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Research Article



SPIRULINA ROLE AS ANTIDIABETIC, ANTI-INFLAMMATORY AND ANTIOXIDANT AGENT IN ALLOXAN INDUCED DIABETIC MALE RABBITS

Mohanad Hasan Mahmood Al-Izzi¹, Marwa Abd-Alsalam Qadir Al-Hashimi¹,
Wedad Mahmood Lahmood Al-Obaidi²

¹Department of Biology, Science College, Tikrit University, Iraq.

² Department of Biology, Education College (AL-Hawiga), Kirkuk University, Iraq.



ABSTRACT

This study was designed in order to estimate the beneficial effects of *Spirulina platensis* microalgae on alloxan induced diabetes mellitus indicated in some immunological (TNF- α) and biochemical parameters (Blood Glucose, Total Cholesterol, Triglycerides, Malondialdehyde and Glutathione) of male domestic rabbits. The study involved (21) blood samples were divided into 3 groups: group one: adult normal healthy male rabbits as control group, group two: adult male rabbits with alloxan induced diabetes mellitus, group three: adult male rabbits with alloxan induced diabetes mellitus treated with spirulina (2000 mg/kg). The results of this study found significant increase in fasting blood glucose concentrations at levels ($P \leq 0.05$) of second group in comparison with control group; while significant decrease in fasting blood glucose concentrations at levels ($P \leq 0.05$) of third group in comparison with diabetic group, and there is significant increase in proinflammatory cytokine TNF- α concentrations at levels ($P \leq 0.05$) of second group in comparison with control group, while significant decrease in TNF- α concentrations at levels ($P \leq 0.05$) of third group in comparison with diabetic group; and there are significant increases in total cholesterol, triglycerides and malondialdehyde concentrations at levels ($P \leq 0.05$) of second group in comparison with control group, while significant decrease in total cholesterol, triglycerides and malondialdehyde concentrations at levels ($P \leq 0.05$) of third group in comparison with diabetic group, and there are significant decreases in glutathione concentrations at levels ($P \leq 0.05$) of second group in comparison with control group, while significant decrease in glutathione concentrations at levels ($P \leq 0.05$) of third group in comparison with diabetic group.

Keywords: Spirulina, Diabetes mellitus, Alloxan, TNF- α , Glutathione.

Introduction

Diabetes mellitus is an endocrinological disorder as a group of heterogeneous metabolic disturbances resulting from deficiencies in insulin actions, insulin secretions or both which leads to chronic abnormal elevated blood sugar and glucose intolerance (1). Chronic hyperglycemia and other metabolic disturbances of diabetes mellitus lead to organ damage and dysfunction involving the eyes, kidneys, nervous and cardiovascular systems (2).

Spirulina is a microscopic blue-green algae –cyanobacteria– from the Oscillatoriaceae family naturally grows in alkaline and warm media; in the sea and fresh water of Asia, Africa, Europe, South and North America(3); spirulina can treat many medical conditions including diabetes mellitus because this algae has antidiabetic, anti-inflammatory, anticancer, antibacterial, antiviral, antifungal and antioxidant properties, due to its content from active molecules such as phycocyanin, zinc, magnesium, manganese, selenium, riboflavin, β -carotene, tocopherol, γ -linolenic acid and phenolic compounds (4,5).

Proinflammatory cytokine TNF- α is produced mainly from macrophages and from adipose tissue participated in autoimmune process of type 1 diabetes mellitus, and type 2 diabetes mellitus by which the damaged pancreatic beta cells is linked with glucotoxicity mediated through TNF- α and other proinflammatory cytokines such as IL-1 β , IFN- γ (6); furthermore advanced glycosylated end products (AGEs) in diabetes mellitus stimulate the production of IL-1 β by macrophages and monocytes (7), which lead to intracellular generation of reactive oxygen species (ROS) which in turn activate NF- κ B which increase the expression of a variety of proinflammatory cytokines including tumor necrosis factors (TNF- α and TNF- β) (8); additionally in diabetes mellitus there is hypercholesterolemia condition which stimulates liver to produce proinflammatory cytokines including TNF- α (9); also reactive oxygen species (ROS) resulted from diabetic condition mediate proinflammatory cytokines expression including TNF- α by activating oxidant-sensitive transcription factors, such as activator protein-1 (AP-1), nuclear factor kappa light chain enhancer of activated B lymphocytes (NF- κ B), Janus kinase signal transducers, and activators of transcription -Jak/Stat- (10).

Malondialdehyde (MDA) is a highly toxic by-product formed by lipid oxidation induced free radicals. MDA is the major metabolite of arachidonic acid reacting both irreversibly and reversibly with proteins and phospholipids (11). Malondialdehyde detaches level in diabetes mellitus(12); indicating lipid peroxidation and oxidative stress due to increasing of free radicals activity in diabetics this together with insulin resistance can lead to activation of stress induced pathways, which play an important role in the development of diabetes mellitus complications (13). Glutathione (γ -L-glutamyl-L-cysteinylglycine-GSH), is a water soluble endogenous tripeptide, from three amino acids glycine, cysteine, and glutamic acid (14). Glutathione (GSH) is a potent antioxidant protecting the cell from oxidative stress. Glutathione is a cofactor for Glutathione peroxidase (GPx) which is a defense mechanism against peroxides, preventing the accumulation of ROS and preventing cellular injury. (15). GSH is also a cofactor for the enzyme dehydroascorbate reductase, which recycles dehydroascorbate back to reduced ascorbic acid (vitamin C); glutathione therefore keeps vitamins C and E in their reduced forms by the reversible oxidation of their sulfhydryl group (16).

Glutathione (GSH) is reduced or depleted in diabetes mellitus condition this occurs due to direct effects of glucose and insulin on glutathione synthesis, and by consumption of cofactors such as NADPH required for GSH (reduced form) regeneration from GSSG (oxidized form) via the polyol pathway; in addition to the effect of changes occurring in GSH dependent enzyme activities, such as glutathione peroxidase, glutamyltranspeptidase, and glutathione S-transferase(17).

Material and Methods:

1. Animals :

The experiment is performed on 21 adult male domestic rabbit (*Oryctolagus cuniculus*) of weighing between (1400-1500) g and age ranges between (7 -8) months obtained from animal market dealer, the animals stayed for 2 weeks for adapted the place and to make sure they are free of disease before

began the experiment. The experiment period was from beginning of April (2017) to mid of July (2017) in Tikrit city.

2. Induction of Diabetes :

Diabetes was induced by single dose (150 mg/kg) of freshly prepared intraperitoneal injection of alloxan (British BDH company). It has dissolved by 1.5 g of alloxan in 10 ml of normal saline(18). After alloxan injection, the rabbits were allowed to drink 5% glucose solution for 24 hours to overcome the drug induced hypoglycemia. Each rabbit of the normal control group was injected with 1 ml of normal saline. The induction of diabetes was confirmed by collecting blood from the external ear vein for glucose analysis by using portable blood glucose monitor and its strips (Rossmax company) every day, for 10 days , and then rabbits with fasting blood glucose levels above 150 mg/dl were considered diabetic (19).

3. Experimental Design :

In the present experiment 21 adult male rabbit were used. The rabbits were divided randomly into three groups. ten male rabbit were included in each group, Equal weights of each group was taken into consideration as much as possible before the start of the study :

Group 1 : (control group) normal control male rabbit were given water and food for 30 days.

Group 2 : (Diabetic group) : they have been injected alloxan150 mg/kg body weight intraperitoneal injection then given food and water for 30 days. .

Group 3 : (Diabetic +Spirulina group) : they have been injected alloxan150 mg/kg body weight intraperitoneally then given 2000 mg/kg body weight spirulina orally concomitantly for 30 days .

4. Determination of Parameters :

The total cholesterol and triglycerides concentration was estimated in serum using kits from BIOLABO France(20).Serum malondialdehyde was measured according to modified method used by (Guidet and Shah,1989from my ph.d. ref.).Glutathione was estimated by the modified method used by (21), and TNF- α were determined by using its kit from (my bioscourse) company (USA) of ELISA technique.

Result and Discussion:

This study showed high significance increase in blood glucose concentration ($p \leq 0.05$) in diabetic group during the experiment period Figure (1) (194.00 ± 17.72 mg/dl) as compared with control group .

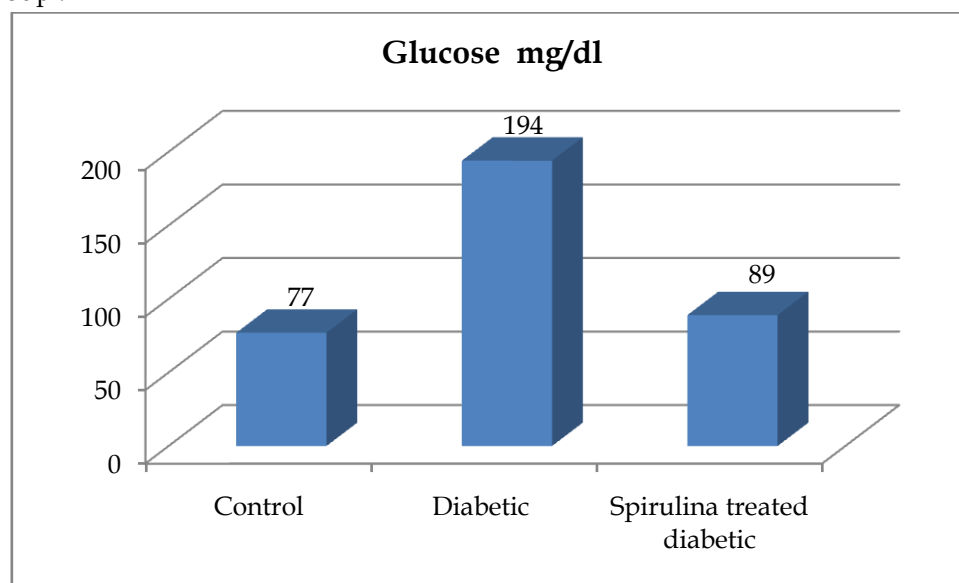


Figure (1): Concentrations of Blood Glucose (mg/dl) in the study groups.

These results coincide with the studies of Matsuura *et al.* (2017)(22); and Alamgeer *et al.* (2016) (23); they found significant increase of blood glucose in alloxan induced diabetic rabbits in comparison with control group.

The current study showed significant decrease in blood glucose concentration ($p \leq 0.05$) in spirulina treated diabetic group during the experiment period Figure (2) (89.00 ± 11.42 mg/dl) as compared with diabetic group, Such significant decrease in blood glucose is supported by other studies such as the study of Aissaoui *et al.* (2017) (24), El-Desoukiet *et al.* (2015) (25) both revealed such significant decrease after oral administration of *Spirulina platensis* extract into alloxan induced diabetic rats.

The antidiabetic effect of *Spirulina platensis* is due to the presence of potent antioxidant bioactive molecules (specially β -carotene and phycocyanin), which increase the insulin secretion from the islet beta cells or promotion of blood glucose transport to the peripheral tissues; this antidiabetic effect also due to the action of peptides and polypeptides that can stimulate insulin secretion generated by the digestion of the spirulina proteins (26); also due to spirulina ability to decrease hexokinase activity in liver and increase glucose-6-phosphatase activity in muscles (27); thus spirulina improving insulin resistance and the uptake of glucose (28); additionally spirulina high fiber composition can reduce glucose absorption (29). This study showed significant increase in blood serum proinflammatory cytokine TNF- α concentrations ($p \leq 0.05$) in diabetic group during the experiment period Figure (3) (199.20 ± 57.81 pg/ml) as compared with control group.

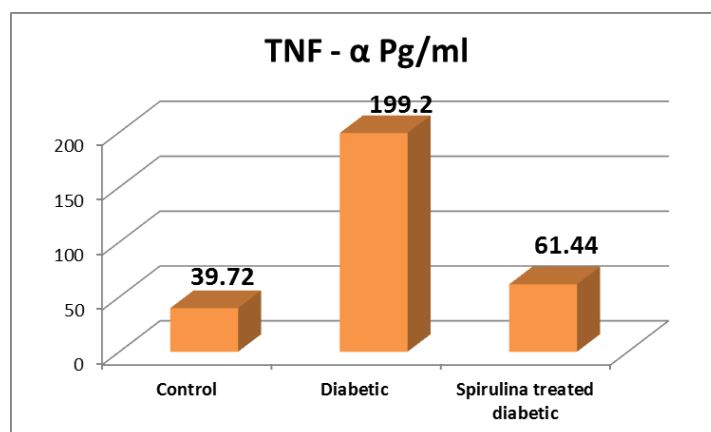


Figure (2): Concentrations of blood serum TNF- α (Pg/ml) in the study groups.

These results agree with the study of Ramadan *la te* (2017)(30); who revealed significant elevation of TNF- α in alloxan induced diabetic rats.

While this study found significant decrease in TNF- α concentrations ($p \leq 0.05$) of male rabbits in diabetic group treated with spirulina in comparison with diabetic group and this agree with the study of Hozayen *et al.* (2016) (29), who revealed such significant decrease after oral administration of spirulina into type 2 diabetic rats.

TNF- α is potent proinflammatory cytokine secreted by several types of cells such as macrophages, monocytes, neutrophils and T lymphocytes. TNF- α expression increased significantly in both types 1 and 2 of diabetes mellitus regarded as one factors of their Pathophysiology (31) in type 2 diabetes TNF- α and other proinflammatory cytokines such as IL-1 beta, IL-6 and INF-gamma play an important role in insulin resistance and pathogenesis (32) of several mechanisms including the down regulation of genes required for normal insulin action, its negative regulation of PPAR α which is an important insulin-sensitizing nuclear receptor, the direct effects on insulin signalling and the induction of elevated free fatty acids via the stimulation of lipolysis (33), additionally TNF- α lowers the activity of 5'AMP-activated protein kinase (AMPK), via increased activity

of protein phosphatase 2C (PP2C), which is one of insulin resistance causes, and in type 1 diabetes TNF- α and INF- γ participate in the apoptosis process of beta cells by activating calcium channels of these cells, and this leads to the mitochondrial dysfunction and activation of caspases leading eventually to death of beta cells (34).

Spirulina contains several active ingredients, importantly phycocyanin and β -carotene that have potent antidiabetic, antioxidant and anti-inflammatory activities (25) and this explain the lowering effect of spirulina on TNF- α levels ; additionally spirulina oral administration elevate serum adiponectin production and this adiponectin hormone in turn inhibit the expression of the proinflammatory cytokine TNF- α (29)

This study showed high significance increase in total cholesterol concentrations ($p \leq 0.05$) in diabetic group during the experiment period Figure (4) (171.10 ± 53.46 mg/dl) as compared with control group.

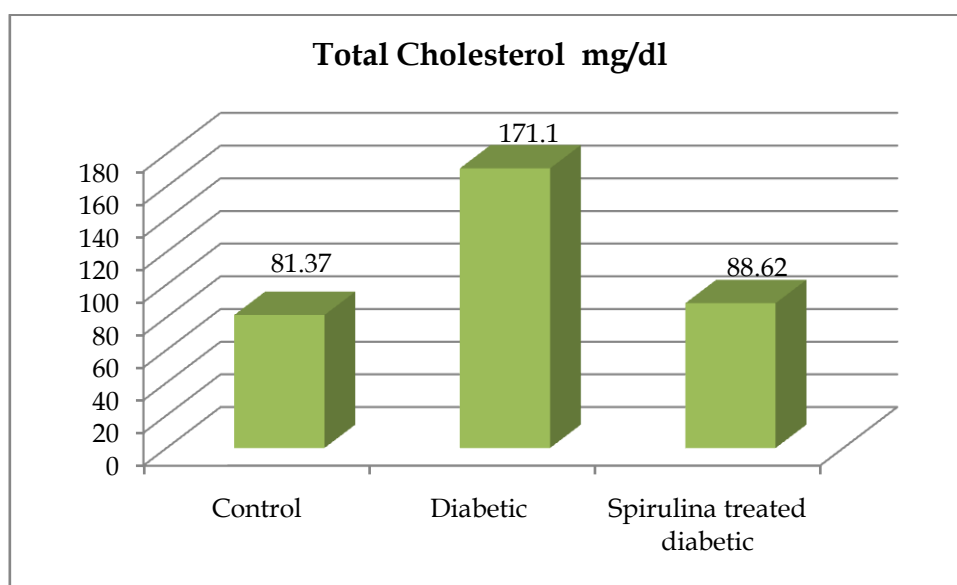


Figure (3): Concentrations of blood serum total cholesterol (mg/dl) in the study groups.

These results agree with with the study of Khulanet *al.* (2015) , who found significant increase of total cholesterol concentrations in alloxan induced diabetic rabbits in comparison with control group.(35)

This study found significant decrease in total cholesterol concentrations ($p \leq 0.05$) of male rabbits in diabetic group treated with spirulina in comparison with diabetic group and this agree with the study of Senthilkumar and John (2008) , who revealed such significant decrease after oral administration of *Spirulina platensis*.(36)

extract into alloxan induced diabetic rats ,The abnormal high concentration of serum lipids in diabetics is mainly due to increase in the mobilization of free fatty acids from the peripheral fat deposits, because insulin inhibits the hormone sensitive lipase production(37); hypocholesteremic effect of spirulina is due to the presence of γ -linoleic acid in spirulina, which prevents accumulation of cholesterol in the body ; in addition to the effect of phycocyanin which is water soluble protein as active ingredient in spirulina responsible for the hypolipidemic activity (38).

The current study showed significance increase in triglycerides concentrations ($p \leq 0.05$) in diabetic group during the experiment period Figure (4) (244.60 ± 49.32 mg/dl) as compared with control group.

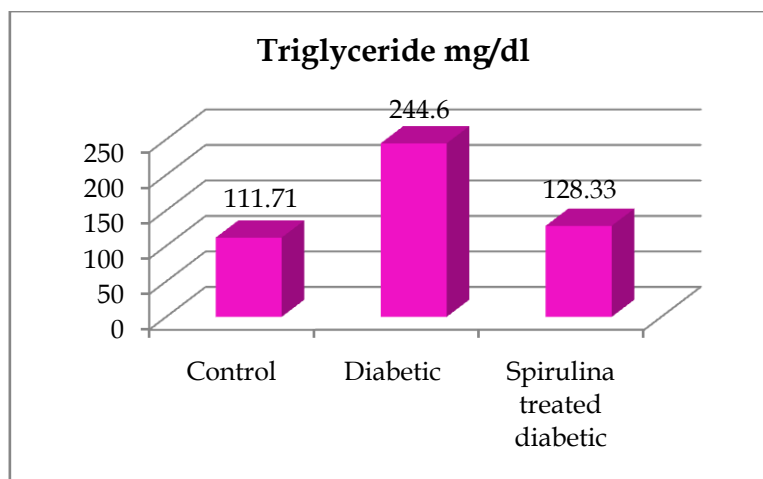


Figure (4): Concentrations of blood serum triglycerides in the study groups.

These results coincide with the study of Khulan *et al.* (2015), who noticed significant increase of blood serum triglycerides in alloxan induced diabetic rabbits in comparison with control group.(35) This study revealed significant decrease in triglycerides concentrations ($p \leq 0.05$) of male rabbits in diabetic group treated with spirulina in comparison with diabetic group and this agree with the study of El-Bazet *al.* (2013), who reported significant decrease in triglycerides concentrations after oral administration of *Spirulina platensis* to type 2 diabetic rats.(38)

Spirulina increase the utilization of glucose, thereby depressing the mobilization of fat; in addition spirulina is effective in normalizing the triglyceride levels through its effect on lipoprotein lipase which is a key enzyme in the metabolism of triglyceride lipoprotein, and via decreasing very low density lipoprotein (VLDL) production or increased VLDL clearance in the peripheral tissues (39).

This study showed high significance increase in blood serum malondialdehyde concentrations ($p \leq 0.05$) in diabetic group during the experiment period Figure (4) ($4.17 \pm 0.86 \mu\text{mol} / \text{L}$) as compared with control group.

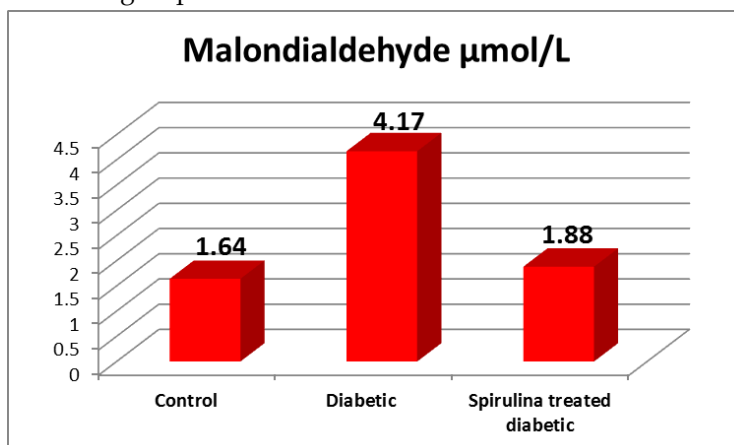


Figure (5): Concentrations of blood serum malondialdehyde in the study groups

These results agree with the study of Owolabiet *al.* (2011), who found significant increase of blood serum malondialdehyde in alloxan induced diabetic rabbits in comparison with control group.(40) This study found significant decrease in blood serum malondialdehyde concentrations ($p \leq 0.05$) of male rabbits in diabetic group treated with spirulina in comparison with diabetic group and this agree with the study of Lee *et al.* (2008)(41), who revealed such significant decrease after oral administration of Spirulina into type 2 diabetes mellitus patients, and the study of (37) who showed such significant decrease after oral administration of spirulina into type 2 diabetic rats.

Treatment with *Spirulina platensis* prevent lipid peroxidation and decrease free radicals production (24) , because spirulina protein content has high antioxidants activity by scavenging the free radicals peroxy, hydroxyl, peroxy nitrite and superoxide , and this inhibit lipid peroxidation (25).

The study showed significance decrease in blood serum glutathione concentrations ($p \leq 0.05$) in diabetic group during the experiment period Figure (4) ($2.19 \pm 0.88 \mu\text{mol/L}$) as compared with control group.

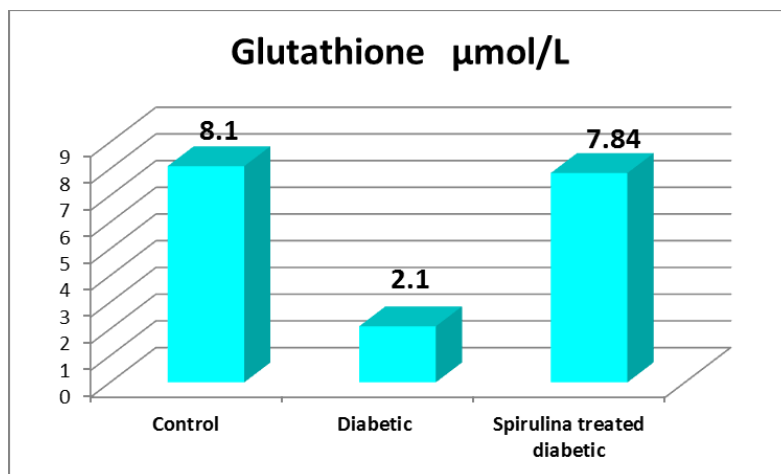


Figure (6): Concentrations of blood serum glutathione in the study groups

These results agree with the study of Ahmadvand and Khosrow beygi (2015) (42) , who found significant decrease of blood serum glutathione in alloxan induced diabetic rabbits in comparison with control group this occur due to oxidative stress resulted from diabetes mellitus which cause consumption of most glutathione used here as an antioxidant agent against diabetic harmful effects (17).

This study found significant decrease in blood serum glutathione concentrations ($p \leq 0.05$) of male rabbits in diabetic group treated with spirulina in comparison with diabetic group and this agree with the study of Senthil *et al.* (2013) (27) who revealed such significant decrease after oral administration of spirulina extract into streptozotocin induced diabetic rats and the study of El-Baz *et al* (2013) (37) who showed such significant decrease after oral administration of spirulina into type 2 diabetic rats, Oral administration of *Spirulina platensis* cause decreasing of free radicals production leading to normalization of glutathione and this occur due to spirulina high contents from protein, essential amino acids, essential fatty acids, minerals, vitamins, carotenoids and other antioxidant active components in addition to spirulina antidiabetic effects (26).

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دور طحلب السبريولينا كعامل مضاد للسكر ومضاد للالتهاب ومضاد للأكسدة في ذكور الارانب المصابة بداء السكر المستحدث بالالوكسان

1 مهند حسن محمود العزي , 1 مروة عبد السلام قادر الهاشمي , 2 وداد محمود لعمود العبيدي

1 قسم علوم الحياة , كلية العلوم , جامعة تكريت , تكريت , العراق

2 قسم علوم الحياة , كلية التربية (الحوبيجة) , جامعة كركوك , كركوك , العراق

الملخص

جرى تصميم هذا البحث لمعرفة التأثيرات الايجابية لطحلب السبريولينا على عدد من المتغيرات المناعية (الحركي الخلوي α -TNF) والكيموحيوية (سكر الدم , الكوليستيرول الكلي , الكليسيريدات الثلاثية , المالونديالدهيد , الغلوتاتايون) في ذكور الارانب المحلية , وشملت الدراسة (21) عينة دم قسمت الى 3 مجاميع : المجموعة الاولى : ذكور ارانب بالغة سليمة كمجموعة سيطرة , المجموعة الثانية : ذكور ارانب بالغة مصابة بداء السكر المستحدث بالالوكسان, المجموعة الثالثة : ذكور ارانب بالغة مصابة بداء السكر المستحدث بالالوكسان ومعالجة بطحلب السبريولينا (2000 ملغم/كغم) , ولقد اظهرت نتائج الدراسة الحالية ارتفاع تركيز الحركي الخلوي α -TNF معنويا عند مستوى ($P \leq 0.05$) في المجموعة الثانية (مجموعة داء السكر) مقارنة مع مجموعة السيطرة , بينما كان انخفاض معنوي عند مستوى ($P \leq 0.05$) بتركيز الحركي الخلوي α -FNT في المجموعة الثالثة (المصابة بداء السكر والمعالجة بالسبريولينا) مقارنة مع مجموعة داء السكر , كما وجدت هذه الدراسة ارتفاعات معنوية عند مستوى ($P \leq 0.05$) لتراكيز مكوني مرتسم الدهون (الكوليستيرول الكلي و الكليسيريدات الثلاثية) وكذلك للمتغير الكيموحيوي المالونديالدهيد في المجموعة الثانية (مجموعة داء السكر) مقارنة مع مجموعة السيطرة , فيما كانت هناك انخفاضات معنوية عند مستوى ($P \leq 0.05$) لهذه المتغيرات الكيموحيوية (الكوليستيرول الكلي , الكليسيريدات الثلاثية , و المالونديالدهيد) في المجموعة الثالثة (المصابة بداء السكر والمعالجة بالسبريولينا) مقارنة مع مجموعة داء السكر , بينما لوحظ انخفاض معنوي عند مستوى ($P \leq 0.05$) لتركيز الغلوتاتايون في المجموعة الثانية (مجموعة داء السكر) مقارنة مع مجموعة السيطرة , ووجد ارتفاع معنوي عند مستوى ($P \leq 0.05$) لتركيز الغلوتاتايون في المجموعة الثالثة (المصابة بداء السكر والمعالجة بالسبريولينا) مقارنة مع مجموعة داء السكر.