of medicine for the treatment of various disease condition and other problems. In homoeopathy it is used as antimal, tonic and in biliary colic, worm colic, diarrhea and dysentery while ethanolic extract (50%) of arial part is used as spasmolytic and diuretic¹⁰. The leaf is applied in the form of paste to eczema and other skin diseases¹¹. However to the best of our knowledge a systematic study on wound healing activity of *Glycosmis Pentaphylla* has not been undertaken. Hence, the present study was undertaken to evaluate the wound healing property of alcoholic extract of *Glycosmis Pentaphylla*.

Materials and Methods

Plant Material: The plant material used in the study, *Glycosmis Pentaphylla* (Rutaceae) was collected from Southern region of India and botanist of MVM College, Bhopal identified the plant. The impurity from plant was removed and leaves were separated from the plant. The leaves of plant were shadow dried at room temperature and powered by mechanical grinder. The powder was sieved by 20mm and 40mm sieve and the intermediate was used for extraction.

Extract Preparation: The powdered plant material was continuously extracted using soxhlet apparatus with petroleum ether to remove oils and fats present. The powder as then extracted with methanol for 24 hrs. After complete extraction, methanolic extract of *Glycosmis Pentaphylla* extract was concentrated under a vacuum to obtain a thick extract that was then dried in a hot air oven to get a free flowing powder.

Ointment Preparation: Preparation of 20g simple ointment base: Wool fat (1g), hard paraffin (1g), cetostearyl alcohol (1g) and white soft paraffin (17g) was mixed and heated gently with stirring then cooled.

- a. 2 gm methanolic leaf extract of *Glycosmis Pentaphylla* was added separately to 20gm of base (10% ointment).
- b. 3 gm methanolic leaf extract of *Glycosmis Pentaphylla* was added separately to 20gm of base (15% ointment)¹².

Experimental animals: 24 young male albino wistar rats weighing between 150-200 grams were used. The rats were divided into four groups (n=6). The animals were kept in separate cages, maintained in air conditioned animal house and fed on a standard diet of mouse food and water ad libitum, accordance with institutional regulations and national criteria for animal experiments at Jawahar lal Nehru Cancer Hospital and Research Center. The project was approved by CPCSEA norms.

Wound healing activity: The surgical materials were sterilized and dorsal fur of the animals was shaved with an electric clipper. The rats were anesthetized with (Xylocaine®) 2% Jelly, Astra Zeneca Pharma India ltd and anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using stencil. A full thickness of the excision wound of circular area of 500mm² and 2mm depth was created along the markings using toothed forceps, scalpel and pointed scissors. The simple ointment, standard drug (0.2% w/w nitrofurazone ointment), methanolic extract ointment 10% w/w of *Glycosmis Pentaphylla* and 15% w/w of *Glycosmis Pentaphylla* were applied topically everyday till the wound was completely healed.

24 animals were divided into groups of four and treated as follows:

Group 1: Simple ointment base was applied once daily and served as vehicle control.

Group 2: Standard drug nitrofurazone ointment (0.2%w/w) was applied once daily (positive control).

Group 3: *G.Pentaphylla* methanolic extract ointment (10%w/w) was applied once daily.

Group 4: *G.Pentaphylla* methanolic extract ointment (15% w/w) was applied once daily

All the above mentioned treatments were started from the day of wound creation and continued till wound was completely healed. The observation of the % wound closure were recorded on day 3th, 6th, 9th, 12th, 15th and 18th and thereafter on alternate days until healing was complete. The changes in healing of wound i.e. measurement of wound on graph paper was expressed as unit (mm²). The percentage of wound closure was calculated. The period of epithialisation was calculated as number of days required for falling the dead tissue remnants of the wound without any residual raw wound. Wound contraction was expressed as percentage reduction of original wound size.

Observations and Results

The mean percentage closure of wound area was calculated on the 0, 3, 6, 9, 12, 15 and 18 post wounding days as shown in Table.No.1. The *G.Pentaphylla* methanol extract 15%w/w Ointment treated animals showed faster epithialisation of wound (17.86± 0.19) then the animals treated with *G.Pentaphylla* methanol extract 15%w/w Ointment (19.03 ±0.59). The period of epithialisation was (16.15± 0.21) in case of standard drug nitrofurazone ointment and (24.29±0.23) in control.

G.Pentaphylla methanol extract 15%w/w ointment treated animals and *G.Pentaphylla* methanol extract 10%w/w ointment animals showed significant wound healing activity (% wound contraction) on 18th day (100% and 98.51, $P < 0.001$) compared to control (82.74, $P < 0.001$).

Discussion and Conclusion

Wound contraction is the process of shrinkage of the area of the wound. The progress of the wound healing induced by *G.Pentaphylla* leaves extract ointment (10% and 15% w/w) as treated groups, simple ointment as control group and nitrofurazone ointment as standard group were shown in Graph No. 1. It is observed that the wound contraction ability of the ointment containing *G.Pentaphylla* extract in different concentrations was significantly greater than that of the control (simple ointment). The 15%w/w methanolic leaf extract containing ointment group showed significant wound healing activity which was comparable to that of the nitrofurazone ointment treated animals. The wound closure time was lesser, as well as the percentage of wound contraction was much more with the 15%w/w methanolic leaf extract ointment treated group.

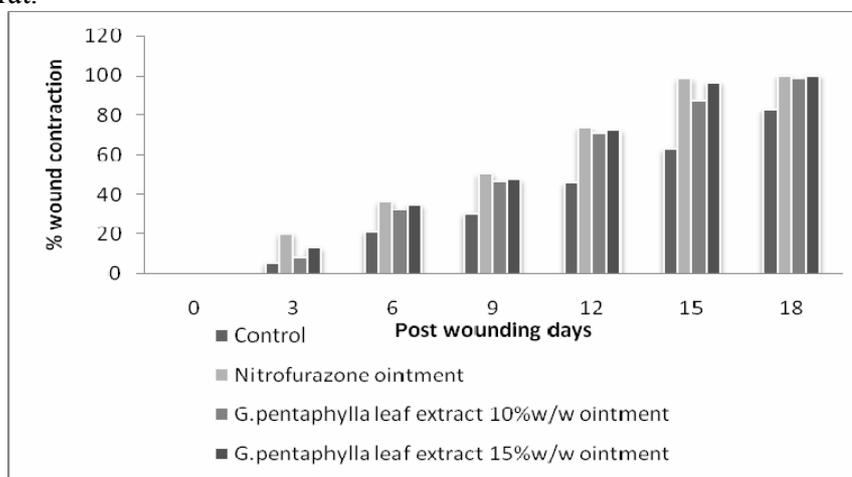
On 18th day 100% contraction was observed in 15% w/w treated group which were almost similar to that of the nitrofurazone ointment group. 10%w/w extract ointment group of animals showed significant wound healing activity on 18th day and achieved 98.51% wound contraction. Our data demonstrate that the 15%w/w methanolic leaf extract of *G.Pentaphylla* may be capable of promoting wound healing activity. However, further investigation required to elucidate their exact mechanism of wound healing activity.

TABLE NO.1: Effect of *G.Pentaphylla* leaves extract ointment (10% and 15% w/w) and Nitrofurazone (0.2%w/w) ointment on healing of excision wound in albino rats.

*indicates significant difference at $P < 0.001$ when compared to control. Values are Mean \pm SEM from 6 animals in each group. Data is analyzed by one way ANOVA

Post wounding days	Simple ointment	Nitrofurazone Ointment (0.2%w/w)	<i>G.Pentaphylla</i> extract 10%w/w Ointment	<i>G.Pentaphylla</i> extract 15%w/w Ointment
0	512.9 \pm 0.46 (0.00)	513 \pm 1.48* (0.00)	507.68 \pm 2.12* (0.00)	509.81 \pm 1.51* (0.00)
3	485.53 \pm 1.49 (5.34)	410.30 \pm 0.61* (20.13)	466.69 \pm 1.49* (8.48)	445.20 \pm 1.18* (12.89)
6	405.21 \pm 1.14 (21.03)	326.59 \pm 1.31* (36.35)	345.40 \pm 0.54* (32.22)	333.64 \pm 0.58* (34.82)
9	358.62 \pm 0.58 (30.14)	254.37 \pm 0.53* (50.51)	273.26 \pm 0.54* (46.46)	270.25 \pm 0.55* (47.27)
12	279.85 \pm 0.72 (45.72)	138.35 \pm 0.47* (73.26)	150.46 \pm 0.57* (70.69)	143.40 \pm 0.43* (72.28)
15	192.16 \pm 0.54 (62.65)	11.32 \pm 0.44* (98.17)	68.22 \pm 0.60* (86.93)	20.50 \pm 0.43* (96.36)
18	89.32 \pm 0.50 (82.74)	0* (100)	9.50 \pm 0.43* (98.51)	0* (100)
Period of epithelialisation	24.29 \pm 0.23	16.15 \pm 0.21*	19.03 \pm 0.59*	17.86 \pm 0.19*

Graph No: 1 Graph shows the effect of *G.Pentaphylla* leaves extract ointment (10% and 15% w/w) and Nitrofurazone (0.2%w/w) ointment on healing of excision wound in albino wistar rat.



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