



## Comparative evaluation of wound healing activity of tilvadi ghrita and durva ghrita on diabetic wound model in rats

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### ABSTRACT



Previous studies proved the wound healing potential of Tilvadi Ghrita (TG) and Durva Ghrita (DG) on normal wounds, so this study was conducted to investigate wound healing activity of TG and DG on diabetic wound model in rats. Forty-eight adult female Wistar rats in eight groups were used. Alloxan was used to induce diabetes in diabetic groups. Excision wound was made on the dorsal shaved surface of rats, and then Framycetin sulfate cream (FSC) 10%, TG and DG were applied in control and treatment groups. While in normal control and diabetic control groups, no medication was done. Wound photographs were taken on alternative days to evaluate wound healing. Percentage wound contraction, the effect on wound size, and epithelization period parameters were studied. Ghrilas were investigated for their preliminary phytochemicals, Physico-chemical properties, skin irritation test, and wound healing activity in normal and diabetic rats. Data were expressed as mean  $\pm$  SEM and analyzed using Two-way ANOVA followed by Bonferroni's multiple range test. Studies revealed that TG and DG do not cause any harmful skin reactions, so both ghrilas were found to be safe for topical application. Both formulations showed better and fast healing as compared to untreated normal and diabetic control groups. After comparative investigation, it was observed that DG possesses higher wound healing potential in normal and diabetic groups with comparison to TG. Wound healing action in normal and diabetic wounds, it endowed due to the presence of different phytoconstituents reported in the literature and proved to be beneficial in the management of wounds.

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### INTRODUCTION

Wounds are the physical injuries to any part of the body that typically involves a break in the continuity of skin and alteration in the normal skin functions along with changes in the skin anatomy. It results in the loss of continuity of epithelium with or without the loss of underlying connective tissue (Deep and Garg, 2015). Wound healing is a complex mechanism that restores normal functions and the structure of damaged tissues. It is a natural process through which body repairs itself damaged tissues without any treatment, but the rate of wound heal-

ing is extremely slow, and there are high chances of microbial infection. A good wound healing agent decreases the demand for other drugs like antibiotics, so there is a reduction in probable side effects of such drugs by their use. Synthetic drug formulations which are available in the market for wound healing is either bacteriostatic or bactericidal in such cases, healing of wound with these formulations are by a natural phenomenon only (Sable and Bhimani, 2012). Wound healing is a normal biological process that includes a series of different overlapping phases that takes place in a sequential manner like inflammatory, proliferative, and maturation phase. Healing of wounds involves the restoration of the integrity of injured tissues (Sandhya and Kumar, 2011). According to the Ayurveda, the wound is the condition that causes discontinuation of the lining membranes of tissue, leaving a scar after healing. The most important objective of wound management is, healing in the shortest time with less pain, minimum discomfort, and scar (Patel and Kurbetti, 2013).

Diabetes mellitus is a chronic disease that affects the normal wound healing process. Diabetic patients suffer from diabetic foot ulcers. Due to the delay in wound healing, there will be a high risk of amputation. Most commonly, the diabetic patient suffers from neuropathy, which could be the reason behind non-healing wounds. Delay in wound healing in diabetic patients is because of the decrease in the ability to fight infection and unable to show adequate inflammatory response (Harold and Marjanaa, 2007). Uncontrolled diabetes mellitus may cause many complications such as diabetic retinopathy, kidney failure, heart attacks, neuropathy, and lower limb amputation, but the most serious complication is diabetic wound. Further, such diabetic non-healing wound leads to loss of body parts. So, all healthcare professionals must focus on the therapeutic wound management of diabetic patients (Nimasajai, 2018). Recently people prefer herbal medicines over synthetic medications for the treatment of different diseases because of their safety, efficacy, and economy. Many Ayurvedic plants showed an effective role in the management and treatment of wounds. Such plants are potent wound healers because they promote wound healing by the natural healing process (Nehete and Nipanikar, 2016). Ayurveda is a traditional Indian system of medicine, in which so many formulations are available for the treatment of wounds without any complications. Recently researcher's attention is directed towards the discovery of a better wound healing agent, which will promote wound healing in a short time with fewer side effects. So there

is extensive scope for research in Ayurveda, which will be beneficial to society (Ghodela and Dudhamal, 2017). Some Ayurvedic formulations possess immense potential for managing and treating the wounds. These formulations are not only cheap but are also safe and affordable. Tilvadi Ghrita (TG) and Durva Ghrita (DG) both Ayurvedic formulations which have traditional history and chemical constituents present in both Ghrithas showed different activities like, antimicrobial, antioxidant, anti-inflammatory and antiulcer (Sultana and Haque, 2010; Nirmala and Selvaraj, 2011; Patil and Patil, 2005; Garg and Khosa, 2008). Natural medicines were used since ancient times, but they do not have scientific data. So, the presence of a wide range of constituents showing anti-microbial, anti-inflammatory, antioxidant, and antiulcer activities in these formulations, including wound healing properties, has prompted us to explore wound healing potential of TG and DG in the diabetic wound model.

## MATERIALS AND METHODS

### Materials

Durva Ghrita was used as test formulation in the present investigation, which composed of Cow's ghee (10%) and whole plant extract of *Cynodon dactylon* (40%). Durva Ghrita was purchased from Vatsal Ayurvedic products Pvt. Ltd; Nashik, Maharashtra-India.

Tilvadi Ghrita was another test formulation; it composed of *Glycyrrhiza glabra* Linn. (25%), *Sesamum indicum* Linn. (25%) And Cow's ghee (50%). This formulation was received as a gift sample from Vedic Ayurveda and Panchakarma Kendra, Baner- Pune, Maharashtra-India.

Ketamine injection (50mg/ml), (Ketajex) was purchased from the local chemist shop-Pune, India. (Baxter Pharmaceuticals, India Pvt. Ltd; Ahmadabad). Alloxan monohydrate was purchased from Explicit Chemicals Pvt. Ltd; Kurkumbh -Pune, Maharashtra-India.

Framycetin sulfate cream (FSC) (10%) (Soframycin®) was purchased from the local chemist shop -Pune (Sanofi India Ltd; Goa).

### Experimental Animals

A normal, healthy adult and nulliparous female Wistar rats weighing from 150-250g were purchased from LACSMI Biofarms Pvt. Ltd; Alephata -Pune (CPCSEA NO- 1277). Animals were selected as per (OECD, 2015). Animals were housed under-maintained standard laboratory conditions (12 hrs light-dark cycle),  $25 \pm 03^\circ\text{C}$  Temperature,  $46 \pm 06$

% relative Humidity. All animals were fed on a standard pellet diet and water *ad libitum* throughout the experiments. Animals were acclimatized to laboratory conditions (for 2 weeks) before experiments were carried out.

### Ethical considerations

The protocol of the study was approved by the Institutional Animal Ethics Committee (IAEC), regulation approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals. (CPCSEA) (Ayush, 2008) And the authors of this manuscript observed ethical issues. Animals were handled according to the guidelines for the care and handling of experimental animals.

### Methods

#### Preliminary phytochemical analysis of the Tilvadi Ghrita and Durva Ghrita

Tilvadi Ghrita and Durva Ghrita were subjected to qualitative chemical analysis for the presence of various phytoconstituents by using standard phytochemical tests (Kokate and Gokhale, 2014; Khandelwal, 2001).

#### Physico-Chemical Evaluation of both Ghrita Formulations

Physico-Chemical Evaluation of both Ghrita Formulations was done as per the methods described in Protocol for testing of Ayurvedic, Siddha and Unani medicines, (Ayush, 2008) for the parameters like colour, odour, taste, saponification value, iodine value, acid value and refractive index (Shaila and Santosh, 2004).

#### Skin Irritation Test

Skin Irritation of the formulation was evaluated as per the (OECD, 2015).

#### Effect of Tilvadi Ghrita and Durva Ghrita on Wound healing in Alloxan induced Diabetic rat model

Wound healing is majorly affected by the presence of Diabetes Mellitus; so, this model is also known as a chronic wound model. Animals were made diabetic by giving a single injection of hypoglycemic drug Alloxan (150 mg/kg) by intraperitoneally after overnight fasting of animals. 5% w/v glucose solution was given to animals to avoid sudden hypoglycemia after the injection of Alloxan. Alloxan solution was prepared by dissolving drugs into a normal saline solution. Doses were calculated and selected according to the weights of individual animals. All the animals of diabetic treatment groups were weighted carefully, and their fasting glucose levels were determined before inducing diabetes. For the measurements of blood glucose level, blood

was withdrawn from the tail vein of rats and measured by the digital Glucometer (Accu-check). Then a single dose of Alloxan was given by the intraperitoneal route. Alloxan was given to diabetic groups only (Nehete and Nipanikar, 2016; Lodhi and Vadnere, 2017).

After 48 hours, animals showing the blood glucose level of more than 150 mg/dL were considered as diabetic and selected for further wound healing activity in diabetic rats. A normal blood glucose level of rats is 70-110 mg/dL (Thakur and Dhamija, 2017).

#### Excision wound model

The Excision wound model was used to study the rate of contraction of the wound, percentage wound contraction, and period of epithelization. This type of wound model is generally used for the study of scar area (Sathish *et al.*, 2013). Before starting of wound healing experiments, animals were weighed individually. Then the dorsal skin of the rats was shaved carefully without causing any abrasions. After 24 hours of shaving, animals were anesthetized by Ketamine injection intraperitoneally, and the shaved area was disinfected by using 70% Ethanol solution. Before wound making, all the surgical instruments were sterilized by autoclaving. Then open circular wound of full-thickness (500 mm<sup>2</sup> area) was made on a predetermined area under aseptic conditions with the help of sterile surgical blades and pointed scissors. Excision wounds were created on the dorsal thoracic region 1.5 cm away from vertebral Column, and 5 cm away from the ears of an anesthetized rat Figure 1. Haemostasis was achieved by blotting the wound area with a cotton swab soaked in normal saline solution. Wounds were kept undressed in the open environment, and each animal was kept separately in the individual cages. Animals were grouped into different groups, according to Table 1, (Deepti *et al.*, 2012; Sharma *et al.*, 2009; Bharathi and Veni, 2010).

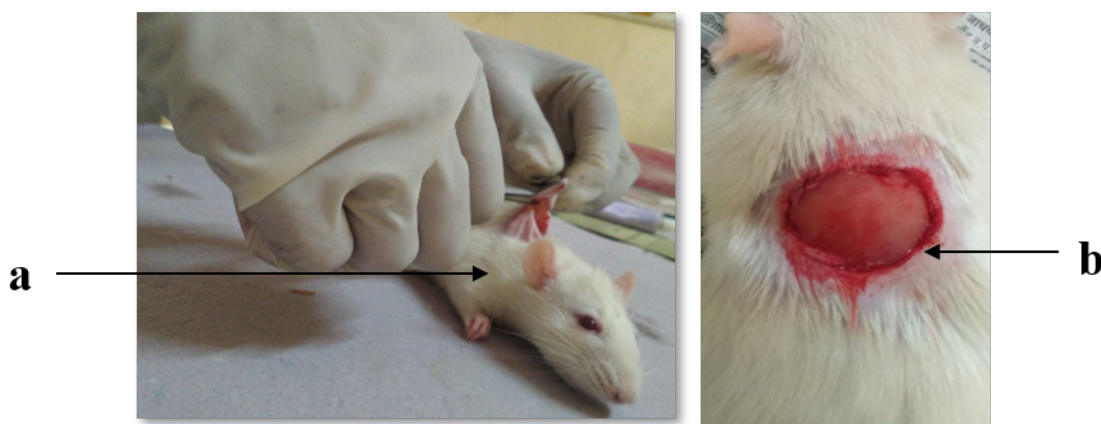
#### Treatment

In all groups, test formulations were applied topically once in a day, according to their respective groups, from the day of creation of wound up to 21<sup>st</sup> day.

#### Evaluation parameters

##### Effect of Formulations on Wound Size

Observation of wound size was obtained by tracing the wounds on transparent paper with the help of permanent marker on zero-day (day of wound creation) and on alternate days on 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, 16<sup>th</sup>, and on 21<sup>st</sup> days of the post wounding day of study. These traced wounds on transparent



**Figure 1: Excision wound making (a) and excision wound model (b)**

**Table 1: Grouping of animals**

Groups	Treatment (n=6)
Normal Control (NC)	Normal Rats with wound, given saline solution (10 ml /kg).
Normal Standard (NS)	Normal Rats with wound were treated with 0.5g Standard drug (Framycetin sulphate) cream topically.
Normal Durva ghrita (NDG)	Normal Rats with wound were treated with 0.5g Durva Ghrita topically.
Normal Tilvadi ghrita (NTG)	Normal Rats with wound were treated with 0.5g Tilvadi Ghrita topically.
Diabetic Control (DC)	Diabetic Rats with the wound (Alloxan Induced).
Diabetic Standard (DS)	Diabetic Rats (Alloxan Induced) with wound were treated with 0.5g Standard drug (Framycetin sulphate) cream topically.
Diabetic Durva ghrita (DDG)	Diabetic Rats (Alloxan Induced) with wound were treated with 0.5g Durva Ghrita topically.
Diabetic Tilvadi ghrita (DTG)	Diabetic Rats (Alloxan Induced) were treated with 0.5g Tilvadi Ghrita topically.

papers were measured using millimeter-scale graph paper. Wound photographs were taken on alternate days (Patel and Kurbetti, 2013).

#### Percentage of wound Contraction

Percentage wound contraction indicates the wound closure; it was studied from the first day till the last day of the experimental model. It was determined by the method described in (Sharma et al., 2009).

Percentage of wound contraction can be calculated by the following formula,

% of wound Contraction =

$$\frac{\text{Initial Wound area} - \text{Wound area on a corresponding day}}{\text{Initial Wound area}} \times 100$$

#### Period of Epithelization

Period of epithelization (falling of eschar) was monitored by assessment of the number of days required for the eschar (dead tissue that sloughs off from the surface of the skin after injury) to fall off from the excision wound surface without leaving a raw wound at the final stage (Dande and Khan, 2012).

#### Statistical analysis

Results data of wound area were analyzed using Two-way ANOVA followed by the Bonferroni's multiple range test and performed by using statistical software package, Graph Pad Prism; version 5.03. Values were expressed as mean  $\pm$  SEM, and the value of \*p <0.05, \*\*<0.01, \*\*\*<0.001 were considered as statistically significant.



## RESULTS AND DISCUSSION

The preliminary phytochemical investigation of the Methanolic extracts of Tilvadi Ghrita and Durva Ghrita revealed the presence of Alkaloids, Tannins, Proteins, Volatile oils, Saponins, and Flavonoids. Physico-chemical parameters of Ghritas are described in Table 2.

### Skin irritation test

To evaluate the safety of DG and TG skin irritation study was carried out on female Wistar rats as per (OECD, 2015). The result of the skin irritation test indicates that both Ghrita formulations Tilvadi Ghrita and Durva Ghrita do not cause any type of harmful skin reactions. There was the absence of any signs of irritation, erythema, and odema. Both formulations Tilvadi Ghrita and Durva Ghrita were found to be safe for topical applications. This study shows that TG and DG are safe for application on the skin.

### Effect on wound size

#### Percentage of Wound contraction

Epithelization Period of TG and DG are described in Table 5

Wound healing is a complicated natural mechanism through which the body restores damaged tissues during injury. Ayurveda system of medicine designed many wound healing formulations with no side effects. Generally, we should focus on the study of a traditional system of medicine scientifically, and we must investigate such formulations for its pharmacological activity. This work was focused on the evaluation of the pharmacological activity of traditional Ayurvedic formulations. If such formulations are validated properly with proper scientific data, it can substitute the modern wound healing agents with no or least side effects. Primary scientific investigation of traditional Ayurvedic products could be used further for the research of some potent wound healing agents (Guo and Dipietro, 2010; Banna and Zorba, 2008).

Herbal medicine in wound management and treatment involves disinfection, debridement, and providing a moist environment to stimulate the establishment of a suitable environment for the natural healing process. The ingredients of TG and DG have been reported to contain several phytochemicals, which are responsible for its various important pharmacological actions (Pandey and Worlikar, 2012; Das and Debnath, 2014).

However, no systematic scientific studies were reported in the modern scientific literature concerning to justify the traditional medicinal claims of TG

and DG. TG and DG showed wound healing activity in normal wounds. This study was undertaken to investigate and rationalize the wound healing activity of TG and DG in diabetic rats (Charde and Fulzede, 2003; Charde and Hemke, 2004).

Skin irritation study did not show any signs of skin irritations during 14 days of study as per (OECD, 2015). Both formulations – Tilvadi Ghrita and Durva Ghrita were found to be safe for topical applications.

Physico-chemical parameters like Saponification value, Iodine value, Acid value, and Refractive index of TG and DG were studied Table 2.

Treatment with a standard FSC drug also showed a significant increase in percentage wound contraction when treated with Durva Ghrita in Diabetic rats showed higher or same percentage wound contraction as Diabetic standard group treated with FSC.

Percentage wound contraction of the TG treated group was found to be slightly lower when compared to DG treated group. Treatment with DG in normal rats exhibited almost a similar percentage of wound contraction like that of the Standard FSC, as mentioned in Table 4. So, DG showed a significant increase percentage of wound contraction.

Treatment with TG in Diabetic rats showed a significant increase in the percentage wound contraction ( $64.44 \pm 3.08$ ,  $79.89 \pm 3.63$ , and  $90.69 \pm 1.74$ ) on days 12, 16, and 21, respectively as compared to Diabetic control group.

Treatment with DG in Diabetic rats showed a significant increase in the percentage wound contraction ( $41.02 \pm 2.01$ ,  $61.7 \pm 2.32$ ,  $85.24 \pm 2.76$ , and  $94.81 \pm 0.49$  on days 08, 12, 16, and 21 respectively as compared to Diabetic control group.

Treatment with a standard drug (Framycetin sulfate) showed a significant increase in percentage wound contraction on 12, 16, and 21 days ( $71.18 \pm 3.98$ ,  $89.35 \pm 1.29$ , and  $97.13 \pm 0.61$ , respectively. In the Normal DG treated group of rats, a significant increase in the percentage wound was observed on 12, 16, and 21 days ( $69.46 \pm 3.73$ ,  $84.84 \pm 3.54$ ,  $95.80 \pm 0.42$ ).

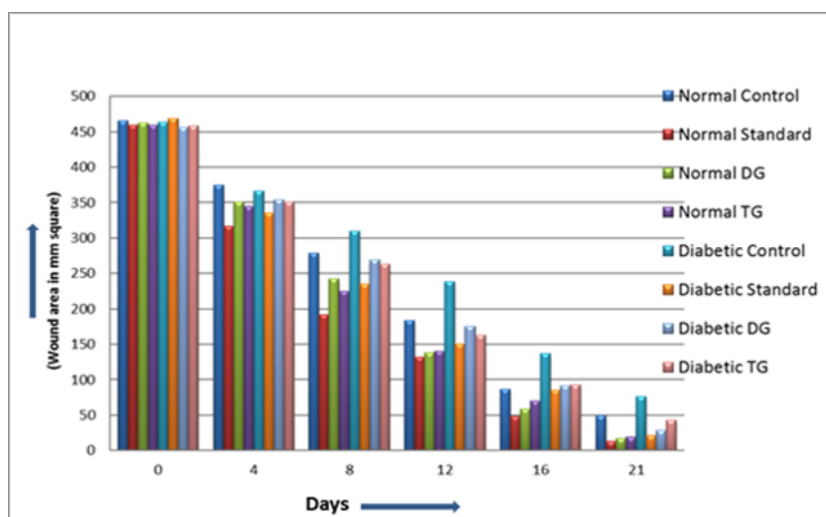
The wound contraction activity of the test formulation TG and DG were initially slow, but it progressively increased day by day in comparison to Normal untreated control and Diabetic control groups. Result data indicate that there was a significant increase in percentage wound contraction in Normal standard, Normal TG, and Normal DG treated groups as compared to Normal control groups. It showed that in Normal rats, Durva Ghrita treated group showed a higher percentage of wound contraction ( $96.23 \pm 0.71$ ) on day 21 compared to Nor-

**Table 2: Physico-chemical parameters of Ghrita formulations**

Parameters	Durva Ghrita (DG)	Tilvadi Ghrita (TG)
Parameters	Durva Ghrita (DG)	Tilvadi Ghrita (TG)
Colour	Greenish-yellow	Brownish-yellow
Odour	Fragrant	Characteristic
Taste	Characteristic	Characteristic
Saponification value	164.17	196.53
Iodine value	26.12	28.19
Acid value	1.23	1.48
Refractive index (40°C)	1.47	1.45

**Table 3: Comparative Effect of Durva Ghrita and TilvadiGhrita on Wound size in Normal and Diabetic Rats**

Groups	Day 0	Day 04	Day 08	Day 12	Day 16	Day 21
NC	465.66(mm <sup>2</sup> ) ± 5.74	374.5(mm <sup>2</sup> ) ± 10.14	278.5(mm <sup>2</sup> ) ± 15.46	184(mm <sup>2</sup> ) ± 15.65	86.5(mm <sup>2</sup> ) ± 8.78	49.5(mm <sup>2</sup> ) ± 8.02
NS	459.17(mm <sup>2</sup> ) ± 13.81	317(mm <sup>2</sup> ) ± 18.70	192.17(mm <sup>2</sup> ) ± 13.97*	132.33(mm <sup>2</sup> ) ± 18.58	48.67(mm <sup>2</sup> ) ± 5.37	13(mm <sup>2</sup> ) ± 2.98
NDG	462.17(mm <sup>2</sup> ) ± 18.97	351.17(mm <sup>2</sup> ) ± 11.00	242.33(mm <sup>2</sup> ) ± 16.97	137.67(mm <sup>2</sup> ) ± 2.95	58.33(mm <sup>2</sup> ) ± 5.92*	17.17(mm <sup>2</sup> ) ± 2.97
NTG	459.5(mm <sup>2</sup> ) ± 18.85	344.67(mm <sup>2</sup> ) ± 12.74	224.67(mm <sup>2</sup> ) ± 17.95*	140.17(mm <sup>2</sup> ) ± 17.65	69.67(mm <sup>2</sup> ) ± 16.85	19.33(mm <sup>2</sup> ) ± 1.78
DC	464(mm <sup>2</sup> ) ± 10.87	366.5(mm <sup>2</sup> ) ± 9.41	310(mm <sup>2</sup> ) ± 6.68***	238.67(mm <sup>2</sup> ) ± 6.62***	137(mm <sup>2</sup> ) ± 18.26**	76(mm <sup>2</sup> ) ± 9.32*
DS	469(mm <sup>2</sup> ) ± 12.53	335.83(mm <sup>2</sup> ) ± 5.63	235.16(mm <sup>2</sup> ) ± 6.79**	150.83(mm <sup>2</sup> ) ± 13.66**	85.83(mm <sup>2</sup> ) ± 14.02	21(mm <sup>2</sup> ) ± 6.69
DDG	456.17(mm <sup>2</sup> ) ± 15.19	354(mm <sup>2</sup> ) ± 12.86	269.17(mm <sup>2</sup> ) ± 13.56**	175.67(mm <sup>2</sup> ) ± 14.46*	91.17(mm <sup>2</sup> ) ± 14.98	28.67(mm <sup>2</sup> ) ± 4.84
DTG	458.83(mm <sup>2</sup> ) ± 9.60	350.83(mm <sup>2</sup> ) ± 12.50	262.83(mm <sup>2</sup> ) ± 16.73*	163.17(mm <sup>2</sup> ) ± 15.19**	92.17(mm <sup>2</sup> ) ± 16.79	42.67(mm <sup>2</sup> ) ± 6.87

**Figure 2: Comparative effect of TG and DG Ghritas on wound size in Normal and Diabetic Rats**

**Table 4: Comparative Effect of Durva Ghrita and Tilvadi Ghrita on Percentage wound Contraction in Diabetic and Normal rats**

Treated Groups	Day 0	Day 04	Day 08	Day 12	Day 16	Day 21
NC	0.0	21.75 % ± 2.68	40.22% ± 3.03	60.4% ± 3.50	71.46% ± 1.85	79.40% ± 1.70
NS	0.0	30.82% ± 4.24	56.73% ± 3.57	71.18% ± 3.98	89.35% ± 1.29*	97.13% ± 0.61*
NDG	0.0	23.82% ± 1.49	47.12% ± 4.41	69.93% ± 1.40	87.23% ± 1.56	96.23% ± 0.71
NTG	0.0	24.85% ± 1.54	50.69% ± 4.35	69.46% ± 3.73	84.84% ± 3.54	95.8% ± 0.42
DC	0.0	13.18% ± 2.15*	29.05% 2.91***	45.38% ± 2.32***	58.36% 4.42***	72.48% ± 2.38**
DS	0.0	28.12% ± 2.17	49.85% ± 1.76**	67.64% ± 3.38**	81.43% ± 3.40**	95.41% ± 1.61**
DDG	0.0	22.03% ± 3.43	41.02% ± 2.01	61.7% ± 2.32	85.24% ± 2.76***	94.81% ± 0.49*
DTG	0.0	23.34% ± 3.18	42.49% ± 4.10	64.44% ± 3.08*	79.89% ± 3.63**	90.69% ± 1.47*

Values are means ±SEM (n = 6). NC: Normal Control, NS: Normal Standard, NDG: Normal Durva Ghrita, NTG: Normal Tilvadi Ghrita, DC: Diabetic control, DS: Diabetic Standard, DDG: Diabetic Durva, DTG: Diabetic Tilvadi ghrita

**Table 5: Epithelization period**

Groups	Epithelization Period
Normal Control	13.33±0.33
Normal Standard	10.83±0.30
Normal DG	12.66±0.21
Normal TG	12.83±0.30
Diabetic Control	20.33±0.33
Diabetic Standard	13.16±0.40
Diabetic DG	15.5±0.42
Diabetic TG	14.5±0.49

mal standard group (97.13±0.61).

In the diabetic wound model (Excision wound model), the test formulation Tilvadi Ghrita and Durva Ghrita showed better and faster healing in both diabetic and normal rats as compared to untreated normal control and diabetic control group Figure 2.

In diabetic groups TG and DG treated rats showed a significant decrease in wound size compared to Diabetic control rats, but Diabetic TG shows a somewhat lower wound reduction response as compared to Diabetic DG Table 3.

Treatment with standard FSC drug also showed a significant increase in percentage wound contraction when treated with Durva Ghrita in diabetic rats showed higher or same percentage wound contrac-

tion as standard diabetic group treated with FSC. Treatment with TG shows a slight low percentage of wound contraction in Diabetic rats compared to Diabetic DG treated rats. The percentage wound contraction of DG treated normal rats was found to be the same as that of percentage wound contraction in standard FSC treated rats. There was a significant reduction in the epithelization period Table 5.

Thus, all the results represent that all normal groups showed a significant decrease in wound size as compared to all Diabetic groups. But DG and TG showed a significant decrease in wound area in normal as well as diabetic treated groups as compared to the Normal control and Diabetic control groups.

Both Ghrita formulations possess a definite wound healing property according to the present investigation. This was demonstrated by a significant

increase in the rate of wound contraction; decrease in wound size by the excision diabetic wound model, with a significant reduction in the epithelization period. Ghrita formulations also facilitated the rate of wound contraction in diabetic and normal excision wound models.

*Glycyrrhiza glabra*, which is a major phytochemical constituent of TG, possesses Antimicrobial, Antibacterial Anti-inflammatory activities. It was also claimed to show wound healing activity in normal wound conditions. So, these studies can help to justify the wound healing activity of *Glycyrrhiza glabra* in the diabetic wound model. Due to its Antimicrobial, Antibacterial and Anti-inflammatory potentials, Tilvadi Ghrita formulation exhibited diabetic wound healing activity (Sultana and Haque, 2010; Nirmala and Selvaraj, 2011; Patil and Patil, 2005).

Til oil (*Sesamum indicum*), another ingredient of TG, also proved effective in treating the wounds in different research studies, it helps in healing the wounds faster than the untreated wounds. Sesame oil extract promotes significant wound healing activity. This was demonstrated by a significant increase observed in the rate of wound contraction (Sharma and Hem, 2016).

DG contains *Cynodon dactylon* extract, which also claimed to possess antiulcer, antibacterial, analgesic, and antipyretic activities by literature survey. Hydro-alcoholic extract of *Cynodon dactylon* also showed wound healing activity in normal wounds. These research findings justify that the *Cynodon dactylon* content of DG may prove helpful in treating the wounds in normal as well as diabetic wounds. Ghee (clarified butter) generally obtained from animal sources. Ayurveda recommends the use of Go Ghrita for the preparation of Ayurvedic Ghrita formulations. Go ghrita was used as a base for both Ghrita formulations, showed enhancement in wound healing itself. It purifies ulcers and helps for wound healings (Banna and Zorba, 2008). Cow's ghee has been reported to exhibit anti-ulcer activity and also effective against infection in the eyes. Ghee contains several saturated and unsaturated fatty acids, which were capable of taking part in metabolic processes involved in the healing of wounds (Biyani et al., 2011).

Thus, all the ingredients of TG and DG claimed to possess wound healing activities, either decreasing the inflammatory phase, by its analgesic and antiulcer activity, or by its antimicrobial property. So it can be stated that both formulations showed wound healing activity of TG and DG in normal and diabetic wounds due to the presence of different phytoconstituents of *Glycyrrhiza glabra*, *Cynodon dacty-*

*lon*, *Sesamum indicum* and Go ghrita.

After comparative investigation of wound healing activity of TG and DG, it was demonstrated that Durva Ghrita claimed to possess higher wound healing potential in normal as well in diabetic groups with comparison to Tilvadi Ghrita. Thus, DG found to be more effective in the treatment and management of normal and diabetic wounds.

## CONCLUSION

Both Ghrita formulations possess a definite wound healing action, as evidenced by present wound healing study. This was demonstrated by a significant increase in the rate of wound contraction, a decrease in wound size, and enhanced epithelization. Wound healing activity of the formulations may be endowed due to the presence of phytoconstituents, which were identified and reported in the literature.

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## Conflict of Interest

The authors declare no conflict of interest.

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