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Original Article

Physiochemical and nutritional evaluation of whole kumquat fruits powder and its protective effect on thyroid hormones and blood sugar levels in diabetic rats

Avaliação físico-química e nutricional de frutas inteiras de kumquat em pó e seu efeito protetor sobre os hormônios da tireoide e os níveis de açúcar no sangue em ratos diabéticos

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Abstract

The present study was conducted to evaluate the chemical composition, antioxidant activity and hypoglycemic effects of whole kumquat (Ku) powder in diabetic rats fed a high-fat-high-cholesterol (HFHC) diet. The antioxidant activities were evaluated using stable 1,1-diphenyl 2-picrylhydrazyl (DPPH) free radical scavenging method, 2,2´-azinobis (3-ethyl benzo thiazoline-6-sulphonic acid) radical cation (ABTS) and Ferric reducing antioxidant power (FRAP). Total phenolic content was (51.85 mg GAE/g) and total flavonoid content was (0.24 mg Cateachin Equivalent, CE/g). DPPH and ABTS values were 3.32 and 3.98 mg Trolox equivalent (TE)/g where FRAP value was 3.00 mM Fe^{2+}/kg dry material. A total of 90 albino rats were used in the present study. Rats group were as follows: normal diet; normal treated (2, 4, and 6% Ku.), diabetic rats (non-treated), diabetic + HFHC diet (non-treated), HFHC (non-treated), Diabetic (treated), HFHC (treated) and Diabetic + HFHC (treated). The diets were followed for 8 weeks. Blood samples were collected at the end of the experiment. Serum glucose was recorded and thyroid hormones (T4, Thyroxine and T3, Triiodothyronine) were conducted. Diet supplemented with Kumquat at different concentrations have a hypoglycemic effect and improve the thyroid hormones of both diabetic rats and HFHC diabetic rats.

Keywords: kumquat, hypoglycemia, total phenolics, total flavonoids, thyroid hormones.

Resumo

O presente estudo foi conduzido para avaliar a composição química, a atividade antioxidante e os efeitos hipoglicêmicos do pó de kumquat (Ku) em ratos diabéticos alimentados com uma dieta rica em gordura e colesterol (HFHC). As atividades antioxidantes foram avaliadas usando o método de eliminação de radicais livres de 1,1-difenil 2-picrilhidrazil (DPPH), 2,2'-azinobis (ácido 3-etilbenzotiazolina-6-sulfônico) radical cátion (ABTS) e antioxidante redutor férrico potência (FRAP). O conteúdo fenólico total foi (51,85 mg GAE / g) e o conteúdo total de flavonoides foi (0,24 mg Cateachin Equivalent, CE / g). Os valores de DPPH e ABTS foram 3,32 e 3,98 mg equivalente de Trolox (TE) / g, em que o valor de FRAP foi de 3,00 mM Fe2 + / kg de material seco. Um total de 90 ratos albinos foi usado no presente estudo. O grupo dos ratos foi o seguinte: dieta normal: tratados normais (2, 4 e 6% Ku.), ratos diabéticos (não tratados), diabéticos + dieta HFHC (não tratados), HFHC (não tratados), diabéticos (tratados), HFHC (tratados) e diabéticos + HFHC (tratados). As dietas foram seguidas por 8 semanas. Amostras de sangue foram coletadas ao final do experimento. A glicose sérica foi registrada e os hormônios tireoidianos (T4, Tiroxina e T3, Triiodotironina) foram conduzidos. A dieta suplementada com kumquat em diferentes concentrações tem um efeito hipoglicêmico e melhora os hormônios tireoidianos tanto de ratos diabéticos quanto de ratos diabéticos com HFHC.

Palavras-chave: kumquat, hipoglicemia, fenólicos totais, flavonoides totais, hormônios tireoidianos.

1. Introduction

Thyroid gland regulates a wide range of physiological activities such as growth, metabolism, homeostasis, and cell proliferation and differentiation through the secretion of thyroid hormones. Thyroid diseases are among the most common endocrine disorders, hypothyroidism is the most common clinical thyroid dysfunction. Hyperthyroidism

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means increased thyroid function and refers to excess metabolic state due to excessive synthesis and secretion of thyroid hormones. Medicinal plants have been identified and used by humans throughout history. The herbs and natural antioxidants are used to treat thyroid disorders including hypothyroidism and hyperthyroidism. (Shokri et al., 2018).

The main product of the thyroid gland is mainly T_4 prohormone (3,5,3,5-tetraiodothyronin), also known as thyroxine, and in less amounts, T_3 active hormone (3,5,3′-triiodo-L-thyronine) (Williams, 2013). Subclinical thyroid disease is defined by abnormal serum TSH, but normal T_3 and T_4 levels and the patients do not always need treatment, while people with clinical thyroid disease have abnormal serum TSH, T_3, and T_4 levels and need treatment (Baskin et al., 2002).

Diabetes mellitus is a disorder that affects the body's ability to make or use insulin. Insulin is a hormone produced in the pancreas that helps transport blood glucose from the blood stream into the cells so they can break it down and use it for fuel. People cannot live without insulin. This can cause severe short-term and long-term consequences ranging from brain damage to amputations and heart disease. (American Diabetes Association, 2007). Citrus fruits have been recommended in traditional herbal medicine as the source of diabetic medication or remedy for diabetes (Baker, 1994).

Diabetes mellitus (DM) is a chronic disease which is caused either by inherited disability or acquired deficiency in production of the hormone insulin and its subsequent inability to regulate the blood glucose level and also where there is sufficient production of insulin but the insulin secreted is unable to regulate the blood glucose levels. The outcome of the above two conditions is that there is an increased level of blood glucose which in turn damages many of the vital organs (kidney, eye, etc.) of the body (Nagappa et al., 2003).

Citrus fruits are abundant in the Indian subcontinent. Lemon, lime, pomelo, sweet lime, and orange, and so forth are cultivated in abundance in different regions of India. The citrus fruits are well recognized for their various ethno-medicinal uses. These properties are attributed to their flavonoids and limonoids which are proven to possess anti-inflammatory and antitumor effects (Middleton Junior et al., 2000; Huang and Ho, 2010). The peels are rich in pectin which is known to possess blood sugar lowering and cholesterol lowering properties (Baker, 1994). Other than these, carotenoids and hydroxycinnamic acids are also abundant in citrus peels (Xu et al., 2006; Manthey and Grohmann, 2001).

Kumquat (In Chinese means golden orange) is one of the smallest citrus fruits , which is characterized by the acidic taste of flesh and soft edible peel, where the fruit can be eaten along with the peel either in the form of row fruit or as juice. It can also be used as pickles and marmalades (Chen et al., 2017). Beside the nutrients, in kumquat there are several phytochemicals in fruits including carotenoids, essential oils, ascorbic acid as well as flavonoids (Wang et al., 2012). Such phytochemicals, with various beneficial biological effects, provide kumquat a great importance as a folk medicine (Lou and Ho, 2017).

Kumquats are consumed preferably in nature, whole and in shell. It is also used to make jellies, mousse, jams, marmalades, liqueurs and cachaça, preparation of syrups, sauces and also, accompaniment salads and for landscaping purposes and ornamentation (Kawaii et al., 1999; Schirra et al., 2008). Citrus japonica has been used as a traditional folk medicine in Asian countries to reduce alcohol intoxication and as antidepressants, so they are used either as medicines or as edible fruit (Liu et al., 2018).

The objective of this study is to evaluate the physicochemical, and nutritional properties of kumquat fruits. In addition, determination the protective effect of different concentration of kumquat fruits on thyroid hormones and blood sugar levels in diabetic rats.

2. Materials and Methods

2.1. Materials

2.1.1. Plant materials

Fresh Kumquat fruits is obtained from the local market Cairo, Egypt. The Kumquat fruits are washed, cutting and dried at 40 °C for 2 days using air oven, then ground in laboratory mill then allowed passing through a mish sieve to obtain a fine powder of Kumquat fruits .

2.1.2. Chemicals

All chemical reagents were obtained from Sigma (St. Louis, MO), unless otherwise specified. All solvents used for compound isolation were HPLC grade. 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (TROLOX) and 2,2´-azino-bis (3-ethyl benzo thiazoline-6-sulphonic acid (ABTS) were purchased from Sigma-Aldrich, Germany.

3. Methods

3.1. Chemical composition of kumquat fruits powder

Chemical composition of Kumquat fruits powder including the contents of moisture, ash, crude protein and crude fat were determined according to AOAC (2007). Carbohydrates were determined by difference (as mentioned in Table 1).

3.2. Determination of minerals

Iron, calcium and potassium and sodium of Kumquat fruits powder were determined using atomic absorption spectrum while, phosphorus was determined by colorimeter method (ammonium molybdate) using spectrophotometer (AOAC, 2000).

3.3. Determination of water-soluble vitamins

An Agilent 1100 chromatographic system (Agilent Ltd., South Queensferry, UK) was used for the analysis and quantitation of water-soluble vitamins in samples according to Hasan et al. (2013). While, ascorbic acid (Vitamin C) was determined according to AOAC (2000).

Table 1. Proximate analyses and vitamins content of whole powder Kumquat.

	Proximate analyses					
	Moisture (%)	**Lipids (%)**	**Protein (%)**	**Ash (%)**	**Crude fiber (%)**	**Total Carbohydrate* (%)**
	9.92	1.27	4.39	2.08	5.09	77.24
Sugar Content (g/100g)	**Sucrose**		**Glucose**		**Fructose**	
	35.98		13.77		14.06	
Mineral Content (mg/100g)	**Ca**		**K**		**P**	**Fe**
	542.64		923.35		140.52	2.28
Water soluble vitamins (mg/100g)	**Thiamine (B_1)**	**Riboflavin (B_2)**	**Niacin (B_3)**	**Cyanocobalamin (B_{12})**	**Ascorbic acid (C)**	
	29.39	0.16	107.26	9.004	232.60	

*Total carbohydrates (%) = 100 – sum of all proximate analyses.

3.4. Determination of Total Phenolic Content (TPC)

The total phenolic content of Kumquat fruits powder was determined according to the Folin-Ciocalteu procedure (Žilić et al., 2012). The total phenolic content was determined by means of a calibration curve prepared with gallic acid, and expressed as milligrams of Gallic Acid Equivalent (mg GAE) per g of sample. Additional dilution was done if the absorbance value measured was over the linear range of the standard curve.

3.5. Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) of the extract of Kumquat fruits powder was determined using the aluminium chloride ($AlCl_3$) method according to a reliable approach using quercetin as the standard Žilić et al. (2012) The results were expressed as milligrams of Cateachin Equivalent (CE) per g of dry material.

3.6. Antioxidant activity

3.6.1. Determination of radical DPPH scavenging activity

Free radical scavenging capacity of Kumquat fruits powder were determined using the stable DPPH• according to Hwang and Thi (2014). The final concentration was 200 µM for DPPH• and the final reaction volume was 3.0 ml. The absorbance was measured at 517 nm against a blank of pure methanol after 60 min of incubation in a dark condition. Percent inhibition of the DPPH free radical was calculated by the following equation:

$$Inhibition\ (\%) = 100 \times \left(A_{control} - A_{sample} \right) / A_{control})$$

Where: $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound), A_{sample} is the absorbance with the test compound. The standard curve was prepared using Trolox. Results were expressed as mg Trolox equivalents (TE / g sample). Additional dilution was needed if the DPPH value measured was over the linear range of the standard.

3.6.2. Determination of radical ABTS scavenging activity

The stock solutions of ABTS• reagent was prepared according to Hwang and Thi (2014) by reacting equal quantities of a 7 mM aqueous solution of ABTS• with 2.45 mM potassium persulfate for 16 h at room temperature (25 °C) in the dark. The working solution was then prepared by diluting 1 ml ABTS• solution with 60 ml of ethanol: water (50:50, v/v) to obtain an absorbance of 1.0 ± 0.02 units at 734 nm using the spectrophotometer. Extracts of Kumquat fruits powder (50 µl) were allowed to react with 4.95 ml of the ABTS• solution for 1 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. Percent inhibition of the ABTS• free radical was calculated by the following equation:

$$Inhibition\ (\%) = 100 \times \left(A_{control} - A_{sample} \right) / A_{control}$$

Where: $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound). A_{sample} is the absorbance with the test compound. The standard curve was prepared using Trolox. Results were expressed as mg Trolox equivalents (TE / g sample). Additional dilution was needed if the ABTS• value measured was over the linear range of the standard.

3.6.3. Ferric Reducing Activity Power (FRAP) assay

The FRAP assay of Kumquat fruits powder was done according to Hwang and Thi (2014).

3.7. Phenolic acids profile

An Agilent 1100 chromatographic system equipped with an auto sampler and a diode-array detector (Agilent Ltd., South Queensferry, UK) was used for the analysis and quantitation of Phenolic acids in sample of Kumquat fruits powder according to Kim et al. (2006).

3.8. Determination of fructose, glucose and sucrose

The Kumquat fruits powder were mixed with 20 ml portion of deionized water and heated to 80 °C under magnetic stirring then sonicated for 30 minutes. The

suspension solutions were passed through a stainless steel mesh sieves before filtering by Whatman filter paper using Buchner funnel. The final volume of solution was made up to 50 ml and stored in refrigerator. All standards and solutions ready to analysis were passed through the 0.45 um desk filter and sonicated for 15 minutes in ultrasonic bath. and then analysis by HPLC System (Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a refractive index detector) according to Salman et al. (2011).

3.9. Biological experiment

3.9.1. Experimental design for biological evaluations

A total of 90 male albino rats with an average weight of (130 : 140g) were purchased from the animal lab of the Research Institute of Ophthalmology, Giza, Egypt. All rats were housed in individual cages in standard conditions (Reeves et al., 1993). High-fat-High-Cholesterol (HFHC) diets (68% control diet, 25% palm oil, 5% cholesterol and 2% colic acid) according to Kitamori et al. (2012). The experimental design was performed in accordance with the guide for the care and use of laboratory animals, as adopted and promulgated by the "Institutional Animal Care Committee". Blood samples were taken at the end of the experiment (49 days) for biochemical analyses (Schermer, 1967; Dhandapani et al., 2002).

3.9.2. Induction of diabetes in rats

Diabetic rats were induced by a single intraperitoneal injection of 45 mg/kg body weight streptozotocin (STZ) dissolved in 0.1 M citrate buffer (pH 4.5). After 7 days of STZ injection, the tail vein blood was collected to determine fasting blood glucose level.. The STZ-treated rats were given 5% glucose water for 24 h following STZ injection, to prevent initial drug induced hypoglycemic mortality. Then, after 72 h STZ injection, blood was drawn from retro-orbital plexus of the surviving rats with heparinized capillaries and the fasting blood glucose levels were estimated according to a previously reported technique (Barham and Trinder, 1972). The rats with a blood glucose level of more than 250 mg/dl were regarded as experimentally induced-diabetic rats.

3.9.3. Biochemical analysis

All biochemical analyses were determined, by colorimetric methods as follow: glucose (Barham and Trinder, 1972), Estimation of serum T3 and T4 was estimated by enzyme-linked-immuno-sorbent-assay (ELISA) method according to Gar-Elnabi et al. (2013)

3.10. Statistical analyses

All the experiments were carried out in triplicate and the mean and standard error values were calculated for all data. Then, the results were subjected to one-way analysis of variance followed by Duncan's significant differences using SAS program (version 9.1.3) software (Cary, NC). Significant levels were defined as ($P < 0.05$) (SAS INSTITUTE, 2004).

4. Results and discussion

4.1. Proximate analysis and vitamins content of whole kumquat powder

The proximate chemical compositions of whole dry kumquat powder are presented in Table 1. Whole kumquat contained 9.92% moisture, 4.39% protein, 1.27% lipids, 5.09% crude fiber, 2.08% ash and 77.23% carbohydrate. As shown in Table 1 sugar types are varied in kumquat fruits where the most dominant type of sugar was sucrose 35.98 g/100g. In addition, kumquats contain modest minerals contents; calcium (Ca), potassium (K), phosphorus (P) and iron (Fe) were 542.64, 923.35, 140.52 and 2.28 mg/100g, respectively. Potassium is an important component of cell and body fluids that help to control the heart rate and blood pressure. Iron is required for red blood cell formation as well for cellular oxidation.

On the other hand, kumquat contained many types of vitamins like Thiamine (29.39 mg/100g, B1), Riboflavin (0.16 mg/100g, B2), Nisin (107.26 mg/100g, B3), Cyanocobalmin (9.004 mg/100g, B12) and Ascorbic acid (232.60 mg/100g, vitamin C). Adequate quantities of antioxidant vitamins such as vitamin C (Wang et al., 2012). Vitamin C is one powerful natural anti-oxidant, which has many essential biological roles like collagen synthesis and wound healing; anti-viral and anti-cancer activities; and helps prevent neurodegenerative diseases such as arthritis, diabetes, *etc*. by removing oxidant free radicals from the body. Furthermore, vitamin C facilitates iron absorption in the food by changing it from its ferrous. Collectively these phytochemical compounds in kumquat fruits help scavenge harmful oxygen derived free radicals from the body and thereby protect us from cancers, diabetes, degenerative diseases and infections. Kumquats contain good quantities of B-complex vitamins. These vitamins function as co-factors for metabolism of carbohydrates, proteins, and fats (Chambial et al., 2013).

4.2. Antioxidant activity and total phenolics of whole kumquat

Antioxidant activity of whole kumquat powder was evaluated using ferric reducing antioxidant power (FRAP) and DPPH free radical-scavenging assays. The FRAP and DPPH assays are simple, rapid, inexpensive tests, and are very useful as routine analyses of antioxidant activity of natural products. Table 2 showed that DPPH and ABTS were 3.32 and 3.98 mg TE/g, respectively. While FRAP was 3.00 mM Fe^{2+}/kg dry matter. Furthermore, phenolic compounds serve as antioxidants due to their free radical scavenging activity or metal chelating ability. Therefore, the high content of phenolic compounds in kumquat might result in the high antioxidant activity (Lou et al., 2014a). The total polyphenols and flavonoids of whole kumquat powder were determined and their contents were 51.85 mg/g and 0.24 mg/g, respectively (Table, 2). Regarding the effect of heat treatment on phenolic compounds and antioxidant activity of citrus peel, Choi et al. (2011), Ho and Lin (2008), Jeong et al. (2004) and Xu et al. (2007) have been reported that high temperature drying might be used as an effective method to release bound phenolic compounds from citrus and increase their antioxidant activity.

Table 2. Radical scavenging activity and phytochemical analysis of whole Kumquat powder.

Phytochemical analysis	Total phenols (mg GAE/g)	Total flavonoids (mg CE/g)	
	51.85	0.24	
Antioxidant activity	**DPPH (mg TE/g)**	**ABTS (mg TE/g)**	**FRAP (mM Fe^{2+}/kg dry material)**
	3.32	3.98	3.00

GAE = Gallic acid Equivalent; CE = Catechin Equivalent; TE = Tolorox Equivalent.

Flavonoids are very important constituents of plants because of the scavenging ability conferred by their hydroxyl groups. The flavonoids may contribute directly to anti-oxidative action. It is known that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when up to 1 g daily is consumed from a diet rich in fruits and vegetables (Tanaka et al., 1998).

Our study illustrated that the whole kumquat powder was subjected to heat treatment (drying of whole kumquat), and it concluded that high temperatures could enhance the extraction efficacy of phenolic content. In preceding studies, the antioxidant activity and total phenolic content of immature calamondin increase significantly after heat treatment at 150 °C for 1.5 h (Lou et al., 2014b). Thus, as shown in Table 2 our study found that the phenolic content is higher than flavonoid content as well as, a proper heat treatment could enhance the antioxidant activity of kumquat and the same trend was found by Lin et al. (2008).

The profiles of flavonoids in kumquats were quite different to citrus in which the most abundant flavonoids are naringin, hesperidin, and polymethoxy flavones, such as nobiletin and tangeretin. No nobiletin and tangeretin were found in kumquat (Lou et al., 2016). Similar data obtained by our study as shown in Table 3 where, naringeen (50.36 µg/g) and rosmarinic (144.10 µg/g) were the dominant flavonoid compounds in kumquat.

4.3. Biological experiment

4.3.1. Effect of administration of whole kumquat powder on the glucose level in HFHC diabetic rats

Many flavonoids derived from citrus fruits have been reported to reduce oxidative stress, improve glucose tolerance, insulin sensitivity and modulate lipid metabolism (Mahmoud et al., 2019). As shown in Table 4 a single intravenous injection of STZ induced an increase of fasting blood glucose levels significantly ($P < 0.05$) to 358.97 mg/dl at the end of the experiment compared to the normal control (84.24 mg/dl). The blood glucose level of diabetic rats fed with a HFHC diet (322.71 mg/dl) was significantly ($P < 0.05$) higher than those of both normal rat and HFHC rats. On the other hand, the glucose levels were always lower in HFHC-diet rats (108.72 mg/dl) than both diabetic rats (358.97 mg/dl) and diabetic rats fed a HFHC-diet (322.71 mg/dl).

The intraperitoneal injection of STZ at doses of 45 mg/kg into rats damages the pancreas, and insulin levels typically fall to 10 – 30% of normal levels leading to hyperglycemia (Ozturk et al., 1996). The present results showed that long-term daily administration of whole kumquat powder significantly decreased fasting blood glucose levels in STZ-induced diabetic rats. These obtained data are in agreement with Tan et al. (2014) who found that kumquat extract reduced blood sugar levels and insulin resistance in diabetic and obese mice. The antihyperglycemic effect of kumquat might be due to simulation of insulin release, like results obtained by Singab et al. (2005) who mentioned that up to different hypotheses of ability of mulberry induced lowering fasting blood glucose level in diabetic rats is probably due to stimulation of insulin release.

Table 3. Phenolic profile of whole Kumquat powder.

Compound	Type of polyphenolic compound	T_R (min.)	µg/g
Pyrogallol	Phenolic compound	4.7	459.80
Gallic	Phenolic compound	5.7	ND
Protochatchuic	Phenolic compound	9.9	22.47
***p*-hydroxybenzoic**	Phenolic compound	15.1	23.18
Gentisic	Phenolic compound	16.8	ND
Catachine	Phenolic compound	18.6	24.16
Chlorogenic	Phenolic compound	20.6	ND
Caffic	Phenolic compound	21.4	8.74
Syrngic	Phenolic compound	23.0	353.08
Vanillic	Phenolic compound	24.8	ND
Ferulic	Phenolic compound	32.4	11.12
Sinapic	Phenolic compound	33.8	112.43
Rutin	Phenolic compound	36.2	15.34
***p*-coumaric**	Phenolic compound	37.2	9.09
Naringeen	Flavonoid compound	38.0	50.36
Hisperdin	Flavonoid compound	38.5	214.13
Rosmarinic	Flavonoid compound	40.0	144.10
Cinnamic	Phenolic compound	42.8	104.62
Qurcetin	Flavonoid compound	43.5	18.10
Apegnin	Flavonoid compound	46.0	28.45
Kaempferol	Flavonoid compound	46.5	3.11
Chyrsin	Flavonoid compound	52.0	4.38

T_R = Retention time, ND = Not detected.

4.3.2. Effect of administration of whole kumquat powder on the thyroid hormones in HFHC diabetic rats

Thyroid hormones thyroxine (T4) and triiodothyronine (T3) are tabulated in Table 5. The present data showed that the T3 and T4 hormones were significantly ($P < 0.05$) decreased in diabetic control (51.56 and 3.08 ng/dl) compared to normal group (85.86 and 5.63 ng/dl). As mentioned before in Table 4, where the fasting blood glucose level was elevated up to 358.97 mg/dl in diabetic non treated group. Thus, the higher the level of fasting blood glucose occurs, the lower the thyroid hormones are founded.

Table 4. Effect of administration whole kumquat powder on blood glucose levels in diabetic rats fed on high-fat / high-cholesterol.

	Glucose concentration (mg/dl)		
	Non-treated with Kumquat (0% Kumquat)		
Normal	$84.24^{b} \pm 3.81$		
Diabetic	$358.97^{g} \pm 4.03$		
HFHC	$108.92^{c} \pm 3.74$		
Diabetic + HFHC	$322.71^{f} \pm 4.25$		
	Treated with Kumquat		
	2% Kumquat	**4% Kumquat**	**6% Kumquat**
Normal	$101.12^{c} \pm 3.76$	$82.59^{ab} \pm 3.93$	$84.99^{b} \pm 4.07$
Diabetic	$144.52^{d} \pm 2.36$	$143.17^{d} \pm 3.54$	$141.68^{d} \pm 4.26$
HFHC	$74.70^{ab} \pm 4.03$	$70.85^{a} \pm 4.31$	$79.94^{ab} \pm 4.35$
Diabetic + HFHC	$185.71^{e} \pm 4.11$	$178.32^{e} \pm 4.46$	$134.82^{d} \pm 4.13$

HFHC = High-fat-High-cholesterol; Each value represents the mean ± Standard Error (S.E.), The mean value with different superscript alphabets indicate significant differences ($P<0.05$) using Duncan test.

Thyroid gland plays a central role in the regulation of metabolism. So, abnormal thyroid function can have a major impact on the control of diabetes. Besides, untreated thyroid disorder can increase the risk of certain diabetic complications and can aggravate many diabetes symptoms. The effect of the thyroid hormones (T3 and T4) on metabolism and the major organ systems of the human body appears in stimulating the enzymes concerned with glucose oxidation and enhancing the rate of uptake of glucose, affecting synthesis, mobilization and degradation of lipids, and lowering blood cholesterol (Idris, 2011).

Thyroid hormones act directly in the insulin secretion. In the hypothyroidism condition there is an increase of insulin secretion stimulated by glucose in the β cells, and the opposite occurs in the hyperthyroidism condition, reducing the secretion of insulin stimulated by glucose (Mitrou et al., 2010; Stanická et al., 2005). Comte et al. (1990) reported that the reduction of gluconeogenesis caused hypothyroidism. Thus the mode of action, as herbal powder and extracts reduced higher levels of thyroid hormones in diabetic rats, this is due to the reduction of gluconeogenesis which led to a reduction in thyroid hormones leading to hypothyroidism (but not vice versa). Regarding the levels thyroid hormones T3 (72.12 ng/dl) and T4 (4.67 ng/dl) in HFHC non-treated diabetic rats, the administration of HFHC diet during the study revealed that significantly ($P < 0.05$) decrement of the thyroid hormones compared to normal rats.

Therefore, it could be concluded that the increase in glucose level led to a reduction in thyroid hormones. More important, hypothyroidism is accompanied by a variety of abnormalities in blood lipid levels. This includes increased total cholesterol and LDL (low-density lipoprotein or "bad") cholesterol levels, and increased triglyceride levels. The abnormal lipid pattern typical of Type 2 diabetes (low HDL, or "good" cholesterol, high triglycerides, and a high proportion of small, dense LDL particles) is usually worsened by hypothyroidism. These changes further raise the already high risk of cardiovascular diseases such as heart disease and stroke among people with diabetes.

Table 5. Effect of administration whole kumquat powder on thyroid hormones (T_3 and T_4) in diabetic rats fed on high-fat / high-cholesterol.

	T_3 (ng/dl)			T_4 (ng/dl)		
	Non-treated with Kumquat (0% Kumquat)			Non-treated with Kumquat (0% Kumquat)		
Normal	$85.86^{de} \pm 1.35$			$5.63^{f} \pm 0.12$		
Diabetic	$51.56^{a} \pm 1.07$			$3.08^{a} \pm 0.07$		
HFHC	$87.06^{de} \pm 2.13$			$5.46^{ef} \pm 0.08$		
Diabetic + HFHC	$72.12^{c} \pm 7.06$			$4.67^{b} \pm 0.09$		
	Treated with Kumquat			Treated with Kumquat		
	2% Kumquat	**4% Kumquat**	**6% Kumquat**	**2% Kumquat**	**4% Kumquat**	**6% Kumquat**
Normal	$91.74^{ef} \pm 0.86$	$94.66^{f} \pm 1.63$	$91.92^{ef} \pm 2.66$	$5.18^{de} \pm 0.16$	$5.29^{def} \pm 0.14$	$5.21^{de} \pm 0.12$
Diabetic	$74.82^{c} \pm 1.79$	$75.13^{c} \pm 1.62$	$76.23^{c} \pm 1.89$	$4.52^{b} \pm 0.13$	$4.72^{b} \pm 0.14$	$4.78^{bc} \pm 0.11$
HFHC	$82.58^{d} \pm 2.79$	$89.03^{def} \pm 3.04$	$92.44^{ef} \pm 1.41$	$5.08^{cd} \pm 0.06$	$5.51^{ef} \pm 0.12$	$5.97^{g} \pm 0.15$
Diabetic + HFHC	$49.12^{a} \pm 2.66$	$59.80^{b} \pm 1.88$	$88.65^{def} \pm 0.77$	$4.54^{b} \pm 0.05$	$4.64^{b} \pm 0.13$	$5.46^{ef} \pm 0.14$

T_3 = Triiodothyronine; T_4 = Thyroxine; HFHC = High-fat/High-cholesterol; Each value under the certain hormone represents the mean ± Standard Error (S.E.); The mean value with different superscript alphabets indicate significant differences ($P<0.05$) using Duncan test.

Another explanation of the reduction of the thyroid hormones (T3 and T4), is the possibilities include caffeic acid phenyl ester induced modulation in de-iodination system, which affects de-iodinase activity through its antioxidant properties (Brzeziβ ska-Slebodziβ ska, 2001; Vrca et al., 2004).

Due to kumquat is a rich source of phytochemicals (flavonoids and phenolic acids) which caused antioxidative activity, it has anti-thyroidal properties, which suggest its potential to ameliorate hyperthyroidism. Same results are found by Chandra and De (2014). The anti-thyroidal role of might be mediated through the inhibition of thyroid peroxidase the key enzyme in thyroid hormone biosynthesis (Cooksey et al., 1986), as it contains the phenolic compound naringin, which inhibits the activity of thyroid peroxidas. Thus, the administration of whole kumquat powder in treated rats, caused hypoglycemic effect in diabetic rats then led to increase the T3 and T4 hormones.

5. Conclusion

Using natural products like kumquat as protective effect on thyroid hormones and blood sugar levels in high-fat-high-cholesterol diabetic rats. Our study concludes that the whole kumquat elevate serum concentration of T3 and T4 in HFHC diabetic rats at the doses of 2% to 6% showing a strong antithyroidal effect. However, we emphasize that further studies are required to identify the precise mechanism of action and isolation of active principle (s) responsible for such activities.

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