

## Anti-Inflammatory Activity of *Sesamum Indicum* L. Seed Extracts in Experimental Animal Models

Kunjumon Dayana<sup>1</sup> and Megaravalli R Manasa<sup>2\*</sup>

<sup>1</sup>Pharmacology department, Pushpagiri institute of medical sciences and research centre (Kerala University of Health Sciences), Thiruvalla, Kerala, India.

<sup>2</sup>Pharmacology department, Karwar institute of medical sciences (Rajiv Gandhi University of Health Sciences), Karwar, Karnataka, India.

\*Corresponding Author E-mail; dr.manasamr@gmail.com

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Inflammation is a defensive mechanism that protects the body from noxious stimuli. Currently available anti-inflammatory drugs are associated with numerous adverse effects. Hence there is a need for novel anti-inflammatory agent with better safety profile. The current study was conducted to investigate the anti-inflammatory activity of the ethanolic and hexane extracts of *Sesamum indicum* L. seeds by carrageenan and formalin induced paw edema respectively in Wistar rats. The animals were divided into 5 groups. Group 1 was given normal saline orally and Group 2 Indomethacin. Groups 3-5 in Carrageenan model were administered ethanolic extract of *Sesamum indicum* L. at three doses - 150, 200 and 250 mg/kg respectively, whereas in Formalin model, they were given hexane extract at the same doses orally. Anti-inflammatory potential was investigated by Carrageenan and Formalin induced models of inflammation. *Sesamum indicum* L. ethanolic extract at 250 mg/kg exhibited a significant inhibition of paw edema at 4th hour while hexane extract at all doses caused significant inhibition of paw edema. The percentage inhibition of edema at 4th hour of hexane extract at 250 mg/kg was comparable to Indomethacin. The ethanolic and hexane extracts of *Sesamum indicum* L. seeds have anti-inflammatory potential. The activity of hexane extract is comparable to indomethacin.

**Keywords:** Anti-Inflammatory; Extract; Indomethacin; Rats; *Sesamum indicum* L.

Inflammation is the defensive response of the body to altered homeostasis due to injury, infection or trauma leading to various systemic and local effects. Although it is a protective mechanism, it can result in complications if left untreated.<sup>1</sup> It may be either acute or chronic, based on the type of the stimulus and the efficacy of the body's response in terminating it.<sup>2</sup> Nonsteroidal anti-inflammatory drugs (NSAIDs) and Glucocorticoids are commonly employed for treating inflammatory conditions. They have several drawbacks such

as short duration of action and adverse effects. NSAIDs are associated with adverse effects such as gastric mucosal damage and ulcer whereas glucocorticoids can cause osteoporosis, muscle weakness, cataract etc on long term use.<sup>3,4</sup> Hence the search for novel anti-inflammatory agents with better safety profile is on-going.

Medicinal plants are an abundant source of new drugs. *Sesamum indicum* L. (Sesame) belongs to the order tubiflorae and family Pedaliaceae. It is known by various names such as gingelly, til,

benne seed etc. It is highly resistant to oxidation and rancidity.<sup>5,6</sup> It has health promoting property. Its seeds are rich source of nutrients such as copper, calcium, iron, magnesium, phosphorous, manganese, vitamin B1, zinc etc.<sup>7</sup> The seeds contain phytochemical compounds like lignans, the important ones are sesamin, sesamol, sesaminol and sesamolol. The biological activities of sesame are attributed to these lignans.<sup>8</sup> The cholesterol lowering property of sesame has been widely reported.<sup>9-12</sup> Previous studies have documented the antioxidant, antiaging, antihypertensive and anticancer activities of sesame.<sup>13-18</sup>

The current study was conducted to investigate the anti-inflammatory activity of the two extracts of *Sesamum indicum* L. seeds (ethanolic and hexane) by two models of inflammation – carrageenan induced and formalin induced paw edema – in Wistar rats.

## MATERIALS AND METHODS

### Preparation of plant extracts

The *Sesamum indicum* L. seeds were sun dried for 5 days. They were powdered in a mortar. The dry powder was taken in Soxhlet apparatus and extraction was done using different solvents like ethanol and n-hexane. Rotary evaporator was used for concentrating the filtrate. After extraction excess solvent was distilled at 80°C. The extract so obtained was stored in sterile bottles.

### Chemicals

The standard drug - indomethacin was purchased from Dabur Pharma Ltd, Tarapur, Thane. Ethanol and other chemicals were purchased from Sigma Aldrich Pvt Ltd, Bengaluru, India. All chemicals were of analytical grade.

### Animals

Albino Wistar rats of either sex weighing 130-150 g were procured from central animal house, Sri Kaliswari college, Sivakasi, India. They were housed in polypropylene cages and maintained at 12hr : 12hr light dark cycle. Standard pellets and water were provided ad libitum. The care and maintenance of experimental animals complied with the The Indian National Science Academy (INSA) guidelines. Institutional Animal Ethical Committee approved the study protocol.

### Experiment

The animals were divided into 5 groups.

Group 1 was given normal saline (0.1 ml/kg per oral) and served as control. Group 2 was administered the standard drug - Indomethacin (20 mg/kg intraperitoneally). For Carrageenan induced paw edema model, the test groups (Groups 3, 4 and 5) were administered ethanolic extract of *Sesamum indicum* L. at three doses - 150, 200 and 250 mg/kg respectively orally, whereas for Formalin induced paw edema model, the test groups were given hexane extract of *Sesamum indicum* L. orally at the same doses (150, 200 and 250 mg/kg respectively). Paw volume of all animals was measured before inducing edema.

### Carrageenan induced paw edema

This test was done following the method of Winter et al.<sup>19</sup> Paw edema was induced by injecting 0.1 ml of freshly prepared 1% w/v suspension of carrageenan in normal saline into the subplantar region of left hind paw. Carrageenan was injected 1 hour after the drug and extract administration. Paw volume was measured at various time intervals (1,2,3,4 hrs) after carrageenan injection using a Plethysmometer. Mean percentage change in paw volume was calculated in all groups and percentage of inhibition of edema was compared with standard.

### Formalin induced paw edema

A sub-plantar injection of 0.1 ml of 2% formalin was given to the left hind paw of each rat after one hour of the drug and extract administration. Paw volume was measured at hourly intervals from 1<sup>st</sup> to 4<sup>th</sup> hour using a Plethysmometer. Mean percentage change in paw volume of all experimental animals was calculated and comparison of percentage of inhibition of edema with standard was done.<sup>20</sup>

The percentage inhibition of paw edema for both the methods was calculated by the formula:  $100 \times (1 - V_t/V_c)$

### Statistical analysis

The data was expressed as mean  $\pm$  standard error of mean (SEM). The data was analysed by one way ANOVA and Dunnett's test as post hoc. P value < 0.05 was considered significant.

## RESULTS

### Effect on Carrageenan induced paw edema in rats

Carrageenan injection into the hind paw of rats resulted in a progressive increase in paw

**Table 1.** Anti-inflammatory activity of *Sesamum indicum* L. ethanolic extract in Carrageenan induced paw edema

Treatment groups	Treatment given	Paw volume in ml (% inhibition of edema)				
		0 hr	1 hr	2 hr	3 hr	4 hr
Group 1 (Control)	Normal saline (0.1 ml/kg)	1.6±0.01	2.8 ± 0.05	3.1±0.09	7.1 ± 0.1	8.1 ± 0.07
Group 2 (Standard)	Indomethacin (20 mg/kg)	1.6±0.05	1.9 ± 0.12 (32)	2.0±0.07 (35)	5.9 ± 0.03 (17)	1.6 ± 0.01* (80)
Group 3	<i>Sesamum indicum</i> L. extract (150 mg/kg)	1.6± 0.03	1.8 ± 0.012 (36)	5.1 ± 0.06	8.1± 0.04	4.1 ± 0.03 (49)
Group 4	<i>Sesamum indicum</i> L. extract (200 mg/kg)	1.6±0.01	1.7± 0.04 (39)	4.6±0.07	7.7±0.03	3.2±0.1 (60.5)
Group 5	<i>Sesamum indicum</i> L. extract (250 mg/kg)	1.6± 0.04	1.1 ± 0.12 (61)	3.4± 0.03	4.4±0.07 (38)	1.9± 0.02* (77)

Data is presented as mean ± SEM. \*= $P < 0.05$ , \*\*= $P < 0.01$  compared with control.

**Table 2.** Anti-inflammatory activity of *Sesamum indicum* L. hexane extract in Formalin induced paw edema

Treatment groups	Treatment given	Paw volume in ml (% of inhibition of edema)				
		0 hr	1 hr	2 hr	3 hr	4 hr
Group 1 (Control)	Normal saline (0.1 ml/kg)	1.5 ± 0.51	3.2 ± 0.89	3.9± 0.01	7.8 ± 0.03	8.9 ± 0.12
Group 2 (Standard)	Indomethacin (20 mg/kg)	1.5 ± 0.80	1.6 ± 0.02 (50)	1.7±1.10 (56)	3.3 ± 1.9 (58)	1.0 ± 0.08* (89)
Group 3	<i>Sesamum indicum</i> L. extract (150 mg/kg)	1.5 ± 0.01	1.6 ± 0.81 (50)	4.4± 0.73	6.4± 0.12 (18)	1.6± 0.11* (82)
Group 4	<i>Sesamum indicum</i> L. extract (200 mg/kg)	1.5 ± 0.62	1.4± 0.43 (56)	3.8± 0.02 (3)	5.1± 0.03 (35)	1.1± 0.01* (88)
Group 5	<i>Sesamum indicum</i> L. extract (250 mg/kg)	1.5 ± 0.53	1.3 ± 0.13 (59)	3.5 ± 0.30 (10)	4.2± 0.18 (46)	1.0 ± 0.02* (89)

Data is presented as mean ± SEM. \*= $P < 0.05$ , \*\*= $P < 0.01$  compared with control

volume. In group 2 rats which were administered Indomethacin (20 mg/kg), there was a significant inhibition of edema when compared to control group ( $p < 0.05$ ). The percentage inhibition was maximum at 4<sup>th</sup> hour and there was 80% inhibition. Among the test group rats (Groups 3, 4, 5), significant inhibition of edema was observed only in Group 5 rats (*Sesamum indicum* L. ethanolic extract 250 mg/kg) at 4<sup>th</sup> hour ( $p < 0.05$ ). The percentage inhibition of edema at 4<sup>th</sup> hour was 77%. (Table 1)

#### **Effect on Formalin induced paw edema in rats**

The subplantar injection of Formalin into hind paw of the animals caused an increase in paw volume which progressed from 1<sup>st</sup> hour to 4<sup>th</sup> hour. There was a significant inhibition of edema in Group 2 rats which were administered Indomethacin (20 mg/kg) ( $p < 0.05$ ). This effect was maximum at 4<sup>th</sup> hour and percentage of inhibition of edema was 89%. The *Sesamum indicum* L. hexane extract at all three doses (150 mg/kg, 200 mg/kg and 250 mg/kg) has caused significant inhibition of edema at 4<sup>th</sup> hour ( $p < 0.05$ ). The percentage of edema inhibition in Group 2, 3 and 4 animals was 82%, 88% and 89% respectively. The percentage inhibition of the extract at 250 mg/kg (Group 5) is comparable to Indomethacin. (Table 2)

### **DISCUSSION**

The current study evaluated the anti-inflammatory activity of ethanolic extract and hexane extract of *Sesamum indicum* L. seed in Carrageenan induced paw edema and Formalin induced paw edema respectively in Albino Wistar rats. Carrageenan induced paw edema is used to study acute and subacute phases of inflammation while formalin induced paw edema is employed to evaluate chronic inflammation in rodents.<sup>21,22</sup>

Carrageenan is a widely used inflammogen because of its greater reproducibility. It is a non-antigenic phlogistic agent without any systemic effects.<sup>23</sup> This model is used as a preliminary screening test for anti-inflammatory compounds as it involves multiple mechanisms.<sup>24</sup> Stimulation of phospholipase A2 by carrageenan initiates the early phase of inflammation while its cytotoxic effects lead to progression of the inflammation.<sup>25</sup> Inflammatory response induced is biphasic. The initial phase (0-1 hr) involves release of mediators

like histamine, serotonin and kinins, while the latter phase (1-5 hr) involves prostaglandin release.<sup>26,27</sup> These cascade of events progress to exudate formation. The biphasic response of this model permits the prediction of the probable biological targets of test compound.<sup>28</sup> This model is frequently utilised for testing both steroidal and non-steroidal anti-inflammatory drugs.<sup>29</sup> The clinically effective anti-inflammatory drugs alter the latter phase. This method has a predictive value for screening compounds which cause inhibition of mediators of acute inflammation.<sup>30</sup> In this study the *Sesamum indicum* L. ethanolic extract at a dose of 250 mg/kg significantly inhibited the paw edema volume in the latter phase which is mediated by prostaglandins. This suggests that *Sesamum indicum* L. ethanolic extract may have a protective role against inflammation induced by Carrageenan.

Formalin-induced paw edema is an appropriate model to investigate chronic anti-inflammation because of its close resemblance to human arthritis.<sup>31</sup> Formalin also induces biphasic inflammation. The early neurogenic phase involves bradykinin and substance-P while the latter inflammatory phase involves histamine, 5-HT, prostaglandins, bradykinin and cytokines like IL-1 $\alpha$ , IL-6, TNF- $\alpha$ , eicosanoids, and Nitric Oxide.<sup>32,33</sup> Based on the ability of the test compound to inhibit the early phase, the latter phase or on both the phases, it can be predicted whether the anti-inflammatory effect involves central or peripheral components. The centrally acting drugs such as opioids cause equal suppression of both the phases, whereas the drugs acting peripherally like NSAIDs and corticosteroids cause exclusive inhibition of latter phase.<sup>34</sup> In the current study, *Sesamum indicum* L. hexane extract has caused significant inhibition of paw edema in a dose dependent manner. The percentage inhibition of the extract at 250 mg/kg was comparable to Indomethacin. These results suggest a possible protective effect of hexane extract of *Sesamum indicum* L. against formalin induced inflammation.

The results of our study are consistent with other studies confirming the anti-inflammatory activity of *Sesamum indicum* L. (sesame).<sup>35-37</sup> Previous studies have reported that Sesamin, which is a major lignan present in *Sesamum indicum* L. seeds is a potent and specific inhibitor of delta 5 desaturase enzyme involved in biosynthesis of

polyunsaturated fatty acids. This leads to dihomolinolenic acid accumulation and displacement of arachidonic acid, thereby reducing the synthesis of pro-inflammatory prostaglandins.<sup>38,39</sup> Hence the anti-inflammatory activity exhibited by *Sesamum indicum* L. seed extracts in the present study may be due its lignan (Sesamin) content.

### CONCLUSION

The findings of our study confirm the anti-inflammatory activity of ethanolic and hexane extracts of *Sesamum indicum* L. seeds in Carrageenan induced inflammation and Formalin induced inflammation models respectively in Wistar rats. Sesamin, the major lignan in *Sesamum indicum* L. seeds can be a potential target for development of novel anti-inflammatory drug.

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### Conflict of Interest

There are no conflict of Interest.

### Funding Sources

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