Anti-Diabetic Activity of Egyptian Celery Apigenin

Amira Ragab El Barky, Amany Abdel hamid Ezz, Karim Samy El-Said, Mohamed EL-Refaay Sadek¹ and Tarek Mostafa Mohamed

ABSTRACT
Diabetes is a metabolic disorder that occurs due to a deficiency in insulin secretion, action, or both. Hyperglycemia, which generated from diabetes, may be harmful to several organs El-Said et al. (2018). Diabetes leads to blindness, kidney disease, nerve weakness, mutilation of fingers or legs and cardiovascular disease Qalawa et al. (2019). Moreover, oxidative stress leads to macro and micro-vascular complications that cause serious toxic effects on various organs Andreassi et al. (2011). Furthermore, free radicals generated as a result of oxidative stress promoted program β-cell death (Rother, 2007). It combines with polyunsaturated fatty acids found in the lipid membrane and leads to lipid peroxidation, which considers a reversal of a lack of antioxidant defenses (Barrera, 2012).

Natural foods supposedly feasible alternatives for the management of diabetes, they can decrease the hazard of diabetes. Apigenin is a non-mutagenic flavone that is a secondary class of flavonoids, which distinguish by a little toxicity Cao et al. (2013). Apigenin has been known by anti-inflammatory, antioxidant and anti-cancer activity Liu et al. (2011). Furthermore, it has anti-oxidative stress and anti-DNA damage (Li et al. 2016). Moreover, it can repress oxidative stress on the melanin apoptosis, which produced by hydrogen peroxide through lowering reactive oxygen species creation Huang et al. (2013). Therefore, the present study was designed to evaluate the potential role of apigenin in managing experimental diabetes in rats.

MATERIALS AND METHODS
Preparation of apigenin from celery
Apigenin has been extracted from Egyptian celery seeds (purchased from a local market, Tanta, Egypt), according to the method described by Javadi et al. (2015), using the soaking method. Celery seeds were soaked for 3 days in 70% ethanol and heated for 60°C, the combined extract has been filtered, the filtrate has been cooled and then, dilute sulfuric acid has been added to the filtrate. The new mixture has been heated for about twenty minutes and then the combined mixture has been filtered to gather yellow precipitate. The precipitate was washed on the filter paper until neutralize and then dried at room temperature (El-Said et al., 2013). The combined extract has been filtered, the filtrate has been cooled, then the combined extract has been filtered, and finally the precipitate was washed on the filter paper until neutralize and then dried at room temperature (El-Said et al., 2013). The precipitate was washed on the filter paper until neutralize and then dried at room temperature (El-Said et al., 2013).

Biochemistry Unit, Chemistry Department, Faculty of Science, Tanta University, Egypt.

Corresponding Author: Amira Ragab El Barky, Biochemistry Unit, Chemistry Department, Faculty of Science, Tanta University, Egypt. Email: amiramaram52@yahoo.com

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Antioxidant activity
The antioxidant activities of apigenin extract have been evaluated by 2, 2-diphenyl-1-picrylhydrazyl method (Wang et al., 2013). The antioxidant activities of apigenin extract have been evaluated by 2, 2-diphenyl-1-picrylhydrazyl method (Wang et al., 2013). The antioxidant activities of apigenin extract have been evaluated by 2, 2-diphenyl-1-picrylhydrazyl method (Wang et al., 2013). The antioxidant activities of apigenin extract have been evaluated by 2, 2-diphenyl-1-picrylhydrazyl method (Wang et al., 2013). The antioxidant activities of apigenin extract have been evaluated by 2, 2-diphenyl-1-picrylhydrazyl method (Wang et al., 2013). The antioxidant activities of apigenin extract have been evaluated by 2, 2-diphenyl-1-picrylhydrazyl method (Wang et al., 2013).
et al. 1998) and phosphomolybdate assay Prieto et al. (1999). Briefly, 100µl of the apigenin extract solution was mixed with 3.0 ml of phosphomolybdenum reagent in glass test tubes, incubation was then performed for about 90 minutes in boiling water bath, the glass test tubes were cooled and the absorbance of the solutions was measured at 695 nm against a blank.

**Experimental**

Eighty white female Wistar albino rats, 3-4 months age and 200-240 g were used in the research. Rats were bought from the Institute of Ophthalmology (Nasser Eye Institute, Egypt). Rats were homed in a special room with a steady temperature and free water. Rats were handled according to the reference of National ethical guidelines for the attendance of laboratory rats as the Animal Ethics Committee (IAEC) of Faculty of Science, Tanta University, Egypt. Diabetes in rats was achieved by injection one dose of citrate buffer streptozotocin intraperitoneal.

**Experimental rats**

Apigenin has been dissolved in water containing 0.1% v/v Dimethyl Sulfoxide (DMSO) for animal treatment according to the earlier report (Ohno et al. 2013). Rats were divided into four equal groups, each of which contained 20 rats as Group (a): Normal healthy rats. Group (b): Normal rats that received 1.5 mg/kg B.wt. of apigenin extract I.P. daily. Group (c): Streptozotocin-induced diabetes in rats, which was injected with a single I.P. dose of STZ, 40 mg kg-1B.wt. and the last group (d): The diabetic group which injected with STZ-induced diabetes and after thirty-five days the rats treated with 1.5 mg/kg B.wt. of apigenin extract I.P. daily. All groups were subdivided into two groups and the experiment lasted six weeks.

**Biochemical parameters**

The glucose level in serum and the activities of α-amylase was determined according to Tietz, (1995) Winn-Deen et al. (1988).

**Determination of Lipid profile**

Both S. Total cholesterol and Triacylglycerol were measured Ellefson and Caraway, 1976; Stein, 1987). Serum VLDL-C values were calculated by the aid of the formula described by Bauer (1982).

**Graphical abstract**

Determination of serum kidney functions test

Both serum Urea and Creatinine concentration as a function of the kidney were determined according to Tietz, (1990) Tietz, (1986).

**Determination of serum L-malondialdehyde concentration**

Serum L-MDA concentration was measured according to Mesbah et al. (2004). In brief, trichloroacetic acid and thiobarbituric acid were added to the serum of experimental rats. The mixture was boiling and then cooled, after that n-butanol was added and centrifuged. The upper clear layer collected and read at 530 nm.

**Tissue samples**

The liver tissues were collected from all experimental rats by the end experimental duration. The liver was homogenized in cold saline. The activity of catalase was determined according to Xu et al. (1997), in brief, 10 µl of the homogenized liver was added to working buffer, mixed very well and the variation in the absorbance after zero and 60 sec. was recorded and measured at 250 nm.

**Determination of liver glycogen content**

One gram of liver tissue was homogenized in strong alkali, the homogenate boiled for about 30 min., ethanol has been added to the solution to precipitate glycogen and absorbance was measured at 492 nm (Togenu et al. 2013).

**Histological examination**

The pancreas tissues were fixed in 10% formalin solution for hematoxylin and eosin stain and the others were processed for immunostain.

**Statistical analysis**

The acquired data were statistically tested by one-way analysis of variance, then Duncan multiple tests have been done. The values of P ≤0.05 were theorized significant.

**RESULTS AND DISCUSSION**

The identification of hydroxyl, alkyl, ether, ester and disubstituted aromatic ring groups of the extracted compound in the FT-IR spectrum indicated the presence of apigenin in celery seeds. Moreover, apigenin exhibit a major absorption peak in the range of 300 to 350 nm, the present
results indicated that UV spectra of apigenin have $\lambda_{\text{max}}$ at 300 nm ($\varepsilon = 13,000$) of highly aromatic ring B-band and $\lambda_{\text{max}}$ at 350 nm of $\alpha, \beta$ unsaturated cyclic ketone in pyranone ring (C=C–C=O). Furthermore, a thermogram of apigenin extract showed that the thermal decomposition occurs in six successive steps. Fig (1, 2, 3).

The obtained data showed that the total antioxidant capacity of the extracted apigenin was increased with the increasing of its concentration. It can be stated that apigenin can scavenge free radicals and could serve as a strong free radical scavenger due to its chemical structure and its ability to donate electrons Fig (4).

Apigenin treatment to diabetic rats showed a significant decrease in serum glucose levels Fig (5). Flavonoids are a major group of phenolic plant ingredient and their activity in the management and protection of diabetes (Cazarolli et al. 2013). They can impact glucose transfer and metabolism in outer tissues and activating insulin release from pancreatic $\beta$-cells Liu et al. (2006).

The obtained results demonstrated that a significant increase in the activity of $\alpha$-amylase has been observed in the serum of diabetic group Fig (6). The rise in the activity of this enzyme, perhaps was due to the releasing of the enzyme from cellular that occurs due to increase injury processes Rajalakshmi et al. (2015). Apigenin have the capacity to repress alpha-amylase activity Han et al. (2003), probably due to their action on carbohydrates binding part of $\alpha$-amylase enzyme that can block the absorption of starch that stimulate hydrolysis of the internal $\alpha$-1,4 glucosidic
linkages in the starch for the repression of postprandial hyperglycemia (Dineshkumar et al., 2010), or direct blockage of the active center of the enzymes (McCue and Shetty, 2004).

The obtained data displayed a significant increment in lipid profile level after four weeks. The increment of serum lipid profile is usually raised in diabetes because of the increase of blood glucose, which considers a hazard agent for heart disease Sakatani et al. (2005). Very-low-density lipoprotein (VLDL) is an excess in the flux of free fatty acids in the liver and at last, the particles are turned into low-density lipoprotein, increased levels of VLDL as a conclusion of reducing clearance and furthermore overproduction in patient of type 1 diabetes mellitus (Andallu et al. 2009).

Otherwise, there was a significant reduction in lipid profile were observed in the diabetic group after six weeks of the experiment Fig (7, 8, 9). The decrease of the lipid profile may be due to STZ-diabetic rats require more energy, so it broke other sources to obtain their energy and hence it broke lipids, this data was in accordance with EL Barky, (2012). Meanwhile, Apigenin treatment significantly increases the lipid profile. Apigenin has the ability to improve the dysregulated lipid balance and could be used to cure many diseases as atherosclerosis and fatty liver (Zhang et al. 2017).

The obtained results displayed a significant increment in urea and creatinine levels Fig (10,11), that consider a biomarker of diabetic nephropathy (Almeida et al. 2012). The altitude of urea in diabetic rats is associated with the greatest protein breakdown. Also, there was a significant increase of the concentration of urea after the end of last duration experiment but its level is less than the first duration which indicated that the diabetic rats broke the amino acid to obtain their energy. The more amino acid was broken the content were decreased, this results confirmed the
Complications of diabetes have been achieved. On the other hand, a significant reduction of the level of urea was observed after the end of the first experimental period in diabetic rats that received apigenin. The obtained data were in accordance with Wang et al. (2014) they assumed that apigenin had the ability to prevent any toxicity resulted from furan which causes troubles in the kidney.

The current study showed that there was a significant increase in serum L-MDA levels in the diabetic group as compared to normal healthy rats Fig (12). The gained results showed that injection diabetic rats with apigenin extract daily has the ability to lower L- MDA level in serum, this decrease may be due to the antioxidant activity of apigenin extract, the existence of number of hydroxyl group (OH) in the structure of apigenin causes an increment of its antioxidant activity and hence has the ability to hinder formation of ROS forming in diabetic rats.

Catalase activities in the liver were found to decrease in diabetic control rats after the end of six weeks, whereas apigenin extract injection enhances the activity to return towards common values again Fig (13). Apigenin has the ability to scavenge free radicals and stimulating antioxidant enzymes, depends on their structure that consist of OH groups which found at the position 4, 5 and 7 of apigenin C-ring (Sharma et al. 2012). Apigenin also has a double bond between the C2 and C3 carbon atoms of the C-ring and the existence of the oxo group in the fourth position of C-ring Jeyabal et al. (2005).

A significant increase in liver glycogen content was observed in diabetic rats that received apigenin daily as compared to both normal healthy and diabetic non-treated group Fig (14). Apigenin extract has the ability to promote glucose uptake and this action was intermediated meantime the classical insulin signal (Cazaroli et al. 2012). Apigenin has the ability to get back the renewal of pancreatic cells which produced in accordance to insulin excretion. So, apigenin extract can activate glycogen formation via increasing liver content of glycogen.

The pathological improvement in the pancreas of diabetic rats that received apigenin extract was confirmed also by both the histological observations and the immunohistochemical findings Fig (15, 16). These results could be attributed to the hypoglycemic action and antioxidant potential of apigenin extract treatment in diabetic rats.

**CONCLUSION**

Apigenin which is a natural bioactive ingredient in celery seeds has a better effect in amelioration biochemical parameters, histochemistry and immune histochemistry in STZ- induced diabetes in female rats so, apigenin consider being an antihyperglycaemic agent.

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