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Antidiabetic effect of spirulina (*Spirulina platensis*) in alloxan induced rabbit model

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ABSTRACT

The increasing prevalence of diabetes mellitus in recent years has increased greatly in Bangladesh. Plant Products such as spirulina can be used as alternative of synthetic drugs to avoid side effects and high cost. So the present study was undertaken to assess the antidiabetic effect of spirulina. We selected 2.5-3 months old rabbits and divided into three groups consisting of 5 rabbits in each group. Group T0 was kept for negative (no alloxan) control, the rest of the group (T1 and T2) were injected with alloxan intraperitonealy at a dose rate of 150 mg /kg body weight. T1 was diabetic control group and T2 was treated with spirulina @1000 mg/kg feed. Observations were recorded to detect induction of diabetics; blood glucose level was measured after 72 hours and body weight, heamoto-biochemical parameters were taken in every week. Blood glucose level increased significantly ($p<0.01$) in all treated groups compared to the control group and the highest induction was recorded in T1 group treated with alloxan but did not received Spirulina. Blood glucose level significantly decreased in the group T2, which were treated with spirulina. Body weight decreased significantly ($p<0.01$) in all alloxan treated groups but increased significantly ($P<0.01$) in the control group after 4 weeks of treatment. Hemoglobin (Hb) and Packed Cell Volume (PCV) decreased in the T1 group and significantly increased in the T2 group. Erythrocyte Sedimentation Rate (ESR) and Total Leukocyte Count (TLC) levels increased in the T1 group and decreased in the T2 group. The level of SGPT, SGOT and Serum creatinine was the highest in group in T1 and lowest in group T2. Taken together, the results revealed that Spirulina have possitive effect on heamato-biochemical parameters and body weight. The data obtained from this study suggests that Spirulina platensis has an antidiabetic effect and can be used to treat hyperglycemia and related complications.

INTRODUCTION

Diabetes mellitus is a multifactorial disease characterized by hyperglycaemia and aggravated basal metabolic rate (Bos and Agyemang 2013). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. The therapeutic extent for the treatment of hyperglycemia include the use of insulin and other agents, such as amylin analogs, and alpha-glucosidase inhibitors such as acarbose and miglitol, voglibiose, sulfonylureas, and biguanides. These drugs have certain adverse effects, such as causing hypoglycemia at higher doses, liver problems, lactic acidosis, and diarrhea (Atkinson, Maclaren, 1994). Therefore, there is a need to look for newer agents that meet the requirement of an ideal antidiabetic compound. From ancient times, plant remedies have been used to help to alleviate diabetes. Plant products are often considered to be minor toxic and more free from side effects than synthetic agents. There is an increasing requirement for natural products with antidiabetic activity to treat diabetic patients. The supervision of antidiabetic agents of plant origin, which are used in traditional medicine, is thus of great significance.

Spirulina, a blue-green algae pertain to the family Oscil- latoriaceae, has been used from ancient

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times as a source of protein and vitamins (Somchit et al., 2014; Rasool and Sabina, 2009). Spirulina is a complete food because of the existance of nutrients that include β-complex, vitamins, minerals, proteins, γ-linoleic acid, nutraceutical pigments and the β-carotene as well as the trace elements that make it helpful for the treatment of various diseases (Keservani et al. 2015). Spirulina have shown to exert metabolic hypolipidemic, hypoglycemic, anti-viral, liver-protecting and blood-vessel relaxing effects along with anti-cancer, anti-inflammatory and anti-oxidant activities (Patteron, 1993). People of all ages can take *S. platensis* for its detergent effect and its ability to help improve digestion and elimination. The United Nations World Food Conference declared spirulina as "the best for tomorrow", and it is accomplished popularity in recent years as a food supplement. Therefore, a large number of works are in progress to find natural sources. Here, the study was undertaken to evaluate the antidiabetic effect of spirulina on alloxan induced (150 mg/kg body weight) diabetes in male New Zealand white rabbits.

MATERIALS AND METHOD

Selection of animal

We selected 15 male New Zealand white rabbits of 2.5 - 3 months of age having 1000 to1100 g body weight. All the rabbit were housed at screen bottomed wire cages placed in rows and bred in the departmental (Physiology & Pharmacology, HSTU) animal house. The animals were fed with pellet at a recommended dose of100 g/kg as advised by ICDDRB. Drinking water was supplied *ad libitum*.

Chemicals

Alloxan monohydrate (NH-CO-NH-COCO.H2O; Sigma Aldrich Chemical, Saint Louis, MO, USA), Blood Glucose determination Kit – Glucolab Active blood glucose system (strip method).

Grouping of experimental animal

The rabbits were divided into three groups consisting of 5 rabbits in each group. The groups were designated as T_0, T_1 and T_2.

Induction of diabetes

Alloxan was dissolved in distilled water. This solution was injected intra-peritonealy and maintained fasting condition for 18 hours before injecting. For induction of diabetes, alloxan was administered at a dose of 150 mg/kg of body weight as recommended previously (Bhaskar and Sufiyan, 2014).

Preparation of Spirulina mixed feed

Each capsule of Spirulina (Spirulina®; Square Pharmaceuticals Limited, Bangladesh) containing 500 mg of *S. platensis* was used in this study. The powder of spirulina was kept in a pannikin after opening from the capsule. The needful amount of Spirulina (1 gm/kg feed) was measured with the help of electric balance. For proper homogenous mixing, small amount of distilled water was added to the spirulina powder to make it a suspension and then the suspension was added drop by drop to the feed and simultaneously the feed was mixed up by a glass rod for homogenous mixing.

Animal treatment

T_0 was kept as an untreated negative control group. The rest of the groups (T_1 and T_2) were injected with alloxan (150 mg/kg b.w.) and T_1 kept as (diabetic control) positive control group. T_2 was treated with Spirulina (1000mg/kg feed).

Observation of rabbits

Body weight and fasting blood glucose level of each rabbits were measured and after 18 hours of fasting alloxan was injected. Body weight and fasting blood sugar level of each rabbit were measured at Day 0 (Pre-treatment) and Day 7, 14, 21 and 28 (post treatment). All blood samples were collected from ear vein and by cardiac puncture.

Hematological and biochemical Test

Blood samples were collected as mentioned above and Hb, PCV, ESR, TLC, SGPT, SGOT and serum creatinine were estimated.

Statistical analysis

Data were analyzed using SPSS v.20 for Windows (SPSS Inc., Chicago, IL, USA). Statistically significant differences between group means were determined by analysis of variance (ANOVA). Data are expressed as mean ± SEM.

RESULTS AND DISCUSSION

The present results exhibited the efficacy of spirulina on diabetes in alloxan induced diabetic rabbit model, which was evident principally by the reduced blood glucose level and also by the improvement of body weight, hematological and biochemical parameters.

Alloxan successfully induced diabetes in rabbits

The study showed that glucose level was the highest in group T_1, which was treated with Alloxan compare to the T_0 group (negative control). This treatment significantly ($p<0.01$) increases the blood glucose level in treated rabbits (T_1 and T_2), conforming to the findings of Akhtar et al., (1981). They reported that Alloxan treatment aggravated the blood glucose level in rabbits.

Spirulina significantly reduced blood glucose level

We observed that glucose level was the lowest in group T_2, which was treated with spirulina compare to the group T_1.

Table 1
Effects of alloxan and spirulina on blood glucose (mmol/L, mean±SE) concentration in alloxan induced diabetic rabbits.

Treatment	T_o	T_1	T_2
Initial	7.28 ± .28	7.80 ± .27	7.10 ± .57
1 week	7.22^{a} ± .18	26.76^{b} ± .54	26.88^{b} ± .30
2 weeks	7.56^{a} ± .28	25.96^{c} ± .35	22.36^{b} ± 1.2
3 weeks	7.40^{a} ± .22	24.88^{c} ± .69	17.64^{b} ± 1.1
4 weeks	7.70^{a} ± .14	24.56^{c} ± .76	12.56^{b} ± 1.2

Values with the different are statistically significant ($P<0.01$). T_0, Control (without treatment); T_1 Alloxan induction (150 mg/kg bw.); T_2, Spirulina treatment (1000 mg/kg feed), n=5

In the group T_2 glucose levels significantly increased as it was in the group T1 but following Spirulina treatment blood glucose level declined sharply and drop to the normal levels, which was comparable to that of group T_0 (Table 1). Our results conformed to the observation of others (Pankaj andVarma, 2013; Abdel-Daim, 2014; Ibrahim andAbdel-Daim, 2015; Abdel-Daim et al., 2016; Ourida Aissaoui et al., 2017). The result also indicated that the dose of Spirulina 1 gm/kg feed orally is essential as a hypoglycemic agent in Alloxan induced diabetic model.

Body weight increased due to alloxan treatment

Body weights of experimental rabbits of all groups were taken on the day 0 and weekly up to 4 weeks. At the end of experiment, the body weight in control rabbits (Group T_o, n=5) was 1102.6 g. On the converse, in diabetic control group (Group T_1, n=5), the body weight was 1021 g. On the other hand, in the Group T_2 that received spirulina mixed feed (1 mg/kg), the body weight was 1086 g (Table 2). The body weight of initial groups were not significant ($p > 0.05$) but in 2nd weeks, 3rd weeks and 4th weeks mean value of body weight were significant ($p<0.01$). The body weight of treated group were increased with their age but in T_1 group it decreased compared to other groups.

Table 2
Effects of spirulina on body weight (g) in alloxan induced diabetic rabbits.

Treatment	T_0	T_1	T_2
Initial	1012.0 ± .5.83	1017.0 ± .3.74	1014 ± 1.87
1 week	1037.4 ± 1.5	1043.2 ± 1.25	1045.2 ±.80
2 weeks	1059.0^{b} ± 5.57	1039.0^{a} ± 1.95	1052.6^{ab} ± 2.18
3 weeks	1080.0^{c} ± 6.12	1032.4^{a} ± 2.29	1060.0^{b} ± 4.47
4 weeks	1102.6^{b} ± 12.24	1021.6^{a} ± 2.66	1086.0^{b} ± 4.30

Values with the different superscripts are statistically significant ($P<0.01$). T_0, Control (without treatment); T_1 Alloxan induction (150 mg/kg bw.); T_2, Spirulina treatment (1000 mg/kg feed), n=5.

Effects of spirulina on hematological parameters

Hb: In control animals Hb was estimated as 12.80±.62. Alloxan induced diabetic rabbit showed significant decrease in Hb value. The lowest value of Hb observed was 10.40±.40. Group T_2 rabbit when fed with SP mixed fed

showed repair of Hb value (Table 3). The decrease in the erythrocyte count and Hb were mainly due to the damaging action of shorented insulin and increased blood glucose level on the erythropoetic tissue (Pankaj and Varma, 2013).

Table 3
Effects of different treatment on Hemoglobin concentration (Hb) (gm/dl) values of rabbits.

Treatment	T_o	T_1	T_2
1 week	11.70 ± .37	12.20 ± .54	12.14 ± .50
2 weeks	12.10 ± .51	11.80 ± .46	11.70 ± .37
3 weeks	12.40 ± .64	11.30 ± .37	11.50 ± .42
4 weeks	12.80^{b} ± .62	10.40^{a} ± .40	11.40^{ab} ± .43

Values with the different superscripts are statistically significant (P<0.05). T_0, Control (without treatment); T_1 Alloxan induction (150 mg/kg bw.); T_2, Spirulina treatment (1000 mg/kg feed).

ESR: In this study ESR was almost same in all groups (Table 4).

Table 4
Effects of different treatment on Erythrocyte Sedimentation Rate (ESR) (mm/1st hour) values of rabbits.

Treatment	T_o	T_1	T_2
1 week	1.777± .029	1.787± .033	1.807 ± .037
2 weeks	1.793 ± .024	1.950 ± .027	1.760 ± .029
3 weeks	1.808 ± .033	2.220 ± .123	1.620 ± .046
4 weeks	1.906 ± .026	2.260 ± .218	1.460 ± .029

PCV: We also determined the effect of the treatment on PCV. The study revealed that PCV was the highest in group T_2, which was treated with spirulina compare to the group T_1 (Table5). This treatment significantly (p<0.05) raised the PCV in treated rabbits, which is in agreement to the findings reported prevously (Pankaj and Varma, 2013).

Table 5
Effects of different treatment on Packed Cell Volume (PCV) (%) values of rabbits.

Treatment	T_0	T_1	T_2
1 week	40.42 ± .57	39.40 ± .51	40.10±.75
2 weeks	40.52^{b} ± .62	38.70^{a} ± .46	41.00^{b} ±.22
3 weeks	40.70^{b} ± .37	38.10^{a} ± .56	42.60^{c} ±.29
4 weeks	41.20^{b} ± .58	36.60^{a} ± .40	43.70^{c} ±.46

Values with the different superscripts are statistically significant (P<0.01). T_0, Control (without treatment); T_1 Alloxan induction (150 mg/kg bw.); T_2, Spirulina treatment (1000 mg/kg feed).

TLC: Significant increase in leucocyte count was observed in group T_1 when compared with group T_0 at incubation period. Diabetic rabbits when treated with Spirulina mixed feed showed decreased TLC from 6.32±0.451 to 5.16±0.213 thousand cells/mm^3 (Table 6). TLC was found to be increased in diabetic subject due to pathophysiological circumstances including autolysis sake by some hydrolytic enzymes released by plasma under stress. (Pankaj and Varma, 2013).

Table 6
Effects of different treatment on Total Leukocyte Count (TLC) (thousand cells/mm^3) values of rabbits.

Treatment	T_o	T_1	T_2
1 week	5.70 ± .264	5.90 ± .400	6.32 ± .451
2 weeks	6.56 ± .331	6.92 ± .357	6.02 ± .369
3 weeks	6.10 ± .427	7.30 ± .438	5.86 ± .399
4 weeks	7.04^{b} ± .163	8.10^{c} ± .187	5.16^{a} ± .213

Values with the different superscripts in the same column are statistically significant (P<0.01). T_0, Control (without treatment); T_1 Alloxan induction (150 mg/kg bw.); T_2, Spirulina treatment (1000 mg/kg feed).

Spirulina treatment improved relevant biochemical parameters

SGOT: In the diabetic positive rabbits the SGOT was found elevated to 136 ± 9.27 (Table 7).

Table 7
Effects on SGOT of rabbits treated with alloxan and spirulina.

Treatment	T_0	T_1	T_2
1 week	68^{a} ± 3.74	98^{b} ± 3.39	99^{b} ± 4.58
2 weeks	71^{a} ± 3.32	114^{b} ± 10.17	90^{a} ± 2.24
3 weeks	79^{a} ± 2.91	124^{b} ± 9.27	88^{a} ± 2.55
4 weeks	84^{a} ± 1.87	136^{b} ± 9.27	83^{a} ± 2.00

Values with the different superscripts are statistically significant (P<0.01). T_0, Control (without treatment); T_1 Alloxan induction (150 mg/kg bw.); T_2, Spirulina treatment (1000 mg/kg feed).

After treatment with *S. platensis* SGOT significantly (P<0.01) reduced to 83 ± 2.00 respectively. Alloxan, a β-cytotoxin, induced 'chemical diabetes' in animals by damaging the insulin-secreting cells of the pancreas and deflect serum biochemical parameters. (Ourida Aissaoui et al., 2017). The SGOT level decreased after *S. platensis* treatment in diabetes (Senthilkumar and John, 2008).

SGPT: In the group T_0, the Level of SGPT was normal, in the group T_1 SGPT level elevated to 94±2.45 (Table 8). After treatment with *S. platensis* SGPT significantly reduced, which was in agreement with findings of Senthilkumar and John, 2008 and Aissaoui et al., 2017.

Table 8
Effects on SGPT of rabbits treated with alloxan and spirulina.

Treatment group	T_o	T_1	T_2
1 week	$30^{a} \pm 3.53$	$53^{b} \pm 5.38$	$54^{b} \pm 5.79$
2 weeks	$32^{a} \pm 3.00$	$67^{b} \pm 5.38$	$47^{a} \pm 3.74$
3 weeks	$35^{a} \pm 2.20$	$80^{b} \pm 3.53$	$39^{a} \pm 4.00$
4 weeks	$35.40^{a} \pm 2.48$	$94^{b} \pm 2.45$	$33^{a} \pm 3.00$

Values with the different superscripts are statistically significant (P<0.01). T_0, Control (without treatment); T_1 Alloxan induction (150 mg/kg bw.); T_2, Spirulina treatment (1000 mg/kg feed).

Serum creatinine: In serum creatinine level, insignificant change was noticed in treated rabbits (group T_0) (Table 9). Diabetic rabbits showed significant elevation in total creatinine (4.10±.187), as compared to normal control group but treatment with *S. platensis* restored the elevated levels to 1.16±.220, supported by the findings of Farouk K. El-Baz et al., 2013.

Table 9
Effects on serum creatinine of rabbits treated with alloxan and spirulina.

Treatment	T_0	T_1	T_2
1 week	$.790^{a} \pm .022$	$2.68^{b} \pm .136$	$2.62^{b} \pm 1.36$
2 weeks	$.828^{a} \pm .033$	$3.16^{c} \pm .143$	$2.06^{b} \pm .117$
3 weeks	$.854^{a} \pm .019$	$3.72^{c} \pm .097$	$1.42^{b} \pm .146$
4 weeks	$.908^{a} \pm .021$	$4.10^{b} \pm .187$	$1.16^{a} \pm .220$

Values with the different superscripts are statistically significant (P<0.01). T_0, Control (without treatment); T_1 Alloxan induction (150 mg/kg bw.); T_2, Spirulina treatment (1000 mg/kg feed).

CONCLUSION

It can be concluded that spirulina have antidiabetogenic effects. The results also revealed that Spirulina have possitive effect on heamato-biochemical parameters and body weight and it can be used as herbal medicine to treat diabetes.

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