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Wound healing prospective of *pongamia glabra, piper nigrum and momordica charantia* on albino rats using anemic burn wound model

Rashmi Shukla, Varsha Kashaw*

SVN Institute of Pharmaceutical Sciences, Swami Vivekanand University, Sagar, Madhya Pradesh, India

ABSTRACT

Objective: The objective of present work was to evaluate wound healing potential of *pongamia glabra, piper nigrum and momordica charantia* using as herbal ointment formulation on albino rats using burn wound model. All the plant has the rich source of alkaloid, glycoside, tannins, carbohydrates, saponins, flavonoids, proteins and amino acids content 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. Burn wound model has been utilized for the evaluation of wound healing potential. Histopathological evaluation has been also carried-out for the physical verification wound healing potential. **Results and Conclusion:** The studies on burn 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 *pongamia glabra, piper nigrum and momordica charantia* has been utilized for wound healing and it is safer for topical application. No toxicity and mortality have been observed during the experimental tenure.

Keywords: Wound healing, *momordica charantia, pongamia glabra, piper nigrum,* burn wound model.

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***Address for Correspondence:**

Dr. (Mrs.) Varsha Kashaw, Professor, SVN Institute of Pharmaceutical Sciences, Swami Vivekananda University, Sagar (M.P.) India

INTRODUCTION

Wounds are the physical injuries resulting in opening or breaking of the skin and appropriate method for healing of wounds is essential for the restoration of disrupted anatomical continuity and disturbed functional status of the skin. In pathology, it specifically refers to sharp injury, which damages the dermis of the skin. Healing of wounds starts from the moment of injury and can continue for varying periods of time depending on the extent of wounding and the process can be broadly categorized into three stages; inflammatory phase, proliferate phase and finally the remodeling phase which ultimately determines the strength and appearance of the healed tissue. Wound healing is a complex regeneration process, which is characterized by intercalating degradation and re-assembly of connective tissue and epidermal layer. Wound healing is a complex and dynamic process with the wound environment changing with the changing health status of the individual or it is the body natural process of regenerating dermal and epidermal tissues. Hyperglycemia has suppressed cell proliferation collagen production[1].

*Pongamia glabra (*Figure 1*)* belong to *Fabaceae* family[2]. 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. It used as bacteriocidal activity against V. cholerae and E. coli, and also used as anti-inflammatory and antipyretic properties[3].

Piper nigrum (Figure 2) belonging to family *piperaceae*[4]. The fruits have small globose drupe and was known as a peppercorn when dried. 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.

Figure 1: Leaves, flowers and seeds of *Pongamia glabra*

Figure 2: Unripe and ripe seeds of *Piper nigrum*

Black pepper is used to improve digestion, stimulate appetite and treat gastrointestinal problems. It is also used to treat cold, cough and sore throats. Antioxidant active chemicals isolated from black pepper include camphene, carvacrol, eugenol, myrcene myristic-acid, myristicin, palmitic-acid and ubiquinone and were responsible for aroma, pungency and medicinal property of the black pepper.

Momordica charantia belong to Cucurbitaceae family[5]. 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 and pith. (Figure 3). *M. Charantia* consists the following chemical constituent charantin, diosgenin, gentisic acid, myristic acid and nerolidol. *Momordica charantia* is used as anthelmentic, anti-mycobacterial, antioxidant, antitumor, wound healing prpperties, antiulcer, antiviral, hypoglycemic and immune-stimulant[6,7].

Figure 3: Fruit of *M. Charantia*

The object of the present paper was to evaluate the wound healing potential of pongamia glabra, piper nigrum and momordica charantia. The anemic burn 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 appression no. is 490/BS/saifia/16 has been preserved for future identification.

Soxhlet Extraction

The crude drugs were dried in shade. The 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 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 defatting 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 and evaluation

Herbal preparations were formulated by combination of three plant extract of momordica charantia, pongamia glabra and piper nigrum. 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[8]. 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[9] 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

S. No.	Content	Quantity (%)	
		F1	F2
1.	MCAQ (1:1)	3.33	5.0
2.	PGAQ (1:1)	3.33	5.0
3.	PNAQ (1:1)	3.33	5.0
4.	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 Pongamiaglabra in 1:1 quantity; PNAQ- Alcoholic and Aqueous extracts of Piper nigrum in 1:1 quantity.

Pharmacological Activity

Anemic wound healing activity

Anemic burn wound model using albino rats was selected for assessing the wound healing potential. This model was employed to study the rate of wound contraction and tensile strength[9-12]. These parameters were selected and albino rats were selected by easy availability and simplicity in handling them.

Selection and 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 10 mg/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 Burn Wound Model

For the anemic incision wound studies, seventy- two anemic 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[13].

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

Creation of Burn Wound

Burn wounds were created on dorsal part of shaved rat's skin surface using concentrate sulfuric acid, exposed for 10 s. After 24 h, dead tissues were excised using sterile surgical blade through a template designed to produce a third degree burn[14]. All groups were treated same as in excision model. In this model, wound contraction and epithelialization period was monitored. A specimen sample from the healed wound of tissue was collected from each rat for histopathological examination[15-16].

Hydroxyproline estimation

Wound tissues were analyzed for hydroxyproline content, a basic constituent of collagen. Tissues were dried in a hot air oven at 60-70°C to constant weight and hydrolyzed in 6 N HCl at 130°C for 4 h in sealed tubes. The hydrolysate was neutralized to pH 7 then subjected to chloramine-T oxidation for 20 min[16]. The reaction was terminated by the

addition of 0.4 M perchloric acid and developed color with Ehrlich reagent at 60°C was read at 557 nm in ultraviolet spectrophotometer.

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 *Momordica charantia*)	PFMC	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 Pongamia glabra*)	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 *Piper nigrum*)	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

PFMC- Petroleum ether Extract of *Momordicacharantia*fruits, **EFMC-**Ethyl acetateExtract of *Momordicacharantia*fruits, **AFMC-** Etahnolic Extract of *Momordicacharantia*fruits, **QFMC-** Aqueous Extract of *Momordicacharantia*fruits,**PLPG-** Petroleum Ether Extract of *Pongamiaglabra*leaves, **ELPG-**Ethyl acetateExtract of *Pongamiaglabra*leaves, **ALPG-** Etahnolic Extract of *Pongamiaglabra*leaves, **QLPG-** Aqueous Extract of *Pongamiaglabra*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.

Pharmacological Activity

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 ± 0.08mmol/L compared to the normal fasting blood sugar level, which was 6.94± 0.52mmol/L before streptozotocin administration. The fasting blood sugar (FBS) levels and were estimated on initial and final day of experimental protocol to confirm the 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 ± 0.08mmol/L compared to the normal fasting blood sugar level, which was 6.94± 0.52mmol/L before streptozotocin administration. The fasting blood sugar levels of different groups are shown in **Table 3 and Figure 4.**

Table 3: Fasting blood sugar level of experimental groups

S. No.	Group	Before STZ induction mmol/L	At day 3 after STZ Induction	At day 10 of the Treatment
1	Control	5.78±0.31	6.32±0.22	5.77±0.29
2	GXII	5.55±0.21	18.05±2.13	21.04±2.88
3	Standard	6.15±0.31	21.54±2.42	14.53±2.25
4	OAQMC	7.94±3.12	15.21±0.89	15.84±3.14
5	OAQ(MC+PN)	7.58±2.46	16.53±2.45	15.08±3.44
6	OAQPG	8.05±2.11	15.71±3.49	16.04±4.25
7	OAQ(PG+PN)	7.76±1.24	15.98±1.88	15.46±0.21
8	OAQ(MC+PG+PN)	7.39±3.50	15.44±2.46	14.77±1.72
9	TF1	7.08±1.25	14.96±2.58	14.15±2.63
10	TF2	6.93±1.11	14.45±3.47	13.84±0.25
11	O3TF1	6.78±3.45	14.2±3.29	13.57±1.85
12	O3TF2	6.39±0.28	13.41±2.74	12.79±2.14

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]**

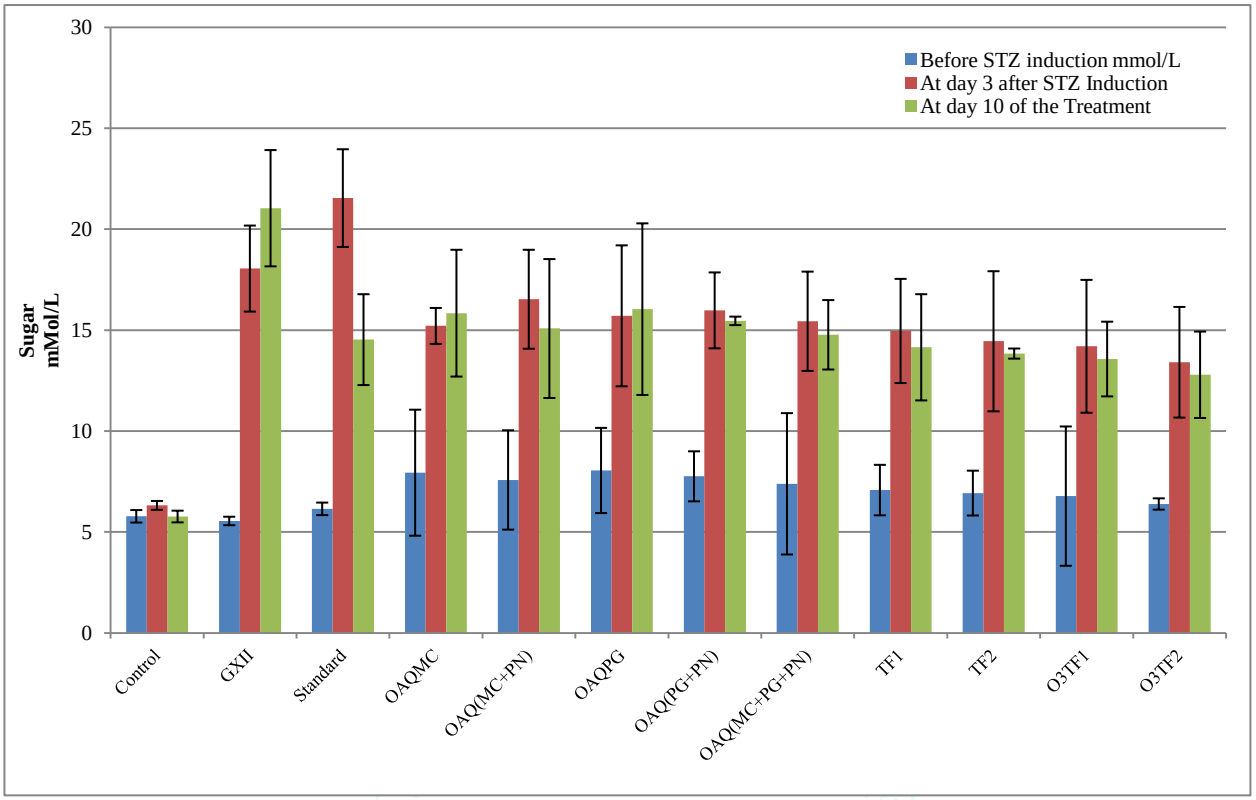

Figure 4: Fasting Blood Sugar Level of Experimental Groups

There is a decrease in RBC count and hemoglobin 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. Anemic 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) increase the RBC count and brought back RBC count towards normal. Hematological parameters after treatment with extracts and formulations were tabulated in Table 5.

Table 5: Hematological parameters after treatment with extracts and formulations

S. No.	Groups	RBC Count (X10^12/L)	Hemoglobin (g/Dl)
1.	Control	8.76±0.65	15.82±0.59
2.	Standard	8.13±0.53**	14.84±0.68**
3.	OAQMC	6.21±0.42	13.2±0.56*
4.	OAQ(MC+PN)	6.23±0.37*	13.26±0.74*
5.	OAQPG	6.19±0.55*	13.17±0.80*
6.	OAQ(PG+PN)	6.35±0.61*	13.43±0.89*
7.	OAQ(MC+PG+PN)	6.77±0.70**	13.62±0.70**
8.	TF1	6.84±0.51**	13.78±0.83**
9.	TF2	6.98±0.64**	13.82±0.99**
10.	O3TF1	7.88±0.80**	14.58±0.42**
11.	O3TF2	8.04±0.23**	14.72±0.19**
12.	GXII	6.18±0.47*	9.82±0.49*

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

Anemic 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 anemia 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.

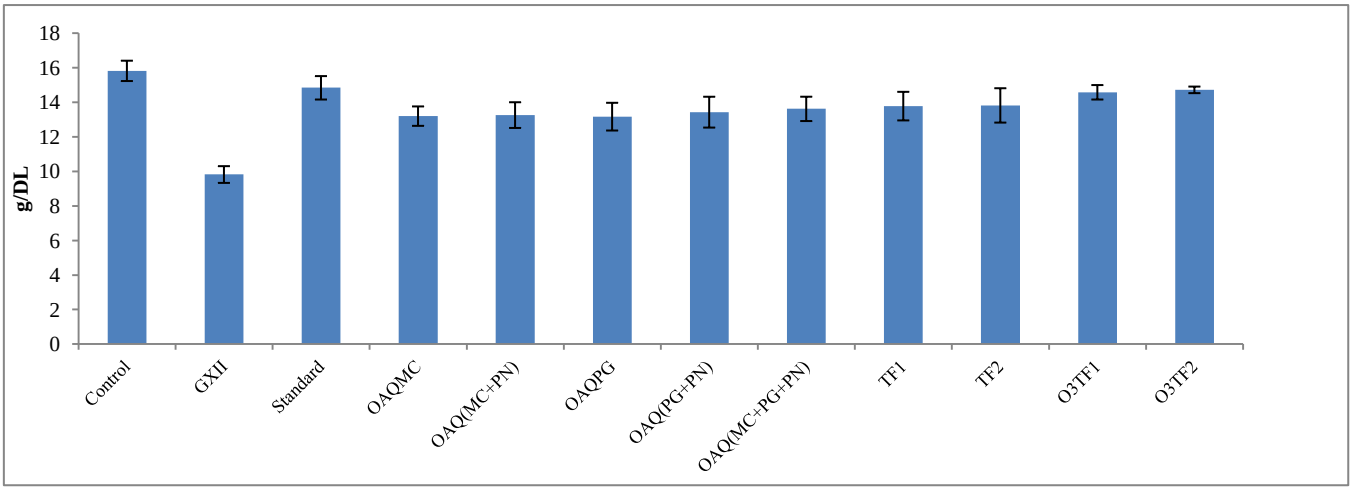

Figure 5: Hemoglobin (g/Dl) level after treatment with various samples

Burn Wound Model[17]

Wound healing activity of extract ointment formulation by burn wound method is represented in Table 6 and Figure 6. Hydroxyproline content of anemic control animals were decreased to significant extent in comparison to the Non-anemic control rat ($p<0.001$). The studies on burn wound healing model reveal that the test group (O3TF1 and O3TF2) showed high hydroxyproline content (stability of collagen) in wound area e.g. 62.43± 0.094 mg/g and 64.12±5.48 mg/g. The other test groups of individual drug ointments at different combinations has shown different hydroxyproline content results e.g. OAQMC and OAQ (MC+PN) shown 58.46±0.225 mg/g and 59.87±0.214 mg/g, OAQPG and OAQ (PG+PN) shown 58.64±0.134 mg/g and 59.02±20.315 mg/g, OAQ (MC+PG+PN) shown 60.39±0.085 mg/g, TF1 and TF2 shown 60.45±0.0112 mg/g and 60.78±0.351 mg/g respectively. Ointment formulations O3TF-1 and O3TF-2 has shown significant hydroxyproline content 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 hydroxyproline content is more when compared to standard. The control (ointment base) has shown 28.02±0.125 mg/g healing.

Table 6: Effect of Various Samples on different parameter of Burn wound model

S. No.	Groups	Hydroxyproline Content (mg/g tissue)	Epithelialization period (mean time in days)	Tensile Strength (g/mm2) 10th day
1	Control	28.02±0.125	18.18±0.345	473.05±6.02
2	GXII	22.68±0.247	18.88±0.259	393.41±3.61
3	Standard	60.41±0.352***	15.5±0.315**	486.35±6.85*
4	OAQMC	58.46±0.225**	17.55±0.420*	496.33±3.69*
5	OAQ(MC+PN)	59.87±0.214**	15.97±0.318**	507.36±2.58*
6	OAQPG	58.64±0.134**	17.75±0.249*	515.44±2.90**
7	OAQ(PG+PN)	59.02±0.315**	16.24±0.405*	520.24±1.77**
8	OAQ(MC+PG+PN)	60.39±0.085**	15.81±0.322**	523.72±4.41**
9	TF1	60.45±0.112**	14.97±0.256***	525.72±6.72**
10	TF2	60.78±0.351**	14.77±0.207***	530.17±5.61***
11	O3TF1	62.43±0.094***	14.08±0.108***	536.33±6.37***
12	O3TF2	64.12±0.107***	13.79±0.159***	565.78±2.89***

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

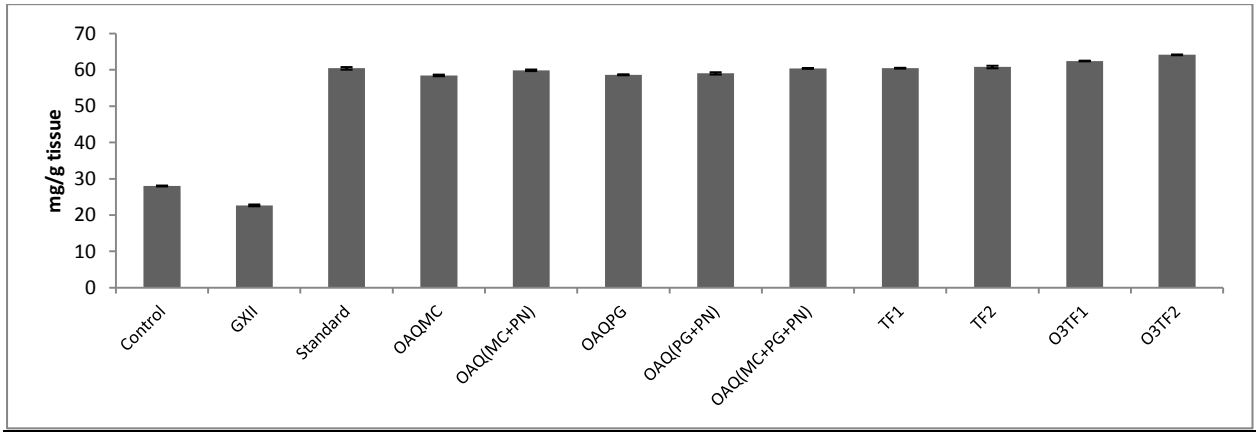

Figure 6: Effect of Various samples on Hydroxyproline content (mg/g tissue) in Burn Wound Model

The mean epithelialization time decreased from 18.23 days in controls, to 14.97 days for TF1, 14.77 days for TF-2, 17.55 days for OAQMC, 15.97 days for OAQ (MC+PN), 17.75 days for OAQPG, 16.24 days for OAQ (PG+PN), 15.81 days for OAQ (MC+PG+PN), 14.08 days for O3TF1, 13.79 days for O3TF2 and 18.88 days for GXII, while standard povidone iodine showed the lowest time of 15.50 days. The overall epithelialization time can be presented as: GXII > Control > OAQPG > OAQMC > OAQ (PG+PN)> OAQ (MC+PN)> OAQ (MC+PG+PN) > Standard > TF1 > TF2 > O3TF1 > O3TF2. Among the prepared herbal formulations, the blended formulation showed the decrease in epithelization period in comparison with controls, individual extracts and showed comparable results to the standard ointment (Figure 7).

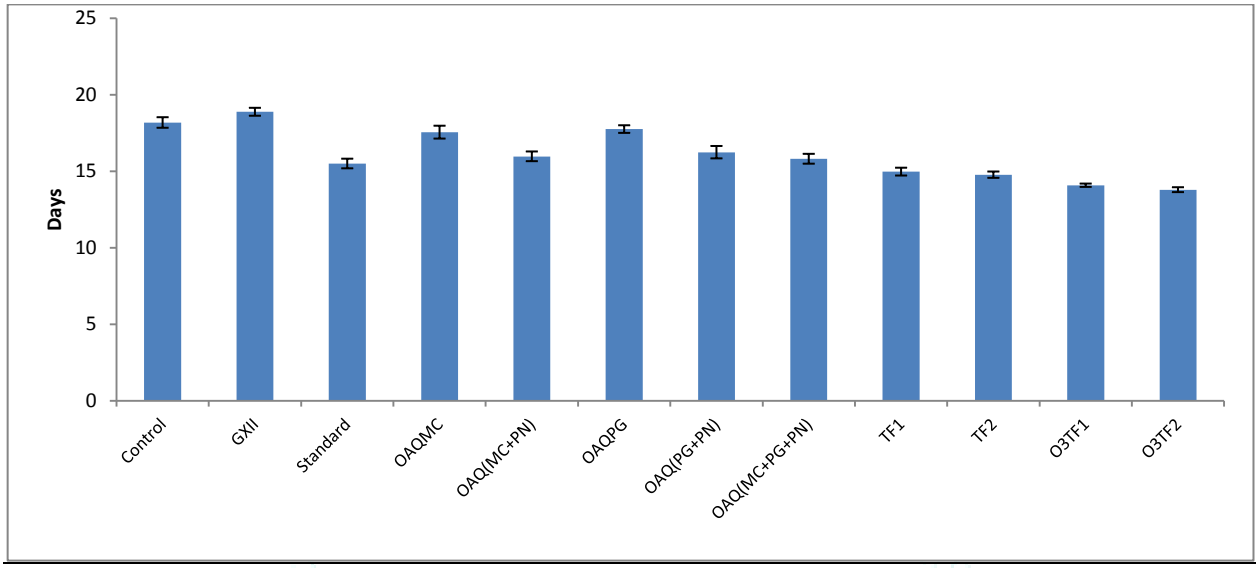

Figure 7: Effect of various samples on epithelialization period (mean time in days) in burn wound model

The data revealed that tensile strength (skin breaking strength) of anemic control animals were decreased to significant extent in comparison to the Non-anemic control rats ($p<0.001$). The studies on burn 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. 536.33±6.37g and 565.78±2.89 g. 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 496.33±3.69 g and 507.362.58 g, OAQPG and OAQ (PG+PN) shown 515.44±2.90 g and 520.24±1.77 g , OAQ (MC+PG+PN) shown 523.72±4.41 g , TF1 and TF2 shown 525.72±6.72 g and 530.17±5.61 g , O3TF1 and O3TF2 shown 536.33±6.37 g and 565.78±2.89 g respectively. Ointment formulations O3TF-1 and O3TF-2 has shown significant tensile strength 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 473.05±6.02g healing (Figure 8)

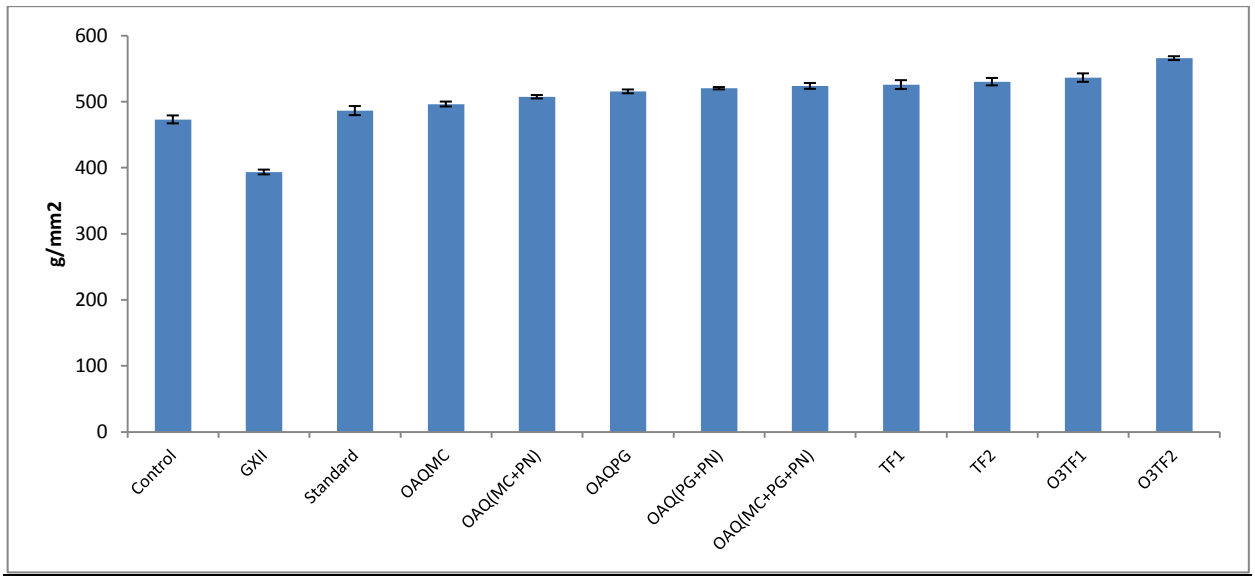

Figure 8: Effect of various samples on tensile strength (g/mm2) in burn wound model

Histopatholgical Changes

Burn Wound Model

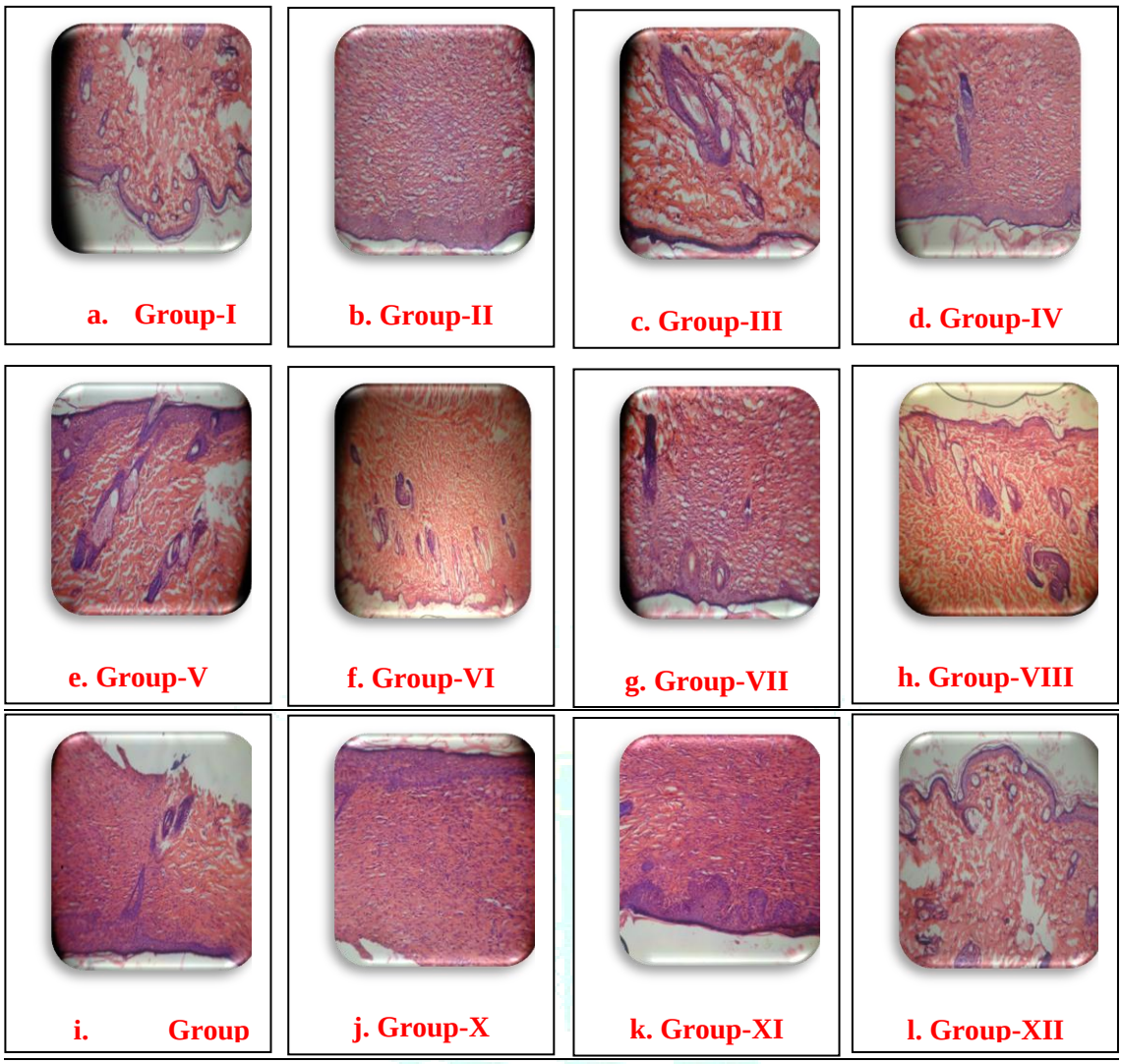

Figure 9 (a-i): Histopathology of skin at day 10 stained with H&E (100X)

Histopathological evaluations showed the burn healing to be better in the (GV, GIII, GIV, GVI, GII, GVII, GIX, GVIII, GX and GXI) with respect to the control group [Figure 9 (a-i)]. A scab formed by necrotic tissue remnants and mononuclear cell infiltration was present in GXII. The regeneration and repairing in the epidermal layer were also observed in various groups. Inflammatory cell infiltration without an epithelial layer was noticed. Vessels were hyper-anemic in the dermis and there were no hair follicles, sebaceous or sweat glands in the groups. The scab was thinner in the Group VIII, Group IX, Group X and Group XI (GIX< GVIII< GXI< GX) group. More improved epithelia layer formation with regeneration and repairing was observed. The dermal fibrosis in the subcutaneous level was also seen. The epithelial layer in the Group VIII, Group IX, Group X and Group XI had a better appearance when compared to control, standard and other groups. Also, granulation tissue in the dermis in the (GIX< GVIII< GXI< GX) group was in better condition when compared with the other groups. The burn wound healing model also provides an *in vivo* approach for studying the healing of burn-related wounds in domestic animals.

CONCLUSION

In the present research work ointment formulations with extract of three herbal drugs was prepared and evaluated for the anemic wound healing activity by burn wound model in anemic albino rat. The three individual drug extracts e.g. *Momordica charantia* fruits, *Pongamia glabra* leaves and *Piper nigrum fruits* has shown wound healing properties.

The extracts prepared by using Soxhlet method were incorporated in the ointment base for formulation. Burn 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 burn wound model that 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

The author declares no conflicts of interest.

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