J Herbmed Pharmacol. 2022; 11(2): 296-304.

http://www.herbmedpharmacol.com doi: 10.34172/jhp.2022.35

Journal of Herbmed Pharmacology

Hydroethanolic *Cyperus rotundus* L. extract exhibits anti-obesity property and increases lifespan expectancy in *Drosophila melanogaster* fed a high-fat diet

Nattapong Wongchum[1,2], Ananya Dechakhamphu[3,4*], Panatda Panya[3,4], Somchai Pinlaor[5], Sitthisak Pinmongkhonkul[6], Alongklod Tanomtong[1]

[1]Biology Program, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Biology Program, Faculty of Science, Ubon Ratchathani Rajabhat University, Ubonratchathani 34000, Thailand
[3]Thai Traditional Medicine Program, Faculty of Thai Traditional and Alternative Medicine, Ubon Ratchathani Rajabhat University, Ubonratchathani 34000, Thailand
[4]Aesthetic Sciences and Health Program, Faculty of Thai Traditional and Alternative Medicine, Ubon Ratchathani Rajabhat University, Ubonratchathani 34000, Thailand
[5]Department of Parasitology, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand
[6]Department of Biology, School of Science, University of Phayao, Phayao 56000, Thailand

ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 7 December 2021
Accepted: 15 February 2022

Keywords:
Herbal medicine
Anti-obesity agent
Oxidative stress
Longevity
Animal model

ABSTRACT

Introduction: *Cyperus rotundus* L. is suspected of having anti-obesity properties. The purpose of this study was to determine the anti-obesity property of hydroethanolic *C. rotundus* extract (HECE) using *Drosophila* as a model organism.

Methods: *In vitro* inhibition of lipase activity by *C. rotundus* extract was investigated. The effects of *C. rotundus* extract on obesity-related characteristics, including body weight, triglyceride content, and lifespan extension were evaluated in *Drosophila* fed a high-fat diet (HFD). The effect of the extract on the reduction of oxidative stress associated with obesity was assessed *in vivo* using antioxidant assays in *Drosophila*.

Results: HECE inhibited lipase activity *in vitro* with an IC_{50} of 128.24 ± 3.65 µg/mL. *In vivo* lipase inhibition experiments demonstrated that feeding *Drosophila* 10 mg/mL HECE or 2 µM orlistat lowered lipase activity by 21.51 ($P<0.05$) and 42.86% ($P<0.01$) and triglyceride levels by 20.67 ($P<0.05$) and 28.39% ($P<0.01$), respectively, compared to those of the untreated group. After 10 mg/mL HECE or 2 µM orlistat supplementation, an increase in the mean survival rate (10.54 ($P<0.05$) and 13.90% ($P<0.01$), respectively) and climbing ability (25.03 ($P<0.01$) and 28.44% ($P<0.01$), respectively) was observed compared to those of flies fed a HFD. The paraquat and H_2O_2 challenge tests revealed that flies fed HECE in a mixed HFD showed increased survival on flies fed a HFD.

Conclusion: This study demonstrates the beneficial effects of dietary HECE supplementation on suppressing pancreatic lipase activity and lowering triglyceride levels and oxidative stress, leading to increased lifespan in *Drosophila* fed a HFD.

Implication for health policy/practice/research/medical education:
This study demonstrates the beneficial effects of dietary hydroethanolic *Cyperus rotundus* extract supplementation on suppressing pancreatic lipase activity and lowering triglyceride levels and oxidative stress. *C. rotundus* extract may be used in the future to develop an anti-obesity drug.

Please cite this paper as: Wongchum N, Dechakhamphu A, Panya P, Pinlaor S, Pinmongkhonkul S, Tanomtong A. Hydroethanolic *Cyperus rotundus* L. extract exhibits anti-obesity property and increases lifespan expectancy in *Drosophila melanogaster* fed a high-fat diet. J Herbmed Pharmacol. 2022;11(2):296-304. doi: 10.34172/jhp.2022.35.

Introduction

Obesity is a significant risk factor for type 2 diabetes, heart disease, and certain types of cancer (1-3). According to the World Health Organization (WHO), being obese and overweight are associated with a higher mortality rate than being underweight (4). On a global scale, there are more obese people than underweight people. Moreover, obesity and diabetes are currently associated with a significantly increased risk of death from COVID-19 infection (5). In 2019, diabetes was the ninth leading

***Corresponding author**: Ananya Dechakhamphu,
Email: ananya.d@ubru.ac.th

cause of death, responsible for an estimated 1.5 million deaths (6). Notably, diabetes significantly reduces an individual's life expectancy (7). By 2030, linear temporal trend analysts predict that 51% of the population will be obese (8). Dietary consumption is the most significant environmental risk factor for the development of chronic metabolic diseases. Diets high in sugar and fat have been linked to obesity and diabetes (9). On the other hand, obesity and diabetes are preventable. Numerous studies have been conducted to determine the efficacy of herbal medicine in preventing obesity and diabetes. A significant proportion of these studies have concentrated on lowering fat and sugar absorption in response to these serious health concerns (10). The advantages of herbal medicines over synthetic drugs include fewer side effects and the ability of active phytochemical ingredients to reduce oxidative stress (11).

Cyperus rotundus L. (Cyperaceae), also known as purple nutsedge, has traditionally been used to treat hyperglycemic disorders such as diabetes. Stilbene dimers found in *C. rotundus* extract were shown to inhibit the activity of α-glucosidase and α-amylase, two carbohydrate digestion enzymes (12). Extracts from the aerial parts of *C. rotundus* inhibited protein glycation, resulting in antihyperglycemic effects in a fructose-mediated model (13). In hyperglycemic rats treated with alloxan, oral administration of 200 and 500 mg/kg of a 70% ethanol extract of *C. rotundus* rhizomes significantly decreased blood glucose levels (14). The tuber extract of *C. rotundus* contains activators of β-adrenoreceptors (ARs), which inhibit obesity in 3T3-F442 adipocytes by stimulating brown adipose tissue thermogenesis (15). A dose of 220 mg/(kg/d) reduced weight gain significantly in Zucker rats (16). Additionally, numerous studies have demonstrated that the rhizomes and tubers of this plant contain antioxidant properties (13,17). These research findings indicate that *C. rotundus* extract may be a promising herbal medicine for the prevention and treatment of obesity and diabetes. However, the *in vivo* effects of *C. rotundus* extract on metabolic enzyme inhibition, oxidative stress reduction, and protection against life expectancy reduction associated with high-energy diets remain unclear.

Drosophila melanogaster, a fruit fly, is an excellent model organism for investigating obesity and metabolic diseases. Similar to humans, *Drosophila* contains insulin-producing cells (IPCs), insulin-like peptides (dILPs), and an insulin receptor (InR) (18-19). *Drosophila* has been used to establish diabetes models that mimic the characteristics of T2D. Specifically, a high-sugar diet model was developed to induce T2D in *Drosophila* (20). In *Drosophila* larvae and adults, insulin-resistant phenotypes such as metabolic dysfunction, increased Dilp mRNA, and impaired insulin signaling activity can be reproduced (21). Additionally, adult *Drosophila* fed a high-sugar diet developed hyperglycemia, insulin resistance, and increased fat accumulation and showed a shorter lifespan (22). A high-fat diet (HFD) model was established in *Drosophila* to induce diabetic phenotypes. According to Birse et al (23), a HFD can result in fat deposition in adipose tissue, fat bodies, and the midgut. Thus, *Drosophila* is an appealing model organism for studying metabolic disorders associated with a high-energy diet. The effects of plant extracts on metabolic disorders caused by high-sugar or HFDs in *Drosophila* have been investigated (24-27). However, there is no report on the effects of *C. rotundus* L. extract in *Drosophila* fed a HFD.

This study aimed to determine the effect of *C. rotundus* extract on metabolic enzyme inhibition *in vivo* using *Drosophila* as the model organism. In *Drosophila* fed a HFD, the effects of *C. rotundus* extract on obesity-related characteristics such as body weight and triglyceride contents were assessed. Additionally, antioxidant assays were performed to determine the extract's effect on reducing oxidative stress associated with obesity in *Drosophila* fed the extract in combination with a HFD.

Materials and Methods

Chemicals and reagents

Porcine pancreatic lipase, *p*-nitrophenyl butyrate (*p*-NPB), *p*-nitrophenol, morpholine propanesulfonic acid, orlistat, and bovine serum albumin (BAS) were purchased from Sigma Aldrich. Ethanol, dimethyl sulfoxide, and tris hydrochloride were purchased from Merck (Germany). Other chemicals used in this study were of analytical grade.

Preparation of plant extracts

The tubers and rhizomes of *C. rotundus* were collected in Singburi province, Thailand. Plant species were identified and authenticated by Mr. Chakkapong Thangthong, Plant Taxonomist, Ubon Ratchathani Rajabhat University, Thailand. A voucher specimen was deposited at Mahasarakham University, Thailand (NW001/2563). Plant materials were dried for 48 hours at 50°C in a hot air oven and ground into a fine powder. Fifty grams of powder was macerated in 500 mL of 70% ethanol for seven days at room temperature (25-30°C). The extracts were obtained through filtration (Whatman filter paper 40) and concentration using a rotary evaporator (Buchi R-210, Flawil, Switzerland). Concentrated extracts were dehydrated at -110°C using a lyophilizer. The dried materials were stored at -20°C until use.

Gas chromatography–mass spectrometry (GC-MS) analysis of hydroethanolic *C. rotundus* extract (HECE)

To determine the phytochemical contents of HECE, a GC-MS instrument (7890B GC/5977B MSD, Agilent Technologies, Santa Clara, CA, USA) was connected to an HP-5MS capillary column of 25 m × 250 m × 0.25 m. The extract was diluted with 70% ethanol, and splitless injection was performed. The oven temperature was set to

60°C for 0 minutes and then increased to 300°C at a rate of 5°C/min. Helium (99.999% purity) was used as the carrier gas, with a flow rate of 1.0 mL/min. The MS temperature of the ion source was 230°C with a 70 eV EI type and a 150°C quadrupole temperature. The results were analyzed in scan mode, starting at mass 35 and ending at mass 550. The data in Figure 1 are summarized in terms of retention time and chemical formula.

In vitro inhibition assay of porcine pancreatic lipase

The extracts' inhibitory activity against porcine pancreatic lipase was determined using a method previously described by Kim et al (28) with minor modifications. To prepare an enzyme buffer, 30 µL of porcine pancreatic lipase solution (2.5 mg/mL in 10 mM morpholine propanesulfonic acid and 1 mM EDTA, pH 6.8) was added to 850 µL of Tris buffer (100 mM Tris-HCl and 5 mM $CaCl_2$, pH 7.0). Next, 100 µL of the plant extracts (100 ug/mL) or orlistat was added, and the mixture was incubated at 37°C for 15 minutes. Then, ten microliters of the substrate (10 mM *p*-NPB in dimethyl-formamide) were added, and the mixture was incubated at 37°C for 30 minutes. The activity of lipase was evaluated by measuring the hydrolysis of *p*-NPB to *p*-nitrophenol at a wavelength of 405 nm using an ELISA reader (Biochrome, England). The inhibitory activity (I) of each compound was determined using the following formula:

$$I\% = \left(1 - \frac{B-b}{A-a}\right) \times 100$$

where A denotes the activity of the enzyme in the absence of an inhibitor, α denotes the activity of the enzyme in the presence of an inhibitor, B denotes the activity of the enzyme in the presence of an inhibitor, and b denotes the activity of the enzyme in the presence of an inhibitor. Orlistat was used as a positive control. The 50% inhibitory concentration (IC_{50}) was determined using the regression equation obtained by plotting the percentage of inhibition versus the extract concentrations (2.5-500 ug/mL).

Drosophila strain, culture conditions, and experimental design

The wild-type *D. melanogaster* Oregon-R-C strain was provided by the Department of Biology, Khon Kaen University. The flies were kept in a conventional wheat cream medium supplemented with yeast powders and maintained in the laboratory at a temperature of 25 ± 1.2°C and a relative humidity of 70-80% under a 12:12 light/dark cycle, with survivors relocated to fresh food vials every 2 days. A HFD was prepared by supplementing the control diet with 15% coconut oil. In this study, we selected a concentration of *C. rotundus* extract of 10 mg/mL based on the longevity results (data not shown), which indicated that this concentration had the greatest effect on *Drosophila* lifespan extension. To prepare HECE-containing diets, 10 mg/mL HECE (HECE10) was added directly to a control diet at 45-50°C to prevent extract degradation. After the HECE was added, it was mixed immediately before the diet became gelatinous and kept at 25°C for 18 hours to allow the steam to completely evaporate. Orlistat-containing diets were prepared by dissolving the powder in 10% DMSO and then adding it at a final concentration of 2 µM to the diet. Orlistat

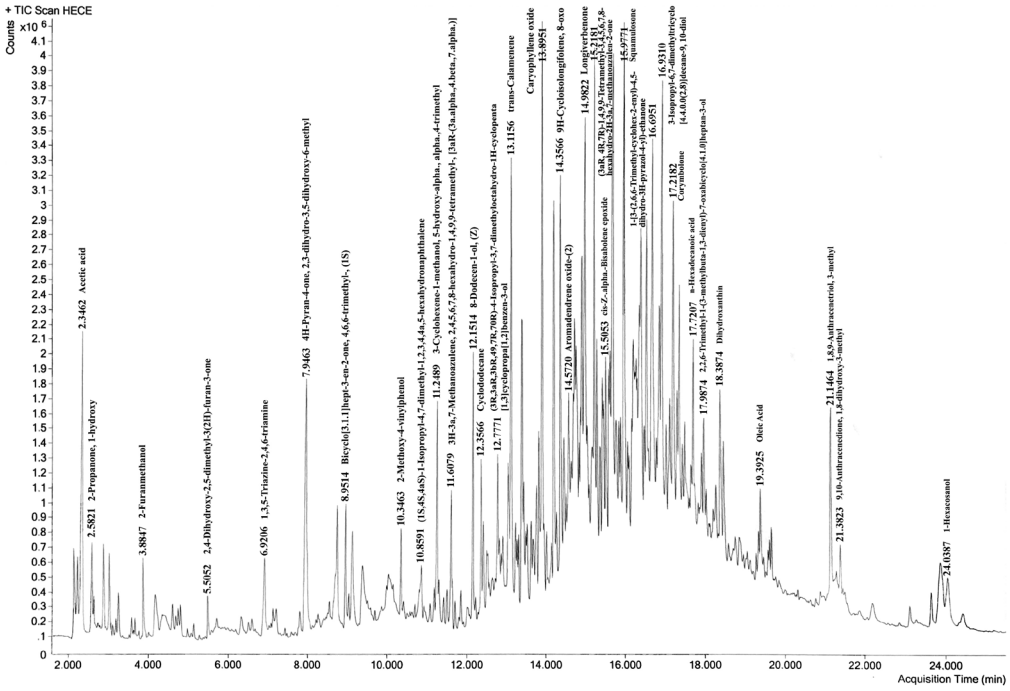

Figure 1. Gas chromatography–mass spectrometry (GC–MS) chromatograms of the hydroethanolic extract from tubers and rhizomes of *C. rotundus*.

concentrations were determined in accordance with the reports of Sieber and Thummel (29). The diets of the other groups were also supplemented with 10% DMSO in the same volume as the orlistat diet.

The experiments were performed with female flies aged 3-5 days. Flies were divided into 5 groups, including the control, HECE10, HFD, HFD+HECE10, and HFD+2 μM orlistat groups. The flies were fed either an experimental or a control diet for 10 days. On day 10, flies were used in each experiment to determine body weight, lipase activities, triglyceride and protein contents, climbing ability, and antioxidant activity. Another experiment was replicated to determine lifespan, with survivors receiving a new feeding vial of the same food every 2 days. The dead flies were counted every 2 days until the final fly died. The research design was approved by the Ubon Ratchathani Rajabhat University's Ethics Committee (Ethical Clearance No. AN63008). The experimental design for this study, as well as the number of flies used in each experiment, are detailed in Figure 2.

Protein content assay

The protein content of the whole-body homogenate was determined according to the Bradford (30) method using Merck's Bradford reagent according to the manufacturer's protocol. Bovine serum albumin was used as the standard, and the concentration was expressed in mg/mL.

Lipase activity assay in *Drosophila*

Flies were homogenized in 300 mL cold 20 mM PBS (Ca^{2+}/Mg^{2+} free, pH 7.0). The debris from homogenates was removed by centrifugation at 1500 g for 5 minutes at 4°C. The supernatant was then used to measure the activity of the enzymes.

The activity of *Drosophila* lipase was measured by mixing 100 μL of supernatant with 850 μL of Tris buffer (100 mM Tris-HCl and 5 mM $CaCl_2$, pH 7.0) and 10 μL of 10 mM *p*-NPB (dissolved in dimethyl formamide). The reaction mixtures were incubated at 37 °C for 30 minutes. The activity of lipase was evaluated by measuring the hydrolysis of *p*-NPB to *p*-nitrophenol at a wavelength of 405 nm using an ELISA reader (Biochrome, England). The enzyme activity was determined using the porcine pancreatic lipase standard curve, which was constructed by plotting the optical density at 405 nm versus the enzyme concentrations (25-500 U/mL). Each sample was examined in triplicate. The enzyme activities were normalized to the protein content in the supernatant.

Body weight and whole-body triglyceride assays in *Drosophila*

Ten days after being reared on the indicated diets, flies were weighed using a microbalance. Whole flies (20 flies/vial) were homogenized in 500 μL of 0.5% Tween 20+1 mM PBS (pH 7.4). The debris from homogenates was removed by centrifugation at 10 000 g for 5 minutes at 4°C. Lipase enzyme activity was inhibited by immediately heating the supernatant at 70°C for 5 minutes. Flies' triglyceride levels were measured using standard enzymatic procedures (Dimension RxL Max; Siemens AG, Erlangen, Germany). Triglyceride levels were calculated as the proportion of triglyceride (mg/dL) to protein (mg/mL).

Climbing ability, antioxidant activity, and lifespan extension assays in *Drosophila*

The assays for climbing ability and oxidant activity were conducted on day 10 following the experiment. Climbing ability was determined as described previously (31). Briefly, for each climbing trial, 20 flies were incubated at 25°C for 30 minutes to adapt. The flies were gently tapped

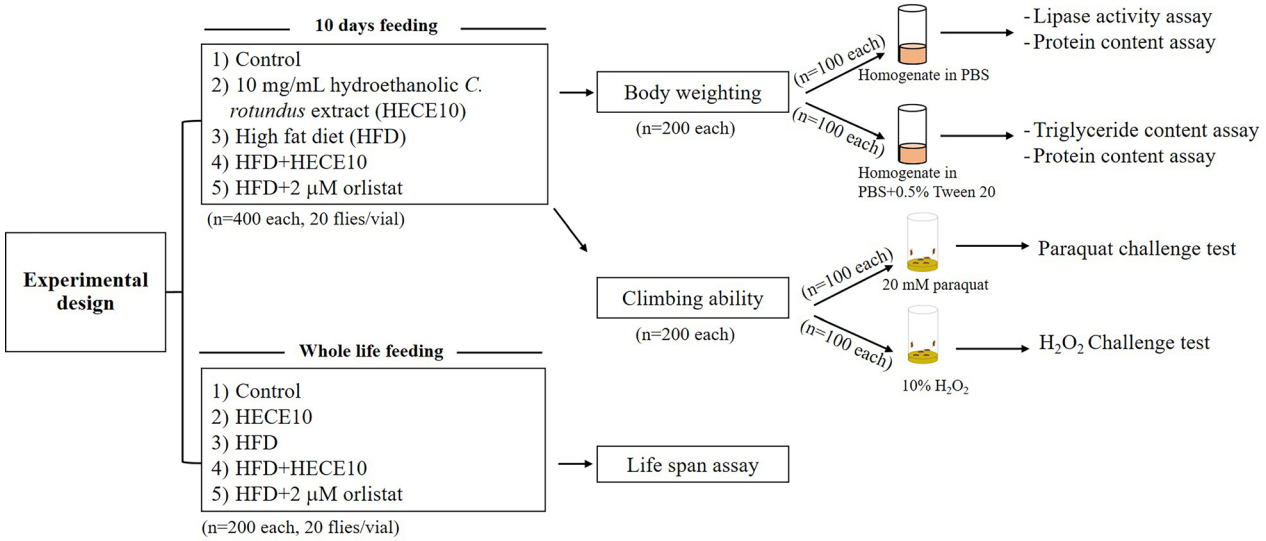

Figure 2. Scheme of the experimental design for studying the effect of *C. rotundus* extract on anti-obesity effects and longevity in *Drosophila* fed a high-fat diet.

to the bottom of the test vial (2.5 × 200 mm) and given 10 seconds to climb back up. The number of flies that climbed 15 cm or more vertically was counted. Each test was conducted three times. Following the climbing assay, flies were tested for antioxidant capacity.

To evaluate resistance to paraquat (superoxide anion) and hydrogen peroxide, fruit flies were starved for 2 hours and then transferred to separate vials containing filter paper saturated with either 20 mM paraquat or 10% hydrogen peroxide prepared with a 6% sucrose solution. Dead files were recorded every 4 hours until the final fly died.

Statistical analysis

SPSS 20.0 was used to conduct statistical analysis of the data (SPSS Inc., Chicago, USA). The *t* test and one-way ANOVA were used to compare means. The Kaplan-Meier test was used to determine the difference between the survival curves. Differences were considered significant when $P<0.05$. *P* values denoted by the symbols *<0.05 and **<0.01 were used to indicate the levels of significance across all assays.

Results

GC–MS chemical profiles of *C. rotundus* extract

The GC–MS chromatograms of a hydroethanolic extract of *C. rotundus* tubers and rhizomes are shown in Figure 1. The most abundant anti-obesity and antioxidant compounds included 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, n-hexadecanoic acid, dihydroxanthin, oleic acid, 1-hexacosanol, carophyllene oxide, 2-methoxy-4-vinylphenol, and oleic acid.

Lipase inhibitory activity of *C. rotundus* extract *in vitro*

The effects of the hydroethanolic extract of *C. rotundus* on lipase activities were measured using spectrophotometry with *p*-NPB as substrates. The extract inhibited lipase at an IC_{50} concentration of 128.24 ± 3.65 μg/mL. The IC_{50} concentration of the standard drug orlistat for lipase inhibition was 0.13 ± 0.009 μg/mL.

Effect of *C. rotundus* extract on lipase inhibition in *Drosophila*

The *in vitro* inhibitory effect of the *C. rotundus* extract on lipase activity was verified *in vivo* using *Drosophila* as a model. The extract properties were evaluated using the standard drug orlistat as a positive control. As illustrated in Figure 3, lipase activity was increased by 36.54% in *Drosophila* fed a HFD compared to *Drosophila* fed a control diet ($P<0.01$). Supplementation with 10 mg/mL *C. rotundus* extract or 2 μM orlistat in a HFD significantly decreased lipase activity by 21.51 ($P<0.05$) and 42.86% ($P<0.01$), respectively, when compared to that in *Drosophila* fed a HFD. When 10 mg/mL *C. rotundus* extract was added to the control diet, no difference in lipase activity was observed when compared to the untreated control.

The effect of *C. rotundus* extract on the body weight and triglyceride levels of *Drosophila*

The effect of *C. rotundus* extract on the body weight and triglyceride levels of *Drosophila* fed a HFD is shown in Figure 4. *Drosophila* body weight was not significantly different when fed a control diet or the experimental diets (Figure 4A). As shown in Figure 4B, the highest level of triglycerides was observed in *Drosophila* fed a HFD (34.48% increase when compared to the control group, $P<0.01$). When *Drosophila* were fed a HFD supplemented with 10 mg/mL HECE or 2 μM orlistat, triglyceride levels were reduced by 20.67 ($P<0.05$) and 28.39% ($P<0.01$), respectively, when compared to those in *Drosophila* fed a HFD. When 10 mg/mL *C. rotundus* extract was added to the control diet, no difference in triglyceride level was observed when compared to the untreated control.

The effect of *C. rotundus* extract on the antioxidant capacity and lifespan of *Drosophila*

The effects of *C. rotundus* extract on the lifespan and antioxidant capacity of *Drosophila* fed a HFD are shown in Figure 5 and Figure 6. As shown in Figure 5A, in *Drosophila* fed a control diet containing 10 mg/mL HECE, the maximum and mean lifespans were increased by 21.24 and 11.09%, respectively, in comparison to those of the untreated control group. *Drosophila* fed a HFD had 59.19 and 52.90% reductions in maximum and mean lifespan rates, respectively, when compared to the control group. When *Drosophila* were fed a HFD supplemented with 10 mg/mL HECE or 2 μM orlistat, the mean lifespan increased by 10.54 ($P=0.025$) and 13.90% ($P=0.010$), respectively, when compared to *Drosophila* fed a HFD alone.

The effect of *C. rotundus* extract on climbing ability in *Drosophila* fed a HFD is shown in Figure 5B. In comparison to all other groups, supplementing *Drosophila* with 10 mg/mL HECE in the control diet resulted in the highest climbing ability. *Drosophila* fed a HFD had a 59.47% reduction in climbing ability when compared

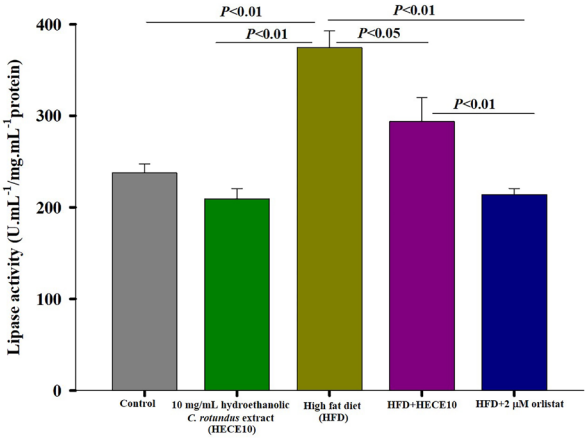

Figure 3. Effect of *C. rotundus* extract on lipase inhibition in *Drosophila* fed a high-fat diet. The bar graphs represent the mean ± SD of lipase activities in *Drosophila* fed a high-fat diet.

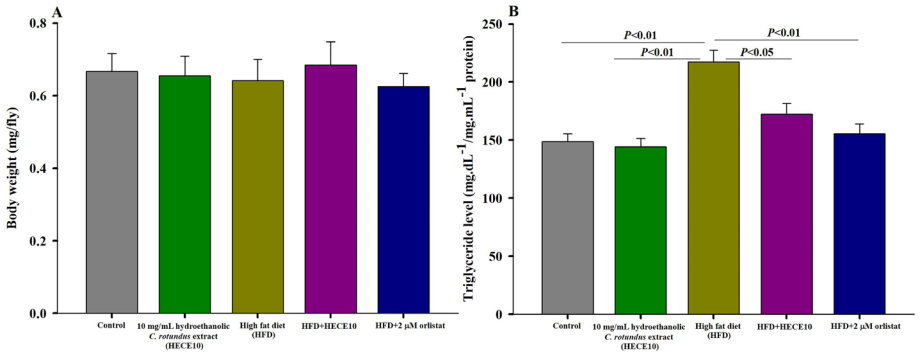

Figure 4. The effect of *C. rotundus* extract on the body weight and triglyceride levels of *Drosophila* fed a high-fat diet. (A) and (B) Mean ± SD of body weight and triglyceride levels in *Drosophila* fed a high-fat diet, respectively.

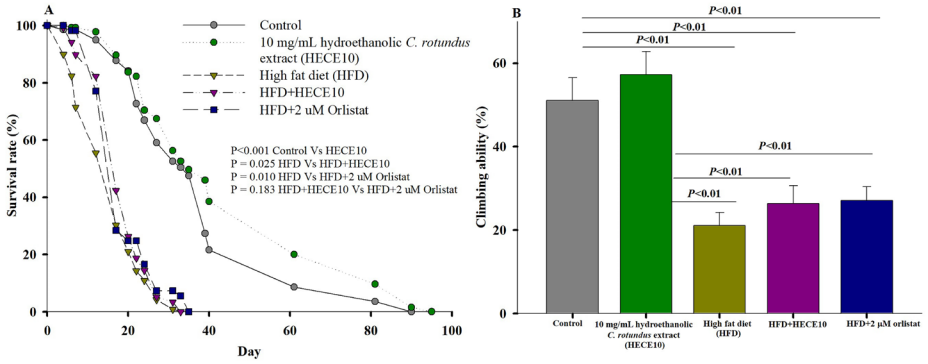

Figure 5. The effect of *C. rotundus* extract on the climbing ability and lifespan of *Drosophila* fed a high-fat diet. The lifespan curves illustrate the survival of *Drosophila* fed a high diet (n=200 per group). The bar graphs represent the mean ± SD of *Drosophila* climbing ability when fed a high-fat diet (n=200 per group).

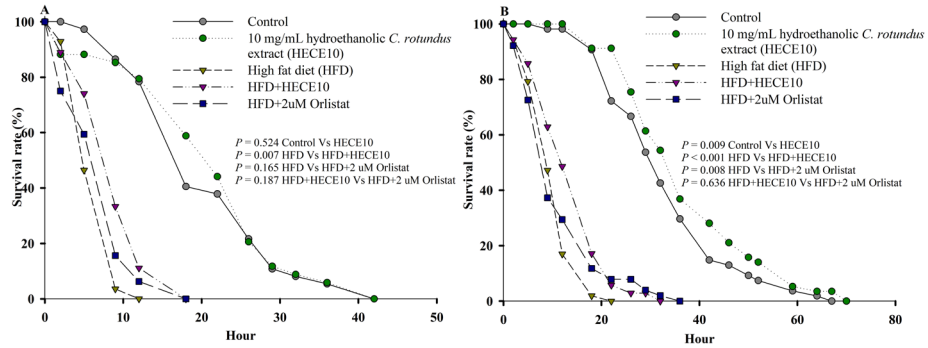

Figure 6. The effect of hydroethanolic *C. rotundus* extract supplementation on the resistance of *Drosophila* fed a high-fat diet to paraquat (A) and hydrogen peroxide (B) exposure (n=100 per group). The survival test was examined using the Kaplan–Meier and log-rank tests. Statistical significance was considered at $P<0.05$.

to the control group. When *Drosophila* were fed a HFD supplemented with 10 mg/mL HECE or 2 μM orlistat, climbing ability increased by 25.03 ($P<0.01$) and 28.44% ($P<0.01$), respectively, when compared to *Drosophila* fed a HFD alone.

The effect of *C. rotundus* extract on resistance to oxidative stress induced by paraquat in *Drosophila* fed a HFD is shown in Figure 6A. The highest mean and maximum survival rates were observed in *Drosophila* fed 10 mg/mL *C. rotundus* extract in the control diet, but statistical significance was not reached when compared to the untreated control. When compared to those of *Drosophila* in the control group, the mean and maximum survival rates of *Drosophila* fed a HFD were reduced by 76.38 and 69.57%, respectively. The mean survival was increased by 42.98 ($P=0.007$) and 20.73% ($P=0.165$) after treatment with 10 mg/mL HECE or 2 μM orlistat.

A similar result was observed in the hydrogen peroxide

challenge test. As shown in Figure 6B, the highest mean and maximum survival rates were observed in *Drosophila* fed 10 mg/mL *C. rotundus* extract in the control diet ($P = 0.009$) when compared to the untreated control. When compared to *Drosophila* in the control group, the mean and maximum survival rates of *Drosophila* fed a HFD were reduced by 69.99 and 66.48%, respectively. When *Drosophila* were fed a HFD supplemented with 10 mg/mL HECE or 2 μM orlistat, the mean survival increased by 38.62 ($P < 0.001$) and 30.78% ($P = 0.008$), respectively, when compared to that of *Drosophila* fed a HFD alone.

Discussion

Consumption of vegetables and fruits has been shown to significantly reduce the risk of developing type 2 diabetes (32), leading to an effective strategy for weight loss and obesity management (33). Here, we evaluated the effect of *C. rotundus* extract on anti-obesity effects and longevity in *Drosophila*. We demonstrated the inhibitory effect of *C. rotundus* on lipid hydrolyzing enzymes *in vitro*, which was consistent with many previous studies on ethanol extracts (12,34,35). According to the GC–MS results, the compounds identified from the extracts, such as 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (36-37), n-hexadecanoic acid (38), dihydroxanthin (39), oleic acid (40), and 1-hexacosanol (41), are reported to have the ability to prevent diabetes and obesity. Hence, based on our findings and other previous reports, we suggest that *C. rotundus* may prevent obesity and diabetes by decreasing dietary fat absorption. To verify this assumption, we investigated the effect of *C. rotundus* extract on the inhibition of lipase in *Drosophila*. We compared the effect of *C. rotundus* extract on *Drosophila* lipase inhibition to that of the standard drug orlistat. As illustrated in Figure 3, feeding *Drosophila* a HFD significantly increased lipase activity. When 10 mg/mL *C. rotundus* extract or 2 μM orlistat was added to HFDs, *Drosophila* lipase activity was significantly decreased. In this study, we found that supplementing a HFD with *C. rotundus* extract significantly decreased triglyceride levels in *Drosophila* compared to those in *Drosophila* fed a HFD alone (Figure 4B). We therefore suggest that the *C. rotundus* extract inhibits pancreatic lipase activity *in vitro* and *in vivo* by reducing dietary fat absorption, leading to lower triglyceride levels and lowering the risk of obesity.

It is well established that high fat consumption results in a shorter lifespan in *Drosophila*. Previous studies have proven that a HFD reduces *Drosophila* life expectancy (42-44). A HFD has been shown to impair *Drosophila*'s climbing ability (43,44). Additionally, it is known that the climbing ability assay can be used to assess *Drosophila*'s physical strength, which serves as an indicator of both muscle and locomotor function (45). In this study, we found that supplementing a HFD with 10 mg/mL *C. rotundus* extract significantly increased the lifespan and climbing ability of *Drosophila* when compared to those of *Drosophila* that were fed only a HFD (Figure 5B), suggesting that *C. rotundus* extract may help reduce the adverse effects of a HFD. The mechanism by which *C. rotundus* extract prolongs life and enhances locomotor function may be related to the reduction in oxidative stress caused by the HFD. Consumption of high-fat and high-sugar diets has been linked to the induction of oxidative stress. A previous study demonstrated that a HFD increases iron absorption, resulting in the formation of hydroxyl radicals via the Fenton reaction (46). Furthermore, dietary supplementation with fat can result in the formation of lipid hydroperoxide (24). Consumption of a high-sucrose diet has been well established as a factor in the induction of oxidative stress (20,46). In addition, antioxidant therapy is another important strategy for the management of obesity and diabetes (32,37). In this study, we investigated whether supplementation with *C. rotundus* extract could reduce free radical-induced mortality in *Drosophila* fed a HFD. As illustrated in Figure 6A and Figure 6B, supplementing *Drosophila* fed a HFD with *C. rotundus* significantly increased survival from paraquat and H_2O_2 exposures when compared to *Drosophila* fed a HFD alone. The effect of the *C. rotundus* extract could be explained by the presence of active ingredients of many compounds that exert antioxidant activity, including 4H-pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl- (47-49), carophyllene oxide (50), 2-methoxy-4-vinylphenol (51), and oleic acid (52,53). Taken together, the increased lifespan, increased locomotor ability, and decreased oxidative stress suggest that supplementation with *C. rotundus* extract may help reduce the adverse effects of a HFD. As a result, we hypothesized that the hydroethanolic extract of *C. rotundus* may have anti-obesity potential and may help reduce the negative effects of long-term HFD consumption. Additional research is necessary to fully understand the pharmacological functions of *C. rotundus* extract.

Conclusion

This study investigated the effects of hydroethanolic *C. rotundus* extract on the anti-obesity activity and lifespan of *Drosophila* fed a HFD. The *C. rotundus* extract inhibited the activity of lipase both *in vitro* and *in vivo*. When compared to those in *Drosophila* fed only a HFD, supplementation with 10 mg/mL *C. rotundus* extract significantly decreased triglyceride levels, increased lifespan, improved climbing ability, and decreased oxidative stress. As a result of this finding, *C. rotundus* extract may be used in the future to develop an anti-obesity drug.

Acknowledgments

We wish to express our gratitude for the instruments provided by the Faculty of Thai Traditional and Alternative Medicine, Ubon Ratchathani Rajabhat University and Department of Biology, School of Science, University of Phayao. We would like to thank Mr. Chakkapong

Thangthong, a Plant Taxonomist at Ubon Ratchathani Rajabhat University, for assisting us with plant identification. We would like to express our gratitude to Assistant Professor Dr. Monthira Monthatong, Faculty of Sciences, Khon Kaen University for providing the wild-type *Drosophila*.

Authors' contributions

NW performed the experiments, analyzed the data and drafted the manuscript. AD designed the experiments, monitored, and edited the manuscript. PP performed the experiments. SP, SP, and AT reviewed the manuscript and supervised the study. All authors reviewed and approved the final manuscript.

Conflict of interests

The authors declare no conflicts of interest.

Ethical considerations

The animal research design was approved by the Ubon Ratchathani Rajabhat University's Ethics Committee (Ethical Clearance No. AN63008).

Funding/Support

This research was supported by a Royal Thai Government Scholarship, Ministry of Higher Education, Science, Research and Innovation (Grant number: 5/2563).

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