Research Article**Evaluation of wound healing prospective of *Momordica charantia*, *Pongamia glabra* and *Piper nigrum* on anemic albino rats using incision wound model****Rashmi Shukla, Varsha Kashaw***

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Received: 10 November 2018

Revised: 14 December 2018

Accepted: 19 December 2018

Abstract

Objective: The objective of present work was to evaluate wound healing potential of *Momordica charantia*, *Pongamia glabra* and *Piper nigrum* using as herbal ointment formulation on albino rats using incision wound model. These plants were contain alkaloidal, glycoside and flavonoid derivatives and were used as anti-diabetic, anti-inflammatory, antitumor, anti-malarial and having wound healing potential. **Materials and Methods:** Extract of all three plants has been separated by the Soxhlet extraction. Herbal ointment formulation has been prepared by mixing the extract of *Momordica charantia*, *Pongamia glabra* and *Piper nigrum* with the wool fat and paraffin. Incision wound model has been utilized for the evaluation of wound healing potential. Histopathological evaluation has been also carried-out. **Results and Conclusion:** The studies on incision wound healing model reveals that all twelve groups showed decreased wound area on the time and there was no mortality observed in the course of study. **Discussion:** These studies have indicated that herbal ointment formulation of *Momordica charantia*, *Pongamia glabra* and *Piper nigrum* has been utilized for wound healing and it is safer for topical application. No toxicity and mortality has been observed during the experimental tenure.

Keywords: Wound healing, *Momordica charantia*, *Pongamia glabra*, *Piper nigrum*, incision wound model

Introduction

A wound is a kind of skin injury in which a tear, cut or puncture occurs in the skin. Pathologically, it reflects a sharp damaging injury to the dermis of the skin or it may be epidermal wounds. The main processes that are involved in wound healing are epithelization, contraction, and connective tissue deposition. Healing processes are governed by the biosynthesis and deposition of new collagens at the site of the wound (Agarwal et al., 2009). The process of wound healing involves the migration of endothelial cells, leading to the neo-vascularization of connective tissues which synthesize extracellular matrices including collagens, and keratinocytes leading to re-epithelialization of the wounded tissue. Wound healing in diabetes (streptozotocin induces it) is a complicated and delayed process in which wound healing follows granulation, collagenation and scar formation. Hyperglycemia suppress cell

proliferation collagen production (Kamer et al., 2010).

Momordica charantia belong to *Cucurbitaceae* family. The fruit has a distinct warty looking exterior and an oblong shape. It is hollow in cross section, with a relatively thin layer of flesh surrounding a central seed cavity filled with large flat seeds (Jayasooriya et al., 2000) and pith. (Figure 1: Fruit of *M. Charantia*). *M. Charantia* consists the following chemical constituents charantin, diosgenin, gentisic acid, momorcharasides, momorcharins, momordenol, multiflorenol, myristic acid and nerolidol. *Momordica charantia* is used as anthelmintic, anti-mycobacterial, antioxidant, antitumor, wound healing, antiulcer, antiviral, hypoglycemic and immune-stimulant (Chia et al., 2011; Leelaprakash et al., 2011; Rajaram et al., 2012; Rawat et al., 2012).

Pongamia glabra (Figure 2) belong to *Fabaceae* family (Sharma et al., 2004). Fruit contains furano-flavonoids, coumestan and pongacoumestan. *P. glabra* has been reported to contain a large number of furano flavonoids e.g. karanjin, pongapin, kanjone, pongamol, and pongaglabrone, along with a number of simple flavonoids and lipid like arachidonic acid (Bandivdekar et al., 2002). It used as bacteriocidal

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DOI: <https://doi.org/10.31024/ajpp.2019.5.2.26>

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Figure 1. Fruit of *M. Charantia*

Figure 2. Leaves, flowers and seeds of *Pongamia glabra*

activity against *V. cholerae* and *E. coli*, as well as anti-inflammatory and antipyretic properties (Krishnan et al., 2013).

Piper nigrum (Figure 3) belonging to family piperaceae (Sunila et al., 2004). The fruits have small globose drupe and was known as a peppercorn when dried (Sudjarwo et al., 2005). Pepper plants grow easily in the shade and require little maintenance until harvest, so they are frequently cultivated for supplemental income on even small farms. Antioxidant active chemicals isolated from black pepper includes camphene, carvacrol, eugenol, myrcene myristic-acid, myristicin, palmitic-acid and ubiquinone were the major chemical compounds responsible for the aroma, pungency and medicinal property of the black pepper.

Black pepper is used to improve digestion, stimulate appetite,

Figure 3. Unripe and ripe seeds of *Piper nigrum*

and treat gastrointestinal problems, including diarrhea, dyspepsia and flatulence. It is also used to treat colds, coughs and sore throats. The object of the present paper is to determine the wound healing potential of *Momordica charantia*, *Pongamia glabra* and *Piper nigrum*. The incision wound healing model has been utilized for the assessment of the wound healing potential.

Materials and methods

Plant material collection and authentication

The fruits of *Momordica charantia* were collected at in the month of July, 2016 from local field areas of Bhopal, Madhya Pradesh. Leaves of *Pongamia glabra* collected from Garden and Fruits of *Piper nigrum* from local market. The specimens were submitted and identified as fruits of *Momordica charantia* (MC) family of Cucurbitaceae, leaves of *Pongamia glabra* (PG) family of Fabaceae, fruits of *Piper nigrum* (PN) family of Pipereacea and authenticated by Dr. Zia ul Hassan, Department of Botany, Saifia Science College, Bhopal. The appession no. is 490/BS/saifia/16 has been preserved for future identification.

Soxhlet extraction

The crude drugs were dried in shade. Then moderately coarse powder of the drugs e.g. *Momordica charantia* (MC), *Pongamia glabra* (PG) and *Piper nigrum* (PN) were subjected to successive soxhlet extraction. Soxhlet extraction has been carried, taking 80 gm of dried coarsely powdered drug was packed in Soxhlet apparatus and defatted with 1000 ml of petroleum ether (40-60°C) till complete defatted. Complete de-fatting ensured by placing a drop by thimble on the filter paper which did not exhibited any oily spot. The defatted material was removed from the Soxhlet apparatus and air dried to remove the last traces of petroleum ether. The defatted material was subjected to extraction by ethyl acetate and then with ethanol as solvent by Soxhlet apparatus and finally with water by maceration process. The completion of extract was confirmed by evaporating a few drops of the extract on the watch glass and ensuring that no residue remained after evaporating the solvent.

The marc was air dried before extracted with the next solvent. Dried marc was macerated with water for 24 h. The extracts were evaporated under reduced pressure at low temperature (30°C) to dryness to yield different extracts, stored in an airtight container in refrigerator for further experimental studies. They were weighed to a constant weight and percentage w/w basis was calculated.

Herbal ointment formulation

Herbal preparations were formulated by combination of three plant extract of *Momordica charantia*, *Pongamia glabra* and *Piper nigrum* with wool fat and paraffin. The alcoholic and aqueous extracts were selected for formulation as they have the higher content of flavonoids and phenolic compounds.

Preparation of Simple Ointment (B.P.) Base

The simple ointment base was prepared by mixing the wool fat, hard paraffin, cetostearyl alcohol and white soft paraffin with gentle heating with stirring (Khan et al., 2013). The obtained ointment base is then cooled and stored. Two formulations were prepared by Fusion method e.g. one containing all three extracts of above mentioned plants parts in equal ratios of alcoholic and aqueous extracts i.e. 3.33% w/w of each extract for the preparation of 10 % w/w ointment in ointment base and the other one containing all three extracts of above mentioned plants parts in equal ratios of alcoholic and aqueous extracts (Sawant et al., 2016) i.e. containing 5.0% w/w of each extract, equal to total 15% w/w in ointment base shown in table 1. The prepared formulations were then evaluated by various parameters e.g. consistency, stability etc.

Table 1. Formulation of ointment

Content	Quantity (%)	
	F1	F2
MCAQ (1:1)	3.33	5.0
PGAQ (1:1)	3.33	5.0
PNAQ (1:1)	3.33	5.0
Ointment base	Q.S.	Q.S.

Where, MCAQ- Alcoholic and Aqueous extracts of *Momordica charantia* in 1:1 quantity; PCAQ- Alcoholic and Aqueous extracts of *Pongamia glabra* in 1:1 quantity; PNAQ- Alcoholic and Aqueous extracts of *Piper nigrum* in 1:1 quantity.

Anemic wound healing activity

Incision wound model using anemic albino rats was selected for assessing the wound healing activity. This model was employed to study the rate of wound contraction, time required for full epithelization and tensile strength. These parameters were selected and albino rats were selected by easy availability and simplicity in handling them.

Procurement of animals

Institutional Animal Ethics Committee (IAEC), Registration number CPCSEA/1413/ PO/ES) has been permitted for animal studies. Albino rats were procured and rats of either sex weighing 150-200 gm were selected and maintained at 24-28°C, housed individually with free access to food and water. They were fed with standard diet and kept in well-ventilated animal house with

alternate dark-light cycle of 12h throughout the studies.

Oral toxicity studies

Albino rats of weight 150-200gm were selected for acute oral toxicity study and it was conducted according to the "Organization for Environmental Control Development" guidelines (OECD: Guidelines 420; Fixed Dose Method) for oral administration of extracts. Eighteen hour overnight fasted animals were subjected to oral administration of extracts at a dose of 2000 mg/kg body weight. All the animals were kept under observation for first 3h for any changes or toxic effects like neurological, gross behavioral and lethality. The animals were observed and confirmed the absence of any toxic effects, hence two dose of combination 10% and 15% ointment were prepared with simple ointment base for topical application and 10mg/ml oral dose taken for study.

Sample Preparation

Sample solutions each of 10 mg/ml of extracts was prepared separately and mixed so as each combination will contain 20 % w/v aqueous extract of *Piper nigrum* - PNAQ.

Anemic incision wound model

For the anemic incision wound studies, seventy-two, albino rats were taken, divided in two main groups: non-anemic group (n=6) and anemic group. The anemic group was further divided into eleven groups of six each (Kumar et al., 2006).

To perform the experiment, the rats were divided into Eleven groups (n=6).

Group I : Control group which received simple vehicle (Ointment base)

Group II: Standard group received Povidone iodine ointment

Group III: Test group received MCAQ extract orally

Group IV: Test group received MCAQ and PNAQ extract orally

Group V : Test group received PGAQ extract orally

Group VI: Test group received PGAQ and PNAQ extract orally

Group VII: Test group received MCAQ, PGAQ and PNAQ orally

Group VIII: Test group received MCAQ, PGAQ and PNAQ formulation F1 (10%) topically

Group IX: Test group received MCAQ, PGAQ and PNAQ formulation F2 (15%) topically

Group X : Test group received MCAQ, PGAQ and PNAQ orally and formulation F1 (10%) topically

Group XI: Test group received MCAQ, PGAQ and PNAQ orally and formulation F2 (15%) topically

Group XII: Anemic Group received no treatment

Induction of re-sutured incisional wound (Kumar et al., 2006)

The anemic animals were anaesthetized with Ketamine (100mg/kg b.w.) before the surgical procedure. Dorsal surface of each animal was shaved with a sterile blade under all aseptic measures. One linear incisional wound of whole skin thickness of length 4cm was made on back of each animal. Parallel and 1cm lateral from the vertebral column on either side. After complete hemostasis the wounds were closed by means of interrupted stitches at 1 cm gap with 4-0 silk (sterile). All the above surgical maneuvers were done with full aseptic measure. Immediately after wounding all the rats were kept in separate spacious clean cages to avoid damage to wound and infection. After certain duration the rats come out of the effect of anesthesia; food and water were given ad libitum after 2 to 3 hours of the day of operation. No local or systemic antibiotics were given in the post-operative period. The animals were inspected daily for any evidence of infection and the animals showing infection were excluded from the study. The day of wounding was referred as day-1.

Tensile strength in incision wound model

The sutures were removed in incision wound model under aseptic measures on 8th post wounding day after anesthetization for further determination of tensile strength. On 10th day the rats

were again anesthetized and each rat is placed on a stack of paper towel on the middle of the board. The amount of the towel could be adjusted in such a way so that the wound is on the same level of tips of the arms. The clamps are then carefully clamped on the skin of the opposite side of the skin of wound at a distance of 0.5 cm away from the wound. The longer pieces of the fishing line are placed on the pulley and finally to the polyethylene bottle and the position of the board is adjusted so that the bottle receive a rapid and constant rate of water from the large reservoir, until the wound began to open. The amount of water in polyethylene bag is weighed and consider as tensile strength of the wound.

Statistical analysis

The statistical analysis of experimental data was done with oneway analysis of variance, and SPSS software (version 11.5). $P < 0.001$ was considered as highly significant.

Results and discussion

Extraction

The extraction was done by successive solvent extraction, to increase the extraction, to achieve separation of compounds in different extracts and decrease the time taken by extraction process the flask and Soxhlet apparatus was covered by cotton to increase the insulation. The drying of extract containing solvent (Petroleum ether, ethyl acetate, ethanol and water) was done by vacuum distillation process. Percent yield of all extract was depicted in table 2.

Table 2. Percentage yield of different extracts

Parts	Solvents	Extract color	Yield (in gm)	% Yield w/w
FMC (fruits of <i>Momordica charantia</i>)	PMMC	Yellowish green	5.54	6.93
	EFMC	Brown	4.72	5.9
	AFMC	Dark Brown	16.05	20.06
	QFMC	Greenish brown	13.08	16.35
LPG (leaves of <i>Pongamia glabra</i>)	PLPG	Greenish brown	1.624	2.03
	ELPG	Brown	2.94	3.675
	ALPG	Dark Brown	6.264	7.83
	QLPG	Brownish Black	5.024	6.28
FPN (fruits of <i>Piper nigrum</i>)	PFPN	Yellowish green	2.304	2.88
	EFPN	Brown	1.664	2.08
	AFPN	Dark Brown	10.158	12.698
	QFPN	Greenish brown	8.904	11.13

PMMC- Petroleum ether Extract of *Momordica charantia* fruits, **EFMC**-Ethyl acetate Extract of *Momordica charantia* fruits, **AFMC**- Etahnolic Extract of *Momordica charantia* fruits, **QFMC**- Aqueous Extract of *Momordica charantia* fruits, **PLPG**- Petroleum Ether Extract of *Pongamia glabra* leaves, **ELPG**- Ethyl acetate Extract of *Pongamia glabra* leaves, **ALPG**- Etahnolic Extract of *Pongamia glabra* leaves, **QLPG**- Aqueous Extract of *Pongamia glabra* leaves, **PFPN**- Petroleum ether Extract of *Piper nigrum*fruits, **EFPN**-Ethyl acetateExtract of *Piper nigrum* fruits, **AFPN**- Etahnolic Extract of *Piper nigrum* fruits, **QFPN**- Aqueous Extract of *Piper nigrum* fruits.

Wound healing activity on anemic rats

Increased fasting blood sugar levels were observed on day 3 after streptozotocin administration. The fasting blood sugars remained high throughout the study period, with a mean value of $14.77 \pm 0.08 \text{ mmol/L}$ compared to the normal fasting blood sugar level, which was $6.94 \pm 0.52 \text{ mmol/L}$ before streptozotocin administration. The fasting blood sugar (FBS) levels and were estimated on initial and final day of experimental protocol to confirm the diabetic and anemic state. A little change in FBS was observed. Increased fasting blood sugar levels were observed on day 3 after streptozotocin administration. The fasting blood sugars remained high throughout the study period, with a mean value of $14.77 \pm 0.08 \text{ mmol/L}$ compared to the normal fasting blood sugar level, which was $6.94 \pm 0.52 \text{ mmol/L}$ before streptozotocin administration. The fasting blood sugar levels of different groups are shown in table 3 and figure 4.

There is a decrease in RBC count and Haemoglobin level after the administration of streptozotocin in negative control group GXII. And these levels are increased with the administration of test extracts and formulations of various selected drugs and combinations, and the P values are also significant. Anaemic rats showed decrease in RBC count whereas the RBC count of the control rats remained the same. Administration of various extracts and combination of drugs significantly ($P < 0.001$)

Figure 4. Fasting blood sugar level of experimental groups

increase the RBC count and brought back RBC count towards normal. Hematological parameters after treatment with extracts and formulations was tabulated in table 4. Anaemic rats showed decrease in Hb level whereas the Hb level of the control rats remained the same shown in figure 5. Administration of various extracts significantly ($P < 0.001$) increase the Hb level and brought back Hb level towards normal. Streptozotocin is able to cause anaemia in animals due to its oxidative stress. Appearance of Heinz bodies in red blood cell morphology proves the presence of anemia and it maybe a type of hemolytic anemia.

Table 3. Fasting blood sugar level of experimental groups

Group	Before STZ induction mmol/L	At day 3 after STZ Induction	At day 10 of the Treatment
Control	5.78±0.31	6.32±0.22	5.77±0.29
Standard	6.15±0.31	21.54±2.42	14.53±2.25
OAQMC	7.94±3.12	15.21±0.89	15.84±3.14
OAQ(MC+PN)	7.58±2.46	16.53±2.45	15.08±3.44
OAQPG	8.05±2.11	15.71±3.49	16.04±4.25
OAQ(PG+PN)	7.76±1.24	15.98±1.88	15.46±0.21
OAQ(MC+PG+PN)	7.39±3.50	15.44±2.46	14.77±1.72
TF1	7.08±1.25	14.96±2.58	14.15±2.63
TF2	6.93±1.11	14.45±3.47	13.84±0.25
O3TF1	6.78±3.45	14.2±3.29	13.57±1.85
O3TF2	6.39±0.28	13.41±2.74	12.79±2.14
GXII	5.55±0.21	18.05±2.13	21.04±2.88

Where, AQ stands for alcoholic and aqueous extract in 1:1 amount, **Group I:** Control group which received simple vehicle (Ointment base), **Group II:** Standard group received Povidone iodine ointment, **Group III:** Test group received MC extract orally [AQMC], **Group IV:** Test group received MC and PN extract orally [OAQ(MC+PN)], **Group V:** Test group received PG extract orally [OAQPG], **Group VI:** Test group received PG and PN extract orally [OAQ(PG+PN)], **Group VII:** Test group received MC, PG and PN orally [OAQ(MC+PG+PN)], **Group VIII:** Test group received MC, PG and PN extract formulation F1 (10%) topically [TF1], **Group IX:** Test group received MC, PG and PN extract formulation F2 (15%) topically [TF2], **Group X:** Test group received MC, PG and PN orally and formulation F1 (10%) topically [O3TF1], **Group XI:** Test group received MC, PG and PN orally and formulation F2 (15%) topically [O3TF2], **Group XII:** Anemic Diabetic Group received no treatment [GXII]

Table 4. Hematological parameters after treatment with extracts and formulations

Groups	RBC Count ($\times 10^{12}/L$)	Haemoglobin (g/Dl)
Control	8.76 $\pm$ 0.65	15.82 $\pm$ 0.59
Standard	8.13 $\pm$ 0.53**	14.84 $\pm$ 0.68**
OAQMC	6.21 $\pm$ 0.42	13.2 $\pm$ 0.56*
OAQ(MC+PN)	6.23 $\pm$ 0.37*	13.26 $\pm$ 0.74*
OAQPG	6.19 $\pm$ 0.55*	13.17 $\pm$ 0.80*
OAQ(PG+PN)	6.35 $\pm$ 0.61*	13.43 $\pm$ 0.89*
OAQ(MC+PG+PN)	6.77 $\pm$ 0.70**	13.62 $\pm$ 0.70**
TF1	6.84 $\pm$ 0.51**	13.78 $\pm$ 0.83**
TF2	6.98 $\pm$ 0.64**	13.82 $\pm$ 0.99**
O3TF1	7.88 $\pm$ 0.80**	14.58 $\pm$ 0.42**
O3TF2	8.04 $\pm$ 0.23**	14.72 $\pm$ 0.19**
GXII	6.18 $\pm$ 0.47*	9.82 $\pm$ 0.49*

Values are expressed as mean $\pm$ SD (n=6), * P<0.001 compared with control group, **P<0.001 compared with negative control group

Figure 5. Haemoglobin (g/Dl) level after treatment with various samples**Table 5.** Effect of herbal formulations and extracts on incision wound model

Groups (n)	Tensile strength (g)
I-Control	472.14 $\pm$ 5.89
II-Standard	485.44 $\pm$ 3.48***
III-OAQMC	496.28 $\pm$ 6.72***
IV-OAQ(MC+PN)	504.32 $\pm$ 3.56***
V-OAQPG	515.54 $\pm$ 2.45**
VI-OAQ(PG+PN)	519.35 $\pm$ 2.77**
VII-OAQ(MC+PG+PN)	523.84 $\pm$ 1.64**
VIII-TF1	526.55 $\pm$ 4.28***
IX-TF2	530.24 $\pm$ 6.59***
X-O3TF1	536.42 $\pm$ 5.48
XI-O3TF2	565.67 $\pm$ 6.24**
XII-GXII	393.12 $\pm$ 2.76**

Note: n =6 animals in each group, values are expressed as Mean $\pm$ SEM, If * =p<0.05, ** =p<0.01, *** =p<0.001 when compare to control.

Incision wound model on anaemic rats

The results of the wound healing activity of extract ointment formulations by incision method are represented in table 5 and figure 6. The results were expressed as mean breaking strength of incision wound area. The data revealed that skin breaking strength of diabetic control animals were decreased to significant extent in comparison to the Non-diabetic control rats (p<0.001). The studies on incision wound healing model reveal that the test group (O3TF1 and O3TF2) showed high breaking strength in wound area from 1st day to 10th day e.g. 565.67 $\pm$ 6.24 and 536.42 $\pm$ 5.48g.

The other test groups of individual drug ointments at different combinations has shown different breaking strength results e.g. OAQMC and OAQ(MC+PN) shown 496.28 $\pm$ 6.72 and 504.32 $\pm$ 3.56, OAQPG and OAQ(PG+PN) shown 515.54 $\pm$ 2.45 and 519.35 $\pm$ 2.77, OAQ(MC+PG+PN) shown 523.84 $\pm$ 1.64, TF1 and TF2 shown 526.55 $\pm$ 4.28 and 530.24 $\pm$ 6.59, O3TF1 and O3TF2 shown 536.42 $\pm$ 5.48 and 565.67 $\pm$ 6.24 respectively.

Figure 6. Tensile strength of wound in different groups of incision wound model.

Ointment formulations O3TF-1 and O3TF-2 has shown significant wound healing activity but lesser than the oral and topical group, which was comparable to that of standard marketed preparations. The O3TF-2 group containing F2 formulation was found more active than the O3TF1 group containing F-1 formulation. The tensile strength is more when compared to standard. The control (ointment base) has shown 339.58 ± 6.52 g healing.

Histopathological observations

The histopathological studies for the incision model revealed figure 7a to 7l that the wounds treated with herbal formulations (Group IX < Group VIII < Group XI < Group X) showed discontinuous epidermis. There is vacuolization with milder strength. In those groups collagenation was also less and the inflammatory cells was absent. The rats of Group XII showed extensive necrosis and collagenation with disturbed epidermis. The reduced necrosis was observed in following order: Group V > Group III > Group IV > Group VI > Group II > Group VII > Group IX > Group VIII > Group X > Group XI.

Hyper-granulation and inflammatory cells are evident in the standard treated animals. In dermis the adnexa were restored, higher degree of fibrosis and collagen tissue were observed. Normal histopathological characteristics were observed in the

control group Group I. During the wound healing the cell population was decreased in the maturation and remodeling phase. And the increase in collagen deposition in the granulation tissues was observed which was forming the scar. Figure 7a to 7l shows the histopathological characteristics of the granulated tissue in various groups e.g. control to treatment groups (GROUP-I to XII).

The hematoxylin and eosin staining were used. The complete loss of collagen and the same was observed in Group VIII, Group IX, Group X and Group XI. The Groups (Group VIII, Group IX, Group X and Group XI) resulted with significant wound-healing activity as they showed the greater decrease in vacuolization period and formation of granulation tissue. The collagen synthesis was comparable to the control group of animals in increased rate of wound contraction aspects.

The inflammatory cells accumulation was lesser in the treatment groups received the combination of oral and topical herbal treatments. They were comparable with the standard groups as having the lesser inflammatory cells. Those was absent in the VIII- XI groups. The groups received the oral treatment of herbal combinations showed more faster healing may be due to the better absorption of the

Figure 7 (a-l). Histopathology of skin at day 10 stained with H&E (100X)

herbal constituents with increased bioavailability. The higher dose of herbal drugs (additively) and the topical application of the ointment formulation showed better results when compared with the standard and control group.

Conclusion

Wound healing in diabetes is a complicated and delayed process in which wound healing follows granulation, collagenation and scar formation. In the present research work ointment formulations with extract of three herbal drugs was prepared and evaluated for the anemic wound healing activity by incision wound model in albino rat. The three individual drug extracts e.g. *Momordica charantia* fruits, *Pongamia glabra* leaves and *Piper nigrum* fruits has shown wound healing properties but the formulation F2 has shown significant results in wound healing.

The extracts prepared by using soxhlet method were incorporated in the ointment base for formulation. Incision wound model utilized for the effectiveness of the three plants and results indicated the effectiveness of herbal ointment F2 show improve wound healing potential. The drug *Momordica* and *Pongamia* when administered orally and topically with *piper* they had shown significant synergistic activity. The formulation will be helpful in anemic wound healing with no side effects and will be beneficial for society and industry with standardization approaches.

The present studies concluded through the incision model that the wound healing property of individual drug extracts *Momordica charantia* and *Pongamia glabra* can be enhanced by the concomitant use of *Piper nigrum* within the oral and topical formulations. The topical formulation containing 15% of extracts of *Momordica charantia*, *Pongamia glabra* and *Piper nigrum* in ointment formulation can be used as marketed formulations and its effect can be enhanced with intake of its oral doses besides the topical application.

Conflicts of interest: Not declared.

References

- Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HD, Goel RK. 2009. Evaluation of wound healing activity of extracts of plantain banana (*Musa sapientum* var. *paradisiaca*) in rats. *Indian Journal of Experimental Biology*, 47(1):32-40.
- Kamer E, Recai Unalp H, Gundogan O, Diniz G, Ortac R, Olukman M, Derici H, Ali Onal M. 2010. Effect of ascorbic Acid on incisional wound healing in streptozotocin-induced diabetic rats. *Wounds*, 22(2):27-31.
- Jayasooriva AP, Sakono P. 2000. Effects of *Momordica charantia* powder on serum glucose levels and various lipid parameters in rats fed with cholesterol-free and cholesterol-enriched diets. *Journal of Ethnopharmacology*, 72(1-2):331-336.
- Chia YY, Yap WaiSum. 2011. In vitro Antimicrobial activity of hexane: petroleum ether extracts from fruits of *Momordica charantia* L. *International Journal of Pharmaceutical and Biological Archives*, 2(3):868-873.
- Leelaprakash G, Caroline Rose, Gautham BM, Jivaji PK, Shivram P. 2011. In-vitro, antimicrobial and antioxidant activity of *Momordica Charantia* leaves. *Pharmacophore*, 2(4):244-252.
- Rajaram P, Mohan N, Vijayan G. 2012. Wound healing property of aqueous extract of seed and outer layer of *Momordica charantia* L. on albino rats. *Indian Journal of Pharmaceutical Science and Technology*, 5(1):1936-1940.
- Rawat P, Gill NS, Arora R. 2012. Study of *Momordica charantia* seeds for their antioxidant Potential. *International Journal of Natural Product Science*, 1:223-227.
- Sharma MC, Joshi C. 2004. Plants used in skin diseases of animal. *Natural Product Radiance*, 3(4): 293-299.
- Bandivdekar AH, Moodbidri SB. 2002. Spermicidal activity of seed oil of *Pongamia glabra*. *Archieve of Andrololgy*, 48(1):9-13.
- Krishnan SK, Pavan KG. 2013. Evaluation of antimicrobial activity of roots of *Pongamia glabra* vent. *International Journal of Pharmaceutical and Chemical Sciences*, 2(1):303-304.
- Sunila ES, Kuttan G. 2004. Immunomodulatory and antitumor activity of *Piper longum* Linn. and piperine. *Journal of Ethnopharmacology*, 90(2-3):339-346.
- Sudjarwo SA. 2005. The potency of piperine as anti-inflammatory and analgesic in rats and Mice. *Folia Medica Indonesiana*, 194.
- Kumar S, Sripriya R, Raghavan VH. 2006. Wound healing potential of *Cassia fistula* on infected albino rat model. *Journal of Surgical Research*, 23:283-289.
- Khan AW, Kotta S, Ansari SH, Sharma RK, Kumar A, Ali J. 2013. Formulation development, optimization and evaluation of aloe vera gel for wound healing. *Pharmacognosy Magazine*, 1: S6-S10.
- Sawant SE, Tajane MD. 2016. Formulation and evaluation of herbal ointment containing Neem and Turmeric extract. *Journal of Scientific and Innovative Research*, 5(4):149-151.