Advances in Bioresearch
Adv. Biores., Vol 6 (1) January 2015:01-05
©2014 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV 7.20 [Poland]

Advances in Bioresearch

ORIGINAL ARTICLE

Ascertaining Anti-Inflammatory and Analgesic Activity in Aqueous Leaf Extract of *Anisomeles indica* (L.) Kuntze

Ruchi Parmar[1], Sumitra Chakraborty[2], Rajashekhar Ingalhalli[1] and Meonis Pithawala[1*]
[1]C G Bhakta Institute of Biotechnology, Uka Tarsadia University,Tarsadi,
Dist Surat-394350, Gujarat, India
[2]Department of Zoology, M. G. Science College, Visnagar, Gujarat, India
Email : meonis_pithawala@yahoo.com

ABSTRACT
The anti-inflammatory properties of aqueous extract of Anisomeles indica leaves were studied in rat model using carageenan induced paw edema. Aspirin 100mg/kg was used as standard drug. The extract of A indica leaves at 250mg/kg were administered orally in rat models 1 hr before induction of carageenan and compared with a negative control group given 10ml/kg distilled water. Using the similar dose of extract the analgesic activity was also studied by tail immersion method in which 100mg/kg diclofenac as standard drug and 10ml/kg distilled water were used for negative control. The results showed maximum anti-inflammatory effect and analgesic effect after 4 hr. The results indicate that Anisomeles indica has potential in phytomedicine.
Keywords: *Anisomeles indica, Anti-inflammatory effect, Analgesic effect, Aspirin, Diclofenac*

Received 12/11/2014 Accepted 01/01/2015

How to cite this article:
Ruchi P, Sumitra C, Rajashekhar I and Meonis P. Ascertaining Anti-Inflammatory and Analgesic Activity in Aqueous Leaf Extract of *Anisomeles indica* (L.) Kuntze.Adv. Biores., Vol 6 [1] January 2015: 01-05. DOI: 10.15515/abr.0976-4585.6.1.15

INTRODUCTION

Anisomeles indica is an ethnomedicinally important aromatic plant belonging to the family Lamiaceae. The plant is commonly known as 'Indian catmint'. It is native to Southeast Asia and distributed throughout India, China, Japan and southwards from Malaysia to Australia [1,2,3,4]. It is used in folk medicine in the treatment of diverse conditions such as inflammatory skin diseases, liver protection, intestinal infection, abdominal pain. Bruised leaves are applied locally in snake bites [5,6]. *A. indica* when applied as mulch significantly reduces emergence and growth of weeds in wheat crops similar to herbicide, without any negative effect on the growth and yield [1].

The crude extracts, essential oils and purified compounds from various parts of the plant such as roots, leaves and flowers have shown to exhibit several bioactivities such as antimicrobial[7,8,9,10,11], antioxidant[11], attenuation of inflammation[10], analgesic[12], anti-inflammatory[13], antiviral[14], anti-platelet aggregation activity[15] and others.

Preliminary chemical screening of *Anisomeles indica* revealed presence of triterpenoids in the entire plant. It has been reported that the plant contains anisomelic acid (terpenoid), ovatodiolide (terpenoid), 4,7- oxycycloanisomelic acid (terpenoid), iso-ovatodiolide, β-sitosterol, stigmasterol, flavones and apigenin yields and essential oils[16]. Fourteen constituents were isolated from the crude extract of the leaves of *Anisomeles indica* which were (1) 7-methoxy-3,4,5,6-tetrahydroxyflavone (pedalitin), (2) apigenin, (3) ovatodiolide, (4) methylgallate, (5) 3,4-dihydroxybenzoic acid, (6) scullarein 7-*O*-d-glucuronide methyl ester, (7) apigenin 7-*O*-glucuronide, (8) desrhamnosylverbascoside (calceolarioside), (9) cistanoside F, (10) betonyoside A, (11) campneoside II, (12) acteoside, (13) isoacteoside, (14)apigenin7-*O*-d-(6-*O*-p-coumaroylglucopyranoside) (terniflorin)[17,13].

Inflammation is a biological response of vascular tissue to harmful stimuli caused by injury, infection, environmental agents, malignancy and cellular changes. It is a protective attempt by the body to remove the injurious stimuli as well as initiate the healing process for the tissue[18]. The inflammatory response

is a complex process that includes activation of white blood cells, the release of immune system chemicals such as complements and cytokines, and the production and release of inflammatory mediators and prostaglandins. Inflammation may be acute or chronic depending on the disease course. Acute inflammation is characterized by heat, erythrema, pain, swelling and loss of function. Chronic inflammation on the other hand results in a progressive shift in inflammatory cells characterized by simultaneous destruction and healing of the injured tissue [19].

Modern system of medicine is consecrated with a number of anti-inflammatory drugs which are effective and provide instant relief. But these synthetic drugs tend to produce a number of unwanted effects like increased risk of causing gastric erosion, haemorrhage. A total safe and effective drug against inflammation is yet to be developed which could fulfill the above requirements. The present study was undertaken with an aim to determine anti-inflammatory and analgesic activity of crude extract of *Anisomeles indica* against experimental animal models (Albino Wistar Rat).

MATERIALS AND METHODS

Plant material:

The plant material growing wildly in the rural areas of South Gujarat, India was collected. The leaves were separated, washed well in order to remove extraneous matter and allowed to dry under shade for 2 weeks. The dried leaves were powdered mechanically.

Experimental Animals:

Experiment was performed using Albino Wistar rats (200-250 g). The animal was obtained from the animal house of Maliba Pharmacy College, Uka Tarsadia University (after the approval of institutional animal ethical committee). The animals were fed with standard rodent cubes and free access to tap water. Animals were exposed to natural lightning.

Test for qualitative determination of bioactive compounds of *A. indica*

Chemical tests for the screening and identification of bioactive chemical constituents in *A. indica* plant under study were carried out using aqueous, methanol, ethanol, petroleum ether, and chloroform extracts.

*Preparation of Extracts***:** 500 gm of air-dried powder of *A. indica* was mixed with 300 ml of organic solvents (distilled water, methanol, ethanol, petroleum ether, and chloroform) in cold for 2 days. The solvent from the total extract was filtered and filtrate was concentrated on water bath for 3 hrs. The filtrate was used for phytochemical analysis [20].

*Phytochemical Analysis***:** The individual extract was subjected to the qualitative phytochemical screening for the presence of some chemical constituents. Phytochemical test were carried out adopting standard procedures[20,21,22,23].Tests were performed for presence of Proteins, Amino acids, Carbohydrate, Steroids, Phenols and Tannins, Saponin, Terpenoid, Alkaloids, Glycosides, Flavonoids, and Fatty acid (Table I) from various solvent extracts of *A. indica*.

Acute toxicity study:

Overnight-fasted Wistar rats (200-250g) of either sex were used for the study. The animals were divided into 2 groups of four animals each. Group A and B received 250 and 400 mg/kg of the extract. The dose was given orally which was prepared by suspending crude powder according to body weight in carboxyl methyl cellulose (CMC). General symptoms of toxicity and mortality in each group were observed within 24 hr. Animals that survived after 24 hr were observed for any signs of delayed toxicity for two weeks [1].

Anti-inflammatory activity:

Carageenan induced paw edema: Wistar rats (200-250 g) were divided into 3 groups of 4 animals each. The test groups received 250 mg/kg crude extract of *A. indica*. The reference group received aspirin (100 mg/kg) while the control group received 3 ml/kg of distilled water. After 1 hr, 0.1 ml, 1% w/v carageenan suspension in normal saline was injected into the subplantar tissue of the right hind paw. The paw thickness was measured after 3hr and after 24hr using a vernier caliper [24]. The percentage inhibition was calculated according to the formula:

$$\% \text{ inhibition} = \left[1 - \frac{dt}{dc}\right] \times 100$$

Where: dt = mean paw size in test models,

dc = mean paw size in control models

Analgesic activity study:

Tail immersion study (thermal stimulus): The Wistar rats were selected by immersing the tail in hot water at temperature 55 ^{0}C ±5 ^{0}C and the basal reaction time was noted. The mice which showed a positive response within a span of 5 seconds for withdrawal of the tail clearly out of water were selected for further studies. The rats selected were weighted (250-300g) and grouped into 3 of 4 in each and the

normal basal reaction time was noted by repeating 5 times. Group 1 served as control and received the dose of DW 10ml/kg body weight. Group 2 received *A. indica* extract 250mg/kg body weight. Group 3 received aspirin antibiotic 100mg/kg body weight. The observations were made before and after administration of respective drugs at 30 min, 1hr, 2hr, 3hr, 4hr, 5hr [25,26].

RESULTS

No adverse effect or mortality was detected in rats up to 400mg/kg of crude extract of *A. indica* during the 24hr observation period. Based on which the respective dose (250mg/kg) was selected for further study. While searching the anti-inflammatory efficacy of leaf extract of the plant using Carageenan induced Rat paw edema method, it was quite evident that a gradual increase in paw volume was observed after carageenan administration and which reached maximum at 3hr and then declined (Table II). The group that received standard drug aspirin showed highly significant effect as compared to control group at 2, 3 and 24 hr. It showed peak effect at 24 hr (94%). While in control group there was no significant change seen. The group exposed with crude extact of *A.indica* showed significant effect as compared to control group. At the dose of 250mg/kg body weight 60% inhibition at 24hr was seen (Fig. I).

During the test for analgesic effect of leaf extract of the plant by tail immersion method, it was observed that the group exposed to Diclofenac showed highly significant analgesic effect at 1hr, 2hr, 3hr, 4hr . Peak effect was observed at 4hr. Control group did not show any significant change in basal reaction time. The group that was fed with crude extract of *A.indica* at a dose of 250mg/kg showed highly significant activity at different time intervals as compared to control group. *A. indica* showed peak effect at 4 hr as compared to control and Diclofenac drug. (Table III, Fig. II)

Table I: Qualitative analysis of different extracts of *A. indica*

Tests	Aqueous extract	Methanol extract	Ethanol extract	Petroleum ether extract	Chloroform extract
Protein	+	+	+	+	+
Amino acid	+	+	+	+	+
Carbohydrate	+	+	+	+	+
Steroid	+	+	+	-	+
Phenol and Tannin	+	+	+	+	+
Saponin	+	+	+	+	+
Terpenoid	+	+	+	+	+
Alkaloid	+	+	+	+	-
Glycoside	-	+	+	-	+
Flavonoid	+	+	+	+	+
Fatty acid	+	+	+	+	+

"+" indicates presence of bioactive compound and
"-" indicates absence of bioactive compound

Table II: Paw edema volume of rats

Groups	0 hr	2hr	3 hr	24 hr
Control (DW)10ml/kg	4.64	7.59	7.71	6.35
Aspirin (100mg/kg)	4.60	6.20	6.20	4.70
A.indica (250mg/kg)	4.76	7.22	7.24	5.45

*all values are expressed in average of each group

Table III: Evaluation of analgesic activity of *A.indica* by tail immersion method

Groups	Reaction time (sec) after administration of drug				
	0hr	1hr	2hr	3hr	4hr
Control(10ml/kg)	6.1	6.4	6.5	6.4	6.1
Diclofenac(100mg/kg)	5.6	8.2	13.1	14.4	15.80
A.indica(250mg/kg)	5.7	9.1	12.9	15.09	16.70

* all values are expressed in average of each group

Fig I: Anti-inflammatory effect of standard drug and *A. indica* at 2hr, 3hr and 24h

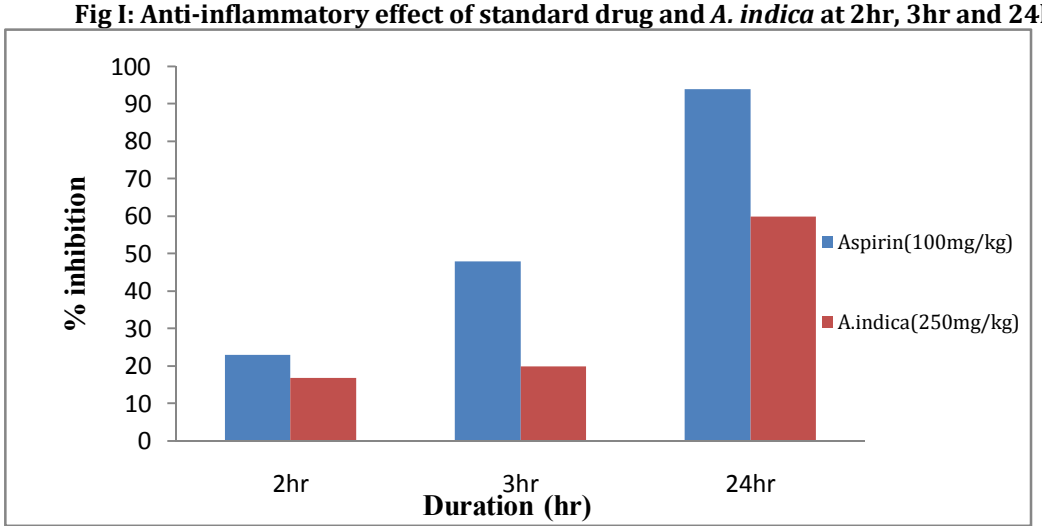
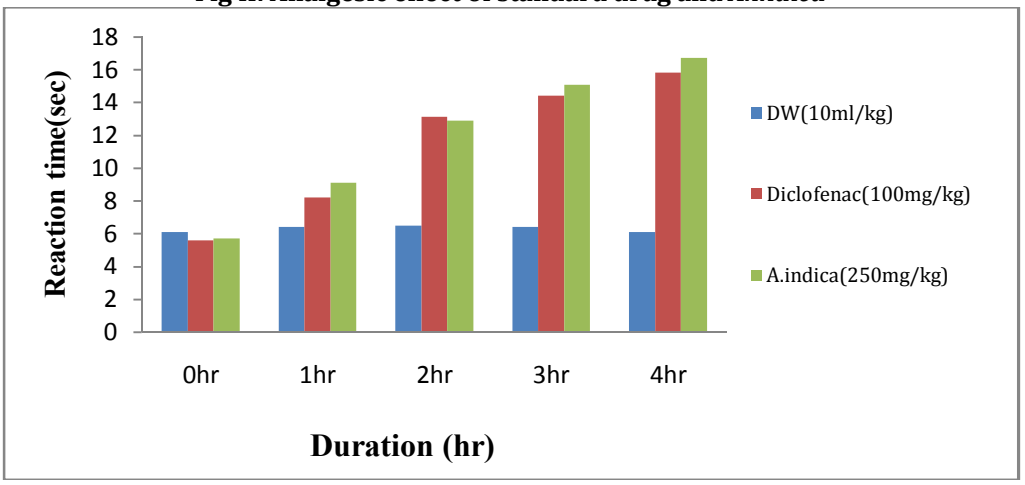


Fig II: Analgesic effect of standard drug and *A.indica*



DISCUSSION

The results of this study suggest that crude extract of *A.indica* leaves has anti-inflammatory and analgesic effect comparable to those of the standard drugs such as Aspirin and Diclofenac. This observation corroborates the findings [12] in which methanolic extract of *A.indica* exhibited systemic and significant anti-inflammatory activity in carrageenan-induced rat paw edema. Carrageenan-induced inflammatory process is believed to be biphasic [27]. The initial phase seen at the 1st hr is attributed to the release of histamine and serotonin [28]. The second accelerating phase of swelling is due to the release of prostaglandin, bradykinin and lysozyme. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents [29]. The anti-inflammatory activity exerted by crude extract of *A.indica* leaves suggests that it could affect kinnin, prostaglandin, bradykinin and lysozyme synthesis. The presence of apigenin, terpenoids and flavonoids in plants has been found to exert active anti- inflammatory and analgesic effects. Studies on the phytochemical analysis of *A.indica*[17,9] have revealed the presence of apigenin, terpenoids and flavonoids in the plant.

In the present study *A.indica* showed analgesic effect comparable to the standard drug (Diclofenac). This finding could be utilized in traditional medicine for the treatment of wounds and other conditions that can cause inflammation, pain etc. Further tests are needed to explore the exact mechanism of action at the molecular level and to know the actual constituents responsible for this activity.

In conclusion, crude extract of *A.indica* leaves could serve as an alternative anti-inflammatory and analgesic therapy in managing severe conditions or as complementary therapy allowing patients to take smaller doses of conventional anti-inflammatory and antibiotic drugs, thereby minimizing the side effects of the standard drugs.

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