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# Hyperglycemia Induced Pathological Shift in Blood Biochemical Parameters of Male Albino Mice and its Repair by Using *Spirulina plantesis* Powder

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## ABSTRACT

The consequence of *Spirulina plantesis* powder on lipid profile in normal and diabetic mice was investigated and presented in present work. Diabetes was induced in male mice (28 ± 5 g) by intra-peritoneal injection of alloxan monohydrate. Both normal and diabetic mice were fed with standard animal food containing 40% of Spirulina *plantesis* powder for three weeks.

In diabetic mice, reduction was recorded in serum triglyceride (TG) from 175.6 ± 3.8 (mg/dL) to 79.8 ± 1.96 (mg/dL) and total cholesterol from 188.2 ± 6.86 (mg/dL) to 108.8 ± 2.64(mg/dL) when mice fed with *Spirulina platensis* powder. A significant increase was recorded in HDL that is 14.8 ± 1.12 (mg/dL) to 27.6 ± 0.16 (mg/dL) where as decreasing trend was recorded in LDL 138.2 ± 8.82 (mg/dL) to 45.24 ± 2.4 (mg/dL) and VLDL 35.20 ± 2.8 (mg/dL) to 15.96 ± 2.14 (mg/dL) These results indicate that *Spirulina platensis* powder recovered the serum lipid profile in diabetic mice by decreasing TG, LDL-C and increasing serum HDL-C. *Spirulina platensis* powder thus proved to be of great worth in managing the diabetic complications.

**Keywords:** *Spirulina platensis powder, Diabetes, Serum triglyceride, Total cholesterol, Diabetic complications.*

## INTRODUCTION

The prevalence of diabetes is mounting and insulin resistance forms the most important factor in its rise. The hyperglycemia resulting in Diabetes leads to multiple complications including retinopathy, nephropathy and regular abscess etc. Judicious changes in life style, controlled dietary intake and physical exercises with proper treatment have been successful in elevating the release of Insulin among diabetics but have not been successful in preventing co-morbidities with cardiovascular involvement (Yazbek, 2010). The improvement in these vital biogenic substances of diabetes is normally treated by synthetic drugs but otherwise traditional and ethnic products like, Jamun, Fenugreek, Gudmar etc have always been in confidence among the diabetics both in urban and rural population (Kothari and Bokaria,2012). Recently Spirulina has also been considered for the treatment of diabetes as an alternative source. Lipids are the different fatty components found in the blood. The lipid profile is the results of a blood test that measures levels of lipids or fats, including cholesterol and triglycerides. Lipids are needed by the body to build cell membranes, make certain hormones and store energy. Triglycerides reflect dietary fat and carbohydrate intake. Cholesterol is made in the liver and can be linked to cardiovascular risk factors. Lipids do not dissolve in water; they are carried in the blood by special proteins made in the liver (Maahs *et al*., 2010).

The lipid profile typically includes:

- Total cholesterol
- High density lipoprotein cholesterol (HDL-C)- frequently called good cholesterol
- Low density lipoprotein cholesterol (LDL-C)-often called bad cholesterol.

HDLs are lipoproteins made mostly of protein and little cholesterol. HDLs can help to clear cholesterol deposits in blood vessels left by another blood component called low-density lipoproteins, or LDLs. (Uko *et al*., 2013).

In the present chapter alterations in different biochemical parameters due to alloxan induced Diabetes (Group II) have been studied and then upon treatment with Spirulina upon experiment animals of Group III have been investigated by supplementing it in powder form for different periods. Animals of Group I (Control) have been compared with alloxan induced diabetic mice (Group II) to estimate the deterioration in Diabetic mice.

## MATERIALS AND METHODS

**Plant material:**

*Spirulina platensis* was procured from Sumanglam Sales Corporation, Jaipur, (Rajasthan). It is a spray dried product in powder form, standard in quality and of scientific grade.

**Experimental animal:**

Swiss albino mice *Mus musculus* weighing about 25±5 gram were obtained from CDRI Lucknow. Mice were maintained at the Animal house of University Department of Zoology, T.M. Bhagalpur University under standard conditions and fed with standard diet. Food and water were given *ad libitum*. Rice husk was used as bedding material and changed daily. Animal handling was performed as per good laboratory practice (Work Manual, CDRI). The mice of 12 weeks of age were acclimatized in the laboratory condition for one week before the experiment (Engle and Rosasco, 1927, Zarrow *et al*., 1964).

**Drugs and Chemicals:**

The drug Alloxan-monohydrate was purchased from Loba Chemicals, Mumbai. All other chemicals used in the entire experiments were of analytical grade.

**Induction of Diabetes:**

Diabetes produced by alloxan results in higher blood sugar levels, which act mainly on release of β-cells. Diabetic animals are used in testing drugs for use in treatment of diabetes mellitus (Huralikuppi, 1991; Williamson *et al*., 1996 and Viana *et al.*, 2004). The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of *beta* cells (Szkudelski, 2001). Thus alloxan induced diabetes mellitus served as a pathological bio model for testing a substance with supposed antioxidant activities *in vivo* (Bartosikova *etal.,* 2003). One of the targets of the reactive oxygen species in DNA of pancreatic islets. Its fragmentation takes place in *beta* cells exposed to alloxan (Takasu *et al.,* 1991and Macedo *et al.,* 2005). A solution of 2% alloxan (40mg/kg) diluted in 0.9% normal saline is used. It is prepared as 5% w/v in distilled water and then injected in experimental mice. Alloxan monohydrate 150mg/kg body weight was

dissolved in normal saline and injected intraperitoneally after 18 hours fasting to induce hyperglycemia in experimental mice (Yanarday and Colak, 1998). Diabetes was induced by intra-peritoneal (i.p) administration of Alloxan-monohydrate (Lenzen and Panten, 1988). The total dose of Alloxan-monohydrate (450 $mgkg^{-1}bw^{-1}$) was administered in three injections at intervals of 48h (150 $mgkg^{-1}bw^{-1}$) each time. (Maeda *et al*., 2000). Mice with blood glucose level of greater than 150mg/dl were considered diabetic and were selected for study (Antia et al., 2005).

The diabetic state was confirmed 48 h after alloxan injection by weight loss, glycosuria (Ajabnoor, 1990) and hyperglycemia (Belfiore *et al., 1990*). The animals, which had blood glucose level more than 200 mg/dl (Boquist *et al*,1983), as well as with the clinical signs of polydypsia (Brownle *et al*., 2001), polyuria (Sundaram *et al*., 1996) and polyphagic (Sochar *et al*.,1985 ) were selected for the experiment.

**Experimental Design:**

The experimental mice were divided into three groups of 10 animals each.

Group-I (Control)

Group-II (Diabetic control)

Group-III (Diabetic fed with *Spirulina platensis)*.

The total experimental protocol was maintained for 21 days after induction of diabetes as per method suggested by Layam and Reddy (2006). Experiments were performed on the frequency of 7, 14 and 21 days for all the test animals.

**METHOD OF BLOOD SAMPLE COLLECTION:**

At the end of experimental protocol, blood samples were obtained from animal by puncture from the tail of the mice; using disposable needles (25 G) fitted with plastic syringe (Lynch etal., 1969) and was carefully transferred to collecting tubes containing 4% sodium citrate. The blood collection tubes were kept cool on ice and transported to the laboratory whereas Lipid profiles were performed (D'amour *et al*., 1965).

**BLOOD BIOCHEMICAL ANALYSIS:**

**Lipid profile:**

Serum was used for the estimation of lipid profile. Total Cholesterol (TC) and HDL were obtained by standard procedure (direct method). LDL and VLDL level were obtained by calculation using prescribed formula. Very Low Density Lipoprotein and Low Density Lipoprotein were calculated by as per Fridevald's equation (Fridewald *et al*., 1979).

**Total Cholesterol Assays (Chod/pOD method):**

**Apparatus-**Test tubes, Micropipette

**Chemical-**Total Cholesterol assays kit contains,$L_1$ – Enzyme reagent 1, $L_2$ – Enzyme reagent 2, .Cholesterol standard

**Procedure:** 2 ml of blood was taken from the tail of the animal by disposable syringe and centrifuged it at 3000 rpm for 15 minute. Serum was separated which was used in all kinds of cholesterol test. Three test tubes ($1^{st}$ as Blank B, $2^{nd}$ as standard S, $3^{rd}$ as test T) were taken.

In the each test tube 0.8 ml $L_1$ and 0.2 ml $L_2$ were taken. 10 µl distilled water, 10 µl standard cholesterol and 10µl serum were added respectively in three test tube. These are shaken and incubated at 37 $^0$C for 5 minute. Optical density was determined by spectrophotometer at 520 nm.

**Reaction Mechanisms:**

Cholesterol + oxygen -- (enzyme cholesterol oxidase)--> cholestenone + hydrogen peroxide

Hydrogen peroxide + 4-aminophenazone + phenol --(enzyme peroxidase)--> colored complex.

**Calculations:**

O.D. of blank = B

O.D. of standard = S

O.D. of test = T

$$\frac{T - B}{S - B} \times 200 \text{ (mg/dl)}$$

**High Density Lipoprotein:**

HDL Cholesterol (High Density Lipoprotein- Cholesterol)

PEG Precipitation method

**Apparatus** – Small test tube, Test tube, Micropipette.

**Chemical**– HDL Cholesterol Kit contains

$L_1$ = Precipitating reagent

S = HDL Cholesterol standard (25 mg/dl)

Kit of total cholesterol

$L_1$ = enzyme reagent 1

$L_2$ = enzyme reagent 2

**Procedure:**

**Sample Preparation:** 0.1 ml L1 (precipitating reagent) and 0.1 ml serum were taken in a small test tube. It was left for 5 minutes at room temperature. Supernatant was used on centrifugation at 3000 rpm for 15 minutes. Three test tubes (1$^{st}$ as Blank B, 2$^{nd}$ as standard S, 3$^{rd}$ as test T) were taken. In each test tube 0.8 ml L1 (enzyme reagent 1) and 0.2 ml L2 (enzyme reagent 2) were taken. Now 50 µl distilled water, 50 µl HDL standard and 50 µl prepared sample on centrifugation were added respectively in three test tubes. These test tubes are incubated at 37$^0$ C for 15 minutes. Optical density was determined by Spectrophotometer at 520 nm.

**Calculations:**

If, O.D. of blank = B

O.D. of standard = S

O.D. of test = T

$$\frac{T - B}{S - B} \times 50 \text{ (mg/dl)}$$

**Very Low Density Lipoprotein:**

VLDL Cholesterol = Serum triglyceride/5

LDL Cholesterol =Total Cholesterol – (HDL-C+ VLDL-C)

## RESULTS AND DISCUSSION

The value of Total cholesterol of control mice (Group-I) varied from 79.84±1.78 mg/dl to 82.18±2.24 mg/dl from experimental days 1 to 21. A significant increase in Total cholesterol was recorded in day 01 (108.94±2.64 mg/dl) on days 07 (138.62±1.98 mg/dl), on days14 (142.42±1.24mg/dl) and days 21 (148.92±1.28mg/dl) in diabetic control mice (Group-II). It was found that at days 21, the level of Total cholesterol decreased from 148.92±1.28mg/dl to 98.65±3.72 mg/dl in Group-III when compared with diabetic control mice (Group-II). Analysis of Variance showed that the source of variance between groups (treatment) and with in groups (residual) were found to be insignificant in day 1 but significant at days 07, days 14 and highly significant at days 21 at 95 % and 99 % of confidence ($p<0.05$, $p<0.01$). The total cholesterol (Table :A) was found to have increased in Group-II due to hyperlipidemia condition and this condition was treated in Group-III by administration of *Spirulina platensis* powder. This leads to significant reduction in blood glucose level and serum lipid level which is in agreement with other studies (Prohaska *et al*.,1985;WHO,ATC,1991;Yazbek,2010;Boitard,2012;Uko *et al*., 2013 and ADA,2014). The increased secretion of insulin stimulates fatty acid biosynthesis and also the incorporation of fatty acids into triglycerides in the liver and adipose tissue (Prohaska *et al*.,1985) Oral food significantly reduces the Total Cholesterol and Triglycerides in serum as compared to the diabetic group. High Density Lipoprotein (HDL) levels were decreased in diabetic group (Maahs *et al*., 2010).

In the present work (Table A), it is found that dietary supplementation of *Spirulina platensis* powder at 200 $mgkg^{-1}bw^{-1}$ (Layam and Reddy, 2006) causes an increase in HDL and decrease in triglyceride**.** The triglyceride was found to be increased in Group-II and this condition was treated in Group-III. The strong anti hyper lipidemic activity of *Spirulina platensis* powder could be through its control of diabetes, as this is a major determinant of Triglycerides and Total Cholesterol levels (Luc and Fruchart, 1991). Serum Triglycerides values of control mice (Group-I) as per experimental protocol varied from 75.30±2.28 mg/dl to 79.20±1.84 mg/dl from day 1to days 21. A significant increase in Serum Triglycerides value was estimated on day 01 (94.30±2.68 mg/dl), days 07 (104.10±1.94 mg/dl), days14 (110.70±1.46 mg/dl) and days 21 (119.80±1.32 mg/dl) in diabetic control mice (Group-II). Among mice of Group-III, it was found that at days 21, the level of Serum Triglycerides decreased from 119.80±1.32 mg/dl to 79.45±3.78 mg/dl when compared with animals of Group-II. Upon Statistical analysis it was found that in Analysis of Variance (ANOVA for single factor) groups (between treatment) and with in groups (residual) were found to be insignificant in day 1 but significant at days 07, days 14 and highly significant at days 21 at 95 % and 99 % of confidence ($p<0.05$, $p<0.01$).

The values of very low density Lipoprotein of control mice (Group-I) varied from 15.06±1.16 mg/dl to 15.84±1.24 mg/dl between days1to days 21. A regular increase was recorded in the values of VLDL on day 01 (18.86±1.12 mg/dl) to days 07 (20.82±1.04 mg/dl), then to days 14 (22.14±1.24 mg/dl) and days 21 (23.96±1.14 mg/dl) among diabetic control mice (Group-II) when compared to the values of control mice (Group I). Analysis of Variance, ANOVA for single factor between groups (treatment) and with in groups (residual) insignificant in day 1 but found significant at days 07, days 14 and highly significant at days 21 at 95 % and 99 % of confidence ($p<0.05$, $p<0.01$). LDL plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to LDL. The decrease of cholesterol and LDL levels achieved by oral dose of *Spirulina platensis* demonstrates a possible protection against hypercholesterolemia (Gingsberg, 1994). Values of High Density Lipoprotein of control mice of Group-I ranged from days 1 to 21 between 23.80±1.24 mg/dl to 26.10±1.46 mg/dl. However, significant increase was observed in HDL values, on first day 01 (22.70±1.28 mg/dl), on days 07 (19.24±1.58 mg/dl), days14 (18.16±1.47mg/dl) and days 21(17.28±1.24 mg/dl) respectively in diabetic control mice of Group-II. The values however days 1 to 21 days among animals of Group-III, recorded increasing trend from 17.28±1.24 mg/dl to 25.26±1.63 mg/dl when compared with mice of Group-II. ANOVA for single factor as between groups of treatment and within residual groups were found to be insignificant in day 1 but significant at days 07, days 14 and highly significant at days 21 at 95 % and 99 % of confidence ($p<0.05$, $p<0.01$). The values of low density Lipoprotein of mice of Group-I) fluctuated from 69.58±1.25 mg/dl to 73.44±1.12 mg/dl from days 1 to 21. There was a significant increase in Low Density Lipoprotein on day 01 (105.12±1.16 mg/dl), days 07 (140.20±1.63 mg/dl), days14 (146.40±1.58mg/dl) and days 21 (155.60±1.78 mg/dl) Group-II. The values normalized at days 21, from 155.60±1.78 mg/dl to 89.28±2.18 mg/dl in Group-III

Statistical analysis showed that the source of variance between groups (treatment) and within groups (residual) were insignificant in day 1 but significant at days 07, days 14 and highly significant at days 21 at 95 % and 99 % of confidence ($p<0.05$, $p<0.01$)

In the present study, total cholesterol and triglycerides were controlled significantly by *Spirulina platensis* powder treatment in diabetic mice. This effect might have been possible due to the control of hyperglycemia. Elevated LDL, VLDL and decreased HDL cholesterol concentrations in diabetic mice appear to be markedly altered favorably by *Spirulina platensis* supplementation.

Similar observations have been found on application of a number of other medicinal plants. The results on such experiments have also been reported to have antihyperglycemic, antihyperlipidemic and insulin stimulatory effects as in the works of Prohaska *et al*., 1985 Gingsberg,1994; Yeh *et al*., 2003; Grover and Yadav,2004; Ramesh *et al*., 2010; Meghnani *et al*., 2010. In the present investigation alloxan monohydrate induced *Mus musculus* for different time duration (7 days, 14 days and 21days) showed decreased protein values (Table A ). The cause of protein depletion may be due to elevation in the activity of proteases and subsequent elevation in the free amino acid content and activity of amino transferees protein depletion may lead to cannibalism by diabetic mother mice (Uko *et al*., 2013). (Fig.A-E). Similar results were reported in diabetic subjects.

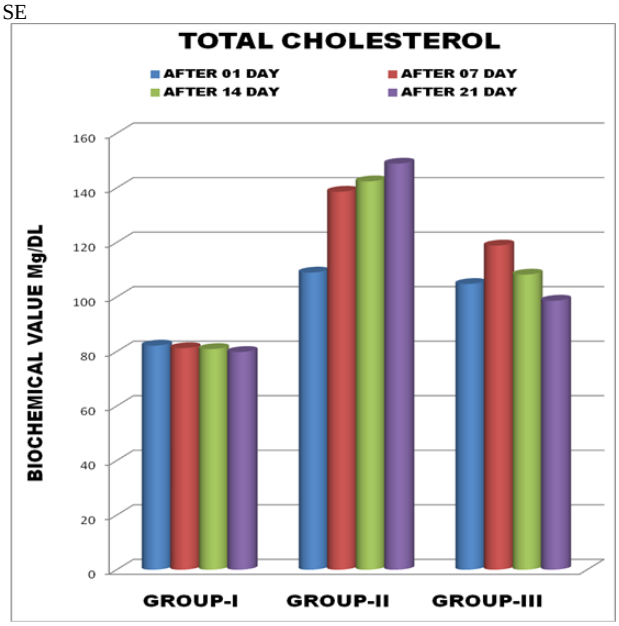
Various theories validating the hypoglycemic effect of *Spirulina platensis* have been proposed. One such theory attributed the effect on its fiber content which leads to reduced

glucose absorption while another theory suggested the possible action of peptides and polypeptides generated by the digestion of *Spirulina* proteins (Rao *et al*., 2010). *Spirulina platensis* is a rich source of good quality proteins. The present study thus concludes that oral administration of the powder of Spirulina platensis powder have significantly reduced plasma lipid levels associated with diabetes mellitus. Thus it can be affirmed that Spirulina powder prevent as well as reverse the plasma lipid profile, thus emphasizing the protective role against diabetes induced hyperlipidemia. Further studies on the active components of *Spirulina platensis* powder and mechanism(s) of its protective effect against diabetic hyperlipidemia are needed.

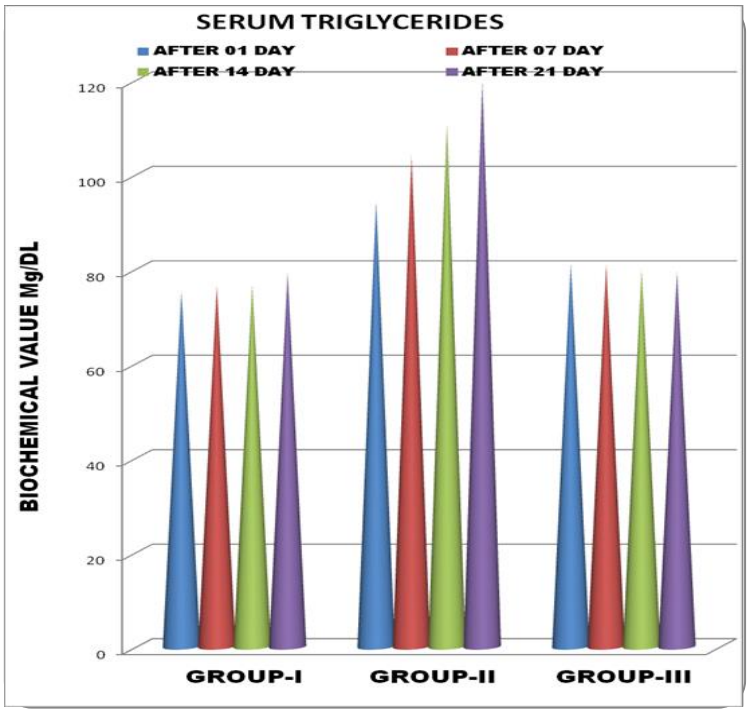
**Table A**
**Comparative analysis of blood biochemical changes in mice due to diabetes and their amelioration by *Spirulina platensis* powder**

| Blood Biochemical Parameter | Groups of Mice | Incubation Period | | | |
|---|---|---|---|---|---|
| | | 1 DAY | 7 DAY | 14 DAY | 21 DAY |
| TOTAL CHOLESTEROL mg/100 ml ± SE | Group-I | 82.18±2.24 | 81.24±3.08 | 80.92±2.68 | 79.84±1.78 |
| | Group-II | 108.96±2.64 | 138.62±1.98 | 142.42±1.24 | 148.92±1.28 |
| | Group-III | 104.78±1.28 | 118.87±3.24 | 108.22±3.94 | 98.65±3.72 |
| SERUM TRI GLYCERIDES mg/100 ml ± | Group-I | 75.30±2.24 | 76.40±3.18 | 76.70±2.72 | 79.20±1.84 |
| | Group-II | 94.30±2.68 | 104.10±1.94 | 110.70±1.46 | 119.80±1.32 |
| | Group-III | 81.20±1.38 | 80.90±3.42 | 79.90±3.92 | 79.45±3.78 |
| VERY LOW DENSITY LIPOPROTEIN mg/100 ml ± SE | Group-I | 15.06±1.16 | 15.28±1.14 | 15.34±1.18 | 15.84±1.24 |
| | Group-II | 18.86±1.12 | 20.82±1.04 | 22.14±1.24 | 23.96±1.14 |
| | Group-III | 16.24±1.24 | 16.18±1.20 | 15.98±1.36 | 15.89±1.32 |
| HIGH DENSITY LIPOPROTEIN mg/100 ml ± SE | Group-I | 23.80±1.24 | 25.60±1.14 | 25.80±1.38 | 26.10±1.46 |
| | Group-II | 22.70±1.28 | 19.24±1.58 | 18.16±1.47 | 17.28±1.24 |
| | Group-III | 21.96±1.26 | 24.68±1.48 | 24.79±1.60 | 25.26±1.63 |
| LOW DENSITY LIPOPROTEIN mg/100 ml ± SE | Group-I | 73.44±1.12 | 70.92±1.42 | 70.46±1.28 | 69.58±1. 25 |
| | Group-II | 105.12±1.16 | 140.20±1. 63 | 146.40±1.58 | 155.60±1.78 |
| | Group-III | 99.06±1.24 | 110.37±3.12 | 99.39±1.38 | 89.28±2.18 |

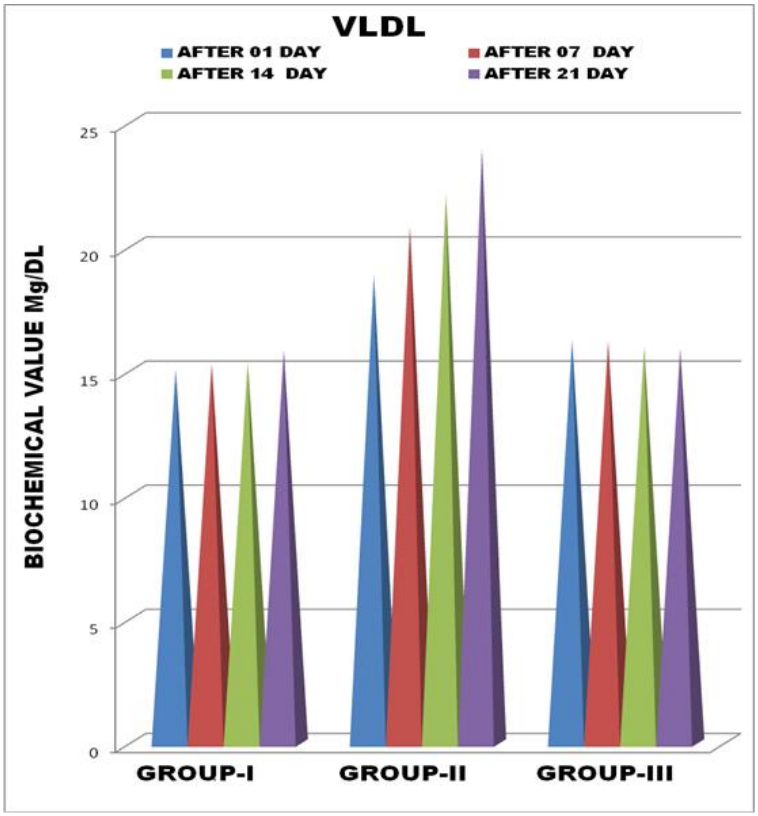
(Group-I- Control; Group-II- Diabetic; Group--III – Diabetic + Spirulina)
N= 10, Values are expressed as mean ± SE



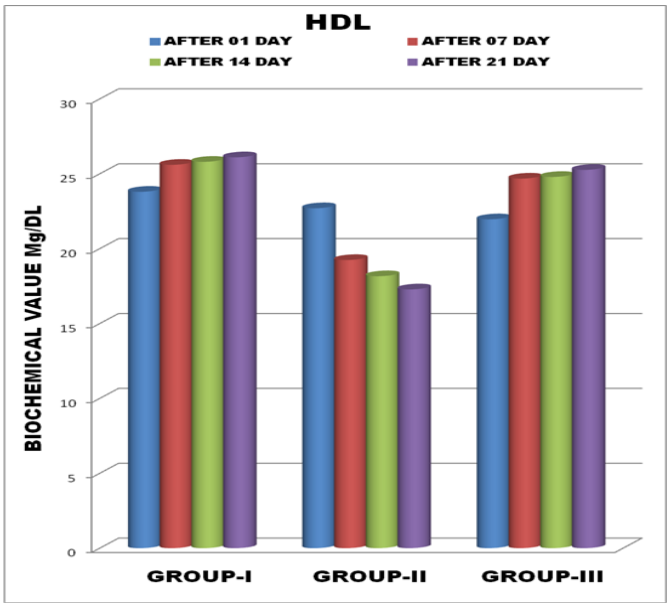

**GRAPH-A: Comparative analysis of biochemical changes in mice due to diabetes and their amelioration by *Spirulina platensis* powder**



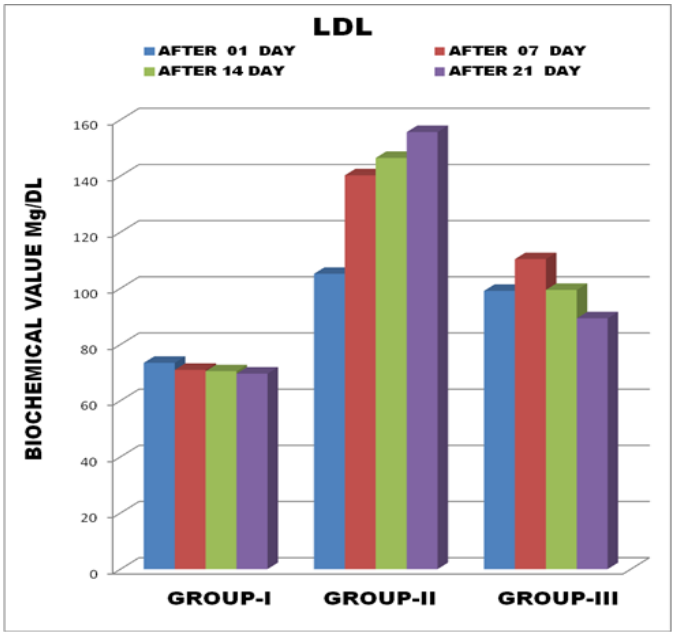

**GRAPH-B: Comparative analysis of biochemical changes in mice due to diabetes and their amelioration by *Spirulina platensis* powder**




**GRAPH-C: Biochemical changes in mice due to diabetes and their amelioration by *Spirulina platensis* powder**




**GRAPH-D: Comparative analysis of biochemical changes in mice due to diabetes and their amelioration by *Spirulina platensis* powder**




**GRAPH-E: Comparative analysis of biochemical changes in mice due to diabetes and their amelioration by *Spirulina platensis* powder**

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