

## Pharmacological Investigation of Shatadhauta Ghrita for the Management of Diabetic Wound Healing

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**ABSTRACT:** Plant and plant products have been utilized with varying success to cure and prevent diseases throughout history. Crude extract mixtures of plant are better than pure isolated chemicals. Several biologically active compounds in a plant work together to produce greater effect than single chemical on its own. Wound healing, as a normal biological process in the human body, it is the process of repair that follows injury to the skin and other soft tissues. Wounds that exhibit impaired healing, including delayed acute wounds and chronic wounds, generally have failed to progress through the normal stages of healing. Such wounds frequently enter a state of pathologic inflammation due to a postponed, incomplete, or uncoordinated healing process. Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease, or pressure.

**Keywords:** Diabetic wounds, herbomineral emulgel, excision model, incision model.

### I. INTRODUCTION:

Wound may be defined as breaking or loss of anatomic and cellular or functional connection of living tissue (Kased et al., 2017). It is an unavoidable part of the human life which is caused by chemical, physical, thermal, immunological or microbial damages to the tissue [1]. Wound healing is natural process, obtained through four highly programmed stages: hemostasis, inflammation, proliferation and remodeling. For a wound to be healed effectively, all of these stages must occur in the appropriate sequence and time frame. Healing involves platelets aggregation, blood clotting, formation of fibrin, a response of inflammation to injury, alteration in the ground substances, angiogenesis and re-epithelialization [2]. The healing process of wound gets worse and time for healing increases in burn injuries due to the obstruction of vascular bed. Due to this obstruction, which hold back the transportation of systemically administered antibiotics; the topical delivery becomes an important component of the burn therapy. Overall the goal of burn therapies is to

promote the healing process by preventing the infection effectively [3].

Topical drug delivery refers to the application of a drug-containing formulation to the skin to treat a cutaneous condition. This system is used when other routes of drug administration (such as oral, sublingual, rectal, and parental) fail, or when a local skin infection, such as a fungal infection, occurs [4]. Topical drug administration is a common treatment method for both local and systemic conditions. In the topical delivery system, the drug is absorbed by the skin and reaches the site of action to provide a therapeutic effect. The rate of drug release from a topical preparation is dependent directly on the physiological features of the carrier. The primary benefit of a topical delivery system is that it avoids the first-pass metabolism [5]. The term microemulsion is based on particle size. Due to their smaller size, the drug particles can easily diffuse through the skin and reach their site of action. The gel will hold the microemulsion for a long time and will aid in the sustained release of the drug. Various fungal infections are growing nowadays which are a major problem for society. Fungal infections such as *Tinea capitis*, *Tinea pedis* and *Tinea corporis* infect the skin severely. A technique such as emulgel can aid in the easy penetration of the drug into the skin and provide a rapid onset of action [6].

Gellified emulsions (either of oil-in-water or water in-oil type) is called emulgel and formed by the addition of a gelling agent. In emulsion the entrapped drugs slowly release from internal phase through external phase and slowly get absorbed through the skin [7]. Thus, emulsion itself is controlled release systems. The same case is for the gel that forms cross linked network in which small particle of drugs are captured. Its release occurs in a controlled manner. Since, emulgel possesses the property of equally emulsions and gel; it acts as dual control release system. Gel is incorporated into emulsion to improve its penetration ability and stability [8]. Further, gel for external use has many good characteristics such as being thixotropic, easily removable, spread easily, greaseless,

soothing, and compatible with numerous excipients, non-staining and miscible or solubilized in water. Emulgels have good stability than other available topical preparation. For example creams have the problem of phase inversion and breaking; hygroscopic nature shown by powders and rancidity is the problem in ointments [9].

## II. MATERIAL AND METHODS:

**Preparation of emulgel containing Shata-Dhauta-Ghrita:** Shata-Dhauta-Ghrita: honey: aloe vera juice: aqueous ochre solution were taken in the ratio of 2:1.5:1.5:1 (S:H: A:O).

### Preparation of the Emulsion

Preparation of the Oil Phase

The prepared Shata Dhauta Ghrita (10–15% w/w) was mixed with Span 80 (Lipophilic surfactant) in a beaker and warmed gently at 40°C.

Preparation of the Gel Base (Aqueous Phase)

Carbopol 940 (1–1.5% w/w) was dispersed in distilled water under continuous stirring to prevent lump formation. Aloe vera juice, honey, and ochre (as a powder) were added to this mixture. Finally, Tween 20 (Hydrophilic surfactant) was added to this aqueous mixture. And the Carbopol was allowed to hydrate completely (usually 1–2 hours).

Formation of Emulgel (Merging Phase)

Both the phases were separately heated to 70°C - 80°C and the Oil Phase (SDG mix) was

added into the aqueous Phase (Gel) while stirring continuously at a moderate speed (approx. 1200 rpm). Carbopol 940 was neutralized by adding a few drops of triethanolamine (TEA) until the desired pH (around 5.5–6.0) was reached, which caused the gel to thicken and turn into a gel-like consistency. Mixing was continued until a homogeneous emulgel was formed. [9].

### Pharmacological screening:

#### Induction of diabetes mellitus (DM)

Rats underwent a fasting period overnight prior to receiving an intraperitoneal injection of streptozotocin (STZ) at a dosage of 60 mg/kg of body weight, prepared in 0.1 M citrate buffer with a pH of 4.5. After a duration of seventy-two hours, blood samples were obtained, and glucose concentrations were measured to verify the onset of diabetes. Only the animals exhibiting hyperglycemia, defined as a blood glucose level exceeding 200 mg/dl, were included in the experiment. [10]

#### Wound Model

Sixty adult male Wistar rats (150–200 gm) were used in this study. Rats were housed separately in a temperature-controlled (22–24 °C) room on a 12 h light/dark cycle, and food and water were provided ad libitum. Type I diabetes mellitus was induced in 60 animals used for this study, then non-diabetic and diabetic animals were further divided into 10 treatment subgroups (Table 1.).

**Table 1: Animal study**

| S. No. | Groups                       | Treatment   |
|--------|------------------------------|---|
| 1      | Non-Diabetic Control Group I | Vehicle treatment   |
| 2      | Diabetic Control Group II    | Vehicle treatment   |
| 3      | Standard Group III           | Silver Sulfadiazine (1 %)   |
| 4      | Test Group IV                | Test formulation emulgel (Combination of shata-dhauta-ghrita, honey, aloe vera and ochre) |
| 5      | Test Group V                 | shata-dhauta-ghrita   |

**Excision wound model:** The rats underwent excision wounds while under anaesthesia. A circular wound measuring approximately 500 sq. mm. was created on the depilated and sterilized dorsal thoracic area of the rats. The wounds were categorized into five groups, each consisting of six

rats. The animals in groups I and II were not treated (Normal control and diabetic control). Group III consisted of animals that served as a reference standard, which were treated with a standard drug. The animals in group IV received treatment with a formulated emulgel, while those in group V were

treated with a formulated SDG, applied topically once daily, starting from the day of the operation until complete epithelialization occurred. The animals were housed individually. Wound measurements were recorded on mm<sup>2</sup> graph paper every two days following the wounding. The percentage of wound closure (% contraction) and the duration of epithelialization (the number of days required for the dead tissue remnants of the wound to fall off without leaving any residual raw wound) were calculated[11].

**Incision wound model:** A 6 cm incision was made through the skin and cutaneous muscles on the depilated back of the rat using a scalpel blade. Subsequently, the incision was closed with interrupted sutures, with stitches placed 1 cm apart, utilizing black surgical thread (no. 000) and a curved needle (no. 11). The continuous threads on both wound edges were tightened for good closure of the wound. After stitching, the animals in group IV received treatment with a formulated emulgel, while those in group V were treated with a formulated SDG daily up to 10 days. The sutures were removed on the 8th day post-wounding, and the breaking strength of the wound, which was 10 days old, was assessed using a tensiometer[12].

#### Wound healing evaluation parameters

**Measurement of wound contraction:** An excision wound margin was traced by following the progressive changes in wound area planimetrically, excluding the day of wounding. The size of wounds was traced on a transparent paper after every 2 days, throughout the monitoring period. The tracing was then shifted to graph paper, from which the wound surface area was evaluated. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking initial size of wound, 500 mm<sup>2</sup>, as 100%, by using the following formula as

% wound contraction = [(initial wound size – specific day wound size)/ initial wound size] × 100

**Epithelialization period:** It was evaluated by noting the number of days required for the Escher to fall off from the wound surface exclusive of leaving a raw wound behind.

**Measurement of tensile strength:** The force required to open the healing action is known as tensile strength. It is used to measure the completeness of healing. It also indicates how

much the repaired tissue resists to breaking under tension and may indicate in part the quality of repaired tissue. The sutures were removed on the 9th day after wounding and the tensile strength was measured on 10th day. For this purpose, the newly formed tissue including scar was excised and tensile strength was measured with the help of tensiometer. In this method, wound-breaking strength was measured as the weight of water at the time of wound breaking per area of the specimen [13].

**Hydroxyproline estimation:** Hydroxyproline is an uncommon amino acid present in the collagen fibres of granulation tissues. Its estimation helps clinically to understand progress rate at which the healing process is going on in the connective tissue of the wound. For the determination of hydroxyproline content, the wound tissues were excised and dried in a hot air oven at 60–70 °C to constant weight and were hydrolysed in 6N HCl at 130 °C for 4 h in sealed glass tubes. The hydrolysate was neutralized to pH 7.0 and was subjected to Chloramine-T oxidation for 20 min. The reaction was terminated by addition of 0.4 Mperchloric acid and color was developed with the help of Ehrlich reagent at 60 °C. The absorbance was measured at 557 nm using a uvspectrophotometer (Shimadzu, Japan). The amount of hydroxyproline in the samples was calculated using a standard curve prepared with pure l-hydroxyproline.

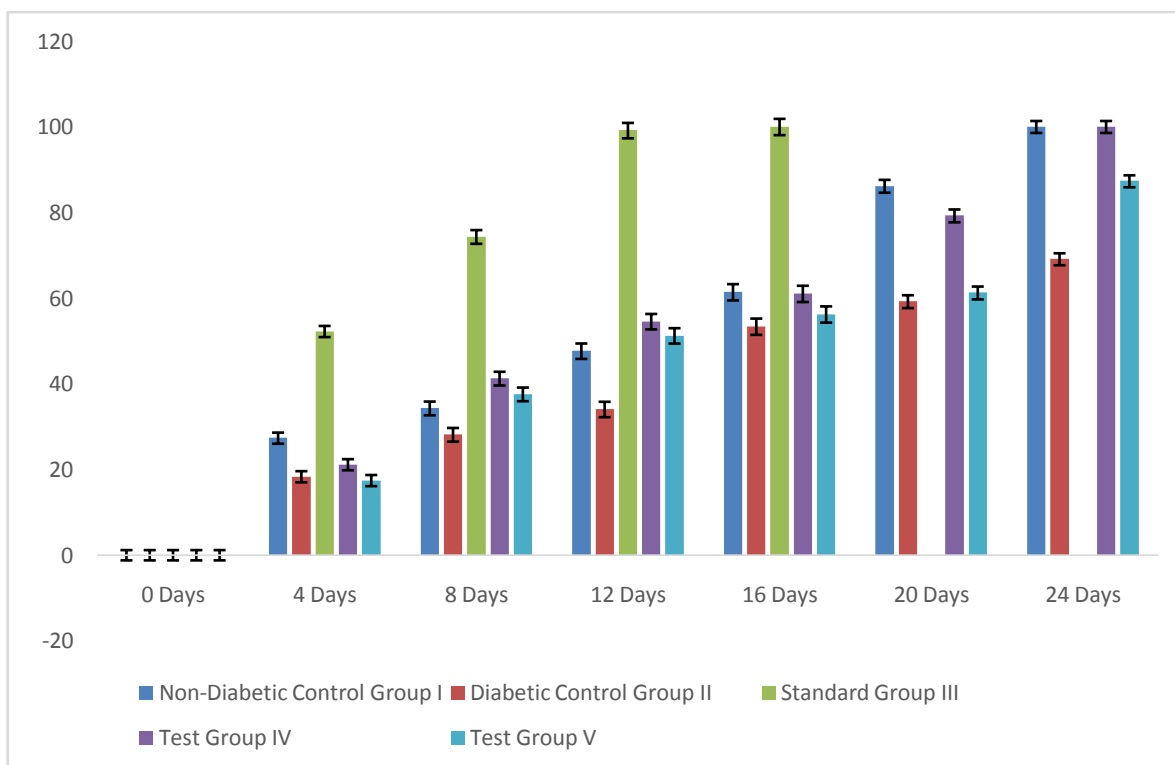
### III. RESULT AND DISCUSSION

#### Excision diabetic wound models

**Wound contraction:** Wound contraction plot shows the percentage of the wound that has closed over 20 days. It was noticed that the Diabetic Control lags significantly behind the others, while the SDG Formulation and Standard Drug showed much steeper recovery curves. On day 1, all the groups treated with STZ (diabetic groups) exhibited significantly increased wound size compared to control animals (P < 0.05). There was decrease in wound size in the control group (non-diabetic) which but it did not show complete healing. The wound size gradually decreased in diabetic animals treated with topical emulgel formulation (Group V) (P < 0.01) and topical SDG (Group IV) (P < 0.01) and both these formulations showed nearly complete wound closure.

**Table 2: Effect of herbomineral gel formulation on % of wound contraction of excision diabetic wound models in rats**

| Group                        | 0 Days | 4 Days    | 8 Days    | 12 Days   | 16 Days   | 20 Days   |
|------------------------------|--------|-----------|-----------|-----------|-----------|-----------|
| Non-Diabetic Control Group I | 0      | 27.34±4.1 | 34.28±3.6 | 61.64±2.2 | 83.40±2.1 | 99.10±0.5 |
| Diabetic Control Group II    | 0      | 18.33±3.1 | 28.12±2.1 | 34.03±2.5 | 53.36±3.3 | 59.21±2.9 |
| Standard Group III           | 0      | 52.24±2.7 | 74.32±2.0 | 87.15±1.8 | 95.90±1.5 | 98.19±1.0 |
| Test Group IV                | 0      | 21.14±0.8 | 41.24±2.6 | 74.54±3.2 | 81.02±25  | 98.24±0.9 |
| Test Group V                 | 0      | 17.43±1.1 | 37.56±2.0 | 59.22±2.9 | 86.21±1.8 | 91.24±0.4 |



**Figure 1: Effect of herbomineral gel formulation on % of wound contraction of excision diabetic wound models in rats (Values are expressed as Mean ± SEM (n=6) \*p<0.05 versus negative control. (One-way ANOVA, followed by Dunnett's post-hoc test for multiple comparisons)**

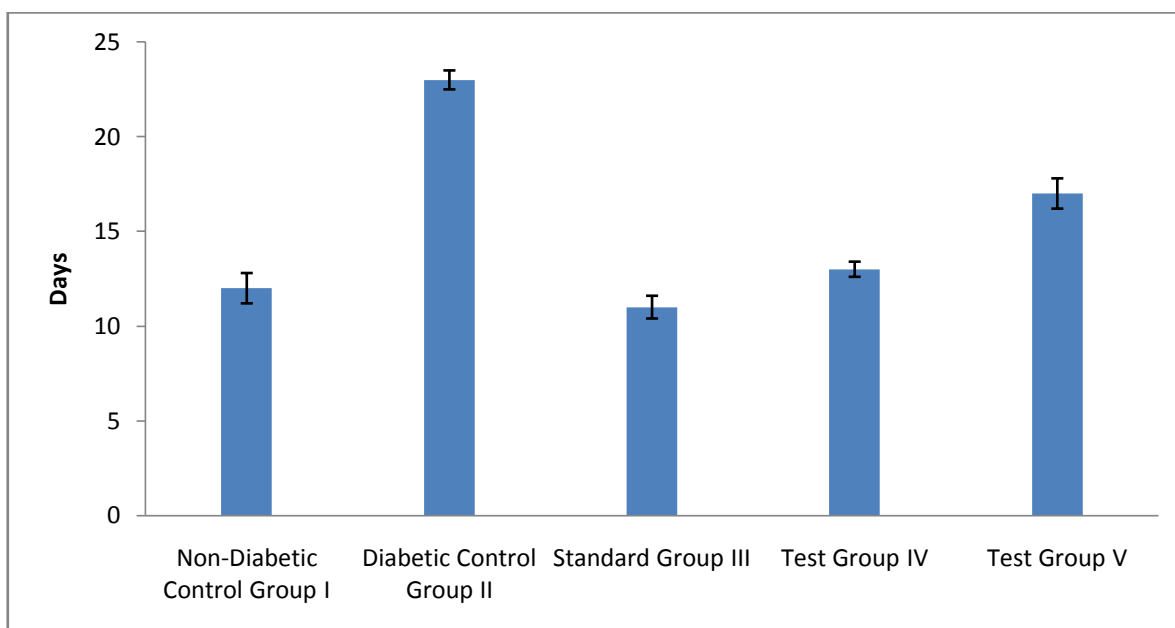
**Epithelialization period:** A bar chart representing the mean number of days required for complete scab shedding defines the epithelialization period. Shorter bars indicate faster healing. The

epithelialization time was measured from the first day. The epithelialization time was found to be significantly (P < 0.01) reduced in Groups IV

containing formulation upto 15 days as compare with standard Group III.

**Table 3: Effect of herbomineral gel on Epithelialization time of excision diabetic wound models in rats**

| Group                        | Epithelialization time (Days) |
|------------------------------|-------------------------------|
| Non-Diabetic Control Group I | 12.1±1.0                      |
| Diabetic Control Group II    | 23.8±1.4                      |
| Standard Group III           | 14.2±0.8                      |
| Test Group IV                | 15.1±0.9                      |
| Test Group V                 | 13.2±0.7                      |



**Figure 2: Effect of herbomineral gel formulation on Epithelialization time of excision diabetic wound models in rats. Values are expressed as Mean ± SEM (n=6) (One-way ANOVA, followed by Dunnett's post-hoc test for multiple comparisons)**

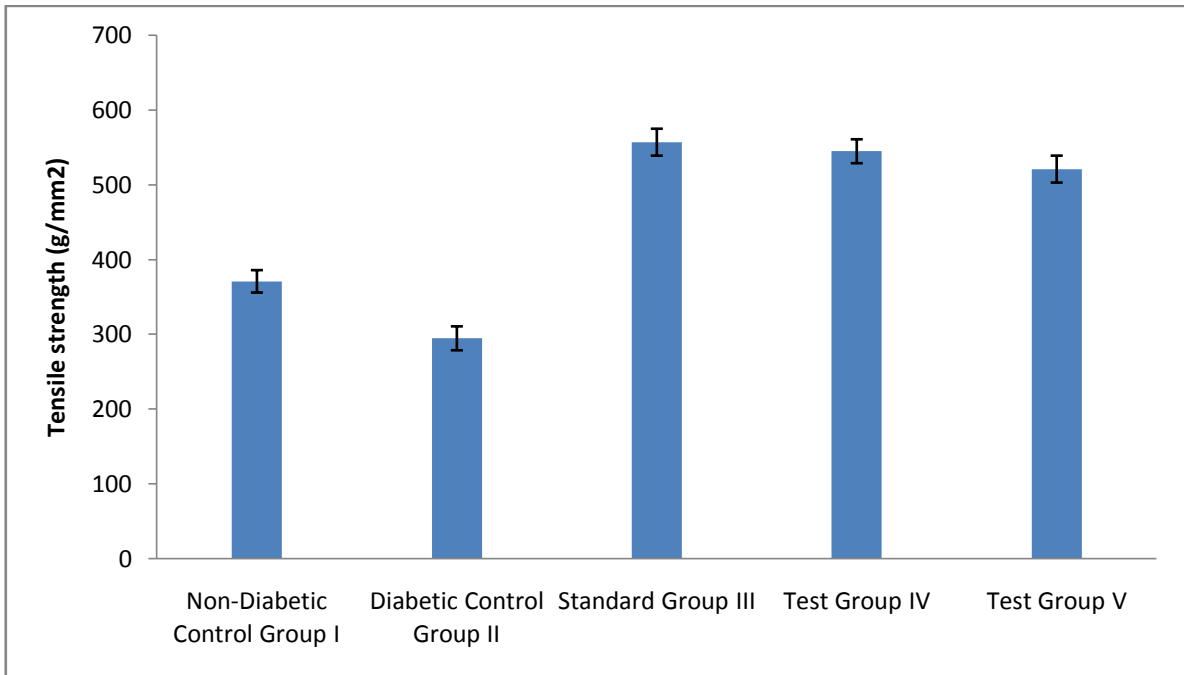
**Incision diabetic wound model:** In evaluating an incision wound model which is primarily used to study the mechanical properties and surgical repair of skin, the following parameters were typically measured:

**Primary Physical Parameter- Tensile (Breaking) Strength:** This is the most crucial parameter for incision models. It measured the minimum force

required to disrupt or pull apart the healed wound, typically on the 10th post-wounding day. Higher tensile strength indicated better collagen maturation and cross-linking. The animals treated with PHF exhibited significant tensile strength (545.21 ± 3.17g) on the post wounding day 10 compared to the reference standard treated group (557.31 ± 3.01g).

**Table 4: Effect of herbomineral gel fromulation on Tensile strength on incision diabetic wound models in rats**

| Group                        | Tensile strength (g/mm2) |
|------------------------------|--------------------------|
| Non-Diabetic Control Group I | 371.21 ± 4.21            |
| Diabetic Control Group II    | 295.04 ± 3.01            |
| Standard Group III           | 557.31 ± 3.01            |
| Test Group IV                | 545.21 ± 3.17            |
| Test Group V                 | 521.32 ± 2.31            |



**Figure 3: Effect of herbomineral gel formulation on Tensile strength on incision diabetic wound models in rats. Values are expressed as Mean ± SEM (n=6) \*\*\*p<0.001 versus negative control, (One-way ANOVA, followed by Dunnett's post-hoc test)**

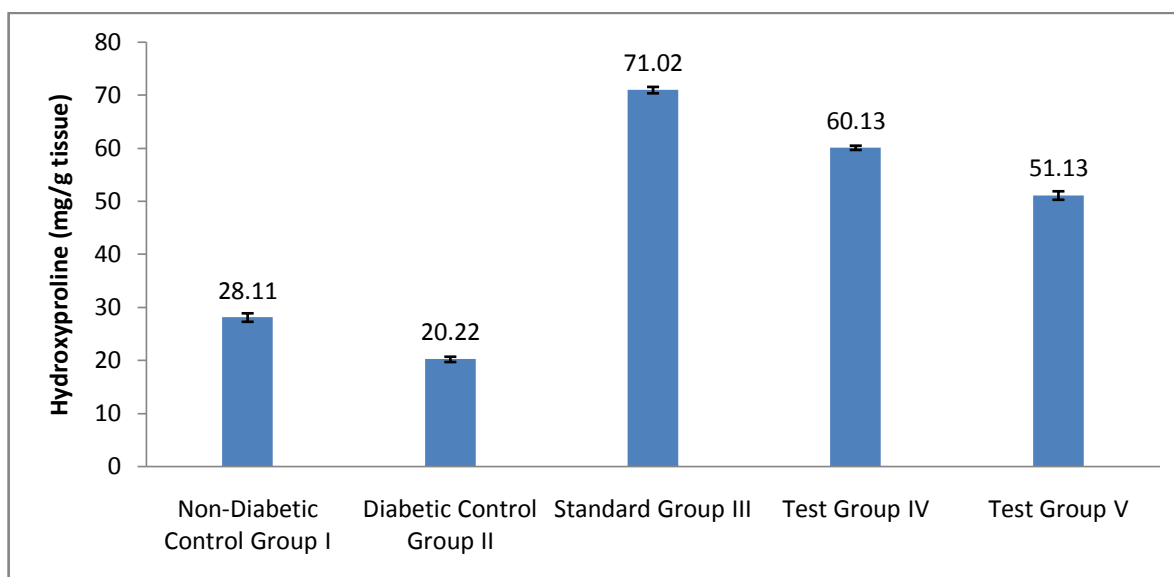
**Biochemical Marker - Hydroxyproline Levels**

A biochemical indicator of collagen content in the tissue. Increased levels typically correlate with higher tensile strength and faster healing. In an incision wound model, hydroxyproline levels serve as a critical biochemical benchmark because this amino acid is almost exclusively found in collagen (comprising roughly 13.5% of mammalian collagen)

The treated group exhibited a notable increase in hydroxyproline content compared to the control group (P < 0.01). The hydroxyproline content in the incision model, as estimated from the results of Group IV, was recorded at 60.13 (mg/g tissue), which was the highest result among other groups and significantly different from the treated group.

**Table 5: Effect of herbomineral gel formulation on Hydroxyproline on incision diabetic wound models in rats**

| Group                        | Hydroxyproline (mg/g tissue) |
|------------------------------|------------------------------|
| Non-Diabetic Control Group I | 28.11 ± 0.001                |
| Diabetic Control Group II    | 20.22 ± 0.002                |
| Standard Group III           | 71.02 ± 0.002                |
| Test Group IV                | 60.13 ± 0.001                |
| Test Group V                 | 51.13 ± 0.002                |



**Figure 4: Effect of herbomineral gel formulation on Hydroxyproline on incision diabetic wound models in rats (Values are expressed as Mean  $\pm$  SEM (n=6) \*\*\*p<0.001 versus negative control, (One-way ANOVA, followed by Dunnett's post-hoc test)**

#### IV. SUMMARY AND CONCLUSION

Drug delivery systems (DDS) are designed to efficiently provide pharmaceuticals throughout the body, considering patient needs and desired therapeutic results. Topical medicine distribution is a prevalent method that use formulations such as emulgels to provide pharmaceuticals locally via the skin. Emulgels are particularly advantageous for the delivery of hydrophobic pharmaceuticals, offering enhancements in stability, drug loading capacity, and dermal penetration. Emulgels combine the characteristics of emulsions and gels. The efficacy of topical medicine absorption is affected by physicochemical characteristics such as molecular weight and physiological variables like skin thickness and blood circulation. Essential components such as carriers, emulsifiers, gelling agents, and penetration enhancers are used in the formulation of emulgels. To improve emulgel formulations for effective drug administration, assessment methods including pH measurement, globule size analysis, and rapid stability testing are essential. In this study groups IV and V showed healing rates similar to Group I (Normal Control), which suggests the formulation effectively overcomes the biochemical hurdles of diabetic pathology (e.g., oxidative stress or poor vascularization). The Test Formulation (Group IV) performed significantly better than the Diabetic Control (Group II), showing healing rates comparable to the Standard Drug. The Diabetic Control group failed to reach 90% contraction even

by Day 20, highlighting the impairment caused by the diabetic state. Both the Emulgel and SDG formulations successfully "rescued" the healing process in diabetic animals.

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