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EXPLORING THE BIOACTIVE COMPOUNDS USING HPTLC ANALYSIS OF BENINCASA HISPIDA LEAVES EXTRACT FOR WOUND HEALING MANAGEMENT

Nilanjan Pahari[a,b*], Beduin Mahanti[a], Mrityunjay Banerjee[c], Biprojit Bhowmik[b], Koyel Mondal[b], Arup Chatterjee[b], AnkritaThapa[d]

[a] School of Pharmacy,Techno India University, Salt Lake, Kolkata, 700091.
[b] Department of Pharmacology, Calcutta Institute of Pharmaceutical Technology and Allied Health Sciences, Banitabla, Uluberia, Howrah,711316
[c]Department of Pharmaceutical Chemistry, Institute of Pharmacy and Technology, Salipur, Cuttack, Odisha, 754202.
[d] Department of Pharmaceutical Technology, University of North Bengal, Matigara, Darjeeling, West Bengal, India, 734013.

***Correspondence author:**paharinilanjan@gmail.com

Abstract: For receiving quality research outcome preparation of plant extract is the initial step. Also, the isolation of bioactive compound has the similar importance. The primary objective of these study is to prepare BHELE, Isolation of the phytochemicals and evaluation of wound healing activity of the ethanolic leaf extract. Proper preparation of plant extracts demands the timely and correct gathering of plants and authentications, proper drying, grinding, and storage of the extract. Extracting medicinal plants involves separating secondary metabolites from inert, non-active substances using the correct solvent and standard extraction procedure. The process of healing wounds is the process of healing that follows trauma to the skin or soft tissues. When a wound or injury is created, an inflammatory reaction takes place inflammatory reaction occurs, and the cells beneath the dermis begin to make additional collagen. After that, the epithelial layer is regenerated, the epithelial layer. Three different phases are involved in the wound healing process, i.e., swelling, inflammation, and proliferation. Regarding the effects of these ethanolic plant leaf extracts on the healing of wounds, no precise scientific data are provided. In this following study, highlights on the wound healing effects of these leaf extract by excision, incision, chemical wound burns and thermal burn model. Dunnet's test was used to assess the results of the current study. $P<0.05$ was thought to be significant. All these models show the significant positive results. The current investigation has shown that, in comparison to a placebo control, the ethanolic extracts of *B. hispida* contain characteristics that stimulate the speed of wound healing.

Keywords: Model of excision, Model of incision, Thermal burn model, BHELE, HPTLC analysis.

1. Introduction:

Medicinal Plants have great importance for the well beings of individuals. The value of medicinal plants hided within their bioactive constituents that creates physiological activities in human body. Currently medicinal plants give considerable significance view due to the attributes of the major phytochemicals source for the development of Novel Medicinal system. [1,2,3] For the processing of any bioactive compound, extraction and pre-extraction procedure are similarly important. The process of preparing a good extract of plants includes the proper and punctual collection of plants, authentications, adequate drying, grinding and storing the extract. The extraction of plants for medicinal use is a procedure to separate secondary metabolites from inactive substances, inert components employing a suitable solvent and conventional extraction methods. [2,4,5] *Benincasahispida* belonging to the family Cucurbitaceae, well known ethnomedicine provides several

pharmacological activities. This climbing tree is grown as a vegetable at wide range in India. It can also be used as a Chinese remedy for treating urinary tract disorders, fever, and cough. [7] From the ancient time these is used as a wonderful pitta medicine that have a wonderful potency in the treatment of epilepsy, Haemorrhage, and insanity. These leaf extract shows significant antiulcer activity in rat model also anti-compulsive, anti-depressant, antidiarrheal, antioxidant, anti-inflammatory, analgesics, antimicrobial, hypoglycemic, hypolipidemic, antiasthmatic, Reno protective effects that are validated in numerous scientific studies [6,7,8,9,10,11]. There isn't any research to verify the effectiveness of wound healing with the ethanolic extracts of these leaves. Therefore, we examine the wound healing characteristics of the extracts from leaves containing ethanol extracted using the standard procedure. Also, we have isolated the bioactive compounds from the BHELE by several analytical techniques (HPTLC).

2. Materials and Methods:

2.1 Plant material:

The leaves that were fresh from *Benincasahispida* were collected from local markets and from the land used for cultivation. The material of the plant has been taxonomically identified as well as authenticated by the Acharya Jagadish Chandra Bose Indian Botanic Garden, Botanical Survey of India, Shibpur, Howrah, West Bengal, where the voucher specimen is preserved in the form of a reference (CIPT /NP 01) to be used in the future.

2.2 Preparation of extract:

The B. hispidaleaves were washed thoroughly under running tap water. Then, they were washed in distillate water. The leaves were then chopped and dried by air in the shade. Then grind the leaves to make coarse powder. The powder was later used to make an extract made from ethanol.

2.3 Preparation of ethanolic extract:

1. The collected plant material was meticulously scrubbed using tap water to remove dirt. The dried plant material was air-dried in the sun for three or four weeks in the shade at room temperature (24 ± 2°C).
2. The dried plant components were sieved after being completely dried, then mechanically grind into a coarse powder.
3. Then petroleum ether (60-80°C) was treated with coarse powder for 72 hours for removal of fatty materials.
4.The extract was processed using alcohol (95 percent) with the help of the Soxhlet device for 36 hours.
5.The extract solution was cleaned with Whatman No. 42 filter paper. The paper was then consolidated under the lower pressure of a vacuum. Finally, the solution dried in a hot oven and store the extract at 4°C.
6. Then the extract is used for the further study.

2.4 HPTLC analysis:

Advanced thin-layer analysis (HPTLC) can be described as a brand-new type of thin-layer chromatography (TLC) kind with more effective separation. Silica gel 60 F254, HPTLC analysis was performed on pre-coated TLC plates (10 cmx10 cm, and 0.2 millimeters thickness) (Merck, Germany). The ethanolic solution of the extracts as well as standards were administered with an automated TLC Sampler 4 (ATS 4, CAMAG, Muttenz, Switzerland) and 100-milliliter HPTLC Syringe. The Plate was made within the twin Trough Chamber (CAMAG), which can be used for 7 centimeters with 25 + 5C using a pre-saturated mobile phase composed of chloroform Acetate and formic acid (5:4:1 V/V/V). After drying, the chromatograms are recorded with TLC Ultraviolet Cabinet 4 (CAMAG). A TLC scanner 4 operated with Vision CATS software (CAMAG with a slit

width of 0.45 millimeters) is used to analyse the chromatograms of various wavelengths, including 254, 366, and 416nm [12].

2.5 Wound healing evaluation:

2.5.1 Preparation of ointment formulation:

1.Topical ointment formulations with BHELE were developed to test the effectiveness of wound healing compared to standard ointment. As per British Pharmacopoeia simple ointment bases are prepared.

2.5 gm wool fat, 5 gm hard paraffin, 5 gm ceto-steryl alcohol, 85 gm white paraffin, and ethyl paraben(preservative) were taken in a beaker and heated at 65 in a water bath until all the ingredients were melted.

3.The mixture was then allowed to cool and homogenized by a homogenizer operating with 1500 rpm for 10 minutes.

4.Ointments with different formulations, BHELE 2.5 percent W/W and the 5% w/w BHELE were made by the quantity extracted BHELE in 100g of the fundamental base for the ointment).

2.5.2 Experimental animal husbandry:

Animal used: The preferred rodent species is the Wistar Albino rat (180-220 gm) of either sex will be used.

Age: When the dosing process begins, the animal must be between 8 to 12 weeks old. The animal's weight should be between 20 and twenty percent average weight of all animals who have received an amount.

Housing / Temperature / Humidity:

Temperature: 22 ˚C ± 3 ˚C

Relative humidity: Other than during the cleaning of rooms, the goal must be at least 50-60%. It should have at minimum 30%, preferentially not exceeding 70%.

Photo-period: Artificial lighting is recommended as it offers 12 hours of light and 12 hours of complete darkness.

Feeding: standard laboratory diets given with a sufficient supply of water.

2.5.3 Acute dermal skin irritation test:

Experimental animals and dosing regimen: 6 rats (180-220 gm) in 3 groups; Group 1 was treated with simple ointment. Group two treated 2.5% formulation of W/W, Group 3 was treated with the 5% formulation of W/W.

Assay end point: Eschar and Erythema formation the formation of edema.

Status of the Regulator: OECD Test Guideline 404 - (TG – 404) (Updated 23 April 2022).

Topical Protocol

1. Test Substance: Ointment formulation applied on 5 cm skin surface

2. Exposure Time: 3 min, 1 hour (skin corrosion), 4 hours (skin irritation)

2.5.4 Wound healing activities:

Animals were split into five distinct groups comprising six (6) animals to serve as the excision and incision wound models. Group, I was treated without treatment and was considered the control group. Group II was utilized as a negative control group. (Ointment treatment base) povidone Iodine ointment (USP) (intestine) for the use for thermal burn models., Silver sulfadiazine I.P. Ointment was applied, group III was used as a standard and the treatment was 2.5 percent (w/w) rough drugs Ointment (BHELE), as well as the Group IV received 5 percent (w/w) rough drug in ointment (BHELE). The treatments were administered every throughout the day.

2.5.5 Excision wound model:

Using the Wistar rat excision wound model and the techniques outlined by Morton and Malone, wound healing activities were assessed [13]. Before making the wounds Ketamine Hydrochloride (100 mg/kg i.m.), was used to anesthetize animals, after that incision was done. The dorsal part of the thoracic area of the rat that was anesthetized was imprinted by 1 cm of vertebral column and 5cm of the inner ear. Fur of dorsal area was clipped with an electric clipperand a circular stainless-steel stencil was used to indicate the projected location of the wound to be produced on the animals' backs. With toothed forceps, a scalpel, and pointed scissors, a wound for excision with 500 mm^2 with a 2-millimeter depth was created per the marks after dabbing the wound using a cotton swab immersed in normal Saline. Haemostasis was achieved. It was then left open. Each surgical procedure was conducted in an aseptic atmosphere. The wounds weren't cleaned for 24 hours, and all rats injured were kept separately in polypropylene cages. On a daily basis, the wound site was treated topically with the extracts and reference medications. It was possible to trace the wound using a transparent newspaper and a permanent marker over the first three, six, and ninth, twelve, fifteen, and eighteenth post-wounding days to determine the wound's closure rate. The wound area was traced over again on a $1mm^2$ graph sheet. The rate of wounds contracted was determined by measuring the changes in the area of the wound was measured. This formula is used to determine the proportion of closure.

$$\text{Wound Closure} = \frac{\text{Wound area of Day (0)} - \text{Wound area of Day (n)} \times 100}{\text{Wound area of day (0)}}$$

Then n = 0, 3^{rd},6^{th}, 9^{th}, 12^{th}, 15^{th}, and 18^{th} days post wound. The epithelialization time was calculated using the days needed to fall the tissue dead remnants, with no residual raw wound.

Procedure:

1. Before creating those wounds, Ketamine HydroChloride (100 mg/kg i.m.) has been used as anaesthetic.
2. The dorsal area of the thoracic area of Rat was anesthetized and imprinted by 1 centimetre of the vertebrae column and 5 centimetres away from the ear.
3. An electronic clipper clipped the fur of the dorsal area and a long stainless-steel circular stencil was used to show the exact site of the wound to be made on the backs of the animals.
4. Utilizing toothed forceps, a scalpel, and sharp scissors, an entire length excision wound with an area of 500 mm^2 and 2mm depth was created in the direction of the lines.
5. The wound was cleaned using a swab of cotton soaked in normal saline and haemostasis was achieved.
6. It was left open. Each surgical procedure was performed in an aseptic setting. The wounds didn't care for 24 hours, and each injured rat was treated separately. On a daily basis, the wound site was treated topically with the extracts and reference medications.

2.5.6 Incision wound model:

Utilizing the techniques outlined by Ehrlich and Hunt, an incision wound model was created. [14]. The ketamine hydrochloride (100 mg/kg i.m.) was used to anesthetize rats before and during the time they were recovering from the cuts they made. Electric clippers were employed to trim the animal's dorsal fur. An incision of a distance between skin tissue and tissue in the rear, which was 5 centimetres long, created the space between vertebrae. A surgeon's thread and an angled needle were used in stitching up the skin divided in half 1cm from each other after the incision. The injuries were not covered. Once daily, the compositions of ointment were applied directly applied to the wound. On the eighth day following the wound, the sutures were removed off and the formulations remained to be applied to the wounds until the tenth post-wound day. On the tenth evening following the final treatment, the strength of wounds was measured. They were sedated and attached to the table; the line was drawn 3 millimetres from the edge of the cut on both sides to measure the strength of the wound. On the other face of the cutting, two forceps were positioned across the line, facing the other. One forceps was connected to supports and the other to a polypropylene graduated vessel suspended by the

thread connected to the pulley. The weights of the standard were cautiously and slowly placed in the container. As weights are added to the container, The pressure at the site of the wound is rapidly increasing, eventually tearing areas of the wound. The process of adding weights to the container halted, When the wound started to bleed, the total weights that were added were weighed and recorded [15].

Procedure:

1. The ketamine hydrochloride (100 mg/kg i.m.) was used to anesthetize rats before and during their healing of the wounds they had made.
2. Electric clippers were used to trim the animals' dorsal fur, and an incision longitudinally into the skin and the cutaneous tissues in the lower back, which was 5 centimetres long, was created between vertebrae.
3. Following the incision, the skin was divided, and it was stitched together one centimetre apart, using a surgical needle and the curly needle.
4. The injuries were not covered by insurance.
5. On a daily basis, the wound was treated topically with the ointment formulations.
6. On the eighth day after the wound was closed, sutures were removed while the formulations continued to be applied to the wounds until the tenth and final day following the injury.
7. On the evening of the 10th day following the previous application, the wound-breaking strength was assessed.

2.5.7 Wound model of chemical burn

The dorsal hairs of the surface were taken off by mechanical means for 24 hours before the incident occurred. After that, 70% ethanol was used to clean the shaved region. Animals were subjected to chemical burn following full anaesthesia recovery, Traumas using a 5x5 cm piece of skin shaved and coated with some drops of powerful hydrochloric acid. The incision site was covered with sterile gauze, after which the pets were kept in separate areas. On the burn, medication was applied once day [16]. With transparent paper as well as Permanent marker closure was determined at 3^{rd} ,6^{rd},9^{th},12^{th},15^{th} and 18 days after injury (17). The formula below calculated the proportion of wound healing caused by chemical burns.

$$\text{Wound Closure} = \frac{\text{Wound area of Day (0)} - \text{Wound area of Day (n)} \times 100}{\text{Wound area of day (0)}}$$

When n is the 3^{rd} ,6^{th},9^{th}, 12^{th},15^{th} ,18^{th}, days of after wounding, the epithelialization period is determined as the time needed to die dead tissue remnants without a leftover open wound.

Procedure:

1. The dorsal hairs of the surface were removed surgically for 24 hrs before the burn began. After that, 70% ethanol was used to clean the shaved region.
2. Animals were given chemical burn injuries by applying a few drops of strong hydrochloric acid to a 5 x 5 cm patch of shaved skin.
3. When the animals were recovered entirely from anaesthetics, the wound was covered with sterile gauze and stored separately in cages.
4. Medication was put to the burn, once daily.
5. The wound's closure rate was determined using transparent paper and permanent markers on days 0, 6, 12, and 18 after wounding.

2.5.8Wound Model of Thermal Burn:

The dorsal hairs on the face were surgically eliminated for 24 hours before the burn started. After that, 70% ethanol was used to clean the shaved region. By pressing a metal rod with ten millimetres in diameter, heated on an uninvolved flame for 30 seconds. The thermal burns were inflicted on the rats' dorsal skin. After the animals had completely recovered from anaesthesia, the burn was wrapped in

sterile gauze, and the animals were kept in separate rooms. The burn was treated with medication. It was applied daily [16]. With transparent paper and a permanent marker, closing wounds were assessed in the first, sixth, and twelve days after wounding [17]. The formula below was used to calculate the wound healing proportion after thermal burns.

$$\text{Wound Closure} = \frac{\text{Wound area of Day (0)} - \text{Wound area of Day (n)} \times 100}{\text{Wound area of day (0)}}$$

In this case, the n represents the 3rd, 6th,9th,12th,15th, 18th, and 6th days after wounding. The epithelialization duration was determined by the amount of time needed to ensure the death of tissue fragments dead without open wounds.

Procedure:

1. The dorsal hairs on the surface were removed surgically for a duration of 24 hrs before when the burn began.After that, 70% ethanol was used to clean the shaved region.
2. The animal's dorsal surface was exposed to burns from thermal radiation when it was pressed against an iron rod with a diameter of 10 millimetres that was heated by the flame in an open area for about 30 seconds.
3. After the complete anaesthesia recovery, the incision was covered in sterile gauze, and the animals were separated from each other.
4. Medication was put to the burn, once daily.
5. On the first, sixth, twelfth, and eighteenth-days following wounding, it was determined by using clear paper and a permanent ink marker.

2.5.9 Isolation of bioactive compounds

The ethanolic extract was spotted on a TLC plate along with different standards. The band of the sample in correspondence with the standard was observed (Fig.1).

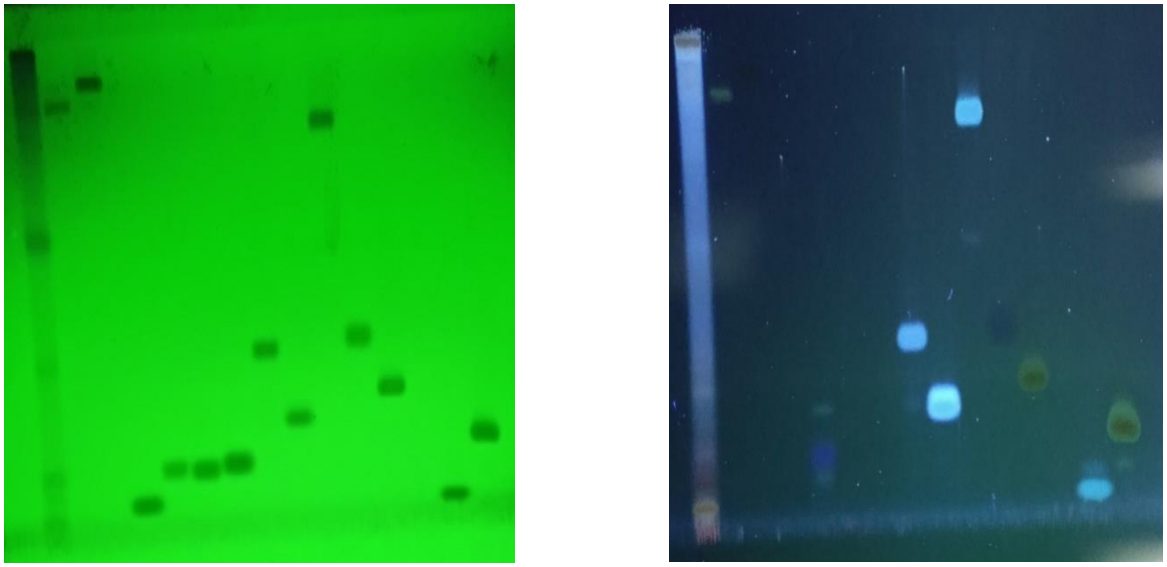


Fig. 1 HPTLC analysis of the fingerprints on the methanolic extracts of *BenincasaHispida* with Chloroform's mobile phase and the formic acids ethyl Acetate and (5:4:1 5:4:1 V/V/V). (a) UV 254 nm, (b) UV 366nm

2.5.10 Statistical analysis:

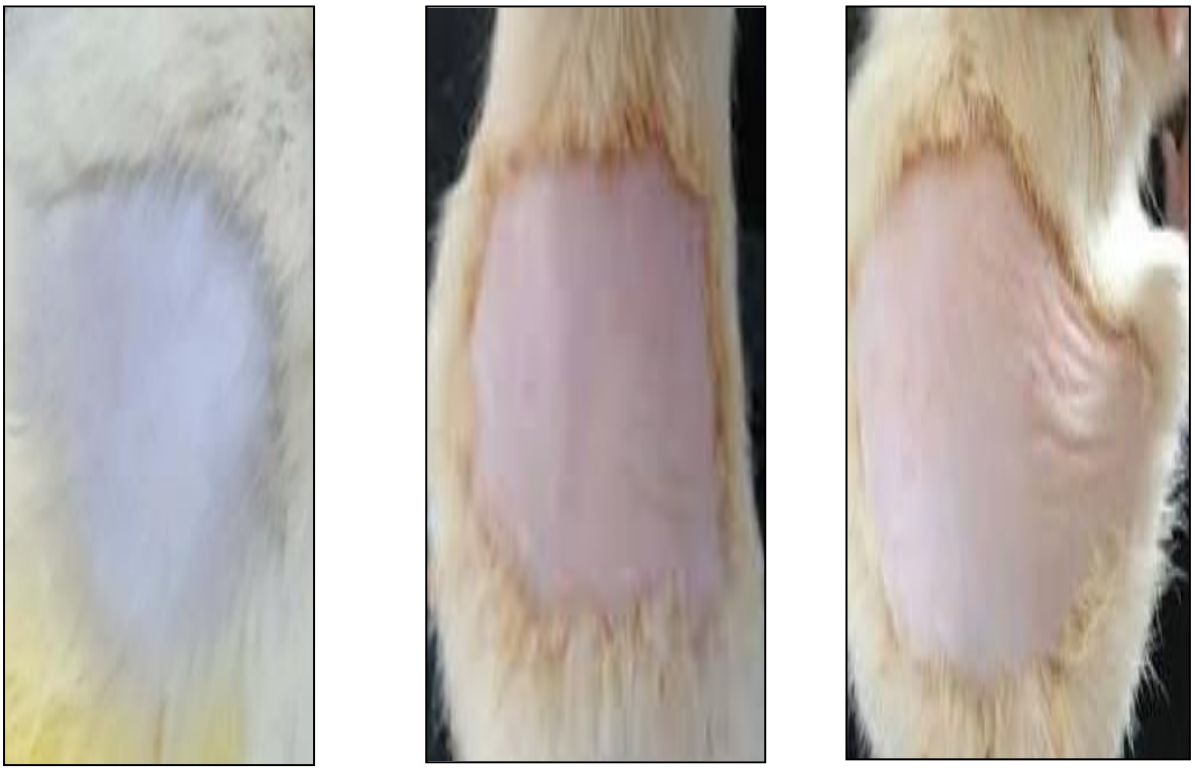
The findings were computed and they were presented as Mean Standard Deviation. One-way analysis of variation (ANOVA) was conducted to find the significant variations in data gathered for the

research. Dunnett's test was employed to determine the importance of the different groups. The word "significant" refers to the significance of the p-value, which is 0.01.

3. Results:

3.1 Acute skin irritation test:

Ethanolic extract from *Benincasahispida*ointment formulation 2.5, 3 % and 2.5 percentage of participants showed "No" signs of irritation or redness, swelling, or any other unusual change on the skin of a rat (Fig.2). Thus, the formula is considered safe to use on the skin of rats.



(a) **(b)** **(c)**

Fig. 2 Acute skin irritation test (a) Simple ointment (b) 2.5 % formulation (c) 5 % formulation

3.2 Model of excision wound

Representation of model of excision wound shown in Fig. 3.

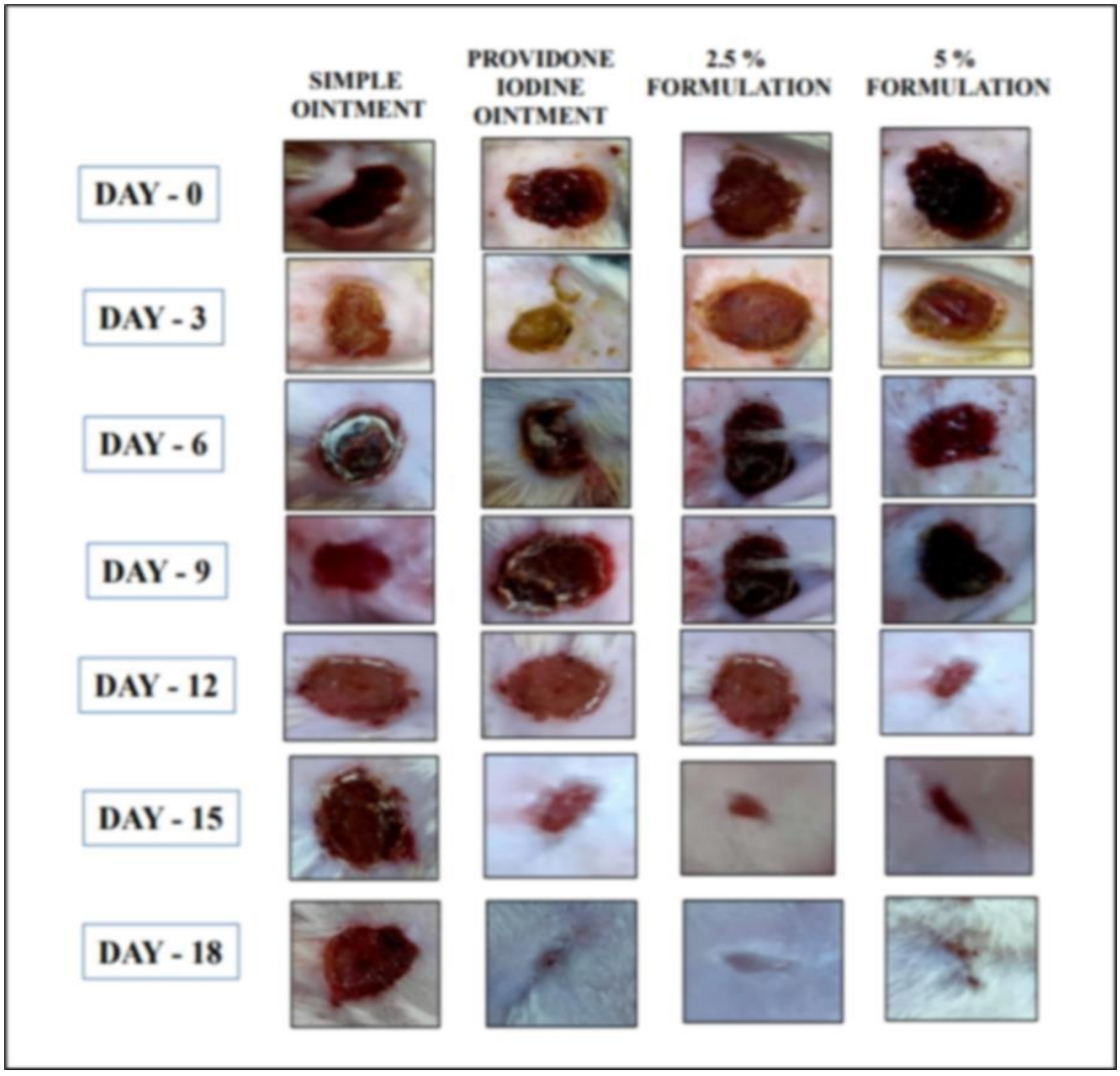


Fig. 3 Photographic representation of model of excision wound

3.2.1 The percentage of wound closure in the excision wound model

The percentage of wound closure in the excision wound described in Table 1.

Table 1 Percentage of wound closure rates

Day	Simple ointment	Povidine iodineointment	2.5% Formulation	5% Formulation
0	0	0	0	0
3	6	6.27	10.51	11.12
6	15	15.78	26.66	28.57
9	21.22	32.11	37.11	49.98
12	30	47.36	50	60
15	42.12	73.11	75.12	78.5
18	50	84.21	85.66	90.71

3.2.2 Statistical analysis of model of excision wound

Statistical analysis of excision wound shown in Table 2.

Table 2: Statistical analysis of excision wound

Day	Simple Ointment	Povidine Iodine Ointment	2.5% Formulation	5% Ointment
0	0	0	0	0
3	4.12±0.82	14.95±0.70	13.83±0.21	14.1±0.141
6	12.95±0.35	20.8±0.28	18.98±0.42	19.9±0.424
9	18.62±0.56	38.6±0.56	31.75±0.77	34.75±0.636
12	42.65±2.56	67.5±2.01**	56.7±0.91*	59.55±1.18**
15	68.87±0.98	94.4±0.84**	80.5±0.13*	93.4±1.313***
18	88.92±0.92	116.15±1.20**	119.3±0.41**	123.2±1.414***

Each group is composed of six animals. The results are presented by means ± S.E.M. ANOVA n=6 followed by a non-paired "Dunnett's"-test P 0.05 *P 0.05 *P 0.05, **P 0.01 and ****P 0.001 is considered statistically significant compared to with the Standard Group treated with Povidone Iodine Ointment.

3.2.3 Graphical Representation

Effect of BHELE on the formulation of ointment on the contraction of excision shown in Fig. **4.**

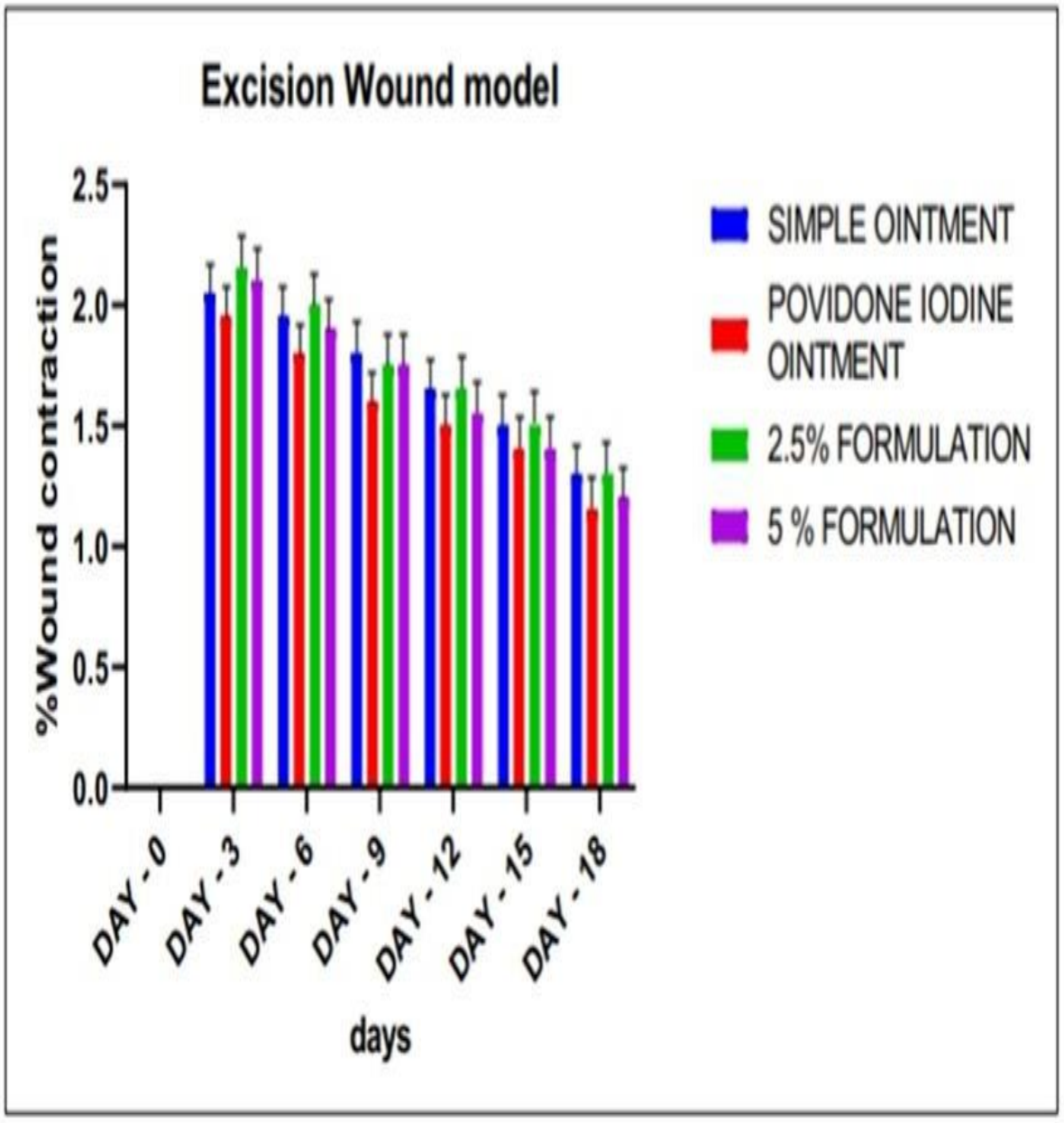


Fig. 4 Effect of BHELE on the formulation of ointment on the contraction of excision wounds percentage

Values are calculated as a function of mean±S.E.M (n=6). Analyzing the statistical data using one-way ANOVA, and then "Dunnett's test".

3.3 Incision wound model

Representation of Incision models with wounds shown in Fig. 5.

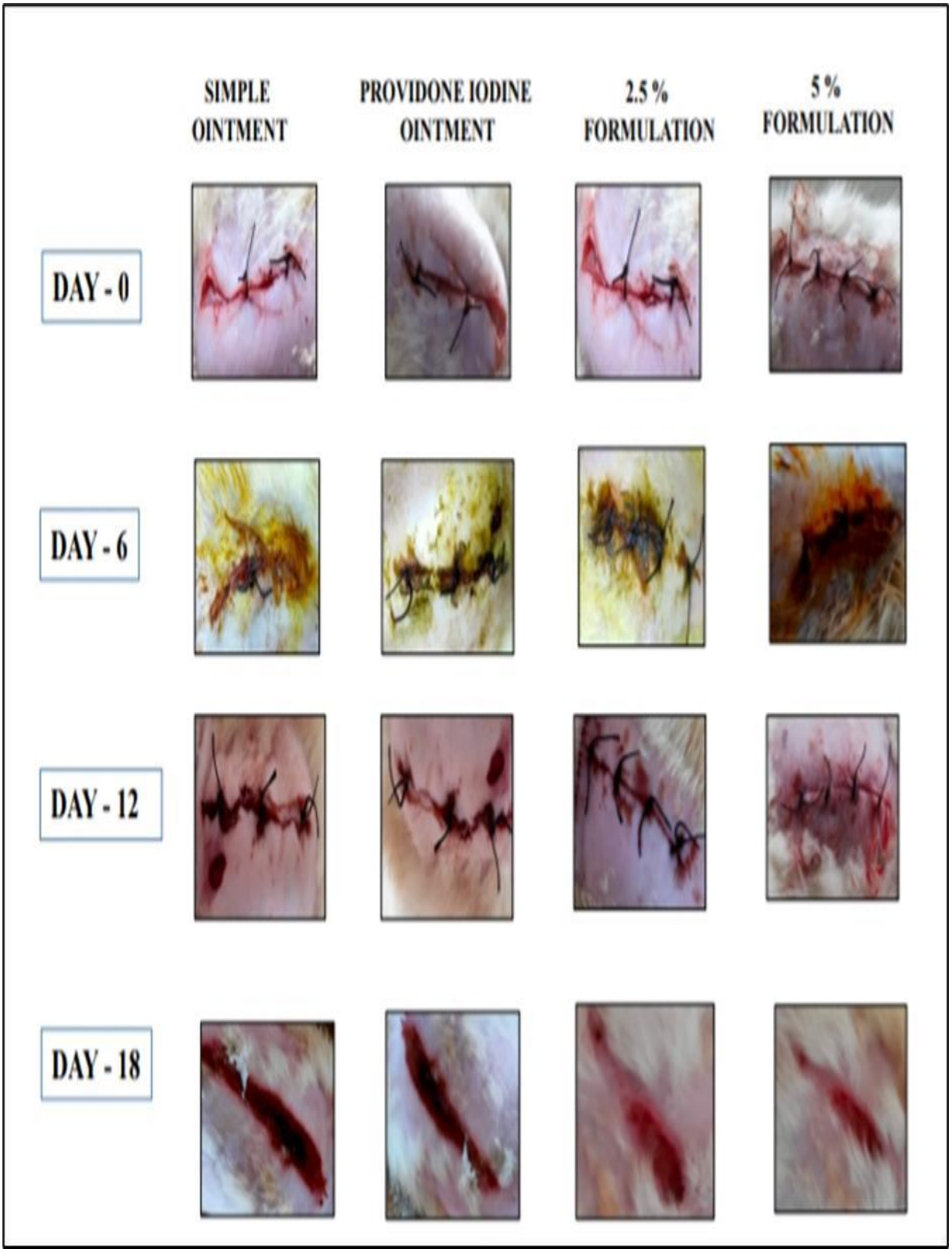


Fig.5 Image representation of incision models with wounds (Day through the day)

3.3.1 Effect of BHELE on the tensile strength of the wound

The impact of BHELE on the power of wound described by Table 3.

Table 3 The impact of BHELE on the power of wound

Group	Treatment	Body Weight	Marking	In g/mm^2	Mean	S.E.M.
I.	Simple ointment	177	H	2.75	2.469	0.6510
		151	B	1.916		
		153	T	2.233		
		184	H+B	2.45		
		179	H+T	2.85		
		162	UM	2.61		
II	Povidine iodine ointment	198	H	4.81	5.597	0.3483
		161	B	5.2333		
		183	T	5.95		
		197	H+B	6.03		
		158	H+T	6.25		
		158	UM	4.56		
III	2.5% formulation	151	H	4.95	4.475	0.700
		176	B	5.9		
		174	T	6.3		
		164	H+B	6.2		
		149	H+T	4.8		
		152	UM	5.2		
IV	5% Formulation	176	H	4.95	3.128	0.816
		158	B	4.56		
		161	T	5.31		
		165	H+B	6.36		
		174	H+T	6.2		
		153	UM	4.83		

The values are expressed in terms of Mean± S.E.M (n=6). The statistical analysis is performed using the multiple-comparative test of Bonferroni.

3.3.2 Graphical representation of tensile strength of incision wound

Effect of BHELE on the formulation of ointment on wounds shown in Fig. 6.

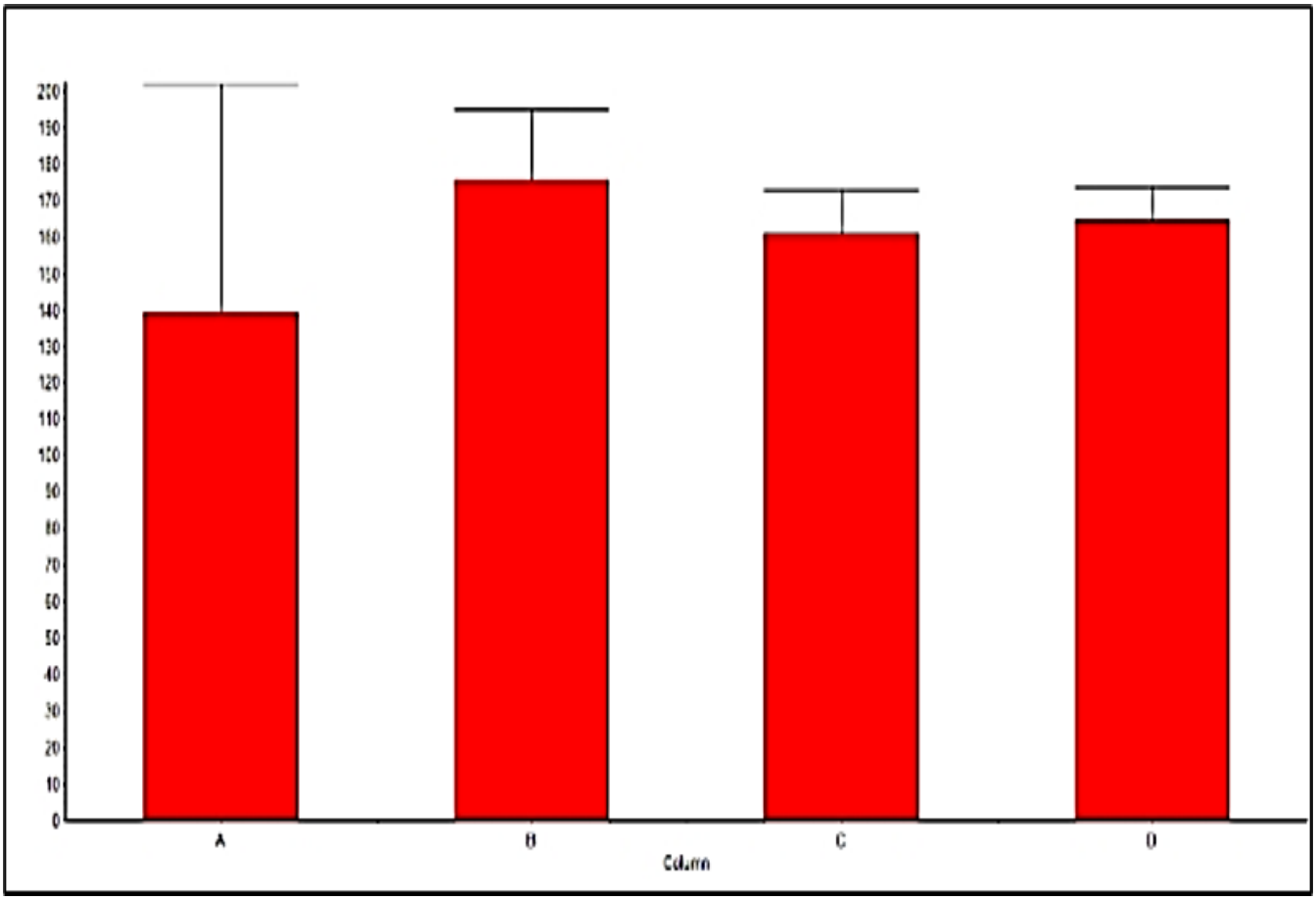


Fig. 6 Effect of BHELE on the formulation of ointment on wounds breaking strength
Values are calculated by using mean±S.E.M (n=6). Statistical data is analysed using ANOVA in one direction and then "Dunnett's"-test.

3.4 Chemical Burn wound model

Photographic representation of chemical burn wound shown in Fig. 7.

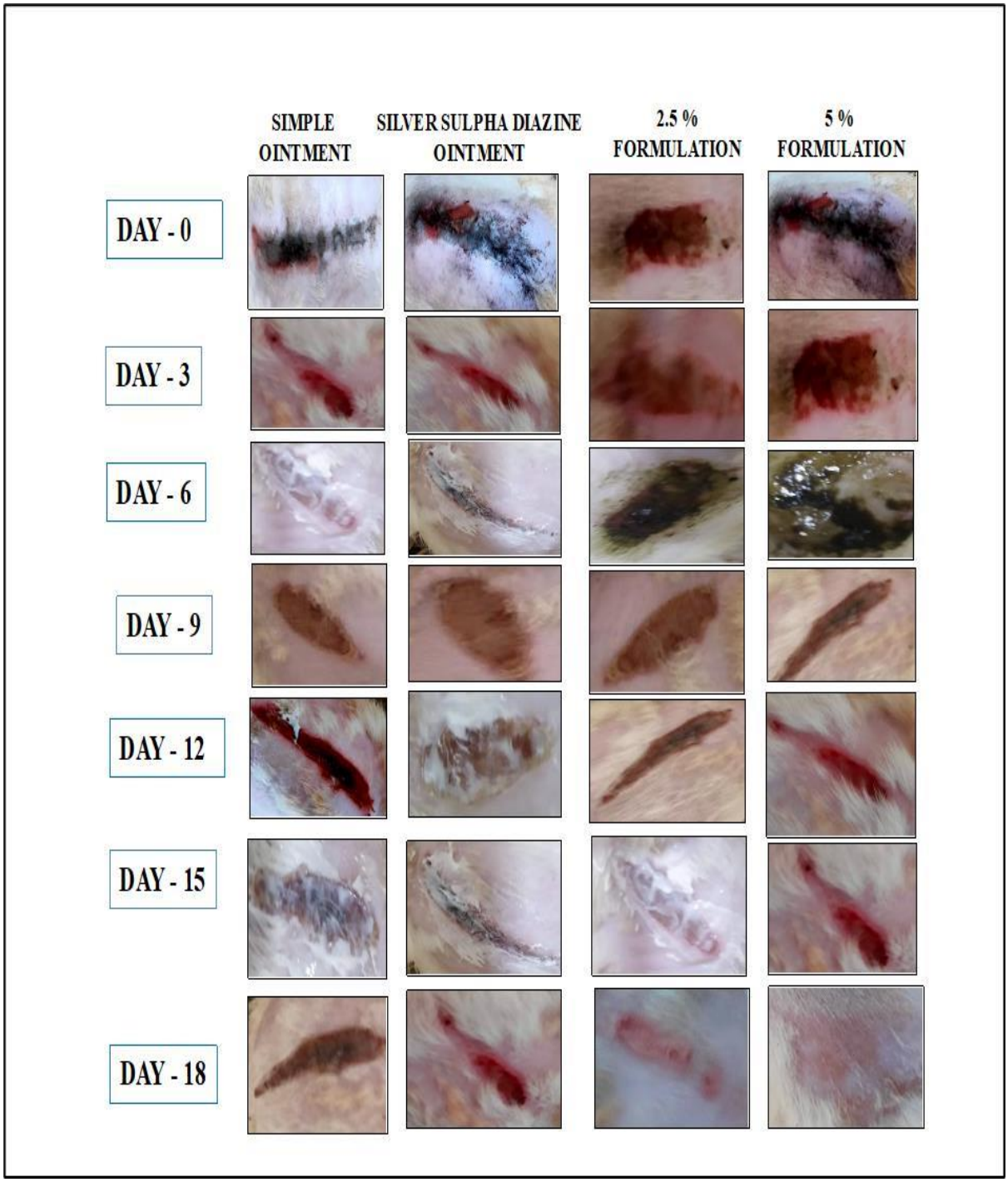


Fig. 7 Photographic representation of chemical burn wound model (Day by day)

3.4.1 Percentage of wound closure in chemical burn wound model

Percentage of wound closure in chemical burn described in Table 4.

Table 4: Percentage of wound closure in chemical burn

Day	Simple ointment	Silver sulfa thiazide ointment	2.5%Formulation	5%Formulation
0	0	0	0	0
3	7	6.1	11.5	10.9
6	14	13.78	24.55	24.55
9	28.5	33.1	50.2	44.5
12	34.41	48.36	56.97	63.22
15	49.65	58.82	70.27	82.22
18	56.12	85.21	81.66	97.71

3.4.2 Statistical analysis of chemical burn wound model

Statistical Analysis of Chemical Burn Wound described in Table 5.

Table 5: Statistical analysis of chemical burn wound model

Day	Simple ointment	Silver sulfa thiazide ointment	2.5%Formulation	5%Formulation
0	0	0	0	0
3	3.22 ± 0.11	13.05 ±0.21	13.2 ±0.28	12.15 ±0.21
6	13.55 ±0.28	21.95± 0.35	19.1±0.42	18.05 ±0.35
9	18.75 ±0.495	40.85± 0.49	38.22 ±0.56	39.85 ±0.63
12	39.91 ±0.60	67.7 ±2.70	51.85 ±0.77	58.7± 0.84
15	70.87 ±0.91	94.5±0.989	75.55 ±1.20	80.55 ±1.06
18	91.12 ±1.27	118.35 ±1.20	116.3 ±1.55	124.3 ±1.41

Each group is comprised of six animals.The data are reported in terms of mean plus S.E.M. ANOVA 6 then followed by an unpaired "Dunnett's"-test **P 0.05 *P 0.05 *P 0.05, **P 0.01 and **P 0.01 and ***P 0.001 is considered to be statistically significant when compared to that of Standard Group treated with Silver Sulfadiazine Ointment.

3.4.3 Graphical representation

Effect of BHELE ointment formulation on chemical wounds shown in Fig. 8.

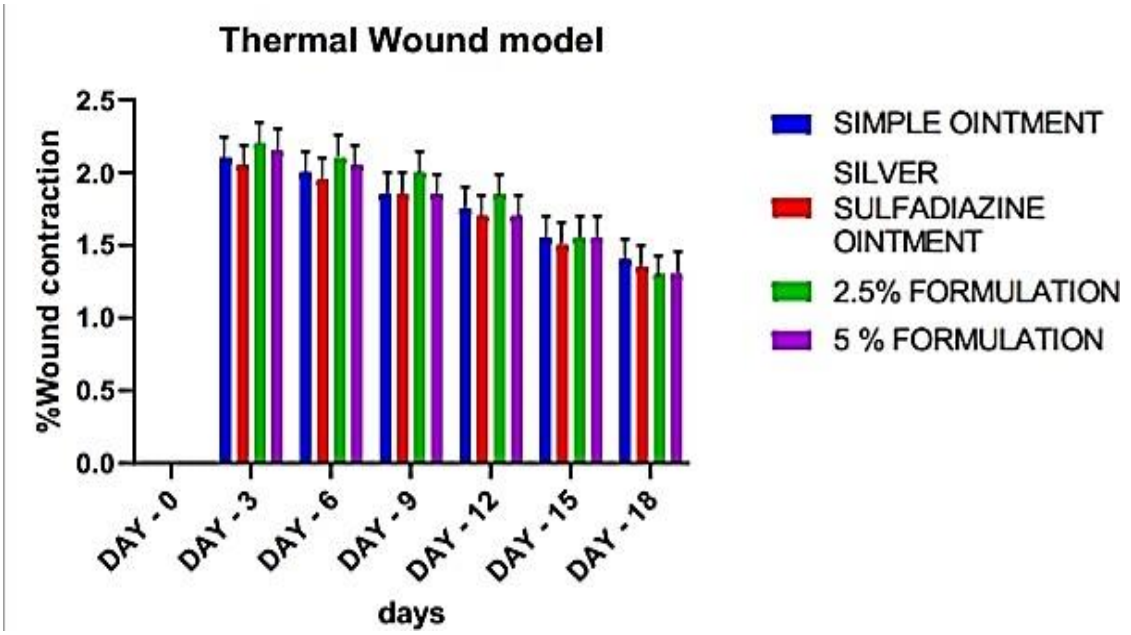


Fig. 8 Effect of BHELE ointment formulation on chemical wounds
Values are calculated as mean ±S.E.M(n=6). They analyze statistical data with one-way ANOVA. They then apply "Dunnett's" test.

3.5 Thermal burn model

Photographic representation of thermal burn wound shown in Fig. 9.

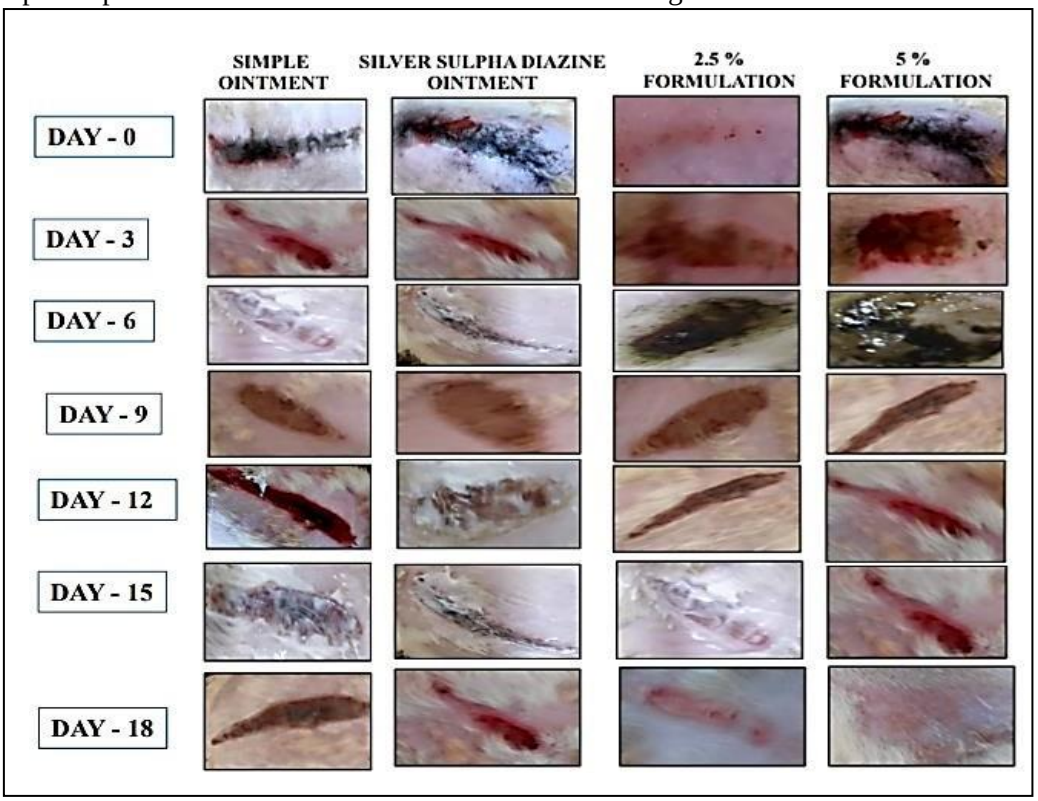


Fig. 9 Photographic representation of thermal burn wound model (Day by day)

3.5.1 Percentageofwound closure in thermal burn wound model

Percentage of wound closure in thermal burn wound described in Table 6.

Table 6: Percentage of wound closure in thermal burn wound model

Day	Simple ointment	Silver sulfa thiazide ointment	2.5%Formulation	5%Formulation
0	0	0	0	0
3	7	6.1	11.5	10.9
6	14	13.78	24.55	26.55
9	28.5	31.1	49.2	43.5
12	36.41	48.36	55.97	63.22
15	47.65	59.82	69.27	80.22
18	58.12	84.21	81.66	96.71

3.5.2 Statistical Analysis of Thermal Burn wound model

Statistical analysis of thermal burn wound described in Table 7.

Table 7: Statistical analysis of thermal burn wound model

Day	Simple ointment	Silver sulfa thiazide ointment	2.5%Formulation	5%Formulation
0	0	0	0	0
3	3.22 ± 0.11	13.05 ±0.21	13.2 ±0.28	12.15 ±0.21
6	13.55 ±0.28	21.95± 0.35	19.1±0.42	18.05 ±0.35
9	18.75 ±0.495	39.85± 0.49	37.22 ±0.56	37.85 ±0.63
12	40.91 ±0.60	65.7 ±2.70	49.85 ±0.77	55.7± 0.84
15	69.87 ±0.91	92.5±0.989	73.55 ±1.20	78.55 ±1.06
18	90.12 ±1.27	116.35 ±1.20	114.3 ±1.55	123.3 ±1.41

Each group is composed of six animals. The results are presented by means ± S.E.M. ANOVA with n=6. Then the unpaired “Dunnett’s test” **P 0.05 *P 0.05 *P 0.05, **P 0.01 and *P 0.001 being statistically significant compared to Standard Group treated with Silver Sulfadiazine ointment.

3.5.2 Graphical representation

Effect of BHELE ointment formulation on thermal wounds shown in Fig. 10.

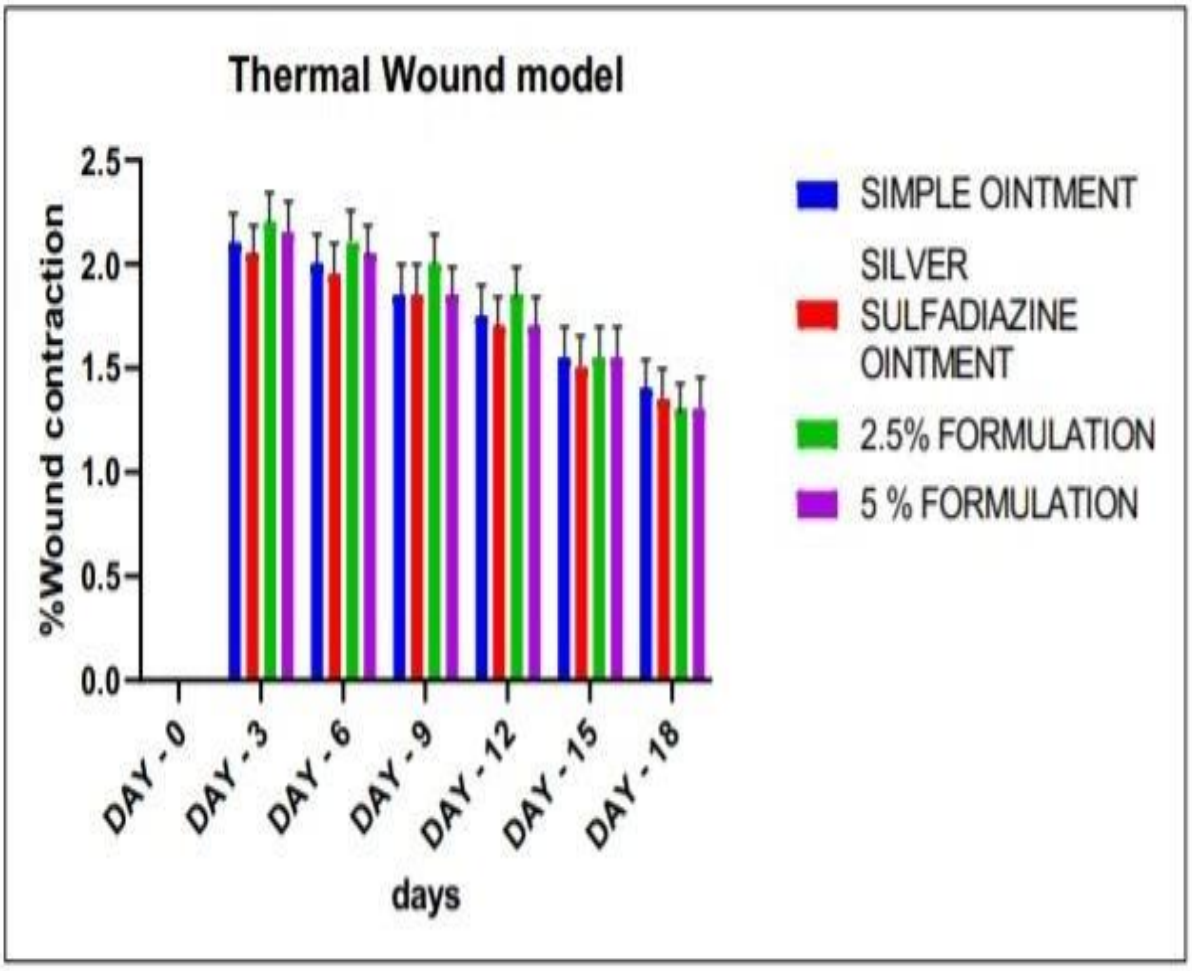


Fig. 10 Effect of BHELE ointment formulation on thermal wounds
Values are calculated as Mean ± S.E.M. (n=6). Analyzing statistical information using one-way ANOVA, followed by "Dunnett's" t-test.

3.6 HPTLC Analysis:

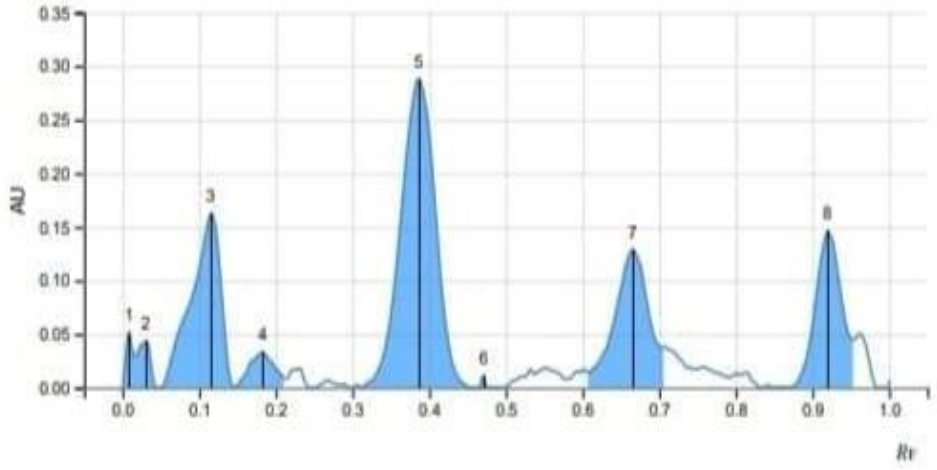


Fig. 112- peak of kaempherol. 4- Peak of naringenin.6-peak of rutin, 8-peak of coumaric acid.

Table 8:

Peak #	Start		Max			End		Area		Manual peak	Substance Name
	R_f	H	R_f	H	%	R_f	H	A	%		
1	0.000	0.0000	0.008	0.0507	5.88	0.015	0.0291	0.00052	1.38	No	
2	0.015	0.0291	0.031	0.0439	5.08	0.044	0.0000	0.00086	2.30	No	
3	0.050	0.0000	0.115	0.1626	18.84	0.146	0.0004	0.00717	19.22	No	
4	0.147	0.0004	0.183	0.0325	3.76	0.211	0.0086	0.00126	3.39	No	
5	0.314	0.0006	0.386	0.2879	33.35	0.456	0.0000	0.01502	40.26	No	
6	0.457	0.0000	0.471	0.0110	1.27	0.483	0.0003	0.00007	0.18	No	
7	0.607	0.0142	0.665	0.1291	14.95	0.706	0.0379	0.00665	17.82	No	
8	0.867	0.0000	0.919	0.1456	16.87	0.953	0.0450	0.00576	15.45	No	

4. Discussion:

Herbal medicine and its derivatives are widely used in many countries in the developing world instead of allopathic medicines to treat various ailments.

In skin irritation test our formulation ointment have no significant change in the applied area of the skin in rat like irritation, redness and swelling were not observed. The procedure is maintained according to the guideline of OECD-404.

The excision method for wounds BHELE Ointment formula and the common referent (Povidone Iodine) treated groups had more excellent wound closing rates than the standard reference (Simple Ointment) group. Our results showed that the closure rate was increased by applying BHELE's Ointment and that the most significant contracture was seen in wounds treated with BHELE five the Ointment. This could be due to the increase in the contractile ability of myofibroblasts or the increase in myofibroblasts entering the wound.

The strength of the wound defines the healing process for wounds, that is, the force required to tear or remove the wound. In the case of wounds, their strength to be broken increases with an increase in collagen, as well as the stability of the fibers. In our study, the BHELE 2.5 percent ointment formulation demonstrated significant improvement in the strength of wounds when compared to the standard.

Thermal burns result from skin contact with hot objects like fire, hot oil, and boiling water, whereas chemical injuries happen if living tissues are exposed to substances that can cause corrosive damage, like bases and solid acids, according to the results of this study, the subjects that were treated with BHELE 5 % ointment, as well as the standard of reference (silver sulfadiazine), showed a significantly more rapid rate of wound healing over the group that was treated with control.

Through HPTLC fingerprinting, presence of rutin, kaempferol and P-coumaric acid was confirmed in the ethanolic extract, which could be the reason for the healing characteristics of the herb.

5. Conclusion:

The study demonstrated that applying topical BHELE Ointment produced the effect of healing wounds across all three models, including those of the Excision, Incision, and thermal as well as chemical Burn wound models, compared with the standard (simple Ointment). This formula, BHELE 5%, demonstrated a greater wound contraction rate in both the wound models that had excision and thermal. For models with wounds that have been incision, the strength in wound healing is higher and results in the formulation of a 2.5 %formula.

HPTLC fingerprinting confirmed the presence of rutin (hRf=1.5), kaempherol (hRf= 4) and p-coumaric acid (hRf= 85).

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Ethics approval:
Ethics approval for this study was obtained from "Calcutta Institute of Pharmaceutical Technology & Allied Health Sciences" animal ethics committee, having approval No. 2075/PO/Re/S/19/CPCSEA, valid till 25/08/2024.

Decelerations of interest:
The authors declare that they have no conflict of interest.

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