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HISTOPATHOLOGICAL CHANGES INDUCED BY DIABETES IN SWISS ALBINO MICE AND THEIR AMELIORATION WITH *SPIRULINA PLATENSIS* SUPPLEMENTATION

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Abstract
Diabetes mellitus is a chronic metabolic condition marked by hyperglycemia and disruptions in the metabolism of carbohydrates, fats, and proteins, frequently resulting in significant organ dysfunction. This study aimed to examine the histological alterations caused by diabetes in Swiss albino mice and to assess the therapeutic effects of *Spirulina platensis* (SP) supplementation. Diabetes was induced with alloxan monohydrate, and the mice were categorized into four groups: control, diabetic control, diabetic mice receiving SP supplementation, and control mice receiving SP supplementation. The research studied fasting blood glucose levels and histological alterations in the liver, kidney, ovary, and pancreas following 21 days of therapy. Histopathological research indicated that SP supplementation alleviated hepatic and renal damage, safeguarded ovarian function, and enhanced pancreatic β-cell integrity. The study indicates that SP has potential as an adjuvant therapy to mitigate organ damage caused by diabetes and enhance reproductive health in diabetic mice.

Keywords: Diabetes mellitus, *Spirulina platensis*, Histopathology, Swiss albino mice, Organ damage, Fasting blood glucose, Liver, Kidney, Ovary, Pancreas.

Materials and Methods

Experimental Animals: Female Swiss albino mice (*Mus musculus*), aged 8 to 10 weeks and weighing 22 to 27 grams, were procured from the Central Drug Research Institute (CDRI) in Lucknow, India. The animals were maintained under laboratory conditions at the Animal House of University Department of Zoology, T.M. Bhagalpur University, Bhagalpur, India. Mice were housed in polypropylene cages with daily changes of rice husk bedding. The temperature was maintained at 22 ± 2°C with a 12-hour light/dark cycle. The animals were acclimatized for 07 days prior to the commencement of the experiment, and were given standard pellet diet and water *ad libitum*.

Induction of Diabetes: Diabetes was induced *via* intraperitoneal injection of alloxan monohydrate (Sigma-Aldrich, USA) at a dosage of 150 mg/kg body weight. Prior to induction, the mice were subjected to overnight fasting while being permitted unrestricted access to water. Diabetes was diagnosed by assessing fasting blood glucose levels with a glucometer (Accu-Chek, Roche, USA) 48 hours post-injection. Mice with FBGL ≥ 200 mg/dl were classified as diabetic and incorporated into the study (Zhang *et al*., 2009).

Experimental Design: The animals were randomly assigned to 04 groups, each consisting of 06 mice (N=06):

Group I (Control): Normal mice receiving a standard diet and water.

Group II (Diabetic Control): Diabetic mice receiving standard diet and water.

Group III (*Spirulina*-treated Diabetic): Diabetic mice fed with SP.
Group IV (*Spirulina*-treated Control): Non-diabetic mice fed with SP at the same dose as Group III.
SP was fed orally once daily for 21 consecutive days, based on previous studies that suggest its safety and efficacy in similar experimental models.

Fasting Blood Glucose Level Measurement: Fasting blood glucose levels were measured at baseline (day 1) and on day 21 using a glucometer (Accu-Chek, Roche). Blood samples were obtained *via* tail vein puncture after an overnight fast. FBGL was expressed in mg/dl.

Histopathological Examination: On the 22nd day, subsequent to euthanasia through cervical dislocation, organs such as the liver, kidney, ovary, and pancreas were taken out, preserved in 10% formalin, and embedded in paraffin. The tissue blocks were sliced to a thickness of 5 µm using a microtome. The sections were stained with Hematoxylin and Eosin (H&E) for histological analysis. The stained tissue sections were examined using a light microscope (Olympus, Japan). Histopathological abnormalities, including vacuolization, necrosis, inflammation, fibrosis, and other pathology alterations, were assessed and recorded in the liver, kidney, ovary, and pancreas.

Ethical Approval: The research proposal (Reg. No. 5873/10) was approved by the Research Committee of the Department, and all experimental procedures were performed according to the guidelines of the Institutional Animal Care Committee and the principles outlined in the Declaration of Helsinki.

Results

Fasting Blood Glucose Level:

Fasting blood glucose levels (FBGL) in the control group (Group I) remained consistent during the experiment period, ranging between 78.33 mg/dl and 78.00 mg/dl. Conversely, diabetic control mice (Group II) demonstrated a substantial elevation in FBGL, reaching 292.33 mg/dl on day 21, indicating of hyperglycemia. Supplementation with SP in diabetic mice (Group III) led to a significant reduction in fasting blood glucose levels, decreasing from 292.33 mg/dl to 141.17 mg/dl by day 21 ($p < 0.01$). The control mice administered SP (Group IV) exhibited no significant alteration in FBGL relative to Group I. The comprehensive data has not been presented.

Histopathological Findings:

***Liver*:**

In the diabetic control group (Group II), liver histology exhibited considerable damage, characterized by hepatocyte necrosis, cytoplasmic vacuolization, and inflammatory cell infiltration. Liver architecture was disrupted, characterized by enlarged sinusoidal gaps and expanded portal tracts containing cellular debris. Binucleated hepatocytes were seen. Conversely, SP supplementation (Group III) demonstrated significant improvement, characterized by less vacuolization, restoration of hepatocyte integrity, and reduced inflammatory infiltration. The liver histology of both the control group (Group I) and the SP-treated control group (Group IV) exhibited normalcy without notable abnormalities. The transverse section of the liver from experimental mice is depicted in Figure 1.

***Kidney*:**

The renal tissue of diabetic control mice (Group II) exhibited damage characterized by tubular necrosis, degeneration, and inflammation in the glomerular and tubular cortex regions. The SP treatment (Group III) markedly enhanced these parameters, resulting in decreased necrosis and inflammation, as well as improved preservation of renal architecture. No notable histological alterations were seen in the kidneys of both control mice (Group I) and SP-treated control animals (Group IV). The transverse section of the kidney of experimental mice is depicted in Figure 2.

***Ovary*:**

Ovarian histology in diabetic mice (Group II) exhibited a decrease in the quantity of developing follicles, accompanied by an increase in atretic follicles. SP supplementation (Group III) reinstated normal ovarian architecture, characterized by an increase in developing follicles and a reduction in atretic follicles. The ovaries of control mice (Group I) and SP-treated control animals (Group IV)

exhibited normal folliculogenesis without notable alterations. The transverse section of the ovary from experimental mice is illustrated in Figure 3.

***Pancreas*:**

Pancreatic tissue of diabetic mice (Group II) exhibited considerable damage, characterized by a reduction in the size of the islets of Langerhans and a significant decline in the number of β-cells. The acinar cells next to the islets exhibited atrophy, and vascular degenerative alterations were apparent. SP supplementation (Group III) facilitated the restoration of pancreatic architecture, resulting in partial regranulation of β-cells and enhanced islet morphology. Control animals (Group I) and SP-treated control mice (Group IV) displayed normal pancreatic histology, characterized by intact islets and acini. The transverse section of the pancreas from experimental mice is depicted in Figure 4.

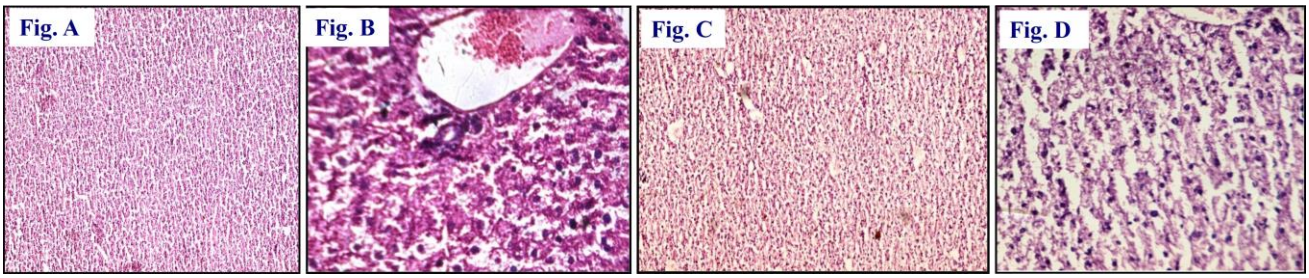

Fig 1. T.S of Liver: A-Group I; B-Group II; C-Group III; D- Group IV)

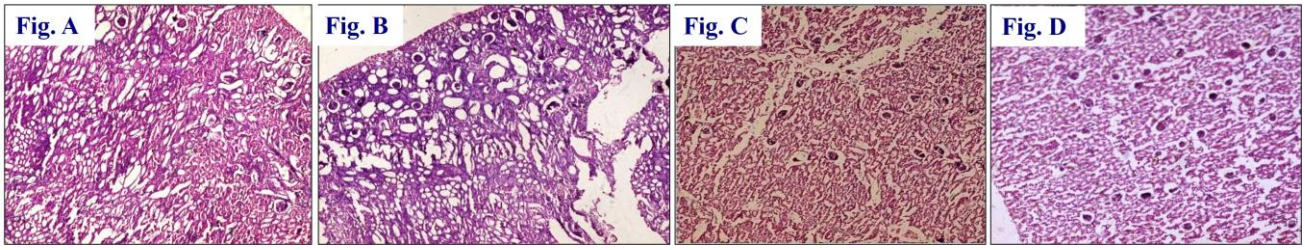

Fig 2. T.S of Kidney: A-Group I; B-Group II; C-Group III; D- Group IV)

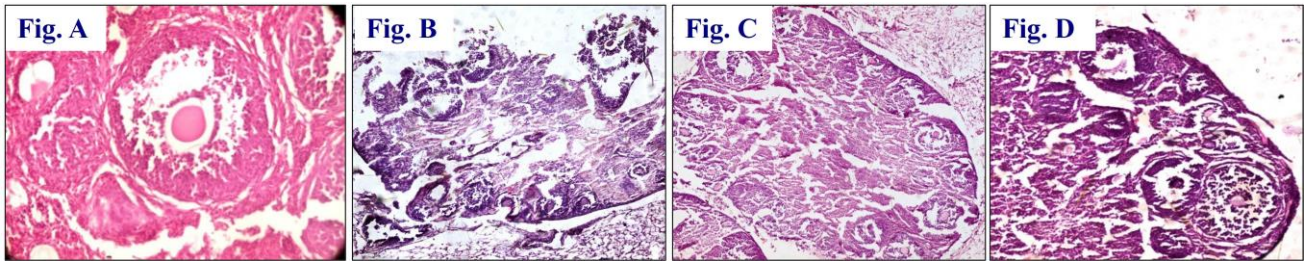

Fig 3. T.S of Ovary: A-Group I; B-Group II; C-Group III; D- Group IV)

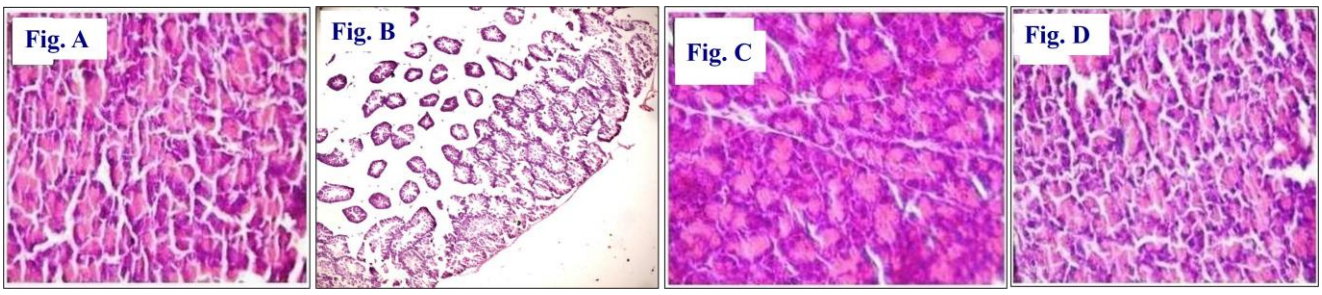

Fig 4. T.S of Pancreas: A-Group I; B-Group II; C-Group III; D- Group IV)

Discussion

Diabetes mellitus is a chronic metabolic condition marked by hyperglycemia due to either insulin insufficiency or resistance. This study examined the therapeutic efficacy of SP supplementation in mitigating the histological alterations caused by diabetes in Swiss albino mice. The findings demonstrate that SP administration markedly improves the histopathological changes detected in multiple organs, such as the liver, kidneys, pancreas, and ovaries. Lee *et al*. (2008) conducted randomized research to assess the effects of *Spirulina* on patients with type 2 diabetes mellitus.

The current investigation revealed that diabetic control mice displayed significantly increased FBGL, aligning with the pathogenesis of diabetes mellitus. SP supplementation in diabetic mice resulted in a notable decrease in FBGL, corroborating its hypoglycemic impact. This aligns with prior research indicating capacity of SP to enhance glucose metabolism (Meineri *et al*., 2009). This decrease may be ascribed to the abundant presence of bioactive substances such phycocyanin and polysaccharides, recognized for their role in regulating insulin secretion and sensitivity (Li *et al*., 2009).

Diabetic mice exhibited significant liver damage characterized by hepatocyte necrosis, vacuolization, and inflammatory cell infiltration, which are hallmark of diabetes-induced hepatopathy (Prashanth *et al*., 2009). These alterations can be ascribed to extended hyperglycemia and oxidative stress, which are recognized to aggravate hepatic injury in diabetic states (Uslusoy *et al*., 2009). Treatment with SP markedly enhanced liver histology by diminishing vacuolization and inflammatory infiltration, indicating a hepatoprotective effect. The protective effect may be attributed to the antioxidant capabilities of *Spirulina*, which mitigate oxidative stress and preserve cellular integrity (McCarty *et al*., 2010). Comparable hepatoprotective properties of *Spirulina* have been documented in other experimental models (Thaakur & Sravanthi, 2010).

Diabetic renal injury is marked by glomerular hypertension, tubular necrosis, and interstitial inflammation, culminating in diabetic nephropathy (Teoh *et al*., 2010). This study demonstrated that diabetic mice experienced considerable renal impairment, which was mitigated by SP administration. The decrease in tubular necrosis and inflammation noted in the SP-treated group indicates its nephroprotective properties. capacity of SP to diminish oxidative stress and inflammation may be pivotal in maintaining renal function under diabetes settings (Kim *et al*., 2010). Moreover, SP has demonstrated the ability to regulate renal function by influencing pathways associated with fibrosis and inflammation (Kutala *et al*., 2008).

Diabetes-induced changes in ovarian function are marked by diminished folliculogenesis and an elevation in atretic follicles (Soleimani *et al*., 2009). In our investigation, SP supplementation resulted in a rise in developing follicles and a decrease in atretic follicles, signifying enhanced ovarian function. This repair may result from Spirulina's capacity to regulate hormonal imbalances and oxidative stress, which frequently impair ovarian function in diabetes settings. Moreover, the antioxidant characteristics of Spirulina may safeguard ovarian cells from oxidative harm, thereby facilitating regular folliculogenesis.

The pancreatic histology of diabetic mice exhibited a diminished size and quantity of islets of Langerhans, alongside a notable drop in the β-cell population, which are characteristic markers of diabetes (Nachnani *et al*., 2010). These modifications can hinder insulin secretion, worsening hyperglycemia. Nonetheless, SP supplementation facilitated the restoration of pancreatic structure, resulting in partial regranulation of β-cells and enhanced islet architecture. This indicates that SP may facilitate β-cell regeneration and enhance insulin secretion, as documented in prior research (Muthuraman *et al*., 2009). The beneficial impact of SP on pancreatic function may be ascribed to its immunomodulatory and antioxidant characteristics.

Conclusion

This study shows that SP supplementation dramatically improves the histological alterations caused by diabetes in Swiss albino mice. The findings indicate significant repair in the morphology of vital organs typically impacted by diabetes, including the liver, kidneys, pancreas, and ovaries.

Specifically, SP therapy diminished hepatocyte necrosis and vacuolization, enhanced renal architecture with reduced tubular necrosis, and facilitated partial regranulation of β-cells in the pancreas. Furthermore, SP supplementation successfully reinstated ovarian function.

These findings indicate the therapeutic potential of SP as an adjunctive strategy for addressing diabetes-related organ damage and reproductive dysfunction. The observed positive effects are presumably facilitated by its antioxidant, anti-inflammatory, and immunomodulatory capabilities. Nevertheless, additional mechanistic investigations are required to elucidate the specific pathways implicated and to refine dosing regimens for its therapeutic use in diabetes treatment.

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