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REGULAR ARTICLE

ROLE OF *CELOSIA POLYGONOIDS* JUSS. IN THE EXPERIMENTAL MODEL OF INFLAMMATION IN WISTAR RATS

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SUMMARY

Inflammation is a prevalent and debilitating disease that affects the human beings vigorously. The study was intended to evaluate the anti-inflammatory activity of whole plant of *Celosia polygonoids* (EECP). EECP constituting phytoconstituents to treat adjuvant induced inflammation rats to minimize the side effects. The anti-inflammatory activity study was carried out by using adjuvant induced model, cotton pellet, croton oil, formaldehyde, histamine and serotonin induced oedema of Wistar albino strain rats. EECP was injected at different doses such as 100 and 300 mg/kg/i.p., and the study was compared with standard drug Indomethacin (10 g/kg). The results obtained from the above methods were subjected to statistical analysis. The study was conducted paw volume for an each interval time and days carried out. The plant has various phytoconstituents such as reducing sugars, flavonoids saponins, starch, and steroids. Significantly one among those phytoconstituents lessens to oedema of the rodents. The EECP showed the maximum inhibitory activity at (300 mg/kg/i.p.) by dose dependent manner. These inhibitions were statistically significant ($p<0.01$). From these results indicate that EECP is a bioactive agent and having significant results in anti-inflammatory and action by inhibition of the exudation, and leukocytes recruitment into the inflamed tissues and bone regeneration calcium deposition.

Keywords: *Celosia polygonoids*; anti-inflammation.

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1. Introduction

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood through the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Ancient literature also mentions herbal medicines for various diseases, for which no scientific proof is available. In the present study of the inflammation may be acute, sub-acute and chronic inflammation. Inflammatory

response occurs in three distinct phases. The first phase is caused by an increased in vascular permeability resulting in exudation of fluids from the blood into the interstitial space, the second phase involves the infiltrations of leukocytes from the blood into the tissue and in third phase granuloma formation and tissue repair. Mediators of inflammation originate either from plasma (e.g. complement proteins. Kinins) or from cells (e.g. histamine, Prostaglandins, Cytokines). The production of active mediators is triggered by microbial products or by host proteins, such as proteins of the complement, kinins and coagulation systems that are themselves are activated by microbes and damaged tissues. Generally the mediators of inflammation are Histamine, Prostaglandins (PGs), Leukotrienes (LTB4), Nitric oxide (NO), Platelet-activation factor (PAF), Bradykinin, Serotonin, Lipoxins, Cytokines, Growth Factors. Large amount of experimental studies and detailed knowledge that arises of mediators of inflammation has been carried out. This paper address the commonly used animal model for the evaluation of anti-inflammatory activity in laboratory practice. It is also giving the principle and procedure behind using each animal model. This paper hopefully fills the expectation to provide the in vivo models in area of inflammation [1].

2. Materials and Methods

Plant Collection

The leaves of *Celosia polygonoids* which predominantly is a habitat in arid places were procured from Alampatti Pudhur, Manaparai district, Tamil Nadu during the month of December-January.

Authentication

The freshly collected plant was then authenticated by the botanist from Botanical Survey of India, Coimbatore, Tamil Nadu, India and a voucher specimen of the plant were submitted in our laboratory (Voucher No. BSI/SC/5/23/06-07/Tech.1260).

Garbling process

Garbling refers to the separation of that portion of the plant to be used from other parts of the plant, dirt and other extraneous matter. This step is often done during the collection process. Although there are machines that perform garbling, usually garbling is performed by hand. After removing all such unwanted adhered materials; the collected materials were then spread over trays and dried under shade, with regular sifting of collected plant materials everyday to avoid growth of fungus. Such shade dried bark/leaves/aerial portions of plant were ground in grinder to powder and then subjected for extraction [2].

Soxhlet extraction

The powdered bark of known quantity was taken in a soxhlet apparatus and extracted with absolute ethanol. The material was extracted continuously for 72 hours. The crude ethanol extract was then concentrated by distilling off the solvent under reduced pressure/vacuum and subjected for further studies [2].

Phytochemical screening

The methods of [3,4,5,6] were used to screen the chemical constituents the EECP. The presence of alkaloid (Dragendroff reagent and Mayer's reagent), flavonoids (Shinoda test), steroids (Liberman Burchard test) and terpenes (Vanillin–sulfuric acid reagent) were assessed.

In vivo study of Anti-inflammatory activity in EECP

Carrageenan induced oedema

Twenty four rats were equally divided into four groups assigned Latin numbers I–IV. Animals were fasted, with free access to water, 16 h before the experiment. Groups I was served as positive control group by inducing the 0.1 ml

of carrageenan dissolved in isotonic saline to the right hind paw, animals in group II were intraperitoneally treated with indomethacin as standard anti-inflammatory drug (10 mg/kg). While groups III and IV was received EECP in two doses; (100 and 300 mg/kg), respectively. Dosing volume was kept constant and was completed with saline when required. The choice of the used doses and time of measurement and sampling was based on pilot studies in our laboratory. Thirty minutes after oral treatment, group I received 0.05 ml saline, while groups II–IV received 0.05 ml carrageenan (1% solution in saline) on the plantar surface of the right hind paw. The right hind paw volume was measured immediately after carrageenan injection by water displacement using modified digital plethysmometer (Tokyo, Japan) [7]. The volume was measured again sequentially 1, 2, 3, 4 and 24th h after carrageenan injection and immediately. The results were expressed as percentage inhibition in relation to the control group. Then the paw volume was tabulated and found the average volumes and the percentage inhibition was calculated by mean paw volume.

Cotton pellet induced granuloma

Twenty four rats were taken equally divided into four groups assigned as I-IV. All groups were implanted the cotton pellet by the following procedure. Cotton pellets were weighing about 10±1 mg were autoclaved up to 20 minutes. Cotton pellets were aseptically implanted in the interscapular distance under the skin on the previously shaved back of the rats which were anesthetized with 25 mg/kg sodium pentobarbital intraperitoneally [8]. Inflammation induced animals were acclimatized. Animals belonging to the group I served as positive control with implanted cotton pellet without any treatment. Group II served as standard drug i.e., Indomethacin (10 mg/kg) and group III and IV served as EECP (100 and 300 mg/kg) respectively and treated the groups up to 7 days by intraperitoneally. After 7 days the animals were sacrificed and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60 0C. The pellets were weighed both moist and dry. Mean weight of the granuloma tissue was recorded. The weight of the pellets taken out from drug administered rats was compared with the weight of the pellets taken out from the control group and indomethacin administered rats was reported by earlier workers [9, 10, 11]. The percentage inhibition also calculated by the mean value of the cotton pellet granuloma.

Croton Oil Induced Ear Oedema

The experiment was performed using a slight modification of the procedure described by [12]. An irritant solution was prepared by dissolving 4 parts croton oil (the irritant) in a solvent mixture of 10 parts ethanol, 20 parts pyridine, and 66 parts ethyl ether. Croton oil irritant solution prepared was applied (0.1 ml) to the inner surface of the right ear of rats. Twenty four rats were equally arranged in four groups numbered I–IV. Irritant solution was injected to right ear disc of the all groups. Group I served and hence received only the irritant solvent mixture and known as positive control group i.e., induced croton oil in the right ear disc without any treatment and the left ear was kept untreated. Group II was served as standard drug i.e., indomethacin (10 mg/kg) by intraperitoneally. Group III and IV were served as EECP in two different concentrations employed; such 100 and 300mg/kg respectively [13]. One hour later, groups II–IV received croton oil solution after the administration of drug. After 4 h, animals were decapitated. An 8-mm cork borer was used to punch out discs from both the treated as well as the control ears. The punches were weighed immediately after decapitation and the difference in weight was used to assess the

inflammatory response. Results were expressed as milligram of tissue i.e., ear disc. Each ear disc was weighed and compared with control. Mean weight of the ear granuloma tissue was recorded. The weight of the ear granuloma taken out from drug administered rats was compared with the weight of the ear granuloma taken out from the control group and indomethacin i.e., standard drug was administered to rats reported by [14]. The percentage inhibition was calculated by the mean value of the ear discs weight taken from the study.

Formaldehyde Induced Oedema

The experiment was slightly varied from the carrageenan induced oedema method. Animals were used twenty four equally divided into four groups. The formaldehyde (2% v/v) solution, 0.02 ml, was injected in the first and third day into the left hind paw just beneath the plantar aponeurosis to induce inflammation. Group I served as the positive control as given the formaldehyde solution prepared by following method. Group II served as the standard drug administration with positive control. Group III and IV served as the EECP (100 and 300 mg/kg/i.p.). The standard drug (10 mg/kg/i.p.) and EECP at the dose of 100 & 300 mg/kg/i.p. administered, once a day, for 7 days. The paw volume was measured by using modified digital plethysmometer for 7 days continuously. The drug administered paw volume results were compared with standard and control group [15]. The percentage inhibition was calculated by the mean value of the paw volume taken from the study. The results were expressed as percentage inhibition in relation to the control group. Then the paw volume was tabulated and found the average volumes and the percentage inhibition was calculated by mean paw volume.

Histamine and Serotonin Induced Oedema

The procedure same as that like carrageenan-induced paw edema, only instead of carrageenan the rats are challenged by a subcutaneous injection of 0.1ml of 1% solution of histamine and serotonin individually into the sub-plantar side of the right hind paw. The paw volume was measured after the treatment and before the treatment. The animals were taken in a four groups and group I was served as positive control and given 0.1 ml of histamine and serotonin individually into right hind paw. Group II was served as standard drug i.e., indomethacin (10 mg/kg/i.p.). Group III and IV was served as EECP (100 and 300 mg/kg/i.p.) respectively. The drug administered paw volume results were compared with standard and control group. The percent inhibition of the inflammation is calculated using the formula and compared with control group.

Percentage inhibition

$$\text{Percentage inhibition} = \frac{Vci - Vt}{Vc} \times 100$$

Vc - Positive Control group Average Paw Volume

Vt - Drug Treated group Average Paw Volume

Statistical analysis

The experimental results were expressed as the mean±SD. Data were statistically evaluated by one way ANOVA followed by Duncan's multiple range post hoc test.

Table. 1. Phytochemical Analysis of EECP

S.NO	Chemical Constituents	EECP
1.	Alkaloids	+
2.	Amino Acids	-
3.	Anthraquinones	-
4.	Flavonoids	+
5.	Glycosides	+
6.	Proteins	-
7.	Reducing sugars	+
8.	Saponins	-
9.	Starch	+
10.	Steroids	+
11.	Tannins	-
12.	Terpenoids	-

Table. 2. Anti-inflammatory activity of EECP - Carrageenan induced method-Paw volume (in ml) and Percentage inhibition (in %)

Groups	1st Hour	2nd Hour	3rd Hour	4th Hour	24th Hour
Positive Control	1.53±0.06	1.66±0.05	1.77±0.03	1.82±0.02	1.93±0.03
Indomethacin (10 mg/kg)	1.06±0.11a (30.944)	0.94±0.06a (43.01)	0.87±0.04b (50.66)	0.78±0.05b,c (56.81)	0.49±0.01a (74.19)
EECP (100mg/kg)	1.20±0.04c,d (21.82)	1.17±0.05e,f (29.51)	1.12±0.05d (36.44)	1.07±0.08c (41.46)	1.02±0.07c (47.30)
EECP (300mg/kg)	1.27±0.03d,e (17.26)	1.22±0.03d (26.50)	1.12±0.04c (36.72)	0.98±0.05g (43.36)	0.79±0.03d,e (51.81)
F	17.939	41.736	58.541	89.307	199.602
df	6, 35	6, 35	6, 35	6, 35	6, 35
P Value	<0.001	<0.001	<0.001	<0.001	<0.001

Values are mean±SD. (N=6), c,d,e,fP<0.05, bP<0.01, aP<0.001, with respect to control. Ns, P>0.05 (Anova followed by new DMRT).

Table. 3. Anti-inflammatory activity of EECP- treatment in Cotton pellet , Croton oil, Histamine & Serotonin induced oedema method

Groups	Cotton pellet weight (in mg)	Ear Disc weight (in mg/kg)	Paw Volume (in ml)	Paw Volume (in ml)
Positive Control	39.66±1.84	16.15±1.26	1.52±0.08	1.31+0.04
Indomethacin (10 mg/kg)	19.79±2.01a (50.10)	5.53±0.89a (65.75)	0.38±0.02a (75.00)	0.34+0.02a (74.04)
EECP (100 mg/kg)	33.61±1.48c (15.25)	15.69±1.48c (2.84)	0.69±0.03b,c (54.6)	0.62+0.04b (52.67)
EECP (300 mg/kg)	31.53±1.22b (20.49)	12.97±1.45b (19.69)	0.67±0.02b (55.92)	0.56+0.03a (57.25)
F	71.551	43.681	278.708	244.481
df	6, 35	6, 35	6, 35	6, 35
P Value	< 0.001	< 0.001	<0.001	<0.001

Values are mean±SD. (N=6), c,dP<0.05, bP<0.01, aP<0.001, with respect to control. Ns, P>0.05 (Anova followed by new DMRT).

Table. 4. Anti-inflammatory activity of EECP- treatment in Formaldehyde induced oedema method

Groups	1st Day	2nd Day	3rd Day	4th Day	5th Day	6th Day	7th Day
Positive control	1.21±0.03	1.23±0.02	1.26±0.01	1.30±0.02	1.35±0.01	1.38±0.01	1.42±0.02
Indomethacin (10 mg/kg)	1.09±0.02	0.89±0.02b (27.64)	0.73±0.03a (42.06)	0.58±0.01a (55.38)	0.50±0.01 b (62.96)	0.38±0.007 a (72.46)	0.38±0.01a (72.46)
EECP (100 mg/kg)	1.20±0.01 (0.82)	1.06±0.03e (13.82)	0.97±0.01 (23.01)	0.84±0.01 d (35.38)	0.70±0.01 (48.14)	0.63±0.02 (54.34)	0.60±0.00d (57.74)
EECP (300 mg/kg)	1.18±0.01 (2.47)	1.04±0.02e (15.44)	0.94±0.01d (25.39)	0.82±0.02c ,d (36.92)	0.66±0.03 f (51.11)	0.58±0.008 g (57.97)	0.57±0.01c (59.85)
F	-	44.873	100.759	174.216	224.378	503.103	512.422
df	-	6, 35	6, 35	6, 35	6, 35	6, 35	6, 35
P Value	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

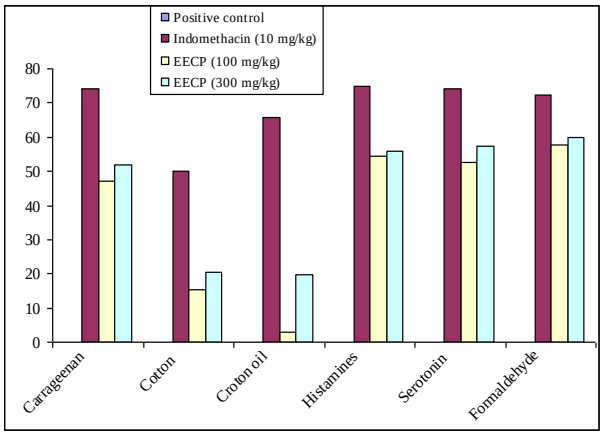
Values are mean±SD. (N=6), c,dP<0.05, bP<0.01, aP<0.001, with respect to control. Ns, P>0.05 (Anova followed by new DMRT).

3. Results and Discussion

Inflammatory diseases affect a significant portion of the population worldwide and have been intensely studied for several decades. Inflammation is protective and defense mechanism of the body. During inflammatory conditions various pathological changes are take place.

The EECP showed that anti-inflammatory activity at the dose of 100 and 300 mg/kg/i.p., when compared with positive control and standard drug indoemthacin (10 mg/kg/i.p.). In carrageenan induced method EECP showed (47.30%, 51.81%) inhibition at 24th hour, carrageenan early is an exudative phase of inflammatory pathology was reported by [16] that involved the action of vasoactive amines, such as histamine, serotonin, and kinins on vascular permeability [17, 18, 19].

Figure. 1. Comparative Evaluation of Anti-inflammatory study on EECP



Subcutaneous injection of carrageenan into the rat paw produces plasma extravasations and inflammation characterized by increased tissue water and plasma protein exudation with neutrophils extravasations and metabolism of arachidonic acid by both cyclooxygenase and lipoxygenase enzyme pathways [20]. It was observed by the increased paw volume under the digital plethysmometer in experimental rats in this study [21] suggested that the early hyperemia of carrageenan-induced oedema results from the release of histamine and serotonin. Thus Carrageenan-induced paw oedema in rats appears to be a biphasic events and the early phase (2.5–3 h) of the inflammation is due to the release of vasoactive amines such as histamine and serotonin. In the later phase (4.5–6 h) is due to the activation of kinin-like substances such as prostaglandins, proteases and lysosome [22]. The first phase showed that the EECP reduced the inflammation by control the proliferation of the histamine and serotonin and in second phase controlled the stimulation of kinin like substances. [23] Was reported that leukocyte adhesion represents one of the first steps in the inflammatory response initiation and it is essential for accumulation of active immune cells at sites of inflammation.

The extracts reduced the paw volume in which first phase the EECP showed the (36.44% & 36.72%) inhibition as that the histamine and serotonin like substances reduced. In the second phase the EECP showed that the (47.30% & 51.81%) inhibition, those results showed that the reduced kinin like substances level. In cotton pellet induced method (15.25%, 20.49%) inhibition on 7th day observation. From the above study we can be concluded that the EECP shows the anti-inflammatory activity and the inhibitory effect is dose dependent. In croton oil induced method (2.84 % & 19.69%) inhibition after 7 hours, at the doses of 100 & 300 mg/kg/i.p., respectively.

In the third method ear oedema the most important mediators are involved as prostaglandins, histamine and serotonin, whereas the lypoxygenase pathway has no important role [24]. The method has certain advantages for natural product testing [25]. But

peripherally acting drugs such as aspirin, indomethacin and dexamethasone only inhibit the later phase [26]. So, the herbal treatment is very suitable to this ear oedema method. Also the EECP showed that the anti-inflammatory activity by reduced ear disc weight. In the formaldehyde induced method the EECP showed that paw volume on 7th day (57.74% & 59.85%) inhibition, which showed that the extract reduced the inflammation as much as so.

In the histamine and serotonin method, this reduced the paw volume (54.6% & 55.92%) and (52.67% & 57.25%) inhibition at 100 & 300 mg/kg/i.p., respectively. Statistical analysis showed that the oedema inhibitions of preparations containing extract is significantly different from the control group at all the concentrations tested and the activity is dose-dependent i.e., 300 mg/kg/i.p.,. These methods revealed that the elevated percentage inhibition of EECP. Although direct evidence of the mechanism of action of extract is not clear. Flavonoids exhibit anti-inflammatory activity was reported [27]. Those flavonoids having deliberately reduced the mediators on inflamed area.

According to this, flavonoids presented in leaves extract activate the immune cells to control the inflamed area. Those results showed that the carrageenan induced inflammation showed that reduced inflammation when compared to other inflammatory models. From these results indicated that EECP is a bioactive agent and having significant results in anti-inflammatory activity by inhibition of the exudation, and leukocytes recruitment into the inflamed tissues.

From the evaluation the EECP has confirmed that having the pharmacological action against the inflammatory condition and it can be used further molecular compound analysis to enumerate the chemical to action.

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