ORIGINAL ARTICLE

https://doi.org/10.3329/jmcwh.v20i2.77290

EFFECTS OF *SPIRULINA PLATENSIS* ON HYPERGLYCAEMIA AND MECHANICAL ALLODYNIA IN STREPTAZOCIN – INDUCED DIABETIC RATS

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ABSTRACT

Background: Diabetic neuropathy is a serious complication of diabetes mellitus and exerts powerful pessimistic effect on patient's life. This neuropathy induced pain is not relieved by taking usual analgesics. At present, several medicinal herbs have gained attention since these exhibit fewer side effects.In this regard, *Spirulina platensis* is one such herb that has various activities beneficial to human health. **Objective:** To note the influence of oral *Spirulina platensis (S.platensis)* on hyperglycaemia and mechanical allodynia following its administration into Long Evans rats that have been induced into diabetic neuropathy. **Materials and method:** This research work was performed between March of 2017 and February of 2019 in the physiology department of BSMMU (Bangabandhu Sheikh Mujib Medical University), Dhaka. Long Evan rats (12 in number, weighing 150-200g) were distributed in to 2 groups based on intervention. Group 1 was given oral *Spirulina platensis*, 400 mg/kg body weight while group 2 received normal saline 5ml/kg body weight once daily and here experiment performed was Von Frey test. The experiment was carried out for 21 days. Mean ± SEM was used to express the data that was analyzed with SPSS (version 16.0). Comparison between the 2 groups was done by means of independent sample 't' test and significance level was taken at $p \leq 0.05$.**Results:** In this study, *Spirulina platensis* demonstrated significant reduction in blood glucose level ($p \leq 0.01$) and higher paw withdrawal threshold ($p \leq 0.001$) of diabetic rats in estimation of random blood glucose level and Von Frey test respectively. **Conclusion**: The present study concluded that, *S. platenis* attenuates hyperglycaemia and mechanical allodynia in rats with diabetic neuropathic pain.

Keywords: Neuropathic pain, Von Frey test, *Spirulina platensis*.

Cite this article: M Ghosh, Tasnim SF, Roy S, Ghosh S, Sathi MN, Jahan N, Mawa J.Effects of Spirulina Platensis on hyperglycemia and mechanical allodynia in Streptazocin-induced diabetic rats.J Med Coll Women Hosp.2024;20(2):56-63.

INTRODUCTION

Although extensive efforts have gone into the development of appropriate diabetes management, the disease itself and complications associated with it are on the rise[1]. The pathophysiology that exists in the case of this disease include reduction in insulin formation by pancreatic β cells or failure of insulin to produce its effect[2]. Patients of diabetes suffer a common complication which is neuropathy[3]. In diabetes induced rats the sensitivity to pain increases even in acute hyperglycemia which increases with increasing time similar to that observed in human[4].

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From the ancient time, different approaches have been adopted to manage pain. Analgesics traditionally in use for pain management follow the World Health Organization (WHO) analgesic ladder, stepping through paracetamol, non-steroidal anti-inflammatory drug (NSAIDs) and finally opioids[4,5]. However, common analgesics do not provide relief of pain in case of neuropathy[5,6]. Current treatment of pain of neuropathy in diabetes include non-steroidal anti-inflammatory drugs, selective serotonin reuptake inhibitors, tricyclic antidepressant, anticonvulsants, opioids, and inhibitor of protein kinase C.Pain relief occurs in a certain number of individuals with the use of these medications. Their side effects, however, reduce their use[6,7]. Various research work have been conducted in recent times throughout the world to find alternatives to the traditional analgesic drugs in order to replace them, or at least to reduce their dosage in an aim to minimize their adverse effects with many herbal products[7-11] [8-12].

Within them *S. platensis* have high nutritional values. *S. platensis* has immunomodulatory[12, 13], cardioprotective[13, 14], renoprotective[14, 15], antihyperlipidimic[15, 16], antioxidant[16, 17], anti-diabetic[17, 18], hepatoprotective[18, 19], neuroprotective[19, 20] roles in various trials involving animal models. Medicinal algae administration have also been reported to reduce pain due to neuropathy (thermal hyperalgesia and mechanical allodynia) significantly[20, 21].

Based on analgesic, antioxidant and antidiabetic role of *S. platensis*, this research work has been designed to observe the influence of *S. platensis* on attenuating hyperglycemia and mechanical allodynia in STZ- induced diabetic rats with neuropathic pain.

MATERIALS AND METHOD

The Physiology department's pain laboratory was the location for conducting this experimental research work and the duration for the study was 2 years (from 2017 March to 2019 February). BSMMU Institutional Review Board provided the necessary ethical approval to conduct the research.

PROCURING AND MAINTAINING OF THE LONG EVAN RATS FOR EXPERIMENT

Long Evan rats (12 in number) of both gender with body weight of about 200±20 gm, obtained from BSMMU animal house, were retained in special cages of plastic with dimensions of 45*30*15 cm^3 (each cage holding 4 rats)[22,23] of the Physiology department's pain laboratory of BSMMU, Dhaka. Sacrifice of all the involved rats was done so that they would not suffer any further. The environment in which the rats were placed included surrounding temperature of 27.5±0.5°C[24] and cycle of light / dark was 12 hours/ 12 hours[22,23]. Timings chosen to carry out the experiment was between 8 am and 4 pm to avoid influence of circadian rhythm[25].

Streptozotocin (STZ) was introduced intraperitonially by means of single injection at a dosage of 50 mg for each kg of body weight (with diluted citrate buffer of pH 4.5) following acclimatization of instruments and environment of the room. This intervention was done for diabetes mellitus induction. In order to ensure the development of diabetes mellitus, the blood glucose level was checked after 2 days following induction. Rats with random blood glucose level of >11 mmol/L were included to proceed further with the experiment.

SOLUTION TO BE ADMINISTERED INTO THE INTERVENTION GROUP

A solution was prepared by dissolving *S. Platensis* powder (400 mg for each kg of body weight) in normal saline (5 ml/kg body weight).

EXPERIMENTAL DESIGN

STZ-induced diabetic Long Evan rats were distributed into 2 groups

based on intervention. Group 1 (n=6) was given oral *Spirulina platensis*, 400 mg/kg body weight for 21 days while group 2 (n=6) received normal saline 5ml/kg body weight once daily for the aforementioned number of days and here experiment performed was Von Frey test. This test for pain was carried out (every 4 days interval) on the rats 1 hour after oral intervention was given and One hour after the dose of oral treatment, all the rats were subjected to the above mentioned pain tests.

BLOOD GLUCOSE ESTIMATION BY RANDOM BLOOD GLUCOSE LEVEL

Random blood glucose was monitored every 4 days interval, random blood glucose level was estimated for all diabetic rats. Blood samples were collected for assessing the blood glucose level.

VON FREY TEST

The procedure for the Von Frey test was done in accordance to studies carried out previously[24,26]. Acclimatization of instrument (for 1 hour per day in wide gauge wire) was done. The test was carried out at an interval of every 4 days on the 12 rats following induction of diabetes mellitus. Each of the rats were put into the wide gauge wire mesh surface (1 hour after intervention) and Von Frey filaments (VFF) were touched (in ascending order) on both the hind paws of the rats (on planter surface between 1st and 2nd metatarsal about 1 cm proximal to the joint of ankle).The recording of the number of times the hind paws were withdrawn was done following application of VFF of varying tensile strength between 2 gm and 18 gm for 3 times with an interval of 30 seconds between each turn. The increasing strength of VFF application was continued upto 18 gm till there was paw withdrawal and incase paws withdrawal did not occur by the tensile strength of 18 gm application. The withdrawal of paws in case of two of the three rounds indicated that further raising the tensile strength is not needed.

RESULTS

Random blood glucose of diabetic rats

As demonstrated in Figure 1, the level of random blood glucose (mean value) in case of the group given *S. platensis* displayed a significant reduction when compared to the control group on day 18 and day 22 with $p \leq 0.05$ and $p \leq 0.01$ respectively

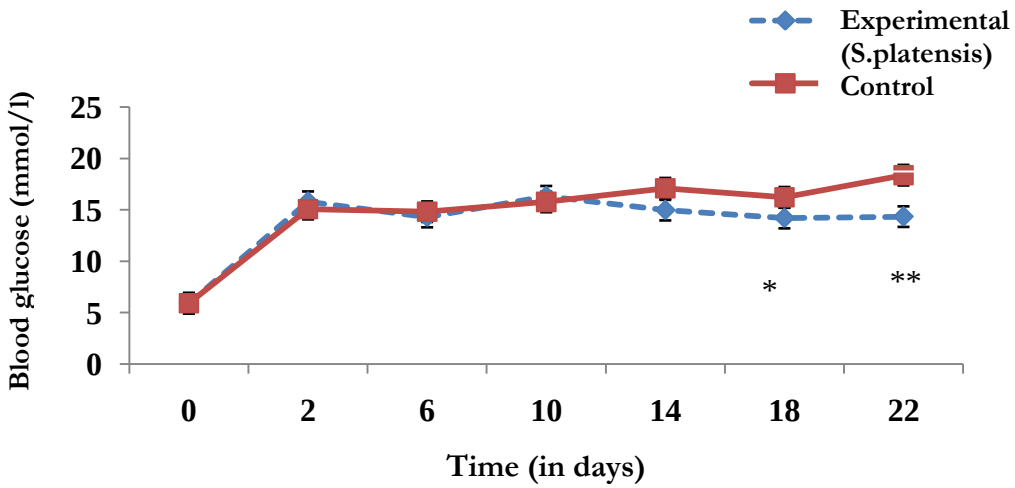

Figure 1: Random whole blood glucose of *S. platensis* (*Spirulina platensis*, 400 mg/kg body weight) in comparison to control, NS (normal saline, 5ml/kg body weight) in different days of Von Frey Test. Each line symbolized for mean±SEM of 6 rats in each group.*/** significant (p≤0.05/p≤0.01)in comparison to control.

MECHANICAL ALLODYNIA

Figure 2 shows the paw withdrawal threshold mean value using Von Frey test as diabetic neuropathic pain model was significantly more in case of the group given *S. platensis* when compared to the control on day 14 ($p \leq 0.05$), 18 ($p \leq 0.05$) and 22 ($p \leq 0.001$).

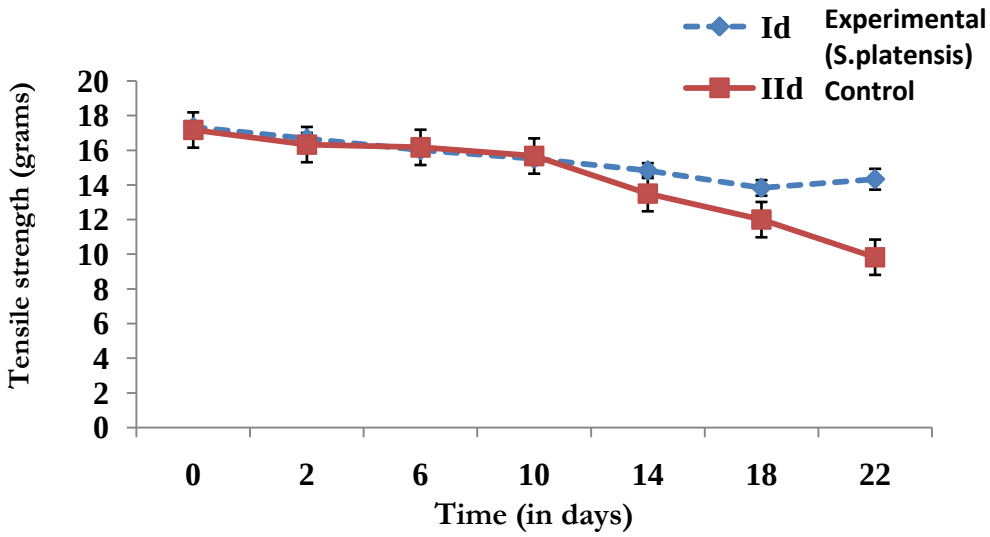

Figure 2: Paw withdrawal threshold of *S. platensis* (*Spirulina platensis*, 400 mg/kg body weight) in comparison to control, NS (normal saline, 5ml/kg body weight) in different days of Von Frey Test. Each line symbolized for mean±SEM of 6 rats in each group.*/*** significant ($p \leq 0.05$/$p \leq 0.001$) in comparison to control.

DISCUSSION

This research has exhibited that there is significant attenuation of blood glucose level in diabetic neuropathic pain induced rats. In this study, *S. Platensis* significantly attenuates hyperglycemia in rats with diabetic neuropathic pain as evidenced by random blood glucose levels being significantly lower in the rats given intervention than that of the control group of rats. Similar outcome has been observed in other research works done on STZ induced diabetic [27,28].

Along with controlling hyperglycemia in this study, *S. platensis* also prevented mechanical allodynia in the diabetes neuropathic pain in case of the experimental rats given *S. platensis* since the threshold of withdrawal of paw were higher in this group in comparison to that of control group. This is in agreement with other studies[22,26].

S. Platensis showed its antihyperglycaemic action may be through potentiated β-cell of pancreas for secretion of insulin or increase transport of glucose to peripheral tissue[29]. It has been reported that *S. platensis* has insulinotropic effects by closing ATP sensitive K^+ channels and also causes membrane depolarization of β-cell of pancreas and increase intracellular calcium. One vivo study reveals that butanol fraction of *S. platensis* lowers blood glucose by increasing gut motility, decreased glucose absorption from GIT and increased plasma insulin levels[30].

A number of researchers have recommended that, in case of diabetes mellitus there is development of oxidative stress due to a loss of balance between antioxidant and oxidants. Neuroinflammation results from the effect of various mediators of inflammation like prostaglandin, bradykinin, substance P, interleukin 6 (IL-6), Interleukin 1β (IL-1β),

and tumor necrosis factor-α (TNF-α),. Inflammation of neuron may be the pathophysiological underlying cause of neuropathy development[31-33]. Signaling pathway p38 MAPK (Mitogen activated protein kinase) is activated by the mediators of inflammation which in turn causes inserting of tetrodotoxin resistant (TTX-R) Na^+ channels at the terminal of peripheral nerve that is affected. This results in decrease in the firing level and enhancement of excitability of the nerve and aggravate pain of neuropathy [31-33].

Additionally glutamate is secreted from nociceptor's activated central terminal resulting in the prolongation of depolarization of post-synaptic neuron of dorsal horn. Transmission of pain therefore intensifies owing to the raised excitability of the dorsal horn neuron. There is also release of ATP that binds with microglial purinoceptors (P2X4) and thus promotes activation of p38 MAPK, contributing to post-synaptic depolarization of dorsal horn neuron prolongation[34]. Mechanical allodynia in the diabetic rats having neuropathic pain of this research may be attributed to the mentioned mechanisms.

S. platensis has the ability of directly suppressing p38 MAPK pathway of islet amyloid polypeptide mediated β cell death[35,36]. Thus, oxidant and / or mediators of inflammation or activation of gene of microglia of the pathway of transmission of pain is suppressed[35,36]. Mechanical allodynia may have been reduced in the experimental rats of this study that were given *S. platensis* by means of the above mentioned mechanism.

CONCLUSION

Diabetes mellitus is a metabolic disorder that may lead to several complications that are often debilitation, affecting the quality of life of those who suffer. Peripheral neuropathy is one such complication. The pain sensitivity is raised in diabetic patients. Our study had aimed to find a means of reducing this pain sensitivity and decreasing the effect of peripheral neuropathy in animal model. The study observed improvement in mechanical allodynia in diabetic rats with peripheral neuropathy upon administration of *S. platensis*. Therefore, further studies in future may be carried out both in animal model and in humans to observe the effectiveness of *S. platensis* in diabetes.

LIMITATION

A larger sample size could be taken due time and financial constraint. Also the lipid profile of the animals could not be done due to the mentioned constraint. Due to time limitation the experiment could not be done for longer period of time.

CONFLICT OF INTEREST

There is no conflict of interest.

FUNDING AGENCY

This research project was not funded by any group or any institution.

CONTRIBUTION TO AUTHORS

Ghosh M and Tasnim SF were contributed from the protocol preparation upto report writing. Roy S, Ghosh S, Sathi MN, Jahan N and Mawa J were involved in the manuscript writing and revision.

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