Comparative Wound Healing Activity of two Polyherbal Formulations GC-01 and GC-02 on Experimentally induced Wounds in Rodents

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ABSTRACT

The present study was undertaken to support the use of herbs in folklore medicine for wound healing activity. In the present investigation, a comparative study and screening was done on two polyherbal formulations GC-01 and GC-02 fortified with different herb extracts in ghee for wound healing activity in experimentally induced wounds in rodents. Wound healing activity was evaluated by using three wound models viz. incision, excision and burn. For the study, standard drug used was Framycetinsulphate cream for incision and excision wound model, while Silver sulfadiazine cream was used for burn wound model. Fresh ointments of 5%, 10% and 15% of polyherbal formulations in simple ointment base were prepared and evaluated by applying topically for a period of 10–21 days depending on the type of study. The parameters assessed were tensile strength in incision wound model whereas in excision and burn wound models reduction in mean wound area, percentage wound contraction, epithelization period, granulation tissue antioxidant status [estimation of superoxide dismutase (SOD) and reduced glutathione (GSH)], free radical (lipid peroxidation), connective tissue formation and maturation (hydroxy proline). The results have shown increased wound breaking strength and levels of hydroxy proline, superoxide dismutase andglutathione in the granulation tissue. Decreased mean wound area and lipid peroxidation was also observed. This could be the result of synergistic/potentiative action of individual medicinal herbs present in GC-01 and GC-02in gheeand diverse array of active principles present in them.

Keywords: Polyherbal formulation, incision, excision and burn wound model, antioxidant status.

INTRODUCTION

Wounds are common and inescapable events of life which are a result of physical, chemical injury or microbial infections.[1] Loss or breaking of cellular and anatomic or disturbances in functional continuity of living tissues are the reasons of wound.[2] It results in a variety of cellular and molecular sequelae, opening or breaking of the skin and is also defined as a breach in the normal tissue continuum.[3] On the other hand, wound healing is a process involving a connective tissue response and an acute inflammatory phase followed by synthesis of collagens and other extra cellular macromolecules which later remodelsto form a scar.

Use of herbal extracts instead of crude herbs started with the aim to control quality and precise dosage for better results. The plant extracts being more efficacious are free from undesirable side effects.

Medicinal plants are used by folklore medicine in India for treatment of cuts, wounds and burns[4] are abundantand due to their specific healing property and non-toxic actions, scientists are keen to evaluate drugs from them.[5]

The present work deals with the systematic study of two polyherbal formulations (PHF), GC-01 and GC-02, containing well known herbs of Indian traditional medicine in ghee, for their

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wound healing potential in incision, excision and burn wound models along with the estimation of anti-oxidant status and biochemical parameters. The present study gives substantial support to the use of these herbs in folklore medicine for wound healing.

MATERIALS AND METHODS

Preparation of ointments of Polyherbal formulations: The polyherbal formulations, GC-01 and GC-02 were procured from Pentacare Ayur Pharma, Bangalore as gift samples. For the study, all the ointments were freshly prepared before topical application. Different concentrations ofPHF(5%, 10% and 15%) were incorporated into simple ointment base I.P. and mixed in a mortar and pestle to obtain uniform consistency.

Animals: Adult, healthy albino Wistar rats of either sex in the weight range of 180–250g were procured from registered breeder during the experiment. Clearance from Institutional Animal Ethics Committee (IAEC) of KLE University's College of Pharmacy, Bangalore was sought (IAEC/04/PA/2011–12) prior to experiment.

Standard drug: Framycetin sulphate cream (1%, Soframycin from Aventis Pharma

Limited) was selected as standard drug for incision and excision wound model whereas Silver sulfadiazine (1%, Burn heal from Curetech Skincare) was used in case of burn wound model.

Chemicals-Nitro blue tetrazolium chloride, Phenazine methosulphate Trichloroacetic acid, Butylated hydroxyl toluene and p-dimethyl amino benzaldehyde were procured from M/s. SD Fine-Chem Limited, Mumbai. N-Butyl alcohol was from M/s. Nice Chemicals Pvt. Ltd., Kochi. Hydroxyproline Standard, Di-thionitro benzoic acid and reduced glutathione were procured from M/s Sisco Research Lab,

Table 1. Composition of formulation of GC-01

Botanical name	Indian name	Family	Quantity
Butea monosperma Lam.	Palashapushpa	Fabaceae	3 gm
Symplocos racemosa Roxb.	Lodhra	Symplocaceae	5 gm
Mimosa pudica Linn.	Lajjalu,	Mimosaceae	4 gm
Curcuma longa Linn.	Haridra,	Zingiberaceae	3 gm
Commiphora mukul	Guggulu,	Burseraceae	500 mg
Piper longum Linn.	Pipalli	Piperaceae	500 mg
Azadarchta indica Linn.	Nimba,	Meliaceae	2 gm
Pongamia glabra	Karanja,	Leguminosae	2 gm
Cocos nucifera Linn.	Narikela	Arecaceae	2 ml
Ghrita	Ghee		8 ml

Table 2. Composition of Formulation of GC-02

Botanical name	Indian name	Family	Quantity (gms)
Myristica fragrans Houtt.	Jatipatra,	Myristicaceae	192
Melia azadarch Linn.	Nimbapatra,	Meliaceae	192
Trichosanthes cucumerina Linn.	Patolapatra,	Cucurbitaceae	192
Picrorhiza kurroa	Katuka	Scrophulariaceae	192
Berberis aristata DC	Darvi,	Berberidaceae	192
Curcuma longa Linn.	Nisha,	Zingiberaceae	192
Hemidesmus indicus R. Br.	Sariva,	Asclepiadaceae	192
Rubia cordifolia Linn.	Manjista,	Rubiaceae	192
Terminalia chebula	Abhya	Combretaceae	96
Vetiveria zizanioides (L.)	Ushira,	Gramineae	96
Madhuca longifolia	Madhuka,	Sapotaceae	192
Pongamia glabra	karanjabeeja,	Fabaceae	192

Each 10ml ghee contains above herbs.

Mumbai. Thiobarbituric acid was obtained from M/s. Spectrochem Pvt. Ltd., Mumbai.

Treatment protocol: The wound healing activity was undertaken in incision, excision and burn wound models. In each model, eight groups of six animals each were used. In all the models group 1 was control (no treatment), group 2 standard (Framycetin/ silver sulfadiazine), groups 3,4 and 5 were treated with 5%, 10% and 15% of GC-01 whereas groups 6, 7 and8 were treated with 5%, 10% and 15% of GC-02 respectively.

For wound healing study in incision wound model, the standard formulation and all test drug ointments (5%, 10% and 15% w/w) were applied topically on wound area once daily from 0 to 10th postoperative days where as in excision and burn wound models the standard formulation and all test drug ointments (5%, 10% and 15% w/w) were applied topically on wound area once daily from 0 to 21 postoperative days or till complete healing, whichever occurred earlier. The effect of GC-01 and GC-02 on various parameters (i) wound tensile strength (ii) physical parameters like mean wound area, percentage wound contraction and epithelization period (iii) biochemical parameters like Malondialdehyde (MDA) and Hydroxy proline were estimated and compared with the standard and also with each other.

Experimental procedure: Adult, healthy albino Wistar rats of either sex in the weight range of 180-250g were used for the study.

Incision wound model: The rats were anaesthetized by administration of ketamine hydrochloride (50 mg/kg b.w., i.p.). On depilated back of the animal, two para-vertebral straight incision of 6 cm length each were made through the entire thickness of the skin, on either side of the vertebral column with the help of sharp sterile scalpel. Care was taken to see that the incisions were atleast 1 cm lateral to the vertebral column. After complete haemostasis the wounds were closed by means of interrupted suture placed at equidistance points about 1 cm apart using 2-zero surgical silk thread and a curved needle (No. 11). Wounds were then mopped with cotton swabs soaked in saline and were caged individually. Animals ingroup 1 received no treatment and served as normal control. Standard (FSC) and all the test drug ointments (5%, 10% and 15% w/w of GC-01 and GC-02) were applied topically to the wound once a day for a period of 10 days. Sutures were removed on 8th day and tensile strength was measured on 10th day by continuous water flow technique of Lee.[6] Three such readings were recorded for a given incision wound and the procedure was repeated on the other wound of the same animal, thus obtaining six readings for each animal. The mean breaking strength in each animal i.e. average of six readings was used to calculate the group mean.

Excision wound model: A round circular seal of 300 mm^2 diameter was impressed on the dorsal thoracic central region 5 cm away from the ears of anaesthetized rats. Full thickness skin from demarked area was excised to get a wound of approximate 300 mm^2. After achieving the full haemostasis wound was blotted with cotton swab soaked in warm saline and animals were placed in their individual cages. The standard formulation (Framycetin), and all test drug ointments were applied as per the protocol.

Burn wound model: Partial thickness burn wound was inflicted on overnight starved animals under light ether anesthesia, by pouring hot molten wax at 800C into a metal cylinder of 300 mm^2 on depilated back of the animal. The molten wax in the cylinder was allowed to solidify which took about 10–12 minutes. Then the metal cylinder with wax adhered to the skin was removed which left a distinctly demarked partial thickness circular burn wound of 300 mm^2 area. Treatment was done as per the protocol.

Assessment of parameters: For assessing parameters in excision and burn wound models, two wounds were induced simultaneously on depilated back of animal. One wound was used for measuring wound contraction and epithelization period whereas the second one was used for various biochemical estimations and anti-oxidant parameters. These estimations were done on 7th post wound day in the granulomatous tissue. Wound contraction[7] was measured by tracing wound on 1 mm^2 graph paper on alternate days. The calculated surface area was then employed to obtain the percentage wound contraction by taking the initial average size of wound 300 mm^2 as 100% by using following equation:

$$\text{Percentage wound contraction} = \frac{\text{Initial Wound Size} - \text{Specific Wound Size}}{\text{Initial Wound Size}} \times 100$$

Epithelialization period[8]: was recorded as the number of days required for the clot (eschar) to fall off from the burn wound surface without leaving a raw wound behind.

Biochemical and antioxidant estimations[9]: On 7^{th} post wounding day of 2^{nd} wound on animal, wounded granulomatous tissue were excised from all the groups of both excision and burn wound modelsin such a way that wounded area was excised without contaminating it with normal skin and then homogenised. This tissue homogenate was used for the estimation of hydroxyproline,[10] superoxide dismutase[11] (SOD), reduced glutathione[12,13] (GSH) and lipid peroxide[14] (MDA).

Statistical analysis: Results were expressed as mean values ± SEM (Standard Error of Mean) and were calculated for each group. Statistical differences between means were determined by Graph Pad Prism (5.0) using one-way ANOVA followed by Tukey's post hoc test. Values $p<0.05$ were considered as statistically significant.

Results

Effect on incision wound model: There was a significant increase in tensile strength of the wound treatedwith polyherbal formulations as compared to that of the control and standard groups. The results are shown in (Table 1). Higher doses of the formulation were found to be more effective. Topical application of 15% GC-01 and 10%, 15% GC-02 produced significant ($p<0.0001$) increase in breaking tensile strength when compared with standard Framycetinsulphate treated group.

Effect on excision wound model: Both the formulations showed faster healing when compared with control group and wound contraction was at a faster rate with the formulations as compared with control group (Table 2). There was a significant decrease in the number of daysrequired for complete healing in the animals treated with5%, 10%, 15% of GC-01 ($p<0.0001$) and5% ($p<0.001$), 10% and 15% of GC-02 ($p<0.0001$) when compared to the control group animals. Higher concentrations of both the formulations have shown lesser epithelialization period or faster wound contraction comparedto standard group. The number of days required for complete healing by 5%, 15% of GC-02 has shown significant result ($p<0.01$, $p<0.0001$) respectively when compared to that of the standard (FSC) group. Percentage wound contraction was found to behighest in10% and 15% of both the formulations compared to lower concentrations and standard group.

Hydroxy proline levels is a measure of collagen index and higher the concentration ofhydroxy prolinefaster the rate of wound healing. Excision wound model has shown a significant increase in hydroxyproline content in 10% ($p<0.01$)and 15% ($p<0.0001$) of GC-01 and all concentrations of GC-02 ($p<0.0001$) formulation treated animals when compared to control group (Fig. 1). The animals received no treatment (group 1) had lowest content of hydroxy proline.

Measurement of malondialdehyde (MDA) is an index of lipid peroxidation. MDA levels were significantly reduced in all the test groups ($p<0.0001$) when compared with the control group and in 5% GC-02 ($p<0.01$) and 10% of GC-02 ($p<0.001$). MDA levels were significantly reduced ($p<0.0001$) in standard groups alsowhen compared with all the test groups as shown in (Fig. 2).

Estimation of antioxidant like SOD and glutathione (GSH)is relevant as it hastens the process of wound healing. All the treatment groups have shown significant increase ($p<0.0001$) in SOD levels when compared with control group. Significant increase was observed in superoxide levels in 15% ($p<0.0001$) of both the formulations when compared with standard group as shown in (Fig. 3).

GSH levels were significantly increased in 5%, 10% and 15% of GC-02($p<0.0001$) and in 10% GC-01($p<0.01$) treated groups when compared with control and standard groups as shown in (Fig. 2).

Effect on burn wound model: Both the formulations showed faster healing of wound (rate of wound contraction) when compared with control group as shown in (Table 3 and Fig. 4). There was a significant decrease in the number of days required for complete healing of wound in the animals treated with 10% and 15% ofGC-01 ($p<0.001$),5%, 10%, 15% of GC-02 ($p<0.0001$) and when compared to control group.Higher concentrations of both the formulations have shown better results. 15% GC-02 was found to be more potent and equivalent to the standard drug.

There was a significant increase in hydroxy proline content in groups of 15% GC-01,10%, 15% of GC-02, formulation($p<0.0001$)and 10% GC-01, 5% of GC-02 ($p<0.01$) when compared tocontrol group.

There was no significant change observed in MDA levels in GC-01 and GC-02 treated animals compared to control as well as standard group.

On comparing different concentrations of both the formulations, there was significant increase in SOD levels of 5% GC- 01 which was comparable to15% GC-01, 10%,15% of GC-02 and 10% GC-01 was compared with 15% of GC-02 ($p<0.0001$).

GSH levels were significantly increased in the treatment groups when compared with control group. Significant

Table 1. Effect of GC 01 and GC 02 on tensile strength in incision wound model on rats a- indicates comparison with control and b- indicate comparison with standard (FramycetinSulphate) treated group.

Groups	Treatment	Tensile strength (g) (Mean±SEM)
I	Control	298.40± 12.56b*
II	Standard	361.30±11.22
III	5% GC-01	355.60±10.16
IV	10% GC-01	375.80±13.56a**
V	15% GC-01	480.80±17.50a***,b***
VI	5% GC-02	389.90±18.73a***
VII	10%GC-02	463.90±15.36a***,b***
VIII	15% GC-02	575.40±8.78a***,b***

Table 2. Effect of GC-01 and GC-02 in Excision wound model in rats a- indicates comparison with control and b- indicate comparison with standard (FramycetinSulphate) treated group.

Groups	Treatment	Mean wound area ±SEM (mm2)						Epithelization Period (days)±SEM
		2ndday	6th day	10th day	14th day	18th day	20th day	
I	Control	306.80±5.73 (5.59±1.76)	207.30±8.38 (36.20±2.57)	72.25±3.88 (57.74±1.19)	22.50±2.10b*** (80.04±0.64)	6.75±1.37 (97.89±0.42)	2.50±0.95 (99.20±0.29)	21.0±0.40b***
II	Standard	244.50±15.28 (24.76±4.70)	186.8±8.34 (42.51±2.56)	55.75±2.75 (82.82±0.84)	7.75±5.66 (97.58±1.74)	0.0±0.0 a*** (100)	0.0±0.0 a*** (100)	15.50±0.28a***
III	5% GC 01	299.00±5.99 (7.99±1.82)	206.5±10.19 (36.45±3.13)	51.25±11.28 (84.20±3.46)	16.0±1.47b** (95.04±0.45)	0.0±0.0 a*** (100)	0.0±0.0 a*** (100)	17.25±0.47 a***
IV	10% GC 01	307.50±6.46 (5.38±1.98)	194.5±3.37 (40.14±1.03)	39.0±1.58 (87.97±0.48)	9.75±1.25a** (96.97±0.38)	0.0±0.0 a*** (100)	0.0±0.0 a*** (100)	16.0±0.40 a***
V	15% GC 01	301.50±5.45 (7.22±1.67)	163.0±17.30 (49.83±5.32)	42.75±2.01 (86.82±0.62)	2.25±0.85 a*** (99.27±0.26)	0.0±0.0 a*** (100)	0.0±0.0 a*** (100)	14.25±0.47 a***
VI	5% GC 02	293.00±19.41 (8.30±3.31)	160.3±14.87 (50.66±4.57)	82.50±9.76 (74.59±3.01)	21.25±1.25 b*** (93.43±0.38)	0.0±0.0 a*** (100)	0.0±0.0 a*** (100)	17.75±0.47 a**,b*
VII	10% GC02	263.00±12.40 (19.07±3.81)	147.8±15.61a* (54.50±4.80)	60.50±11.0 (81.36±3.38)	9.25±2.59 a** (97.12±0.79)	0.0±0.0 a*** (100)	0.0±0.0 a*** (100)	17.25±0.47 a***
VIII	15% GC 02	253.30±25.31 (22.05±2.78)	121.3±9.81 a***,b* (62.65±3.01)	60.50±11.0 (81.36±3.38)	0.25±0.25 a*** (99.89±0.07)	0.0±0.0 a*** (100)	0.0±0.0 a*** (100)	12.0±0.70 a***,b***

Figure: 1

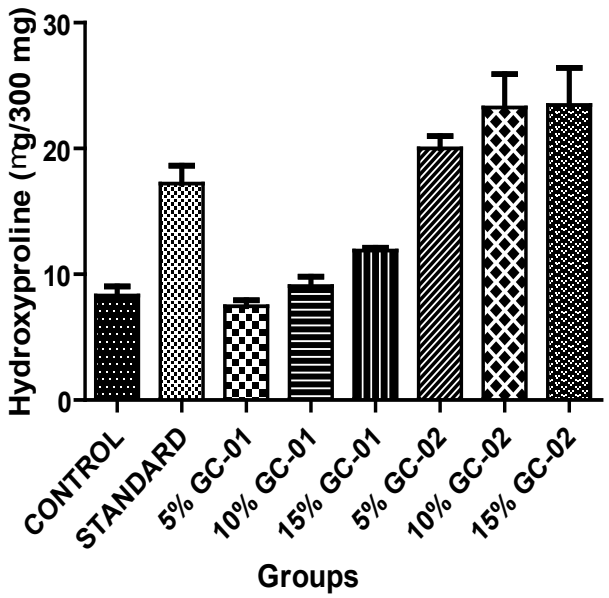
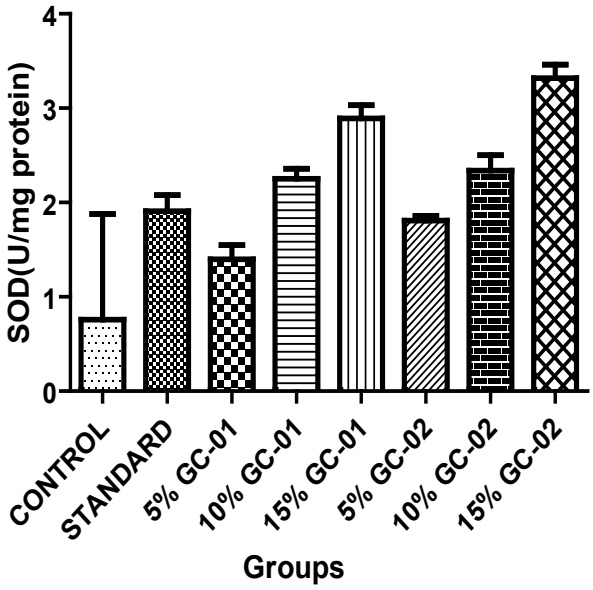


Figure: 2

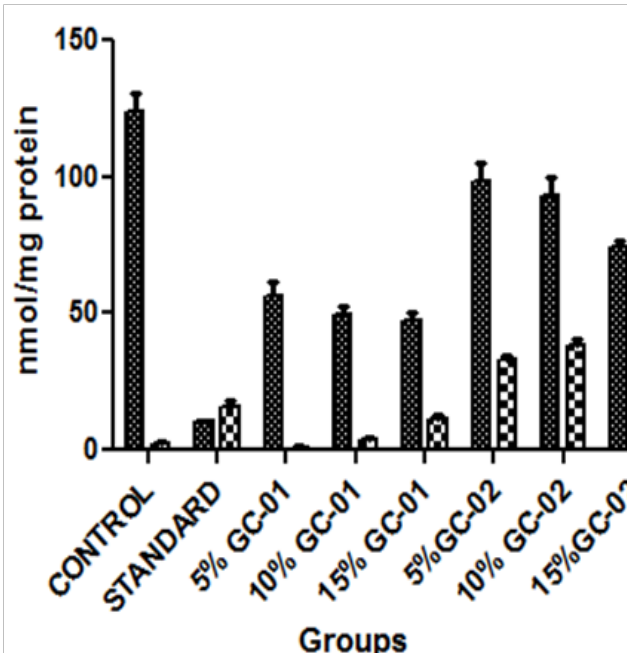


increase was observed in GSH levels of 5%, 10% and 15% of GC-01 and5% GC-02 formulation ($p<0.0001$) when compared with standard group. Standard group has shown significant increase ($p<0.0001$) when compared with 5%, 10%, 15% GC-01 and 5% GC-02.The various biochemical and anti-oxidant parameters in burn wound model are listed in (Table 4).

Discussion

Wounds in severe caseswhen left untreated can pose a major health problem, sometimes even leading to death. The phenomenon of restoring damaged tissue as closely as possible to normal state is known as wound healing resulting in restorage of tissue integrity. The process consists of acute inflammatory response, proliferation,

Figure: 3



remodelling of connective tissue, synthesis of extracellular matrix protein, acquisition of tensile strength, contraction and epithelization. The basic principle of optimal wound healing involves minimal tissue damage and provision of adequate tissue perfusion and oxygenation, proper nutrition and wound healing environment to restore the continuity and function of the affected part.

GC-01 and GC-02 are polyherbal formulations having a combination of herbs processed in gheewith reportedphytoconstituents like flavonoids, steroids, curcumin, diterpenoids, azadiractin, β-sitosterol, berberine, chebulinic acid,etc. Since some of these active constituents are known to contain growth promoting factor which enhances the wound healing process, the present claim of this formulation under study as a wound healing agent may be attributed to these active principles solely or synergistically. Further, the process of wound healing has two components, first is the formation of new tissue and other is the protection from microbial infections duringhealing[2]. Ghee promoteswound healing while herbs present in the PHF GC-01 and GC-02 offers both components. This may be the reason to formulate the present polyherbal formulations GC-01 and GC-02 in ghee, used extensivelyin Ayurveda for wound healing.

Tensile strength of wound represents the effectiveness of wound healing(the force required to open the healing skin) and is used to measure the completeness of healing.[15] In incision wound model, the increase in tensile strength of skin in treated wounds may be due to elevated collagen levels and stabilization of collagen fibers. Significant wound healing activity was observed in the animals treated with GC-01 and GC-02 polyherbal formulations on 10th post wounding day. From this, it may be inferred that the formulations not only increases collagen synthesis per cell but also aided in cross linking of the protein.

Hydroxy proline is the result of collagen breakdown and its measurement gives an index of collagen turnover.[16] In the present study, both the formulations have shown dose dependent faster healing and increase in levels of hydroxyproline, superoxide dismutase and glutathione. Increased turnover of these leads to rapid healing with concurrent increase in tensile strength. Free radicals and oxidative reaction products produce tissue damage. Overproduction of reactive oxygen species (ROS) results in oxidative stress thereby causing cytotoxicity and delayed wound healing. Therefore, elimination of ROS could be an important strategy in healing of chronic wounds.[17] ROS also play an important role in the failure ofischaemic wound healing while antioxidants improve healing in ischemic skin wounds.[18]

Hence, estimation of antioxidants like superoxide dismutase (SOD) and glutathione (GSH) in granulation tissues were also relevant as these anti-oxidants hasten the process of wound healing by destroying the free radicals. In the present study, both the formulations have shown significant increase in the levels of SOD and GSH , indicating preventive phenomenon of the formulations against oxidative damage, thereby contributing to wound healing.

Elevated levels of MDA, a marker of free radical damage may be attributed to impaired wound healing in immune-compromised rats.[19] Lipid peroxidation is an important process of several types of injuries like burn, inflicted wound and skin ulcers. A drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils, increasing the strength of collagen fibers by an increase in circulation, thereby preventing the cell damage and promoting DNA synthesis.[18] MDA levels were significantly reduced by the formulations under study in excision wound model suggesting protection to damaged tissues whereas there was no significant change in MDA levels in burn wound model. There are reports suggesting that lipid peroxidation process begins immediately after severe burn and was continued during the whole 48 h post burn period and marked increase in plasma levels of MDA depends according to the extent of injury.[20] Since, there are no significant changes in MDA levels in this model, the protection in burn may be due to elevation of other parameters like hydroxyproline, SOD and glutathione.

In addition to this, it is a widely known fact that burn wound after healing leaves a scar on skin

Table 3. Effect of GC-01 and GC-02 formulations on burn wound model in rats a- indicates comparison with control and b- indicate comparison with standard (Silver sulfadiazine cream) treated group.

Groups	Treatment	Mean wound area(mm2) ±SEM						Epithelization Period (days)±SEM
		2ndday	6th day	10th day	14th day	18th day	20th day	
I	Control	316.50±3.01 (2.61±0.92)	232.50±5.57 (28.44±2.71)	178.80±3.68b*** (44.90±1.13)	104.50±6.86b*** (67.80±2.11)	56.75±4.60b*** (82.66±1.41)	21.85±0.95b*** (93.24±0.29)	21.75±1.10b***
II	Standard	306.0±4.20 (5.84±1.29)	174±3.13 (46.44±0.96)	92.50±4.80 (71.51±3.01)	5.50±5.63 (98.20±1.73)	1.25±0.75 a*** (99.50±0.23)	0.0±0.0 a*** (100)	14.00±0.70a***
III	5% GC 01	295.3±7.82 (9.13±2.40)	171.0.±16.01a* (47.37±2.41)	110±6.86a** (66.10±2.11)	21.00±2.48a*** (93.51±0.76)	13.0±1.95 a*** (95.91±0.59)b*	0.0±0.0 a*** (100)	19.25±0.75 a***,b***
IV	10% GC 01	289.3±7.82 (10.98±2.40)	197±15.58 (39.37±4.79)	94.25±14.95a** (70.97±4.59)	16.25±6.46a*** (94.97±1.98)	13.25±2.28 a*** (95.84±0.70)b*	0.0±0.0 a*** (100)	17.75±0.47 a***,b*
V	15% GC 01	294±3.58 (9.53±1.10)	179.0±16.01 (44.90±4.92)	113.8±17.91a* (64.9±5.50)	28.50±5.20 a*** (91.20±1.59)	4.00±1.82 a*** (98.72±0.55)	0.0±0.0 a*** (100)	15.00±0.40 a***
VI	5% GC 02	282.50±10.70a* (13.00±3.29)	189.0±16.66 (41.80±4.12)	89.75±12.96a*** (72.30±3.98)	20.50±4.80 a*** (93.60±1.47)	9.75±3.30 a*** (96.96±1.01)	0.0±0.0 a*** (100)	15.00±0.40 a**
VII	10% GC02	261.30±10.80a*** (19.59±3.32)b**	185.50±13.93 (42.90±4.28)	92.0±13.64a*** (71.60±4.19)	9.70±3.75 a*** (96.90±1.15)	3.00±1.29 a*** (99.00±0.39)	0.0±0.0 a*** (100)	14.50±0.64 a***
VIII	15% GC 02	289.50±5.33 (10.91±1.63)	161.30±14.04a* (51.30±4.31)	84.75±10.10a*** (73.90±3.10)	6.75±2.68 a***,b* (97.80±0.82)	1.25±0.75 a*** (99.57±0.23)	0.0±0.0 a*** (100)	13.75±0.62 a***

Figure 4: Photographs showing effect of GC-01 and GC-02 in burn wound healing

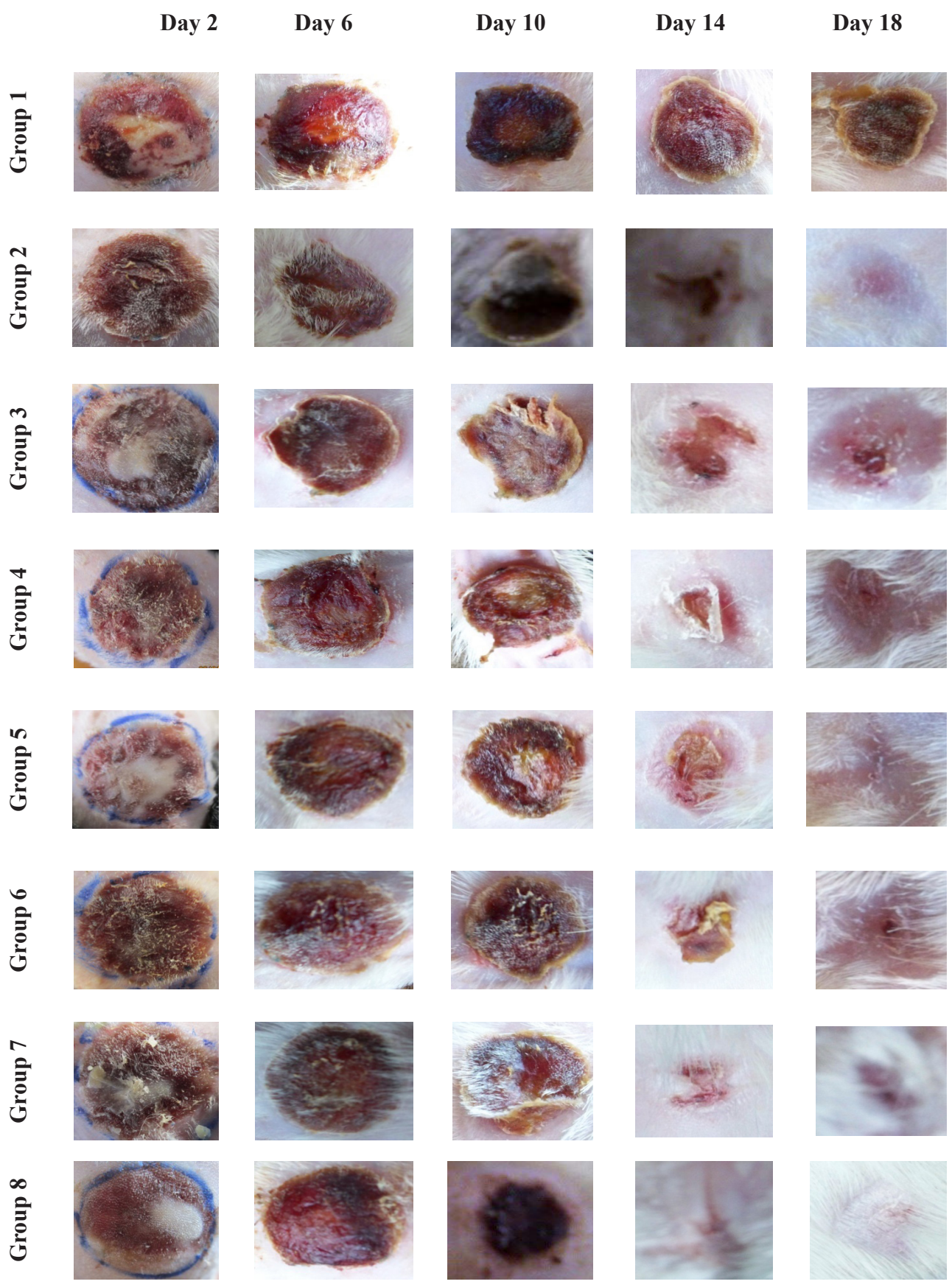


Table 4. The various biochemical and anti-oxidant parameters in burn wound model are as follows a- indicates comparison with control and b- indicate comparison with standard (Silver sulfadiazine cream) treated group.

Groups	Treatment	Biochemical parameters	Anti-oxidant parameters	
		Hydroxyproline (µg/300mg)	Glutathione (nmol/mg protein)	SOD (U/mg protein)
I	Control	9.52±0.98b*	3.38±0.47b***	1.54±0.19 b*
II	Standard	23.49±1.57	43.69±2.44	7.14±0.85
III	5% GC 01	18.16±1.63	9.60±0.95b***	4.94±1.58
IV	10% GC 01	25.38±4.92 a**	16.60±0.91 b**	12.26±1.47 a***,b*
V	15% GC 01	29.75±2.46a***	23.26±0.87a*	19.23±0.87 a***,b***
VI	5% GC 02	26.97±2.08a**	26.76±1.25 a***,b***	5.76±1.00
VII	10% GC02	30.28±1.36 a***	37.49±1.05 a***,b***	18.97±0.89 a***,b***
VIII	15% GC 02	31.92±2.58 a***	41.00±1.77 a***,b***	27.19±0.72 a***,b***

while Silver sulfadiazine is devoid of thisandit can be noted that the higher concentrations of the formulations (15%w/v) of GC-01 and GC-02 also leftno scar on skin which is a highly acceptable observation during clinical trials.

Recent studies have shown that phytochemical constituents like flavonoids and triterpenoids are known as promote the wound healing process mainly due to astringent and antimicrobial properties, which appear to be responsible for wound contraction and increase in rate of epithelization.[21]

The potent wound healing activity of GC-02 compared to GC-01 may be due to the presence of additional herbs like *Rubia cordifolia, Trichosanthes cucumerina, Terminali achebula, Vetiveria zizanioides, Madhuca longifolia*, berberis aristata as these herbs prove to have anti-oxidant activity contributing to wound healing. In addition, the herbs which are in common in GC-01 and GC-02 are found to be more in quantity in GC-02 formulation which may be attributing to the more potent wound healing property of GC-02.

Since the formulations contain many herbs from folklore medicine and reported phytoconstituents of the herbs viz. flavonoids, steroids, curcumin, diterpenoids, triterpenes, azadiractin, β-sitosterol, berberine, chebulinic acid, etc are well known to play active role in wound healing and anti-inflammatory activity. Also, the constituents are known to contain growth promoting factor which enhances the healing process, the increase in wound healing activity may be due to these constituents in addition to ghee. Moreover, some of the herbs in the formulations like *Curcuma longa, Azadirachta indica, Butea monosperma, Commiphora mukul, ghrita* etc. have already been reported for their wound healing, anti inflammatory, anti arthritic, antiseptic and anti-microbial properties. Thus, the above findings could be attributed to the effective wound healing property of both GC-01 and GC-02 polyherbal formulations thereby justifying the use of these herbs in indigenous system of medicine.

CONCLUSION:

The polyherbal formulations GC-01 and GC-02 in ghee when compared for wound healing potency, GC-02 was found to be superior to GC-01 may be due to the presence ofadditional herbs compared to GC-01 exhibiting their synergistic potential. Hydroxy proline content in healed tissue was found to be greater in GC-02 than in GC-01 treated healed wound. The active constituents of both the formulations in ghee may be responsible for potential wound healing activity. From, these results it can be concluded that both formulations GC-01 and GC-02 have potentially beneficial effect as wound healing agents.

REFERENCES

1. Kotade K, Mohammed A. Wound healing activity of *Sesamum indicum L* seed and oil in rats. Ind J Exp Biol. 2008; 46: 777–82.
2. Prasad V, Dorle AK. Evaluation of ghee based formulation for wound healing activity. J Ethnopharmacol. 2006; 107: 38–47.
3. Shanbhag TV, Sharma C, Sachidananda A, Bairy LK,Shenoy S, Shenoy G. Wound Healing Activity Of Alcoholic Extract Of *Kaempferia Galanga* in Wistar Rats. Ind J Physiol. Pharmacol. 2006; 50(4): 384–90.
4. Karodi R, Jadhav M, Rub R, Bafna A. Evaluation of the wound healing activity of a crude extract of *Rubia cordifolia* L. (Indian madder) in mice. IJARNP 2009; 2(2): 12–18.
5. Govindarajan R., Vijayakumar M., Rao, CV, et al.,Healing potential of *Anogeissus latifolia* for dermal wounds in rats. ActaPharm. 2004; 54:331–38.

6. Lee KH, Tong TG. Mechanism of action of retinyl compound on wound healing II and effect of active retinyl derivative on granulation formation. J Pharma Sci 1970; 59: 1195–97.
7. Morton JJ,Malone MH. Evaluation of vulnerary activity by an open wound procedure in rats. Arch Int Pharmacodyn Ther. 1972; 196: 117–26.
8. Shanmuga PK, Gnanamani A, Radhakrishnan N, Babu M. Healing potential of *Datura alba* on burn wounds in albino rats. J Ethnopharmacol. 2002; 83: 193–99.
9. Shukla A, RasikAM. Asiaticoside-induced elevation of antioxidant levels in healing wounds. Phyto Res. 1999; 13: 50–54.
10. Neuman RE, Logan MA. The determination of hydroxyproline. J BiolChem 1950; 184: 299–306.
11. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Ind J Biochem Biophys. 1984; 21 :130–32.
12. Rajeshkumar UK, Amteshwar SJ, Singh N, Sharma BK. Ameliorative role of Atorvastatin and Pitavastatin in L-Methionine induced vascular dementia in rats. BMC Pharmacol. 2008; 8: 14.
13. Beutler RG, Duron O, Kelly B: Reduced glutathione estimation. J Lab Clin Med. 1963; 61:82.
14. Pulla RAC, Lokesh BR. Studies on spice principles as antioxidants in the inhibition of lipid peroxidation of rat liver microsomes. Mol. Cell. Biochem. 1992; 111: 117–24.
15. Fulzele SV, Satturwar PM, Joshi SB, Dorle AK. Wound healing activity of Hingvadyaghrita in rats. Indian Drugs 2002; 39: 606–09.
16. Agrawal PK, Singh A, Gaurav K,Goel S. Evaluation of wound healing activity of extracts of plaintain banana (*Musa sapientum*) in rats. Ind J. Exp. Biol. 2009;47: 32–40.
17. Dissemond J, Goos M, Wagner SN. The Role of Oxidative Stress in the Pathogenesis and Therapy of Chronic Wounds. Hautarzt . 2002; 53: 718–22.
18. Senel O, Centinkale O, Ozbay G, Ahcioglu F, Bulan R. Oxygen Free Radicals Impair Wound healing in Ischaemic Rat Skin. Ann Plas Surg.1997; 39: 516–20.
19. Gupta A, Singh R L, Raghubir R. Antioxidant Status during Cutaneous Wound healing in Immunocompromised Rats. Mol Cell Biochem 202; 241: 1–7.
20. Marciniak A, Szpringer E, Lutnicki K, Bełtowski J. Inflluence of Early Excision of Burn wound on the Lipid peroxidation in selected rat tissues. Bull. Vet. Inst. Pulawy. 2002; 46: 255–65.
21. Tsuchiya H, Sto M, Miyazaki T, Fujiwara S, Tanigaki S. The Antibacterial Activity of Phytochemical Flavanones against Methicillin resistant Staphylococcus Aures. J Ethnopharmacol. 1996; 50: 27–32.