Original Article

Spirulina platensis Attenuates Cold Allodynia and Chemical Allodynia in Streptozotocin induced Diabetic Rats

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Abstract

Background: *Diabetic neuropathic pain exerts strong as well as negative influence on both survival and quality of life of the patient. This pain is difficult to treat with conventional analgesics. Now a days, different medicinal herbs are highlighted due to their wide range of beneficial application with less side effects. In this regards Spirulina platensis is well known for its wide range of biological activities.*

Objective: *To assess the effects of oral administration of Spirulina platensis on cold allodynia and chemical allodynia in diabetic neuropathic pain in Long Evans rats.*

Materials and Methods: *This study was conducted in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka from March 2017 to February 2019. Twenty four (24) Long Evans rats, having 150 to 200 grams body weight (on the basis of treatments), the rats were divided into Group I (normal saline 5 ml/kg body weight) and Group II (Spirulina platensis 400 mg/kg body weight). All the supplementations were given once daily for consecutive 21 days orally and last doses were given one hour before the pain evaluation tests. Further on the basis of experiments, previously grouped rats were divided into subgroup 'a'(cold tail immersion test), and 'b' (formalin test). Data were expressed as mean±SEM and were statistically analyzed by SPSS (Version 16) using independent sample 't' test to compare mean value between two groups. (p 0.05 was considered as the level of significance).*

Results: *Spirulina platensis showed significantly higher tail flick latency (p 0.001) in cold tail immersion test. S. platensis demonstrated significantly lowered jerking frequency (p 0.001,p 0.01,p 0.001, respectively); the duration of licking (p 0.001,p 0.05,p 0.001, respectively) and the duration of flexing (p 0.001, non-significant 0.01, respectively) in early, inter and late phases of formalin test in compared to those of control.*

Conclusion: *S. platensis attenuates cold allodynia and chemical allodynia diabetic neuropathic pain in rat model.*

Key words: *Neuropathic pain, Cold tail immersion test, Formalin test, Spirulina platensis.*

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Introduction

Worldwide diabetes mellitus is an arising crisis and has turned into a global burden. Globally about 371 million people diagnosed with diabetes mellitus and prevalence rate is 8.3% as per the Diabetes Atlas 2012.[1] It is caused by inherited and/or acquired deficiency in the production of insulin by β cells of pancreas or by ineffectiveness of insulin produced.[2] Neuropathic pain is one of the most familiar complications of uncontrolled chronic hyperglycemia.[3]

Different oxidants particularly reactive oxygen species (ROS) including hydroxyl radical are increasedin diabetes mellitus and antioxidative capacity is decreased due to reduction of gene expression of super oxide dismutase (SOD), catalase and reduced glutathione (GSH). This oxidative stress (oxidant and antioxidant imbalance) causes secretion of various inflammatory mediators augmenting neuroinflammation which plays a vital role in the pathogenesis of neuropathy.[4-7]

Spirulina platensisis a microscopic filamentous cyanobacterium (world's largest natural protein source), has been suggested as

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an important medicinal herb.[8-12] S. platensisprevented decrement of total antioxidative power in CCI induced neuropathic pain, reduced inflammatory mediators (IL-1β, TNF-α, IL-6) in serum of collagen induced arthritic rats.[13,14]

S. platensis has also showed immunomodulatory, cardioprotective, renoprotective, antihyperlipidimic, antioxidant, protective against heavy metals, anti-diabetic, hepatoprotective, neuroprotective effects in different animal models.[15-23] Diabetic neuropathic pain (mechanical allodynia and thermal hyperaigesia) was also shown to be significantly decreased after administration of this medicinal algae.[13, 24]

Based on analgesic and antioxidant properties of S. platensis, the present study has been aimed to evaluate the effect of S. platensis on cold allodynia and chemical allodynia in Streptozotocin (STZ) induced diabetic neuropathic pain rats.

Materials and Methods

Animals- This experimental study was conducted in the Pain laboratory in the department of Physiology, BSMMU from March 2017 to February 2019. The study protocol was approved by the Institutional Review Board of BSMMU.

Procurement and maintenance of animals: We obtained a total of 12 Long Evans rats of both sexes having 200±20 gm body weight from the animal house of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka and housed them in specially constructed plastic cages (45X30X15 cm3) with 3 to 4 rats per cage under a 12/12 hours light/dark cycle in the pain laboratory of Department of Physiology, BSMMU. [25,26] The rats were familiarized to the laboratory condition for 7 days before beginning of the experiment. Our team made efforts to reduce the sufferings and number of animals used. For this reason, 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±0.5°C.[27] Standard laboratory food and cooled boiled water ad libitum were provided for all the rats throughout the experimental period.[28] All the experiments were performed at daytime (between 08:00 and 16:00 hours) to avoid the circadian influences.[29] After room environmental and instrumental acclimatization, all the rats were treated with single intraperitonial injection of Streptozotocin (STZ) (50 mg/kg body weight) diluted in citrate buffer (pH 4.5) to induce diabetes mellitus, which was ensured biochemically by checking increased random whole blood glucose >11 mmol/l, 2 days after induction.

Dose schedule: Powder of S. Platensis [Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh] at the dose of 400 mg/kg body weight was dissolved in 5 ml/kg body weight of normal saline NS) (Popular infusion limited, Bangladesh) and their solutions were prepared.

Experimental design: On the basis of treatments, all the diabetic rats were divided into group I (experimental, S. platensis for 21 consecutive days, n=12) and group II (control, only NS for 21 consecutive days, n=12). On the basis of pain tests applied, rats in each group were further divided into subgroup 'a' (cold tail immersion test, n=6), 'b' (formalin test, n=6). One hour after the dose of oral treatment, all the rats were subjected to the above mentioned pain tests. This test was done every 4 days interval after diabetes mellitus induction.

Cold tail immersion test: Cold tail immersion test procedure was performed according to previously published procedures.[30] After 7 days of room environmental acclimatization, 12 diabetic rats were separated for cold tail immersion test [Ia (6), IIa (6)]. Then 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 a plexiglass mechanical restraining cage for 5 minutes to adjust to the cage environment with the tail hanging freely. Then 400 ml of cold water maintained at 10°C was taken in a 500 ml glass beaker with a laboratory thermometer placed inside. The distal 5 cm of the freely hanging tail of the rat was immersed into the cold water and latency period of the tail-flick was recorded. The mean of the measurements obtained from three (3) similar successive maneuvers, performed at five (5) minute intervals, was taken as the baseline latency. Then on the day of experiment one (1) hour after the last dose of treatment (NS / S. platensis) another tail immersion test was done and mean of 3 tail withdrawal latencies at 5 minutes interval was noted as test latency (TL). This test was done every 4 days interval after diabetes mellitus induction.To minimize tissue damage, a maximum latency of 20 seconds was considered as cut off time. The antinociceptive effect was expressed as percentage of the maximum possible effect (% MPE) as follows: %MPE = [(TL - BL)/ (Cut of time - BL)] ×100

Formalin test: Formalin test procedure was performed according to the methods of previous publications.[31, 32] After 7 days of room environmental acclimatization, 12 diabetic rats were separated for formalin test [Ib (6), IIb (6)]. Then each rat was acclimatized in the observation cage of the plexiglass formalin test box (30 X 30 X 30 cm3) for 1 hour daily for 7 consecutive days. Then on the day of experiment (at day 21), one hour after the last dose of treatment (NS / S. platensis), the rat was restrained by a thick towel and the right hind paw was exposed. Fifty (50) µl of dilute formalin (0.2%) was injected subcutaneously into the planter aspect of the rat's right hind paw with an insulin syringe. Immediately the rat was placed in the observation cage of the formalin box and the pain behaviors was observed for following 60 minutes. Within this period, the first 5 minutes (1st to 5th) was considered as the early phase, middle 10 minutes (6th to 15th) as the interphase and last 45 minutes (16th to 60th) as the late phase. 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 time through a mirror fixed below the observation cage at 450 angle.

Results

Cold allodynia

In this study, we evaluated the effect of S. platensis on cold allodynia by cold tail immersiontest as diabetic neuropathic pain model. As shown in Figure 1, mean value in tail flick latency in different days of cold tail immersion test is significantly

higher in experimental group (with S.P) on day 14 (p≤0.01), 18 (p≤0.001) and 22 (p≤0.001) in comparison to that of control.

%MPE = in % Maximum possible effect
Time (in days)

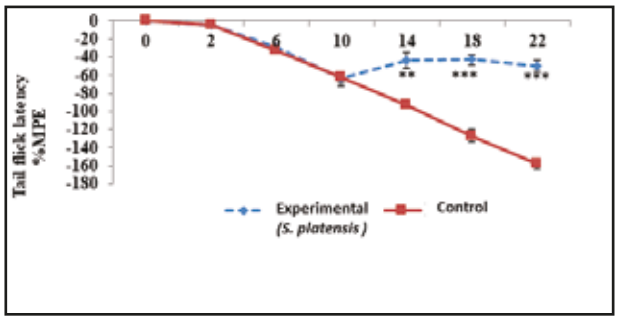

Figure 1: Cold allodynia of S. platensis (Spirulina platensis, 400 mg/kg body weight) in comparison to control, NS (normal saline, 5ml/kg body weight) in tail flick latency in different days of cold tail immersion test. Comparison was done on percentage of maximum possible effect (%MPE). Each line symbolized formean±SEM of 6 rats in each group.

/* significant (p≤0.01/p≤0.001)in comparison to control.

Chemical allodynia

The effects of oral administration of S. platensison chemical allodynia in the early (1st to 5th minutes), inter- (6th to 16th minutes) and late (16th to 60th minutes) phases of formalin test, as model of diabetic neuropathic pain, were observed. The neuropathicpain behaviors were separately analyzed as total frequency of jerking, total duration of licking and total duration of flexing in all phases. S. platensis demonstrated significantly lowered jerking frequency (p 0.001,p 0.01,p 0.001, respectively); the duration of licking (p 0.001,p 0.05,p 0.001, respectively) and the duration of flexing (p 0.001, non-significant 0.01, respectively) in early, inter and late phases of formalin test in compared to those of control. (Figure 2,3,4).

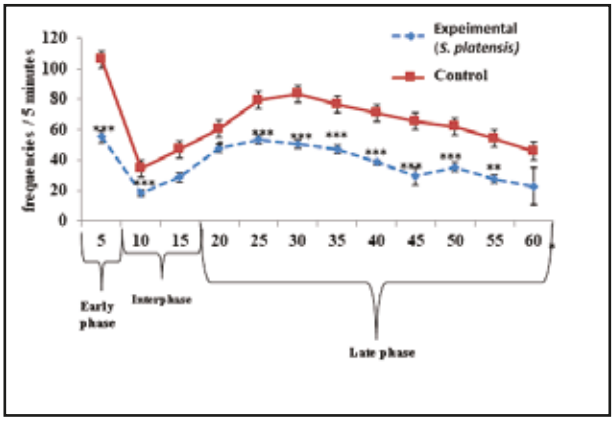

Figure 2: Chemical allodynia of S. platensis (Spirulina platensis, 400 mg/kg body weight) in comparison to control, NS (normal saline, 5ml/kg body weight)injerking time score in different phases of formalin test. Each line symbolized for mean±SEM of 6 rats in each group.

/* significant (p≤0.01/p≤0.001) in comparison to control.

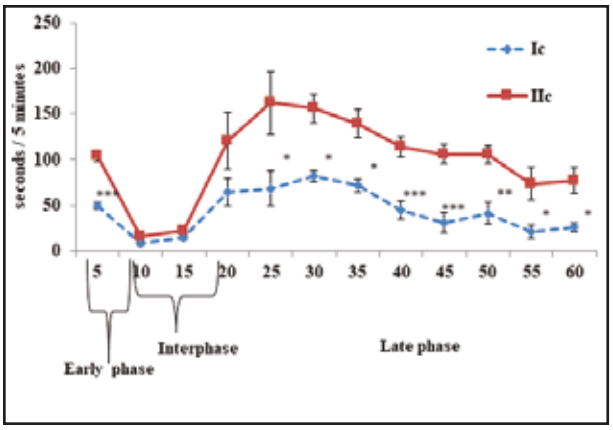

Figure 3: Chemical allodynia of S. platensis (Spirulina platensis, 400 mg/kg body weight) in comparison to control, NS (normal saline, 5ml/kg body weight) inflexing time score in different phases of formalin test. Each line symbolized for mean±SEM of 6 rats in each group.

*/ **/*** significant (p≤0.05/p≤0.01/p≤0.001) in comparison to control.

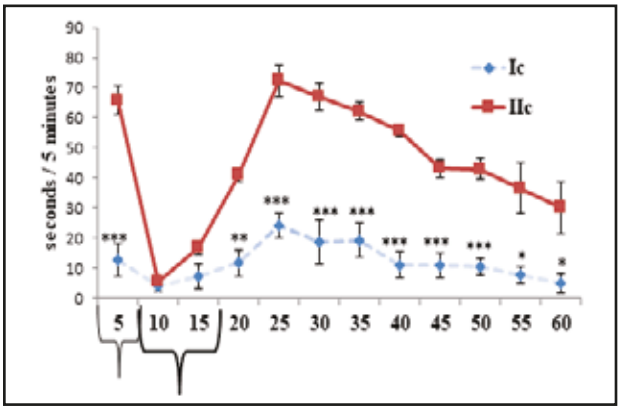

Figure 4: Chemical allodynia of S. platensis (Spirulina platensis, 400 mg/kg body weight) in comparison to control, NS (normal saline, 5ml/kg body weight) inlicking time score in different phases of formalin test. Each line symbolized formean±SEM of 6 rats in each group.

*/ **/*** significant (p≤0.05/p≤0.01/p≤0.001)in comparison to control.

Discussion

In this study, S. Platensis significantly prevented development of cold allodynia in rats with diabetic neuropathic pain as evidenced by non-decrement of tail flick latency in cold tail immersion testin the experimental rats when compared to those of controls. No comparable study was available to support this finding.

Along with cold allodynia in the present study, S. platensis also inhibited development of chemical allodynia in rats with diabetic neuropathic pain as evidenced by significant

decrement of jerking, flexing and licking in all phases of formalin test in the experimental rats when compared to those of controls.Similar finding was reported by other authors in streptozotocin induced diabetic rats.[33,34]

Several researchers have suggested that, in diabetes mellitus,-different oxidants specially reactive oxygen species (ROS) including hydroxyl radical are increased[4,5,6] and antioxidative capacity is decreased due to reduction of gene expression of super oxide dismutase (SOD), catalase and reduced glutathione (GSH)[7]. This oxidative stress (oxidant and antioxidant imbalance) causes secretion of various inflammatory mediators augmenting neuroinflammation which plays a vital role in the pathogenesis of neuropathy.[4-7] These inflamed nerves release more and more mediators including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), bradykinin, substance P, nerve growth factor, prostaglandin (PG) and interleukin-6 (IL-6)7followed by activation of signalling pathway p38 MAPK resulting in insertion of tetrodotoxin resistant (TTX-R) Na+ channels in injured peripheral nerve terminal[7,35]. Ultimately there is decrement of activation threshold and increment of excitability of these peripheral nervescausing enhancement of Ca2+ channels trafficking in central terminal of nociceptor[35]. In addition, these TTX-R Na+ channels were also found to be involved in the response of cold sensitive fibers to noxious cold(≤120C), which might be the cause of cold allodynia in our rats.[36.37]

In addition, activated central terminal of nociceptor releases glutamate causing prolonged post synaptic depolarization of dorsal horn neuron (DHN). It has also been suggested that formalin injection in formalin test causes accumulation of PG, TNF-α, IL around peripheral end of nociceptors as well as release of glutamate from central end of nocicepter which might be the cause of chemical allodynia in our rats.[38,39] This glutamate causes relief of physiological Mg2+ block of N-methyl D-aspartate (NMDA) receptors resulting in increased excitability of DHN with subsequent intensification of pain transmission.[35]

It has been reported that S. platensis prevented decrement of total antioxidative power in CCI induced neuropathic pain, reduced inflammatory mediators (IL-1β, TNF-α, IL-6) in serum of collagen induced arthritic rats and downregulated microglial activation directly in arthritic rats with induced neuropathic pain.[13,14,40] In addition, it has been found that C-phycocyanin (an important component of S. platensis) possesses the ability to scavenge hydroxyl radical (one of ROS) directly.[41,42] Moreover, the biliprotein of S. platensis was shown to reduce PG in inflammatory tissue homogenate of mice, to reduce gene expression of NMDA in mice.[43,44] Therefore suppressing oxidants and / or inflammatory mediators and / or inflammatory signaling pathway and / or gene expression of NMDAin pain transmission pathway might be the cause ofcold allodynia, chemical allodyniain our rats with S. platensis.

Conclusion

S. platensis attenuates cold allodynia and chemical allodynia diabetic neuropathic pain in rat model. Even though, further studies are needed to find out exact component and molecular mechanism responsible for these valuable effects of S. platensis.

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