1121
Arab Univ.
J. Agric. Sci.,
Ain Shams Univ., Cairo
Special Issue, 26(2A), 1121-1134, 2018

# HYPOGLYCEMIC AND HYPOLIPIDEMIC EFFECTS OF *SPIRULINA PLATENSIS*, PHYCOCYANIN, PHYCOCYANOPEPTIDE AND PHYCOCYANOBILIN ON MALE DIABETIC RATS

**[84]**

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**Keywords:** *Spirulina platensis*, Phycocyanin, Phycocyanobilin, Diabetes Mellitus, Hypoglycemic, Hypolipidemic, Insulin Resistance

## ABSTRACT

This study aimed to evaluate hypoglycemic and hypolipidemic activities of *Spirulina Platensis* and its bioactive components (phycocyanin (PC), phycocyanopeptide (PCP) and phycocyanobilin (PCB)) on male diabetic Rats compared to controls and glibenclamide drug. For this reason, male Albino rats were equally divided into seven groups designated as normal control, diabetic control, diabetic + glibenclamide (Glyburide) drug (600 µg kg−1 body weight), diabetic + Spirulina biomass suspension (50 mg/ml/ $kg^{-1}$ body weight), diabetic + phycocyanin (50 mg $kg^{-1}$ body weight), diabetic + phycocyanopeptide (49 mg $kg^{-1}$ body weight) and diabetic + phycocyanobilin (982 µg $kg^{-1}$ body weight). The results show a statistically significant reduction ($P < 0.05$) level of fasting blood glucose, insulin resistance and lipids levels in diabetic animals administration with *Spirulina Platensis*, phycocyanin, phycocyanopeptide and phycocyanobilin compared with diabetic control. Also, there were an increase in HDL–cholesterol levels and β-cell function in these treatments. Histopathologically, diabetic rats treated with spirulina, PC, PCP induced a slight improve of pancreatic cells and an obvious recovery of pancreatic cells. The expression of insulin secretion from cells (β-cells) of diabetic rats was improved in the groups treated with Spirulina, phycocyanin, phycocyanopeptide. While, diabetic rats treated with phycocyanobilin recorded insulin levels lower than them. From this study it can be concluded that *Spirulina Platensis*, phycocyanin, phycocyanopeptide and phycocyanobilin possessed hypoglycemic, insulin sensitivity and hypolipidemic effects. Hypoglycemic and hypolipidemic effects of *Spirulina Platensis* may be attributed to phenolic compounds and phycocyanin. The antidiabetic effect of PC is most likely due to its ability to reduction of insulin resistance, enhance β-cell function and recovery of β-cells. The effect of PC may be attributed to selenium-binding phycocyanopeptide or/ and phycocyanobilin responsible for the antioxidant activity and chromium-binding phycocyanopeptide which activates insulin receptors.

## INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by increased blood sugar levels **(Okigbo et al 2009)**. It was resulting from a defect in insulin secretion, insulin action or both. Increased oxidative stress, impaired antioxidant defense systems and consequently lipid peroxidation are major participants in the development and progression of DM and its complications **(Rudge et al 2007**). Type-2 diabetes (T2DM), also known as non-insulin-dependent DM, accounts for nearly 90% of all DM cases **(Gannon 2001 and Mu et al 2012).**

Natural products are gaining increased importance in drug discovery and development. *Spirulina platensis* is possessed high content of protein content **(Oliveira et al 2009),** in addition to vitamins such as C, A, E and B-complex, essential amino acids, minerals, γ-linolenic acid, and antioxidants such as β-carotenoids and phycocyanin (**Jaime- Ceballos et al 2006)**.

**(Received 8 October, 2017)**
**(Revised 5 November, 2017)**
**(Accepted 12 November, 2017)**

Phycocyanin (PC) is the major protein compound in cyanobacteria such as *Spirulina platensis* contributing to approximately 15-40% of their dried biomass. PC acts as antioxidant, antiinflammatory, neuroprotective, hepatoprotective and anti-cancer **(Chaiklahan et al 2011; Eriksen, 2008; Ou et al 2010; Pento´n-Rol et al 2011 and Romay et al 2003)**. It contains an open-chain tetrapyrrole chromophore known as phycocyanobilin, which is covalently attached to the apoprotein called (phycocyanopeptide) **(Terry et al 1993**). Several investigations demonstrated that phycocyanin exhibit antioxidant activity (**Romay et al 1998 and Bhat and Madyastha, 2001**). The present work evaluated the effect of orally administered *Spirulina platensis*, phycocyanin, phycocyanopeptide and phycocyanobilin on some biochemical parameters related to hypoglycemic effect, insulin resistance, β-cell function in male diabetic rats.

## MATERIALS AND METHODS

### Materials

*Spirulina platensis* was obtained from National Research Centre (NRC), Unit algae technology, Giza, Egypt. Male albino rats (150 -190g) were obtained from Animal house, Agriculture Research Center, Cairo, Egypt. Cholesterol, high density lipoprotein cholesterol (HDL-C), triglyceride and glucose kits were obtained from Egyptian company for Biotechnology, Cairo, Egypt. Ultrasensitive rat insulin ELISA kit was purchased from Shanghai Korian Biotech Company, Yangpu Dist.Shanghai, China.

### Methods

### Preparation of phycocyanin (PC) from *Spirulina platensis*.

A number of separation methods were applied for extraction and purification of phycocyanin (PC) from dried *Spirulina platensis*. These methods included freezing and thawing, sonication, centrifugation, precipitation using ammonium sulfate, dialysis and gel filtration chromatography (**Setyaningsiha et al 2015 and Kumar et al 2014)**.

### Breaking of phycocyanin to its component phycocyanobilin and phycocyanin peptide

Breaking thioether bond between phycocyanobilin and phycocyanopeptide according to **Robort et al (1979).**

### Determination of chromium and selenium content

Chromium and selenium levels in *Spirulina platensis,* phycocyanin, phycocyanopeptide and phycocyanobilin were determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

### Biological experimental design

Male albino rats were fed on a standard diet and drinking tap water. They were kept for one week to be acclimatized to the environmental conditions. The animals were divided into seven groups (6 rats for each) as follows:

**Group 1:** Normal control **(C)** (non- diabetic rats).

**Group 2:** Diabetic control **(DC)** rats as negative control; diabetic rats were induced by Streptozotocin (STZ), each rat was injected intraperitoneally with STZ (45 mg/kg body weight). After 3 days of injection fasting serum glucose level was measured (>300 mg/dl).

Hyperglycemic rats were classified as follows:

**Group 3:** diabetic rats treated orally with Glyburide medicine **(D+G)** as positive control (600µg/kg body weight) daily for 30 days.

**Group 4:** diabetic animals administered orally daily for 30 days with (50 mg/ kg body weight) spirulina biomass suspension **(D+S**).

**Group 5:** Diabetic animals were given orally phycocyanin (50 mg/kg body weight) **(D+ PC)** daily for 30 days**.**

**Group 6:** Diabetic rats were given orally phycocyanin peptide (49 mg/kg body weight) **(D + PCP)** daily for 30 days**.**

**Group 7:** Diabetic rats orally treated daily with phycocyanobilin (982 µg/kg body weight) (**D + PCB)** for 30 days**.**

After 30 days of experiment, rats were fasted overnight and then blood samples were collected and centrifuged at 3000 rpm for 15 minutes at 4°C to separate the serum for biochemical analysis. Pancreatic tissue samples were carefully dissected out for histological studies.

### Biochemical assay

Fasting serum glucose, serum total cholesterol, triglycerides and serum HDL-cholesterol were estimated using commercial kits according to **Tietz, (1990), Roeschlau, et al (1974), NCEPR, (1995) and Mgowan, et al (1983).** LDL-cholesterol was calculated using the total cholesterol level in con-

junction with the triglyceride level and HDL-cholesterol level according to **Makni et al (2008)** using the formula:

***LDL-cholesterol = total cholesterol – (triglyceride /5 + HDL-cholesterol)***

Insulin was determined by using a rat-specific Insulin-kit ELISA according to **Finlay and Dillard (2007)**. Insulin resistance and β-cell function were evaluated by Homeostatic Model Assessment (HOMA) score for insulin resistance and β-cell function (HOMA-IR and HOMA-β) **(Okoduwa et al 2017)** as follows:

***HOMA-IR = insulin level (m IU/L) x blood glucose (mmol/L)/22.5***

***HOMA-β = (20 x insulin level (m IU/L) / blood glucose (mmol/L)) -3.5***

**Histological analyses**

The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin (H&E) stains **(Banchroft et al 1996)** for histopathological examination through the electric light microscope.

**Statistical analysis**

Values presented the means ± standard deviations (SD) for six replicates. Analysis was assessed using the Statically Analysis System Software System for windows **(SAS, 2008).** One way analysis of variance (ANOVA) was performed to evaluate the significant differences between sample means, with significant level being considered at $p<0.05$. Means comparisons were assessed by Duncan´s test.

## RESULTS AND DISCUSSION

**Biochemical analysis**

**1- Fasting serum glucose and insulin**

Data in **Table (1)** demonstrate the levels of fasting serum glucose and insulin in male diabetic animal treated for 30 days with *spirulina Platensis* biomass, phycocyanin, phycocyanopeptide and phycocyanobilin. There was a significant increase in serum glucose levels in diabetic control (371 mg/dL) when compared with normal rats (83 mg/dL). On the other hand, administration of alga biomass, phycocyanin, phycocyanopeptide and phycocyanobilin significantly decreased glucose levels (168, 128, 112 and 122 mg/dL), respectively. The observed results indicated that levels of glucose in rats orally given phycocyanin, phycocyanopeptide and phycocyanobilin lower than rats given glibenclamide drug (positive control). From the results obtained, it is obvious that the *Spirulina platensis* statistically decreased glucose value significantly in STZ- induced diabetic rats. Similar results were reported by **Drapeau et al 2001; Senthilkumar and John, 2008).** These data were in agreement with **Layam et al (2006)** who found that given of diabetic animals *Spirulina platensis* significantly reduced the blood glucose level which due to high fiber content of S. platensis that interfered with the absorption of glucose. Another theory was based on effect of peptides and polypeptides generated by the digestion of spirulina proteins **(Mani et al 2000)**. Also, **Yu et al (2016) and Sarmadi and Ismail (2010)** mentioned that the hypoglycemic effect of phycocyanopeptide is closely related to their amino acid composition, structure, and sequences in phycocyanopeptide.

In addition, **Takai (1991)** reported that several therapeutically important compounds/ molecules like β- carotene, phycocyanin, γ- linolenic acid identified in *S.platensis* are shown to possess immnuomodulatory and biomodulatory functions. Also, **Layam et al (2006)** proved the same effect in diabetic animals given 15 mg/kg Spirulina for 45 days. Oxidative stress can cause oxidation and damage to many cellular components like DNA, lipids and proteins **(Jones, 2008),** and development of insulin resistance **(Whiteman et al 2010)**. The antioxidant activity of Spirulina due to its active constituents such as β-carotene, vitamin B, vitamin C, vitamin D, vitamin E, ω-3 and ω-6, phenolic compounds and phycocyanin **(Huang et al 2007)**.

**Table (1)** show that the level of serum insulin was significantly decreased in streptozotocin diabetic rats (6.56 mIU/L**)** when compared with normal rats (15.06 mIU/L**)**. While, the levels of serum insulin were elevated in the animal groups were treated with phycocyanin, phycocyanopeptide, phycocyanobilin and spirulina biomass (12.25, 12.91, 10.27 and 12.17 mIU/L), respectively. In the same time, group administration with phycocyanobilin recorded the lowest insulin levels in comparison with other treatments.

**Table 1.** Effect of *spirulina Platensis*, phycocyanin, phycocyanopeptide and phycocyanobilin on fasting serum glucose and insulin in diabetic male rats after 30 days treatments

| Group | Fasting serum glucose (mg/dL) | Fasting Serum insulin (mIU/L) |
|---|---|---|
| **Normal Control** | $83^{e} \pm 2.65$ | $15.06^{a} \pm 0.05$ |
| **Diabetic Control** | $371^{a} \pm 2.52$ | $6.56^{e} \pm 0.49$ |
| **Diabetic+ glibenclamide** | $165^{b} \pm 5.29$ | $11.90^{c} \pm 0.09$ |
| **Diabetic +Spirulina** | $168^{b} \pm 5.57$ | $12.17^{c} \pm 0.21$ |
| **Diabetic+ phycocyanin** | $128^{c} \pm 3.21$ | $12.25^{c} \pm 0.27$ |
| **Diabetic + phycocyanopeptide** | $112^{d} \pm 7.51$ | $12.91^{b} \pm 0.89$ |
| **Diabetic +phycocynobilin** | $122^{c} \pm 3.51$ | $10.27^{d} \pm 0.12$ |

Each value presents the mean ± SD. Different letters refer to significant difference (P≤ 0.05)

The amount of insulin was reduced in diabetic control due to STZ which is known for its selective β- cell cytotoxicity. It's specifically induces DNA strand breakage in β- cells of pancreas causing diabetes mellitus **(Kumar et al 2012)**. STZ is uptake in beta cells through Glut2 transporter, increased oxidative stress attribute to generation of nitric oxide (NO) and reactive oxygen species (ROS) **(Sanchez-Liraa et al 2017).** The reduction of insulin levels was due to beta cells damage compared to healthy group. The administration of phycocyanin and phycocyanopeptide brought the insulin level back to near normal levels compared to negative control which had lower levels ($P < 0.05$), this mean that phycocyanopeptide enhanced beta cell functions as shown in **Table (1)**. This data was in harmony with **Mani et al (2000)** who found that decrease of blood glucose due to effect of peptides produced by the digestion of proteins in spirulina.

In the present study, it was observed that given of phycocyanin could reverse the above mentioned diabetic effects. The possible action by which phycocyanin lead its hypoglycemic activity might be through potentiation of the pancreatic emission of insulin from β- cell or due to enhanced transfer of blood glucose to the peripheral tissue. In addition, **Chen et al (2017)** mentioned that phycocyanin possessed antioxidation and anti-inflammatory activities, this mean that phycocyanin play as a scavenger for free radicals to prevent pancreatic harm induced by diabetes. Also, the existence of chromium in Spirulina makes it a highly beneficial adjuvant therapy (**Gupta, et al 2010).** Chromium binds to peptide in phycocyanin known as Apo-LMWCr that in turn binds to insulin receptor and enhances the activity **(Piñero-Estrada et al 2001).**

**2- β-cell function (HOMA-β score) and insulin resistance (HOMA-IR score)**

The results in **Table (2)** show the impact of *spirulina Platensis*, phycocyanin, phycocyanopeptide and phycocyanobilin on insulin resistance and β- cell function in diabetic rats. It was observed that pancreatic β-cell function was decreased in negative control (2.88 mg/dL) when compared with normal rats (61.88 mg/dL). The HOMA-β score was observed to be highest value in the diabetic animals given phycocyanopeptide (38.15 mg/dL) followed by phycocyanin (30.99 mg/dL), phycocyanobilin (26.78 mg/dL) and spirulina alga (22.63 mg/dL) in comparison with diabetic control (2.88 mg/dL). Insulin resistance was increased in diabetic control (6.00 mg/dL) when compared with normal rats (3.08 mg/dL). HOMA-IR score was decreased in all treatments compared to diabetic control. It is suggested that insulin resistance has been improved in treated rats. In diabetic rats, the present data indicated a marked increase in glucose value and insulin resistance (371 and 6 mg/dL) as compared to normal rats (83 and 3.08 mg/dL). Administration of STZ caused rapid destruction of pancreatic β-cells in rats, which led to impaired glucose stimulated excretion of insulin and insulin resistance, both of which are marked features of T2DM (**Farswan et al 2009).** The increase of glucose level in blood may be attributed to decrease in transport of glucose to peripheral tissues, increase in glycogen breakdown and glucose production in liver (**Beck-Nielsen, 2002).** The hypoglycemic activity is also confirmed by histopathological examination of pancreas.

**Table 2.** Effect of *spirulina Platensis*, phycocyanin, phycocyanopeptide and phycocyanobilin on insulin resistance and β- cell function in diabetic rats

| Group | Insulin resistance (HOMA-IR score) (mg/dL) | β-cell function (HOMA-β score) (mg/dL) |
|---|---|---|
| **Normal Control** | $3.08^{d} \pm 0.04$ | $61.88^{a} \pm 0.76$ |
| **Diabetic Control** | $6.00^{a} \pm 0.40$ | $2.88^{f} \pm 0.53$ |
| **Diabetic+ glibenclamide** | $4.84^{b} \pm 0.12$ | $22.51^{e} \pm 1.04$ |
| **Diabetic +Spirulina** | $5.04^{c} \pm 0.08$ | $22.63^{c} \pm 1.34$ |
| **Diabetic+ phycocyanin** | $3.87^{c} \pm 0.20$ | $30.99^{b} \pm 0.32$ |
| **Diabetic + phycocyanopeptide** | $3.57^{d} \pm 0.00$ | $38.15^{d} \pm 5.62$ |
| **Diabetic +phycocynobilin** | $3.10 \pm 0.05$ | $26.78 \pm 1.22$ |

Each value presents the mean ± SD. Different letters refer to significant difference (P≤ 0.05).

In comparison with diabetic control, the present study showed a highly significant decrease in glucose level and insulin resistance of diabetic animals administration of *spirulina platensis*. It can be hypothesized that the possible mechanism of phycocyanin, chlorogenic acid and chromium antihyperglycemic action may be due potentiating the pancreatic excretion of insulin from islet β-cells and/or due to entry of blood glucose to peripheral tissue. By its ability to scavenge free radicals, phycocyanin contain phycocyanopeptide binding with selenium and phycocyanobilin prevent STZ-induced oxidative stress and protect β-cells resulting in increased insulin secretion and decrease in the elevated blood glucose levels. In addition, chromium binding with phycocyanin act as activator of insulin receptors so decreased insulin resistance.

**3- Chromium and selenium levels in *spirulina platensis*, phycocyanin, phycocyanopeptide and phycocyanobilin**

The obtained results of this work show that both selenium and chromium were bonded with phycocyanopeptide **(Table 3)**. The present data indicate that selenium and chromium contents were higher in phycocyanopeptide (0.13 and 2.39 ppm) compared to phycocyanobilin (0.04 and 0.15 ppm), respectively.

**Table 3.** Chromium and selenium Level in *spirulina platensis*, phycocyanin, phycocyanopeptide and phycocyanobilin

| | *Spirulina Platensis* | Phycocyanin | Phycocyanopeptide | Phycocyanobilin |
|---|---|---|---|---|
| **Chromium (ppm)** | 0.92 | 2.61 | 2.39 | 0.15 |
| **Selenium (ppm)** | 0.02 | 0.19 | 0.13 | 0.04 |

It is important to note that selenium and chromium were bonded to peptide, these caused that phycocyanopeptide and phycocyanin possessed hypoglycemic activity, and this confirmed by biological and histopathological examination. These results were supported by **Gupta et al (2011) and Bhat and Madyastha (2001)**. They reported that phycocyanin act as natural antioxidant for efficient management of tributyltin (TBT) and peroxy nitrite induced oxidative damage. Also, **Cherdkiatikul and Suwanwong (2014)** mentioned that the two components of phycocyanin (phycocyanopeptide and phycocyanobilin) were possessed strong antioxidant activity. **Huang et al (2007)** reported that antioxidant activity of phycocyanin attribute to selenium found in C-PC. **Zhai et al (2017)** reprted that the selenium possessed antioxidant effect and plays an important role in protecting against oxidative stress.

**4- Lipid profile levels (Total-cholesterol, Triglycerides, HDL-cholesterol and LDL- cholesterol)**

The data recorded in **Table (4)** illustrated the values of total-cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol after 30 days of treatment in treated and untreated diabetic rats. Data indicated that serum total-cholesterol, triglycerides and LDL-cholesterol were increased in diabetic control when compared with normal control. Insulin resistance in diabetic disease (type 2) is also correlating with high of lipid profile in blood and atherosclerosis **(Hozayen, et al 2016)**. The abnormal increase mount of blood lipids of diabetic rats is mainly attribute to increase in the mobilization of free fatty acids from the peripheral fat deposits, because insulin inhibits the hormone sensitive lipase production **(Udayakumar et al 2009).**

The levels of serum cholesterol were significantly ($p < 0.05$) decreased from 234.85 mg/dL in diabetic control to 158.57, 146.51, 137.60 and 139.01 mg/dL in the rats administered Spirulina alga, phycocyanin, phycocyanopeptide and phycocyanobilin, respectively. These results are in agreement with **Nagaoka et al (2005)** who observed that both, *S. platensis* concentrates or phycocyanin and other pigment extracted from *Spirulina* were caused hypocholesterolemic activity in rats.

**Table 4.** Effect of *spirulina platensis*, phycocyanin, phycocyanopeptide and phycocyanobilin on lipid profile (total cholesterol, triglycerides, HDL and LDL) in diabetic male rats

| Group | Total cholesterol (mg/dL) | Triglycerides (mg/dL) | HDL – cholesterol (mg/dL) | LDL – cholesterol (mg/dL) |
|---|---|---|---|---|
| **Normal Control** | $90.26^{d} \pm 5.83$ | $54.03^{f} \pm 5.05$ | $40.13^{a} \pm 1.03$ | $39.32^{e} \pm 4.12$ |
| **Diabetic Control** | $234.85^{a} \pm 4.45$ | $184.12^{a} \pm 4.58$ | $19.75^{c} \pm 1.16$ | $178.23^{a} \pm 3.17$ |
| **Diabetic+ glibenclamide** | $149.84^{b} \pm 4.96$ | $120.60^{b} \pm 1.01$ | $38.35^{b} \pm 2.18$ | $91.37^{c} \pm 4.20$ |
| **Diabetic +Spirulina** | $158.57^{b} \pm 3.24$ | $102.38^{c} \pm 3.57$ | $38.93^{b} \pm 2.15$ | $99.16^{b} \pm 3.30$ |
| **Diabetic+ phycocyanin** | $146.51^{c} \pm 2.45$ | $98.27^{d} \pm 0.58$ | $40.42^{a} \pm 4.25$ | $86.44^{c} \pm 5.71$ |
| **Diabetic + phycocyanopeptide** | $137.60^{c} \pm 2.44$ | $95.87^{e} \pm 0.05$ | $42.12^{a} \pm 3.27$ | $76.30^{d} \pm 3.34$ |
| **Diabetic +phycocynobilin** | $139.01^{c} \pm 3.51$ | $113.36^{d} \pm 4.62$ | $41.07^{a} \pm 3.03$ | $75.27^{d} \pm 6.29$ |

Each value presents the mean ± SD. Different letters refer to significant difference ($P \leq 0.05$).

On the other hand, the highest value of serum high-density lipoprotein (HDL)- cholesterol (42.12 mg/dL) was found in animals group given phycocyanopeptide this may be due to selenium binding with peptide as suggested by **Yang et al (2014)**. No significant difference was detected between the positive diabetic control (diabetic rats given glibenclamide drug) and rats treated *spirulina platensis* in total cholesterol and HDL-cholesterol. Also, the levels of serum LDL-cholesterol were decreased from 178.23 mg/dL in negative control to 91.37, 99.16, 86.44, 76.30 and 75.27 mg/dL in the rats given glibenclamide drug, Spirulina alga, phycocyanin, phycocyanopeptide and phycocyaobilin, respectively. The levels of serum triglycerides (TGs) were lower levels in diabetic + phycocyanin (98.27 mg/dL) and diabetic + phycocyanopeptide (95.87) groups when compared with diabetic control.

These results are confirmed with **Kay (1991)** who observed that *Spirulina platensis* decreased total cholesterol levels and increased cholesterol associated to high density lipoproteins, suggesting that *Spirulina* was protected the cardiovascular system. **Blé-Castillo et al (2002)** and **Torres-Durán et al (1999)** observed that *S. maxima* have ability to prevent the changes induced by $CCl_4$ in liver lipids in rats. The existence of antioxidants such as phycocyanin and polyphenols compounds in *Spirulina* can reduce serum lipid amount in *spirulina platensis*.

In the present investigation, *spirulina platensis* possess hypolipidemic activity is agreement with the authors who mentioned that the amounts of phenolic acids, α-tocopherol and β -carotene presents in *Spirulina* extracts induced the antioxidant activity (**Colla et al 2004; Olguin et al 2001; Alonso and Maroto, 2000; Quoc et al 1994 and Cohen et al 1993**). These compounds have been studied in respect to its therapeutic properties such as its ability to lowering blood cholesterol levels (**Kurushima et al 1995).** In addition, the results of the present study are in accordance with **Lee et al (2008)** who reported that Spirulina had an impact of on triglyceride level of diabetes mellitus (type 2) patients.

**Histopathological Results**

Pancreatic beta cells are highly susceptible to oxidative stress due to their restricted capacity for DNA repair induced by oxidative stress **(Lenzen, 2008 and Acharya and Ghaskadbi, 2010).** Hyperglycaemia generates free radicals that in turn induce oxidative stress, a deleterious condition for the pancreatic beta cells, which leads to cellular damage and apoptosis. In experimental diabetes model in rats, antioxidant compounds act as scavenger activity diminish insulin resistance and decrease the glucose levels **(Palsamy et al 2010).** These Compounds with antioxidant impacts has been studied in diabetes mellitus-type1 (DM1) and diabetes mellitus-type 2 (DM2) and demonstrated that they are useful to partially recover pancreatic beta cell function by improving glucose levels, insulin secretion and insulin resistance **(Sanchez-Lira et al 2017).**

Microscopically, the pancreatic sections of non-diabetic rats stained with H&E revealed no histopathological changes, normal pancreatic acini and normal islets of Langerhan's **(Fig. 1).** The pancreatic acini consist of pyramidal cells containing basal rounded nuclei. Meanwhile, Pancreas of rats from group 2 (STZ- diabetic rats) showed necrosis of islets cells of Langerhan's (**Figs. 2 & 3)**, cystic dilatation of pancreatic duct and vacuolation in islet cells **(Fig. 2).**

Streptozotocin (STZ) experimentally produces diabetes via β-cell death by the mechanism of DNA damage in rat's islets. As the result of streptozotocin action, B cells undergo the destruction by necrosis (**Senthil et al 2013).**

However, pancreas of animals given glibenclamide showed vacuolation of some cells of islets of Langerhan's **(Fig. 4)**. Also, observed reduction of necrotic areas in islet of Langerhans. While the effect of (STZ) still present in pancreatic tissue and changes similar to those observed in diabetic animals including vacuole. In diabetic animals given with *Spirulina platensis* suspension (group 4), the pancreatic sections showed slight improvement in the minimization of the vacuolated islets cells **(Fig. 5)**. Diabetic rats given alga marked regeneration of the pancreatic cells. Similar observations were noticed by **El-Desouki et al (2015)**, who found that administration of spirulina (2g/kg body weight) for 21 days to diabetic rats caused marked recovery of the pancreatic cells.

In addation, group treated with phycocyanin showed an obvious regeneration of pancreatic cells to almost normal structure **(Fig. 6)**. The results showed that oral administration of phycocyanin (50 mg/kg) for 4 wk protected against streptozotocin. Also, a histology of a pancreatic section of rats administered phycocyanopeptide **(Fig. 7)** and phycocyanobilin **(Fig. 8)** showed marked improvement of pancreatic cells.

The islets of diabetic rats under study administered with phycocyanopeptide are present with a large proportion of islet cells this showed that treated with phycocyanopeptide caused marked regeneration of the pancreatic islets. This mean that phycocyanin, phycocyanopeptide and phycocyanobilin protect against streptozotocin. This compound improves the antioxidant defence in pancreatic cells, may due to their powerful antioxidant properties, protect cells from free radicls damage and reverse degeneration changes in β-cells of streptozotocin-induced β- cells necrosis. This observation was confirmed the biochemical data in the present study and several studies indicated the cyanobacterial proteins in spirulina are the molecules of high potency to work as antioxidants by scavenging peroxyl, hydroxyl, peroxynitrite, superoxide radicals, and as inhibitors of lipid peroxidation **(Bhat and Madyastha, 2001).**

Other studies suggested that spirulina contains several active ingredients, notably phycocyanin and β-carotene that have antioxidant and anti-inflammatory activities **(Senthil et al 2013)**. Also, **Zheng et al (2013)** found that phycocyanin and phycocyanobilin protective of kidney in mice by prevent oxidative stress. **Ercan (2016)** found that the water-soluble fraction of *Spirulina platensis* was decreased the serum glucose level**.** Several articles have described that phycocyanin possessed antioxidant and anti-inflammatory properties **(Romay et al 1998; Piñero -Estrada et al 2001; Romay et al 2003; Zhou et al 2005; Samarakoon and Jeon, 2012 and El-Tantawy., 2015)**. Further studies have demonstrated that phycocyanobilin, which have structural similarities to bilirubins, play as antioxidants **(Bhat and Madyastha 2001).** Generally, several studies reported that the effect of phycocyanin as hypoglycemic agents. but the action of its as antidiabetic did not understand. Our results explaning the hypoglycemic and hypolipidemic effect of bioactive pigment phycocyanin in *spirulina platensis* attributed to selenium and chromium were bonded with phycopeptide phycocyanobilin.

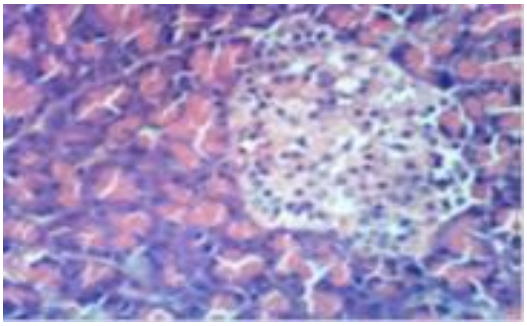

**Fig. 1.** Pancreas of rat from group 1 showing no histopathological changes. Notice normal pancreatic acini and normal islets of Langerhan's **(H & E X 400).**

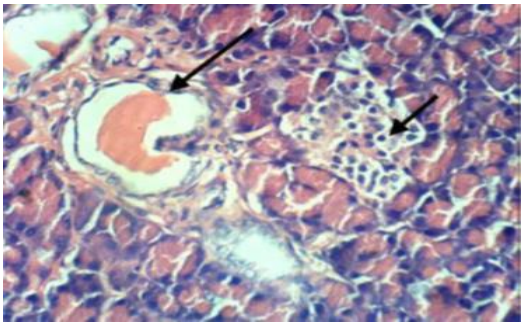

**Fig. 2.** Pancreas of rat from group 2 showing necrosis of cells of islets of Langerhan's (small arrow) and cystic dilatation of pancreatic duct (large arrow) **(H & E X 400).**

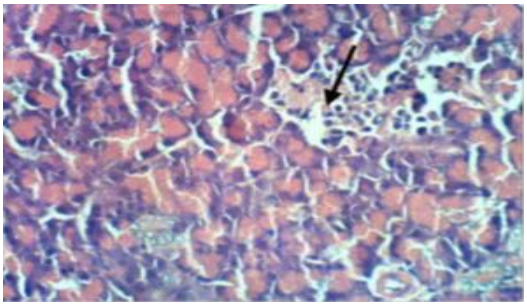

**Fig. 3.** Pancreas of rat from group 2 showing necrosis of cells of islets of Langerhan's **(H & E X 400).**

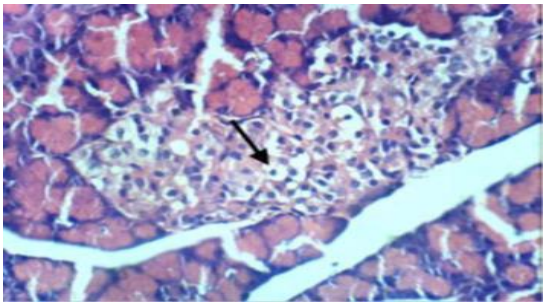

**Fig. 4.** A section of pancreas of animals treated with glibenclamide drug (group 3) showing vacuolation of some cells of islets of Langerhan's **(H & E X 400).**

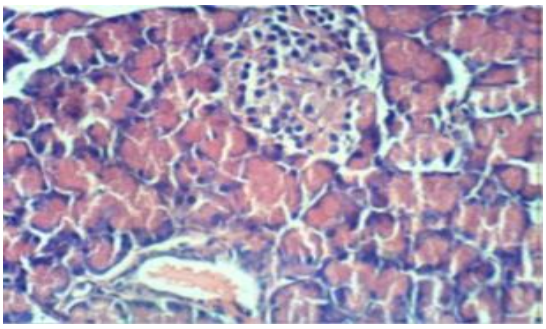

**Fig. 5.** A pancreatic section of a diabetic rat administered spirulina (group 4) showing marked improvement of pancreatic islet and acinar cells **(H & E X 400).**

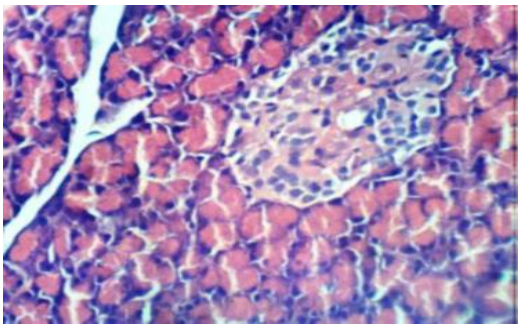

**Fig. 6.** Pancreas of diabetic rat treated with phycocyanin (group 5) showing no histopathological changes and the normal structure of pancreatic islet **(H & E X 400).**

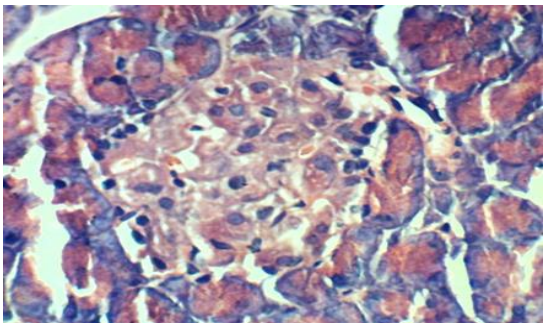

**Fig. 7.** A section of pencreas of diabetic rat administered phycocyanopeptide (group 6) showing normal appearance of acini and islet of Langerhans and showing no histopathological changes **(H & E X 400).**

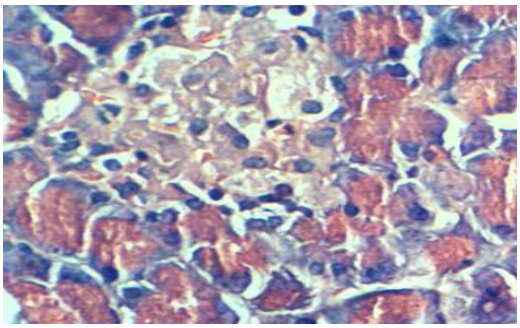

**Fig. 8.** Pancreas of rat from (group 7) showing no histopathological changes **(H & E X 400).**

## CONCLUSION

In the present study, we have concluded that *Spirulina platensis* possessed hypoglycemic and hypolipidemic effects which helps the diabetic rats to control the blood glucose levels. *Spirulina platensis* is a promising agent as a functional food for the management of diabetic rats. Bioactive compounds which extracted from *Spirulina platensis* like phycocyanin, phycocyanopeptide and phycocyanobilin act as antioxidant and can be used as antidiabetic agent instead of insulin and hypoglycemic drugs to decrease the side effects of these drugs. We can conclude the antioxidant effect of *spirulina platensis* is attributed to phycocyanin and phenolic compounds. The antioxidant effect of phycocyanin is attributed to selenium-binding phycocyanopeptide and chromo-phore in phycocyanobilin. The hypoglycemic effect of phycocyanin is attributed to chromium -binding phycocyanopeptide which act as an activator of insulin receptors.

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