

COMBINED WOUND HEALING ACTIVITY OF *GYMNEMA SYLVESTERE* AND *TAGETES ERECTA* LINN

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ABSTRACT

To screen the wound healing activity of carbopol gels prepared from hydro alcoholic extracts of *Gymnema sylvestere* (GE) and *Tagetes erecta* Linn. (TE) in excision wound model and burn wound models in albino mice. Formulations of the extracts was done in the form of gels of carbopol individually and also in combination in equal ratio. In excision and burn wound models, the so treated animals showed significant reduction in period of epithelization and wound contraction and combined gel showed accelerated wound healing activity may be because of synergism. The enhanced wound healing activity of hydro alcoholic extracts may be due to free radical scavenging action and the phytoconstituents (flavonoids) present in it which either due to their individual or additive effect fastens the process of wound healing. Presence of flavonoids in alcoholic extracts was confirmed by phytochemical investigation and TLC methods.

Keywords: *Gymnema sylvestere* and *Tagetes erecta* Linn., wound healing activity and hydro alcoholic extract

INTRODUCTION

A wound is a disruption of tissue integrity that results in damage and is typically associated with loss of function. Wound healing can be defined as a complex dynamic process that results in the restoration of anatomic continuity and function¹. Healing of wounds usually takes place in a direction away from its normal course and under healing, over healing or no healing of wounds is common. Management of under healing of wounds is a complicated and expensive programme and research on drugs that increase wound healing is a developing area in modern biomedical science.²

Many ayurvedic medicinal plants have a very important role in process of wound healing. Plants are more potent healers because they promote the repair mechanisms in the natural way. The healing process can

be physically monitored by assessing the rate of contraction of the wound.³ *Gymnema sylvestere* (Asclepiadaceae) is native to central and western India. It is a potent antidiabetic plant used in folk, ayurvedic and homeopathic systems of medicine. It is used in the treatment of asthma, eye complaints, inflammation and snake bite. It possesses antimicrobial, anti-inflammatory, antihyperlipidemic, hepatoprotective and sweet suppression properties.^{4,5} GE leaves contain triterpene saponins oleanane and dammarene classes, oleanane saponins are gymnemic acids and gymnema saponins, while dammarene saponins are gymnemasides.⁶ *Tagetes erecta* Linn. (Asteraceae) commonly known as marigold is a common garden plant. The leaves are reported to be effective against piles, kidney troubles, muscularpain, ulcers, and wounds.

The pounded leaves are used as an external application to boils and carbuncles.⁷ It is reported to have antioxidant, antimycotic, analgesic activity and 18 active compounds are identified by GC-MS, many of them are Terpenoids.⁸⁻¹⁰

Both the plants especially leaves are having good wound healing activity individually¹¹. The present study has been undertaken to ascertain the combined wound healing effect of carbopol gels of GE and TE leaves on experimentally induced wounds in mice.

MATERIALS AND METHODS

Plant material

Leaves of *Gymnema sylvestre* and *Tagetes erecta* Linn were collected from local areas of Hyderabad, shade dried and were authenticated from botany department, Osmania University, Hyderabad.

Preparation of extracts

100g of leaves of both the plants were powdered to coarse form. The powdered materials were loaded in soxhlet extractor and defatted with petroleum ether (40-60°C). The marc was dried and extracted with ethanol (50 % v/v) in a same extractor up to three cycles. Finally the extracts were concentrated to semi solid mass using rotary evaporator under vacuum. The traces of solvent were removed by keeping the dried extract in to a desiccator. *Gymnema sylvestre* extract was labeled as GSE and *Tagetes erecta* Linn extract was labeled as TEE.

Phytochemical studies

The individual extracts were subjected to qualitative chemical investigation for the identification of the phytoconstituents: sterols, alkaloids, glycosides, saponins, carbohydrates, flavonoids and tannins.¹²

Thin layer chromatography (TLC)¹³⁻¹⁵

TLC was performed for both the extracts by using suitable solvent system. Mobile phase for GEE is n-butanol-water-methanol

(10:10:1) and for TEE is ethyl acetate: formic acid: glacial acetic acid: water (100: 11: 11: 26). Pre coated silica gel is acted as stationary phase in both the experiments.

EXPERIMENTAL

Animals: Healthy adult albino mice weighing between 25-40 g were procured from the institute. The animals were caged individually after wounding for treatment till completion of wound healing. In each group of different models six animals were used. The experimental protocol was approved by institutional animal ethics committee and animals were maintained under standard conditions in an animal house approved by committee for the purpose of control and supervision on an experiment on animals (CPCSEA).

Chemical

Metrozyl gel was procured from hetero pharmacy (Hyderabad) and carbopol, methyl paraben and propyl paraben were procured from SD fine chemicals Pvt. Ltd. (Mumbai).

Toxicity Studies¹⁶

Toxicity studies of alcoholic extract were carried out in oral doses of 100 to 2000 mg/kg- body weight using albino mice. After test extract administration, animals were observed 72 hr. period. The number of deaths was expressed as a percentile and the LD₅₀ was determined by probate a test using the death percentage versus the log dose. Study protocol was approved from the Institutional Animal Ethics Committee (IAEC).

Selection of gel base and formulations¹⁷

A water soluble base like carbopol containing methyl paraben (0.01%) and propyl paraben(0.1%) was selected as base for both the extracts individually and also in combination in equal ratio for local application as gel (carten sjet all).

Carbopol gel, 2.5%: Carbopol: 2.5g, methyl paraben: 0.01g, propyl paraben: 0.10gms, distilled water: 97.50ml.

GSE gel, 2.5%: GSE: 2.5g, carbopol: 2.5g, methyl paraben: 0.01g, propyl paraben: 0.10g, distilled water: 97.50ml.

TEE gel, 2.5%: TEE: 2.5g, carbopole: 2.5g, methyl paraben: 0.01g, propyl paraben: 0.10g, distilled water: 97.5ml

Combined extract, CE gel, 2.5%: GSE: 1.25g, TEE: 1.25g, carbopole gel: 2.5g, methyl paraben: 0.01g, propyl paraben: 0.10g, distilled water: 97.50ml.

Mix vigorously to get gels of uniform consistency.

EVALUATION OF WOUND HEALING ACTIVITY¹⁸⁻²⁰

Excision model

Randomly collected mice of both sex, weighing between 25-40 g. Divided them into five groups of six in each and are placed in different cages. Treatment groups: Group I: carbopol gel, Group II: GSE gel, Group III: TEE gel, Group IV: CE gel, Group V: Standard (metrozyl gel)

For the excision wound study each group containing six animals was selected. A circular wound of about 10mm diameter was made on depilated dorsal thoracic region of mice under light ether anesthesia in aseptic condition and observed throughout the study. Animals were housed individually. Group-I animals are applied with 2.5% of carbopol gel. Group II & III are applied with 2.5% of GSE and TEE gels respectively. Group IV is applied with 2.5% of CE as thin layer twice daily. Group V animals are applied with metrozyl gel twice daily as thin layer. Wound area can be measured on 2,4,6,8,10,12,14,16,18,20,22nd post wounding days. % of wound contraction was calculated from the day of measurement of wound area and epithelization period was also calculated.

Burn wound model

Wax is heated to a temperature above 100⁰c and is poured as a drop on the mice skin to create a wound on the dorsal thoracic region 1cm away from the vertebral column and 5cm away from the ear. Area of the wound was measured in sqmm by placing a transparent polythene graph over the wound and then traced the area of the wound on it. This is taken as initial wound area reading. Group-I animals are applied with 2.5% of carbopol gel. Group II & III are applied with 2.5% of GSE and TEE gels respectively. Group IV is applied with 2.5% of CE as thin layer twice daily. Group V animals are applied with metrozyl gel twice daily as thin layer. Wound area can be measured on 2,4,6,8,10,12,14,16,18,20,22nd post wounding day. % of wound contraction was calculated from the day of measurement of wound area and epithelization period was also calculated.

RESULTS AND DISCUSSION

GSE and TEE were subjected for the qualitative analysis to detect chemical constituents using standard procedures:

Identification Test	GSE	TEE
Test for alkaloids		
• Dragendroff's test	+	+
• Mayer's test	+	--
• Wagner's test	+	+
Test for saponins		
• Foam Test.	+	+
Test for Glycosides		
• Killer-killiani test	+	-
• Libermann burchard's test	+	-
Test for anthraquinone glycoside		
• Borntrager's test	-	-
Test for flavonoids		
• Ferric chloride test	+	+
• Shinoda test	-	-
• Sodium hydroxide test	+	+
• Lead acetate test	+	+
Test for tannins:		
Heavy metal test and FeCl ₂	+	+

Table 1: Qualitative analysis for different chemical constituents

TLC results: For GSE, R_F values of Gymnemic acid-A: 0.6, Gymnemic acid-B: 0.49, Gymnemic acid-C: 0.30, Gymnemic acid-D: 0.25 and for TEE, R_F Value of Quercetin- 0.93.

treated with GSE and TEE when compared with control. Even group treated with combined extract has shown much significant increase in % wound contraction when compared with control and it has shown synergistic effect when compared to groups

Groups	% Wound contraction					Epithelialization period, days
	2 nd day	4 th day	6 th day	8 th day	10 th day	
I	51.1±6.26	68.63±4.52	72.9±6.06	79.55±6.61	79.55±6.61	18.57
II	36± 0	66.30±5.82	77.84±6.62	89.51± 9.9	89.51 ± 9.9	14.33
III	38.3 ±3.49	70.3 ±5.76	83.4±5.55*	93.7 ± 6.10	93.7 ± 6.10	11.56
IV	43.85±3.28*	72.59 ±6.4	81.8 ±6.39	91.63 ±6.8	91.63 ±6.8	11.15
V	47.66±4.6	52.34 ±5.4	75.17±5.32	81.51±5.93	81.51±5.9*	16.66

treated with GSE and TEE.

Table 2: Excision wound model

Groups	% Wound contraction					Epithelialization period, days
	2 nd day	4 th day	6 th day	8 th day	10 th day	
I	35.69± .19	49.78±1.93	66.40±3.04*	76.43±4.5	86±1.66	19.83
II	41.50± .93	52.72±3.16	72.87± 0.9	82.74±3.48	94.66±1.9*	16.76
III	38.19 ± 5*	60.86 ± 4.5	74.85 ±2.12	90.18±3.97*	96.12±3.45	15.47
IV	36.85±4.38	56.50± 2.3	79.23± 1.87	94.5± 1.72	98.67±1.46	12.22
V	38.36±4.43	54.86±5.6	72.55± 8.72	93.24 ±3.37	96.66±2.98	14.39

Table 3: Burn wound model

* Significant at P<0.001,P-value was calculated by comparing with control by ANOVA test, Values are expressed as mean ±SEM (n=6)

The significant increase in the wound-healing activity was observed in the animals treated with the GSE, TEE and CE compared with those who received the control treatments. Table 2 showed the effects of the hydro alcoholic extracts of *Gymnema sylvestre*, *Tagetes erecta* and combined extract of both the plants on wound healing activity in mice with excision wounds. Significant increase in % wound contraction is observed in groups

The significant increase in the wound-healing activity was observed in the animals treated with the GSE, TEE and CE compared with those who received the control treatments. Table 3 showed the effects of the hydro alcoholic extracts of *Gymnema sylvestre*, *Tagetes erecta* and combined extract of both the plants on wound healing activity in mice with burn wounds. Significant increase in % wound contraction is observed in groups treated with GSE and TEE when compared with control. Even group treated with combined extract has shown much significant increase in % wound contraction when compared with control and it has shown

significant synergistic effect when compared to groups treated with GSE and TEE.

The qualitative tests used to identify phytochemical constituents of the *Gymnema sylvestre*, *Tagetes erecta* showed the presence of alkaloids, saponins, flavanoids and tannins. *Gymnema sylvestre* showed the presence of glycosides also. The thin layer chromatography of the hydro alcoholic extract of GSE showed showed 3 spots. The specific staining of the thin layer slides showed the presence gymnemic acids in GSE. The thin layer chromatography of the hydroalcoholic extract of TEE showed spot of quercetin.

CONCLUSION

The present study has demonstrated that hydro alcoholic extract of *Gymnema sylvestre*, *Tagetes erecta* and their combined extract have properties that render it capable of promoting accelerated wound healing activity compared with placebo controls. Wound contraction, qualitative tests and TLC support further evaluation of synergistic wound healing effect of these two plants and even stability studies of their carbopol gels in the topical treatment and management of wounds.

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