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Research Article

ASSESSMENT OF WOUND HEALING ACTIVITY OF POTENT HERBAL EXTRACTS GEL IN ALBINO WISTAR RAT

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ABSTRACT

Objectives: The purpose of this study was to formulate and determine the wound healing activity of gel containing the blend of potent herbal extracts from various plants such as *Curcuma longa* L., *Tridax procumbens* L., and *Jatropha curcas* L.

Methods: The crude extracts were obtained by the Soxhlet extraction and maceration method. Preliminary phytochemical screening was done for all extracts. Two different concentrations of extract gels (HF1 and HF2) were prepared using Carbopol 934 as a gel base. Prepared gels were further evaluated for different parameters such as appearance, pH, viscosity, spreadability, extrudability, skin irritation test, and stability studies. Excision wound model used to determine wound healing activity in albino Wistar rat. Animals were divided into four groups and each group contains six animals (n=6). Soframycin was used as a standard treatment. Prepared formulations were applied to wounds for all 14 days of study. Wound contraction rate was measured at specified day's intervals during the study.

Results: Preliminary phytochemical analysis confirmed the presence of the bioactive phytoconstituents. Both the gel formulations showed good gelling properties and homogeneity. The pH of both gels lies in the normal pH range of human skin and there is no skin irritation. Formulations were found within the specified limit in stability studies. The obtained results of wound contraction rate were higher in Group IV which was treated with HF2 gel formulation compared to the control group (*p<0.05).

Conclusion: It was concluded that the prepared herbal gel formulation shows a promising wound healing effect compared to synthetic medicament.

Keywords: Wound healing, *Curcuma longa* L., *Tridax procumbens* L., *Jatropha curcas* L., Soxhlet extraction, Maceration, Excision wound model, Carbopol 934.

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INTRODUCTION

Wide range of herbal medicines used in India since ancient times. Active phytochemical constituents of different species of medicinal plants play an important role in the treatment of various diseases and disorders such as diabetes, hypertension, wounds and burn, and inflammatory diseases [1,2]. Synthetic drugs have multidrug resistance and several adverse effects on health, ultimately world shifting their trend and start using herbal extract over synthetic medications [3]. India cultivates and harvests such a potent herb and provides it to the whole world [4].

The wound damages the normal skin and breakdown its protective function [5]. The wound is a very challenging clinical issue if it is not treated with the correct treatment. Sometimes, wounds become more infectious and lead to mortality which is mostly seen in the developing countries where there are no proper hygienic condition and clean environment [6]. Unhealed wounds continuously produce inflammatory mediators that lead to pain and swelling at the wound site. Wound care is a complex, process, and much costlier when we treat with synthetic drugs, and still, they have several side effects. Medicinal plants consist of active medicaments for wound healing in many clinical situations due to their cheap price, easily available, non-toxic, ease of use, and patient compliance [7].

Wound healing is a complicated and long-term process, which includes interactions with immunological and biological systems. Wounds are mostly considered acute wounds and chronic wounds [8]. In acute wounds, the process of healing includes the initial inflammation and immunological reactions, accumulation of collagen and fibroblast, angiogenesis, contraction of the wound area, and scar remodeling. In the inflammatory process, various inflammatory mediators involve like PGE2. In cells, proliferation fibroblast plays an important role in re-epithelization. Collagen is an important component of extracellular tissue in wound healing [9-11].

In chronic wounds, more clinical challenges occur, and the healing rate is slow. This is because of some underlying conditions such as microbial infections and several disease states. Due to infection at the wound site and endotoxins production, repeatedly immunological reaction occurs which leads to inflammation at the wound site and healing slowed [8].

Topical gel formulations are used for healing wounds because gel being lipophilic easily penetrate the skin layer and can show promising result in wound healing [12]. Gelling agents such as various carbomers mostly Carbopol are more acceptable gel base because it is biocompatibility and has low or negligible toxicity [13]. Herbal extracts gels are best suitable for healing wounds because they act as humectants and produce a protective layer over the wound surface [7].

The main purpose of this study was to formulate and determine the wound healing potential of various herbal extracts gel, which contains three different medicinal plant extracts such as *Curcuma longa* L., *T. procumbens* L., and *Jatropha curcas* L. These herbal extracts containing gel is prepared using a biodegradable gelling agent i.e., Carbopol 934 and other biocompatible excipients. The wound healing potency of gel was pharmacologically evaluated by excision wound model in albino Wistar rat and determined the wound contraction rate at specified days of study.

MATERIALS AND METHODS

Plant materials

Three different medicinal plant materials were used, including the dried rhizome of *C. longa* L. (Zingiberaceae), whole dried

plant of *T. procumbens* L. (Asteraceae), and dried latex of *J. curcas* L. (Euphorbiaceae).

Curcuma longa L.

C. longa L. dried rhizome is also called turmeric in English and Haldi in the Hindi language. Turmeric is a perennial herb and member of the Zingiberaceae family. *C. longa* L. has a long traditional use as medicine in Chinese traditional medicine and in Ayurveda. It is used as a potent anti-inflammatory agent and treatment of other diseases. It is also used in various cosmetics and dermatological treatments. The active constituents of turmeric are flavonoids such as curcumin, desmethoxycurcumin, and bisdemethoxycurcumin [14-16].

Tridax procumbens L.

The whole plant of *T. procumbens* L. another name is coat button in English and Ghamra in Hindi. It is belonging to the Asteraceae family. It is a wild herb distributed throughout India and has an ancient heritage of traditional medicine. The active constituent of *T. procumbens* is a flavonoid called procumbenetin, this medicinal herb is used in various clinical treatments in wound healing, diabetes, cancer, and as an antimicrobial agent [17-20].

Jatropha curcas L.

J. curcas L. is a shrub and belongs to the Euphorbiaceae family. *J. curcas* L. produce latex after breaking the aerial parts and it is having lots of medicinal importance. Latex contains a curcacyline chemical constituent that helps to stop gum bleeding and make a healthy tooth, also it is having a beneficial role in ulcer treatment. The latex of Jatropha is traditionally used as a hemostatic agent (Figs. 1-3) [21-23].

Fig. 1: Rhizome of *Curcuma longa*

Fig. 2: Whole plant of *Tridax procumbens*

Collection and authentication of the plant material

The dried rhizomes of *C. longa* L. were collected from the local market. The whole plant of *Tridax procumbens* L. and latex of *Jatropha curcas* L. were collected from the botanical garden of Vishal Institute of Pharmaceutical Education and Research. The plant materials were authenticated by Dr. S.S. Rahangdale, Department of Botany, B. J. College, Ale, Pune. The identified and authenticated plant materials were used for further extraction process.

Chemicals and reagents

Carbopol 934, glycerol, propylene glycol, and triethanolamine (Loba Chemie, Mumbai, Maharashtra, India), methyl paraben and propyl paraben (Suprim Chemicals, Mumbai, Maharashtra, India), ethanol, acetone, and petroleum ether (S D Fine-Chem Limited), and purified water and soframycin cream 1% (Mfg. by Sanofi India Limited). All analytical grade reagents were used for other phytochemical estimations and other analytical interpretations.

Preparation of extracts

Extraction of *C. longa* was done by the Soxhlet extraction method [24]. Dried rhizome powder of turmeric was placed in Soxhlet apparatus using acetone as an extracting solvent, extraction was carried out at 70°C for 6 h. The excess acetone was evaporated using a rotatory evaporator to get the semisolid extract. Extract further dried using hot air oven, after drying extract was a store at room temperature and dry place. *T. procumbens* extract was prepared using the hydroalcoholic maceration method [25]. The whole plant was washed with water to remove unwanted particles and dried by keeping for sun drying, after completely dry plant material grind to make a fine powder. Petroleum ether was used for defatting and mixed with hydroalcoholic solvent ethanol: water (1:1) for 24 h maceration process. Filter the extract using a muslin cloth and liquid extract dried using a hot air oven. Collected latex of *J. curcas* filtered to remove unwanted particles and then dried using a hot air oven. After complete dry latex powder was triturated with mortar and pestle to make a fine powder, latex powder was stored at room temperature and dry place.

Preliminary phytochemical screening of prepared extracts

Phytochemical estimation of the extracts was carried out after successive extraction according to the standard protocol. The prepared plant extracts were subjected to preliminary phytochemical screening to identify the presence of various phytochemicals, that is, carbohydrates, proteins and amino acids, vitamins, oil and fat alkaloids, terpenoids, glycosides, steroids, triterpenoids, flavonoids, saponins, and tannins [26-28].

Preparation of herbal extracts gel

Two different concentrations of herbal extracts gel (HF1 and HF2) were prepared using simple mixing of extracts and gel base. A 5 g of Carbopol dispersed in 200 ml of purified water and keep aside for overnight soaking. Prepared extracts were dissolved in propylene glycol

Fig. 3: Plant of *Jatropha curcas*

and glycerol to make a uniform blend. Mix the weighed quantity of gel base and extract blend together using a mechanical agitator (Table 1), while mixing add the preservatives, that is, methyl paraben and propyl paraben. Triethanolamine is used to adjust the pH of formulation in the range near human skin [29]. Stir until getting homogenized and good consistency gel.

Evaluation of herbal extracts gel

Prepared gel formulations were evaluated for various organoleptic and physicochemical parameters.

Appearance

Both gels were tested for homogeneity and visual inspection after gel preparations. They were tested for their color, odor, and texture.

pH

The pH of the herbal gel formulations was determined using a digital pH meter. A 1 g gel dissolved in 10 ml of purified water and keep aside for 20 min, then pH takes with triplicate readings.

Viscosity

The viscosity of prepared gel formulations was determined using a Brookfield viscometer with an LV-4 spindle.

Spreadability

The spreadability of gel formulations was determined using the glass slide method. The formulation was applied on one glass slide and another glass slide was kept over it with an attached known weight. Movement time and length of movement of one slide over another were calculated and determined the spreading index for gel [30].

Extrudability

The gel formulation was filled in 25 g collapsible tubes. The extrudability was determined by the weight of gel required to extrude 0.5 cm of ribbon in 10 s [31].

Skin irritation test

A 2 g of gel formulation apply to the skin of five different people and observed for any irritation or redness on site of administration.

Stability studies

Both gel formulations were kept in a collapsible tube in a stability chamber for accelerated stability testing at 40±2°C/75% RH±5% RH for 6 months as per ICH guidelines. Formulations were evaluated for appearance, pH, and viscosity on the initial day and after 6 months [32].

Animal and treatments

Male albino Wistar rats (150–200 g) were procured from the animal house of Vishal Institute of Pharmaceutical Education and Research and were free access to water and food. Animal studies were conducted according to the ethical guidelines of CPCSEA. Approval was obtained from Institutional Animal Ethical Committee (IAEC) for wound healing activity in animals with project reference no: VIPER/IAEC/UG/2015-01. All rats acclimatize at 22°C and are divided into four groups, each group containing six animals (n=6). Group I animals were untreated and considered a control. Group II animals received 1% soframycin cream which considers a standard treatment. Groups III and IV received treatment of prepared herbal extracts gel formulation HF1 and HF2, respectively. All treatments are given to animals for the whole study period, that is, 14 days of study.

Table 1: Composition of herbal extract gel formulations

Serial number	Ingredient	Quantity (%)	
		HF1	HF2
1	*Curcuma longa* L. extract	0.6	1.0
2	*Tridax procumbens* L. extract	0.45	0.75
3	*Jatropha curcas* L. extract	0.45	0.75
4	Propylene glycol	3.0	5.0
5	Glycerol	2.0	3.0
6	Methyl paraben	0.5	0.5
7	Propyl paraben	0.5	0.5
8	Triethanolamine	1.5	1.5
9	Carbopol 934 gel base	Q.S to 100	Q.S to 100

Excision wound model

Grouped animals acclimatize to the working environment, animals were anesthetized with the help of a chloroform chamber. Hairs were removed from the dorsal thoracic central region and marked the excision area measured at around 2 cm^2 with a marker pen. Full-thickness marked area was excised to produce the wound. The wound area was cleaned with a cotton swab soaked in ethanol. Animals were kept in an open environment to reduce the effect of anesthesia and then shift to a clean room for further wound healing activity assessment [33].

Assessment of wound healing

Wound contraction rate was measured for all groups daily for 14 days of the study and recorded the data of surface area changes in cm^2. The change of surface area in post-wound days compared with initial days and calculate the wound contraction rate (%) using the following equation [34].

$$\text{Wound contraction rate } (\%) = \frac{\text{Initial day wound size } (W_0) - \text{Treatment days wound size } (W_t)}{\text{Initial day wound size } (W_0)} \times 100$$

Statistical analysis

Experimental data are expressed as the mean ± standard error of the mean. Statistical analysis was performed by one-way ANOVA followed by Student's *t*-test using GraphPad Prism version.9.3.1 software. p value was (*p<0.05) considered statistically significant compared to the control group.

RESULTS AND DISCUSSION

The result shows the good yield of extracts after successive extraction of dried rhizomes of *C. longa* and whole plant of *T. Procumbens* using the Soxhlet extraction and hydroalcoholic maceration method. Furthermore, the latex of *J. curcas* after complete drying. Phytochemical screening of all extracts shows the presence of various phytoconstituents such as carbohydrates, proteins and amino acids, vitamins, oil and fat, alkaloids, terpenoids, glycosides, steroids, flavonoids, saponins, and tannins, as shown in Table 2. All core phytoconstituents show presence in all extracts. Phenolic compounds have a potential role in wound healing.

The blend of extracts gel was prepared using Carbopol 934 gelling agent and other excipients. Both gel formulations were evaluated and found satisfactory results. The appearance of the formulations was found good

Table 2: Phytochemical screening of prepared extracts

Serial number	Phytochemical tests	Herbal extracts		
		Curcuma longa	*Tridax procumbens*	*Jatropha curcas*
1	Carbohydrates	+	+	–
2	Proteins and amino acids	+	+	+
3	Oil and fat	+	+	–
4	Vitamins	+	–	–
5	Alkaloids	+	+	+
6	Glycosides	+	+	+
7	Steroids	+	+	+
8	Triterpenoids	+	+	–
9	Tannins	+	+	+
10	Saponins	+	+	+
11	Flavonoids	+	+	+

+: Present, –: Absent

Table 3: Stability studies of herbal extracts gel

Formulations	Storage duration	Color and appearance	pH	Viscosity (cps)
HF1	Initial	Brownish- yellow smooth gel	6.12	2615
	6 months	Brownish-yellow smooth gel	6.09	2608
HF2	Initial	Brownish-yellow smooth gel	6.25	2542
	6 months	Brownish-yellow smooth gel	6.18	2533

Table 4: Day-wise wound contraction area in cm^2

Groups	Animal treatment	Wound area (cm^2)			
		1st day	5th day	9th day	14th day
Group I	Control	2.0±0.02	1.6±0.05	1.1±0.03	0.6±0.04
Group II	Standard	2.0±0.06	1.4±0.03	0.8±0.01	0.1±0.02
Group III	HF1	2.0±0.02	1.4±0.05	0.7±0.04	0.2±0.03
Group IV	HF2	2.0±0.04	1.2±0.01	0.5±0.02	0.0±0.01

*P<0.05 compared to control. Values are expressed as mean±SEM (n=6).
SEM: Standard error of mean

and smooth texture gel when touched. The color of formulations was the brownish-yellow and pungent odor. The pH of both formulations was 6.12 and 6.25, which was within the human skin pH range. The viscosity of both gel formulations was in the acceptable range of gel formulations; it was 2615 and 2542 cps for HF1 and HF2, respectively. The spreadability of both formulations was found at 15.25 and 16.54 g.cm/s. Both gel formulations were shows good extrudability and easily come out from the collapsible tube after applying minimum pressure to the tube. There was neither significant adverse effect nor irritations at the site of administration on human skin observed, formulations are completely safe for topical use on human skin. After a 6-month stability study, the formulation was found stable, and no significant change was observed at stress condition of accelerated stability at 40°C ± 2°C/75% RH ± 5% RH (Table 3).

Wound healing rate

The rate of wound healing for all groups (i.e., Group I: Control, Group II: Standard, Group III: HF1, and Group IV: HF2 treatments) was measured for 14 days (Table 4 and Fig. 4) with the interval of 4 days, 1st day consider as the initial day of treatment, 5th and 9th day of post-wound treatment, and final 14th day of wound healing study (Fig. 5). Group IV which was treated with formulation HF2 shows good wound healing compared with the control and standard groups.

The wound contraction rate % of all groups shows above 90% wound contraction except the control group and Group IV, that is, HF2 formulation shows 100% wound healing on day 14th.

The herbal extracts gel formulations show good results in wound healing compared to marketed synthetic product, that is, 1% soframycin cream. Herbal products play a crucial role in various diseases over synthetic treatments and have no or negligible adverse effects.

The efficiency of the local application of extracts such as *C. longa* and *T. procumbens* on wounds and burns has been reported by many researchers in their work. *C. longa* is a potential herbal remedy for chronic and acute wounds, as evidence of their anti-inflammatory and antibacterial activity [14,15]. The wound healing potential of *T. procumbens* is well known for decades. The whole plant of *T. procumbens* is used in various traditional medicines due to the presence of different active phytochemical constituents [17,18]. Latex of *J. curcas* promoted wound healing after the cell injury, also it is having antioxidant and antibacterial activity [35].

Polyherbal gel formulations are the modern trend for the preparation of topical formulations such as anti-inflammatory gels and wound healing gels [30]. This kind of gel contains various potent active constituents which work actively in different processes and mechanisms. The combinations of multiple herbal extracts work synergistically with less or no side effects compared to synthetic medications [36].

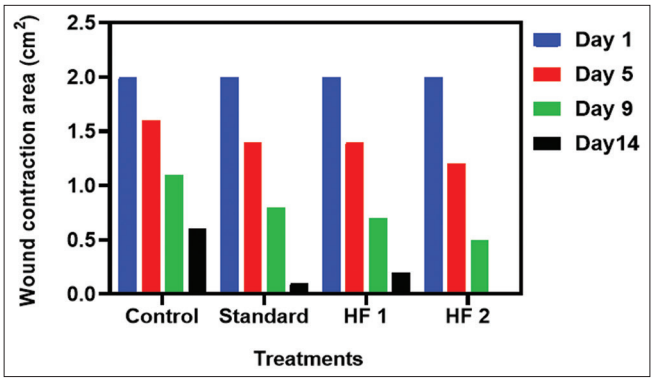

Fig. 4: Post-wound treatment days versus wound contraction area in cm^2

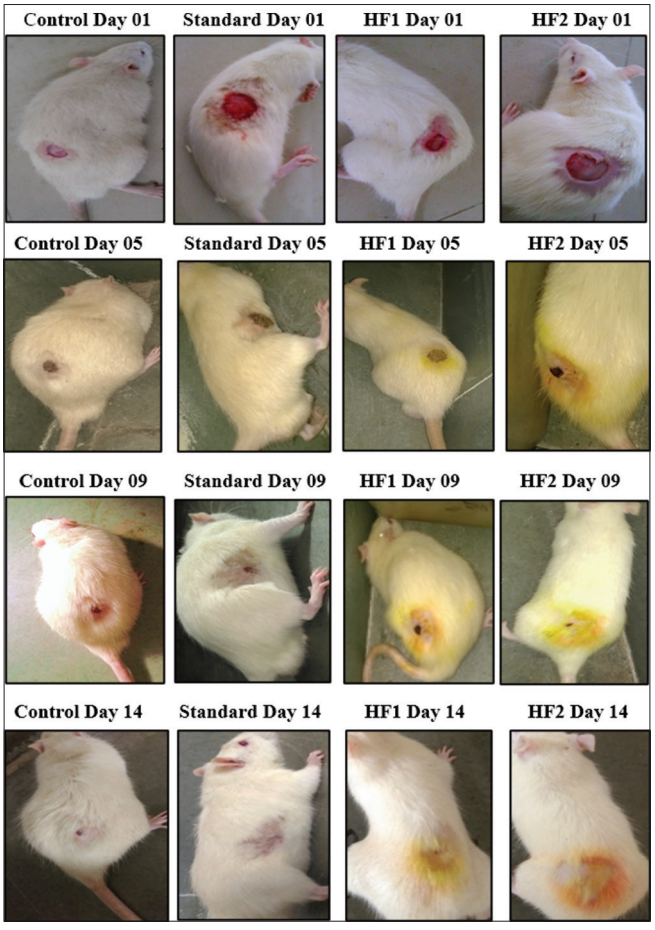

Fig. 5: The 1st, 5th, 9th, and 14th days wound contraction

CONCLUSION

Herbal extracts gel containing the extracts of *C. longa, T. procumbens,* and *J. curcas* promotes wound healing in albino Wistar rats with a comparison of the synthetic formulation. The wound contraction rate

was higher in the treatment of gel containing a higher concentration of extracts.

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AUTHORS' CONTRIBUTIONS

The coauthor assists me in the preparation of the article by reviewing it.

CONFLICTS OF INTEREST

No conflicts of interest.

AUTHORS FUNDING

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