



Research Article

EVALUATION OF NEWER POLY HERBAL FORMULATIONS FOR WOUND HEALING AND ANTI-INFLAMMATORY ACTIVITY

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ABSTRACT

According to Herbalism, independent medicinal herbs are not sufficient to obtain the required pharmacological effect, but when we use these herbal compounds in combination at appropriate proportions will help to obtain maximum pharmacological effects with minimal toxic effects. In this present study, efforts were made to develop an intervention with a poly herbal formulation which was prepared from ethanolic leaf extracts of *Chromolaena odorata*, *Tridax procumbens*, *Euphorbia hirta* Linn. The impact of poly herbalism is assessed for its wound healing property by using excision wound model and anti-inflammatory property by using formalin induced paw oedema model. For wound healing assessment two polyherbal formulations F1 and F2 are prepared with 2% and 4% W/W. These formulations were examined for stability studies. F1(*p<0.005) and F2 (**p<0.001) have shown significant wound closure and faster epithelialisation when compared to standard Povidone-Iodine (5%) ointment (**p<0.001). For assessing anti-inflammatory activity, the poly herbal formulations are prepared as 2% (F1) and 4% (F2) solutions. The doses from each formulation are given as 100 mg/kg, 200 mg/kg and 300 mg/kg to different groups of animals. Both the poly herbal formulations have shown significant results F1(*p<0.05, **p<0.005) and F2 (***p<0.0005, ****p<0.0001) respectively when compared to standard Indomethacin 4 mg/kg dose (****p<0.0001). This study has revealed that poly herbal formulations have shown better anti-inflammatory and wound healing properties due to the synergism of the phytochemicals present in these plant extracts which may lead to future developments of potential poly herbal formulations in the treatment of wounds and inflammatory mediated diseases.

KEY WORDS: Poly Herbal Formulations, Wound Healing, Anti Inflammatory, Excision Wound Model, Formalin, Paw Oedema

INTRODUCTION

Topically applied herbal medicines have established considerable attention because of their extensive use and well-defined benefits / risk ratio¹. Topical application of cream and ointment at damaged tissue will enhance the penetration of dosage form and rapid release of drug directly into the site of action². The concept of poly herbalism has mentioned in “Sarangdhar Samhitha”. This stated that products with a combination of plant extracts are more beneficial compared to alone. Independent plant extracts contain phyto constituents but are in less quantity which are not enough to produce appropriate pharmacological effect³. Generally wound healing is a dynamic process which plays a vital role in replacing the devitalized tissue structures and preventing the invading pathogens into these damaged tissues and restoring its integrity. It is a complex process which involves four phases that are hemostasis, inflammation, proliferation and tissue remodeling and finally closure of wound⁴. Conventional treatment of wounds and inflammation consist of Non-Steroidal Anti-Inflammatory drugs (NSAIDS), Antibiotic and Corticosteroids which have many drawbacks. To overcome those drawbacks phytochemicals are considered as upcoming alternative in treating wounds and inflammatory diseases. In this present study, efforts were made to validate the wound healing and anti-inflammatory efficiency of ethanolic leaf extracts of *Chromolaena odorata*, *Tridax procumbens* and *Euphorbia hirta* Linn leaves⁵.

The wound healing efficiency is mainly due to antioxidant properties of plants which enhances conserving the fibroblast and keratinocyte proliferation on those wounds⁶. *Chromolaena odorata* is one of the widespread and abundant perineal scandent semi-woody flowering shrub in the family Asteraceae which originated from Central and South America and have spread throughout Africa and Tropical Asia. Traditionally it is used as an antimalarial remedy, on soft tissue wounds, burns, dental alveolitis and various skin infections^{7,8}. *Tridax procumbens*, which is also called as coat button belonging to the family Asteraceae. It is a thin crawling flowering weed widely distributed in most parts of the world. *Tridax procumbens* is known for medicinal values such as antimicrobial as well as antioxidant. It is traditionally used in the treatment of wound, dysentery, hemorrhage, and skin infections. *Tridax procumbens* have shown antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Bacillus megaterium*. It is also used as an anticoagulant and antifungal agent⁹. *Tridax procumbens* is a potent source of antioxidants which helps in treating wounds and related infections¹⁰. *Euphorbia hirta* Linn is a small annual herb belonging to the family Euphorbiaceae. This plant is abundantly available in hotter parts of tropical countries. *Euphorbia hirta* Linn can grow up to 40 cm height with slender stems often reddish in colour with yellowish hairs on tender parts. Its leaves are 5 cm in length with greenish or reddish colour. Stem and leaves can produce milky secretion. This plant can be used in treatment of colic troubles, dysentery, cough, asthma, worms, vomiting, hypertension and

oedema. It is also used as antidote, sedative, and antioxidant^{11, 12}. Leaf extracts of these three plants are rich in alkaloids, flavonoids, tannins, saponins, terpenes, carotenoids and essential oils, which impart good antioxidant potential to these plants. Due to diverse pharmacological properties and their easy availability, these plant products are selected for preparation of newer polyherbal formulation in this study^{7,8,9,10,11}.

MATERIALS AND METHODS

Collection of Plant Materials

Chromolaena odorata, *Tridax procumbens* and *Euphorbia hirta* Linn plant leaves were collected from a local garden and authenticated by Pilikula Nisargadhama botanical garden, Vamanjoor, Mangalore. Leaves of all three plants were cleaned with distilled water and dried under shade for about two weeks. The dried leaves of all three plants were coarsely powdered separately and used for the extraction process.

Preparation of Leaf Extracts

Powdered leaves of *Tridax procumbens* were macerated with 70% ethanol for 48 hours. Macerate was filtered using Whatman filter paper followed by evaporation to obtain semi solid extract¹⁰. Powdered leaves of *Euphorbia hirta* Linn were cold macerated with ethanol for 72 hours. Macerate was filtered using Whatman filter paper and followed by evaporation to obtain semi solid extract¹¹. Powdered leaves of *Chromolaena odorata* were macerated with absolute ethanol for 48 hours with occasional stirring. Macerate was filtered using Whatman No.1 filter paper followed by evaporation to obtain semi solid extract¹³.

Formulation of Ointment Base

Required quantities of stearic acid and white wax were weighed and melted in a water bath. To the melted mixture a weighted quantity of yellow petroleum jelly, propylene glycol and water were added and stirred until the mixture turned into creamy consistency. Triethanolamine was added drop wise while stirring. This ointment base is used to formulate poly herbal ointments¹⁴.

Formulation of Polyherbal Herbal Ointment

The semisolid leaf extracts of *Chromolaena odorata*, *Tridax procumbens* and *Euphorbia hirta* were used at 2%, 4% W/W respectively in the formulation of polyherbal ointments (F1, F2), by using trituration method. Formula for poly herbal ointments is given in Table 1.

Drugs and Chemicals

Povidone-Iodine (5%), Indomethacin, Ethanol, Stearic acid, White wax, Yellow petroleum jelly, Polypropylene glycol, Triethanolamine, Formalin, Chloroform and Spirit are the drugs and chemicals which are used in the present study.

Experimental Animals

Wistar strain Albino rats of either sex, weighing about 200-250g are used for the studies. The experiments were conducted after getting approval from the Institutional Animal Ethical committee

(Application no: KCP/IAEC/F140/142/2021). The albino rats are maintained in a controlled animal house of Karavali College of Pharmacy, Department of Pharmacology, Mangalore. Animals were housed under suitable conditions, temperature 25-26°C, humidity 50-65%, 12-hour light-dark cycle, ventilation and nutrition. Animals were acclimatized with experimental conditions by placing them in clean and dry polypropylene cages with sterile paddy husk bedding before conducting the experiment and will continue throughout the experiment. The cages were cleaned and replaced by fresh husks every 48 hours to avoid contamination. The animals were accessed freely to food and water consumption, and they were monitored carefully and observed for any behavioural changes throughout the experiment.

Experimental Design for Excision Wound Model

Animals are divided into four groups. Each group contains five animals and named as control, standard, F1 and F2 treated groups. Animals were anaesthetized using chloroform, the fur were removed on the dorsal portion with the help of hair removal cream and applied the sterile disinfectant on the dorsal surface for decontamination. Then the wound is created around 5cm² on the dorsal surface by excising the skin with the help of sterile surgical blade, scissors and forceps. Aseptic conditions were maintained properly throughout the experiment. Animals were kept separately in sterile polypropylene cages after post wounding. The polyherbal ointments (F1, F2) are applied twice daily on the surface of the wound. The animals which are left without applying any medication are considered as the control group, and the standard group is treated with Povidone-Iodine (5%) ointment. The parameters studied is percentage wound closure by tracing the wound on mm² graph paper on post wounding day 3, 5, 7 and 9⁴.

$$\% \text{ Wound closure} = 1 - A_d/A_0 \times 100$$

A_d = Wound size on respective days, A_0 = Wound size on day zero

Experimental Design for Formalin Induced Paw Oedema Model

Animals are divided into eight groups. Each group contains 5 animals and denoted as control, standard, test 1, test 2, test 3, test 4, test 5 and test 6. Control group and standard group animals are treated orally with 1ml of normal saline solution and Indomethacin (4 mg/kg) respectively. Poly herbal formulations F1 was prepared as a solution, from which 100 mg/kg, 200 mg/kg, 300 mg/kg is injected orally to test groups 1, 2, and 3 respectively. Poly herbal formulation F2 was prepared as a solution, from which 100 mg/kg, 200 mg/kg and 300 mg/kg is injected orally to test groups 4, 5 and 6 respectively by using gastric cannula before 30 minutes of injection of formalin solution. Both polyherbal formulations were prepared as solution form by using normal saline as a solvent. Formalin 0.1ml solution was administered with the help of an insulin syringe into the sub plantar region of the dorsal surface of the left hind paw of rats. The paw volume was measured at 30, 60, 90 and 120 minutes using graph paper⁴.

$$\% \text{ Inhibition of inflammation} = 1 - S_t/S_c \times 100$$

S_t = Paw size at corresponding time, S_c = Paw size of control group at corresponding time

Table 1: Composition of polyherbal ointments

Extracts used	Quantity of extracts and Ointment base in (grams)	
	F1 (2%)	F2 (4%)
Ethanolic extract of <i>Chromolaena odorata</i>	2	4
Ethanolic extract of <i>Tridax procumbens</i>	2	4
Ethanolic extract of <i>Euphorbia hirta</i> Linn	2	4
Ointment base (Q.s)	100	100

Table 2: Effect of polyherbal ointment on excision wound model of rat

Days	Wound size (cm ²)			
	Control	Standard	F1 (2%)	F2 (4%)
0	5.00 ± 0	5.10 ± 0.8**	5.00 ± 0*	5.20 ± 0.1**
3 rd	4.80 ± 0.1	3.92 ± 0.44**	4.18 ± 0.31*	3.82 ± 0.49**
5 th	4.52 ± 0.24	2.50 ± 1.01**	2.60 ± 0.96*	2.02 ± 1.25**
7 th	3.90 ± 0.55	1.26 ± 1.32**	1.34 ± 1.28*	0.22 ± 1.84**
9 th	2.80 ± 1.1	0.26 ± 1.27**	0.34 ± 1.23*	0.00 ± 1.4**
% Wound closure on day 9	42	94	93	100

(Values are expressed as Mean ± SD, n=5, Results are significant *p<0.005, **p<0.001 as compared to control)

Table 3: Effect of polyherbal formulations on formalin induced paw oedema

Time (Minutes)	Paw Size (cm)							
	Control	Standard	F1 (100 mg/kg)	F1 (200 mg/kg)	F1 (300 mg/kg)	F2 (100 mg/kg)	F2 (200 mg/kg)	F2 (300 mg/kg)
0	0.50±0	0.10±0.14****	0.42±0.02*	0.42±0.02**	0.40±0.03**	0.34±0.05***	0.22±0.09****	0.12±0.13****
60	0.52±0.007	0.10±0.14****	0.48±0.014*	0.42±0.02**	0.41±0.03**	0.36±0.05***	0.24±0.09****	0.13±0.13****
90	0.54±0.014	0.12±0.14****	0.48±0.21*	0.44±0.03**	0.42±0.02**	0.36±0.06***	0.26±0.09****	0.14±0.14****
120	0.56±0.021	0.12±0.14****	0.48±0.021*	0.45±0.038**	0.42±0.02**	0.38±0.063***	0.28±0.098****	0.16±0.141****
% Inhibition at 120 minutes	0	78	14	17	25	32	50	71

(Values are expressed as Mean ± SD, n= 5, Results are significant *p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001 as compared to control)

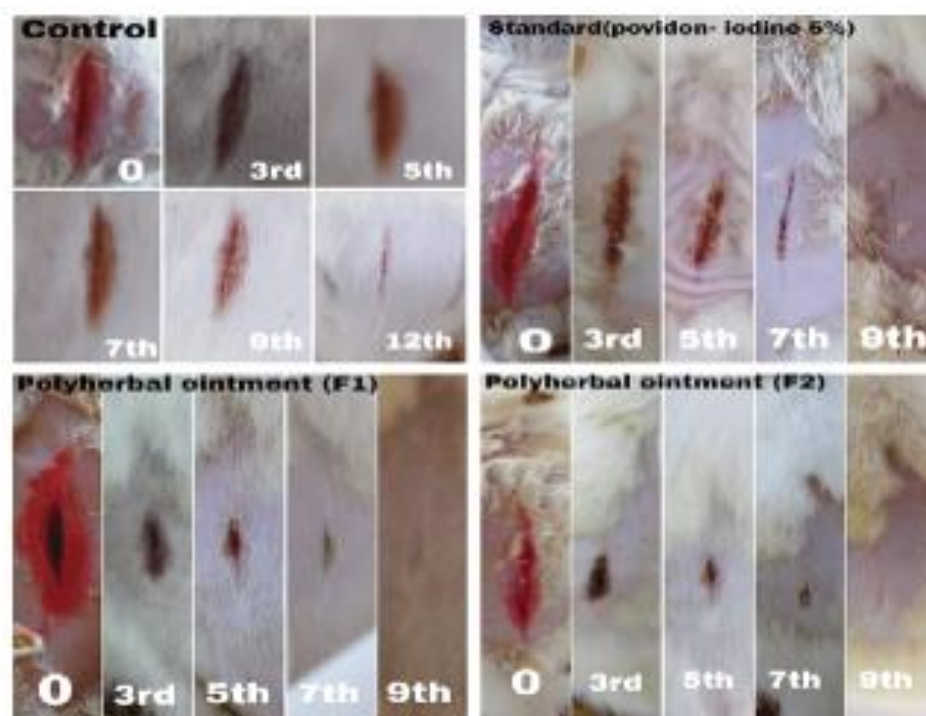


Figure 1: Wound healing effect on various treatment groups

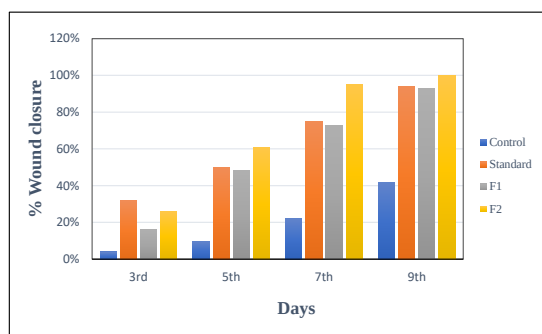


Figure 2: Percentage wound closure

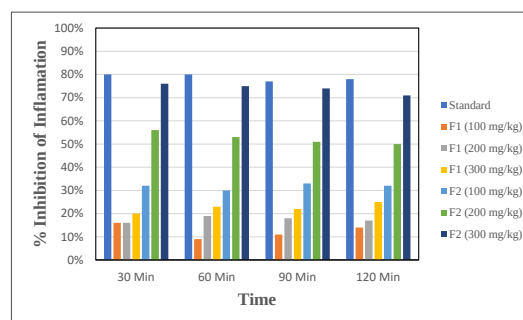


Figure 3: Percentage inhibition of inflammation

RESULTS AND DISCUSSION

Evaluation of Physicochemical Parameters of Polyherbal Ointment

Both poly herbal ointments F1(2%) and F2 (4%) are dark green in colour with characteristic herbal odor. Formulations are in semisolid form, free from gritty particles and non-irritant. Skin irritancy test studies have shown no oedema or erythema appearance on site of application for about a week. The pH of formulation was determined as 6.0 which is compatible with skin because of the pH range is within 5.4-6.0.

Phytochemical Screening

Phytochemical screening studies conducted for ethanolic extract of *Tridax procumbens*, have revealed the presence of secondary metabolites like glycosides, alkaloids, saponins, phytosterols, tannins, flavonoids, steroids and phenols. Study of phytochemical screening of ethanolic extracts of *Chromolaena odorata* has proved the presence of glycosides, tannins, saponins, flavonoids, cardiac glycosides, phenols and steroids. Ethanolic extracts of *Euphorbia hirta* Linn upon screening for phytochemicals have shown the presence of alkaloids, flavonoids, steroids, tannins and phenols.

Statistical Analysis of Results

Results are analyzed by using one way ANOVA followed by Dunnett's multiple comparison tests graph pad prism (Version 9). The results are expressed as mean $\pm$ SD.

Analysis of Results from Excision Wound Model

Around 5 cm² sized wounds were created for each animal on day 0. Animals were treated with respective treatment and reduction in wound size were observed on day 3, 5, 7 and 9 which have been shown in Figure 1. The wound size in control rats on day 3, 5, 7, 9 and 12 were found to be 4.80 ± 0.1 cm², 4.52 ± 0.24 cm², 3.90 ± 0.55 cm², 2.80 ± 1.1 cm² and 2.10 ± 1.1 cm² respectively. Similarly, povidone-iodine (5%) treated rats have shown wound sizes of 3.92 ± 0.44 cm², 2.50 ± 1.01 cm², 1.26 ± 1.32 cm² and 0.26 ± 1.27 cm² on 3, 5, 7 and 9 days respectively. Wound size of rats treated with poly herbal ointment F1(2%) were found to be 4.18 ± 0.31 cm², 2.60 ± 0.96 cm², 1.34 ± 1.28 cm² and 0.34 ± 1.23 cm² on 3, 5, 7 and 9 days respectively. Wound size of rats treated with poly herbal ointment F2 (4%) were found to be 3.82 ± 0.49 cm², 2.02 ± 1.25 cm², 0.22 ± 1.84 cm² and 0.00 ± 1.4 cm² on 3, 5, 7 and 9 days respectively. F2 (4%) formulation of poly herbal ointment is more significant (**p<0.001) when compared to that of F1 (2%) formulation of poly herbal ointment (*p<0.005) which are shown in Table 2. Percentage closure of wound in control and standard group on the 9th day of study were found to be 42% and

94% respectively. Whereas percentage wound closure of F1(2%) poly herbal ointment and F2 (4%) poly herbal ointment treated groups on the last day of study were found to be 93% and 100% respectively which have been shown in Figure 2.

Analysis of Results from Formalin Induced Paw Oedema

The change in paw size of control, standard, F1 and F2 treated groups at 30, 60, 90 and 120 minutes respectively are given in Table 3. On the basis of statistical analysis, F1(100 mg/kg, 200 mg/kg and 300 mg/kg) treated groups have shown significant (**p<0.005 and *p<0.05) inhibition of inflammation when compared to control group. Whereas poly herbal formulations F2(100 mg/kg, 200 mg/kg and 300 mg/kg) have shown significant (***p<0.005 ****p<0.0001) inhibition of inflammation when compared to control group. Poly herbal formulation F2 can be considered as more effective than F1. The percentage inhibition of inflammation at 120 minutes of all the doses of two formulations and standard were depicted in Figure 3. Percentage inhibition of inflammation of Poly herbal formulation-F1(100 mg/kg, 200 mg/kg and 300 mg/kg) were found to be 14%, 17% and 25% respectively at 120 minutes. Whereas percentage inhibition of inflammation by poly herbal formulation-F2 treated groups F2(100 mg/kg, 200 mg/kg and 300 mg/kg) were found to be 32%, 50% and 71% respectively at 120 minutes. Percentage inhibition of inflammation of the standard group was found to be 78% at 120 minutes.

The extracts used in this poly herbal formulations are rich in phytoconstituents like flavonoids, phenols, amino acids, terpenoids, glycosides, alkaloids, saponins, tannins and essential oils, which impart excellent antioxidant property to the formulations. This helps in accelerating healing capacity against wounds and inflammation when compared to conventional allopathic medication.

CONCLUSION

Polyherbal formulation are a type of formulation of multiple extracts of plants in a single dosage form where the combination is beneficial as it shows the additive or synergistic effect on the healing process. From the above discussions we concluded that poly herbal formulation F2 (4%) has shown better results when compared to poly herbal formulation F1(2%) and standard (Povidone-Iodine 5%) on wound healing activity. The dose given from poly herbal formulation F2(300 mg/kg) has shown better anti-inflammatory activity when compared to standard drug Indomethacin. The efficiency of poly herbal formulation could be mainly attributed to its potential antioxidant, antibacterial, free radical scavenging and anti-inflammatory activity. From the above results we can conclude that our poly herbal formulations are safe and effective, and it can be supported as traditional medicine for the management of severe wounds and

inflammatory conditions. It is advisable to conduct clinical studies to prove that it is clinically significant when compared to NSAIDS and Corticosteroids.

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