



Journal of  
**Pharmacology and  
Toxicology**

ISSN 1816-496X



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## Studies on the Analgesic, Antipyretic and Ulcerogenic Properties of *Spirulina fusiformis* in Mice

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**Abstract:** This study was intended to evaluate the analgesic, antipyretic and ulcerogenic properties of aqueous suspension of *Spirulina fusiformis* (400/800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) in different experimental standard models in mice. For comparison purpose, non-steroidal anti-inflammatory drug Indomethacin (10 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) was used as a standard. The results showed that *Spirulina fusiformis* possesses significant analgesic and antipyretic effect with the absence of gastric damage at different dose levels in mice.

**Key words:** *Spirulina fusiformis*, analgesic, antipyretic, gastric damage, indomethacin

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### INTRODUCTION

*Spirulina*, blue green algae, has been used since ancient times as a source of food because of its high protein and nutritional value (Rasool *et al.*, 2006). The chemical composition of *Spirulina* indicates that it has phenolic acids, tocopherols and  $\beta$ -carotene, which are known to exhibit antioxidant properties (Sharma *et al.*, 2007). *Spirulina fusiformis* possess potent antiviral activity, anticancer effects, strengthens immune system and metalloprotective effects (Premkumar *et al.*, 2004). Its safety for human consumption has also been established through numerous toxicological studies (Hirahashi *et al.*, 2002). Moreover in our laboratory, we have already reported that the *Spirulina fusiformis* possess anti-inflammatory effect against adjuvant-induced arthritis in mice (Rasool *et al.*, 2006). Since analgesic and antipyretic properties associated with gastric damage are the most important features of anti-inflammatory drugs. Therefore, the present study was undertaken to identify the analgesic, antipyretic and ulcerogenic properties of *Spirulina fusiformis* at different dose levels (400/800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) in standard models in mice. The standard non-steroidal anti-inflammatory drug, Indomethacin, was used as a reference drug for purposes of comparison.

### MATERIALS AND METHODS

#### Animals

The study was performed with Swiss albino mice, 25-30 g, of either sex. The mice were brought from Tamil Nadu Veterinary College, Chennai, India. The mice were acclimatized for a week in a light and temperature-controlled room with a 12 h dark-light cycle. The mice were fed commercial pelleted feed from Hindustan Lever Ltd. (Mumbai, India) and water was made freely available. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, Ministry of Culture, Chennai. Experimental protocol was approved by our Departmental Ethical Committee.

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## **Drug**

The commercially available *Spirulina fusiformis* (a fine dark blue-green spray-dried powder) was obtained from RECON, Ltd., Bangalore, India and was dissolved in 2% gum acacia solution to give an aqueous suspension. This aqueous suspension of *Spirulina fusiformis* was used at a different dose levels (400/800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) orally (Rasool and Varalakshmi, 2006). Indomethacin (10 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) obtained from Tamilnadu Dadha Pharmaceuticals, Chennai, India was dissolved in 2% gum acacia solution and was administered orally (Rasool and Varalakshmi, 2006). All other reagents used were standard laboratory reagents of analytical grade and were purchased locally.

## **Analgesic Test**

### **Acetic Acid-Induced Writhing Response in Mice**

This test was conducted using the method described by Witkin *et al.* (1961). The muscular contractions were induced in mice by intra peritoneal injection of 0.6% solution of acetic acid (10 mL kg<sup>-1</sup> b.wt.<sup>-1</sup>). Immediately after administration of acetic acid, the animals were placed in glass cages and the number of stretchings per animal was recorded during the following 30 min. A significant reduction in the number of writhings by any treatment as compared to control animals was considered a positive analgesic response. *Spirulina fusiformis* (400/800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) and Indomethacin (10 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) suspended in 2% gum acacia solution were administered 30 min before the acetic acid injection.

### **Hot-plate Reaction Time in Mice**

The method of Williamson *et al.* (1996) was used. Mice were placed individually in a 2 l glass beaker placed on a thermostatically controlled hot plate maintained at 55°C. The pain threshold is considered to be reached when the animals lift and lick their paws or attempt to jump out of the beaker. The time taken for the mice to react in this fashion was obtained using a stopwatch. The animals were first tested for the paw-lick or jump response and only those that reacted after 4 sec were used for the experiment. Mice were tested in groups of six per dose 30 min after oral administration of *Spirulina fusiformis* (400/800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) and Indomethacin (10 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>). Control animals received equal volume of normal saline and the experiment was repeated.

## **Antipyretic Test**

The mice were injected subcutaneously with 10 mL kg<sup>-1</sup> of 20% aqueous suspension of baker's yeast and the rectal temperatures were recorded initially and at 18 h *Spirulina fusiformis* (400 and 800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) and Indomethacin (10 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) were administered orally after the 18 h reading. When the increase of temperature was at its peak, it was measured at hourly intervals up to 5 h after administration of drugs (Mukerjee, 1996).

## **Ulcerogenic Test**

Animals of six groups of 6 mice in each were kept fasting for 16 h and the test compounds were then administered orally. *Spirulina fusiformis* was administered at dose levels of 800/1600 mg kg<sup>-1</sup> b.wt.<sup>-1</sup> and Indomethacin at a dose of 20 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>. Animals were killed 3 h after the administration of the drugs and the stomachs were removed, cut along the lesser curvature and the gastric mucosa was washed with normal saline and scored according to the scale, 0: no lesion, 0.5: hyperaemia, 1: one or two lesions, 2: severe lesions, 3: very severe lesions, 4: mucosa full of lesions (Cashin *et al.*, 1977).

## **Statistical Analysis**

Results were expressed as mean±SD and statistical analysis was performed using ANOVA to determine significant differences between groups, followed by Student's Newman-Keul's test. p< 0.05 implied significance.

## RESULTS

### Analgesic Test

The treatment of animals with *Spirulina fusiformis* (400/800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>, p.o.) produced 26.3 and 44.1% significant inhibition in abdominal writhes induced by acetic acid, whereas Indomethacin ((10 mg kg<sup>-1</sup>, p.o.), used as a standard drug produced 46.1% inhibition. The inhibition produced by *Spirulina fusiformis* (800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>, p.o.) was found nearly similar to Indomethacin. As shown in Table 1, the administration of *Spirulina fusiformis* (400/800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>, p.o.) produced 56.8 and 125.6% increased reaction time, respectively; however Indomethacin (10 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>, p.o.) provided 148% increased reaction time in the hot plate test when compared to controls.

### Antipyretic Test

Administration of brewer's yeast to mice produced a significant increase in rectal temperature 18 h after yeast injection (p<0.05). *Spirulina fusiformis* (400/800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup> p.o.) and Indomethacin (10 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>, p.o.) caused a significant decrease in rectal temperature (Fig. 1).

### Ulcerogenic Effect

As shown in Fig. 2, the oral administration of Indomethacin (20 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) after 16 h fasting induced gastric lesions, whereas *Spirulina fusiformis* at both the dose levels (800/1600 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) induced only slight, but not statistically significant inhibition of mucosa damage.

Table 1: Effect of *Spirulina fusiformis* and Indomethacin on hot plate reaction time in mice

	<i>Spirulina fusiformis</i> (400 mg kg <sup>-1</sup> b.wt. <sup>-1</sup> )	<i>Spirulina fusiformis</i> (800 mg kg <sup>-1</sup> b.wt. <sup>-1</sup> )	Indomethacin (10 mg kg <sup>-1</sup> b.wt. <sup>-1</sup> )
Control (Reaction time in sec)	(Reaction time in sec)	(Reaction time in sec)	(Reaction time in sec)
6.25±0.38	9.8±0.76*	14.1±0.53*	15.5±0.73*

Mice were tested 30 min after oral administration of *Spirulina fusiformis* (400/800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) or Indomethacin (10 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>). Comparisons are made with control groups. The results are given as mean±SD; number of animals used (n = 6) The symbols represent statistical significance at: \*p<0.05

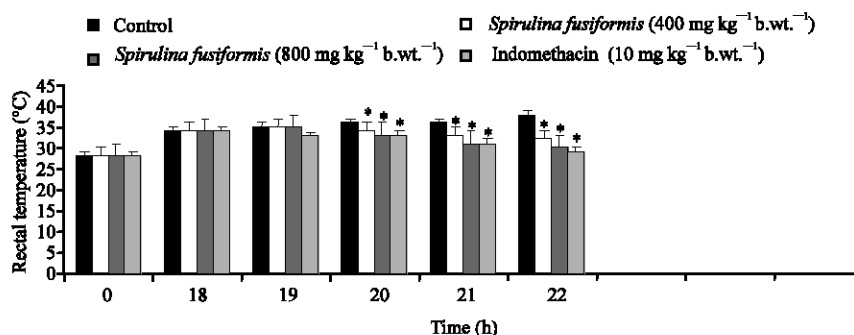


Fig. 1: Antipyretic effects of *Spirulina fusiformis* and Indomethacin in mice. Pyrexia was induced by subcutaneous injection of bakers yeast (10 mL kg<sup>-1</sup>), after obtaining the rectal temperature of the mice at 0 h. *Spirulina fusiformis* and Indomethacin, were administered orally 18 h after bakers injection. Rectal temperature of normal mice and those treated were measured at hourly intervals up to 5 h after the administration of drugs. Each point and vertical bar represent the mean and SD (n = 6). Comparisons are made with control groups, the symbols represent statistical significance at: \*: p<0.05

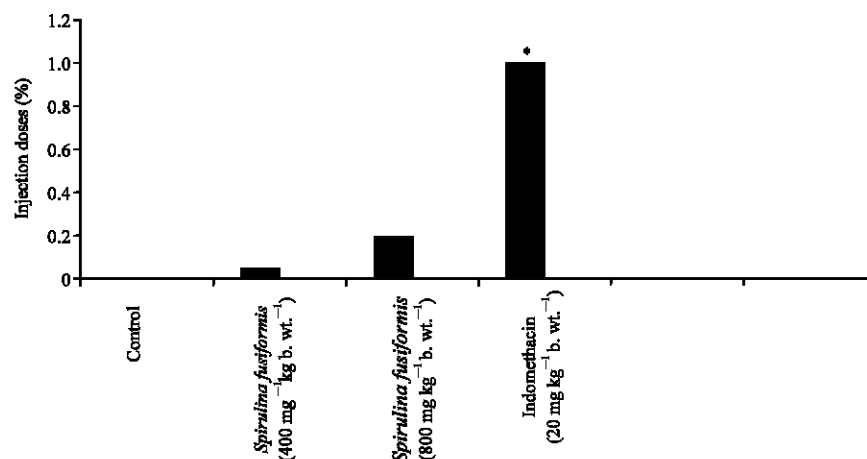


Fig. 2: Ulcerogenic action of *Spirulina fusiformis* and Indomethacin in mice. *Spirulina fusiformis* and Indomethacin were administered orally after 16 h fasting. Comparisons are made with control groups. Number of animals used (n = 6). The symbol represents statistical significance at: \*: p<0.05

## DISCUSSION

We first proved that *Spirulina fusiformis* possess anti-inflammatory effect against adjuvant-induced arthritis, an experimental model for human rheumatoid arthritis in mice (Rasool *et al.*, 2006), since analgesic and antipyretic properties associated with gastric damage are the most important features of anti-inflammatory drugs. Therefore, in this present study, we investigated the analgesic, antipyretic and ulcerogenic properties of *Spirulina fusiformis* in different experimental models in mice. The results outlined in our present study lead us to confirm that *Spirulina fusiformis* exerts a significant analgesic, antipyretic with the absence of gastric damage at different dose levels in mice.

The writhing response of the mouse to an intraperitoneal injection of noxious chemical is used to screen for both peripherally and centrally acting analgesic activity. Acetic acid causes pain by liberating endogenous substances and many others that excite pain nerve endings (Raj, 1996). Indomethacin and other non-steroidal anti-inflammatory drugs can inhibit number of writhes in this model by inhibition of cyclooxygenase enzyme in peripheral tissues, thus interfering with the mechanism of transduction in primary afferent nociceptors by blocking the effect or the synthesis and/or release of inflammatory mediators (Panthong *et al.*, 2007). In order to evaluate the analgesic activity, *Spirulina fusiformis* was studied against acetic acid induced writhings in mice. As shown in Table 2, *Spirulina fusiformis* showed analgesic activity at both the dose levels (400/800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>). Induction of the acetic acid writhing in mice is an effect of the acute inflammatory reaction related to the increase in levels of prostaglandins E<sub>2</sub> and F<sub>2 $\alpha$</sub>  in the peritoneal fluid (Deraedt *et al.*, 1976). The hot plate test has been found to be suitable for evaluation of centrally acting analgesics. In the hot plate test, *Spirulina fusiformis* (400/800 mg kg<sup>-1</sup> b.wt.<sup>-1</sup>) showed a significant analgesic action 30 min after its administration. From the results it is evident that the *Spirulina fusiformis* showed a significant analgesic effect in both hot plate test and acetic acid induced writhing response. Analgesic effect of *Spirulina fusiformis* in both models confirms that it has been acting through both peripheral and central mechanism.

Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection, or other disease states. Antipyretic are drugs, which reduce the elevated body

Table 2: Effect of *Spirulina fusiformis* and Indomethacin on acetic acid -induced writhing response in mice

Control	<i>Spirulina fusiformis</i> (400 mg kg <sup>-1</sup> b.wt. <sup>-1</sup> )	<i>Spirulina fusiformis</i> (800 mg kg <sup>-1</sup> b.wt. <sup>-1</sup> )	Indomethacin (10 mg kg <sup>-1</sup> b.wt. <sup>-1</sup> )
(No. of writhings in 30 min)	(No. of writhings in 30 min)	(No. of writhings in 30 min)	(No. of writhings in 30 min)
59.1±3.06	43.5±2.25*	33±1.78*	31.8±1.16*

*Spirulina fusiformis* and Indomethacin were administered orally 30 min before the acetic acid injection. Comparisons are made with control groups. The results are given as mean±SD; number of animals used (n = 6). The symbols represent statistical significance at: \*p<0.05

temperature. Since antipyretic activity is commonly mentioned as a characteristic of drugs or compounds which have an inhibitory effect on prostaglandin-biosynthesis (Vane, 1987). In this present study, the yeast-induced pyrexia in mice was employed to investigate the antipyretic activity of *Spirulina fusiformis*. It was found that the *Spirulina fusiformis* caused a significant decrease in rectal temperature similar to Indomethacin. The production of prostaglandins, particularly the most potent pyretic agent, PGE<sub>2</sub>, appears to be a final pathway responsible for fever production induced by several pyrogens. It is therefore suggested that the antipyretic effect of *Spirulina fusiformis* occurs in a similar fashion as Indomethacin or other non-steroidal anti-inflammatory drugs, by inhibition of prostaglandin biosynthesis in the central nervous system. This result seems to support the view that the *Spirulina fusiformis* has some influence on prostaglandin-biosynthesis because prostaglandin is believed to be a regulator of body temperature (Milton, 1982).

Concerning the anti-inflammatory activity found in *Spirulina fusiformis*, one can hypothesize that it leads to adverse gastric effects, as commonly observed by non-steroidal anti-inflammatory drugs like Indomethacin due to the inhibition of the enzyme cyclooxygenase (COX) (Peura, 2004). The main side effect of non-steroidal anti-inflammatory drugs is their ability to produce gastric lesions (Pagella *et al.*, 1983).

In this study, *Spirulina fusiformis* did not induce any adverse effect on gastric mucosa, indicating non ulcerogenic activity; whereas Indomethacin treated mice produced small erosions. The above results associate the anti arthritic, analgesic and antipyretic actions of the *Spirulina fusiformis* with absence of gastric damage. For further support, the research has begun to investigate the intervention of *Spirulina fusiformis* at the cyclo-oxygenase-2 mediate prostaglandin synthesis.

In conclusion, present results indicate that *Spirulina fusiformis* presents an important analgesic, antipyretic and gastroprotective properties, which might be attributed, at least in part, to the presence of phenolic acids, tocopherols and β-carotene.

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