Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:

Potential wound healing activity of the different extract of *Typhonium trilobatum* in albino rats

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ARTICLE INFO

Article history:
Received 19 October 2012
Received in revised form 25 October 2012
Accepted 12 November 2012
Available online 28 December 2012

Keywords:
Hybanthus enneaspermus
Different extracts
Wound healing activity

ABSTRACT

Objective: To establish the wound healing activity of methanolic, ethyl acetate and chloroform extracts of plant of *Typhonium trilobatum*. **Methods:** Two models were performed to evaluate the wound healing activity *i.e.* excision and incision models. In incision model the parameter which is carried out was breaking strength of the wounded skin. In excision model the percentage wound contraction and period of epithelialization were established for three extracts. Reference standard drug was povidone iodine ointments for comparison with other groups. **Results:** From the observation in both two models, methanolic and ethyl acetate extract were found greater wound healing activity than chloroform extract in terms of breaking strength in incision model and percentage wound contraction, period of epithelialization in excision model than that of other groups. **Conclusions:** The results indicate that the different extracts of *Typhonium trilobatum* has significant wound healing activity.

1. Introduction

Medicinal plants are important for pharmacological research and drug development, not only when plant constituents are used directly as therapeutic agents, but also as starting materials for the synthesis of drug or as models for pharmacologically active compounds. They plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns. A wound which is disrupted state of tissue caused by physical, chemical, microbial or immunological insult ultimately heals either by regeneration or fibroplasias[1]. Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury, an inflammatory response occurs and the cells below the dermis begin to increase collagen production[2]. Later, the epithelial tissue is regenerated. Wound healing consists of an orderly progression of events that re-establish the integrity of the damaged tissue[3]. There are three main phases of wound healing *viz.*, inflammatory, proliferative and remodeling phase. The inflammatory phase begins immediately after injury with vasoconstriction that favors and releases inflammatory mediators. The proliferative phase is characterized by granulation tissue formation mainly by fibroblasts and angiogenesis. The remodeling phase is characterized by reformulation and improvement in the components of the collagen fiber that increases the tensile strength.

Typhonium trilobatum is a genus in the Araceae family endemic to tropical Asia, the South Pacific, and Australia. It consists of approximately 50 species that are typically found growing in wooded areas[4]. It has been valued in Ayurveda and Unani systems of medicine for possessing variety of therapeutic properties. Most of the plant parts of *Typhonium trilobatum* are used in traditional systems of medicine in India. According to Ayurveda, the rhizome is used with effect for treating vomiting, cough, asthma, excessive expectoration, pyogenic sore throat, headache, gastric ulcer, abcess, snake bite. This study was designed to explore the healing effects of topically applied *Typhonium trilobatum* extracts in rat intraoral rout[5,6].

2. Material and methods

2.1. Plant collection

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The whole plant was collected from rural belt of Westbengal, medinipore district, during the month of July and August 2010 in the early morning. The plant was identified, confirmed and authenticated by Prof. Sushil Ku. Mallick taxonomist in the Department of Botany, S.V.M Autonomous College, Bhubaneswar. After authentication the plant was collected in bulk and washed under running tap water to remove adhering dirt and soil particles. The plants were dried under shade at room temperature, after washing. The dried materials were made into coarse powder by grinding in mechanical grinder and passed in sieve no 40 and used for further study.

2.2. Preparation of extracts and its fraction

The coarse powder of the plant was taken in soxhlet apparatus and extracted with methanol. The extraction with each solvent is done for 72 h and the temperature was maintained in between 37-40 ℃ to prevent the loss of thermosensitive constituent of the plant. The methanolic extract is mixed with water (1:1) and used for fractionation with chloroform and ethylacetate by the use of separating funnel. The liquid extracts and fractionation were concentrated separately under vacuum and resulting dried extracts were preserved in a desiccator until further use.

2.3. Animals

Healthy adult albino rats of wister stain weighing 180 to 250 g were taken from the Laboratory Animal bread center, School of pharmaceutical sciences, SOA university. The animals were kept under 12:12 h day and light schedule with temperature between 18 to 20 ℃. the animals were housed in large spacious hygienic cages during the experimental period. The animals were provided with food and water regularly. CPCSEA number: 955/A/06/CPCSEA (CPCSEA stands for Committee for the Purpose of Control and Supervision of Experiments on Animals, India).

2.4. Wound healing properties of plant extract of Typhonium trilobatum

2.4.1. Excision wound model (without infection) non diabetic wound formation

Five group of healthy wister stain albino rat of either sex weighing 150-200 g containing six animals in each group were anaesthetized by anaesthetic ether in desiccators .The rats were depilated on the back and cutaneous circular wound of 8mm diameter were inflicted on the pre -shaved sterile dorsal surface of the animal by cutting . in each group each animal received one wound . Animals were housed individually in metallic cages[7]. The wound was left undressed to the open environment. Then the treatment was started in the following manner:

Group I : Solvent control (PEG-400)
Group II : Standard (Povidone iodine 5% w/w ointment)
Group III: Methanol Extract (100 mg/mL)
Group IV: Chloroform fraction (100 mg/mL)
Group V : Ethyl acetate fraction (100 mg/mL)

Application of drugs was done once a day after cleaning with surgical cotton wool.

2.4.2. Excision wound model(without infection) with diabetes

And another five group were induced diabetes by introducing alloxen 140 mg/kg, 72 h were left for diabetes to set in. The rats were depilated on the back and cutaneous circular wound of 8 mm diameter were inflicted on the pre -shaved sterile dorsal surface of the animal by cutting in each group each animal received one wound. Animals were housed individually in metallic cages[8]. The wound was left undressed to the open environment. Then the treatment was started in the following manner:

Group I : Solvent control (PEG-400)
GroupII : Standard (Povidone iodine 5% w/w ointment)
GroupIII : Methanol extract (100 mg/mL)
GroupIV : Chloroform fraction(100 mg/mL)
Group V : Ethyl acetate fraction (100 mg/mL)

Application of drugs was done once a day after cleaning with surgical cotton wool.

Parameters used to assess wound healing activity:

To assess the area of the healing wound the surface area was measured by tracing the boundary on semitransparent paper and calculation was done using a graph paper.

Histopathological studies wound tissues stained with erosion and hematoxylin were studied for anglogenesis, fibrogenesis, and epithelisation. Results were analyzed by students t test. P values less than 0.001 were taken as significant.

2.4.3. Excision wound model (with infection) with diabetes.

The methodology used for wound formation and diabetes formation was the same as that of excision wound model of without infection. But it was infected with a loop full of inoculums of mixed microorganisms comprising of staphylococcus aureus and *E.coli*. The mixed microorganisms were prepared by mixing 1mL of each from 106 cfu/mL of *S.aureus* and *E.coli* cultures. 48 h were left for infection to set in and then the treatment was started in following manner to the different groups, till the wound was completely healed.

Group I : Solvent control (PEG-400)
GroupII : Standard (Povidone iodine 5% w/w ointment)
GroupIII : Methanol extract (100 mg/mL)
GroupIV : Chloroform fraction(100 mg/mL)
Group V : Ethylacetate fraction (100 mg/mL)

Application of drugs was done once a day after cleaning

with surgical cotton wool. The parameters used to assess wound healing activity by this model is similar as that used in case of Excision wound model (without infection).

2.4.4. Excision wound model(with infection) non diabetes

The methodology used for wound formation was same as that of excision wound model of without infection. But it was infected with a loop full of inoculums of mixed microorganisms comprising of staphylococcus aureus and *E.coli*. The mixed microorganisms were prepared by mixing 1mL of each from 10^6 cfu/mL of *S.aureus* and E.coli cultures forty eight h were left for infection to set in and then the treatment was started in following manner to the different groups, till the wound was completely healed[9].

Group I : Solvent control (PEG-400)
GroupII : Standard (Povidone iodine 5% w/w ointment)
GroupIII : Methanol extract (100 mg/mL)
GroupIV : Chloroform fraction(100 mg/mL)
Group V : Ethyl acetate fraction (100 mg/mL)

Application of drugs was done once a day after cleaning with surgical cotton wool.

2.4.5. Incision wound model(without infection)

Five group of rats containing three animals in each group were anaesthetized and one paravertebral long incisions were made through the skin and cutaneous muscle at a distance of about 1.5 cm from the midline on each side of the depilated back of the rat. Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment[10,11]. All the groups were treated in the same manner as mentioned in the case of excision wound model. No ligature was used for stitching.

After the incision was made, the parted skin was kept together and stitched with black silk at 0.5cm intervals ; surgical thread (No.000) and a curved needle (No.11) were used for stitching. The continuous threads on both wound edges were tightened for good closure of the wound .The wound was left undressed. Then the treatment was started in the following manner:

Group I : Solvent control (PEG-400)
GroupII : Standard (Povidone iodine 5% w/w ointment)
GroupIII : Methanol extract (100 mg/mL)
GroupIV : Chloroform fraction(100 mg/mL)
Group V : Ethyl acetate fraction (100 mg/mL)

Application of drug was done once a day after cleaning with surgical cotton wool. All the drugs are administered for nine days to the respective groups; when wounds were cured thoroughly the sutures were removed on the ninth day and tensile strength was measured[12].

2.4.6. Incision wound model(with infection)

The methodology used for wound formation was same as that of incision wound model without infection. All the rats were infected with a loop full of inoculum of mixed microorganisms comprising of staphylococcus aureus and E.coli.The mixed microorganisms were prepared by mixing 1mL of each from 106 cfu/mL of *S.aureus* and *E.coli* cultures.

Forty eight h were left for infection to set in and then the treatment was started in the following manner to the different groups till the wound was completely healed.

Group I : Solvent control (PEG-400)
GroupII : Standard (Povidone iodine 5% w/w ointment)
GroupIII : Methanol extract (100 mg/mL)
GroupIV : Chloroform fraction(100 mg/mL)
Group V : Ethylacetate fraction (100 mg/mL)

Application of drug was done once a day after cleaning with surgical cotton wool. All the drugs are administered for 9 days to the respective groups; when wounds were cured thoroughly the sutures were removed on the ninth day and tensile strength was measured by Tensiometer by the same procedure as described in the incision model without infection.

The parameter used to assess wound healing activity by this model is similar to that used in case of incision model without infection.

2.4.7. Incision wound model (without infection) with diabetes.

Five group of rats containing three animals in each group were produce diabetes by introducing 140 mg/kg alloxen to each rat and 72 h were left the diabetes to set in then the rat were anaesthetized and one paravertebral long incisions were made through the skin and cutaneous muscle at a distance of about 1.5 cm from the midline on each side of the depilated back of the rat. Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment[10,11]. All the groups were treated in the same manner as mentioned in the case of excision wound model. No ligature was used for stitching.

After the incision was made, the parted skin was kept together and stitched with black silk at 0.5 cm intervals ; surgical thread (No.000) and a curved needle (No.11) were used for stitching.The continuous threads on both wound edges were tightened for good closure of the wound. The wound was left undressed.

Then the treatment was started in the following manner:

Group I : solvent control (PEG-400)
GroupII : standard (Povidone iodine 5% w/w ointment)
GroupIII : chloroform extract (100 mg/mL)
GroupIV : Methanol extract(100 mg/mL)
Group V : Aqueous extract (100 mg/mL)

Application of drug was done once a day after cleaning with surgical cotton wool. All the drugs are administered for nine days to the respective groups; when wounds were cured thoroughly the sutures were removed on the ninth day and tensile strength was measured[12].

The tensile strength was measured by using the

tensiometer.Wound tissues strained with eosin and haematoxlin were studied for anglogenesis, fibrogenesis and epithelisation.

Results were analyzed by students *t* test. *P* values less then 0.001 were taken as significant. The mean and SEM values were calculated and tabulated in Table 4.

The Tensiometer consists of a 6×12 inch wooden board with one arm of 4 inch long, fixed on each side of the possible longest distance of the board .The board was placed at the edge of a table. A pulley with bearing was mounted on the top of one arm. An alligator clamp with 1cm width was tied on the tip of the other arm by fishing line (2016 test monofilament) in such a way that the clamp could reach the middle of the board. Another alligator clamp was tied on a longer fishing line with a polyethylene bottle on the other end. The tensile strength of a wound represents the degree of wound healing. Usually wound promotes a gain in tensile strength. The instrument used for measurement is called a Tensiometer, as explained above. This was designed on the same principle as thread testing in the textile industry one day before performing the experiment (measurement of tensile strength) the sutures were removed from the stitched wound[12].

The suture were removed on the ninth day after wounding and the tensile strength was measured on the tenth day . The sample drugs along with standard and control were administered throughout the period, once daily for 9 days . on the tenth day the rats were again anesthetized and each rat were placed on a stack of paper, towels on the middle of the board. The number of the towels could be adjusted in such a way that the wound was on the same level as the tips of the arms.

The clamps were then carefully attached to the skin on the opposite sides of the wound at a distance of 0.5cm away from the wound. The longer pieces of the fishing line were placed on the pulley and finally on to the polyethylene bottle and the position of the board was adjusted so that the bottle receive a rapid and constant rate of water from a large reservoir until the wound began to open. The amount of water in the polyethylene bag was weighed and considered as an indirect measure of the tensile strength of the wound[13]. The mean determination of tensile strength on the two pavavertebral incisions on both sides of the animals were taken as the measure of the tensile strength of the wound for an individual animal the tensile strength of the extracts treated wound were compared with controls. The tensile strength increment indicates better wound healing profile.

2.4.8. Incision wound model (with infection) with diabetes.

The methodology used for wound formation and diabetes indusing was the same as that of incision wound model without infection. All the rats were infected with a loop full of inoculum of mixed microorganisms comprising of *staphylococcus aureus* and *E.coli*. The mixed microorganisms were prepared by mixing 1mL of each from 106 cfu/mL of *S.aureus* and *E.coli* cultures.

48 h were left for infection to set in and then the treatment was started in the following manner to the different groups till the wound was completely healed.

Group I : solvent control (PEG-400)
GroupII : standard (Povidone iodine 5% w/w ointment)
GroupIII : chloroform extract (100 mg/mL)
GroupIV : Methanol extract(100 mg/mL)
Group V : Aqueous extract (100 mg/mL)

Application of drug was done once a day after cleaning with surgical cotton wool. All the drugs are administered for 9 days to the respective groups; when wounds were cured thoroughly the sutures were removed on the ninth day and tensile strength was measured by Tensiometer by the same procedure as described in the incision model without infection. The parameter used to assess wound healing activity by this model is similar to that used in case of incision model without infection.

2.5. Histopathological study

For histological studies, granulation tissues were fixed in 10% neutral formalin solution for 24 h and dehydrated with a sequence of ethanol-xylene series of solutions 19, 20. The materials were in filtered and embedded with paraffin (40-60 ℃). Microtome sections were taken at 10 μm thickness. The sections were processed in alcohol-xylene series and stained with hematoxylin-eosin dye. The histological changes were observed under a microscope.

2.6. Statistical analysis

The results were expressed as mean±standard error mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test and $P < 0.001$ was considered significant.

3. Results

3.1. Excision wound model

The measurement of the progress of the wound healing induced by (Povidone iodine 5%w/w ointment),Methanol extract, Chloroform fraction, Ethyl acetate fraction in the concentration of 100 mg/mL and respective control group (PEG400) in excision wound model without infection and without diabetic rat in the Table 1. The % wound contracture on on 9th 12th, 15th, 18th, post wound healing days. It was observed that there is complete wound healing on the 21th post wound healing day post wounding day registered 60.95, 78.47, 88.75, 95.60, 100; 60.84, 72.52, 87.44, 94.77, 100; 67.93, 86.01, 92.09, 95.58, 100 with respect to aqueous, methanol and chloroform extract, while standard drug at the same day demonstrated 67.68, 76.84, 85.53, 92.45, 100 of

wound contracture respectively. The solvent control group showed 36.93, 47.24, 64.45, 82.53, 87.42 wound contracture on parallel post wounding days. The studies revealed the skin wound treated with all extracts to respective group showed significant (P<0.05 to P<0.001) activity when compared to solvent control on 3rd, 6th, 9th, 12th, 15th, 18th post wounding day and is comparable with positive control (povidone iodine 5% w/w ointment). It was observed that there is complete healing of wound on the 18 to 21days of post wounding with all extract. The % of wound contraction on 18th in methanolic extract, chloroform fraction, ethyl acetate fraction were found to be 95.60, 94.77, 95.58 respectively. The degree of wound contraction on 18th post wounding day with all extract and its fractions were in the order of methanolic

Table 1
Effect of plant extract and its fraction of Typhonium trilobatum on excised wound (without infection) non diabetic rats.

Group	Wound contraction (mm^2)							Period of epithelialisation
	0	3	6	9	12	15	18	21	
PEG 400	110.21±6.96 (0.00)	90.66±5.92 (17.74)	80.16±2.52 (27.26)	69.5±2.62 (36.93)	58.13±3.40 (47.24)	39.16±3.49 (64.45)	19.23±2.16 (82.53)	13.83±0.47 (87.42)	24.13±0.30
Povidone iodine 5%	103.55±6.54 (0.00)	68.10±5.70a (34.10)	49.16±2.08c (52.39)	33.32±1.91c (67.68)	23.83±1.92c (76.84)	14.83±2.27c (85.53)	7.66±1.28c (92.45)	0 (100)	20.16±0.30
Methanolic extract	87.5±3.81a (0.00)	67.33±6.71a (23.05)	48.33±2.09c (44.76)	34.16±1.37c (60.95)	18.83±1.47c (78.47)	9.83±1.32c (88.75)	3.83±0.70c (95.60)	0 (100)	20.00±0.42
Chloroformfraction	89.83±3.86a (0.00)	68.31±4.68a (24.15)	50.16±2.31c (44.15)	35.16±1.66c(60.84)	24.66±1.83c (72.52)	11.25±2.26b (87.44)	4.66±1.08c (94.77)	0 (100)	21.16±0.44
Ethyl acetate	109.66±7.94 (0.00)	72.22±4.47a (34.14)	55.33±1.94c (49.54)	35.16±1.49c (67.93)	15.33±1.62c (86.01)	8.66±1.82c (92.09)	4.83±1.16c (95.58)	0 (100)	18.16±0.25
F(4,25)	3.13*	3.51*	37.23**	69.61**	62.44**	24.03**	21.35**		

Values are expressed as mean ± SEM; n= 6, One Way ANOVA followed by Dunnet's t-test F-values denotes significance at *P<0.05, **P<0.01, t-value denotes significance at a: P<0.05; b: P<0.01; c: P<0.001 respectively; Number of parenthesis indicate percentage of wound contraction.

Table 2
Effect of plant Extract and its Fraction of Typhonium trilobatum on excised wound (without infection) diabetic rats.

Group	Wound contraction (mm^2)								Period of epithelialisation (days)
	0	3	6	9	12	15	18	21	
PEG 400	86.23±3.04 (0.00)	69.16±2.90 (19.79)	60.83±2.66 (29.45)	56.13±2.28 (34.90)	43.16±2.27 (49.94)	33.33±1.40 (61.33)	23.33±1.62 (72.92)	12.16±0.744(85.87)	26.66±0.30
Povidone iodine 5%	72.16±4.43a (0.00)	48.24±2.95c (33.14)	26.83±2.99c (62.81)	13.16±1.19c (81.75)	8.16±1.30c (88.67)	3.83±1.01c (94.67)	0 (100)	0	20.16±0.30
Methanol extract	78.24±3.94 (0.00)	42.16±2.65c (46.11)	33.83±3.02c (56.75)	25.83±2.05c (65.97)	16.33±1.33c (78.11)	9.16±1.42c (87.27)	3.66±0.88c (94.29)	0 (100)	20.00±0.36
Chloroform Fraction	82.33±4.97 (0.00)	59.22±2.64a (28.06)	50.83±3.43b (38.25)	42.34±2.11c (48.56)	31.24±1.63c (62.04)	16.16±1.56c (80.35)	6.83±1.44c (91.68)	0	19.83±0.47
Ethyl acetate	81.16±4.73 (0.00)	49.24±2.82c (39.32)	34.16±2.53c (57.90)	23.83±1.35c (70.62)	17.24±1.59c (78.73)	10.16±1.64c (87.45)	4.83±1.35c (94.01)	0	18.16±0.16
F (4,25)	1.47	14.50**	33.12**	89.19**	68.83**	63.78**	55.98**		

Values are expressed as mean ± SEM; n= 6, One Way ANOVA followed by Dunnet's t-test F-values denotes significance at *P<0.05, **P<0.01, t-value denotes significance at a: P<0.05; b: P<0.01; c: P<0.001 respectively; Number of parenthesis indicate percentage of wound contraction.

Table 3
Effect of plant extract and its fraction of Typhonium trilobatum on Excised wound (with infection) with diabetes rat.

Group	Wound contraction] (mm^2)							Period of epithelialisation
	0	3	6	9	12	15	18	
PEG 400	76.24±1.93 (0.00)	69.5±3.03 (8.84)	58.16±2.41 (23.71)	46.23±1.39 (39.35)	35.16±1.77 (53.86)	24.83±1.55 (67.40)	13.16±1.92 (82.70)	26.16±0.30
Povidone iodine 5%	61.24±3.09a (0.00)	42.66±2.41c (30.33)	26.5±2.27c (56.71)	11.83±2.10c (80.66)	5.16±1.13c (91.55)	0 (100)	0 (100)	15.18±0.36
Methanol Extract	108.33±4.36c (0.00)	63.83±2.27 (41.03)	53.83±1.60 (50.26)	42.5±6.20 (60.71)	27.83±5.65c (74.25)	11.66±2.12c (89.17)	4.66±1.14 (95.63)	20.50±0.42
Chloroform Fraction	52.33±2.23c (0.00)	43.66±1.91c (16.56)	37.23±1.75c (28.84)	27.16±4.83c (48.08)	15.33±2.95c (70.68)	4.16±1.16c (92.02)	0 (100)	17.66±0.42
Ethyl acetate	53.35±2.58c (0.00)	37.33±2.15c (30.02)	25.66±2.23c (51.90)	15.66±1.87c (70.64)	8.5±2.09c (84.06)	2.5±0.42c (95.30)	0 (100)	16.83±0.40
F(4,25)	62.11**	36.16**	58.48**	83.25**	51.71**	86.93**		

Values are expressed as mean ± SEM; n= 6, One Way ANOVA followed by Dunnet's t-test F-values denotes significance at *P<0.05, **P<0.01, t-value denotes significance at a: P<0.05; b: P<0.01; c: P<0.001 respectively; Number of parenthesis indicate percentage of wound contraction.

Table 4
Effect of plant extract and its fraction of Typhonium trilobatum on Excised wound (with infection) non- diabetic rat

Group	Wound Contraction (mm^2)							Period of epithelialisation
	0	3	6	9	12	15	18	
PEG 400	67.16±3.64(0.00)	54.16±2.08(18.61)	45.22±1.59(31.92)	33.66±1.83(49.13)	23.5±1.52(64.25)	17.66±1.71(72.94)	10.23±1.35(84.00)	25.11±0.36
Povidone iodine 5%	57.5±2.21a(0.00)	38.66±5.94c(32.76)	20.5±1.89c(64.34)	10.66±1.62c(81.45)	3.5±0.22c(93.90)	0(100)	0	20.00±0.36
Methanol extract	60.50±2.32(0.00)	40.5±6.13b(33.05)	36.16±2.13b(40.22)	19.66±4.19c(67.47)	7.45±0.73c(87.63)	3.83±0.54(93.61)	0(100)	21.24±0.36
Chloroform Fraction	57.33±2.31a(0.00)	37.25±8.58c(35.02)	22.33±1.28c(61.04)	12.35±1.03c(78.44)	3.83±0.47c(93.30)	0(100)	0	20.18±0.36
Ethyl acetate	69.33±3.67(0.00)	43.34±8.05b(37.48)	20.50±1.66c(70.35)	14.16±1.83c(79.49)	4.66±1.28c(93.19)	0(100)	0(100)	20.16±0.36
F(4,25)	3.51*	9.39**	41.61**	40.04**	40.72**			

Values are expressed as mean ± SEM; n= 6, One Way ANOVA followed by Dunnet's t-test F-values denotes significance at *P<0.05, **P<0.01, t-value denotes significance at a: P<0.05; b: P<0.01; c: P<0.001 respectively; Number of parenthesis indicate percentage of wound contraction.

Table 5
Effect of plant extract and its fraction of Typhonium trilobatum on incised wound without infection and with infection in non diabetic rats.

Group	Breaking strength (g)	
	Without infection	With infection
PEG 400	416.66±16.66	316.66±16.66
Povidone iodine 5% w/w	625.66±30.95c	616.66±33.33c
Methanolic extract	508.33±27.13a	491.66±15.36c
Chloroform fraction	508.33±23.86a	466.66±16.66c
Ethyl acetate fraction	516.66±21.08a	450.66±18.25b

Values are expressed as mean ± SEM; n= 6 , t-value denotes significance at a: P<0.05; b: P<0.01; c: P<0.001.

Table 6
Effect of plant extract and its fraction of Typhonium trilobatum on Incised wound without infection and with infection in diabetic rats.

Group	Breaking strength(g)	
	Without infection	With infection
PEG 400	366.66±24.72	358.33±20.06
Povidone iodine 5% w/w	650.66±18.25c	633.33±16.66c
Methanolic extract	525.66±30.45b	508.33±15.36c
Chloroform fraction	541.66±20.06c	525.66±25c
Ethyl acetate fraction	525.33±30.95c	525.66±30.95b

Values are expressed as mean ± SEM; n= 6 , *t*-value denotes significance at a: $P<0.05$; b: $P<0.01$; c: $P<0.001$.

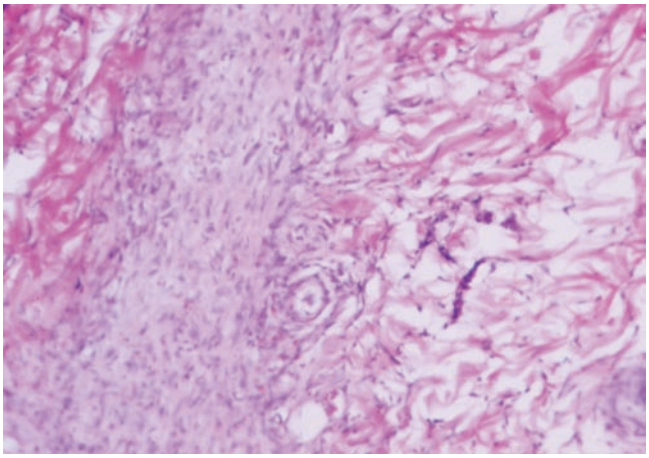

Figure 1. Without infection and non diabetes (ethyl acetate fraction).

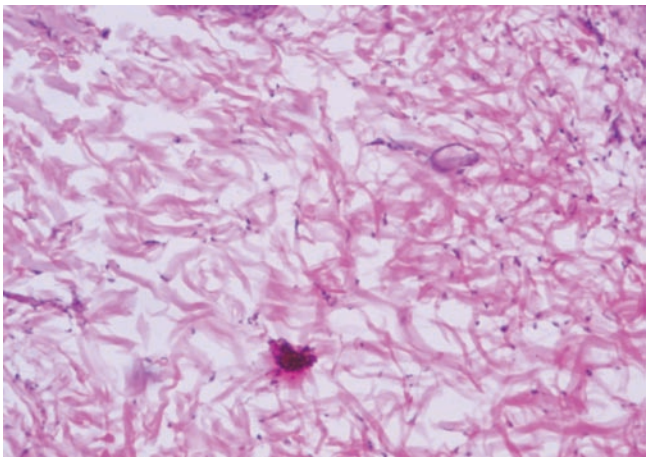

Figure 2. Without infection and non diabetes (solvent)

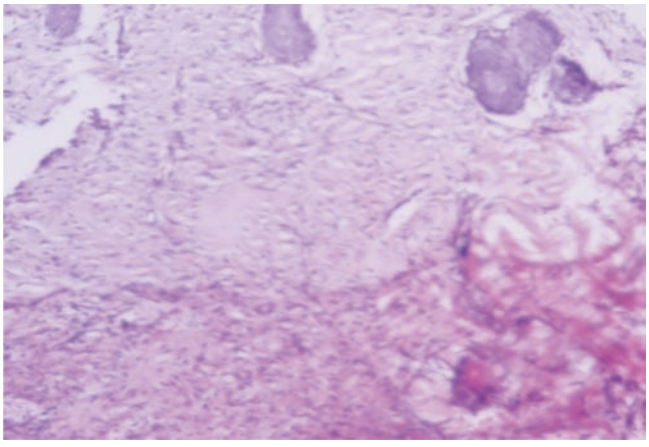

Figure 3. Without infection and diabetes (Chloroform fraction).

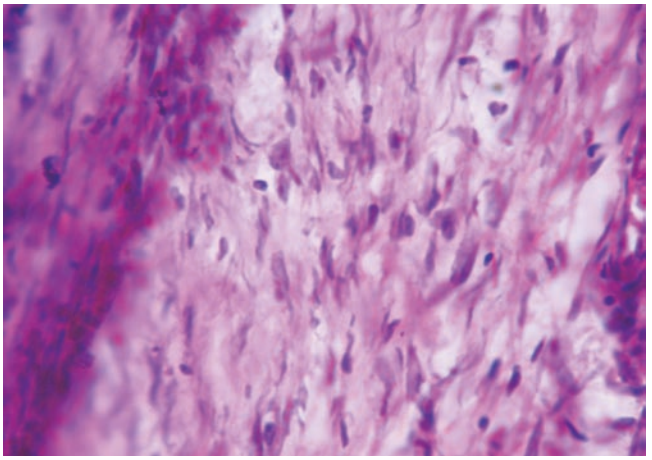

Figure 4. Without infection and diabetes (solvent).

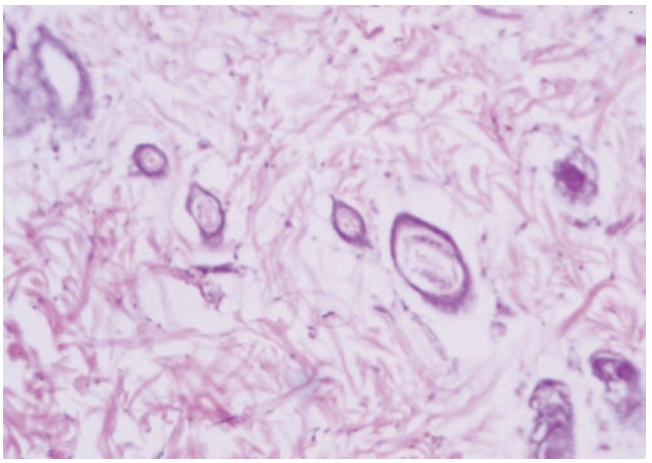

Figure 5. With infection and diabetes (Ethyl acetate fraction).

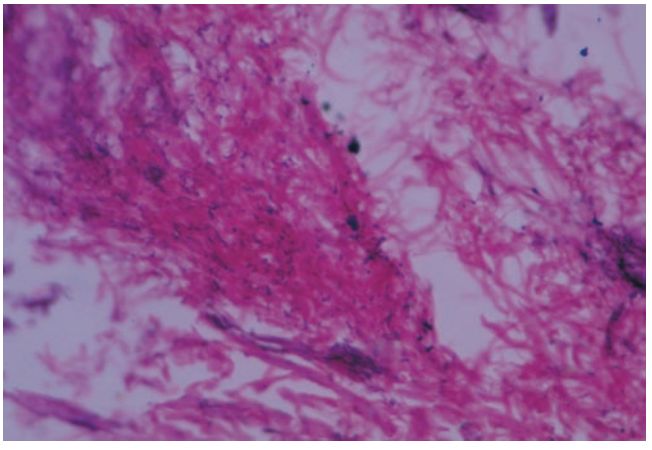

Figure 6. With infection and diabetes (Solvent).

extract>ethylacetate fraction>chloroform fraction. However on the same post wounding day povidone iodine 5% w/w ointment and PEG -400 treated group registered 92.45% and 82.53% wound contraction respectively. The skin biopsy of the excised uninfected wound collected on 21st day of solvent control group shows, ulcer covered by debris, stroma reveals presence of granulation tissue, edema, congested vessels and scattered mixed inflammatory cell. There is no evidence of granuloma, dysplasia or malignancy. The skin biopsy reports for ,methanolic extract, treated group shows healing of ulcer with fibrosis where as stroma reveals mild inflammation with lymphoid cells and mild to moderate oedema. Epithelialization is complete with normal histology of epidermis (Figure 1, 2). There is no evidence of granuloma , dysplasia and malignancy. The healing of the ulcer by use of plant extract may be due to the in-vivo antimicrobial activity of the phytoconstituent like flavonoids, terpenoids, present in the extract[14]. This phytoconstituent are also

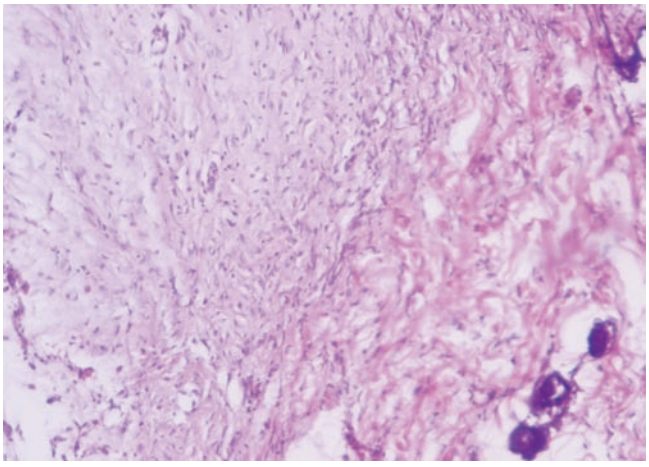

Figure 7. With infection and non diabetes (Ethyl acetate fraction).

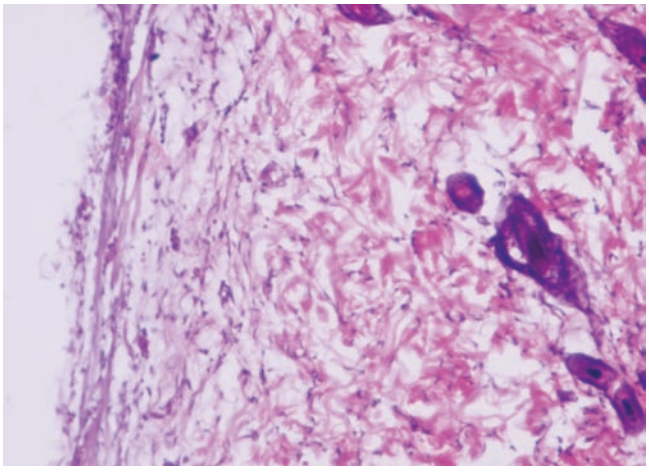

Figure 8. With infection and non diabetes (solvent).

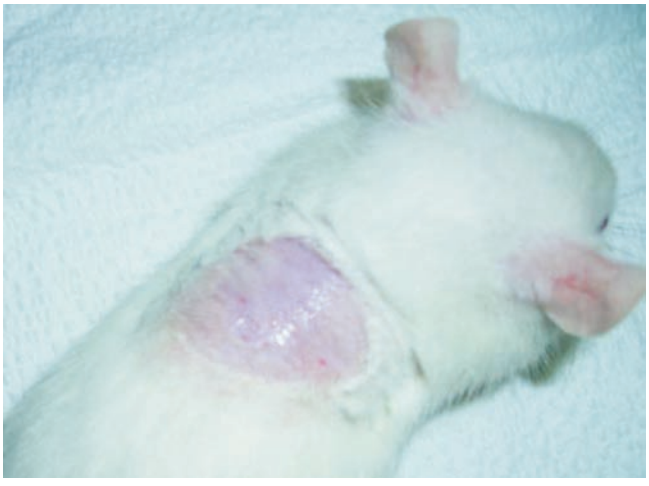

Figure 9. Excision wound formation on skin.

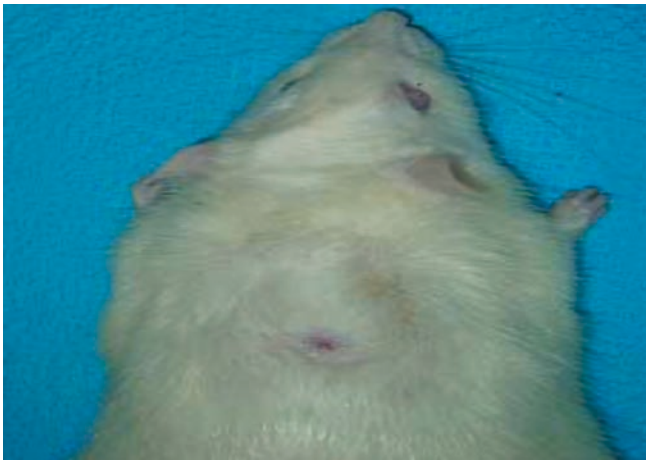

Figure 10. Excision wound healing activity.

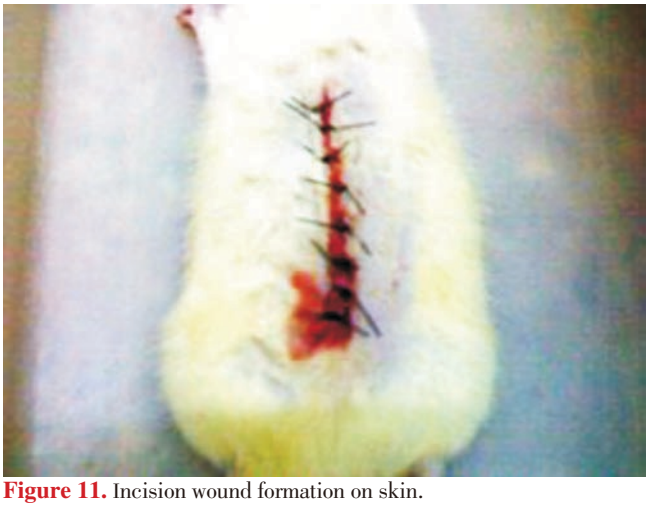

Figure 11. Incision wound formation on skin.

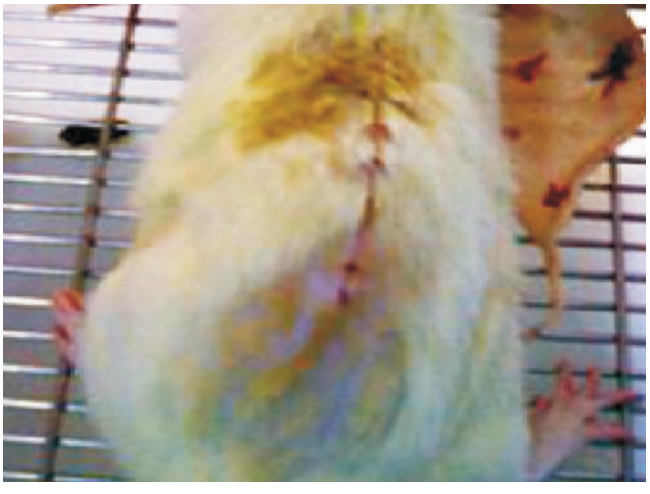

Figure 12. Incision wound healing activity.

known to promot wound healing activity mainly due to antimicrobial property,which seem to be responsible for wound contraction and increased rate of epithelialisation(Ya c, *et al* and Goren N, *et al*).

In case of excision wound model without infection and with diabetic rats in the Table 2. The % wound contracture on 9th, 12th, 15th, 18th, and 21st post wounding day registered 65.97, 78.11, 87.27, 94.29, 100; 48.56, 62.04,80.35, 91.68,100; 70.62, 78.73, 87.45, 94.01, 100; with respect to methanolextract,chloroform fraction,ethylacetate fraction while standard drug at the same day demonstrated 81.75, 88.67, 94.67, 100, 100%of wound contracture respectively. The solvent control group showed 34.90, 49.94, 61.33, 72.92, 85.87% wound contracture on parallel post wounding days. The studies revealed the skin wound treated with all extracts to respective group showed significant ($P<0.05$ to $P<0.001$) activity when compared to solvent control on 3rd, 6th, 9th, 12th, 15th, 18th post wounding day and is comparable with positive control (povidone iodine 5% w/w ointment).It was observed that there is complete healing of wound on the 18 to 21days of post wounding with all extract. The % of wound contraction on 18th in methanolic extract,chloroform fraction,ethylacetate fraction were found to be 94.29%, 91.68%, and 94.01% respectively. The degree of wound contraction on 18th post wounding day with all extracts were in the order of Methanol extract>ethylacetate fraction>chloroform fraction. However on the same post wounding day povidone iodine 5% w/w ointment and PEG -400 treated group registered 100% and 72.92% wound contraction respectively. The biopsy of skin of excised wound of methanolic extract treated group without infection on 18th day of treatment showed healed ulcer, fibrosis and epithelisation (Figure 3, 4). No inflammatory cell collection or granuloma is detected. The healing of the ulcer by use of plant extract may be due to the *in-vivo* antimicrobial activity of the phytoconstituent like flavonoids, terpenoids, present in the extract[15]. This phytoconstituent are also known to promot wound healing activity mainly due to antimicrobial property,which seem to be responsible for wound contraction and increased rate of epithelialisation (Ya C, *et al* and Goren N, *et al*) .

In case of excision wound model with infection and with diabetic rat in the Table 3. The studies revealed the skin wound treated with Extract and fraction to respective group showed activity when compared to solvent control and positive control (Povidone iodine 5%w/w ointment) on 9th 12th, 15th, 18th and 21th post wound healing days registered 60.71, 74.25, 89.17, 95.63, 100; 48.08, 70.68, 92.02, 100, 100; 70.64, 84.06, 95.30, 100, 100; with respect to methanol extract, chloroform fraction, ethyl acetate fraction, while standard drug at the same day demonstrated 80.66,91.55,100,100,100; % of wound contracture respectively. The solvent control group showed 39.35, 53.86, 67.82.70, 89.60;% wound contracture on parallel post wounding days. The studies revealed the skin wound treated with all extracts to respective group showed significant ($P<0.05$ to $P<0.001$) activity when compared to solvent control on 3rd, 6th, 9th, 12th, 15th, 18th post wounding day and is comparable with positive control (povidone iodine 5% w/w ointment).It was observed that there is complete healing of wound on the 18 to 21 days of post wounding with all extract.The percentage of wound contraction on 18th post wounding day was found to be 95.63, 100, 100 % for methanolic extract chloroform fraction, ethyl acetate fraction treated group respectively by local application. The degree of wound contraction on 18th post wounding day with all extracts were in the order of chloroform fraction, ethyl acetate fraction>methanol extract.. However on the same post wounding day povidone iodine 5% w/w ointment and PEG -400 treated group registered 100% and 82.70% wound contraction respectively. The biopsy of excised wound with infection on 18th day of treatment showed focal ulceration covered by fibrinoid material.Stroma reveals pump fibroblasts and granulation tissue (Figure 5, 6). No significant inflammatory reaction is seen. Intact portion of epidermis and dermis shows normal histology. The data in the observation table revealed that the extent of wound contraction in case of infected excised model is less than excised uninfected model. The healing of the ulcer by use of plant extract may be due to the in-vivo antimicrobial activity of the phytoconstituent like flavonoids, terpenoids, present in the extract[16]. This phytoconstituent are also known to promot wound healing activity mainly due to antimicrobial property,which seem to be responsible for wound contraction and increased rate of epithelialisation(Ya C, *et al* and Goren N, *et al*) .

IV)In case of excision wound model with infection and non diabetic rat in the Table 4. The studies revealed the skin wound treated with Extract and fraction to respective group showed activity when compared to solvent control and positive control (Povidone iodine 5%w/w ointment) of 9th, 12th, 15th, 18th post wound healing days registered 67.47, 87.63, 93.61, 100, 100; 78.44, 93.30, 100, 100, 100; 79.49, 93.19, 100, 100, 100; with respect to methanol extract, chloroform fraction, ethyl acetate fraction, while standard drug at the same day demonstrated 81.45, 93.90, 100, 100, 100; %of wound contracture respectively. The solvent control group showed 49.13, 64.25, 72.94, 84.00, 92.23;% wound contracture on parallel post wounding days. The studies revealed the skin wound treated with all extracts to respective group showed significant ($P<0.05$ to $P<0.001$) activity when compared to solvent control on 3rd, 6th, 9th, 12th, 15th, 18th post wounding day and is comparable with positive control (povidone iodine 5% w/w ointment).It was observed that there is complete healing of wound on the 18 to 21 days of post wounding with all extract and its fractions. The percentage of wound contraction on 15th post wounding day was found to be 93.61, 100, 100 % for methanolic extract and chloroform fraction, ethyl acetate fraction treated group respectively by local application . The degree of wound contraction on 15th post wounding day with all extracts and its were in the order of chloroform fraction,ethyl acetate fraction>methanol extract. However on the same post wounding day povidone iodine 5% w/w ointment and PEG -400 treated group registered 100% and 72.94% wound contraction respectively. The data in the observation table revealed that the extend of wound

contraction in case of infected excised model is less than excised uninfected model. The biopsy of excised wound with infection on 18th day of treatment showed healing of ulcer with normal epithelisation with fibrosis over it (Figure 7, 8) .There is no evidence of granuloma, dysplasia and malignancy .In case of solvent control group the biopsy report showed incomplete wound healing with persistence of ulceration.Therefore it is suggested that all extracts have wound healing activity in excised infected model (Figure 11, 12). But methanol extract shows less activity chloroform fraction and ethyl acetate fraction shows high activity. The healing of the ulcer by use of plant extract may be due to the *in-vivo* antimicrobial activity of the phytoconstituent like flavonoids, terpenoids, present in the extract[17]. This phytoconstituent are also known to promot wound healing activity mainly due to antimicrobial property,which seem to be responsible for wound contraction and increased rate of epithelialisation(Ya C, *et al* and Goren N, *et al*) .

3.2. Incision wound model

All the extract of *Typhonium trilobatum* was studied to assess the wound healing properties in albino rats by incision wound model (without infection) and (with infection) in non diabetic and the result are reported in Table 5. In the incision wound (without infection) and (with infection) studies, there was a increased tensile strength on 10th post wounding day with all extracts when compared with solvent control (PEG-400). The tensile strength was observed on 10th post wounding day with ethyl acetate fraction, methanolic extract, chloroform fraction were found to be 516 gm,510 gm, 508 gm in group without infection) and 450 gm, 466 gm, 491 gm in group with infection. The order of increase in tensile strength for extract and its fraction were in order of ethyl acetate fraction>chloroform fraction>methanolic extract in (without infection) and methanolic extract>chloroform fraction>ethyl acetate fraction in (with infection) group. The positive control povidone iodine 5% w/w ointment and solvent control (PEG -400) registered tensile strength up to the extent of 625 gm and 416 gm respectively in (without infection) group and 616 gm and 316 gm in (with infection) group. It is worth mentioning here that all test extract and its fraction obtained from the plant of *Typhonium trilobatum* possess wound healing activity in both without infection and with infection group in non diabetic rats. The biopsy of incised wound (without infection) and (with infection) in non diabetic rats show healed ulcer. Stroma reveals fibrous tissue and mild non-specific inflammation[18]. Intact portion of skin shows normal histology. Normal appearing epidermis and dermis. No inflammatory reaction or granuloma is seen.

All the extract of *Typhonium trilobatum* was studied to assess the wound healing properties in albino rats by incision wound model (without infection) and (with infection) in diabetic rat and the result are reported in Table 6. In the incision wound (without infection) and (with infection) studies, there was a increased tensile strength on 10th post wounding day with all extracts when compared with solvent control (PEG-400). The tensile strength was observed on 10th post wounding day with chloroform fraction, ethyl acetate fraction, methanolic extract were found to be 541 gm, 530 gm, 525 gm in group without infection and 525 gm, 528 gm, 508 gm in group with infection. The order of increase in tensile strength for different extract were in order of chloroform fraction>ethyl acetate fraction>methanolic extract in (without infection) and ethyl acetate fraction>chloroform fraction>methanolic extract in (with infection) group[19, 20]. The positive control povidone iodine 5% w/w ointment and solvent control (PEG -400) registered tensile strength up to the extent of 650 gm and 366 gm respectively in group without infection and 633 gm and 358 gm in group with infection. It is worth mentioning here that test extracts and its fractions obtained from the plant of *Typhonium trilobatum* possess wound healing activity in both without infection and with infection group in non diabetic rats. The biopsy of incised wound (without infection)and (with infection)in non diabetic rats show healed ulcer (Figure 9, 10). Stroma reveals fibrous tissue and mild non-specific inflammation. Intact portion of skin shows normal histology. Normal appearing epidermis and dermis. No inflammatory reaction or granuloma is seen.

4. Discusion

Wound healing is stepwise process, which consists of different phases such as hemostasis, inflammation, proliferative and remodeling or maturation. The genetic response regulating the body's own cellular resistance mechanisms contributes to the wound and its repair[21]. Therefore, in this study two different models were used to establish the healing potentials of methanolic, chloroform and ethyl acetate extracts of *Typhonium trilobatum* on various phases.

In incision wound, the increase in tensile strength of treated wounds may be due to the increase in collagen concentration and stabilization of the fibres. A healing tissue synthesizes collagen, which is a constituent of growing cell[22-27]. Increase in blood vessels and may be for antioxidant property of that plant. From the observations, it was evident that *Typhonium trilobatum* possesses a definite potential healing action. The breaking strength of the incision wounds was increased in ethyl acetate and chloroform extract treated groups.

In excision wound healing model (without infection and without diabetes, without infection and with diabetes, with infection and without diabetes, with infection and with diabetes), the methanol and ethyl acetate extract of the plant *Typhonium trilobatum* showed significant increase in percentage closure by enhanced epithelialization. This enhanced epithelialization may be due to the effect of *Typhonium trilobatum* extracts on enhanced collagen synthesis. The higher breaking strength indicates better healing of wounds. Thus it supports the wound healing activity of *Typhonium trilobatum*. Further study has to be

need for active constituents of this plant mainly Flavonoids which can help for wound healing. The study reveals that methanolic, ethyl acetate and chloroform extracts treated groups possess good wound healing properties which may be attributed to the individual or combined action of phytoconstituents like, flavanoids, alkaloids, saponins and tannins.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are thankful to the Managing Trustee of School of Pharmaceutical Sciences College.

Reference

[1] Padmaa M Paarakh, Chansouria J P N, KhosaR L. Wound healing activity of Annona muricata extract. *J Pharm Res* 2009; **2**: 404-406.

[2] Lambole Vijay, Upendra Kumar. Effect of Moringa oleifera Lam. on normal and dexamethasone suppressed wound healing. *Asian Pacific Journal of Tropical Biomedicine* 2012; **2**: 219-S223.

[3] Krishna Murti, Upendra Kumar. Enhancement of wound healing with roots of Ficus racemosa L. in albino rats. *Asian Pacific Journal of Tropical Biomedicine* 2012; **2**: 276-80.

[4] Mohammed Rahmatullah, Lutfor Rahman, Fatema Rehana, Marjina Akter Kalpana, Mst. Afsana Khatun et al. A Scientific Evaluation of Medicinal Plants Used in the Folk Medicinal System of Five Villages in Narsinghdi District, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*2010; **4**: 55-64.

[5] Das.P, Palai.S.K, Samantaray.S. In vitro somatic embryogenesis in *Typhonium trilobatum* schott. *Plant Growth Regulation* 1999; **27**: 193-97.

[6] The wealth of India, raw material. "council of scientific and industrial research" 2005.

[7] Boominathan., R., Parimaladevi., B. Mandal., S.C. and Ghoshal., S.K; Anti-inflammatory evaluation of Ionidium suffruticosam Ging in rats, J. *Ethnopharmacol.*, 2004, **91** (2-3), 367-70.

[8] Bartkova J., Grøn B., Dabelsteen E., and Bartek J; Cell-cycle regulatory proteins in human wound healing. *Archives of Oral Biology*, 48(2), 2003, 125-132.

[9] BS Nayak and Lexley M Pinto Pereira. Catharanthus roseus flower extract has wound-healing activity in Sprague Dawley rats. BMC Complementary and Alternative Medicine. *BMC Complementary and Alternative Medicine* 2006; **6**: 1-6.

[10] Mohd Asif Khan, Amit Saxena, Farheen Tabassum Fatima, Gaurav Sharma, Veerana Goud, Asif Husain. Study of wound healing activity of Delonix regia flowers in experimental animal models. Am. *J. PharmTech Res*. 2012; **2**: 380-90.

[11] Hiranand Dewangan, Manju Bais, Vinay Jaiswal and Vinod Kumar Verma. Potential wound healing activity of the ethanolic extract of Solanum xanthocarpum schrad and wendl leaves. Pak. *J. Pharm. Sci.* 2012; **25**: 189-194.

[12] A. A. Zahra, F. A. Kadir, A. A. Mahmood, A. A. A hadi, S. M. Suzy, S. Z. Sabri, I. I. Latif and K. A. Ketuly. Acute toxicity study and wound healing potential of Gynura procumbens leaf extract in rats. *Journal of Medicinal Plants Research* 2011; **5**: 2551-2558.

[13] Manish Pal Singh and Chandra Shekhar Sharma. Wound healing activity of Terminalia Chebula in experimentally induced diabetic rats. *Int.J. PharmTech Res*.2009; **1**: 1267-1270.

[14] Nalesh Malviya and Sanjay Jain. Wound healing activity of aqueous extract of radix paeoniae root. *Acta Poloniae Pharmaceutica* ñ *Drug Research*2009; **66**: 543-47.

[15] Matadeen Bharti, R. C. Saxena, Omrao Singh Baghel, Rahul Saxena and K. G. Apte. Wound healing activity of leaf of Nyctanthes arbor-trisitis (linn.). *IJPSR* 2011; **2**: 2694-98.

[16] Chitra Shenoy, M B Patil, Ravi Kumar and Swati Patil. Preliminary phytochemical investigation and wound healing activity of allium cepa linn (liliaceae). *International journal of pharmacy Pharmacy and Pharmaceutical Sciences*2009; **2**: 167-75.

[17] A. A. Zahra, F. A. Kadir, A. A. Mahmood, A. A. Al hadi, S. M. Suzy, S. Z. Sabri, I. I. Latif and K. A. Ketuly. Acute toxicity study and wound healing potential of Gynura procumbens leaf extract in rats. *Journal of Medicinal Plants Research* 2011; **5**: 2551-58.

[18] K Ilango and V Chitra. Wound Healing and Anti-oxidant Activities of the Fruit Pulp of Limonia Acidissima Linn (Rutaceae) in Rats. *Tropical Journal of Pharmaceutical Research June* 2010; **9** (3): 223-230.

[19] G. K. Dash and P. N.Murthy. Studies onWound Healing Activity of Heliotropium indicum Linn. Leaves on Rats. *International Scholarly Research Network Volume* 2011; **1**: 1-8.

[20] Vandana Panda, Madhav Sonkamble and Swati Patil. Wound healing activity of Ipomoea batatas tubers (sweet potato). *Functional Foods in Health and Disease* 2011; **10**: 403-15.

[21] Lodhi S, Pawar R, Jain A, Singhai A. Wound healing potential of Tephrosia purpurea (Linn.) Pers. in rats. *J. Ethnopharmacol* 2006, **108**: 204-10.

[22] Pattanayak SP, Sunita P. Wound healing, anti-microbial and antioxidant potential of Dendrophthoe falcata (L.f). Ettingsh. *J Ethnopharmacol* 2008; **120**: 241-247.

[23] Sharma S, Sharmaa MC, Kohli DV. Wound healing activity and formulation of Ether-Benzene-95% ethanol extract of herbal drug Madhuca Longifolia leaves in Albino rats. *J Optoelectr Biomed Mat* 2010; **1**: 13 - 15.

[24] Jayasutha J, Monic Josephine Nithila S. Evaluation of wound healing activity of ethanolic extract of Aristolochia bracteata and Cassia tora on Wistar Albino rats. *Int J Pharm Tech Res* 2011; **3**: 1547-50.

[25] Suruse P, Kale MK, Gunde M, Amnerkar N, Pathak AK. Evaluation of wound healing activity of arisaema leschenaultii blume. In rats. *Pharmacia Lettre* 2011; **3**: 200-6.

[26] Akram E, Masoud K, Vahid A. Evaluation of the antibacterial and wound healing activity of Quercus persica. *J Basic App Sci* 2012; **8**: 118-123.

[27] Nayak BS, Lexley M Pinto Pereira. Catharanthus roseus flower extract has wound-healing activity in Sprague Dawley rats. *BMC Complement Alter Med* 2006; **6**: 41.