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A comparative evaluation study of anti-inflammatory activity of *Saraca asoca* and its commonly used substitute plants

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ABSTRACT

This study was carried out to compare anti-inflammatory activity of methanolic extract of *Saraca asoca* and its commonly used substitute plants *Kingiodendron pinnatum,* and *Cynometra travancorica.* Chopped bark of each plant where blundered and extracted with methanol, the antioxidant activity were evaluated using Swiss Albino mice. Carrageenan induced paw edema. The intraplantar injection of carrageenan in Swiss Albino mice produced a local inflammatory response and reached a maximum intensity of edema at 3rd hour. After 3rd hour, the edema was significantly reduced by 65.88% in animal group treated with higher dose of *Saraca asoca* compared to control. *Kingiodendron pinnatum* and *Cynometra travancorica* showed 60 and 67.06% of percentage inhibition respectively. Formalin induced paw edema- The extract treated group showed remarkable reduction in paw edema on 4th day by 32.87, 48.56, 46.71% at lower dose and 52.13, 52.59 and 53.52% respectively in animal groups treated with higher doses of each extract compared to control. The group treated with Diclofenac (10 mg/kg) showed 58.02% reduction in paw thickness on 4th day. The data obtained from the study indicate that the substitute plants *Kingiodendron pinnatum* and *Cynometra travancorica* were much effective as *Saraca asoca* in reducing inflammation gives their curable role as an anti-inflammatory drug.

INTRODUCTION

Ayurveda is a system of traditional medicine native to India [1]. *Saraca asoca* (Roxb.), De. wild or *Saraca indica* are commonly used binomial Latin name of Asoka tree [2]. It is an indigenous plant used in large quantities in ayurvadic medicines, belonging to Caesalpinaceae family and these are the preferred species in Ayurveda as 'Asokam' [3]. Asoka is one of the foremost plants utilized from antiquity till to date. The bark of *Saraca asoca* is the useful part and an important raw drug in 'Asokaristam' and several other medicinal preparations. According to Ayurveda, it is a sacred tree of India, famous for its use in treating gynecological disorders and is especially relied upon as an astringent to treat menorrhagia [4]. The tannins contained in the bark provide the main astringent action for halting excessive menstrual bleeding, and also for bleeding haemorrhoids, bleeding ulcers, and haemorrhagic dysentery. 'Asokarishtam', the fermented formulation of 'Asokam' is used as a tonic for menorrhagia. The inhibitory activity of prostaglandin H_2 synthetase was reported for the methanolic extract of Asoka[5]. Moreover, oxytocic efficacy of a phenolic glycoside isolated from this plant has also been reported [6]. Some of the compounds such as tannin, catechol, ketosterol and organic calcium have been isolated from the bark [7, 8]. Due to the wide spectrum properties of *Saraca asoca*, the plant becomes over exploited and the size of natural populations has been dwindling over the years in the country.

Substitution of herbs achieved many goals though basic idea was to provide similar therapeutic effects as that of original drug [9]. Accordingly, the most essential criteria for substitution are the pharmacological activity rather than morphology or phytoconstituents. The annual consumption of 'Asokam' in the Ayurvedic drug industry in Kerala is about 105 tones/year [10]. Due to the wide use of this tree, it has been almost depleted from its natural habitat. International Union for Conservation of Nature and Natural Resources (IUCN) has listed this species under the threat category, 'Globally Vulnerable' [11]. The scarcity of this tree has led to substitution with the bark of other related or unrelated trees. The drug is widely adulterated with the bark of *Polyalthialongifolia*. Other Caesalpiniaceae members particularly *Kingiodendron pinnatum* and *Cynometra travancorica* are commonly used as substitutes. The medicinal properties of these species are not well known. Hence, the work is

carried out to assess the anti-inflammatory activity of *Saraca asoca* and its substitutes, *Kingiodendron pinnatum,* and*Cynometra travancorica.*

Inflammation is the body's basic response to injury, in which white blood cells and chemicals protect the body from infection and repair injury. The Immune system sensing inflammation or irritation creates a protein chain called Circulating Immune Complex (CIC) which is specifically tagged to infection. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Edema formation, leukocyte infiltration and granuloma formation represent such components of inflammation [12]. Edema formation in the paw is the result of a synergism between various inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow [13]. Several experimental models of paw edema have been described. Carrageenan-induced paw edema 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 [14], whereas prostaglandins are detectable in the late phase of inflammation. The study analysed the comparative accounts on the anti-inflammatory effects of *Saraca asoca* and its substitute plants.

MATERIALS AND METHOD

Materials

Collection of plant samples

The bark of *Saraca asoca, Kingiodendron pinnatum* and *Cynometra trvancorica* were obtained from Kerala Forest Research Institute, Peechi, Kerala.

Animals

Swiss albino mice (aged 7 days) were purchased from Small Animal Breeding Station, College of Veterinary, Agricultural University, Thrissur, Mannuthi, Kerala. The animals were maintained under standardized environmental conditions (22-28^{0}C, 60-70% relative humidity, 12 hrs dark/light cycle) and fed with standard mice feed (Sai Durga Feeds and Foods) and water *ad libitum*. All the animal experiments were carried out in Al Shifa College of Pharmacy by the prior permission of Institutional Animal Ethics Committee (IAEC).

Methods

Preparation of plant sample

The barks of each plant is freshly collected, chopped in to small pieces and washed thoroughly with distilled water, shade and air dried at room temperature for 7 days. The dried samples then pulverized using electric blender and the powdered sample of 150g of each plant was extracted using 500ml of methanol in Soxhlet apparatus for 48 hours at 50. The apparatus was intermittently shaken and the extracts were filtered. The solid mass obtained after evaporation and dryness of the solvent, stored in desiccator for further use.

Determination of anti-inflammatory activity of each extract

Carrageenan induced paw edema

Swiss Albino mice were made into 8 groups with 6 animals in each group. Group 1st remained as control (without any treatment), group 2nd was received standard Diclofenac, 3rd, 4th,5th, 6th, 7th and 8th group were received 200 and400mg/Kg of each extract orally for five days. On the 6th day exactly after one hour when animals received drug, they were injected with 0.02 ml of freshly prepared 1% carrageenan in 0.1% carboxy methyl cellulose for each groups on sub plantar region of the right paw to induce inflammation [15]. The thickness of paw was measured using Vernier Caliper before and after carrageenan injection and also in 1st, 2nd, 3rd, 4th, 5th and 24th hour.

Formalin induced paw edema

Swiss Albino mice were divided and given the dosages as above. Freshly prepared 2% formalin (0.02 ml) solution was injected on sub planar region of the right paw to induce inflammation. The oral treatment of each extracts were started one hour prior to formalin injection and continued for 6 consecutive days [16]. The paw thickness was measured using Vernier Caliper before and after formalin injection and continued the measurement of thickness for next 6 days.

RESULTS

Carrageenan induced paw edema

Animals treated with prepared extracts of *Sacaca asoca*, *Kingiodendron pinnatum* and *Cynometra travancorica* of doses200 and400 mg/Kg were showed in the figure 1, 2 and 3. Image 1 shows 2nd and 6th day of treatment. The percentage inhibition of each extract is given in the table 1.The intraplantar injection of carrageenan in Swiss Albino mice produced a local inflammatory response and reached a maximum intensity of edema at 3rd hour. After 3rd hour, the edema was significantly reduced by 65.88% in animal group treated with higher dose of *Saraca asoca* compared to control. *Kingiodendron pinnatum* and *Cynometra travancorica* showed 60 and 67.06% of inhibition respectively.

Formalin induced paw edema

The formalin induced chronic inflammation in Swiss Albino mice models which was treated with extract prepared with *Saraca asoca*, *Kingiodendron pinnatum* and *Cynometra travancorica* of doses 200 and 400 mg/Kg was measured and showed in the figure 4, 5 and 6. The percentage inhibition of each extract is given in the table 1. The treated group showed remarkable reduction in paw edema on 4th day by 32.87, 48.56, 46.71% at lower dose and 52.13, 52.59 and 53.52% respectively in animal groups treated with higher doses of each extract compared to control. The group treated with Diclofenac (10 mg/kg) showed 58.02% reduction in paw thickness on 4th day.

DISCUSSION

Inflammation is an immunologic effort to inactivate or destroy invading organisms or remove irritants. Inappropriate activation of our immune system can result in inflammation leading to rheumatoid arthritis (RA). Various studies reported that *Saraca asoca* showed anti-inflammatory action by acting on the cyclo-oxygenase (COX) pathway. According to modern medicine, most diseases are caused by the over production of free radicals and associated inflammatory events. Various reports suggest that the cornification of uterus have a strong and persistent link with chronic inflammation. Although inflammation is an essential response to injury or infection, chronic inflammation is harmful and causes tissue damage. Inflammation promotes the production of free radicals, which is a contributing factor to the onset of most of the degenerative diseases including haemorrhage. The primary

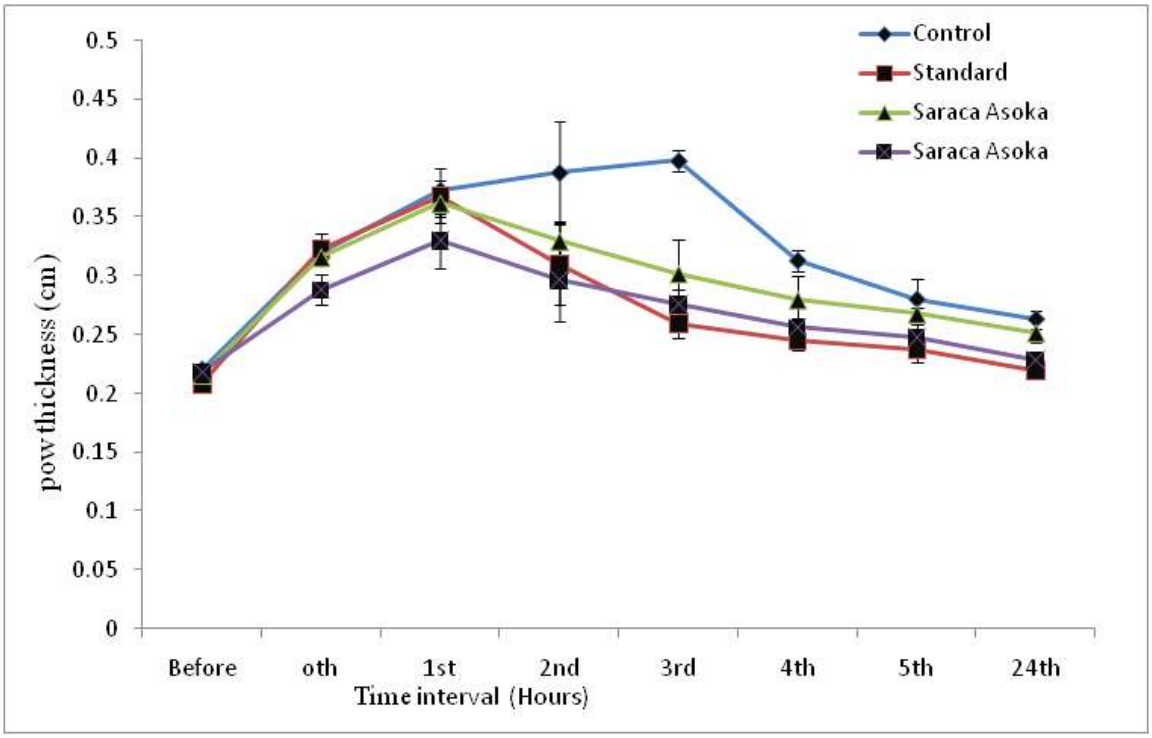

Fig. 1 : Effect of extract prepared with *Saraca asoca* on carrageenan induced paw edema in Swiss albino mice. Values are mean ± SD; for six animals in each group.

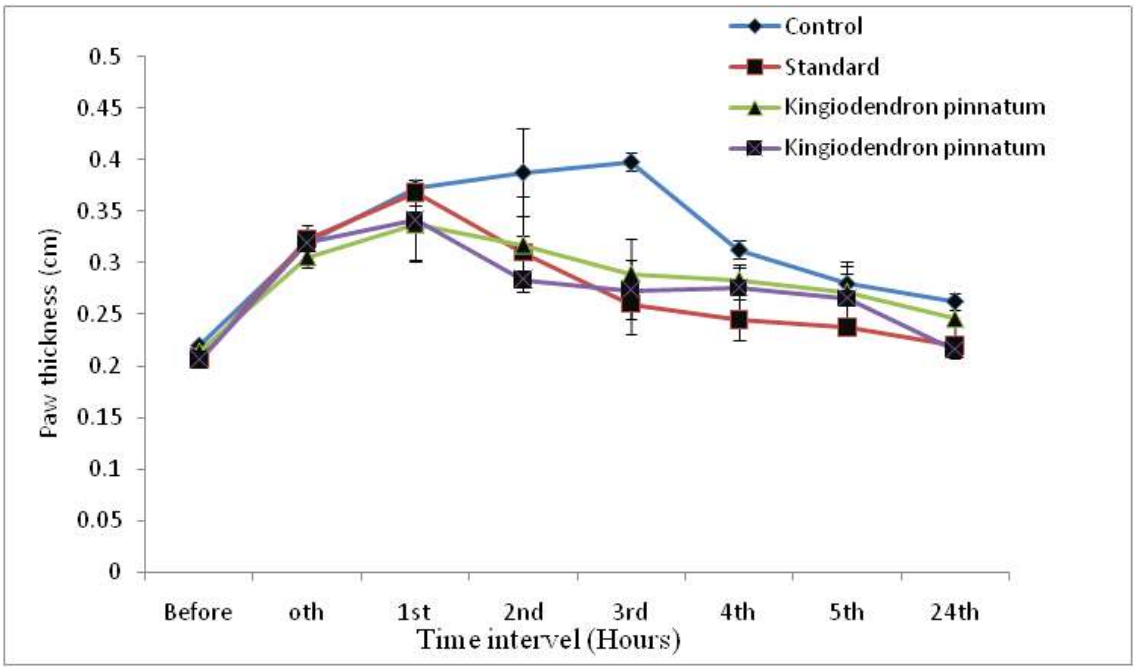

Fig. 2 : Effect of extract prepared with *Kingiodendron pinnatum* on carrageenan induced paw edema in Swiss albino mice. Values are mean ± SD; for six animals in each group.

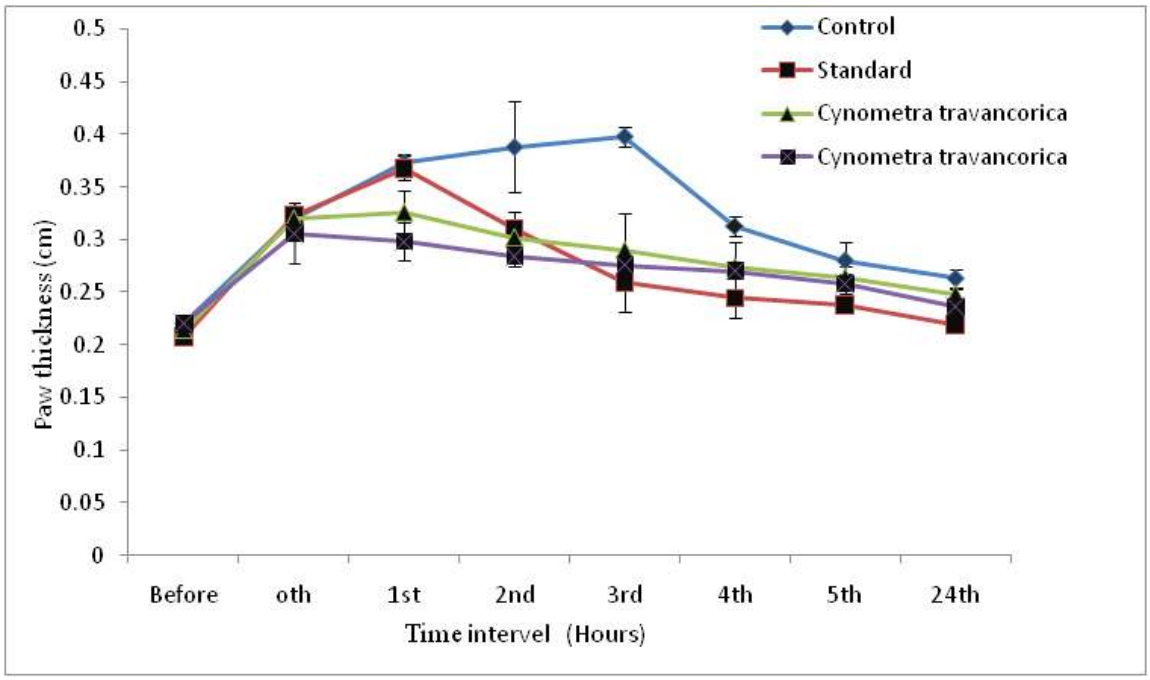

Fig. 3 : Effect of extract prepared with *Cynometra travancorica* on carrageenan induced paw edema in Swiss albino mice. Values are mean ± SD; for six animals in each group.

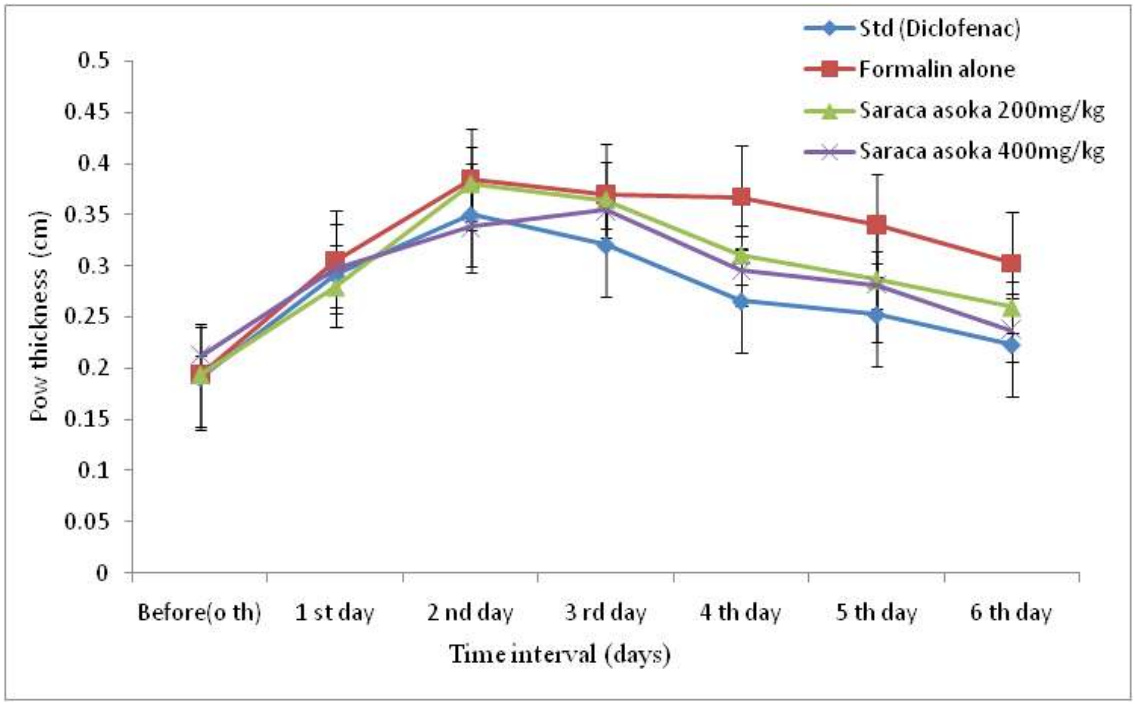

Fig. 4 : Effect of extract prepared with *Saraca asoca on* formalin induced paw edema in Swiss albino mice. Values are mean ± SD; for six animals in each group.

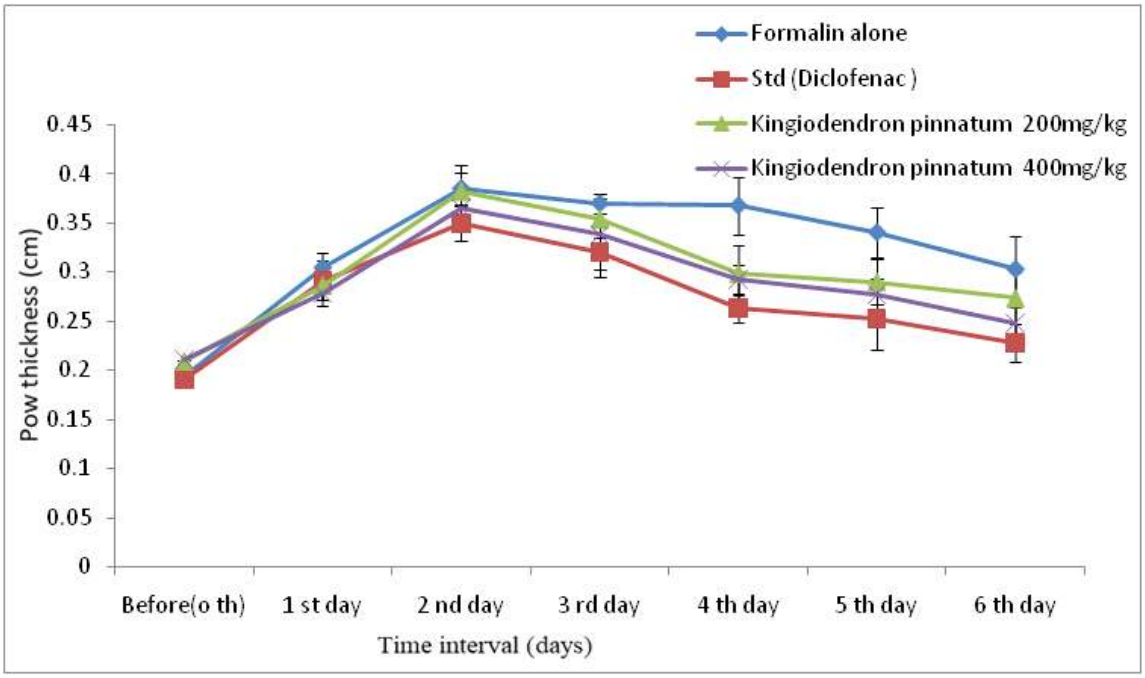

Fig. 5 : Effect of extract prepared with *Kingiodendron pinnatum* on formalin induced paw edema in Swiss albino mice. Values are mean ± SD; for six animals in each group.

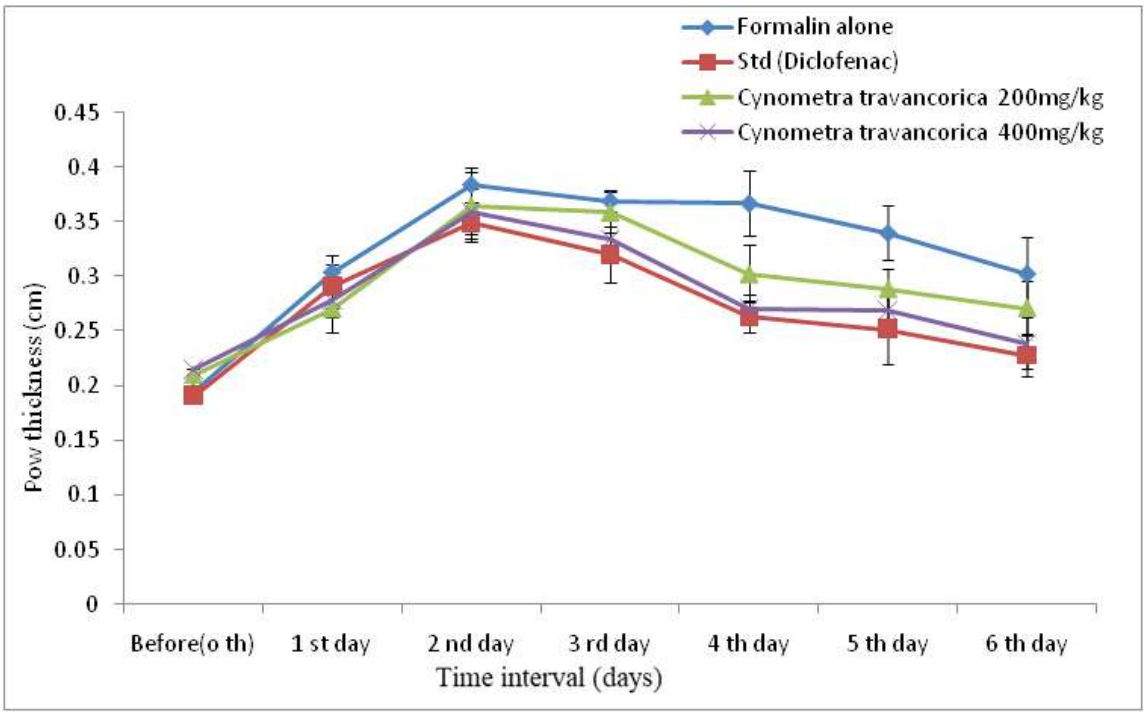

Fig. 6 : Effect of extract prepared with *Cynometra travancorica* on formalin induced paw edema in Swiss albino mice. Values are mean ± SD; for six animals in each group.

Table 1 : Percentage inhibition of each extract in anti-inflammatory activity

Treatment	% inhibition in carrageenan induced inflammation	% inhibition in formalin induced inflammation
Standard diclofenac 10 mg/Kg	69.41%	58.02%
Saracaasoca 200 mg/Kg	49.41%	32.87%
Saracaasoca 400 mg/Kg	65.88%	52.13%
Kingiodendronpinnatum 200 mg/Kg	55.29%	48.56%
Kingiodendronpinnatum 400 mg/Kg	60%	52.59%
Cynometratravancorica 200 mg/Kg	55.29%	46.71%
Cynometratravancorica 400 mg/Kg	67.06%	53.52%

precursor of this pathway is arachidonic acid which is a 20-carbon fatty acid and is present as a component of the phospholipid of cell membrane. It is released from there by the action of phospholipase A_2 and other acyl hydrolases. At the site of disease and inflammation, the two enzymes such as COX_1 and COX_2 will produce prostaglandins (PGs), thromboxanes (Tx) and prostacyclin from Arachidonic acid. These are serving as inflammatory mediators. COX_1 is described as a house keeping enzyme that regulate the normal cellular process such as gastric site protection, vascular homeostasis, platelet aggregation and kidney function. COX_2 is constitutively expressed in tissue such as the brain, kidney and bone during the state of inflammation. PGs act by binding with cell membrane receptors like G-protein coupled receptors, which subsequently activate or inhibit adenylyl cyclase (Ac) or phospholipase C (PlC). This causes an enhanced formation of diacylglycerole (DAG) and IP_3. In the study, it is revealed that the extract prepared with *Cynometra travancorica* showed a significant percentage of inhibition in

inflammatory response.

CONCLUSION

In conclusion, the indiscriminate use and unscientific extraction of *Asoka* bark has led to acute scarcity of the genuine raw drug and this in turn has led to cost escalation and wide spread adulteration of the drug. The data generated from the study thus provides scientific basis for the use of substitute plants and in therapeutic preparations of *Saraca asoca*. Nowadays almost all Ayurvedic preparations have been adulterated. These adulterations lead to a wide variation in quality control. The result is that, Ayurveda has not been able to capitalize its wealth by promoting its use. Thus the quality assurance is the integral part of all systems of medicine to ensure quality medicine today.

The substitute plants *Kingiodendron pinnatum* and *Cynometra travancorica* were much effective as *Saraca asoca* in reducing inflammation give their curable role as an anti-inflammatory drug. In the carrageenan (acute) and formalin (chronic) induced inflammation in mice, the three preparations of each extract produces nearby percentage of inhibition when compared with standard.

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