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ANTIOXIDANT ACTIVITY AND INHIBITORY EFFECT OF 2,4,4'-TRIHYDROXYCHALCONE ON DIGESTIVE ENZYMES RELATED TO OBESITY

Hadeil M. Alsufiani[1,2]*, Ghaynat A. Aldhaheri[1], Ulfat M. Omar[1,3], Taraji M. Bahdilah[1], Rasha A. Mansouri[1]

[1]Biochemistry Department, Faculty of Sciences, [2]Experimental Biochemistry Unit, King Fahad Medical Research Center, [3]Princess Dr. Najla Bint Saud Al-Saud Center for Excellence Research in Biotechnology, King Abdulaziz University, Jeddah, Saudi Arabia

*Corresponding author, E-mail: halsufiani@kau.edu.sa

Abstract: The aim of this study was to investigate the antioxidant activity and inhibitory effect of 2,4,4'-trihydroxychalcone on digestive enzymes related to obesity, including sucrase, α-amylase, and lipase. The in vitro antioxidant activities of three concentrations of 2,4,4'-trihydroxychalcone (100, 300, and 500 µg/ml) were determined using 2,2-diphenyl-1-picrylhydrazyl radical scavenging, reducing power, and ferrous ion chelating assays. Moreover, in vitro inhibition of lipase, α-amylase, and sucrase enzyme activities by 2,4,4'-trihydroxychalcone was determined using a specific assay for each enzyme. 2,4,4'-Trihydroxychalcone has been shown to have antioxidant properties and inhibits sucrase, α-amylase and lipase activities. These findings suggest that 2,4,4'-trihydroxychalcone demonstrated an antioxidant activity and can effectively inhibit the key enzymes related to obesity.

Key words: 2,4,4'-trihydroxychalcone; DPPH radical scavenging activity; reducing power; ferrous ion chelating activity; sucrase; α-amylase; lipase

Introduction

Obesity is a global epidemic associated with significant morbidity and mortality in adults (1). Worldwide, 40% of women and 39% of men aged ≥18 years are overweight (2). In Saudi Arabia, the prevalence rates of overweight and obesity are increasing, with an overall prevalence of 35% (3). As obesity causes the development of metabolic disorders such as diabetes mellitus, hypertension, cardiovascular diseases, and inflammation-related pathologies, reducing the incidence of overweight and obesity is of considerable importance to public health (4).

A positive energy balance resulting from a chronic disparity between the intake of energy and its expenditure leads to weight gain and eventually obesity (5). Between 45% and 65% of the total caloric intake is accounted for by carbohydrates in their various forms, making them the most important energy source (6). The major dietary carbohydrates are of plant origin and can be found in grains, tubers, and legumes (7). The digestion of these starchy foods begins in the mouth by the action of salivary α-amylase, which hydrolyses the α-1,4 bonds in starch. The products of this process are shorter polysaccharides and maltose (8). The

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digestion processes of shorter polysaccharides and maltose continue in the small intestine by the action of pancreatic α-amylase and α-glucosidases, producing glucose (8,9). Moreover, sucrase (isomaltase) catalyses the sucrose hydrolysis to yield glucose and fructose (8). In addition to carbohydrates, fats represent approximately 35% of the daily energy intake where dietary triacyl glycerides are the predominant fat in foods and are hydrolysed by enzymes called lipases (6). Pancreatic lipase removes fatty acids at carbon 1 and 3, producing a mixture of 2-monoacylglycerol and free fatty acids, thereby facilitating their uptake (8).

To reduce the energy intake through gastrointestinal mechanisms without altering any central mechanism, reduction of dietary carbohydrate and fat absorption from the intestine through the inhibition of their digestive enzymes appear to be effective in reducing obesity (10). Although α-amylase inhibitors such as acarbose and pancreatic lipase inhibitors such as orlistat are safe drugs for weight loss, they have unpleasant side effects. Therefore, the interest in studying the inhibition of digestive enzymes by plant derived phytochemicals has recently increased (10). Among the different classes of phytochemicals, phenolic compounds have been increasingly recognised to be the most active inhibitors of α-amylase, sucrase and lipase enzymes (10).

Chalcones are a class of flavonoids that belongs to phenolic compounds (11). They are naturally occurring bioactive compounds and known to have several medicinal and pharmaceutical applications (12). Over the last recent years, few studies started investigating the inhibitory effect of chalcones on enzymes but this area still largely unexplored (13-15). 2,4,4'-Trihydroxychalcone (isoliquiritigenin) is one of the chalcone family that is found in liquorice (*Glycyrrhiza uralensis*), shallots, and soybeans (16,17). This chalcone has been reported to exhibit anti-inflammatory and anti-tumour properties (18). However, the antioxidant activity and inhibitory effect of this compound on digestive enzymes related to obesity are still largely unexplored and might be an excellent target for the development of safe and effective anti-obesity drug. Therefore, the aim of this study was to investigate the antioxidant activity and inhibitory effect of three concentrations of 2,4,4'-trihydroxychalcone (100, 300, and 500 μg/ml) on digestive enzymes related to obesity, including sucrase, α-amylase, and lipase.

Materials and methods

Chemicals

2,4,4'-trihydroxychalcone (cat.no.961-29-5), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (cat. no.1898-66-4), quercetin (cat.no.117-39-5), Vitamin C (cat.no.50-81-7), Sucrase (≥300 U/mg solid) (cat.no.9001-57-4), α-amylase (≥1000 U/mg) (cat.no.9000-90-2), Porcine pancreatic lipase (PPL, type II ≥100 U/mg) (cat.no.9001-62-1), orlistat (cat.no.96829-58-2) and Acarbose (cat.no.56180-94-0) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trichloroacetic acid (TCA) (cat.no.76-03-9), potassium ferricyanide (cat.no.13746-66-2), ferric chloride (cat. no.7705-08-0), Ferrozine (cat.no.63451-29-6), Ethylenediaminetetraacetic acid (EDTA) (cat. no.60-00-4), ferrous chloride (cat.no.7758-94-3), sucrose (cat.no.57-50-1), 3,5-dinitrosalicylic acid (cat.no.609-99-4), sodium phosphate dibasic (cat. no.7558-79-4), Sodium phosphate monobasic (cat. no.10049-21-5), sodium chloride (cat.no.7647-14-5), potassium sodium tartrate tetrahydrate (cat. no.6381-59-5), sodium hydroxide (cat.no.1310-73-2), p-Nitrophenyl α-D-Glucopyranoside (cat.no.3767-28-0), Sodium Carbonate (cat. no.497-19-8), Tween 80 (cat.no.9005-65-6) were purchased from E. Merck (Darmstadt, Germany). 2,4-Dinitrophenyl butyrate (cat.no.24273-19-6) was purchased from cymitquimica. All chemicals and reagents used in all experiments were of analytical grade or purer.

Measurement of the antioxidant activity of 2,4,4'-trihydroxychalcone

DPPH Radical-scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was used to determine the radical scavenging activity of 2,4,4'-trihydroxychalcone (19). To achieve this, 0.15 mM of the DPPH radical was prepared in methanol, and 1 ml of the solution was mixed with 1 ml of different concentrations of the 2, 4, 4'-trihydroxychalcone solution (100, 300, and 500 μg/ml). The reaction mixture was left at

room temperature for 15 min. The absorbance of the mixture was recorded at 517 nm. The negative control was prepared in a similar way but without 2,4,4'-trihydroxychalcone. Different concentrations of vitamin C and quercetin (100, 300, and 500 µg/ml) were used as positive controls. The experiment was repeated four times for two independent days, and the average absorbance was calculated. The percentage of the DPPH scavenging activity was then calculated using the following equation:

% DPPH radical scavenging activity = $[(A_0 - A_1)/A_0] \times 100\%$,

where A_0 is the absorbance of the negative control and A_1 is the absorbance of the 2,4,4'-trihydroxychalcone or positive controls.

Reducing power assay

The reducing power of 2,4,4'-trihydroxychalcone was determined using Oyaizu's method (20). At different concentrations, 2.5 ml of 2,4,4'-trihydroxychalcone (100, 300, and 500 µg/ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1.0%), and the mixture was incubated in a water bath at 50°C for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture and then centrifuged for 10 min at 3000 rpm. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%), and the absorbance was then measured at 700 nm. The negative control was prepared in a similar way but without 2,4,4'-trihydroxychalcone. Different concentrations of vitamin C (100, 300, and 500 µg/ml) were used as positive controls. The experiment was repeated four times for two independent days, and the average absorbance was calculated. The absorbance values were then used to indicate the reducing power of the antioxidants.

Ferrous ions-chelating activity

The chelating activity of ferrous ions by 2,4,4'-trihydroxychalcone was determined using the method of Dinis et al. (21). Different concentrations of 2,4,4'-trihydroxychalcone (100, 300, and 500 µg/ml) at 0.4 ml were added to a solution of ferrous chloride (0.2 ml, 2.0 mM). The reaction was started by adding ferrozine (0.4 ml, 5 mM), and the total volume was adjusted to 4 ml with methanol. The mixture was then shacked and incubated for 10 min at 37°C. The absorbance of the mixture was recorded at 562 nm. The negative control was prepared in a similar way but without 2,4,4'-trihydroxychalcone. Different concentrations of ethylenediaminetetraacetic acid (EDTA; 100, 300, and 500 µg/ml) were used as positive controls. The experiment was repeated four times for two independent days, and the average absorbance was calculated. The percentage inhibition was then calculated using the following equation:

% Chelating activity of ferrous ions = $[(A_0 - A_1)/A_0] \times 100\%$,

where A_0 is the absorbance of the negative control and A_1 is the absorbance of 2,4,4'-trihydroxychalcone or the positive controls.

Measurement of digestive enzymes activity

Sucrase inhibition assay

The effect of 2,4,4'-trihydroxychalcone on sucrase activity was examined using Honda and Hara's method (22). Different concentrations of 2,4,4'-trihydroxychalcone (100, 300, and 500 µg/ml) at 50 µl were mixed with a sucrase solution (50 µl, 4.8 U/µl) and incubated for 10 min at 37°C. The enzyme reaction was started by adding the sucrose solution (100 µl, 60 mM). After 30 min, the reaction was stopped by adding a 3,5-dinitrosalicylic acid reagent (200 µl), and the mixture was incubated in a boiling water bath for 5 min. After the addition of distilled water (2.0 ml), the absorbance of the mixture was read at 540 nm using an ultraviolet (UV)-visible spectrophotometer. The negative control was prepared in a similar way but without 2,4,4'-trihydroxychalcone. The experiment was repeated four times for two independent days, and the average absorbance was calculated. The enzyme activity inhibition was calculated as follows:

Inhibition (%) = $[(A_{control} - A_{sample})/A_{control}] \times 100\%$,

where $A_{control}$ is the absorbance of the negative control and A_{sample} is the absorbance of 2,4,4'-trihydroxychalcone.

α-Amylase inhibition assay

The α-amylase inhibition activity of 2,4,4'-trihydroxychalcone was estimated using the method of Oboh et al. (23) with some modifications. The α-amylase enzyme (40 µl, 5 U/ml) was mixed with a sodium phosphate buffer (0.36 ml,

0.02 M, pH 6.9 with 0.006 M NaCl) and 0.2-ml 2,4,4'-trihydroxychalcone (100, 300, and 500 µg/ml), and incubated for 20 min at 37°C. The starch solution in the sodium phosphate buffer (300 µl, 1%) was then added, and the mixture was re-incubated for 20 min. The reaction was stopped by adding a 3,5-dinitresalicylic acid reagent (0.2 ml). The mixture was then mixed well and kept in a boiling water bath for 5 min. After the addition of distilled water (6 ml), the absorbance of the mixture was read at 540 nm using a UV-visible spectrophotometer. The negative control was prepared in a similar way but without 2,4,4'-trihydroxychalcone. Different concentrations of acarbose (100, 300, and 500 µg/ml) were used as positive controls. The experiment was repeated four times for two independent days, and the average absorbance was calculated. The enzyme activity inhibition was calculated as follows:

Inhibition (%) = $[1 - (A_{sample}/A_{control})] \times 100$,

where $A_{control}$ is the absorbance of the negative control and A_{sample} is the absorbance of 2,4,4'-trihydroxychalcone or the positive controls.

Lipase inhibition assay

The lipase inhibition activity of 2,4,4'-trihydroxychalcone was estimated using the method of Won et al. (24) with some modifications. A reaction mixture containing porcine pancreatic lipase (0.1 ml, 200 units/ml in 0.1 M potassium phosphate buffer, pH 6.8, 1% tween 80) and 0.1 ml of 2,4,4'-trihydroxychalcone (100, 300, and 500 µg/ml) was prepared. After adding 0.5 ml of 25 mM 2,4-dinitrophenyl butyrate as a substrate, the reaction was started. Then, the reaction mixture was kept at 37°C during the assay. After 1 minute of incubation, 2,4-dinitrophenol was released by the lipase and measured at 360 nm using a UV-visible spectrophotometer. The negative control was prepared in a similar way but without 2,4,4'-trihydroxychalcone. Different concentrations of orlistat (100, 300, and 500 µg/ml) were used as positive controls. The experiment was repeated four times for two independent days, and the average absorbance was calculated. The enzyme activity inhibition was calculated as follows:

Inhibition (%) = $[1 - (A_{sample}/A_{control})] \times 100$,

where $A_{control}$ is the absorbance of the negative control and A_{sample} is the absorbance of 2,4,4'-trihydroxychalcone or the positive controls.

Statistical analysis

To assess the differences in antioxidant activity and inhibitory effect between the three different concentrations of 2,4,4'-trihydroxychalcone on α-amylase and lipase enzymes, two-way analysis of variance (ANOVA) was performed followed by the Tukey test. One-way ANOVA followed by the Bonferroni test was used to test the differences of the three different concentrations of 2,4,4'-trihydroxychalcone in terms of their inhibitory effects on the sucrase enzyme. All independent analyses were replicated in quadruplicates, for which the results were expressed as mean ± SEM. All statistical analysis was performed using the statistical software GraphPad Prism 8. The statistical significance levels were based on $p < 0.05$.

Results

The antioxidant activity of 2,4,4'-trihydroxychalcone

DPPH radical scavenging activity

Figure 1 shows the DPPH radical scavenging activity of the three different concentrations of vitamin C, quercetin, and 2,4,4'-trihydroxychalcone. At a concentration of 100 µg/ml, vitamin C showed the highest scavenging effect (97.2%), followed by quercetin (95.7%) and then 2,4,4'-trihydroxychalcone (57.4%). At a concentration of 300 µg/ml, vitamin C showed the highest scavenging effect (97.6%), followed by quercetin (95.9%) and then 2,4,4'-trihydroxychalcone (61.88%). The same trend was found when the concentration was increased to 500 µg/ml where vitamin C still had the highest scavenging effect, followed by quercetin and then 2,4,4'-trihydroxychalcone (97.88%, 96.3%, and 74.7%, respectively).

In addition, the results showed the relationship between the concentration of the three compounds and their DPPH scavenging activity (Figure 1). For vitamin C and quercetin, the results showed that their scavenging activity did not changed with the increment in the concentrations. In contrast, 2,4,4'-trihydroxychalcone showed a significant gradual increase from 57.4% at 100 µg/ml to 74.7% at 500 µg/ml.

Reducing power assay

Figure 2 shows the reducing power of vitamin C and 2,4,4'-trihydroxychalcone where increasing absorbance indicates an increase in reducing power. At each concentration (100, 300, and 500 μg/ml), vitamin C showed higher reducing power than 2,4,4'-trihydroxychalcone. In addition, the results of vitamin c showed that the significant difference in reducing power occurred only at concentrations of 100 μg/ml and 500 μg/ml. On the other hand, the results of 2,4,4'-trihydroxychalcone showed a direct proportional increase with the concentration.

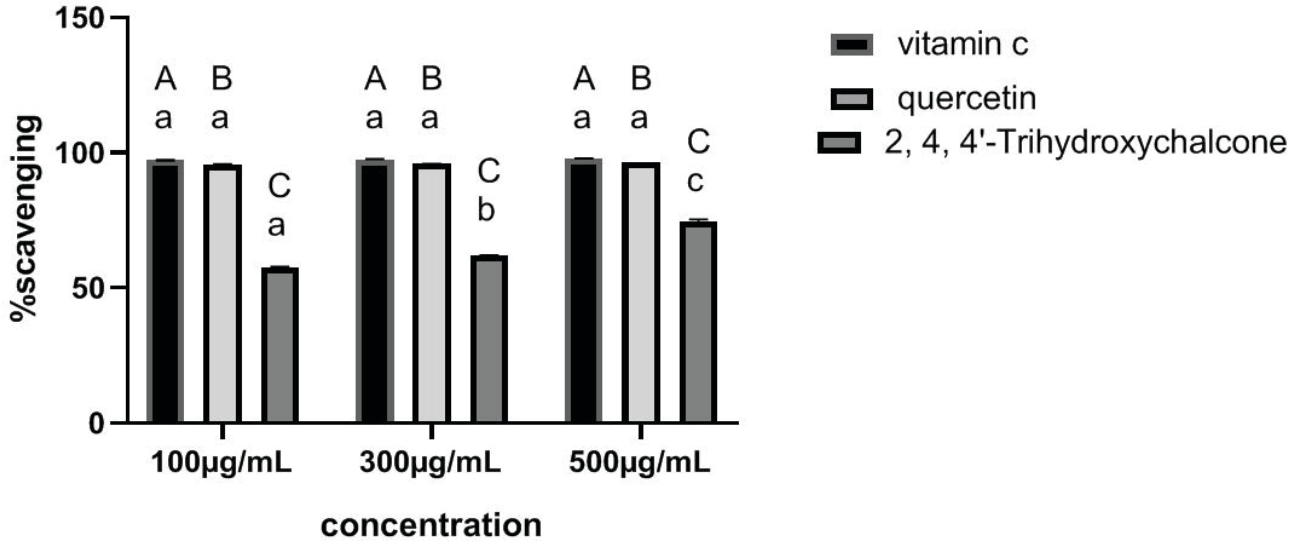

Figure 1: DPPH radical scavenging activities of vitamin C, quercetin, and 2,4,4'-trihydroxychalcone

Different upper-case letters denote significant differences ($p < 0.05$) between the different types of compounds at the same concentration. Different lower-case letters denote significant differences ($p < 0.05$) between the different concentrations of the same compound. Values are presented as mean ± SEM. Error bars shows the SEM. DPPH: 2,2-diphenyl-1-picrylhydrazyl, SEM: standard error of the mean.

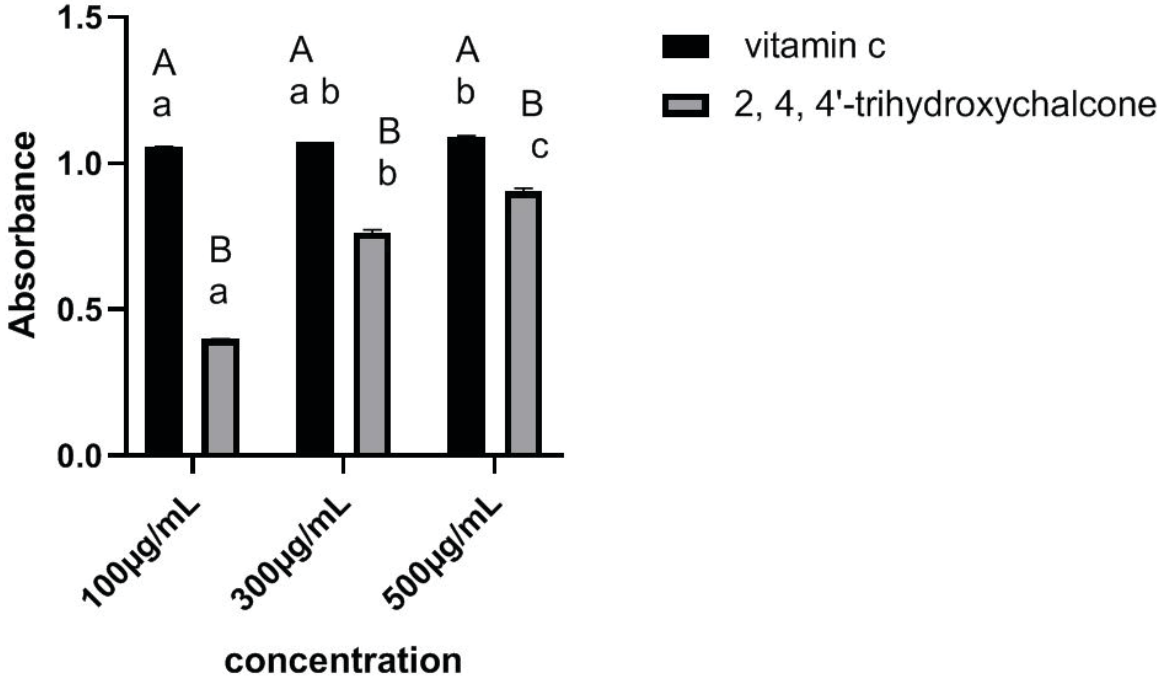

Figure 2: Reducing power of vitamin C and 2,4,4'-trihydroxychalcone

Different upper-case letters denote significant differences ($p < 0.05$) between the different types of compounds at the same concentration. Different lower-case letters denote significant differences ($p < 0.05$) between the different concentrations of the same compound. Values are presented as mean ± SEM. Error bars shows the SEM. SEM: standard error of the mean.

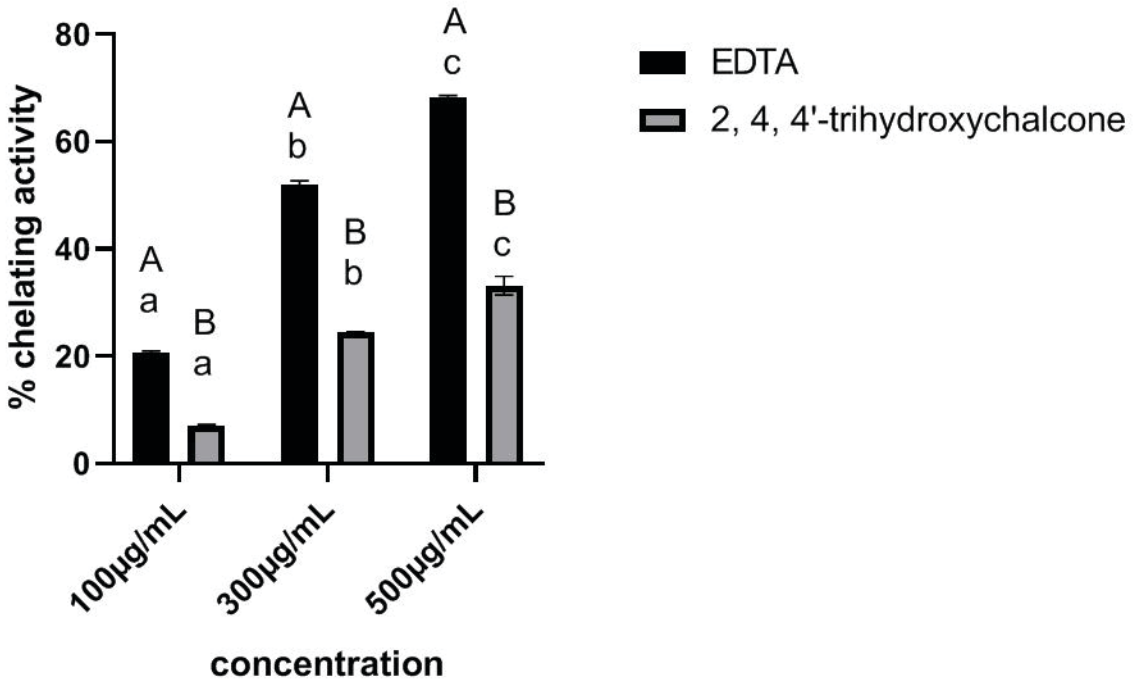

Figure 3: The Ferrous ions-chelating activity of EDTA and 2,4,4'-trihydroxychalcone

Different upper-case letters denote significant differences ($p < 0.05$) between the different types of compounds at the same concentration. Different lower-case letters denote significant differences ($p < 0.05$) between the different concentrations of the same compound. Values are presented as mean ± SEM. Error bars shows the SEM. EDTA: ethylenediaminetetraacetic acid, SEM: standard error of the mean.

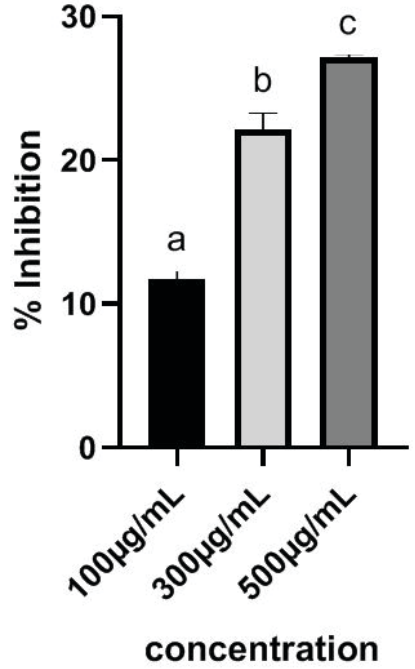

Figure 4: Sucrase inhibition activity by 2,4,4'-trihydroxychalcone.

Different lower-case letters denote significant differ-ences ($p < 0.05$) between the different concentrations. Values are presented as mean ± SEM. Error bars show the SEM. SEM: standard error of the mean.

Ferrous ions-chelating activity

The chelating activities of the ferrous ions of EDTA and 2,4,4'-trihydroxychalcone (100, 300, and 500 µg/ml) are shown in Figure 3. Results of 100 µg/ml showed that EDTA has a higher chelating activity (20.58%) than 2,4,4'-trihydroxychalcone (7.125%). A concentration of 300 µg/ml also showed that EDTA has higher chelating activity (52.04%) than 2,4,4'-trihydroxychalcone (24.42%). Similarly, the same trend was observed with 500 µg/ml where the chelating activities of EDTA and 2,4,4'-trihydroxychalcone were 68.22% and 33.20%, respectively.

In addition, the results showed a proportional relationship between the compound's concentration and the chelating activity. The EDTA results showed that the chelating activity was increased from 20.58% at 100 µg/ml to 68.22% at 500 µg/ml. Likewise, the results for 2,4,4'-trihydroxychalcone showed that the chelating activity was increased from 7.125% at 100 µg/ml to 33.20% at 500 µg/ml.

Digestive enzymes activity

Sucrase inhibition

The sucrase inhibition activities of the different concentrations of 2,4,4'-trihydroxychalcone (100, 300, and 500 µg/ml) are shown in Figure 4. The inhibitory activity was found to be proportional to the increment in the concentration where the results showed that 100, 300, and 500 µg/ml of 2,4,4'-trihydroxychalcone lead to 11.73%, 22.17%, and 27.12% sucrase inhibition, respectively α-Amylase inhibition.

The inhibitory effects of α-amylase at different concentrations of acarbose and 2,4,4'-trihydroxychalcone (100, 300, and 500 µg/ml) are shown in Figure 5. 2,4,4'-Trihydroxychalcone showed a higher inhibitory effect (23.87%) than acarbose (7.12%) at 100 µg/ml. In contrast, when the concentrations of acarbose and 2,4,4'-trihydroxychalcone were increased to 300 and 500 µg/ml, the two compounds exhibited similar inhibitory effects.

Moreover, the results showed a proportional correlation between 2,4,4'-trihydroxychalcone and acarbose in terms of concentration and inhibitory activity. The results for acarbose showed an increase in inhibitory effect from 7.12% at 100 µg/ml to 37.55% at 500 µg/ml. Similarly, 2,4,4'-trihydroxychalcone showed an increase in inhibitory effect from 23.87% at 100 µg/ml to 37.96% at 500 µg/ml.

Lipase inhibition

The results, as shown in Figure 6, indicate that the different concentrations of orlistat and 2,4,4'-trihydroxychalcone (100, 300, and 500 µg/ml) inhibit lipase activity. Orlistat showed a significantly higher inhibitory activity (63.58%) than 2,4,4'-trihydroxychalcone (57.71%) at 100 µg/ml.

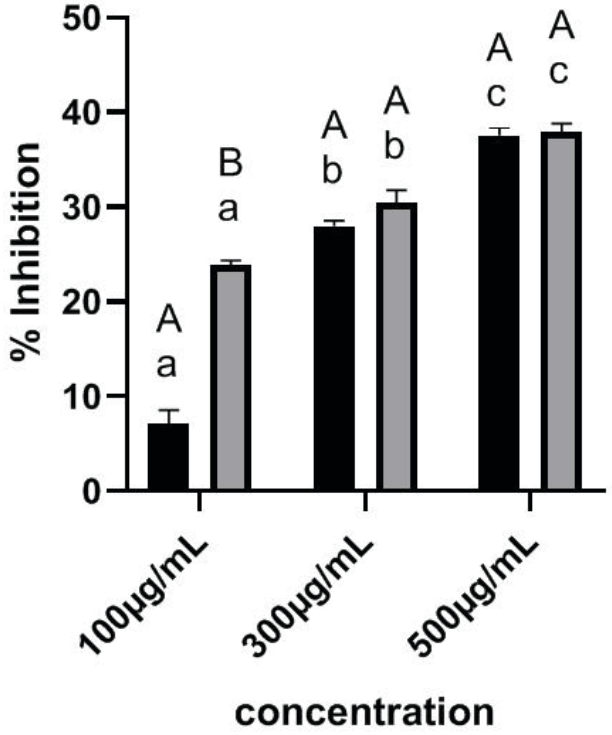

Figure 5: α-Amylase inhibition activities of acarbose and 2,4,4'-trihydroxychalcone

Different upper-case letters denote significant differ-ences ($p < 0.05$) between the different compounds at the same concentration. Different lower-case letters denote significant differences ($p < 0.05$) between the different concentrations of the same compound. Values are pre-sented as mean ± SEM. Error bars show the SEM. SEM: standard error of the mean.

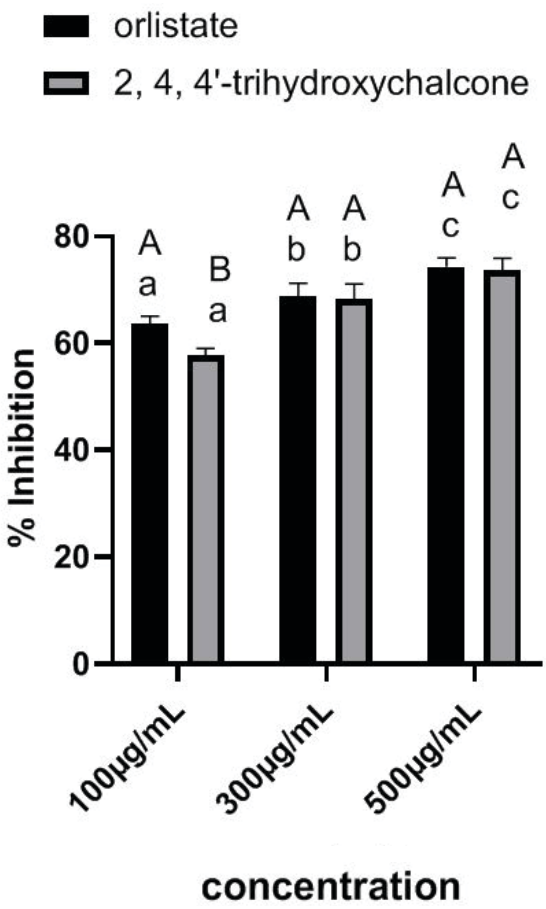

Figure 6: Lipase inhibition activities of orlistat and 2,4,4'-trihydroxychalcone

Different upper-case letters denote significant differences ($p < 0.05$) between the different compounds at the same concentration. Different lower-case letters denote significant differences ($p < 0.05$) between the different concentra-tions of the same compound. Values are presented as mean ± SEM. Error bars show the SEM. SEM: standard error of the mean.

By contrast, no significant differences were found in lipase inhibition between orlistat and 2,4,4'-trihydroxychalcone at higher concentrations (300 and 500 µg/ml).

In addition, the concentrations and inhibitory activities of 2,4,4'-trihydroxychalcone and orlistat were found to be proportvionally associated. The inhibitory effect of orlistat was increased from 63.58% at 100 µg/ml to 74.21% at 500 µg/ml. Similarly, the inhibitory effect of 2,4,4'-trihydroxychalcone was increased from 57.71% at 100 µg/ml to 73.64% at 500 µg/ml.

Discussion

The present study investigated the antioxidant activity and inhibitory effect of 2,4,4'-trihydroxychalcone on obesity-related digestive enzymes.

The antioxidant activity of 2,4,4'-trihydroxychalcone

DPPH Radical scavenging activity

The results of the present study showed that 2,4,4'-trihydroxychalcone has a DPPH radical scavenging activity. This finding is in line with previous studies that investigated the DPPH scavenging activity of several extracts containing flavonoids (25-29). The DPPH radical is a commercially oxidising radical that is reduced by antioxidants. In this assay, the violet colour of DPPH changes into a pale-yellow colour because of the acceptance of DPPH to the hydrogen atoms donated by antioxidants [30]. This explains the high dense yellow colour presented in this study which indicates the high scavenging activity of 2,4,4'-trihydroxychalcone.

The results also showed a proportional relationship between the 2,4,4'-trihydroxychalcone concentration and the DPPH scavenging activity. These results agree with those of previous studies that showed that increasing concentrations of extracts containing flavonoids increased the DPPH scavenging activity (30-35).

Reducing power assay

In this study, a reduced power activity was observed for 2,4,4'-trihydroxychalcone. Finding in the present study is consistent with findings of previous studies that investigated the reducing power of several plant flavonoids (25,29,36). The presence of 2,4,4'-trihydroxychalcone in the assay resulted in the reduction of Fe^{3+} to Fe^{2+} via the electron donation of the compound. The yellow colour of the test solutions changed to various shades of light green to dark blue, indicating the amount of Fe^{2+} complex formed (37).

The reducing power of 2,4,4'-trihydroxychalcone was gradually increased with the increase in its concentration. Several previous studies showed similar results where the reducing power was increased by increasing the concentration of extracts containing flavonoids (32-35).

Ferrous ions-chelating activity

The activity of 2,4,4'-trihydroxychalcone in chelating ferrous ions was confirmed in this study. Similarly, findings of previous studies revealed the chelating activity of ferrous ions by different plant extracts containing flavonoids (35,38-40). In this test, Ferrozine is capable of forming red complexes with Fe^{2+} [38]. In the presence of 2,4,4'-trihydroxychalcone, the formation of these red complexes was decreased. This could be explained by the inference of 2,4,4'-trihydroxychalcone with the formation of the ferrous and ferrozine complex, which suggests that it has a chelating activity and captures ferrous ions before ferrozine binding (41,42).

Moreover, the chelating of ferrous ions by 2,4,4'-trihydroxychalcone was gradually increased with the increase in its concentration. This is supported by previous studies which reported that the chelating activities of extracts containing flavonoids on ferrous ions were increased when the concentrations of the extracts increased (35,40).

Digestive enzyme activity

Sucrase inhibition

In this assay, sucrase hydrolysed sucrose to glucose and fructose. Then, these reducing sugars reduced 3,5-dinitrosalicylic acid (DNS) to 3-amino-5-nitrosalicylic acid (a reddish-brown compound) (43,44). In the presence of 2,4,4'-trihydroxychalcone, the formation of this reddish-brown compound was reduced, indicating the inhibition of sucrase enzyme. To our knowledge, this is the first study to investigate the effect of a

chalcone compound on sucrase activity. However, our results are in accordance with the findings of a previous study which stated that flavonoids extracted from tamarix gallica inhibited sucrase activity up to 30% (45).

The results also showed a direct correlation between the 2,4,4'-trihydroxychalcone concentration and the inhibition activity. A similar pattern of results was observed in previous studies (46,47).

α-Amylase inhibition

The DNS colorimetric method was used to measure amylase activity by using starch as the substrate. Under alkaline conditions, the colour of the solution was changed from yellow to reddish brown when the reducing sugar (glucose) reduced 3,5-dinitrosalicylic acid to 3-amino-5nitrosalicylic acid (48-49). In the presence of 2,4,4'-trihydroxychalcone, the formation of the reddish-brown compound was reduced, indicating that 2,4,4'-trihydroxychalcone binds to α-amylase enzyme and reduces its activity up to 40%. This result is in line with those of a previous study which reported that different flavonoids extracted from tartary buckwheat inhibited α-amylase activity up to 45% (50). Similar inhibition % were also reported with synthetic chalcone compounds (51).

When comparing the effect of 2,4,4'-trihydroxychalcone with that of an acarbose drug, 2,4,4'-trihydroxychalcone demonstrated a greater inhibitory effect than acarbose at 100 µg/ml and similar inhibitory effects when their concentrations were increased to 300 and 500 µg/ml. The use of acarbose was associated with several gastrointestinal symptoms, including flatulence, abdominal distension, and diarrhoea (52). Accordingly, 2,4,4′-trihydroxychalcone could be considered a good source of natural compound in slowing down the breakdown and absorption of carbohydrates.

In addition, the results of the present study showed that the inhibition activity of α-amylase was directly proportional to the concentration of 2,4,4'-trihydroxychalcone. A similar result was previously reported by different studies which found an increase in the inhibitory effect of flavonoids on α-amylase with the increase in its concentration (50,53-55).

Lipase inhibition

In the lipase activity assay, 2,4-dinitrophenyl butyrate was used as the substrate. Upon hydrolysis, this substrate liberates the strongly absorbing 2,4-dinitrophenolate anion (DNP), which can be measured spectrophotometrically (56). In the presence of 2,4,4'-trihydroxychalcone, the formation of DNP was reduced, indicating that 2,4,4'-trihydroxychalcone binds to lipase enzyme and reduced its activity up to 70%. Studies that investigated lipase inhibition using several flavonoid-containing extracts from cinnamon, cardamom, gloves, Rosa damascene and white and blue poppy seeds or using other chalcone compounds extracted from licorice have reported similar % inhibition (57, 55, 58, 59).

In the intestine, lipase inhibitors block the action of this enzyme, and as a result, the conversion of dietary triglyceride to monoglyceride and fatty acid is reduced. Therefore, dietary fat is less likely to be absorbed (60). Orlistat is the only over-the-counter weight-loss drug approved by the Food and Drug Administration and European Medicines Agency that inhibits gastric and pancreatic lipases in the lumen of the gastrointestinal tract and therefore decreases the absorption of dietary fats (61). At long-term clinical trials in adults, orlistat reduced dietary fat absorption by approximately 30% and weight gain by approximately 5% (62). Orlistat has several side effects, including gastrointestinal problems (fatty stool and faecal incontinence), interfering with nutrients and drugs, and liver damage (61). When comparing the effect of 2,4,4'-trihydroxychalcone with that of orlistat drug, orlistat demonstrated a greater inhibitory effect than 2,4,4'-trihydroxychalcone at 100 µg/ml, but the two showed similar inhibitory effects when their concentrations were increased to 300 and 500 µg/ml. Thus, 2,4,4′-trihydroxychalcone could be a promising natural alternative to orlistat.

Along with its increased concentration, 2,4,4'-trihydroxychalcone exhibited increased lipase inhibitory activity. This result agrees with previous results that showed that extracts containing flavonoids had an increased inhibitory effect as their concentrations increased (55,58).

Limitations and future research

This study has limitations, as α-amylase and lipase were derived from porcine pancreas, not human pancreas. Another limitation is that sucrase was derived from baker's yeast rather than from human pancreas. Future studies should examine the mode by which 2,4,4'-trihydroxychalcone inhibits enzymes and investigate the effects of 2,4,4'-trihydroxychalcone on digestive enzymes in animals and humans.

Conclusion

The findings stated that 2,4,4'-trihydroxychalcone demonstrated an antioxidant activity and can effectively inhibit the key enzymes related to obesity.

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