



International Journal of Research in Pharmacy and Life Sciences

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Research Article

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Anti Inflammatory Activity of *Symphytum Officinale* Linn Root on Wistar Albino Rats

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ABSTRACT

Acute inflammation is a short-term process, usually appearing within a few minutes or hours and ceasing upon the removal of the injurious stimulus. It is characterized by five cardinal signs [Dolor (pain), Calor (heat), Rubor (redness), Tumor (swelling) and Functio laesa (loss of function)]. *Symphytum officinale* Linn belongs to the family boraginaceae. It is a herb commonly known as Comfrey. Many reports have demonstrated the usefulness of the extracts of this plant in many diseases and conditions like lung disorders, gastritis, and stomach ulcer and bleeding. The present study was carried out to assess the possible anti-inflammatory effect of methanolic extract of roots of *Symphytum officinale* Linn (MERSO) using egg white-induced edema in rats (acute model of inflammation). The anti-inflammatory effect of MERSO was evaluated in acute inflammation model using 30 Wistar albino rats and divided into five groups including normal saline 10 ml/kg orally, diclofenac 5 mg/kg IP and MERSO 250,500 & 700mg/kg b.wt orally. Thirty minutes post treatment, inflammation was induced by injecting 0.1 ml of fresh egg albumin into the sub plantar surface of the right hind paw and mean increase in paw edema was measured 60 min, 120 min, 180 and 240 min after induction of inflammation using plethysmometer. Suppression of paw inflammation by either diclofenac or MERSO and the percentage of inhibition of paw edema were assessed. The data obtained from this study reported that oral administration of extract significantly ($P < 0.05$, $P < 0.01$) inhibited raw egg albumin- induced rat paw oedema as compared to control group. Maximum inhibitory effect (33.53%) was observed at a dose of 750mg/kg, at the end of 240 minutes when compared to control group

Keywords: Acute inflammation; *Symphytum officinale* Linn; Egg white-induced edema.

ARTICLE INFO

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Article History: Received 15 February 2016, Accepted 25 March 2016, Available Online 24 May 2016

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Manuscript ID: IJRPLS2974



PAPER-QR CODE

Citation: D. Yashwanth Kumar, et al. Anti Inflammatory Activity of *Symphytum Officinale* Linn Root on Wistar Albino Rats. *Int. J. Res. Pharm, L. Sci.*, 2016, 4(1): 47-50.

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1. Introduction

Acute inflammation is usually of sudden onset and short duration following the injury of tissues. The damage may be purely physical, or it may involve the activation of an immune response¹. Three main processes includes increased blood flow due to dilation of blood vessels (arterioles) supplying the region, increased permeability of the capillaries, allowing fluid and blood proteins to move into the interstitial spaces and migration of neutrophils (and perhaps a few macrophages) out of the venules and into interstitial spaces².

Symphytum officinale Linn (common name: Comfrey) is a herb belongs to the family boraginaceae. Comfrey is native to Europe and temperate Asia and is common throughout England. Russian comfrey or blue comfrey has been introduced in Shimla at India. It is generally found in wet areas and along road sides and in ditches. Traditionally it is applied externally for the treatment of inflammation, cuts, bruises, sprains, sores, eczema, broken bones, pulled ligaments and muscles, arthritis, and boils. Roots or leaves were taken internally against lung disorders, gastritis, and stomach ulcer and bleeding. It is also used as analgesic, astringent, demulcent, diuretic, expectorant and hemoptysis (bronchial or pulmonary hemorrhage). As a part of our research studies on the medicinal plants we investigated the analgesic activity of methanolic extract of root of *Symphytum officinale* Linn and herein, report the results of our examinations.^{3,4}

2. Materials and Methods

1.1. Materials

1.1.1. Collection of plant material

The roots of *Symphytum officinale* Linn was collected in the month of March 2013 from Tirumala hills, Tirumala, Chittoor Dt, A.P, India. It was authenticated by Dr. K. Madhava chetty, Department of botany, Sri Venkateswara University, Tirupati, A.P, India.

1.1.2. Chemicals

All the chemicals used for the study are of analytical grade.

1.1.3. Selection of experimental animals

Wistar albino rats of either sex (150-200 gm) were used in the study. Animals were housed individually in polypropylene cages in a ventilated room under ambient temperature of 22 ± 2 C and 45-65 % relative humidity, with a 12 hour light followed by 12 hour dark. All the animals were acclimatized for at least 7days to the laboratory conditions prior to experimentation. Tap water and food pellets were provided ad libitum. Food pellets was with held overnight prior to dosing. All rats were handled and maintained strictly as per guidelines of "Guide for the care and Use of Laboratory animals".(Institute of Laboratory Animals Resources, National Academic Press 1996: NIH Publication number # 85-23, revised 1996).

1.2. Methods

1.2.1. Extraction of *Symphytum officinale* Linn roots

Symphytum officinale Linn. roots was shade dried and made into coarse powdered which was passed through a# 40 mesh sieve to get uniform particle size and was extracted using methanol by continuous hot percolation process using soxhlet apparatus⁵.

2.2.2. Preliminary phytochemical analysis

The methanolic extract of roots of *Symphytum officinale* Linn (MERSO) is subjected to preliminary phytochemical analysis to test for presence or absence of various phytochemical constituents used standard procedures⁶.

2.2.3. Anti inflammatory activity

Raw egg albumin-induced paw edema test

Wistar albino rats were divided into five groups, either of sex. Group I: Control group received normal saline 10ml/kg body weight, Group II: Standard group received Diclofenac 5mg/kg body weight. Group III, IV& V: Test groups 1, 2 &3 received MERSO 250,500 &750mg/kg body weights. All the doses of test drug were administered per orally and standard drug was administered intraperitonially. Animals were weighed and marked. Marks were made both on the hind paw (right and left) just beyond the tibio-tarsal junction. The initial paw volume (both right and left) of each rat was noted by mercury displacement method. The animals were then treated with vehicle, Diclofenac sodium, and methanolic extract of roots of *Symphytum officinale* Linn. After 30 min, acute inflammation was induced by the sub – planter administration with 0.1 ml of raw egg albumin in the left hind paw of the rats. The paw volumes were measured at 60 min, 120min, 180min, 240minutes, by mercury displacement method using Plethysmometer.^{7,8,9}

2.2.4. Statistical analysis

Statistical analysis was carried out using Instat 3 software. All results were expressed as Mean \pm S.E.M. The statistical analysis of all the results was done using one way analysis of variance (ANOVA) followed by Dunnett's test.

3. Results and Discussion

3.1. Preliminary phytochemical analysis of methanolic extract *Symphytum officinale* Linn root.

It was found that methanolic extract contained reducing sugars, gums, proteins. Amino acids, flavonoids and tannins.

Table 1: Preliminary phytochemical analysis

S.No	Compound	Methanolic extract
1	Reducing agents	+
2	Gums	+
3	Proteins	+
4	Amino acids	+
5	Cardiac glycosides	–
6	Flavanoids	+
7	Tannins	+
8	Starch	–
9	Alkaloids	–
10	Saponin Glycosides	–
11	Steroids	–

3.2. Anti- Inflammatory Activity

The inflammatory response is a physiological characteristic of vascularized tissues¹⁰. Exudation, which is a consequence of increase vascular permeability, is considered as a major feature of acute inflammation¹¹. Egg albumin-induced paw edema in rats is an in vivo model of inflammation used to screen agents for anti-inflammatory effect¹². The characteristic swelling of the paw is due to edema formation. Inhibition of increased vascular permeability and hence the attendant edema modulate the extent and magnitude of the inflammatory reaction. The paw edema that was induced by injection of egg albumin is

peaked after 30min and then progressively declined with time. The anti-inflammatory activity of MERSO was evaluated by egg albumin-induced paw edema method. Oral administration of methanolic extract of roots of *Symphytum officinale* Linn (250mg/kg, 500mg/kg and 750mg/kg) significantly (P<0.05, P<0.01) inhibited raw egg albumin-induced rat paw oedema as compared to control group as illustrated in table 2 & figure 1. Maximum inhibitory effect (33.53%) was observed at a dose of 750mg/kg, at the end of 240 minutes when compared to control group. Diclofenac sodium (5mg/kg) inhibited the paw oedema by 51.38% as depicted in table 3

Table 2: Anti – inflammatory effect of methanolic extract of roots of *Symphytum officinale* Linn on egg-albumin induced paw edema (volume in ml) in rats.

Groups	Dose (mg/kg)	0 min	60 min	120min	180 min	240 min
Control (Normal saline)	10ml	3.66±0.3	4.08±0.23	3.83±0.10	3.25±0.11	3.25±0.11
Test-1	250	2.75±0.21*	3.30±0.21*	3.25±0.11**	2.83±0.10*	2.83±0.10*
Test -2	500	2.5±0.25**	3.25±0.11*	3.08±0.08**	2.75±0.11*	2.75±0.11**
Test -3	750	2.41±0.15**	3.25±0.11*	2.75±0.11**	2.25±0.11**	2.16±0.1**
Standard (Diclofenac sodium)	5	2.66±0.24*	2.5±0.18**	2.16±0.1**	1.75±0.11**	1.58±0.08**

n=6. The observations are mean ± SEM. *P<0.05, ** P<0.01, as compare to control. (ANOVA followed by Dunnett’s test). Test = Methanolic extract of roots of *Symphytum officinale* Linn.

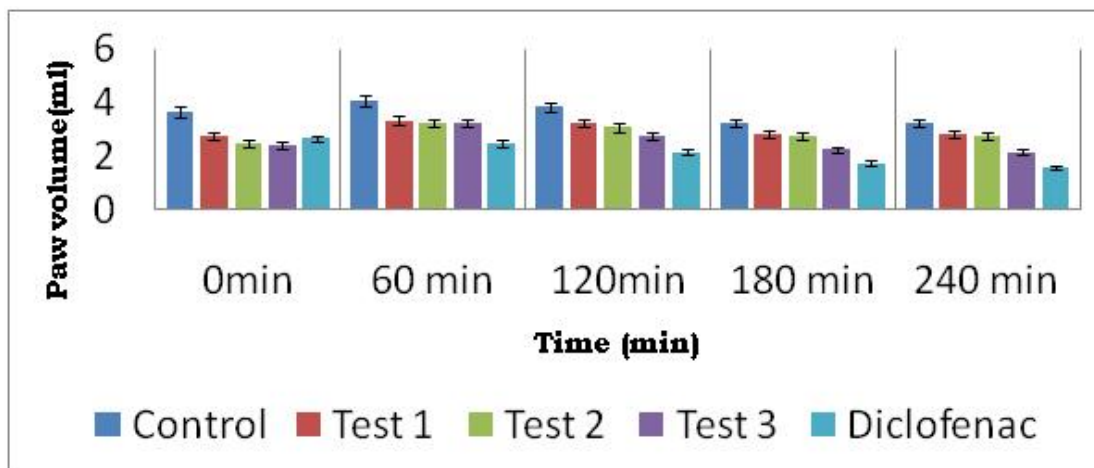


Figure 1: Anti – inflammatory effect of methanolic extract of roots of *Symphytum officinale* Linn. On egg-albumin induced paw edema in rats.

Table 2: Percentage inhibition of methanolic extract of roots of *Symphytum officinale* Linn on egg-albumin induced paw edema in rats.

Groups	Dose (mg/kg)	0 min (% inhibition)	60 min (% inhibition)	120 min (% inhibition)	180min (% inhibition)	240 min (% inhibition)
Control (Normal saline)	10ml	-	-	-	-	-
Test-1	250	24.86	19.11	15.14	12.92	12.92
Test -2	500	31.69	20.34	19.58	15.38	15.38
Test -3	750	34.15	20.34	28.19	30.76	33.53
Standard (Diclofenac sodium)	5	27.32	38.72	43.60	46.15	51.38

Test = Methanolic extract of roots of *Symphytum officinale* Linn.

4. Conclusion

The oral administration of extract at doses of 250mg/kg, 500mg/kg and 750mg/kg significantly ($P < 0.05$ - 0.01) inhibited raw egg albumin induced paw edema in rats. It is concluded that, the Methanolic extract of roots of *Symphytum officinale* Linn possess anti-inflammatory activity. Further studies involving the purification of the chemical constituents of the plants, receptor oriented mechanism and the investigations in the biochemical pathways, may result in the development of a potent agent with better therapeutic index.

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