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Wound Healing Activity of Anti-Haemorrhoidal Ointment on Rats

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Keywords: Anti-haemorrhoidal, *Cynodon dactylon*, *Emblica officinalis*, *Tamarindus indica*, *Terminalia chebula* and *Terminalia belerica*

ABSTRACT

Wounds may be defined as loss or breaking of cellular and anatomic or functional continuity of living tissues. It is a dynamic process, involving sequence of events which take place in an orderly way i.e. inflammatory repair, closure, remodelling and final healing and also refers to the replacement of dead tissue by visible tissue. Plants have the immense potential for the management and treatment of wounds. Hence, anti-haemorrhoidal ointment was prepared and various doses (Dose 1, Dose 2 and Dose 3) were applied topically on the rats. The experiments were undertaken with the help of excision wound model and the effect of anti-haemorrhoidal ointment was compared to that of the Standard Povidone ointment. Anti-haemorrhoidal ointment was prepared by using plant materials like *Cynodon dactylon*, *Emblica officinalis*, *Tamarindus indica*, *Terminalia chebula*, *Terminalia belerica* etc. Animals were treated by external application of different formulations at a final concentration of 10 mg/kg body weight. Group I (Control group) animals were treated with normal ointment (5% w/w), Group II (Standard group) animals were treated with (0.5g) 5% Povidone Ointment, Group III consisted of animals to which Dose 1 of the formulation was applied while Group IV and Group V animals were applied with Dose 2 and Dose 3 respectively. Test formulations were applied to respective groups twice a day for 16 days starting from the day of wounding. 100% of wound healing was recorded in the Standard group on the 16th day, therefore, the comparisons were done accordingly. In the control group, 76.40± 0.88% wound contraction was recorded. Group III applied with the Dose 1 reflected wound contraction of 91± 1.15% whereas those animals applied with Dose 2 and 3 reflected wound contraction of 93± 1.73% and 98± 1.52% respectively. Thus, the results suggest that as compared to control ones the formulation showed excellent wound healing properties.

INTRODUCTION

Wounds may be defined as loss or breaking of cellular and anatomic or functional continuity of living tissues (Yeo, *et. al*., 2000). It is the restoration of the integrity of the injured tissues. It is a dynamic process, involving sequence of events which take place in an orderly way i.e. inflammatory repair, closure, remodelling and final healing and also refers to the replacement of dead tissue by visible tissue.

Phases of Wound Healing comprises of Inflammatory Phase, Fibroblast Proliferation Phase, Maturation Phase, Epithelisation, Contraction Phase and Granulation Tissue Production Phase. There are different attributes for wound healing like physical, mechanical, biochemical and histological. Here, we have considered physical attributes in our study. Physical attributes like wound contraction, epithelisation and scar remodelling can be monitored by measuring the total wound area, open wound area and noting the physical changes in the scar e.g. size, shape and color etc. Excision wound model is the ideal one to study these attributes. The area measurement not only gives the rate of healing but can distinguish between contraction and epithelisation (Ramirez, *et. al*., 1969). The extent of epithelisation is determined by measuring the raw wound area from total wound area. Different methods are available for measuring the wound area. This may be traced on a paper, weighed and compared with that of a reference piece of same thickness and unit area on the same can be retraced on the graph paper to measure the area directly. The completion of epithelisation can be assessed by noting the time for complete covering of the raw surface of the wound. Granuloma study is another physical attributes of wound healing studies which can be assessed by quantifying the granuloma itself by noting its overnight dried weight (Lee, 1968).

Role of Plants

Plants have the immense potential for the management and treatment of wounds. In most of the countries, a large number of plants are used by tribal and folklore for the treatment of wounds and burns. These natural agents induce healing and regeneration of the lost tissue by various mechanisms. These phytomedicines are not only cheap and affordable but are also safe. The presence of wide range of life-sustaining constituents in plants has urged scientists to examine these plants with a view to determine potential wound healing properties (Nayak, *et. al.*, 2006).

Many phytopharmaceutical laboratories are now concentrating their efforts mainly to identify the active constituents and modes of action of various medicinal plants (Hwang, *et. al.*, 2000). The medicinal property of these plants lies in bioactive constituents that produce definite physiological action on the human body (Akinmoladun, *et. al.*, 2007). These constituents include various chemical classes like alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds (Edeoga, *et. al.*, 2005). For the discovery of new potent drugs, the screening of herbal extracts has been of great interest to the scientists (Kosger, *et. al.*, 2009). A number of reports concerning the antibacterial, anti-inflammatory, and wound healing activity of various plants have appeared in the literature, but the vast majority have yet to be explored. Numerous pharmacological reports are available on number of plants employing different wound healing models and its underlying molecular mechanism for the validation of their traditional claims and development of potent, safe and effective and globally accepted herbal drugs for wounds (Reddy, *et. al.*, 2012).

Various plants of wound healing property and also contain flavonoids as active constituents have been found. Tannins promote the wound healing through several cellular mechanisms, chelating of the free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation of capillary vessels and fibroblasts (Pawar, *et. al.*, 2012).

Excision Wound Model

In this model, a standard wound is made by cutting a circular skin in dorsal thoracic region of the experimental animals. Usually, a wound of 500 mm^2 areas is made with the help of marking of margin on pre-shaved area with an indelible ink and rubber seal. The wound measurement can also be carried out by putting sutures on the margin of the wound or after marking the margin with indelible ink. It is also possible to study the phase of the wound healing in this model. This is done by taking photographs of the wounds at different time intervals in the process of wound healing. The technique of photography has to carefully standardize to keep a constant distance between the camera lens and wound surface throughout the study. Percentage closure of original wound area after a predetermined lap at time (in days) is taken as a parameter. However, this result can be also quantified as the half time, in which time is taken by the wound to heal 50% of the day 1 wound area. It is more appropriately described as wound half closure time. By this

method, it is possible to differentiate the process of construction from epithelisation. Like Percentage of wound contraction and Period of epithelisation.

MATERIALS AND METHODS

Shaving blade, Povidone ointment, various doses of anti-haemorrhoidal formulation etc.

Animals

Albino rats weighing between 200 – 250g of either sex were used for the study. They were housed in the Animal House of the college (Sharad Powar College of Pharmacy) under the standard environmental conditions of temperature (25± 2^{o}C), Humidity (55± 10%) and Light (12:12 hrs. Light/Dark cycle; lights on at07: 00 hrs). Rats were supplied with standard pellet diet (Goldmuhar Brand Rat Feed supplied by Lipton, India Ltd.) and tap water *ad libitum*. The animals were handled and acclimatized to laboratory conditions 24 hours before conducting the experiments. All the experiments were conducted between 09: 00 and 18:00 hours. The parental administrations were given by disposable syringe and strict aseptic conditions were followed during the administration. The institutional animal ethics committee has approved the experimental protocols and was performed in accordance with the guidelines for the care and use of laboratory animals as adopted and promulgated by institutional animal ethical committee (SPCP/2013/653-1 by CPCSEA).

Preparation of Ointment as a Base

Simple Ointment IP Formula consisted of White Bees Wax (20 g), Hard Paraffin (30 g), Cetosteryl alcohol (50 g), white Soft Paraffin (900 g). Thus, ointment was prepared by taking weighed quantities of White bees wax (2%), hard paraffin (3%), cetosteryl alcohol (5%) and white soft paraffin (90%) as mentioned above and melted.

Preparation/Plants used for the Formulation:

1 g crude drug contains *Berberis aristata* (90 mg), *Cassia fistula* (100 mg), *Cynodon dactylon* (80 mg), *Emblica officinalis* (120 mg), *Tamarindus indica* (80 mg), *Terminalia chebula* (100 mg), *Terminalia belerica* (100 mg), *Sphaeranthus indicus* (110 mg), *Syzigium cumini* (100 mg), *Holarrhena antidysentrica* (50 mg) and *Mesua ferrea* (70 mg).

Preparation of Anti-haemorrhoidal ointment

Weighed quantities (10%) of above-mentioned aqueous anti-haemorrhoidal crude drug formulations i.e. 50, 100 and 200 mg/kg comprising of Dose 1, 2 and 3 respectively, were mixed with simple ointment (as a base) and spatula. The formulated ointments were preserved in the refrigerator.

Procedure:

Before proceeding towards the animal experiments, it was necessary to undertake the safety and efficiency of the formulation. Thus, the acute toxicity studies were conducted.

Acute toxicity studies:

Acute oral toxicity studies were carried out according to OECD guidelines 423. The animals of both sexes were selected by random sampling technique and divided into 5 groups of 3 animals each. A single oral dose (200, 400mg, 600mg, 800mg and 1000mg/kg) of each extract was administered orally at the dose level up to 1000mg/kg body weight. The animal groups were observed for appearance of toxic symptoms including behavioral changes, locomotion, muscle spasm, loss of righting reflex, tremor, convulsions and mortality for 24 hrs and further supervised for a period of 14 days for occurrence of toxic symptoms and mortality. However, from the first day till the 14^{th} one, there were no such adverse symptoms as mentioned above. There was no change in their behavior or their living skills also, in fact they remained unaffected completely. However, a bit of sluggishness was observed at higher doses.

The procedure was carried out as given in the thesis submitted by Shaikh, (2011) with some modifications.

Animals were under light ether anesthesia throughout the surgical procedure. An impression of 2.5 cm diameter (500 sq. mm.) as described by Morton and Malone was made after leaving at least 5 mm space from the ears. The skin of the impressed area was excised carefully to the complete thickness and a wound of 500 sq. mm. was formed. Homeostasis was achieved by application of the normal saline solution. The rats were kept individually in separate cages. The physical attributes of wound healing like wound closure (contraction) and epithelisation were recorded. The wound contraction was studied by tracing the raw wound area on a transparent

paper on 4th, 8th, 12th and 16th day. The criterion for complete epithelisation was fixed as formation of scar with absence of raw wound area. The raw wound area was measured planimetrically with the help of sq.mm. scale graph paper. The percentage wound closure was calculated by using the following formula.

Percentage closure = 1- Ad/Ao x 100

Where, Ad = Wound area on day zero (500 sq.mm.)

Ao = Wound area on corresponding days.

The number of days for complete epithelisation was noted. In the present study, no animals showed visible signs of infection.

Experimental Design

The animals were divided into 6 groups each consisting of 6 animals.

Group 1. Control group: Applied topically (0.5g) simple ointment.

Group 2. Standard group: Applied topically (0.5g) 5% Povidone Ointment.

Group 3. Dose 1 group: Applied topically (0.5g) Anti-haemorrhoidal Crude drug formulation ointment (10% of 50 mg/kg of rat body weight).

Group 4. Dose 2 group: The procedure here remains the same except for the concentration of the formulation which is 10% of 100 mg/kg of the rat body weight.

Group 5. Dose 3 group: Here also the procedure remains the same except for the concentration of the formulation which is 10% of 200 mg/kg of the rat body weight.

Observations:

Table 1: Effect of Anti-Haemorrhoidal Crude Drug Formulation on Excision Wound Model

Sr. No.	Treatment	% Wound Contraction On				Epithelization Time (Days)
		4th Day	8th Day	12th Day	16th Day	
1.	Control	24.6 ± 2.08	52.8 ± 3.05	66.8 ± 1.73	76.40 ± 0.88	20± 2.08
2.	Standard	39.8 ± 1.73	69.8 ± 3.05	91.6 ± 2.51	100	14± 1.08
3.	Dose 1	25.8 ± 1.15	59± 2.08	74± 2.30	91± 1.15	16± 2.36
4.	Dose 2	27.8 ± 2.16	75± 2.16	84± 2.64	93± 1.73	15± 1.64
5.	Dose 3	39.2 ± 2.30	73± 2.08	82.8 ± 3.60	98± 1.52	13± 1.59

Values are expressed as Mean Values ± Standard Error at N = 6, ($p < 0.5$).

RESULTS AND DISCUSSION

Animals were treated by external application of different formulations at a final concentration of 10 mg/kg body weight. Group I (Control group) animals were treated with normal ointment (5% w/w), Group II (Standard group) animals were treated with (0.5g) 5% Povidone Ointment, Group III consisted of animals to which Dose 1 of the formulation was applied while Group IV and Group V animals were applied with Dose 2 and Dose 3 respectively. Test formulations were applied to respective groups twice a day for 16 days starting from the day of wounding. Wound healing property was evaluated by wound contraction percentage and closure time. The wound area was measured every fourth day by placing a transparent paper over the wound and tracing it out; area of this impression was calculated using the graph sheet, and wound contraction was expressed as percentage of contraction. Wound closure time was recorded when the total wound was healed completely.

As shown in Table 1, the % wound contraction on 4th, 8th, 12th and 16th day in Control Group was 24.6± 2.08, 52.8± 3.05, 66.8± 1.73 and 76.40± 0.88% respectively whereas in Standard it was 39.8± 1.73, 69.8± 3.05, 91.6± 2.51 and 100% on the 4th, 8th, 12th and 16th day respectively. Group 3 applied with the Dose 1 of the formulation showed the % wound contraction of 25.8± 1.15, 59± 2.08, 74± 2.30 and 91± 1.15 % while Group 4 applied with Dose 2 of the formulation showed % wound contraction of 27.8± 2.16, 75± 2.16, 84± 2.64 and 93± 1.73 % on the 4th, 8th, 12th and 16th day respectively. Group 5 applied with Dose 3 of the formulation reflected the % wound contraction of 39.2± 2.30, 73± 2.08, 82.8± 3.60 and 98± 1.52 % on the 4th, 8th, 12th and 16th day respectively.

As mentioned above, 100% of wound healing was recorded in the Standard group on the 16th day therefore, the comparisons are done accordingly. In the control group, 76.40± 0.88% wound contraction was recorded. Group III applied with the Dose 1 reflected wound contraction of 91± 1.15% whereas those animals applied with Dose 2 and 3 reflected wound contraction of 93± 1.73 and 98± 1.52% respectively. Thus, the results suggest that as compared to control ones the Formulation showed excellent wound healing properties but slightly lesser than that of the Standard Povidone ointment. The rate of wound healing was faster in case of the Standard and parallel to that of Dose 3 hence, it can be considered as the most effective one among the three doses of the formulation. The enhanced capacity of wound healing with the various doses of the formulation could be explained on the basis of the anti-inflammatory effects as documented in the literature (Geethalakshmi, *et. al.*, 2013). In wound healing studies, the wound closure time and wound contraction were taken as parameters. In all doses of the formulation wound closure time was 16 days. Results obtained in this study confirm the wound healing activity of the formulation.

CONCLUSION

In conclusion, while plant-based traditional medicine has been used throughout generations, the efficacy of such treatments requires experimental backup and scientific verification. In this study, anti-haemorrhoidal formulation was selected based on ethnopharmacological information. The formulation shows a positive influence on wound healing and therefore, has a beneficial role in wound healing; further, the synergistic effect of antioxidant activity accelerated the wound healing process. It exhibited good wound healing activity probably due to the presence of

phenolic and flavonoid constituents. It significantly enhanced the rate of wound contraction and the period of epithelialization comparable to Povidone.

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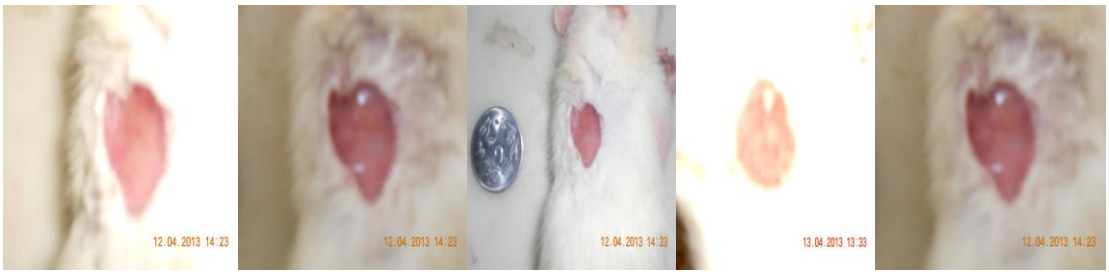
REFERENCES

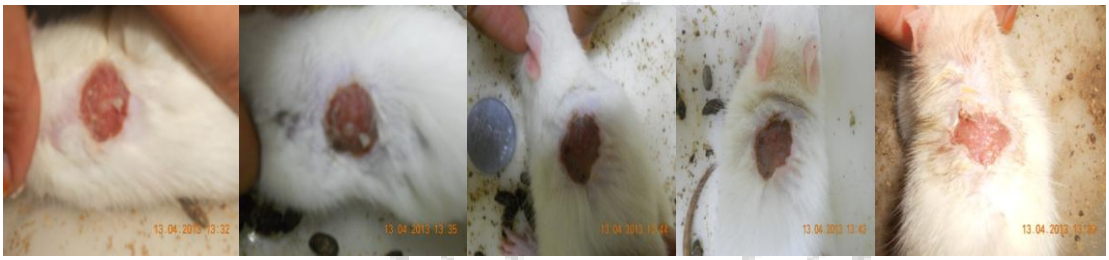
1. Akinmoladun, A. C.; Ibukun, E. O. and Afor, E. (2007). Chemical Constituents And Antioxidant Activity Of *AlstoniaBoonei*.*African Journal of Biotechnology*: 1197–1201.
2. Edeoga, H. O.; Okwu, D. E. and Mbaebie, B. O. (2005).Phytochemical Constituents of Some Nigerian Medicinal Plants.*African Journal of Biotechnology* **4**: 685–688.
3. Geethalakshmi, R.; Sakravarthi, C.; Kritika, Kirubakaran, M. A. and Sarada, D. V. L. (2013).Evaluation of Antioxidant and Wound Healing Potentials of *Sphaeranthusamaranthoides*Burm.f.*BioMed Research International* **2013**: 1-7.
4. Hwang, J. K.; Kong, T. W.; Baek, N. and Pyun, Y. R. (2000). A- Glycosidase Inhibitory Activity of HexagalloylglucoseFrom The Galls of *QuercusInfectoria*. *PlantaMedica*, **66**: 273–274.
5. Kosger, H. H.; Ozturk, M.; Sokmen, A. and Bulut, E. (2009). Wound Healing Effects Of *ArnebiaDensiflora* Root Extracts On Rat Palatal Mucosa. *European Journal of Dentistry***2** : 96-99.
6. Lee, K. H. (1968). Studies on the mechanism action of salicylates II, effect of vitamin A on wound healing retardation action of aspirin. *J Pharmacol Sci*. **57** : 1238-1240.
7. Nayak, B. S. and Pinto, P. L. M. (2006).*CatharanthusRoseus* Flower Extract Has Wound-Healing Activity In Sprague Dawley Rats. *Bmc Complementary and Alternative Medicine* **6** : 41.
8. Pawar, R. S. and Fedelic, A. T. (2012).Plants That Heal Wounds.A Review.*HerbaPolonica***58** (1) : 48-65.
9. Ramirez, A.; Soroff, A. R.; Schwartz, H. S. and Moofley, M. S. (1969). Experimental wound healing in man. *Surg* .*Gynecolobstet***128** : 283-93.
10. Reddy, A. K.; Priyanka, B.; Saranya, C. and Ashok, K. C. (2012). Wound healing Potential of Indian Medicinal Plants. *International Journal of Pharmacy Review and Research*.**2** (2): 75-87.
11. Shaikh, M. Y. (2011). Ph. D. Thesis - Evaluation of antidiabetic and wound healing property *of Ichnocarpusfrutescens* L. Reg.No.09: 254.
12. Yeo, J. H.; Lec, K. W.; Kim, H. C.; Lyum, Y.; Kim, A. and Kim, S. Y. (2000). The effect of PVA / Chitosan/ Fibroin – blended spongy sheets on wound healing in rats. *Biolpharm Bull*. **23**: 1220-23.

Wound Healing Activity of the Anti-Haemorrhoidal Crude Formulation on Excision Wound Model

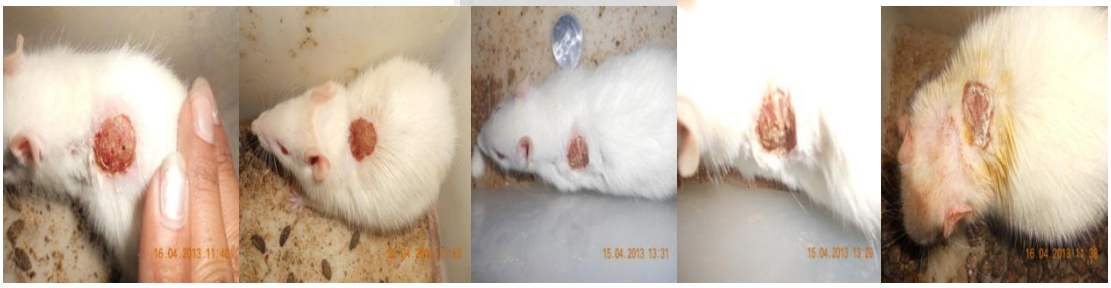
Control **Standard** **Dose 1** **Dose 2** **Dose 3**



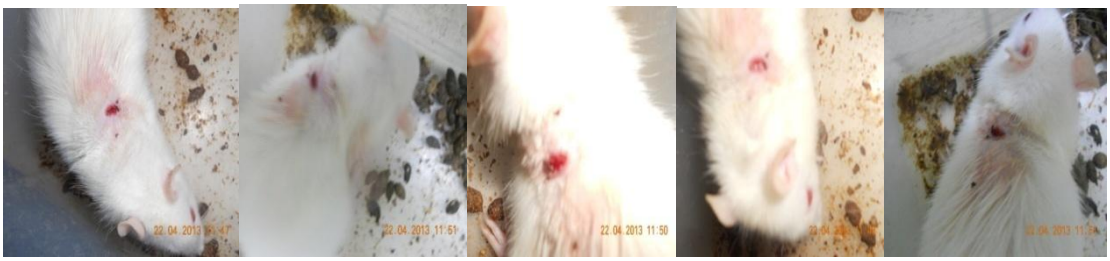
0 Day



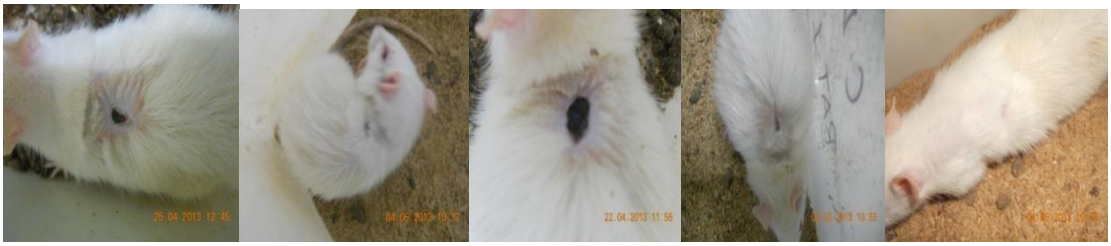
4th Day



8th Day

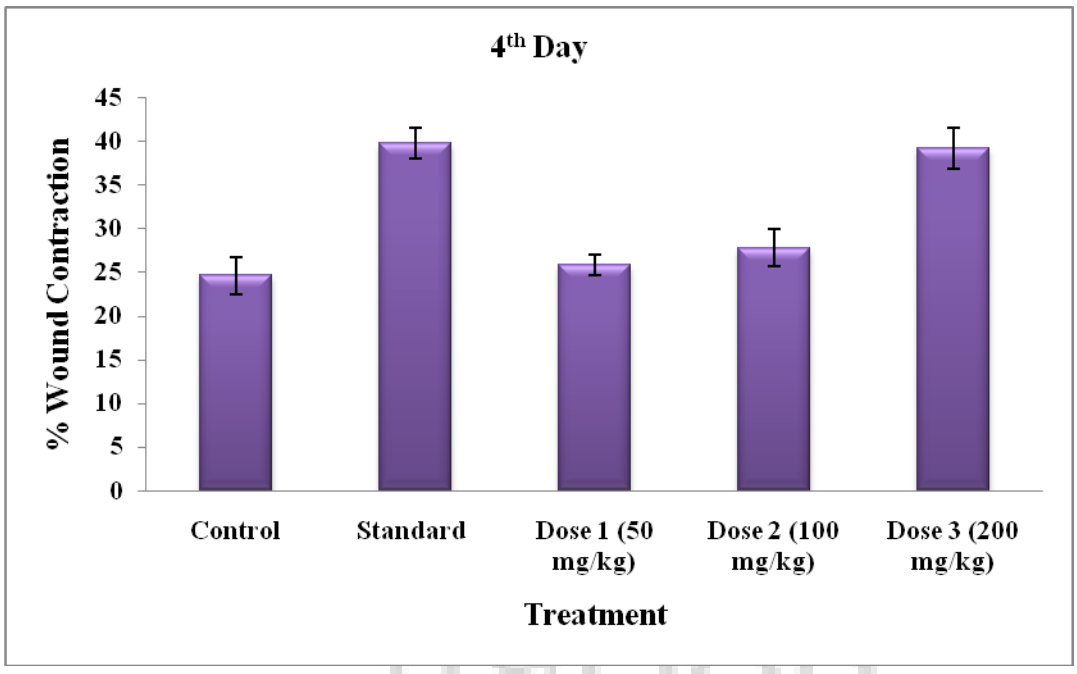


12th Day

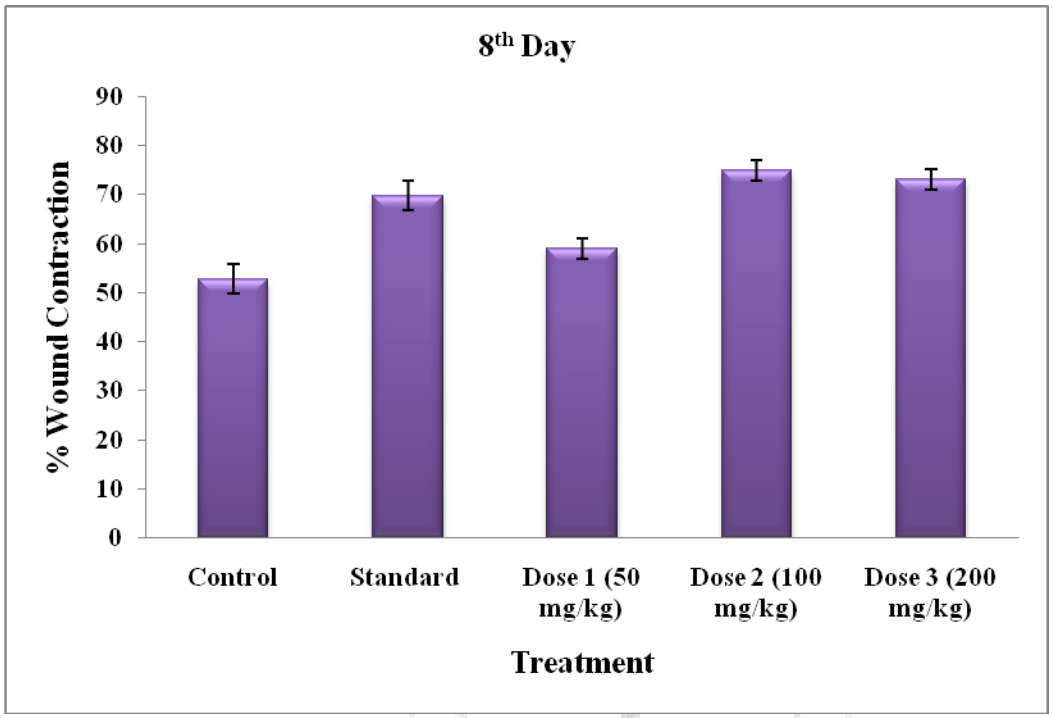


16th Day

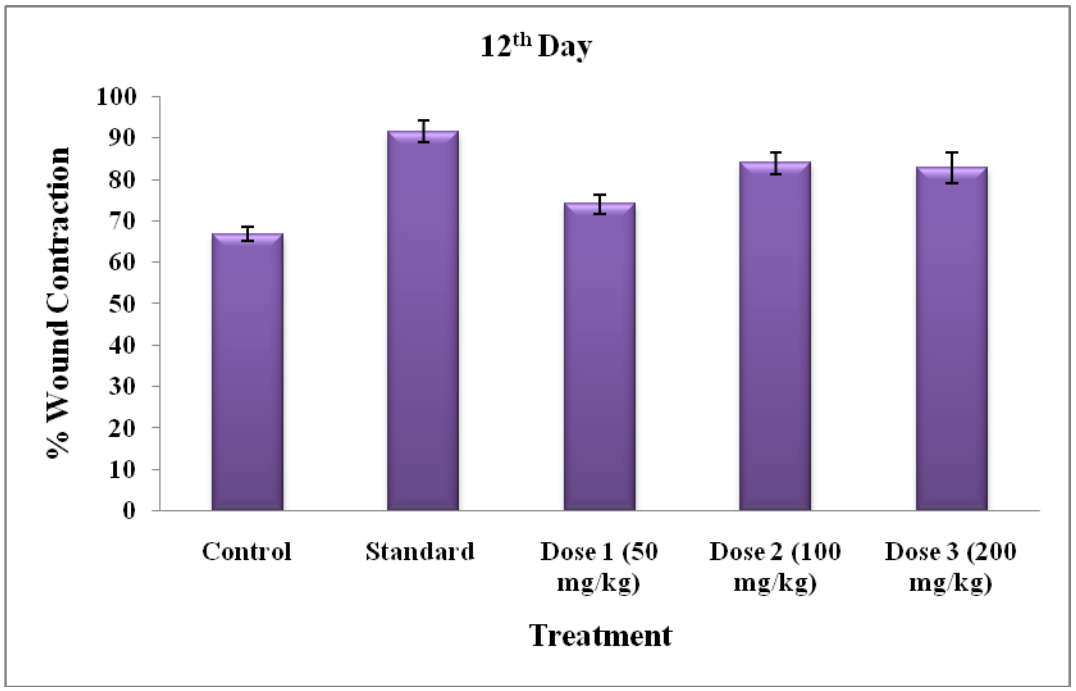
Graph 1. Effect of Anti-haemorrhoidal Crude Drug Formulation on Excision Wound Model on the 4th Day



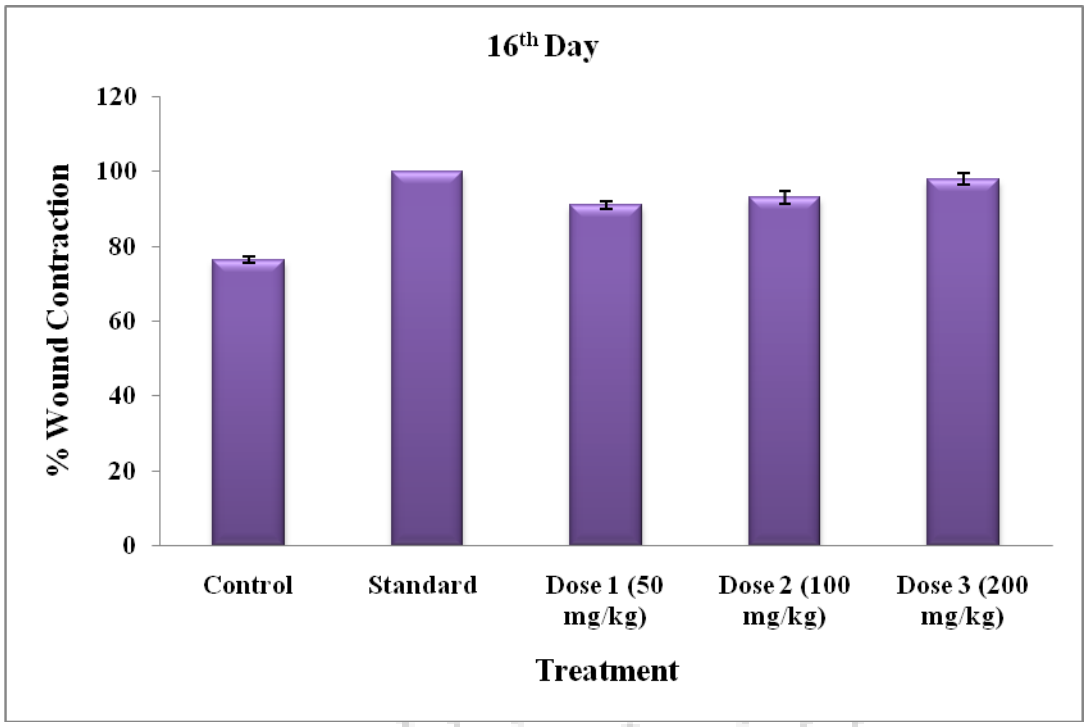
Graph 2. Effect of Anti-haemorrhoidal Crude Drug Formulation on Excision Wound Model on the 8th Day



Graph 3. Effect of Anti-haemorrhoidal Crude Drug Formulation on Excision Wound Model on the 12th Day



Graph 4. Effect of Anti-haemorrhoidal Crude Drug Formulation on Excision Wound Model on the 16th Day



Graph 5. Effect of Anti-haemorrhoidal Crude Drug Formulation on Excision Wound Model for Epithelization Time (Days)