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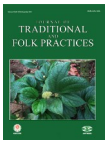

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In vivo anti-inflammatory effect of root of *Ampelocissus indica* (L.) Planch (*Chembravalli*)

V S Nithya*[1],V C Indulekha[2] and A Helen[3]

[1] Department of Dravyaguna Vijnana, Shri Dhanwantry Ayurveda College Chandigarh, 160047 Kerala, India
[2] Department of Dravyaguna Vijnanam, Govt. Ayurveda College Thiruvananthapuram, 695001 Kerala, India
[3] Department of Biochemistry, University of Kerala, Karyavattom, Thiruvananthapuram 695581 Kerala, India

*nithyasarangi3@gmail.com

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Abstract

Ampelocissus indica (L.) Planch, Vitaceae locally known as *Chembravalli,* is medicinal plant used for inflammatory skin ailments and documented in Hortus Malabaricus, and traditional Ayurveda books. The objective of the study is to conduct acute toxicity of root of *A. indica* and to evaluate its anti-inflammatory activity. Acute toxicity of *A. indica* was conducted as per OECD-425.Decoction of *A. indica* (AI-8.64ml/kg, AI-4.32ml/kg) and ethanolic extract (AIE-500mg/kg) were orally given to rats with Carrageenan induced rat paw edema and also in other rats with Cotton pellet induced granuloma; paw volume, weight of granuloma, histopathology, serum TNF_α and IL1β were assessed. Oral administration of decoction AI-8.64ml/kg, AI-4.32ml/kg, and AIE-500mg/kg significantly reduced inflammation by reducing paw volume, serum TNF_α and IL-1β.AI-8.64ml/kg reduced significantly weight of granuloma and serum TNF α, IL1β. No acute toxicity was found up to 2000mg/kg. The present finding suggested that *A. indica* has a protective effect on experimental inflammation in rats.

Keywords: : *Ampelocissus indica* (L.)Planch, Anti inflammatory, *Chembravalli*, Hortus Malabaricus, Paw edema

1. Introduction

Inflammation is the response of immune system to harmful stimuli, such as pathogens, damaged cells, toxic compounds or irradiation, and acts by removing injurious stimuli and initiating the healing process. Inflammation is therefore a defense mechanism that is vital to health. Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. However, uncontrolled acute inflammation may become chronic, contributing to a variety of chronic inflammatory diseases (Chen *et al*, 2017)**.** The early phase of acute inflammation involves the cellular influx associated with the release of mediators such as histamine and serotonin, which are primarily released from mast cells, followed by the production of bradykinin and prostaglandins. During an inflammatory response, several pro inflammatory mediators are released, including interleukin-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α) and cyclooxygenase- 2 (COX-2). These mediators have important role in the initiation and amplification of inflammatory processes (Coura *et al*, 2015). The NF-κB transcription factor plays an important roles in inflammation, immune response, survival and apoptosis processes. This pathway regulates pro-inflammatory cytokine production and inflammatory cell recruitment, which contribute to the inflammatory response(Chen *et al*, 2017). Current anti-inflammatory medications; steroidal and non steroidal anti-inflammatory drugs (NSAIDs) having several adverse effects. This creates an emerging interest in natural herbal remedies and dietary supplement for inflammation (Jitta *et al*, 2019).

According to the WHO report, about 70–80% of the world's population relies on nonconventional medicine mainly from herbal sources in their primary health

care (Amdekar *et al*, 2012). The need of the hour is therefore to explore such knowledge of traditional medicines for the benefit of humanity. *A.* indica(Al*)* is locally known as *Chembravalli* documented in Hortus Malabaricus (Rheed, 2003) and some Keraliya traditional books like *Chikitsamanjari* (Namboothiri, 2015), *Arogyakalpadrumam* (Varier,2011), *Yogamrutam* (Namboothiri,2010) and *Vaidyatarakam* (Narayanan,1974) prescribed for inflammatory skin ailments and wound healing. According to *Arogyakalpadrumam* the plant is used to cure *visarpa* (erysipelas), *Nelkarappan* (a kind of chronic skin disorder affecting infants), *Gudakushta*(a kind of ulcer affecting on perianal area) and *vrana* (ulcer) (Varier, 2011) also the plant is used to cure *vidradi* (abscess) as per the textbooks *Chikitsamanjari* (Namboothiri,2015) and *Yogamrutam* (Namboothiri,2010). The anti-oxidant (Sasikumar *et al*, 2016), anti diabetic and diuretic properties (Sunilson *et al,* 2004) of the plant and its fractions has been explored earlier. Since the traditional knowledge claims the cure of inflammatory skin ailments and wounds; the present study intended to see the anti-inflammatory effect of *A. indica* in Wistar rats

Carrageenan-induced paw edema is one of the most popular and highly sensitive and reproducible test for new non-steroidal anti-inflammatory drugs (Dzoyem *et al*, 2017). The cotton pellet granuloma method has been widely employed to access the transudative, exudative and proliferative components of sub-acute inflammation (Ashok *et al*, 2010).

2. Materials and methods

2.1. Plant material

The root tuber of the *A. indica* was collected from its natural habitat in Chippanchira (Thiruvananthapuram district, Kerala state, India) (Fig.1.) The plant was authenticated by Dr. Mathew Dan, Principal Scientist & Head Plant Genetic Resource Division, KSCSTE-Jawaharlal Nehru Tropical Botanical Garden Palode, Thiruvananthapuram, Kerala, India. (Fig. 2.) and the voucher specimen (specimen no: DGAVC201/19) was deposited at the department of Dravyaguna vijnana of Govt. Ayurveda College Thiruvananthapuram, Kerala, India.

Fig .1 Root tuber of *Ampelocissus indica* (L.)Planch

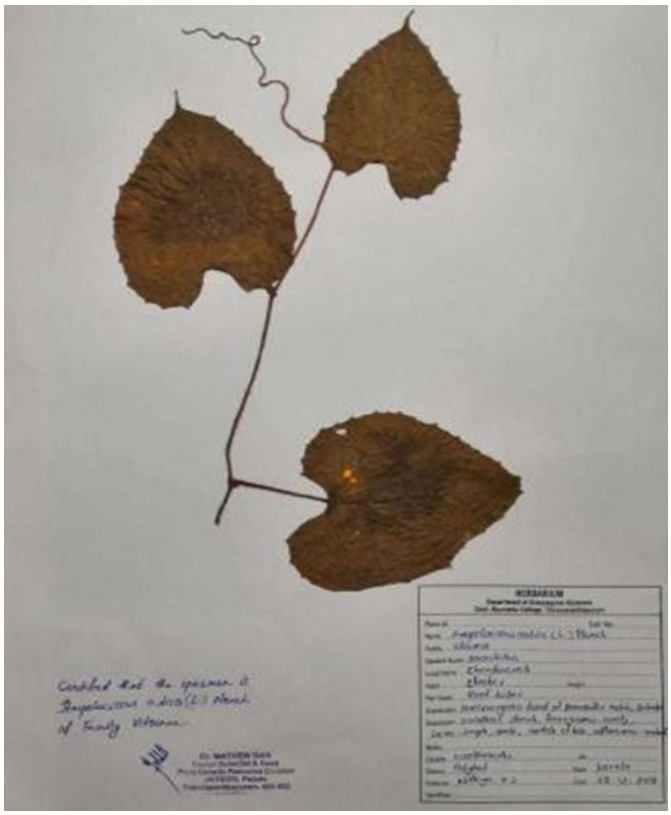

Fig. 2 Authenticated sample of *Ampelocissus indica* (L.) Planch

2.2 Preparation of decoction

Fresh root tuber of *A. indica* was cleaned and dried in shade. 48g of coarsely powdered root was boiled with 768ml water and reduced to 96ml as per Ayurveda pharmacopeia of India (Government of India ,2008).

2.3 Preparation of ethanolic extract

100g of coarsely powdered tubers of *A. indica* was taken in a conical flask and reflexed for one hour in 98% of 1000ml ethanol. The extract was filtered and evaporated on a rotary evaporator under reduced pressure to obtain the viscous residue and evaporated to dryness over a water bath.

2.4 Biological activity assays

2.4.1. Animals and preparation of test samples for bioassay

Female Wistar rats (150 ± 50g) were purchased from the Animal house of Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram and housed under standard condition (12h light / 12h dark cycle at 25± 2˚C) with free access to standard laboratory food and water. Animal welfare and experimental procedures were carried out strictly in accordance with the Guide for the Care and use of laboratory animals and approved by Institutional Animal Ethics Committee and CPCSEA committee (2015/GO/Re/S/18/CPCSEA 12/06/2018) in Government Ayurveda College Thiruvananthapuram.

Carrageenan purchased from Sigma Corporation (USA) was suspended in 1% saline. Antibodies for ELISA and FITC conjugated antibodies were purchased from Abcam, UK. ELISA plates were purchased from NUNC, Denmark.

2.5. Acute toxicity of *A. indica* as per OECD guidelines 425

Acute toxicity of *A. indica* was evaluated as per fixed single dose procedure (limit test) by the Organization for Economic Corporation and Development- OECD guidelines 425(O. E. C. D.2001). Five healthy nulliparous female Wistar albino rats weighing 150g to 200g were selected and deprived of feed overnight and 3 h after the administration of decoction. 2000 mg/kg of decoction was administered orally, and were observed individually for mortality and toxic symptoms at 10 min, 30 min, 1 h, 2 h, 4 h and 6 h and once daily thereafter for 14 days.

2.6. Establishment of carrageenan-induced hind paw edema model and drug administration

The rats were randomly divided into 6 groups (n=5), namely normal, Carrageenan control, indomethacin 5mg/ kg (standard drug), AI-8.64ml/kg (normal dose of *A.indica* decocotion), AI-4.32 ml/kg (half dose of decoction of *A. indica*) and AIE -500 mg/kg (ethanolic extract of *A. indica*). The decoction AI- 8.64ml/kg, AI-4.32ml/kg and the ethanolic extract AIE-500mg/kg, indometacin -5mg/kg, were administered orally an hour prior to the carrageenan injection. The rats in control group administrated by were distilled water. Carrageenan (0.1mL of 1% solution in normal saline) was injected subcutaneously into plantar fascia of the right hind paw. Measured paw volume at time points, i.e., 0, 1, 2, 3, 4 and 5hr after carrageenan challenge using a Plethysmometer (Misra *et al*, 2018). Blood samples were collected 5hour after induction of inflammation and centrifuged immediately at 3000rpm for 10min.The serum was transferred and stored at -20^0 C

2.7. Cotton pellet induced granuloma model and drug administration

Rats after overnight fasting, sterile cotton pellet (20 ± 1 mg) was implanted subcutaneously in the inter-scapular region and after the implantation the wound was closed by interrupted suture. The procedure was done under anesthesia using ketamine hydrochloride injection (40mg/kg i.p). Animals were given the test and standard drug orally once a daily for 7 consecutive days from the day of implantation. On the 8th day, the rats were sacrificed and wet cotton pellets with granuloma were dissected out. Then, they were dried in hot air oven at 60°C for 24 h and again the dry weight has determined (Misra *et al*, 2018). Blood was extracted via cardiac puncture and centrifuged at 3500 rpm for 15 minutes at 4℃. Serum was frozen at -17℃ until analyzed. The skin area around granulomatous tissue were taken out and stored in 10% neutral-buffered formaldehyde.

2.8. Analysis of IL-1β and TNF-α

TNF-α, IL-1β levels in serum were quantified using ELISA method (Engvall and Perlmann, 1971) and (0.5ml of o-dianisidine in methanol(1%) + 21ml of citrate phosphate buffer (PH5.0) + 2.4µl 30% H_2O_2) was used as a substrate and the absorbance of the colored HRP product was measured spectrophotometrically at 490nm by an automated microplate reader (Thermo Multiskan Spectrum).

2.9 Histopathological analysis

Skin tissue slices were embedded in paraffin and thin sections at 4mm thickness was made using a semi-automated microtome. The sections were stained with Hematoxylin and Eosin, and observed under light microscope to identify the inflammatory changes. Images were captured at 10 and 40 magnifications using DP71 digital camera system Histopathology was evaluated qualitatively with help of a qualified veterinary pathologist.

2.10 Statistical analyses

Comparison of paw volume at different time was done by repeated measure of ANOVA with Post hoc multiple comparison tests. Analysis of granuloma weight was by ANOVA and Tukey's multiple comparison. The TNFα and IL-1β were analyzed by One-way ANOVA.

3. Results and discussion

3.1 Acute toxicity study

Throughout the observation period neither incidence of mortality nor animals found in a moribund condition. Body weight gain and animal behavior were normal for 14days. Thus decoction of *A. indica* is safe up to the dose of 2000 mg/kg, in tested rats.

3.2 Carrageenan induced paw edema model

Three hours after carrageen injection, carrageenan control group showed marked paw odema, but the drug treated groups; indomethacin-5mg/kg,AI-8.64ml/kg, AI -4.32ml/kg andAIE-500mg/kg;demonstrated significant amelioration on edema comparing with the control group ($p<0.01$) as illustrated in Fig 3.The edema reduction continued up to five hours.

3.3 Cotton pellet induced granuloma model

There was significant reduction of weight of granuloma in indomethacine 5 mg/kg, AI8.64 ml/kg when compared with control group as illustrated in Fig 4. No significant reduction in AI. 4.32 ml/kg and AIE 500 mg/kg.

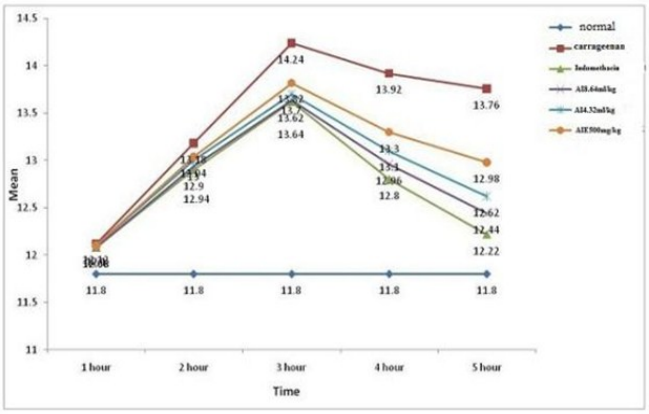

Fig.3. Line diagram of mean paw volume

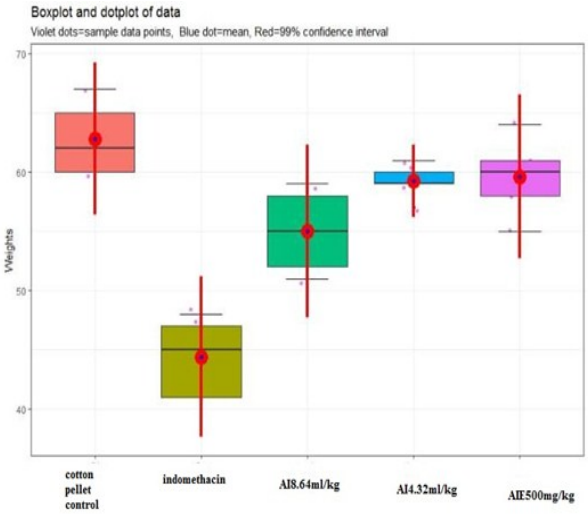

Fig. 4. Boxplot showing the assessment of mean weight of granuloma.

3.4 Analysis of IL-1β and TNF-α

In carrageenan induced paw edema the levels of IL-1β and TNFα in the treated groups; indomethacin 5mg/kg, AI-8.64ml/kg, AI-4.32ml/kg and AIE-500mg/kg group were significantly reduced in comparison to the control group ($p < 0.05$) depicted in Table 1. Highest reduction was found in group treated with indomethacin 5mg/kg and AI 8.64ml/kg.

In cotton pellet induced granuloma rats also the levels of IL-1β and TNFα in the treated groups;indomethacin 5mg/kg, AI-8.64ml/kg, AI-4.32ml/kg and AIE-500mg/kg showed significant ($p<.05$) reduction when compared with cotton pellet control group is shown in Table 1. Highest reduction was found in group treated with indomethacin-5mg/kg and AI-8.64ml/kg.

3.5 Histopathological analysis

Microscopic sections of skin as depicted in Plate 1, shows variable degree of sub acute inflammation often predominated by histiyocytes. Occasional giant cells were seen indicating a granulomatous reaction found in control group which was absent in treated groups. The extent and severity of lesions could not be graded.

In this study, we elucidated the anti-inflammatory potential of *A indica.* Inflammation was described as "the succession of changes which occurs in a living tissue when it is injured provided that the injury is not of such a degree as to at once destroy its structure and vitality" or "the reaction to injury of the living microcirculation and related tissues" (Punchard *et al*, 2004).

The carrageenan-induced paw edema in rats, with the advantage of high stability, convenience in operation, short time period and obvious manifestations, is a classical model to estimate the anti-inflammatory activity of natural products(Alqasoumi *et al*, 2012). Therefore, we applied this model to investigate the acute anti-inflammatory effect. Carrageenans are polysaccharides that induce inflammatory responses and clinical symptoms such as edema, erythema and hyperalgesia appeared immediately after the injection of carrageenan (Morris,2003). The development of these symptoms resulted from the action of pro-inflammatory agents such as bradykinin, histamine, complement and reactive oxygen, which can be generated *in-situ* at the site of insult or by infiltrating cells like neutrophils also inflammatory cytokines, such as IL-1β, IL-6, TNF-α and inflammatory proteins and

Table 1. Serum biochemical analysis

Group	Paw edema		Cotton pellet granuloma	
	IL-1β	TNF-α	IL-1β	TNF-α
Normal control	0.082±0.003	0.092±0.003	0.0871±0.003	0.0953±0.003
Carrageenan control & cotton pellet control	2.29±0.085[a]	1.66±0.061 [a]	0.2973±0.011[a]	0.3372±0.012 [a]
AI4.32ml/kg	1.24±0.046[a,b]	1.1275±0.042 [a,b]	0.2860±0.010[a,b]	0.3085±0.011 [a,b]
AI8.64ml/kg	1.21±0.045 [a,b]	0.9246±0.034 [a,b]	0.1999±0 .007[a, b]	0.2501±0.009 [a,b]
AIE 500mg/kg	1.57±0.0588 [a,b]	1.07±0.039 [a,b]	0.2870±0.010[a,b]	0.2993±0.011 [a,b]
Indomethacin 5mg/kg	0.747± 0.0278 [a,b]	0.719±0.026 [a,b]	0.1814±0.006[a,b]	0.2030±0.007 [a,b]

*Values expressed as the average of 6 values ± SEM in each group, a-The statistical difference compared with normal control at ($p<0.05$). b- Statistical difference compared with carrageenan control at ($p<0.05$).

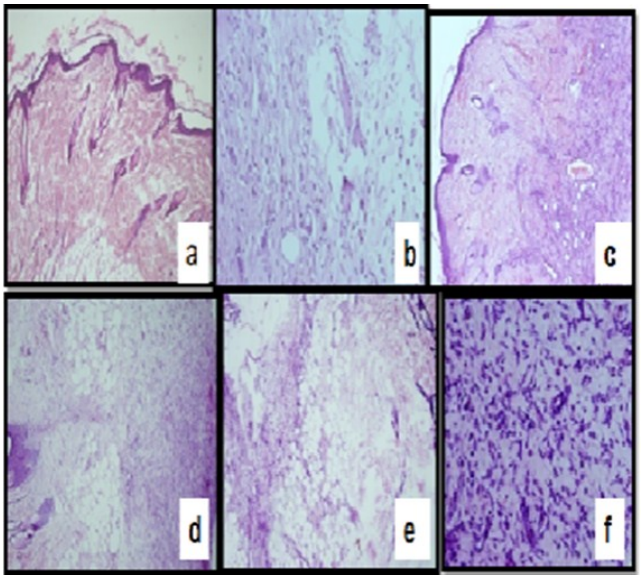

Plate. 1. Histopathological analysis of skin tissue around the granuloma. a- normal rat skin (magnification 10X), b- cotton pellet control (magnification 40X),c- indomethacin5mg/kg (magnification 10X), d- AI8.64ml/kg (magnification 40X), e- AI 4.32ml/kg (magnification 10X), f- AIE500mg/kg magnification 40X)

enzymes. These molecules can potentially serve as biomarkers for diseases diagnosis, prognosis and therapeutic decision making (Chen *et al*, 2017).

Carrageenan evokes biphasic edema, the first phase of which is mediated by the release of histamine and serotonin from mast cells and the second phase of which involves neutrophil infiltration release of prostaglandin E2, cytokines such as IL-1β, IL-6, IL-10 and TNF-α which can be attributed to the action of inducible cyclooxygenase (COX- 2)(Coura2015). The second phase is continued until 5 h after the induction of inflammation.

Indomethacine was the reference drug for carrageenan-induced paw edema model in this study. After 4-5 hours; all the drug treated groups produce significant reduction in paw volume ($p<.01$), when compared with carrageenan control group. Indomethacin 5mg/kg treated group and AI- 8.64ml/kg group showed high reduction in paw volume, also the AI-4.32ml/kg and AIE-500mg/kg group has significant reduction in paw volume when compared with control group. So based on the above reports, it can be inferred that the anti-inflammatory effect of *A indica* may be due to the inhibition of the enzyme cyclooxygenase leading to the inhibition of prostaglandin synthesis.

The cotton pellet granuloma method has been widely employed to access the transudative, exudative and proliferative components of subacute inflammation (Ashok *et al*, 2010). In this study the Indomethacin 5mg/kg group and AI-8.64ml/kg group elicited significant reduction on the weight of granuloma at $p<.01$, when compared with cotton pellet control group. AI-4.32ml/kg and AIE-500mg/kg were not elicited significant reduction on the weight of granuloma.

TNF-α and IL-1β are pro-inflammatory cytokines, involved in the interaction with various target cells as well as diverse immunological functions. These cytokines also mediate immunity and inflammation. Large amounts of TNF-α and IL-1β were released by macrophages (Park *et al*, 2018). The drug treated groups significantly reduced ($p<.05$) TNF-α and IL-1β level in rat serum when compared with control group. Indomethacin 5mg/kg and AI-8.64ml/kg treated group showed high reduction in serum TNF-α and IL-1β than AI-4.32ml/kg and AI-E500mg/kg even though they are significant when compared with the control group. Reduction of cytokine can be attributed to the anti-inflammatory activity.

Histopathological analysis was done on the skin tissue around the granuloma. All the groups showed signs of inflammation when compared with normal control. Occasional giant cells were seen indicating a granulomatous reaction found in control group which was absent in treated groups. The extent and severity of lesions could not be graded.

In acute oral toxicity test, no mortality recorded even at the highest dose level administered. This proves that drug is safe up to the dose 2000mg/kg.

Flavonoids are reported as antioxidants and scavengers of free radical (Alqasoumi *et al*, 2012). The antioxidant activity of *A.indica* is proven(Sasikumar2016).so the flavonoids present in the drug *A.indica* may be the main phytochemical responsible for the observed anti-inflammatory property of the drug.

4. Conclusion

The decoction and extract of *A indica* have anti-inflammatory effects and the probable mechanism is down regulation of cytokines such as IL-1β and TNF-α. The acute toxicity study proved that, the drug is safe up to the dose 2000mg/kg.

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