

POTENTIAL ROLE OF SPIRULINA PLATENSIS IN MAINTAINING BLOOD PARAMETERS IN ALLOXAN INDUCED DIABETIC MICE

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ABSTRACT

Objective: This study was designed to evaluate the effect of supplementation of *Spirulina platensis* on altered blood parameters in alloxan induced diabetic mice.

Methods: Diabetes mellitus was induced by intra-peritoneal administration of alloxan-monohydrate. Mice were divided into four groups of six mice in each group. Group-I was normal, group-II was diabetic, group-III was diabetic control fed with *Spirulina platensis* and group-IV was control mice fed with *Spirulina platensis* powder. The dose of 15 mgkg⁻¹bw at a single dose per day for three weeks was given orally to concerned test animal.

Results: The effects on erythrocyte count, percent haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and leucocyte count were estimated in diabetic mice and compared with other groups. WBC and MCV were significantly increased ($p < 0.05$), whereas rest other hematological parameters were significantly decreased ($p < 0.05$) in diabetic control non-treated mice, while these parameters in group-III were comparable with normal control group.

Conclusion: The findings indicated that the administration of *Spirulina platensis* tended to bring the parameters significantly towards the normal ($p < 0.05$) for group-III but insignificant for group-IV ($p < 0.05$). The *Spirulina platensis* proves to maintain blood parameters against alloxan induced diabetes mellitus.

Keywords: Blood parameters, *Spirulina platensis*, *Mus musculus*, Alloxan, Diabetes mellitus.

INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases that characterized by hyperglycemia. It is a condition in which the body does not produce enough or properly respond to insulin, a hormone produced by the beta cells of pancreas. Insulin enables cells to absorb glucose in order to turn it to energy. Increased blood glucose level in the blood, often leads to various complications[1,2]. DM is creating disturbances in carbohydrate, fat and protein metabolism[3]. It is characterized by hyperglycemia, polydipsia and polyuria. It causes retinopathy, nephropathy and neuropathy[4]. It is the most common endocrine disorder. It is estimated that more than 200 million people will have DM worldwide and 300 million will subsequently have the disease by 2025[5].

Spirulina platensis (SP) is a genus of filamentous cyanobacteria (Blue Green Algae) with a coil-like shape. It is rich in proteins, lipids, carbohydrates and some vital elements like zinc, magnesium, manganese, selenium, β -carotene, riboflavin, tocopherol and α -linoleic acid[6,7,8].

It is used for treatment of many diseases like Allergy, Rhinitis. It shows Immunomodulation Effects[9], Anti-Inflammatory Activity[10], Antioxidant effects[11], Anticancer effects[12], Anti-viral effects[13].

Diabetic models have provided considerable insight into physiological and biochemical derangement of the diabetic state[14]. Various hematological parameters and the immune system were also reported to be altered due to DM[15]. Anemia is also caused in diabetic patients due to the hemolysis of red blood cells (RBCs)[16].

Synthetic drugs used for the treatment of diabetes may produce serious adverse effects[17]. A lot of money and time are required to synthesize a new drug to be in the market after appropriate clinical trials, therefore, there is a need to explore and characterize more of these abundant natural plants for the management of diabetes. Medicinal plants used to treat hyperglycemic conditions are of considerable interest for ethno-botanical community[15-18]. For quite some years now, plants have been recommended for the treatment of diabetes[19-23]. Meanwhile, medicinal plants are commonly used in under developed countries as alternative therapies which are effective in controlling plasma glucose level with minimal side.

It has been reported that ingestion of medicinal plants or drugs can alter the normal hematological values[24]. Therefore, hematological parameters could be an important tool in the assessment of deleterious effect of drugs as well as medicinal plants[25]. Herbal drugs are widely prescribed today because of their minimal adverse effects and low cost[26]. Researches on medicinal plants are very popular in the medical sciences because of their availability and increase usage throughout the world. Most of these plants have not been identified and fully authenticated for their proper use in specific diseases[27].

The current treatment for diabetes mellitus have many drawbacks including undesirable side-effects and the high cost of long-term treatment. SP has been studied for treatment of variety of diseases by many workers but detail literature related to haematological changes is still not adequate. Considering the therapeutic potential of SP, the aim of the present investigation is to study and evaluate the altered blood parameters in alloxan induced diabetic mice.

MATERIALS AND METHODS

Plant material

SP powder for all experiments has been procured from Sunova Spirulina, India. It is a spray dried product, standard in quality and a part of bulk production by the industry.

Experimental animal

Swiss albino mice *Mus musculus* weighing about 22-27 gram were obtained from CDRI Lucknow, India. Mice were maintained at the animal house of University Department of Zoology, T.M. Bhagalpur University, Bhagalpur, India under standard condition and fed with standard diet. The animals were housed throughout the experiment in polypropylene cages (six animals housed per cage). Animals were maintained under controlled conditions of temperature ($23 \pm 1^\circ\text{C}$), humidity ($50 \pm 15\%$) and normal photoperiod (12 to 12 h light-dark cycles). The animals were allowed free access to standard dry pellet diet and water *ad libitum*. Rice husk was used as bedding material and changed daily. The research proposal was approved by Departmental Research Committee of University Department of Zoology, T.M. Bhagalpur University, Bhagalpur, India. All the animals were kept as accepted principles for laboratory animal use and care. The mice were

acclimatized for one week before the experiment and then used in experiment at about 12 weeks of age.

Drugs and Chemicals

The drug Alloxan-monohydrate purchased from Loba chemical, Mumbai, India. All other chemicals were analytical grade and used as such without further testing.

Induction of Diabetes

The animals were fasted for 16 to 18 h with free access to water prior to the induction of diabetes. Diabetes was induced by intraperitoneal (i.p) administration of Alloxan-monohydrate[28]. The total dose of Alloxan-monohydrate (450 mg/kg¹bw) was administered in three injections at intervals of 48h (150 mg/kg¹bw) each time. The mice with blood glucose level above 200 mg/dl were then selected for the study.

Experimental Design

The experimental mice were divided into four groups of 06 animals. The total experimental protocol was maintained for 21 days after induction of diabetes.

Group-I (Control)

Group-II (Diabetic control)

Group-III (Diabetic control mice fed with SP)

Group-IV (Control mice fed with SP)

Haematological Analysis

The total RBC and Leucocytes (WBC) count were done by using Thoma Ziess haemocytometer[29,30]. Haemoglobin concentration (gram/100ml) was determined by Sahlis haemoglobinometer[29]. The Packed Cell Volume was estimated by the method of Wintrobe using Van-allen-microhaematocrit tubes by centrifuging it for 30 minutes at 3000 rotation per min[31]. Mean corpuscular volume, Mean corpuscular haemoglobin and Mean corpuscular haemoglobin

concentration were determined by using the formula as suggested by Hyde[32]. Collected blood in tubes containing EDTA was used to assess changes in white blood cell (WBC), packed cell volume (PCV) and haemoglobin (Hb)[33,34].

$$MCV(\mu m^3) = \frac{PCV(\%)}{\text{Total RBC million/mm}^3} \times 10$$

$$MCH(pg) = \frac{\text{Hb in gram/100ml}}{\text{RBC million/mm}^3} \times 10$$

$$MCHC(\%) = \frac{\text{Hb (gm \%)}}{\text{PCV (\%)}} \times 100$$

Statistical analysis

The results are presented as means \pm SEM. The statistical methods used to analyse the data in this study were unpaired Student's t-test (two-tailed) and two-way analysis of variance (ANOVA). Comparisons with p values < 0.05; <0.01 were considered to be statistically significant. Analysis of Variance (ANOVA) and standard error of mean were calculated using standard formula[35]. a= Significant difference compared between Group-I against Group-II (p<0.05, p<0.01); b= Significant difference compared between Group-II against Group-III (p<0.05, p<0.01).

RESULTS

Erythrocyte Count (RBC Count)

The control mice showed 5.88 \pm 0.22 to 5.89 \pm 0.334 million RBC per cubic millimetre (Figure-1). Significant decrease in erythrocyte count was observed at days 14 and days 21 in group-II (p<0.05; p<0.01) when compared with group-I. Control mice when fed with SP powder showed insignificant change in erythrocyte count.

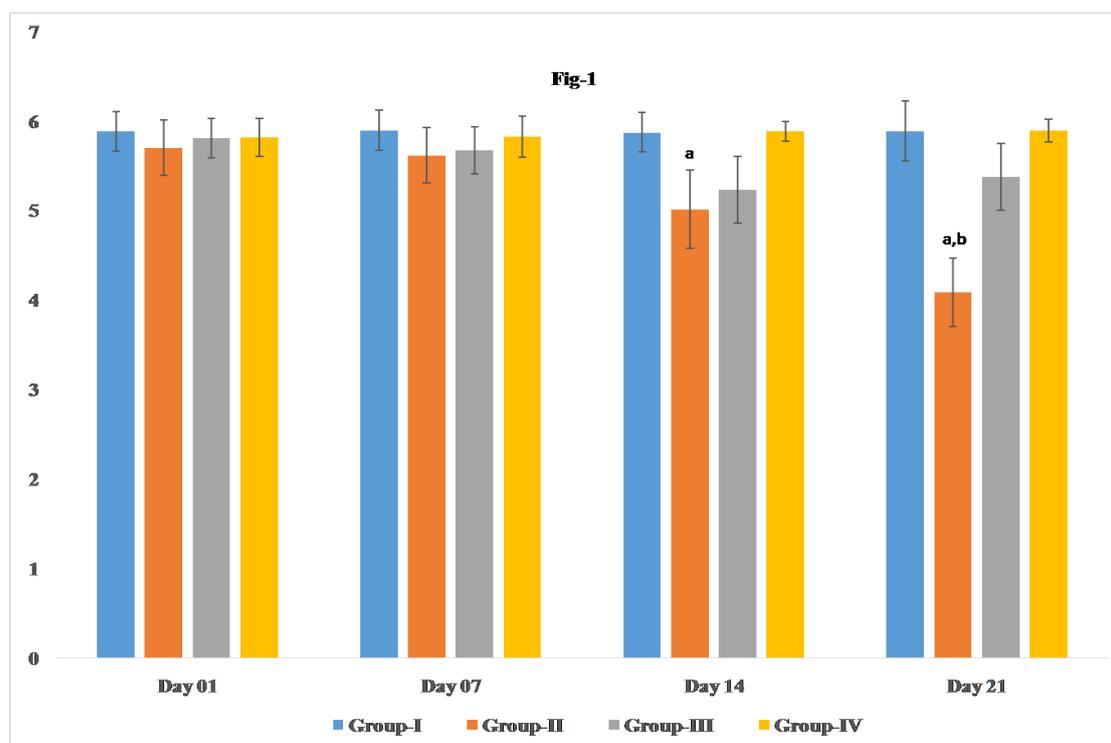


Fig. 1: Effect of treatment with *Spirulina platensis* powder for three weeks on RBC Count (10⁶/mm³)

N= 6, Values are expressed as mean \pm SEM. ^{a,b}Values in same column with different superscripts are significantly different at P<0.05 and P<0.01 respectively.

Leucocyte Count (WBC Count)

The control animals showed 6.18 ± 0.061 to 6.08 ± 0.09 thousand cells/mm³ WBC during experiment (Figure -2). Significant increase in leucocyte count was observed in group-II when compared with group-I at incubation period (7 days, 14 days and 21 days). Diabetic mice when treated with SP powder showed leucocyte count decreased from 6.91 ± 0.06 to 6.23 ± 0.14 thousand cells/mm³. Control mice when fed with SP powder showed insignificant change in leucocyte count ($p < 0.05$, $p < 0.01$).

Percent Haemoglobin (Hb %)

The control group showed 9.79 ± 0.41 to 9.76 ± 0.22 gram/100ml of haemoglobin during experiments (Figure-3). Significant decrease in haemoglobin (Hb %) was observed in group-II at days 14 and days 21 when compared with group-I ($p < 0.05$, $p < 0.01$). Diabetic mice when treated with SP powder showed that there is increase in haemoglobin (Hb %) from 5.95 ± 0.63 g/100 ml to 9.69 ± 0.07 g/100 ml. Control mice when fed with *Spirulina platensis* powder showed insignificant changes in haemoglobin ($p < 0.05$, $p < 0.01$).

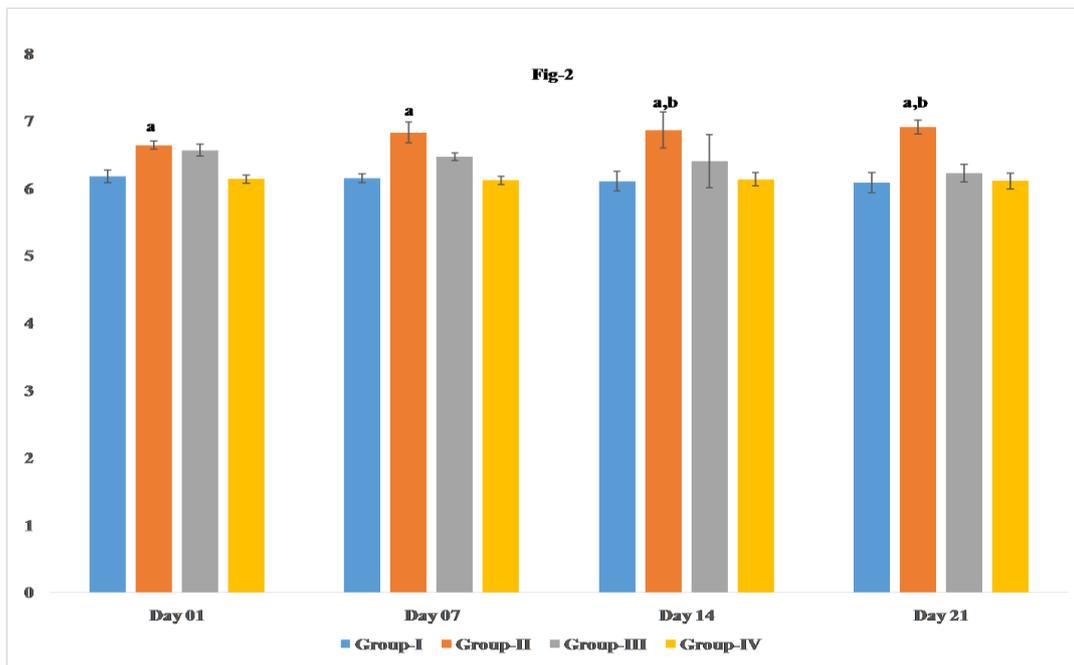


Fig. 2: Effect of treatment with *Spirulina platensis* powder for three weeks on WBC Count (10³/mm³)

N= 6, Values are expressed as mean ± SEM. ^{a,b}Values in same column with different superscripts are significantly different at $P < 0.05$ and $P < 0.01$ respectively.

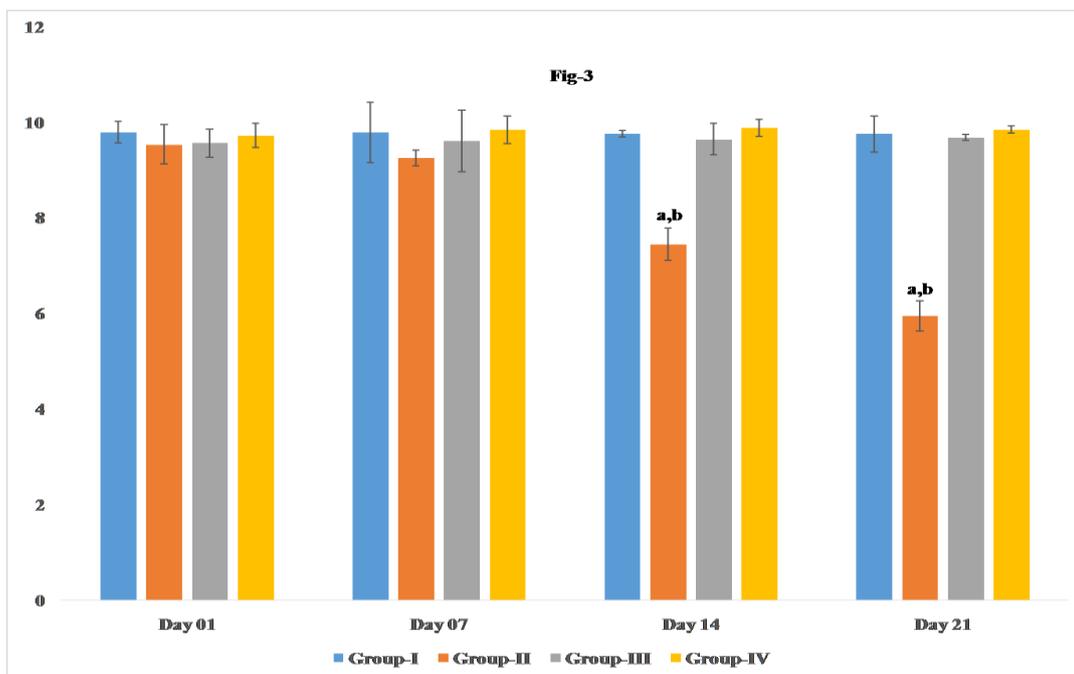


Fig. 3: Effect of treatment with *Spirulina platensis* powder for three weeks on Blood Haemoglobin (g/100ml)

N= 6, Values are expressed as mean ± SEM. ^{a,b}Values in same column with different superscripts are significantly different at $P < 0.05$ and $P < 0.01$ respectively.

Packed Cell Volume (PCV %)

The PCV % of Group-I ranged from 37.81±0.43 to 38.02±0.32 during all experiments (Figure-4). Packed cell volume (%) was found to be reduced significantly with the increase of exposure period (day 01, 07, 14 and 21) in alloxan induced diabetic mice. Diabetic mice when treated with SP powder showed elevated packed cell volume (PCV %) in all exposure period (day 01, 07, 14 and 21). Control mice when fed with SP powder showed insignificant change in packed cell volume (%). The minimum value of packed cell volume (PCV %) recorded was 35.08±1.22% at 21 days of exposure period in group-II.

Mean Corpuscular Volume (MCV)

Mean corpuscular volume of control animal showed 64.32±2.57-64.74±4.045 μm^3 during experiments (Table-1). Significant increase in mean corpuscular volume was observed in group-II when compared with group-I at days 14 and 21 respectively ($p<0.05$; $p<0.01$). Treated diabetic mice showed mean corpuscular volume 66.85±0.37 μm^3 at days 21. Control mice when fed with SP powder showed insignificant change in mean corpuscular volume.

Mean Corpuscular Haemoglobin (MCH)

The control animal showed 16.67±1.05 to 16.62±1.212 picogram of MCH. Alloxan induced diabetic mice showed significant decrease in MCH value (Table-2). The lowest value of MCH observed was 14.77±2.95 picogram. Control mice when fed with SP powder showed repair of MCH value.

Mean Corpuscular Haemoglobin Concentration (MCHC)

Declining trend was observed in MCHC value in diabetic mice. The decrease was more pronounced with the increase of incubation period (Table-3). The lowest value (19.99±1.99) of MCHC (%) was recorded at 21st day after the induction of diabetes. Significant decrease in MCHC was observed in group-II when compared with group-I at incubation period (14 days and 21 days). Diabetic mice when treated with SP powder showed MCHC increased from 19.99±1.99 to 23.90±0.12 of MCHC (%) at days 21. Control mice when fed with SP powder showed insignificant change in MCHC (%) ($p<0.05$, $p<0.01$).

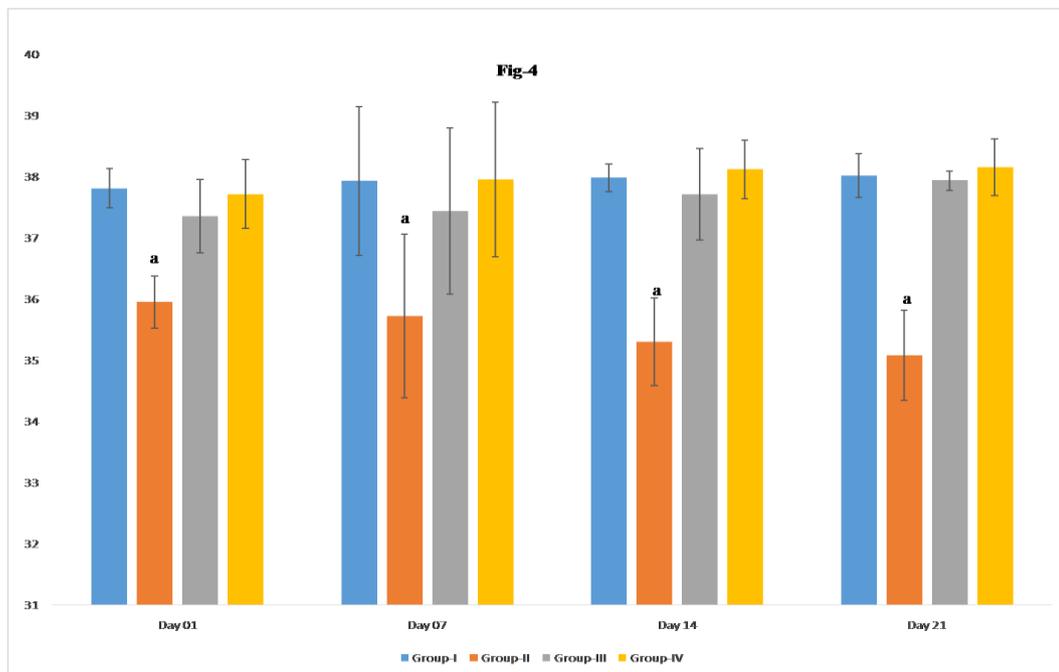


Fig. 4: Effect of treatment with *Spirulina platensis* powder for three weeks on PCV (%)

N= 6, Values are expressed as mean \pm SEM. a,bValues in same column with different superscripts are significantly different at $P<0.05$ and $P<0.01$ respectively.

Table 1: Effect of treatment with *Spirulina platensis* powder for three weeks on MCV (μm^3)

Group of Mice	Mean Corpuscular Volume (MCV) (μm^3)			
	Day 01	Day 07	Day 14	Day 21
Group-I	64.319±2.568	64.339±1.988	64.704±1.721	64.736±4.047
Group-II	64.059±5.055	64.811±5.165	67.698±5.206 ^{ab}	70.593±9.973 ^{ab}
Group-III	64.074±2.322	64.098±3.300	65.305±4.697	66.852±0.371
Group-IV	64.929±3.262	64.929±3.262	64.763±1.975	64.720±1.367

N= 6, Values are expressed as mean \pm SEM. a,bValues in same column with different superscripts are significantly different at $P<0.05$ and $P<0.01$ respectively.

Table 2: Effect of treatment with *Spirulina platensis* powder for three weeks on MCH (pg)

Group of Mice	Mean Corpuscular Haemoglobin (MCH) Content (pg) \pm SEM			
	Day 01	Day 07	Day 14	Day 21
Group-I	16.669±1.052	16.769±1.142	16.624±0.933	16.623±1.218
Group-II	16.774±1.054	16.674±1.252	16.531±1.608 ^{a,b}	14.766±2.946 ^{a,b}
Group-III	16.602±1.079	16.503±1.079	16.521±1.093	16.552±1.313
Group-IV	16.727±0.578	16.621±0.578	16.718±0.734	16.709±0.681

N= 6, Values are expressed as mean \pm SEM. a,bValues in same column with different superscripts are significantly different at $P<0.05$ and $P<0.01$ respectively.

Table 3: Effect of treatment with *Spirulina platensis* powder for three weeks on MCHC (%)

Group of Mice	Mean Corpuscular Haemoglobin Concentration (MCHC) %			
	Day 01	Day 07	Day 14	Day 21
Group-I	25.913±1.164	25.828±0.875	25.716±0.874	25.671±0.649
Group-II	25.562±1.074	25.904±1.451	21.143±1.445 ^a	19.991±1.992 ^a
Group-III	25.623±1.012	25.688±0.828	23.587±0.616	23.900±0.119
Group-IV	25.801±1.271	25.948±0.220	25.936±0.334	25.816±0.870

N= 6, Values are expressed as mean ± SEM. ^{a,b}Values in same column with different superscripts are significantly different at P<0.05 and P<0.01 respectively.

DISCUSSION

Blood parameters are key factors in diagnosing the actual physiological status of animals. An organism must keep its blood composition and constituent relatively constant under natural conditions to function properly[36]. It is thus natural to expect the alteration in blood parameter in diabetic subjects[37].

Alloxan monohydrate is known to induce diabetes by partial destruction of pancreatic beta cells of islet of langerhan[38,39]. This results in depletion of insulin levels and hyperglycemia leading to DM. The alloxan-treated mice, therefore, appear to represent a good laboratory model for DM. There is possibility for the survival of a few beta-cells and this has been proved by several workers who observed antihyperglycemic activity with oral hypoglycemic agents like glibenclamide, tolbutamide etc. in alloxan-induced diabetic mice[40-42].

Flavonoids[43-45], triterpenoid[46,47], tannins[48,49], saponins[50] and glycosides of different plant origin showed a promising antidiabetic activity as demonstrated in diabetic animal models.

In the present study, the time response of % Hb, RBC Count, WBC Count, PCV, MCV, MCH and MCHC in blood of control as well as in diabetic mice were studied (Figure:1-4; Table:1-3). The decrease in the erythrocyte count and haemoglobin concentration was mainly due to the damaging action of decreased insulin and increased blood glucose level on the erythropoietic tissue. The present study further reveals decrease in erythrocyte count at all incubation period. The change in value of RBC count was in accordance with the reduced hemoglobin content. This may be due to decrease in the number of erythropoietin sensitive centres committed to RBC precursors. The lower Hb content may be due to the lack of time for synthesis of RBC. Lower Hb content may also be due to the insufficient supply of iron from source[51]. The reduction in hemoglobin content may be related to decrease amount of RBC number which indicates hemolysis, hemorrhage and reduced erythropoiesis in pathophysiological conditions. In group-III diabetic mice were treated, this might be that SP powder which is rich source of iron contributed to the elevated levels of hemoglobin. Vitamin B₁₂ and Folic acid deficiency causes maturation of erythrocytes ceases in the process of erythropoiesis[52,53].

Similarly, significant decrease in the haemoglobin content and packed cell volume were also observed in mice due to pathophysiological condition when treated with heavy metals[54] tartrazine[55], food colours[56], chemical dye[57], sodium benzoate[58] and fluoride[59]. These chemicals also caused elevation in blood glucose levels.

A decreased MCHC value was observed in diabetic mice when compared to control group. The MCHC expresses the concentration of haemoglobin in the cytoplasm of the erythrocytes. Increased level of blood glucose and decreased level of insulin decline capacity to manufacture haemoglobin at the required rate in bone marrow, so the haemoglobin content of each cell has reduced MCHC value[60].

Similarly, significant decrease in MCHC was also observed in laboratory mammal having increased blood glucose and cholesterol level when exposed to selenium[57], tannery[61].

In the present investigation, an increase in the total WBC count was recorded at all the incubation period. The leucocytes are the mobile units of the body's defensive mechanism. The cause of enhancement

of WBC content may affect the defensive mechanism against the pathophysiological conditions in the body[62,63].

Studies have shown that SP powder feeding at experimental dose (15 mg/kg-1bw) of diet for continuously three weeks was not toxic for Swiss albino mice. Experiments have also been carried out for the toxicological evaluation of single cell proteins (*Spirulina sp.*), mainly in rats as well as the target (farm) animals[64].

WBC was found to be increased in diabetic subject due to pathophysiological conditions including autolysis caused by some hydrolytic enzymes released by plasma under stress[65]. SP strengthened hemopoetic system by supplying various constituent thus helps to control MCH, MCHC which was found to be decreased in diabetic subject.

Reactive oxygen species has also been implicated in the mechanism of red cells damage[66,67]. DM is usually accompanied by increased production of free radicals[68,69]. Excessively high levels of free radicals cause damage to cellular proteins, membrane lipids and nucleic acids and eventually cell death. The anaemic condition that occurs in DM has been reported to be due to the increased non-enzymatic glycosylation of red blood cell membrane proteins[70]. Oxidation of these proteins and hyperglycaemia in DM causes an increase in the production of lipid peroxides, a marker of oxidative stress in diabetes which consequently have toxic effects on cells through degradation to highly toxic hydroxyl radicals[71-73] that lead to haemolysis of red blood cell[70,71]. However, treatment of diabetic animals with SP improved the levels of packed cell volume, haemoglobin concentration and red cell count. This suggests that the SP may contain some phytochemicals that can stimulate increased protein synthesis or mobilization which are largely produced in the liver and also secretion of erythropoietin in the stem cells of the animals. Erythropoietin is a glycoprotein hormone which stimulates stem cells in the bone marrow to produce red blood cells[74,75]. The stimulation of this hormone enhances rapid synthesis of red blood cell which is reflected by increased levels red blood cell and packed cell volume.

Alloxan-induced diabetic mice showed significantly reduced blood levels when compared to the normal control group. Alloxan is a well known chemical has been reported to suppress the immune system by destroying certain cells and organs in the body[74] as was observed in this present study. The alteration of these parameters could be attributed to change in the number of leucocytes which may account for poor defensive mechanisms against infection, thus may have consequential effects on the immune system and phagocytic activity of the animals[76,77].

CONCLUSION

It is concluded that oral SP treatment might normalize some hematological parameters. Thus, the present study provides a scientific evidence for the use SP as anti-diabetic agent. The antioxidant activity of the SP might also contribute towards the anti-diabetic effect of the SP by providing protection against the cytotoxic effect of free radicals generated by the alloxan or diabetes itself[78,79]. This study indicates that SP might attenuate some disturbed hematological parameters of diabetic mice. In this study, the hemoglobin level in diabetic control mice decreased, which is in agreement with other studies[80,81]. So, it is clear from the data that SP may not have adverse effect on the bone marrow, kidney and hemoglobin metabolism. The results of this study showed that SP possess anti-diabetic activity and improved the levels of erythrocyte

indices in alloxan-induced diabetic animals at all doses.

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