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Comparative evaluation of anti-inflammatory activities of three Indian medicinal plants (*Alstonia scholaris* Linn, *Swertia chirata, Swietenia macrophylla* Linn.)

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Abstract
Inflammation is a defensive reaction of the body against infections and injuries. Acute inflammation can be conventionally described as a vascular and cellular event. Chronic inflammation causes tissue destruction brought by activated macrophages by release of variety of biological active substances. Inflammation plays a major role in most chronic illnesses, including neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease, though relatively little knowledge about their mode of action is available. There is a growing interest in pharmacological evaluation of various plants used in Indian traditional system of medicine. Carrageenan, from the Irish word "carraigin" meaning Irish moss, refers not only to a species of red alga chondrus crisous found along rocky areas of the Atlantic coast of the British Isles, Europe, and North America, but also refers to its mucopolysaccharides extracts, discovered by the British pharmacist Stanford in 1862. In the present study, the anti-inflammatory activities of three Indian medicinal plants were investigated with reference to standard drugs indomethacin. Both the aqueous and ethanolic extract significantly reduced carrageenan induced acute inflammation in animal models. The ethanolic extracts were more potent than the aqueous extracts. Of the three plants *Swietenia macrophylla* (both aqueous and ethanolic extracts) showed maximum protection followed by Swertia chirata. Although active inflammatory compound have already been reported from these plants, the study emphasizes on the comparative assessment of anti-inflammatory activities of these three plants with reference to standard drug indomethacin.

Keywords: Inflammation, three plants (*Alstonia scholaris*, *Swietenia macrophylla* and *Swertia chirata*.), aqueous and ethanolic extracts

Introduction
Inflammation is a defensive reaction of the body against infections and injuries. Edema formation, leukocyte infiltration and granuloma formation represent typical features of inflammation. Non-steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs, and immune-suppressant drugs, which have been usually used in the relief of inflammatory diseases worldwide for a long time, are often associated with severe adverse side effect, such as gastrointestinal bleeding and peptic ulcer. Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defense reaction to eliminate or limit the spread of injurious agent as well as necrosed cells. Acute inflammation can be conventionally described as a vascular and cellular event. In vascular events, alterations include hemodynamic changes such as transient vasoconstriction, persistent progressive vasodilatation, followed by local hydrostatic pressure, stasis, lucocytes migration and vascular changes in which accumulation of edema fluid. In cellular events, phagocytosis, that is engulfment of solid particulate material by cells, causes the inflammation. Chronic inflammation causes tissue destruction brought by activated macrophages by release of variety of biological active substances. It would appear that the extracts had a suppressive effect on these events. Inflammation plays a major role in most chronic illnesses, including neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease, though relatively little knowledge about their mode of action is available. There is a growing interest in pharmacological evaluation of various plants used in Indian traditional system of medicine. Carrageenan, from the Irish word "carraigin" meaning Irish moss, refers not only to a species of red alga chondrus crisous found along rocky areas of the Atlantic coast of the British Isles, Europe, and North America, but also

refers to its mucopolysaccharides extracts, discovered by the British pharmacist Stanford in 1862. The name was later changed to carrageenan so as to comply with the "an" suffix for polysaccharides. Structurally, the carrageenans are a complex group of polysaccharides made up of repeating galactose- related monomers and are of three main types; lambda, kappa, and iota. Each has their own gel characteristics which are all thermally reversible. The lambda from does not gel strongly at room temperature and is injectable to induce an inflammatory response. Inflammatory induced by carrageenan is acute, nonimmune, well-researched, and highly reproducible. Cardinal signs of inflammation are edema, hyperalgesia, and erythema developed immediately following subcutaneous injection, resulting from action of proinflammatory agents like bradykinin, histamine, tachykinins, complement and reactive oxygen, andf nitrogen species. Such agents can be generated in situ at the site of insult or by infiltrating cells. Neutrophils readily migrate to sites of inflammation and can generate proinflammatory reactive oxygen and other species. The inflammatory response is usually quantified by increase in paw size (edema) which is maximal around 5 hours postcarrageenan injection. The development of edema in the paw of the rat after the injection of carrageenan is due to release of histadin, serotonin and prostaglandin like substances. According to an estimate made by the WHO around 80% of the world's population in developing countries rely on traditional plant medicines for their primary health care needs, of which a major portion involves the use of plant extracts or their active. Recently many natural medicines divided from plants, marine organism, etc. were considered effective and safer for the treatment of various diseases including inflammation activity of niloticane isolated from the bark of A. nilotica. Moreover, the inhibitory effect of a single dose (500 mg/kg) of A. nilotica extract on carrageenan induced paw edema has also been mentioned. In t5he present study, inhibitory effects of the aqueous extracts of A. nilotica pods were investigated in more details using acute and chronic inflammation models namely xylene induced ear edema, carrageenan-induced paw edema and cotton pellet-induced granuloma.

Materials and Methods

Table 1: Groups in the present of study

Group I (control)	Carrageenan treated
Group II (negative control)	Saline treated
Group III	Indomethacin (standard drug)
Group IV	Carrageenan + Aqueous alstonia scholaris treated
Group V	Carrageenan + Aqueous Swertia chirata treated
Group VI	Carrageenan + Aqueous Sweitenia macrophylla
Group VII	Carrageenan + Ethanolic alstonia scholaris
Group VIII	Carrageenan + Ethanolic Swertia chirata
Group IX	Carrageenan + Ethanolic Sweitenia Macroplylla

Materials

- **Chemicals**

Indomethacin (Signal, Aldrich), lambda carrageenan (sigma chemical Co.), sodium chloride (Merck), ethanol (Merck) will be purchased from Chaulia chemicals, Medinipur (enlisted supplier).

- **Collection of plant materials**

Dried plant parts of *Alstonia scholaris Linn, Swertia Chirata, swetenia macroplylla Linn* were obtained commercially from M/s. united chemicals & Allied products, Calcutta, India. Tha plants were identified and authenticated by the dept. of Botany, Vidyasagar University.

- **Animals**

Males swiss albiono mice (20±2 g) were obtained commercially from enlisted supplier of Vidyasagar University and maintained in standard laboratory conditions. They were given standard laboratory diet and water *ad* libitum. All animal experiments are approved by the University Animal Ethics Committee, dept. of Physiology with community health, Vidyasagar University, Paschim Medinipur, India and were in accordance with the guidelines of the committee for the purpose of control and supervision of experiments on Animal (CPCSEA), Government of India.

- **Aqueous extract**

Plant parts were collected, washed thoroughly and dried in shade. It was then crushed and taken in a round-bottomed flask with distilled water and refluxed in a water bath for 1 hour at 90-95 degree centigrade. The supernatant obtained were combined and filtered through a whatman No. 1 filter paper. The filtrate was concentrated at low temperature by lyophilization. The residue obtained was designated as aqueous extract.

- **Ethanol extract**

The plant parts of *Alstonia scholaris Linn, Swertia chirata, Swetenia macrophylla Linn* A fine powder with a mechanical grinder. The powder plant material (300g) will be soaked in 80% ethanol and allowed to stand for 3 days. The extract will be concentrated to dryness and stored at -4 degree centigrade until use.

- **Toxicity study**

24 mice were divided into six groups of four animals each. One group served as a control and receive 0.9% Nacl alone (10 ml/kg) given intraperitoneally (*i.p.*), while the remaining groups were treated with increasing doses of the aqueous extract 100mg?kg, 500mg?kg and 100 mg/kg (*i.p.*), respectively. The mortality rate within a 24 hours period will be determined according to the method described by Miller and Tainter.

- **Acute inflammation**

Carrageenan- induced rat paw edema is used widely as a working model of inflammation in the search for new anti-inflammatory drug. The anti-inflammatory activity of the aqueous and ethanolic extracts of Alstonia scholaris Linn (bark), Swertia chirata (stem), Swetenia macroplylla Linn (seed) was evaluated by carrageenan induced rat paw edema method.

Swiss albino mice (20±2 g) obtained from commercial suppliers were used anti inflammatory activity was measured using carrageenan induced rat paw edema assay. The rats were divided into 9 groups of 5 animals each (plant extracts were dissolved in sterial distilled water and administrated intra peritoneally at different dose levels). Group I was treated with carrageenan (1% w/v) in saline. Rats of group II were given normal saline and treated as negative control. Rates in group III were administrated indomethacin (10mg/kg bw) and considered as standard. Rats from group IV to group IX were given aqueous and ethanolic extracts of the plants. Since the acute toxicity of the plant extracts were estimated by the trial

and error method. Anti- inflammatory activity will be measured as the percentage reduction in edema level when drug was present, relatives to control. Acute paw edema was induced by injecting 0.1 ml of 1% (w/v) carrageenan solution, prepared in normal. After 1 hour, 0.1 ml, 1% carrageenan suspension in 0.9% Nacl solution were injected into the sub-plantar tissue tissue of the right hind paw. The linear paw circumference will be measured at hourly interval for 3 h. The perimeter of paw was measured by using screw gauge. Measurements were taken at 0, 1, 2, 3 and 4 hours after the administration of the carrageenan.

The anti-inflammatory activity was calculated by using the relation

$$\% \text{ inhibition of edema} = \frac{C\text{-}T * 100}{C}$$

C- Perimeter of control paw edema

- **T-perimeter of test paw edema**

Used by planichamy (1990) were C and T denotes increase in paw diameter of control and drug treated animals. The results are expressed as percentage inhibition.

- **Statistical analysis**

Results of anti-inflammatory activity were expressed as mean increase in paw diameter ± SD. The significance of difference between means was determined by student's t test and values of $p< 0.05$ are considered significant.

Result and discussion

Table 2: studies on acute toxicity

N=5 (aqueous extracts)	Alstonia scholaris		Swertia chirata		Sweitenia macrophylla	
	Live	Dead	Live	Dead	Live	Dead
100 mg/kg bw	5	0	5	0	5	0
500 mg/kg bw	5	0	5	0	3	2
1000 mg/kg bw	5	0	5	0	5	0

Table 3: Anti-inflammatory activities of aqueous extracts of Alstonia scholaris, Swertia chirata and Sweitenia, macrophylla

N= 5 (in mm)	0 hours	30 min	1 hour	2hours	3 hours	4 hours
Carrageenan (control)	2.01±0.07	3.34±0.06	3.9±0.03	3.99±0.09	3.95±0.95	3.9±0.02
Carrageenan + indomethacin (10mg/kg)	2.05±0.05	3.0±0.05	2.96±0.07	2.25±0.04	2.2±0.06	2.03±0.7**
Carrageenan + alstonia scholaris (100mg/kg *bw*)	2.03±0.01	3.28±0.05	2.7±0.04	2.65±0.03	2.49±0.03	2.12±0.01**
Carrageenan + swertia chirata (100mg/kg *bw*)	2.05±0.02	3.25±0.02	3.28±0.07	2.72±0.01	2.45±0.03	2.1±0.06**
Carrageenan+ *Swietenia macrophylla*(100 mg/kg *bw*)	1.99±0.02	2.65±0.08	2.55±0.07	2.15±0.02	2.15±0.08	2.12±0.02**

All values are expected as Mean SD.** $p< 0.05$ compared control.

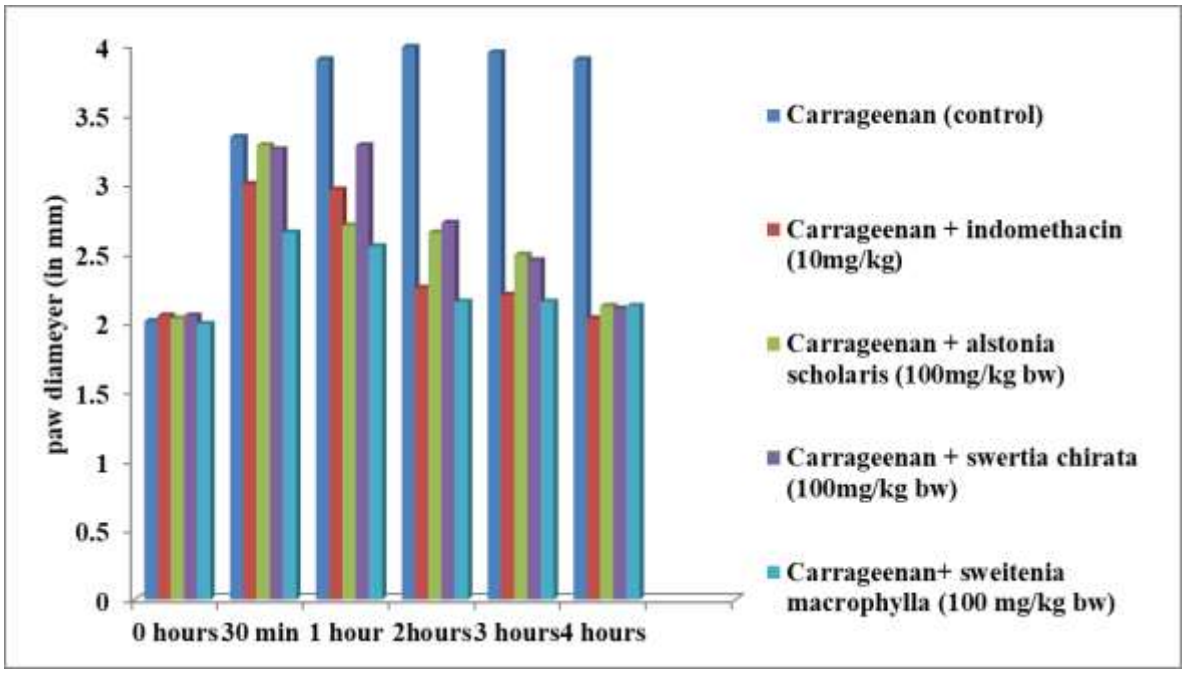

Fig 1: Anti-inflammatory activities of aqueous extracts of *Alstonia scholaris*, *Swertia chirata* and *Swietenia, macrophylla*

Table 4: Percentage inhibition of inflammation of aqueous extracts of *Alstonia scholaris*, *Swertia chirata* and *Swietenia macrophylla.*

% Inhibition	30 min	1 hour	2 hours	3hours	4 hours
Carrageenan + alstonia scholaris (100mg/kg *bw*)	6.28	17.9	24.5	24.3	24.35
Carrageenan + swertia chirata (100mg/kg *bw*)	3.29	22.3	33.33	37.97	40.5
Carrageenan+ *Swietenia macrophylla*(100 mg/kg *bw*)	4.19	27.94	38.59	43.54	45.64
Carrageenan + indomethacin (10mg/kg)	11.3	24.1	43.6	44.3	47.94

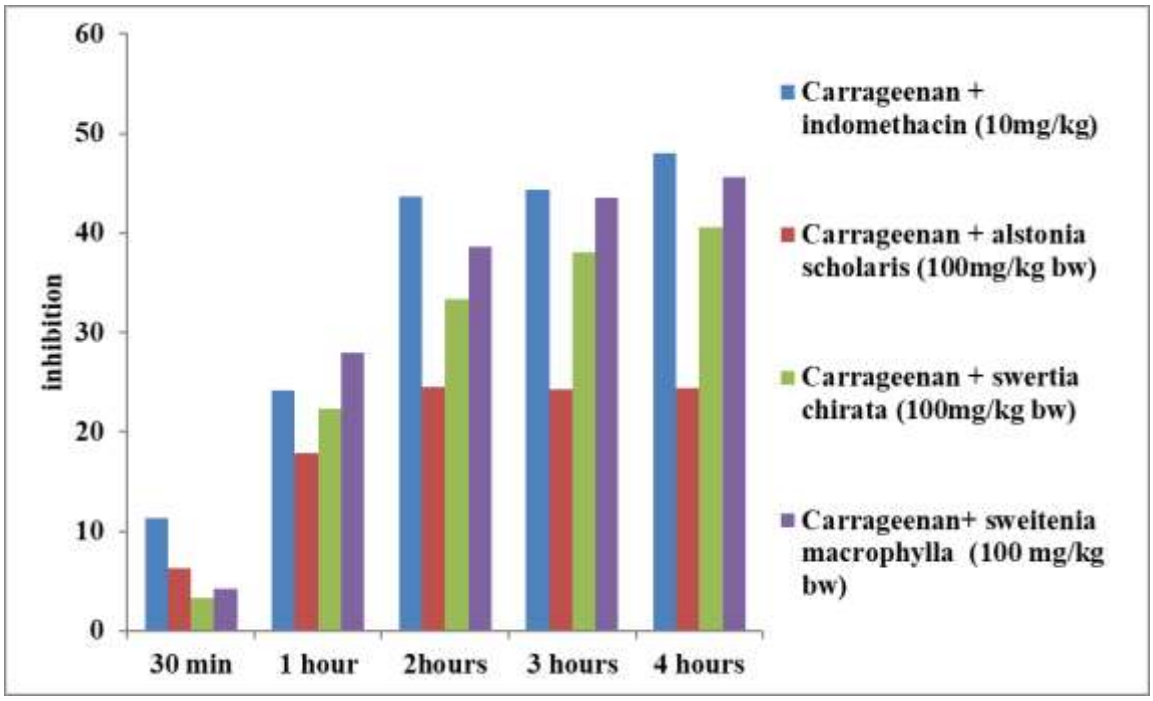

Fig 2: percentage inhibition of inflammation of aqueous extracts of *Alstonia scholaris*, *Swertia chirata* and *Swietenia macroplylla*

Table 5: Anti-inflammatory activities of ethanolic extracts of *Alstonia scholaris*, *Swertia chirata* and *Swietenia*, *macrophylla*.

N= 5 (in mm)	0 hours	30 min	1 hour	2hours	3 hours	4 hours
Carrageenan (control)	2.01±0.07	3.34±0.06	3.9±0.03	3.99±0.09	3.95±0.95	3.9±0.02
Carrageenan + indomethacin (10mg/kg)	2.05±0.05	3.0±0.05	2.96±0.07	2.25±0.04	2.2±0.06	2.03±0.7**
Carrageenan + alstonia scholaris (100mg/kg *bw*)	2.03±0.01	3.28±0.05	2.7±0.04	2.65±0.03	2.49±0.03	2.12±0.01**
Carrageenan + swertia chirata (100mg/kg *bw*)	2.05±0.02	3.25±0.02	3.28±0.07	2.72±0.01	2.45±0.03	2.1±0.06**
Carrageenan+ *Swietenia macrophylla*(100 mg/kg *bw*)	1.99±0.02	2.65±0.08	2.55±0.07	2.15±0.02	2.15±0.08	2.12±0.02**

All values are expected as Mean SD.** $p< 0.05$ compared control.

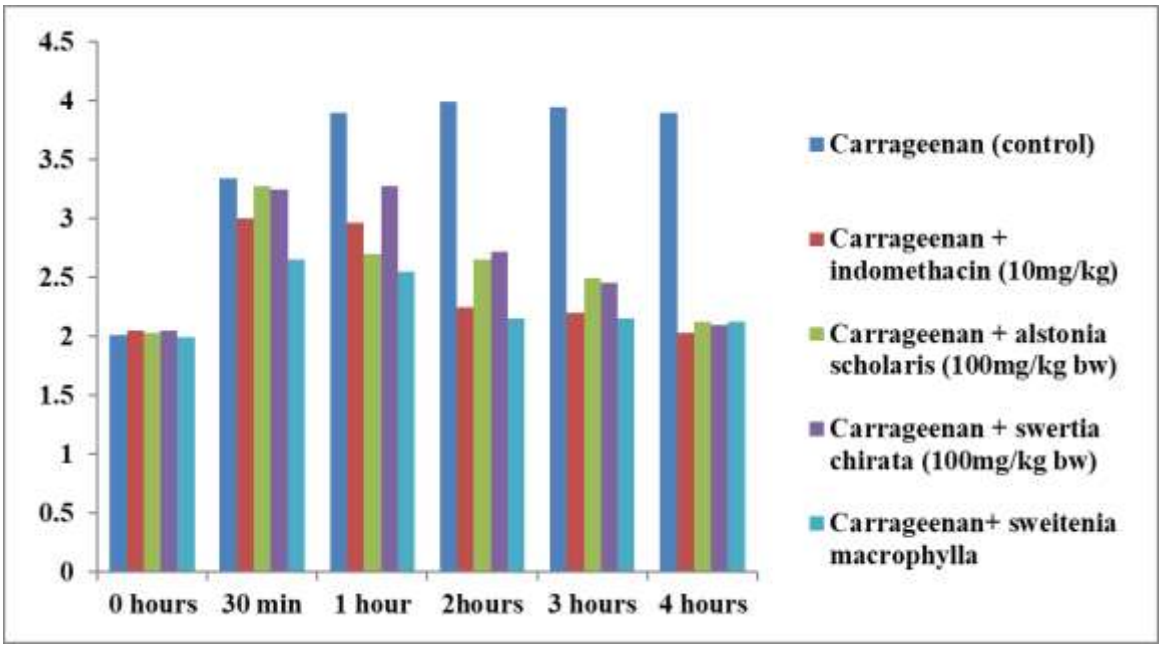

Fig 3: Anti-inflammatory activities of ethanol extracts of *Alstonia scholaris*, *Swertia chirata* and *Swietenia macrophylla*

Table 6: percentage inhibition of inflammation of ethanolic extracts of *Alstonia scholaris*, *Swertia chirata* and *Swietenia macroplylla*.

% Inhibition	30 min	1 hour	2 hours	3 hours	4 hours
Carrageenan + alstonia scholaris (100mg/kg *bw*)	1.8	30.7	33.5	36.9	45.6
Carrageenan + Swertia chirata (100mg/kg *bw*)	2.69	13.3	31.8	37.9	46.15
Carrageenan+ *Swietenia macrophylla*(100 mg/kg *bw*)	2.69	34.6	46.1	45.5	45.6
Carrageenan + indomethacin (10mg/kg)	10	24.1	43.6	44.3	47.9

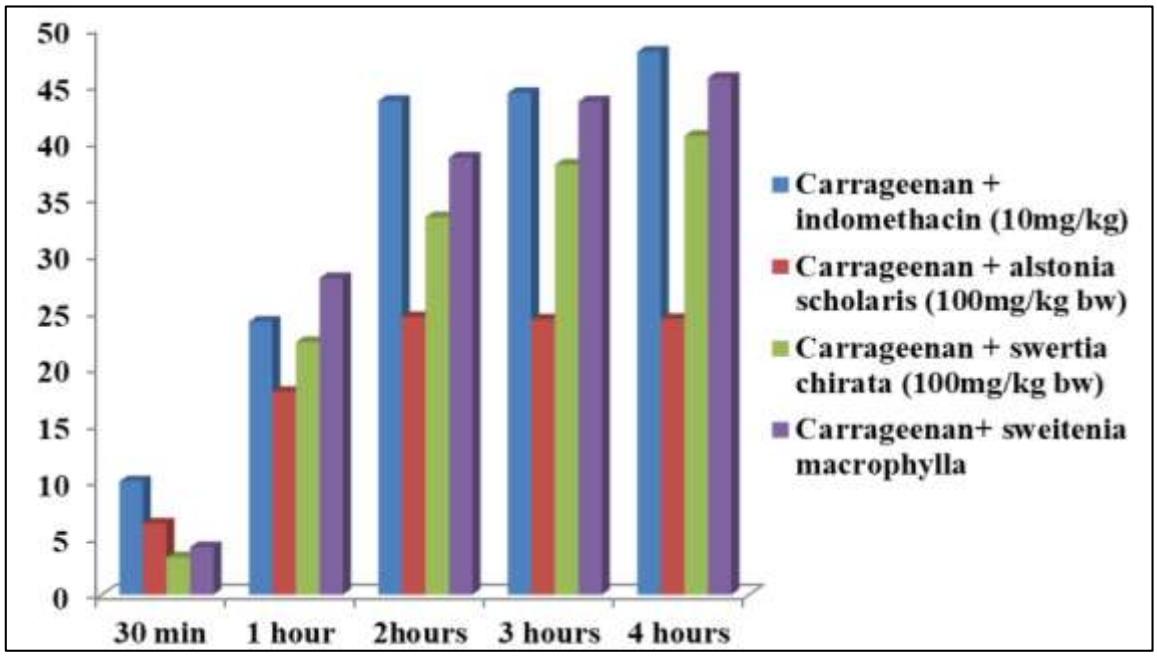

Fig 4: percentage inhibition of inflammation of ethanolic extracts of *Alstonia scholaris*, *Swertia chirata* and *Swietenia macroplylla*.

Inflammation is a severe response by living tissue to any kind of injury. There can be four primary indicators of inflammation: pain, redness, heat or warmness and swelling. When there is injury to any part of the human body, the arterioles in the encircling tissue dilate. This gives a raised blood circulation towards the area (redness). Vasoactive chemicals also increase the permeability (increase pore size) of these arterioles which allows blood cells, chemicals substance, blood protein and fluid to accumulate in that region. This fluid accumulation causes swelling and many compress nerves in the area resulting in pain. In addition, prostaglandins that might also result in irritation of the nerves and further contribute to pain. Most people who take inflammatory drugs have no side effects, or only minor types. When take approximately, the advantages usually far outweighs the possible adverse effects, can occur. There are a number of anti-inflammatory herbs that could help to achieve similar result without the harmful effect. Carrageenan induced edema is a biphasic response. The first phase is initiated by the release of histamine, serotonin and kinins, whereas second phase is mediated by the releasing of prostaglandins. It is reported that second phase of edema is sensitive to drugs like hydrocortisone, indomethacine and phenyl butazone. Indomethacin is cyclooxygenase inhibitors, the ethanol extract has activity which is aompareable to indomethacin and can be said to inhibit the cyclooxygenase enzyme but lipooxygenase inhibitors also possess significant anti-inflammatory activity against carrageenan induced paw edema, so inhibition of carrageenan induces paw edema by the crude extract could also be due to its inhibitory activity on the lipooxygenase enzyme.

Conclusion

In the present study, the anti-inflammatory activities of three Indian medicinal plants were investigated with reference to standard drugs indomethacin. Both the aqueous and ethanolic extract significantly reduced carrageenan induced acute inflammation in animal models. The ethanolic extracts were more potent than the aqueous extracts. Of the three plants *Swietenia macrophylla* (both aqueous and ethanolic extracts) showed maximum protection followed by Swertia chirata. Although active inflammatory compound have already been reported from these plants, the study emphasizes on the comparative assessment of anti-inflammatory activities of these three plants with reference to standard drug indomethacin.

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