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Research Article

ANTI-INFLAMMATORY POTENTIAL OF *CASSIA ITALICA* (MILL) LAM. EX. FW. ANDREWS LEAVES

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ABSTRACT

The methanolic extract of the leaves of *Cassia italica* was investigated for its anti inflammatory activity in animal models. The extract at different doses used showed good anti inflammatory activity which has been done significantly, by the formation of oedema induced by carrageenan and formalin. These results were also comparable to Indomethacin, the reference drugs used in this study. The results from present study indicate the efficacy of the methanolic extract as a therapeutic agent in acute as well as chronic inflammatory conditions. Thus it could be concluded that *Cassia italica* leaves extracts possess significant anti-inflammatory properties.

Keywords: Anti-inflammation, *Cassia italica*, Indomethacin, Good anti inflammatory activity and therapeutic agent.

INTRODUCTION

Inflammation is fundamentally a protective response, ultimate goal of which is to get rid of the noxious things, but sometimes it may be potentially harmful and needs pharmacological treatment to control its symptoms[1]. Inflammation is the body's way of dealing with infections, maintaining a subtle balance between the beneficial effects of inflammation cascades to restrict the infection and potential for long-term tissue destruction [2,3] and involves a complex array of enzyme activation, mediator release, fluid extra vacations, cell migration, tissue breakdown and repair[4]. If not controlled, inflammation can lead to development of diseases such as chronic asthma, rheumatoid arthritis and rheumatoid bowel disease, etc [5,6,7]. Till date a very few anti-inflammatory drugs from herbal origin have been found and a number of plants from ethno-medicinal databases are under laboratory investigations across the world[8].

Inflammation is the protective mechanism of the local microcirculation to tissue injury which caused by physical trauma, noxious stimuli by chemical agents, heat, antigen-antibody reaction and microbial effect. The signs and symptoms of inflammation include redness, swelling, heat, pain and loss of function of the affected area[9]. Inflammation is the main process to remove the invaded microorganism and irritants from the affected part of the body and also it set for tissue repair. Various inflammatory mediators (amines such as histamine, serotonin and lipids such as prostaglandins and small peptides such as Kinins) involved in the triggering mechanism to cause inflammation and the specific chemical mediators vary with the type of inflammatory process[10,11]. Currently uses anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary [12]. Since time immemorial mankind believing plants are the drugs to cure various types of health problem. In Indian traditional system like Ayurveda, Siddha and Unani are predominantly based on the use of medicinal plants as a drug to cure various diseases. Moreover, herbal drugs are playing a major role in the world because of their safety, efficacy and cost effectiveness [13,14]. Even though, significantly trendy of several herbal drugs in general, they are still unacceptable treatment modalities for inflammatory diseases. The limiting factors that contribute to this eventuality are (i) lack of standardization of the herbal drugs; (ii) lack of identification of active ingredient(s)/ principle(s) (iii) lack of randomized controlled clinical trials (RCTs) and (iv) lack of toxicological evaluation [15]. A massive number of plants and formulations have been claimed to have anti-inflammatory activity.

Medicinal herbs have been used as a form of therapy for the relief of pain throughout history. The treatment of rheumatic disorder is an area in which the practitioners of traditional medicine enjoy patronage and success. Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy. Therefore, ascribable importance has been given globally to develop plant-based anti-inflammatory drugs effective against a variety of inflammatory disorders. The present review is aimed at compiling data based on reported works on promising phytochemicals from medicinal plants that have been tested in inflammatory models.

Plants in the Caesalpinaceae have significant economic values. Plants in the Leguminosae, especially in Caesalpinaceae family are increasingly being used not only as herbal remedies in complementary and alternative medicine, but also in conventional therapy in many parts of the world for many years, especially in Africa and India where they are widely distributed [16]. *Cassia* species have been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values. They are well known in folk medicine for their laxative and purgative uses [17,18,19]. Besides, they have been found to exhibit anti-inflammatory [20], the ethanolic extract of the whole plant parts of *Cassia italica* was investigated for bioactivities: namely anti-inflammatory, antipyretic, analgesic and prostaglandin released by rat peritoneal leucocytes, antineoplastic and antiviral [21]. *Cassia italica* leaves are used in the treatment of gas troubles and skin diseases.

MATERIALS AND METHODS

Plant material

Fresh leaves of the selected plant *C. italica* having medicinal value were collected from Sathiyanathapuram, Theni District, Tamilnadu, India. The plant materials were taxonomically identified and authenticated by the Botanical Survey of India.

Preparation of the plant extract

The plant was freshly collected and was shade dried until all the water molecules were evaporated (15 - 30 days). After drying the plant leaves were ground into fine powder using mechanical grinder and then transferred into airtight containers for future studies. The fine powder (of about 50g in 250ml of methanol) is then subjected to soxhlet apparatus for the extraction of pure form of the plant leaf extract. The extract was filtered and the filtrate was concentrated at 30ºC under reduced pressure in a rotary evaporator. The crude extract was then dissolved in 1ml of methanol and evaporated and used for further experiments.

Carrageenan induced paw edema

Carrageenan induced inflammation is a useful model to detect oral action of anti-inflammatory agents [22]. The development of edema in the paw of the rat after the injection of Carrageenan is due to release of histamine, serotonin and prostaglandin like substances [23].

Experimental design

Wistar albino rats weighed around 150 to 175gm were categorized into 6 experimental groups for this study.

Group - I: Normal (control) rats.

Group - II: Carrageenan (0.1ml of 1% Carrageenan) induced rats.

Group – III: Pretreated inflammation: extract (250mg/kg body wt/day) was administered to the rats one hour before the induction of carrageenan.

Group - IV: Pretreated Inflammation: extract (500mg/kg body wt/day) was administered to the rats one hour before the induction of carrageenan.

Group - V: Pretreated Inflammation: extract (750mg/kg body wt/day) was administered to the rats one hour before the induction of carrageenan.

Group - VI: Standard drug Indomethacin (10mg/kg body wt/day) was administered to the rats one hour before the induction of carrageenan.

Procedure [24]

The animals of Group-I were treated as control rats and the animals of Group-II were induced inflammation by subaponeurotic injection of 0.1ml of 1% w/v carrageenan in normal saline in the left hand paw. Animals of Group-III received 250mg extract/kg body weight of rats one hour before the carrageenan injection. Animals of Group-IV received 500mg extract /kg body weight of rats one hour before carrageenan injection. Animals of Group-V received 750mg extract/kg body weight of rats one hour before the carrageenan injection. Animals of Group-VI received 10mg of Indomethacin/kg body weight of rats one hour before the carrageenan injection. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark.

The paw volume was measured at 0, 1, 2, 3, 4 and 5hrs after carrageenan injection using Digital Plethysmometer. The difference between initial and subsequent readings gave the actual edema volume.

Formalin induced paw edema

Formalin induced paw edema is one of the most suitable test procedures to screen chronic anti-inflammatory agents as it closely resembled human arthritis [25]. The rat formalin test causes a local injury in the paw, which is used as a model for tonic pain [26]and localized inflammatory pain [27]. There are two phases of responses, while the stimulus during the early phase is a direct chemical stimulation on nociceptors that during the late phase, involves inflammation. Formalin induced pain is caused primarily by peripheral tissue inflammation [28]. A central sensitization of dorsal horn neuron occurs during inflammatory pain. Acute inflammation may last for relatively shorter duration, ranging from few minutes to few days. Exudation of fluid and plasma proteins, emigration of leukocytes and predominantly neutrophils, are characteristic changes [29].

Animals

Wister albino rats (150-180gm) were used in these experiments. They were housed in polypropylene cages under standard conditions of temperature (23±1°C), relative humidity (55±1%) and periodical exposure of 12hrs/light and 12hrs/dark cycle and fed with standard pellet and water.

Experimental design

The following 6 groups of animals were categorized and experimented during this study.

Group - I: Normal (control) rats.

Group - II: Formalin (0.1ml of 2% formalin) induced rats.

Group - III: Pretreated inflammation: extract (250mg/kg body wt/day) was administered to the rats one hour before the induction of formalin.

Group - IV: Pretreated Inflammation: extract (500mg/kg body wt/day) was administered to the rats one hour before the induction of formalin.

Group - V: Pretreated Inflammation: extract (750mg/kg body wt/day) was administered to the rats one hour before the induction of formalin.

Group - VI: Standard drug Indomethacin (10mg/kg body wt/day) was administered to the rats one hour before the induction of formalin.

Procedure [30]

The Wister rats weighing between 150-180gm were categorized into 6 groups and fasted for 24hours before starting the experiment. The animals of Group-I were treated as control rats and the animals of Group-II were induced inflammation by subaponeurotic injection of 0.1ml of 2% w/v formalin in normal saline in the right hind paw. Animals of Group-III received 250mg extract/kg body weight of rats one hour before the formalin injection. Animals of Group-IV received 500mg extract/kg body weight of rats one hour before the formalin injection. Animals of Group-V received 750mg extract/kg body weight of rats one hour before the formalin injection. Animals of Group-VI received 10mg of Indomethacin/kg body weight of rats one hour before the formalin injection. The thickness of the paw was measured using a vernier caliper before and after the injection of formalin and thereafter thickness of the paw was measured at every 30 minutes interval up to four hours. Percentage inhibition of paw thickness was calculated using the formula.

Increase in paw volume in control / treatment

$PC / PT = Pt - P_0$

% of inhibition = PC - PT X 100 / PC

Where, Pt = Paw volume at time't', P_0 = Initial paw volume, PC= Increase in paw volume of control group and PT = Increase paw volume of the treatment groups.

Statistical comparison

One way ANOVA (Tukey) was followed to compare treated Groups with normal control (Group- I) and negative control (Group-II). Data are expressed in Mean ± SE. (n = 6, animals in each group).

RESULTS

Inflammation is generally considered as a primary physiological defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. [31] defined that inflammation is a common phenomenon and a reaction of living tissues towards injury. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses [32]. Inflammation volume reaches its maximum, approximately 3hours post treatment after which it begins to decline**.** The knowledge of these mediators involved in different phases is important for interpreting mode of drug action [33].

The methanol leaf extract of C. italica was evaluated for Carrageenan and formalin induced paw oedema anti-inflammatory activity in experimental animal model and the results are summarized in Tables 1 & 2. Doses of 250, 500 and 750mg/kg of the extract were significant inhibited in different reaction time. The inhibition was observed during the different exposures. The reduction was statistically significant at ($p<0.05$). Where as compared to standard drug Indomethacin, it was purely dose dependent.

Table 1: Anti-inflammatory efficiency of methanol leaf extract of *C. italica* on carrageenan induced paw oedema

Treatment	Exposure schedule					
	At 0hr	After 1hr	After 2hrs	After 3hrs	After 4hrs	After 5 hrs
Control	1.139±0.180	1.139±0.180	1.139±0.180	1.139±0.180	1.139±0.180	1.139±0.180
Carrageenan	1.208±0.099	1.289±0.299	1.604±0.084	2.273±0.048	2.611±0.180	3.379±0.310
250mg/kg	1.352±0.114^{NS}	2.102±0.330*	2.102±0.330^{NS}	2.468±0.384^{NS}	2.435±0.103^{NS}	1.590±0.032***
500mg/kg	1.241±0.062^{NS}	1.509±0.097^{NS}	1.898±0.081^{NS}	2.222±0.182^{NS}	2.370±0.180^{NS}	2.083±0.173*
750mg/kg	1.121±0.123^{NS}	1.453±0.120^{NS}	2.482±0.103^{NS}	2.843±0.033^{NS}	1.250±0.067***	2.083±0.173*
Indomethacin	1.312±0.028	1.450±0.156^{NS}	0.981±0.014^{NS}	1.452±0.089***	1.3450.0341***	1.211±0.214***

Table 2: Anti-inflammatory efficiency of methanol leaf extract of *C. italica* on formalin induced paw oedema.

Treatment	Exposure schedule							
	At 0min	After 30min	After 60min	After 90min	After 120min	After 150min	After 180min	After 210min
Control	1.139±0.180	1.168±0.039	1.292±0.0.032	1.378±0.026	4.45±0	1.428±0.024	1.438±0.034	1.428±0.03
Formalin	1.208±0.099	4.75±0.29	5.10±0.34	5.32±0.67	5.47±0.59	5.57±0.57	5.89±0.67	6.24±0.64
250mg/kg	1.312±0.028	4.63±0.04	4.87±0.13	5.34±0.4	6.16±0.87	6.56±1.10	6.64±1.35	5.10±0.34
500mg/kg	1.352±0.114^{NS}	0.962±0.016*	1.044±0.015**	1.076±0.013***	6.25±0.41	1.032±0.018***	0.994±0.025***	0.908±0.030***
750mg/kg	1.241±0.062^{NS}	0.996±0.023	1.102±0.030	1.15±0.024*	6.42±1.62	1.128±0.030**	1.088±0.027***	1.028±0.027***
Indomethacin	1.121±0.123^{NS}	0.898±0.020**	0.968±0.022***	0.932±0.033***	5.61±0.65	0.926±0.028***	0.904±0.033***	0.852±0.018***

Values are representing in Mean±SEM of six animals One way ANOVA Tukey

NS- Non significant, *- significant at P<0.01, ***- significant at P<0.05.

DISCUSSION

Many plants are used as medicinal agents traditionally for various diseases. But it is important to study the scientific evidences for their medicinal value. In this study pharmacological evaluation of anti-inflammatory activities of methanolic extract of *C. italica* was carried out using different experimental models.

The results observed in the rat paw oedema assay showed a significant inhibitory activity of the tincture in carrageenan induced paw inflammation, for the administrated doses of 250, 500 and 750 mg/kg (Table 1&2). This test model basically reflects the action of prostaglandins involved in the inflammation process induced by carrageenan [34,35]. Oedema formation in paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or mediators that increase blood flow [36]. The development of oedema after the injection of carrageenan has been described as a biphasic event [23], the early phase, observed around 1h is related to the production of 5-hydroxytryptamin, histamine, bradykinin and cyclooxygenase products; and the late phase is due to neutrophyl infiltration, as well as to the continuing of the production of arachidonic acid metabolites [37,38]. Moreover, due to the role of reactive species in the inflammatory process [39] and recent reports about the relation between periodontal disease/stomatitis and impaired antioxidant status [40,41,42], the scavenging effects of the tincture on ROS (HOI, O2 I_, HOCl, ROOI and H2O2), and RNS (ONOO_ and INO) could be considered of great importance for the plant anti-inflammatory effect (Fig. 1).

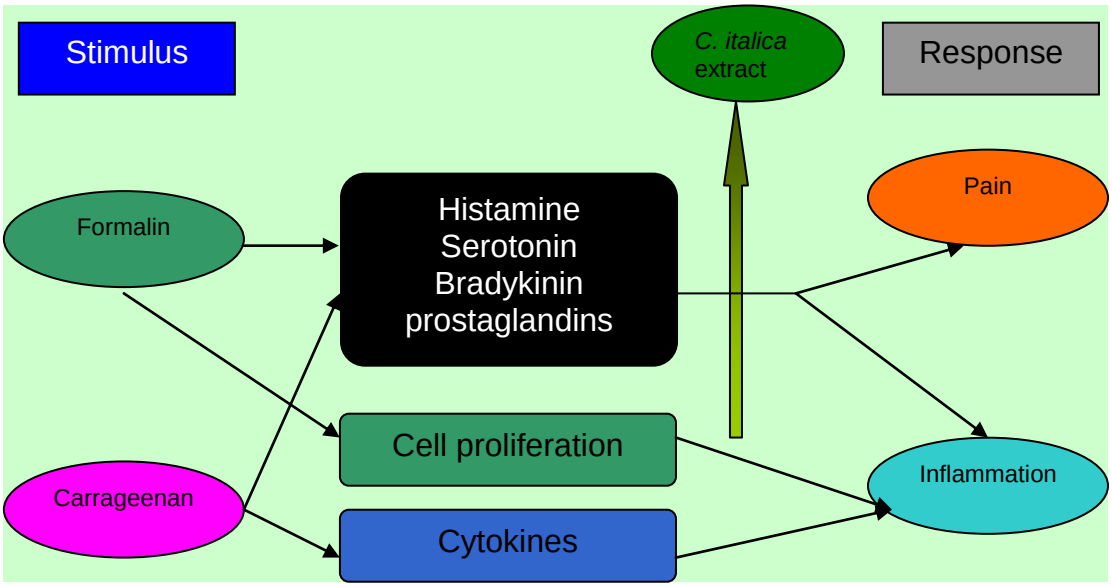

Fig. 1: Probable mechanism of the anti-inflammatory activity of the methanol leaf extract of *C. italica*.

It has been reported that second phase of oedema is sensitive to most clinically effective anti-inflammatory drugs, which has been frequently used to access the anti-oedematous effect of natural products [43]. Prostaglandin plays a major role in the development of second phase of reaction that is measured after 3hrs. These mediators take part in the inflammatory response and are able to stimulate nociceptor and thus induce pain [23,22].

Inflammation is a tissue reaction of infection and irritation caused by the pathogens and foreign substance respectively and thus it is an integral part of host defense mechanism. Inflammation has been recognized as a simple allergic reaction for many decades, as it is currently being considered to underlie patho-physiology of a much broader spectrum of diseases [44]. Inflammation is a complex process and Reactive oxygen species (ROS) play an important role in the pathogenesis of inflammatory diseases [45]. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can easily be induced [46]. The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses [47]. Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary.

Usually most anti-inflammatory and analgesic drugs possess antipyretic activity. In general, non steroidal anti-inflammatory drugs produce their antipyretic action through the inhibition of prostaglandin synthesis within the hypothalamus [48]. In Indian system of medicine, certain herbs are claimed to provide relief to pain and inflammation. The claimed therapeutic reputation has to be verified in a scientific manner. One such drug yielding plant, *C. italica* was taken for the present study. The methanol leaf extract of *C. italica* exerts anti-inflammatory effect on both the acute and chronic inflammations that are induced in rat models. Plants, which belonging to Caesalpinaceae family are rich in flavonoids and bioflavonoids are known for their anti-inflammatory activity.

The results observed in the rat paw oedema assay showed a significant inhibition against carrageenan and formalin induced paw inflammation, when the rats were administrated 250, 500 and 750mg of extract/kg body weight of the rat. Similar results were obtained in *Cassia fistula* [49,50], *Cassia occidentalis* [51] and *Buchanania lanzan* [52].

Carrageenan induced inflammation is a useful model for the estimation of orally active anti-inflammatory effects [33]. Carrageenan is the sulphated polysaccharide obtained from seaweed, which is widely used phylogistic agent, which shows signs and symptoms of inflammation that can be assessed as increase in paw thickness in mouse as a result of increased inflammation (oedema) and increased vascular permeation. The carrageenan induced paw edema model in rats is known to be sensitive to cyclooxygenase (COX) inhibitors [53].

Inflammation produced by Carrageenan is a triphasic response. In the first phase of inflammation, histamine, serotonin and other primary mediators are involved. They cause the oedema and redness. In the second phase, different cytokinase get released in response to the inflammation and the secreted mediators at the localized site. In the third phase, the cyclooxygenase (COX) enzyme plays pivotal role leading to the production of prostaglandins, which induces pain [54,46,47].

Flavonoids are known to target prostaglandin, which is involved in the late phase of inflammation [55]. Prostaglandins have two major actions: they are mediators of inflammation and they also sensitize nerve endings, lowering their threshold of response to stimuli of both mechanical and chemical, allowing the other mediators of inflammation, e.g. histamine, serotonin, bradykinin, to intensify the activation of the sensory endings [56]. Based on these reports, it can be inferred that the inhibitory effect of the extract of *C. italica* on carrageenin-induced inflammation in rats may be due to inhibition of these mediators responsible for inflammation.

The current study results have confirmed the weight lowering potential of *C. italica,* which at least could be partially attributed to the presence of tannins found in the plants. However, saponins are also known to inhibit growth rate [57,58]. Our results are in full agreement with earlier studies, where tannins were reported to be involved in growth regulations [59]. The tannins present in the extracts, used during this study, could potentially inhibit the activity of lipases found in mice, thereby lowering their body fat content. Previous studies using tannins from grape seed extract showed their potential hypolipidemic and anti-hypercholesteromic effects [60].

Phytochemicals such as flavonoids, steroids, glycosides, alkaloids, saponins and anthraquinones have been reported to exhibit both acute, chronic inflammatory and anti-pyretic activity in rats [61,62] and present study also reports the presence of these phytochemicals in the methanol leaf extract of *C. italica*. Similar result also concludes this work[63]. The present study shows that *C. italica* extract exhibits significant anti-inflammatory activity against these inflammatory models. As this plant contains phytochemical constituents such as, flavonoids, phenols, etc., it has received considerable attention in recent years due to diverse pharmacological properties that are also responsible for the anti-inflammatory activity. The presences of bioactive constituents indicated are responsible for the observed anti-inflammatory activity [64].

In conclusion, Pharmacognosic parameters could be useful to detect the authenticity of this medicinally useful plant. Furthermore, the methanolic extract of the leaves have potent anti-inflammatory activity.

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