WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 4, 1410-1423. Research Article ISSN 2277– 7105

# EFFICACY OF CALCIUM ALGINATE FIBRES IN WOUND HEALING IN WISTAR RATS

**Dr. Rishabh Joshi*, Dr. Pankaj Bansode and Dr. (Brig.) Chandrashekhar Joshi**

Department of General Surgery, Bharati Vidyapeeth Deemed University Medical College, Pune, Maharashtra, India.

Article Received on 10 Feb. 2017,
Revised on 02 March. 2017,
Accepted on 22 March 2017
DOI: 10.20959/wjpr20174-8232

***Corresponding Author**
**Dr. Rishabh Joshi**
Department of General Surgery, Bharati Vidyapeeth Deemed University Medical College, Pune, Maharashtra, India.

## INTRODUCTION

Wound is described as a discontinuation in the epithelial integrity of the skin or living tissue. Wound healing is a cascade of well-organized biochemical and cellular events resulting in growth and regeneration of wounded tissue. The search for wound healing agents is one of the oldest challenges in medicine. There are various methods of wound management available; however there is no consensus on which is the best method for wound management.

Alginates have been used in various forms for 50 years, and yet they remain a poorly understood and probably underused dressing. Compared to many modern dressings, the literature is sparse and inconclusive. Alginate dressings are derived from seaweed. They are highly absorbent and biodegradable. The high absorption is achieved via strong hydrophilic gel formation. This limits the wound secretions and minimizes bacterial contamination. Alginate fibres trapped in a wound are readily biodegraded.[1] They have been successfully applied to cleanse a wide variety of secreting lesions. Alginate dressings maintain a physiologically moist microenvironment that promotes healing and the formation of granulation tissue. Alginates are also believed to have a bioactive effect on the wound healing by promoting the pro-inflammatory signals in wounds by activating the macrophages leading to release of various cytokines. Alginates can be rinsed away with saline irrigation, so removal of the dressing does not interfere with healing granulation tissue. This makes change of dressing virtually painless. Alginate dressings are very useful for moderate to heavily exudative wounds.[2]

Alginates act as calcium ion (Ca) donors as they contain mannuronic (M) or guluronic (G) groups with a high Calcium content. The major uses of alginates include its use by the pharmaceutical industry in the production of controlled-release agents, as bio-adhesive systems, tablet disintegrants, suspending agents and implants.[3] Annually more than 20,000 tons of alginate are used for above and other purposes.[3]

According to Gacesa[4], most alginate is obtained commercially from three of the 265 reported genera of the marine brown algae, Phaeophyceae. The majority is extracted from members of the genus Macrocystis that includes the giant kelp (Macrocystis pyrifera) harvested of the west coast of the USA. In northern Europe alginates are extracted from horsetail kelp (Laminaria digitata) and sugar kelp (L. saccharina) collected from waters off the Outer Hebrides and the west coast of Ireland.[3]

The Ancient Romans, Greeks, Egyptians and Polynesians discovered and used the virtues of seawater to obtain therapeutic, preventive and curative benefits. They used it to treat various wounds, bruises and swellings. The oils in seaweed have been used from a long time to recuperate from illness. Though the extraction of alginates from seaweeds is a relatively recent invention, it has been used for centuries for a variety of purposes.[3] They were considered to be of help in detoxifying the body and in the renewal of damaged skin cells. Seaweed was known as “Mariner’s Cure” or “Sailor’s Cure” by ancient mariners.

The function of alginates within the algae is thought to be primarily skeletal[5], with the gel conferring the strength and flexibility required to withstand tidal activity in the water in which the seaweed grows. Certain species of bacteria – including Azobacter vinelandii and Pseudomonas aeruginosa – are known to produce alginates which form a protective coating around the organism but these are not used commercially.[3]

The claimed higher efficacies of calcium alginate dressings with respect to wound healing outcomes have attracted a lot of interest in them from many Surgeons and other medical personnel. It is also cost-effective in lot of ways as the number of times a dressing is to be done is minimal and thus helping the cause in more ways than one. Previously several research groups have reported protective effects of calcium alginate. However there is paucity of reports on effect of alginate on wound healing. The objective of present investigation was to evaluate the wound healing potential of Calcium alginate fibres in excision wound model in laboratory rats. For this wound surface area, wound contraction, wound index and histopathological examination were compared between the groups.

This study will also pave the path for further research in future where larger study group will be taken with human subjects having a variety of wounds to further strengthen the significance of use of calcium alginate dressing as a potent wound healing agent.

## MATERIAL AND METHODS

### Animals

Male Wistar rats weighing between 230-250g were procured from National Institute of Bioscience, Pune, India. The animals were housed at an ambient temperature (25±2°C) and relative humidity (45-50%) and light and dark cycle (12 h light/dark). The animals had access to pellet diet (Sourced from Chakan oil mills, Pune) and water *ad libitum* throughout the experimental period. The animals were acclimatized for a period of 1 week and were kept under pathogen-free conditions.

### Research protocol approval

The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA), India.

### Excision wound model

Animals were anaesthetized with a dose of 80 mg/kg of ketamine (i.p.) and then the back of the animals were shaved. An impression was made on the back of the neck on the anaesthetized rat. Excision wounds of size 500 $mm^2$ and depth 2 mm were made by cutting out a layer of skin from the shaven area. Haemostasis was achieved by blotting the wound with cotton swab soaked in normal saline.[5] The entire wound was left open. The study comprised three different groups containing six animals in each group. The test dressing of calcium alginate fibres of appropriate size as per the wound was applied locally.

### Study Groups

**Group No I: Vehicle control animals-** received injury for wound formation but will not receive any treatment locally.

**No. of animals=** 6.

**Group No II: Calcium Alginate Fibre Dressing treated animals-** received injury for wound formation and treatment with Calcium Alginate Dressing on the wound and no any other topical application or debridement.

**No. of animals=** 6.

**Group No III: Povidone iodine (Betadine) treated animals-** received injury for wound formation and treatment with povidone iodine (betadine) only each three times a day.

**No. of animals=** 6.

**Measurement of wound area**

The changes in wound area were observed and recorded on day 0, 5, 10, 15 and 20. Wound area was measured by using image J software to determine the area.

**Measurement of wound contraction**

Wound contraction was calculated as percentage of the reduction in original wound area size. It was calculated by using the following formula:

$$\textbf{Percentage wound contraction} = \frac{\text{Initial area of wound} - \text{Nth day area of wound}}{\text{Initial area of wound}} \times 100$$

**Histopathological examination**

A specimen sample of tissue was isolated from the skin of each group of rat on day 10, 15 and 20 to evaluate for the histopathological alterations. Samples were fixed in 10% buffered formalin, processed and blocked with paraffin and then sectioned into 5 µm and stained with haematoxylin and eosin (H and E). Photomicrographs were captured at a magnification of 100X and 400X. Sections were analysed and scored as mild (+), moderate (++) and severe (+++) for epidermal or dermal remodelling. Scab formation, congestion, capillary formation, fibroblast proliferation, infiltration of macrophages, oedema in dermis was analysed to score the epidermal or dermal remodelling.

**Figure 1: Animal wound and its measurements.**

## Statistical Methods

Statistical comparisons were made between drug-treated groups and vehicle control animals.

### *t* -test

A *t*-test compares the means of two groups. The *t* test compares one variable (pain score) between two groups. The *t*-tests (and related nonparametric tests) compare exactly two groups.

### Bonferroni's multiple comparison test

Bonferroni's multiple comparison test can be used to determine which means amongst a set of means differ from the rest. When we have more than two groups, it is inappropriate to simply compare each pair using a *t*-test because of the problem of multiple testing. The correct way to do the analysis is to use a one-way analysis of variance (ANOVA) to evaluate whether there is any evidence that the means of the populations differ. If means of the populations differ, we might then be interested in investigating which of the means are different. This is where the Bonferroni's multiple comparison test is used. The Bonferroni's test compares every mean with every other mean.

## SOFTWARE

All the statistical calculations were done through Graph pad Prism software, Version 6 for windows.

## OBSERVATION AND RESULT

### Effect of calcium alginate fibre dressing on wound area in excision wound

The wound area ($mm^2$) in all animal groups was measured on days 0, 5, 10, 15 and 20. At day 0, wound area showed none significant difference which indicated the same size of wound area before treatment started in each group. There was no significant difference in the wound area of **Group I** (556.66±48.44 $mm^2$) when compared with **Group II** (553.33±5.16 $mm^2$) on day 0. The significant reduction ($p<0.0001$) in wound area was observed in the **Group II** treated rats on 5$^{th}$ day (203.33±5.16 $mm^2$) when compared with **Group I** (356.66±12.11 $mm^2$). This reduction in wound area was more significant ($p<0.001$) in **Group II** treated rats on 15$^{th}$ day than on 5$^{th}$ day when compared with **Group I**.

**Table 1: Effect of Calcium alginate fibre dressing on wound area ($mm^2$).**

| Time | Group I | | Group II | | Group III | |
|---|---|---|---|---|---|---|
| (Days) | Mean | SD | Mean | SD | Mean | SD |
| 0 | 556.66 | 48.44 | 553.33 | 5.16 | 565.00 | 5.477 |
| 5 | 356.66 | 12.11 | 203.33 | 5.16 | 315.00 | 5.48 |
| 10 | 223.33 | 12.11 | 16.67 | 19.66 | 90.000 | 10.95 |
| 15 | 90.00 | 6.32 | 0.000 | 0.000 | 18.33 | 14.72 |

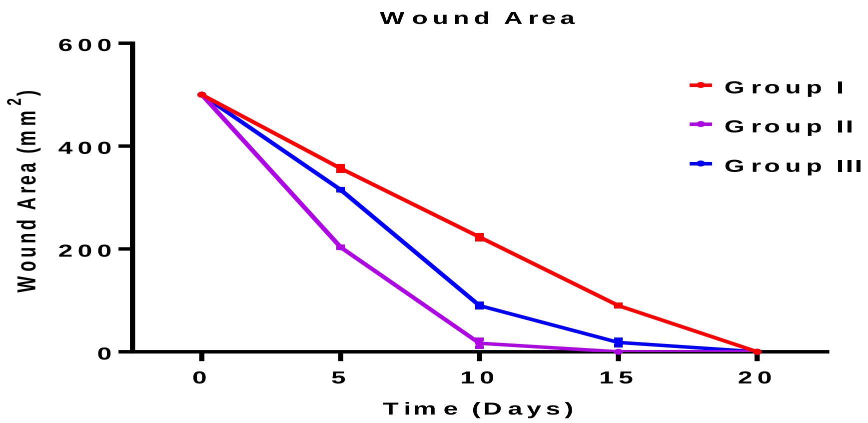



**Figure 2. Effect of Calcium alginate fibre dressing on wound area.**

**Table 2. Bonferroni's multiple comparisons of Calcium alginate fibres treatment on wound area (mm$^2$).**

| Bonferroni's multiple comparisons test | Significant? | P Value |
|---|---|---|
| **At 0 DAY** | | |
| Group I vs. Group II | No | > 0.9999 |
| Group I vs. Group III | No | > 0.9999 |
| Group II vs. Group III | No | > 0.9999 |
| **At 5 DAY** | | |
| Group I vs. Group II | Yes | < 0.0001 |
| Group I vs. Group III | Yes | < 0.0001 |
| Group II vs. Group III | Yes | < 0.0001 |
| **At 10 DAY** | | |
| Group I vs. Group II | Yes | < 0.0001 |
| Group I vs. Group III | Yes | < 0.0001 |
| Group II vs. Group III | Yes | < 0.0001 |
| **At 15 DAY** | | |
| Group I vs. Group II | Yes | < 0.0001 |
| Group I vs. Group III | Yes | < 0.0001 |
| Group II vs. Group III | No | 0.1246 |
| **At 20 DAY** | | |
| Group I vs. Group II | No | > 0.9999 |
| Group I vs. Group III | No | > 0.9999 |
| Group II vs. Group III | No | > 0.9999 |

**Effect of Calcium alginate fibres on percentage of wound contraction**

The percentage of wound healing in all animal groups was measured from days 0 to day 5, 10, 15 and 20. Application of Calcium alginate fibres significantly increased ($p<0.0001$) the wound contraction rate on 5$^{th}$ day and was found to be 59.33±1.03, whereas in Group I rats, the rate of wound contraction was only 28.67±2.42. This rate of wound contraction was more significantly increased (100±00, $p<0.001$) after the administration of Calcium alginate fibres over a period of 15 days when compared with vehicle control Group I rats (82.00±1.26) on the same day. Group III, Povidone iodine also showed significant wound contraction over a period of 20 day. However, Calcium alginate fibres showed highly significant wound contraction compare to Povidone iodine ($p<0.0001$).

**Table 3. Effect of Calcium alginate fibre dressing on percentage of wound contraction.**

| Time | Group I | | Group II | | Group III | |
|---|---|---|---|---|---|---|
| (Days) | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| 0 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 5 | 28.67 | 2.42 | 59.33 | 1.03 | 37.00 | 1.09 |
| 10 | 55.33 | 2.42 | 96.66 | 3.93 | 82.00 | 2.19 |
| 15 | 82.00 | 1.26 | 100.00 | 0.000 | 96.33 | 2.94 |
| 20 | 100.00 | 0.00 | 100.000 | 0.000 | 100.00 | 0.00 |

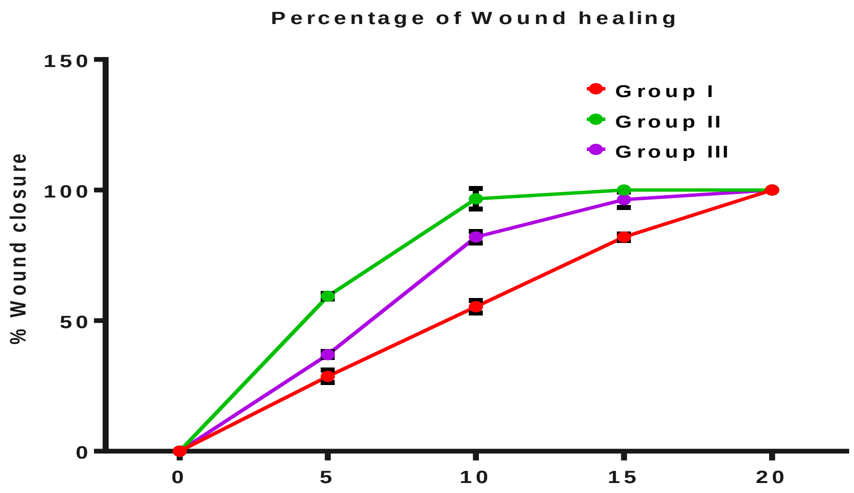



**Figure 3. Effect of Calcium alginate fibres treatment on percentage of wound contraction.**

**Table 4. Bonferroni's multiple comparisons test for percentage of wound contraction.**

| Bonferroni's multiple comparisons test | Significant? | P Value |
|---|---|---|
| **At 0 DAY** | | |
| Group I vs. Group II | No | > 0.9999 |
| Group I vs. Group III | No | > 0.9999 |
| Group II vs. Group III | No | > 0.9999 |
| **At 5 DAY** | | |
| Group I vs. Group II | Yes | < 0.0001 |
| Group I vs. Group III | Yes | < 0.0001 |
| Group II vs. Group III | Yes | < 0.0001 |
| **At 10 DAY** | | |
| Group I vs. Group II | Yes | < 0.0001 |
| Group I vs. Group III | Yes | < 0.0001 |
| Group II vs. Group III | Yes | < 0.0001 |
| **At 15 DAY** | | |
| Group I vs. Group II | Yes | < 0.0001 |
| Group I vs. Group III | Yes | < 0.0001 |
| Group II vs. Group III | No | 0.0013 |
| **At 20 DAY** | | |
| Group I vs. Group II | No | > 0.9999 |
| Group I vs. Group III | No | > 0.9999 |
| Group II vs. Group III | No | > 0.9999 |

**Effect of Calcium alginate fibres on histopathological examination in excision wound**

On day 20, wound tissue from Group I showed marked inflammation with minimal collagen fibres and absence of granulation tissue corresponding to the delayed wound healing. The corresponding histopathological photograph is shown in Figure 4.

Group II showed keratinization, abundant collagen deposition, fibroblasts, few adipocytes and occasional inflammatory cells. This was consistent with signs of good wound healing. It also showed the presence of few macrophages and absence of oedema. The corresponding histopathological photograph is shown in Figure 5.

Group III showed minimal inflammatory cells with fibroblasts and collagen deposition. Also formation of granulation tissue with evidence of angiogenesis was seen. The corresponding histopathological photograph is shown in Figure 6.

**Table 5. Effect of Calcium alginate fibres treatment on wound healing processes and healing phases in rats.**

| Group | Scab formation | Congestion | Capillary formation | Fibroblast proliferation | Infiltration of macrophages | Edema |
|---|---|---|---|---|---|---|
| | - | - | ++++ | - | - | - |
| **Group I** | ++++ | +++ | + | +++ | +++ | ++ |
| **Group II** | ++ | ++ | +++ | + | ++ | + |
| **Group III** | +++ | +++ | ++ | ++ | +++ | ++ |

- : no abnormality detected,

+ : damage/ active changes up to less than 25%,

++ : damage/ active changes up to less than 50%,

+++ : damage/ active changes up to less 75%,

++++ : damage/ active changes up to more than 75%.

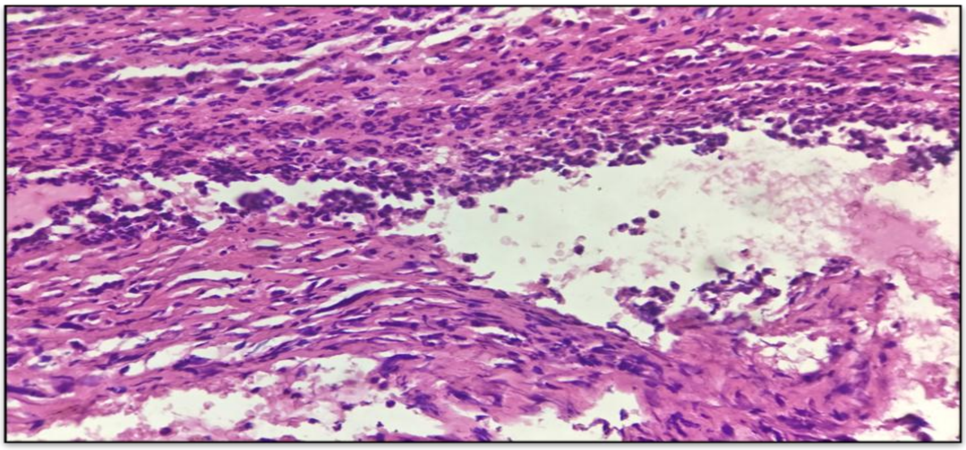


**Figure 4: Photomicrographs of the sections of skin from rats stained with haematoxylin (H) and eosin (E) in the excision model. Images (400× magnification) are typical and representative of Group I.**

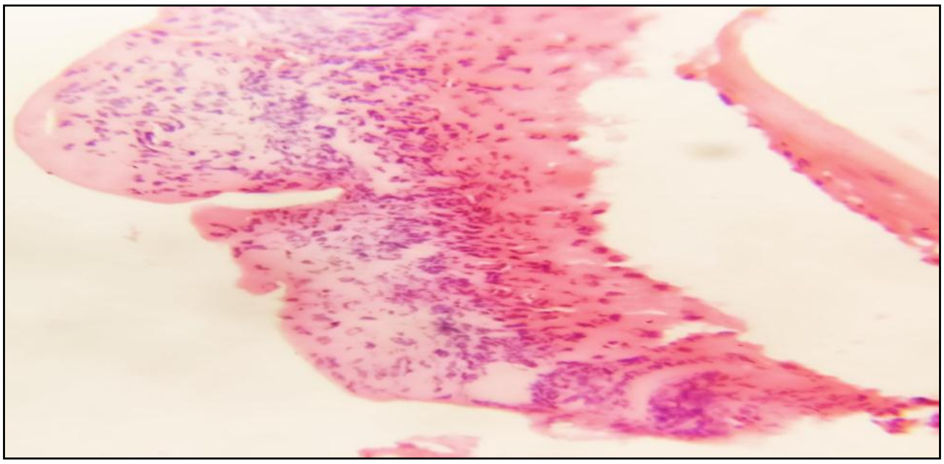


**Figure 5: Photomicrographs of the sections of skin from rats stained with haematoxylin (H) and eosin (E) in the excision model. Images (400× magnification) are typical and representative of Group II.**

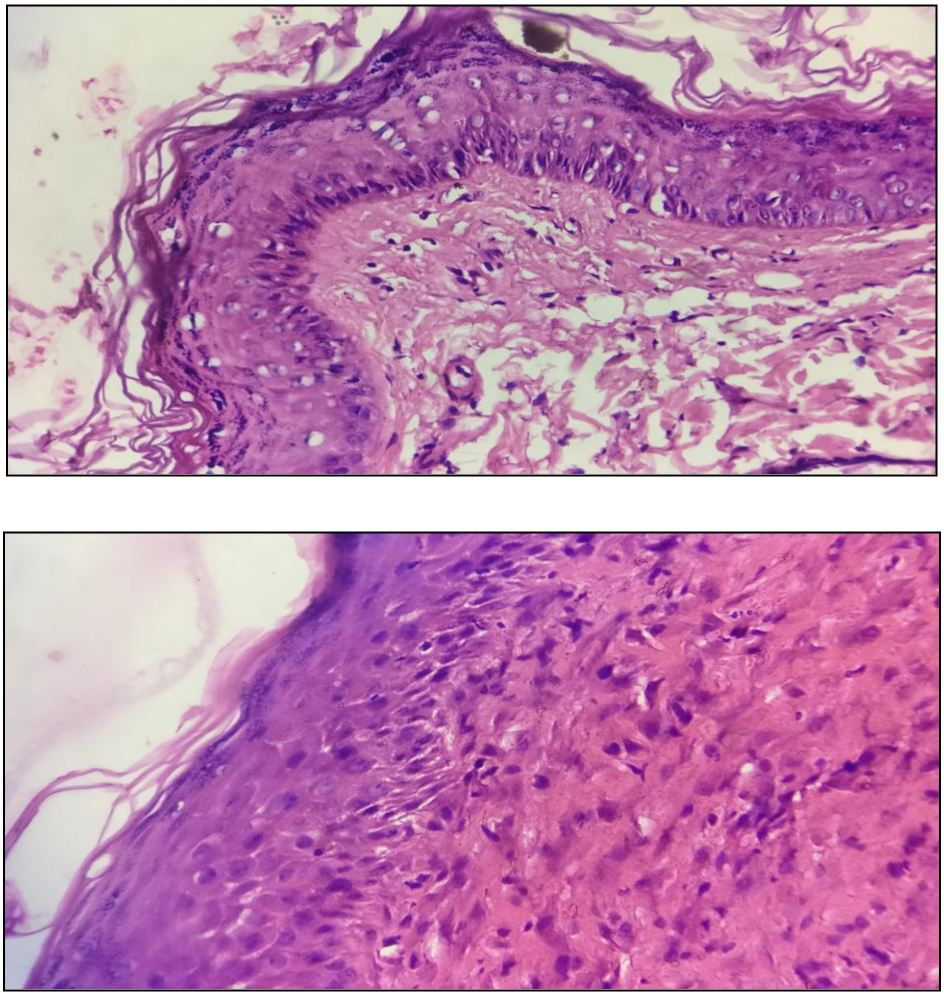
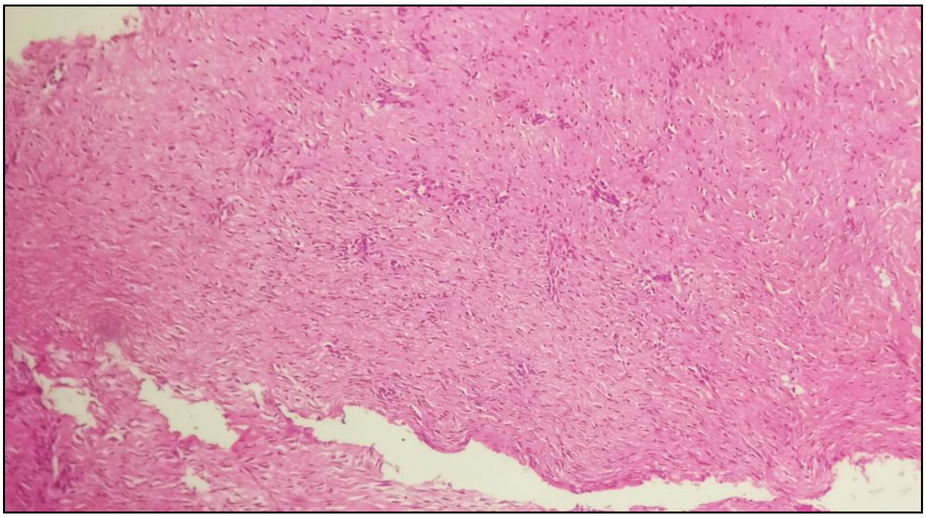
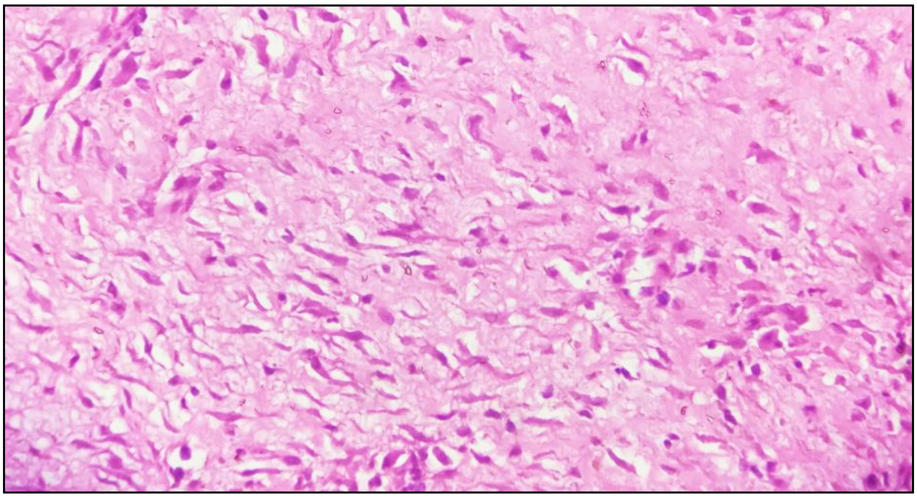


**Figure 6: Photomicrographs of the sections of skin from rats stained with haematoxylin (H) and eosin (E) in the excision model. Images (400× magnification) are typical and representative of Group III.**





## DISCUSSION

The focus of this study was on the evaluation of efficacy of calcium alginate fibre dressing on wound healing in wounds on Wistar rats. For evaluation, 4 parameters i.e. wound contraction, decrease in wound area, percentage of wound healing and improvements in wound index scoring were studied. The results obtained showed statistically significant improvements in the all parameters in wounds treated with calcium alginate fibre dressing.

a) At day 0, wound area was almost same in all groups. Very significant reduction ($p<0.0001$) in wound area was observed in the **Group II** i.e. Calcium alginate dressing treated rats on 5$^{th}$ day (203.33±5.16 mm$^2$) when compared with **Group I or Group III** rats. This reduction in wound area was even more significant ($p<0.001$) in **Group II** treated rats on 15$^{th}$ day and wounds were totally healed.

b) The percentage of wound contraction in the rats treated with Calcium alginate fibre dressings showed significant increase ($p<0.0001$) on 5th day ($59.33\pm1.03$), which was much higher then that recorded in the other 2 groups. On 15th day also the percentage of wound contraction more significantly increased ($100\pm00$, $p<0.001$) in the Group II rats. Group III, Povidone iodine also showed significant wound contraction but that was over a period of 20 days which was higher then the Group II rats which were treated with calcium alginate fibre dressing.

The above results showed that with respect to reduction in wound area and percentage of wound contraction, the results showed a significant improvement in the rats that were having calcium alginate fibre dressing.

c) In Comparison with the study carried out by **O'Donoghue *et al*[6]** in 1997 who conducted prospective controlled trial on a group of 51 patients amongst whom 30 patients were randomised to the calcium alginate group and 21 patients were assigned to the paraffin gauze group. All patients underwent harvesting of the split skin graft and resultant donor sites were treated according to the assigned group. The donor sites of Twenty one of the patients dressed with calcium alginate were completely healed at day 10, while only seven in the paraffin gauze group were healed ($p < 0.05$). In our study, all rats in the calcium alginate dressing group on day 15th showed complete healing while in the other two groups none of the rats showed complete wound healing which is almost as statistically significant as in the study carried out by **O'Donoghue *et al.***

d) The Improvement in Arbitrary Wound Index on treatment with calcium alginate fibres for 20 days resulted in significant decrease ($1.556\pm0.7429$, $p<0.0013$).

e) On Histopathological assessment also, the samples taken from the Group II rats showed good keratinization, abundant collagen formation, improved angiogenesis and far better epidermal remodelling in comparison with other two groups. The persistent inflammatory response was minimal and the absence of necrosed tissue was observed on Day 15th wounds of Group II rats when compared with the other 2 groups.

Thus the histopathological evidence was also signifying the advantage that calcium alginate fibre dressing had over the other two groups.

As already mentioned before, wound healing is a complex set of overlapping events for repairing the damaged tissue to its normal state and involves epithelization, contraction and connective tissue deposition.[7] Rate of wound healing mainly depends upon the type and extent of damage, general health status and tissue reparability. Moreover, the association of various diseases like diabetes, immune-compromised conditions as well as malnourishment, ageing, local infection also leads to delay in healing. Hence, there is a need to have agents that can accelerate the wound healing process and shorten the duration for healing and improve the quality of life of the patients.

Our study demonstrates that treatment with calcium alginate fibres accelerates the wound healing process and it was observed that the wound contraction begins within 4 days of treatment. Thus, treatment with Calcium alginate fibres significantly shortens the period of wound contraction and reduces the period of normal natural wound healing process. In a clinical study by **Naik *et al.*** (2013)[8] reported similar effect of calcium alginate and betadine dressings in wound healing.

## CONCLUSION

Management of wounds has always been a challenging issue. There is a need for application of newer and advanced modalities in management of wounds. In particular, we wish to emphasize the potential benefit of a safe, easily-applicable and effective treatment.

Our *in vivo* study investigating wounds in healthy Wistar rats showed that it is possible to enhance the rate of wound healing with Calcium alginate fibres application. The current study provides new evidence that Calcium alginate fibres shows beneficial effect in the acute wound microenvironment. Calcium alginate fibres could prove to be a cost-effective treatment of acute wounds.

Our study revealed

- Application of calcium alginate
- Significantly decreases wound area
- Decreases epithelization period
- Increases wound contraction

Local application of Calcium alginate fibres improves wound healing as evident from biochemical studies, clinical signs observed in animals and further confirmed on histopathological analysis.

## REFERENCES


1. Gilchrist T, Martin AM. Wound treatment with Sorbsan--an alginate fibre dressing. Biomaterials. 1983; 4(4): 317-20.
2. Motta GJ. Calcium alginate topical wound dressings: a new dimension in the cost-effective treatment for exudating dermal wounds and pressure sores. Ostomy/wound management. 1989; 25: 52-6.
3. Thomas S. Alginate dressings in surgery and wound management--Part 1. Journal of wound care. 2000; 9(2): 56-60.
4. Gacesa P. Alginates. Carbohydrate polymers. 1988; 8(3): 161-82.
5. Goswami S, Kandhare A, Zanwar AA, *et al.*. Oral l-glutamine administration attenuated cutaneous wound healing in Wistar rats. International wound journal. 2014.
6. O'Donoghue JM, O'Sullivan ST, Beausang ES, *et al.*. Calcium alginate dressings promote healing of split skin graft donor sites. Acta chirurgiae plasticae. 1997; 39(2): 53-5.
7. Cannavo M, Fairbrother G, Owen D, *et al.*. A comparison of dressings in the management of surgical abdominal wounds. Journal of wound care. 1998; 7(2): 57-62.
8. Segal HC, Hunt BJ, Gilding K. The effects of alginate and non-alginate wound dressings on blood coagulation and platelet activation. Journal of biomaterials applications. 1998; 12(3): 249-57.