



**ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF SPONDYLON SOFT GEL  
CAPSULE IN EXPERIMENTAL ANIMAL MODELS**

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**ABSTRACT**

The present study was done to investigate the anti-inflammatory and analgesic effect of Spondylon Soft Gel Capsule (SPD), herbal combination in oil base, when compared with standard drug, Indomethacine (IND). We have determined the anti-inflammatory and analgesic activity of Spondylon Soft Gel Capsule at the doses of 3, 6, 12 and 24 mg/kg b. wt. and Indometnacine 10 mg/kg b. wt. by oral administration to healthy animals. The drug was studied for anti-inflammatory activity in carrageenan-induced hind paw edema in rats and the paw volume was measured plethysmometrically at 0 to 4 hr after treatment. The medicines were also evaluated for analgesic activity using Eddy's hot plate method Swiss albino mice. The SPD, significantly ( $P < 0.05$ ) reduced carrageenan-induced paw edema in rats and analgesic activity evidenced by increase in the reaction time by Eddy's hot plate method in albino mice. The 12mg/kg showed a good anti-inflammatory and analgesic effect comparative to the standard drugs, indomethacin. The present results indicated that 12mg/kg exhibited more significant activity than other doses 3, 6 and 24 mg/kg in the treatment of pain and inflammation.

**KEYWORDS:** *Anti-inflammatory, Carrageenan, Analgesic, Hot plate, Spondylon Soft Gel Capsule, Experimental animals.*

**1. INTRODUCTION**

Human beings have relied on natural products as a resource of drugs for thousands of years. Plant-based drugs have formed the basis of traditional medicine systems that have been used for centuries in many countries such as Egypt, China, and India.<sup>[1]</sup> Today plant-based drugs continue to play an essential role in health care. It has been estimated by the World Health Organization that 80% of the population of the world rely mainly on traditional medicines for their primary health care.<sup>[2]</sup>

Spondylosis is a broad term meaning degeneration of the spinal column from any cause. In the more narrow sense it refers to spinal osteoarthritis, the age-related wear and tear of the spinal column, which is the most common cause of spondylosis. It progresses with age and often develops at multiple interspaces. Spondylotic changes can result in stenosis of the spinal canal, lateral recess, and foramina. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs also to treat pain and inflammatory in spondylosis conditions.<sup>[4]</sup> But they have common side effects like ulcer, bleeding, and renal disorders.<sup>[5]</sup> Therefore, medicinal plants are the common source of therapeutically active chemical substances with lesser side effects.<sup>[6]</sup>

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine.

Spondylon Soft Gel capsules (SPD) is an ethical proprietary product developed by Nagarjuna Herbal Concentrates Ltd. It is a herbal remedy effective in Cervical spondylosis, Ankylosing spondylosis, Brachial neuralgia and Pain relief. This is a completely herbal combinations processed in oil base. This method follows the principle of incorporating the efficacy of herbal ingredients through repeating the process (Avarthy). Objective of the study is to ascertain the efficacy and dosage of Spondylon Soft Gel capsule through anti-inflammatory and analgesic activity.

**2. MATERIALS AND METHODS**

**2.1. Raw material collection**

All the ingredients used for this medicine were collected from raw material store of Nagarjuna Herbal Concentrats Ltd, Thodupuzha, Kerala, India. The herbs were identified and authenticated at the Herbarium of Pharmacognosy department, Nagarjuna Herbal Concentrats Ltd, Thodupuzha, Kerala, India. For the

preparation of medicine 8 raw materials used for Kashayam (decoction) and 8 raw materials for Kalkam (Paste).

### 2.1.1. Preparation of medicine

The process is as per the preparation of Aavathy (Repeating the process), a pottenciation process mentioned in the Ayurvedic classical texts. All the raw materials were cleaned, washed and dried. The raw materials used for preparing the Kashayam (decoction) were divided into 21 parts. The raw materials for Kalkam (Paste) are powdered and also divided into 21 parts. From these, 3 parts were taken together for preparing the decoction for three Avarthees at a time. This was then added with 16 times of water and reduced to one fourth through boiling. On completion it was filtered and divided into three parts. One part of this kashayam along with Sesame oil and one part of Kalkam were boiled up to the proper consistency (Chalipakam). When the consistency is reached, milk is added up to thrice the quantity of oil and boiled again till it reaches the next consistency (Chikkanapakam). Then this was allowed to settle the sediments and filtered. This process was repeated up to 21 times with the remaining materials in the same oil. The final product was filtered till all the residues were separated and used for the study.

## 2.2. Experimental animals

Female Wistar rats weighing 120-170 g and Female Swiss mice weighing 20-30g bred in Nagarjuna Herbal Concentrates Ltd., Thodupuzha, Kerala, India, were used in the present study. The animals were housed in polyacrylic cages (38 × 23 × 10 cm) with not more than 3 animals per cage and maintained under standard laboratory conditions with natural dark and light cycle.

They were allowed free access to standard dry rat/mice diet and water *ad-libitum*. All animal for the experiments were approved by the Institutional Animal Ethics Committee, Nagarjuna Herbal Concentrates Ltd., and were maintained in accordance with the guidelines of the CPCSEA.

### 2.2.1. Anti-inflammatory activity

Female Wistar rats (120 - 170 g) kept at the laboratory Animal house of the Nagarjuna Herbal Concentrats Ltd. Idukki, Kerala, India were used. The animals were maintained under standard environmental conditions and had free access to standard diet and water. Anti-inflammatory activity was measured using carrageenan induced rat paw oedema assay.<sup>[7]</sup> 16 hour fasted rats were selected and classified into ten groups. Group I to serve as edema control receiving 0.5% tween 20, Group II was treated with SPD (3mg/kg), Group III was treated with SPD (6mg/kg), Group IV was treated with SPD (12mg/kg), Group V was treated with SPD (24mg/kg), and the Group IV was treated with standard drug, IND (10mg/kg). The initial Paw volume was measured before inducing the oedema. The right hind paw of the rat was induced with oedema by injecting 0.1ml of 1% solution

of Carrageenan in sterilized normal saline. The medicines were administered orally 30 min before the injection of Carrageenan. The paw volume was then measured at 60, 120, 180 and 240 minutes by the mercury displacement method using a Plethysmograph.

### 2.2.3. Analgesic property

Female Swiss albino mice (20 - 30 g) kept at the laboratory Animal house of the Nagarjuna Herbal Concentrats Ltd. Idukki, Kerala, India were used. The hot plate test was used to calculate analgesic activity by the method explained by Eddy and Leimbach<sup>[8]</sup> with minor modifications. Mice were retained on a hot plate having a stable temperature of  $55 \pm 1^\circ\text{C}$ . The time taken for either paw licking or jumping was recorded. Each mouse was individually placed on the hot plate in order to find the animal's reaction to electrical heat-induced pain (licking of the forepaws and eventually jumping). Group I to serve as normal control receiving 0.5% tween 20, Group II was treated with SPD (3mg/kg), Group III was treated with SPD (6mg/kg), Group IV was treated with SPD (12mg/kg), Group V was treated with SPD (24mg/kg), and the Group IV was treated with standard drug, Ind (10mg/kg). The latency until mice showed first signs of discomfort (hind paw lifting, hind paw licking, or jumping) was recorded, before (baseline), and response was determined at 30, 60, and 90min after the treatment.

## 2.3. Data Analysis.

Data were analyzed using statistical software GraphPad Prism version 7. Two-way repeated measure ANOVA test used to assay the differences in volume of paw edema in rats and increased latency response in pain was measured in mice. Data are shown as mean  $\pm$  S.D. All data were considered significant at  $P < 0.05$ .

## 3. RESULTS

### 3.1. Anti-inflammatory activity

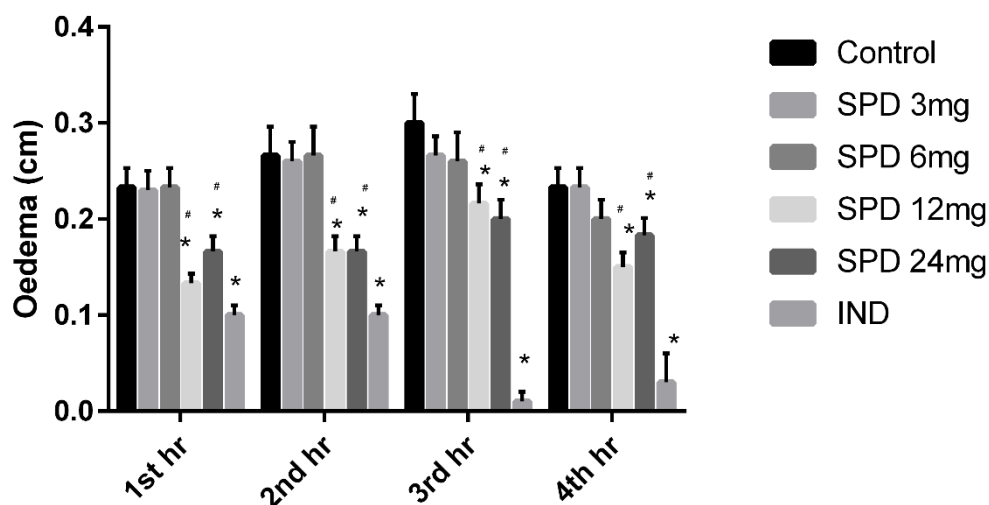
The SPD (3, 6, 12 and 24 mg/kg, p.o.) showed a significant inhibition of carrageenan-induced rat paw edema from 1<sup>st</sup> hour to 4<sup>th</sup> hours following drug administration, compared to the control group (Fig I). The maximum inhibition of paw edema by SPD was observed as 42.91% in 1<sup>st</sup> hr and 37.59% in 2<sup>nd</sup> hr ( $p < 0.05$ ) at the dose of 12mg/kg. Indomethacine (IND) 10 mg/kg p.o. showed inhibition of 57.08% at 1<sup>st</sup> hour and 63.84% at 2<sup>nd</sup> hour after the treatment. Indomethacine inhibit the paw oedema very effectively through out the experiment. SPD also decrease the paw edema in rats, induced by carrageenan.

### 3.2. Analgesic property in mice

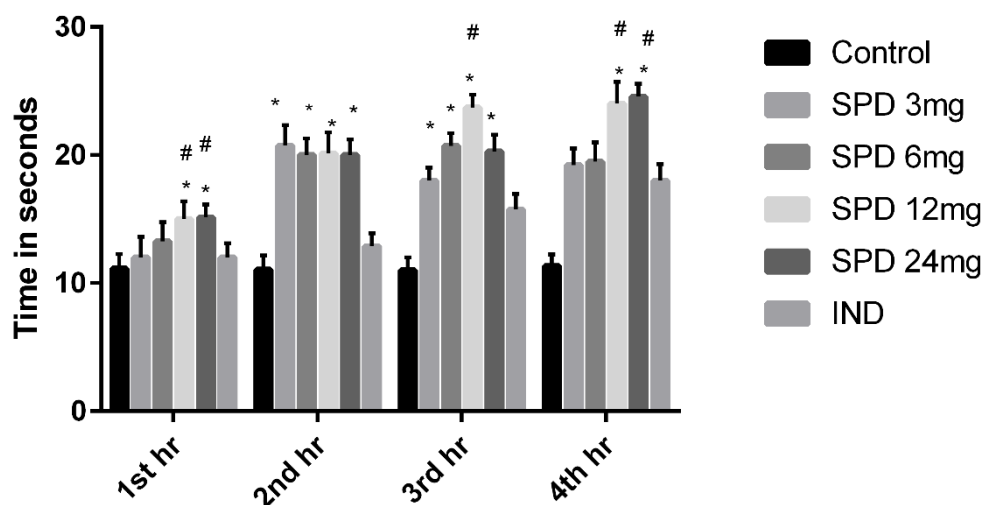
The results were in Figure II, shows that the treatment of mice increased the latency response in the hot plate test from 30 to 90 minutes. On the other hand, SPD significantly influence the reaction time of the animals to the hot plate. In SPD the maximum efficacy was  $23.71 \pm 1.6$  and  $24.00 \pm 1.7$  minutes in 3<sup>rd</sup> and 4<sup>th</sup> hr respectively at the dose of 12mg/kg, which near with the

dose 24mg/kg. Doses of 3 and 6mg/kg shows low latency when compare with 12 and 24mg/kg.

Indomethacine, which have optimum latency at the doses of 10mg/kg in 18.23minutes.



**Figure 1:** Effects of normal control, Indomethacine (10 mg/kg), and Spondylon (3, 6, 12 and 24mg/kg b.wt.) on carrageenan induced paw edema in rats. Data are presented as mean  $\pm$  S.D. \* indicates statically significant ( $p < 0.05$ ), compared to respective control. # indicates statically higher activity ( $p < 0.05$ ), compared to other doses of the same drug.



**Figure 1I:** Effects of normal control, Indomethacine (10 mg/kg), and Spondilon (3, 6, 12 and 24mg/kg b.wt.) on the results of the hot plate test in mice. Data are presented as mean  $\pm$  S.D. \* indicates statically significant ( $p < 0.05$ ), compared to respective control. # indicates statically higher activity ( $p < 0.05$ ), compared to other doses of the same drug.

#### 4. DISCUSSION

In this study, we evaluated the anti-inflammatory activity of Spondylon Soft Gel Capsule in carrageenan-induced paw edema model. The carrageenan-induced paw edema model is used to screen the anti-inflammatory activity of a drug in the acute phase of inflammation. Edema induced by carrageenan is believed to be biphasic.<sup>[9,10]</sup> The first phase (1 hour) involves the release of serotonin and histamine and the second phase (> 1 hour) is mediated by cyclooxygenase products. Continuity

between the two phases is provided by kinin.<sup>[11]</sup> The SPD were significantly inhibited the edema formation in both the first and second phases. The anti-edematous activity of the medicines in the first phase could be due to the possible suppression of histamine signaling by the mast cell stabilizing effect<sup>[12,13]</sup>, and direct inhibition of histamine H1 receptor and histidine decarboxylase gene transcriptions.<sup>[14]</sup> Another possible explanation could be the corticotrophic action of drugs as evidenced by a raise in plasma cortisol levels<sup>[15]</sup>, which antagonizes nuclear

factor-kappa-B (NFkB).<sup>[16]</sup> In the present study, the anti-edematous activity of the medicines persisted in the second phase with the maximal effect observed at 2<sup>nd</sup> hour in both medicines and at the dose of 12mg/kg body weight.

Three anti-nociceptive models hot plate models was used to evaluate the analgesic activity of SPD since tests of analgesic drugs commonly measure nociception and involves the reaction of animals to painful stimuli. The stimulus may be thermal (hot plate tests), chemical (acetic acid-induced writhing or formalin tests) or mechanical (tail or paw pressure tests).<sup>[17]</sup> For this study we use hot plate method. In hot plate model, the paws of mice were very sensitive to temperatures at  $55 \pm 1^\circ\text{C}$ .<sup>[18]</sup> In this model, increase in pain reaction time (PRT) or latency period indicates the level of analgesia of drug.<sup>[19]</sup> The drugs showed a significant increase in Pain Reaction Time (PRT). When we compare the PRT the drugs in deferent doses 12 and 24mg/kg significantly increased the pain reaction time ie, drug shows a dose depended activity. In this model, sensory nerves sensitise the nociceptors and the involvement of endogenous substances such as prostaglandins are minimized.<sup>[20]</sup> From the results, though the medicines showed good analgesic actions in hot plate models.

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