Original Article

# Preparation and Evaluation of Wound Healing Activity of New Polyherbal Formulations in Rats

**Ansar M. Patel*[1], S.M. Kurbetti[2], R.V.Savadi[3], V.A. Thorat [4], V. V. Takale [5], S.V. Horkeri[6]**

[1]Research Scholar, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan-333001, India.
[2]Dept. of Pharmacognosy and Phytochemistry, SGMR Pharmacy College Mahagaon, Gadhinglaj, Kolhapur, Maharashtra, India.
[3]Principal, Arvind Gavali College of Pharmacy, Satara, Maharashtra, India.
[4]Dept. of Pharmacognosy and Phytochemistry, SGMR Pharmacy College Mahagaon, Gadhinglaj, Kolhapur, Maharashtra, India.
[5]Research Scholar, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan-333001, Maharashtra, India.
[6]Dept. of Pharmacognosy and Phytochemistry, SGMR Pharmacy College Mahagaon, Gadhinglaj, Kolhapur, Maharashtra, India.

**Address for Correspondence**

Research Scholar, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu, Rajasthan-333001, India.

Tel:+91-9860002258
**E-mail:** ansarpatel2007@rediffmail.com

## ABSTRACT

The aim of present study was to evaluate the wound healing activity of newly prepared polyherbal formulations ( i ,e Ointment, Gel and Liquid ) in Excision and Incision wound models in rats. The wound-healing activity was assessed by the rate of wound contraction, period of epithelialization and skin-breaking strength. In Excision wound model the polyherbal Ointment treated group exhibit 99.78±0.11% reduction in wound area on 20$^{th}$ day when compared to standard Betadine Ointment and controls which was 98.55±0.44 % and 90.12±0.27% and faster rate of epithelization (16.55 ± 0.46). In the incision wound model, there was a significant increase in tensile strength (538.10± 1.52) was observed in ointment treated group. In all cases, there was a progressive decrease in wound area with time, indicating an efficacy of the formulations in healing the induced wounds. Our present study reveals that the new polyherbal formulations posses potent wound healing activity, which could be a good choice of remedy for wound healing.

**Keywords**: Incision wound model, Excision wound model, Polyherbal Formulations, Betadine Ointment.

## INTRODUCTION

Wounds may be defined as cut or breaks in continuity of any tissue. Proper healing of wounds is essential for the restoration of disrupted anatomical

continuity and disturbed functional status of the skin[1]. Wound may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue [2]. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Restoration of damaged tissue, wound or fracture is an important process which play a vital role in survival of life [3]. Wound healing involves continuous cell–cell and cell–matrix interactions that allow the process to proceed in three overlapping phases viz. inflammation (0–3days), cellular proliferation (3–12 days) and remodeling (3–6 months) (Glynn, 1981; Clark, 1996; Martin, 1996) [1]. Healing of wounds is one of the important areas of clinical medicines explained in many Ayurvedic texts under the heading "Vranaropaka". According to the Ayurveda, Vrana (wounds or ulcers) is the discontinuation of lining membrane that after healing leaves a scar for life closely resembling the modern definition [4]. Similarly, inflammation is considered to be an early phase in the pathogenesis of wounds termed Vranashotha. The objective in wound management is to heal the wound in the shortest time possible, with minimal pain, discomfort, and scarring to the patient [5]. Certain factors that affects the wound healing process includes bacterial infection, nutritional deficiency, drugs, sterility, obesity and movement of wound edges [6].

Plants and their extracts have immense potential for the management and treatment of wounds. The phyto-medicines for wound healing are not only cheap and affordable but are also safe as hyper sensitive reactions are rarely encountered with the use of these agents [2]. These natural agents induce healing and regeneration of the lost tissue by multiple mechanisms. However, there is a need for scientific validation, standardization and safety evaluation of plants of the traditional medicine before these could be recommended for healing of the wounds.

## MATERIALS AND METHODS

### Materials

All crude drugs (*Erythrina indica, Bergenia ciliata, Cissampelos pareira)* were provided by S.G. Phyto Pharma Pvt. Ltd. Kolhapur, Maharashtra. which were authenticated as per Ayurvedic standards as well as our Pharmacognostic authentification was also included to establish proper selection. And also all the Chemicals like Liquid paraffin, Hard paraffin, Glycerin, Triethanolamine, Petroleum jelly, Cetostearyl alcohol, PEG 6000, Methylparaben , ethanol, Carbomer934P were supplied by the SG. Phyto Pharma Pvt. Ltd. Kolhapur, Maharashtra.

### Methods

### Preparation of extracts

Air dried coarsely powdered plant materials of (Erythrina indica, Bergenia ciliata & Cissampelos pareira) were extracted with ethanol (95%) using soxhlet apparatus for 4-5 hrs. All the extracts were concentrated at low pressure by rotary flash evaporator and finally air-dried.

### Formulation

The dried ethanolic extracts of *Erythrina indica, Bergenia ciliata & Cissampelos pareira (5 gm each)* were taken for the preparation of 100gm Ointments, 100gm Gel and 100ml Liquid formulations. The Ointment and Gel formulations were prepared by using an Ointment base and Gel base. Standard method of fusion was used, where the solid fats were melted and mixed by continuous trituration. The required quantity of the ointment base and gel base was weighed and melted at a temperature of about $70^{O}C$ in a hot water bath. The designated

quantity of the extract (s) were respectively added to the melted base at 40$^{O}$C and the mixed, stirred gently and continuously until a homogenous dispersion is obtained. For the Liquid Formulation, the weighed quantity of dried ethanolic extracts was dissolved in 50 ml of 95% ethanol and then added the distilled water to make the final volume 100 ml.

### Animals

Healthy wistar rats of either sex and of approximately the same age, weighing about 150-250 g were used for the study. They were fed with standard diet and water *ad libitum*. They were housed in polypropylene cages and maintained under standard conditions. CPCSEA approval reference number :- (Tatyasaheb Kore College of Pharmacy, Warnanagar, Tal-Panhala, Dist- Kolhapur –416113 ) (Regd. No. 1090/PO/ac/07/CPCSEA)

### Grouping of Animals

Animals were divided in to nine groups, each group consisting of 6 rats.

**Group I** : Treated with plain ointment base and served as control-I.

**Group II** : Treated with standard ointment i.e. Betadine ointment.

**Group III** : Treated with **new polyherbal ointment formulation** (Test-I).

**Group IV** : Treated with Gel base and served as control-II.

**Group V** : Treated with standard drug i.e. Framycetin sulphate cream.

**Group VI** : Treated with **new polyherbal Gel formulation** (Test-II).

**Group VII** : Treated with Water for injection and served as control-III.

**Group VIII** : Treated with standard drug i.e. Wockadine liquid.

**Group IX** : Treated with **new polyherbal Liquid formulation** (Test-III).

### Excision wound model[7]

Excision wounds were used for the study of rate of contraction of wound and epithelization. Animals were anaesthetized with diethyl ether and the hairs on the skin of the back, shaved with sterilized razor blades. A circular wound of about 300 mm$^2$ area and 2 mm depth was excised on depilated dorsal thoracic region of excised rats, 5 cm away from ear. The entire wound was left open. The treatment was done topically in all the cases. The wounds were traced on transparent tracing paper by permanent marker on the day of wounding and subsequently on alternate days until healing were complete. Wound areas were measured on days 1, 4, 8, 16 and 20 for all groups.

### Incision wound mode l[8]

Two 6-cm long paravertebral incisions were made through the full thickness of the skin on either side of the vertebral column of the rat. Wounds were closed with interrupted sutures, 1 cm apart with the help of black silk surgical thread and a curved needle (no.11). The sutures were removed on the seventh day. Wound-breaking strength was measured in anesthetized rats on the tenth day after wounding by continuous constant water supply technique.

### Statistical Analysis

The data was statistically analyzed by one-way ANOVA followed by Bonferroni's Multiple Comparison Test with equal sample size. The difference was considered significant when $p<0.001$. All the values were expressed as mean ± standard error mean (S.E.M) .

## RESULTS

The studies on excision wound healing model reveals that all the nine groups showed decreased wound area from day to day. However on 20$^{th}$ post wounding day,

Group-I animals showed 90.12±0.27% of healing, where as Group-II and Group-III animals showed 98.55±0.44% and 99.78±0.11% of healing. Group IV animals showed 91.02±0.77% of wound healing, while Group V and Group VI animals showed 98.20±0.48% and 97.65±0.48% of wound healing. On the other hand, Group VII animals showed 90.05±1.13% of wound healing while Group VIII and Group IX animals showed 98.40±0.46% and 99.20±0.23% of wound healing, (table 1). All readings are found to be statistically significant and comparable with control. The epithelization time i.e. time at which complete scar formation occur, also suggest that all polyherbal formulations i,e. Ointments , Gel and Liquid treated groups were found to be significant and comparable with control (table 2). On the basis of the results obtained in the present investigation, it is concluded that the new polyherbal ointment, polyherbal Gel and polyherbal Liquid has significant wound healing activity. Wound healing activity of new polyherbal ointment formulation was found to be better than the standard Betadine ointment group.

## DISCUSSION

Wound healing involves different phases such as contraction, epithelization, granulation, collagenation which are concurrent but independent to each other. Hence in the present study two different wound models were used. In the excision wound model, animals treated with the polyherbal ointment showed better and fast healing compared to the standard and control groups. Also there was significant decrease in the epithelization period. In the incision wound model, there was significant increase in the tensile strength of the 10 days old wound due to treatment with polyherbal ointment.

## CONCLUSION

Literature survey revealed that the above plants were used traditionally for various ailments, especially for its wound healing property. Extensive scientific studies were not performed on these plants. Its wound healing property was not under taken for any scientific study. All formulations i.e. Polyherbal Ointment, Polyherbal Gel and Polyherbal Liquid prepared from the ethanolic extracts of *Erythrina indica, Bergenia ciliata & Cissampelos pareira* showed the better wound healing activity in rats. Further experiments have to be conducted in other animal models for wound healing activity. Based on above tests, trails on human being can be performed.

## REFERENCES


1. Karodi R, Jadhav M, Rub R and Bafna A. Evaluation of the wound healing activity of a crude extract of *Rubia cordifolia* L. (Indian madder) in mice. *Int J Applied Research Nat Prod* 2009; Vol 2(2) : 12-18.
2. Rajinder Raina , Shahid Pravez, Verma PK and Pankaj NK. Medicinal plants and their role in wound healing. *Vet Scan* 2008; Vol 3(1): 1-7.
3. Jaiswal S, Singh S, Singh B and Singh H. Plants used for tissue healing of animals. *Indian J Nat Prod Resour* 2004; Vol 3(4): 284-292.
4. Kumar B, Vijaykumar M, Govindarajan R, *et.al*. Ethnopharmacological approaches to wound healing. *Exploring medicinal plants of India*. 103-113.
5. Swati Rawat and Akhilesh Gupta. Development and study of wound healing activity of an Ayurvedic formulation. *Asian J. Res. Pharma. Sciences* 2011; Vol 1(1):26-28.
6. Karl M, Lacrix JV and Preston, HH. Canine surgery, 4th edition, California: American Veterinary Publications; 1995: 42-45.
7. Narendra Nalwaya, Gaurav Pokharna, Lokesh Deb and Naveenkumar Jain . Wound healing activity of latex of Calotropis

gigantae. *Int J P P Sci* 2009; Vol. 1(1): 176-181.
8. Agarwal PK, Sing A, Gurav K, *et al*. Evaluation of wound healing activity of extracts of *Plantain banana* in Rats. *Indian J Exp Biol* 2009; Vol. 47: 32- 40.

**Table 1.** Effect of new polyherbal formulations on healing of excision wound model (Significant levels are mentioned)

| Group | Percentage wound contraction on post wounding days | | | | | Epithelialization in days |
|---|---|---|---|---|---|---|
| | 4th | 8th | 12th | 16th | 20th | |
| Group I | 17.32±0.26 | 34.42±0.27 | 58.66±0.28 | 81.06±0.26 | 90.12±0.27 | 22.5 ± 0.92 |
| Group II | 32.22±0.50*** | 63.70±0.49*** | 78.05±1.05*** | 89.12±0.39*** | 98.55±0.44*** | 17.80 ± 0.56*** |
| Group III | 33.52±0.49*** | 67.81±0.52*** | 82.55±0.68*** | 93.40±0.55*** | 99.78±0.11*** | 16.55 ± 0.46*** |
| Group IV | 18.20±0.47 | 35.97±0.36 | 61.50±0.50 | 82.25±0.51 | 91.02±0.77 | 21.52 ± 0.54 |
| Group V | 31.85±0.49*** | 64.05±0.58*** | 83.44±0.56*** | 91.80±0.96*** | 98.20±0.48*** | 17.50 ± 0.43*** |
| Group VI | 30.36±0.88*** | 63.91±0.72*** | 81.90±0.41*** | 92.30±0.59*** | 97.65±0.48*** | 18.20 ± 0.56** |
| Group VII | 18.76±0.46 | 38.20±0.41 | 67.87±0.89 | 83.52±0.45 | 90.05±1.13 | 22.20 ± 0.65 |
| Group VIII | 30.92±0.60*** | 64.89±0.85*** | 83.50±0.68*** | 92.15±0.64*** | 98.40±0.46*** | 17.68 ±0.77*** |
| Group IX | 32.15±0.74*** | 66.05±0.85*** | 87.21±0.59*** | 94.03±0.47*** | 99.20±0.23*** | 17.15 ± 0.58*** |

**Table 2.** Effect of new polyherbal formulations on healing of incision wound model

| Sr. No | Groups | Tensile strength of skin (g) |
|---|---|---|
| 1 | Group -I | 359.60 ± 0.92 |
| 2 | Group -II | 499.10 ± 1.27*** |
| 3 | Group- III | 538.10± 1.52*** |
| 4 | Group- IV | 348.30 ± 0.94 |
| 5 | Group -V | 491.10 ± 0.85*** |
| 6 | Group -VI | 483.90± 0.78*** |
| 7 | Group VII | 351.30 ± 0.92 |
| 8 | Group VIII | 502.50± 0.90*** |
| 9 | Group IX | 494.50 ± 0.95*** |

Values are Mean ± S.E.M. of six animals in each group. *p<0.001 as compared to control.

 ISSN 2321 – 2748

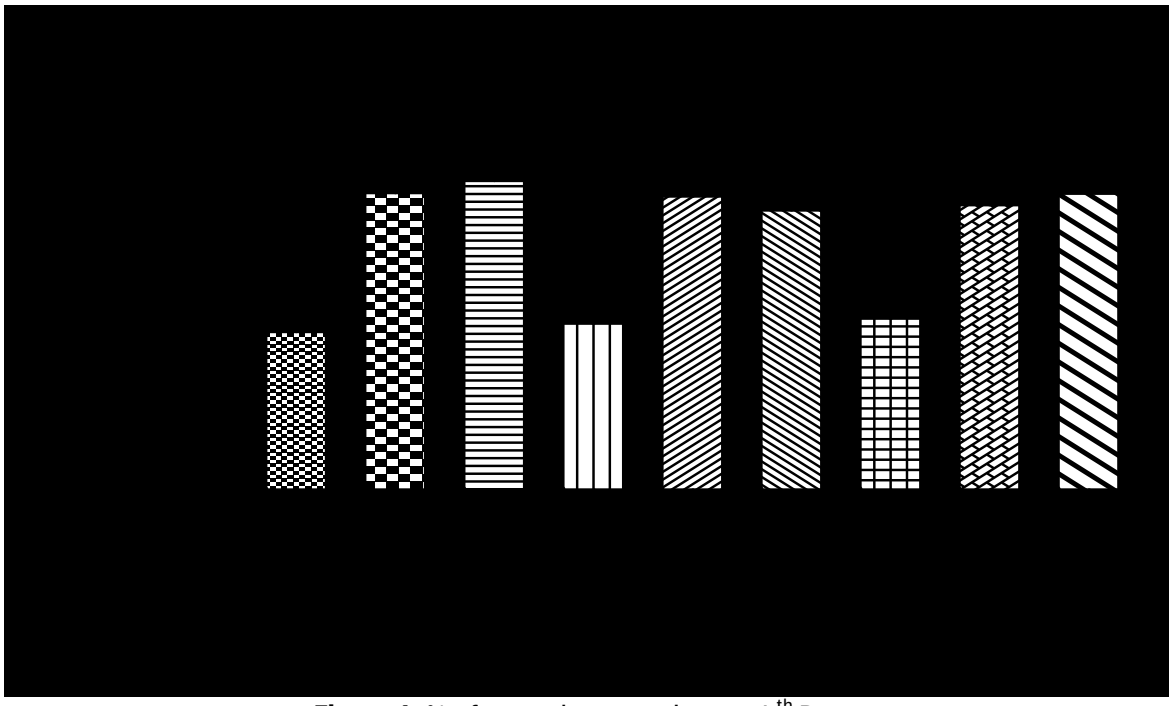

**Figure.1**. % of wound contraction on 4 $^{th}$ Day.

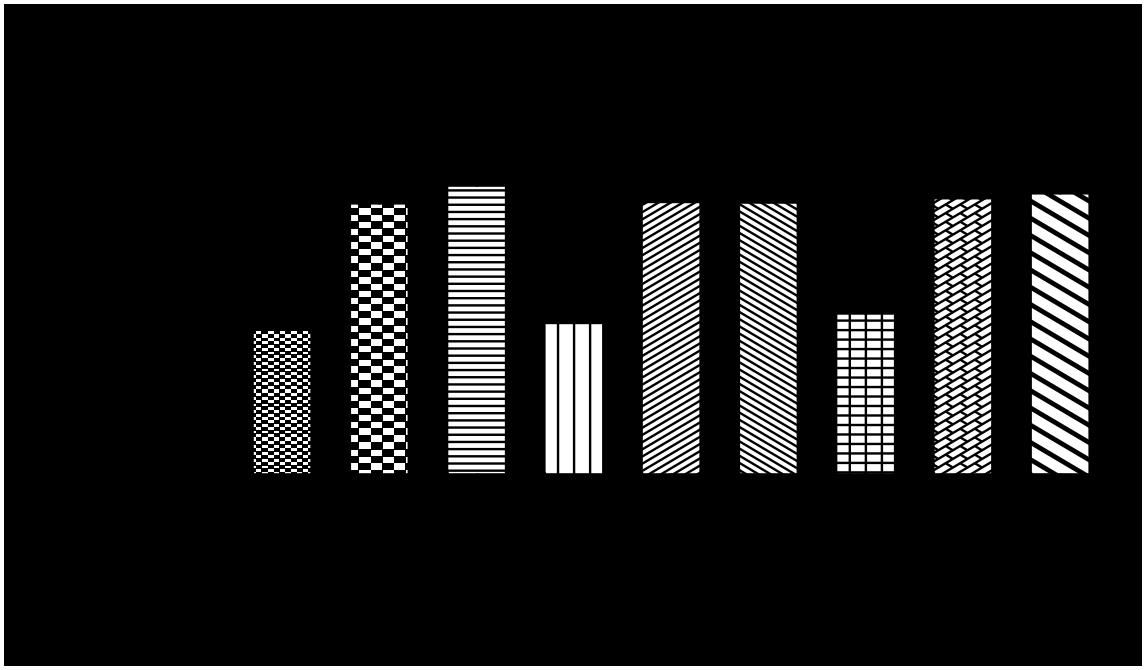

**Figure.2**. % of wound contraction on 8$^{th}$ Day.

 ISSN 2321 – 2748

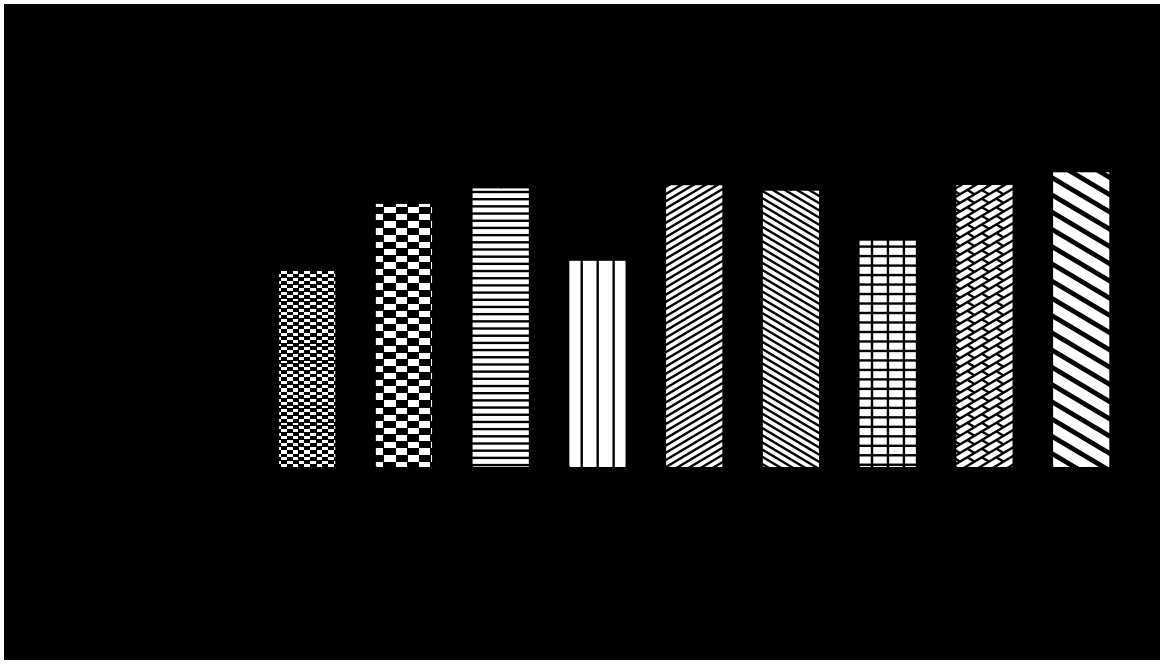

**Figure.3**. % of wound contraction on 12$^{th}$ Day.

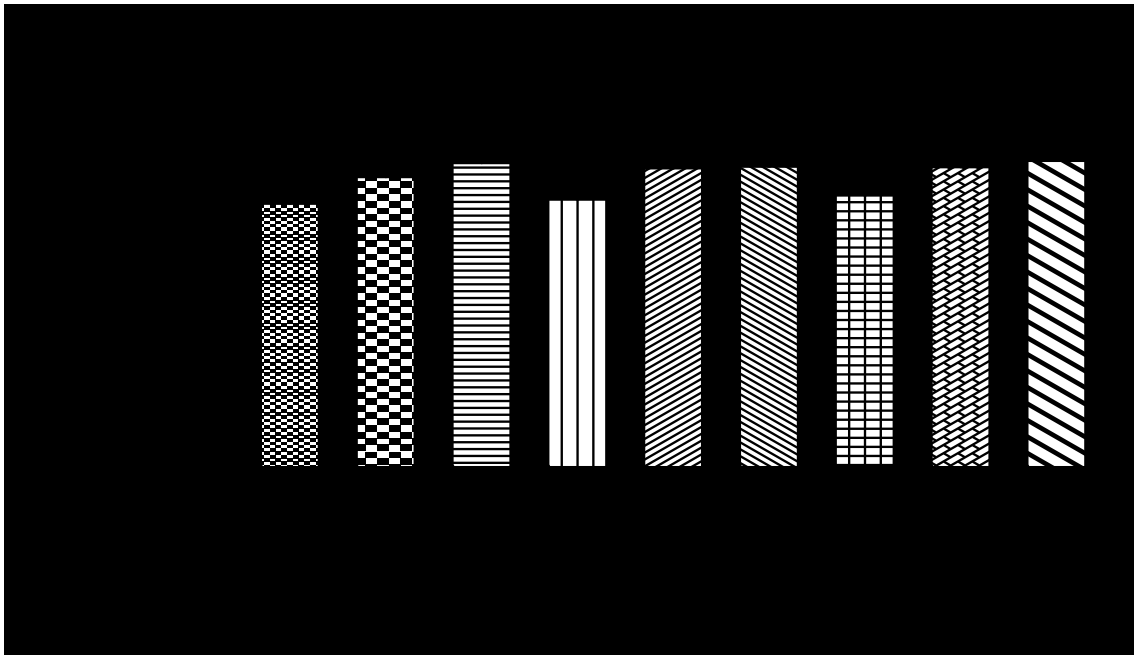

**Figure.4**. % of wound contraction on 16$^{th}$ Day

 ISSN 2321 – 2748

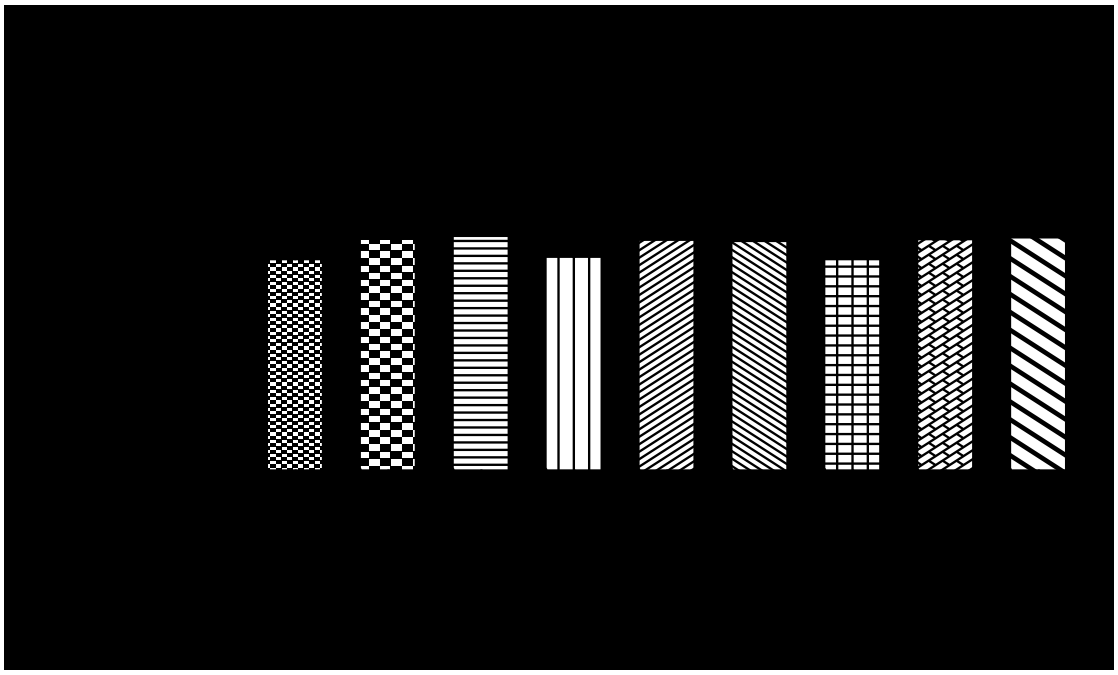

**Figure.5**. % of wound contraction on 20th Day

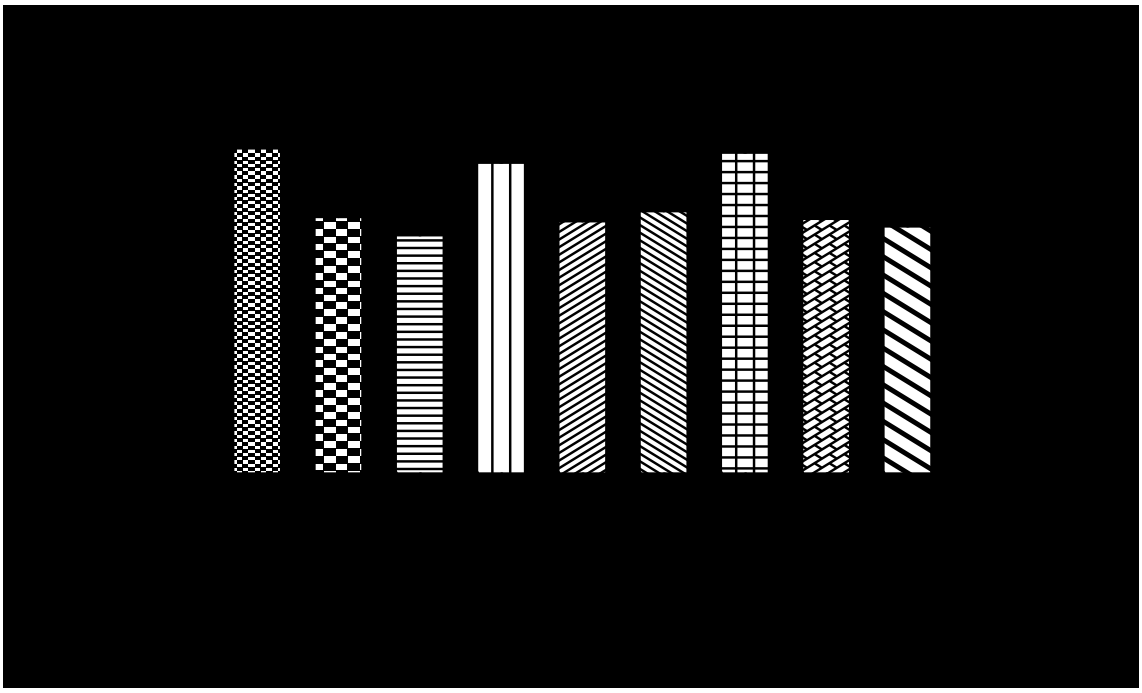

**Figure.6**. Epitheliazation in days

 ISSN 2321 – 2748

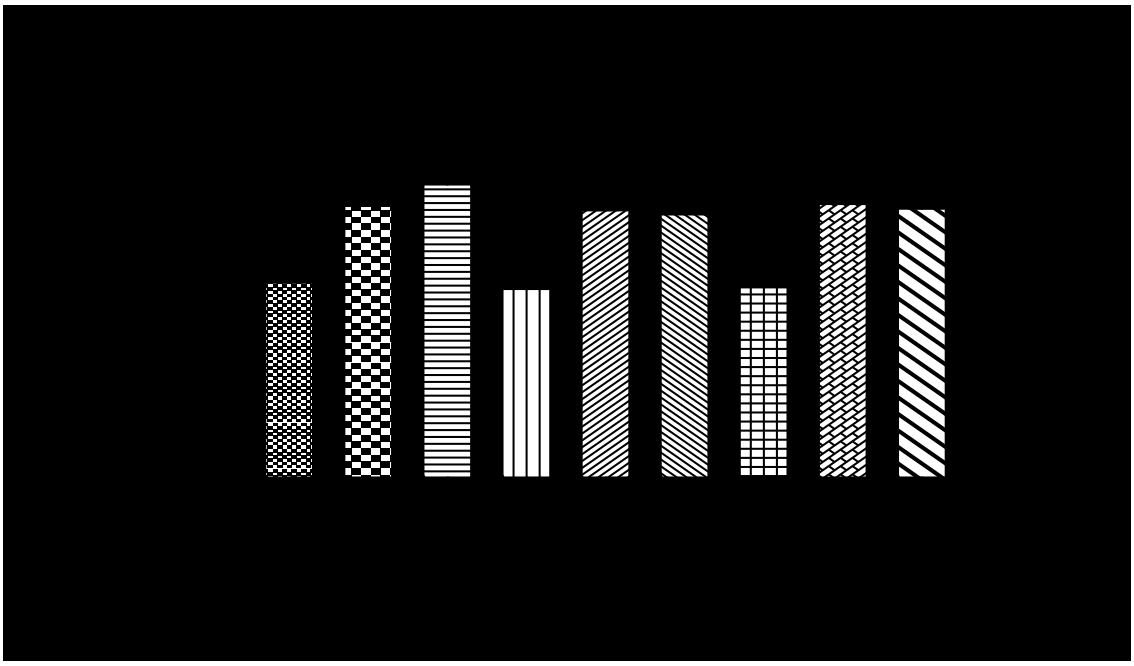

**Figure.7**. Tensile strength of skin in gms