

INVESTIGATING SKIN REGENERATING ABILITY OF *BERGINIA CILIATA* EXTRACT IN COMBINATION WITH OTHER BIOPOLYMERS IN MAMMALIAN MODEL

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Abstract

When wounds are made, the skin and underlying tissues are damaged and provide an entry point for bacteria and other pathogens. Wound healing is essential for tissue repair and recovery. This study evaluated the wound-healing potential of a novel hydrogel dressing made from PVA/Pectin/Gelatin combined with *Bergenia ciliata* rhizome extract using an APTES crosslinker. Sixteen Swiss albino mice were divided into four groups: BC0 (control/untreated), BC1 (hydrogel with 100 μ l of extract), BC2 (hydrogel with 300 μ l of extract) and BC3 (hydrogel only). Full thickness 5 mm wounds were created and body weight, feed intake and wound size were measured on days 3, 6, 9, 12 and 14. The wound contraction was highest in BC2 (99%), followed by BC1 (97%), BC3 (73%), and BC0 (59%). Blood tests indicated no adverse effects. Histological examination showed improved epidermal growth, blood vessels, follicles and glands in treated groups.

Introduction

The skin is the largest organ of the human body and acts as a barrier to the harmful agents, pathogens and environmental stressors, helps to maintain body homeostasis and sensory functions. It has three significant layers: the epidermis, dermis, and hypodermis. The epidermis protects from microbial invasion, fluid loss and mechanical injury, while the dermis houses connective tissues which aid in skin strength, sensation and nutrient supply (McGrath and Uitto, 2023; Mohamed and Hargest, 2022; Yousef, Alhajj, and Sharma, 2022). Injuries to the skin integrity such as burns, cuts, ulcers will compromise the protective mechanisms and can lead to

infection, inflammation, fluid depletion and impaired healing (Desconsi et al., 2026).

Wound healing is a complex process that includes hemostasis, inflammation, proliferation, angiogenesis and tissue remodeling. Angiogenesis and tissue regeneration are controlled by growth factors like vascular endothelial growth factor (VEGF), platelet-derived growth factor, and fibroblast growth factor, which stimulate cell proliferation, collagen production, and vascularization (Omorphos et al., 2021; Sedighi et al., 2023). Any delay or abnormality in wound healing can contribute to chronic wounds, infections, and scar formation (Kangal, & Kopitnik, 2025). Thus, the

development of efficient and affordable wound healing therapies continues to be a major research interest in the field of regenerative medicine.

The biocompatibility, moisture retention, non-toxicity, and similarity to the extracellular matrix make hydrogels very suitable for use in wound management. These properties make hydrogels highly suitable for maintaining a moist environment, absorbing wound exudates, preventing infections, and supporting tissue regeneration (Liang et al., 2021). Natural and synthetic polymers like polyvinyl alcohol (PVA), gelatin, pectin, alginate, and chitosan are used to make hydrogels. Natural polymers, such as gelatin and pectin, offer great biodegradability, bioactivity and cell compatibility, while PVA possess excellent mechanical strength and stability (Kamoun et al., 2021; Xiang and Cui, 2021). Gelatin has a similarity with proteins found in the extracellular matrix (ECM) and it is a good promoter of cell adhesion and tissue regeneration; pectin has gelling properties, adhesive properties, and biocompatibility, which enhance the efficiency of wound healing (Groult et al., 2021; Morello et al., 2023).

Cross-linking agents, like amino propyl triethoxy silane (APTES), can be introduced to enhance the strength of the hydrogel network, increase the vascularization and oxygen supply to the wound area, and accelerate healing (Ara et al., 2022). Besides biomaterials, medicinal plants have been traditionally used in wound healing due to their antimicrobial, antioxidant,

and anti-inflammatory properties (Miranda et al., 2021). Of these, *Bergenia ciliata*, a medicinal plant of the Saxifragaceae family, is well known for its wound healing properties. It possesses useful phytochemicals such as flavonoids, tannins, phenols, and alkaloids that account for its antioxidant, antimicrobial, anti-inflammatory, and regenerator properties (Shah et al., 2020). *Bergenia ciliata* has been shown to promote fibroblast activity, angiogenesis, and tissue regeneration, rendering it very effective in wound and burn treatment (Faiz et al., 2023).

Therefore, the present study was designed to investigate the skin regeneration potential of novel hydrogels prepared from *Bergenia ciliata* extracts combined with multiple polymers and cross-linked with APTES for enhanced wound healing on mammalian skin.

Methodology

2.1 Hydrogel preparation

Procedure for hydrogen formation is as follow:

2.1.1 *Bergenia ciliata* extract preparation

Bergenia ciliata plant dried rhizome was ground to fine powder in a blender. 75 grams of powder were mixed in 500ml alcohol and stored in air tight glass bottle in dark place for 15 days. Then the extract prepared was filtered through muslin cloth followed by Whatman filter paper no 1. 80% ethanolic-aqueous extract was selected for angiogenic and wound healing purpose. (H.Imran, 2023)

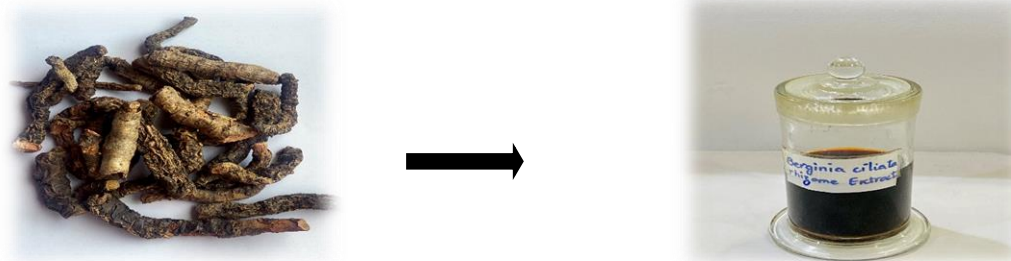


Figure Figure2.1: Rhizome of *Bergenia ciliata* and its ethanolic extract

2.1.2 PVA (Poly Vinyl Alcohol), pectin and gelatin solution preparation

0.5 grams of PVA powder was dissolved in pre heated water, and stirred at 650 rpm at 50°C on magnetic hot plate stirrer for about two hours until homogeneous aqueous solution of PVA was formed (Zang and Zang, 2020).

0.3 g of pectin powder was dissolved in 15 ml distilled water and stirred at 650 rpm at 50°C on magnetic hot plate stirrer for an hour to form a homogenous mixture (Groult, Buwalda et al. 2021)

0.3 g of gelatin was dissolved in 15 ml distilled water and stirred for constant blending for

about an hour until a homogeneous aqueous solution of gelatin was formed.

Both pectin and PVA solutions were mixed at 650 rpm and at 50°C on magnetic hot plate stirrer. After an hour previously made gelatin solution was added to this mixture and stirred for an hour on same speed and temperature. This solution was labelled as BC1. Two more sets of same solution (PVA, Pectin and Gelatin) were prepared following the above-mentioned procedure and labelled as BC2 and BC3.

2.1.3 Incorporation of Cross linker and Plant Extract

In BC1 and BC2 solution, 100 µl and 300 µl of *Bergenia ciliata* extract was added respectively and stirred for an hour. Then 25 µl of APTES cross linker was added in each solution and again stirred for 1 hour. The gels formed were the final forms of required PVA/Pectin/Gelatin 100 µl and 300 µl *Bergenia ciliata* Hydrogels respectively.

In solution BC 3, no *Bergenia ciliata* extract was added only 25 µl of APTES cross linker was added and stirred for 1 hour the gel formed is the final form of required PVA/Pectin/Gelatin Hydrogel.

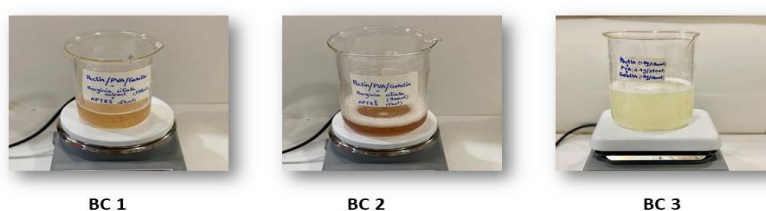


Figure 2.2: Preparation of BC 1, BC 2, and BC 3 Hydro gel on magnetic hot plate stirrer

2.1.4 Gel Casting

5ml from each gel was poured into separate petri plates, bubbles were removed by tapping on surface, each Petri plates were labeled and placed in drying oven at 50°C for 24 hours then the casted gels were placed in separate zipper bags to avoid contamination.

2.2 Mice Rearing

Male Swiss albino mice *Mus musculus* were allowed to adjust the laboratory condition for 7 days before experiment was started (Belachew et al., 2020). For rearing mice, standard parameters of temperature 24 ± 2 °C, humidity at $50\% \pm 10\%$ and 12:12 hr dark–light cycles in

animal house were maintained. Daily fresh feed and fresh water were given to mice. Major components of diet were 5% fat, 3.5% fiber and 13% protein.

2.3 Experimental Design

For experiment, 16 male mice of 19-33 g bodyweight were randomly separated into four groups namely BC 0, BC 1, BC 2 and BC 3. Each group contained 4 mice. Group I labelled as BC 0 was control group containing untreated mice. Group BC 1, BC 2 and BC 3 were treated with different formulations of hydrogels as shown in table 2.1.

Table 2.1: Experimental groups with their respective hydrogel composition

Experimental group	Sample Applied	Group Labeling
Group 1	No treatment	BC 0
Group 2	PVA/Pectin/Gelatin 100 µl Plant extract	BC 1
Group 3	PVA/Pectin/Gelatin 300 µl Plant extract	BC 2
Group 4	PVA/Pectin/Gelatin	BC 3

2.4 Excision of Wound

Mice in each group were given anesthesia. The skin on the posterior dorsal side of each mouse was exposed by removing its fur with blade and sanitized with 70% alcohol. At predefined region double excision wound was given by removing 5 mm of skin by using biopsy punch.

After ten minutes of anesthesia, the animals recovered usual sensitivity to their surroundings. Each mouse was kept in separate box to prevent them from eating wounds of each other. On regular basis cage and food dishes were cleaned and sanitized to prevent wounds from infection.



Figure 2.3: Mice with double excision wound

2.5 Wound treatment with Hydrogel

After wound excision, mice were treated with novel *Bergenia ciliata* hydrogel according to their groups as shown in table 2.1. Hydrogels were applied daily on wound area by using cotton buds, for a period of 2 weeks/14 days.

2.8 Collection of Blood Sample

When mice healed completely on 15th day, mice were given deep anesthesia and blood samples were collected by heart puncture technique. Blood samples were immediately transferred and stored in separate EDTA tubes

2.6 Feed and weight measurements

Weight of mice, weight of feed consumed and weight of feed given to each mouse were also measured.

2.9 Biochemical Examination

By using sampling techniques biochemical analysis, CBC (complete blood count) of blood samples was done.

2.7 Wound size measurement & Calculation of Wound Size Reduction

To detect degree of wound contraction wound size of each mouse was measured using digital Vernier caliper on day 1, 3, 6, 9, 12 and 14. Measurements were done to detect the degree of wound contraction.

%wound reduction

$$= \frac{\text{wound size on day 0} - \text{wound size on } nth \text{ day}}{\text{wound size on day 0}}$$

Where n is the number of days 1, 3, 6, 9, 12, 14. By using this formula we can trace the reduction in the size of wound.

2.10 Histological Analysis of skin

Perfect small flattened segments of mice skin from recovered area were removed and fixed in 10% formalin. Then gradual dehydration was performed in 30%, 50%, and 70% alcohol. Xylene was used as clearing agent. The skin sample was then placed in warm paraffin wax to make blocks and sectioning was done using microtome. Skin sections were subjected to Hematoxylin and Eosin staining. Canada balsam was used for mounting of slides and xylene dipped cover slip was used to protect the stained section from damage. At last the slides were observed at 100x.



Figure 2.4: Grafted segment of healed skin of mice

RESULTS

3.1 Statistical Analysis

Numerical data collected during the study was evaluated by using SPSS (version 26) and results were presented in form of Mean $\pm$ S.E. one-way ANOVA test was applied followed by Tukey Post Hoc test. Results were presented in form of table and graphs.

3.2 Biometric analysis

Feed intake and body weight of mice were measured at regular intervals, no negative effect of *Bergenia*/PVA/Pectin/Gelatin on Body weight and feed intake of control and treated groups was observed. Slow behavior of mice was observed during first two days later on they recovered from the state of inactivity and illness. There was no random increase or decrease in body weight. Body weight and feed intake followed a linear progression.

Table 3.1.: This table demonstrate calculated data, representing the body weight at alternate days in treated and control groups.

Groups* days	BC 1	BC 2	BC 3	BC 0
Day 0	27.7a $\pm$ 1.80a	29.2a $\pm$ 2.82a	23.9a $\pm$ 2.07a	25.7a $\pm$ 2.29a
Day 3	28.7a $\pm$ 1.86a	29.8a $\pm$ 2.77a	24.2a $\pm$ 1.90a	25.9a $\pm$ 2.40a
Day6	29.8a $\pm$ 1.86a	30.5a $\pm$ 2.71a	24.8a $\pm$ 1.73a	26.5a $\pm$ 2.49a
Day 9	31.0a $\pm$ 1.93a	31.3a $\pm$ 2.73a	25.6a $\pm$ 1.99a	27.3a $\pm$ 2.49a
Day12	32.0a $\pm$ 1.99a	32.1a $\pm$ 2.71a	26.6a $\pm$ 1.86a	28.1a $\pm$ 2.45a
Day 14	32.6a $\pm$ 2.00a	32.7a $\pm$ 2.71a	27.0a $\pm$ 1.61a	28.8a $\pm$ 2.37a

Numerical values are illustrated as (Mean $\pm$ S.E) as $p \geq 0.05$ Alphabets as Superscripts presents insignificant difference among control and treated groups.

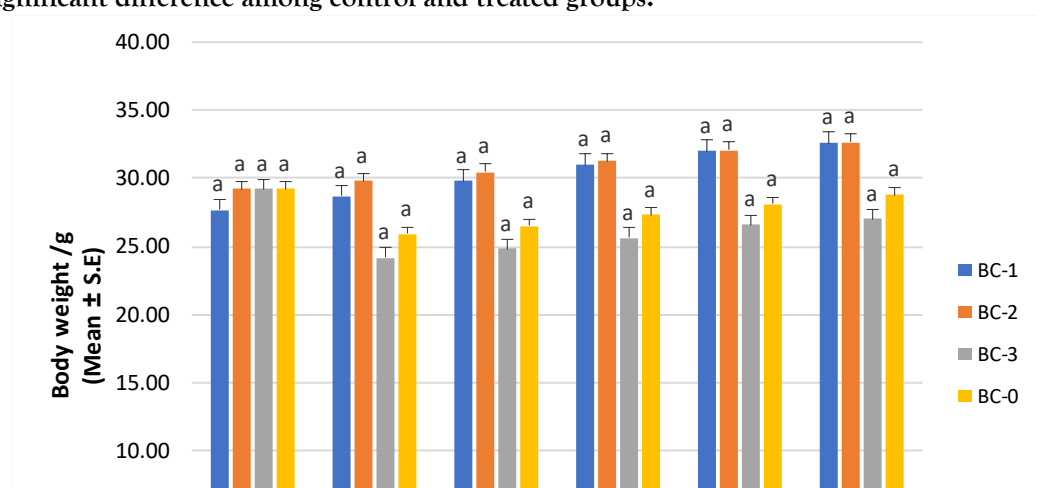


Figure 3.1: Graphical representation of body weight (g) in hydrogel treated in comparison with control group.

(Alphabets as superscripts shows that there is insignificant difference in bodyweight of treated and control groups. The α value is greater than 0.05 of One-Way ANOVA).

Table 3.2: The calculated data represents the Feed intake at alternate days in treated and control groups.

Groups Days	BC 1	BC 2	BC 3	BC 0
DAY 0	7.2 ^a ± 0.82	5.98 ^{ab} ± 1.18	3.95 ^b ± 0.50	4.61 ^b ± 0.47
DAY 3	5.66 ^a ± 0.54	5.28 ^a ± 1.35	4.13 ^a ± 0.74	4.22 ^a ± 0.92
DAY 6	5.33 ^a ± 0.55	6.21 ^a ± 1.36	4.96 ^a ± 0.75	4.73 ^a ± 0.93
DAY 9	6.38 ^a ± 1.47	6.65 ± 0.92	5.51 ^a ± 0.90	6.43 ^a ± 0.38
DAY 12	6.26 ^a ± 1.48	5.75 ^a ± 0.93	4.36 ^a ± 0.91	6.05 ^a ± 0.39
DAY 14	4.51 ^a ± 1.49	6.68 ^a ± 0.94	5.77 ^a ± 0.92	7.32 ^a ± 0.40

Numerical values are illustrated as (Mean ± S.E); Superscripts presents insignificant difference ($p \geq 0.05$) from each other.

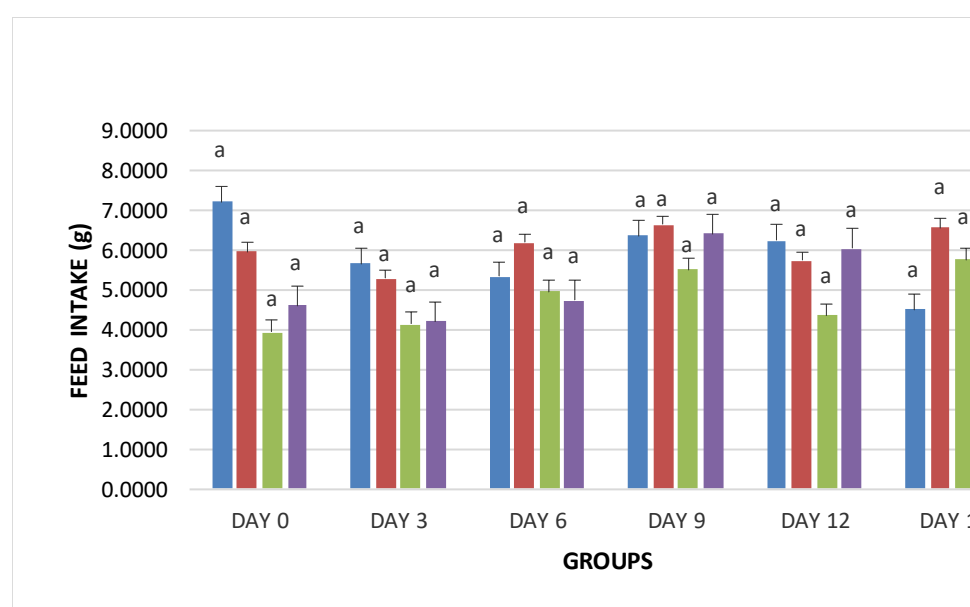


Figure 3.2: Graphical representation of Feed intake (g) in hydrogel treated and control groups

3.3 Wound reduction Examination

Mortality rate was zero during the experiment. Pictures of wound regions were captured. Wound reduction % of control and treated groups were measured and statistically analyzed by using one way ANOVA and represented as mean±SE as shown in table 3.3. Significant difference appeared in control and treated groups at alternate days ($P \leq 0.05$). Post Hoc

test was applied for further evaluation of wound size reduction % of control and treated groups. Healing occurred rapidly in group BC 1 and BC 2 as compared to BC 3 and BC 0. The control group showed slow healing in comparison to treated groups. Healing percentage in control group was 55.93% which occurred because of metabolic process and immune system of mice.

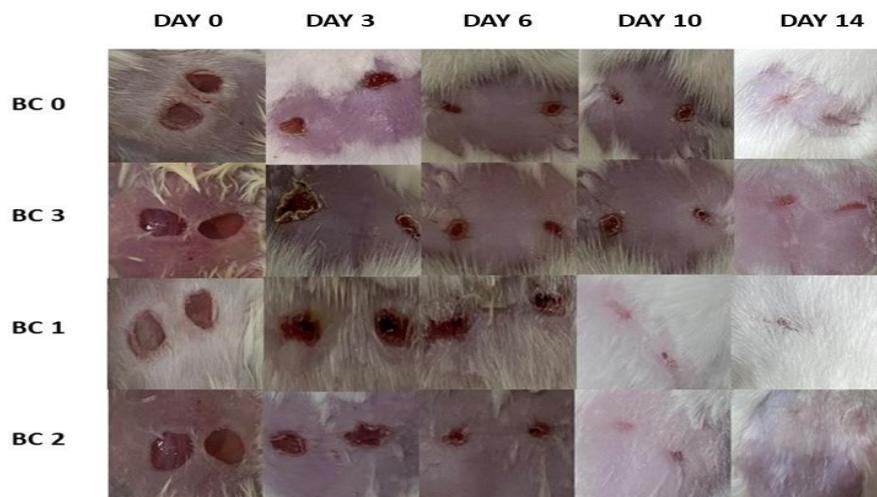


Figure 3.3: Wound size of treated groups in contrast to control group at alternate days

Table 3.3: This table demonstrates calculated data, representing the wound size reduction % at alternate days in treated and control groups

Groups	BC 0	BC 1	BC 2	BC 3
Days				
day 3	16.35 ^{ab} ± 3.82	20.18 ^a ± 2.55	9.965 ^b ± 2.49	12.99 ^{ab} ± 3.20
day 6	23.78 ^a ± 4.83	23.02 ^a ± 3.60	30.17 ^a ± 1.76	20.88 ^a ± 2.59
day 9	39.94 ^a ± 3.97	51.90 ^a ± 5.02	53.08 ^a ± 4.74	47.20 ^a ± 4.20
day 14	55.93 ^b ± 4.73	83.61 ^{ab} ± 11.21	92.87 ^a ± 2.77	78.18 ^{ab} ± 7.43

Numerical values are illustrated as (Mean ± S.E); Superscript alphabets represent significant difference ($p \geq 0.05$) from each other.

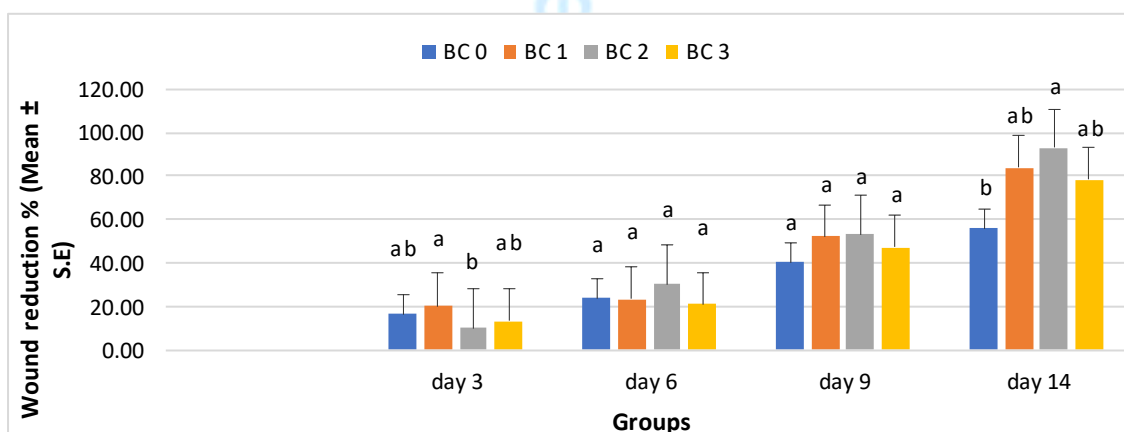


Figure 3.4: Graphical representation of Wound reduction % of hydrogel treated in comparison with control group. Superscript alphabets of different groups represent the difference among the groups

3.4 Complete Blood Count (CBC)

The complete blood count (CBC) was done to investigate the difference that occurs in differentials of blood in treated and control groups.

3.4.1 White blood cells

There was no statistically significant difference in WBCs count of experimental groups and control group ($P \geq 0.05$) showing that treatment has no noticeable impact on WBC.

count of treated groups as compared to control group.

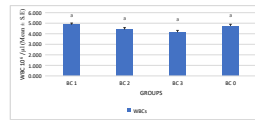


Figure 3.5. Histogram represents the WBC count among control and treated groups. (WBC values are illustrated as (Mean $\pm$ S.D); Superscripts represents no significant difference between groups at significance level ≥ 0.05 followed by Tukeys' s Post-Hoc test).

3.4.2 Lymphocytes

Statistically significant difference ($P \leq 0.05$) occurred among treated (BC 1=78.40 $\pm$ 1.16, BC 2= 76.20 $\pm$ 1.59, BC 3= 75.60 $\pm$ 1.21) and control

groups (BC 0= 73 $\pm$ 1.37) when it came to lymphocytes count showing that treatment had noticeable impact on treated groups lymphocytes count compared to control group.

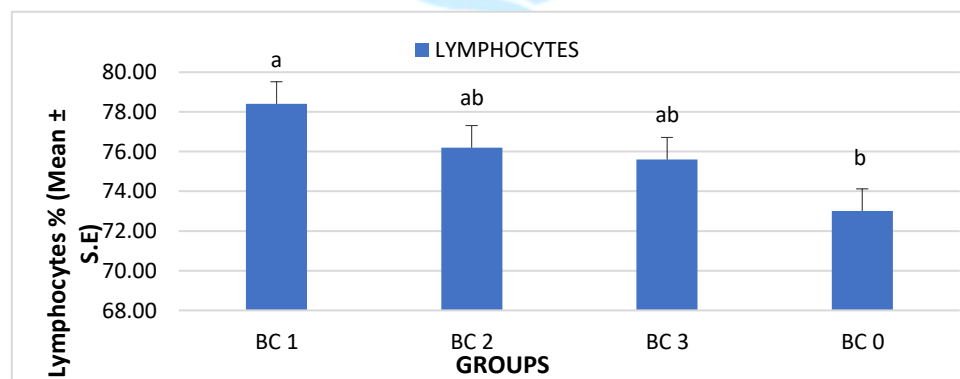


Figure 3.6. Histogram represents the Lymphocytes concentration among control and treated groups. (Lymphocytes values are illustrated as (Mean $\pm$ S.E); Superscripts of groups represents significant difference $p \leq 0.05$ between groups).

3.4.3 Eosinophils

Statistically no significant difference occurred in eosinophil count of treated groups (BC 1, BC 2, and BC 3) and control group BC 0 ($P \geq$

0.05) showing that treatment had no noticeable impact on eosinophil count of hydrogel treated groups as compared to control group.

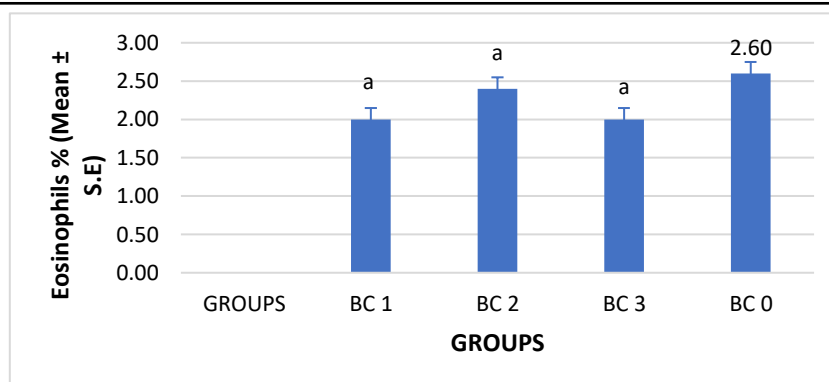


Figure 3.7: Histogram represents the Eosinophil concentration among control and treated groups. (Eosinophil values are illustrated as (Mean $\pm$ S.E); Superscripts represents no significant difference between groups at significance level ≥ 0.05 followed by Tukey's Post-Hoc test).

3.4.4 Monocytes

Treatment had no noticeable impact on monocytes count as compared to control group

as no significant difference was observed in monocyte count of experimental groups and control group ($P \geq 0.05$).

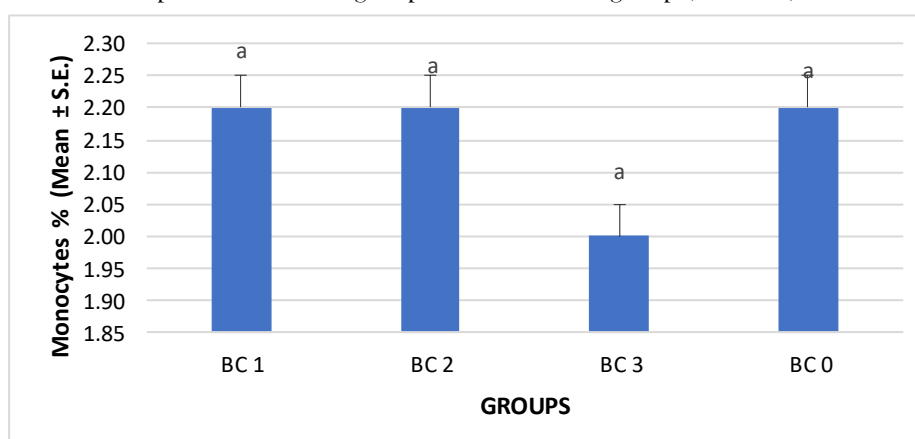


Figure 3.8. Histogram represents the Monocyte count among control and treated groups. (Monocytes values are illustrated as (Mean $\pm$ S.E); Superscripts represents no significant difference between groups at significance level ≥ 0.05 followed by Tukey's Post-Hoc test).

3.4.5 Hemoglobin

There was no significant difference in experimental (BC 1, BC 2, and BC 3) and control groups BC 0 ($P \geq 0.05$). In treatment

groups, hydrogel treatment have no noticeable impact on WBC count as compared to control group.

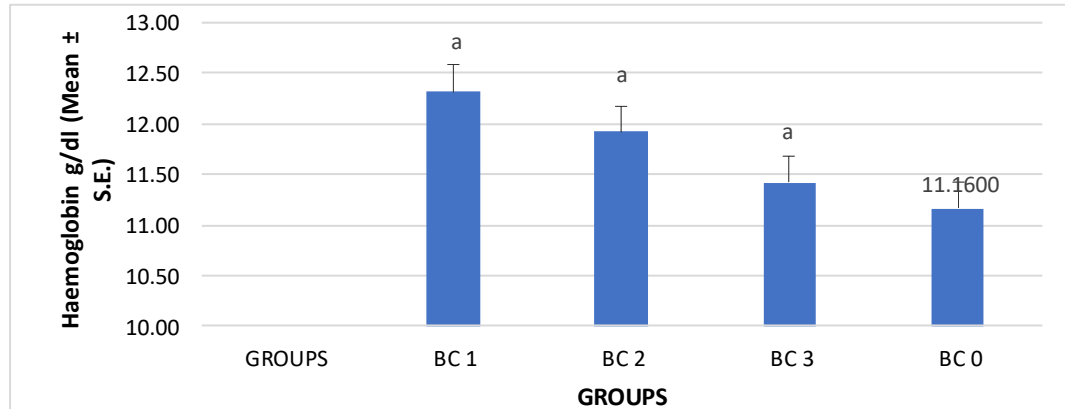


Figure 3.9. Histogram represents the Hemoglobin count among control and treated groups. (Hemoglobin values are illustrated as (Mean ± S.E); Superscripts represents no significant difference between groups at significance level ≥ 0.05 followed by Tukey's Post-Hoc test).

3.4.6 Platelets

Statistically significant difference ($P \leq 0.05$) occurred among treated and control groups when it came to platelets count showing that

treatment had noticeable impact on platelets count of treated groups as compared to control group.

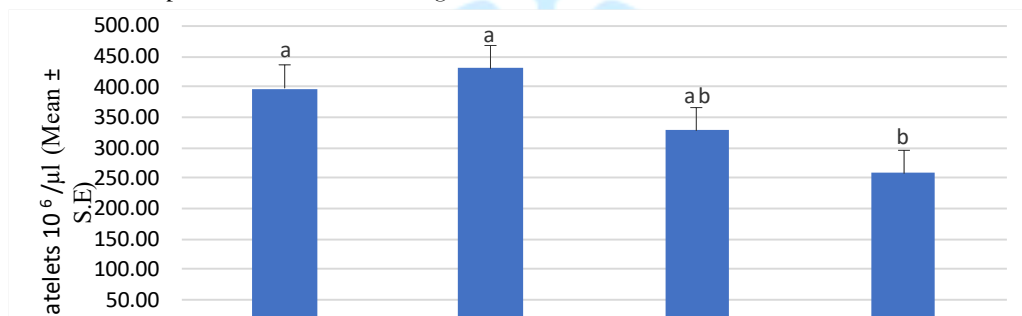


Figure 3.10. Histogram represents the Platelets count among control and treated groups. (Platelets values are illustrated as (Mean ± S.E); Superscripts represents significant difference between groups at significance level ≤ 0.05 followed by Tukey's Post-Hoc test).

3.4.7 Neutrophils

There was no significant difference in experimental groups BC 1, BC 2, BC 3 and control group BC 0 ($P \geq 0.05$) as shown in

figure 3.11. It shows that treatment had no noticeable impact on neutrophil count as compared to control group.

Z

Figure 3.11. Histogram represents the Neutrophil concentration among control and treated groups. (Neutrophil values are illustrated as (Mean ± S.E); Superscripts represent no significant difference between groups at significance level ≥ 0.05 followed by Tukey's Post-Hoc test).

Table 3.4: Quantification of Blood cells in treated and control groups

Groups	BC 0	BC 1	BC 2	BC 3
Parameters				
Hemoglobin (g/dl)	11.16 ^a ± 0.48	12.32 ^a ± 0.20	11.92 ^a ± 0.33	11.42 ^a ± 0.51
WBC (μ/L)	4.74 ^a ± 0.32	4.86 ^a ± 0.26	4.42 ^a ± 0.27	4.16 ^a ± 0.15
Platelets (μ/l)	258.2 ^b ± 19.3	397 ^a ± 62.3	431.6 ^a ± 8.05	328.6 ^{ab} ± 37.8

Eosinophils (%)	2.6 ^a ± 0.24	2.0 ^a ± 0.00	2.4 ^a ± 0.24	2.0 ^a ± 0.31
Neutrophils (%)	22 ^a ± 1.37	18.4 ^a ± 0.92	19 ^a ± 1.76	20.4 ^a ± 1.20
Lymphocytes (%)	73 ^b ± 1.37	78.4 ^a ± 1.16	76.2 ^{ab} ± 1.59	75.6 ^{ab} ± 1.20
Monocytes (%)	2.2 ^a ± 0.20	2.2 ^a ± 0.20	2.2 ^a ± 0.20	2.0 ^a ± 0.31

Numerical values are illustrated as (Mean ± S.E); Superscript alphabets represents significant difference between groups.

According to One Way ANOVA significant difference occurs in Platelets and Lymphocytes with p value ≤ 0.05. Hemoglobin, WBCs, Eosinophils, Neutrophils, Lymphocytes,

Monocytes have p value ≥ 0.05 which shows there is no significant difference followed by Tukeys Post Hoc test.

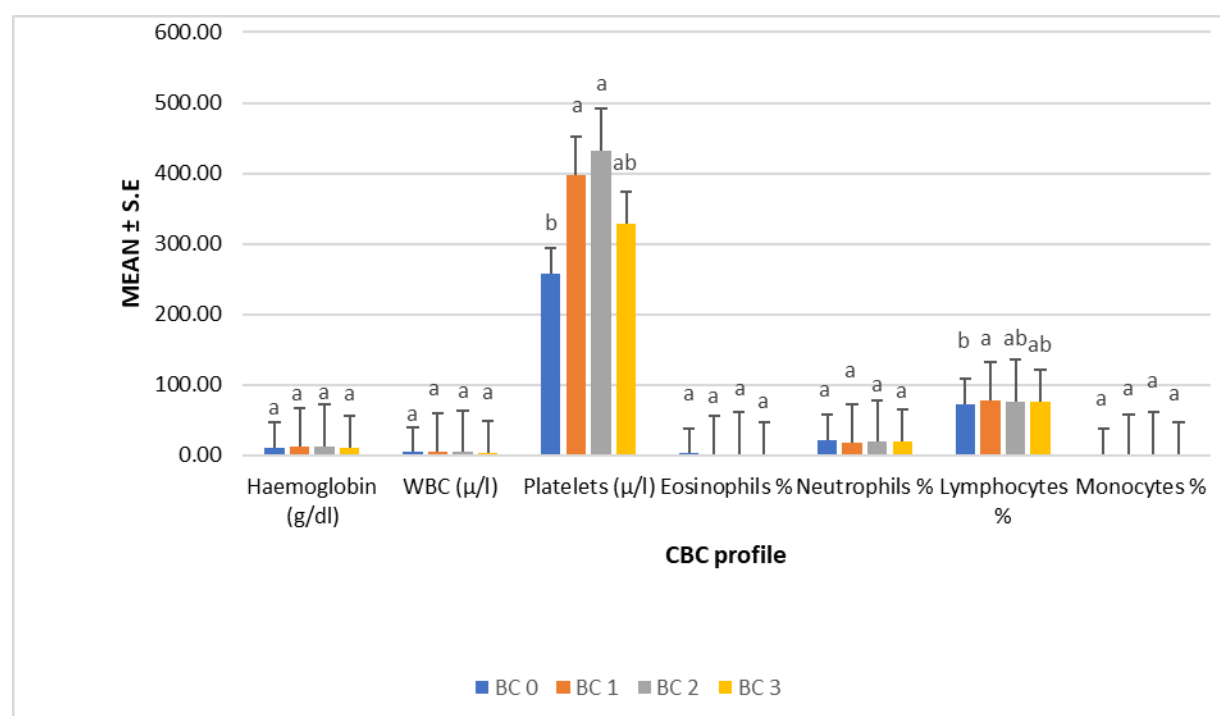


Figure 3.12. Graphical representation of Mean Quantification of blood cells in hydrogel treated in comparison with control group. Superscripts represents variation occur in platelets and lymphocytes.

3.5 Histological Examination

Epidermis, dermis and hypodermis of experimental groups was similar to that of normal skin as shown in figure 3.13. In control and BC 3 there was less cell growth near

epidermis. BC 1 and BC 2 showed various elements like epidermis, dermis, hair follicles, and Blood vessels etc. Angiogenesis was more visible in treated groups.

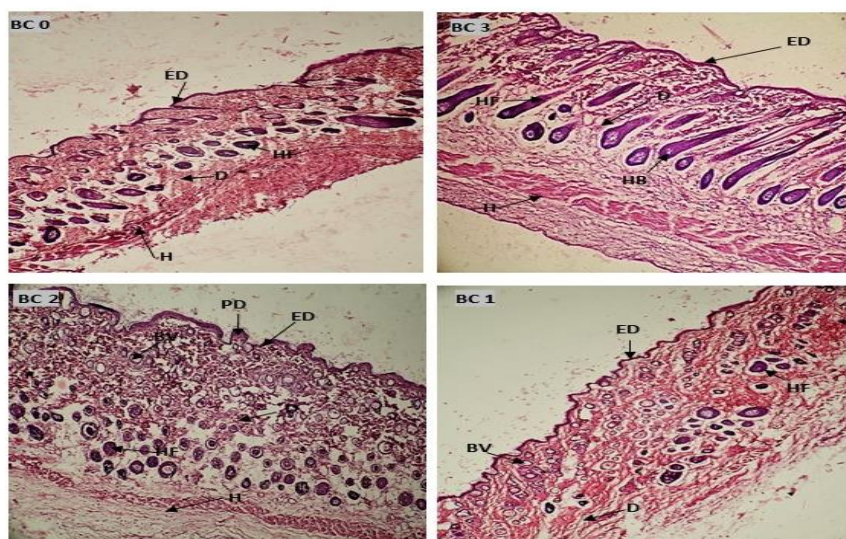


Figure 3.13 Histological sections of Hematoxylin and Eosin stained skin of control and treated groups observed at 10x.

Abbreviations: ED, Epidermis; D, Dermis; BV, Blood vessel; HF, Hair follicle; HB, Hair bulb; H, Hypodermis, PD papillary dermis.

DISCUSSION

This research was designed to prepare an ointment that would have the potential to heal the wounds faster and show best results by increasing the process of angiogenesis and reepithelization. According to Riaz et al., 2025, ideal dressing of wound should be porous enough to allow diffusion of gases to keep the wounds surrounding moist as the dry environment of wound slow down the healing process. It should have ability to absorb the discharge of wound, heal the wound fast, alleviate discomfort, prevent infection and promote angiogenesis. For this study we formulated herbal medicinal plant *Bergenia ciliata* rhizome extract cross linked with natural hydrogels (PVA, Pectin and Gelatin) by APTES and hypothesized that their combination will enhance the healing potential of skin wound.

According to our experimental results the groups that were given the treatment of polyvinyl hydrogel loaded with pectin and gelatin along with *Bergenia ciliata* extracts showed maximum healing ability among all the tested groups. BC 3 group (treated with PVA, Pectin and Gelatin only) showed visible healing potential in comparison to no treatment group. So, hydrogel treated groups showed improved

healing and regeneration ability as compared to control group which was not treated with hydrogel. Liang et al., 2021, says that the hydrogels reduce the inflammation of wounds by maintaining the pH of wound micro environment. Hydrogels heal wounds faster because of chemical and physical resemblance of hydrogels with the extracellular matrix (Gounden & Singh, 2024). According to the findings of Kim and Lee, 2017, hydrogel formed from polyvinyl alcohol and pectin is efficient enough to reduce the size of wound and enhance regeneration process in rats as compared to control. Their findings were compatible to this research.

The investigations carried out by Jeong et al., 2022, showed that the hydrogels formed from PVA and gelatin also have a significant positive impact on wound healing process. The mechanical strength and porous nature of such hydrogels enhance the process of regeneration by facilitating quick adhesion and proliferation. Their study showed that such hydrogels are nontoxic with no side effect on cell activities. When PVA, Pectin and gelatin hydrogels are combined with rhizome extract of *Bergenia ciliata* it shows excellent healing results as in group BC 1 and BC 2. These results are compatible with the study carried out by Kour et al., 2021, which revealed that *Bergenia ciliata* extracts play an important role in wound healing. They worked on ethanolic extract of

Bergenia ciliata and found that *Bergenia ciliata* extracts promote the process of collagenation and re-epithelialization.

The hematological analysis revealed that the *Bergenia ciliata*-loaded PVA/pectin/gelatin hydrogels promoted wound healing without significant systemic hematological changes. No significant differences observed in total WBC count, neutrophils, eosinophils or hemoglobin indicated that the hydrogel formulation caused no systemic inflammation, allergic response, toxicity, or hematological stress. The results suggest the treatment was mainly local and targeted inflammation and tissue repair at the wound site, rather than causing systemic immune responses. *Bergenia ciliata* has been reported to have considerable antioxidant, antimicrobial and anti-inflammatory properties due to the presence of bergenin, gallic acid, catechin, rutin etc. polyphenolic compounds which have beneficial effects on wound healing and anti-inflammatory damages (Sinha et al., 2001; Kour et al., 2021).

The fact that neutrophils were not markedly elevated may also be an indication of rapid resolution of the inflammatory phase as the presence of neutrophils for extended periods is typically correlated with delayed healing and tissue damage. Likewise, when there was no difference in eosinophil counts, it indicated that there were no hypersensitivity or allergic responses caused by the hydrogel matrix. The treatment was not found to have a negative effect on erythrocyte physiology or integrity, as evidenced in the stability of hemoglobin. Similar results have been documented in studies showing that *Bergenia ciliata* formulations promoted wound healing through enhanced re-epithelialization, collagen formation, angiogenesis, and reduced inflammatory cytokines like TNF- α and IL-6 (Kour et al., 2021; Riaz et al., 2025).

On the other hand, difference in lymphocytes and platelets could be associated with better wound repair mechanisms. During the later stages of healing, lymphocytes have an important role in immune regulation, activation of fibroblasts, collagen synthesis, and tissue remodeling. Platelets are also of importance due to their role in initiating hemostasis and secreting growth factors that are important for angiogenesis and granulation

tissue formation. Thus, any changes in these parameters in treated groups could be attributed to the activation of physiological repair processes linked to faster healing of wounds. The hydrogel system could also have played a role, due to its ability to create a moist environment and promote continuous delivery of bioactive phytochemicals to the wound. The overall results indicate that the use of hydrogels loaded with *Bergenia ciliata* significantly accelerates wound healing via localized anti-inflammatory, anti-oxidant and tissue regeneration effects, without inducing any adverse systemic hematological changes (Ahmad et al., 2018; Kour et al., 2021).

REFERENCES

- Ahmad, M., Butt, M. A., Zhang, G., Sultana, S., Tariq, A., & Zafar, M. (2018). *Bergenia ciliata*: a comprehensive review of its traditional uses, phytochemistry, pharmacology and safety. *Biomedicine & Pharmacotherapy*, 97, 708-721.
- Ara, C., Jabeen, S., Afshan, G., Farooq, A., Akram, M. S., Islam, A., ... & Khan, R. U. (2022). Angiogenic potential and wound healing efficacy of chitosan derived hydrogels at varied concentrations of APTES in chick and mouse models. *International Journal of Biological Macromolecules*, 202, 177-190.
- Desconsi, D., Zanetti, A. A. P. M., Miranda, R. M. L. D., Araujo, J. P., Lopes, C. T., Herdman, T. H., & Serafim, C. T. R. (2026). Concept, Content, and Clinical Validation of the Nursing Diagnosis Impaired Skin Integrity (00046): An Integrative Review. *International Journal of Nursing Knowledge*, 20473087261443260.
- Faiz, S., Waseem, D., Haq, I. U., Taqi, M. M., Mohsin, S. A., Irshad, N., ... & Fatima, H. (2023). Comparative appraisal of in vitro biological profile and in vivo wound healing attributes of bergenin and *Bergenia ciliata* (Haw.) Sternb. *Journal of Ethnopharmacology*, 304, 115993.

- Gounden, V., & Singh, M. (2024). Hydrogels and wound healing: current and future prospects. *Gels*, 10(1), 43.
- Groult, S., Buwalda, S., & Budtova, T. (2021). Pectin hydrogels, aerogels, cryogels and xerogels: Influence of drying on structural and release properties. *European Polymer Journal*, 149, 110386.
- Jeong, H., Lee, D. Y., Yang, D. H., & Song, Y. S. (2022). Mechanical and cell-adhesive properties of gelatin/polyvinyl alcohol hydrogels and their application in wound dressing. *Macromolecular Research*, 30(4), 223-229.
- Kamoun, E. A., Loutfy, S. A., Hussein, Y., & Kenawy, E. R. S. (2021). Recent advances in PVA-polysaccharide based hydrogels and electrospun nanofibers in biomedical applications: A review. *International Journal of Biological Macromolecules*, 187, 755-768.
- Kangal, M. K. O., & Kopitnik, N. L. (2025). Physiology, wound healing. In *StatPearls [Internet]*. StatPearls Publishing.
- Kim, J., & Lee, C. M. (2017). Wound healing potential of a polyvinyl alcohol-blended pectin hydrogel containing Hippophae rhamnoides L. extract in a rat model. *International journal of biological macromolecules*, 99, 586-593.
- Kour, H., Raina, R., Verma, P. K., Khan, A. M., Bhat, M. A., & Nashiruddullah, N. (2021). Evaluation of the wound healing activity of ethanolic extract of *Bergenia ciliata* (Haw.) Sternb. rhizome with excision wound model in Wistar rats. *Journal of Ethnopharmacology*, 281, 114527.
- Liang, Y., He, J., & Guo, B. (2021). Functional hydrogels as wound dressing to enhance wound healing. *ACS nano*, 15(8), 12687-12722.
- McGrath, J. A., & Uitto, J. (2023). Structure and Function of the Skin. *Rook's textbook of dermatology*, 1-50.
- Miranda, J. J. M. (2021). Medicinal plants and their traditional uses in different locations. In *Phytomedicine* (pp. 207-223). Academic Press.
- Mohamed, S. A., & Hargest, R. (2022). Surgical anatomy of the skin. *Surgery (Oxford)*, 40(1), 1-7.
- Morello, G., De Iaco, G., Gigli, G., Polini, A., & Gervaso, F. (2023). Chitosan and pectin hydrogels for tissue engineering and in vitro modeling. *Gels*, 9(2), 132.
- Omorphos, N. P., Gao, C., Tan, S. S., & Sangha, M. S. (2021). Understanding angiogenesis and the role of angiogenic growth factors in the vascularisation of engineered tissues. *Molecular biology reports*, 48, 941-950.
- Riaz, A., Ali, S., & Summer, M. (2025). Evaluation of Burn Wound Healing and Anti-inflammatory Potential of Semi-synthetic *Bergenia ciliata*-loaded Carboxymethyl Cellulose Hydrogel: A Biochemical Perspective. *Chemistry & Biodiversity*, 22(12), e00925.
- Riaz, S., Waheed, H., Ahmad, F., Khan, M. I., & Shanableh, A. (2025). Natural and synthetic biomaterials, structural matrices-based wound dressings: Key properties, material correlation, and adaptability. *Regeneration Repair Rehabilitation*, 1(4), 47-65.
- Sedighi, M., Namdari, M., & Mahmoudi, P. (2023). An overview of angiogenesis and chemical and physiological angiogenic factors: short review. *Journal of Chemical Health Risks*.
- Shah, S. S. S. S., Dawood, S., Ibrahim, K., Muhammad, I., & Sohail, A. J. (2020). *Bergenia ciliata* as antibacterial agent. *GSC Biological and Pharmaceutical Sciences*, 12(2), 037-045.
- Sinha, S., Murugesan, T., Maiti, K., Gayen, J. R., Pal, M., & Saha, B. P. (2001). Evaluation of anti-inflammatory potential of *Bergenia ciliata* Sternb. rhizome extract in rats. *Journal of pharmacy and pharmacology*, 53(2), 193.
- Xiang, L., & Cui, W. (2021). Biomedical application of photo-crosslinked gelatin hydrogels. *Journal of Leather Science and Engineering*, 3, 1-24.

Yousef, H., Alhajj, M., & Sharma, S. (2022). Anatomy, skin (integument), epidermis. StatPearls. *Preprint*. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK470464/> (Accessed: 21 June 2023), 29262154.

