ANTIOXIDANT EFFECT OF BEE POLLEN ON IMMUNE STATUS OF HYPERGLYCEMIC RATS

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ABSTRACT

The aim of the current study was to evaluate the ability of bee pollen (BP), a known antioxidant-rich food supplement, to prevent or minimize the immune insufficiency complications associated with diabetes mellitus (DM) in rat model. A total of 32 adult male Wistar Albino rats divided into 4 groups (8 rats each) were used in the current study. One group were fed basal diet only for 60 successive days and used as non- diabetic control group (G I), while the other groups were used for induction of DM model after injection with streptozotocin (STZ) 65 mg/kg. One of the diabetic rat groups received only basal diet without BP supplementation for the same period and used as diabetic control group (G II). The other diabetic groups were subdeivided into 2 subgroups, which received basal diet supplemented with BP at a concentration of 1% and 2% for 60 successive days and assigned as G III and G IV, respectively. The obtained results revealed that at the end of the experiment there are still significant increases ($P \leq 0.001$) in glucose levels in groups II, III and IV in comparison to G I. However, non-significant changes were recorded in glucose levels G III & IV as compared G II. With regard to oxidative stress, significant reduction ($P \leq 0.01$) were recorded in the levels of evaluated antioxidant parameters (SOD, CAT and GSH-PX) in G II as compared to G I. However, significant increases ($P \leq 0.01$) and ($P \leq 0.001$) were recorded in the levels of antioxidant parameters in G III and G IV, respectively as compared to G II of hyperglycemic rats. With regards to immune status, significant reduction of CIC ($P \leq 0.01$), phagocytic activity of neutrophils ($P \leq 0.01$) and IFN-gamma ($P \leq 0.001$) were recorded G II as compared to G I. On the other hand, significant increase were recorded in the levels of IgG ($P \leq 0.01$) and IgM ($P \leq 0.001$) in G II as compared to G I. However, significant improvement of CIC ($P \leq 0.05$ and $P \leq 0.01$), Phagocytic activity ($P \leq 0.05$ and $P \leq 0.01$), IgM ($P \leq 0.05$ and $P \leq 0.01$), IgG ($P \leq 0.05$ and $P \leq 0.01$) and IFN-gamma ($P \leq 0.01$ and $P \leq 0.001$) were recorded among G III and G IV, respectively as compared to G II. In conclusion, an association between oxidative stress and immune insufficiency were recorded in the present study in diabetic rats Moreover, an obvious improvement of immune status parameters were recorded after supplementation of diabetic rats with bee pollen as an antioxidant-rich food supplement.

Key words: Diabetes Mellitus, bee pollen, antioxidant, immune insufficiency.

INTRODUCTION

Diabetes mellitus (DM) is a syndrome of metabolic disease characterized by hyperglycemia. It results from deficiency of insulin secretion or insulin action or both, with the subsequent abnormal metabolism of carbohydrate, protein, and lipid (Kuzuya *et al.*, 2002). DM is a prooxidant state characterized by increased reactive oxygen species (ROS) that promote oxidative stress (Devaraj *et al.*, 2008). Oxidative stress may be defined as an imbalance between production and degradation of ROS. One of the major hypotheses to explain the onset of diabetic complications is a DM-induced increase in oxidative stress (Kochar and Umathe, 2009).

Several evidences supported the concept that acute hyperglycemia in DM works through the production of an oxidative stress. Some studies reported that antioxidants can hinder some of the effects induced by hyperglycemia, which suggests that the action of acute hyperglycemia is mediated by the production of free radicals. In addition, it has been reported that during an oral glucose challenge study, a reduction of antioxidant defenses was observed (Konukoglu *et al.*, 1997; Ceriello *et al.*, 1998 and Tessier *et al.*, 1999). Sources of oxidative stress in DM include non enzymatic (glucose autoxidation, non enzymatic glycosidation of proteins), enzymatic (NADPH oxidase, nitric oxide synthase) and mitochondrial pathways (Johansen *et al.*, 2005).

Immune insufficiency is one of the serious complications of DM that leads to further complication from increased susceptibility to infection. (Mustschen *et al.*, 1992 and Esper *et al.*, 2009). Several previous studies have showed that DM-associated oxidative stress is responsible for quantitative and qualitative changes of immune cells particularly B-cells which result in inactivation of functional active proteins with subsequent immune insufficiency (Krapfenbaner *et al.*, 1998, Wen *et al.*, 2002 and Yavari and Azizova, 2012).

Beehive products have been used since ancient times as dietary supplements because of their perceived health-promoting effects on the human body. Bee pollen (BP) has been used for many years in both traditional medicine and supplementary nutrition, as well as in alternative diets, mainly due to its nutritional properties and health benefits (Bonvehi and Jorda, 1997; Isla *et al.*, 2001; Kroyer and Hegedus, 2001; Almeida-Muradian *et al.*, 2005). BP is an agglomerate of pollen grains from various botanical sources, which are collected by the bees and mixed with nectar and secretion from the hypopharyngeal glands such as β-glycosidase enzymes. BP is referred to as the "only perfectly complete food", as it contains all the essential amino acids needed for the human organism. Its nutritional composition consists of proteins, lipids, sugars, fibers, mineral salts, aminoacids and vitamins. In addition to this, pollen also has high contents of polyphenolic substances, chiefly flavonoids with antioxidant and antimicrobial activity (Kroyer and Hegedus, 2001; Campos *et al.*, 2003, Garcia *et al.*, 2001; Basim *et al.*, 2006).

Treatment and control of DM has been a long existed goal. In this regard, apart from therapeutic interventions such as oral glucose lowering drugs and insulin, dietary supplements are a potential intervention that have been recently investigated as an aid for the control of DM (Liatis *et al.*, 2009). Most of previous trials were concerned with the lowering of the glucose level and correction of the associated metabolic syndrome. However, further investigation is needed to determine whether is it possible to prevent the onset of DM complications or to slow down its progression by preemptive therapy prior to the development of tissue damage. Therefore the aim of the current study was to evaluate experimentally the ability of the local organic Bee Pollen, as an antioxidant-rich food supplement, to prevent or minimize immune insufficiencies associated with diabetes mellitus in rat model.

MATERIALS and METHODS

Bee Pollen sample

Samples of BP used in the present study originated from the beehive of Al-Taif region, Makkah province, KSA. These samples were harvested in September 2011 as yellow granules and dried for use as food additive. Chemical analysis of BP including moisture, crude protein, crude fat, and ash were determined on dry weight basis according to Horwitz (2000). Total carbohydrates were determined by difference as 100-(protein+ total fat+ ash).

Experimental animals:

Adult male Wistar albino rats weighing (185±16 gm) were used in the present study. Animals were obtained from Laboratory Animal Centre, Umm Al-Qura University, Makkah, KSA. Biological investigation has been carried out in the animal house facility of the faculty of Applied Medical Sciences, Umm Al-Qura University, where animals were housed in a clean polypropylene cages with not more than four animals *per* cage and maintained under standard laboratory conditions (temperature 25 ± 2 ºC with 12/12 h dark/light cycle). They were fed standard basal diet (12%casein, 10% corn oil, 0.2% choline chloride, 1% vitamin mixture, 5% cellulose, 4% salt mixture, and up to 100% corn starch) according to N.R.C., 1995 and water *ad libitum*. The animals were acclimatized to laboratory conditions for one week prior to the experiment. All procedures described were reviewed and approved by the Animal care and use Bioethical Committee of Medical Sciences, Umm Al-Qura University, KSA.

Induction of diabetic rate and experimental design:

Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal Streptocytocin (STZ) injection in a dose of 65 mg/kg body weight (Ravi *et al.*, 2004). After 3 days, fasting blood glucose levels were measured and the animals showing blood glucose level ≥225 mg/dL were used for the study (Ewart *et al.*, 1975). Proved hyperglycemic rats were divided into three groups (8 rats each). In addition a group of 8 normal rats without diabetes were included as a control. Investigated animal groups were fed on various diets for 60 days. The first group

included normal rats fed only on standard basal diet and served as negative control group (G I). The second group was diabetic rats fed only on standard basal diet and served as positive control group (G II). The third and fourth groups included diabetic rats fed on standard basal diet plus 1% and 2% of BP and assigned as G III and G IV, respectively. At the end of the experiment, biological evaluation of the different diets was carried out by determination of body weight gain% (BWG%) and food efficiency ratio (FER) according to Chapman *et al.* (1950). All rats were weighted once weekly.

Blood and organs sampling:
At 24 h of the last feeding, blood samples were collected from all investigated animal groups. The animals were anesthetized in a chamber containing diethyl ether. Two blood samples were collected from each animal into a heparin containing tube and plane tube, respectively. For serum production, collected blood samples in plane tubes were centrifuged at 3000 rpm for 10 minutes. The produced serum was collected and stored at -20°C until further analysis while packed RBC was used for the estimation of antioxidant enzymes.

Neutrophils were isolated from the freshly collected whole blood samples of control and diabetic rats by the standard procedure of dextran (Sigma, St. Louis, USA) sedimentation followed by centrifugation on Ficoll-Hypaue (Pharmacia, Uppsala, Sweden) as described elsewhere (Nurun Nabi *et al.*, 2005).

Food efficiency parameters:
In addition to body weight gain (BWG), food efficiency parameters in term of food intake and food efficiency ratio were evaluated in different investigated rat groups using the methods Chapman *et al.* (1950).

Biochemical and immunological analysis:
Enzymatic colorimetric method used to determine blood glucose in serum samples according to Sugiura and Hirano (1977) using glucose test kit (Cayman Chemical Company, Ann Arbor, Michigan, USA).

Levels of immunoglobulins, circulating immune complexes (CIC), IFN-gamma and neutrophils phagocytic activity were measured in the current study to evaluate immune status of investigated rats. Imunoglobulins quantification was performed by conventional radial immunodiffusion according to method of Mancini (1970) using commercial kits for rat sera (ICN Biomedicals, Aurora, OH). Measurements were based on an 18-h timed diffusion for IgG and end-point determinations for other Igs (Everson, 2005). IFN-gamma was measured in serum using Rat-IFN-gamma Sandwich ELISA kit (Thermo Fisher Scientific Inc., Rockford, IL, USA). The phagocytic activity of collected neutrophils from all investigated animals was evaluated by Zymosan colorimetric format of CytoSelect™ 96-Well Phagocytosis Assay. The assay uses pre-labeled Zymosan particles as a phagocytosis pathogen. The assay was performed as instructed by the manufacturer (Cell Biolabs, Inc., San Diego, CA, USA). CIC concentration was measured in the serum of all investigated rats by Grinevich method (CIC-enriched fraction, precipitated with 3.5% polyethylene glycol-PEG) as previously described (Ovsenyan *et al.*, 2004).

Antioxidant enzyme assessment:
The RBCs was lysed by mixing chilled water and RBC. Lysate was used for the estimation of antioxidant enzymes namely catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px). Catalase activity was determined spectrophotometrically by the method of Aebi (1984). SOD activity was determined spectrophotometrically according to the method of McCord and Fridovich (1969). GPx was assayed by the method of Pagila and Valentine (1967).

Statistical Analysis
The results were expressed as mean ± standard deviation (SD). Statistical analysis of the results was performed using the statistical package software - statistical package for social science (SPSS), version 16 for windows (SPSS, 2008). Paired-sample t-test was used to compare the parameters between control and diabetic rat groups. A *P*-value less than 0.05 was considered statistically significant.

RESULTS

Chemical composition and effect of BP on food efficiency parameters:
In the present study bee pollen was analyzed for the content of protein, fat, ash, moisture and total carbohydrate. The results as given in table 1 showed that protein, fat, ash, moisture, carbohydrate and energy of pollen grains were 18.5, 4.5, 7.7, 6, 63.3 and 367.7%, respectively. Regarding food efficiency parameters, significant reduction ($P \leq 0.01$) were reported in the BWG, FI and FER of the diabetic rat group fed on non BP-supplemented diet (G II) as compared to non diabetic control group (G I). On the other hand, significant increases ($P \leq 0.05$) were reported in these parameters in diabetic rat groups fed of BP-supplemented diets (G III & G IV) as compared to diabetic rat group fed on non BP-supplemented diet (G II) as shown in table 2.

Table 1: Chemical composition of Al-Taif bee pollen (g/100g w/w).

Nutrient contents	Protein	Fat	Ash	Moisture	CHO	Energy (Kcal)
Pollen grains	18.5%	4.5%	7.7%	6%	63.3%	367.7

Table 2: Effect of bee pollen on body weight gain (BWG%), food intake (FI, g) and food efficiency ratio (FER) of hyperglycemic rats.

Rat Groups		BWG %	FI	FER
Non diabetic rats (G I)		13.5±2.02	16.28±2.28	0.044±0.01
Diabetic rats	NO BP (G II)	3.27±0.79**	11.24±2.15**	0.01±0.006**
	BP (1 %) (G III)	8.69±1.94*	15.2±1.5*	0.03±0.01*
	BP (2%) (G IV)	9.14±2.18*	15.9±1.81*	0.03±0.006*

Data are expressed as Mean ± SD of eight experiments. A P-value less than 0.05 was considered statistically significant. Parameters of G II were compared to that of G I, and parameters of G III and G IV were compared to that of G II. *($P \leq 0.05$) significant change; **($P \leq 0.01$) significant change.

Effect of BP supplementation on oxidative stress of hyperglycemic rats:
The current results showed that at the end of the experiment there are still significant increases ($P \leq 0.001$) in glucose levels in both diabetic rat group fed on diabetic rat group fed on non BP-supplemented diet (G II) and diabetic rat groups fed of BP-supplemented diets (G III and IV) as compared to non diabetic control group (G I). Non significant changes were recorded in blood glucose levels of diabetic rat groups fed of BP-supplemented diets (G III & IV) as compared to diabetic rat group fed on diabetic rat group fed on non BP-supplemented diet (G II, table 3). With regard to oxidative stress, significant reduction ($P \leq 0.01$) were recorded in the levels of evaluated antioxidant parameters (SOD, CAT and GSH-PX) in diabetic rats fed non BP-supplemented diets (G II) as compared to non diabetic control group (G I). However, significant increases ($P \leq 0.01$) and ($P \leq 0.001$) were recorded in the levels of antioxidant parameters in diabetic rat groups fed BP-supplemented diets (G III and G IV, respectively) as compared to diabetic rat group fed on non BP-supplemented diet (G II, table 4).

Table 3: Glucose level in different investigated groups of normal and diabetic rats.

Groups	Non diabetic rats (G I)	Diabetic rats (No BP) (G II)	Diabetic rats (BP, 1%) (G III)	Diabetic rats (BP, 2%) (G IV)
Glucose (mg/dl)	101.5±4.9	240.5±12.8***	232.9±10.8***	229.1±8.8***

Data are expressed as Mean ± SD of eight experiments. A P-value less than 0.05 was considered statistically significant. Parameters of G II, G III and G IV were compared to that of G I, ***($P \leq 0.001$) significant change.

Table 4: Effect of bee pollen on erythrocyte superoxide dismutase (SOD), catalase (CAT) activities and glutathione peroxidase (GSH-Px) (U/mg protein) of hyperglycemic rats.

Groups		SOD	CAT	GSH-Px
Non diabetic rats (G I)		3.14±0.53	35.18±4.13	54.65±3.8
Diabetic rats	No BP (G II)	0.98±0.11**	25.86±2.89 **	18.59±2.79**
	BP (1%) (G III)	3.36±0.4**	35.32±5.76 **	54.31±5.58**
	BP (2%) (G IV)	4.15±0.45***	47.19±3.52 ***	67.15±3.25***

Data are expressed as Mean ± SD of eight experiments. A P-value less than 0.05 was considered statistically significant. Parameters of G II were compared to that of G I, and parameters of G III and G IV were compared to that of G II. **($P \leq 0.01$) significant change; ***($P \leq 0.001$) significant change.

Effect of BP supplementation on immune status of hyperglycemic rats:

Significant reduction of phagocytic activity of neutrophils ($P \leq 0.01$) and levels of CIC ($P \leq 0.01$), and IFN-gamma ($P \leq 0.001$) were recorded in diabetic rat group fed on non BP-supplemented diet (G II) as compared to non-diabetic control group (G I). On the other hand, significant increase were recorded in the levels of IgG ($P \leq 0.01$) and IgM ($P \leq 0.001$) among G II as compared to G I (Table 5). However, after BP supplementation, the results revealed significant improvement ($P \leq 0.05$) and ($P \leq 0.01$) of CIC, Phagocytic activity, IgM and IgG and ($P \leq 0.01$) and ($P \leq 0.001$) of IFN-gamma were recorded among 1% and 2% BP-supplemented diabetic rats (G III and G IV), respectively as compared to diabetic rats of G II fed on non BP-supplemented diet (Table 5).

Table 5: Effect of bee pollen on some immunity indices (mg/dl) of hyperglycemic rats.

Groups		CIC (mg/ml)	Phagocytic activity (%)	IgM (mg/ml)	IgA (mg/ml)	IgG (mg/ml)	IFN-gamma (ng/ml)
Non diabetic rats (G I)		0.59±0.07	95.38±7.9	22.88±3.3	17.13±2.3	69.63±7.7	359.9±20.7
Diabetic rats	No BP (G II)	0.91± 0.09**	50.75± 4.8**	48.25±5.1***	19.25±2.3	149.75±21.1**	659.3±27.2***
	BP (1%) (G III)	0.76±0.05*	76.75±5.6*	34.87±3.5*	20.63±3.8	99.38±12.6*	492.3±35.5**
	BP (2%) (G IV)	0.65±0.06**	89.13±7.3**	26.87±2.8**	20.87±4.3	74.75±7.1**	405.9±20.7***

Data are expressed as Mean ± SD of eight experiments. A P-value less than 0.05 was considered statistically significant. Parameters of G II were compared to that of G I, and parameters of G III and G IV were compared to that of G II. *($P \leq 0.05$) significant change; **($P \leq 0.01$) significant change; ***($P \leq 0.001$) significant change.

DISCUSSION

One of the serious complications of diabetes, that is believed to be induced by the associated oxidative stress, is the immune insufficiency with the subsequent increased susceptibility to infection and aggravation of further complications of diabetes mellitus in affected patients (West, 2000; Esper *et al.*, 2009 and Kochar and Umathe 2009). The question of the current study was whether supplementation of diabetic patient with adequate antioxidants is able to prevent diabetic complications?. Although it may not be possible to completely reverse diabetic complications with antioxidants once they have been established, the trial to minimize these complications or to slow down its progression by preventative antioxidant therapy prior to the development of tissue damage is a point of interest that require further investigations. Therefore, the current study aimed to evaluate the ability of bee pollen, a known antioxidant-rich food supplement, to minimize or prevent the immune insufficiency complication associated with diabetes mellitus.

In the current study, type 2 diabetic (T2D) rat model was induced using STZ. Animal models of T2D mellitus provide the opportunity to investigate the pathophysiology as well as to evaluate the potential strategies for treatment and prevention of the disease and related complications (Kahn *et al.*, 1999). Induction of diabetes with STZ is associated with a characteristic loss of body weight, which is due to increased muscle wasting and loss of tissue proteins (Swanston *et al.*, 1990). In the current study, significant reduction in body weight gain of all diabetic groups were recorded as compared to non diabetic control group (G I). However, significant improvement in the body (BWG%) were recorded in diabetic rat groups fed of BP-supplemented diets (G III, G IV) as compared to that of diabetic rat group fed on non BP-supplemented diet (G II). Regarding other food efficiency parameters, current work revealed significant reduction in the food intake (FI) and food efficiency ratio (FER) in diabetic rat group fed on non BP-supplemented diet (G II) as compared to non diabetic control group (G I). On the other hand, non-significant changes of these parameters were recorded in diabetic rat groups fed of BP-supplemented diets (G III, G IV) as compared to G I. However, significant improvement of FI and FER were noticed in G III and G IV as compared to G II (Table 2). This could be attributed to the effect of high nutritive value of BP that contains complex chemical composition (carbohydrate, protein, fat, amino acids, vitaments and minerals), which make it an excellent nutritional source (Mouroa and Alencer 2009). This was confirmed by the food analysis findings of the local organic BP used in the current study which revealed that BP is a very carbohydrates and proteins-rich food (63.3%, and 18.5%, respectively) with a total energy of 367.7 Kcal/100g weight (Table 1).

Glucose level was significantly higher in all induced diabetic groups as compared to non diabetic control group. Insignificant improvement of the glucose level was observed in diabetic rat groups fed of BP-supplemented diets. These findings however, could be explained by fact that STZ-induced T2D is linked to impaired insulin sensitivity (insulin resistance) coupled with a failure of pancreatic beta cells to compensate by adequate insulin secretion. When hyperglycemia occurred, the insulin secretion function presented only light impairment and relatively insulin deficiency (Fujimoto, 2000 and Saini *et al.*, 1996). The high carbohydrate and fat diet, represented by the currently used BP supplementation in diabetic rat groups (63.3% and 4.5%, respectively) would aggravate hyperglycemia due to substance competition among fatty acids and glucose (Zhang *et al.*, 2003). The nature of insulin resistance, as it was the case in the current T2D model, characterized by reduced capability of glucose utilization, which is only stimulated by insulin. Hence no food supplement alone could improve hyperglycemia.

Increased oxidative stress and depleted antioxidant defenses are well established as occurring in diabetes. (West, 2000; Evans *et al.*, 2002 and Scott and King, 2004). The current results were in agreement with previous observations as significant reduction in oxidative stress markers (SOD, GSH-Px and catalase) were recorded in diabetic group (G II) as compared to non diabetic control group (G I) as shown in table 4. GSH-Px, an enzyme whose main biological role is to protect the organism from oxidative damage by free radicals, is key indicator of oxidative stress (Johnson *et al.*, 2000). Optimum level of glutathione is required in body which in turn potentiates GSH-Px activity to stay healthy. GSH-Px activity represents the initial protective response required for adjusting the hydrogen peroxide (H_2O_2) concentration under normal physiological conditions as well as after oxidative insult (Lee *et al.*, 2008). Superoxide dismutases (SOD) are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen. In addition, catalase is a ubiquitous enzyme that destroys the reactive oxygen species (H_2O_2) formed during oxidative stress (Cederberg *et al.*, 2000). During hyperglycemia, the induced reduction in the $NADPH/NADP^+$ ratio, resulting from inhibition of G_6PD or stimulation of NADPH oxidase, can increase oxidative stress by two mechanisms, first, by decreasing the regeneration of the important cellular antioxidant, reduced glutathione (GSH) from oxidized glutathione (GSSG), and second, by decreasing availability of NADPH, thereby decreasing activity of catalase (Kashiwagi *et al.*, 1996 and King and Loeken, 2004). Notably, supplementation with bee pollen, a known antioxidant-rich food supplement, of diabetic rats significantly minimized the oxidative stress and improved the antioxidant parameters in supplemented diabetic rats as compared to non-supplemented diabetic ones.

Immune insufficiency, manifested by significant reduction of CIC, phagocytic activity of neutrophils and IFN-gamma as well as hyperimmunoglobulinemia of IgG and IgM, were evident in the current study among diabetic rats as compared to non diabetic control ones. Intensive generation of ROS by neutrophils and other white blood cells is reported to induce excessive lipid peroxidation which leads to quantitative and qualitative changes of lipid bilayer of immune cells plasmatic membranes particularly B-cells (Krapfenbaner *et al.*, 1998 and Wen *et al.*, 2002). Moreover, ROS start the process of free radical oxidation of functional active proteins, which is one of the reasons of inactivation of enzymes and

immunoglobulins (Yavari and Azizova, 2012). This results in disturbance of the normal function of immune system and immune insufficiency which facilitate the generalization of inflammatory processes, development of complications, decline or lack of any clinical effect of basic therapy and increase of lethality of diabetes mellitus (Wen *et al.*, 2002 and Shanmigam *et al.*, 2003). Moreover, It has been reported that duration of diabetes mellitus and diabetic complications are characterized by derangement of cellular differentiation of T-lymphocytes and depression of their proliferative activity, hyperimmunoglobulins of all classes and presence of circulating immune complexes (CIC) in blood stream (Akinlade *et al.*, 2004 and Niccoloff *et al.*, 2004). One of the parameters representing immune status and autoimmune process is the level of CIC in blood. It is believed that CIC play a large role in pathogenesis of diabetic late complications. Circulation of CIC in body for long time, even at insignificantly increased level, can cause their deposition in tissues, high platelet aggregation and adhesions, which results in microcirculatory injury, vessel congestion, tissue damage and necrosis (Yavari and Azizova, 2012).

In humoral immunity, B-cells are responsible for protecting against foreign bodies or infections. The function of the B-cells is mediated by immunoglobulins, proteins that have an antibody function, of which Immunoglobulin G (IgG) is the most abundant and widely distributed. Immunoglobulins act as recognizing blocks or structures that identify a foreign body or infection and initiate an immune response (Young *et al.*, 2008). Any alteration to its structure can hinder the function of immunoglobulins and affect its contribution in the humoral immunity. The hyperimmunoglobulin class G and M as recorded in the current study among diabetic rat group fed on non BP-supplemented diet as compared to non diabetic ones could be explained by the fact that short-term hyperglycemia depresses immunity through nonenzymatic glycosylation of circulating immunoglobulin (Black *et al.*, 1981). Glycosylation of immunoglobulins decreases their ability to fix complement and increase their half life with the subsequent increase of their concentration in the blood (Austin *et al.*, 1987).

Interferon gamma, which is a well-described macrophage activating factor, is generally considered a pro-inflammatory production of reactive oxygen intermediates and reactive nitrogen intermediates and believed to be involved in the pathogenesis of diabetes (Flesch *et al.*, 1994 and Ohmori and Hamilton 1994). The currently recorded significant increase of IFN-gamma in diabetic group as compared to non diabetic group is in agreement with previous reports that have shown an increase in the activities of IFN-γ in the insulitis process (Cardozo *et al.*, 2003 and Foulis *et al.*, 2005).

Interestingly, the results of the current study revealed significant improvement of all studied immune parameters among diabetic rat groups fed of BP-supplemented diets as compared to diabetic rat group fed on non BP-supplemented diet. The improvements of the selected immune parameters were significantly higher in 2% BP-supplemented diabetic rats as compared to the 1% BP-supplemented diabetic ones. In conclusion, DM is a metabolic disorder associated with increased oxidative stress and depleted antioxidant defenses. The currently recorded association of the increased oxidative stress manifested by depletion of antioxidant parameters and the produced immune insufficiency in diabetic rats support the theory that DM-associated oxidative stress is responsible for quantitative and qualitative changes of immune cells with subsequent immune insufficiency. Moreover, this theory was even confirmed by the obvious improvement of immune status parameters after supplementation of diabetic rats with a known antioxidant-rich food supplement as BP.

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تأثير حبوب لقاح النحل كمضاد للأكسدة على الحالة المناعية للفئران المصابة بداء السكري

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تهدف هذه الدراسة الي تقييم قدرة حبوب لقاح النحل (BP)، والذي يعرف كمكمل غذائي غني بمضادات الأكسدة، وذلك لمنع أو تقليل مضاعفات نقص المناعة لدي الفئران المصابة بداء السكري. تم استخدام ٣٢ من ذكور الجرذان البيضاء قسمت إلى ٤ مجموعات (كل مجموعه تتكون من ٨ فئران). مثلث المجموعة الاولي (الضابطة السالبة) ٨ فئران وتم تغذيتها علي الوجبة الأساسية لمدة ٦٠ يوما متتالية، أما المجموعات الاخرى استخدمت لعمل نموذج لداء السكري بالفئران بعد حقنها بمادة استربتزوتوسين بنسبة ٦٥ ملجم/كم من وزن الجسم. استخدمت احدى هذه المجموعات الثلاث (المجموعة الثانية) كمجموعة ضابطة موجبة حيث تغذت علي الوجبة الأساسية فقط لنفس الفترة الزمنية. اما المجموعة الثالثة والرابعة تغذت علي وجبة اساسية مضاف لها حبوب لقاح النحل بتركيز ١٪ و ٢٪ على التوالي لنفس الفترة. أظهرت النتائج التي تم الحصول عليها في نهاية التجربة ان هناك زيادات معنوية ($P\leq0.001$) في مستويات الجلوكوز في المجموعات الثانية والثالثة والرابعة بالمقارنة بالمجموعة الاولي (الضابطة السالبة). من ناحية اخري لم تسجل النتائج تغييرات معنوية في مستويات الجلوكوز بالنسبة للمجموعة الثالثة والرابعة مقارنة بالمجموعة الثانية (الضابطة الموجبة). كما لوحظ انخفاض معنوي ($P\leq0.01$) في عوامل مضادات الاكسدة (SOD, CAT and GSH-PX) في المجموعة الثانية بالمقارنة بالمجموعة الاولي، بينما سجلت النتائج زيادة في مستويات مضادات الأكسدة بدرجة معنوية للمجموعة الثالثة ($P\leq0.01$) والرابعة ($P\leq0.001$) بالمقارنة بالمجموعة الثانية. فيما يتعلق بالحالة المناعية، وجد انخفاضا معنويا ($P\leq0.01$) في مستويات CIC و نشاط الخلايا البلعمية ($P\leq 0.01$) و IFN-gamma ($P\leq0.001$) في المجموعة الثانية بالمقارنة بالمجموعة الاولي. كذلك وجد ارتفاع معنوي في مستويات (IgM) و (IgG) ($P\leq0.001$) ($P\leq0.01$) علي التوالي في المجموعة الثانية بالمقارنة بالمجموعة الاولي. من ناحية اخري لوحظ تحسن معنوي في مستوي CIC ($P\leq0.05$ and $P\leq0.01$) و نشاط الخلايا البلعمية ($P\leq0.05$ and $P\leq0.01$) و IgM ($P\leq0.05$ and $P\leq0.01$) و IgG ($P\leq0.05$ and $P\leq0.01$) وتحسن معنوي في مستوي IFN-gamma ($P\leq0.01$ and $P\leq0.001$) في المجموعة الثالثة والرابعة بالمقارنة بالمجموعة الثانية على التوالي. وفي الختام، انتهت الدراسة الى وجود ارتباط واضح بين مستوى مضادات الأكسدة لدي الفئران المصابة بداء السكري وقصور الحالة المناعية لهذي الفئران. وقد تأكد ذلك بتسجيل تحسن واضح في الحالة المناعية للفئران المصابة باستخدام حبوب لقاح النحل كمكمل غذائي غني بالمضادات للأكسدة.

الكلمات المفتاحية: *داء السكري - حبوب لقاح النحل - مضادات الاكسدة - القصور المناعي*