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Wound healing in diabetic rats: Comparative efficacy of topical Insulin, Jatyadi formulations and Silver sulphadiazine

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Abstract

To compare the effect of topical insulin, silver sulfadiazine and two ayurvedic formulations (jatyadi-ghrita and jatyadi-tail) on excision wound in experimentally induced diabetic rats. Diabetes Mellitus was induced by single intraperitoneal (i.p.) injection of 150mg/kg alloxan in wistar rats weighing 150-200gm.Study animals were divided into 6 groups of six rats each. Group I- Non Diabetic Control (no treatment), Group II - Diabetic Control (no treatment), Group III – Topical Insulin, Group IV – Silver Sulfadiazine, Group - V Jatyadi-tail and Group VI- Jatyadi-Ghrita. An excision wound was inflicted in all rats by cutting full thickness of a pre-determined area (1 X 1 cm) on the depilated back of the rat under ether anesthesia. Drug treatment was given topically once daily. The healing was assessed by parameters like epithelization period and wound contraction. The results were analyzed using One-way ANOVA followed by Tukey's test by SPSS Software Version 20.

Epithelization was delayed in diabetic control animals (18 days) in comparison to non diabetic control animals(12 days) and was hastened in all drug treated groups (insulin-8 days; silver sulfadiazine-10 days; jatyadi tail-11days and jatyadi ghrita-15 days) in comparison to diabetic control animals. There was significant improvement in wound contraction in Groups III-V ($p<0.05$from day 8 onwards) as compared to diabetic control group.

Topical insulin has comparable wound healing efficacy to silver sulfadiazine in alloxan induced diabetic rats.Jatyadi tail also has shown very promising results in this model.

Keywords: topical insulin; wound; diabetic rats; jatyadi tail; ghrita.

Introduction

Wound is defined as loss or breaking of anatomic and cellular continuity of living tissue. Wound healing process is a biological process instigated by trauma and causes scar formation. It occurs in few different stages such as coagulation, epithelization, granulation, collagenation and remodeling of tissue. The objective of wound management is to heal the wound in express time possible, with very nominal pain, discomposure and scarring in patient with wound.

Diabetic wounds are slow non-healing wounds that can persist for weeks in spite of adequate and appropriate care. Such wounds are difficult and tough to manage. The exact pathogenesis of poor wound healing in diabetic wounds is not clearly understood. Evidence from studies involving both human and animal models reveals several abnormalities in the various phases of the wound healing process. Approximately 15% of all patients with diabetes will, at some time, have non-healing wounds, despite treatment and meticulously-controlled diet, and this is the leading cause of lower extremity amputation[i].

Managing the wounds in diabetics is important because they are common, painful and can result in amputation of affected parts or death in severe cases. Diabetic wounds result in significant morbidity, prolonged hospitalization, and enormous health-care expenses. Most of the early treatment modalities include topical application of silver sulfadiazine, povidone iodine etc mainly aimed at preventing infection. Topical application of Insulin has been shown to accelerate wound healing in diabetic rats.[ii]In Ayurveda practice, numbers of preparations are used to treat diabetic injuries and whether these can be used as alternatives to our conventional treatment options needs to be studied. This study would give us a clue whether topical insulin and these ayurvedic formulations can be used as alternatives to our conventional treatment options for diabetic wounds.

Hence this study was planned to compare the effect of standard drug silver sulfadiazine with topical application of Insulin and two ayurvedic formulations (Jatyadi Ghrita and Jatyadi Tail) in experimentally induced diabetic rats.

Material and Methods

The study was started after getting approval from Institutional Animal Ethics Committee (Approval Letter No. IAEC /BVDUMC/07/2009-2010 dated 2/9/2009) and in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Animals

No- 48
Species – Albino Wistar Rats
Sex – Either
Weight – 150-200 gms.
Source – Central Animal House recognized by CPCSEA

Experimental conditions

Food –Standard food pellets were used as a basal diet during the experimental period.
Water – The control and experimental animals were provided Aquaguard drinking water ad libitum.

Drugs and chemicals

Insulin (I)– It is an antidiabetic agent which was used as a topical preparation in this study. Biphasic isophane insulin injection I.P. (Human Mixtard) was used. It is Monocomponent Human Insulin (rDNA origin) which contains 30% soluble insulin and 70% NPH insulin. Human Mixtard was manufactured by Torrent Pharmaceutical Ltd and distributed by Abott India Ltd under licenceof Novo Nordisk India Private Ltd.
Jatyadi tail (JT)- Traditional oil based formulation which as per the classical text in ayurveda acts as antiseptic, fungicidal and a good healer used in boils, cuts, wounds, burns, piles & fistula. JatyadiTail of AryaVaidya Pharmacy (Coimbatore) Limited, Kerala, India was used.
Jatyadi Tail ingredients: Jati – Myristicafragrans; Nimba – Neem – Azadirachtaindica; Patola – Stereospermumsuaveolens; Naktamala – leaves of Pongamiapinnata; Sikta – Honey bee wax; Madhuka – Licorice – Glycyrrhizaglabra; Kushta – Saussurealappa; Haridra – Turmeric – Curcuma longa; Daruharidra – Berberisaristata; Manjishta – Rubiacordifolia; Katurohini – Picrorhizakurroa; Padmaka – Prunuspuddum; Lodhra – Symplocosracemosa; Abhaya – Terminaliachebula; Nilotpala - Nymphaeastellata; Tutthaka – Copper sulphate; Sariva – Hemidesmusindicus; Naktamalabeeja – Seeds of Pongamiapinnata; Taila& Water.
Jatyadi Ghrita (JG)-It is a ghee based formulation useful for wounds, painful ulcers, insect bite wounds, wounds caused by heat or fire, diabetic ulcers and deep wounds by external application as per the classical texts of ayurveda. JatyadiGhrutham from AshtavaidyanThaikkatuMoossVaidyaratnamOushadshala, Kerala, India was used.
Jatyadi Ghrita Ingredients:Jati –Jasminumsambac; Nimbapatra – neem leaves; Patolapatra- Trichosanthesdioica; Katuka – Picrorrhizakurroa; Darvi – Berberisaristata, Nisha – Curcuma longa; Sariva – Hemidesmusindica; Manjishta – Rubiacordifolia; Abhaya – Terminaliachebula; Siktaka – Honey bee wax; Tuttha – Purified blue vitriol; Madhuka – Glycyrrhizaglabra; Naktahva – Pongamiapinnata; Sarpi – ghee; water.
The above combination is heated till herbal ghee is prepared.
Silver sulfadiazine (SSD)-The preparation of 1% silver sulfadiazine cream (SSD) of Universal Twin Labs, Solan (H.P.) India, was used as standard drug.
Alloxanmonohydrate(Sigma-Aldrich)- Dry powder, freshly made solution in Normal Saline was used to induce diabetes mellitus in experimental animals.
Anaesthetic ether(NarsonPharma, Chittoor, Andhra Pradesh,India)
Dextrose (Emkay Labs, India)

Instruments

Glucometer (Health Pro, Infopia company private limited, Korea) was used to check Blood Sugar levels and glucose strips of Alixir medical systems private limited, Pune, India were used.
Digital weighing machine (Master, Manufactured by Ace Corporation, Pune)

Experimental model

Study was divided into 3 parts.
Induction of diabetes
To develop excision wound model
To evaluate wound healing activity of various drugs

Alloxan induced diabetes mellitus model

Alloxan monohydrate was first weighed individually for each animal according to their weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting it in a dose of 150 mg/kg body weight intraperitonially.[iii]After 1 hour of alloxan administration, the animals were given feed ad libitum and 10 % dextrose solution was also given in a feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation. Hyperglycemia was confirmed by the elevated glucose levels in plasma, determined at 72 hrs and then on 7th day after injection. Blood glucose level was measured by glucometer. The diabetic rats (glucose level >200 mg/dl) were separated and divided into five different groups (Groups II –VI) for experimental study, with each group containing six animals. Group I contained non-diabetic rats and served asnon-diabetic control.

Study design

All animals were divided into 6 groups (n=6).

Table 1: Study groups

Group	Treatment
I	Non-Diabetic Control (NDC)
II	Diabetic control (DC)
III	Insulin(Insulin)
IV	Jatyadi-Tail (JT)
V	Jatyadi-Ghrita (JG)
VI	Std- Silver Sulfadiazine ointment(SSD)

Drugs were applied topically covering the wounds completely in the animals of Group III to Group VI as per above table. Drugs were

appliedevery day with sterile gauze till complete healing of the wounds.Insulin was instilled into the wound topically in the dose of 1U/100 gm[ii].

To develop excision wound model

An excision wound wasinflicted,on all overnight starved Wistar rats, by cutting full thickness of a pre-determined area (1 X 1 cm) on the depilated back of the rat under ether anaesthesia[iv]. The concerned area of the wound to be created was outlined on back of the animals with marker pen using square stencil. The entire wound was left open. Animals were closely observed for signs of any infections and those which showed any signs of infection were separated, excluded from the study and replaced.

To assess wound healing activity of various drugs

Animals were inspected daily and the healing was assessed based on the physical parameters like epithelization period and wound contraction .Apart from the drugs under investigation no local/systemic chemotherapeutic cover was provided to animals.

Epithelization period:
It was monitored by noting the number of days required for the eschar to fall off from the burn wound surface without leaving a raw wound behind.
Wound contraction:
It was assessed by noting the progressive changes in wound area planimetrically, excluding the day of the wounding. The size of the wounds was traced on a transparent paper every two days, throughout the monitoring period. The tracing wasthen transferred to 1 mm^2 graph sheet, from which the wound surface area was evaluated. The evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound, as 100%, by using the following:

$$\% \text{ of wound contraction} = \frac{\text{Initial wound size-specific day wound size}}{\text{Initial wound size}} \times 100$$

Statistical Analysis-
The results of the study were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons by using SPSS Software Version 20. Values with $P < 0.05$ were considered significant.

Results and Discussion

Wound healing is characterized by three stages viz. inflammation, proliferation and remodeling. The proliferative phase typically demonstrates angiogenesis, collagen deposition, granulation tissue formation, epithelization and wound contraction. In angiogenesis, new blood vessels grow from endothelial cells. In fibroplasias and granulation tissue formation, fibroblast grows and forms a new provisional extracellular matrix by excreting collagen and fibronectin. In epithelization, epithelial cells crawl across the wound bed and cover it. Fibronectin, the major glycoprotein secreted by fibroblast has important functions of chemo-attraction for macrophages, fibroblast and endothelial cells, promoting re-epithelization and acting as a transduction agent in wound contraction. Wound contraction occurs by myofibroblasts, which establish grip on the wound edges bringing them in apposition.
Diabetic wounds are slow non-healing wounds that can persist for weeks in spite of adequate and appropriate care. Such wounds are difficult and tough to manage. Though the exact pathogenesis of poor wound healing in diabetic wounds is not clearly understood, evidence from studies involving both human and animal models reveal several abnormalities in the various phases of the wound healing process. Approximately 15% of all patients with diabetes will, at some time, have non-healing wounds, despite treatment and meticulously-controlled diet, and this is the leading cause of lower extremity amputation.
Wound healing deficits in diabetes are diverse ,multifactorial, complex and interrelated[v].This defect is believed to be caused by impaired blood flow and oxygen release from blood sugar , decreased collagen and fibronectin synthesis from protein malnutrition, impaired local immune and cell defence , and decreased anabolic activity and decreased insulin and growth hormone.Collagen, fibrin and keratin accumulate advanced glycation Amadori end products which affect binding of regulatory molecules, susceptibility to proteolysis, and finally decrease the ability for protein cross-linkage[vi]. Di Girolamo et al[vii] postulated that defects in wound healing are caused by the hyperglycosylation of the locally synthesized fibronectin. However hyperglycemia affects whole range of neutrophil functions, which include migration, chemotaxis adherence and phagocytic and bactericidal activity[viii].
Managing the wounds in diabetics is important because they are common, painful and can result in amputation of affected parts or death in severe cases.Diabetic wounds result in significant morbidity, prolonged hospitalization, and enormous health-care expenses. Most of the early treatment modalities include topical application of silver sulfadiazine, povidone iodine etc mainly aimed at preventing infection. Topical application of Insulin has been shown to accelerate wound healing in diabetic rats[13]. In ayurvedic practice, numbers of preparations are used to treat diabetic injuries and whether these can be used as alternatives to our conventional treatment options needs to be studied.
Hence in this study we planned to compare the effect of silver sulfadiazine with topical application of Insulin and two ayurvedic formulations (Jatyadi Ghrita and Jatyadi Tail) in experimentally induced diabetic rats. In our study, we used Alloxan[ix] to develop diabetes in experimental animals. It is a chemical agent used to induce diabetes. Alloxan has been used to induce experimental diabetes due to the selective destruction of the insulin-producing pancreatic beta-islets. Alloxan induces a multiphasic blood glucose response when injected into to an experimental animal, which is accompanied by corresponding inverse changes in the plasma insulin concentration followed by sequential ultrastructural beta cell changes ultimately leading to necrotic cell death.

Figure 1: Effects of Topical Insulin, Jatyadi Tail, Jatyadi Ghrita and Silver Sulphadiazine on Duration of epithelization

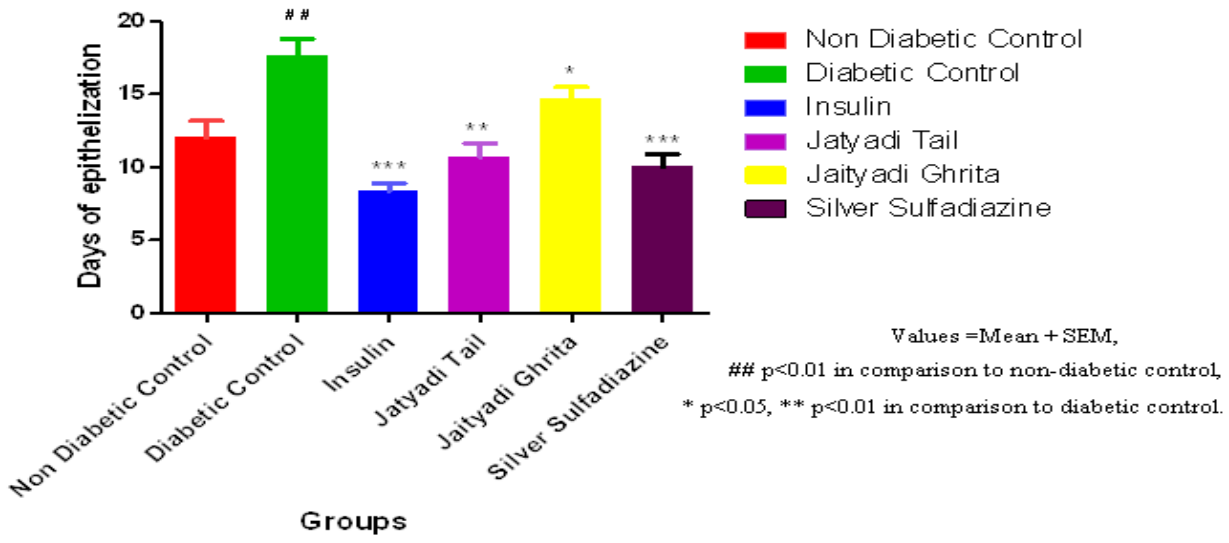
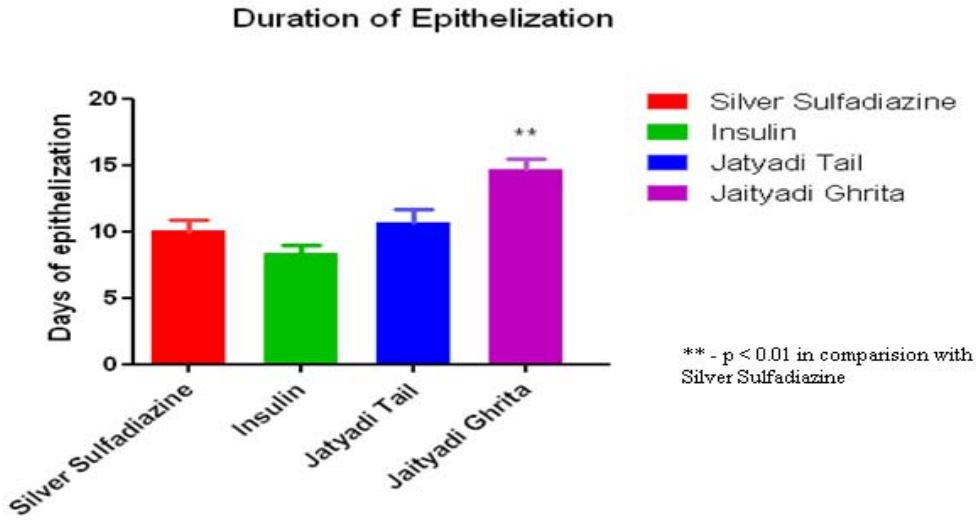


Figure 2: Comparison of efficacy of Topical Insulin, Jatyadi Tail and Jatyadi Ghrita with Silver Sulfadiazine (Wound Epithelization)



In the present study, silver sulfadiazine (SSD) significantly (p< 0.001) reduced the period of epithelization of the wound (10.0 +0.9 days) in comparison with the diabetic control group (17.7+1.0 days). Topical Insulin (p< 0.001, 8.3+0.6 days) and both the ayurvedic drug formulations, Jatyadi Ghrita (p< 0.05) and Jatyadi Tail (p< 0.01) also significantly reduced the period of epithelization of the burn wound (14.7+0.8 and 10.7+1.0 days respectively) as compared to diabetic control group wounds. Wound epithelization activity of topical insulin and the ayurvedic formulation Jatyadi Tail is comparable with that of topical silver sulfadiazine on excision

wounds in diabetic rats. Wound epithelization activity of the ayurvedic formulation Jatyadi ghrita is significantly less ($p<0.01$) as compared with that of topical silver sulfadiazine on excision wounds in diabetic rats.

In the present study, as compared to nondiabetic control group, there was significant reduction in percentage of wound contraction ($p< 0.01$) in drug treated groups on day 4, 8, 12 & 16. Topical Insulin application showed wound healing properties in this Alloxan induced diabetic rat model. As shown in Table 2, the wound was contracted by 70 % on Day 8 after topical Insulin application as compared to only 37% in diabetic control group. Also this study shows that topically applied Insulin took only 8.33 ± 0.61 days in comparison with diabetic control which took 17.67 ± 1.08 days for the eschar to fall off leaving no raw wound behind. It showed statistically significant reduction in period of epithelization and statistically significant rise in wound contracture ($p< 0.01$) from day 4 to right upto complete wound healing i.e. day 16. It was observed by Xuelian Chen et al[x] that topical insulin application decreased neutrophil infiltration by inhibiting MIP-2 expression and advanced neutrophil resolution. They also reported that insulin promotes neutrophil functions; which suggest there is a delicate regulation of insulin on wound inflammatory response during the healing process. In diabetic patients, Maria H.M. et observed that topical insulin cream markedly improved wound healing, representing an attractive and cost-free method for treating this devastating complication of diabetes[ii]. They observed that topical insulin accelerates wound healing in diabetes by enhancing the anti-serine-threonine kinase (AKT) and extracellular signal-regulated kinase (ERK) pathways. Apikoglu-Rabus S[xi] et al demonstrated that topical insulin application to cutaneous wounds accelerates wound healing in rats with or without acute diabetes.

Table 2: Effects of Topical Insulin, Jatyadi Tail, Jatyadi Ghrita and Silver Sulphadiazine on Percentage of wound contraction

Days	Study Groups					
	NDC	DC	Insulin	JT	JG	SSD
Day 4	21.17 ± 8.83	3.05 ± 7.70	35.95 ± 3.66**	30.09 ± 6.35	27.39 ± 5.23	28.66 ± 4.47
Day 8	75.28 ± 3.57	37.36 ± 7.97##	69.92 ± 4.68**	64.55 ± 4.39*	51.77 ± 6.23	66.22 ± 4.32**
Day 12	93.66 ± 1.74	66.15 ± 7.95##	95.67 ± 1.31**	87.24 ± 3.08*	78.94 ± 5.45	97.56 ± 0.94**
Day 16	99.56 ± 0.44	78.20 ± 6.70##	100.00 ± 0.00**	99.77 ± 0.23**	93.84 ± 1.64**	100.00 ± 0.00**

Values indicate Mean + SEM, ## $p < 0.01$ as compared to Nondiabetic control, * $p<0.05$, ** $p<0.01$ in comparison to diabetic control. NDC - Non diabetic control; DC - Diabetic Control; JT - Jatyadi Tail; JG –Jatyadi Ghrita; SSD - Silver sulfadiazine

Jatyadi Tail showed significant increase ($p< 0.05$) in wound contracture from day 8 to day 12 and also on day 16 ($p< 0.01$). Also as compared to Diabetic control group, it took only 10.67 ±0.99 days for epithelization, demonstrating its importance in diabetic wound healing. In traditional Indian medicinal treatment there are several Ayurvedic formulations mentioned which have been claimed as potential wound healing agents like Madhu Ghrita and Jatyadi Taila. Jatyadi Taila (JT) is a medicated oil formulation (Taila) popularly used in the treatment of various topical wounds. Treatment for Diabetic Foot Ulcer i.e., Doorvadi Taila and Jatyadi Taila is well explained in Ayurvedic Classics. Jatyadi Taila has definite antidiabetic activity[xii,xiii,xiv,xv]The phytochemical evaluation of JT has revealed presence of flavonoids, essential oils, tannins, glycosides, steroids and alkaloids. Tannins & phytosterols promote the wound healing process with increased capillary formation & fibroblast proliferation enhancing the rate of epithelization[xvi]. Previous study done with JT in excision wound model has also shown promising results. Topical application of JT on excision wounds has caused significantly faster reduction in wound area as compared to the application of modern topical formulation (Neosporin) and untreated control wounds. Animals treated with JT showed significant increase in protein, hydroxyproline and hexosamine content in the granulation tissue when compared with the untreated controls [xvii].Ingredients of JT have been studied extensively for their antimicrobial, anti-inflammatory and antiseptic activity which may be responsible for its efficacy in wound healing. There are promising results of Jatyadi tail in partial thickness burn wound model and its comparable effectiveness to silver sulfadiazine in burn wound healing[xviii].

Table 3: Comparison of efficacy of Topical Insulin, Jatyadi Tail and Jatyadi Ghrita with Silver Sulfadiazine (Wound Contracture)

	Study Groups			
	SSD	INSULIN	JT	JG
Day – 4	28.66 ± 4.47	35.95 ± 3.66	30.09 ± 6.35	27.39 ± 5.23
Day – 8	66.22 ± 4.32	69.92 ± 4.68	64.55 ± 4.39	51.77 ± 6.23 *
Day – 12	97.56 ± 0.94	95.67 ± 1.31	87.24 ± 3.08	78.94 ± 5.45 **
Day - 16	100.00 ± 0.00	100.00 ± 0.00	99.77 ± 0.23	93.84 ± 1.64

Values =Mean + SEM, * p<0.05, ** p<0.01 in comparison to Silver Sulfadiazine. JT - Jatyadi Tail; JG –Jatyadi Ghrita; SSD - Silver sulfadiazine

Jatyadi Ghrita showed increase in wound contracture but it was significant (p< 0.01) only on day 16. Also as compared to Diabetic control group, it took only 14.67±0.84 days for epithelization. Thus it also shows wound healing activity in diabetic rats but is comparatively less than the other two experimental drugs- topical insulin and Jatyadi tail and standard drug silver sulfadiazine. Jatyadi Ghrita[xix,xx] is the best remedy to heal all kind of wounds of the body, these might be small wounds like in anal fissure or the worst wounds by diabetic carbuncles- what so ever Jatyadi Ghrita can heal up all kind of wounds. The chronic wounds- which could not be healed even by using different anti-septic and antibiotic herbs for months and years - shows miraculous results for the healing of such wounds. Jatyadi Ghrita is a time tested classical combination of ayurveda and it has proven results as natural cure of the wounds. When used internally it helps in conditions like Ulcerative Colitis/ gastric ulcers by repairing the internal wounds quickly. It is mainly indicated in Fresh wound, Non healing wounds, Diabetic ulcers, Bedsores, Wounds in immune-compromised conditions, Ulcerative colitis, Gastric ulcers. Previous studies done with Jatyadi ghrita in dushtavrana (wound with foul smell, intense discharge, pain and long time for healing) as dressing, for fissure-in-ano and chronic wounds[xxi] have shown promising results in those respective models.

Thus in the present study, Topical Insulin, Jatyadi tail have shown comparable efficacy to standard treatment drug silver sulfadiazine in full thickness excision wound healing in diabetic rats. About 100% wound contracture was observed with SSD, Insulin and Jatyadi Tail groups on day 16. The efficacy of Jatyadi Ghrita in this model is seen comparatively less (p<0.05 on day 8 and p<0.01 on day 12) than standard treatment drug silver sulfadiazine. The results can be verified in clinical trials to approve the utility of topical insulin and Jatyadi tail as effective options for treatment of diabetic wound injuries.

Conclusion

Topical Insulin, Jatyadi Tail and Jatyadi Ghrita have wound healing activity on excision wound injury in alloxan induced diabetic rats. They significantly reduced the epithelization period and increased wound contracture in this model.

Protective effect of topical insulin and the ayurvedic formulation Jatyadi Tail was comparable with that of topical silver sulfadiazine (SSD) on excision wounds in diabetic rats. In these groups, it took approximately 9 days for the eschar to fall as compared to nondiabetic control and diabetic control groups where it took more than 12 days to do so. About 100% wound contracture was observed with SSD, topical Insulin and Jatyadi Tail groups on day 16 after induction of excision wound.

Protective effect (wound epithelization activity) of the ayurvedic formulation Jatyadi Ghrita was significantly less as compared with that of topical silver sulfadiazine on excision wounds in diabetic rats. Jatyadi Ghrita showed increase in wound contracture but it was significant only on day 16 of the induced wound.

Author's Contributions

VM has made substantial contributions to conception and design, acquisition of data, interpretation of data and drafting the manuscript. PD was involved in conception and design,drafting the manuscript and revising it critically for important intellectual content and has given final approval of the version to be published. VP has made substantial contributions to conception of research proposal and availability of resources for the research work. HP was involved in acquisition of data, interpretation of data and drafting the manuscript. SH contributed in designing the protocol, acquisition of data and drafting the manuscript. All authors read and approved the final manuscript.

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References

[1]. Reiber GE,Vileikyte L et al. 'Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings.' Diabetes Care 1999;22: 157–62.

[2]. Lima MHM, Caricilli AM et al. 'Topical Insulin Accelerates Wound Healing in Diabetes by Enhancing the AKT and ERK Pathways: A Double-Blind Placebo-Controlled Clinical Trial'PLoS One. 2012; 7(5): e36974.

[3]. Kannur DM, Hukkeri VI, Akki KS. Antidiabetic activity of Caesalpiniabonducella seeds extracts in rats. Fitoterapia.2006;77:546–9.

[4]. Sikarwar MS, PatilMB.Antidiabetic activity of Pongamiapinnata leaf extracts in alloxan-induced diabetic rats,Int J Ayurveda Res. 2010 Oct-Dec; 1(4): 199–204.

[5]. HemahDevi, Shankar Jothietal.Wound healing activity of terminaliaarjunain albino wistar rats. International Journal of Phytopharmacology.2012;3(3): 234-240.

[6]. Shanbhag T, Shenoy S, Rao CM. Wound healingprofile of Tinosporacordifolia. Indian drugs 2005;42(4): 217–221.

[7]. Greenhalgh DG, Wound healing and Diabetes Mellitus. Clinical Plastic Surg,2007;30: 37-45.

[8]. Reiser KM,Nonenzymaticglycation of collagen in aging and diabetes.Proceedings for society of experimental Biology and Medicine.1998;218(1): 23-37.

[9]. Di Girolamo N, Underwood A et al. Functional activity of fibronectin in patients with Diabetes Mellitus. Diabetes, 1993; 42 (11):1606-13.

[10]. Wall SJ,Samson MJ et al.Elevated matrix metalloproteinase 2 and 3 production from human diabetic fibroblasts. British journal of Dermatology.2003; 149 (1): 13-6.

[11]. AnkurRohilla,Shahjad Ali.Alloxan Induced Diabetes: Mechanisms and Effects International Journal of Research in Pharmaceutical and Biomedical Sciences,2012; 3 (2): 819-23.

[12]. Xuelian Chen, Xiong Zhang. Effect of Topical Insulin Application on Wound Neutrophil Function Wounds. 2012;24(7):178-84.

[13]. Apikoglu-Rabus S, Izzettin FV,et al.Effect of topical insulin on cutaneous wound healing in rats with or without acute diabetes. Clinical and Experimental Dermatology,2010; 35(2):180-5

[14]. Sharangadhar- SharangadharSamhita, translated by Prof. K R Srikanta Murthy, madyamaKhanda –Ch-9, sl-168-171,Varanasi: Chowkambaorientalia; 7th edition, 2007, pg-132.

[15]. BaishajyaRatnavali – Sri Govinda Das, Hindi Commentator,Analysis, Appedices By AmbikadattaShastri, edited by RajeshwaraDattaShastri-Varanasi : Chowkambaorientalia; 2005,18th edition, Ch-47th ,sl-64-68, pg-851.

[16]. Bavaprakash – BavaprakashNighantu, translated by Dr.Amrithpal Singh –ch-2, sl-51-53, Varanasi: Chowkambaorientalia; 1st edition,2007, pg-58.

[17]. Dr. BrahmanandaNayak ,Management of infection: In Ayurvedaline , Published By Dr.Sitharam Prasad Bangalore, 2009-10, pg 412.

[18]. Sathish HS et al. Review of clinical observational studies conducted on tissue healing at IPGT and RA, Jamnagar International Journal of Research in Ayurveda and Pharmacy2011 2 (1): 13-17.

[19]. Shailajan S, Menon S, et al.Wound healing efficacy of JatyadiTaila: in vivo evaluation in rat using excision wound model.J Ethnopharmacol 2011; 138(1): 99-104.

[20]. DhandePP, Raj Simpy, et al. Burn Wound Healing Potential of JatyadiFormulations in Rats, Research Journal of Pharmaceutical, Biological and Chemical Sciences 2012 ,3 (4):747

[21]. AcharyaVagbhata, AshtangaHridaya, Uttar tantra, 25th chapter, Shloka no 67, KavirajAtridevGupt. Varanasi: ChaukhambhaPrakashan; 2008:741

[22]. Govind Dash, Bhaishajyaratnavali, 47th chapter, Shloka no 53, KavirajDr.AmbikaduttShastri. Edition no 15. Varanasi: Chaukhambha Sanskrit Sansthan; 2002: 596.

[23]. Lad MD, Bandgar SB. Management of nonhealing wound with NyagrodhadiKwatha and JatyadiGhrita (classical ayurvedicpreperations) Global Journal of Research on Medicinal Plants & Indigenous Medicine 2012; 7 (1): 301–308.