Physiological and molecular study on the anti-obesity effects of pineapple (*Ananas comosus*) juice in male Wistar rat

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Abstract The present study was performed to assess anti-obesity effects of raw pineapple juice in high fat diet (HFD)-induced fatness. Based on food type, rats were divided into normal diet and HFD groups. When animals of HFD group become obese, they were given pineapple juice along with either HFD or normal diet. Blood biochemistry, liver and muscle gene expressions were analyzed. HFD induced significant elevations in body weight, body mass index (BMI), body fat accumulation, liver fat deposition and blood lipids while juice restored these parameters near to their normal values. Juice significantly decreased serum insulin and leptin while adiponectin was increased. Juice administration downregulated the increment of FAS and

SERBP-1c mRNA expression in liver and upregulated HSL and GLUT-2 expressions. The muscular lipolytic CPT-1 expression was upregulated by juice treatment. Pineapple juice, therefore, may possibly be used as anti-obesity candidate where it decreased lipogenesis and increased lipolysis.

Keywords Pineapple juice · Rat · Obesity · Gene expression · Histopathology

Introduction

Obesity has achieved outbreak rate where over than one billion worldwide are overweight and no less than 0.3 billion of them are diagnosed as corpulent. The predominance of obesity in youngsters and youths is about 5–14% in males and from 3 to 18% in women [1]. Many reasons could induce corpulence including hereditary components, medical reasons and psychiatric illness however the central cause is the combination between extreme intake of high-energy food and absence of physical activities [2]. Obesity predisposes to serious diseases including Type 2 diabetes, osteoarthritis and cardiovascular diseases. Therefore, it decreases life probability and causes many public and economic problems [3]. The home grown supplements and eating routine based treatments for weight reduction are recorded as the most widely recognized integral and elective prescription modalities. There are assortments of common items being utilized for an elective treatment of stoutness. These include medicinal plants, either as pure compounds or as extracts. Many plants contain a large variety of components with unique properties that prompt the researchers to study their efficacy in dealing with obesity, diabetes and other chronic diseases [4].

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Pineapple (*Ananas comosus*) fruit is rich in nutrients include calcium, potassium as well as vitamin C and vitamin A. Fresh pineapple is rich in bromelain which may act as anti-inflammatory [5]. Interestingly, pineapple fruits contain eight phenolic compounds, including gallic acid, gentisic acid, syringic acid, vanillin, ferulic acid, sinapic acid, isoferulic acid and o-coumaric acid [6]. These phenolic compounds exhibit an antioxidant activity and anti-inflammatory function as well as inhibit the hydrolytic and oxidative enzymes in human cells [7].

Pineapple has progressed toward becoming a favorable fruit in obesity and dyslipidemia treatment as it contains a high amount of bromelain, which has a role in lipolysis and diminishing the severity of cardiovascular syndromes. Fresh fruits have been known for their defensive impact against cardiovascular diseases. They have the benefit of containing high amounts of specific dietary fiber, which decreases cholesterol uptake by the organism [8]. Fat binding capacity (FBC), important for the detection of anti-obesity property in food, was very high in pineapple. A couple of clinical investigations demonstrate the helpful impacts of bromelain in stoutness treatment [9]. The present study was therefore, conducted to investigate anti-obesity effects of pineapple raw juice in HFD-induced obese rats. It aimed also to expound the mechanisms behind this effect through testing the mRNA expression of fat metabolism-related enzymes.

Materials and methods

Juice preparation

Pineapple fruit used in this experiment was purchased from PANDA store in Taif city, Saudi Arabia and it was imported from Philippine (Del Monte Gold type). The composition mentioned on the label included protein 1 g/100 g, total fat 0 g/100 g, cholesterol 0 g/100 g, carbohydrates 14 g/100 g, sugars 10 g/100 g, sodium 0 g/100 g, calcium 20 mg/100 g and vitamin C 54 mg/100 g. The Fresh fruits were peeled, and the edible portion was squeezed. The juice was filtered through double-layer gauze and the filtrate was divided into 10 mL aliquots in 15 mL Falcon tubes and stored at -80°C until used [10]. The filtrate was offered to rats with drinking water in a dosage of 15% (v/v) [11]. We did not analyze the chemical composition of the filtrate and depended on the label composition of the fruit and on the analyses previously conducted [12, 13].

Animals and experimental design

Twenty-four adult male Wistar rats (7 weeks old) were obtained from experimental animal center, Faculty of Pharmacy, King Abdul-Aziz University, Saudi Arabia. The rats were retained under controlled states of temperature ($23 \pm 2^{\circ}\text{C}$), humidity ($50 \pm 5\%$) and 12 h light-dim cycle. Rats were acclimatized for a week before beginning the experiment. They were kept in sanitized polypropylene cages containing sterile husk as bedding with free access to standard pellets as basal diet and water ad libitum. All procedures approved by the Animal Care Committee of Taif University (#1-437-4742).

After one-week of acclimatization, rats were divided randomly into two main groups. GI (control, $n = 6$) that was kept on normal diet. HFD group ($n = 18$) that was fed on HFD (1 kg ration contained 164 g casein, 303.1 g corn starch, 90 g dextrin, 115 g sucrose, 58.9 g cellulose, 190 g butter oil, 10 g soya bean oil, 2.1 cysteine, 2.9 g choline tartrate, 35 g mineral mix and 11.7 g vitamin mix) [14]. Animals of this group gained free access to water for 7 weeks until the point that obesity was affirmed by high BMI and elevated serum triacylglycerol. HFD group were divided into 3 groups (each group contained 6 rats). Untreated obesity group that received HFD with normal water (GII), GIII as an obesity group received normal diet (ND) + raw pineapple juice (15% V/V) in drinking water using bottles that were changed every day; and GIV kept to feed on HFD + raw juice as in GIII. The BW and food consumption for each group were recorded weekly throughout the experimental period (8 weeks).

Sampling

Sampling was done according to Ahmed et al. [4]. At the completion of the experiment, rats were fasted for 12 h and were anaesthetized using diethyl ether. BW and nose-anal length were measured for calculating BMI. Blood samples were collected from medial canthus of the eyes. Clotted blood was centrifuged, and sera were preserved at -80°C for biochemical analysis. Rats were sacrificed by head decapitation, liver and renal adipose tissue weights were recorded. A slice of liver and muscle tissues were immersed immediately in liquid nitrogen and was preserved in -80°C for gene expression analysis. Another portion was kept in 10% neutral buffered formalin for histopathological inspection using oil-red and H&E stains.

Food intake and body gain

Animal BW, BW gain (g/day/rat) and food intake were weekly measured throughout the experimental period. Food intake was measured by calculating the difference

between the weight of food offered and that persisted in the food bin.

Serum biochemical assays

Serum total cholesterol (TC), triacylglycerol (TAG), HDL-cholesterol and glucose were measured using spectrophotometer and commercial kits (HUMAN Gesellschaft für Biochemica und Diagnosticam bH, Wiesbaden, Germany). ELISA kits (R&D, Inc., USA) was used to measure serum insulin, adiponectin and leptin according to the manufacturer's guide.

Analysis of gene expression

RNA extraction and cDNA synthesis

Total RNA was prepared from 100 mg of tissue using QIAzol reagent (QIAGEN Inc., Valencia, CA) according to the manufacturer's guidelines as detailed previously. RNA quality was checked by electrophoresis while RNA concentration and pureness was determined using spectrophotometer at both 260 nm and 280 nm. The OD260/280 ratio of RNA samples were from 1.8 to 2.0. Two µg of total RNA were reverse transcribed using oligo-dT primer and reverse transcriptase enzyme (SibEnzyme Ltd. AK, Novosibirsk, Russia) as described by the manufacturer's manual. The obtained cDNA was preserved at -20°C until analysis.

Semi-quantitative-PCR

To examine the expression of some genes involved in energy metabolism, semi-quantitative PCR were done using primers listed in Table 1 (Macrogen Company,

GAsa-dong, Geumcheon-gu, Korea). The genes included fatty acid synthase (FAS), sterol regulatory element-binding protein-1c (SREBP-1c), hormone sensitive lipase (HSL), glucose transporter-2 (GLUT-2), and carnitine palmitoyltransferase I (CPT-I). PCR were conducted in a final volume of 25 µl using PCR GoTaq[®] Green Master Mix (Promega Corporation, Madison, WI) with annealing temperatures and number of cycles as described in Table 1 using β -actin gene as a reference. PCR products were electrophoresed in 1% agarose gel (Bio Basic INC. KonradCres, Markham Ontario) for 30 min stained by ethidium bromide and photographed under UV. Band intensity was measured densitometrically by NIH image program (<http://rsb.info.nih.gov/ni-image>).

Histopathological examination

Liver and renal adipose tissues from all groups were fixed in 10% neutral buffered formalin for 24 h, washed under running tap water and preserved in 70% ethanol for general histological studies. The samples were dehydrated in ascending concentrations of ethanol, cleared in xylene, embedded in Paraplast Plus[®], and sectioned at 5 µm thickness. Sections were mounted on glass slides and stained using hematoxylin and eosin (HE). Processing and staining methods are described in Bancroft et al. [15]. Other portion of hepatic tissue (2 mm thick) was fixed in 10% neutral buffered formalin for 24 h and processed [16] for lipid droplets demonstration by oil-red O staining.

Statistical analysis

Data were statistically analyzed using ANOVA test, and Scheffe's protected least significant difference test by SPSS software (SPSS version 13.0, IBM, Chicago, IL, USA).

Table 1 Primer sequence, GenBank accession numbers (or reference), and PCR conditions

Gene, accession number	Primer sequence (5'-3')	Annealing ($^{\circ}\text{C}$) cycles number	Product size
β -actin (V01217)	F-ATGTACGTAGCCATCCAGGC R-TCCACACAGAGTACTTGCGC	56 $^{\circ}\text{C}$, 30 cycles	628 bP
FAS (NM017332)	F-GAGCTGCGGCTACGTGGCTA R-GCCGCCGTGAGGTTGCTGTT	61 $^{\circ}\text{C}$, 28cycles	340 bP
SREP1-c (L16995)	F-GGAGCCATGGATTGCACATT R-AGGAAGGCTTCCAGAGAGGA	58 $^{\circ}\text{C}$, 28cycles	191 bP
HSL (U4001)	F-TGCCCAGGAGTGTGTCTGAG R-AGGACACCTTGCTTGAGCG	61 $^{\circ}\text{C}$ 35 cycles	313 bp
GLUT-2	F-AAGGATCAAAGCCATGTTGG R-GGAGACCTTCTGCTCAGTGG	55 $^{\circ}\text{C}$, 28 cycles	330 bp
CPT-1	F-TATGTGAGGATGCTGCTTCC R-CTCGGAGAGCTAAGCTTGTC	52 $^{\circ}\text{C}$, 32 cycles	628 bp

Table 2 Obesity and the biochemical parameters as means $\pm$ standard errors for GI, GII, GIII, and GIV with their comparisons according to ANOVA test

Item	GI	GII	GIII	GIV
Initial BW (g)	278.83 $\pm$ 13.93	381 $\pm$ 13.6	369.14 $\pm$ 11.0	359.33 $\pm$ 14.7
Final BW (g)	331.5 $\pm$ 9.31 ^a	519 $\pm$ 18 ^b	398 $\pm$ 2.19 ^c	425 $\pm$ 23.4 ^d
BMI	0.62 $\pm$ 0.02 ^a	0.82 $\pm$ 0.03 ^b	0.69 $\pm$ 0.1a	0.75 $\pm$ 0.03 ^b
Food Consumption (g/d))	27.78 $\pm$ 0.9 ^a	17.96 $\pm$ 0.3 ^b	25.87 $\pm$ 1.1 ^b	18.49 $\pm$ 1.8 ^c
Perirenal fat weight (g)	1.65 $\pm$ 0.09 ^a	2.89 $\pm$ 0.12 ^b	1.21 $\pm$ 0.8 ^c	2.25 $\pm$ 0.3 ^d
Liver weight (g)	2.7 $\pm$ 0.11 ^a	2.076 $\pm$ 0.01 ^b	2.76 $\pm$ 0.4 ^a	2.84 $\pm$ 0.2 ^c
Adipocyte size (μm^2)	598.1 $\pm$ 12.86 ^a	4186.74 $\pm$ 777.5 ^b	638.03 $\pm$ 14.99 ^c	1876.68 $\pm$ 78.31 ^d
Blood glucose level	110.33 $\pm$ 0.55 ^a	173.66 $\pm$ 6.375 ^b	133.83 $\pm$ 4.135 ^a	147.33 $\pm$ 6.142 ^c
Serum Insulin	25.08 $\pm$ 1.09 ^a	47.80 $\pm$ 3.89 ^b	30.96 $\pm$ 2.52 ^c	38.82 $\pm$ 1.01 ^d
Serum Leptin	11.73 $\pm$ 0.85 ^a	22.22 $\pm$ 1.68 ^b	15.38 $\pm$ 0.96 ^c	18.54 $\pm$ 1.20 ^d
Serum Adiponectin	17.02 $\pm$ 1.59 ^a	10.25 $\pm$ 0.96 ^a	14.73 $\pm$ 1.14 ^c	13.90 $\pm$ 1.48
Serum Triacylglycerol	75.14 $\pm$ 7.597 ^a	170.2 $\pm$ 3.817 ^b	97.26 $\pm$ 10.3 ^c	130.9 $\pm$ 11.05 ^d
Serum Total Cholesterol	59.88 $\pm$ 1.72 ^a	84.72 $\pm$ 5.19 ^b	63.12 $\pm$ 3.401 ^a	74.07 $\pm$ 6.12 ^c
HDL-C	22.01 $\pm$ 1.738 ^a	17.34 $\pm$ 0.652 ^b	26.13 $\pm$ 0.578 ^c	18.44 $\pm$ 2.206 ^b
LDL-C	22.83 $\pm$ 2.53 ^a	33.34 $\pm$ 5.90 ^b	17.53 $\pm$ 4.28 ^c	29.44 $\pm$ 7.68 ^b
VLDL	15.03 $\pm$ 1.51 ^a	34.03 $\pm$ 4.32 ^b	26.12 $\pm$ 2.06 ^c	26.18 $\pm$ 2.21 ^c
Atherogenic index	0.52 $\pm$ 0.04 ^a	0.99 $\pm$ 0.02 ^b	0.56 $\pm$ 0.04 ^a	0.86 $\pm$ 0.06 ^c

Values with different letters are significantly different at * $p < 0.05$

The difference at $p < 0.05$ was regarded as statistically significant. Data were shown as means $\pm$ standard errors (SEM).

Results and discussion

In the present study, the anti-obesity efficacy of pineapple juice was explored by measuring food intake, BW gain and lipid profiles in the juice-treated obese rats. Meanwhile, the mRNA expression of some genes involved in fat and energy metabolism was also examined in liver and muscle to gain insights into the mechanism underlying the anti-obesity properties of pineapple juice. After initiation of obesity using HFD, the obese rats received normal diet with fresh pineapple juice administration. Moreover, to assess the anti-obesity effect of the pineapple juice in the presence of HFD, a group of obese rat was maintained on HFD together with pineapple juice administration.

The results showed that the body heaviness of untreated obesity GII was increased significantly than control GI. This increase was significantly reduced by feeding normal diet with juice (GIII). In addition, receiving juice in combination with HFD (GIV) significantly reduced BW in comparison to the untreated obesity GII (Table 2). The weights of perirenal fat were increased significantly in untreated obesity GII compared to the control. Meanwhile, weights of perirenal fat were decreased significantly in groups received juice with either normal diet (GIII) or HFD (GIV) (Table 2). Interestingly, the dimensions of

adipocytes in sections stained with H & E was increased in untreated obesity GII related to control GI. Meanwhile, juice administration induced significant decreases in adipocyte size in obesity groups. The effect was more obvious in rats received ND (GIII) than in those with HFD (GIV) (Fig. 1).

Biochemically, levels of glucose, insulin, leptin, triacylglycerol, total cholesterol, LDL-cholesterol, atherogenic index, and VLDL were elevated by HFD feeding (GII). Conversely, the adiponectin level was significantly decreased in the untreated obesity GII. However, juice supplementation showed decreased glucose, insulin, leptin, triacylglycerol, total cholesterol, LDL-cholesterol, and VLDL levels, and elevated adiponectin level in blood

Fig. 1 Upper panel: Effect of pineapple juice on lipid accumulation in liver (oil-red O-stained hepatic sections). (A) sections from GI, notice absence of red droplets in hepatocytes. (B) sections from GII, notice variable-sized red droplets (arrowheads) in hepatocytes. (C) Sections from GIII; notice the reduction in number of large (arrowhead), and small (arrow) red droplets in hepatocytes. (D) sections from GIV; notice the slight reduction in number of large (arrowhead), and small (arrow) red droplets in hepatocytes. Scale bars: 50 μm . Lower panel: Effect of pineapple juice on lipid accumulation and size of adipocytes (hematoxyline and eosin-stained adipose tissue). (E) sections from GI; notice normal sized adipocytes ($589.10 \pm 12.86 \mu\text{m}^2$). (F) Sections from GII; notice large sized adipocytes ($4186.47 \pm 777.50 \mu\text{m}^2$). (G) Sections GIII; notice the nearly normal sized adipocytes ($638.03 \pm 14.99 \mu\text{m}^2$). (H) Sections from GIV; notice the reduction of adipocytes size ($1876.68 \pm 78.31 \mu\text{m}^2$) but is still larger than that of control. Scale bars: 50 μm

compared to HFD feeding GII (Table 2). In other words, administration of pineapple juice significantly ameliorated HFD-induced BW pick up, visceral adiposity (represented by increased perirenal fat weight) (Table 2), dyslipidemia, hyperleptinemia, hyperglycemia, hyperinsulinemia and hypoadiponectinemia in rats, without effect on food consumption. The juice effect was more prominent in GIII (fed normal diet) compared to GIV (fed HFD). The obtained results were in accordance with those of Priya and Dhar-amveer [17] who demonstrated that treatment with hydro-alcohol *Ananas comosus* extract decreased serum total cholesterol, LDL-cholesterol and VLDL. Moreover, Xei et al. [8] have stated that pineapple decreased total cholesterol, triacylglycerol and LDL-cholesterol in rat and mice. The effect was referred to the existence of bromelain that having lipolytic and proteolytic activity and to the contents of high amount of fiber in raw juice which induced hypo-cholesterolemic effect [8].

The pineapple extract was found to increase HDL level compared to obese group and this indicated that pineapple could be able to diminish the risk of lipid-associated diseases [18]. These findings are in accordance with the current results as decreasing serum cholesterol in groups received juice (GIII, GIV) was accompanied with a decrease in its LDL fraction that is the potential risk factor for cardiovascular diseases. It thus decreases the atherogenic index possibly via the improvement of LDL catabolism through hepatic receptors [19]. The adipocytes secrete a variety of adipocytokines including leptin and adiponectin. Leptin is produced by *Ob* gene acting as a protein hormone and an adipocytokine. It is a key controller of appetite and energy spending, adipose tissue mass and BW in humans and rodents. Its serum level is positively correlated to amount of triacylglycerol stored in adipocytes [20]. In both humans and animal, adiponectin assumes a fundamental part in the control of carbohydrate and fat metabolism in insulin-sensitive tissues by enhancing insulin sensitivity [21]. There is a positive association between serum leptin and adiposity in rats [22]. The reduced serum concentration of leptin in the obese groups (GIII, GIV) received pineapple juice in comparison to the untreated obesity group (GII) can be elucidated by the decrease of visceral fat. This is in line with an earlier study stated that HFD significantly increased serum and liver total cholesterol and triacylglycerol levels [23]. In the present experiment, plasma leptin concentration was reduced by juice administration (GIII and GIV) compared to the untreated obesity group (Table 2). Histological examinations showed that the adipocytes size was significantly reduced in juice-treated rats [Fig. 1(B), Table 2]. The obtained results proposed that reduction in serum leptin following juice treatment could be referred to the reduction in fat accumulation in white adipose tissue. In

addition, HFD is recognized to upregulate fatty acids synthesis, and the delivery of free fatty acids to the liver [24]. It decreases β -oxidation of free fatty acids resulting in fat deposition in the liver [20]. The histological finding indicated that there was a fatty degeneration of hepatocytes and accumulation of fat globules in liver tissues of obese rats, which was not seen in the control group. A prominent lipid accumulation was detected by oil-red O stain in HFD only fed group matched to the control group. This accumulation was not found in the rats received ND and juice while it was low in the group received HFD with juice compared to untreated obesity group (Fig. 1), suggesting that the juice may regulate adipocytes fat storage and mobilization through modulating serum level of leptin. It was worthy noted that juice treatment significantly reduced leptin, glucose and insulin serum levels in obese rats (GIII, GIV) (Table 2). Taking into consideration that the increased glucose and insulin in rats fed HFD is an indicator of obesity-induced insulin resistance, the current results proposed that pineapple might protect against obesity-induced insulin resistance development [8].

Compared to control group, liver histology showed the presence of fatty degeneration in hepatocytes. A prominent lipid deposition was seen by oil-red O stain in HFD group (GII). It was not observed in the group taken ND with juice and was low in a group received HFD with juice. Meanwhile, the adipocyte size of renal fat in HFD rats was greater than in control. Juice restored the cell size near to normal. The decrease in both body fat weight and adipocyte size were associated with a reduction in liver fat deposition (Fig. 1). Similarly, Ahmed et al. [11] showed that a decrease in BW of obese rat received pomegranate juice was accompanied with a decrease in liver fat deposition. Obtained results stated that pineapple juice enhanced lipolysis of hepatic fat.

To clarify the molecular mechanisms underlying the anti-steatotic effect of the pineapple juice, the expression of the main enzymes that control lipid metabolism and energy homeostasis in the liver was investigated. We inspected the mRNA expression of certain enzymes implicated in the pathways of fatty acids biosynthesis including fatty acid synthase (FAS), sterol regulatory element-binding protein 1 and 2 (SREBP-1c) and those are associated with the lipolysis including carnitine palmitoyl transferase-I (CPT-1), and hormone sensitive lipase in liver and muscle. As fatty liver or hepatic steatosis is regarded as one of the serious hepatic complications associated with obesity, our results showed deposition of fat globules in liver tissues of obese rats. A hepato-protective effect of pineapple juice was reported in obese rats received juice with either normal diet or even HFD. This protective effect was represented as down-regulation of the over expression of the main lipogenic gene FAS mRNA in liver tissue. This

Fig. 2 Effect of pineapple juice on FAS (A), SREBP-1c (B), HSL-1c (C) and GLUT-2 (D) mRNA expressions in hepatic tissue of rat. Representative blots and results of densitometric analyses of at least five independent experiments are shown. Values are mean $\pm$ SE. GI is control group fed on normal diet with no further treatment, GII

untreated obesity group, GIII is an obesity group fed normal diet (ND) with juice and GIV is an obesity group fed HFD with juice. * $p < 0.01$ versus GI (A) § $p < 0.01$ versus GII (B) * $p < 0.05$ versus GI (C) and § $p < 0.05$ versus GII (D)

result was also shown as decreases in fat contents, BMI and BW and suppression in fat deposition of liver tissue. Similarly, using of oleic-acid-dihydroxyamphetamine conjugation in treatment of obese rat resulted in inhibition of the hepatic FAS expression compared to non-treated obese rats and thus reduced BW [Fig. 2(A)]. Due to its vital role, FAS could be considered as a therapeutic target for treating fatty liver and dyslipidemia [25].

Lipogenic transcription factor, SREBP-1c plays a main regulator for fat metabolism as it controls the expressions of enzymes for TAG uptake and fatty acid synthesis [26]. Obtained results demonstrated that the expressions of SREBP-1c mRNA was increased in liver and muscles of HFD only fed rats, and this upregulation was countered after administration of pineapple juice with either ND or HFD [Fig. 2(B)]. These results agreed to that of Kim et al.

Fig. 3 Effect of pineapple juice on FAS (A), SREBP-1c (B) and CPT-1 (C) mRNA expressions in muscles. Representative blots and results of densitometric analyses of at least five independent experiments are shown. Values are mean $\pm$ SE. * $p < 0.05$ versus GI. $\S p < 0.05$ versus GII

[27] who reported that administration of *Aster spathulifolius* extract (ASE) by rats with HFD induced obesity resulted in suppression of the expression of lipogenesis-related genes SEBP-1C and FAS.

Hepatocyte GLUTs play an important role in the glucose uptake from the portal blood circulation to be storage as glycogen [28]. GLUT-2 a glucose transport protein is expressed mainly in the hepatic, pancreatic beta cells, and

on the kidney as well as small intestine epithelia [29]. In this study, the expression of Glut-2 mRNA was upregulated in HFD fed obese rats compared to the control group. The administration of pineapple raw juice either with ND or with HFD restored the expression [Fig. 2(C)]. In liver, the expression mode of glucose transporters is controlled not only by glucose and insulin levels but also by IL-6, which elevates in case of obesity and diabetes [30]. In fatness, insulin resistance drives an elevation in GLUT-2 levels that may lead to metabolic dysfunction in NAFLD [31]. Normal control mice significantly suppressed expression of liver GLUT2 relative to untreated obese animals [32]. Overexpression of hepatic GLUT2 suggested the dysregulation of glucose homeostasis and the increase of liver glucose output [33]. Obesity showed to suppress the mRNA expression of HSL, while pineapple juice was shown to upregulate HSL expression over than control. This upregulation was higher in the group received juice with normal diet than that received juice with HFD [Fig. 2(D)]. Concerning the expression of FAS and SERPB-1c in muscle, both genes showed patterns as that in liver where HFD induced their up-regulation and pineapple juice inhibited this increment restoring the expression near to normal [Fig. 3(A, B)]. The first step of β -oxidation is the transportation of long-chain fatty acids into the mitochondrial matrix which is controlled by CPT system [34]. The present study revealed that HFD induced down regulation of CPT-1 expression in muscles while pineapple juice, restored this gene expression indicating the enhancement of fatty acid oxidation [Fig. 3(C)].

It is worthy to note that pineapple contain high amount of vitamin C which plays a role as antioxidant-based treatments to counteract obesity fat accumulation complications. Moreover, the phenolic compounds in pineapple [6] could contribute the anti-obesity effect of pineapple juice observed in this experiment. Gallic acid reduced body weight and serum triacylglycerol in obese mice [35]. Syringic acid possesses anti-obesity and anti-steatotic effects via the regulation of lipid metabolism [36]. Ferulic acid was found to diminish the risk of HFD-induced obesity by modulation of enzymatic, hormonal and inflammatory responses [37].

In conclusion, pineapple juice has suppressed HFD-induced obesity via decreasing body serum lipid, weight gain and hepatic lipid accumulation. The juice has also induced a decrease in number and size of adipocytes in HFD-induced obese rats. It seemed to exert these effects by modulation of fat metabolism through downregulating lipogenesis and upregulating fatty acid oxidation at transcriptional level of lipid metabolism-related genes. These findings suggest that pineapple juice could be an actual anti-obesity candidate with no obvious harmful effects. Further studies are needed in order to separate active

ingredients and to determine their individual efficiency in obesity therapy.

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