

Antihyperglycemic activity of quince (*Cydonia oblonga* Mill.) fruit extract and its fractions in the rat model of diabetes

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Abstract:

Introduction: Diabetes mellitus is a metabolic disease that has affected approximately 10% of population worldwide. *Cydonia oblonga* Mill. (*C. oblonga*), commonly called quince, contains diverse phytochemical constituents with a broad range of pharmacological activities. The current study is aimed to investigate the antihyperglycemic effect of aqueous extract of *Cydonia oblonga* Mill. fruit (ACO) in streptozotocin-induced diabetic rats and to identify the active fraction.

Methods and Results: Diabetes was induced in rats by a single intraperitoneal (i.p.) injection of 60 mg/kg streptozotocin. The antihyperglycemic activity of different concentrations of ACO (80, 160 and 240 mg/kg body weight daily for a period of 28 days) was evaluated in the diabetic rats by measuring their fasting blood glucose (FBG). Furthermore, the antihyperglycemic effects of two major fractions of ACO were evaluated for the identification of active fraction. Finally, the chemical composition of the active fraction, methanolic fraction (MF), was examined by gas chromatography-mass spectrometry (GC-MS) assay. The oral administration of ACO on diabetic rats resulted in a significant collapse in FBG in a dose-dependent manner. In addition, the MF was the active fraction and exhibited antihyperglycemic activity in diabetic rats during the experiment. The main component of MF was identified as 5-hydroxymethylfurfural or 5-HMF (a well-known natural compound) that may be responsible, at least partly, for the antidiabetic and antihyperglycemic effects of quince.

Conclusion: Our results have demonstrated for the first time that quince possesses antihyperglycemic effect in diabetic rats and the MF of the aqueous extract is active fraction.

Keywords: Antidiabetes; *C. oblonga*; 2,5-dimethyl-4-hydroxy-3(2H)-furanone; GC-MS; 5-Hydroxymethylfurfural; Polyphenol

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1. Introduction

Diabetes mellitus is one of the most common chronic diseases, which is rapidly increasing in nearly all countries. The increasing prevalence of diabetes is one of the most serious public health problems and it has been estimated that 552 million people will have diabetes by

2030 worldwide [1]. The metabolic disease characterized by chronic hyperglycemia, which in turn results in diabetes complications such as cardiovascular diseases, neuropathy, nephropathy, and retinopathy [2, 3]. There is a growing trend of using herbal preparations in the glycemic control of diabetic patients and in the prevention and management of this progressive disease

[4]. This is because of their effectiveness, minimal undesirable side effects and comparatively low costs. In this regard, World Health Organization (WHO) commends the use of natural products for diabetic management [5].

Quince (*Cydonia oblonga* Mill.) belongs to the Rosacea family and is one of the medicinal plants that have been used in Iranian and Turkish traditional medicines for the management of diabetes [6-8]. Quince is a native plant in Iran and Turkey, and is popular for its nutritional and medicinal uses. Its fruit has a variety of valuable phytochemicals and has been used as a dietary source. The fruit of quince exhibited a broad spectrum of pharmacological activities such as antioxidant effect due to diverse phenolic compounds and flavonoids [9, 10]. It has been also reported that the fruit possesses anti-bacterial, anti-fungal, anti-hemolytic, anti-allergic, anti-ulcerative, anti-inflammatory, hepatoprotective, cardioprotective, and geno-protective effects [6, 11-17]. Recently, two research groups described antiproliferative activity of quince fruit against human kidney and colon cancer cells and suggested that a quince-rich regimen may be a promising agent for cancer prevention and treatment [18, 19]. In this regard, we have previously demonstrated the preventive effect of quince fruit against hepatocellular carcinoma in rats [20].

We have previously shown that the aqueous extract of quince fruit prevented diabetes-related complications in streptozotocin-induced diabetic rats [8]. Our results revealed that quince fruit possesses hypolipidemic, hepatoprotective, and renoprotective effects in diabetic rats. Since hyperglycemia triggers a cascade of events that eventually leads to the diabetes complications, it can be concluded that the ameliorating effects of quince in these abnormalities may be originated from its antihyperglycemic activity [21]. Hence, the present study was designed to evaluate the potential antihyperglycemic activity of aqueous extract of *Cydonia oblonga* Mill. fruit (ACO) in streptozotocin-induced diabetic rats. We have also evaluated the antihyperglycemic effects of two major fractions of ACO to identify the active fraction and to examine the chemical composition of the active fraction by gas chromatography-mass spectrometry (GC-MS) assay.

2. Materials & Methods

2.1. Chemicals

Streptozotocin, gallic acid, quercetin, methanol and chloroform were purchased from Sigma-Aldrich Co. (Taufkirchen, Germany).

2.2. Preparation of ACO

Cydonia oblonga Mill. fruits were collected in October 2015 from Shahriar, Alborz province, Iran and were authenticated by Mr. Mohammad Kamalinejad, a qualified botanist at, Shahid Beheshti University of

Medical Sciences (8054, voucher specimen in Shahid Beheshti University of Medical Sciences Herbarium, Tehran, Iran). The fresh fruits were cleaned with their peels and the extraction was carried out by the maceration of 100 g fruit with 900 mL distilled water for 30 min. The resulting extract was evaporated by placing in water bath 90°C. Finally, the extract was filtered and was kept at -20°C until use. The extract was dissolved in distilled water to receive desired concentrations just before use. The moisture level of the extract was determined by weight loss after placing 2 g of the final extract in an oven at 60–65°C for 72 h. The final extract contained 24% water [22].

2.3. Standardization of Extract

Total polyphenol content was determined by spectrophotometry, using gallic acid as the standard based on the Folin-Ciocalteu method [23]. Total phenolic content was 49.13 ± 1.23 mg gallic acid equivalents (GAE) per gram of ACO (mg of GAE/g of plant extract). Total flavonoid content was measured with the aluminum chloride colorimetric assay [24]. Quercetin was used as the standard and flavonoid contents were expressed as mg of quercetin equivalent per gram of ACO. Flavonoid contents were 15.34 ± 0.93 mg quercetin equivalents (QE) per gram of ACO (mg of QE /g of plant extract).

2.4. Preparation of ACO fractions

To prepare the methanolic fraction (MF), the fruit extract (5 g) was re-extracted with methanol (35 mL) under shaking for 4 h at an ambient temperature in a conical flask. The mixture was filtered and the filtrate was concentrated under a reduced pressure to get prepared for the experiment. Afterward, the semi-solid residue of the first fractionation (1.02 g) was further extracted with moderately polar solvent, chloroform [25].

2.5. Animals

Male Sprague-Dawley rats weighing 200 to 250 g were housed in ventilated plastic cages over PWI 8-16 hardwood bedding. There were 12 air changes per h, 12 h light photoperiods, an environmental temperature of 21–23°C, and a relative humidity of 50–60%. The animals were fed a standard normal chow diet and given tap water ad libitum. In the present study, the principles of laboratory animal care (NIH publication No. 85-23, revised 1985) were followed.

2.6. Induction of diabetes in rats

Experimental diabetes was induced in rats subsequent an overnight fast, by a single intraperitoneal (i.p.) injection of 60 mg/kg streptozotocin freshly prepared in citrate buffer (0.1M, pH 4.5) [26]. Non diabetic control animals

were injected with vehicle only. Diabetes was confirmed 4 days after injection by measuring the tail vein blood glucose level. Only the animals with fasting blood glucose (FBG) levels ≥ 250 mg/dl were selected for the study.

2.7. Experimental protocol and study design

Initially, the three doses of 80, 160 and 240 mg/kg body weight daily were chosen based on our previous study [8]. Normal and diabetic rats were divided into six groups of six rats ($n = 6$). Group-1 non diabetic rats served as normal control group; Group-2 served as untreated diabetic control; Group-3 diabetic rats were treated with glibenclamide as a positive control at 10 mg/kg body weight. Groups- 4, 5, 6 diabetic rats were treated with ACO at 80, 160, and 240 mg/kg body weight, respectively. The different concentrations of the extract and glibenclamide were administered orally once daily using an intragastric tube for 4 weeks. The hypoglycemic effects of two major fractions of ACO were evaluated in diabetic rats by the measurement of FBS at time 0 and intervals of 1, 3, 5, and 7 h after single oral administration of the fractions (240 mg/kg body weight).

2.8. Determination of blood glucose level

Blood samples were collected from the tail vein of the overnight (12–15 h) fasting rat for the measurement of blood glucose level using a standardized glucometer (ARKAY, INC., Japan) [8].

2.9. GC-MS analysis of bioactive compounds

The MF of the extract was subjected to GC-MS for the identification of bioactive volatile compounds. The analysis was carried out on an Agilent model, 6890 GC interfaced to a 5975C mass detector employing the following conditions:

HP-5MS capillary column (30m $\times$ 0.25mm $\times$ 0.25 μ m film thickness; Agilent, USA) was used. For the GC-MS detection, an electron ionization system with ionization

energy of 70 eV was adopted. Helium gas (99.999%) was the carrier gas at a flow rate of 1 mL/min. The oven temperature was operated as follows: 50°C for 2 min, then raising at the rate of 5°C/min up to 280°C maintained for 10 min. Injector temperature and injection volume were 250°C and 1 μ L, respectively. The relative percentage amount of each component was calculated by comparing its average peak area to the peak area of the most abundant component. Identification of compounds was based on comparisons of the relative retention indices and mass spectra with those of the Wiley and NIST library data standards of the GC-MS system. Retention indices were determined by analyzing a solution covering the homologous series of normal alkanes (C7–C22) and then calculated as described by van Den Dool and Kratz [27].

2.10. Statistical analysis

The homogeneity of variances was tested using Levene's test. The results were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD as the post hoc test. FBG data in days 0 and 28 in each group were statistically preceded by means of Student's t-test. The data were presented as the mean $\pm$ SD ($n=6$). The minimum level of significance chosen was $P < 0.05$.

3. Results

3.1. Effects of ACO on FBG levels

Our results showed that the oral administration of different concentrations of ACO on diabetic rats caused a significant decrease in FBG in a dose-dependent manner and the concentration of 240 mg/kg body weight/day was more effective than the other two concentrations ($P < 0.001$). However, the different concentrations of ACO were not as effective as glibenclamide, a standard antihyperglycemic drug ($P < 0.001$) (Table 1 and Fig. 1).

Table 1. Hypoglycemic effects of the different concentrations of ACO in streptozotocin-induced diabetic rats

	Fasting Blood Glucose (mg/dl)		
	Day 0	Day 14	Day 28
Normal rats	120 $\pm$ 10	102 $\pm$ 21	115 $\pm$ 18
Diabetic rats	373 $\pm$ 21***	377 $\pm$ 31***	376 $\pm$ 18***
+ Glibenclamide	380 $\pm$ 20 ^{NS}	258 $\pm$ 31 ^{###}	174 $\pm$ 16 ^{###}
+ ACO (80 mg/kg)	382 $\pm$ 36 ^{NS}	369 $\pm$ 29 ^{NS, ΦΦΦ}	343 $\pm$ 18 ^{NS, ΦΦΦ}
+ ACO (160 mg/kg)	385 $\pm$ 37 ^{NS}	338 $\pm$ 13 ^{NS, ΦΦΦ}	306 $\pm$ 28 ^{###, ΦΦΦ}
+ ACO (240 mg/kg)	382 $\pm$ 19 ^{NS}	330 $\pm$ 33 ^{NS, ΦΦΦ}	236 $\pm$ 23 ^{###, ΦΦΦ}

Data are presented as mean $\pm$ SD.

*** $P < 0.001$ compared to normal group in corresponding time

$P < 0.001$ compared to diabetic group in corresponding time

ΦΦΦ $P < 0.001$ compared to glibenclamide group in corresponding time

NS: Not Significant

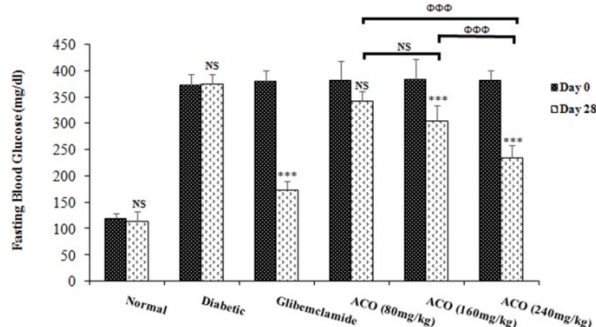


Figure 1. Hypoglycemic effects of the different concentrations of ACO in diabetic rats at the start and end of the experiment. Data are presented as mean±SD. *** P < 0.001 compared to day 0 in corresponding group; ΦΦΦ P < 0.001 compared to the other concentration at the same time; NS: Not Significant.

3.2. Effects of ACO fractions on FBG levels

In the current study, the effects of ACO fractions on the glucose levels of diabetic rats were evaluated for a

period of 7 h. The fractions were only used as an oral single-dose (240 mg/kg) and FBG was excellently decreased in MF-treated rats; whereas chloroform fraction (CF) didn't indicate any significant effect on the glucose levels (Fig. 2).

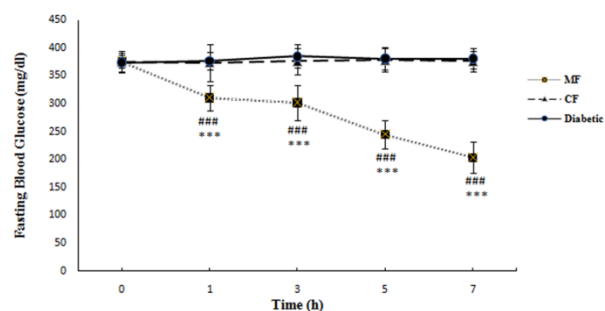


Figure 2. Hypoglycemic effects of two major fractions of ACO in diabetic rats. Data are presented as mean±SD. MF: methanolic fraction-treated diabetic rats. CF: chloroform fraction-treated diabetic rats. ### P < 0.001 compared to diabetic group in corresponding time; *** P < 0.001 compared to CF-treated rats in corresponding time.

Table 2. Identified compounds from the methanolic fraction of *Cydonia oblonga* Mill. fruit extract

No.	Retention Time (min)	Name of compound	Molecular formula	MW	Area (% of maximum peak)	Area (%)	RI ^a
1	6.504	Furfural	C ₅ H ₄ O ₂	96	13.48	4.6	858
2	10.103	5-Methyl furfural	C ₆ H ₆ O ₂	110	2.96	1.01	978
3	10.527	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	C ₆ H ₈ O ₄	144	1.92	0.65	991
4	13.434	Furaneol	C ₆ H ₈ O ₃	128	4.91	1.68	1090
5	15.831	Dihydroxymaltol	C ₆ H ₈ O ₄	144	20.06	6.9	1100
6	17.229	Desulphosinigrin	C ₁₀ H ₁₇ NO ₆ S	279	4.41	1.51	1214
7	18.777	5-Hydroxymethylfurfural	C ₆ H ₆ O ₃	126	100.00	34.2	1271
8	20.207	3-Methyl-2-furoic acid	C ₆ H ₆ O ₃	126	24.76	8.5	1341

^a Retention Indices were calculated by a homologous series of normal alkanes (C7–C22)

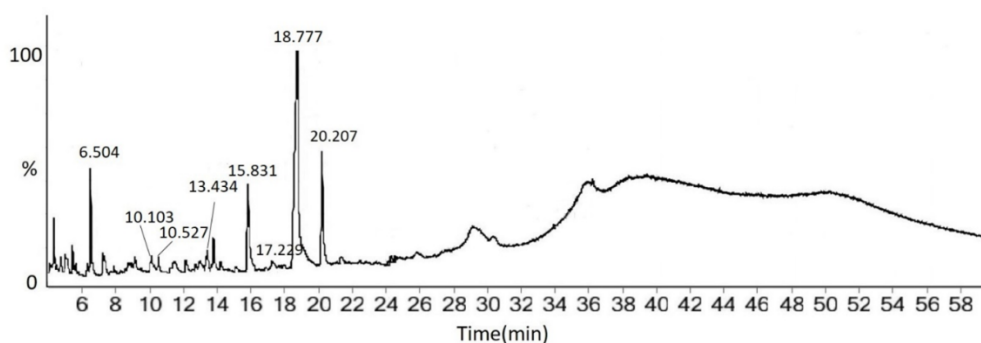


Figure 3. GC-MS chromatogram of the methanolic fraction of *Cydonia oblonga* Mill. fruit extract

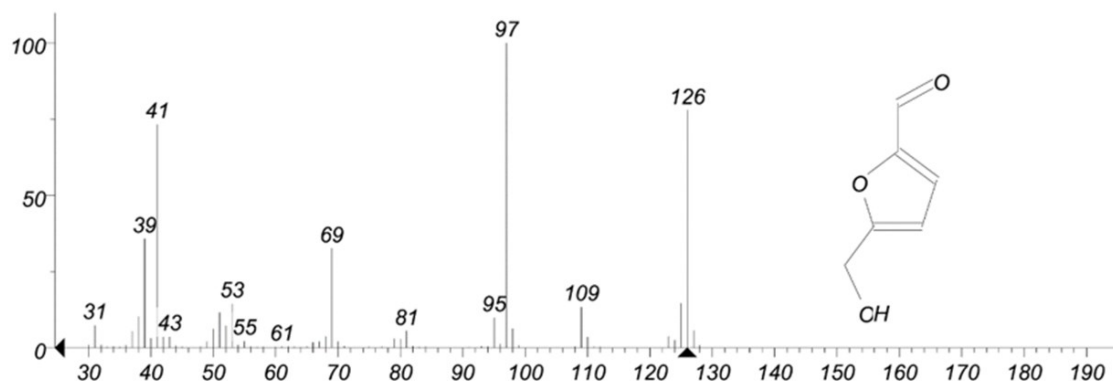


Figure 4. Mass spectrum of 5-HMF

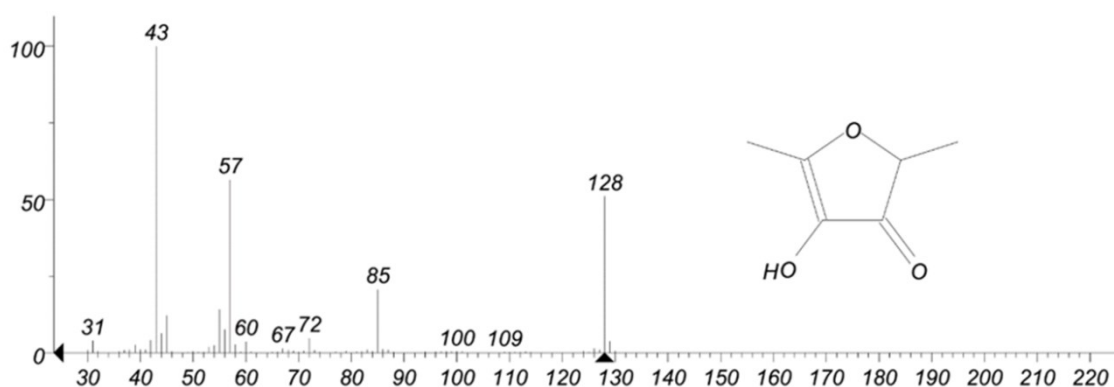


Figure 5. Mass spectrum of DMHF

3.3. GC-Mass analysis of the methanolic fraction of ACO

Based on the GC-MS analysis, the MF of the fruit extract contained more than twenty compounds. The chemical components of those were identified, the percentage of peak area of each constituent and their retention times and indices were summarized in the Table 2. The total ion chromatogram of the MF and the mass spectra of two main components were depicted in Figs. 3-5, respectively. The main components of the MF were identified as furfural (4.6%), dihydroxymaltol (6.9%), 5-hydroxymethylfurfural or 5-HMF (34.2%), and 3-methyl-2-furoic acid (8.5%). Among them, the most abundant compound found was 5-HMF.

4. Discussion and Conclusion

This is the first report showing antihyperglycemic activity of *Cydonia oblonga* Mill. fruit in streptozotocin-induced diabetic rats. Hyperglycemia is the defining feature of diabetes mellitus that can result in microvascular and macrovascular diabetic

complications which might be disabling or even life-threatening [2]. Therefore, many attempts have been made to control the glucose levels of diabetic patients and glycemic control is the basic principal of diabetes management. In addition to different classes of antihyperglycemic drugs, herbal medicines have been utilized to manage diabetes mellitus and its co-morbid conditions throughout human history.

Aslan *et al.* (2010) revealed that the methanolic extract of quince leaves at the dose of 500 mg/kg body weight twice daily for 5 consecutive days had antihyperglycemic property in streptozotocin-induced diabetic rats [7]. The results of our previous study indicated that ACO (80, 160 and 240 mg/kg body weight daily for 6 weeks) attenuated dyslipidemia in diabetic rats and efficiently reduced serum triglyceride, total cholesterol, and LDL-C that was accompanied with the elevated levels of HDL-C. The fruit extract also significantly reduced the biochemical markers of liver and renal damage in diabetic rats, including ALT, AST, ALP, urea, and creatinine [8]. Since the elevated levels of glucose in diabetes lead to the development and progression of diabetes complications [28], it can be suggested that the

hypolipidemic, hepatoprotective, and renoprotective effects of ACO in diabetic rats may be arisen from the antihyperglycemic activity of the fruit.

It is evident from our results that ACO exhibited significant antihyperglycemic effect in a dose-dependent manner and the highest dose of the ACO (240 mg/kg) caused a significant decrease of about 38.2% in FBG after 4 weeks of treatment. In addition, the doses of 80 and 160 mg/kg exhibited 10.2% and 20.5% decline in FBG, respectively. On the other hand, glibenclamide, a standard oral hypoglycemic agent, showed a better antihyperglycemic effect compared to the different doses of ACO and presented 54.2% reduction in FBG at the end of the experiment (Fig. 1).

In the second part of the study, the antihyperglycemic effects of ACO derived fractions were evaluated to identify the active fraction in diabetic rats. In order to achieve the fractions, two different organic solvents, methanol and chloroform, were used due to their different polar natures. The MF showed a potent antihyperglycemic effect compared to chloroform fraction during the experiment. So, it was concluded that MF contains active constituents responsible for the antihyperglycemic effect of ACO.

Previous studies have identified numerous volatile compounds in quince fruit that impart characteristic odor to the fruit [29-31]. So in the current study, GC/Mass analysis was used for the detection of the active constituents of MF. The results of our investigation revealed that this fraction contains 5-hydroxymethylfurfural known as 5-HMF. 5-Hydroxymethyl-2-furancarboxaldehyde (5-HMF) is a naturally occurring compound of plant origin found in different natural resources. The present study is the first report showing that *Cydonia oblonga* Mill. fruit contains 5-HMF at a high concentration. The compound exhibited various biological properties, including antioxidant, cytoprotective, anti-atherosclerosis, and antitumor activity [32-35]. Furthermore, this active compound protected human vein epidermal cell against glucose and hydrogen peroxide-induced liver damage in mice [36]. It has been also reported that 5-HMF inhibited high glucose-induced oxidative stress in human umbilical vein endothelial cells and it was concluded that 5-HMF can be a promising agent for the prevention of diabetes and its associated vascular diseases [37]. Considering 5-HMF has inhibited the toxic consequences of glucose and our results showed the antihyperglycemic activity of ACO; it can be suggested that 5-HMF may be one of the active components responsible for the glucose-lowering effect of quince fruit. Data in our study will definitely encourage future studies to increase our understanding

on the antidiabetic and antihyperglycemic potential of 5-HMF.

2, 5-dimethyl-4-hydroxy -3(2H)-furanone (DMHF), also known as furaneol is another compound detected in the MF of the extract. DMHF is an aroma compound found in many fruits and processed foodstuffs, including pineapples, mangos, grapes, raspberries, and strawberries and is widely used as a food flavoring [38]. DMHF exhibited antioxidant, anti-cataract, anti-carcinogenic, antifungal, and antimicrobial effects in different *in vivo* and *in vitro* models [38]. As shown in Table 2, six additional natural compounds were identified in quince fruit. However, GC/Mass analysis only detects volatile components of the fraction and other groups of compounds were not identified. Therefore, further complementary studies are required to establish what kinds of molecules are responsible for the antidiabetic and antihyperglycemic effects of quince fruit and to fully explain the mechanisms underlying the effect.

The different kinds of phenolic acids and flavonoids are present in quince fruit, including caffeoylquinic acids, kaempferol 3- glucoside, rutin, kaempferol-3-rutinoside, and quercetin 3-galactoside [9]. It has also been reported that this fruit contains 21 amino acids, vitamin C, pectin, tannins, and minerals such as calcium, sodium, potassium, and phosphorus [6, 39]. Polyphenolic compounds are natural phytochemicals that have been used to treat and prevent diabetes and affect various aspects of this chronic disease [40]. Polyphenols indicated remarkable antihyperglycemic effects in *in vitro* and *in vivo* models through different mechanisms. These natural compounds play beneficial roles in carbohydrate metabolism and glucose homeostasis by decreasing intestinal absorption of dietary carbohydrate and modulation of the enzymes involved in glucose metabolism [41]. In addition, they also improve β -cell function and stimulate insulin secretion [40, 42]. It has been also reported that phenolic acids, flavonoids, and tannins inhibited α -glucosidase and α -amylase, which are responsible for the digestion of dietary carbohydrates to glucose [40-43]. In this study, the extract was prepared from whole fruit and about 5% of the total weight related to polyphenols. Therefore, it can be concluded that the antihyperglycemic activity of quince fruit may be, at least partly, mediated through the hypoglycemic effects of polyphenols. Additionally, several studies reported the preventive effects of polyphenols against complications induced by diabetes [44, 45]. So, it can be said that the protective effects of quince fruit in our earlier study may be mediated by 5-HMF and/or polyphenols that present in the fruit [8].

To conclude, these findings clearly demonstrated that the fruit of quince has antihyperglycemic activity in

diabetic rats and the MF of the aqueous extract is the active fraction. Therefore, this valuable fruit can be considered as a suitable natural product for the management of diabetic patients and their glycemic control. This activity may be mediated through 5-HMF and/or polyphenols. Further studies to find out the active constituents and synergy among different compounds present in quince for controlling hyperglycemia would be complementary to this study.

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Conflict of interest

There is no conflict of interest.

Ethics

All experiments were conducted according to the ethical standards and protocols approved by the Committee of Animal Experimentation of Zanjan University of Medical Sciences, Zanjan, Iran (protocol approval number: ZUMS.REC.1392.221).

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