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# Histological Changes in Testis of Diabetic Mice and Role of *Spirulina platensis* Powder in Their Recovery

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## ABSTRACT

The effect of *Spirulina plantesis* powder on Testis histology 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. Histology of reproductive organ (Testis) of different test groups animals (Group-I, Group-II and Group-III) after 21 day exposure and recovery periods have been shown in Fig-1 A,B to Fig- 4 A,B

In the present study, The HE stained 5µ section of Testis of alloxan monohydrate induced diabetes group (Group-II) animals after 7day,14day and 21day (Fig-1B and 2 A and 2B) showed substantive damages in normal histological architecture marked by Cytoplasmic vacuolization, cellular infiltration of acute inflammatory cells were seen in diabetic mice (Group-II) and it is found to be pronounced with increased exposure period 07 days (Fig-6.1B), 14 days( Fig-6.2 A) and 21 days (Fig-6.2 B). The different abnormalities appeared were found to have mitigated upon treatment with Spirulina in the powder form along with the food. However, additional work should be conducted to evaluate the molecular mechanisms underlying losses observed during present work and the mechanism of Spirulina action.

**Keywords:** *Alloxan monohydrate, Intra-peritoneal injection, Spirulina platensis powder, HE stained section, Cytoplasmic vacuolization, cellular infiltration.*

## INTRODUCTION

The testes are paired ovoid organ and they develop early in embryonic life in the abdominal cavity and later descend into the scrotum (Clouthier *et al*., 1996). Each testis is covered by two membranes visceral layer tunica vaginalis and the tunica albuginea. The tunica albuginea is thickened on the posterior surface of the testes to form the media sternum testes (Dym and Cavicchia, 1978) from which fibrous septa penetrate the gland, dividing it into about pyramidal compartments called the testicular lobules (De Rooij and Russell, 2000). Each lobule is occupied by one to four seminiferous tubules. The parenchyma of the testes is composed of many seminiferous tubules of varying size. Each one is surrounded by an outer thinner layer of connective tissue and lined by spermatogenic epithelium, surrounding a central lumen. The germinal epithelium lies on a basement membrane consists of Sertoli cells and spermatogenic cells (Van Pelt *et al*.,2002). Sertoli cells are bigger, somewhat triangular cells with broad bases and indistinct they are attached by their broad bases to the basement membrane and reaching the lumen by their apices (Spradling *et al*., 2001). Sertoli cells are fewer in number and each one has large oval nucleus, pale cytoplasm and ill-defined boundaries. The spermatogenic cells are composed of proliferating cells of varying phases in

mostly one layer directly on the basement membrane (Spradling *et al.*, 2002). They are small rounded mother cells having deeply stained rounded from which the primary spermatocytes are produced by mitosis .The primary spermatocytes are also large rounded cells which remain nearer to the spermatogonia cells and are arranged into 3to 4 layers . They have large spherical nuclei compared to nuclei of other kinds of spermatogenic cells. From each primary spermatocyte four secondary spermatocytes are produced. Secondary spermatocytes are also rounded cells but are smaller than the primary spermatocytes. They are located more towards lumen. Each secondary spermatocyte forms two spermatids (Anjamrooz *et al*.,2007). The spermatids are located in small groups near the lumen of the tubule and have irregular shape and densely stained nuclei . Each spermatid gets transformed in a sperm or spermatozoon (Clouthier *et al*.,1996). Spermatozoa appear in the lumen of the tubules and directed with their head toward Sertoli cells . The interstitial cells are large and ovoid with rounded nuclei, they are found mostly in groups between the seminiferous tubules. The spermatid are rounded cells with scanty cytoplasm and in mice have oval nuclei. In mice spermatogenic cells at similar stages of development occupy the same level in the germinal epithelium of a seminiferous tubule. Produce spermatozoa (spermatogenesis) and secrete sex hormones (Neves *et al*., 2002). In general, approximately at least 50% of the seminiferous tubules must contain spermatogenesis for a mouse to be considered as fertile (Izadyar *et al*., 2002).Development of the germ cells begins with the spermatogonia at the periphery of the seminiferous tubule and advances towards the lumen over spermatocytes I (primary spermatocytes), spermatocytes II (secondary spermatocytes), spermatids and finally to mature sperm cells (Hutson *et al*., 1983). The sertoli cell is essential for spermatogenesis as it provides support for the developing sperm cells moving them towards the lumen of the somniferous tubule as they develop until maturity when they are released. The Sertoli cell also reduces motility and capacitation (initiation of the acrosome reaction) of the sperm cells so viability is maintained (Foresta *et al*., 2002).Spermatozoa are produced in seminiferous tubules in the testes. They start off as spermatogonia, undergoing mitosis becoming a type A spermatogonium or a type B spermatogonium. Type B spermatogonia become *primary spermatocytes*. Primary spermatocytes go through a meiotic division to become *secondary spermatocytes*, which undergo another meiotic division to become spermatid (Adewole *et al*., 2007).

Diabetes mellitus is a disease of different etiology; it may arise as a result of abnormalities in a number of essential factors as stress; pregnancy or disorder in β cells function in synthesis and less or irregular secretion of insulin (Newsome *et al*., 2003). Experimentally, the disease can be induced through the destruction of β cells in the islets of Langerhans by diabetogenic agents such as alloxan monohydrate (Lenzen and Panten, 1988). Diabetes is known to affect large number of metabolic pathways by altering the activities of various enzymes involved in these pathways.

The present paper deals with investigations on the effect of diabetes on histological changes occurring in the histoarchitecture of testes and their possible remedial recovery by using food supplementation of Spirulina powder for 21 days.

## 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:**

Alloxan is a well- known Diabetogenic agent widely used to induce diabetes in animals (Viana *et al.*, 2004). It is a urea derivative which causes selective necrosis of the pancreatic islet β-cells. It is used to produce experimental diabetes in animals such as rabbits, rats, mice and dogs (Huralikuppi, 1991). 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 (Williamson *et al*., 1996). Alloxan and its reduction product dialuric acid establish a redox cycle with the formation of superoxide radicals. These radicals undergo dismutation to hydrogen peroxide. There after highly reactive hydroxyl radicals are formed by Fenton reaction. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of *beta* cells (Szkudelski, 2001).

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.

**Histological Studies**

**Processing of tissues:**

The casting and embedding were done with the help of moulds. Two L-shaped blocks were placed on a metallic plate, which acts as a base of the mould and molten wax was poured into it. The tissues were placed in the mould filled with wax and left to solidify. After solidification the blocks of the wax were removed and properly labeled for microtomy. The slides were subsequently stained by a haematoxylin and eosin. The slides were cleaned beyond the area of tissue implantation, dried and mounted in DPX and examined first under low power and then high power.

**Histology of Testis:**

For the histological study, sections of Testis were taken from different groups and for different incubation period (7, 14 and 21 days). The groups are control (Group I), diabetic (Group-II), and diabetic fed with Spirulina (Group III). This was undertaken to analyze the extent of damage and the efficacy of hyperglycemic treatment of Spirulina powder

## RESULTS AND DISCUSSION

Histology of reproductive organ (Testis) of different test groups animals (Group-I, Group-II and Group-III) after 21 day exposure and recovery periods have been shown in Fig 1 A,B to Fig 4 A,B. In the present study, HE stained 5µ section of Testis of alloxan monohydrate induced diabetes group (Group-II) animals after 7day,14day and 21day (Fig-1B and 2 A and 2B)showed substantive damages in normal histological architecture. There is, however, an increase in Testis weight due to glucose over-utilization and subsequent enhancement in glycogen synthesis, lipogenesis and protein synthesis (Meyer *et al*., 1998). These changes may lead to serious micro vascular complications, which involve a series of metabolic changes in the pathogenesis of diabetic reprotoxicity (Ramchandran *et al*., 2002). Cytoplasmic vacuolization, cellular infiltration of acute inflammatory cells were seen in diabetic mice (Group-II) and it is found to be pronounced with increased exposure period 07 days (Fig 1B), 14 days( Fig 2 A) and 21 days (Fig 2 B). It is well established that in severe diabetes a catabolic response develops in tissues, such as the liver, muscle and adipose tissue, with the prevalence of catabolic over anabolic processes. However, in other tissues, such as the kidneys, the reverse may be true.

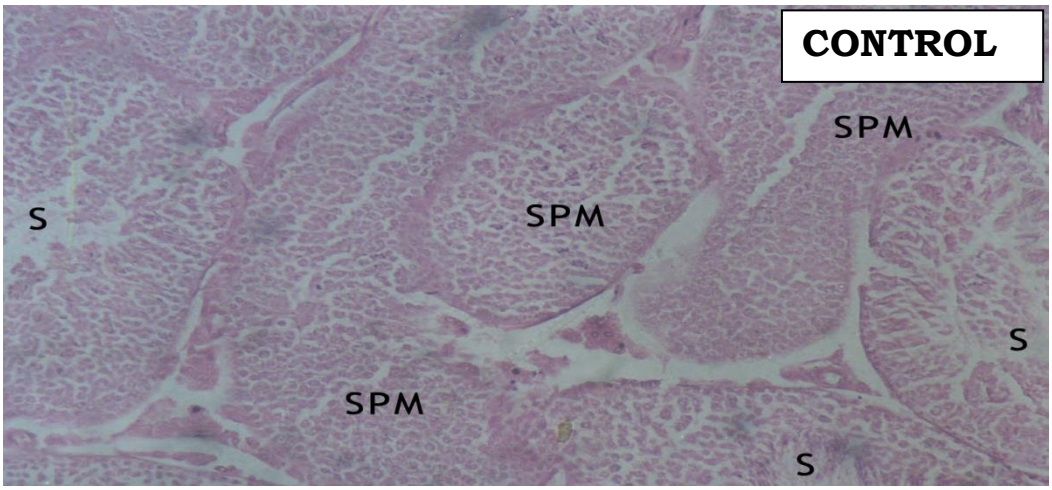
Zhang and Wang (1984) suggested that the cytoplasmic vacuolization is mainly a consequence of considerable disturbance in lipid inclusions and fat metabolism occurring during histological changes. In the present study, the vacuolization of the cytoplasm of the Testicular cells appeared at first in the germ cell of the peripheral zone of the testis, extending gradually toward the centre. This may be due to the direction of the blood supply. Damage of cells due to vacuolation was also noted by James *et al.*, (2006) in similar conditions and corrected giving Spirulina with diet. Sustained hyperglycemia in diabetes causes microvascular dysfunction; the transmembrane proteins translocate

glucose into the cells. Intracellular glucose and its metabolites give rise to vasoactive peptides and elevate intra glomerular pressure (West, 1982).

*Spirulina platensis* powder is a glucose sensitizer on Leydig cell outcomes of Diabetic reprotoxicity. As anti-hypertensive agents, it produces beneficial effects in reducing proteinuria (Cooper, 1991). Antihypertensive agents, dyslipidemic agents like *Spirulina platensis* powder is expected in delaying the progression of disease in diabetic subjects.It is further suggested here that the mode of action of *Spirulina platensis* powder may be caused by their contents of alkaloids and other phytonutrients or constituents through reducing the increased blood glucose level, thereby preventing hyperglycemia during diabetes and reducing lipid profile to almost normal and suppressing the oxidative stress together with converting Testis histomorphology caused by diabetes to normal histoarchitectural pattern. At the light microscopic level, the seminiferous tubules showed a normal arrangement of cellular components of germ cells and Sertoli cells in the control animals. The testes of control animal had shown normal seminiferous tubule, Leydig cells and spermatogenesis. Sertoli cells were found in good condition. The outline of seminiferous tubules of control group animals was smooth (Fig. 1A). Compared to the control (Group I) among Diabetic mice (Group II), the seminiferous epithelium of the treated animals was disrupted with broad spaces between the cellular components showing the presence of copious vacuoles frequently associated with degenerating germ cells. Testes among Diabetic mice exhibited degeneration of the spermatogenic cells accompanied with absence of spermatozoa (Fig.2B). Testes after 14 days of Diabetes showed disarrangement of spermatogenic cells and reduction in some seminiferous tubules, dilatation and congestion of blood vessels (Fig.2A).

Diabetic mice after 21 days showed degeneration in the spermatogonia (Fig.2B). Leydig cells between seminiferous tubules were diminished after 14 and 21 days. (Fig.2A and Fig.2 B). *Spirulina platensis* treatment increased the number of spermatogenic cell in mice of Group III when compared to that of diabetic animals Group II, it was found to be in tandem with the exposure periods. The Testis configuration was retained and Leydig cells were partially rejuvenated in *Spirulina platensis* treated diabetic group of days 7 (Fig 3A). Some abnormal Leydig cells are also present in few slides of days 21 (Fig 2B). The spermatogenic cells are seen to be recovering in days 21 (Fig 4A and 4B) treatment with Spirulina powder in stipulated dose. The present study showed that significant alterations in histological patterns in the testes occurred in Diabetic mice. Changes accompanied by the accumulation of immature cells within the tubular lumen. The primary impact of diabetes was degeneration in the seminiferous tubules and in process of spermatogonia (Fig 1B and 2 A and B). Leydig cells between seminiferous tubules were also diminished. Germinal epithelium ruptured at several places. Deformations of the Sertoli cells, epithelial sloughing, tubular atrophy, and abnormal germ cells have been in case of hyperglycaemia as reported by Annunziata *et al*., 2005**.**The aim of the present study was to investigate the adverse effects of diabetes on histological structure of male albino mice and their subsequent by treatment with Spirulina (Group III).The observed scenes were found to have enhanced Spermatogenesis as in terms of increase of spermatids number by Spirulina powder food supplementation that checks the degenerative changes in testes, since it profoundly contains compounds that accelerate the testicular function (Ojeda and Urbanski,1994 and Waffa *et al.*, 1999).

Testis is an insulin-dependent tissue involved in the transport of glucose in the cells and thus gets severely affected due to increased blood glucose (DuPlessis *et al.*, 2010). Renal glucose uptake is markedly increased during diabetes and is inversely correlated with renal uptake, which is reduced during diabetes (Meyer *et al.*, 1998). The present study is thus an attempt to provide information on the effects of Diabetes and their treatment with *Spirulina platensis* powder on the testicular histoarchitectural parameter. The different abnormalities appeared were found to have mitigated upon treatment with Spirulina in the powder form along with the food. However, additional work should be conducted to evaluate the molecular mechanisms underlying losses observed during present work and the mechanism of Spirulina action.



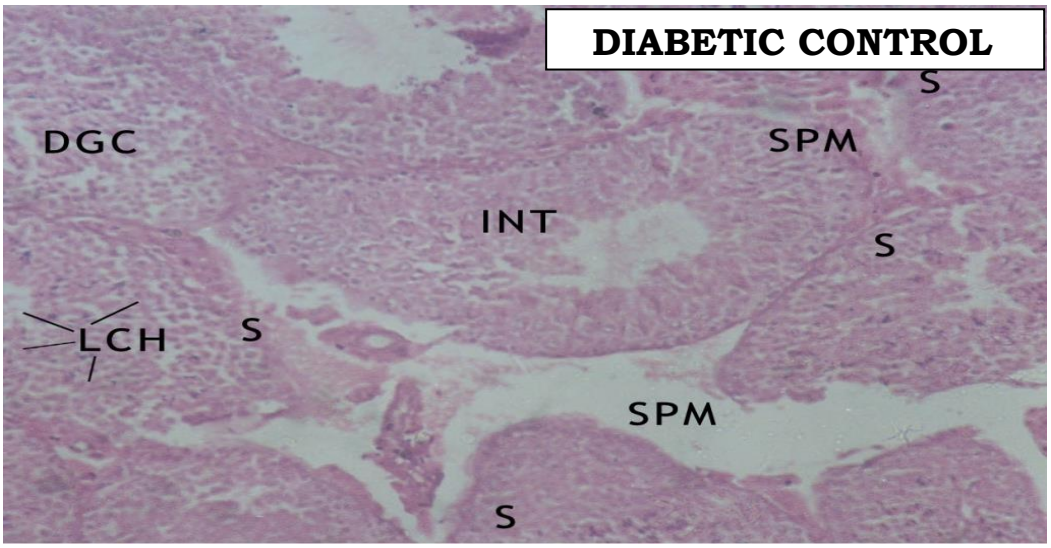




**Plate 1A. Testicular autopsy of Control mice after showing normal spermatogenesis with presence of spermatids (SPM) and Spermatozoa (S),the lumen with normal interstitum.**

**1B. Testicular autopsy of Diabetic control mice showing of presence of slaughed off germinal cells (DGC) along withspermatids (SPM) and spermatozoa (S) interstitum(INT) slightly enlarged.**

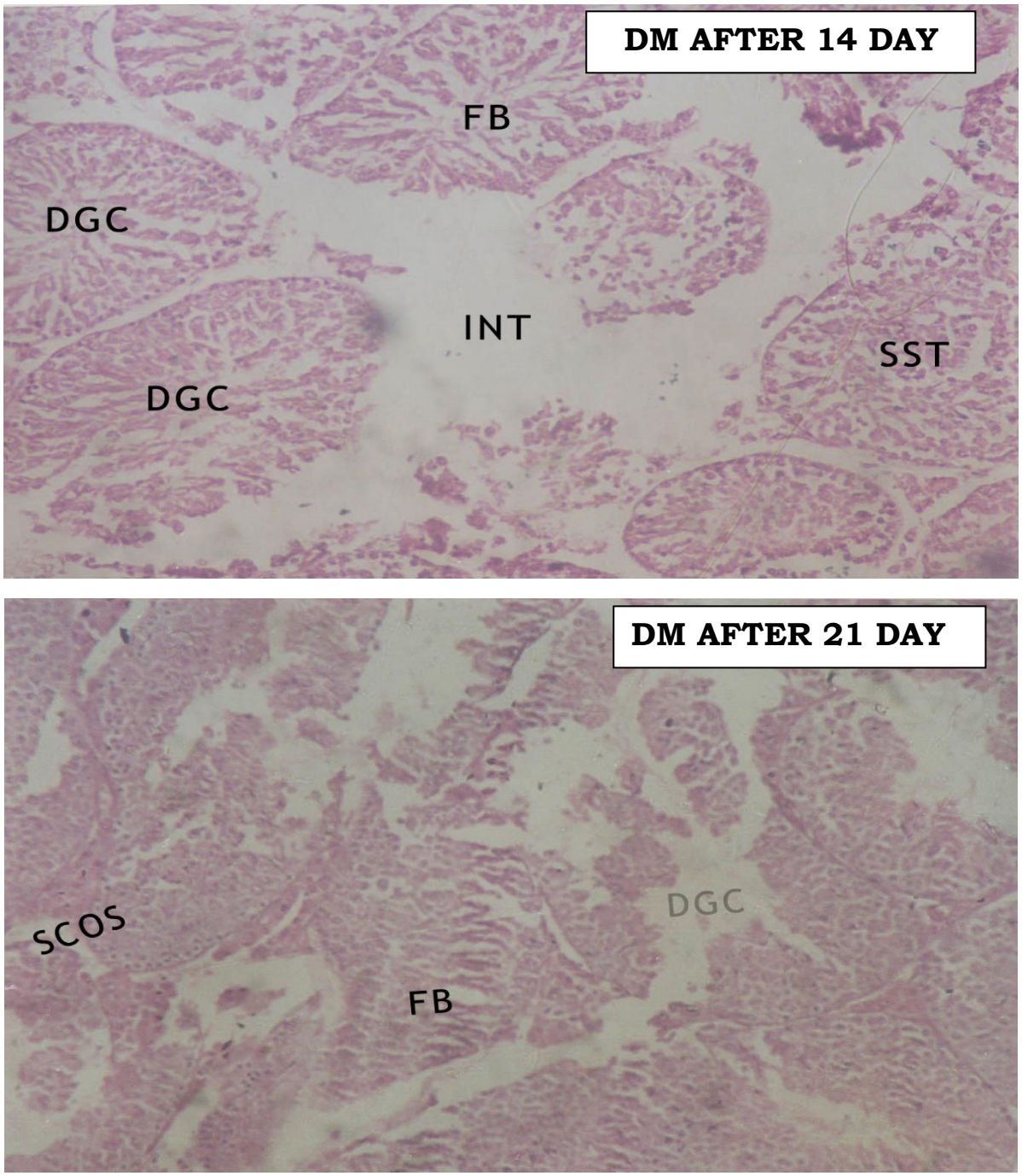
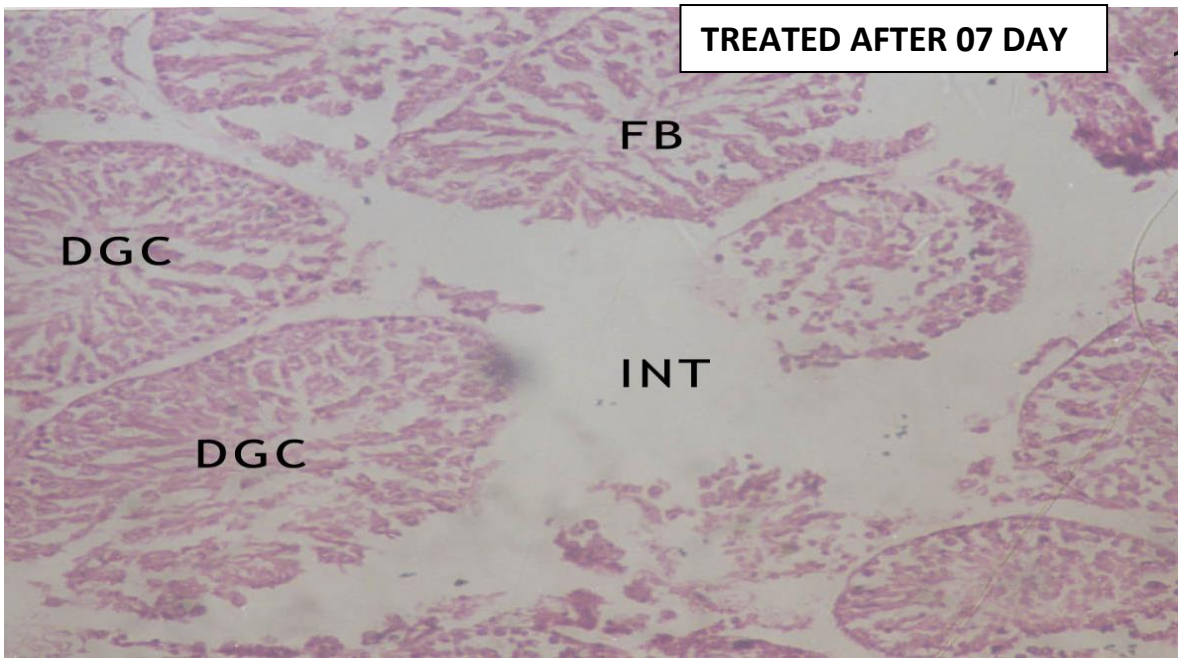



**Plate-2A: Testicular autopsy of Diabetic mice after 14 day showing maturation arrest at secondary spermatocyte stage (SST), interstitium apparently enlarged.**

**B: Testicular autopsy of Diabetic mice after 21 days showing seminiferous tubules were mostly ghost like cells(DGC) and some of them shows maturation arrest at primary spermatocyte state(SST), interstitum highly enlarged with good no. of fabriloblast (FB).**



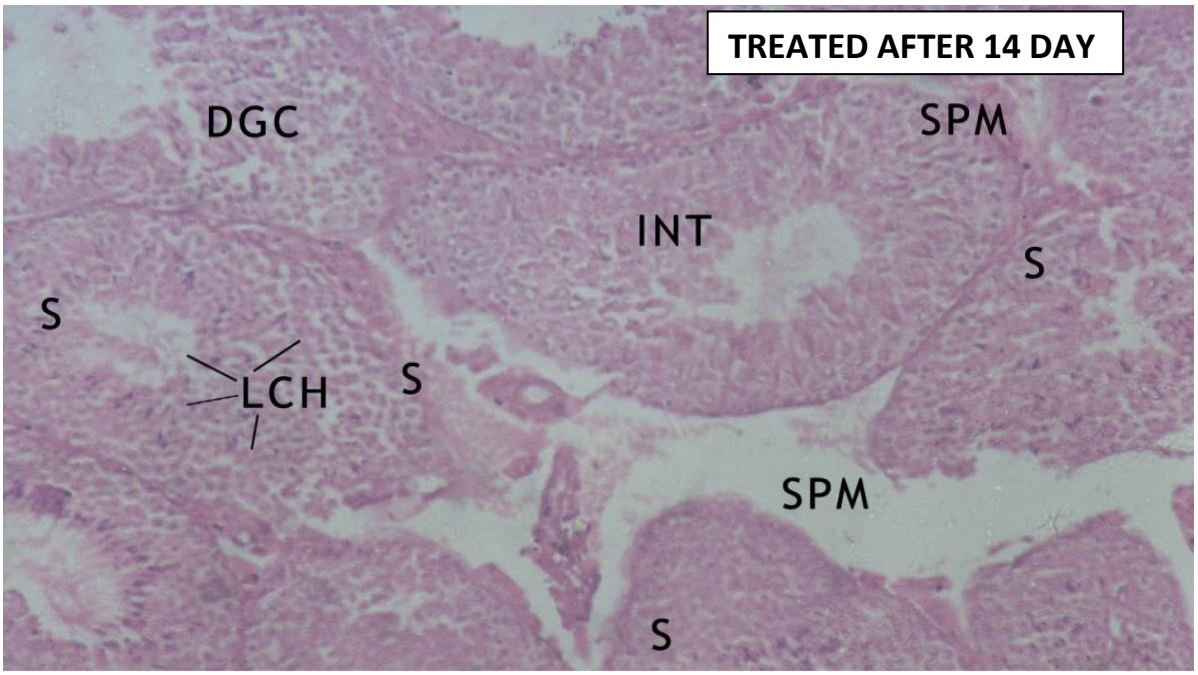




**Plate 3A. Testicular autopsy of Diabetic mice treated with *Spirulina platensis* after 07 Day showing normal spermatogenesis with presence of ghost like cell (DGC), reduced fibrous tissue(FB) and the lumen with normal interstitum (INT).**

**3B. Testicular autopsy of Diabetic mice treated with *Spirulina platensis* after 14 Day showing normal spermatogenesis with presence of ghost like cell (DGC), reduced fibrous tissue(FB) and the lumen with normal interstitum filled with ledig cell (LCH).**

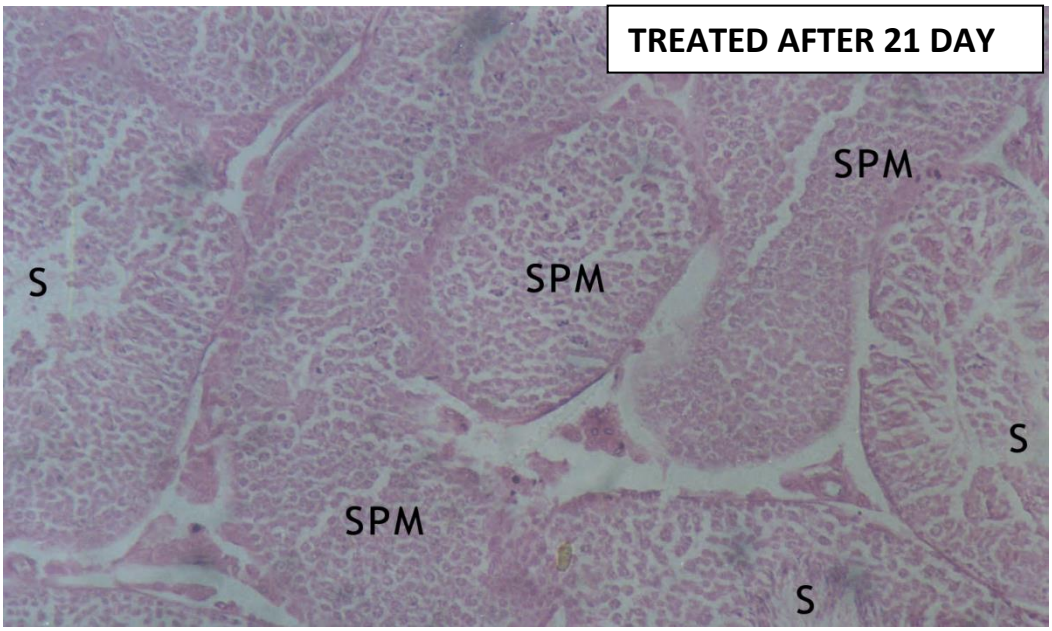



**Plate 4A. Testicular autopsy of Diabetic mice treated with *Spirulina platensis* showing normal spermatogenesis with presence of spermatids (SPM) and Spermatozoa (S),the lumen with normal interstitum.**

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