Efficacy of *Spirulina platensis* in improvement of the reproductive performance and easing teratogenicity in hyperglycemic albino mice

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**Introduction**

Diabetes mellitus (DM) is a metabolic syndrome in which pancreatic β-cells fail to produce insulin resulting in hyperglycemia that affects many organs and contributes to several complications. During pregnancy, DM can cause reproductive abnormalities, abortions, congenital anomalies, alterations of fetal growth, neonatal morbidity, and mortality. Several epidemiological and experimental studies have confirmed that the likelihood of developing DM is higher among offspring of diabetic mothers. DM is associated with functional and developmental disorientations of the reproductive system in laboratory animals.

Animal models have already been created using alloxan (AXN), a cytotoxic drug toward pancreatic β-cells to investigate the reproductive performance in diabetes and fetal development during pregnancy. Female mice with AXN induced DM showed altered secretion of gonadotropins due...
Spirulina platensis (SP) is a unicellular cyanobacterium belonging to cyanophyceae class, oscillatoriaceae family; this cyanobacterium is characterized by spiral chains of the cells enclosed in a thin sheath. It contains very potent naturally occurring antioxidant, free radical scavenging agents.[6] SP is nontoxic, bioavailable, and provide significant multiorgan protection against many drugs and chemicals induced toxic assaults.[6] Its active constituents exhibit anti-inflammatory, neuroprotective, hepatoprotective, immunomodulatory, and anticancer activities.[10]

To the best of our knowledge, there are no reports that describe effects of SP in diabetes associated developmental abnormalities such as alteration in implantation sites, viable embryos, gestation length. In the present study, it has been investigated about therapeutic effect of SP on functional and developmental aspects of mice that developed in hyperglycemia conditions in utero.

**Materials and Methods**

**Plant Material**

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

**Animals**

Swiss Albino mice *Mus musculus* weighing 22–27 g were obtained from Central Drug Research Institute, Lucknow, India. Mice were maintained at the animal house of host University under standard conditions. Animals were fed with standard diet (Aashirwad Ltd., Chandigarh, India) and water *ad libitum*. Animals were maintained under controlled conditions (temperature [23 ± 1°C], humidity [50 ± 15%]), and normal photoperiod (12 h light-dark cycle). Eleven weeks old mice were acclimatized for 1-week prior to experimental studies. Rice husk was used as bedding material and changed daily. The research proposal (Reg. No. 5873/10) was approved by the Research Committee of the Department and all experimental procedures were performed in accordance with the guidelines of Institutional Animal Care Committee and the principles outlined in the Declaration of Helsinki.

**Drugs and Chemicals**

AXN-mono hydrate was purchased from Spectrochem, India. All other chemicals and biochemical reagents were of analytical grade.

**Induction of Diabetes**

Animals were fasted for 16–18 h with free access of water prior to induction of diabetes. DM was induced by intra-peritoneal (i.p.) administration of AXN (450 mg kg⁻¹ bw) in three injections at intervals of 48 h (150 mg kg⁻¹ bw each time). Mice with blood glucose level above 200 mg/dl were considered as diabetic[11] and selected for further experimental investigations.

**Experimental Design**

The experimental mice were divided into four groups containing six mice in each group.

- **Group I**: Control (normal saline, 10 ml/kg i.p.)
- **Group II**: Diabetic control; diab (AXN 150 mg kg⁻¹ bw for three times)
- **Group III**: Diab + SP (diabetic control mice fed with 15 mg of SP)
- **Group IV**: Ctrl + SP (control mice fed with 15 mg of SP).

**Preparation and Administration of Spirulina Platensis Suspension**

The suspension was prepared by supplementing 1.5 g of SP in 50 ml of distilled water. SP suspension was fed orally during entire tenure of experiment using infant feeding catheter (3 mm size) attached to a sterile syringe. Catheter was inserted into the gastric region of mice, and 0.5 ml of suspension containing of 15 mg of SP was discharged gradually into each test animals.

**Determination of Fasting Blood Glucose Level**

Fasting blood glucose level (FBGL) was determined using glucometer (Accu-Chek Active, Mumbai, India) and compatible blood glucose strips at different time points during the course of the study.

**Estrous Cycle**

The virgin female mice were divided into four groups, each containing 20 mice (*n* = 20). Vaginal smears were prepared and examined at regular time points during the duration of the study. Mice showing normal full cycle (4–5 days) were considered as normal. For the study of estrus cycle, vaginal smear of all the female mice were examined twice a day until the completion of the treatment by introducing 2–3 drops of physiological saline in vagina and final drop obtained in the dropper was examined microscopically.

**Fertility Test**

On 8th day of post-DM induction, each female in estrus was placed in cage having one nondiabetic male mouse during the dark period of the cycle to allow overnight mating. Similar experimental setup was set up for other groups also. Next morning, male and female animals were separated, and vaginal smears were examined for the presence of sperms. The day when sperm was found in the vaginal smear was considered day 0 of gestation period and thereafter female mice were observed daily till delivery. Laparotomy was performed on day 8 of pregnancy to determine the implantation sites. Mice that did not deliver were anesthetized and sacrificed by decapitation. Uteri were removed and implantation sites, resorptions, and deformities were enumerated.[12]

**Statistical Analysis**

The statistical analysis of the data was done using SPSS. Data expressed as mean ± standard error of mean (SEM). Comparisons between two groups with normality and
homogeneity of variances were performed by two-tailed unpaired Student’s t-test. One-way analysis of variance (ANOVA) was used, followed by the post-hoc Tukey multiple comparison test to analyze data for control mice when the SP treated diabetic group was included. The levels of significance were set at $P < 0.05$ (almost significant) and $P < 0.01$ (significant).

**Results**

**Effects of Spirulina Platensis Administration on Fasting Blood Glucose Level**

FBGL of control animals ranged from 78.33 ± 3.88 to 83.02 ± 3.58 mg/dl during experimental period which later increased significantly ($P < 0.01$) to 273% at 21 days in diabetic mice [Figure 1a]. Decrease in the FBGL was observed when diabetic mice fed with SP ($P < 0.01$). FBGL decreased to 61% in diab + SP at 21 days. However, SP treated control mice showed an insignificant change in FBGL.

**Estrous Cycle Phases of Different Groups of Mice**

Control animals showed 11 ± 0.89 h for estrus phase, 10 ± 0.89 h for metestrus phase, 65 ± 0.89 h for diestrus phase and 103.5 ± 2.74 h for total period of cycle which were increased by 91%, 180%, 15.38%, and 32.36%, respectively in diabetic mice when compared with control [Figure 1b]. A significant decrease ($P < 0.05$) was witnessed in proestrus phase (74.28%) of diabetic when compared to control. Control mice when fed with SP showed insignificant change in proestrus phase.

**Litters Count, Implantation Sites and Gestation Length of Test Groups of Mice**

Control animals showed 11.00 ± 0.63 implantation sites and 10.43 ± 0.75 l count per animal. The numbers of implanted embryos were decreased to 22.72% in diabetic whereas SP treatment increased the value to 11.76% [Figure 2]. Gestation length of control mice was found to 19.50 ± 0.55 days that were increased to 14.51% days in diabetic ($P < 0.01$) following treatment with SP, gestation length decreased nearly to normal.

**Preweaning Development of Test Groups of Mice**

Diabetic mice with defective forelimb and hind limb including digits [Figure 3a and b], puffiness of the body and venations in the ear [Figure 3c and d], retinopathy with altered gross morphology [Figure 3e and f], pattern baldness (alopecia) in $F_1$ mice [Figure 3g], deformed fetus [Figure 3h-k], and fetus with abnormal length and weight during 18 days of gestation length were observed [Figure 3l].

**Litters in Fertility Studies**

Control animals ($n = 40$) showed 10.43 ± 0.32 implantation site as compared to diabetic that possessed 6.58 ± 0.24 implantation site per animal. When mice were fed with SP at fixed dose (15 mg kg$^{-1}$ bw d$^{-1}$), implantation sites were found to be 7.83 ± 0.22 which corresponds to 37% increase compared to diab. Percentage implantational loss was highest in diabetic control (58.9%) which was reduced to 21% in diab + SP. Live pups at birth in diabetic mice were decreased to 83.51% on

Figure 1: (a) Fasting blood glucose level (mg/dl). $n = 10$, data are presented as mean ± standard error of mean, (b) therapeutic effects of *Spirulina platensis* on estrous cycle phases of different groups of test mice. $n = 20$, values are expressed as mean ± standard error of mean; superscripts ** are significantly different at $P < 0.01$ when compared to control. One-way analysis of variance was used followed by the post-hoc Tukey multiple comparison test.

Figure 2: Therapeutic potential of *Spirulina platensis* in modulating fertility and preweaning development studies of different groups of mice. (a) Effects on litters counts, implantation sites and gestation length of test groups of mice. Data are presented as mean ± standard error of the mean of 40 animals. **$P < 0.01$ when the value are compared with Group I. One-way analysis of variance was used followed by the post-hoc Tukey multiple comparison test. (b) Preweaning development in test groups of mice. Data are expressed as mean ± standard error of mean of 20 mice.
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the other hand, number of dead fetus of 0 day age increased to 40% in MDM group when compared to normal mice. Resorption rate (36.14%) and abnormal fetus (66.67%) were increased in diab when compared to ctrl [Table 1].

\( F_0 \) Generations During Fertilities Studies

Mice (n = 40) of \( F_0 \) generation were employed for fertilities studies. Reproductive performance was shown by 36 control animals that decreased to 31 in diabetic mice out of 40. Feeding of diabetic mice with SP increased their numbers to 33. Fertilities rate were observed minimum (77.5%) in diabetic group, and these were increased to 82.5% in diab + SP. Death during delivery was higher (3.23%) in diabetic mice as compared to control that showed no mortality. Mortality rate was also decreased from 4 to 3 as a result of administration of SP to diabetic mice [Table 2].

Discussion

AXN induced hyperglycemia is considered as valid experimental model to study the reproductive performance in diabetic mice. AXN selectively obliterated the pancreatic insulin secreting \( \beta \)-cells, leaving less active cells, and resulting in a diabetic state. Some \( \beta \)-cells of islets of langerhans remained active thus helping to survive in spite of increasing FBGL.

The reproductive toxicity was assessed based on an array of parameters such as estrus cycle stages (proestrus, estrus, metestrus, and diestrus) of various duration, implantation sites, implantational loss, fertilities studies, live pregnant at delivery, live pups at birth, survival rate (0–4 days), gestation length, and preweaning development. Cyclic changes were observed in the estrous cycle, gave a reasonable index of the ovarian activity and synthesis of estrogen and progesterone. The control mice exhibited regular and normal duration of each phases of estrous cycle. In contrast, diabetic mice exhibited significant increase in the length of estrous cycle, duration of estrus, diestrus and metestrus with concomitant decrease in the duration of proestrus phase (\( P < 0.01 \)) which were further regained by SP supplementation. The appearance of prolonged diestrus indicates secretion of estrogen from ovary must be affected. It might be due to less secretion of gonadotropins, thus causing hormonal imbalances.

Similar results had been reported when mice treated with a carbamate fungicide mancozeb,\(^{13}\) cyclophosphamide.\(^{14}\) Similar
findings like increased BGL and decreased insulin level were also noted in test mice. Such biochemical changes act on the hypothalamus that affects the ovary that in turn alter estrous cycle and folliculogenesis.\cite{13} The number of implantation sites was significantly ($P < 0.01$) reduced by progression of hyperglycemic state. This condition was however ameliorated by SP supplementation [$\text{Figure 4}$].

Diabetic mice have shown an increased BGL from day 1 onward and continued until the end of the experiment. Similarly, induction of diabetes using AXN leading to hyperglycemic condition have been reported by a number of researchers.\cite{10,16} The results on the increased FBGL were indicative of the curative effect of SP in induced diabetic mice models. Treatment with SP for 21 days resulted in utmost reversal of the hyperglycemic condition in diabetic mice. In this regard, SP has already been established for its hypoglycemic effect in human subjects.\cite{18,17}

SP contains satisfactory amount of trace metal mixtures (chromium, magnesium, manganese, and zinc) which improve glucose tolerance by assisting glucose transport and regulation of glucose homeostasis. Dietary fibers present in SP help in improving blood sugar level by slowing down the absorption of sugar.\cite{18}

In one of the study, embryos of mice when injected with glucose caused NTD.\cite{21} In a diabetic condition, a high proportion of glucose is metabolized through the polyol pathway, where aldose reductase reduces glucose to sorbitol. Sorbitol may accumulate in tissue and cause damage through osmotic effects. The increased availability of glucose in experimental diabetic pregnancy is paralleled by an increase in embryonic sorbitol concentration. MDM caused an increase in both sorbitol content and aldose reductase activity in fetus.\cite{19}

ROS reacts with lipids causing altered cell membrane fluidity.\cite{19} These mechanisms have been suggested to be part of the pathogenesis of diabetic embryopathy. MDM is associated with increased oxidative stress (OS) in the embryo during organogenesis and induced malformations.\cite{15,16}

The beneficial effect of giving Vitamin C and E to the pregnant diabetic mice in the management of diabetic embryopathy have already been established.\cite{21-24} Orally fed natural antioxidant-Vitamin C (100 mg kg$^{-1}$ bw for 15 days) showed 49.14% reduction of FBGL in AXN diabetic rat.\cite{21} Similarly, diabetic rat when fed with dietary antioxidant (0.5% Vitamin C and 0.5% Vitamin E supplemented diet for 10 days) did not lower down the FBGL to the steady levels but there was significant reduction ($P < 0.05$) as compared to DM group. FBGL reduced up to the mark when the double concentration of Vitamin C keeping Vitamin E as usual was given.\cite{23}

Thirty percentage increase in placental weight was observed in MDM, but supplementation of 1% Vitamin C and 0.5% Vitamin E with food restored the placental weight by approximately 35% in 20 days gestational diabetic rats. In the MDM groups, 23% malformations and 32% resorptions were observed which were successively decreased by Vitamin C treatment.\cite{23} Results were in agreement with other workers who suggested that Vitamin C may prevent the accumulation of sorbitol intracellularly by inhibiting the aldose reductase enzyme and maintaining glucose homeostasis.\cite{24} SP contains antioxidant (Vitamin C and E) and blood sugar lowering agents whose treatment resulted in an improvement of pregnancy

### Table 1:

| Parameters (per animal) | Group I ($\pm$SEM) | Group II ($\pm$SEM) | Group III ($\pm$SEM) | Group IV ($\pm$SEM) |
|-------------------------|---------------------|---------------------|---------------------|---------------------|
| Implantation sites      | $10.43\pm0.32$      | $6.58\pm0.24^*$     | $7.83\pm0.22^*$     | $11.03\pm0.31$      |
| Implantational loss (%)  | $3.6\pm0.03$        | $58.9\pm0.63^*$     | $21.0\pm0.12^*$     | $3.4\pm0.05$        |
| Live pups at birth      | $9.2\pm0.04$        | $1.5\pm0.01^*$      | $4.95\pm0.02^*$     | $9.85\pm0.01$       |
| Dead fetus              | $0.25\pm0.01$       | $0.35\pm0.01$       | $0.33\pm0.02$       | $0.28\pm0.01$       |
| Resorption              | $0.53\pm0.01$       | $0.83\pm0.02^*$     | $0.55\pm0.01$       | $0.53\pm0.01$       |
| Abnormal fetus          | $0.10\pm0.01$       | $0.30\pm0.02^*$     | $0.30\pm0.02$       | $0.10\pm0.02$       |
| Survival rate (0-4 days)| $8.55\pm0.36$       | $1.28\pm0.04^*$     | $4.40\pm0.05^*$     | $9.10\pm0.06$       |

**n=40:** Values are expressed as meansSEM for individual experimental animal; **Values are significantly different at $P<0.01$ when compared to control. One-way ANOVA was used followed by the post-hoc Tukey multiple comparison tests. Group I: Ctrl (control), Group II: Diab (diabetic control), Group III: Diab+SP (diabetic control mice fed with SP), and Group IV: Ctrl+SP (control mice fed with SP). SEM=Standard error of mean, ANOVA=Analysis of variance

### Table 2:

| Parameters | Group I ($\pm$SEM) | Group II ($\pm$SEM) | Group III ($\pm$SEM) | Group IV ($\pm$SEM) |
|------------|---------------------|---------------------|---------------------|---------------------|
| Number per group | 40                  | 40                  | 40                  | 40                  |
| Pregnant per group| 36                  | 31                  | 33                  | 37                  |
| Fertility (%)      | 90                  | 77.5                | 82.5                | 92.5                |
| Percent survival of litter count | 95.67\(\pm0.52\) | 83.50\(\pm0.55^*\) | 92.50\(\pm0.55\) | 95.83\(\pm0.41\) |
| Live pregnant at delivery | 36                  | 30                  | 32                  | 37                  |
| Death during delivery (%) | 0                   | 3.23                | 3.03                | 0                   |
| Death during study | 1                   | 4                   | 3                   | 0                   |

**n=40:** Percentage survival of litter count is expressed as meansSEM; **Values are significantly different at $P<0.01$ when compared to control. One-way ANOVA was used followed by the post-hoc Tukey multiple comparison tests. Group I: Ctrl (control), Group II: Diab (diabetic control), Group III: Diab+SP (diabetic control mice fed with SP), and Group IV: Ctrl + SP (control mice fed with SP). SEM=Standard error of mean, ANOVA=Analysis of variance
outcome, manifested as decreased rates of malformations and resorptions in the treated group of mice.\(^{108}\)

In relation to physical development, the time taken for ear unfolding, upper incisor development, and eye opening was observed among new born and found to be insignificant. Some worker reported delay in the duration of development as well as neonatal growth that might be related to intrauterine disturbances during pregnancy caused by MDM.\(^{225}\)

Medicinal plants/plant extracts containing secondary metabolites like phenolic compounds, alkaloids, flavonoids, saponins, terpenoids, and phytosterol were found to be effective in the management of diabetic complications.\(^{226}\) In this regard, *Tinospora cordifolia* and *Ipomoea aquatica* (whole leaf powder) were investigated to attenuate embryopathy, OS and protection in the fetal brain and liver, as evidenced by increased levels of antioxidant molecules and reductions of ROS in streptozotocin-diabetic rats.\(^{227}\)

The placenta represents an important line of defense against any embryopathic agent within the fetomaternal unit. Both human and animal studies have confirmed that phytomolecules can easily cross the placental barrier.\(^{227}\) The teratological part of the present experiment showed a prominent reduction in the malformations rate and resorptions rate after treatment. However, SP administration remarkably reduced the hyperglycemia-generated detrimental tendencies. On the other hand, SP preserved the significant number of fetuses after having raised the number of implantations, probably referring to the antioxidant activity, thus allowing preparing a proper local environment that supports postimplantational development. In contrast to the identified external anomalies in ctrl and diab + SP; no skeletal dysmophia were seen in these groups, suggesting that chronic hyperglycemia is directly involved in skeleton mal-development. In this context, progeny from diabetic animals are frequently subjected to paralysis of limb, puffiness of the body, venations in the ear, alopecia, retinopathy, altered gross morphology, and deformity in the fetus.

From this study, it can be concluded that oral therapeutic treatment with SP suspension to pregnant diabetic mice modulates reproductive performance, alleviate maternal hyperglycemic state and attenuate diabetic teratogenesis. Results clearly unmasked therapeutic effects of SP against diabetic embryopathies and warrants further investigation at wider dose regimens in other test animals.

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**Conflicts of Interest**

There are no conflicts of interest.

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