

Effects of *Spirulina platensis* on pain and inflammation in long Evans rats

Nahida Sultana Nipa¹, Taskina Ali², Shabnam Akhter² and Kazi Rafiq^{3*}

¹Department of Physiology, North East Medical College, Sylhet, Bangladesh

²Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh

³Department of Pharmacology, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh

Abstract: *Spirulina platensis* (*S. platensis*) is a traditional herb that has been reported to have a lot of medicinal values. This study was designed to observe the effects of *S. platensis* on different types of pain and inflammation in comparison to diclofenac sodium. Three groups of Long Evans rats (n=21 in each group) of both sexes were used. Group I was treated orally with normal saline (5ml/kg/day for 21 days), group II was treated with diclofenac sodium (10mg/kg/day for 7 days) and group III with *S. platensis* (400mg/kg/day for 21 days). Effects of *S. platensis* on pain were assessed by tail immersion test (nociception pain), formalin test (nociception and inflammatory pain), Von Frey test (neuropathic pain) and the effects on inflammation were assessed by formalin induced paw edema test. Macroscopic and microscopic examinations of rats' stomachs were done to observe anti-ulcerogenic effect. *S. platensis* showed potent (statistically significant) analgesic effects in all 3 models of pain (tail immersion test, formalin tests, Von Frey test) as well as anti-inflammatory effects in formalin induced paw edema test. Interestingly, anti-ulcerogenic effect of *S. platensis* was almost similar to that of negative control and was significantly different with positive control. In conclusion, these data indicate that *S. platensis* possess analgesic, anti-inflammatory and anti-ulcerogenic potential.

Keywords: Pain, inflammation, gastric ulcer, *Spirulina platensis*, diclofenac sodium.

INTRODUCTION

Pain is frequently the first symptom that triggers the urge to seek the attention of a physician from which diagnosis are guided (Abbott and Fraser, 1998). Following tissue damage or injury, the biological system responds by initiating pain and subsequent removal of the stimuli or self-withdrawal (Julius and Basbaum, 2000). Pain can be categorized into nociception, inflammatory, neuropathic, and functional pain (Schölz and Woolf, 2002; Woolf, 2004). Early sensation to pain is a function of the nociception phenomenon that alerts the body to respond via activated nociceptors (Woolf, 2004). The nociceptors respond to mechanical, thermal, chemical, or electrical stimuli once there is enough sensation to tap on the pain threshold (Julius and Basbaum, 2000; Woolf, 2004).

Withdrawal response is aimed at preventing tissue damage but can however function in tissue healing process if an initial withdrawal response has not prevented tissue damage (Woolf, 2004). Pro inflammatory cytokines, growth factors, kinins, prostanoids, proteases, and ions diffuse into the extracellular fluid from damaged tissue and could stimulate nociceptors to initiate pain and subsequent activation of pain pathways within the nervous system (Schölz and Woolf, 2002). This generated pain, known as inflammatory pain, originates from the activation of nociceptors and nervous system sensitization from inflammatory mediators. Healing property of pain is protective, but too much inflammation is detrimental

because of its tissue damaging consequence. This excessive inflammatory pain causes sufferings and increases morbidity.

On the contrary, neuropathic pain originates from pathology in any part of the nervous system, which reflects both peripheral and central sensitization mechanisms (Woolf and Mannion 1999; Campbell and Meyer, 2006).

There are a variety of approaches for treating pain and inflammation, in addition to our immune mechanism. Analgesics used to treat pain traditionally follow the World Health Organization (WHO) analgesic ladder, stepping through paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs), and finally opioids. Among them, diclofenac sodium (DS) is a commonly prescribed NSAID to treat pain associated with inflammation. Orthodox analgesics have many side effects (Rainsford *et al.*, 2008). It has been observed that, oral DS could produce significant analgesia both after single administration of a high dose (100mg/kg) (Subhan *et al.*, 2008) as well as after a much lower dose (10 mg/kg/day) for 7 consecutive days (Santos *et al.*, 2004) in animal models of pain. Strikingly, both of these dose schedules of DS analgesic were associated with adverse effects like gastric ulcer. Recently many studies have been conducted worldwide in search of alternative approach to modern analgesics in order to replace them, reduce their dosage, or minimize their adverse effects using different herbal products (Maroon *et al.*, 2010;

*Corresponding author: e-mail: krafiq73@yahoo.com

Anand and Bley, 2011; Hussein *et al.*, 2013; Golechha *et al.*, 2014; Nesa *et al.*, 2014). Among them *S. platensis* (world's largest natural protein source), has been suggested as an important medicinal herb (Vonshak, 1997; Teitze, 2004; Moorhead *et al.*, 2006; Habib and Parvin, 2008; Henrikson, 2010; Sixabella *et al.*, 2011; Neekhara *et al.*, 2014). Two species of this blue green algae are most commonly used as nutritional supplements, *Spirulina platensis* (*S. platensis*) and *Spirulina maxima* (*S. maxima*) (Sixabella *et al.*, 2011; Tefera *et al.*, 2016; Wu *et al.*, 2016). Though both of them have similar nutritional values, *S. platensis* has higher growth rate in any given temperature compared to *S. maxima* (Oliveira *et al.*, 1999). Apart from high nutritional value, *S. platensis* has also shown immuno-modulatory (Hayashi *et al.*, 1994), cardio-protective (Khan *et al.*, 2005), reno-protective (Gaurav *et al.*, 2010), anti-hyperlipidemia along with anti-oxidant property (Makhlouf and Makhlouf, 2012), protective against heavy metals poisoning (Abdel-Daim *et al.*, 2013), anti-diabetic (El-Baz *et al.*, 2013), hepato-protective (Yoshinari *et al.*, 2014; Sharoud, 2015), neuro-protective (Alam and Hendawi, 2015), and anti-viral (Chen *et al.*, 2016) effects in different animal models. In accordance with several preclinical studies, *S. platensis* was also reported to be effective as anti-cancer (Liu *et al.*, 2000), preventive against allergy and rhinitis (Mao *et al.*, 2005; Cingi *et al.*, 2008), preventive of arsenic poisoning (Misbahuddin *et al.*, 2006), preventive of anaemia (Selmi *et al.*, 2011), hepato-protective (Mazokopakis, 2014), and cholesterol-lowering effects (Mani *et al.*, 2015) in different clinical studies. In addition, 400mg/kg body weight/day *S. platensis* was shown to produce significant analgesia (Elgendy and Diam, 2014) and anti-inflammatory effects (Joventino *et al.*, 2012) after ≥ 3 weeks' oral administration in rats. Moreover, no adverse effect was seen even after 5000 mg/kg body weight/day oral *S. platensis* for 14 days in rats (Chen *et al.*, 2016). However, to the best of our knowledge, no study has yet been reported on the effect of *S. platensis* on neuropathic pain, inflammation, gastric ulcer, and its comparison with that of orthodox analgesic. On the basis of this background, the present study was designed to evaluate the effects of oral administration of *S. platensis* (400mg/kg body weight/day for 21 consecutive days) on pain and inflammation in Long Evans rats as well as to compare these effects with those of DS (10mg/kg body weight/day for 7 consecutive days).

MATERIALS AND METHODS

The study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka and the Department of Pharmacology, Bangladesh Agricultural University (BAU), Mymensingh from March 2016 to February 2017. The study protocol was approved from the Institutional Review Board of BSMMU.

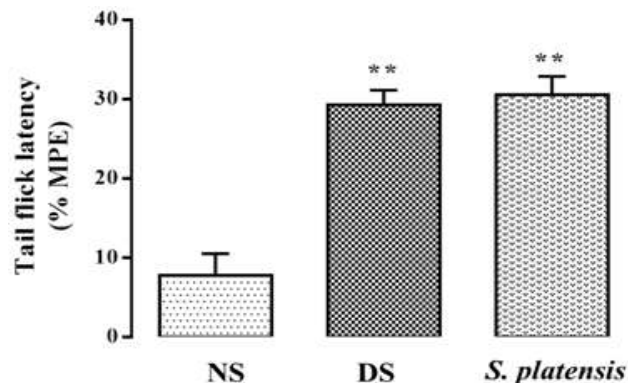


Fig. 1: Analgesic effect of *S. platensis* (*Spirulina platensis*, 400mg/kg body weight) and positive control, DS (diclofenac sodium, 10mg/kg body weight) in comparison to negative control, NS (normal saline, 5 ml/kg body weight) in tail flick latency of tail immersion test. Comparison was done on percentage of maximum possible effect (%MPE). Each bar symbolizes mean \pm SE of 7 rats in each group. **Significant ($P \leq 0.01$) in comparison to negative control.

Procurement and maintenance of animals

We obtained a total of 63 Long Evans rats of both sexes having 200 \pm 20gm body weight from the Bangladesh University of Health Sciences, Dhaka, Bangladesh and housed in specially constructed plastic cages (45X30X15 cm³) with 3 to 4 rats per cage under a 12/12 hours light/dark cycle (Ali *et al.*, 2012; Tamaddonfard *et al.*, 2013) in the pain laboratory of Department of Physiology, BSMMU. The rats were familiarized to the laboratory condition for 7 days before commencement of the experiment. Our team made efforts to reduce the sufferings and number of animals used. Each animal was used only once and sacrificed immediately after the experiment. Corresponding to the thermo-neutral zone for rodents, the ambient room temperature was maintained at 27.5 \pm 0.5 $^{\circ}$ C, (Refinetti and Horvarth, 1989). Standard laboratory food (Islam *et al.*, 2001) and cooled boiled water *ad libitum* were provided for all the rats throughout the experimental period. All the experiments were performed at daytime (between 08:00 and 16:00 hours) to avoid the circadian influences (Moallem *et al.*, 2008) in accordance with the international guidelines on the use of laboratory animals and on the basis of codes for ethics in animal research in the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b).

Dose schedule

Ten (10) mg/kg body weight (Hasani *et al.*, 2011) powder of diclofenac sodium (DS (Biopharma, Bangladesh) and 400mg/kg body weight (Neekhara *et al.*, 2013) powder of *S. Platensis* [Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh] were dissolved in 5ml/kg body weight of normal saline (NS) (Popular infusion limited, Bangladesh) and their solutions were prepared.

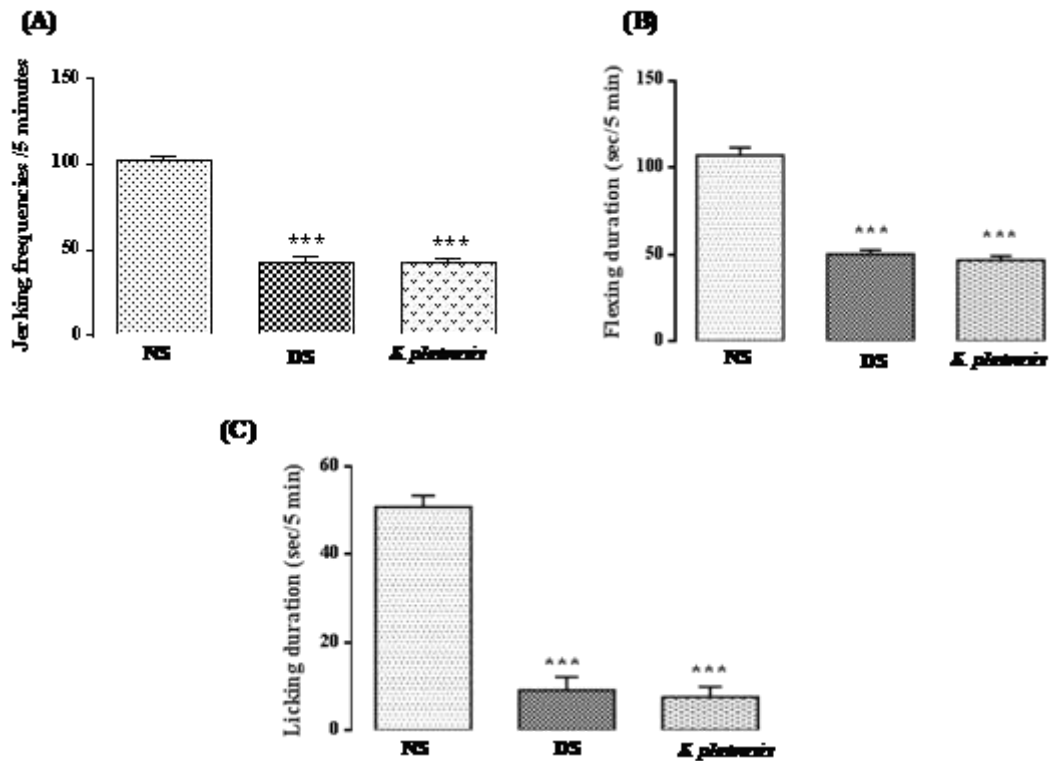


Fig. 2: Analgesic effect of *S. platensis* (*Spirulina platensis*, 400 mg/kg body weight) and positive control, DS (diclofenac sodium, 10 mg/kg body weight) in comparison to negative control, NS (normal saline, 5 ml/kg body weight) in early phase of formalin test (1st to 5th), assessed by jerking (A), flexing (B) and licking (C). Each bar symbolized for mean±SE of 7 rats in each group. ***Significant ($P \leq 0.001$) in comparison to negative control.

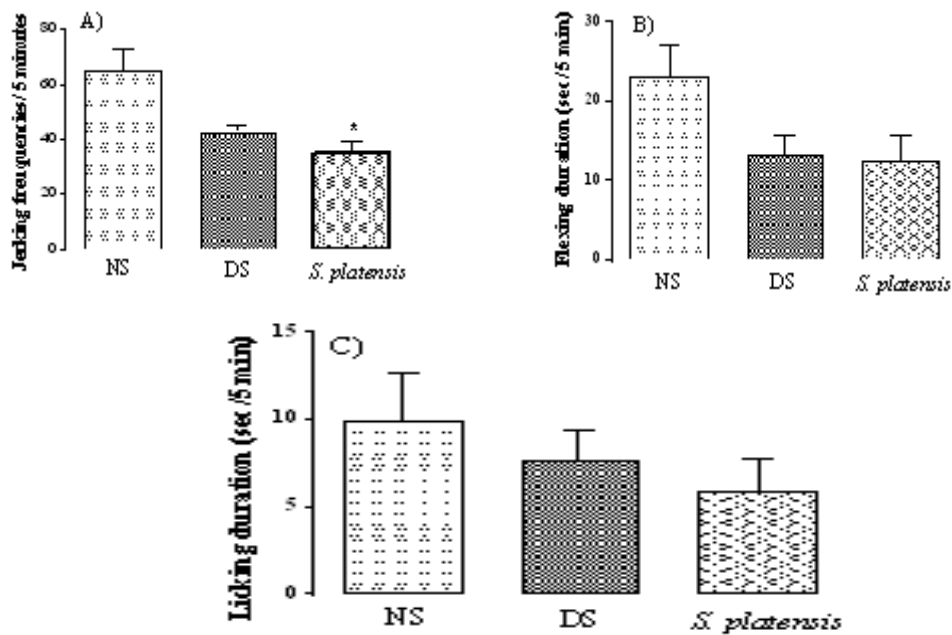


Fig. 3: Analgesic effect of *S. platensis* (*Spirulina platensis*, 400 mg/kg body weight) and positive control, DS (diclofenac sodium, 10 mg/kg body weight) in comparison to negative control, NS (normal saline, 5 ml/kg body weight) in interphase of formalin test (6th to 15th) assessed by jerking (A), flexing (B) and licking (C). Each bar symbolized for mean ±SE of 7 rats in each group. *Significant ($P \leq 0.05$) in comparison to negative control.

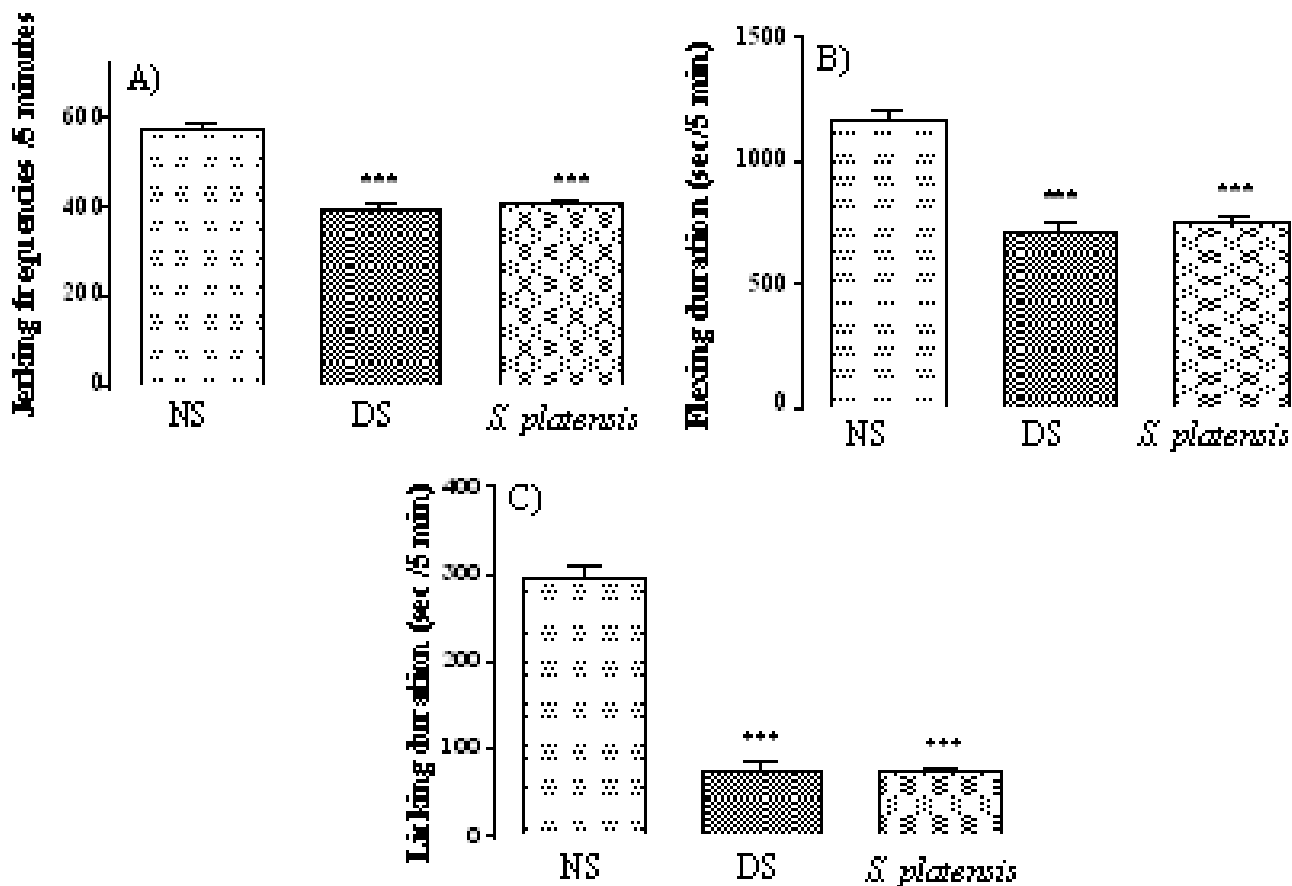


Fig. 4: Analgesic effect of *S. platensis* (*Spirulina platensis*, 400 mg/kg body weight) and positive control, DS (diclofenac sodium, 10 mg/kg body weight) in comparison to negative control, NS (normal saline, 5 ml/kg body weight) in late phase of formalin test (16th to 60th) assessed by jerking (A), flexing (B) and licking (C). Each bar symbolized for mean \pm SE of 7 rats in each group. ***Significant ($P \leq 0.001$) in comparison to negative control.

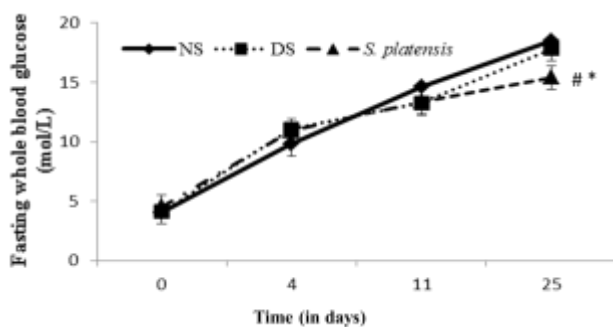


Fig. 5: Anti-hyperglycemic effect of *S. platensis* (*Spirulina platensis*, 400mg/kg body weight) in comparison to positive control, DS (diclofenac sodium, 10mg/kg body weight) and negative control, NS (normal saline, 5ml/kg body weight) in fasting whole blood glucose level. Each line symbolized for mean \pm SE of 7 rats in each group. *Significant ($P \leq 0.05$) in comparison to NS, #Significant ($P \leq 0.05$) in comparison to DS.

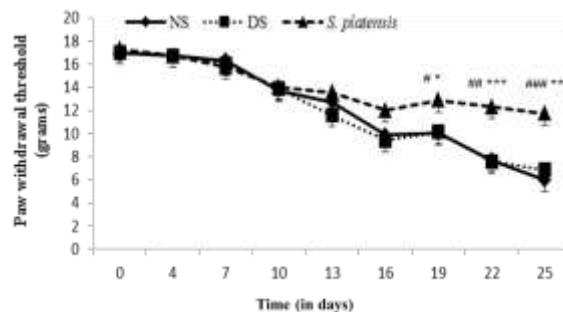


Fig. 6: Analgesic effect of *S. platensis* (*Spirulina platensis*, 400 mg/kg body weight) in comparison to positive control, DS (diclofenac sodium, 10 mg/kg body weight) and negative control, NS (normal saline, 5 ml/kg body weight) in paw withdrawal threshold of Von Frey test. Each line symbolized for mean \pm SE of 7 rats in each group. */***Significant ($P \leq 0.05$ / $P \leq 0.001$) in comparison to NS; #/##/###Significant ($P \leq 0.05$ / $P \leq 0.01$ / $P \leq 0.001$) in comparison to DS.

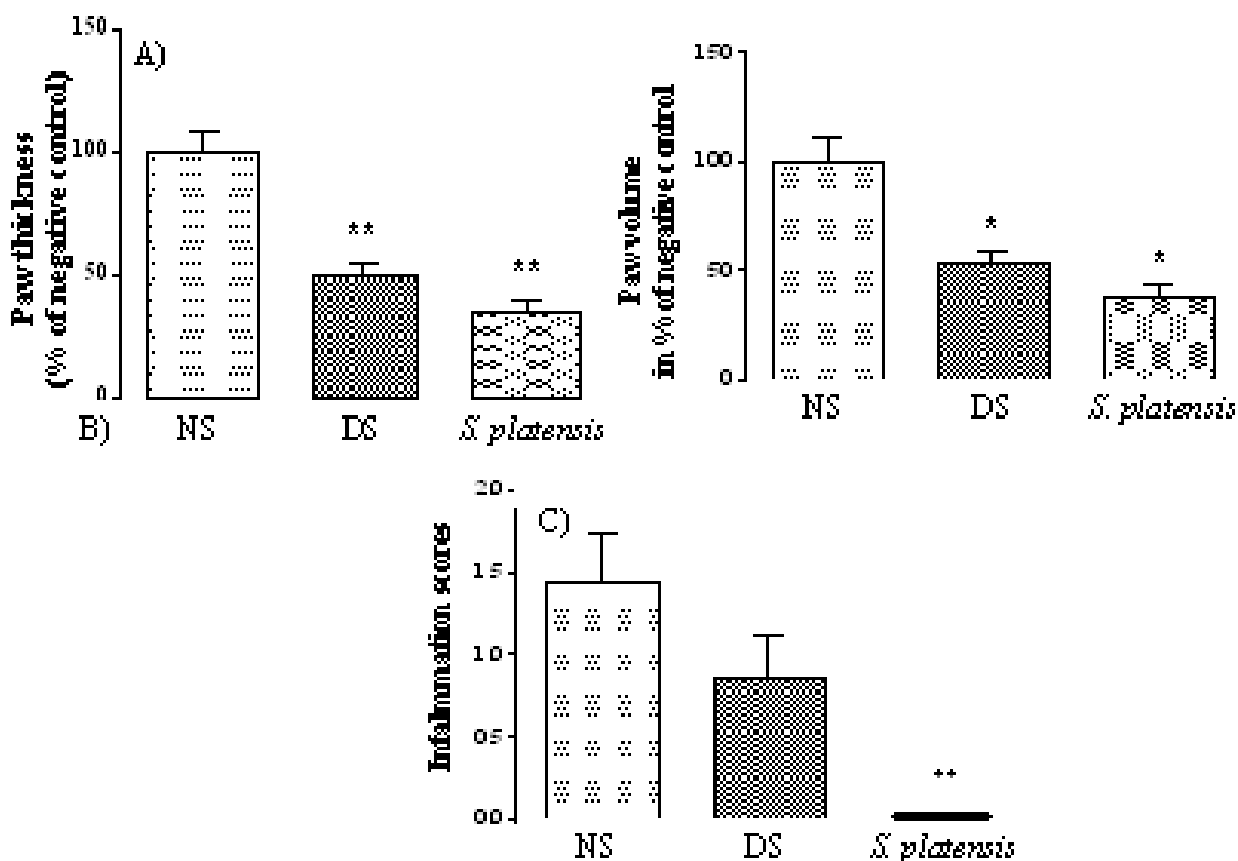


Fig. 7: Anti-inflammatory effects of *S. platensis* (*Spirulina platensis*, 400 mg/kg body weight) in comparison to positive control, DS (diclofenac sodium, 10 mg/kg body weight) and negative control, NS (normal saline, 5 ml/kg body weight) in paw thickness (A), paw volume (B) and inflammation score (C) in paw edema test. Each bar symbolized for mean \pm SE of 7 rats in each group. **/***Significant ($P \leq 0.05$ / $P \leq 0.01$) in comparison to negative control.

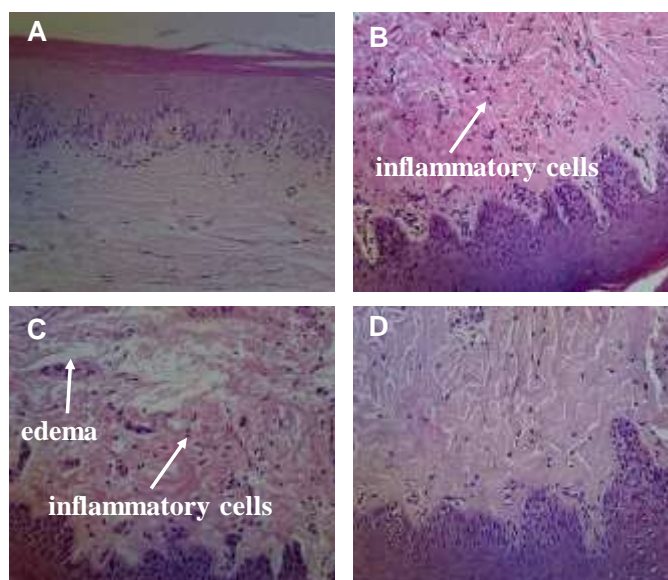


Fig. 8: Anti-inflammatory effects of *S. platensis* (*Spirulina platensis*, 400 mg/kg body weight) in comparison to positive control, DS (diclofenac sodium, 10 mg/kg body weight) and negative control, NS (normal saline, 5 ml/kg body weight) in Hematoxylin and Eosin stained paw tissues. (A) Left paw, (B) Formalin induced right paw after NS, (C) Formalin induced right paw after DS, and (D) Formalin induced right paw after *S. platensis*. All figures were magnified by 20X. The arrows indicate inflammatory cells and edema.

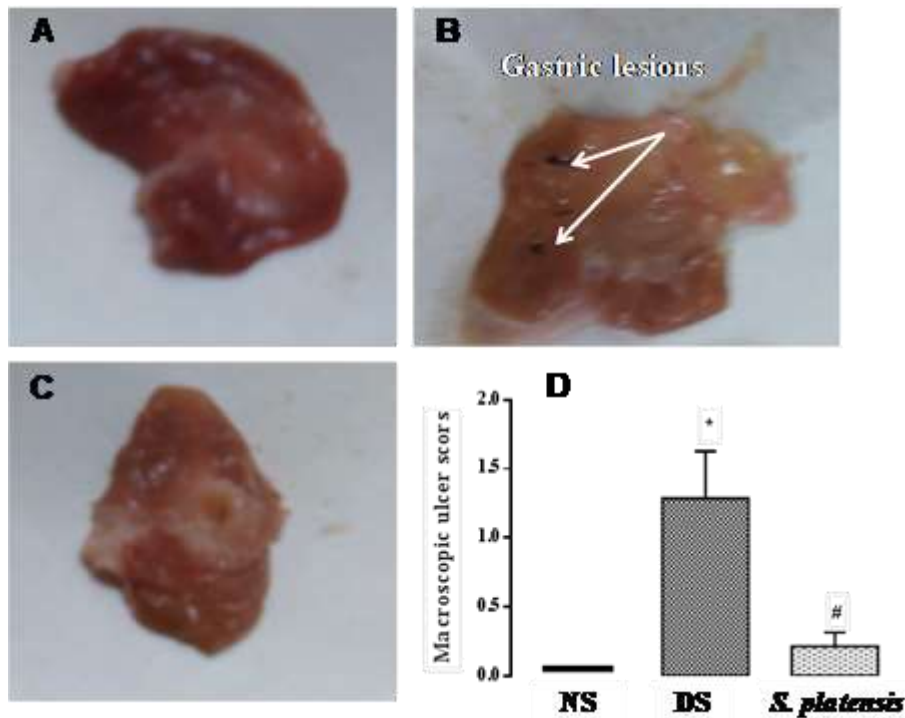


Fig. 9: Anti-ulcerogenic effects of *S. platensis* (*Spirulina platensis*, 400mg/kg body weight) in comparison to positive control, DS (diclofenac sodium, 10mg/kg body weight) and negative control, NS (normal saline, 5ml/kg body weight) in macroscopic (naked eye) examination of gastric ulcer. Morphological appearance was magnified by 2X. Gastric ulcer morphological appearance (A) NS, (B) DS, (C) *S. platensis*, and (D) Macroscopic scoring. The arrows indicate gastric lesions. Each bar symbolized for mean±SE of 7 rats in each group. *Significant ($P \leq 0.05$), in comparison to negative control; #Significant ($P \leq 0.05$), in comparison to positive control.

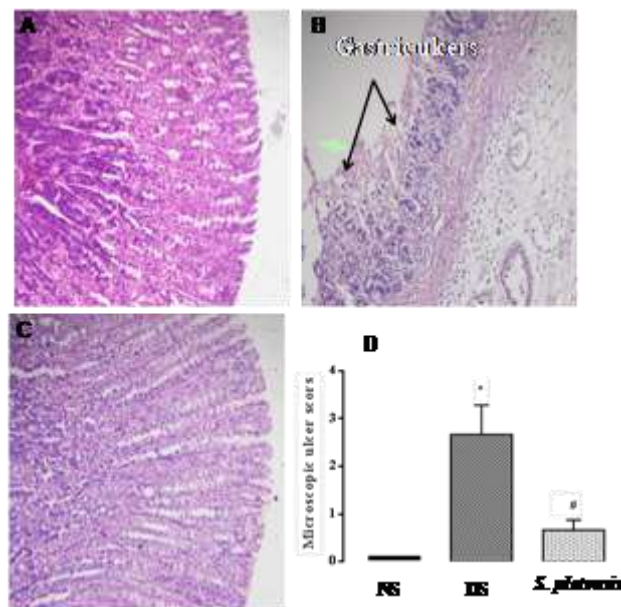


Fig. 10: Anti-ulcerogenic effects of *S. platensis* (400 mg/kg body weight) in comparison to positive control, DS (diclofenac sodium, 10 mg/kg body weight) and negative control, NS (normal saline, 5 mg/kg body weight) in microscopic examination of gastric ulcer in Hematoxylin and Eosin stained stomach tissues. Histological appearance (A) NS, (B) DS, (C) *S. platensis* and (D) Microscopic scoring. Histological figures were magnified by 20X. The arrow indicates gastric lesions. Each bar symbolized for mean±SE of 7 rats in each group. *Significant ($P \leq 0.05$), in comparison to negative control; #Significant ($P \leq 0.05$) in comparison to positive control.

Experimental design

On the basis of oral treatments, all the rats were divided into group I (negative control, only NS for 21 consecutive days, n=21), group II (positive control, DS for 7 consecutive days, n=21) and group III (experimental, *S. platensis* for 21 consecutive days, n=21). On the basis of tests applied, rats in each group were again divided into subgroup 'a' (tail immersion test, n=7), 'b' (formalin test, paw edema test, n=7) and 'c' (Von Frey test, n=7). One hour after the last dose of oral treatment, all the rats were subjected to the above mentioned pain and inflammatory tests.

Tail immersion test

Tail immersion test procedure was performed according to previously published procedures (Le Bars *et al.*, 2001; Vogel *et al.*, 2002; Lin *et al.*, 2005; Steinmiller *et al.*, 2007; Ahmadi *et al.*, 2010; Hasani *et al.*, 2011; Pandhare *et al.*, 2012). After 7 days of room environmental acclimatization, 21 rats were separated for tail immersion test [Ia (7), IIa (7), IIIa (7)]. Each rat was placed in a plexiglass mechanical restraining cage for 10 minutes/day, for another 7 consecutive days, for instrumental acclimatization. Then on the very first day of study, at 8.00 am, each rat was kept individually in that cage for 5 minutes for adjustment with the tail hanging freely. Then a 500ml glass beaker containing 400ml of water (preheated to 52±0.1°C) was taken and a laboratory thermometer was placed inside. The distal 5cm of the freely hanging tail of the rat was immersed into that preheated water and latency period of the tail-flick was recorded. The mean of the measurements obtained from 3 similar consecutive maneuvers, performed at 5 minutes interval, was taken as the baseline latency (BL). Then on the final experiment day (at day 7 or 21), just one hour after the last dose of treatment (NS/ DS/ *S. platensis*), another tail immersion test was done and mean of 3 tail withdrawal latencies at 5 minutes interval was recorded as test latency (TL). To reduce the tissue damage, a highest latency of 15 seconds was considered as cut off time. The antinociception effect was expressed as percentage of the maximum possible effect (% MPE) as follows:

$$\%MPE = \left[\frac{TL - BL}{\text{Cut off time} - BL} \times 100 \right]$$

Formalin test

Formalin test procedure was performed according to the methods of previous publications (Abbott *et al.*, 1995; Yashpal and Coderre, 1995; Franca *et al.*, 2001; Ali *et al.*, 2012). After 7 days of room environmental acclimatization, 21 rats were separated for formalin test [Ib (7), IIb (7), IIIb (7)]. Then each rat was placed in the observation cage of the plexiglass formalin test box (34X34X34 cm³) for 1 hour daily for 7 consecutive days, for instrumental acclimatization. Then on the day of experiment (at day 7 or 21), just one hour after the last dose of treatment (NS/ DS/ *S. platensis*), fifty (50) µl of

dilute formalin (2.5%) was injected subcutaneously into the exposed planter aspect of the restrained rat's right hind paw, with an insulin syringe. Then the rat's pain behaviors were observed for following 60 minutes placing it into the observation cage of the formalin box. This 60 minutes observation period was recorded as, the early phase [first 5 minutes (1st to 5th)], the interphase [middle 10 minutes (6th to 15th)] and the late phase [last 45 minutes (16th to 60th)]. With the help of a mirror fixed below the observation cage (at 45^o angle), observation was made by counting (with a stop watch) the total frequency of jerking, total duration of flexing and total duration of licking of the injected paw during this period.

Formalin induced paw edema test

Anti-inflammatory effects of DS and *S. platensis* were determined in the rats of subgroup Ib (7), IIb (7) and IIIb (7) by formalin induced paw edema model (Ali *et al.*, 2012). Here the indicator of inflammation severity was assessed by the amount of paw edema resulted from subcutaneous injection of 50µl of 2.5% formalin in the planter aspects of rats' right hind paw.

Edema thickness (Lee and Jeong, 2002; John and Shobana, 2012): Before the administration of subcutaneous formalin in the right hind paw, each rat was placed in a large glass desiccator and 3 to 4 ml of di-ethyl ether (99%) was poured into it. For the following 5 to 10 minutes the rat was observed closely and the ventilation was maintained by intermittent opening and closing of the desiccator's lid. Then anesthetized state was assessed by observing regular slow and deep breathing pattern and its well-being was assured by apex beat palpation. Then the paw thickness of both hind paws of the anesthetized rat was measured by a simple measuring tape and the basal paw thickness difference was determined by subtracting the left paw thickness from the right paw one. Similar maneuvers was done 3 hours after subcutaneous administration of dilute formalin (50µl; 2.5%) in the right hind paw and test paw thickness difference was calculated. Then the edema thickness was determined by subtracting the *baseline* value from the *test* one.

Basal paw thickness difference = before formalin, paw thickness (right – left)

Test paw thickness difference = after formalin, paw thickness (right – left)

Edema thickness = paw thickness difference (test – basal)

Edema volume (Ferridoni *et al.*, 2000; Franca *et al.*, 2001; Ali *et al.*, 2012): After completion of the test paw thickness measurement, each rat was again placed in the large glass desiccator and about 10ml of di-ethyl ether (99%) was poured to anaesthetize it deeply. Death of this deeply anesthetized rat was ensured by decapitation. Then both the hind paws of the sacrificed rat were cut just above the ankle joints (4cm from tip toe) by a sharp scissor. A water plethysmo meter was used to measure the volumes of animal's paws and the edema volume was

determined by subtracting the left paw volume from right one.

Left paw volume = height of water with left paw (after immersion – before immersion)

Right paw volume = height of water with right paw (after immersion – before immersion)

Edema volume = paw volume (right – left)

Paw histology (Amborgi, 1960; Hussein *et al.*, 2013; Ma *et al.*, 2013): After edema thickness and edema volume measurement, both hind paws were preserved in 10% formalin and paw histology was done with hematoxylin-eosin staining as described by Amborgi (1960). Histopathological scoring of paw tissues were rated according to Hussain *et al.* (2013) and Ma *et al.* (2013): score 0= no inflammation (no polymorphonuclear infiltration and no edema); score 1= mild inflammation (mild polymorphonuclear infiltration and mild edema); score 2= moderate inflammation (moderate polymorphonuclear infiltration and moderate edema); score 3= severe inflammation (severe polymorphonuclear infiltration and severe edema).

Von frey test

Von Frey test procedure was performed according to the previously described method (Rafiq *et al.*, 2009; Detloff *et al.*, 2010; Kumar *et al.*, 2010; Akinola *et al.*, 2012; Detloff *et al.*, 2014; Loubano-Voumbi *et al.*, 2015). After 7 days of room environmental acclimatization, 21 rats were separated for Von Frey test [Ic (7), IIc (7), IIIc (7)]. All of the rats were then placed in a wide gauge wire mesh surface for 1 hour daily, for 7 consecutive days, for instrumental acclimatization. At the 3rd day of instrumental acclimatization, each rat was treated with single intraperitoneal injection of alloxan monohydrate 150mg/kg to induce diabetes mellitus, which was ensured biochemically by checking increased fasting whole blood glucose (>150mg/dl or 8.3mmol/l), at the 4th to 5th day of induction (6th to 7th day of instrumental acclimatization).

Then the hyperglycemic rats (>150mg/dl or 8.3mmol/l) were treated with either NS (n=7; for 21 days) or DS (n=7; for 7 days) or *S. platensis* (n=7; for 21 days) orally. At the day of experiment (day 7 or 21), just 1 hour after the last dose of treatment, each rat was placed individually on the wide gauge wire mesh surface. Then the calibrated Von Frey filaments (VFF) was touched in an ascending order on planter surface of both hind paws of the rat between first and second metatarsal approximately one cm proximal to the ankle joint.

Each VFF (of varying tensile strengths of 2 to 18gm) was applied three times at 30 seconds intervals and the number of hind paw withdrawal was recorded. The next larger VFF was applied unless paw withdrawal occurred in at least two of the three. If the rat failed to withdrawal

its paw at maximum force of 18gm, then no more VFF (of increased tensile strength) was applied, to prevent tissue injury.

Examination of gastric lesions

Macroscopic examination (Vogel *et al.*, 2002; Adinortey *et al.*, 2013; Morjan *et al.*, 2013): After sacrifice, we made a surgical incision through the linea alba of each rat to collect stomach tissue. We placed the stomach tissue on a filter paper (saline soaked) to make an incision longitudinally along its greater curvature. Observations were made for presence or absence of gastric lesions which we scored according to the methods of Adinortey *et al.* (2013): score 0= normal colored stomach; score 0.5= red coloration; score 1= spot ulcers; score 1.5= hemorrhagic streaks; score 2= ulcer with area > 3 but < 5mm².

Microscopic/ histopathological examination: We used the methods of Amborgi (1960) and Morzan *et al.*, (2013). After naked eye examination, the stomach was preserved in 10% formalin and gastric histology was done with hematoxylin-eosin staining as described by Amborgi (1960). Histopathological scoring of gastric lesions was rated according to Morjan *et al.* (2013): score 0 = no mucosal lesions; score 1 = mucosal edema, congestion and neutrophilic infiltration; score 2 = surface mucosal erosion; score 3 = > 2 gastric ulcers; score 4 = > 2 gastric ulcers.

STATISTICAL ANALYSIS

All the data were expressed as mean ± SEM. One-way analysis of variance (One-way ANOVA), followed by Bonferroni post hoc test, was used to analyze the data statistically, by the help of analytical software Graphpad Prism (Version 6). In the interpretation of results, $P \leq 0.05$ was considered as the level of significance.

RESULTS

Tail immersion test

We evaluated the effect of *S. platensis* on pain by tail immersion test, as an acute somatosensory model of nociception pain. As shown in fig. 1, *S. platensis* increased the tail flick latency significantly ($P \leq 0.01$) in comparison to that of negative control, however, this result was not significant when compared to positive control

Formalin test

The effects of oral administration of *S. platensis* on the early (1st to 5th minutes), inter- (6th to 16th minutes) and late (16th to 60th minutes) phases of formalin test, as model of nociception, central analgesic activity and inflammatory pain, were observed. The pain behaviors were separately analyzed as total frequency of jerking,

total duration of licking and total duration of flexing in all phases. fig. 2 and 4, in early and late phases of formalin test, *S. platensis* showed significant ($P \leq 0.001$) analgesia of all 3 variables in comparison to those of negative controls, however, these results are not significant when compared to positive control.

In addition, this medicinal herb also showed significant ($P \leq 0.05$) lowering of jerking in interphase in comparison to negative control as showed in fig. 3. However, there was no difference in comparison to flexing and licking.

Von frey test

The effect of oral administration of *S. platensis* on tactile allodynia in the Von Frey test was observed. Here paw withdrawal threshold of alloxan induced diabetic rats (fig. 5) was analyzed as a variable of neuropathic pain, as mentioned in fig. 6. In this study, the paw withdrawal threshold was significantly higher in *S. platensis* treated rats in comparison to those of positive as well as of negative controls, on day 19 ($P \leq 0.05$), 22 ($P \leq 0.01$) and 25 ($P \leq 0.001$), as presented in fig. 6.

Formalin induced paw edema test

We assessed the effect of oral administration of *S. platensis* on the amount of edema formed in paw, 3 hours after formalin injection, as a model of inflammation. Here the inflammatory changes were separately analyzed as edema thickness, edema volume and histological inflammation score. As illustrated in fig. 7 and 8, *S. platensis* showed significant lowering of paw thickness ($P \leq 0.01$), paw volume ($P \leq 0.05$) as well as histological inflammation score ($P \leq 0.01$) in the paw edema model in comparison to those of negative controls. However, this lowering of all three inflammatory variables was not significant when compared to positive control.

Gastric ulcer

We also observed this medicinal herb's effect on gastric ulcer, by naked eye as well as histopathological examinations of stomach. Here ulcer scores were significantly lower ($P \leq 0.05$) in *S. platensis* treated group in comparison to those of positive controls in both examinations, as presented in fig. 9 and 10. The differences were not significant when compared to negative control.

DISCUSSION

Pain management is one of the most common and yet difficult tasks in clinical practice. Many potent pain medications and anti-inflammatory agents have been developed that possess adverse effects, resulting to search for safer conceptual innovations (Rainsford *et al.*, 2008). Our data demonstrated that *S. platensis* could elicit strong analgesia in rats subjected to the acute thermal (nociception), persistent chemical (nociception and

inflammatory) as well as mechanical (neuropathic) pain stimuli and strong anti-inflammation in chemical induced paw edema model.

The formalin test for the assessment of nociception and inflammatory pain in animal model signifies an early phase (the first 5 minutes) by direct activation of nociceptors, an interphase (middle 10 minutes) due to the activation of the analgesic system in brain stem and spinal cord, and a late phase (last 45 minutes) manifestation of pain due to indirect activation of nociceptors by inflammatory mediators (Henry *et al.*, 1999). The tail immersion test assesses nociception pain behaviors in animal models, which is among the common and useful methods (Steinmiller and Young, 2007; Ahmadi *et al.*, 2010) to elucidate the central nervous system (CNS) mediated antinociception responses, which signifies mainly the changes above the spinal cord level. In the present study *S. platensis* exerted its analgesic effect against nociception pain either by modulating the pain transmission in the CNS or by inhibiting the pain perception at peripheral nociceptor level. Though the precise mechanism of our findings cannot be explained from our study, but it has been reported that, *S. platensis* might enhance serotonin and GABA activity in the CNS (Alam and Hendawi, 2015) to activate the inhibitory interneurons or post synaptic neurons in the spinal cord (Bardin, 2011; Hall, 2016). Moreover, in the present study, there was significant reduction of jerking frequency in the interphase of formalin test following the use of *S. platensis* in comparison to that of negative control, as shown in fig. 3A. As interphase (6th to 15th minutes) of formalin test has been suggested to be due to active inhibition of the pain pathway mediated by spinal mechanisms (Henry *et al.*, 1999), so it may be proposed that this medicinal herb may also cause analgesia against nociception pain by inhibiting the spinal pathway of pain perception.

In the present study, administration of *S. platensis* significantly lowered inflammation as well as inflammatory pain when compared to that of negative control as evidenced by lowering the edema thickness, edema volume and histological inflammation score of paw, in addition to jerking, flexing and licking in the late phase (16th to 60th minutes) of formalin test. These findings were in consistent with the findings of other investigators of different countries (Kumar *et al.*, 2010; Pak *et al.*, 2012; Hwang *et al.*, 2013; Quader *et al.*, 2013; Elgendy and Diam, 2014; Neekhara *et al.*, 2014; Patro *et al.*, 2014; Ali *et al.*, 2015). Though we could not explain the exact mechanism of our findings from this study, but it has been suggested by other researchers that *S. platensis* has the ability to scavenge free radicals and increase glutathione level (Romey *et al.*, 1998; Bhata and Madyasthab, 2000; Riss *et al.*, 2007; Hassanen *et al.*, 2015), to inhibit peroxynitrite (ONOO⁻) and superoxide in mitochondria

(Bhata and Madyasthab, 2001; Zheng *et al.*, 2013), which inhibits expression of N-methyl-D-aspartate (NMDA) receptor, tumor necrosis factor- α (TNF- α), interleukin-1 α (IL-1 α) and cyclooxygenase-2 (COX-2) genes in different inflamed tissues (Kumar *et al.*, 2010; Hwang *et al.*, 2013), to suppress the inducible nitric oxide synthase (iNOS) followed by decreased NO production, that modulates the c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) pathways (Li *et al.*, 2002; Khan *et al.*, 2005). So, it may be proposed that this medicinal herb may cause anti-inflammation and inflammatory analgesia in our study by any of the above-mentioned mechanisms.

For the assessment of neuropathic pain in rodents, Von Frey test is a standard method, where tactile allodynia is assessed by paw withdrawal threshold (Lambert *et al.*, 2009; de Sousa *et al.*, 2014). In our study we remarkably noticed that, oral administration of *S. platensis* could reduce the tactile allodynia in neuropathic pain in diabetic rats, as evidenced by significantly higher paw withdrawal threshold in the experimental group compared to those of positive as well as negative controls, after 19 day of diabetes induction (fig. 6). To the best of our acquaintance, this is the first data showing analgesic effect of *Spirulina platensis* on neuropathic pain in rats. However, no relevant study was available to support or explain our observations regarding *S. platensis*. Only Patro *et al.* (2014) had proposed that *S. platensis* may suppress the peripheral sensitization via modulation of glial activation in spinal cord, which might be the cause of our exceptional observation of this herb's analgesic effect on neuropathic pain. The anti-hyperglycemic role of this medicinal herb (El-Baz *et al.*, 2013) may potentiate its analgesic effect on neuropathic pain due to diabetes mellitus, as shown in our study.

Another mentionable finding of our study was that, the 21 days *S. platensis* administration was as effective as 7 days DS administration in lowering nociception pain, inflammatory pain, neuropathic pain and inflammation, as indicated by non significant differences of all of their variables between experimental group and positive controls, as shown in fig. 1 to 8.

Interestingly, in our study, 21 days oral *S. platensis* did not produce any gastric ulcer, whereas 7 days oral DS did, as evidenced by macroscopic as well as microscopic examination of stomach (fig. 9 and 10). As far our knowledge goes (similar to our finding on neuropathic pain) this is the first data showing anti-ulcerogenic effect of this nutritional herb. However, no relevant study was available to support as well as to explain our observation regarding this beneficial effect of *S. platensis*.

CONCLUSION

S. platensis could reduce pain (nociception, inflammatory, neuropathic) and inflammation and its analgesic and anti-

inflammatory effects after 21 days administration is as effective as 7 days diclofenac sodium administration. In addition, 21 days administration of *S. platensis* did not show ulcerogenic effect however, 7 days administration of diclofenac sodium showed ulcerogenic effect. Although, further studies are required in order to determine the exact component and molecular mechanism responsible for these beneficial effects of *Spirulina platensis*.

ACKNOWLEDGEMENTS

Authors would like to thank Ms. Tahmina Yeasmin of the Department of Anatomy, Faculty of Basic medical and Paraclinical sciences, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, for technical support.

Ethics approval

The protocol for the *in vivo* experiments has been approved by the Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

REFERENCES

- Abbott FV, Franklin KB and Westbrook RF (1995). The formalin test scoring properties of the first and second phases of the pain response in rats. *Pain*, **60**(1): 91-102.
- Abbott FV and Fraser MI (1998). Use and abuse of over-the-counter analgesic agents. *J. Psychiatr. Neurosci.*, **23**(1): 13-34.
- Abdel-Daim MM, Abuzead SM, Halawa SM (2013). Protective role of *Spirulina platensis* against acute deltamethrin-induced toxicity in rats. *PLoS ONE*, **8**(9): 1-7.
- Adinortey MB, Ansah C, Galyunon I and Nyarko A (2013). In vivo models used for evaluation of potential antigastrointestinal ulcer agents. *Ulcers* 796405.doi: 10.1155/2013/796405.
- Ahmadi A, Khalili M, Hajikhani R, Barghi L and Mihandoust F (2010). Synthesis and determination of chronic and acute thermal and chemical pain activities of a new derivative of phencyclidine in rats. *Iran. J. Pharm. Res.*, **9**(4): 379-385.
- Akinola O, Gabriel M, Suleiman AA and Olorunsogbon F (2012). Treatment of alloxan-induced diabetic rats with metformin or glitazones is associated with amelioration of hyperglycemia and neuroprotection. *Open Diabetes J.*, **5**: 8-12.
- Alam RTM and Hendawi MY (2015). Protective efficacy of *Spirulina platensis* against cadmium induced neurotoxicity in rats. *Global Veterinaria*, **14**(4): 490-499.
- Ali EA, Barakat BM and Hassan R (2015). Antioxidant and angiostatic effect of *Spirulina platensis* suspension

- in complete Freund's adjuvant-induced arthritis in rats. *PLoS ONE*, **10**(4): 1-13.
- Ali T, Javan M, Sonboli A and Semnianian S (2012). Evaluation of the antinociception and anti-inflammatory effects of essential oil of *Nepeta pogonosperma* Jamzad et Assadi in rats. *DARU* **20**(1): 1-8.
- Ambrogi LP (1960). Manual of histologic and special staining techniques. 2nd ed. London (Blakiston Division): McGraw-Hill.
- Anand P and Bley K (2011). Topical capsaicin for pain management: Therapeutic potential and mechanisms of action of the new high-concentration capsaicin 8% patch. *Br. J. Anaesth.*, **107**(4): 490-502.
- Bardin L (2011). The complex role of serotonin and 5-HT receptors in chronic pain. *Behav. Pharmacol.*, **22**(5-6): 390-404.
- Barrot M (2012). Tests and models of nociception and pain in rodents. *Neuroscience*, **211**: 39-50.
- Bhata VB and Madyasthab KM (2000). C-Phycocyanin: A potent peroxy radical scavenger *in vivo* and *in vitro*. *Biochem. Biophys. Res. Commun.*, **275**(1): 20-25.
- Bhata VB and Madyasthab KM (2001). Scavenging of peroxynitrite by phycocyanin and phycocyanobilin from *Spirulina platensis* protection against oxidative damage to DNA. *Biochem. Biophys. Res. Commun.*, **285**(2): 262-266.
- Campbell JN and Meyer RA (2006). Mechanisms of neuropathic pain. *Neuron*, **52**(1): 77-92.
- Chen YH, Chang GK, Kuo SM, Huang SY, Hu IC, Lo YL and Shih SR (2016). Well-tolerated *Spirulina* extract inhibits influenza virus replication and reduces virus-induced mortality. *Sci. Rep. Apr.*, **12**: 6: 24253.
- Cingi C, Conk-Dalay M, Cakli H and Bal C (2008). Effects of *Spirulina* on allergic rhinitis. *Eur. Arch. Otorhinolaryngol.*, **265**(10): 1219-1223.
- De Oliveira MACL, Monteiro MPC, Robbs PG and Leite SGF (1999). Growth and chemical composition of *Spirulina maxima* and *Spirulina platensis* biomass at different temperatures. *Aquacult. Inter.*, **7**(4): 261-275.
- De Sousa MVP, Ferraresia C, de Magalhaesb AC, Yoshimura EM and Hamblin MR (2014). Building, testing and validating a set of home-made von Frey filaments: A precise, accurate and cost effective alternative for nociception assessment. *J. Neurosci. Methods*, **232**: 1-5.
- Detloff MR, Clark LM, Hutchinson KJ, Kloos AD, Fisher LC and Basso DM (2010). Validity of acute and chronic tactile sensory testing after spinal cord injury in rats. *Exp. Neurol.*, **225**(2): 366-376.
- Detloff MR, Smith EJ, Molina DQ, Ganzer PD and Houle JD (2014). Acute exercise prevents the development of neuropathic pain and the sprouting of non-peptidergic (GDNF- and artemin-responsive) c-fibers after spinal cord injury. *Exp. Neurol.*, **255**: 38-48.
- El-Baz F, Aly HF, El-Sayed AB and Mohamed AA (2013). Role of *Spirulina platensis* in the control of glycemia in DM2 rats. *Int. J. Sci. Enginer. Res.*, **4**(12): 1731-1739.
- Elgendy H and Diam AM (2014). Antinociception effect of *Spirulina platensis* in streptozotocin induced diabetic rats: 14AP6-6. *European. J. Anaesthe.*, **31**: 230.
- Eli I and Svensson P (2009). The multi-dimensional nature of pain. In: Bergenholtz G, Horsted-Binslev P, Reit C, editors. Textbook of endodontology. Chichester:iley-Blackwell. pp. 277-289.
- Ferridoni M, Ahmadiani A, Semnianian S and Javan M (2000). An accurate and simple method for measurement of paw edema. *J. Pharmacol. Toxicol. Methods*, **43**(1): 11-14.
- Franca DS, Souza ALS, Almeida KR, Dolabella SS, Martinelli C and Coelho MM (2001). B vitamins induce an antinociception effect in the acetic acid and formaldehyde models of nociception in mice. *Eur. J. Pharmacol.*, **421**(3): 157-164.
- Gaurav D, Preet S and Dua KK (2010). Protective effect of *Spirulina platensis* on cadmium induced renal toxicity in wistar rats. *Archives. App. Sci. Res.*, **2**(1): 390-397.
- Golechha M, Sarangal V, Ojha S, Bhatia J and Arya DS (2014). Anti-inflammatory effect of *Embllica officinalis* in rodent models of acute and chronic inflammation: involvement of possible mechanisms. *Inte. J. Inflamma.*, 178408. doi:10.1155/2014/178408.
- Grosser T, Symth E and Fitzgerald GA (2011). Anti-inflammatory, anti-pyretic and analgesic agents; pharmacotherapy in Gout. In: Brunton LL, Chabner BA, Knollmann BC, editors. Goodman and Gilman's: The pharmacological basis of therapeutics. 12th ed. McGraw-Hill medical publishing division. pp.960-1005.
- Gupta AK, Parasar D, Sagar A, Choudhary V, Chopra BS, Garg R, Ashish and Khatri N (2015). Analgesic and anti-inflammatory properties of gelsolin in acetic acid induced writhing, tail immersion and carrageenan induced paw edema in mice. *PLoS ONE*, **10**(8): 1-16.
- Habib MAB, Parvin M, Huntington TC and Hasan MR (2008). Review on culture, production and use of *Spirulina* as food for humans and feeds for domestic animals and fish. FAO Fisheries and Aquaculture Circular no. **1034**: 1-33.
- Hall JE (2016). Textbook of Medical Physiology. 13th ed. Pennsylvania: Elsevier Saunders.
- Hassanen MR, Mahfouz MK, Farid AS and Fadlullah AH (2015). Biochemical effects of *Spirulina platensis* against oxidative stress caused by doxorubicin. *Benha. Veter. Med. J.*, **28**(2): 147-154.
- Hasani A, Soljakova M, Jakupi M and Ustalar-Ozgen S (2011). Preemptive analgesic effect of midazolam and diclofenac in rat model. *Bosn. J. Basic. Med. Sci.*, **11**(2): 113-118.
- Hayashi O, Katoh T and Okuwaki Y (1994). Enhancement of antibody production in mice by

- dietary *Spirulina platensis*. *J. Nutr. Sci. Vitaminol.*, **40**(5): 431-441.
- Henrikson R (2009). Earth food *Spirulina*. 7th ed. Hawaii.
- Henry JL, Yashpal K, Pitcher GM and Coderre TJ (1999). Physiological evidence that the 'interphase' in the formalin test is due to active inhibition. *Pain*, **82**(1): 57-63.
- Hussein SZ, Mohd Yusoff K, Makpol S and Mohd Yusof YA (2013). Gelam honey attenuates carrageenan-induced rat paw inflammation via NF-kB pathway. *PLoS ONE* **8**(8): e72365.
- Hwang JH, Chen JC and Chan YC (2013). Effects of C-phycocyanin and *Spirulina* on salicylate-induced tinnitus, expression of NMDA receptor and inflammatory genes. *PLoS ONE*, **8**(3): e58215.
- Islam KMN, Rahman ASM and Al-Mahmud KA (2001). Manual for care and use of laboratory animals. Animal research branch. icddr,b, Dhaka.
- John NAA and Shobana G (2012). Anti-inflammatory activity of *Talinum fruticosum* L. on formalin induced paw edema in albino rats. *J. Appl. Pharma. Sci.*, **2**(1): 123-127.
- Joventino IP, Alves HG, Neves LC, Pinheiro-Joventino F, Leal LK, Neves SA, Ferreira FV, Brito GA and Viana GB (2012). The Microalga *Spirulina platensis* presents anti-inflammatory action as well as hypoglycemic and hypolipidemic properties in diabetic rats. *J. Complement. Integr. Med.* **9**: Article 17. doi: 10.1515/1553-3840.1534.
- Julius D and Basbaum AI (2001). Molecular mechanisms of nociception. *Nature*, **413**(6852): 203-210.
- Khan M, Shobha JC, Mohan IK, Naidu MU, Sundaram C, Singh S, Kuppusamy P and Kutala VK (2005). Protective effect of *Spirulina* against doxorubicin-induced cardiotoxicity. *Phytother. Res.*, **19**(2): 1030-1037.
- Kumar N, Kumar P and Singh S (2010). Immunomodulatory effect of dietary *Spirulina platensis* in type II collagen induced arthritis in rats. *Res. J. Pharmaceu. Biolo. Chem. Sci.*, **1**(4): 877-885.
- Kumar N, Laferriere A, Yu JAC, Leavitt A and Coderre TJ (2010). Evidence that pregabalin reduces neuropathic pain by inhibiting the spinal release of glutamate. *J. Neurochem.*, **113**(2): 552-561.
- Lambert GA, Mallos G and Zagami AS (2009). Von Frey's hairs - a review of their technology and use - a novel automated von Frey device for improved testing for hyperalgesia. *J. Neurosci. Methods*, **177**(2): 420-426.
- Le Bars D, Gozariu M and Cadden SW (2001). Animal models of nociception. *Pharmacol. Rev.*, **53**(4): 597-652.
- Lee IO and Jeong YS (2002). Effects of different concentrations of formalin on paw edema and pain behaviors in rats. *J. Korean. Med. Sci.*, **17**(1): 81-85.
- Li XL, Xu G, Chen T, Wong YS, Zhao HL, Fan RR, Gu XM, Tong PC and Chan JC (2009). Phycocyanin protects INS-1E pancreatic beta cells against human islet amyloid polypeptide induced apoptosis through attenuating oxidative stress and modulating JNK and p38 mitogen-activated protein kinase pathways. *Int. J. Biochem. Cell. Biol.*, **41**(7): 1526-1535.
- Lin JA, Lee MS, Wu CT, Yeh CC, Lin SL, Wen ZH and Wong CS (2005). Attenuation of morphine tolerance by intrathecal gabapentin is associated with suppression of morphine-evoked excitatory amino acid release in the rat spinal cord. *Brain Res.* **1054**(2): 167-173.
- Liu YF, Xu LZ, Cheng N, Lin LJ and Zhang CG (2000). Inhibitory effect of phycocyanin from *Spirulina platensis* on the growth of human leukemia K562 cells. *J. Appl. Phycol.* **12**(2): 125-130.
- Loubano-Voumbi G, Diaw M, Ouedraogo V, Sow AK, Seck A, Boumba LMA, Ba A and Samb A (2015). Molecular analysis of *Clerodendrum formicarum* effects in painful diabetic neuropathy in rat. *World. J. Neurosci.*, **5**(4): 258-269.
- Ma Y, Li Y, Li X and Wu Y (2013). Anti-inflammatory effects of 4-methylcyclopentadecanone on edema models in mice. *Int. J. Mol. Sci.*, **14**(2): 23980-23992.
- Makhlouf R and Makhlouf I (2012). Evaluation of the effect of *Spirulina* against Gamma irradiation induced oxidative stress and tissue injury in rats. *Int. J. Appl. Sci. Eng. Res.*, **1**(2): 152-163.
- Mani UV, Desai S and Iyer U (2015). Studies on the long-term effect of *Spirulina* supplementation on serum lipid profile and glycosylated proteins in NIDDM patients. *J. Nutraceut. Function. Med. Food*, **2**(3): 25-32.
- Mao TK, Van de Water J and Gershwin ME (2005). Effects of a *Spirulina*-based dietary supplement on cytokine production from allergic rhinitis patients. *J. Med. Food*, **8**(1): 27-30.
- Maroon JC, Bost JW and Maroon A (2010). Natural anti-inflammatory agents for pain relief. *Surgo. Neurol. Int.*, **1**: 80.
- Mazokopakis EE, Papadomanolaki MG, Fouteris AA, Kotsiris DA, Lampadakis LM and Ganotakis ES (2014). The hepatoprotective and hypolipidemic effects of *Spirulina* (*Arthrospira platensis*) supplementation in a Cretan population with non-alcoholic fatty liver disease: a prospective pilot study. *Ann. Gastroenter.* **27**(4):387-394.
- Misbahuddin M, Islam AZMM, Khandker S, Mahmud AI, Islam N and Anjumanara (2006). Efficacy of *Spirulina* extract plus zinc in patients of chronic arsenic poisoning: A randomized placebo-controlled study. *Clini. Toxicol (Phila.)*, **44**(2):135-141.
- Moallem AS, Hosseinzadeh H and Farahi S (2008). A study of acute and chronic anti-nociception and anti-inflammatory effects of thiamine in Mice. *Iran. Biomed. J.*, **12**(3):173-178.
- Moorhead K, Capelli B and Cysewski GR (2006). *Spirulina: Nature's super food*. 1st ed. Kailua-Kona: Cyaotech Corporation, 1-14

- Morjan S, Laham SA and Atieh R (2013). Gastroprotective efficacy of folic acid and omeprazole in indomethacin-induced gastropathy in rat. *Int. J. Phamaco. Phyto. Res.*, **5**(2): 113-119.
- Neekhara S, Jain S, Jain SA, Garg NK, Jain A, Jain V, Jain P and Jain A (2014). Anti-nociception activity of *Spirulina platensis* in mice. *IJP*, **1**(8):507-510.
- Nesa L, Munira S, Mollika S, Islam MM, Choin H, Chouduri AU and Naher N (2014). Evaluation of analgesic, anti-inflammatory and CNS depressant activities of methanolic extract of *Lawsonia inermis* barks in mice. *Avicenna. J. Phytomed.*, **4**(4): 287-296.
- Pak W, Takayama F, Mine M, Nakamoto K, Kodo Y, Mankura M, Egashira T, Kawasaki H and Mori A (2012). Anti-oxidative and anti-inflammatory effects of *Spirulina* on rat model of non-alcoholic steatohepatitis. *J. Clin. Biochem. Nutr.*, **51**(3): 227-234.
- Pandhare RB, Songameswarran B, Mohite PB and Khanage SG (2012). Attenuating effect of *Sesbania sesban* (*L*) Merr extract on neuropathic pain in streptozotocin-induced diabetic rats: an evidence of neuroprotective effects. *Rev. bras. farmacogn.*, **22**(2): 190-201.
- Patro N, Sharma A, Kariaya K and Patro I (2014). *Spirulina platensis* protect neuron via suppression of glial activation and peripheral sensitization leading to restoration of motor function in collagen induced arthritic rats. *Indian. J. Exp. Biol.*, **49**(10): 739-741.
- Quader SH, Islam SU, Saifullah A, Majumder MFU and Hannan PDJ (2013). In-vivo studies of the anti-inflammatory effects of *Spirulina platensis*. *The Pharma. Innovation. J.*, **2**(4): 70-80.
- Rafiq K, Sherajee SJ, Nishiyama A, Sufiun MA and Mustofa M (2009). Effects of indigenous medical plants of Bangladesh on blood glucose level and neuropathic pain in streptozocin-induced diabetic rats. *Afr. J. Pharma. Pharmacol.*, **3**(12): 636-642.
- Rainsford KD, Kean WF and Ehrlich GE (2008). Review of the pharmaceutical properties and clinical effects of the topical NSAID formulation diclofenac epolamine. *Curr. Med. Res Opini.*, **24**(10):2967-2992.
- Refinetti R and Horvath SM (1989). Thermopreferendum of the rat: inter- and intra-subject variabilities. *Behav. Neural. Biol.*, **52**(1):87-94.
- Riss J, Decorde K, Sutra T, Delage M, Baccou JC, Jouy N, Brune JP, Oreal H, Cristol JP and Rouanet JM (2007). Phycobiliprotein C-phycoerythrin from *Spirulina platensis* powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters. *J. Agric. Food. Chem.*, **55**(19): 7962-7967.
- Romay C, Ledon N and Gonzalez R (1998). Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation. *Inflamm. Res.*, **47**(8):334-338.
- Santos LH, Feres CAO, Melo FH, Coelho MM, Nothenberg MS, Oga S and Tagliati CA (2004). Anti-inflammatory, antinociception and ulcerogenic activity of a zinc-diclofenac complex in rats. *Braz. J. Med. Biol. Res.*, **37**(8): 1205-1213.
- Scholz J and Woolf CJ (2002). Can we conquer pain? *Nat. Neurosci.*, **5** suppl: 1062-1067.
- Selmi C, Leung PS, Fischer L, German B, Yang CY, Kenny TP, Cysewski GR and Gershwin ME (2011). The effects of *Spirulina* on anemia and immune function in senior citizens. *Cell. Mol. Immunol.*, **8**(3): 248-254.
- Sharoud MNM (2015). Protective effect of *Spirulina* against paracetamol-launched hepatic injury in rats. *J. Exp. Biolo. Agri. Sci.*, **3**(1): 44-53.
- Sixabela PSS, Chivandi E, Badenhorst M and Erlwanger KH (2011). The effects of dietary supplementation with *Spirulina platensis* in growing rats. *Asian. J. Animal. Vet. Adv.*, **6**(6): 609-617.
- Steinmiller CL and Young AM (2007). Pharmacological selectivity of CTAP in a warm water tail-withdrawal antinociception assay in rats. *Psychopharmacology (Berl.)*, **195**(4):497-507.
- Subhan N, Alam MA, Ahmed F and Shahid IZ (2008). Antinociception and gastroprotective effect of the crude ethanolic extracts of *Exoecaria agallocha* linn. *Turk. J. Pharm. Sci.*, **5**(3):143-154.
- Tamaddonfard E, Samadi F and Egdami K (2013). The effects of vitamin B12 and diclofenac and their combination on cold and mechanical allodynia in a neuropathic pain model in rats. *Vet. Res. Forum.*, **4**(1):19-24.
- Tefera G, Hailu D and Tsegaye Z (2016). Importance of arthrospira [*Spirulina*] in sustainable development. *Int. J. Curr. Trend. Pharmacobiol. Med. Sci.*, **1**(2):60-68.
- Tietze HW (2004). *Spirulina: Micro Food, Macro Blessings*. 4th ed. India (New delhi): Jain Publishers.
- Vogel HG, Vogel WH, Scholkens BA, Sandow J, Muller G and Vogel WF (2002). *Drug discovery and evaluation pharmacological assay*. 2nd ed. Berlin: Springer.
- Vonshak A (1997). *Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology*. 1sted. London: Taylor and Francis Ltd., **252**
- Woolf CJ and Mannion RJ (1999). Neuropathic pain: aetiology, symptoms, mechanism and management. *Pain*, **353**(9168): 1959-1964.
- Woolf CJ, American College of Physicians, American Physiological Society (2004). Pain moving from symptom control toward mechanism-specific pharmacologic management. *Ann. Intern. Med.*, **140**(6): 441-451.
- Wu Q, Liu L, Miron A, Klimova B, Wan D and Kuca K (2016). The antioxidant, immunomodulatory and anti-inflammatory activities of *Spirulina*: an overview. *Arch. Toxicol.*, **90**(8): 1817-1840.
- Yashpal K and Coderre TJ (1998). Influence of formalin concentration on the antinociception effects of anti-inflammatory drugs in the formalin test in rats:

- Separate mechanisms underlying the nociception effects of low- and high-concentration formalin. *Eur. J. Pain.*, **2**(1): 63-68.
- Yoshinari O, Shiojima Y and Igarash K (2014). Hepatoprotective effect of germanium-containing *Spirulina* in rats with D-galactosamine- and lipopolysaccharide-induced hepatitis. *Br. J. Nutr.*, **111**(1): 135-140.
- Zheng J, Inoguchi T, Sasaki S, Maeda Y, McCarty MF, Fujii M, Ikeda N, Kobayashi K, Sonoda N and Takayanagi R (2013). Phycocyanin and phycocyanobilin from *Spirulina platensis* protect against diabetic nephropathy by inhibiting oxidative stress. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, **304**(2): R110-120.