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Anti-Diabetic Activity of Egyptian Celery Apigenin

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Abstract

Diabetes is a metabolic disorder that occurs due to a deficiency in insulin secretion, action or both of them. Apigenin is a potent antioxidant, found mainly in celery. Therefore, this study aimed to display the biological activity of apigenin and it is a role in lowering blood glucose levels. Apigenin has been extracted from celery and administrated daily to streptozotocin-diabetic rats for six weeks. Apigenin significantly minimizes blood glucose level, the activities of α-amylase, lipid profile and malondialdehyde in serum. On the other hand, liver glycogen has been elevated in diabetic rats that treated with apigenin. The histopathological and immunohistochemical results confirmed that apigenin can decrease degenerative changes in the pancreatic β-cells. So, this study, depicts that apigenin considers a hypoglycemic agent with the potency to normalize odd in the biochemical parameter of diabetes and keep the normal architecture of the islet cells of the pancreas.

Key words: Apigenin, Diabetes, Oxidative stress, Streptozotocin.

Abbreviation: DM: Diabetes mellitus, FT-IR: Fourier transform infrared, STZ: streptozotocin, UV: Ultraviolet Spectroscopy, TGA: Thermal gravimetric analysis. TC: total cholesterol, TAG: Triacylglycerol and VLDL: very low density lipoprotein.

Introduction

Diabetes is a metabolic disorder resulted from 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 was obtained and then placed in the oven at 30°C.

Identification apigenin

Dried apigenin was powdered and analyzed as a potassium bromide pellet by Fourier transform infrared (FT-IR) (Model-JASCO FT-IR4100LE, made in Japan; Range: 4000-400 cm^{-1}). The apigenin extract was measured by UV-Visible double beam spectrophotometer in the range of 200 nm-800 nm for maximum wavelength determination. Finally, apigenin was heated using the Shimadzu TG-50 thermogravimetric analyzer for TGA analysis with heating rate 10°C /min under nitrogen atmosphere in the range of 25-800°C (all the identification methods have been done in Microanalysis unit of faculty of science, Tanta University, Egypt).

Antioxidant activity

The antioxidant activities of apigenin extract have been evaluated by 2, 2-diphenyl-1-picrylhydrazyl method (Wang

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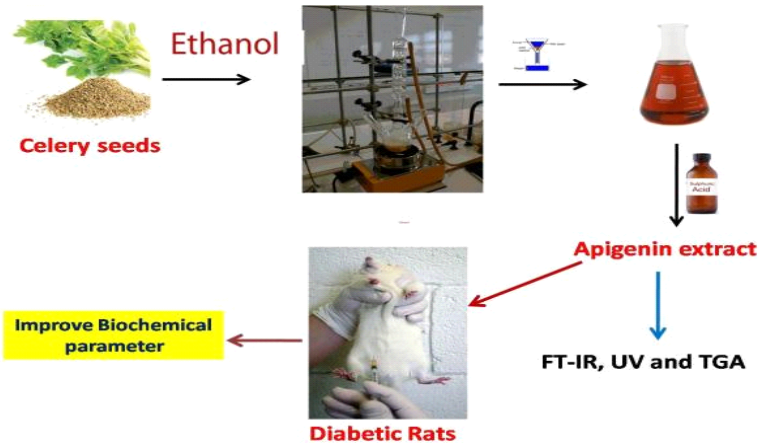

Graphical abstract

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 with1.5 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).

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 λ max at 300 nm (ε = 13,000) of highly aromatic ring B-band and λ max at 350 nm of α, β 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 β-cells Liu *et al.* (2006).

The obtained results demonstrated that a significant increase in the activity of α- 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 α-amylase enzyme that can block the absorption of starch that stimulate hydrolysis of the internal α-1,4 glucosidic

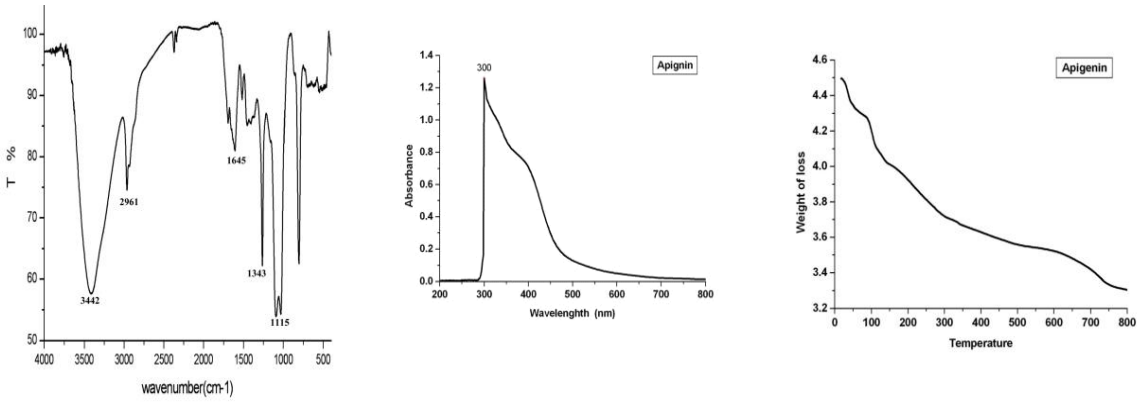

Fig (1, 2, 3): FT-IR spectrum, UV and TGA of apigenin extract.

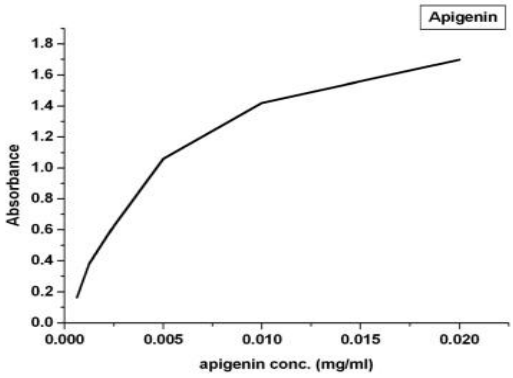

Fig (4): Total antioxidant activity of apigenin extract.

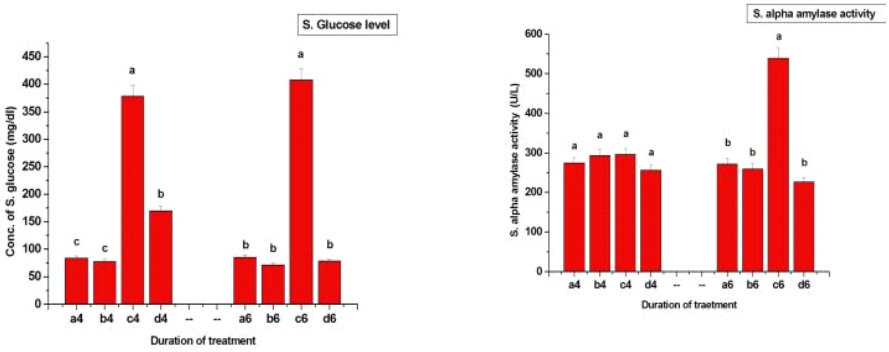

Fig (5, 6): Serum glucose level and α-amylase activity.

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 inpatient 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

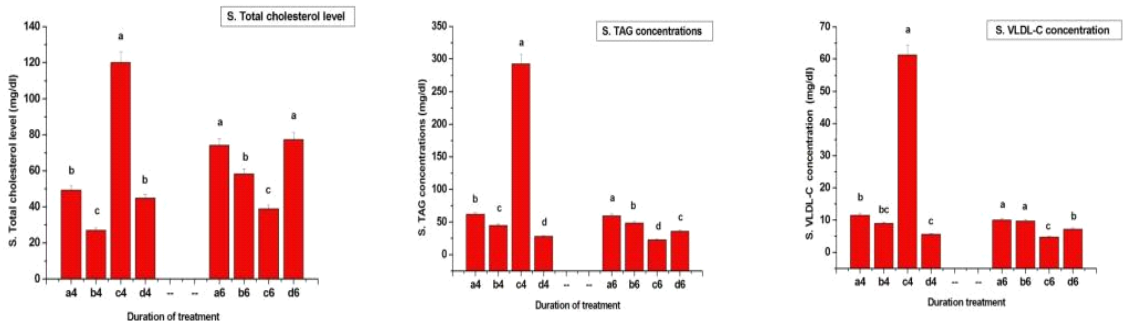

Fig (7, 8 , 9): Serum total cholesterol, TAG and VLDL.

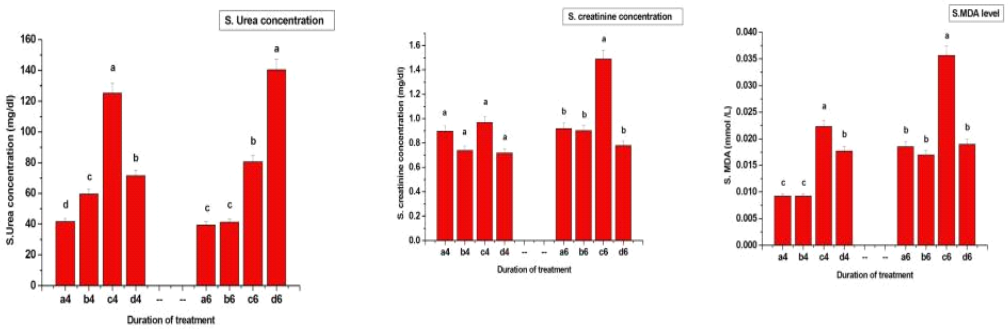

Fig (10, 11, 12): Serum urea, creatinine and L-MDA.

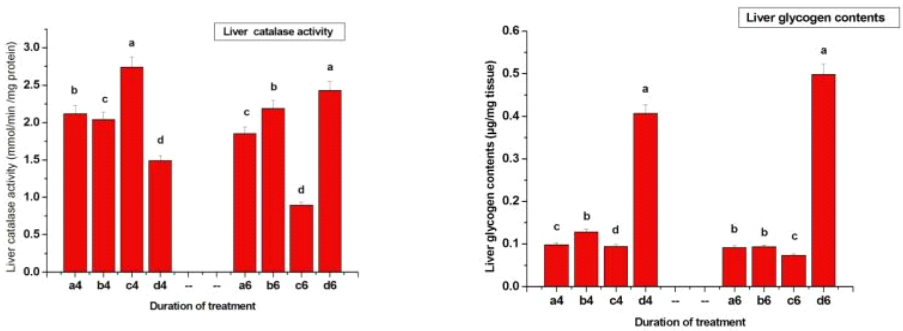

Fig (13, 14): Liver catalase activity and glycogen level.

Mean values with different superscript letters in the same column are significantly different at ($P \leq 0.05$).

Control normal group and normal apigenin group (a4, a6, b4 and b6), diabetic group (c4,c6) and diabetic apigenin (d4,d6).

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 protect 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).

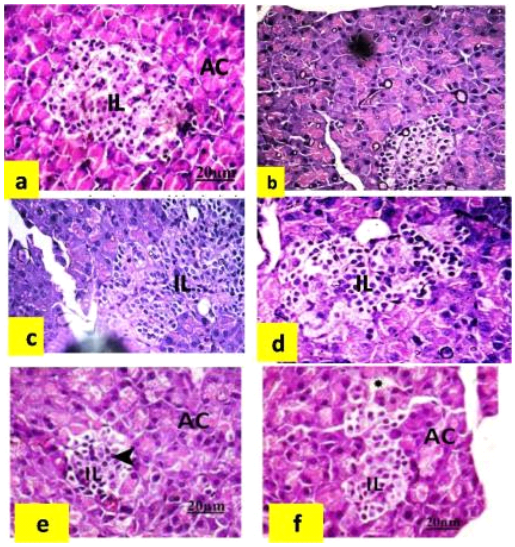

Fig (15): Photomicrograph of pancreatic tissue stained with hematoxylin and eosin (G.a) normal healthy group, (G. b) rats that were received apigenin extract. (G. c, d) diabetic rats group and (G. e, f) diabetic rats diabetic rats which were received apegnin extract.

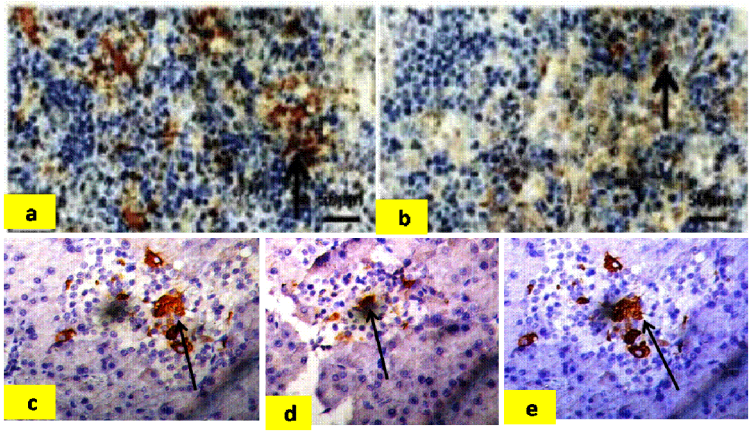

Fig (16): Photomicrograph of the pancreatic tissue immunostained with insulin antisera (a) normal healthy pancreas, (b) diabetic pancreas and (c, d, e) diabetic pancreas received apigenin. Group (a, b) cited from EL Barky *et al.* (2016).

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 (Cazarolli *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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REFERENCES

Almeida, D., Braga, C., Novelli, E. (2012). Evaluation of lipid profile and oxidative stress in STZ-induced rats treated with antioxidant vitamin. Braz. arch. biol. technol. 55: 527-536.

Andallu, B., Vinay Kumar, A., Varadacharyulu, N. (2009). Lipid abnormalities in streptozotocin-diabetes: Amelioration by Morus indica L. cv Suguna leaves. Int J Diabetes Dev Ctries. 29(3): 123–128.

Andreassi, M., Barale, R., Iozzoand, P. (2011). The association of micronucleus frequency with obesity, diabetes and cardiovascular disease. Mutagenesis, 26: 77-83.

Barrera, G. (2012). Oxidative stress and lipid peroxidation products in cancer progression and therapy. Oncology, 2012: 1-21.

Bauer, J. (1982). Clinical Laboratory Methods" 9th Ed, the C.V. Company Waistline Industrial Missouri 63116 Chapter 33: p.555.

Cao, X., Liu, B., Cao, W. (2013). Autophagy inhibition enhances apigeninduced apoptosis in human breast cancer cells. Chin J Cancer. Res. 25:212–222.

Cazarolli, L., Kappel, V., Pereira, D. (2012). Anti-hyperglycemic action of apigenin-6-C-β-fucopyranoside from Averrhoa carambola. Fitoterapia. 83: 1176–1183.

Cazarolli, L., Zanatta, L., Alberton, E. (2008). Flavonoids: prospective drug candidates. Mini Rev Med Chem. 8(13):1429–40.

Dineshkumar, B., Mitra, A., Manjunatha, M. (2010). Comparative study of alpha amylase inhibitory activities of common antidiabetic plants of Kharagpur 1 block. Int J Green Pharm. 4:115-21.

EL Barky, A. R. (2012). Biochemical influence of alpha-lipoic acid on lipid peroxidation and antioxidant enzymes in blood and tissues of streptozotocin induced diabetes in rats. Ms. Thesis, faculty of Veterinary Medicine, Moshthor, Benha University.

El Barky, A. R., Hussein, S., Alm Eldeen, A., Mohamed, T. M. (2016). Anti-diabetic activity of Holothuria thomasi saponin. Biomed. Pharmacother. 84: 1472–1487.

Ellefson, R., Caraway, W. (1976). Fundamentals of Clinical Chemistry. Ed Tietz NW, 506.

El-Said, K., Ezz, A., El Barky, A. R., Mohamed, T. M. (2018). The potential role of male bone marrow mesenchymal stem cells of diabetic female rats. Diabetes Manag. 8(6):137-146.

Han, L., Sumiyoshi, M., Zheng, Y. (2003). Anti-obesity action of Salix matsudana leaves (part 2): Isolation of anti-obesity effectors from polyphenol fractions of Salix matsudana, Phytother Res. 17: 1195.

Huang, C., Lii, C., Lin, A. (2013). Protection by chrysin, apigenin and luteolin against oxidative stress is mediated by the Nrf2-dependent up-regulation of heme oxygenase 1 and glutamate cysteine ligase in rat primary hepatocytes. Arch Toxicol. 87:167–178.

Javadi, I., RashidiNooshabadi, M., Goudarzi, M. (2015). Protective effects of celery (*Apiumgraveloens*) seed extract on bleomycin-induced pulmonary fibrosis in rats. J BabolUniv Med Sci. 17(1):70–76.

Jeyabal, P., Syed, M., Venkataraman, M. (2005). Apigenin inhibits oxidative stress-induced macromolecular damage in N-Nitrosodiethylamine (NDEA)-induced hepatocellular carcinogenesis in wistar albino rats. Mol. Carcinog. 44: 11-20.

Li, F., Yao, Y., Huang, H. (2016). Apigenin attenuates diabetes-associated cognitive decline in rats via suppressing oxidative stress, nitric oxide synthase and anti-apoptotic pathway. Int J ClinExp Med. 9(6):8945–8952.

Liu, D., Zhen, W., Yang, Z. (2006). Genistein acutely stimulates insulin secretion in pancreatic beta-cells through a cAMP dependent protein kinase pathway. Diabetes. 55(4):1043–50.

Liu, Q., Chen, X., Yang, G. (2011). Apigenin inhibits cell migration through MAPK pathways in human bladder smooth muscle cells. Biocell. 35: 71-79.

McCue, P. and Shetty, K. (2004). Inhibitory effects of rosmarinic acid extracts on porcine pancreatic amylase *in vitro*. Asia Pac J Clin Nut r. 13: 101-106.

Mesbah, L., Soraya, B., Narimane, S. (2004). Protective effect of flavonides against the toxicity of vinblastine cyclophosphamide and paracetamol by inhibition of lipid-peroxydation and increase of liver glutathione. Haematol. 759-67.

Ohno, M., Shibata, C., Kishikawa, T. (2013). The flavonoid apigenin improves glucose tolerance through inhibition of microRNA maturation in miRN A103 transgenic mice. Sci Rep. 3:2553–2559.

Prieto, P., Pineda, M., Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of Vitamin E1. Anal. Biochem. 269: 337–341.

Qalawa, S. and Abd Elazeem, N. (2019). Association between Type II diabetic patient's compliance with self-care, self-efficacy and blood glucose control in Port-Said City. IOSR-JNHS journal. 8 (1): 36-44.

Rajalakshmi, K., Christian, G., Shanmuga Priya, P. (2015). Validation of anti-diabetic potential of avirai kudineer a siddha herbal formulation-A review, J. Dental Med. Sci. 4: 07–15.

Rother, K. (2007). Diabetes treatment-bridging the divide. 3. N Engl J Med. 356: 1499-501.

Sakatani, T., Shirayama, T., Uzaki, Y. (2005). The association between cholesterol and mortality in heart failure. Comparison between patients with and without coronary artery disease, Int. Heart J. 46: 619-629.

Sharma, P., Jha, A., Dubey, R. (2012). Reactive oxygen species, oxidative damage and antioxidative defense mechanism in plants under stressful conditions. J. Bot. 2012: 1-26.

Stein, E. (1987). Lipids, lipoproteins and apolipoproteins. In: Fundamentals of Clinical Chemistry, 3rd ed. NW Tietz, ed. WB Saunders Philadelphia, 448.

Tietz, N. (1986). Textbook of Clinical Chemistry. WB saunders, Philadelphia, 1271-1281.

Tietz, N., E.D. (1990). Clinical Guide to Laboratory Tests. 2nd ed. WB Saunders, Philadelphia. 566.

Tietz, N., ed. (1995). Clinical Guide to Laboratory Tests. 3rd ed. WB Saunders, Philadeiphia.268-273.

Togenu, M., Austin, U., Joyce, A. (2013). Anti diabetic effect of aqueous leaf extract of Heinsia crinata on key glycolytic enzymes and glycogen in streptozotocin induced diabetic rats. Academia Journal of Scientific Research. 1: 109-114.

Wang, E., Chen, F., Hub, X. (2014). Protective effects of apigenin against furan-induced toxicity in mice. Food Funct. 5: 1804.

Wang, M., Li, J., Rangarajan, M. (1998). Antioxidative phenolic compounds from sage (*Salvia officinalis*). J. Agric. Food Chem. 46: 4869–4873.

Winn-Deen, E., David, H., Sigler, G. (1988). Development of a Direct Assay for alpha-Amylase, Clin. Chem. 34(10): 2005-2008.

Xu, J., Yuan, X., Lang, P. (1997). Determination of catalase activity and catalase inhibition by ultraviolet spectrophtometry. Chinese Environ. Chem. 16(1): 73–76.

Zhang, K., Song, W., Li, D. (2017). Apigenin in the regulation of cholesterol metabolism and protection of blood vessels. Exp Ther Med. 13(5): 1719–1724.

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