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WOUND HEALING POTENTIAL OF *TRIANTHEMA PORTULACASTRUM* LINN. IN WISTAR ALBINO RATS

YADA D[*1], K. S. S. L. RAMYA[2] AND K. SWETHA[3]

1: Associate Professor, Department of Pharmaceutical Chemistry, Malla Reddy College of Pharmacy, Maisammaguda, Dhulapally, Secunderabad, Telangana-500100

2: Assistant Professor, Department of Pharmacology, Malla Reddy College of Pharmacy, Maisammaguda, Dhulapally, Secunderabad, Telangana-500100

3: M. Pharmacy (Pharmacology) scholar, Malla Reddy College of Pharmacy, Maisammaguda, Dhulapally, Secunderabad, Telangana-500100

***Corresponding Author: Dr. Divya Yada: E Mail: yada.divya@gmail.com**

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ABSTRACT

Inflammation contributes as a key factor for retarding the process of dermal wound healing. *Trianthema portulacastrum* Linn. reported to possess antioxidant, antifungal, anti-inflammatory and antibacterial properties, which could make TP a promising wound healing agent. In the current study Wound healing potential of Chloroform extract of TP using excision and incision wound model was studied. Wistar albino rats were randomly divided into four groups, containing six animals in each group; group I served as control untreated, group II was standard group, treated with Soframycin skin cream, group III treated with Chloroform extract with 100mg/kg, and group IV treated with Chloroform extract with 200mg/kg. All the groups were topically applied their respective treatments, once daily, till the complete healing achieved. Wound healing was assessed by analyzing % wound closure, epithelialization period, tensile strength. 100mg/kg and 200mg/kg extracts significantly accelerated the wound healing process dose-dependently in both wound models, evidenced by the faster rate of wound contraction, epithelialization, high tensile strength compared to the control group. There exists a dose-dependant amelioration of wound healing by re-epithelialization of the wounded skin sample in Chloroform extract of *Trianthema*

portulacastrum treated groups. These results implicate potential medicinal value of Chloroform extract of *Trianthema portulacastrum* to heal dermal wounds.

Keywords: *Trianthema portulacastrum*, wound healing activity, Incision model, Excision wound model, chloroform extract

INTRODUCTION:

Nature is the source of traditional herbal medicine and still the primary health care system **[1]**. The presence of various life strengthening phytoconstituents in plants made scientists to work on these plants in order to explore their benefits in treating certain infective diseases and management of chronic wounds.

A wound is developed as the result of physical disruption of the skin, that leads to loss or breaking of cellular and anatomic or functional continuity of healthy tissue **[2]**. Wound healing consists of three stages: inflammation, proliferation, and remodeling. The inflammatory phase makes the area ready for healing and immobilizes the wound by causing it to swell and become painful, and results in restricted movement. The fibroblastic phase results in rebuilding the structure, and then the remodeling phase provides the final form. The proliferative phase is marked by angiogenesis, collagen deposition, granulation tissue formation, epithelialization and wound contraction. In angiogenesis, new blood vessels originate from endothelial cells. Collagen, the principal component that strengthens and supports extracellular tissue, contains substantial amounts of hydroxyproline, has been used as a biochemical marker for tissue collagen **[3]** in fibroplasia and granulation tissue formation. Where In epithelialization, epithelial cells proliferate and spread across the wound surface and contraction of the myofibroblasts results in Wound contraction. Platelets release growth factors and other cytokines **[4]**. Chronic wounds fail to heal in spite of adequate and appropriate care. Currently, debridement, irrigation, antibiotics, tissue grafts and proteolytic enzymes were been available to treat chronic wounds, which possess major drawbacks and unwanted side effects. In this point of view, there exists a scope for continuing research on plant-based medications.

Trianthema portulacastrum Linn. (also known as *Trianthema monogyna* Linn.; family: Aizoaceae), also known as horse purslane, carpetweed, giant pigweed, Punarnava, Gadabani and Labuni, has historically been valued by Indian and African cultures for its numerous medicinal

effects **[5-7]**. The fleshy nature of leaves makes them suitable for use as a wound dressing. In the Gold Coast, plant applied as wound dressing or as poultice. In India, the plant is used for edema of the liver and spleen, asthma, severe cough, amenorrhea, dropsy and uteralgia. The present study reveals the wound healing potential of Trianthema portulacastrum using incision and excision wound models in rats.

MATERIALS AND METHODS:

Collection of Plant material and Plant Extraction: The whole plant of *Trianthema portulacastrum* was collected from the forests of Maisammaguda, Secunderabad situated in the state of Telangana (India) and shade dried and powdered mechanically. The plant specimen was authenticated by botanist of Osmania University and authenticated voucher specimen Number 145 of the plant has been preserved in department for future reference. The dried plant were then milled to coarse powder mechanically and successively extracted with petroleum ether, chloroform, ethyl acetate and Methanol in Soxhlet's apparatus and method of maceration was allowed for water at a duration of 72 hours. The crude extracts were evaporated to dryness under vacuum and dried in vacuum desiccators. Later Stored in refrigerator. Preliminary phyto chemical investigation was performed **[8]**. Based on the presence of Phyto constituents, Chloroform extract of *Trianthema portulacastrum* was selected for screening the wound healing potential in experimental animals.

Animals: An ethical approval of this experimental study was obtained from the Institutional Animal Ethical Committee with an approval number: CPCSEA/ IAEC/JLS/ 11/11/19/14. Albino rats with an average body weight from 150 to 250 g were utilized in this study. They were procured from Sanzyme Bio-analytical lab, Plot no. 8 Sys.No.542, Kothur(V), Shameerpet, R.R. dist. The rats were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles at 25 ± 3°C and35-60 % humidity). Standard pelletized feed and tap water were provided *ad-libitum*.

EXPERIMENTAL DESIGN:

The animals were starved for 12h prior to wounding. All the rats were anaesthetized using pentobarbitone (30mg/kg). The rats were divided into four groups (n = 6). Animals were depilated at the dorsal thoracic region before wounding.

Group I: Control group left untreated.

Group II: Standard drug (Soframycin skin cream) applied topically once a day for 16days

Group III: Chloroform Extract of *Trianthema portulacastrum* (100mg/kg), applied topically once a day for 16 days.

Group IV: Chloroform Extract of *Trianthema portulacastrum* (200mg/kg), applied topically once a day for 16 days.

Excision wound model [9] An impression was made on the dorsal thoracic region 1cm away from vertebral column and 5cm away from ear using a round seal of 2.5cm diameter on the anaesthetized rat. The skin of impressed area was excised to the full thickness to obtain a wound area of about 500 mm^2 diameters. Hemostasis was achieved by blotting the wound with cotton swab soaked in normal saline. Contractions, which contribute for wound closure in the first two weeks, were studied by tracing the wound on a transparency paper initially. Then an impression was taken on a millimeter scale graph paper, scar area after complete epithelization and time required for complete epithelization in days was evaluated to calculate the degree of wound healing. The following parameters i.e., percentage of wound closure, period of re-epithelization time, wound area was studied. The observation of the percentage wound closure were recorded on 2^{nd}, 4^{th}, 6^{th}, 8^{th}, 10^{th}, 12^{th}, 14^{th} and 16^{th} day of post wounding day and also for re- epithelization and size and shape of scar area.

Incision wound model [10] In the incision model, the rats were anesthetized by anesthetic ether and two longitudinal para vertebral incisions of 6cm length were made through the skin and cutaneous muscle at a distance of about 1.5cm from the midline on each side of the depilated back. After the incision, the parted skin was sutured 1cm apart using a surgical thread (No. 000) and curved needle (No. 11). The wounds were left undressed. The extracts were applied topically once a day, till complete healing. The sutures were removed on eighth post-wound day. The tensile strength of the 10^{th}day old wound was measured by using Tensiometer **[11]**.

Measurement of wound closure The wound closure rate was assessed by tracing the wound on 2, 4, 6, 8, 10, 12, 14 and 16 post wounding days using transparent paper and a permanent marker. The wound areas recorded were measured using 1mm scale of graph paper. Changes in wound area were evaluated giving an indication of the rate of wound contraction and epithelialization period. The evaluated surface area was used to calculate the percentage of wound

contraction, taking initial size of the wound as 100 % **[12]** as shown below:

% wound closure = (Initial wound size - nth days of wound size) /(Initial wound size)X100

Epithelialization period measurement Falling of scab leaving no raw wound behind was taken as end point of complete epithelialization and the days required for this was taken as period of epithelialization **[13]**. The results of epithelialization period are tabulated in **Table 4**.

Tensile strength The tensile strength of a wound represents the degree of wound healing. Usually wound–healing agents promote a gain in tensile strength. The sutures were removed on the 9th day of wounding and the tensile strength was measured on the 10th day **[14-16]**. The mean tensile strength on the two paravertebral incisions on both sides of the animals was taken as the measures of the tensile strength of the wound for an individual animal. The tensile strength of different extracts and ointment-treated wounds were compared with control groups. The tensile strength increment indicates better wound healing stimulation by the applied drug. Tensile strength was calculated using the following formula **[17]**.

Tensile strength=Breaking Strength(g)/Cross-section area of skin(mm^2)

Tensiometer In the present study local made Tensiometer was used, which consists of a wooden board to which four nails were fixed. To one end the nail thread tied which is fixed, were as to another end easy movement of thread was allowed with help of pulley, to the edge of thread weighing balance was attached. Two clamps were tied to the thread in each side. The rats were anesthetized individually and were placed on wooden board between nails. The clamps were then carefully attached to the skin on the opposite sides of the wound at a distance of 0.5 cm away from the wound. Analytical weights were placed on the weighing balance by increasing the weights until the healed wound opens. Thus tensile strength of wound was measured.

RESULTS AND DISCUSSION:

Wound contraction Topical applications of chloroform extracts of *Trianthema portulacastrum* (100mg/kg and 200 mg/kg) whole plant showed pronounced effect on the wound healing process on the rats. The progress of wound area **(Table 1, Figure 1)**, wound contraction induced by treatment of (100mg/kg and 200 mg/kg) whole plant extract of *Trianthema portulacastrum*, control and Soframycin skin cream is shown in **(Table 2, Figure 2)**. The 200 mg/kg chloroform extract treated group showed

significant wound contraction starting from the second day and highly significant difference were observed from 10th day onward in comparison with the control group. As shown in Table there was no significant difference in activity between the 200 mg/kg and 100 mg/kg extract. But, higher rate of wound closure was observed with 200 mg/kg chloroform extract. 200 mg/kg chloroform extract has shown comparable efficacy with Soframycin skin cream. The maximum rate of wound contraction was seen on the 10th, 12th, 14th and 16th day. The animals treated with 100 mg/kg chloroform extract showed significant wound contraction from 8th day onward as compared to control group. Significant wound contraction was also observed for Soframycin skin cream treated group from 6th day onward as compared to control group with highly significant wound contraction on 12th ,14th and 16th days. The maximum rate of wound contraction for Soframycin skin cream was seen on the 12th, 14th and 16th day.

Epithelialization period The period of epithelialization was 17.83±1.11, 13.00±0.45, 14.80±0.96 and 13.56±0.40 for control group, standard drug, 100 mg/kg and 200mg/kg extract respectively. 200mg/kg extract treated rats showed faster rate of epithelialization compared to control group. There was no significant difference of epithelialization period between 200mg/kg and 100mg/kg extract and standard drug. Both standard drug and 200mg/kg chloroform extract showed significant difference of epithelialization period as compared to control group **(Table 3)**.

Wound healing (incision model) In incision wound model **(Table 4)**, 100 mg /kg and 200 mg/kg whole plant extracts of *Trianthema portulacastrum* and Soframycin skin cream treated animals showed significant increase in breaking strength (390.8±1.19, 409.6±3.27 and 428.3 ±2.46*) when compared to the control group (164 ±0.93), graphically shown in **Figure 3**.

Biochemical parameters: *Trianthema portulacastrum* treatment produced significant wound healing activity, which may be due to its angiogenic and mitogenic potential. Its prohealing activity was marked, as all the parameters observed were significantly affected **(Table 5)**. A healing tissue synthesizes collagen, which is a constituent of growing cell. Concentration of hydroxyproline is a measure of concentration of collagen. Higher the concentration of hydroxyproline indicates faster rate of healing wound. Biochemical analysis showed increased hydroxyproline content, which is a reflection of increased cellular proliferation

and there by increased collagen synthesis. Increased hexosamine content reflects the stabilization of collagen molecules by enhancing electrostatic and ionic interactions. Collagen not only confers strength and integrity to the tissue matrix but also plays an important role in homeostasis and in epithelialization at the latter phase of healing. Hence enhanced synthesis of hydroxyproline and hexosamine in treated rats provide strength to repaired tissue and also healing pattern. The result showed potent wound healing capacity as evident from the wound contraction; increased tensile strength and increased biochemical parameters in healing tissue have thus validated the ethnotherapeutic claim.

Table 1: Effect of chloroform extract of whole plant *Trianthema portulacastrum* on process of wound induced in rats (excision model)

Treatment	Wound area (mm^2) post-wounding days (SD± Mean)								
	0	2	4	6	8	10	12	14	16
Control	500	486.16± 2.04	378.33 ± 2.94	342.16 ± 2.31	325.0 ± 1.78	299.50 ± 1.37	243.83 ± 3.25	189.66 ± 3.61	141.66 ± 2.94
Standard	500	419.83 ± 3.18***	321.0 ± 2.09***	237.83 ± 1.16***	203.83 ± 2.31***	134.16 ± 1.72***	82.50 ± 3.33***	41.50 ± 2.81***	26.83 ± 2.13***
CETP (100mg/kg)	500	457.83± 2.56*	332.50 ± 2.25*	264.66 ± 1.36*	247.66 ± 3.07*	201.50 ± 1.87*	163.83 ± 2.63*	104.66 ± 2.58*	66.50 ± 1.37*
CETP (200mg/kg)	500	426.33± 2.06**	324.33 ± 2.58**	252.16 ± 1.83**	214.33 ± 2.06**	151.67 ± 3.12**	115.83 ± 2.31**	68.33 ± 2.06**	39.16 ± 1.94**

Values were expressed as Mean ±SD (n=6). Using Dunnett's-test, an intergroup deviation between various groups was analyzed by graph pad Prism software & Data were analyzed by using One way ANOVA. *p<0.05, **p<0.01, and *** p<0.001 in comparison to control group

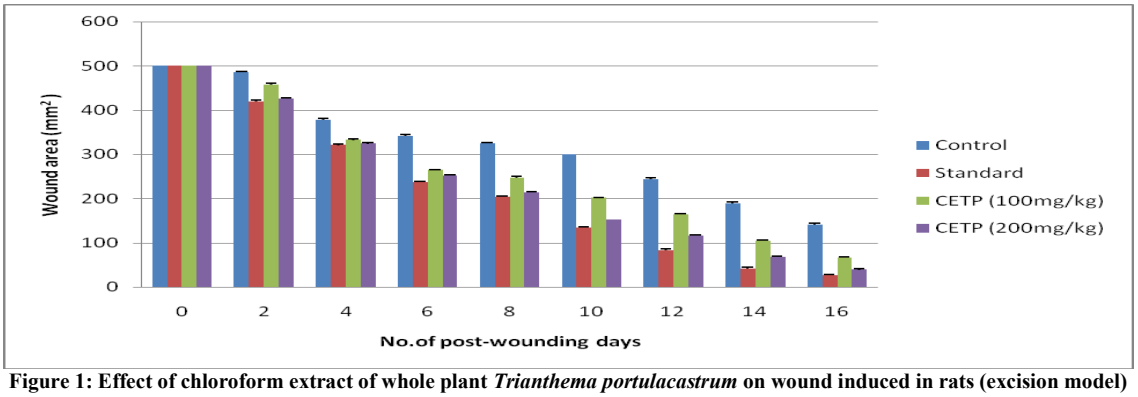

Figure 1: Effect of chloroform extract of whole plant *Trianthema portulacastrum* on wound induced in rats (excision model)

Table 2: Effect of Chloroform Extract of *Trianthema portulacastrum* whole plant on progress of % wound closure (excision model)

Treatment	% of wound closure post-wounding days (SD± Mean)								
	0	2	4	6	8	10	12	14	16
Control	0	2.76	24.33	31.56	35	40.1	51.23	62.06	71.66
Standard	0	16.03	35.8	52.43	59.23	73.16	83.5	91.7	94.63
CETP (100mg/kg)	0	8.43	33.5	47.06	50.46	59.7	67.23	79.06	86.7
CETP (200mg/kg)	0	14.73	35.13	49.56	57.13	65.76	76.83	86.33	92.16

Values were expressed as Mean ±SD (n=6). Using Dunnett's-test, an intergroup deviation between various groups was analyzed by graph pad Prism software & Data were analyzed by using One way ANOVA. *p<0.05, **p<0.01, and *** p<0.001 in comparison to control group.

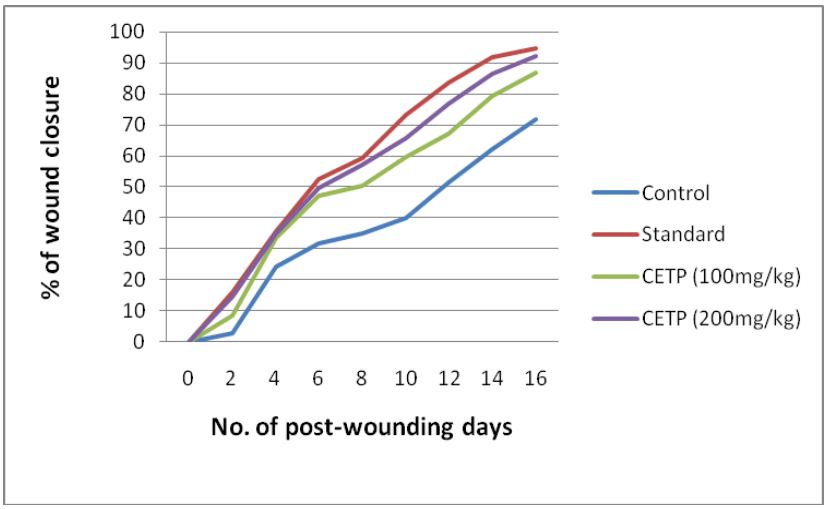

Figure 2: Effect of Chloroform Extract of *Trianthema portulacastrum* whole plant on % wound closure (excision model)

Table 3: Effect of Chloroform Extract of *Trianthema portulacastrum* whole plant on Period of epithelialization (no. of days)

Groups	Period of epithelization No. of days (mean ±SEM)
Group I: Control	17.83±1.11
Group II: Standard	13.00±0.45***
Group III: CETP (100mg/kg)	14.80±0.96*
Group IV: CETP (200mg/kg)	13.56±0.40**

Values were expressed as Mean ±SD (n=6). Using Dunnett's-test, an intergroup deviation between various groups was analyzed by graph pad Prism software & Data were analyzed by using One way ANOVA. *p<0.05, **p<0.01, and *** p<0.001 in comparison to control group

Table 4: Effect of Chloroform extract of *Trianthema portulacastrum* whole plant whole plant on wound, induced in rats(incision model)

Groups	Tensile strength (g) (mean ±SEM)
Group I: Control/ Disease induced	164 ±0.93
Group II: Standard	428.3 ±2.46*
Group III: CETP (100mg/kg)	390.8±1.19**
Group IV: CETP (200mg/kg)	409.6±3.27***

Values were expressed as Mean ±SD (n=6). Using Dunnett's-test, an intergroup deviation between various groups was analyzed by graph pad Prism software & Data were analyzed by using One way ANOVA. *p<0.05, **p<0.01, and *** p<0.001 in comparison to control group.

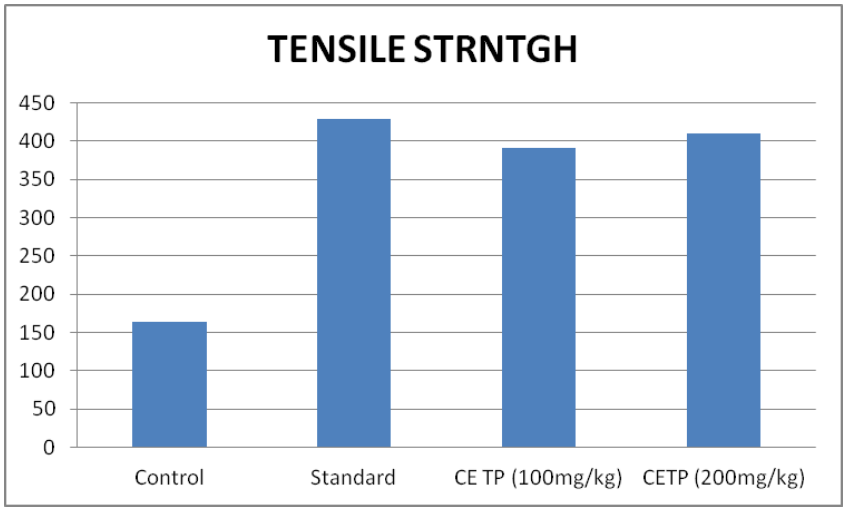

Figure 3: Effect of chloroform extract of *Trianthema portulacastrum* whole plant whole plant on wound, induced in rats (incision model)

Table 5: Effect of chloroform extract of *Trianthema portulacastrum* whole plant on biochemical parameters

Groups	Hydroxyproline content (mg/g tissue)	Hexosamine (mg/g tissue)	Total Protein (mg/g tissue)
Control	23.87±0.01	0.43±0.56	106.7±0.97
Standard	73.46±0.04***	0.79±0.05***	121.76±0.24***
CETP(100mg/kg)	65.06±0.03*	0.67±0.21*	110.1±0.09*
CETP(200mg/kg)	71.53±0.12**	0.73±0.54**	117.4±0.18**

Values were expressed as Mean ±SD (n=6). Using Dunnett's-test, an intergroup deviation between various groups was analyzed by graph pad Prism software & Data were analyzed by using One way ANOVA. *p<0.05, **p<0.01, and *** p<0.001 in comparison to control group

CONCLUSION

The progress of the wound healing induced by chloroform extract of whole plant *Trianthema portulacastrum* (100mg/kg and 200 mg/kg) as treated groups, untreated as control group and Soframycin ointment as standard group were studied. It is observed that the wound contraction ability of the *T. portulacastrum* extract in different concentrations was significantly greater than that of the control. The 200mg/kg chloroform extract containing group showed significant wound healing activity which was comparable to that of the Soframycin ointment treated animals. The wound closure time was lesser, as well as the percentage of wound contraction was significant with the 200mg/kg chloroform extract containing group as comparing to control. The results of the study showed that Chloroform Extract of whole plant *Trianthema portulacastrum* effectively stimulates wound contraction; increases tensile strength as compared with the control group. These findings could justify the inclusion of this plant in the management of wound healing.

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