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Original Article

Evaluation of Anti-Inflammatory and Anti Arthritic Activity for Different Extracts of Aerial Parts of *Cassia grandis linn.*

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ABSTRACT

This work has done for the investigation of anti-inflammatory activity and anti arthritic activity of the different extracts (methanolic,ethylacetate and hexane) of dried aerial parts of *Cassia grandis* linn. by oral administration at dose of 100,200 mg/kg/day of body weight to healthy animals. These three extracts were studied for their anti-inflammatory activity by using egg albumin induced paw edema and anti-arthritic activity by using formaldehyde induced arthritis in rats and the mean increase in paw volume and % inhibition in paw volume were measured by using vernier calipers at different time intervals after egg albumin (1%w/v) and formaldehyde (1%w/v) induced injection. In egg albumin induced paw edema model although all the drug treated groups showed a decrease in paw thickness as compared to the control, the difference was significant in hexane extract at a dose of 200mg/kg followed by ethyl acetate 200mg/kg and methanol extract 200mg/kg. In formaldehyde induced arthritis an increase in joint diameter was seen in all animals through out the observation period. The difference was significant in hexane extract at a dose of 200mg/kg followed by ethylacetate 200mg/kg and methanolic extract 200mg/kg.

keywords : Anti-inflammatory activity, Anti-arthritic activity, egg albumin, diclofenac, formaldehyde, *Cassia grandis*

1. INTRODUCTION

Inflammation is a local response of living mammalian tissues to injury. It is a body defence reaction in order to eliminate or limit the spread of injurious agent. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such components of inflammation. [1] Oedema formation in the paw is the result of a synergism between various inflammatory mediators that increase

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vascular permeability and/or the mediators that increase blood flow. [2] Several experimental models of paw oedema have been described. Carrageenan-induced paw oedema is widely used for determining the acute phase of inflammation. Histamine, 5-hydroxytryptamine and bradykinin are the first detectable mediators in the early phase of carrageenan-induced inflammation, [3] whereas prostaglandins are detectable in the late phase of inflammation. [4] A large number of Indian medicinal plants are attributed with various pharmacological activities because they contain a diversified class of phytochemicals. It is believed that current analgesia-inducing drugs such as opoids and non-steroidal anti-inflammatory drugs are not useful in all cases, because of their side-effects and potency. [5] As a result, a search for other alternatives seems necessary and beneficial. Medicinal plants having a wide variety of chemicals from which novel anti-inflammatory agents could be discovered. Scientific studies are required to judge their efficacy. Traditional and folklore medicines play an important role in health services around the globe. About three quarters of the world population relies on plants and plant extracts for healthcare. India has an extensive forest cover, enriched with plant diversity. Several plants have been used in folklore medicine. [6, 7] The rational design of novel drugs from traditional medicine offers new prospects in modern healthcare.Ayurveda the traditional medicinal system in India; describes certain plants which strengthen the host immune system.

Cassia grandis L. (Family: Leguminosae) is a deciduous or semi-deciduous spreading tree. It is well known as a Pink shower. [8] Several studies on the various parts of this plant have been reported as in-vitro antioxidant, purgative and in treatment of skin disorders etc. The pulp from the pods is very strong smelling with a bitter and astringent taste, which has laxative properties. It is sometimes used in veterinary practices also hence known as Horse Cassia. The juice from the pods is reported to strengthen the blood. The phytochemical studies revealed the presence of flavonoids, anthraquinones and sterols. [9-12]

2. MATERIALS AND METHODS

Collection of plant:

The Plant material of *Cassia grandis* were collected in,visakhapatnam(dist) andhrapradesh,India in the month of February 2016. The plant species was authenticated by Department of botany, Andhra university, Visakhapatnam.The vocher specimen (22210) were deposited in the herbarium, college of pharmaceutical sciences, Andhra university

EXTRACTION PROCESS

The collected leaves and stem bark were dried under shade and powdered. The powdered materials were carried out successive maceration using different solvents such as hexane, ethylacetate and methanol.

Procedure:

Plant material (crushed or cut small or moderately coarse powder)

⇓

Placed in a closed vessel

⇓

Allowed to stand for seven days shaking occasionally

⇓

Liquid strained off

⇓

Solid residue (marc) pressed (recover as much as occluded solution)

⇓

Strained and expressed (liquids mixed)

Toxicity studies:

The HECG,EACG and MECG Extracts were given at a dose of 100mg/kg, 200 mg/kg/day body weight per day an acute toxicity study relating to the determination of LD50 was performed.

Selection of animals:

Wistar albino rats of either sex weighing between 150-200 g were obtained from Teena Biolabs, Hyderabad, Andhra Pradesh, India. The animals were housed under standard environmental conditions (temperature of 25 $\pm$ 2^0c with an alternating 12h light-dark cycle and relative humidity of 50 ±15%), one week before the start and also during the experiment as per the rules and regulations of the Institutional Animal Ethics committee and of the Regulatory body of the government (Regd no.516 / PO / c / 01 / CPCSEA). They were fed with standard laboratory diet (supplied by Krish Scientists shoppe, Bengaluru) and water *ad libitum* during the experiment.

The rats were given doses orally with extracts at different dose levels 18 h and 2 h prior to the induction of egg albumin and formaldehyde subcutaneously (*SC*) into the subplantar tissue of the hind paw of each rat, 0.1 ml of 1% egg albumin and formaldehyde suspension.

Experimental Protocol

The drug effects were estimated by comparing the maximal oedema response during 6 hrs in the drug as extract treated group with that of vehicle treated group as control. Group I normal rats treated with Drug vehicle (5% NACMC) and served as normal control and Group II rats were treated with diclofenac 10mg/kg b.wt. All the doses were administered orally according to the body weight of the animals.

Aim

To evaluate the anti-inflammatory activity of different Cg extracts

Materials and Methods

Wistar albino rats of either sex (150-200 g, n=6), egg albumin and the vernier calipers were used for measuring paw thickness.

The Cg extracts at different doses were administered *p.o* in sodium CMC 18 h and 2 h prior to the induction of oedema

by egg albumin injection and monitored the oedema progression as described.

The extracts were administered orally in the following order

Group-I Received – negative control
Group-II Received – standard
Group-III Received – MECG-100mg
Group-IV Received – MECG-200mg
Group-V Received – EACG-100mg
Group-VI Received – EACG-200mg
Group-VII Received – HECG-100mg
Group- VIII Received – HECG-200mg

Pharamacological screening [13]

Egg albumin induced paw in rats

The paw edema method by winter et al, 1962[14] was used. Young male wistar albino adult rats were used. The acute inflammation of the hind paw was induced in each of the rats by injecting 0.1ml/kg body weight of fresh egg albumin into the sub plantar surface of the right hind paw. The paw volume is measured by using vernier calipers apparatus. The test groups animals received 100 and 200 mg/kg body weight, p.o., of Cg extracts 30 min before inducing inflammation with the injection of egg albumin. The negative control group received 1 ml/kg body weight, p.o.of 5% NaCMC. The paw volume of all groups were measured before and 1,2,3, and 4 h after induction of edema. Inflammation was assessed as the difference between the zero time volume of the treated paw and the volume at the various time after the administration of the phlogistic agent. The odema rate and inhibition was calculated by using following ratio.

Edema rate (ER)% = V_t-V_0/V_0

Inhibtion rate (IR)%= E_c-E_t/E_c

Where V_0 is the volume before egg albumin injection in ml; V_t is the volume day after egg albumin injection(ml); E_c is the odema ratio of control group; E_t is the odema rate of treated group.

Statistical analysis:

The statistical analysis of all the result was carried out using one-way ANOVA followed by Dunnette's multiple comparision test

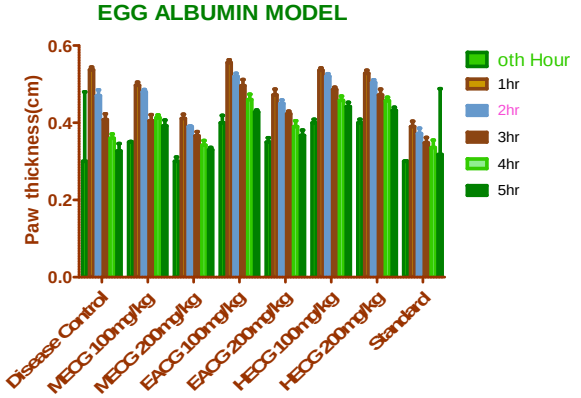

Fig 1: Egg albumin model

Table 1: Effect of *Cassia grandis* extracts on egg albumin induced paw edema in rats

Groups	DOSE (mg/kg) bodyweight	Edema rate in percentage				
		1h	2h	3h	4h	5h
Negative control	1 ml egg albumin	79.08±0.02	77.25±0.01	76.16±0.006	74.08±0.008	73.0±0.003
Standard (Diclofenac)	10mg/kg	30.29±0.013(61.6)*	24.13±0.012 (57.851)*	16.19±0.014 (55.2)*	12.10±0.04 (47.14)*	6.12±0.002(57.8)*
MECG	100mg/kg	42±0.01 (46.83)*	38±0.009(33.62)*	30±0.001 (17.03)*	18.0±0.006(22.01)*	12.0±0.05(33.3)*
MECG	200mg/kg	37±0.03 (53.16)*	30.5±0.04(46.72)*	22.00±0.01(39.15)*	14.0±0.005(39.1)*	8.0±0.25(11.11)*
EACG	100mg/kg	39±0.02 (60.63)*	32±0.09(43.89)*	24.00±0.04(33.62)*	15.00±0.05(35.00)*	7.0±0.002(22.22)*
EACG	200mg/kg	35±0.072(55.6)*	29±0.02(49.34)*	21.00±0.37(41.92)*	13.00±0.42(43.67)*	5.0±0.025(44.44)*
HECG	100mg/kg	34±0.02 (56.96)*	30±0.08(47.59)*	21.50±0.46(40.54)*	14.5±0.05(37.17)*	10.5±0.01(14.28)*
HECG	200mg/kg	32±0.34 (59.49)*	26±0.47(54.58)*	18±0.28(50.22)*	14.3±0.33(38.04)*	8.0±0.005(11.11)*

A values are expressed in Mean ± S.E.M. (n=6); values in parentheses indicate the percentage inhibiton rate. Difference between groups were statistically analyzed by one-way annova Dunnette's multiple comparision tests*P<0.05,P<0.01,***P<0.001 are considered significant compared to control**

Anti-arthritic activity

Arthritis is a chronic, systemic inflammatory disease predominantly affecting the joints and peri-articular tissues. Arthritis still remains a formidable disease, being capable of producing severe crippling deformities and functional disabilities. Arthritis is classified as an inflammatory arthritis, the disease comprises of 3 basic inter-related processes like inflammation, synovial proliferation and joint tissue destruction. Arthritis factor containing immune complexes found in the joints activate the pathological process. Tumour necrosis factor alpha (TNF-alpha) is the product of macrophages has been demonstrated to play an important role in the pathogenesis of Arthritis. Conventional treatments for arthritis, including Nonsteroidal Anti-inflammatory Drugs (NSAID's), disease modifying anti-rheumatoid drugs (DMARD's) and corticosteroids, aim to reduce the patient's pain and joint inflammation, minimize loss of function and decrease the progression of joint damage. However, such treatments are rarely totally effective and some pharmacological therapies have the potential to cause side effects. Even though several studies have been performed, still an efficient medicine is not found out. The mediators bind to specific receptors, causing gene transcription, and form complicated signaling interactions which contribute to the progression of inflammatory arthritis, e.g. leukocyte infiltration, cytokine networks formation, cartilage catabolism elevation and anabolism suppression. The onset of arthritis is rapid, typically developing 10-13 days after immunization with homologous or heterologous type II collagen, peaking at about days 15-20 and then gradually declining. The resulting polyarthritis is characterized by marked cartilage destruction associated with immune complex deposition on articular surfaces, bone resorption, periosteal proliferation and moderate to marked

synovitis and periarticular inflammation. Many immune cell populations, participate in the ongoing inflammatory process, suggesting the presence of multiple cellular targets for immunotherapy of arthritis. The progress has been made in understanding immune and inflammatory processes and hence these autoimmune changes are receiving increased attention in drug discovery and development. In the articular chondrocytes in the synovial joint, HIF- 1α promotes homeostatic pathways, and HIF-2α promotes degradative pathways that foster osteoarthritis. HIF-2α promotes chondrocyte hypertrophy, a terminal differentiation state characterized by a unique gene expression program. This switch to hypertrophy seems to be a relatively early signal to ignite and drive osteoarthritis in stressed cartilage. Therefore, we have employed a disease progression model using formaldehyde-induced arthritis in rats to provide an understanding of the relationship between target modulation and efficacy in the animal model. As part of this research, significant attention has been paid to the natural drug as these drugs elicit few side effects and are inexpensive. The objective of this study was to investigate the anti-arthritic effect and mechanisms of action Cg (*Cassia grandis)* and compare the mode of interactions existing, in the hunt of better therapies against arthritis and provide scientific evidence to folkloric claim of the plant using , in vitro pharmacological models.

Materials and Methods

Wistar albino rats of either sex (150-200 g, n=6), Formaldehyde and the vernier calipers were used for measuring paw thickness.

The Cg extracts at different doses were administered *p.o* in sodium CMC 18 h and 2 h prior to the induction of oedema by formaldehyde injection and monitored the oedema progression as described.

The extracts were administered orally in the following order

Group-I Received – negative control
Group-II Received – standard
Group-III Received – MECG-100mg
Group-IV Received – MECG-200mg
Group-V Received – EACG-100mg
Group-VI Received – EACG-200mg
Group-VII Received – HECG-100mg
Group- VIII Received – HECG-200mg

Formaldehyde induced arthritis:

The animals were divided into eight groups each of six animals each (n=6) and the baseline ankle joint diameter was measured by using vernier calipers on the day 0 of the experiment. Group 1 received the formaldehyde and served as the negative control group [15]. Group 2 received the standard drug Diclofenac sodium (10mg/kg, i.p). Group 3 and 4 received the methanolic extracts and group 5 and 6 received the ethyl acetate extracts in doses of 500mg/kg body weight p.o. and Group 7 and 8 received the hexane extracts of Cg in dose of 500mg/kg body weight p.o. 30 minutes after oral administration of vehicle/drugs, arthritis was induced by subplantar administration of 0.1 ml formaldehyde (2%v/v) in to the right hind paw of all the animals [16, 17]. This was designated as day1.vehicle/drug treatment was continued for duration of 9 more days. Formaldehyde (0.1ml 2% v/v) was again injected into the same paw on third day. The increase in paw thickness and paw volume was measured on days 0,2,4,6,8 and 10, 30 minutes after administration of the respective vehicle/drug treatment. The body weight changes were recorded every day weighing balance. Arthritis was assessed by measuring the mean increase in paw thickness and edema volume over a period of 10 day.

The percentage inhibition of right paw edema was calculated by the following formula.

% inhibition= $(V_c-V_t)\times100/V_c$

Where V_c = paw edema volume of control group

V_t = paw edema volume of the test group

Statistical analysis:

The results were expressed an Mean ± S.E.M. the data were analyzed by one way annova followed by Dunnette's multiple comparision tests. The level of significance was set as $p<0.05$. All statistical tests were carried out using prism 6.0 (graph pad, san diego CA, USA) statistical software.

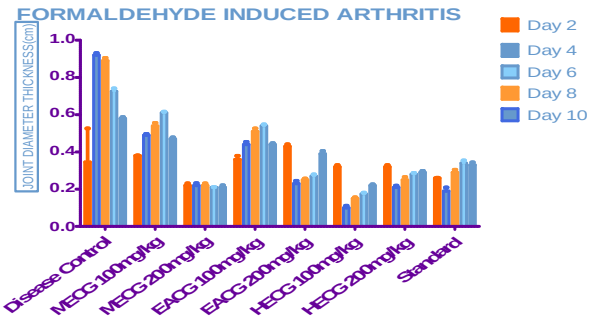

Fig 2: Formal dehyde induced arthiritis

Table 2: Mean changes in paw thickness and percent inhibition in Formaldehyde-induced arthritis in rats.

TREATMENTS	DOSE (mg/kg)	Paw thickness					%inhibition
		Day 2	Day 4	Day 6	Day 8	Day 10	
Normal control		0.11±0.06	0.30±0.14	0.34±0.16	0.25±0.09	0.17±0.09	74.87
Negative control	0.9% NACMC (1ml)	0.346±0.014	0.581±0.014	0.726±0.007	0.89±0.009	0.92±0.004	-
Standard (Diclofenac)	10mg/kg	0.268±0.012	0.334±0.015	0.34±0.012	0.29±0.011	0.19±0.016	85.61
MECG	100mg/kg	0.38±0.014*	0.47±0.015*	0.61±0.011*	0.54±0.007*	0.49±0.007*	62.84
MECG	200mg/kg	0.22±0.001*	0.21±0.28	0.21±0.28	0.22±0.27	0.22±0.27	68.64
EACG	100mg/kg	0.36±0.001	0.44±0.009	0.54±0.007	0.51±0.008	0.44±0.011	66.67
EACG	200mg/kg	0.43±0.019	0.39±0.0014	0.27±0.02	0.25±0.016	0.23±0.09	75.91
HECG	100mg/kg	0.32±0.18**	0.22±0.218**	0.174±0.02**	0.15±0.016**	0.10±0.25**	73.18
HECG	200mg/kg	0.32±0.01**	0.29±0.0014**	0.28±0.027**	0.25±0.016**	0.21±0.009**	80.91

Values are expressed in Mean±S.E.M (n=6) *p<0.05,p<0.01 are considered significant when compared to control**

3. RESULTS AND DISCUSSIONS

Sub-plantar injection of 1% egg albumin (0.1 ml) produced marked, sustained and time sustained and time related increase and decrease in the right hind paw odema of the control group. Paw swelling or edema was reached peak 1evel at 2nd hr after the injection of egg albumin and gradually decreased in the following hours.

In egg albumin induced paw oedema model an increase in paw thickness was seen in all animals throughout the observation period till 2 hrs. Although all drug treated groups showed a decrease in paw thickness as compared to the control, the difference was significant in CGHE Extracts at a dose of 200mg/kg followed by ethyl acetate 200mg/kg and methanolic extract. The order of activity is CGHE-200mg/kg>CGEA 200mg/kg>CGHE-100mg/kg>CGME-200mg/kg>CGEA-100mg/kg>CGME-100mg/kg. All the extracts at higher dose level showed reduction in joint swelling on 2nd hour. Egg white induced paw oedema is known to be mediated through the release of 5-hydroxytryptamine. Egg white, essentially a foreign albumin presented to the intercellular space, cause inflammation and oedema.

In formaldehyde induced arthritis an increase in joint diameter was seen in all animals throughout the observation period. Although all drug treated groups showed a decrease in joint swelling as compared to the control, the difference was significant in CGHE Extracts at a dose of 200mg/kg followed by ethyl acetate 200mg/kg and methanolic extract. The order of activity is CGHE200mg/kg>CGEA200mg/kg>CGHE100mg/kg>CGME200mg/kg>CGEA100mg/kg>CGME-100mg/kg.

All the extracts at higher dose level showed reduction in joint swelling on days 9 and 10. Formaldehyde induces arthritis by denaturing of proteins at the site of administration, which leads to the development of immunological reaction against the degraded products

The results suggested that the aerial parts of *Cassia grandis* possessing significant anti-inflammatory activity and Antiarthritic activity . The preliminary phytochemical examination suggested that the aerial parts possess flavonoids, phenols, tannins, sterols and cardiac glycosides.

Flavonoids are known to inhibit the enzyme prostaglandin synthase, more specifically the endoperoxidase and reported to produce anti-inflammatory effects. Thus we conclude that the crude extract of *cassia grandis* produces significant anti inflammatory activity.

It is therefore worth study further to isolate the pure molecules responsible for anti inflammatory activity and anti arthritic activity

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