ORIGINAL ARTICLE

Evaluation of Anti-Hyperlipidaemic Activity of a Mixture of *Zinger officinale*, *Allium sativum*, Citrus Lemon, Honey, and *Malus domestica* Vinegar (ZACAH) Extracts in Rats Fed with High Cholesterol Diet

Kokila Vani Perumal[1], Hasnah Bahari[1], NorShafarina Shari[2], Kasturi Kanniappan[2], Khairul Kamilah Abdul Kadir[3], Zunoliza Abdullah[3], Mohd Radzi Ahmad[3], Ibrahim Kalle Kwaifa[1,4], Siti Hadizah Jumidil[1], Azrina Zainal Abidin[1,2], Sabariah Md Noor[1], Yong Yoke Keong[1], Santhra Segaran Balan[1,2]

[1] Faculty Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[2] Faculty of Health and Life Sciences, 40100 Shah Alam, Selangor, Malaysia.
[3] Institut Penyelidikan Perhutanan Malaysia (FRIM), 52109 Kepong, Selangor.
[4] School of Medical Laboratory Sciences, College of Health Sciences, Usmanu Danfodiyo University (UDU), Sokoto, North-Western 2346, Nigeria

ABSTRACT

Introduction: The incidence and complications of hyperlipidemia associated co-morbidities and mortalities are grossly increasing globally. Intervention with natural products preparation has been identified as an alternative for the management of hyperlipidaemia and its related cardiovascular complications. This study investigated the anti-hyperlipidaemic activities of a mixture of *Zinger officinale, Allium sativum*, Citrus lemon, honey, and *Malus domestica* vinegar (ZACAH) extracts in in Sprague Dawley (SD) rats fed with high cholesterol diet. **Methods:** Thirty-six male SD rats were randomly distributed into 6 groups, including normal chow diet-fed (NC), high cholesterol diet (HCD), HCD+ Simvastatin (standard drug) while the remaining three groups were fed with HCD + ZACAH extracts at different doses (1, 3 and 5mg/kg body weight) for 18 weeks. Simvastatin at 10 mg/kg of bodyweight was used as control. High-performance liquid chromatography (HPLC) was used to determine phenolic compounds present in ZACAH extracts, elastase inhibitory assay was determined using spectrometric with a substrate (N-Succ-(Ala)3-pnitroanilide (SANA) while 2,2-diphenyl-1-picrylhydrazyl (DPPH) was performed based by the method described by Blois, 1958. **Results:** In vitro; ZACAH extracts had oxygen radical absorbance capacity (ORAC) value of 2000 µmol TE/100 mL, total phenolic content (TPC) of 7537 ± 54.5%, DPPH free radical scavenging activity of 27.34 ± 2.71%, elastase inhibitory assay of 29.29 ± 1.65% and lipoxygenase inhibitory assay of 98.58 ± 1.42%. In vivo, ZACAH extracts showed decreased bodyweight, adipose tissue, improved lipid profiles and hepatic biochemical enzymes. **Conclusion:** These results suggested that ZACAH extracts supplementation improved hyperlipidaemia in SD rats and might be a promising adjuvant for the treatment of hyperlipidaemia.
Malaysian Journal of Medicine and Health Sciences (2022) 18(5): 55-63. doi:10.47836/mjmhs18.5.9

Keywords: ZACAH extracts, Hyperlipidaemia, High cholesterol diet

Corresponding Author:
Santhra Segaran Balan, Msc
Email: santhra@msu.edu.my
Tel: +60 16-2612383

INTRODUCTION

Hyperlipidaemia is characterized by the excess amount of lipids concentration in the bloodstream (1). According to American Health Rankings, the unhealthy level of cholesterol is considered when it reaches more than 240 mg/dL. Hyperlipidaemia has been identified as a risk factor to the formation of cardiovascular diseases (CVDs) (2). According to WHO, it was estimated that 2.6 million deaths are reported every year due to the increased cholesterol levels. Globally, 39% of adults (37% men, 40% of females) have a high amount of cholesterol in the data reported in 2008 (3). Hyperlipidaemia is associated with elevated levels of total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and subsequently dropped in the concentration of high-density lipoprotein (HDL). Cholesterol is an essential structural component of cell membranes functioning in the controlling a constant membrane fluidity and permeability. Physiologically, cells are protected from the intracellular excessive deposition of cholesterol by regulating its synthesis, influx and efflux (4). 3-Hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase is the main

rate-limiting step enzyme for the synthesis of cholesterol. While cholesterol influx is facilitated by the LDL receptor, the efflux is majorly regulated by ATP-binding cassette transporter A1 (ABCA1) (5). Alteration in this mechanism leads to excessive deposition of cholesterol, which may subsequently result in end-organ damage, characterized by increased necrosis and cell death (6).

Several pharmacological approaches for the prevention of hyperlipidaemia have recently been reported. Statin family are considered as the gold standard drugs for the treatment of hyperlipidaemia, leading to CVDs. However, these pharmacological drugs have been investigated to have several side effects, including liver dysfunction, liver poisoning, anti-inflammation effects (7, 8), constipation, insomnia and heart attack (9). Therefore, alternative sources from plant extracts is needed that can effectively and safely treat hyperlipidaemia without adverse effects as well as low toxicity (9, 10, 11). More importantly, the phenolic compounds from the plants have been shown to have a high cholesterol-lowering effect that helps to prevent excess cholesterol absorption (12). Therefore, the herbal mixture has desirable effects on various metabolic parameters. The content of ZACAH extracts have been used in the past centuries for its beneficial effects on health (13, 14). Most of the constituents of ZACAH extracts have been widely used as foodstuff and traditional medicine globally (15). The medicinal properties of ZACAH extracts have been used in many countries for numerous therapeutic applications, including hyperlipidaemia and obesity (16). Also, several compounds available in ZACAH extracts have been reported to show various biological activities, including hyper and hypolipidemia, and hyper and hypocholesterolaemia effects, antioxidant potential (17), antimicrobial activity (18), anti-ulcer, antiplatelet and hypotensive (16). This present study proposed that ZACAH extracts work as a natural cholesterol-lowering agent that interferes with the mevalonate synthesis pathway through the inhibition of HMG-CoA reductase activities. Presently, there is an insufficient study on the anti-hyperlipidaemic activity of this herbal mixture. This present study therefore is aimed to evaluate the potential effects of ZACAH extracts on serum biomarkers in SD rats fed with HCD. The parameters investigated, including TC, TG, LDL and HDL, serum aspirate aminotransferase (AST), serum alanine aminotransferase (ALT), and serum creatinine.

MATERIALS AND METHODS

Ethical consideration

The ethical approval for this study was obtained from the Animal Ethics Committee, Management & Science University (MSU-RMC-02/FR01/08/L3/018).

Preparation of ZACAH mixture

ZACA extracts is a mixture of Zingiber Officinale (ginger), Allium Sativum (garlic), Citrus lemon, Honey, and Malus domestica (apple) vinegar provided by Natural Tone Trading with a combined ratio of 1:1:1:1:1. It has been registered by the Ministry of Health of Malaysia (No: J06P1150420-016615) and Agri-Food & Veterinary Authority of Singapore (AVA) (No: V-021-2015-06-01094). The nutritional composition as shown in Table I.

Table I: Nutritional composition of ZACAH

Composition	Per 100 g
Calories	140 kcal
Total Fat	0 g
Saturated Fat	0 g
Trans Fat	0 g
Cholesterol	0 mg
Sodium	10 mg
Total Carbohydrate	34 g
Dietary Fibre	0 g
Sugar	9 g
Protein	1 g

In Vitro

ORAC (Oxygen radical absorbance capacity) assay

Trolox was used as a control standard. The fluorescenes of Fluorescein disodium was recorded when 2,2′-azobis (2-amidino-propane) dihydrochloride (AAPH) was added. The ORAC value expressed as Trolox equivalents (TE) per gram (μmol TE/g) (19).

Determination of total phenolic content (TPC)

TPC analysis was used to determine the concentration of polyphenols present in the sample equivalent to Gallic acid standard. TPC was carried out with Folin-Ciocalteu reagent by a method described by Singleton and Rossi (1965). About 0.5mL of diluted sample was added into 2.5mL of 0.2mol/L of Folin-Ciocalteu reagent for 4 minutes. About 2mL of a saturated sodium carbonate solution was added and incubated for 2hrs at room temperature. The absorbance was measured at a wavelength of 760nm, and the results were expressed as gallic acid equivalent per 100g of the sample (mg/100g GAE).

Determination of Elastase inhibitory assay

Elastase inhibitory assay was determined using spectrometric with a substrate (N-Succ-(Ala)3-pnitroanilide (SANA). The release of p-nitroaniline was observed for 15 min at 25°C and the absorbance was measured at a wavelength of 410nm. The reaction mixture consists of substrates such as 0.2 M Tris-HCl buffer (pH 8.0), 1μg/mL elastase, 0.8 mM SANA (ESIV; elastase substrate IV, Calbiochem) and the remaining inhibitors dissolved in 70% ethanol or dimethyl sulfoxide (DMSO) and were incubated at 25°C for 15 min. The substrate was added and the reaction was started. All the elements presented in the blank except enzyme and oleanolic acid were represented as a positive control

(20). The calculation as below:

Percentage of inhibition (%) = (1 – B/A) x 100
*A – Enzyme activity without inhibitor
*B – Activity with the presence of inhibitor

Determination of DPPH radical scavenging assay
DPPH assay was used to predict the antioxidant potential of the sample by inhibiting lipid peroxidation. DPPH was performed based on the method described by Blois (1958). About 0.5 mL of 0.5 M acetic acid buffer solution, 1 mL of 0.2 mM DPPH in ethanol, and 1.5 mL of 50% (v/v) ethanol aqueous solution were mixed and added to the ZACAH sample. After 30 mins incubation, the absorbed were measured at 517nm. The activity are expressed as the ratio of the absorption decrease of DPPH (%) to the control DPPH solution (100%) in the absence of the sample (21).

Identification of phenolic compounds
HPLC was used to determine phenolic compounds present in ZACAH extracts by using HPLC system (Waters 2535 quaternary gradient pump, Waters 2707 auto sampler and Waters 2998 Pda, Phenomenex Gemini NX C18 (5µm, 4.6 mm X 250 mm) with a gradient system where Solvent A (water that containing 0.1% formic) and Solvent B (Acetonitrile). The samples were measured by UV absorption spectra. The data were analysed by comparing the retention time and UV absorption spectra (22).

In Vivo

Experimental animals
A total of 36 male SD rats (150 to 200g) were used in this study. The rats were housed at 28±2°C temperature, maintained 12:12h light/dark cycle and humidity of 50±10% and allowed food and water ad libitum. All the experimental procedures for animal care were performed under the National Institutes of Health (NIH) guidelines and approved by the Committee on Animal Care of the Management & Science University (MSU) institute (MSU-RMC-02/FR01/08/L3/018). All the Male Sprague Dawley rats were purchased from KRK Seri Enterprise (001328850-K), Selangor, Malaysia.

Preparation of high-cholesterol diet (HCD)
The HCD contained 8.62kcal/g. It consisted of 500g pellets, 200g egg yolk, 50g ghee, and 100g corn oil. All the ingredients were mixed well and cooled in the refrigerator at 2 to 4°C before fed to the rats. The preparation was used to feed the SD rats in PC and treatment groups through oral gavage throughout the experimental period.

Experimental design
After a week of acclimatization, the rats were distributed into 6 groups:
Group A: Negative control (NC) rats that received a normal chow diet throughout the experimental period.
Group B: Positive Control (PC) rats that received HCD for the period of the study.
Group C: Drug control (DC) rats that received HCD along with 10mg/kg of Simvastatin through oral gavage, daily for 18 weeks.
Group D: Treatment I (TX1) rats that received HCD along with 1mg/kg bodyweight of ZACAH extracts via oral gavage, daily for 18 weeks.
Group E: Treatment 2 (TX2) rats that received HCD along with 3mg/kg bodyweight of ZACAH extracts by oral gavage, daily for 18 weeks.
Group F: Treatment 3 (TX3) rats that received HCD along with 5mg/kg bodyweight of ZACAH extract by oral gavage, daily for 18 weeks.

Measurement of body weight (g) and caloric intake (kJ)
The body weight was recorded weekly by subtracting the final body weight from initial body weight for each rat. The caloric intake was calculated before feeding to the rats. The amount of calories consumed per rats was calculated on the next day and it was deducted from the amount caloric that was given to get the daily caloric intake.

Samples collection
After the experimental period, the rats fasting for 12 hours and were sacrificed using ketamine/xylazine anaesthesia. The blood sample was collected by cardiac puncture. The blood was centrifuged at 3000 rpm for 15 minutes, serum was separated and kept at -20°C for biochemical assay. Liver, kidney, retroperitoneal adipose tissue (RpWAT), visceral fat, and gonadal fat were removed, washed with saline, and weighed. The parts of the liver, kidney, RpWAT tissue were fixed in 10% NBF (neutral buffered formalin) for histological examination.

Biochemical analysis
The TC, TG, LDL, HDL, serum AST, ALT, and serum creatinine was measured using Alere Cholestech LDX® Analyzer (219284, United State).

Statistical analysis
Results were expressed as mean ± standard error (SEM) by using SPPS version 25. Before SPSS, all data were tested for normality. Data were analysed by one-way ANOVA test, followed by post hoc test least significant difference (LSD) test. Correlations between parameters were assessed by the Pearson test. The significance differences were considered at $P<0.05$.

RESULTS

ORAC value, Elastase inhibitory activity, Lipoxygenase inhibitory assay, DPPH free radical scavenging activity and total phenolic of ZACAH extracts
ORAC assay was carried out to assess the antioxidant

levels of the content of ZACAH extracts. The higher antioxidant activity presented in higher ORAC values (23). The ORAC value of ZACAH extracts is 2000 µmol TE/100mL. Elastase inhibitory activity exhibited 29.29 ± 1.65 % at 25mg/mL of sample, while lipoxygenase inhibitory assay showed 98.58 ± 1.42% effects of ZACAH extracts. DPPH assay used to analyse the scavenging effect of ZACAH extract. About 5% of the concentration was used to calculate the value. ZACAH extracts had the lowest DPPH (27.34 ± 2.71 %). The TPC was expressed as gallic acid equivalent per 100g of the sample (mg/100g GAE) (Table II).

Table II: ORAC value, Elastase inhibitory activity, Lipoxygenase inhibitory assay, DPPH free radical scavenging activity and total phenolic of ZACAH extracts

ORAC value (µmol TE/100mL)	ZACA extracts is 2000 µmol TE/100mL
Elastase inhibitory activity (%)	29.29 ± 1.65
Lipoxygenase inhibitory assay (%)	98.58 ± 1.42
DPPH (%)	27.34 ± 2.71
TPC (mg/100g GAE)	7537 ± 54.5

Data expressed as a percentage of radical scavenging activity, indicated mean value of triplicate wells in triplicate experiments, Standard error of mean (SEM). High (H): 70 – 100%, Moderate (M): 50 – 69%, Low (L): 0 – 49%. Data expressed as a percentage of Elastase inhibitory activity, indicated mean value of triplicate wells in triplicate experiments, Standard error of mean (SEM) High (H): 55 – 100%, Good (G): 30 -54%, Moderate (M): 10 – 29%, Low (L): 1 – 9%**.** Abbreviation: ORAC**:** Oxygen Radical Absorbance Capacity, DPPH: 2,2-diphenyl-1-picrylhydrazyl, TPC; total phenolic content

Identification of Phenolic Compounds of ZACAH extract

The identification of ZACAH extracts was carried out by using the HPLC method. Retention time (RT) and mass spectra were compared to determine the peak. Peak with RTs (min) of 39.08 was identified as Hesperidin (Fig 1).

Effect of ZACAH Extracts of bodyweight and caloric intake

There was no significant difference of the initial bodyweight of the rats in all groups. Table III showed the mean final bodyweight of all the groups. The consumption of high cholesterol diet in PC group significantly increased in body weight compared with the consumption of normal chow diet in the NC group. The PC group has the highest bodyweight as compared to the NC group. All ZACAH treated groups (TX1, TX2, TX3) significantly reduced body weight as compared to the PC group. Among ZACAH treated groups, TX3 showed the lowest in bodyweight.

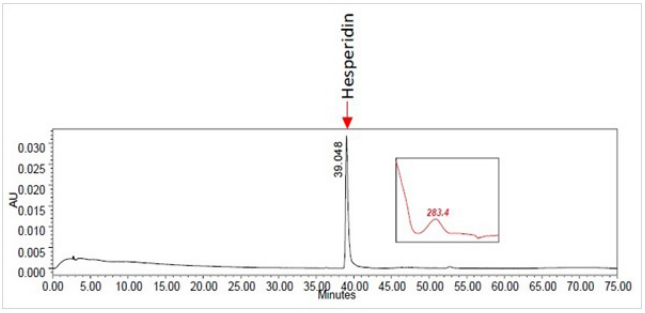

Figure 1: High-performance liquid chromatogram (HPLC) of ZACAH extracts

The NC group were fed with normal chow diet whereas the remaining groups received normal chow diet and HCD. The PC group showed significantly higher level of caloric intake in initially as compared to NC group. All ZACAH treated groups (TX1, TX2, TX3) showed no changes in caloric intake compared to the PC group. The PC group showed increased in final caloric intake compared with the NC group. All ZACAH treated groups (TX1, TX2, TX3) showed no changes in caloric intake compared to the PC group.

Effect of ZACAH Extracts on Organ Weight

The PC group showed an increased in liver weight, RpWAT weight, visceral weight, kidney weight, as compared to the NC group. DC group has the highest liver weight, RpWAT weight, visceral weight, gonadal weight, kidney weight, compared with the PC group. All ZACAH treated groups (TX1, TX2, TX3) reduced in liver weight, RpWAT weight, visceral weight, gonadal weight, kidney weight as compared to the PC group. Among ZACAH treated groups,TX3 showed the lowest liver weight, RpWAT weight, visceral weight, and kidney weight (Table IV).

Effect of ZACAH Extracts on Lipid Profile

The PC group showed the highest TC, TG and LDL compared with the NC group All ZACAH extracts treated groups (TX1, TX2, TX3), including the DC group, showed a reduction in TC and LDL compared with the PC group, and the lowest TC and LDL values were

Table III: Effects of ZACAH Extracts on bodyweight and caloric intake

Parameter		NC	PC	DC	TX1	TX2	TX3
Bodyweight (g)	Initial	289.87 ± 15.82	289.79 ± 10.76	285.50 ± 15.30	281.77 ± 17.68	274.18 ± 15.43	275.41 ± 11.35
	Final	456.75 ± 8.13^{b}	627.16 ± 3.22^{a}	585.41 ± 6.56^{b}	563.57 ± 6.99^{b}	505.92 ± 8.13^{b}	487.95 ± 8.89^{b}
Caloric intake (kcal)	Initial	1440.63 ± 87.86 $^{b, c}$	3098.75 ± 347.26 ^{a}	3089.28 ± 225.18 ^{a}	3693.56 ± 391.04 ^{a}	3482.03 ± 446.88 ^{a}	3792.35 ± 466.50 ^{a}
	Final	1087.66 ±3.33^{b}	6125.53 ± 2.45^{a}	6036.03 ± 6.87^{a}	6029.52 ± 8.21^{a}	6027.40 ± 6.74^{a}	6051.11 ± 5.93^{a}

Data were presented as mean± SEM. Means values with the different letter were significantly different at p<0.05. Abbreviation: NC; negative control, PC; positive control, DC; drugs control, TX; treatment I, 2 and 3.

Table IV: Effects of ZACAH Extracts on organ weight

Organ weight (g)	NC	PC	DC	TX1	TX2	TX3
Liver	14.69 ± 0.76^{c}	15.95 ± 0.85^{c}	19.17 ± 1.43^{a,b}	15.83 ± 1.02^{c}	14.88 ± 0.57^{c}	14.20 ± 1.23^{c}
Adipose tissue						
RpWAT	6.00 ± 1.62^{b}	14.49 ± 1.24^{a}	19.39 ± 3.19^{a}	5.37 ± 1.23^{b}	3.81 ± 1.11^{b}	3.21 ± 1.25^{b}
Visceral fat	1.95 ± 0.87	2.89 ± 0.86	3.51 ± 1.21^{b}	1.83 ± 0.45	1.01 ± 0.30	0.97 ± 0.77^{a}
Total WAT	12.98 ± 2.79^{b}	26.05 ± 2.93^{a}	33.77 ± 3.97^{a}	13.09 ± 2.44^{b}	9.84 ± 2.27^{b}	7.62 ± 0.89^{b}
Kidney	2.99 ± 0.18^{b}	3.24 ± 0.14	3.57 ± 0.22a	3.19 ± 0.29	3.14 ± 0.17	3.02 ± 0.10

Data were presented as mean± SEM. Means values with the different letter were significantly different at p<0.05. Abbreviation: NC; negative control, PC; positive control, DC; drugs control, TX; treatment I, 2 and 3.

observed in ZACAH extracts TX3 group respectively. The TG value in PC group was elevated compared to the NC group, however, TX1, TX2, TX3 and DC treated groups which showed decreased TG value compared with the PC group. The PC group showed decreased in HDL value compared with the NC group. The HDL values for all ZACAH extracts treated groups were shown to be increased compared with the PC group, with ZACAH extracts TX3 treated group recorded the highest HDL value (Table V).

Effect of ZACAH Extracts on Liver and Kidney Function Test

The PC group showed increases in AST, ALT and Creatinine compared with the NC group. All ZACAH treated group (TX1, TX2, TX3), including DC group, showed a reduction in Creatinine, AST, and ALT with the PC group. Among ZACAH treated groups, TX3 has the
lowest levels of Creatinine, AST, and ALT (Table VI).

DISCUSSION

Several animal models of hyperlipidaemia have been developed to mimic hyperlipidaemia-like condition in human, which would help to develop cost-effective anti-hyperlipidaemia treatments. The rats fed with a high cholesterol/fat diet among the other animals are considered very useful based on the research involving metabolic disorders (24). The HCDs Rats have been reported to develop visceral adiposity, hyperglycaemia, lipidaemia, diabetes mellitus and liver steatosis, leading to hyperlipidaemia and obesity. Prolonged rats fed with HCD resulted in hyperlipidaemia, attributed with high body weight and fat mass (16).

Various active compounds available in plant extracts can exert therapeutic activities, hence, they can be utilized for therapeutic benefits in the treatment and prevention of metabolic disorders (16) . Hyperlipidaemia is a common metabolic disease caused by long term consumption of a cholesterol-rich diet. Simvastatin has been recognized for the treatment of hyperlipidaemia (25), but is attributed to various adverse effects, such as liver damage, muscle pains, weakness, and nausea. These create opportunities for utilizing natural-based products for plants to combat metabolic disorders, including hyperlipidaemia and obesity, without adverse side effects. In this present study, Elastase inhibitory activity exhibited 29.29 ± 1.65 % at 25mg/mL of

Table V: Effect of ZACAH extracts on lipid profiles

Lipid profile (mmol/L)	NC	PC	DC	T1	T2	T3
TC	1.75 ± 0.06^{b}	2.47 ± 0.13^{a,c}	2.02 ± 0.13^{b}	2.25 ± 0.13^{a}	2.17 ± 0.07^{a}	1.85 ± 0.14^{b}
HDL	0.53 ± 0,02^{b,c}	0.33 ± 0.02^{a}	0.32 ± 0.03^{a}	0.58 ± 0.05^{b,c}	0.60 ± 0.04^{b,c}	0.67 ± 0.07^{a,b,}c
LDL	0.78 ± 0.07^{b,c}	1.57 ± 0.1^{5a}	1.23 ± 0.16^{a}	1.03 ± 0.15^{b}	0.90 ± 0.17^{b}	0.77 ± 0.17^{b,c}
TG	0.88 ± 0.09 ^{b}	1.58 ± 0.21^{ac}	1.03 ± 0.17^{b}	1.43 ± 0.09^{a}	1.10 ± 0.17^{b}	0.74 ± 0.13^{b}

Data are shown as mean ± SEM. Means values with different letter were significantly different at p<0.05. Abbreviation: NC; negative control, PC; positive control, DC; drugs control, TX; treatment I, 2 and 3, TC; total cholesterol, HDL; high-density lipoprotein; LDL; low-density lipoproteins, TG; total triglycerides

Table VI: Effect of ZACA extracts on liver and kidney function tests

	NC	PC	DC	TX1	TX2	TX3
Creatinine (umol/L)	25.83 ± 1.99	29.00 ± 1.75^{b}	24.33 ± 2.14	24.50 ± 1.65	24.67 ± 1.28	24.17 ± 0.54^{a}
AST (U/L)	132.00 ± 8.83^{b}	194.00 ± 6.21^{a}	107.00 ± 8.57^{b}	161.17 ± 2.39^{b}	94.50 ± 8.78^{b}	82.67 ± 7.82^{b}
ALT (U/L)	48.67 ± 6.95^{b}	129.67 ± 4.45^{a}	35.17 ± 12.25^{b}	32.83 ± 7.66^{b}	22.17 ± 1.56^{b}	22.83 ± 2.36^{b}

Data are presented as mean± SEM. Means values with the different letter were significantly different at p<0.05. Abbreviation: AST; aspirate aminotransferase, ALT; alanine aminotransferase, NC; negative control, PC; positive control, DC; drugs control, TX; treatment I, 2 and 3

sample. Elastase is associated with anti-hyperlipidaemic properties.

Lipoxygenase inhibitory assay showed 98.58 ± 1.42% effects of ZACAH extracts, indicating that it has the best antioxidant properties, which play essential role in the treatment of degenerative diseases (26). The phenolic compounds have been reported to show much therapeutic potential to prevent associated diseases (27). DPPH assay was used to analyse the scavenging effect of ZACAH extracts and 5% concentration was used to calculate the value.

ZACAH extracts had the lowest DPPH (27.34 ± 2.71 %). The lower the DPPH values, the higher the antioxidant effect in ZACAH extracts (27). The TPC was calculated as gallic acid equivalent per 100g of the sample (mg/100g GAE). Phenolic compounds are responsible for antioxidant activity (28). HPLC has been utilized to determine the phytochemical compound present in ZACAH extracts. Hesperidin is a bioflavonoid that is found in ZACAH extracts and has been investigated to have both anti-oxidative and anti-inflammatory activities that can lower the cholesterol level (29).

In this study, the body weight and caloric intake were investigated in the experimental SD rats. This study observed increased bodyweight in HCD group compared with NC group but there was no significant difference observed from the NC group compared to all ZACAH treated groups. This might be due to consumption of HCD alone throughout the study. We found that ZACAH treated SD rats, including DC group have significantly reduced body weight. Although ZACAH extracts lead to weight reduction but there were no changes observed in caloric intake. The study done by Karimi et al. (2015) and Bahari et al. (2020) stated that gained in bodyweight are fully depended on the caloric intake, implicated by the higher energy uptake and in the intestinal barrier. Hyperlipidaemia is associated with changes in organs weight, including adipose tissue, liver, and kidney. High dosage of ZACAH extracts showed an efficient reduction in liver weight. This result indicated that hesperidin contained in ZACAH extracts lessens the liver damages caused by the consumption of HCD. These results were similar to the previous study where the liver weight was reduced in the rats fed with Hesperidin (30). Moreover, hesperidin improved lipid metabolism. Lipid is stored in the form of triglycerides in the adipose tissue that secrete and regulate adipokines as well as cytokines.

Excessive levels of lipid caused rapid adipose tissues hypertrophy. In our findings, ZACAH treated groups showed decreased adipose tissue compared with PC groups. Hence, hesperidin was identified to inhibit adipose tissue hypertrophy in rats fed with HCD.

Antihyperlipidemic effect of ZACAH extracts at 5mg/kg bodyweight was relatively the same with the effect of standard drug simvastatin. This therapeutic effect may be attributed to the presence of active phytochemicals, such as hesperidin. Hesperidin has been investigated to interfere with HMG-CoA reductase, and hence, may be responsible for antihyperlipidemic (30). There was no significant change observed in the kidney weight, among the groups and no toxic effects or mortality was recorded in all the animals throughout the experimental period.

In this present study, the PC group showed the highest TC, TG and LDL compared with the NC group. All ZACAH extracts treated groups (TX1, TX2, TX3), including the DC group, showed a reduction in TC and LDL compared with the PC group. The TG value in the PC group was increased compared to the NC group, however, TG values were also observed to be increased in both ZACAH extracts TX1 and TX2 treated groups compared with the PC group. This study also reported that the PC group showed decreased in HDL value compared with the NC group, while the HDL values for all ZACAH extracts treated groups were shown to be increased compared with the PC group. However, the HDL value in DC group was relatively reduced compared with the PC group. HCD supplementation resulted in hyperlipidaemic changes is associated with increased TGs, TC, LDL and subsequent reduction in serum concentration of HDL. Hence modification of lipid profiles could be utilized as an index of Hyperlipidaemia and obesity. Additionally, changes in the lipid profile might be due to the activations of gastric lipases enzymes, intestinal absorption of fat, and fat lipolysis. Indeed, elevated levels of TC was reported to be among the risk factor for developing coronary heart disease. In other way, high HDL concentration has been investigated to help in mobilizing excess cholesterol to the liver, where it would be converted to bile for excretion (16). In this present study, the PC group exhibited increased lipid levels, indicating that diets-rich cholesterol plays a vital role in increasing TC, LDL, TG, and decreasing HDL.

This study demonstrated that ZACAH extracts decrease the TC, LDL and TG levels while promoting the availability of HDL levels, possibly due to the present of hesperidin. The results of our findings agreed with previous studies, in which the TC, TG and LDL levels were significantly reduced and subsequently increased in the HDL levels (31). The high level of TGs observed in other treated groups may be due to cholesterol taken, which had been shown to reduce oxidation of fatty acid, and subsequently increases the hepatic and plasma triglyceride concentrations (16). Also, hesperidin improved hyperlipidaemia by inhibiting cholesterol synthesis and absorption through the mevalonate biosynthetic pathway. Our findings investigated that ZACAH extracts administration could prevent hyperlipidaemia in SD rats fed with HCD.

The ALT, AST activities were measured to determine the hepatic damage. The increase in ALT and AST enzymes indicate hepatic damage (32). Hepatic damage is caused by fat accumulation in the liver. In this study, the creatinine activities were measured to determine renal damage.

Our findings revealed that ZACAH extracts treated groups, including the DC group showed decreased AST, ALT, and creatinine activities compared with the PC group. Hesperidin was also reported to have the ability to minimize the level of AST, ALT, and creatinine in serum (23). Knowledge on the molecular mechanisms involved in the pathogenesis of hyperlipidaemia, change in the lifestyle and regular exercise are the necessary strategies to be developed to reduce the incidence of CVDs associated with hyperlipidaemia and obesity (33).

CONCLUSION

Interestingly, hesperidin and other phytochemicals contained in ZACAH extracts showed positive effects on body weight, organ weight, controlling lipid profile, and blood toxicity. Administration of ZACAH extracts would decrease the level of TC, LDL, TG, AST, ALT, and creatinine and increase the levels of HDL in HCD-induced hyperlipidaemia SD rats. Administration of ZACAH extracts at 5mg/kg body weight showed strong therapeutic effects in SD rats fed with HCD. We suggested that ZACAH extracts can be utilized as a potential therapeutic alternative for the treatment of hyperlipidaemia. More research is needed to investigate the specific anti-hyperlipidaemic compound available in ZACAH extracts in the animal study, which can further be replicated to humans without any adverse effects.

ACKNOWLEDGEMENTS

The authors would like to thank the Management & Science University for the grants (SG-001-012019-FHLS), the Natural Tone Trading Company and Universiti Putra Malaysia.

REFERENCES

1. Karam I, Yang YJ, Li JY. Hyperlipidemia Background and Progress. SM Atherosclerosis Journal. 2017; 1(1): 1–8.
2. Chen J, Deng J, Zhang Y, Yang J, He Y, Fu W, Xing P, tong Wan H. Lipid-lowering effects of Danhong injection on hyperlipidemia rats. Journal of ethnopharmacology. 2014 Jun 11;154(2):437-42. doi: 10.1016/j.jep.2014.04.023
3. World Health Organization (2020). Raised cholesterol- Global health Obsevatory (GHO) data. Available at: http://apps.who.int/gb/NCDs/pdf/A_NCD_2-en.pdf
4. Han Q, Yeung SC, Ip MS, Mak JC. Dysregulation of cardiac lipid parameters in high-fat high-cholesterol diet-induced rat model. Lipids in health and disease. 2018 Dec;17(1):1-0. doi: 10.1186/s12944-018-0905-3.
5. Weibel GL, Drazul-Schrader D, Shivers DK, Wade AN, Rothblat GH, Reilly MP, et al. Importance of evaluating cell cholesterol influx with efflux in determining the impact of human serum on cholesterol metabolism and atherosclerosis. Arteriosclerosis, thrombosis, and vascular biology. 2014 Jan;34(1):17-25. doi: 10.1161/ATVBAHA.113.302437.
6. Li W, Wu N, Shu W, Jia D, Jia P. Pharmacological preconditioning and postconditioning with nicorandil attenuates ischemia/reperfusion-induced myocardial necrosis and apoptosis in hypercholesterolemic rats. Experimental and therapeutic medicine. 2015 Dec 1;10(6):2197-205. doi: 10.3892/etm.2015.2782
7. Pinal-Fernandez I, Casal-Dominguez M, Mammen AL. Statins: pros and cons. Medicina Clнnica (English Edition). 2018 May 23;150(10):398-402. doi: 10.1016/j.medcli.2017.11.030.
8. Thompson PD, Panza G, Zaleski A, Taylor B. Statin-associated side effects. Journal of the American College of Cardiology. 2016 May 24;67(20):2395-410. doi: 10.1016/j.jacc.2016.02.071.
9. Sung YY, Yoon T, KiM SJ, Yang WK, KiM HK. Anti-obesity activity of Allium fistulosum L. extract by down-regulation of the expression of lipogenic genes in high-fat diet-induced obese mice. Molecular medicine reports. 2011 May 1;4(3):431-5. doi: 10.3892/mmr.2011.451
10. Perumal KV, Ja'afar NL, Balan SS, Abidin AZ, Arapoc DJ, Shafie NH, Bahari H. Preventive effect of Elateriospermum tapos seed extract against obese Sprague Dawley rats. Advances in Traditional Medicine. 2020 Mar;20(1):107-13. doi:10.1007/s13596-019-00394-w
11. Balan SS, Abidin AZ, Perumal KV, Ahamad Lotafi AH, Danabala S, Manimaran M, et al. Effect of Elateriospermum tapos extract as coadjuvant in ameliorating maternal obesity on female offspring at weaning. Malaysian Journal of Microscopy. 2019 Dec 2;15(1).
12. Shree R, Ghosh AK, Bharti SK. Effect of dietary Withenia somnifera and Commiphora weightii on induced hyperlipidemic wistar rats. The Pharma Innovation Journal. 2018; 7(4): 302–313.
13. Ling Y, Shi Z, Yang X, Cai Z, Wang L, Wu X, et al. Hypolipidemic effect of pure total flavonoids from peel of Citrus (PTFC) on hamsters of hyperlipidemia and its potential mechanism. Experimental gerontology. 2020 Feb 1;130:110786. doi: 10.1016/j.exger.2019.110786
14. Cappello AR, Dolce V, Lacopetta D, Martello M, Fiorillo M, Curcio R, et al. Bergamot (Citrus bergamia Risso) flavonoids and their potential benefits in human hyperlipidemia and atherosclerosis: an

overview. Mini reviews in medicinal chemistry. 2016 May 1;16(8):619-29. doi: 10.2174/1389557515666150709110222.
15. Yang C, Li L, Yang L, Lu H, Wang S, Sun G. Anti-obesity and Hypolipidemic effects of garlic oil and onion oil in rats fed a high-fat diet. Nutrition & metabolism. 2018 Dec;15(1):1-8. doi: 10.1186/s12986-018-0275-x
16. Saravanan G, Ponmurugan P, Deepa MA, Senthilkumar B. Anti-obesity action of gingerol: effect on lipid profile, insulin, leptin, amylase and lipase in male obese rats induced by a high-fat diet. Journal of the Science of Food and Agriculture. 2014 Nov;94(14):2972-7. doi: 10.1002/jsfa.6642
17. Asdaq SM. Antioxidant and hypolipidemic potential of aged garlic extract and its constituent, s-allyl cysteine, in rats. Evidence-Based Complementary and Alternative Medicine. 2015 Oct;2015: 1-7. doi: 10.1155/2015/328545.
18. Karuppiah P, Rajaram S. Antibacterial effect of Allium sativum cloves and Zingiber officinale rhizomes against multiple-drug resistant clinical pathogens. Asian Pacific journal of tropical biomedicine. 2012 Aug 1;2(8):597-601. doi:10.1016/S2221-1691(12)60104-X
19. Ou B, Hampsch-Woodill M, Prior RL. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. Journal of agricultural and food chemistry. 2001 Oct 15;49(10):4619-4626. doi: 10.1021/jf010586o.
20. Kim JH, Byun JC, Hyun CG, Lee NH. Compounds with elastase inhibition and free radical scavenging activities from Callistemon lanceolatus. Journal of Medicinal Plants Research. 2009 Nov 30;3(11):914-20.
21. Aoshima H, Tsunoue H, Koda H, Kiso Y. Aging of whiskey increases 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity. Journal of agricultural and food chemistry. 2004 Aug 11;52(16):5240-5244. doi: 10.1021/jf049817s.
22. De Brum TF, Zadra M, Piana M, Boligon AA, Frιhlich JK, De Freitas RB, et al. HPLC analysis of phenolics compounds and antioxidant capacity of leaves of Vitex megapotamica (Sprengel) Moldenke. Molecules. 2013 Jul;18(7):8342-57. doi: 10.3390/molecules18078342.
23. Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. Journal of agricultural and food chemistry. 2004 Jun 16;52(12):4026-4037. doi: 10.1021/jf049696w.
24. Marques C, Meireles M, Norberto S, Leite J, Freitas J, Pestana D, et al. High-fat diet-induced obesity Rat model: a comparison between Wistar and Sprague-Dawley Rat. Adipocyte. 2016 Feb 3;5(1):11-21. doi: 10.1080/21623945.2015.1061723
25. Krysiak R, Zmuda W, Okopien B. The effect of short-term simvastatin treatment on plasma adipokine levels in patients with isolated hypercholesterolemia: a preliminary report. Pharmacological reports. 2014 Oct 1;66(5):880-884. doi: 10.1016/j.pharep.2014.05.012
26. Soccio M, Laus MN, Flagella Z, Pastore D. Assessment of antioxidant capacity and putative healthy effects of natural plant products using soybean lipoxygenase-based methods. An overview. Molecules. 2018 Dec;23(12):3244. doi: 10.3390/molecules23123244.
27. Azlan A, Wei Kok Y, Eng Khoo H. Antioxidants Content and Activity of Polyphenol Rich Mixtures. Journal of Engineering and Applied Sciences. 2018; 13(9): 6973–6979. doi:10.36478/jeasci.2018.6973.6979
28. Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. Mutation Research/Fundamental and Molecular mechanisms of mutagenesis. 2005 Nov 11;579(1-2):200-213. doi: 10.1016/j.mrfmmm.2005.03.023.
29. Xiong H, Wang J, Ran Q, Lou G, Peng C, Gan Q, et al. Hesperidin: A therapeutic agent for obesity. Drug design, development and therapy. 2019 Nov 12;13:3855-3866. doi: 10.2147/DDDT.S227499.
30. Wang X, Hasegawa J, Kitamura Y, Wang Z, Matsuda A, Shinoda W, et al. Effects of hesperidin on the progression of hypercholesterolemia and fatty liver induced by high-cholesterol diet in rats. Journal of pharmacological sciences. 2011;117(3):129-138. doi: 10.1254/jphs.11097fp
31. Selvaraj P, Pugalendi KV. Efficacy of hesperidin on plasma, heart and liver tissue lipids in rats subjected to isoproterenol-induced cardiotoxicity. Experimental and Toxicologic Pathology. 2012 Jul 1;64(5):449-452. doi: 10.1016/j.etp.2010.10.012
32. Lasker S, Rahman MM, Parvez F, Zamila M, Miah P, Nahar K, et al. High-fat diet-induced metabolic syndrome and oxidative stress in obese rats are ameliorated by yogurt supplementation. Scientific reports. 2019 Dec 27;9(1):1-5. doi: 10.1038/s41598-019-56538-0.
33. Kwaifa IK, Bahari H, Yong YK, Noor SM. Endothelial dysfunction in obesity-induced inflammation: molecular mechanisms and clinical implications. Biomolecules. 2020 Feb;10(2):291. doi: 10.3390/biom10020291.
34. Bahari H, Abidin AZ, Balan SS, Perumal KV, Rosli NS, Lotafi AH, et al. The effects of Elateriospermum tapos against obese maternal rat in mitigating obesity development among their adult female offspring. Pharmacognosy Magazine. 2020 Oct 1;16(72):706-712. doi:10.4103/pm.pm_142_20
35. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958 Apr;181(4617):1199-1200. doi:10.1038/1811199a0

36. Karimi G, Sabran MR, Jamaluddin R, Parvaneh K, Mohtarrudin N, Ahmad Z, et al. The anti-obesity effects of Lactobacillus casei strain Shirota versus Orlistat on high fat diet-induced obese rats. Food & nutrition research. 2015 Jan 1;59(1):29273. doi: 10.3402/fnr.v59.29273
37. Psarros C, Economou EK, Koutsilieris M, Antoniades C. Statins as pleiotropic modifiers of vascular oxidative stress and inflammation. The Journal of Critical Care Medicine. 2015 Apr;1(2):43-54. doi: 10.1515/jccm-2015-0007.
38. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965 Jan 1;16(3):144-158.