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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *BARLERIA LONGIFLORA* L.f. AND *POTENTILLA ANSERINA* LINN

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ABSTRACT

Aim: Evaluation of aqueous and ethanol extracts of leaves of *Barleria longiflora* L.f. and whole plant of *Potentilla anserina* Linn for anti-inflammatory activity using Carrageenan induced paw oedema in rats. **Materials and method:** Animals were divided into 10 groups of 6 rats. Groups 1 and 2 served as Carrageenan induced control and standard (Diclofenac 5 mg/Kg, i.p.) respectively. Groups 3 and 4 were treated, orally with aqueous extract of *Barleria longiflora* L.f. with 100 and 400 mg/kg b.w, respectively. Groups 5 and 6 were administered, orally, ethanol extract of *Barleria longiflora* L.f. with 100 and 400 mg/kg b.w, respectively. Groups 7 and 8 were received, orally, aqueous extract of *Potentilla anserina* Linn of 100 and 400 mg/kg b.w, respectively. Groups 9 and 10 were administered, orally, ethanol extract of *Potentilla anserina* Linn of 100 and 400 mg/kg b.w, respectively. The paw volume was measured at 0, 1, 2, 3 and 4 hr after carrageenan injection. The actual edema volume was calculated. The data was expressed as mean ± S.E.M. The statistical analysis was done by means of ANOVA followed by Dunnett's post hock test. **Results**: The aqueous and ethanol extracts of leaves of *Barleria longiflora* L.f. and whole plant of *Potentilla anserina* Linn exhibited significant reduction of edema in the rats. **Conclusion:** Ethanol extract of whole plant of *Potentilla anserina* Linn revealed superior anti-inflammatory activity.

KEYWORDS: *Barleria longiflora* L.f., *Potentilla anserina* Linn, inflammation, carrageenan, edema.

INTRODUCTION

Inflammation is a body defence mechanism which eliminates or limits the spread of the injurious agent. It serves to destroy, dilute, or wall off the offender and sets into motion a series of events that try to heal and reconstitute the damaged tissue.[1] Non-steroidal anti-inflammatory drugs (NSAIDs) make up one of the largest groups of drugs used for pain and inflammation. Currently available anti-inflammatory agents are associated with unwanted side effects- gastrointestinal damage and have their own limitations.[2] Ayurveda is a primitive Indian system of remedies that makes usage of herbs. The plant *Barleria longiflora* L.f. is commonly known as "White long flowered nail dye," have been traditionally used to treat cough, inflammations, dropsy, kidney stone problems.[3] It consists of four anthraquinones, a pentacyclic titerpene arnidiol, campesterol, stigmasterol and β-sitosterol.[4] *Potentilla anserina* Linn is also called as "Silverweed". It is used as astringent, anti-inflammatory, antispasmodic, haemostatic, for diarrhoea, leucorrhoea, dysmenorrhoea, arthritis, cramps, kidney stones, bleeding piles; as a mouth wash in pyrrhoea, gingivitis and sore throat.[5] The plant contains anthocyanins—cyaniding and delphinidin. Aerial parts contains tannins (2–10%). The plant has choline, betaine, histidine, an essential oil and vitamin E. The maximum amounts of tannins occur in the root stock (up to 17.5% on dry basis). The hydroalcoholic extract of the herb (1: 5) contain 0.3 to 0.8% of tannin. The tannin fraction exhibited anti-mutagenic effect. The flowers and young shoots contain flavonoids, quercetin, terniflorin, tribuloside and (−)-catechin. The plant also contains stigmasterol, beta-sitosterol and campesterol; (−)-epicatechol gallate, (±)-catechol, (−)-epicatechol, (−)-epigallocatechol and (−)-epigallocatechol gallate have been isolated from aerial parts.[5] The current investigation was carried out to assess the anti-inflammatory activity of aqueous and alcoholic extracts of leaves of *Barleria longiflora* L.f. and whole plant of *Potentilla anserina* Linn [(Aq(BL), E(BL)] and [(Aq(PA), E(PA)] using Carrageenan induced paw oedema in rats.

MATERIALS AND METHOD

Plant material and Preparation of extracts[6]

The leaves of *Barleria longiflora* L.f. (Balsaminaceae) and whole plant of *Potentilla anserina* Linn (Rosaceae) were collected from Tirupati, India and were authenticated by Dr. K. Madhava Chetty, Sri Venkateshwara University, Tirupati, India. The authenticated aqueous and ethanolic extracts of leaves of *Barleria longiflora* L.f. and whole plant of *Potentilla anserina* Linn were obtained from "Green Chem", Bangalore-560071. The percentage yield

of extracts is as follows: *Barleria longiflora* L.f.; Aqueous: 18% w/w, Ethanol 10% w/w, *Potentilla anserina* Linn*;* Aqueous: 19% w/w, Ethanol 12% w/w.

METHODOLOGY

Determination of anti-inflammatory activity of [(Aq(BL), E(BL)] and [(Aq(PA), E(PA)].

Carrageenan induced paw oedema.[7], [8]

Albino rats (Wistar strain) of either sex weighing between 180-200gm body weights were selected for the experimental study. They were divided into 10 groups of six animals each. Group 1 rats served as Carrageenan control (0.1ml of 1% w/v, s.c, hind paw), group 2 rats received standard drug Diclofenac 5 mg/Kg, b.w, i.p., group 3 and 4 rats were administered, orally with aqueous extract of *Barleria longiflora* L.f. of 100 and 400 mg/kg b.w, respectively. Group 5 and 6 rats were administered, orally, ethanol extract of *Barleria longiflora* L.f. of 100 and 400 mg/kg b.w, respectively. Group 7 and 8 rats were administered, orally, aqueous extract of *Potentilla anserina* Linn of 100 and 400 mg/kg b.w, respectively. Group 9 and 10 rats were administered, orally, ethanol extract of *Potentilla anserina* Linn of 100 and 400 mg/kg b.w, respectively. After 1 hr 0.1 ml of 1% w/v Carrageenan was injected into sub plantar region of right hind paw. The paw volume was measured at 0, 1, 2, 3 and 4hr after carrageenan injection. The left paw volume was measured initially at 0 hr for all the groups which gives the normal paw volume. The difference between the left and right paw values gives the actual edema volume which was compared with carrageenan control. The inhibition of inflammation was calculated using the formula,

$$\% \text{ inhibition} = 100\ (1-Vt/Vc),$$

Where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

Statistical analysis

The data was expressed as mean ± S.E.M. The statistical analysis of results was done by means Analysis Of Variance (ANOVA) followed by Dunnett's post hock test. The P value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

As seen from table 1, in the rats administered with vehicle (Distilled water), the sub plantar injection of Carrageenan produced a local edema that increased progressively from 0.1567 ml after the first hour to reach a maximum 0.6533 ml within four hour. The extracts of *Barleria*

longiflora L.f. leaves (100, 400 mg/kg) and whole plant of *Potentilla anserina* Linn (100, 400 mg/kg) from post Carrageenan injection caused a dose dependent and significant reduction of edema in the rats. Diclofenac (5mg/kg) produced a significant decrease in edema when compared with the Carrageenan treated group.

Inflammation is a body defence mechanism which eliminates or limits the spread of the injurious agent. It serves to destroy, dilute, or wall off the offender and sets into motion a series of events that try to heal and reconstitute the damaged tissue.[1] Non-steroidal anti-inflammatory drugs (NSAIDs) make up one of the largest groups of drugs used for pain and inflammation. Currently available anti-inflammatory agents are associated with unwanted side effects- gastrointestinal damage and have their own limitations.[2] Hence there is a need for the study on herbal drugs for treating inflammation for which the present study was carried out. The most widely used primary test to screen new anti-inflammatory agents is to measure the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent. Carrageenan-induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase (1-2) of carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages. NSAIDS were used as anti-inflammatory agents as they inhibit these different inflammatory mediators by inhibiting cyclooxygenases (COX-1 and COX-2).[9] Diclofenac sodium inhibits the prostaglandin synthesis.[10] The results showed that administration of extracts inhibited the edema starting from the first hour and during all phases of inflammation which is probably inhibition of different steps and chemical mediators of inflammation. The inhibitory activity was shown by the aqueous and alcoholic extracts of *Barleria longiflora* L.f (100, 400 mg/kg) and *Potentilla anserina* Linn (100, 400 mg/kg). The alcoholic extracts have higher activity when compared to aqueous extracts. E(LBL) and E(PA) exhibited significant increase in percentage inhibition of paw volume. The E(PA) indicated the remarkable rise in percentage inhibition. Previous phytochemical studies indicated presence of β-sitosterol,[1] (in *Typha angustifolia*), flavonoids,[11] (in *Tecoma stans*), phenolic compounds[12] (in *Polyalthea longiflora*), tannins[13] (in *Ficus religiosa*) are responsible for anti-inflammatory activity. Previous literature search and prior studies have indicated presence of β-sitosterol[4] in *Barleria longiflora* L.f. and tannins, flavonoids, β-sitosterol in *Potentilla anserina* Linn[5]. Hence these

phytoconstituents may be responsible for anti-inflammatory activity of leaves of *Barleria longiflora* L.f. and whole plant of *Potentilla anserina* Linn.

Table 1: Percentage inhibition of paw volume of different extracts of Barleria longiflora L.f. and Potentilla anserina Linn

Groups	0 hour		1 hour		2 hour		3 hour		4 hour	
	Paw edema (ml)	% Reduction of volume	Paw edema (ml)	% Reduction of volume	Paw edema (ml)	% Reduction of volume	Paw edema (ml)	% Reduction of volume	Paw edema (ml)	% Reduction of volume
Carrageenan Control	0.1567± 0.022	-	0.4183±0.014	-	0.4883± 0.024	-	0.5683±0.047	-	0.6533± 0.059	-
Diclofenac 5 mg/kg	0.1567± 0.022	-	0.2500±0.002***	39.87	0.2667±0.021***	44.31	0.2367±0.012***	56.67	0.2183±0.007***	65.08
Aq (BL) 100mg/kg	0.2050± 0.009	-	0.3450±0.039	15.89	0.3700±0.023***	23.88	0.3400±0.010***	38.70	0.3283±0.025***	47.65
Aq (BL) 400mg/kg	0.1783± 0.018	-	0.3050± 0.025*	26.38	0.3350±0.029***	31.59	0.3017±0.030***	46.23	0.2883±0.012***	53.53
E (BL) 100mg/kg	0.2083± 0.025	-	0.3367± 0.019	18.53	0.3467±0.013***	28.01	0.3183±0.017***	41.19	0.3117±0.006***	49.84
E (BL) 400mg/kg	0.1983± 0.042	-	0.2933± 0.019**	29.06	0.3167±0.013***	34.74	0.2817±0.018***	49.27	0.2667±0.011***	56.97
Aq (PA) 100 mg/kg	0.2150± 0.037	-	0.3217± 0.022*	23.36	0.3450±0.021***	28.80	0.3183±0.020***	42.96	0.2800±0.025***	54.68
Aq (PA) 400 mg/kg	0.1733± 0.034	-	0.2800± 0.045**	33.42	0.2983±0.014***	38.56	0.2733±0.019***	50.96	0.2633±0.016***	58.52
E (PA) 100 mg/kg	0.2333± 0.012	-	0.2967± 0.011**	28.22	0.3417±0.014***	29.15	0.2917±0.015***	46.23	0.2700±0.019***	56.51
E (PA) 400 mg/kg	0.2217± 0.015	-	0.2700±0.010***	35.23	0.2850±0.015***	41.19	0.2517±0.026***	54.91	0.2417±0.014***	59.97

n=6, values are mean ± S.E.M, one way ANOVA followed by Dunnet's post hoc test. Significance at *P<0.05,P<0.01,***P<0.001 v/s control. Aq (BL)-Aqueous extract of leaf of *Barleria longiflora* L.f. E (BL)- Ethanolic extract of leaf of *Barleria longiflora* L.f. Aq (PA)-Aqueous extract of *Potentilla anserina*. E (PA)- Ethanolic extract of *Potentilla anserine.***

CONCLUSION

Ethanol extract of leaves of *Barleria longiflora* L.f. and whole plant of *Potentilla anserina* Linn showed strong anti-inflammatory activity than aqueous extracts of the plants when compared with Diclofenac as standard. Among *Barleria longiflora* L.f. and *Potentilla anserina* Linn, *Potentilla anserina* Linn showed significant anti-inflammatory activity.

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