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Core structure of flavonoids precursor as an antihyperglycemic and antihyperlipidemic agent: an *in vivo* study in rats

Mahmoud Najafian[1], Azadeh Ebrahim-Habibi[2], Parichehreh Yaghmaei[1]✉, Kazem Parivar[1] and Bagher Larijani[2]

Science and Research Branch, Islamic Azad University, Tehran, Iran; Endocrinology and Metabolism Research Center, Tehran University of Medical Sciences, Tehran, Iran

***trans*-Chalcone is the core structure of naringenin chalcone, located halfway in the biosynthesis pathway of flavonoids. Flavonoids have been reported as mammalian alpha-amylase inhibitors, a property which could be useful in the management of postprandial hyperglycemia in diabetes and related disorders. As a mammalian alpha-amylase inhibitor *in vitro*, the putative beneficial effect of *trans*-chalcone on diabetes was tested in a streptozotocin-induced rat model of diabetes type 1, and the results analyzed with commonly used statistical methods. Significant reduction of blood glucose levels and beneficial effect on dyslipidemia were observed in diabetic rats, as well as reduction of disturbing consequences of diabetes such as high urine volume and water intake. *trans*-Chalcone was observed to have a weight loss-inductive effect, alongside with a reduction in food intake, which is suggestive of a therapeutic potential of this compound in overweight and obese patients.**

Keywords: *trans*-chalcone, diabetes, benzylideneacetophenone, alpha-amylase inhibitor, hyperglycemia, hyperlipidemia

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INTRODUCTION

According to the International Diabetes Federation (IDF) (http://www.diabetesatlas.org), the global prevalence of diabetes is predicted to grow from 6.6% in 2010 to 7.8% in 2030, and the global prevalence of impaired glucose tolerance from 7.9% in 2010 to 8.4% in 2030. Translation of these percentages to numbers would give 439 million people suffering from diabetes in 2030, and 472 million with impaired glucose tolerance. Diabetes is cited in the IDF report as being the fourth or fifth leading cause of death in most high-income countries and an epidemic disease in economically developing and newly industrialized nations. Its chronic characteristic leads to progressive complications which affect life quality, and as such needs to be attended to in preventive and therapeutic means.

Among the multiple molecular targets that are aimed at in diabetes, hydrolase enzymes acting in carbohydrate digestion are currently gaining more attention, since postprandial hyperglycemia is known to be an important factor in diabetic complications (Ceriello, 2005; Rebolledo & Actis Dato, 2005). The first inhibitor of α-glucosidase to be commercialized, acarbose, has been shown to be safe and maintain its effect in the long-term (Rosak & Mertes, 2009). It may also reduce the risk of cardiovascular disease by influencing insulin and pro-insulin as risk factors of cardiovascular dysfunction (Rosak & Mertes, 2009). The acarbose effect in the control of postprandial hyperglycemia is suggested to make this compound a potential preventive aid in metabolic syndrome complications (Yamagishi *et al.,* 2005). Acarbose is also an inhibitor of α-amylase, although in micromolar concentrations (Yoon & Robyt, 2003), and other well-known inhibitors of α-amylase have been shown to be effective in the control of postprandial hyperglycemia, while devoid of the diarrhea side effect (Boivin *et al.,* 1988; Lankisch *et al.,* 1998) associated with acarbose (Godbout & Chiasson, 2007).

Classical inhibitors of mammalian α-amylase include protein and carbohydrate-like structures, the latter mainly based on acarbose (Machius *et al.,* 1996). In recent years, small molecules have also been studied in this regard, and among these flavonoids have been reported to exhibit such activity (Kim *et al.,* 2000; Tadera *et al.,* 2006; Lo Piparo *et al.,* 2008).

Flavonoids are a widely studied group of natural polyphenolic compounds which have been attributed a variety of therapeutical properties such as: antiatherosclerotic, anti-inflammatory, antitumor, antithrombogenic, antiosteoporotic, and antiviral (Nijveldt *et al.,* 2001). Flavonoid-rich fractions of plants have also been reported to be effective as antihyperglycemic and antihyperlipidemic agents in animal models of diabetes (Aslan *et al.,* 2007; Li *et al.,* 2007; Sharma *et al.,* 2008). *trans*-Chalcone is a precursor of flavonoids in plants (Verhoeyen *et al.,* 2002; Ferrer *et al.,* 2008). Based on the similarity of its structure to that of the flavonoids (*trans*-chalcone could be considered a simplified flavonoid), its inhibitory effect toward mammalian α-amylase was investigated. The compound was found to be effective in the micromolar range (Najafian *et al.,* 2010). The current study was therefore designed to observe whether this compound was also effective *in vivo*. Rats with streptozotocin-induced diabetes (usually considered as a model of type 1 diabetes mellitus) were treated with four doses of the compound, which showed actual benefit in lowering serum glucose and lipid levels.

✉e-mail: yaghmaei_p@srbiau.ac.ir

Abbreviations: D, diabetic rats; DO, diabetic control group; DCh2, DCh8, DCh16, and DCh32, diabetic rats receiving chalcone 2, 8, 16, or 32 mg/kg respectively; ND, non-diabetic rats; NDO, non-diabetic control rats; NDCh2, NDCh8, NDCh16, NDCh32, non-diabetic rats receiving chalcone respectively, 2, 8, 16, or 32 mg/kg; HDL, high-density lipoproteins; LDL, low-density lipoproteins; STZ, streptozotocin; TG, triglycerides; VLDL, very low-density lipoproteins

MATERIAL AND METHODS

Chemicals. *trans*-Chalcone (benzylideneacetophenone) was from Sigma Chemical Co. (St. Louis, MO, USA) and streptozotocin from Pharmacia & Upjohn (Kalamazoo, MI, USA).

Animals. Male adult Wistar rats (2.5 months) weighting 200 ± 15 g were used in these experiments. The animals were housed as six rats per cage at room temperature (22–24 °C) with lights on from 08:00 to 20:00 h. Rats received standard rodent diet: maintenance diet Letica, Panlab S.L. (Barcelona, Spain); 61.4% (w/w) carbohydrate (100% starch), 3.9% fibre, 15.1% protein and 2.7% fat, and tap water. Food and water were *ad libitum*.

Diabetes induction. Diabetic condition was induced by the use of a single dose of streptozotocin (STZ) (70 mg/kg body weight) dissolved in a citrate buffer (0.1 mol/l), pH 4.5. STZ was administered intraperitoneally on the first day of experiment according to existing protocols (Portha *et al.,* 1974; 1989; Tormo *et al.,* 2004), and the blood glucose levels were measured after 2 days. The ethical aspect of the experimental protocol was approved by the ethical committee of the Science and Research Branch of Islamic Azad University (Tehran, Iran).

Treatment with *trans*-chalcone. Rats were divided into two classes (non-diabetic rats (ND) and diabetic rats (D)) and each class divided into five groups (n = 6), as defined below:

(I) Non-diabetic control group (NDO): rats in this group received grape seed oil (O) orally for 24 days through a gastric cannula in a single dose (0.5 ml) at 8:30 a.m. (II) Diabetic control group (DO): rats in this group received grape seed oil (O) as group I. (III) Non-diabetic groups receiving *trans*-chalcone (NDCh): rats in these groups received *trans*-chalcone at 2, 8, 16, 32 mg/kg body weight (respectively, NDCh2, NDCh8, NDCh16, NDCh32) dissolved in grape seed oil, orally for 24 days through a gastric cannula in a single dose (0.5 ml) at 8:30 a.m. (IV) Diabetic groups receiving *trans*-chalcone (DCh): rats in these groups received *trans*-chalcone at 2, 8, 16, 32 mg/kg body weight (respectively, DCh2, DCh8, DCh16, and DCh32) as above. Throughout the manuscript, chalcone doses of 2, 8, 16, 32 mg/kg body weight are shown as Ch2, Ch8, etc.

Measured parameters. As rats were fed at night *ad libitum*, ingestion of food and water, as well as urine volume was measured every morning at 9:00 a.m. Blood glucose levels were measured in 2 μl blood samples extracted from the tail of the animal every two days on mornings at 9:00 a.m. with a glucometer (One Touch Profile, Life Scan). Serum insulin levels were determined three times during the experiment period, that is at day one, day 12 and day 24, with the use of insulin kits (DRG, France), using double-antibody enzyme-linked immunosorbent assay (ELISA). At the end of experiment period, the body weight of all animals was measured and they were sacrificed under light ether anesthesia. The abdomen was cut open, and blood collected from heart. Blood samples were placed on ice and centrifuged within 15 min after collection at 3000 × *g* for five minutes. Serum was stored at –20 °C for less than 1 week before subsequent analyses. Standard biochemical methods were used to measure cholesterol (Deeg & Ziegenhorn, 1983), triglycerides (Cole *et al.,* 1997), high-density lipoproteins (HDL) (Wiebe & Warnick, 1997), low-density lipoproteins (LDL) (Bachorik, 1997) concentrations, and α-amylase activity (Kruse-Jarres *et al.,* 1989). Estimates of very low density lipoprotein (VLDL) were calculated from the formula VLDL-C = triacylglycerol/5 (Friedewald *et al.,* 1972)

Statistical analysis. Standard deviation was calculated in all cases and results expressed as mean ± SD. The data were analyzed by one-sample Kolmogrov-Smirnov test and then by the Levene's test. One way analysis of variance (ANOVA) followed by Tukey's *post hoc* test for multiple comparisons were used to compare difference between experimental groups. The criterion for statistical significant was $P<0.05$.

RESULTS AND DISCUSSION

Glucose

Administration of STZ resulted in high levels (405.6 ± 41.9 mg/dl) of blood glucose after 2 days in the treated group in contrast with the non-diabetic control group in which blood glucose levels were in the range of 104.8 ± 11.9 mg/dl.

In our previous *in vitro* experiments, *trans*-chalcone, being virtually insoluble in aqueous medium was dissolved in dimethyl sulfoxide (Najafian *et al.,* 2010). Given the hydrophobic character of the compound, it was necessary to choose a suitable hydrophobic vehicle for its administration to rats, hence the choice of grape seed oil. Grape seed ethanolic extract has been suggested to

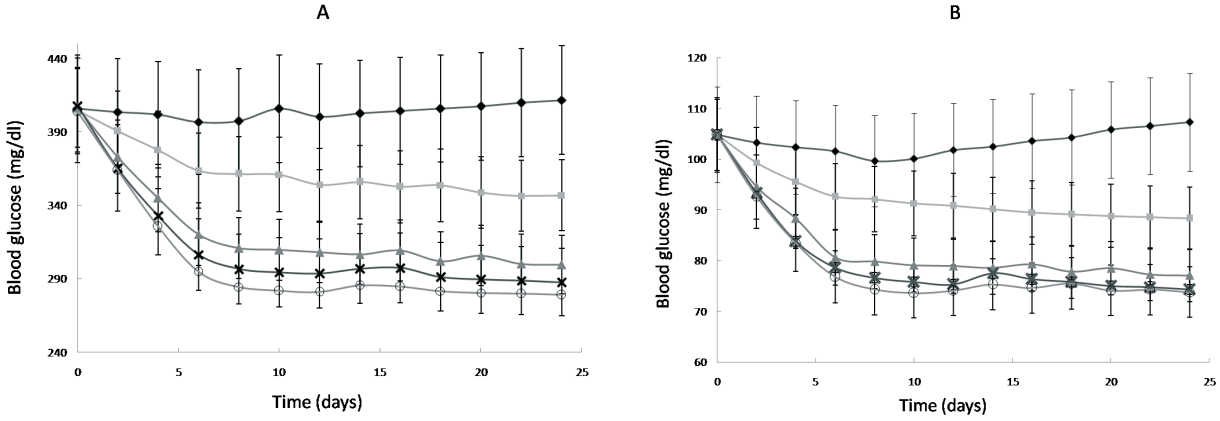

Figure 1. Effect of *trans*-chalcone on blood glucose in diabetic and non-diabetic rats
Rats were given grape seed oil (◆) or chalcone dissolved in oil of doses 2 (■), 8 (▲),16 (**x**), and 32 (○) mg per kg body weight for 24 days. Values are means ± S.D. **(A)** Diabetic rats. **(B)** Non-diabetic rats.

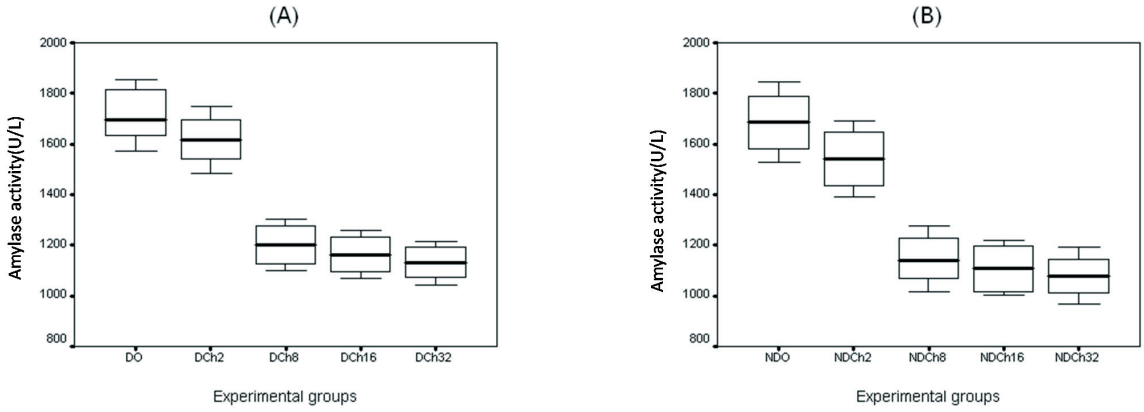

Figure 2. Effect of *trans*-chalcone treatment on α-amylase activity in diabetic and non-diabetic rats
(A) Diabetic rats. Using one way ANOVA with Tukey–Kramer test at $P<0.05$, the following results were obtained for the data**:** DCh8 is significantly different from DO and DCh2; DCh16 is significantly different from DO and DCh2, DCh32 is significantly different from DO and DCh2. **(B)** Non-diabetic rats. Using one way ANOVA with Tukey–Kramer test at $P<0.05$ following results were obtained: NDCh8 is significantly different from NDO and NDCh2; NDCh16 is significantly different from NDO and NDCh2; NDCh32 is significantly different from NDO and NDCh2.

be beneficial in a rat model of type 2 diabetes (Hwang *et al.,* 2009), and grape seed oil content analysis has shown the presence of phytosterol (in concentrations between 5179 and 5480 mg/kg) (Navas, 2009). However, the main component of grape seed "oil" seems to be linoleic acid, as observed in different varieties (Tangolar *et al.,* 2007; Navas, 2009). In the current study, 0.5 ml daily administered grape oil was found to be devoid of any effect on blood glucose levels of both diabetic and normal rats. In fact, these two groups showed unchanged blood glucose levels: in the non-diabetic control group (NDO) and in the diabetic control group (DO) blood glucose remained constant throughout the experimental period at about 104.8–107.3 mg/dl and 405.6–411.6 mg/dl respectively (Fig. 1).

A decrease in glycemia was observed from day 2 after *trans*-chalcone administration at all the doses used. In each group this decrease occurred rapidly until day 6 and the blood glucose levels reached then were maintained until the end of the treatment (day 24). The effect appears to be dose-dependent, but there was no significant difference between the two doses of 16 and 32 mg/kg (Fig. 1). An interesting finding was that blood glucose levels were also decreased in the non-diabetic rats taking chalcone (Fig. 1B).

In this study, animals were not subjected to fasting before taking blood samples, thus the α-amylase inhibitory property of *trans*-chalcone could have a role in the observed effect on the blood glucose levels. In a recent report, an effect of *trans*-chalcone and some of its derivatives has been shown on transient hyperglycemia induced by oral glucose gavage in normal rats. In that case, some chalcone derivatives were found to act in a similar manner as insulin (Alberton *et al.,* 2008), suggesting the possibility of multiple targeting for these compounds.

α-Amylase

trans-Chalcone was found to be a competitive inhibitor of mammalian α-amylase *in vitro* (Najafian *et al.,* 2010); but given the complexity of *in vivo* systems, it could not be assumed to have the same effect when administered orally in a living organism. This is why the serum α-amylase activity was also measured in this study (Fig. 2). Interestingly, α-amylase activities of the diabetic and non-diabetic groups are comparable, as is the decrease in this activity, which was found to be significantly higher for the Ch8, Ch16 and Ch32 doses in comparison with the Ch2 dose while the difference between the three mentioned doses was not significant. This is possibly indicative of a saturation in the inhibitory effect of *trans*-chalcone toward α-amylase. At any rate, this experiment shows that at least part of this compound's effect is exerted through inhibition of α-amylase *in vivo*.

Insulin

Serum insulin levels of diabetic and non-diabetic rats sampled three times during the experiment are shown in Fig. 3**.** After STZ administration, serum insulin levels were significantly reduced in diabetic rats compared with non-diabetic rats, that is, 2.45 ± 0.26 *vs.* 3.85 ± 0.41 ng/ml ($P<0.01$)**.** In both control groups of diabetic and non-diabetic rats administered grape seed oil alone, the serum insulin levels remained nearly constant throughout the experiment. On the other hand, in both the diabetic and non-diabetic groups, given chalcone was administrated, halfway through the experiment (day 12)**,** the serum insulin levels were significantly reduced. For example in DCh2 and NDCh2 a reduction was observed from 2.45 ± 0.26 to 1.61 ± 0.18 ng/ml ($P<0.01$) and from 3.85 ± 0.41 to 2.95 ± 0.18 ng/ml ($P<0.01$), respectively. In all groups this decline in serum insulin level was observed to be dose-dependent of chalcone from 2 mg/kg to 16 mg/kg, while there was no significant difference between Ch16 and Ch32. From day 12 of experiment, until the last day (day 24) the serum insulin levels increased slightly.

It is interesting to note a similar observation made about the effect of a well studied food product containing an α-amylase inhibitor, namely kidney bean (*Phaseolus vulgaris*), on rats. In that study, insulin plasma levels decreased when rats were fed kidney beans, while the amount of mRNAs for the insulin receptor and GLUT-4 increased in a specific muscle type (Knott *et al.,* 1992). The effect observed in the current study must be associated with the presence of chalcone and is independent of the pathological condition of diabetic rats, since it is also observed in non-diabetic rats, although the effect is less pronounced. Considering the fact that some chalcone derivatives act similarly to insulin-like compounds (Al-

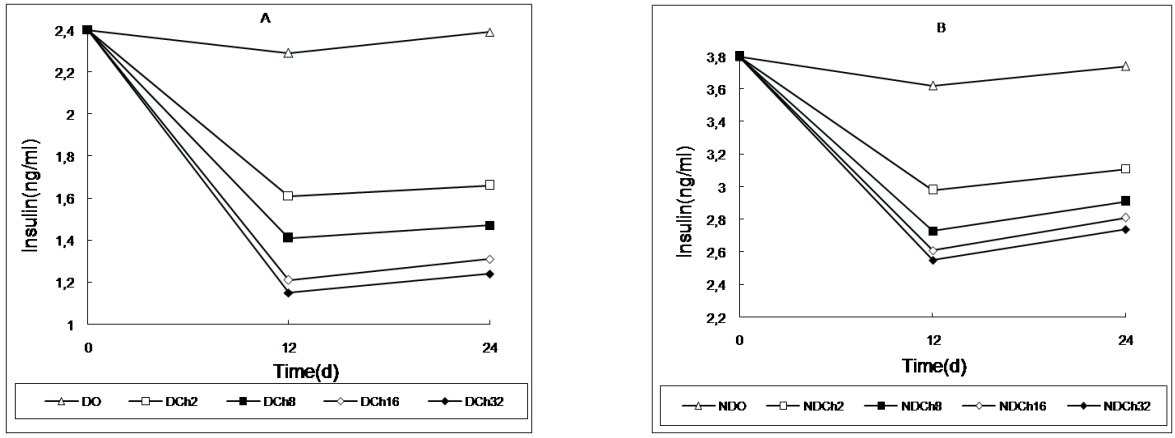

Figure 3. Effect of *trans*-chalcone treatment on plasma insulin levels in rats
(A) Diabetic rats. **(B)** Non-diabetic rats.

Table 1. Effect of chalcone treatment in serum lipids of streptozotocin-induced diabetic rats
Data are expressed as mean ± S.D. for six rats. a, significantly different from diabetic control group (DO); b, significantly different from DCh2; c, significantly different from DCh8; d, significantly different from DCh16 using one way ANOVA with Tukey–Kramer test at $P<0.05$.

Serum level (mg/dl)	NDO	DO	DCh2	DCh8	DCh16	DCh32
Cholesterol	85.5 ± 11.5	125.5 ± 17.2	103.8 ± 14.9	95.5 ± 15.1[a]	93.3 ± 17.3[a]	95.1 ± 15.6[a]
Triglycerides	63.5 ± 8.4	119.6 ± 18.9	89.5 ± 14.2	76.6 ± 10.7[a]	78.3 ± 12.6[a]	275.5 ± 34.4[a,b,c,d]
VLDL-C	12.7 ± 1.7	23.9 ± 3.8	17.9 ± 2.8	15.3 ± 2.1[a]	15.6 ± 2.5[a]	55.1 ± 6.9[a,b,c,d]
LDL-C	22.5 ± 4.6	41.6 ± 9.2	27.6 ± 4.6[a]	22.3 ± 5.4[a]	25.6 ± 6.0[a]	25.5 ± 5.7[a]
HDL-C	44.5 ± 9.6	23.3 ± 5.8	34.5 ± 7.3	38.0 ± 7.8[a]	41.6 ± 8.2[a]	40.1 ± 8.4[a]

berton *et al.,* 2008) it could be suggested that the effect observed on plasma insulin could be a result of interactions between *trans*-chalcone and insulin receptors, possibly leading to down-regulation of insulin secretion. This is of course a pure speculation and needs further evidence.

Lipids

Dyslipidemia is observed as a consequence of both type 1 (Verges, 2009) and type 2 (Gosavi *et al.,* 2006) diabetes, and could contribute to an increased incidence of cardiovascular diseases if untreated. However, it is usually observed that when type 1 diabetic patients have well-controlled glycemic levels, trigycerides (TG) and low density lipoproteins (LDL) become normal or slightly decrease, while a slight increase in high density lipoproteins (HDL) could also occur (Verges, 2009). In the current experiment, blood lipid levels of diabetic rats were also found to be elevated (Table 1). In the diabetic control group, in contrast to the non-diabetic control group, a marked elevation was observed in the concentration of serum cholesterol (47%), TG (88%), very low density lipoproteins (VLDL) (88%), and LDL (85%), while the concentration of HDL was reduced by 48%. This lipid

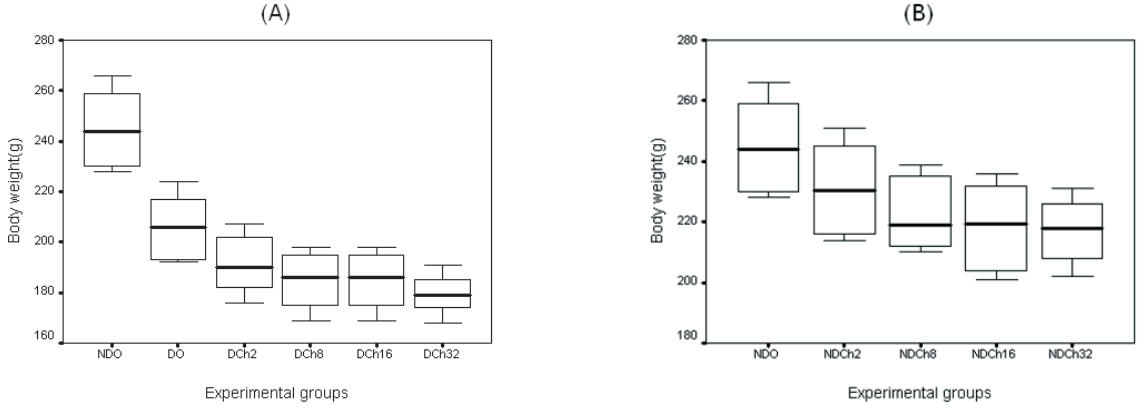

Figure 4. Effect of *trans*-chalcone treatment on body weight in diabetic and non-diabetic rats
(A) Diabetic rats. Using one way ANOVA with Tukey–Kramer test at $P<0.05$ following results were obtained for the data: DO is significantly different from NDO; DCh2 is significantly different from NDO; DCh8 is significantly different from NDO and DO; DCh16 is significantly different from NDO and DO; DCh32 is significantly different from NDO and DO. **(B)** Non-diabetic rats. Using one way ANOVA with Tukey–Kramer test at $P<0.05$ NDCh16 was found to be significantly different from NDO and NDCh32 significantly different from NDO.

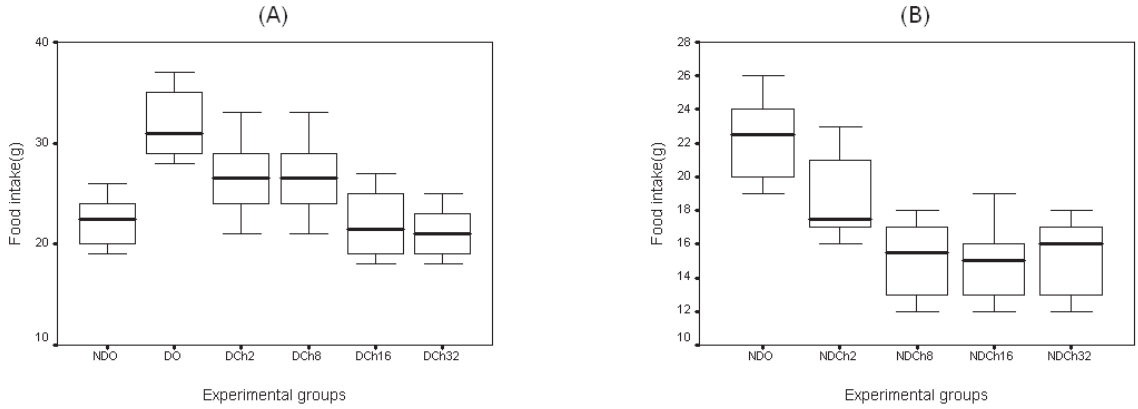

Figure 5. Effect of *trans*-chalcone treatment on food intake in diabetic and non-diabetic rats
(A) Diabetic rats. Using one way ANOVA with Tukey–Kramer test at $P<0.05$ following results were obtained for the data: DO is significantly different from NDO; DCh8 is significantly different from DO; DCh16 is significantly different from DO; DCh32 is significantly different from DO. **(B)** Non-diabetic rats. Using one way ANOVA with Tukey–Kramer test at $P<0.05$, NDCh8 was found to be significantly different from NDO; NDCh16 significantly different from NDO, and NDCh32 significantly different from NDO.

profile was remarkably improved after administration of chalcone, which is in accordance with the other effects of this compound observed in this study. It is interesting to note that the three doses Ch8, Ch16 and Ch32 had a similar effect on cholesterol, LDL and HDL concentration, and that Ch8 and Ch16 had a similar effect on triglycerides and VLDL concentration. On the other hand, the Ch32 dose acted in a reverse manner, and resulted in elevated levels of triglycerides and VLDL (more than two-fold), which suggests that this higher dose of chalcone would interfere in the lipid metabolism in some other way than what is observed for the lower doses.

As chalcone affected the blood glucose levels and insulin of normal rats, blood lipid levels of non-diabetic rats were also modified upon its administration (Table 2). It could be observed that in the experimental groups, the use of all doses (from 2 to 32 mg/kg) had a similar reducing effect on the concentrations of cholesterol, triglycerides, VLDL, and LDL, while no significant effect was found on HDL. This effect on lipid profile is slightly different from the effect on blood glucose, possibly because it would be a more indirect result of chalcone interactions with its putative targets *in vivo*. It should be noted that the 32 mg/kg dose had no deleterious effect on the lipid profile, in contrast to its effect in diabetic rats.

Body weight

Use of chalcone was found to induce weight loss in both diabetic and non-diabetic rats. Results of body weight measurements done at day 24 are shown in Fig. 4. As a known consequence of type 1 diabetes, the body weight in the diabetic control group was significantly reduced in comparison with the non-diabetic control group (206.3 ± 13.1 *vs.* 245.1 ± 15.8, $P<0.01$). When treated with chalcone, a dose-dependent body weight loss was observed in both diabetic and non-diabetic rats, although it was similar for the Ch16 and Ch32 doses. Weight loss has been suggested to be desirable in both type 1 and type 2 diabetic patients. Although this is more frequently a problem in patients with type 2 diabetes, since this condition is more likely to be associated with overweightness, unhealthy weight loss practices have also been found in youth with type 1 diabetes. As insulin intake is known to induce body weight increase, skipping insulin doses could occur in type 1 diabetic youth trying to lose weight (Lawrence *et al.,* 2008). In this regard, the therapeutic potential of a compound able to lower glucose levels while also inducing weight loss would be interesting. This effect of chalcone could be related to its α-amylase inhibitory property, or some kind of appetite-suppressing effect (as shown in the next section, chalcone treatment results in lower food intake).

Food intake

The effect of chalcone on food intake could be suggested to be part of its interaction with multiple targets. As shown in Fig. 5, food intake was significantly increased in the diabetic control group (DO) compared with the non-diabetic control group (NDO) (31.8 ± 3.5 g *vs.* 22.3 ± 2.7 g, $P<0.01$). Upon administration of chalcone, food intake was reduced in all groups receiving the treatment, but while the effect was more pronounced for doses exceeding 2 mg/kg, the three doses Ch8, Ch16 and Ch32 had nearly the same effect, a trend that was also observed for the chalcone effect on cholesterol and LDL. The fact that this effect is also observed in normal rats should be emphasized, since chalcone could be suggested to possess an appetite-suppressing effect, and thus be potentially effective in patients who would need weight loss.

Water intake

As a well-known consequence of becoming diabetic, water intake was observed to significantly increase in the diabetic control group (DO) compared with NDO (98.0 ± 11.3 ml *vs.* 34.5 ± 4.8 ml, $P<0.001$) (Fig. 6). Chalcone administration decreased the amount of water taken by the diabetic groups, again with Ch8, Ch16 and Ch32 acting similarly and more strongly than Ch2. As expected, chalcone had no effect on the water intake in the non-diabetic groups.

Urine volume

In diabetic rats (DO), urine volume was significantly increased relative to the non-diabetic control (NDO) (72.6 ± 8.3 ml *vs.* 17.3 ± 2.7 ml, $P<0.001$) (Fig. 7), again

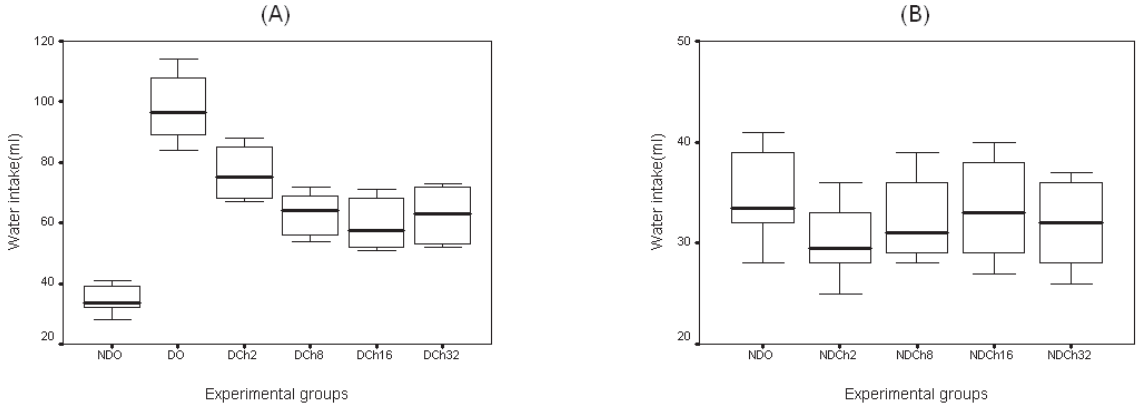

Figure 6. Effect of *trans*-chalcone treatment on water intake in diabetic control and non-diabetic rats
(A) Diabetic rats. Using one way ANOVA with Tukey–Kramer test at $P<0.05$ following results were found for the data: DO is significantly different from NDO; DCh2 is significantly different from NDO and DO; DCh8 is significantly different from NDO and DO; DCh16 is significantly different from NDO , DO and DCh2; Dch32 is significantly different from NDO and DO. **(B)** Non-diabetic rats. Water intake in control and experimental groups were found to have no significant difference using one way ANOVA with Tukey–Kramer test at $P<0.05$.

Table 2. Effect of chalcone treatment on serum lipids in non-diabetic rats.
Data are expressed as mean ± S.D. for six rats. a, significantly different from non-diabetic control group (NDO) using one way ANOVA with Tukey–Kramer test at $P<0.05$.

Serum level (mg/dl)	NDO	NDCh2	NDCh8	NDCh16	NDCh32
Cholesterol	85.5 ± 11.5	71.1 ± 10.5[a]	70.6 ± 9.4[a]	71.3 ± 10.4[a]	72.6 ± 8.1[a]
Triglycerides	63.5 ± 8.4	52.3 ± 7.7[a]	51.6 ± 6.5[a]	50.5 ± 5.2[a]	52.1 ± 8.3[a]
VLDL-C	12.7 ± 1.7	10.5 ± 1.5[a]	10.3 ± 1.3[a]	10.1 ± 1.1[a]	10.4 ± 1.6[a]
LDL-C	22.5 ± 4.6	17.1 ± 3.2[a]	16.5 ± 4.6[a]	17.3 ± 4.3	17.5 ± 4.5
HDL-C	44.5 ± 9.6	43.6 ± 8.6	44.3 ± 9.4	45.1 ± 7.2	44.5 ± 8.9

as a well-known consequence of diabetes. Administration of chalcone reduced urine volume in a dose-dependent manner, but without any significant difference between Ch16 and Ch32. This is the same trend observed in the effect of chalcone on blood glucose, which is directly related to the urine volume.

Again, no significant effect was detected on the urine volume of non-diabetic rats upon administration of chalcone.

Unwanted effect

At the end of the experiment, the gastrointestinal tract of sacrificed animals was found to show anomalies in the groups treated with higher doses of chalcone (especially DCh32 and NDCh32). The gut and intestine were swollen and their walls got thinner, so that their content was visible from the outside. Chalcone is a yellow compound, and yellow aggregates were observed in the gut. This could be indicative of a bioavailability problem,

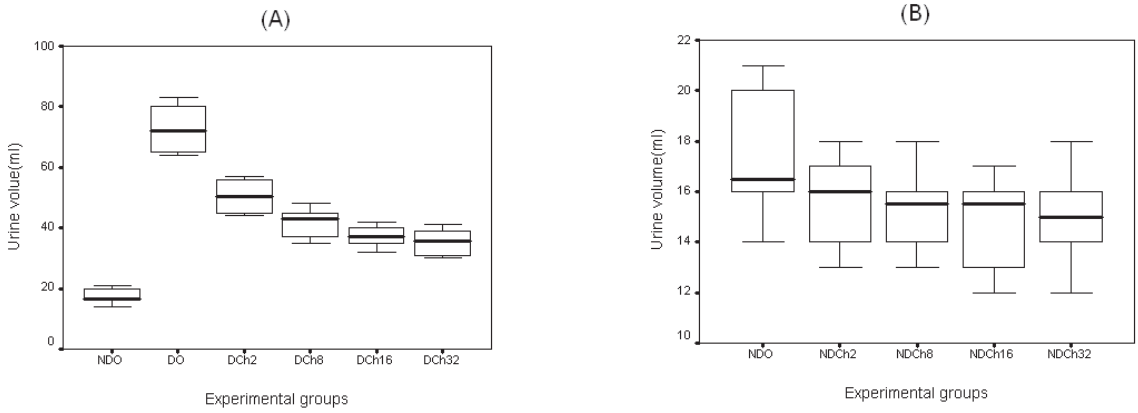

Figure 7. Effect of *trans*-chalcone treatment on urine volume in diabetic rats.
(A) Diabetic rats. Using one way ANOVA with Tukey–Kramer test at $P<0.05$ 05 following results were found for the data: DO is significantly different from NDO; DCh2 is significantly different from NDO and DO; DCh8 is significantly different from NDO and DO; DCh16 is significantly different from NDO, DO and DCh2; DCh32 is significantly different from NDO, DO and DCh2. **(B)** Non-diabetic rats. Urine volume in control and experimental groups have no significant difference using one way ANOVA with Tukey–Kramer test at $P<0.05$.

which is suggestive of the need to design better-absorbable chalcone derivatives, or use of intermediate dosages.

CONCLUSION

trans-Chalcone was shown to possess α-amylase inhibitory property *in vivo*, in a rat model of type 1 diabetes, and to be able to correct to a great extent the complications associated with the diabetic state. Significant reducing of hyperglycemia, as well as normalization of lipid profile were obtained when using a dose of 8 mg/kg, alongside with a reduction of water intake and urine volume. Weight loss and decrease of food intake, also observed in the case of a peptidic α-amylase inhibitor from *Phaseolus vulgaris* (Tormo *et al.,* 2004), which occur in both normal and diabetic rats, is another interesting result, and suggestive of the potential use of *trans*-chalcone as an effective agent for the treatment and even the prevention of obesity. Reduced blood glucose levels of normal rats is also another point of interest, as sharp increases of glucose levels in postprandial states are considered a risk factor in the transition from glucose intolerance toward diabetes, and metabolic syndrome complications among which cardiovascular problems are the most serious (Beisswenger *et al.,* 2004; Chiasson *et al.,* 2004; Yamagishi *et al.,* 2005). The observed reduction of insulin levels could be suggestive of an insulin-like effect of the compound, or as stated in the case of acarbose, a possible secondary effect that would be the result of its main mechanism of action (Rosak & Mertes, 2009). Overall, a comparison of the results obtained here with the ones reported about other α-amylase inhibitors shows a good correlation; on the other hand, in reports of chalcone derivatives' effects, insulin mimetic properties and inhibition of TG secretion (Casaschi *et al.,* 2004; Ogawa *et al.,* 2005) are also mentioned, which are suggestive of a possibility for *trans*-chalcone to act on multiple targets, but this assumption needs further verification.

In conclusion, *trans*-chalcone is one of the few small molecules with α-amylase inhibitory property that shows beneficial effect in the treatment of diabetes and related disorders, and could be proposed as a lead compound in this regard.

Competing interests

The authors declare that they have no competing interests.

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