Antioxidants and antidiabetic effects of fortified cake with zucchini (*Cucurbita pepo* L.) flowers on alloxan-induced diabetic rats

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Abstract: Zucchini (*Cucurbita pepo* L.) flowers currently discarded as waste, although it is a rich source of bioactive and nutraceutical compounds. This study was assessed to determine therapeutic effects of treating with zucchini flowers fortified cake at two levels (10% and 20%) on blood glucose, brain glucose, lipid profile, lipid peroxidation and the antioxidant defense system of brain of alloxan-induced diabetic rats. Thirty five male albino rats weighting 200 ±5 g were used and divided into 5 groups, each of 7 rats for 30 days. The first group fed on basal diet, served as a normal control group. Twenty eight rats were injected by alloxan with a single intravenously (40 mg/kg b.w) to induce diabetes and randomly classified to four groups, diabetic (untreated) , the other three groups treated with cake with 100% wheat flour and fortified cake with 10% and 20% zucchini flowers powder . Results revealed that both two levels 10% and 20% of zucchini flowers fortified cake were found to normalize many parameters which were shifted to pathological values as a consequence of the alloxan-induced diabetes: serum glucose in blood and brain were decreased. As well as, there was a significant increase in HDL-C and a significant decrease in total cholesterol, TG, LDL-C and VLDL-C. In addition, Acetylcholinesterase **(**AChE), catalase (CAT) and glutathione (GSH) activities, which were lowered in brain of diabetic animals, were restored by both treatments (10% and 20%), and consequently, level of lipids peroxidation (LPO) was reduced in brain of treated groups, as compared to diabetic (untreated) animals. In this study, the high levels of blood and brain glucose and oxidative damage associated with diabetes were ameliorated with treatment with zucchini flower fortified cake. The protective effect of zucchini flowers are mainly attributed to antioxidant properties and the presence of bioactive and nutraceutical compounds.

Keywords: Zucchini flower, alloxan; antioxidant activities, lipid peroxidation, diabetes, brain

Introduction:

Zucchini (Cucurbita pepo L) flowers, which are consumed widely in many countries and play an important role in the diet of different people. Zucchini flowers are known to be a great source of phytochemicals, essential amino acids, folic acid, vitamins B1 and B2 and minerals **(Talavera 1999; Sotelo *et al.* 2007; Mlcek and Rop 2011; and Fedchenkova *et al.*, 2015),** as well as antioxidant compounds such as carotenoids, polyphenols and ascorbic acid **(Urrutia-Hernández 2011; and Aquino-Bolanos *et al.*, 2013),** and are often prescribed for those who are anemic, lethargic and pregnant. The bright yellow color, soft texture and delicate, slightly sweet flavor of zucchini flowers have made them a favored constituent in different areas (**Tarhan *et al.*,. 2007).** Nowadays the plant cultivated mainly for the flowers that are used in prepared different forms such as main dishes, salads, soups, dressings, creps, pasta, quesadillas, and also in evolving recipes offered in deluxe restaurants, and consumed without broth elimination, which is logical since they have no antinutritional factors **(Sotelo *et al.* 2007),** because of its high aerobic rate, a zucchini flower leftover fresh for one day at room temperature **(Villalta *et al.* 2004).** Thus to

effectively used available zucchini flower, processing requires are needed with mild approach that enables to use the natural properties of the flower.

For various reasons in recent years, the popularity of medicinal plants in diabetic control has increased. The reason for using these plants as medicine due to containing a wide variety of free radical scavenging molecules, such as phenolic compounds, vitamins, and some other endogenous metabolites that possess antioxidant activities **(Zheng and Wang 2002& Goel, 2013**). In ancient times flowers were fundamentally consumed for their pharmacological characteristics rather than their nutritional value. Nowadays, several studies detected the chemical compositions of edible flowers, showing the presence of important bioactive and nutraceutical compounds including dietary fiber, carotenoids, fatty acids, phenolic acids, flavonoids, isothiocyanates, polyols, sterols, vitamins, essential mineral elements, amino acids, phytoestrogens and prebiotics/probiotic **(Kaisoon *et al.* 2011; Cavaiuolo *et al.* 2013; Lim 2014& Koike *et al.* 2015)**. Edible flowers include tens of inflorescences of different shapes, colors and sizes consumed in various forms around the world to improve the appearance, color and nutritional value and sensory qualities of foods **(Kelley *et al.* 2001; Lim 2014 &Koike *et al.* 2015)**.

Diabetes mellitus the most chronic metabolic disorder of multiple etiologies is characterized by hyperglycemia, glucosuria and negative nitrogen balance and is primarily caused due to absolute deficiency and deprecated production of insulin. It is the most prevalent disease in the world affecting 25% of the population and afflicts 150 million people and is predicted to rise to 300 million by 2025 **(Sharma *et al.* 2014).** Reactive oxygen species (ROS) which incentives to cellular damage by the oxidation ability have been involved in the pathogenesis of diabetes mellitus **(Brownlee 2001, Kowluru *et al.* 2007).** During diabetes, persistent hyperglycemia increases the production of ROS through glucose oxidation, thus leading to the disturbance of cellular functions, oxidative damage to membranes and increased susceptibility to lipid peroxidation **(Hunt *et al.* 1990& Wolff *et al.* 1991).** Plurality obtainable conventional drugs that used for diabetes management are bedeviled by prohibitive costs, need for know-how in prescription and administration and various side effects, which are precursors of diabetes complications **(Njagi *et al.*, 2015)**, thus natural foods and food-derived antioxidants have received growing attention because they are known to function as chemo-preventive agents with a safe solutions better than synthetic drugs against oxidative damages **(Valavanidis, 2004; Carrasco-Pancorbo *et. al.* 2005; and Perez-Bonilla, 2006.).** Therefore, this work was designed to assess the antidiabetic and antioxidant potential effect of zucchini flowers fortified cake by studying its role on lipid peroxidation and enzymic antioxidant in brain of alloxan-induced diabetic rat.

Material and Methods

Materials

Chemicals

Alloxan, Casein, minerals, vitamins, cellulose and DL-methionine were obtained from El- Gomhoria Company for Trading Drugs, Chemicals and Medical Instruments, Cairo, Egypt.

Plant material

Fresh zucchini flowers were collected from the Agricultural Research Center in Gizaa city, Egypt

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Animals

Thirty five adult male albino rats of Sprague Dawley Strain, 8-9 weeks of age (weighing 200 ±5 g) were obtained from Laboratory of Animal Colony, Helwan, Egypt. The animals were housed in acrylic cages and kept under standard environmentally controlled, clean-air room with temperature of 24 ± 5°C, illumination (12 h light/12 h dark cycles), a relative humidity of 60 ± 4%, for 1 week in order to adapt, The animals were fed on standard diet, which formulated according to **NRC (1995)** and water ad libitum.

Methods

Preparation of zucchini flowers powder

The collected zucchini flowers were rinsed in clean water and dried at room temperature for two weeks. The dried flowers sample was ground into powder using a mortar and pestle, according to **Russo, (2001)**.

Preparation of cake

Cake was prepared according to the common method of (**Penfield and Campbell, 1990**). Cake was carried out by using wheat flour (72%), samples replaced separately with 10 and 20% zucchini flowers powder.

Proximate analysis

Control cake (100% wheat flour 72% extraction) and cake fortified with 10% and 20% zucchini flowers powder were analyzed for the moisture, fat, protein, ash, and fiber contents. The carbohydrates as nitrogen free extract (NFE) calculated by difference (100 - (ether extract + protein + ash + fiber) were determined as described in **AOAC, (2000).**

Induction of diabetes

Diabetes was induced in overnight-fasted rats by a single intravenously injection of a freshly prepared aqueous solution of alloxan monohydrate, at the dose rate 40 mg/kg body weight according to **Nayeemunnisa, (2009).** Blood was extracted from the tail vein for glucose analysis after 48 hours of alloxan injection and rats with fasting blood glucose ranging from 210-220 mg/dl, showing clear signs of polyuria, polyphagia and polydipsia, considered diabetic, separated and used for the study. Rats with fasting blood glucose less than 200 mg/dl were rejected.

Experimental design

The rats were divided into five groups, each consisting of 7 animals.

Group 1: Normal control group fed on standard diet only.

Group 2: positive control fed on standard diet only (diabetic).

Group 3: Diabetic + fed on basal diet with cake 100% wheat flour (72%) extraction.

Group 4: Diabetic + fed on basal diet with fortified cake with 10% zucchini flowers powder as treated group.

Group 5: Diabetic + fed on basal diet fortified cake with 20% zucchini flowers powder as treated group.

Measurements of body weight and feed intake

During the experimental period (30 days), the diets consumed and rats were weighed individually at weekly by using a Triple Beam Balance. And the body weights were recorded to calculate weekly body weight gains. Feed intake was recorded daily

Collection of Blood: At the end of the experiment, the rats were fasted overnight, then the rats were anaesthetized and sacrificed, and blood samples were collected from the aorta.

The blood samples were centrifuged and serum was separated and collected in clean bottles and stored at 4˝C until required.

Measurement of Blood Glucose

Blood glucose concentration estimated by colorimetric method of **Burrin and price, (1985)**

Assay of serum Cholesterol and Triglyceride

Total cholesterol and high density lipoprotein cholesterol (HDL-c) and triglycerides were determined by colorimetric methods of **Allian *et al.* (1974)**, **Fnedewaid, (1972) and Fossati and Prencipe, (1982),** respectively .The determination of VLDL (very low density lipoproteins) and LDL (low density lipoproteins) were carried out according to the method of **Fnedewaid, (1972)** by calculation as follows: VLDL (mg/dl) = Triglycerides /5
LDL (mg/dl) = Total cholesterol –HDL-VLDL.

Assay of antioxidant parameters in brain

The brains were quickly removed and washed in ice-cold saline. Cerebral hemispheres were dissected out and carefully separated at 0ºC with bent forceps and scalpel, weighing with an electric balance in mammalian Ringer solution and immediately used for further assay procedures.

Lipid peroxidation was determined by measuring malondialdehyde (MDA) according to **Ohkawa *et al.* (1979).**

Acetylcholinesterase **(**AChE) activity was determined colorimetrically according to **Hestrin, (1949).** Isoenzymatic specturm of AChE was resolved by polyacrylamide gel electrophoresis, as develop by **Davis, (1964), and Ornstein, (1964).** One hundred percent (w/v) homogenates of brain tissue were prepared in deionized distilled water and centrifuged at 7000 rpm for 1 h and the supernatants were used for electrophoresis. Gels were prepared by polymerization of acrylamide monomers. Raymond's buffer system (pH 8.5) was used **Raymond, (1964).** Ten microlitres of the test samples were spotted on the gel for electrophoresis. The tubes were run at 6 million amps per tube for 2 h using Raymond's buffer system. After the electrophoretic run the gels were incubated for AChE activity and stained.

Catalase (CAT) activity was estimated by measuring the rate of decomposition of H_2O_2. Ten percent (W/V) tissue homogenate was prepared, centrifuged for 90 min and the resulting supernatant was used for determining CAT activity **Aebi, (1983).** Agar gel electrophoresis was employed to study isoforms of CAT and the relative activity measured. Ten percent (W/V) brain tissue homogenate in 0.1M phosphate buffer pH 7.0 was prepared and centrifuged at 2000 rpm for 30 min. 20 µl of the clear supernatant was spotted on the filter paper strip embedded in centrally made slots of the 2.5% solidified agar gel spread over 245x70mm glass plate with 3 mm height frame. Constant current of 10 mA (Electroselenium Ltd, Essex, England) was employed**.** Electrophoresis was carried out at 7 ºC for 16 hours. After the run, the gels were removed washed in phosphate buffer and soaked in 0.6 M H_2O_2 in 0.2 M phosphate buffer (pH 7). In few minutes at the region where CAT has migrated, O_2 bubbles were liberated due to enzymatic hydrolysis of H_2O_2. As the bubbles accumulated a pearly granular region became visible on the gel representing activity bands.

Glutathione (GSH) level was determined according to reported method of **Tietze, (1969).**

Statistical analysis: Data was subjected to analysis of variance using the statistical package for social sciences SPSS (Statistic Program Sigmastat, Statistical Soft-Ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and $p<0.05$ was used to indicate significance between different groups **(Snedecor and Cochran, 1967).**

Results

Proximate composition of control and zucchini flowers powder fortified cake (dry basis %)

Table (1) depicts proximate composition of control and fortified cake with two levels of zucchini flower powder (10% and 20%). The control cake (100% wheat flour) contains (15.80, 11.86, 10.76, 1.55, 0.59 and 59.44) of moisture, ether extract, protein, ash, crude fiber and nitrogen free extract, respectively. While 10% zucchini flower fortified cake contains (17.77, 12.62, 12.45, 1.65, 0.89 and 54.62) of moisture, ether extract, protein, ash, crude fiber and nitrogen free extract, respectively, and the level of 20% zucchini flower fortified cake contains (20.98, 13.59, 12.66, 1.69, 0.89, 50.19) of moisture, ether extract, protein, ash, crude fiber and nitrogen free extract, respectively. As determined in this study, indicated ether extract, protein, ash, crude fiber tendency to increase according to the increments of zucchini flower powder in cake samples due to the higher content of zucchini flowers in protein and ash which reflects to essentials amino acids and different minerals compared to control cake (100% wheat flour).

Parameters %	Control cake	Fortified cake with zucchini flowers powder (ZFP)	
		10%	20%
Moisture	15.80	17.77	20.98
Ether extract	11.86	12.62	13.59
Protein	10.76	12.45	12.66
Ash	1.55	1.65	1.69
Crude Fiber	0.59	0.89	0.89
Nitrogen free extract	59.44	54.62	50.19

Table (1): Proximate composition of zucchini flowers powder (ZFP) fortified cake (dry basis %).

Values are expressed as mean ± SD, n = 3.

Effect of treatment with zucchini flowers powder (ZFP) fortified cake on the Feed intake and body weight gain of alloxan-induced diabetic rats

The results of feed intake and body weight gain of alloxan-induced diabetic rats treated with cake fortified with 10% and 20% zucchini flower powder are shown in Table (2) revealed that diabetic (untreated) group showed significant decrease in feed intake and body weight gain % compared to normal control group. The treated groups with 10% and 20% zucchini flowers fortified cake reversed the effect of alloxan monohydrate as it showed significant increase in feed intake and body weight gain compared to diabetic (untreated) group.

Table (2): Feed intake and body weight gain (%) of alloxan-induced diabetic rats treated with zucchini flowers powder (ZFP) fortified cake

Groups	Parameters	
	Feed intake (g/ day)	Body weight gain %
Normal control	17.34± 0.83 [a]	20.70± 0.89 [a]
Diabetic (untreated)	11.70± 1.09 [d]	16.30± 1.44 [c]
Diabetic + treated (control cake)	13.70± 1.77 [c]	17.00± 1.81 [b]
Diabetic + treated (10% ZFP cake)	16.50± 1.77 [b]	17.40± 1.77 [b]
Diabetic + treated (20% ZFP cake)	16.82± 1.77 [b]	17.58± 1.77 [b]

Values are expressed as mean ± SD; n = 7, Values in the same column having different superscripts letters are significantly ($p<0.05$) different and vice versa. ZFP: Zucchini flowers powder

Effect of treatment of zucchini flowers powder (ZFP) fortified cake on glucose levels of serum and brain of alloxan-induced diabetic rats

Induction of diabetes led to increase in the blood glucose level as shown in Table (3). The blood glucose levels of diabetic (untreated) group were significantly higher (198.60 mg/dl) after diabetes induction ($p<0.05$) as compared to control and treated groups with zucchini flowers powder. Feeding on the fortified cake was observed to reduce the level as the experiment progressed. At the end of the experiment, feeding of the cake with 20% zucchini flowers powder led to 50.25 % reduction in the blood glucose level. So no significant differences were observed between control and treated groups with 10% and 20% zucchini flower cake. Thus, ZFP treatment resulted in a significant enhancement of blood glucose levels in diabetes rats similarly as normal group. As well as brain glucose was enhanced during diabetes and in zucchini flowers cake-fed rats, a decline in glucose content was observed (Table 3). Feeding with cake that fortified with zucchini flowers powder at both levels (10% and 20%) reversed brain glucose to near of normal control group.

Effect of treatment with different levels of zucchini flowers powder (ZFP) fortified cake on lipid profile of alloxan-induced diabetic rats

The results on the lipid profile of alloxan-induced diabetic rats are shown in Table 4. A highly significant ($p <0.05$) increase in total cholesterol level was recorded in diabetic (untreated) rats group, when comparing with normal control group. Conversely, a high significant decrease in total cholesterol level was observed in treated groups feeding with zucchini flowers powder cake, when compared with diabetic (untreated) group. Serum triglycerides levels were also elevated in the diabetic rats (untreated) group when compared to normal control rats group (168.26 vs 96.73 mg/dl).There was a significant decrease on TC, TG, LDL-c, Total lipids and VLDL-c levels of treated groups that feeding on zucchini flowers cakes (10% and 20%) when compared with diabetic rats (untreated) ($p<0.05$). However there is no significant difference between normal control group and treated group that fed on 20% zucchini flowers. But there is marked increase in level of HDL-c of both treated groups of zucchini flowers cake when compared to diabetic (untreated) group significance ($p<0.05$).

Table 3. Glucose levels of serum and brain of alloxan-induced diabetic rats treated with zucchini flowers powder (ZFP) fortified cake

Groups	Parameters	
	Brain glucose µM /g	Serum glucose mg/dl
Normal control	1.09 ± 0.03 [d]	98.00± 4.68 [c]
Diabetic (untreated)	2.60 ± 0.05 [a]	198.60± 4.93 [a]
Diabetic + treated (control cake)	2.02 ± 0.02 [b]	117.00± 3.16 [b]
Diabetic + treated (10% ZFP cake)	1.49 ± 0.04 [c]	99.80± 0.83 [c]
Diabetic + treated (20% ZFP cake)	1.19± 0.02 [c]	98.80± 0.61 [c]

Values are expressed as mean ± SD; n = 7, Values in the same column having different superscripts letters are significantly ($p<0.05$) different and vice versa, ZFP: Zucchini flowers powder

Table 4. Lipid profile of alloxan-induced diabetic rats treated with zucchini flowers powder (ZFP) fortified cake

Groups	Parameters					
	Total lipids g/l	TC mg/dl	TG mg/dl	LDL-C mg/dl	HDL-C mg/dl	VLDL-C mg/dl
Normal control	2.67 ±0.33 [c]	96.73 ±0.83 [c]	147.18 ±1.58 [d]	20.19 ±0.46 [d]	47.90 ± 1.14 [a]	29.43 ± 0.31 [d]
Diabetic (untreated)	4.25 ±0.52 [a]	168.26 ±10.96 [a]	216.00 ±11.40 [a]	87.63 ±12.26 [a]	34.43 ± 3.53 [c]	40.20 ±2.28 [a]
Diabetic + treated (control cake)	2.95 ±0.19 [b]	108.12 ±7.54 [b]	173.95 ±3.55 [b]	25.51 ±8.69 [c]	42.85 ± 1.95 [b]	34.76 ±0.69 [b]
Diabetic + treated (10% ZFP cake)	2.78 ±0.18 [b]	104.13 ±5.36 [b]	172.20 ±4.38 [b]	28.89 ±6.78 [b]	44.80 ± 2.49 [a]	34.44 ±0.87 [b]
Diabetic + treated (20% ZFP cake)	2.68 ±0.12 [c]	100.13 ±5.31 [c]	159.44 ±4.35 [c]	21.45 ±6.76 [c]	46.80 ± 2.38 [a]	31.88 ±0.87[c]

Values are expressed as mean ± SD; n = 7, Values in the same column having different superscripts letters are significantly ($p<0.05$) different and vice versa. TC: Total Cholesterol, TG: Triglycerides, LDL-C: Low density lipoprotein cholestrol, HDL-c: High density lipoprotein cholesterol, V LDL-c: Very low density lipoprotein chohestrol. ZFP: Zucchini flowers powder

Effect of treatment with zucchini flowers powder (ZFP) fortified cake on the activities of brain antioxidant enzymes of alloxan-induced diabetic rats.

Table (5) shows the alterations occurring in LPO, CAT, AChE and GSH levels in different groups of rats. The brain lipid peroxidation is significantly higher in diabetic (untreated) rats compared to normal control and treated with different levels of zucchini flower powder fortified cake. Treatment of diabetic rats with zucchini flowers powder fortified cake had a very high significant influence on AChE, CAT and GHS activities ($p<0.05$) comparing with untreated diabetic rats. CAT activity was decreased in the diabetic (untreated) group compared to that in both of control group and treated with

zucchini flowers cake groups (P < 0.05). Furthermore, zucchini flowers cake treatment significantly increased CAT level compared to the untreated diabetic group (Table 5). Induction of diabetes led to a reduction of GSH level in the brain tissues of the diabetic rats (untreated). Glutathione (GHS) level was significantly depleted in diabetic rat group (untreated). However treatment with zucchini flowers powder with 10% and 20% cake increased the GSH levels by 30.15% and 35.07% which were statically significant (P<0.05).

Table 5. Lipid peroxidation, acetylcholinesterase, catalase activity and glutathione activity in the brain of alloxan-induced diabetic rats treated with zucchini flowers powder (ZFP) fortified cake

Groups	Parameters			
	LPO nmol	AChE nmol	CAT nmol	GSH mg/g
Normal control	190.4 ± 8.24 ^{e}	5.89 ± 0.4 ^{a}	72.13± 5.22 a	16.4 ± 1.4 ^{a}
Diabetic (untreated)	321.5 ± 11.72 ^{a}	3.94 ±0.4^{c}	37.25± 3.47 ^{c}	9.22 ± 0.6 ^{d}
Diabetic + treated (control cake)	288.67 ± 9.34 ^{b}	4.46 ± 0.7 ^{b}	64.33± 6.35 ^{b}	11.2 ± 0.2 ^{c}
Diabetic + treated (10% ZFP cake)	266.35 ± 8.12 ^{c}	4.69 ± 0.6 ^{b}	65.14± 7.16 ^{b}	13.2 ± 0.2 ^{b}
Diabetic + treated (20% ZFP cake)	253.35 ± 7.12 ^{d}	5.05 ± 0.5 ^{b}	68.62± 7.16 ^{b}	14.2 ± 0.2 ^{b}

Values are expressed as mean ± SD; n = 7, Values in the same column having different superscripts letters are significantly (p<0.05) different and vice versa, LPO: Lipid peroxidation, AChE: Acetylcholine esterase, CAT: Catalase activity, GSH: Glutathione activity, ZFP: Zucchini flowers powder

Discussion

The results of the current study showed that alloxan at a dose of 40 mg/kg body weight, apparently, caused considerable damage to pancreatic ß-cells that function in the regulation of insulin secretion and thus resulting in a significant (p≤0.05) increase in blood glucose levels **(Verma *et al.*, 2010).** Oxidative stress plays a role in the development of diabetic complications (**Brownlee 2001 and Kowluru *et al.*, 2007).** It has been reported that the toxic effect of alloxan in the pancreas is followed by its rapid uptake by the ß-cells and ROS generation **(Munday, 1988; and Das *et al.*, 2012).** One of the most main sources of oxygen free radicals in diabetes is glucose oxidation, first action is oxidize to reactive ketoaldehydes and superoxide anion radicals, then the superoxide anion undergoes dismutation to hydrogen peroxide and, if not destroyed by antioxidant defense systems, it could generate extremely reactive hydroxyl radicals **(Jiang *et al.* 1990).**

The observed weight loss could be partially related with the decrease in the feed intake. Weight loss has been correlated to several health benefits in the diabetic patients **(Erukainure *et al.* 2012)**. Interestingly, it has been shown that blood glucose and brain

glucose levels are decreased by feeding with the zucchini flower fortified cake at levels of 10% and 20% compared to untreated diabetic animals. Lower blood glucose levels may be due to a regenerative effect that certain compounds from the zucchini flower could have acted on pancreatic β-cells. The therapeutic efficiency of zucchini flower is attributed to the various phytochemicals with antioxidant activities (**Mlcek and Rop 2011; UrrutiaHernández 2011; Aquino-Bolanos *et al.*, 2013; and Fedchenkova *et a.*, 2015).**

Diabetes has been reported to be associated with dyslipidemia as evidenced by high total cholesterol, particularly a high low density lipoprotein and a decrease high density lipoprotein , and high triglycerides **(Elleuch *et al.*, 2010),** hyperglycemia increase the glycation and the atherogenicity of LDL-C (**Ochuko *et al.*, 2013).** The observed hypolipidemic activity as indicated by reduced total cholesterol, low density lipoprotein cholesterol (LDL-C), triglyceride (TG), and increased HDL-C levels, portrays a protective effect of the zucchini flowers fortified cake against diabetes.

Maintenance of the antioxidant status has a major role in pancreatic β-cells survival and in preservation of islet secretory capacity. Antioxidant enzymes are the main scavengers of free radicals and under oxidative stress they may act as compensatory mechanisms by increasing their activity in various tissues. Antioxidants play an important role in scavenging the free radicals and protect the human body from oxidative stress **(Baynes and Thorpe, 1994).** Recently, several reports have accumulated to indicate that diabetes has detrimental effects on brain function. The MDA concentrations were determined in order to evaluate oxidant damage to lipids in all the groups, the increase of lipid peroxidation revealed by higher levels of MDA after alloxan-induced diabetes sustain either an intensive production of ROS and a decrease in the activity of antioxidant defense systems. The significant increased level of MDA in the brain tissues of the diabetic (untreated) rats group reflects an increase in lipid peroxidation. Thus, indicating a reduction in enzymatic antioxidant defense systems in diabetic (untreated) rats. These results agree with the earlier reports of **Cui *et al.*, (2008),** and **Arnal *et al.*, (2010)**, they reported an increased MDA level in brain tissues of diabetic rats. GSH activity is also a major endogenous antioxidant, which counteracts free-radical mediated damage and an indicator of oxidative stress **(Erukainure *et al.*, 2011).** It forms an important substrate for other enzymes which is involved in the free-radical scavenging. Its observed reduction in brain tissues of diabetic (untreated) rats further reflects oxidative stress. Its increased level in the treated group suggested the antioxidant potentials of cakes fortified with different zucchini flowers powder.

Abnormalities affecting the level of AChE have been reported in various neurological diseases including diabetes. Various studies have reported alleviation in activities of cholinesterases in brain and erythrocyte membrane during diabetes **(Ragoobirsingh *et al.*, 1992; Chavez and Salceda 2001& Rizvi and Zaid 2001).** From the values given in Table 5, it is clear that the activity level of AChE was decreased in the cerebrum on diabetic rats. As the rats experimented after 48 hours of alloxan administration, the observed decline in enzyme activity is related to early stage of diabetes induced by alloxan. These results are in parallel with that obtained by (**Nayeemunnisa and Tarannum, 2009).**

The reduced CAT and GSH levels in the brain tissues of the treated diabetic groups could also be protective potentials of the zucchini flowers fortified cake that counteract the oxidative stress in brain tissue. Their increased synthesis as observed in the diabetic (untreated) group corresponds to previous studies by **Onyema *et al.*(2005)** and **Erukainure *et al.*, (2011)** which indicated that these enzymes are synthesized in response to oxidative

stress. These observed changes showed that the fortified cake with zucchini flowers powder at level of 10% and 20% acted as an effective antioxidant, thus safeguarding the brain tissues against diabetes-induced oxidative damage. This could be attributed to the phytochemicals and antioxidants compounds of zucchini flowers (**Mlcek and Rop 2011; Aquino-Bolanos *et al.*, 2013 and Fedchenkova *et al.*, 2015).** The antioxidant properties of zucchini flowers have been reported and may be exploited as potential novel antioxidants (**Goel, 2013).**

It was elucidated that diabetes mellitus caused oxidative damage and lipid peroxidation in the brain tissue and increase the plasma glucose levels, and these effects were significantly ameliorated by feeding on zucchini flower powder fortified cake. The results suggested that zucchini flower has a significant role in silencing diabetic disorders by its antioxidative effects, that reversed hyperglycemia.

The research revealed that feeding on zucchini flower cake to diabetic rats increased AChE, CAT and GSH activities and alleviated lipid peroxidation as confirmed by diminished MDA levels. These findings suggest the protective role zucchini flower which may be due to the antioxidant action of polyphenols, carotenoids and vitamin C from the flower that act by neutralizing ROS (**UrrutiaHernández, 2011 and Aquino-Bolanos *et al.*, 2013).**

Conclusions

From the current work, it can be concluded that feeding on zucchini flower powder fortified cake at level of 10% and 20% produces significant antidiabetic effect by controlling the blood glucose and brain glucose levels. In addition it possesses potent antihyperlipidemic effect, lowers both total cholesterol, triglycerides and low density lipoprotein cholesterol and at the same time increases HDL-cholesterol in alloxan-induced diabetic rats. Furthermore, it revealed a significant antioxidant effect by increasing AChE, ACT and GHS activities and alleviating the lipid peroxides level**.** Thus, treatment with zucchini flower powder should be considered in the treatment of diabetic complications. Moreover zucchini flower supplementation can be beneficial for humans in order to reduce the harmful effects of diabetes, such as oxidative damage and decreased blood glucose levels.

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التأثيرات المضادة للأكسدة ولمرض السكري للكيك المدعم بزهور نبات الكوسة على الفئران المصابة بالسكر

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الملخص

زهور الكوسة تعتبر من مخلفات الإنتاج الغير مستغله بالرغم من غناها بالمركبات التغذوية الفاعلة. أجريت هذه الدراسة لتقييم التأثيرات العلاجية للمسحوق المجفف لزهور الكوسة المدعم للكيك بنسبتي 10% و20% على نسبة جلوكوز المخ والسكر بالدم ومشتقات الدهون والإنزيمات المضادة للأكسدة في أنسجة المخ للفئران المصابة بالسكري. استخدمت الدراسة 35 من ذكور فئران الألبينو وزن 200جم تقريبا لمدة 30 يوم قسمت إلى 5 مجموعات/ 7فئران لكل مجموعة. المجموعة الضابطة القياسية السليمة و4 مجموعات مصابة بالسكري (حقنت بالألوكسان 40 ملليجرام/كجم وزن) تم تقسيمها إلى مجموعة السكري الغير معالجة القياسية، ومجموعة تغذت على كيك 100% دقيق قمح، مجموعتين تغذت على كيك مدعم 10% و20% مسحوق زهور الكوسة مستبدل من دقيق القمح على الترتيب. أظهرت نتائج الفئران التي تغذت على غذاء مدعم بزهور الكوسة المجفف بنسبتي 10% و 20% تحسن طبيعي معنوي في العديد من القياسات الحيوية الكيميائية امتدت إلى القياسات النسيجية للمخ للفئران المصابة. كما أوضحت النتائج انخفاض مستوى الجلوكوز بالدم وفي أنسجة المخ مع زيادة معنوية في مستوى الليبوبروتين مرتفع الكثافة وانخفاض الكولسترول ومشتقاته مع خفض نسب الإنزيمات المضادة للأكسدة في أنسجة المخ (GSH ،CAT، AChE) مستوى الدهون المتأكسدة (LPO) انخفض معنويا في المجموعات المعالجة مقارنة بمجموعة السكري الضابطة. توصي الدراسة بضرورة استخدام مسحوق زهور الكوسة بتدعيم منتجات المخبوزات والمنتجات الغذائية المناسبة ضمن النظام الغذائي لمرضى السكري لما له من فعالية في وقاية أنسجة المخ من الضرر التأكسدى الناجم عن مرض السكر، وزيادة مضادات الأكسدة الطبيعية بالجسم مع تحسين التأثيرات الحيوية الغذائية بفعالية.

الكلمات المفتاحية: زهور الكوسة، ألوكسان، مضادات الأكسدة، الدهون المتأكسدة، السكري، المخ.