EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 4
Issue: 4
730-734
Year: 2017

ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *BASELLA ALBA* IN ACUTE AND SUB-ACUTE MODEL

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Article Received on 16/02/2017 **Article Revised on 11/03/2017** **Article Accepted on 31/03/2017**

ABSTRACT
The present study is carried out to investigate the anti-inflammatory potential of Methanolic extract of *Basella alba* (BA). Anti-inflammatory activity was evaluated by using Egg Albumin, Turpentine Oil & Formaldehyde as phlogistic agents. The animals were treated with doses 250mg/kg and 500mg/kg of extract and Diclofenac Sodium at a dose of 10mg/kg is used as a standard drug. The BA showed a significant anti-inflammatory activity in a dose dependent manner in all the models when compared with the standard treatment. The extract (500mg/kg) exhibited maximum antiinflammatory activity i.e., 46.26%, 44.34%, 46.38% (P<0.001) like standard Diclofenac 47.03%, 46.54%, 48.55% in Egg albumin, Turpentine oil and Formaldehyde induced methods respectively. Based on the above results, we conclude that the BA has significant anti-inflammatory activity and might prove efficacious for further design and development of agents with significant biological activity.

KEYWORDS: Basella alba, anti-inflammatory activity, phlogistic agents.

INTRODUCTION
Herbal therapy is also known as herbalism, which play a major role in treatment of so many diseases in so many countries & in traditions.[1] Inflammation is a complex process, which is frequently associated with pain and involves occurrences such as: the increase of vascular permeability, increase of protein denaturation and membrane alteration. When tissue cells become injured they release kinins, prostroglandins and histamine.[2] Rheumatoid arthritis a ravaging disease is a major public health burden in about 1% of the population worldwide. As the currently used drugs are associated with severe side effects, the urge to develop new chemical entities with potent biological activity from natural sources with lesser side effects has become mandatory. Traditional medicine using plant extracts continue to provide health coverage for over 80% of the world's population especially in developing countries.[3] Many medicinal plants have been investigated for novel drugs or templates for the development of new therapeutic agents.[4] Various species from the genus alba have been reported to possess anti-inflammatory activity.

The childhood arthritis is the fifth most common chronic disease of the childhood and the most common of the paediatric rheumatic disease, affecting as many people in America. Despite of the use of the conventional methods in pain management ,persistent pain continuous to be predominant problem in children.[5] The aim of the present study is to identify the identify the anti inflammatory activity of the plant extract in animal models. to identify the better molecule which useful to human use.

Basella alba (Telugu: *bachhali* ; Family**:** *Basellaceae*) is a vine spinach, leaves are thick, rugose, succulent and green to purple colour. Basella Alba is advantageous for treating Ulcers and Abscesses, Rheumatic pain and Swellings.[6] Medicinal plants contain numerous biologically active compounds such as carbohydrates, proteins, enzymes, fats and oils, vitamins, alkaloids, quinines, terpenoids, flavonoids, carotenoids, sterols, simple phenolic glycosides, tannins, saponins, polyphenols etc. Basella alba contains Vitamin A, Vitamin E, Vitamin K, flavonoids, saponins and β-Carotene. The plant is reported to treat against laxative, rubefacient, skin diseases, burns, ulcers, diarrhoea, diuretic[7] and cancer. The present study was under taken to evaluate anti inflammatory activities of Methanolic leaf extract of *Basella alba.*

MATERIALS AND METHODS
Collection of plant material & Extraction: The leaves of Malabar spinach were collected from surrounding

villages of Kakinada A.P. The plant authentication was done by Dr. A.Srinivasa Rao, Dept. of Botany, P.R Degree College, Kakinada, East Godavari District, Andhra Pradesh, India & the voucher was preserved. The plant material was thoroughly cleaned, shade dried at room temp. for 23 days & then pulverized to a coarse powder and shifted. 95% Methanol was added to coarsely powered (2kg) plant material & extracted by using soxhlet apparatus. The extract was concentrated by distillation under reduced pressure & evaporated to dryness.[8]

Experimental animals

Healthy adult albino rats of wistar strains weighing 150-250gm of either sex were used in this study. The animals were kept properly in polypropylene cages under standard laboratory conditions (12/12hr light/dark cycle at 25 ± 5^0c). The rats were fed a commercial diet & water ad libitum & were divided into 5 groups. The experimental protocol was approved by the Institutional Animal Ethical Committee (Approval no: 4/2016/CPCSEA).

METHODOLOGY

Research on inflammation has become the focus of global scientific study because of its implication in virtually all human and animal diseases. Plant based drugs used in the practice of traditional treatment of diseases including inflammation have become the focus of current research because they are cheap and have great therapeutic potential without much of the side effects associated with synthetic drugs.[10]

Measuring anti inflammatory agent by a substance to reduce local oedema induced in the rat paw by a phlogistic agent. In this study Egg albumin, Turpentine oil & Formaldehyde were used as irritants.

Egg albumin induced paw oedema is similar to carrageenan induced acute inflammation & is a biphasic system. The early phage is due to release of histamine, serotonin & increase prostaglandins (PG) synthesis in the damaged tissue surroundings. The second phage is occurred from 3 to 5 hours after administration by PG release & mediated by bradykinin, leukotriene's, polymorphonuclear cells.[11] The test formulation showed the better anti-inflammatory activity & this is may be due to the inhibition of release of histamine, serotonin, kinins & this also retarded the release of PG like substances.

Turpentine oil in the sub plantar region causes paw oedema that was maintained throughout the observation period i.e., 7 days as it is subacute model due to the triphasic release of inflammatory mediators. The initial phase is due to the release of histamine & serotonin, intermediate phase is due to kinin like substances and the late phase is due to cyclooxygenase & lipoxygenase products. Kinin is considered to be the main mediator of granuloma as it causes vasodilatation and increases vascular permeability in the early stages of inflammation.[12] BA exhibited maximum inhibition of paw oedema during the late phase of inflammation which suggest a prominent cyclooxygenase/ lipoxygenase inhibitory activity.

Formaldehyde induced paw oedema is one of the most suitable test procedure to evaluate the anti-inflammatory & anti-arthritic agents as it closely resembles human arthritis. Formaldehyde is reported to produce inflammation through proliferation and migration of fibroblasts, which are mainly concerned with the formation of connective tissue.[13] The paw swelling due to 2%v/v formaldehyde was maintained throughout the observation period i.e.10 days due to the release of histamine, serotonin, and PG. Histamine and PG are the key mediators in inflammatory hyperalgesia which are mediated through the activation of local pain receptors and nerve terminals producing hypersensitivity in the area of injury.[14] Inhibition of paw oedema may be due to the ability of the BA to inhibit histamine, serotonin & PG. In all the experimental procedures, the results showed a significant inhibition of paw oedema by BA & standard drug when compared with the control. Further studies are required on isolation of potent chemical constituents of the plant & to investigate the mechanism of antiinflammatory activity.

Egg albumin induced rat paw oedema

Five groups of adult rats (n=6) were used in this study. Animals were fasted over night with free access to water before the experiment. On the day of experiment, base line paw volume was recorded by using a digital plethysmometer (UGO Basile, 7140 Italy). Thereafter group-I (normal rats) received the vehicle (Distilled water 5ml/kg). Group-II (control rats) received the inducing agent & vehicle. Group-III (standard rats) received diclofenac sodium (10mg/kg) along with inducing agent. Group-IV & V received extract at doses of 250mg/kg & 500mg/kg respectively along with inducing agent. 1hr after administration of vehicle/drugs, oedema was induced by administration of 0.1ml of fresh undiluted egg albumin solution into the subplantar region of right hind paw. Paw volume of each rat from all groups was measured at 0, 1, 3, 6, 12 & 24hr after phlogistic agent administration. From the mean oedema volume, the percent inhibition of oedema was calculated by using following formula:

% Inhibition of oedema = 100 (VC-VT/VC)

Where, VC = Mean paw oedema volume of control group

VT = Mean paw oedema volume of treated group

Turpentine oil induced rat paw oedema

Grouping of animals & drug treatments was same as above. 30min after administration of the vehicle/drug, oedema was induced by administration of 0.05ml turpentine oil into the subplantar region of right hind paw of animal. Paw volume of each rat from all groups was measured on 0, 1, 3 & 7th day after phlogistic agent

administration. From the mean oedema volume, the percent inhibition of oedema was calculated.

% Inhibition of oedema = 100 (VC-VT/VC)

Where, VC = Mean paw oedema volume of control group

VT = Mean paw oedema volume of treated group

Formaldehyde induced rat paw oedema

Grouping of animals & drug treatments was same as above. Drugs/vehicles were administered for a duration of 10days. 30min after administration of the drug/vehicle, oedema was induced by administration of 0.1ml of 2%v/v Formaldehyde into the subplantar region of right hind paw of all animals on days 1 and 3.[9] Increase in paw oedema volume was measured on 0, 1, 3, 7 and 10th day, 30min after administration of the respective vehicle/drug. From the mean oedema volume, the percent inhibition of oedema was calculated.

% Inhibition of oedema = 100 (VC-VT/VC)

Where, VC = Mean paw oedema volume of control group

VT = Mean paw oedema volume of treated group

Statistical Analysis

The statistical significance was measured by using one way analysis of variance (ANOVA) & followed by Dunnett's comparison test. All the data are presented as mean ± SEM & $p < 0.001$ was considered as significant.

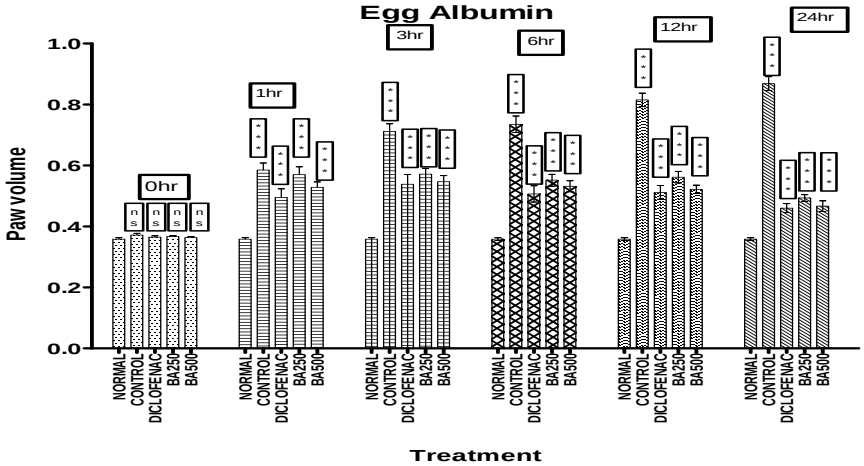
RESULTS

The phlogistic agents induced inflammation was significantly inhibited by the treatment given when compared with the standard drug. BA exhibited significant anti-inflammatory activity in a dose dependent manner.

Egg albumin induced paw oedema: The effect of BA on egg albumin induced paw oedema was depicted in the table 1. The BA at a dose of 500mg/kg showed significantly greater inhibitory activity (46.26%) against standard diclofenac sodium (47.03%).

Table 1: Anti-inflammatory activity of BA on Egg albumin induced paw oedema
Percentage inhibition of paw oedema

Group	Dose	0hr	1hr	3hr	6hr	12hr	24r
Diclofenac	10mg/kg	2.12%	15.39%	24.34%	31.08%	37.24%	47.03%
BA	250mg/kg	1.28%	2.58%	19.66%	24.92%	31.09%	43.19%
	500mg/kg	1.61%	9.69%	23.20%	27.68%	35.98%	46.26%

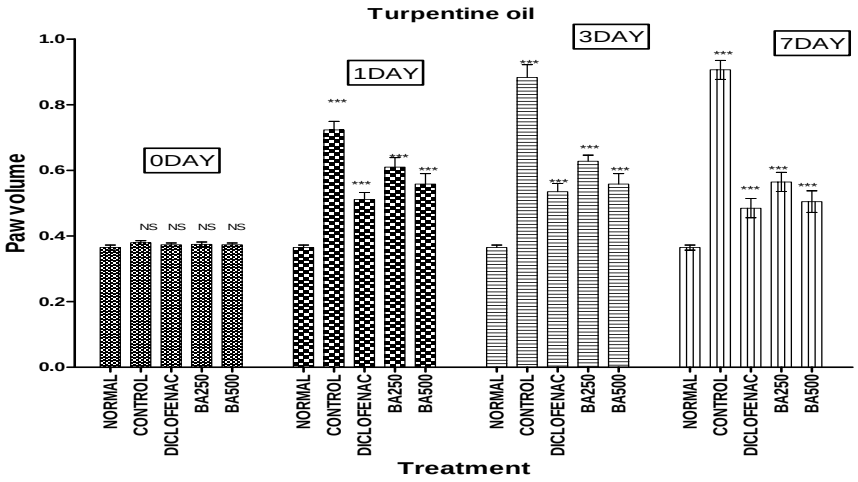


All values are expressed as mean ± SEM, n=6, one way Analysis of variance (ANOVA) followed by Dunnett's multiple comparison test; ***p<0.001 as compared to control group; ns=non-significant.

Turpentine oil induced paw oedema: The inhibitory activity on turpentine oil induced paw oedema is shown in table 2. The BA at a dose of 500mg/kg showed inhibitory activity of 44.34% against standard (46.54%).

Table 2: Anti-inflammatory activity of BA on Turpentine oil induced paw oedema
Percentage inhibition of paw oedema

Group	Dose	0 DAY	1 DAY	3 DAY	6 DAY
Diclofenac	10mg/kg	1.74%	29.24%	39.46%	46.54%
BA	250mg/kg	1.32%	15.68%	28.84%	37.48%
	500mg/kg	1.73%	22.84%	36.80%	44.34%

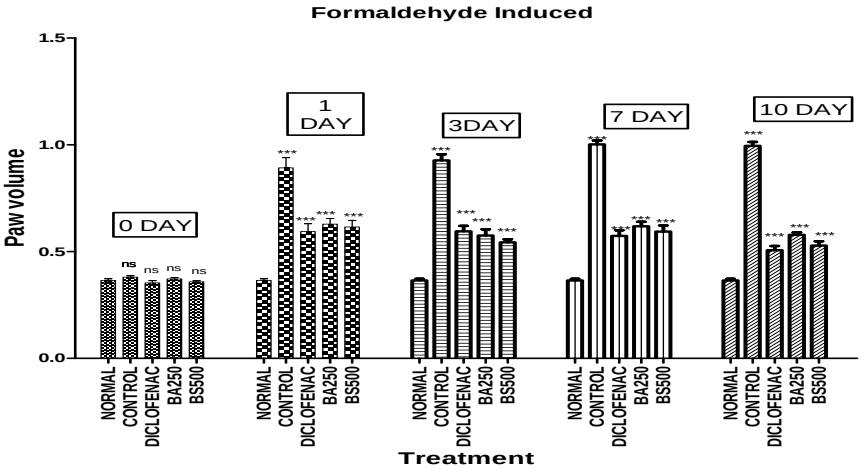


All values are expressed as mean ± SEM, n=6, one way Analysis of variance (ANOVA) followed by Dunnett's multiple comparison test; ***p<0.001 as compared to control group; ns=non-significant.

Formaldehyde induced paw ooedema: As shown in table 3 the BA at a dose of 500mg/kg showed greater inhibitory activity (46.38%) against standard (48.55%).

Table 3: Anti-inflammatory activity of BA on Formaldehyde induced paw oedema
Percentage inhibition of paw ooedema

Group	Dose	0 DAY	1 DAY	3 DAY	7 DAY	10 DAY
Diclofenac	10mg/kg	7.05%	33.45%	35.79%	42.92%	48.55%
BA	250mg/kg	2.4%	29.53%	30.61%	38.43%	41.37%
	500mg/kg	5.68%	31.05%	34.03%	40.93%	46.38%



All values are expressed as mean ± SEM, n=6, one way Analysis of variance (ANOVA) followed by Dunnett's multiple comparison test; ***p<0.001 as compared to control group; ns=non-significant.

DISCUSSION

The phlogistic agents induced inflammation was significantly inhibited by the treatment given when compared with the standard drug. BA exhibited significant anti-inflammatory activity in a dose dependent manner. The BA at a dose of 500mg/kg showed significantly greater inhibitory activity (46.26%) against standard diclofenac sodium (47.03%). The BA at a dose of 500mg/kg showed inhibitory activity of 44.34 % against standard (46.54%). The BA at a dose of 500mg/kg showed greater inhibitory activity (46.38%) against standard (48.55%).

CONCLUSION

Interestingly, the test compound showed potential anti inflammatory activity like standard Diclofenac sodium and justified the traditional use of Basella alba in the treatment of various types of pain & inflammation in all experimental models. Based on the above results, we conclude that the Methanolic extract of *Basella alba* has significant anti-inflammatory activity and might prove efficacious for further design and development of agents with significant biological activities. Further studies are required to identify lead molecule for the anti inflammatory action in paediatric and human use.

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