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EVALUATION OF WOUND HEALING ACTIVITY OF POLYHERBAL GEL FORMULATION

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ABSTRACT

The aim of present study was Evaluation of Wound Healing activity of Polyherbal Gel Formulation. The different concentration of gel was prepared by using corbopol 940 as a gel base. Azadirachta Indica leaves extract, Tridax procumbens leaves extract, and honey by using partial thickness burn wound model in winster rats. All animals were divided into six groups. Group I was control, Group II was treated by 1% of Azadirachta Indica gel, Group III was treated by 1% of Tridax procumbens gel, Group IV were treated by 1% Honey gel . Group V were treated by polyherbal gel and Group IV were treated by Marketed Preparetion (Reference Standard). For 15 days all gel applied topically. The wound healing activity evaluated by physical parameters namely wound contraction and epithelialization. The polyherbal gel shows significant effect on wound healing activity.

KEYWORDS: Wound healing activity, polyherbal gel, wound contraction and epithelialization.

INTRODUCTION

Now a days pathology, wounds remain a challenging clinical problems with early and late complications presenting a frequent cause of morbidity and mortality. In an attempt to reduce the wound burden, much effort has focused on understanding the physiology of healing and wound care with an emphasis on new therapeutic approaches and the continuing development of technologies for acute and long-term wound management. Wounds are still major problem in world or a developing countries, because of poor hygienic conditions, often imposing severe complications and high cost for therapy. Wound healing is the set of coordinated

responses to tissue injury. It represents a dynamic physiological process initiated and remodeling phases. Wound care is often complex, frequently time-consuming, sometimes confusing and nearly always expensive. Plant products are widely used as medicaments for wound healing in many clinical situations due to their cost effectiveness, widespread availability, non toxicity, ease of use, fewer side effects, and patient compliance.[1]

There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low costs. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown.[2] Even the World Health Organization (WHO) approves the use of plant drugs for different diseases. Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety.[3]

In the present study, an indigenous herbal formulation containing Azadirachta Indica - Leaves, Tridax procumbens-Leaves, Honey which claims to have the potential in the treatement of wounds, burns, etc; was selected for the evaluation of wound healing activity in wound models in albino rats.

MATERIALS AND METHODS

Plant material: Leaves of Azadirachta Indica, Tridax procumbens, Honey was collected from own farm of Buldhana district (M.S.) India. The leaves was authenticated by department of botany Shivaji Senior College Chikhali. Dist-Buldhana.

Preparation of Extract: The powdered leaves were used for extraction. The powder is extracted in soxhlet apparatus with 90% ethanol. The extraction procedures were carried out till a sufficient quantity of extract was obtained. The solvent was removed by distillation method.[4]

Animal used: Wister male rats weighing 150-200 gm were procured from ANURADHA COLLEGE OF PHARMACY, CHIKHLI, DIST. BULDANA and will be used for the present study. The experimental design will be approved by Institutional Animal Committee and the study were performed according to the Committee for Control and Supervision of Experiments on Animals (CPCSEA) guidelines for the use and care of animals.

Chemicals: Ethanol, Carbopol 940, Triethanolamine (Ozon International Mumbai), Heal Gel (Orden Health Care Global Pvt. Ltd, Pune) were used for the study.

Preparation of gel

Carbopol 940 (1gm) and the measured quantity of extract (to prepare 1% gel) were dispersed in 80 ml of distilled water with continuous mixing using a magnetic stirrer at 800 rpm for 1 h. Glycerol 5 ml was added to the mixture under continuous stirring. The mixture was then neutralized by drop-wise addition of tri ethanolamine. Mixing was continued until a transparent gel was formed. Fresh gel formulation was prepared for each treatment.[5]

Physical Evaluation of Gel

- **Evaluation of the Formulation (in vitro)**

The prepared gel formulations incorporating herbal extracts i.e. Azadirachta indica Tridax procumbens, extracts and Honey were subjected for the in vitro evaluation and stability studies by using the various parameters.

- **Physical evaluation**

The colour, appearance and the feel on application of the prepared herbal gel formulations were noted and the results are shown in Table.

- **Subjective properties**

Subjective properties such as consistency, texture and irritation are observed and shown in Table.

- **pH measurement**

The pH of the gel was determined by using a digital pH meter (Systronics pH meter type 335) 5 gm gel dissolved in 50 ml water and pH was determined by dipping the glass electrode completely into gel solution system so as cover the electrode. Then instrument reading in terms of pH are tabulated in the Table The pH was studied for 15 days.[6]

- **Stability testing**

Since the period of stability testing can be as long as two year, it is time consuming expensive. Therefore it is essential to device a method that will help rapid prediction of long term stability of drug. The accelerated stability testing is defined as the validated method by which the product stability may be predicted by storage of the product under condition that accelerated the change in defined and predictable manner. The stability studies of formulated gels were carried out at 4^0C, 25^0C, 45^0C and at a room temperature for the period of one month. The effect of temperature, humidity and time on the physical characterization of the

gels was evaluated for assessing the stability of prepared formulation. The result are shown in.

- **Experimental Procedure**

Burn wound model

A Partial thickness Burn wound model was employed as per.[7] The rats were anaesthetized with diethyl ether and the hair on the back will be shaved with a sterile blade. The shaved area was disinfected with 70% (v/v) ethanol. Then burn wound will be created by pouring hot molten wax (2gm) at (80c). The wax was poured on the shaven back of the animal through a metal cylinder of 300mm2 circular opening. The wax will be allowed to remain on the skin till it gets solidified. Immediately after the injury and on subsequent days, all the gel will be daily applied topically for 15 days or till complete epithelialization whichever will earlier.[8] (After animal recovered completely from anesthesia, they were kept in individual cages and followed all norms of good laboratory practice in carrying the animals.[9]

The animals were randomly divided into 6 groups and each group containing 6 animals the treatments of each gel (500mg/rats) were applied topically once a day.[10]

1. **Group I:** Control group.
2. **Group II:** Test group Treated with *Azadirachta indica* gel. (Formulation I).
3. **Group III:** Test group Treated with *Tridax procumbens* gel. (Formulation II).
4. **Group IV:** Test group Treated with Honey gel. (Formulation III).
5. **Group V:** Test group Treated with (polyherbal) gel. (Formulation IV).
6. **Group VI:** (Referance Standard) Heal Gel (Silver sulphadiazine 1% w/w).

Assessment of burn healing

Animal will be inspected every alternate day and the healing will be assessed based on physical parameters namely.

1. **Wound contraction and**
2. **Epithelialization**

1. **Wound contraction:** Wound contraction will be monitored by measuring the progressive changes in row wound area, plan metrically on a transparent paper, from which the wound surface area were evaluated. The tracing was then transferred to 1 mm^2 graph sheet, from which the wound surface area was evaluated. The evaluated surface area was

then employed to calculate the percentage of wound contraction, taking the initial size of the wound, 300 mm^2, as 100% by using the following equation.

Wound contraction (%) = Initial wound size – Specific day wound size / Initial wound size X 100

2. Epithelialization: Time taken for till epithelialization were measured by recording the days.[9]

Statistical analysis

Experimental data are expressed as Mean +_(SEM) standard error of mean. Statistical analysis was performed by using one way ANOVA followed by Dunnet test.

RESULTS AND DISCUSSION

Gels: The results of various physical parameters for evaluation of the prepared herbal gel formulation are reported below.

All results of different parameters of evaluation are recorded. The physical parameter such ad color, appearance, feel on application are observed and shown in **Table1.**

Table 1: Physical evaluation.

Physical Parameters	Formulation I	Formulation II	Formulation III	Formulation IV
Color	Dark green	Dark green	Light yellow	Dark green
Appearance	Translucent	Translucent	Translucent	Translucent
Feel on application	Smooth	Smooth	Smooth	Smooth

The color of prepared herbal gels was dark green, dark green, and light yellow as the color of extracts was green and yellow. Apperances of gel was translucent and it was smooth on application.

The subjective properties such as consistency, texture and irritation are observed and shown in **Table 2.**

Table 2: Subjective Properties.

Parameters	Formulation I	Formulation II	Formulation III	Formulation IV
Consistency	Good	Good	Good	Good
Texture	Smooth	Smooth	Smooth	Smooth
Irritation	--	--	--	--

The subjective properties such as consistency was good and texture of prepared herbal gel was found to be smooth. No skin irritation was there on application of gel to the skin surface. So it can be used safely.

pH of the Gels: The pH value of gel formulation were studied at room temperature are change in pH is observed and shown in **Table 3.**

Table 3: pH of the Gels.

	pH of formulation			
Time in days	**Formulation I**	**Formulation II**	**Formulation III**	**Formulation IV**
0	6.38	6.51	6.42	6.48
3	6.41	6.47	6.43	6.51
6	6.39	6.53	6.41	6.53
9	6.38	6.50	6.38	6.50
12	6.41	6.52	6.35	6.46
15	6.40	6.47	6.40	6.48

Prepared herbal gel incorporating the medicinal plant extract was studied by using digital pH meter Systronics. (pH meter type 335). The pH was studied for 16 days at room temperature. All four formulations were in range of 6.35-6.53 pH at initial phase. As we go from epidermis to dermis, Ph of the skin increases and attained the neutral value i.e. 7. So gel formulation having pH range 6.2 to 7 are desirable to skin.

Stabilility testing

Result of stability study shows a good stability even at different temperature.

Table 4: Stability testing.

Formulation	**Initial Color**	**4^0 C, 25^0 C, 45^0C and at a room temperature**			
		I^{st} week	II^{nd} week	III^{rd} week	IV^{th}week
Formulation I	Dark green	+	+	+	+
Formulation II	Dark green	+	+	+	+
Formulation III	Faint yellow	+	+	+	+
Formulation IV	Dark green	+	+	+	+

Where + Indicates there is no change in colour or lump in formulation.

The prepared herbal gel formulations were subjected to accelerated stability testing **Table 4**. The prepared herbal gel were store at 4^0C, 25^0C, 45^0C in refrigeration, room temperature and at different humidity condition. The physical parameter were evaluated during study period the result of study preparation are physically stable at 45^0C.

PHARMACOLOGICAL STUDIES

Wound contraction

Wound contraction is another parameter used to assess wound healing. Significant wound contraction was shown in **Table 5.**

Wound contraction in (%)

Gr. No.	Treatment	Area of wound during different days of observation (%)				
		3	6	9	12	15
1	Control	08.80 ±0.9180	17.80 ±0.9085	36.14 ±0.1430	55.65 ±0.9939	68.58 ±0.7791
2	Formulation I	13.08** ±0.7367	27.58** ±0.7063	49.91** ±0.7829	69.80** ±0.7914	81.25** ±0.7822
3	Formulation II	09.92* ±0.9296	20.36* ±0.7389	39.30* ±0.9207	56.47* ±0.7488	69.14* ±0.7497
4	Formulation III	10.47** ±0.9941	21.58** ±0.8983	40.08** ±0.7588	58.42** ±1.2080	74.47** ±0.7822
5	Formulation IV	13.19** ±0.7252	30.25** ±0.1886	53.36** ±0.8942	72.91** ±0.7286	89.35** ±0.4155
6	Referance Standard	15.08 ±0.9407	31.58 ±0.9292	55.14 ±0.8542	75.47 ±0.8428	90.43 ±0.7691

[values are expressed as mean ± SEM, N= 6, *p<0.05, **p<0.01 Vs Group 6 One way ANOVA followed by Dunnets test]

In pharmacological evaluation the wound healing activity was studied using a partial thickness burn wound model in rats. The burn wound will be created and initial wound area was measured and all the gel will be daily applied topically. The animals were maintained under good hygienic conditions. The animals were divided into 6 groups. The observations were made on 3 day, 6 day, 9day, 12 day and 15day repectively and the observations were mentioned in **Table 5**.

Marketed Heal Gel showed 90.43% wound contraction after 15 days with respect to initial wound size 300 mm2 as 100%, were as Formulation –I (*Azadirachta Indica*) showed 81.25% wound contraction, formulation II (*Tridax Procumbens*) 69.14% wound contraction, Formulation-III (Honey) 74.47% wound contraction, Formulation IV(polyherbal) 89.35%

wound contraction and control group showed 68.58% wound contraction as showed in **Table 5**. Thus due to concentration of extracts activity increased.

Wound contraction in (%)

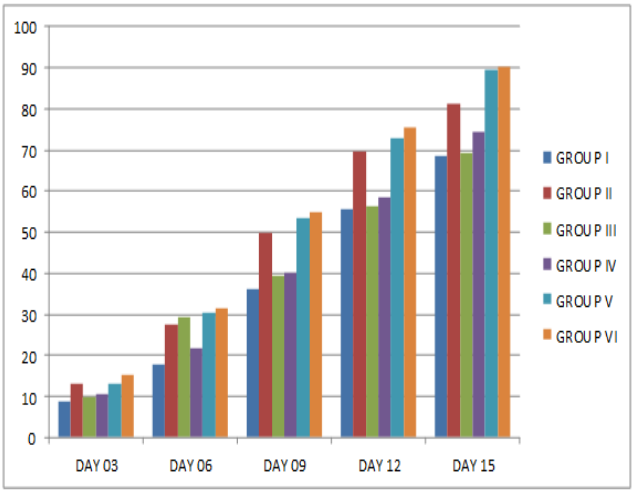

Fig. 1: Evaluation of wound healing activity.

Epithelialization Period (Days)

Table 6 Epithelialization Period

Group no.	Groups	Epithelialization period (days)
1	Control	38.36 ±1.77
2	Formulation - I	28.77 ±1.22
3	Formulation – II	32.10 ±0.86
4	Formulation – III	31.25 ±0.33
5	Formulation – IV	26.32 ±2.22
6	Reference Standard	25.20 ± 2.10

The mean epithealization period of marketed SSD cream showed 25.20 ± 2.10 days where as the mean epithealization period of Formulation –I(*Azadirachta Indica*) was 28.77 ±1.22 days, Formulation – II (*Tridax Procumbens*) was 32.10 ±0.86 formulation –III(Honey) was 31.25 ±0.33, formulation- IV (polyherbal)was 26.32 ±2.22 days as showed in **Table 6.**

CONCLUSION

From the above results it can be concluded that the prepared herbal gel shows significant wound healing activity and the combination of *Azadirachta indica*, *Tridax procumbens* and Honey had synergistic wound healing activity.

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