

# Anti-obesity effect of *Carica papaya* in high-fat diet fed rats

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**Abstract.** The present study evaluated the anti-obesity properties of papaya in high-fat (HF) diet fed rats. In the *in vitro* portion of the present study, the effects of papaya juice on pancreatic lipase enzyme activity was assessed, and it was shown that papaya exhibited an inhibitory effect on these enzymes. In the *in vivo* portion of the study, papaya was found to reduce the expression levels of markers of obesity, inflammation and oxidative stress in rats. Obesity was induced in 28 male Sprague Dawley rats by feeding them a HF diet for 12 weeks. The anti-obesity effects of papaya was evaluated by feeding papaya juice orally in with two experimental doses: 0.5 ml (HFL) and 1.0 ml (HFH) per 100 g of body weight. The HF diet resulted in significant increases in the body weight, serum triglyceride, serum total cholesterol and serum low-density lipoprotein cholesterol levels, as well as a decrease in serum high-density lipoprotein cholesterol levels. The HF diet also induced adipocyte hypertrophy, lipid accumulation and increased malondialdehyde levels. Papaya reversed all of these changes and significantly increased serum superoxide dismutase and decreased serum cytokine (interleukin-6) levels. The protein expression of levels PPAR $\gamma$  in the HF group was significantly increased compared with the other groups, but was decreased significantly in the HFH group. Histological observations of epididymal adipose tissue provided evidence for the lipid-lowering effects of papaya. The results of the present study demonstrate that papaya has the potential to reduce the risk of obesity associated with adiposity, anti-inflammation and anti-oxidation.

## Introduction

The World Health Organization defines obesity as accumulation of excessive fat in the body (1), which is most commonly

caused by overconsumption of fat-rich diets (2,3). Consumption of excessive quantities of fat can result in the accumulation of visceral fat and an increase in body weight (2). Obesity has been cited as a public health problem that is also associated with an increased risk of metabolic diseases, such as cardiovascular disease, diabetes mellitus, dyslipidaemia, metabolic syndrome and several types of cancer (4,5), therefore, controlling it is essential for improving personal health.

Papaya is widely grown in several regions around the world, especially in central and South America, Asia and other tropical countries. It is an economically significant crop, since it is the fourth most traded tropical fruit following bananas, mangoes and pineapples (6). Papaya possesses several medicinal properties, including antioxidant, anti-hypertensive and hepatoprotective properties (7). A previous study demonstrated that papaya fruit aqueous extracts lowered lipid peroxidation, increased glutathione levels, and increased the activity of catalase and superoxide dismutase, as well as improving the immune status, as reflected by an increase in IgG and IgM levels in acrylamide intoxicated rats (8). Furthermore, papaya leaf possesses hypoglycaemic and hypolipidemic effects on rats fed a high cholesterol diet (9). Ripe papaya possess carotenoids and vitamin C (7):  $\beta$ -carotene, a subtype of carotenoids, exhibits an anti-hyperlipidaemic effect on spontaneously hypertensive rats, and dietary  $\beta$ -carotene is associated with a decreased serum lipid profile in hypercholesterolemic rats fed a high cholesterol diet (10). In addition, accumulation of  $\beta$ -carotene in 3T3-L1 adipocytes increases the expression of genes associated with insulin sensitivity and reactive oxygen species levels in insulin-resistant adipocytes (11).

Based on the properties of papaya extracts described above, it was hypothesized that papaya may exhibit potential as a treatment for obesity. In addition, there are several studies regarding the anti-obesity effects of papaya, therefore, its beneficial effects on obesity, inflammation and oxidative stress in obese rats fed a high-fat (HF) diet were investigated. The doses of papaya juice used in the present study can be easily implemented in a nutritional diet for humans. Therefore, the use of the papaya fruit may be a promising alternative dietary remedy against obesity.

## Materials and methods

**Plant material and preparation.** In the present study, two cultivars of papaya (Holland and Khak-Dam) were used, due to their popularity and wide consumption in Thailand. The

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papaya were purchased from a supermarket in Phitsanulok, Thailand, and selected for their uniformity of shape, size and external skin colour. The peel and seeds were removed and the flesh was cut into small pieces and crushed into a juice.

*$\beta$ -carotene and vitamin C extraction.* For extraction of vitamin C, 100 ml papaya juice was mixed with 3% meta-phosphoric acid and 8% acetic acid in a mechanical shaker at 180 rpm for 30 min, and then the mixture was centrifuged at 9,000 x g for 10 min at 4°C.

For  $\beta$ -carotene extraction, 10 ml papaya juice was mixed with hexane, acetone and absolute ethanol using a magnetic stirrer for 30 min, then 15 ml distilled water was added to the mixture to separate by phase. Following separation, the supernatant solution was stored at -80°C.

*High-performance liquid chromatography (HPLC) analysis.* Chromatographic analysis of vitamin C and  $\beta$ -carotene in papaya juice was performed on an HPLC device (Shimadzu LC-10ADvP; Bara Scientific Co., Ltd.) using a Luna 5u C18(2) 100A column (250x4.6 mm) (Phenomenex). In vitamin C, the mobile phase was composed of methanol and buffer solution (0.1 M  $\text{KH}_2\text{PO}_4$ ) pH 4.4 (60:40). The injected volume was 20  $\mu\text{l}$  at a flow rate of 1.0 ml/min, with the absorbance peak recorded at 245 nm.

In  $\beta$ -carotene, the mobile phase was composed of acetonitrile, methanol and 2-propanol (20:30:50, v/v). The injected volume was 50  $\mu\text{l}$  at a flow rate of 1.0 ml/min, with the absorbance peak recorded at 450 nm.

*In vitro pancreatic lipase inhibition assay.* The *in vitro* pancreatic lipase assay was slightly modified according to a previously described method (12). Briefly, porcine pancreatic lipase (Sigma-Aldrich; Merck KGaA) was dissolved in distilled water to a final concentration of 1 mg/ml. The stock of 1% (w/v) 4-nitrophenyl laurate (Sigma-Aldrich; Merck KGaA) was used as the lipase substrate and dissolved in 5 mM sodium acetate (pH 5.0) containing 1% Triton X-100. To initiate the reaction, the reaction mixture containing 80  $\mu\text{l}$  assay buffer, 30  $\mu\text{l}$  orlistat or papaya, and 4-nitrophenyl laurate were mixed and incubated at 37°C for 2 h before centrifugation at 23,000 x g for 2.5 min at 25°C. The absorbance was measured at 400 nm. In a microplate reader (BioTek Synergy HT; Bio-Tek Instruments, Inc.). The results are expressed as percentage inhibition, and were calculated from  $(\text{Ablank} - \text{Asample}) / \text{Ablank} \times 100$ , where Ablank is the absorbance of the control and Asample is the absorbance of orlistat or papaya juice.

*Animals and experimental protocol.* All experimental procedures were approved by the Ethics Committee of the Centre for Animal Research, Naresuan University (Phitsanulok, Thailand) (approval no. NUA580174). A total of 28 male Sprague Dawley rats weighing 80-100 g were obtained from the National Laboratory Animal Centre, Mahidol University (Bangkok, Thailand). The rats were kept in a temperature controlled environment ( $22 \pm 10^\circ\text{C}$ ) with a relative humidity of  $55 \pm 10\%$  and a 12 h light-dark cycle. A commercial pellet diet and water were provided *ad libitum*, and after 1 week of acclimatization, the rats were fed either the normal diet or HF diet for 8 weeks to induce obesity. The standard diets were purchased from Perfect

Companion Group Company. The HF diets were prepared by mixing the control diet with 1.5% cholesterol, 20% palm oil and 0.25% cholic acid as previously described (13). The rats were randomly divided into four experimental groups as follows ( $n=7$  rats/group): Group 1, Control (C) group; these rats were fed a normal diet. Group 2, HF diet group; these rats were fed a HF diet. Group 3, low-dose (HFL) group; these rats were fed a HF diet with 0.5 ml/100 g of body weight papaya juice. Group 4, high-dose (HFH) group; these rats were fed a HF diet with 1.0 ml/100 g of body weight papaya juice.

In week 8, the rats in the HFL and HFH groups received papaya juice daily via oral feeding. Their food intake was recorded daily, and their body weight was measured weekly throughout the experiment. After 12 weeks, all the rats were fasted for 12 h overnight, and then anesthetized with an intraperitoneal injection of thiopental sodium (50 mg/kg). Cardiac puncture was then performed to collect 10-12 ml blood. Death was confirmed by the cessation of heartbeat and absence of reflexes. Epididymal, perirenal and mesenteric adipose tissues were removed immediately, weighed and stored at -80°C until analysis. A portion of the epididymal adipose tissue of each individual rat was fixed in 10% neutral buffer formalin at room temperature for 24 h for histological analysis.

*Serum biochemical analysis.* Blood was centrifuged at 800 x g for 30 min at 4°C to obtain the serum, and then frozen at -80°C. The levels of serum cytokines, including tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ; cat. no. EZRTNFA; Sigma-Aldrich; Merck KGaA), interleukin-6 (IL-6; cat. no. EZRIL6; Sigma-Aldrich; Merck KGaA), leptin (cat. no. EZRL-83K; EMD Millipore) and insulin (cat. no. EZRMI-13K) were quantified using ELISA kits. The levels of serum triglyceride (TG; Triglycerides liquicolor mono; cat. no. 10720P), total cholesterol (TC; Cholesterol liquicolor cat. no. 10017) and high-density lipoprotein (HDL; HDL liquicolor cat. no. 10084) were measured using commercial kits (all from HUMAN Gesellschaft fur Biochemica und Diagnostica GmbH). Low-density lipoprotein (LDL) levels were determined using as follows:  $\text{LDL} = \text{TC} - [\text{HDL} + (\text{TG}/5)]$ .

*Determination of lipid peroxidation.* Lipid peroxidation was determined by measuring malondialdehyde (MDA) levels as previously described (14). Thiobarbituric acid (90 mM) and 3 M trichloroacetic acid were added to the serum samples, and then incubated at 95°C for 60 min. The samples were immersed into an ice bath for rapid cooling, and the peroxidation products formed in the samples with thiobarbituric acid were measured at 532 nm with malondialdehyde used as a standard, and the results are expressed as nmol of thiobarbituric acid reactive substances (TBARS)/mg protein.

*Determination of superoxide dismutase and glutathione reductase levels.* Plasma superoxide dismutase and glutathione reductase levels were measured spectrophotometrically using commercial ELISA kits (superoxide dismutase assay kit, cat. no. 706002; glutathione reductase assay kit; cat. no. 703202; Cayman Chemical Company) according to the manufacturer's protocol, and the results are expressed as U/L.

*Histological analysis.* Epididymal adipose tissues were embedded in paraffin, 8  $\mu\text{M}$  sections were obtained, and

stained with haematoxylin for 2 min and eosin for 1 min at room temperature. Images of the histological sections were obtained at magnifications of x10 and x20. The size of the adipocytes were calculated using Image-J version 1.50e (National Institutes of Health). The mean area of the adipocytes was calculated in 20 adipocytes from 3 randomly selected fields of view per sample.

**Western blot analysis.** Protein extraction from adipose tissue was performed by adding proteinase inhibitor (cat. no. 539131; EMD Millipore) and RIPA lysis buffer (cat. no. R0278, Sigma-Aldrich; Merck KGaA) to form a mixture, which was then homogenised by sonication on ice for 1 min. The supernatant was centrifuged at 20,000 x g for 15 min at 4°C and the protein concentration from the epididymal adipose tissue was measured using a bicinchoninic acid assay (cat. no. 23227; Thermo Fisher Scientific, Inc.). Total proteins were loaded on a 15% SDS-gel, resolved using SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane using wet transfer. Anti-PPAR $\gamma$  rabbit polyclonal antibody (cat. no. 07-466; EMD Millipore) and anti- $\beta$ -actin antibody (cat. no. AF7018; Affinity Biosciences) was dissolved in 3% non-fat dry milk (1:1,000) and incubated overnight at 4°C, then washed with Tris-buffered saline with Tween 20 buffer. Subsequently, peroxidase-conjugated AffiniPure goat anti-Rabbit IgG (H+L) (cat. no. 111-035-003; Jackson ImmunoResearch, PA, USA) was also dissolved in 3% non-fat dry milk (1:1,000) for 2 h at room temperature.

**Statistical analysis.** All data are presented as the mean  $\pm$  the standard error of the mean. The data were analysed using GraphPad Prism Version 6.0 (GraphPad Software, Inc.) and compared using a one-way ANOVA with a Tukey's multiple comparison test.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**Quantification of  $\beta$ -carotene and vitamin C levels in Holland and Khak-dam papaya juices.** Quantification of  $\beta$ -carotene and vitamin C in the two popular cultivars of papaya in Thailand; Holland and Khak-dam, are presented in Table I. The  $\beta$ -carotene content in the Holland papaya was  $48.45 \pm 1.40$   $\mu\text{g/g}$  and the vitamin C content was  $255.90 \pm 4.56$   $\mu\text{g/g}$ . In the Khak-dam papaya, the  $\beta$ -carotene content was  $37.22 \pm 0.97$   $\mu\text{g/g}$  and the vitamin C was  $156.60 \pm 2.10$   $\mu\text{g/g}$ .

**Effect of papaya on pancreatic lipase inhibition assay.** The percentage of inhibitory activity on pancreatic lipase using papaya juice from Holland cultivar was greater than that of the Khak-Dam at the same fruit juice concentration ( $40.37 \pm 3.87$  and  $26.41 \pm 3.49\%$ , respectively) as shown in Table I. Based on the higher levels of  $\beta$ -carotene, vitamin C and the percentage of pancreatic lipase inhibition, the Holland cultivar was used for the animal experiments.

**Effects of the papaya juice on body weight, food intake and adipose tissue weight.** At the beginning of the experiment, the initial body weight of all the experimental groups did not differ significantly (Table II), whereas at the end of 12 weeks,

Table I. Quantification of  $\beta$ -carotene, vitamin C and percentage of pancreatic lipase activity in Holland and Khak-dam papaya juices.

Compounds	Holland cultivar	Khak-dam cultivar
$\beta$ -carotene, $\mu\text{g/g}$	$48.45 \pm 1.40$	$37.22 \pm 0.97^a$
vitamin C, $\mu\text{g/g}$	$255.90 \pm 4.56$	$156.60 \pm 2.10^a$
Pancreatic lipase inhibition, %	$40.37 \pm 3.87$	$26.41 \pm 3.49$

<sup>a</sup> $P < 0.05$  vs. Holland papaya. Data is expressed as the mean  $\pm$  the standard error of the mean.  $n = 6$ .

the HF group exhibited significantly increased body weight compared with the C group (Table II). The final body weight and body weight gain in the treatment group was significantly decreased by 7.73% in the HFL group and 12.49% in the HFH group compared with the HF group, and the food intake amongst the four groups did not differ significantly. The mass of adipose tissues, including epididymal, perirenal and mesenteric fat pads, were significantly decreased by 17, 10 and 6% in the HFL group, and decreased by 15, 18 and 11% in the HFH group, respectively, compared with the HF group (Table II).

**Effects of papaya juice on the serum lipid profiles.** The serum levels of TG, TC and LDL-C in the HF group were significantly increased compared with the C group. In the HFL and HFH groups, these parameters were notably lower. The serum levels of HDL-C in the HF, HFL and HFH groups were significantly lower compared with the C group (Table II).

**Effect of papaya on inflammatory cytokines.** There were no significant differences in the serum levels of TNF- $\alpha$  amongst the C and treated groups. The serum levels of IL-6 in the HF group were significantly increased compared with the C group, and the levels in the HFL and HFH groups were significantly reduced compared with the HF group (Table II).

**Effect of papaya on serum leptin and insulin levels.** The serum levels of leptin did not differ significantly between the C and treated groups. The insulin levels in the HF group were significantly increased when compared with the C group, and the HFL and HFH groups exhibited reduced insulin levels compared with the HF group (Table II).

**Effect of papaya on lipid peroxidation.** Using the TBARS method, the results of lipid peroxidation from polyunsaturated fatty acids was used to determine the degree of lipid oxidation. The results showed that the total MDA content was significantly increased in the HF group when compared with the C group, whereas in the rats treated with papaya juice, the total MDA content was significantly decreased compared with the HF group (Table II).

**Effect of papaya on serum antioxidant status.** SOD activity was significantly reduced in the HF group compared with the C group, and was significantly increased in the HFL group

Table II. Effects of papaya juice on body weight, food intake, tissue weight and biochemical parameters.

Factors	C	HF	HFL	HFH
Initial weight, g	218±29.83	236.5±33.26	215.5±34.31	221.83±35.01
Final weight, g	465.83±11.13	536±33.24 <sup>c</sup>	509.33±33.57 <sup>a</sup>	471.33±15.04 <sup>e</sup>
Weight gain, g	247.8±8.36	299.5±16.26 <sup>a</sup>	293.8±8.64	249.5±14.92 <sup>d</sup>
Food consumption, g	16.62±3.65	20.81±3.27 <sup>c</sup>	21.14±2.37 <sup>c</sup>	20.95±2.75 <sup>c</sup>
Calorie intake, kCal/day	59.91±2.87	113.41±3.88 <sup>c</sup>	115.23±2.82 <sup>c</sup>	114.19±3.27 <sup>c</sup>
Blood pressure, mmHg	132.45±12.33	134.83±11.76	133.27±16.23	129.2±17.93
Plasma glucose, mg/ml	199.13±67.49	164.29±23.42	177.86±24.53	175.29±32.89
Insulin, ng/dl	0.41±0.15	5.58±1.02 <sup>b</sup>	1.83±0.97 <sup>d</sup>	3.58±1.82
HOMA-IR	4.61±1.59	59.55±13.03 <sup>a</sup>	22.58±15.15	38.04±23.69
Leptin, ng/ml	1.75±0.54	2.15±0.33	2.78±0.91	2.16±0.79
Epididymal fat, %	0.78±0.08	0.83±0.1	0.69±0.05 <sup>d</sup>	0.71±0.06 <sup>d</sup>
Perirenal fat, %	0.91±0.05	1.11±0.15 <sup>a</sup>	1±0.07	0.93±0.12 <sup>d</sup>
Mesenteric fat, %	0.55±0.07	0.61±0.07	0.57±0.04	0.55±0.07
Atherosclerosis index	0.26±0.11	0.67±0.08 <sup>c</sup>	0.7±0.12 <sup>c</sup>	0.66±0.06 <sup>c</sup>
Serum triglyceride, mg/dl	52.89±4.86	96.90±15.68 <sup>a</sup>	82.63±5.94	68.88±7.01
Serum cholesterol, mg/dl	73.45±3.19	274.0±38.84 <sup>c</sup>	211.4±34.29 <sup>b</sup>	216.4±23.00 <sup>b</sup>
Serum LDL cholesterol, mg/dl	34.79±2.76	234.9±36.21 <sup>c</sup>	178.0±33.27 <sup>b</sup>	187.6±22.13 <sup>b</sup>
Serum HDL cholesterol, mg/dl	28.09±0.96	19.76±2.11 <sup>b</sup>	16.88±2.06 <sup>c</sup>	15.00±1.43 <sup>c</sup>
Serum TNF- $\alpha$ , pg/dl	47.28±2.82	45.69±3.95	45.57±3.22	46.89±2.01
Serum IL-6, pg/dl	57.61±24.01	217.6±82.72	40.32±7.23 <sup>d</sup>	28.32±10.56 <sup>d</sup>
MDA, nmol of TBARS/mg protein	1.52±0.19	3.20±0.34 <sup>c</sup>	1.51±0.21 <sup>f</sup>	1.40±0.26 <sup>f</sup>
SOD, U/l	21.88±0.53	15.96±1.68	28.80±2.65 <sup>f</sup>	30.41±2.02 <sup>a,f</sup>
GR, U/l	0.04±0.00	0.03±0.00	0.05±0.01	0.04±0.00

<sup>a</sup>P<0.05, <sup>b</sup>P<0.01, <sup>c</sup>P<0.001 vs. C; <sup>a</sup>P<0.05, <sup>c</sup>P<0.01, <sup>f</sup>P<0.001 vs. HF. C, control; HF, high-fat; HFL, HF diet treated with 0.5 ml papaya juice/100g body weight; HFH, HF diet treated with 1 ml papaya juice/100 g body weight; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; LDL, low density lipoprotein; HDL, high density lipoprotein; TNF- $\alpha$ , tumour necrosis factor- $\alpha$ ; IL-6, interleukin-6; MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; GR, glutathione reductase.

when compared with the HF group. In the HFH group, SOD activity was significantly increased compared with both the C and HF groups (Table II), while the activity of GR showed no difference in any of the groups (Table II).

*Effect of papaya on epididymal adipose tissue.* Histological examination of the epididymal adipose tissue showed that the adipocyte size of the epididymal fat in the HF, HFL and HFH groups were enlarged when compared with the C group, and this effect was visible by the naked-eye. Furthermore, the epididymal adipocyte size was quantified by analysing images, which showed that the size in the HF group was significantly higher when compared with the C group, and the HFH group exhibited reduced adipocyte hypertrophy compared with the HF group (Fig. 1).

*Effect of papaya on PPAR $\gamma$  expression in the epididymal adipose tissue.* PPAR $\gamma$  is one of the key regulators of lipid metabolism (15), and thus was measured in the epididymal adipose tissue using western blotting. The protein expression levels of PPAR $\gamma$  in the HF group was significantly increased compared with the C group, however, supplementation of papaya juice (1.0 ml/100 g body weight) significantly decreased PPAR $\gamma$  expression to levels close to that observed in the C group (Fig. 2).

## Discussion

Chronic consumption of a HF diet has been shown to increase the prevalence of obesity (3). The largest component of dietary fats are TGs, and these are hydrolysed to free fatty acids and monoglycerides by pancreatic lipase, a key enzyme involved in the digestion of fat (16). Free fatty acids from triglycerides are transported to the blood system and delivered to the adipose tissue and liver, leading to lipid accumulation and the development of obesity. Inhibition of pancreatic lipase reduces digestion and the absorption of fat (16,17). Therefore, it is one of the most common treatments for obesity. In the present study, the results showed that papaya inhibited pancreatic lipase activity in the *in vitro* study. This effect is beneficial in inhibiting or delaying the digestion of lipids and, consequently, the absorption of fatty acid (17). Thus, the effects of papaya on a HF diet induced obese rats was assessed. The results of the present study were similar to previous studies, which showed that a HF diet increased body and adipose tissue weight, and caused obesity (3,18). In addition, in the present study, it was shown that a HF diet was associated with hyperlipidaemia, which results in elevated levels of lipids in the blood, such as triglycerides, total cholesterol and/or LDL-cholesterol.

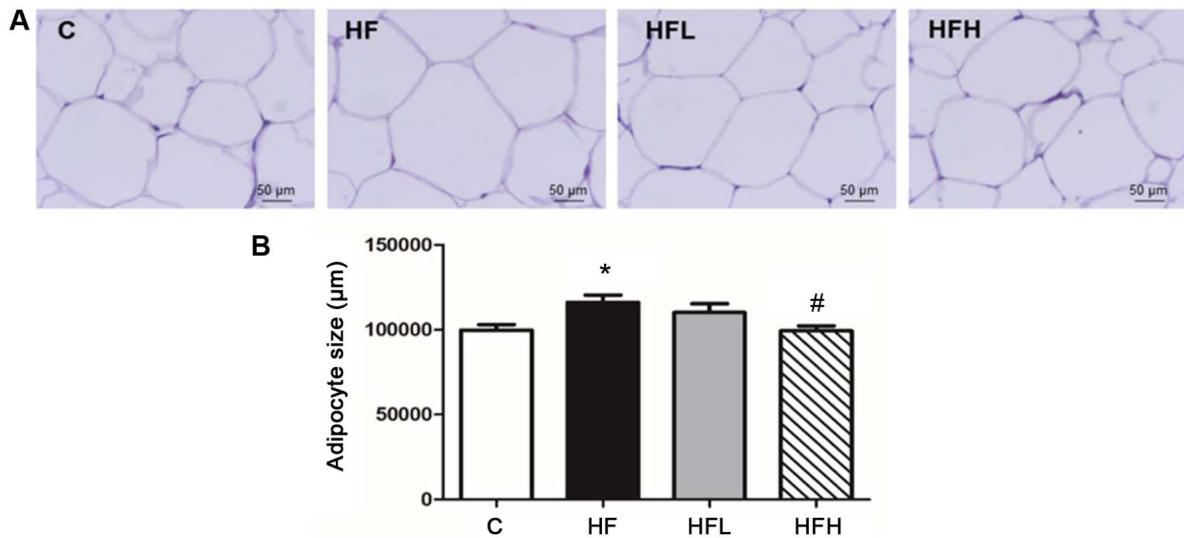


Figure 1. Effects of administration of papaya juice on epididymal adipose tissue in HF diet induced-obese rats. (A) Adipocytes from epididymal fat depots were stained using haematoxylin and eosin. (B) Adipocyte size in each group. Data are expressed as mean  $\pm$  the standard error of the mean (n=7). \*P<0.05 vs. C; #P<0.05 vs. HF. C, Control; HF, high-fat; HFL, HF diet treated with 0.5 ml papaya juice/100g body weight; HFH, HF diet treated with 1 ml papaya juice/100 g body weight.

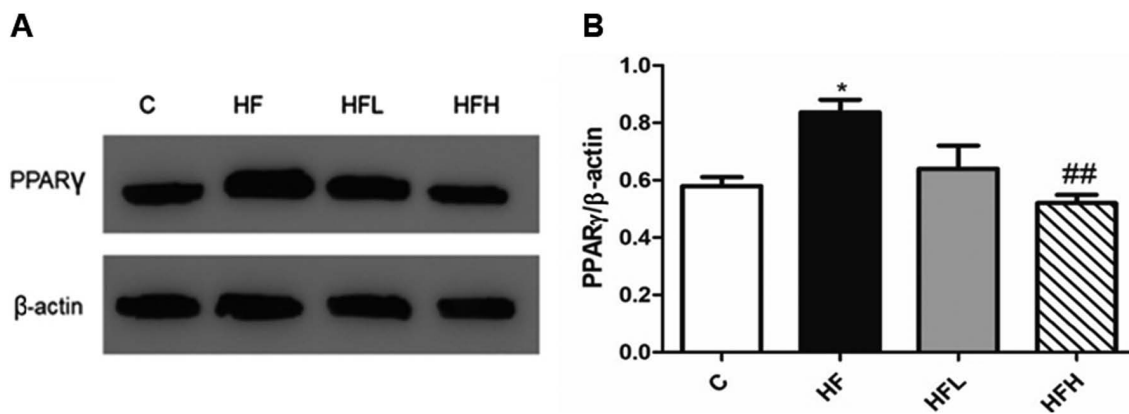


Figure 2. Effect of papaya juice administration on PPAR $\gamma$  expression in the epididymal adipose tissue in HF diet-induced obese rats. (A) Protein expression levels of PPAR $\gamma$ .  $\beta$ -actin was used as the loading control. (B) Densitometry analysis of PPAR $\gamma$  protein expression levels. Data are expressed as the mean  $\pm$  the standard error of the mean (n=7). \*P<0.05 vs. C; ##P<0.01 vs. HF. C, Control; HF, high-fat; HFL, HF diet treated with 0.5 ml papaya juice/100g body weight; HFH, HF diet treated with 1 ml papaya juice/100 g body weight.

Treatments using either 0.5 or 1.0 ml papaya juice showed that it significantly decreased body and adipose tissue weight, whilst also reducing TG, total cholesterol and LDL cholesterol levels in the serum. The reduction of serum lipid profiles indicated that papaya may decrease lipid transport to blood circulation, which resulted in the reduction of lipid accumulation in tissues. These results support the hypothesis that papaya may reduce the extent of obesity induced by a HF diet, by inhibiting intestinal absorption of dietary fat via the inhibition of pancreatic lipase activity. Additionally, the results of the present study showed that adipose tissue weight was decreased, resulting in a reduction of body weight.

In the present study, the histological results of the epididymal adipose tissue showed that the size of adipocytes were smaller in the HF diet fed rats treated with papaya compared with the HF group. Interestingly, papaya efficiently reduced both adipocyte hypertrophy and adipose tissue weight. Lipid accumulation in adipose tissue is a major cause

of the production of ROS, which leads to oxidative stress. A previous study reported that obese mice exhibited increased release of H<sub>2</sub>O<sub>2</sub> from white adipose tissue, whereas no increase was found in the muscles and the aorta (19). A study in 3T3-L1 murine adipocytes exposed to H<sub>2</sub>O<sub>2</sub>, found that the adipocytes produced ROS (20). Similarly, an increase in adipocyte tissue resulted in an increase in free radical levels in HF diet induced obesity as observed in the present study. MDA levels in the serum were increased in the obese rats and decreased in the rats treated with papaya. A previous study reported that polyphenol-rich extracts from papaya reduced the production of ROS and the secretion of IL-6 in adipose cells exposed to H<sub>2</sub>O<sub>2</sub> (21). The mRNA expression levels of TNF- $\alpha$  increased in the white adipose tissue of obese mice, and also increased the expression levels of IL-6 and MCP-1 in 3T3-L1 adipocytes exposed to ROS by incubation with H<sub>2</sub>O<sub>2</sub> (19,20), therefore, these results demonstrate that ROS production is associated with adipocytokines.

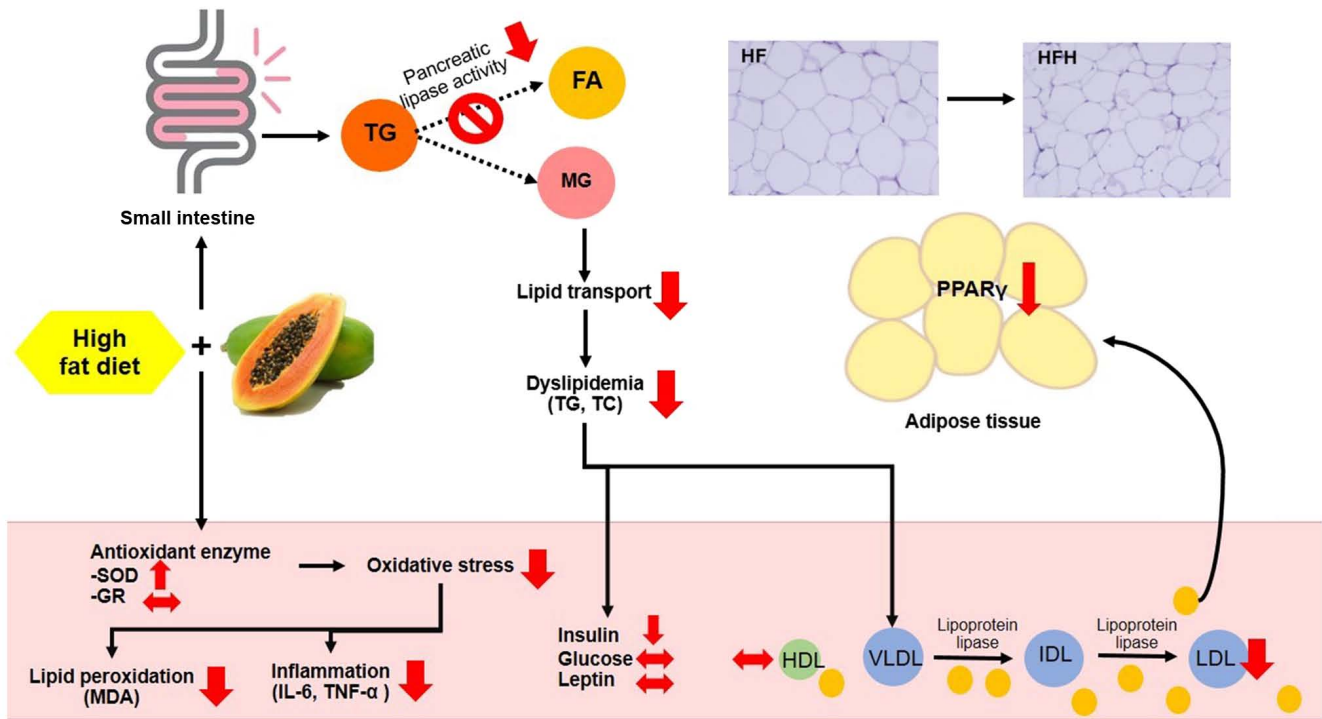


Figure 3. Schematic diagram of the potential mechanism of the observed anti-obesity effects of *Carica papaya* in HF fed rats. Papaya may prevent high-fat diet induced obesity, by inhibiting intestinal absorption of dietary fat via the inhibition of pancreatic lipase activity. A HF diet induced adipocyte hypertrophy, lipid accumulation and increased MDA levels, whereas supplementation of papaya juice reversed all these parameters. Papaya significantly increased serum superoxide dismutase and decreased serum IL-6 levels. Papaya juice may have regulated fat storage mediated via the PPAR $\gamma$  pathway in the adipose tissue. The results demonstrated that papaya juice exhibits potential for use as a nutritional supplement to reduce the risks of obesity. SOD, superoxide dismutase; GR, glutathione reductase; MDA, malondialdehyde; TNF- $\alpha$ , tumour necrosis factor- $\alpha$ ; HDL, high-density lipoprotein; VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; TG, triglyceride; TC, total cholesterol; FA, fatty acid; MG, monoglyceride; HF, high-fat; HFL, HF diet treated with 0.5 ml papaya juice/100 g body weight; HFH, HF diet treated with 1 ml papaya juice/100 g body weight.

Obesity is caused by the accumulation of free fatty acids in the adipose tissue which can result in enlarged adipocytes and/or hypertrophy. Adipogenesis is the process of adipocyte differentiation that transforms preadipocytes to adipocytes, and is dependent on peroxisome PPAR- $\gamma$ , which is a transcription factor (15). The results of the present study were similar to previous studies, which showed that a HF diet increased the expression of PPAR $\gamma$ , both at the mRNA and protein expression levels, and also resulted in enlarged adipocytes and/or hypertrophy (22). One of the characteristics of obesity is low-grade chronic inflammation in which adipose tissue releases several inflammatory mediators (23). As the adipocytes enlarge, the blood supply to them is reduced, causing hypoxia. Adipose tissue is not only a lipid storage site, but also functions as a key endocrine organ. Therefore, during hypoxia, macrophages filter into the adipocytes and stimulate secretion of pro-inflammatory cytokines and adipocytokines, such as TNF- $\alpha$  and IL-6 (24). In the present study, TNF- $\alpha$  and IL-6 levels in the serum were measured using ELISA. The results showed that the HF diets resulted in a marked increase in serum IL-6 levels. The elevated IL-6 levels were significantly decreased when the diet of the rats was supplemented with 0.5 or 1.0 ml papaya juice, compared with the untreated HF diet fed rats. In addition, a previous study showed that  $\beta$ -carotene decreased pro-inflammatory cytokines such as nitric oxide, TNF- $\alpha$  and IL-1 in mice (25). In the present study, the results of HPLC analysis found that papaya contains both vitamin C and  $\beta$ -carotene. Therefore, vitamin C and/or  $\beta$ -carotene from

papaya may likely underlie the reduction of pro-inflammatory cytokines.

Obesity can induce systemic oxidative stress through various biochemical mechanisms, including reducing antioxidant defence or increasing chronic inflammation (26). Papaya is a good source of antioxidant phytochemicals, such as vitamin C, carotenoids and vitamin E, which serve as antioxidants reducing oxidative stress (27,28). MDA was measured as a biomarker of oxidative stress and activity of the antioxidant system. The results of the present study showed that papaya improved the imbalance of oxidative stress generation and ability to detoxify or repair the damage caused by decreased levels of MDA and increased levels of antioxidants. These results are similar to several studies which have shown the potent antioxidant properties of papaya (8,29). Furthermore, papaya reduced the size of the adipocytes, and the expression of PPAR $\gamma$  in obese rats, which resulted in decreased levels of pro-inflammatory cytokines in the serum, decreased levels of MDA and increased anti-oxidant levels. The beneficial effects of several natural products on reducing obesity are attributed to the presence of significant quantities of bioactive compounds, which possess antioxidant and anti-inflammatory properties (18). Additionally, these bioactive compounds significantly decrease the levels of TBARS, and increase SOD and glutathione reductase levels. As a rich source of antioxidant activity, papaya can decrease the serum levels of TBARS, which cause oxidative damage to lipid components in cell membranes (14). In addition, an increase in the activity

of SOD was observed when papaya was administered. SOD is one of the first lines of defence in the detoxification of products resulting from oxidative stress (30). An increase in SOD activity following supplementation of papaya, may imply that papaya can stimulate SOD, and this may result in counteracting the effects of potentially harmful substances.

Based on these findings, it was shown that papaya juice reduced lipid absorption as well as the anti-obesity, anti-dyslipidaemia and anti-inflammatory effects in obese rats. The proposed schematic is shown in Fig. 3. In conclusion, papaya juice may be a promising alternative treatment and/or dietary supplement for obese individuals.

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### Availability of data and materials

The datasets used and/or analysed during the present study are available from the corresponding author on reasonable request.

### Authors' contributions

POE and WD performed the experiments, analyzed, interpreted the data and wrote the manuscript. WM and IP conceived and designed the study, supervised the study, interpreted the data and drafted the manuscript. ST designed and supervised the study, interpreted the data, discussed the results, wrote and revised the manuscript. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

All experimental procedures were approved by the Ethics Committee of the Centre for Animal Research, Naresuan University (Phitsanulok, Thailand) (approval no. NUA580174).

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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