

## Trauma Rejuvenation and Anti-Inflammatory Activity of *Ervatamia Coronaria* (L.) Root

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### Abstract

The present study deals with the evaluation of Trauma Rejuvenation and Anti-inflammatory activity of ethanolic extract of *Ervatamia Coronaria* roots. The ethanolic extract was subjected to screen for Trauma Rejuvenation activity using Excision trauma model. The anti-inflammatory activity was carried out by using carragenan induced rat paw edema method. The different doses range 100 mg/kg, 200mg/kg & 400 mg/kg show anti-inflammatory activity in range of 50-60%. The extract show prominent anti-inflammatory activity as compared to that of standard (indomethacin). The extract show good anti-inflammatory activity on carragenan induced rat paw edema method.

**Keywords:** *Ervatamia Coronaria*, Anti-inflammatory activity, Trauma Rejuvenation

### 1. Introduction

*Ervatamia Coronaria* Stapf (*Tabernaemontana divaricata*) belonging to the family Apocynaceae, is a glabrous, evergreen tree indigenous to India and is cultivated in gardens for its ornamental and fragrant flowers (refer figure 1). This species has been extensively investigated and a number of chemical constituents such as alkaloids, triterpenoids, steroids, flavonoids, phenyl propanoids and phenolic acids were isolated from leaves, roots and stems of the plant. In Indian traditional system of medicine, this plant material is widely used as a purgative, tonic to the brain, the spleen and the liver; in the treatment of cancer, wounds and inflammations. The plant extract was also found to possess analgesic, antipyretic and anti-inflammatory properties (Khare C.P. 1990).

*Ervatamia Coronaria* has four typical characteristics including: (i) evergreen shrub forms shaped like symmetrical mounds 6-feet high, (ii) horizontal branches having the appearance of an attractive, almost horizontal shrub, (iii) large, shiny, deep green leaves, 6 or more inches in length and 2 inches wide, and (iv) waxy blossoms with white, five-petal pinwheels, gathered in small clusters on the stem tips. (Rathi R., Chakrvarti P. S., 2016). Milky juice of *Ervatamia Coronaria* leaves posses anti-inflammatory activity and also applied to trauma. The flowers of plant mixed with oil used in skin disease and the roots of *Ervatamia Coronaria* plant are used in toothache & also used as a vermicide.



**Figure 1: Ervatamia Coronaria plant**

Tannins are major phytoconstituents present in Ervatamia Coronaria which may be responsible for Trauma Rejuvenation activity. (Bjarnsholt, T., et al. 2008).

## **2. Material and Methods**

*Plant material and Preparation of Herbal Extract:* The roots of Ervatamia Coronaria was collected from local market and authenticated in National Bureau of Plant Genetic Resources (Indian Council of Agricultural Research), New Delhi. The roots of Ervatamia Coronaria were dried under shade and then subjected to crushed and grinded and allow to undergo continuous heat extraction. The powdered roots of Ervatamia Coronaria was extracted exhaustively with 95% ethanol in a soxhlet apparatus by continuous heat extraction. The percent yield of Ervatamia Coronaria roots extract is 9.24%. The dry extracts were subjected to various chemical tests to detect presence of different photochemical constituents.

*Animals:* In the present study male Wister rats (150-200 gm) were used for the study. At the commencement of the study the weight variation of animals used were kept minimal and not exceeding  $\pm 20\%$  of the mean weight of each animal. They were individually housed and maintained on normal standard diet (Gold Muhor Brand, Lipton India Limited) and water ad libitum. Temperature was maintained at  $22^{\circ}\text{C} (\pm 30^{\circ}\text{C})$  with 12 hours light and 12 hours dark cycle throughout the course of the study. (GE & Evans WC 1987).

*Trauma Rejuvenation Activity:* Excision trauma model was used for the determination of Trauma Rejuvenation activity. For the excision model the animals were grouped six animals in each group. Group 1 served as control, Group 2 served as standard treated with 5% povidone iodine ointment topically, Group 3 served as test group treated with 2% Ervatamia Coronaria ethanolic root extract ointment topically. Group 4 served as test group treated with 4% Ervatamia Coronaria ethanolic root extract ointment topically, Group 5 served as test group treated with 8% Ervatamia Coronaria ethanolic root extract ointment topically. (Arnold, M. and Barbul, A 2006).

Excision trauma model was used to monitor trauma contraction and trauma closure time. Under light ether anesthesia an impression of 500 sq mm was made on the shaved back of the rat. Animals were kept in separate cages. The day on which wound made was considered as day 0 (zero). After complete homeostasis trauma was cleaned by hydrogen peroxide and treated for 20 days (refer Figure 2). Tracing of the trauma was done at every 4th day and mean % trauma closure is calculated (Pradeep T.D. et al 2009).

**% Wound contraction:** Percent wound contraction represents the rejuvenation of trauma. Trauma area was determined by tracing the trauma and calculating the diameter of trauma.

$$\text{Area of wound} = \pi r^2$$

Where r = radius of the trauma

After calculating the area of trauma the % trauma contraction was determined by the following equation.

$$\% \text{ trauma contraction} = \frac{\text{wound area on day 0} - \text{wound area on day n} \times 100}{\text{wound area on day 0}} \times S$$

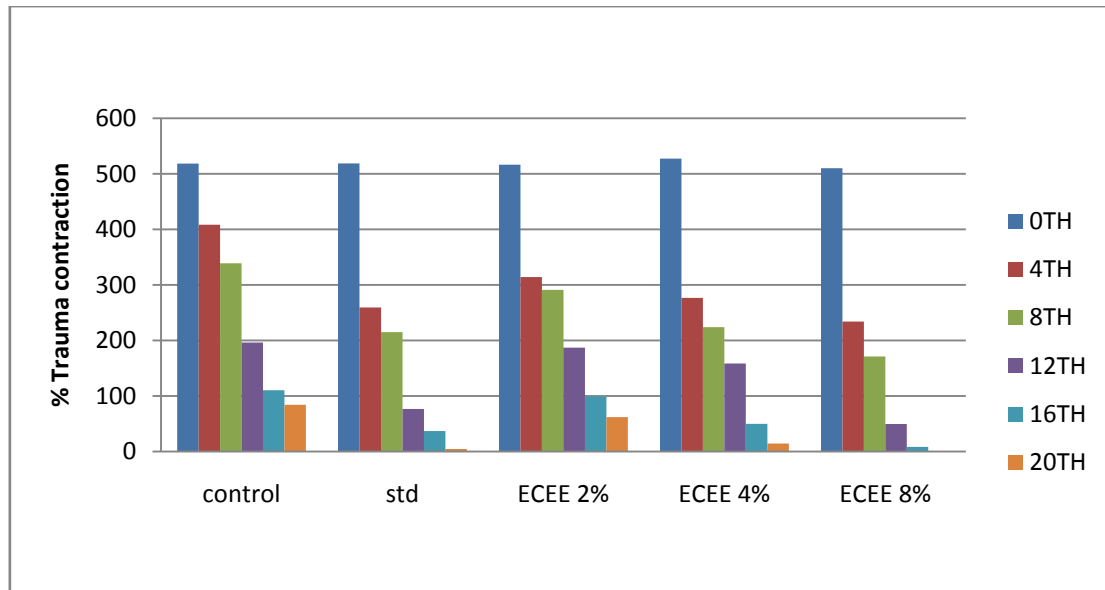
Where n = number of days 4<sup>th</sup>, 12<sup>th</sup>, 16<sup>th</sup>, day and 20<sup>th</sup> day.

**Period of Epithelization:** Period of epithelization represents the total period for trauma rejuvenation. The period of epithelization was calculated as the number of days required for falling of the dead tissue remnants without any residual raw wound (Dnyaneshwar D et al. 2009). From the observation table we have found that the *Ervatamia Coronaria* ethanolic (root) extract shows the significant activity when compared to control and standard. Period of Epithelization and % wound contraction were used as evaluation parameters in excision model, 8% ointment of ethanolic extract of *Ervatamia Coronaria* (root) produces the results which are comparable with the standard. Povidone iodine 5% ointment was used for the standard treatment. The result of 8% ointment of ethanolic extract of *Ervatamia Coronaria* was highly significant (p, 0.01). 4% ointment of ethanolic extract of *Ervatamia Coronaria* also produces significant result in comparison to control (p, 0.01) (Refer Table1).

**Table 1: Effect of *Ervatamia Coronaria* Ethanolic (root) extract ointment on the % Trauma Contraction and Period of Epithelization**

Day	Control Group	Standard Group	ECEE 2% Group	ECEE 4% Group	ECEE 8% Group
0	518.50 ±1.67	518.67±3.73	516.33±2.06	527.50±3.15	510±1.23
4	408.26±7.33(21.28)	259.50±9.48(49.96)	313±13(39)	276.74±12.83(47)	233.96±8.28(54.12)
8	339.02±17.13(34.63)	215.02±4(58.46)	291±9(43.58)	223.85±16.08(57.56)	171.16±7.26(66.43)
12	196.28±15.80(62.14)	76.79±7.18(85.19)	187±4(63.7)	158.64±6.31(69.92)	49.48±3.41(90.29)
16	110.35±6.25(78.71)	36.92±6.06(92.88)	99±5(80.68)	49.74±4.71(90.570)	8.40±2.66(98.35)
20	84.25±5.28(83.75)	4.74±0.78(99.08)	62±3(87.85)	14.52±3.33(97.24)	±0(100)
<b>Epithelization on Period (Days)</b>	21	18	20	19	16

ECEE- *Ervatamia Coronaria* ethanolic (root) extract ointment \*\*\*P <0.001, \*\*P<0.01,\*P, 0.05, n= 6 animals in each group.



**Figure 2: Effect of *Ervatamia Coronaria* Ethanollic (root) Extract on 5% Trauma Contraction.**

*Anti-inflammatory activity:* Anti-inflammatory activity of *Ervatamia coronaria* root extract was studied by carrageenan induced paw edema method. The animals were divided into 3 groups containing 6 animals in each group. Group 1 (control) untreated group, Group 2 topical application with *Ervatamia coronaria* extract, Group 3 topical application of indomethacin for comparison of anti-inflammatory activity. One hour after the application of *Ervatamia coronaria* extract 0.1 ml of carrageenan (1%) was injected into sub-planar region of hind paw of rat. A similar volume of saline solution was injected into the other hind paw. The paw volume was measured before injection of carrageenan or saline by the digital plethysmometer and the time course of edema formation was followed over 4 hours. In separate groups of animals, indomethacin 10 mg/kg was administered orally as standard drugs, saline solution as solvent controls. The ethanolic root extracts at different doses was administered orally 30 min. before carrageenan injection. The volume increase of the inflamed paw was estimated by subtracting the volume of the contralesional paw. The anti-inflammatory effect of the drugs was evaluated as the degree of edema inhibition (Fizquez B et al 1996).

### 3. Evaluation parameters:

- *% Inhibition in edema*
- *Anti-inflammatory Activity*
- *Carragenan induced paw edema*

Percent Inhibition in edema represents the inhibition of the inflammation. Edema was determined by measuring the paw volume by digital plethysmometer. (Mahata, M.A. and Patil B.M., 2007).

$$\% A = \% I_e - I_e$$

Where %  $I_e$  and  $I_e$  are the mean inflammation values reached in control and experimental groups respectively. The values were expressed as mean  $\pm$  SEM from 6 animals. The results were subjected to statistical by using one-way ANOVA followed by Dennett's test to

calculate the significant difference if any among the groups.  $p < 0.05$  was considered as significant. (Refer Table 2)

**Table 2: Effect of *Ervatamia Coronaria* Ethanolic [root] Extract on Paw Volume**

Time (hour)	Paw volume (ml) Mean $\pm$ SEM, n=6 and Edema Inhibition				
	Control(0.1 ml) Group	Standard(10mg/kg) Group	ECEE(100mg/kg) Group	ECEE(200mg/kg) Group	ECEE(400mg/kg) Group
0	2.67 $\pm$ 0.07	1.38 $\pm$ 0.12 <sup>***</sup> (48.31)	2.63 $\pm$ 0.076(1.49)	2.36 $\pm$ 0.045 <sup>*</sup> (11)	2.03 $\pm$ 0.035 <sup>***</sup> (24)
1	3.35 $\pm$ 0.13	1.08 $\pm$ 0.13 <sup>***</sup> (67.76)	2.53 $\pm$ 0.065 <sup>*</sup> (24.47)	2.30 $\pm$ 0.045 <sup>**</sup> (31.34)	1.30 $\pm$ 0.15 <sup>***</sup> (61.19)
2	3.81 $\pm$ 0.079	0.88 $\pm$ 0.15 <sup>***</sup> (76.90)	2.58 $\pm$ 0.084 <sup>**</sup> (32.28)	2.16 $\pm$ 0.033 <sup>***</sup> (43.30)	1.62 $\pm$ 0.062 <sup>***</sup> (57.48)
3	3.63 $\pm$ 0.056	1.30 $\pm$ 0.15 <sup>***</sup> (64.18)	2.58 $\pm$ 0.11 <sup>**</sup> (28.92)	2.15 $\pm$ 0.047 <sup>***</sup> (40.77)	1.72 $\pm$ 0.053 <sup>***</sup> (52.61)

ECEE-*Ervatamia Coronaria* ethanolic (root) extract ointment.

\*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ , n= 6 animals in each group.

#### 4. Result and Discussion

The present study deals with evaluation of Trauma Rejuvenation and Anti-inflammatory activity of ethanolic extract of root of *Ervatamia Coronaria*. The method used to determine trauma rejuvenation is based on excision trauma model. 8% ointment value of *Ervatamia Coronaria* extract produces the results which are comparable with the standard povidone iodine 5% ointment is used for the standard treatment. 4% ointment of ethanolic extract of *Ervatamia Coronaria* also produced significant result in comparison to control ( $p < 0.01$ ). The anti-inflammatory study was performed by carragenan induced rat paw edema method. The ethanolic extract of *Ervatamia Coronaria* root [200-400 mg /kg] shows a dose dependent inhibition of edema. At lower dose 100 and 200 mg /kg less significant effect was detected at all time periods. However at higher dose [400mg/kg] it produced significant and long duration inhibition in edema volume. Approximately 50-60% inhibition was observed during, which found to be almost consistent at subsequent time intervals with the magnitude of 60% at 2<sup>nd</sup> hour. As a result *Ervatamia Coronaria* ethanolic [root] extract posses the significant anti-inflammatory activity when compare to the standard and control groups.

#### 5. Conclusions

The *Ervatamia Coronaria* plant extract possess Trauma Rejuvenation action by improving regeneration and organization of the new tissue due to presence of Tannins. Thus it is concluded that the root of *Ervatamia Coronaria* shows significant Trauma Rejuvenation and anti-inflammatory activity.

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