Screening of Aqueous And Ethanolic Leaf Extracts of *Barleria Longiflora* L.f. For Anti-Inflammatory Activity

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ABSTRACT

This study was designed to evaluate, aqueous and ethanol leaf extracts of *Barleria longiflora* L.f. for anti-inflammatory activity using Formalin induced paw oedema in rats. Animals were divided into 6 groups of 6 rats. Groups 1 and 2 served as Formalin 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. of 100 and 400 mg/kg b.w, respectively. Groups 5 and 6 were administered, orally with ethanol extract of *Barleria longiflora* L.f. of 100 and 400 mg/kg b.w, respectively. The paw volume was measured at 0, 1, 2, 3 and 4 hr after Formalin 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. The aqueous and ethanol leaf extracts of *Barleria longiflora* L.f. exhibited significant reduction of edema in the rats. Ethanol leaf extract of *Barleria longiflora* L.f. revealed surpassing anti-inflammatory activity.

Keywords: *Barleria longiflora* L.f., inflammation, formalin, edema.

INTRODUCTION

Pain and inflammation remains one of the world's major health problems[1]. It is a common complaint in most patients suffering from disease conditions. Inflammation is a host defense mechanism to combat or overcome the invading pathogen or the foreign particles[2]. Excessive inflammatory response has damaging effects, such as septic shock, which can lead to multiple organ dysfunction syndrome and death[3]. The side effects of steroidal and non steroidal anti-inflammatory drugs currently used for management of chronic inflammatory diseases may be more difficult to manage than the disease itself[4]. Novel potent anti-inflammatory drugs without considerable side effects from the natural sources are under investigation[3]. Despite the progress in modern medicine, it has been reported that more than 70% of the developing worlds population still depends on complementary and alternative systems of medicine, otherwise known as traditional medicine[5]. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine[6]. Thus the present investigation was carried out to evaluate the anti-inflammatory potential of aqueous and ethanol extracts of leaves of *Barleria longiflora* L.f. The plant *Barleria longiflora* L.f. have been traditionally used for treating cough, inflammations, dropsy, kidney stone problems[7]. The phyto constituents reported in this plant include anthraquinones, a pentacyclic titerpene arnidiol, campesterol, stigmasterol and β-sitosterol[8].

MATERIALS AND METHOD

Plant material and Preparation of extracts:[9]

The leaves of *Barleria longiflora* L.f. (Balsaminaceae) 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. were obtained from "Green Chem", Bangalore-560071. The percentage yield of extracts of *Barleria longiflora* L.f. obtained was Aqueous: 18% w/w, Ethanol 10% w/w.

Qualitative phytochemical investigation of aqueous and ethanolic extracts of *Barleria longiflora* L.f. [(Aq (BL), E (BL)]:[10, 11]

The dry extracts were screened for the presence of various phytoconstituents responsible for the medicinal properties of the drug like alkaloids, glycosides, carbohydrate, tannins – phenolic compounds, proteins, amino acids, fats, oils, flavonoides, saponins and steroids etc.

Methodology:

Formalin induced paw oedema[12, 13]

Albino rats (Wistar strain) of either sex weighing between 180-200gm body weights were selected for the experimental study. They were divided into six groups of six animals each. Group 1 rats served as Formalin control (0.1ml of 2% v/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 leaf extract of *Barleria longiflora* L.f. of 100 and 400 mg/kg b.w, respectively. Group 5 and 6 rats were administered, orally with ethanol leaf extract of *Barleria longiflora* L.f. of 100 and 400 mg/kg b.w, respectively. After 1 hr 0.1 ml of 2% v/v Formalin was injected into sub plantar region of right hind paw. The paw volume was measured at 0, 1, 2, 3 and 4hr after Formalin 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 Formalin control. The inhibition of inflammation was calculated using the formula,

$$\% \text{ inhibition} = 100 \, (1 - V_t/V_c),$$

Where 'V_c' represents edema volume in control and 'V_t' edema volume in group treated with test extracts.

STATISTICAL ANALYSIS:

The data was expressed as mean $\pm$ 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, Alkaloids, Saponins, Flavonoids, Glycosides, Tannins, Phenolic compounds and steroids were present and carbohydrate and protein were absent.

As seen from table 2, in the rats administered with vehicle (Distilled water), the sub plantar injection of Formalin produced a local edema that increased progressively from 0.4333 ± 0.030ml after the first hour to reach a maximum 0.9417 ± 0.056 within four hour. The [(Aq(BL), E(BL)] at doses of 100 and 400 mg/kg each from the third hour post formalin injection caused a dose dependent and significant (P<0.001) reduction of edema in the rats. Diclofenac (5mg/kg) produced a significant (P<0.001) decrease in edema at third and forth hour when compared with the Formalin treated group.

Despite progress within medical research during the past decades, the treatment of many serious diseases remains problematic[14]. Chronic Inflammatory diseases remain one of the world's major

health problems[15]. Inflammation is generally considered as an essentially protective response to tissue injury caused by noxious physical, chemical or microbiological stimulus. It is a complex process involving various mediators, such as prostaglandins, leukotrienes and platelet activating factor[16]. Anti-inflammatory drugs are agents that reduce inflammation. It has been found that conventional synthetic NSAIDs accelerate damage and erosion of joint cartilage, advancing the osteoarthritis process. These NSAIDs are also known to cause liver and kidney damage with long term use[5]. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though little knowledge about their mode of action is available[6] because they are cheap, abudently available and relatively less toxic[17]. 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 agent measure the ability of a compound to reduce local edema induced in the rat paw by injection of formalin. The development of edema in the rat paw after the injection of formalin is a biphasic event. The initial phase of the edema is due to the release of histamine and serotonin and the edema is maintained during the plateau phase by kinin-like substance[18] and the second accelerating phase of swelling due to the release of prostaglandin-like substances. Inhibition of edema observed in the formalin model may be due to the ability of extracts to inhibit these chemical mediators of inflammation[19]. NSAIDS were used as anti-inflammatory agents as they inhibit these different inflammatory mediators by inhibiting cyclooxygenases (COX-1 and COX-2)[20]. prostaglandin synthesis was inhibited by Diclofenac sodium[21]. The inhibitory activity was shown by the aqueous (100,400 mg/kg) and ethanolic extracts (100,400 mg/kg) of *Barleria longiflora* L.f. The ethanolic extract has higher activity when compared to aqueous extract. The percentage inhibition for *Barleria longiflora* L.f. aqueous extract (100,400 mg/kg) was 43.29, 54.27 and etanolic extract (100,400 mg/kg) was 49.79, 58.41.

Phytochemical investigation of aqueous and ethanolic extracts of *Barleria longiflora* L.f. has revealed the presence of various phytoactive constituents such as alkaloids, saponins, flavonoids, glycosides, tannins, phenolic compounds and steroids. Previous literature studies revealed the presence of β-sitosterol[22], (in *Typha angustifolia*), flavonoids[3], (in *Tecoma stans*), phenolic compounds[23], (in *Polyalthea longiflora*), and tannins[24] (in *Ficus religiosa*) may be responsible for anti-inflammatory activity. These plant extracts also contain the similar phyto constituents which was responsible for the activity.

Phenolic compounds such as flavonoids, tannin and steroid are a major group of compounds that act as anti-inflammatory agents. Phenolics are compounds possessing one or more aromatic rings

with one or more hydroxyl groups. They are broadly distributed in the plant kingdom and are the most abundant secondary metabolites of plants, with more than 8,000 phenolic structures currently known, ranging from simple molecules such as phenolic acids to highly polymerized substances such as tannins[25]. Phenolic compounds possess ideal structure chemistry for anti-inflammatory due to these characteristics: (1) Planar ring system which is essential in flavonoid molecules to display the activity; (2) Unsaturation in the C ring as ketonic carbonyl at C_4 and/ C_2-C_3 double bond; (3) Hydroxyl groups in B ring and at C_5 and C_7 of A ring are crucial; (4) The number and position of hydroxyl groups as the catechol group at ring B; (5) The flavones and flavonols having a hydroxyl group at $4^{'}$ position of B ring showed higher activity; (6) The non-glycosylation of molecule, aglycones have a better effect than glycosides.[26, 27] There is a relationship between phenolic structure and pro inflammatory mediators. Three main structural characters precipitate to flavonoids NO inhibitory potential:(a) a C_2-C_3 double bond, (b) a bulky group as a substituent lowered or nullify the inhibitory effect of compound (aglycones have bigger effect than glycosides), (c) 7 and 4′ OH-groups, but this last feature should be accompanied by any of the aforementioned[28] Phenolic compounds work same as NSAIDs, additionally some of them retard other pro-inflammatory mediators besides COX by inhibiting their activity or gene expression. Besides, some phenolic compounds can up/down regulate transcriptional factors, like nuclear factor-kB (NFkB) or Nrf-2, in inflammatory and antioxidant pathways[29, 30] Steroids exhibit the anti-inflammatory activity by regulating stability of pro-inflammatory mRNA signalling including COX-2[31].

Table 1: Qualitative phytochemical screening of [(Aq(BL), E(BL)]

Chemical tests	*Barleria longiflora* L.f.	
	Aqueous extract	Ethanol extract
1)Test for Triterpenoids /Steroids		
Liebermann Burchard Test	+	+
2) Test for Glycosides		
Keller Killiani Test	+	+
Legals test	+	-
3) Test for Saponins		
Foam test	+	+
4) Test for Alkaloids		
Dragendorffs test	+	+
5) Test for Flavanoids		
Ferric Chloride test	+	+
Alkaline reagent test	+	+
Lead Acetate Solution test	+	+
6) Test for Tannins/phenols		
$KMNO_4$ test	+	+

5% $FeCl_3$	+	+
7) Test for Proteins		
Biuret test	-	-
8) Test for Free amino acids		
Ninhydrin Test	-	-
9)Test for Carbohydrates		
Molischs test	-	-
10) Test for fat and oil		
Saponification test	-	-

- Negative; + positive

Table 2: Effect of Aq(BL) AND E(BL), On formalin induced paw oedema (percentage inhibition)

Groups	0 hour		1 hour		2 hour		3 hour		4 hour	
	Paw edema (ml) Mean ± SEM	% ROV	Paw edema (ml) Mean ± SEM	% ROV	Paw edema (ml) Mean ± SEM	% ROV	Paw edema (ml) Mean ± SEM	% ROV	Paw edema (ml) Mean ± SEM	% ROV
Control	0.4333 ± 0.030	-	0.675 ± 0.017	-	0.7833 ± 0.024	-	0.8667 ± 0.047	-	0.9417 ± 0.045	-
Diclofenac 5 mg/kg	0.3083 ± 0.023	-	0.395 ± 0.063***	41.69	0.4167 ± 0.058***	46.95	0.37 ± 0.061***	56.59	0.3117 ± 0.042***	68.94
Aq(BL) 100mg/kg	0.3583 ± 0.023	-	0.5717 ±0.047$	15.84	0.575 ± 0.070*	26.34	0.5167 ±0.065***	38.49	0.5167 ± 0.065***	43.29
Aq(BL) 400mg/kg	0.35 ± 0.031	-	0.5333 ± 0.030$	20.24	0.5383 ± 0.028**	30.82	0.4417 ± 0.074***	48.62	0.4233 ± 0.076***	54.27
E(BL) 100mg/kg	0.3583 ± 0.020	-	0.5417 ± 0.050$	19.90	0.5667 ± 0.053*	27.50	0.4917 ± 0.063***	40.82	0.445 ± 0.073***	49.79
E(BL) 400mg/kg	0.35 ± 0.025	-	0.5083 ± 0.030$	24.37	0.5217 ± 0.030**	32.74	0.405 ± 0.046***	52.26	0.3833 ± 0.049***	58.41

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.

CONCLUSION:

Ethanol extract of leaves of *Barleria longiflora* L.f. showed strong anti-inflammatory activity than aqueous extracts of the plants when compared with Diclofenac as standard.

ACKNOWLEDGEMENT:

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