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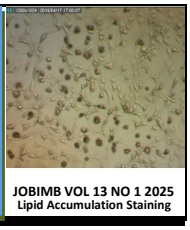

Anti-Obesity and Anti-Diabetic Potential of Fermented Terengganu Cherry Juice

Nur Suraya Ashikin Rosli[1], Uswatun Hasanah Zaidan[1], Mohd Ezuan Khayat[1,4], Muhajir Hamid[2] and Mohd Badrin Hanizam Abdul Rahim[1,3,4]*

[1]Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[2]Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[3]NaturMeds, Institut Biosains, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[4]Agribiotechnology Group, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

*Corresponding author:
Mohd Badrin Hanizam Abdul Rahim,
Department of Biochemistry,
Faculty of Biotechnology and Biomolecular Sciences,
Universiti Putra Malaysia,
43400 UPM Serdang,
Selangor,
Malaysia.
Email: badrin@upm.edu.my

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Abstract

As readily available medications for obesity and diabetes are reported to cause unwanted side effects, many natural alternatives to control obesity and diabetes are brought forward. Terengganu cherry (*Lepisanthes fruticosa* (Roxb.) Leenh) is an underutilized fruit in Malaysia that has limited study on its health benefits. Hence, this study aims to investigate the potential anti-obesity and anti-diabetic properties of fermented juice extract of Terengganu cherry (FTCJ). For anti-obesity effects, the impact of FTCJ and PTCJ on adipogenesis was assessed using 3T3-L1 cell differentiation assays. FTCJ inhibited lipid accumulation by 50.45%, 48.40%, and 67.12% at concentrations of 0.25 mg/mL, 0.5 mg/mL, and 0.75 mg/mL, respectively. Although PTCJ exhibited greater lipid accumulation reduction than FTCJ, this effect may be attributed to its cytotoxicity, as observed in cytotoxicity assays. For anti-diabetic effects, α-amylase and α-glucosidase inhibition assays were conducted. Both FTCJ and PTCJ showed no inhibitory activity against α-amylase. However, PTCJ demonstrated significantly higher α-glucosidase inhibition (99.11%) compared to FTCJ (31.72%) at 1.0 mg/mL. These findings suggest that while PTCJ exhibits stronger effects in both adipogenesis inhibition and α-glucosidase inhibition, its potential cytotoxicity raises concerns. In contrast, FTCJ exhibits promising anti-obesity effects with lower cytotoxicity, highlighting its potential as a natural alternative for managing obesity and diabetes. Further studies are recommended to explore its mechanisms and potential applications.

INTRODUCTION

According to the latest reports by the National Institute of Health Malaysia, 54.4% of adult Malaysians and 30.5% adolescents within the age of 5-17 are either overweight or obese [1, 2]. Although genetic factors and psychological stresses could contribute to the cause of obesity, unhealthy diet and sedentary lifestyle are still deemed to be the main factors [3]. When there is a lack of balance between energy intake (unhealthy diet) and energy expenditure (sedentary lifestyle), the number and size of adipocyte cells will also increase, causing more fat to be stored and along with it, weight gain [4]. It is suggested that regulating adipogenesis, a cell differentiation process that transforms preadipocytes into mature adipocytes, may help control weight gain[5]. Adipogenesis also triggers the change of cell morphology, cell secretory capacity, and insulin sensitivity induction [6]. Various transcription factors and genes are involved in the differentiation process of preadipocytes to mature adipocytes. Among them are the peroxisome proliferator-activated receptor γ (PPARγ) as well as the CCAAT/enhancer binding proteins (C/EBPs), which are the main adipogenesis regulators or markers for adipogenesis. Without these two regulators, it is impossible for mature adipocytes to form [7].

Obesity is a major risk factor for the development of type 2 diabetes (T2D). Excess fat, particularly visceral fat around the abdomen, leads to insulin resistance, in which the body's cells become less responsive to insulin. As a result, the pancreas has to produce more insulin to maintain normal blood sugar levels. Over time, this increased demand can strain the pancreas, leading to impaired insulin production [8]. The combination of insulin resistance and insufficient insulin production eventually leads to the development of T2D. Additionally, obesity-related inflammation and altered fat metabolism contribute to the disruption of normal glucose regulation [9].

Although there are well-known drugs available, such as orlistat, sibutramine, and rimonabant to treat obesity, these medications are reported to bring unfavourable side effects such as insomnia, headaches, and poor bowel control to some patients [10]. Furthermore, these medications can be expensive, particularly for low- and middle-income populations. Therefore, many natural and plant-based resources have been extensively studied for their potential abilities to inhibit adipogenesis. One of the most famous *in vitro* models for studying adipocyte development and function is the 3T3-L1 cell line, which exhibits similar phenotype characteristics to those of human adipocytes differentiated [11]. Among these plant-based products, fermented fruit juices have garnered much attention over recent years.

Terengganu Cherry (*L. fruticosa*) is a native plant to Southeast Asia, mainly in Malaysia, Indonesia, and the Philippines. Locally, it has several different names, including Ceri Terengganu, Buah Johor, and Perupok. The edible fruit from this plant is non-seasonal and can either be eaten fresh or blended to make a juice. The Terengganu Cherry is reported to have a high moisture content and is also low in calories. To add, its ash content is also high, which indicates that this fruit contains a lot of minerals [12]. The process of fermentation for beverages has been widely used throughout the world for centuries. Microbial fermentation is typically achieved by introducing probiotics to beverages, with Lactococcus, Lactobacillus, and Streptococcus being among the common bacteria used.

The commonly detected yeasts include *Candida* and *Saccharomyces* [13]. Besides its preservation purposes, fermentation also significantly contributes to enhancing sensory characteristics and promotes numerous health benefits. It is suggested that the addition of probiotics improves the immune system, decreases lactose intolerance, and also lowers cholesterol levels in the blood [14]. Additionally, there have been reports stating that the probiotics added in the beverages cause a significant increase in the bioactive compounds present. This increases the nutrient content as well as the nutritional value of the original product, which consequently further increases its health benefits [3].

Hence, this study aimed to investigate the potential inhibition properties of fermented Terengganu Cherry juice (FTCJ) towards adipogenesis. Considering that obesity is greatly associated with type 2 diabetes mellitus, the potential inhibition properties of FTCJ towards starch-hydrolysing enzymes (α-amylase and α-glucosidase) were also determined. It is hypothesized that FTCJ would have significant anti-obesity and anti-diabetic properties. Therefore, this study seeks to explore the effect of FTCJ on the 3T3-L1 cell viability, adipocyte cell development as well as the anti-diabetic properties of FTCJ against α-amylase and α-glucosidase enzymes. By bridging the knowledge gap regarding the health benefits of fermented fruit juices, this study has the potential to contribute to the development of natural therapeutic strategies to combat diabetes and obesity, two of the most pressing health issues worldwide.

METHODOLOGY

Chemicals

3T3-L1 fibroblasts were purchased from American Type Culture Collection (VA, USA). Dulbecco's Minimal Eagle's Medium (DMEM), trypsin-EDTA, penicillin-streptomycin antibiotic, dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS), MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), and human insulin were purchased from Nacalai Tesque (Kyoto, Japan). Fetal bovine serum (FBS) was obtained from Tico Europe (Netherlands). Oil red O, α-amylase, α-glucosidase, p-nitrophenyl-α-D-glucopyranoside (PNPG), and 3,5-dinitrosalicylic (DNS) acid were procured from Sigma-Aldrich (MA, USA).

Sample Preparation

Fermented Terengganu Cherry Juice (FTCJ) and pasteurized Terengganu Cherry juice (PTCJ) were provided by Dr. Musa Al Bakri Abdul Manan from Malaysian Agriculture and Research Development Institute (MARDI), Serdang, Malaysia. The procedure of the beverage fermentation can be referenced in a different study [15]. The prepared 100% fruit juices were first freeze-dried (Thermo Electron Corporation, USA) to yield dried FTCJ and PTCJ samples, and then they were dissolved in sterile distilled water. Sample solutions were then filtered with a 0.2-mm filter before being subjected to cells to ensure further sterilization for viability and differentiation assays.

Cell Growth and Maintenance

The 3T3-L1 preadipocytes were cultured in DMEM containing high glucose (4500 mg/L), supplemented with 10% of FBS and 3.7 g/L of sodium bicarbonate, in an atmosphere of 5% carbon dioxide (CO_2) at 37 °C. When reaching 70-80% confluency, the cells were sub-cultured twice a week in order to prevent the culture from becoming over-confluent. Cells were maintained in T75 flask during routine subculturing. Cells with passage numbers between 3 and 13 were used for the experiment.

Cell Viability Assay

The cell viability assay was done using the MTT cytotoxicity method with a few modifications [16]. Briefly, the 3T3-L1 preadipocyte cells were seeded in a 96-well plate and allowed to reach confluence. Cells were grown in high-glucose DMEM + 10% FBS. Once confluency was reached, cells were treated with FTCJ and PTCJ at varying concentrations (0.5 – 1.5 mg/mL). Untreated cells were used as a control. After 24 h, the medium was removed and the cells were washed with PBS (1X, pH 7.4). In each well, 150 µL of fresh medium containing 0.5 mg/mL of MTT solution was added before the cells were incubated at 37 °C for 4 h. After the incubation period, the medium containing MTT solution was removed, and the formazan crystals formed were solubilized with DMSO. Cells were incubated again at room temperature for 30 min to allow solubilisation to occur. Absorbance was then determined at 540 nm by a microplate reader (Tecan Group Ltd., Switzerland). DMSO was used as the blank solution.

Determination of the Optimal Day for 3T3-L1 Differentiation into Adipocytes

The differentiation of 3T3-L1 cells followed a previously described method [17]. The 3T3-L1 cells were cultured in high-glucose DMEM and 10% FBS, incubated at 37 °C in a 5.0% CO_2 incubator. 3T3-L1 cells were grown in 24-well plates and left to confluence. After 2 days post-confluence (day 0), differentiation

was induced by supplementing the cells with differentiation medium containing 10 µg/mL insulin, 0.5 mM IBMX (3-isobutyl-1-methylxanthine), and 1 µM dexamethasone. The medium was replaced every 2 days with fresh medium containing only low-glucose DMEM, 10 µg/mL insulin, and samples until day 10. For the optimisation process, the cells were harvested every day for 10 days to observe their daily growth. The cells were then subjected to Oil Red O staining (described below). The cell was then destained using isopropanol to quantify lipid accumulation via absorbance reading (490 nm, UV-visible spectrophotometer (Labomed, Inc., USA)). Differentiation day with the highest absorbance signifies the highest lipid accumulation, thus chosen for cell maturity.

Adipogenesis Inhibition Assay

The 3T3-L1 cells were grown in 24-well plates and left to confluence. The cell differentiation followed the previously described protocol above. To test the inhibitory effect of samples towards the cells' adipogenesis, FTCJ and PTCJ at concentrations ranging from 0.25 – 0.75 mg/mL were also added to the medium from day 0 until day 6 of differentiation. Untreated cells were used as a control. On day 8 of differentiation, the cells were stained with Oil Red O as described below, and lipid accumulation was observed using a light microscope (20X magnification).

Determination of Lipid Accumulation via Oil Red O Staining

Lipid accumulation inside the cells was observed through Oil Red O staining following a previously described method [17]. The cell culture medium was first removed, and the cells were washed with 1X PBS. To fix the cells, 1 mL of 4% formalin was added and incubated for 15 minutes. Oil Red O stain was then added, and the samples were incubated for 30 min. The cells were washed five times with sterile distilled water prior to microscopic observation. For quantitative analysis, isopropanol was added to the samples and left to incubate for 15 min to solubilise the Oil Red O stain, and finally, the absorbance was read at 490 nm using a UV-visible spectrophotometer (Labomed, Inc., USA). Isopropanol was used as the blank solution.

α-Amylase Inhibitory Activity Assay

The α-amylase inhibitory activity assay was performed according to a previously described method, which utilized the 3,5-dinitrosalicylic acid method with a few minor modifications [18]. Two hundred microliters of sample solutions (FTCJ and PTCJ) with a concentration range of 0.25–1.5 mg/mL were added to test tubes. Then, 200 µL of α-amylase solution (2 U/mL) premixed in 0.02 M sodium phosphate buffer (with the addition of 0.006 M NaCl at pH 6.9) was also added. The mixture was left incubated at 30 °C for 15 min. Afterwards, 200 µL of 1% starch solution was added to each tube and re-incubated at 30 °C for 15 min. The termination of the enzymatic reaction occurred via the addition of 200 µL of DNS reagent.

The mixture was boiled in a water bath at 90 °C for 10 min and then allowed to cool to room temperature before being diluted to 5 mL. Absorbance was measured using a UV-visible spectrophotometer (Labomed, Inc., USA) at 540 nm. The mixture without the FTCJ and PTCJ samples was used as the negative control to demonstrate 100% enzyme activity, while a blank reaction was prepared using a similar procedure but without the addition of the enzyme solution.

The percentage of α-amylase inhibition activity was calculated using the formula below.

$$\% \text{ of } \alpha\text{-amylase inhibition} = \frac{(\text{Absorbance negative control} - \text{Absorbance sample})}{(\text{Absorbance negative control})} \text{ x } 100\%$$

α-Glucosidase Inhibitory Activity Assay

This assay was carried out following a standard constructed method [19]. First, 60 µL of the FTCJ and PTCJ sample solution with the concentration range of 0.25 – 1.5 mg/mL was added to a 96-well plate and mixed with 50 µL of 0.2 U/mL α-glucosidase solution. The enzyme solution was prepared by dissolving α-glucosidase powder in 0.1 M potassium phosphate buffer at pH 6.8. The mixture was incubated at 37 °C for 20 min. Thereafter, 50 µL of 0.005 M PNPG was added, and the mixture was re-incubated at 37 °C for an additional 20 min. To stop the reaction, 160 µL of 0.2 M sodium carbonate was added. Absorbance was recorded at 405 nm by using a microplate reader (Tecan Group Ltd., Switzerland). The mixture without the FTCJ and PTCJ samples was used as the negative control to demonstrate 100% enzyme activity, while a blank reaction was prepared using a similar procedure but without the addition of the enzyme solution. The percentage of α-glucosidase inhibition activity was calculated through the formula below.

$$\% \text{ of } \alpha\text{-glucosidase inhibition} = \frac{(\text{Absorbance negative control} - \text{Absorbance sample})}{(\text{Absorbance negative control})} \text{ x } 100\%$$

Statistical Analysis

The experiments were conducted using a completely randomized design. All numerical data were subjected to one-way analysis of variance (ANOVA), and the significant difference between means was determined using Tukey's multiple range test in GraphPad Prism software version 8.1.1.

RESULTS AND DISCUSSION

The Effect of FTCJ and PTCJ on 3T3-L1 Cell Viability

Fig. 1 illustrates the effect of FTCJ and PTCJ on the viability of 3T3-L1 preadipocyte cells during cell proliferation. The MTT assay, which was used in this study, is a frequently used method in cytotoxicity and cell proliferation studies. Exposure to FTCJ was observed to have no cytotoxic effect on the cells at any of the tested concentrations, where the cells were 78.36 ± 9.25%, 96.31 ± 6.75%, 91.24 ± 4.10%, and 96.66 ± 7.23% viable at concentrations of 0.5, 0.75, 1.0, and 1.5 mg/mL, respectively. However, concentrations of PTCJ at 1.0 and 1.5 mg/mL were seen to drastically reduce the cell viability from 100% (control) to 34.35 ± 19.88% and 2.31 ± 1.56%, respectively.

A similar trend of cell viability reduction was observed when 3T3-L1 cells were subjected to different concentrations of bog bilberry anthocyanin extract (BBAE) [20]. In their study, the viability of the cells was reduced to less than 20% at the BBAE concentration of 0.8 mg/mL and higher. Anthocyanins are a type of plant secondary metabolite or pigment responsible for giving fruits and vegetables their red, blue, or purple color [21]. Considering that ripe Terengganu cherry is red in colour, the presence of anthocyanins in the fruit is plausible. This possibility may also explain the cytotoxicity effect of PTCJ towards the cells in the present study. To prove this, however, a study is needed to identify the bioactive compounds of Terengganu cherries.

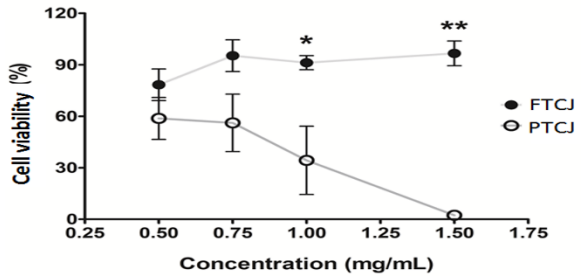

Fig. 1. The effect of FTCJ and PTCJ on 3T3-L1 cell viability. Results are expressed as mean ± standard error of mean (SEM) (n=4). A one-way ANOVA followed by Tukey's post-test was performed; *p<0.05, **p<0.01, ***p<0.001. FTCJ: fermented Terengganu cherry juice, PTCJ: pasteurized Terengganu cherry juice**.**

As depicted in **Fig. 1**, there was a stark difference between the effect of PTCJ and FTCJ on cell viability. This may be due to the effect of the fermentation process done towards FTCJ, in which some of the bioactive compounds originally present might have been reduced. Said compounds may also be responsible for causing the reduction in its cytotoxicity effect. A similar case of reduction was reported in a study on goji berries, where the level of carotenoids present in the fruit was reduced after undergoing microbial fermentation [22]. A 50% decrease in anthocyanin level was also observed on fermented mulberry [23]. In this study, it can be inferred that a high concentration (>0.75 mg/mL) of PTCJ is toxic to the cell. Therefore, the following study on the inhibitory effect of PTCJ and FTCJ on 3T3-L1 cell differentiation was conducted at non-toxic concentrations of PTCJ and FTCJ, specifically 0.25, 0.5, and 0.75 mg/mL.

Optimisation of 3T3-L1 Cells Differentiation

Optimisation of 3T3-L1 cell differentiation was carried out to observe the day the preadipocyte cells of 3T3-L1 would differentiate into mature adipocytes for cell harvesting purposes. Shown in **Fig. 2**, the absorbance reading for the cells peaked on day 8. The result obtained through this optimisation corroborated with other studies, which also mentioned that their cells became mature on day 8 [24, 25]. Hence, for this reason, day 8 was chosen as the day to harvest the cells for observation and staining in the cell differentiation assay.

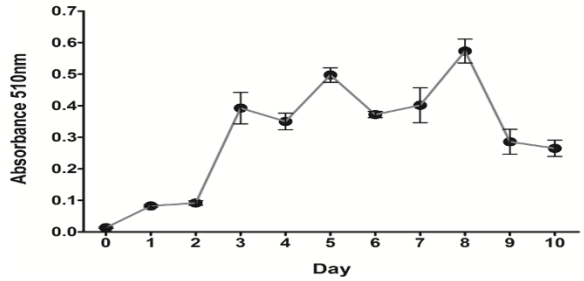

Fig. 2. Optimisation of 3T3-L1 cells differentiation. Data represented as mean ± SEM (n=4).

The Effect of FTCJ and PTCJ on 3T3-L1 Cell Differentiation

In this study, 3T3-L1 preadipocyte cells were treated with insulin, IBMX, and dexamethasone to induce adipocyte cell differentiation. When the preadipocyte cells were differentiated into mature adipocytes, they would be accumulating lipid within the cells which could be observed through Oil Red O staining, a fat-soluble dye (**Fig. 3**). Meanwhile, lipid accumulation was measured through the absorbance reading of eluted Oil Red O. FTCJ and PTCJ were added into the media at the concentrations of 0.25, 0.5, and 0.75 mg/mL to test on their ability to inhibit the adipogenesis process.

Fig. 4 unveiled the effect FTCJ and PTCJ had on cell differentiation and lipid accumulation. Both FTCJ and PTCJ are capable of reducing lipid accumulation and simultaneously inhibiting adipogenesis by at least 50% compared to control (untreated samples). The highest adipogenesis inhibition was observed when the cells were treated with 0.75 mg/mL of PTCJ, with a percentage of lipid accumulation of 21.04 ± 0.5% compared to the control. The PTCJ concentrations of 0.25 mg/mL and 0.5 mg/mL, meanwhile, showed 31.13 ± 1.23% and 25.17 ± 1.50% lipid accumulation, respectively.

PTCJ dose-dependently inhibited adipocyte cell differentiation more effectively than FTCJ, with lipid accumulation percentages of 49.55 ± 8.21%, 51.50 ± 3.20%, and 32.88 ± 2.26% at concentrations of 0.25 mg/mL, 0.5 mg/mL, and 0.75 mg/mL, respectively. However, this could be attributed to the mild cytotoxic effect that PTCJ may have on the cell. Since FTCJ did not previously exhibit any signs of cytotoxicity, the reduction of lipid accumulation in cells treated with this sample may be caused by its ability to inhibit 3T3-L1 adipocyte cell differentiation.

Previously, Kowalska et al. [17] studied the inhibition ability of cranberries. Apart from its lipid-accumulation inhibition effect, cranberry was also reported to be able to down-regulate PPARγ, C/EBPα, and SPREBP1, which are the transcriptional factors of the adipogenesis process. These factors are important as they are responsible for stimulating adipocyte cell differentiation [7]. Thus, the downregulation of these factors is suggested to be the key factor in reducing lipid accumulation and cell differentiation in adipocyte cells.

Many bioactive compounds, such as resveratrol, anthocyanin, and quercetin, were reported in previous literature with their ability to decrease the expression of adipogenesis-related transcription factors [26]. Nevertheless, to date, the purification and identification of bioactive compounds from the Terengganu cherry sample has yet to be reported. Due to this reason, the exact mechanism as to how FTCJ managed to inhibit 3T3-L1 cell differentiation is not yet fully understood.

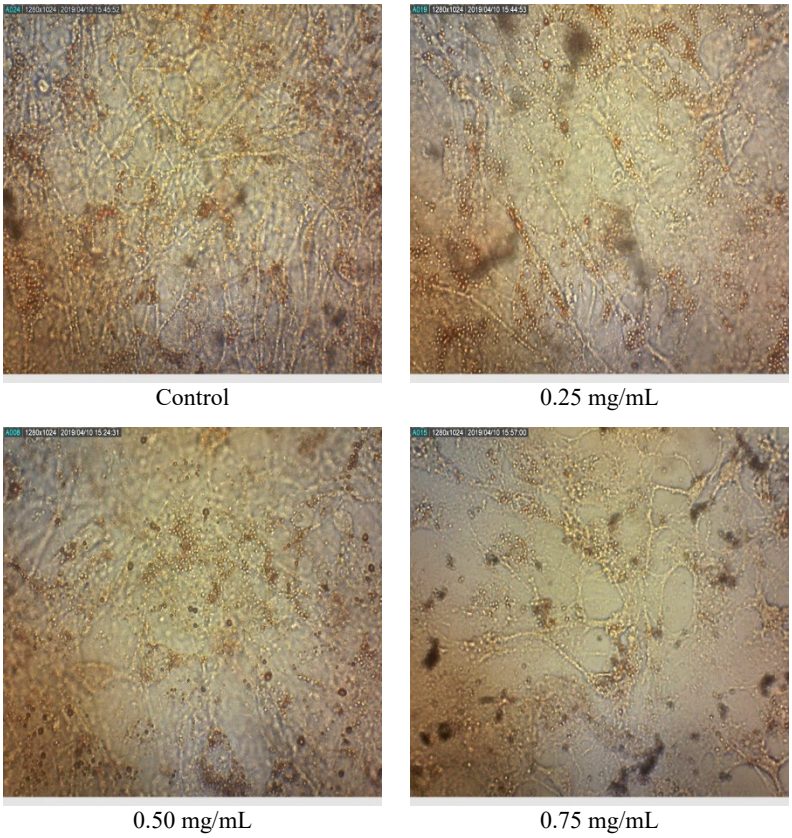

Fig. 3. The effect of different concentrations of FTCJ on the differentiation of 3T3-L1 cells. The 3T3-L1 preadipocytes were allowed to grow to confluence before being differentiated into adipocytes in differentiation medium supplemented with FTCJ (0.25, 0.5, and 0.75 mg/mL) for 8 days. No sample treatment was done for the control. Oil Red-O staining was carried out on day 8.

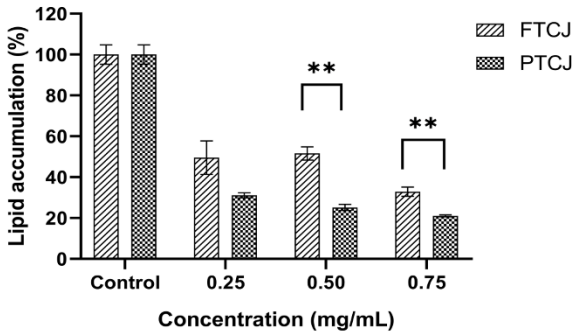

Fig. 4. Effect of FTCJ and PTCJ on the lipid accumulation within matured 3T3-L1 adipocytes. Results are expressed as mean ± SEM (n=4). A one-way ANOVA followed by Tukey's post-test was performed; *p<0.05, **p<0.01, ***p<0.001. FTCJ: fermented Terengganu cherry juice, PTCJ: pasteurized Terengganu cherry juice

The Potential Effect of FTCJ and PTCJ in Inhibiting α-Amylase Activity

The α-amylase inhibition assay was carried out to determine the inhibitory effect of FTCJ and PTCJ on α-amylase enzyme activity. As displayed in **Fig.** 5, both FTCJ and PTCJ exhibited negative inhibition towards α-amylase in a dose-dependent manner for the concentration range of 0.25 to 1.5 mg/mL. PTCJ showed higher values at -14.16 ± 0.98%, 15.81 ± 1.49%, -16.01 ± 1.21%, -18.06 ± 2.18%, and -17.55 ± 1.08% for the concentration of 0.25, 0.5, 0.75, 1.0, and 1.5 mg/mL, respectively. The highest concentration of FTCJ at 1.5 mg/mL showed the value of -13.91 ± 0.42% of inhibition. These results indicated that α-amylase activity is not suppressed by these two samples at all tested concentrations. Based on this observation, it is suspected that both fermented and pasteurized Terengganu cherries may have activated the enzyme instead of inhibiting it.

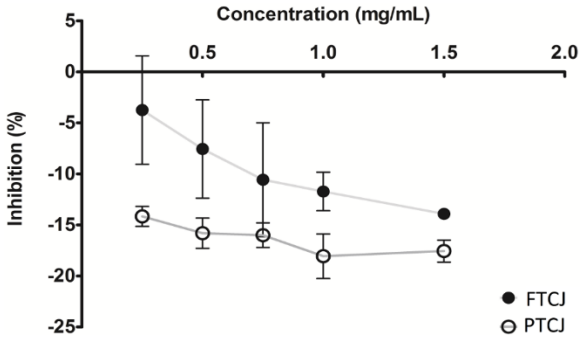

Fig. 5. The effect of FTCJ and PTCJ on α-amylase inhibition activity. Results are expressed as mean ± SEM (n=5). A one-way ANOVA followed by Tukey's post-test was performed; * p<0.05, **p<0.01, ***p<0.001. FTCJ: fermented Terengganu cherry juice, PTCJ: pasteurized Terengganu cherry juice.

Recently, lignin isolated from *Canna edulis* Ker was found to be able to increase the activity of α-amylase concentration-dependently [27]. This study is in agreement with previous research, which found that the novel activation effect of lignin towards α-amylase was due to the spontaneous formation of a hydrogen-bonding complex between the two, affecting the enzyme's conformation 28]. In plants, lignin is a polyphenolic

material that has importance in the formation of cell walls. Additionally, it is one of the beneficial dietary fibers in our diets [29]. Lignin was reported to be present in various kinds of fruits. In cherry pomace, lignin accounted for 49.57% of its dry matter (DM), which was higher than the amounts of cellulose, hemicellulose, and pectin in the fruit. Similarly, black currant possessed a high amount of lignin, which accounted for 53.84% of 0 g DM [30]. In addition, rambutan, which belongs to the same family as the Terengganu cherry (*Sapindaceae*), also has a high lignin content at 35.34 ± 2.05% (w/w), compared to cellulose and hemicellulose [31]. In the present study, both fermented and pasteurized Terengganu cherry juices may have the presence of lignin, which is an activator of α-amylase. This could possibly explain the negative inhibition of α-amylase when being tested with the samples.

The Potential Effect of FTCJ and PTCJ in Inhibiting α-Glucosidase Activity

The potential effect of FTCJ and PTCJ in inhibiting α-glucosidase activity was studied by using α-glucosidase inhibition assay. As shown in **Fig. 6**, both samples displayed positive inhibitory activity against α-glucosidase in a dose-dependent manner. PTCJ exhibited a significantly high percentage (>80%) of enzyme inhibition even at the lowest concentration tested (0.25 mg/mL). At the highest concentration (1.5 mg/mL), maximum enzyme inhibition was obtained with 99.29 ± 0.19% inhibition compared to the control (100% enzyme activity). FTCJ, however, showed lower inhibitory activity compared to PTCJ with the inhibition percentages of 0.99 ± 11.39%, 3.27 ± 9.34%, 26.56 ± 6.97%, 31.71 ± 6.03% and 48.94 ± 3.84% for the concentrations of 0.25, 0.5, 0.75, 1.0, and 1.5 mg/mL, respectively.

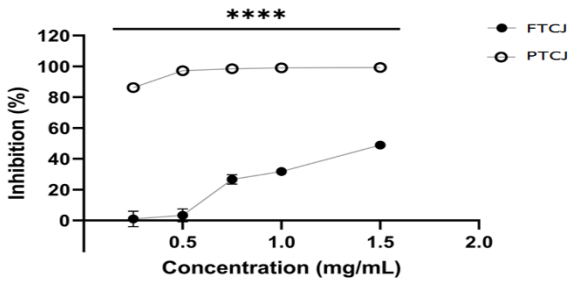

Fig. 6. The effect of FTCJ and PTCJ in inhibiting α-glucosidase activity. Results are expressed as mean ± SEM (n=5). A one-way ANOVA followed by Tukey's post-test was performed; *p<0.05, **p<0.01, ***p<0.001, ****P<0.0001; FTCJ: fermented Terengganu cherry juice, PTCJ: pasteurized Terengganu cherry juice.

The ability of natural products to inhibit the enzyme α-glucosidase can be attributed to the presence of secondary metabolites in plants. Anthocyanins, catechin, and resveratrol are among the bioactive compounds or phytochemicals that are reported to be responsible for the inhibitory activity of this enzyme [32–34]. In a previous study, α-glucosidase inhibitory activity of Terengganu cherry leaves was reported to be greatly contributed by the presence of proanthocyanidins, a polymer that was made up by (epi)catechins and (epi)gallocatechins [35].

Besides Terengganu cherry leaves, proanthocyanidins were also present and responsible for the inhibition of α-glucosidase in persimmon, okra (*Abelmoschus esculentus*), and Chinese bayberry leaves [34, 36, 37]. In light of these findings, it is suspected that proanthocyanidins may also be present in the fruit of the Terengganu cherry and could be a potential enzyme inhibitor for this study. However, we do not rule out the possibility that there might be other bioactive compounds than proanthocyanidins involved in this process. In addition, it is also suspected that the lower inhibition activity of FTCJ compared to PTCJ may be caused by the reduction of some bioactive compounds present during the fermentation process, as stated earlier. To compare the two anti-diabetic assays carried out, FTCJ and PTCJ were shown to be better inhibitors of α-glucosidase than α-amylase. This present study is in agreement with previous research that suggested plant phytochemicals are strong α-glucosidase and weak α-amylase inhibitors [38, 39]. Instead of acting as the cause of malabsorption, α-glucosidase inhibitors aim to delay the digestion and absorption of glucose in the small intestine, while simultaneously reducing the level of glucose in the blood. The ability of FTCJ and PTCJ to inhibit α-glucosidase is a good indicator that Terengganu cherry can potentially be used to treat postprandial hyperglycemia. Furthermore, Terengganu cherries also have a low carbohydrate and calorie content, making them an even better choice for a diet [12].

CONCLUSION

Our data demonstrated that fermented and pasteurized Terengganu cherry juices were able to reduce 3T3-L1 cell differentiation and adipogenesis. While PTCJ exerts cytotoxicity effects towards the cells that might have caused the reduction in viable cells, FTCJ, on the other hand, may have the ability to inhibit adipogenesis. Moreover, both PTCJ and FTCJ have demonstrated the ability to inhibit α-glucosidase. Comparison between pasteurized and fermented Terengganu Cherry juices was also observed, where the fermentation process was suspected to reduce the number of bioactive compounds present in the fruit. We believe that this is the first study to investigate the effect of the juice extract from the Terengganu cherry on adipogenesis and the inhibition of starch hydrolases. Thus, these findings could serve as a platform for understanding the potential anti-obesity and anti-diabetic properties of this fruit. This study, however, lacks data on the identification of Terengganu Cherry bioactive compounds that could be responsible for the observed results. Hence, more elaborate *in vitro* and *in vivo* studies are needed in the future to confirm the possibility of using the Terengganu cherry, including its juice extract, as a means to reduce obesity and diabetes.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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