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EVALUATION OF WOUND HEALING POTENTIAL OF VARIOUS WOUND HEALING CREAMS IN FRESH, DIABETIC AND BURN WOUND MODELS

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ABSTRACT

Introduction: The present study was designed to investigate comparative efficacy of various wound healing creams WHCs (F1 to F7) with placebo cream and marketed formulations (Mupirocin cream, Silver Sulfadiazine Cream and Jatyadi oil) using excision, incision and burn wound models in normal and diabetic rats. **Materials and Methods:** In-vivo excision, incision and burn wound models in normal and diabetic rats were used in order to assess comparative efficacy of various wound healing creams WHCs (F1 to F7) with marketed formulations. In case of the excision and burn wound models, wound contraction and period of epithelization were studied. Histopathological study was conducted in order to assess fibroblast proliferation, collagen formation, angiogenesis and epithelialization of wound. In incision wound model, tensile strength of wound was evaluated. **Result:** WHC [Formulation No. 4 (F-4)] showed significantly better wound healing activity than placebo cream, other various WHCs, Mupirocin Cream, Silver Sulfadiazine Cream and Jatyadi Oil. F-4 also showed complete epithelization and good collagen deposition as compared to Placebo Cream, other various WHCs, Mupirocin Cream, Silver Sulfadiazine Cream and Jatyadi Oil. **Conclusion:** F-4 showed statistically significant better wound healing activity in excision, incision and burn wound models in normal and diabetic rats as compared to placebo cream, other various WHCs, Mupirocin Cream, Silver Sulfadiazine Cream and Jatyadi Oil.

KEYWORDS: Burn wound model, Excision Model, Incision Model, Wound healing potential, diabetic wound healing model, Silver Sulfadiazine Cream, Jatyadi Oil.

INTRODUCTION

Wound is defined as a loss or breaking of cellular and anatomic or functional continuity of living tissues[1] and Wound healing involves platelet aggregation, blood clotting, formation of fibrin, an inflammatory response to injury, angiogenesis and re-epithelization.[2-4] This complex cascade of event starts from the moment of injury and continues for varying periods of time depending on the severity of wounding. Normal wound healing contains four highly integrated and overlapping phases of cellular and biochemical activities including hemostasis, inflammation, proliferation and maturation or remodeling.[5] In spite of tremendous advances in pharmaceutical drug industry, the availability of drugs capable of stimulating the process of wound repair is still limited.[6,7] Only 1–3% of the drugs listed in Western pharmacopoeias are intended for use on wounds; on the other hand, at least one-third of herbal remedies are applied as wound healing agents.[7,8] Many traditional practitioners across the world have valuable information of many plants for treating wounds and burns. The presence of bioactive constituents in plants has urged researchers to screen medicinal plants with a view to determine potential wound healing activities and isolate chemical entities associated with wound healing.[9] Wound healing process is promoted efficiently by the use of traditional remedies which are mainly based on plant sources. These remedies have been shown to affect one or more stages of the wound healing process. In this context, traditional medicines can provide a vast source for the discovery of original drug leads.[10-12]

There is need for new cost-effective therapies with better efficacy for wound healing. Medicinal plants are important sources of new chemical substances that have beneficial therapeutic effect.[13] Taking in to consideration the need of today's world, Ari Healthcare Pvt. Ltd. has conceptualized and developed the wound healing cream (WHC). Based on the traditional documents on Indian medicine, various new wound

healing formulations were developed which contains Jatyadi oil, *Ficus religiosa*, *Ficus benghalensis*, *Centella asiatica*, *Shorea robusta*, *Glycyrrhiza glabra*, *Azadirachta indica*, *Pongamia glabra*, and Yashad Bhasma (Classical Ayurvedic Formulation). The study was planned to evaluate wound healing activity of WHC in comparison with various wound healing cream (WHCs) (F1 to F7), Placebo Cream, marketed formulations i.e. Mupirocin Cream, Silver Sulfadiazine Cream and Jatyadi Oil by using excision, incision and burn wound models in normal and diabetic rats.

MATERIALS AND METHODS

Preparation of formulations

WHCs were conceptualized and developed by Ari Healthcare Pvt. Ltd. It contains Jatyadi Oil, *Ficus religiosa*, *Ficus benghalensis*, *Centella asiatica*, *Shorea robusta*, *Glycyrrhiza glabra*, *Azadirachta indica*, *Pongamia glabra* and Yashad Bhasma (Classical Ayurvedic Formulation). Seven different variants of WHCs (F1 to F7) were prepared by using different concentrations. Also placebo cream was prepared. Jatyadi oil, Silver Sulfadiazine Cream and Mupirocin cream were purchased from the market.

Experimental animals

Institutional Animal Ethics Committee approval was taken (MMCOP/IAEC/001/2013) before initiation of this study. Male Wistar Rats were procured and acclimatized for 5 days. Animals were maintained at 25± 2^{o}C and relative humidity of 45 to 55% and under standard environmental conditions (12 h light and 12 h dark cycle). The food and water were provided *ad libitum.*

Excision wound model in normal rats

Rats were anaesthetized with ketamine 50-60 mg/kg. i.p and xylazine 5-10 mg/kg. i.p. Animals were shaved on right side. The area of the wound was outlined with methylene blue using circular stencil. A full thickness excision wound of around 300 mm^2 and 2 mm in depth was created to each animal. The entire wound was left open. Treatments were given to respective group as per study design (Table No. I). Treatments were given topically twice a day. Wound area was measured and recorded on day 1, 3, 7, 11 and 15. Wound area was traced using transparent graph paper and permanent marker. Assessment of the wound area was done. Photograph of day 1, 9, 13, were taken. Day of complete epithelization was recorded for each animal. Skin sample of animals were collected and histopathological studies were performed to observe Epithelization, neoangiogenesis, neutrophil infiltration, collagen deposition etc. as wound healing parameters.

Incision wound model in normal rats

Rats were anaesthetized with ketamine 50-60 mg/kg i.p. and Xylazine 5-10 mg/kg i.p. Animals were shaved on right side and placed in their respective group (Table No. 1). Paravertebral straight incision of 6 cm length was made through the entire thickness of the skin. The wound was closed by means of interrupted sutures placed at approximately 1 cm apart. The treatment was done topically twice daily. The wound breaking strength was estimated on 10th day. The wound breaking strength was evaluated by constant water flow technique. Each anesthetized animal was secured to the operation table and a line was drawn on either side of the wound 3 mm away from the line. Two allice forceps were firmly applied to the line facing each other. One of the forceps was fixed while the other was connected to a freely suspended light weight polypropylene graduated container through a string run over to a pulley. Water was allowed to flow from the reservoir slowly and steadily into the container. A gradual increase in weight was transmitted to the wound site for pulling apart the wound edges. As and when the wound edges just opened up, the water flow was arrested and the volume of water collected in the container was noted. The average reading of the group was taken as an individual value of breaking strength.

Table No.1: Study design: Excision and incision wound models.

Gr. No.	Group Description	No. of Animals (N)
I	Normal Control (Base of formulation)	6
II	Formulation No.1 (F-1)	6
III	Formulation No.2 (F-2)	6
IV	Formulation No.3 (F-3)	6
V	Formulation No.4 (F-4)	6
VI	Formulation No.5 (F-5)	6
VII	Formulation No.6 (F-6)	6
VIII	Formulation No.7 (F-7)	6
IX	Positive control (Mupirocin cream)	6
X	Jatyadi Oil	6

Induction of diabetes

Animals were weighed and their fasting blood glucose level was determined before induction of diabetes. The animals were then injected with single dose of freshly prepared streptozotocin 50 mg/kg in cold 0.1 M citrate buffer pH 4.5 in tail vein to induce diabetes. Control animals were injected with 0.1 M citrate buffer. Fasting blood glucose was measured three days later to confirm diabetes status of the animals. Blood was collected by retro-orbital puncture method. After induction of

diabetes the same procedure that of normal rat for conducting different models was followed.

Burn wound model in normal rats

Rats were anaesthetized with ketamine 50-60 mg/kg i.p. and Xylazine 5-10 mg/kg i.p. Animals were shaved on the neck region and placed in their respective groups (Table No. 2). Partial thickness burn wounds were inflicted by pouring hot molten wax (2 gm) at 80ºC. The wax was poured with the spoon and spreading is restricted using the stencil. The wax was allowed to remain on the skin till it got solidified. The treatment was given topically two times a day. Wound area was measured on day 1, and every alternate day till the day of epithelization. Measurement was done using transparency sheet and permanent marker. Recordings of the transparency were traced on the graph paper and marked area on the graph paper were measured and recorded. The day of Escher falling without any residual raw wound was considered as the day of epithelization.

Table No. 2: Study design: Burn wound model in normal animals (rats).

Gr. No.	Group Description	No. of Animals (N)
I	Normal Control (Base of formulation)	6
II	Formulation No.1 (F-1)	6
III	Formulation No.2 (F-2)	6
IV	Formulation No.3 (F-3)	6
V	Formulation No.4 (F-4)	6
VI	Formulation No.5 (F-5)	6
VII	Formulation No.6 (F-6)	6
VIII	Formulation No.7 (F-7)	6
IX	Positive control (Silver sulfadiazine cream)	6
X	Jatyadi oil	6

Burn wound model in diabetic rats

Induction of diabetes mellitus in rats was done as described earlier. Rats were anaesthetized with ketamine 50-60 mg/kg i.p. and Xylazine 5-10 mg/kg i.p. Animals were shaved on the neck region and placed in their respective group (Table No.3). Partial thickness burn wounds were inflicted by pouring hot molten wax (2 gm) at 80ºC. The wax was poured with the spoon and spreading was restricted using the stencil. The wax was allowed to remain on the skin till it got solidified. The treatment was given topically two times as day. Wound area was measured on day 1 and every alternate day till the day of epithelization. Measurement was done using transparency sheet and permanent marker. Recordings of the transparency were traced on the graph paper and marked area on the graph paper were measured and recorded. The day of eschar falling without any residual raw wound was considered as the day of epithelization.

Table No. 3: Study design: Burn wound model in diabetic rats.

Gr. No.	Group Description	No. of Animals (N)
I	Diabetic Negative Control (Formulation Base)	6
II	Diabetic Control (Formulation Base)	6
III	Formulation No.1 (F-1)	6
IV	Formulation No.2 (F-2)	6
V	Formulation No.3 (F-3)	6
VI	Formulation No.4 (F-4)	6
VII	Formulation No.5 (F-5)	6
VIII	Formulation No.6 (F-6)	6
IX	Formulation No.7 (F-7)	6
X	Positive control (Silver sulfadiazine cream)	6
XI	Jatyadi Oil	6

Excision wound model in diabetic rats

Induction of diabetes mellitus in rats was done as described earlier. Rats were anaesthetized with ketamine 50-60 mg/kg. i.p and xylazine5-10 mg/kg. i.p. Animals were shaved on a right side and placed in their respective group (Table No. 4). The area of the wound was outlined with methylene blue using circular stencil. A full thickness excision wound of around 300mm^{2}and 2 mm in depth was created to each animal. The entire wound was left open. Treatments were given to respective group as per study design. Treatments were given topically twice a day. Wound area was measured on day-1, 3, 7, 11 and 15 and recorded. Wound Area was traced using transparency and permanent marker. Wound area was measured using graph paper. Photograph of day 1, 9 and13 were taken. Day of complete epithelization was recorded for each animal. Skin sample of animals were collected and histopathological studies were performed considering Epithelization, neoangiogenesis, neutrophil infiltration, collagen deposition etc. as wound healing parameters.

Table No. 4: Study design: Excision and incision wound models in diabetic rats.

Gr. No.	Group Description	No. of Animals (N)
I	Diabetic Negative Control (Formulation Base)	6
II	Diabetic Control (Formulation Base)	6
III	Formulation No.1 (F-1)	6
IV	Formulation No.2 (F-2)	6
V	Formulation No.3 (F-3)	6
VI	Formulation No.4 (F-4)	6
VII	Formulation No.5 (F-5)	6
VIII	Formulation No.6 (F-6)	6
IX	Formulation No.7 (F-7)	6
X	Positive control (Mupirocin Cream)	6
XI	Jatyadi Oil	6

Incision wound model in diabetic rats

Induction of diabetes mellitus in rats was done as described earlier. Rats were anaesthetized with ketamine 50-60 mg/kg i.p. and Xylazine 5-10 mg/kg i.p. Animals were shaved on right side placed in their respective group (Table No 4). Paravertebral straight incision of 6 cm length was made through the entire thickness of the skin. The wound was closed by means of interrupted sutures placed at approximately 1 cm apart. The treatment was done topically twice daily. The wound breaking strength was estimated on the 10^{th} day. The wound breaking strength was evaluated by constant water flow technique. Each anesthetized animal was secured to the operation table and a line was drawn on either side of the wound 3 mm away from the line. Two allice forceps were firmly applied to the line facing each other. One of the forceps was fixed while the other was connected to a freely suspended light weight polypropylene graduated container through a string run over to a pulley. Water was allowed to flow from the reservoir slowly and steadily into the container. A gradual increase in weight transmitted to the wound site pulling apart the wound edges. As and when the wound was just opened up, the water flow was arrested and the volume of water collected in the container was noted. The average reading of the group was taken as an individual value of breaking strength.

RESULTS

Burn model in normal rats

Among all groups, group V Formulation No.4 (F-4) took less days (13) for complete wound healing in burn model in normal rats. Formulation No. 4 (F-4) was statistically significant effective wound healing agent as compared to placebo cream. Also Silver sulfadiazine cream and Jatyadi Oil was statistically significant wound healing agents as compared to placebo group. When compared between groups, Formulation No. 4 (F-4) was statistically significant wound healing agent than Jatyadi Oil and Silver sulfadiazine cream in burn model in normal rats. The details are presented in Table No. 5 and figure no 1.

Table No. 5: Effect of topical application of various wound healing formulations, placebo cream, Silver Sulfadiazine cream (Positive control) and Jatyadi oil on days required for epithelization in burn model.

Gr. No.	Treatment	Days of Epithelization (Mean ± SEM)
I	Normal Control (Base of formulation	23 ± 0.70
II	Formulation No.1 (F-1)	21 ± 0.31
III	Formulation No.2 (F-2)	19 ± 0.61
IV	Formulation No.3 (F-3)	18 ± 0.80
V	Formulation No.4 (F-4)	13 ± 0.21**#
VI	Formulation No.5 (F-5)	21 ± 0.56
VII	Formulation No.6 (F-6)	16 ± 1.32*
VIII	Formulation No.7 (F-7)	17 ± 0.79
IX	Positive control (Silver sulfadiazine cream)	16 ± 0.33*
X	Jatyadi Oil Formulation	19 ± 0.81

**P<0.01 significant as compared to placebo using one-way ANOVA followed by Dunnet test.

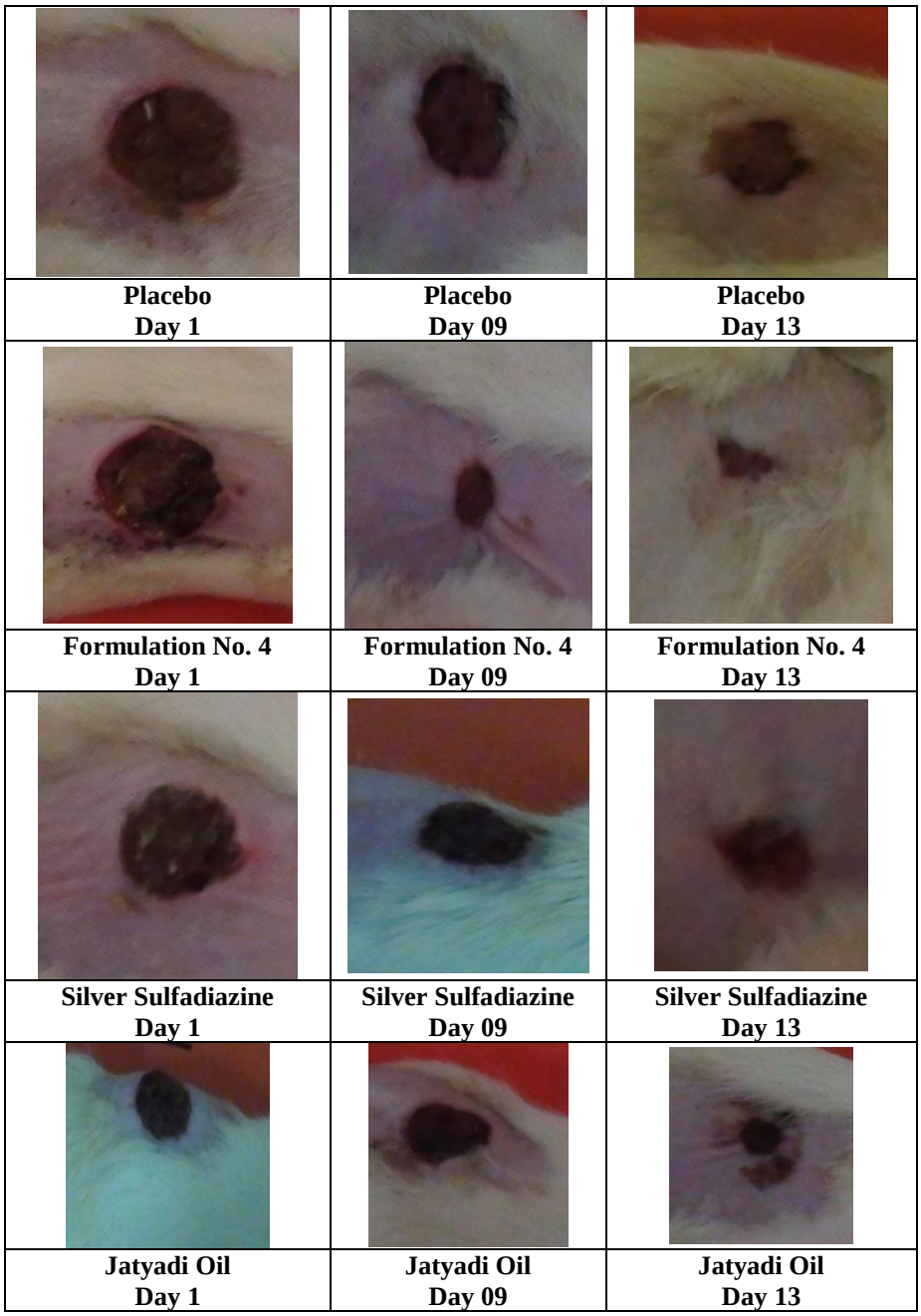

Figure. 1: Effect of topical application of various wound healing formulations, placebo cream, silver sulfadiazine cream (positive control) and Jatyadi oil on days required for epithelization in burn model.

Excision Wound Model in Normal Rats

Among all the formulation studied, Formulation No. 4 (F-4) required less days (15) for complete wound healing. Formulation no. 4 (F-4) was statistically significant wound healing agent as compared to placebo. When compared between groups, formulation no 4(F-4) was statistically significantly better wound healing agent than Jatyadi Oil. Also Formulation 4 (F-4) was better than Mupirocin cream in terms of wound healing activity. The details are presented in Table No.6.

Table No. 6: Effect of topical application of various wound healing formulations, placebo cream, Mupirocin cream (positive control) and Jatyadi oil on days required for epithelization in excision model.

Gr. No.	Treatment	Days of Epithelization (Mean ± SEM)
I	Normal Control (Base of formulation)	27 ± 1
II	Formulation No.1 (F-1)	26 ± 1
III	Formulation No.2 (F-2)	18 ± 1**
IV	Formulation No.3 (F-3)	17 ± 1***
V	Formulation No.4 (F-4)	15 ± 0***
VI	Formulation No.5 (F-5)	25 ± 2
VII	Formulation No.6 (F-6)	19 ± 2 *
VIII	Formulation No.7 (F-7)	16 ± 1***
IX	Positive control (Mupirocin cream)	18 ± 2**
X	Jatyadi Oil	23 ± 2$^{\#}$

**P<0.05 **P<0.01 ***P<0.001significant as compared to placebo using one-way ANOVA followed by Bonferroni post test. $^{\#}$ P<0.05 Significant as compared to Formulation-4 using one-way ANOVA followed by Bonferroni post test.*

Incision Wound Model in Normal Rats

The wound breaking strength of formulation no. 4 (F-4) was higher (388.00 ± 46.38) as compared to any other formulation used in this study. When compared between groups, formulation no. 4. (F-4) was having statistically more significant wound breaking strength than Mupirocin cream. The details are presented in Table No. 7.

Table No. 7: Effect of topical application of various wound healing formulations, placebo cream, Mupirocin cream (positive control) and Jatyadi oil on wound breaking strength.

Gr. No.	Treatment	Wound breaking Strength (Mean ± SEM)
I	Normal Control (Base of formulation)	309.17 ± 19.85
II	Formulation No.1 (F-1)	256.67 ± 12.02
III	Formulation No.2 (F-2)	253.33 ± 8.43
IV	Formulation No.3 (F-3)	296.67 ± 18.56
V	Formulation No.4 (F-4)	388.00 ± 46.38**
VI	Formulation No.5 (F-5)	278.33 ± 46.76
VII	Formulation No.6 (F-6)	310.00 ± 30.22
VIII	Formulation No.7 (F-7)	250.00 ± 19.66
IX	Positive control (Mupirocin cream)	248.33 ± 19.56
X	Jatyadi Oil Formulation	313.33 ± 19.78

***P<0.01 significant as compared to Mupirocin using one-way ANOVA followed by Bonferroni post test.*

Histopathology

In histological analysis of the wound, the wounds of rats from control group had not been fully restored and inflammatory cells in scar tissue were observed. Re-epithelialization of the wounds showed incomplete. When compared with that in control, the progress from granulation formation to re-epithelialization was accelerated in F-2, F-3, F-4, F-6, F-7, Mupirocin cream and Jatyadi Oil groups.

Among all the formulations tested, Formulation No. 4 (F-4) was better in terms of wound healing parameters viz. complete epithelization, more blood vessels and more collagen fibers.

Microscopic observations of the wounds from animals treated with Formulation No. 4 (F-4) showed complete re-epithelialization and a normal epidermis covered the wound area, collagen fibers were thick and were denser as compared to that of Placebo Cream, Mupirocin Cream and Jatyadi oil. The cutaneous annexes, such as sebaceous glands and hair follicles in the centre of the scar tissue were also well formed.

This indicated that Formulation No. 4 (F-4) stimulates and accelerates the activities towards faster wound healing and could play a role in the early stage of the wound healing process as compared to Placebo Cream, Mupirocin Cream and Jatyadi oil. Results are shown in the figure no 2-5.

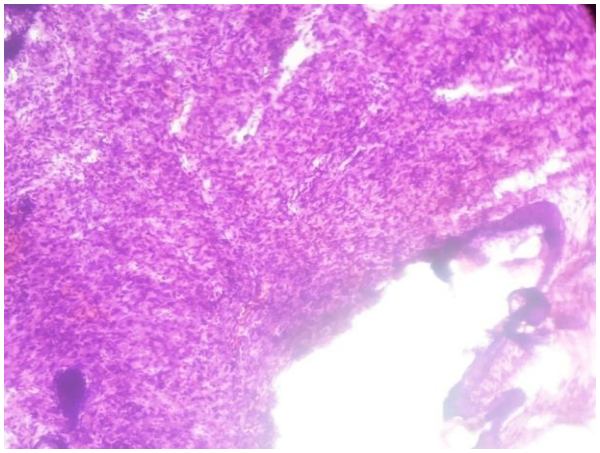

Figure 2: Histopathological study of placebo cream treated wound in rat (incomplete epithelialization and presence of fibroblasts. H & E 20X).

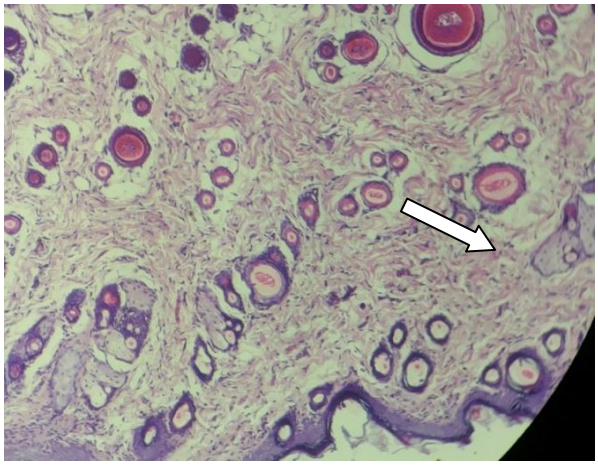

Figure 3: Histopathological study of formulation-4 (F-4) treated wound in rat (skin –complete epithelialization and presence of thick collagen fibers. H&E 20X).

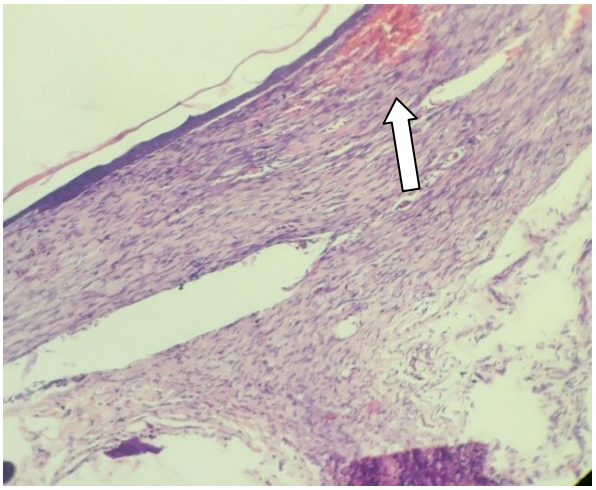

Figure 4: Histopathological study of Mupirocin cream treated wound in rat (presence of inflammatory cells, new blood vessels and hemorrhages. H & E 20X).

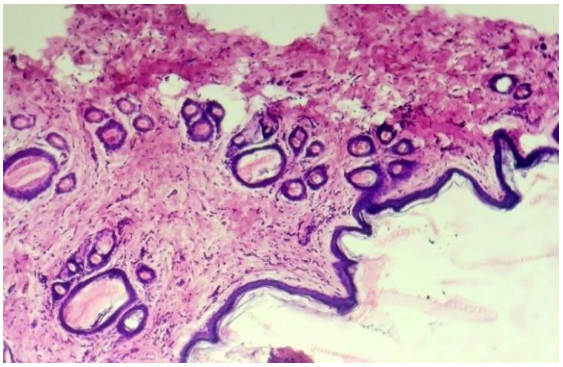

Figure 5: Histopathological study of Jatyadi oil treated wound in rat (skin –incomplete epithelialization, presence of few inflammatory cells. H & E 20X).

Burn model in diabetic rats

Among all groups, group VI (Formulation No. 4) took less days (15) for complete wound healing in burn model in diabetic rats. Formulation No. 4 was statistically significant effective wound healing agent as compared to placebo cream. Also, Silver sulfadiazine was statistically significant wound healing agent as compared to placebo group. When compared between groups, Formulation No. 4 (Amarantha Wound Healing Cream) was statistically significantly better wound healing agent than Jatyadi Oil and Silver sulfadiazine in burn model in diabetic rats. The details are presented in Table No. 8 and figure no 6.

Table No. 8: Effect of topical application of various Wound Healing formulations, Placebo Cream Silver Sulfadiazine cream (Positive control) and Jatyadi oil on days required for epithelization in Burn Model.

Gr. No.	Treatment	Days of Epithelization (Mean ± SEM)
II	Diabetic Control (Formulation Base)	27 ± 0.30
III	Formulation No.1 (F-1)	22 ± 0.15
IV	Formulation No.2 (F-2)	19 ± 0.25
V	Formulation No.3 (F-3)	17 ± 0.80
VI	Formulation No.4 (F-4)	15 ± 0.31**#
VII	Formulation No.5 (F-5)	22 ± 0.56
VIII	Formulation No.6 (F-6)	16 ± 1.43*
IX	Formulation No.7 (F-7)	17 ± 0.79
X	Positive control (Silver sulfadiazine cream)	18 ± 0.33*
XI	Jatyadi Oil Formulation	21 ± 0.73

***P<0.01 significant as compared to placebo using one-way ANOVA followed by Dunnet post test. #P<0.01 significant as compared to Silver Sulfadiazine and Jatyadi oil using one-way ANOVA followed by Bonferroni Test.*

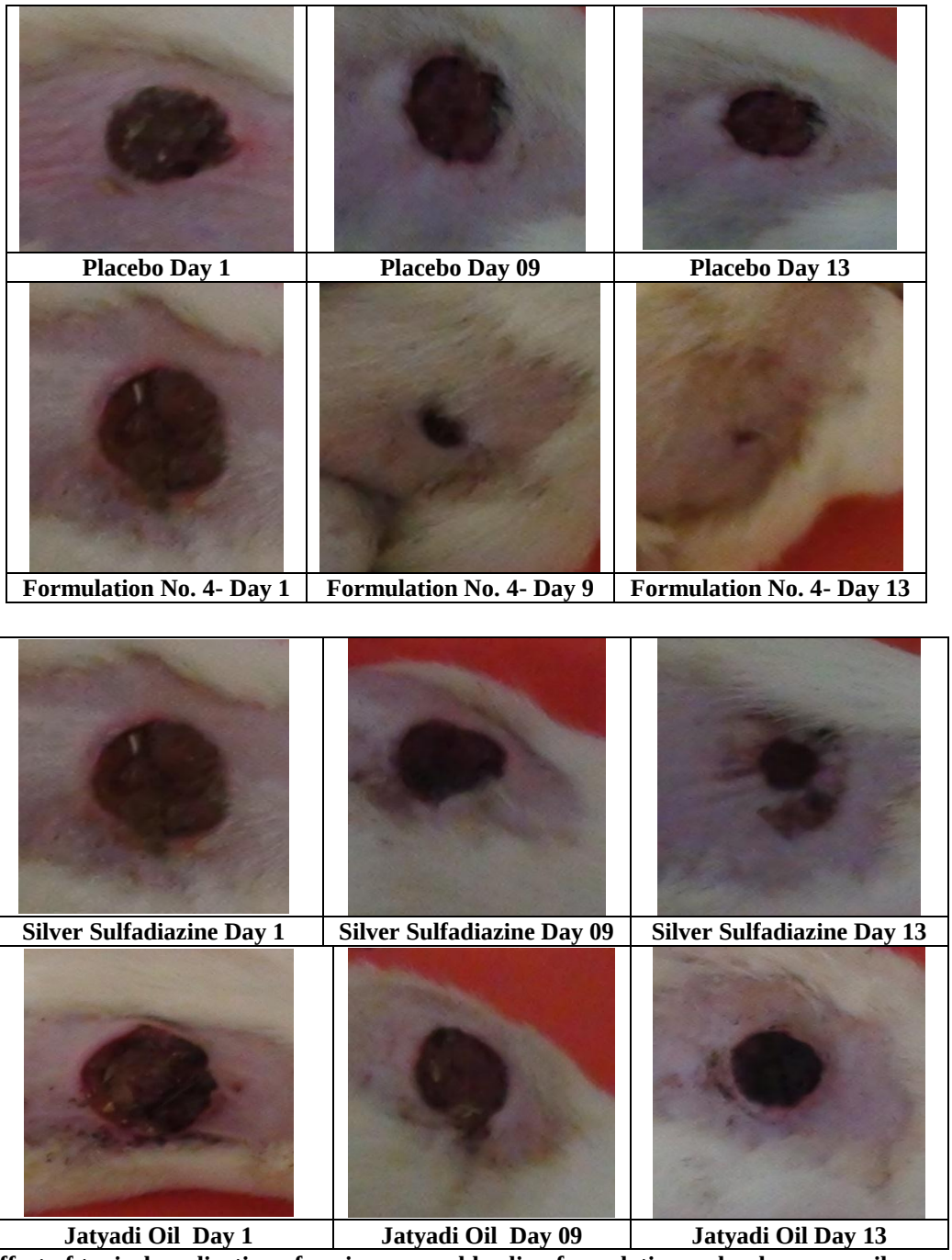

Figure 6: Effect of topical application of various wound healing formulations, placebo cream silver sulfadiazine cream (positive control) and Jatyadi oil on days required for epithelization in burn model.

Excision wound model in diabetic rats

Among all the formulation studied, formulation no. 4 required less days (15) for complete wound healing. Formulation No. 4 was statistically significant wound healing agent as compared to placebo cream. When compared between groups, Formulation No 4 was statistically significantly better wound healing agent than Jatyadi oil. Also Formulation No 4 was better than Mupirocin cream in terms of wound healing activity. The details are presented in Table No. 9.

Table No. 9: Effect of topical application of various wound healing formulations, Placebo Cream, Mupirocin cream (Positive control) and Jatyadi oil on days required for epithelization in excision model.

Gr. No.	Treatment	Days of Epithelization (Mean ± SEM)
II	Diabetic Control (Formulation Base)	26 ± 1
III	Formulation No.1 (F-1)	23 ± 1

IV	Formulation No.2 (F-2)	17 ± 1**
V	Formulation No.3 (F-3)	18 ± 1**
VI	Formulation No.4 (F-4)	15 ± 1**#
VII	Formulation No.5 (F-5)	23 ± 2
VIII	Formulation No.6 (F-6)	18 ± 2 **
IX	Formulation No.7 (F-7)	17 ± 1**
X	Positive control (Silver sulfadiazine cream)	19 ± 2**
XI	Jatyadi Oil Formulation	24 ± 2

***P<0.01 significant as compared to placebo using one-way ANOVA followed by Dunnet test.*
 # P<0.01 significant as compared to Jatyadi oil using one-way ANOVA followed by Bonferroni Test.

Incision wound model in diabetic rats

The wound breaking strength of Formulation No. 4 was higher (395.00 ± 16.18) as compared to any other formulation used in this study. When compared between groups, Formulation No. 4 was having statistically more significant wound breaking strength than Placebo cream, Mupirocin cream and Jatyadi Oil. The details are presented in Table No. 10.

Table No. 10: Effect of topical application of various Wound Healing formulations, Placebo Cream, Mupirocin cream (Positive control) and Jatyadi oil on wound breaking strength.

Gr. No.	Treatment	Wound breaking Strength (Mean ± SEM)
II	Diabetic Control (Formulation Base)	289.17 ± 14.35
III	Formulation No.1 (F-1)	266.27 ± 11.32
IV	Formulation No.2 (F-2)	273.11 ± 5.93
V	Formulation No.3 (F-3)	256.76 ± 16.51
VI	Formulation No.4 (F-4)	395.00 ± 16.18**
VII	Formulation No.5 (F-5)	259.43 ± 32.76
VIII	Formulation No.6 (F-6)	287.00 ± 23.23
IX	Formulation No.7 (F-7)	263.00 ± 12.66
X	Positive control (Mupirocin Cream)	259.23 ± 18.26
XI	Jatyadi Oil	309.23 ± 17.87

***P<0.01 significant as compared to placebo using one-way ANOVA followed by Dunnet test.*

Histopathology

In histological analysis of the wound of rats from control group it was observed that wound was not fully restored and inflammatory cells in scar tissue were observed. Re-epithelialization of the wounds showed incomplete. Among all the formulations tested, Formulation No. 4 (Amarantha Wound Healing Cream) was better in terms of wound healing activity. As compared to positive control (Mupirocin cream), Formulation No. 4 showed complete epithelization, more blood vessels and more collagen fibers. Results are shown in the figure. 7-10.

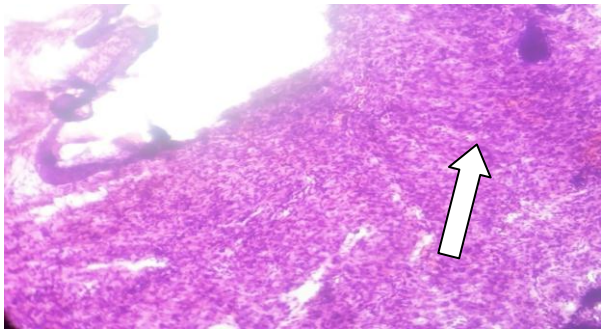

Figure No.7: Histopathological study of placebo cream treated wound in rat (incomplete epithelialization and presence of fibroblasts. H & E 20X).

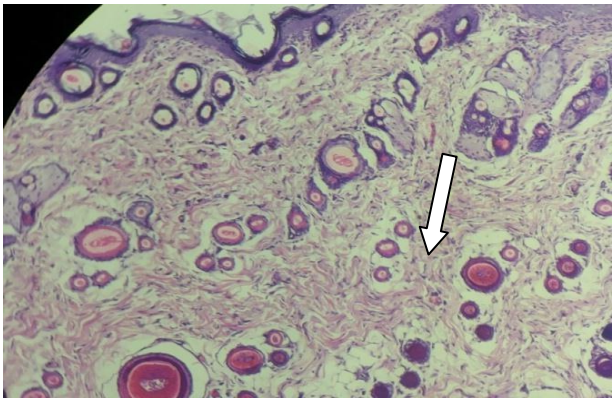

Figure No. 8: Histopathological study of formulation-4 treated wound in rat (skin –complete epithelialization and presence of thick collagen fibres. H & E 20X).

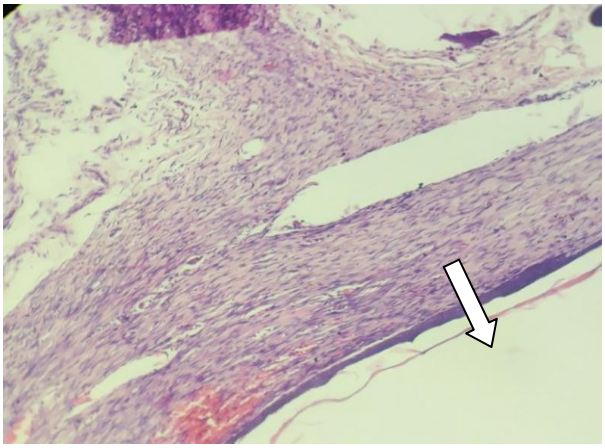

Figure No. 9: Histopathological study of Mupirocin cream treated wound in rat (presence of inflammatory cells new blood vessels and hemorrahages and incomplete epithelization. H & E 20x).

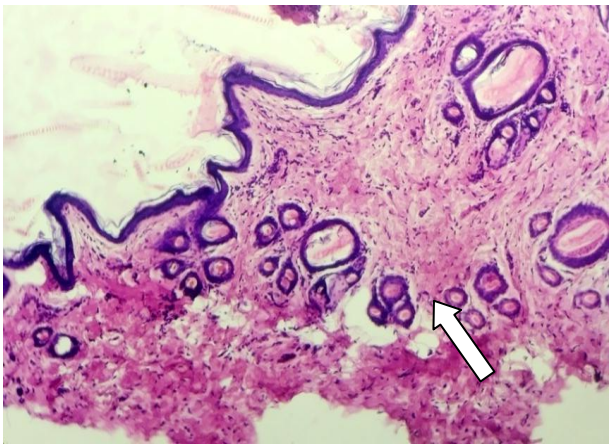

Figure. 10: Histopathological study of Jatyadi oil treated wound in rat (skin –incomplete epithelialization, presence of thick collagen fibers. H & E 20X).

Microscopic observations of the wounds from animals treated with Formulation No. 4 showed complete re-epithelialization and a normal epidermis covering the wound area, collagen fibers were thick and were denser as compared with that of placebo control and Jatyadi oil. The cutaneous annexes, such as sebaceous glands and hair follicles in the center of the scar tissue were also well formed.

DISCUSSION

In the present study it was observed that formulation no. 4 was statistically significant wound healing agent as compared to Placebo Cream, Mupirocin Cream and Jatyadi oil in Excision Wound Model. The histopathological observations of wounds confirmed that formulation no. 4 is having better wound healing activity than Placebo Cream, Mupirocin Cream and Jatyadi Oil as complete epithelization, thick and denser collagen fibers, well-formed cutaneous annexes, such as sebaceous glands and hair follicles were observed in samples of skins rats. The wound breaking strength of formulation no 4 was also statistically significantly better than Mupirocin cream. The wound healing action of Formulation No.4 was superior to various wound healing creams, Placebo cream, Mupirocin Cream and Jatyadi oil.

The results of the study showed that except formulation no.1, 5 and Jatyadi Oil, all other formulations showed statistically significant decrease in days required for complete re-epithelization as compared to placebo in excision model in diabetic rat. This activity in formulation no. 2, 3, 4 and 7 is even better than the Jatyadi oil formulation. Moreover, formulation No. 4 showed statistically significant increase tensile strength in incision wound model as compared to Mupirocin Cream and Jatyadi Oil. Formulation no. 4 also showed complete epithelization and good collagen deposition in the histopathological studies as compared to placebo cream, Mupirocin Cream and Jatyadi Oil.

It was also observed that Formulation No. 4 (Amarantha Wound Healing Cream) is statistically significant wound healing agent than Jatyadi Oil and Silver sulfadiazine in burn model in normal and diabetic rats.

The better efficacy of Formulation No. 4 over other formulations may probably be due to the phytoconstituents present in the plant or could be a function of either the individual or the additive effects of the phytoconstituents present in formulation no. 4.

It has been observed in scientific studies that most of the ingredients of the Formulation No. 4 (Amarantha Wound Healing Cream) possess anti-bacterial and anti-fungal properties, which help in keeping the wound sterile during wound healing process. Also it has been established in the scientific studies conducted on individual ingredient of the Formulation No. 4 (Amarantha Wound Healing Cream) that almost all the ingredients have wound healing property, which help to accelerate the healing process. Jatyadi oil is one of the important ingredients of formulation no. 4. Jatyadi Oil has been used to treat *Dagdha* (burns & scalds). Also Mandukaparni (*Centella asiatica*) is useful in partial thickness burns. Mandukaparni has shown to have burn wound healing activity in mice. It has ability to heal wounds by increasing synthesis of collagen and intracellular fibronectin contents. It also possesses wound healing and anti-microbial properties. Yashtimadhuka (*Glycyrrhiza glabra*) is useful in burn wound infection caused due to variety of organism such as *Pseudomonas aeruginosa*, *Staph. aureus*, etc. Ashvattha (*Ficus religiosa*) and Nimba (*Azadirachta indica*) are used to treat burns. Yashad Bhasma (Ayurvedic classical formulation) one of the important ingredients of formulation no. 4, improves the binding power of the cells of skin, soft tissues, improves cell migration and cell regeneration and hastens wound healing process.

Thus synergistic activity of ingredients of formulation no 4 would have exerted the superior results in healing of excision, incision and burn wounds in normal and diabetic rats.

CONCLUSION

These finding concludes that Formulation No.4 is statistically significant better wound healing agent as compared to various wound healing creams, Placebo Cream, Silver sulfadiazine and Jatyadi oil in burn wound models in normal rat and diabetic rat. Also Formulation No.4 is statistically significant better wound healing agent as compared to various wound healing creams (F-1 to F-3, F5, F6, F7) Placebo Cream, Mupirocin Cream and Jatyadi Oil in excision and incision would models in normal rat and diabetic rat. Thus formulation no. 4 (Amarantha Wound Healing Cream) is best alternative solution in treating cut, wounds and burns.

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