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Pineapple Juice Ameliorates the High Fat Diet- induced Alterations in Cardiac Gene Expression Pattern in Male Rats

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Background: Obesity is a public health problem worldwide. It results from energy imbalance where energy intake exceeds energy expenditure. It's associated with a variety of diseases including cardiovascular dysfunctions.

Aims: The current study aimed to evaluate the potential role of pineapple juice to ameliorate the high fat diet (HFD)-induced obesity and its associated cardiovascular dysfunction.

Study Design: Animals were divided into 2 groups: a control ($n=6$) group fed a regular diet and tap water, and HFD-fed ($n=12$) group that was fed HFD and tap water *ad libitum* for 10 weeks. After induction of obesity, HFD-fed group was subdivided into 2 groups each of six animals: Obese, non-treated group; gained free access to normal drinking water and HFD; pineapple group, given HFD and pineapple juice (15% in drinking water, vol /vol). The treatment was continued for 5 weeks. Animals' body weight was recorded weekly throughout the experimental period.

Place and Duration of Study: Department of Biotechnology, College of Science, Taif University, between May 2016 and September 2016.

Methodology: At the end of the experimental period, animals were sacrificed, serum was prepared

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and assayed for triacylglycerol, total cholesterol and nitric oxide (NO) levels, and cardiac tissues were collected for analysis of the possible alterations in cardiac gene expression pattern in HFD-induced obese male wistar rat using RT-PCR.

Results: Pineapple juice treatment significantly ($P=0.05$) reduced HFD-induced increases in body weight, serum triacylglycerol and cholesterol. It also inhibited the obesity-induced upregulation of ET-1 (Endothelin-1), TNF- α , NF κ B, AT1 receptor (Angiotensin II receptor type 1), iNOS and ACE (Angiotensin Converting Enzyme) mRNA expression ($P=0.05$), while it reversed the obesity-induced decrease of eNOS mRNA expression and NO production ($P=0.05$). Thus, pineapple juice can ameliorate the obesity-induced alterations of cardiac gene pattern at transcriptional level.

Conclusion: Pineapple juice might be a candidate therapeutic approach for treating HFD-induced obesity and its associated cardiovascular dysfunctions.

Keywords: Pineapple juice; obesity; cardiovascular dysfunction, rats.

1. INTRODUCTION

Obesity is a widespread chronic metabolic disorder. It results from an energy imbalance where energy intake exceeds energy expenditure, characterized by an excessive fat accumulation in the body and hyperlipidemia [1, 2]. It has become dramatically prevailing over the past few decades [3]. It predisposes for several chronic diseases such as cardiovascular diseases [4], type 2 diabetes [5], hypertension [6] and cancer [7,8]. Abdominal obesity is one of the main predisposing factor for insulin resistance, type 2 diabetes mellitus and could be ended by death [9] Obesity adversely affect cardiac function and could result in severe cardiomyopathy [10]. This effect has been observed in several animal models of obesity including high fat diet (HFD)-fed rat [11]. Thus treating obesity is of great health importance.

As obesity is linked to various detrimental health problems, it is considered as a global epidemic [12,13]. It is essential to tackle this problem and find out strategies to reduce body weight and fat levels in these obese individuals. This can be executed through surgical, pharmacological or lifestyle interventions [14]. Pharmaceutical treatment of obesity may result in undesired side effects from the medicines [15]. Meanwhile, surgical approaches of obesity treatment could induce nutritional deficiency [16]. Because, the surgical and medicinal treatments of interventions and of obesity are often associated with many hazardous side effects, it is more worthy to think about the nutritional therapy which maybe the safest and most cost-effective way to control the body weight [17,18]. This coincides with an increased concern in complementary and alternative medicine [19]. Moreover, plant food supplements and dietary therapies for weight loss are among the most

common complementary and alternative medicine modalities [20]. Thus, there is a resurgence of interest in studying the natural sources with body weight lowering properties that having minimal side effects [21].

Among plant products that known to have anti-obesity activities is pineapple (*Ananas comosus*) extracts. Mature fruit of pineapple contains a proteolytic enzyme; bromelin, 14% sugar, and considerable amount of citric acid, malic acid, vitamin A, B and C [22]. Pineapple also contains good amount of potassium, calcium, Phosphorus and Chlorine, [22,23]. It has an antioxidant, anti-cancer and anti-inflammatory activities, [22,24]. Phytochemical analysis indicated that pineapple leave extract contains alkaloids, flavonoids, saponins and tannins [25]. The current study aimed to evaluate the effects of pineapple juice in reversing the obesity induced-cardiac dysfunction.

2. MATERIALS AND METHODS

2.1 Animals and Experimental Design

Eighteen adult male Wistar rats of 7 weeks old (140-150 g) were purchased from experimental animal center, Faculty of Pharmacy, King Saud University, Saudi Arabia. The animals were housed in the animal facility of the College of Science, Taif University, Saudi Arabia at 22°C and 55% humidity with 12 h-light/ dark cycle. All procedures were approved by the Animal Care Committee of Taif University.

2.2 Induction of Obesity

Rats were left for one week to attain acclimatization. Then, animals were divided into two main groups; a control group of six rats was

fed a standard pellet diet ad libitum and gained free access to water and high fat diet (HFD)-fed group of 12 rats that was fed high fat diet and gained free access to water for 10 weeks. The composition of HFD is indicated in Table 1 and was based on our previous study [26]. Obesity was confirmed by the increase in blood lipid parameters and body weight.

Table 1. Composition of HFD diet used to induce obesity

Ingredients	Food (g/kg)
Normal pellet diet (NPD)	365
Beef tallow	310
Casein	250
Cholesterol	10
Vitamins and Mineral Mix	60
D, L. Methionine	3
Yeast powder	1
Sodium chloride	1

2.3 Preparation of the Juice

Fresh Pineapple was purchased from local market in Taif City. 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.

2.4 Animal Treatment

High fat-fed rats were gained free access to HFD throughout the experimental period. After induction of obesity, they were subdivided into the following two groups each of six animals: Obese, non-treated group; gained free access to normal drinking water and HFD; Pineapple group, given Pineapple juice and HFD. The Pineapple juice was given in drinking water at a dose 15% (vol /vol). The dose of Pineapple juice was chosen based on our previous study on Pomegranate [26]. Water bottles were changed every other day. The treatment was continued for 5 weeks. Animals' body weight was recorded weekly from onset and throughout the experimental period.

2.5 Sampling

At the end of the experiment, animals were fasted for 10 h, anaesthetized using diethyl ether, and blood samples were collected from medial canthus of the eyes. Blood samples were allowed to clot, centrifuged and serum samples

were collected and stored at - 80°C until used for biochemical assays. Then rats were sacrificed by head decapitation. Immediately, heart tissue samples were collected, snap frozen in liquid nitrogen then stored at - 80°C until used for analysis of gene expression.

2.6 Biochemical Assays

Total cholesterol (TC) and triacylglycerol (TAG) levels of serum were measured spectrophotometrically using specific commercial kits (HUMAN Gesellschaft für Biochemica und Diagnosticam bH, Wiesbaden, Germany) following the manufacturer's instructions. Serum level of nitric oxide was assayed using available kit (Item No. #780022, Cayman Chemical, Michigan, USA) according to the manufacturer's instructions.

2.7 Analysis of Gene Expression

2.7.1 RNA extraction and cDNA synthesis

Total RNA was extracted from about 100 mg of each heart tissue sample using QIAzol lysis reagent (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions and as detailed previously [27]. The prepared RNA was electrophoresed to check its integrity. RNA concentration and purity were determined spectrophotometrically at 260 nm and 280 nm. The ratio of the OD_{260/280} of all RNA samples was 1.8-2. Four micrograms of the total RNA were reverse transcribed with oligo-dT primer and Moloney murine leukaemia virus Virus (M-MuLV) reverse transcriptase (GoScript™ Reverse Transcriptase Cat. # A5000; Promega Co, Madison, USA) according to the manufacturer's instructions. Briefly, 4 µg total RNA were mixed with 0.5 µg oligo-dT primer, the volume was completed to 5 µL nuclease-free water. The RNA-primer mix was incubated at 70°C for 5 minutes, immediately chilled in ice for 5 minutes. Then the reverse transcription reaction mix was prepared (on ice) consisting of 4 µl GoScript™ 5X Reaction Buffer, 4 µl 25 mM MgCl₂, 1 µl PCR Nucleotide Mix, 20 units recombinant RNasin® Ribonuclease Inhibitor, 1 µl GoScript™ Reverse Transcriptase. the volume was brought upto 15 µL using nuclease-free water. This mix was combined with the 5 µl of RNA and primer mix, incubated in the thermal cycler at 25°C for 5 minutes for annealing, at 42°C for 1h for extension (cDNA synthesis step) and at 70°C for 15 minutes for inactivation of the reverse

Table 2. Primer sequence, GenBank accession number (or reference), and PCR conditions used in this study

Gene, accession number/reference	Primer sequence (5`-3`)	Annealing (°C), cycles number	Product size
β -actin (V01217)	F-ATGTACGTAGCCATCCAGGC R-TCCACACAGAGTACTTGCGC	56 °C, 25 cycles	628 bP
Endothelin1 (NM_012548)	F-ATCATCTGGGTCAACACTC R-GAATCTCCTGGCTCTCTG	51 °C, 28 cycles	727 bP
TNF- α (HQ201305)	F-CGCTCTTCTGTCTACTGAAC R-AGGTACATGGGCTCATACCA	53 °C, 28 cycles	457 bP
NF- κ B	F-TACCATGCTGTTTTGGTTAC R-TCAAGCTACCAATGACTTTC	51 °C, 28 cycles	208 bP
AT1 receptor [28]	F-GCACACTGGCAATGTAATGC R-GTTGAACAGAACAAGTGACC	55 °C, 28 cycles	385 bp
eNOS [NM_021838]	F-CTGGCAAGACCGATTACACGA R-CGCAATGTGAGTCCGAAAATG	55 °C, 28 cycles	423 bP
iNOS [U03699]	F-TCTGTGCCTTTGCTCATGAC R-CATGGTGAACACGTTCTTGG	54 °C, 28 cycles	340 bP
ACE [U03708]	F-CACTGGAGCCTGATCTGACA R-AGGGTGCCACCAAGTCATAG	56 °C, 28 cycles	399 bP

transcriptase. The resultant cDNA was preserved at -20°C until used.

2.7.2 Semi-quantitative polymerase chain reaction (PCR)

The expression of some genes involved in regulation of cardiovascular functions was tested by semi-quantitative PCR using specific primers for these genes (Table 2). The tested genes were endothelin-1 (ET-1), tumor necrosis factor- α (TNF- α), nuclear factor NF- κ B (NF κ B), Angiotensin II receptor, type 1 (AT1 receptor), endothelial constitutive nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS) and angiotensin converting enzyme (ACE). In addition, as a reference gene, β -actin expression was also tested. The used primers were designed using Oligo-4 program and nucleotide sequences published in the Genbank (Table 2). Primers were synthesized by MacroGen (MacroGen Company, GAsa-dong, Geumcheon-gu, Korea). PCR was conducted in a final volume of 25 μ L using PCR GoTaq® Green Master Mix (Promega Corporation, Madison, WI) as previously described [26] with a modification of the annealing temperatures that corresponding to each primer (Table 2). The number of cycle was 25-28 cycles (Table 2). As a reference, expression of β -actin mRNA was tested using specific primers (Table 2). PCR products were electrophoresed on 1.5% agarose A (Bio Basic INC. KonradCres, Markham Ontario) gel, stained with ethidium bromide staining, visualized under UV light and photographed. The intensities of the

bands were quantified densitometrically using NIH image program (<http://rsb.info.nih.gov/nih-image>).

2.8 Statistical Analysis

Statistical analysis for the obtained results was performed using analysis of variance (ANOVA) and Scheffe's protected least significant difference test using SPSS software (SPSS version 13.0, IBM, Chicago, IL, USA) with $P = 0.05$ regarded as statistically significant. The results were expressed as means \pm standard errors of means (SEM).

3. RESULTS

3.1 Effect of Pineapple Juice on Body Weight and Lipid Profile

To induce obesity, animal were fed on high fat diet (HFD). There was a significant, clear difference between the control group fed on the normal diet and the HFD-fed group which had higher body weight than control as shown in Table 3. Moreover, body weight was significantly decreased in response to pineapple juice administration as presented in Table 3 where the body weight in the group received the juice was lower than that of obese, non-treated group and further it was reduced compared to before onset of juice treatment. In addition, serum total cholesterol (TC) and triacylglycerol (TAG) levels were higher in obese group compared to control. Both TC and TAG were reduced in response to juice treatment (Table 3).

Table 3. Body weight gain, lipid profile in control, Ob and Ob + pineapple juice rats groups

	Control	Ob	Ob+ Pineapple juice
Initial BW (g)	269.3±3.8	265.1±5.4	
Week10 BW (g)	362.8±6.4	404.1±2.7 [*]	
Final BW (after 5 weeks of juice treatment) (g)	355.2±9.1	419.25± 1.9 [*]	380.5±2.1 [†]
TAG (mg/dL)	55.1±3.1	96.6±6.2 [*]	50.2±2.9 [§]
TC (mg/dL)	55.4±2.5	65.6±2.4 [*]	58.3±1.4 [§]

Body weights, triacylglycerol (TAG), and total cholesterol (TC) in rats fed normal diet (control), high fat diet (Ob) and high fat diet in the presence of pineapple juice treatment (Ob+ Pineapple juice). Data presented are means± standard errors of means (SEM). ^{*}P= 0.05 Vs control group, [†]P= 0.05 Vs Ob group

3.2 Effect of Pineapple Juice Treatment on the Expression of Genes Related to Cardiovascular Function in Heart Tissue

Among the tested genes, the vasoconstrictor gene, endothelin-1(ET-1) was upregulated (1.7 fold increase) in obesity group. This upregulation was inhibited in juice-treated obesity group (Fig. 1).

In the same context with ET-1, obesity significantly induced mRNA expression of the inflammatory cytokine, tumor necrosis factor (TNF)- α (2.5 folds) and its downstream signaling molecule, and nuclear factor kappa- β (NF- κ β) (7 folds) as shown in Fig. 2 and Fig. 3 respectively.

These inductions were significantly abrogated in response to pineapple juice treatment. We also tested the effect of HFD on mRNA expression of Angiotensin II receptor, type 1 (AT1 receptor). AT1 receptor mediates the cardiovascular effects of angiotensin II including vasoconstriction, regulation of aldosterone synthesis and secretion, increased vasopressin secretion and thus controlling blood pressure. The obtained results showed that HFD significantly stimulated AT1 mRNA expression (4.3 folds) compared to control. This stimulation was significantly inhibited after administration of pineapple juice (Fig. 4).

Going in a harmony with these findings, feeding HFD significantly inhibited the mRNA expression of endothelial nitric oxide synthase (eNOS) to about 30% of its level in control group. This inhibition is significantly counteracted by pineapple juice treatment (Fig. 5A). The inhibition of eNOS expression in obesity group was associated with reduced production of the vasodilator molecule, nitric oxide (NO). Meanwhile, administration of pineapple juice significantly restored the NO production (Fig. 5 B).

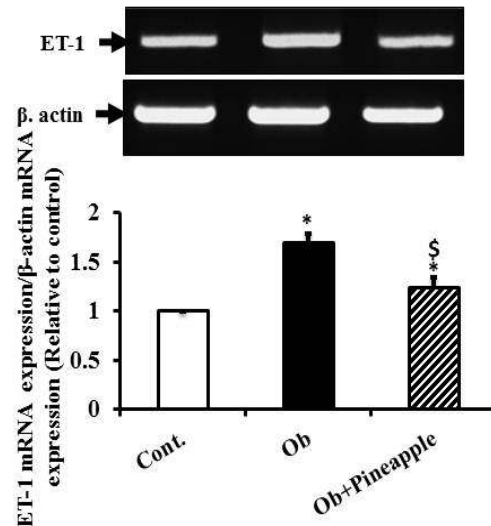


Fig. 1. Effect pineapple juice on Endothelin-1(ET-1) mRNA expression in cardiac tissue of rat

RT-PCR was performed as described in material and method section. Representative agarose gel photographs and results of densitometric analyses of at least five independent experiments are shown. Values are means ± SE. Cont, control group; Ob, obesity, non-treated group; Ob+ pineapple; obese group treated with pineapple juice. ^{*}P = 0.05 vs. Control group. [§]P < 0.05 vs. Obesity, non-treated group

In contrast to the constitutively expressed eNOS, the inducible form of NO synthase (iNOS) which produces large amounts of NO and known to be induced by cytokines such as TNF- α [29, 30], was induced in obesity group (2.8 folds) compared to control. This induction of iNOS was inhibited in the pineapple juice treated group (Fig. 6). Similarly, angiotensin-converting enzyme (ACE), the central component of the renin-angiotensin system, which has a hypertensive effect controlling blood pressure was upregulated in obesity group while this upregulation was reversed in response to pineapple juice treatment (Fig. 7).

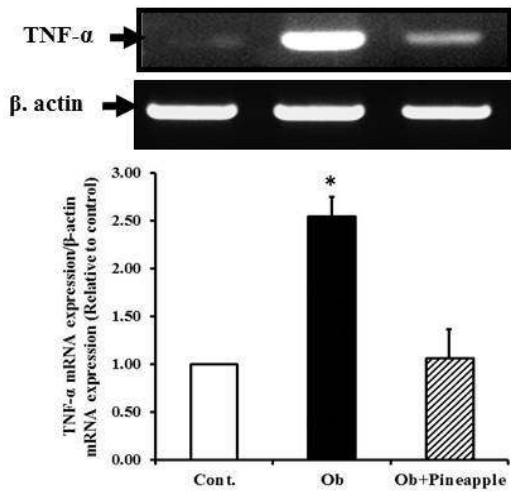


Fig. 2. Effect pineapple juice on tumor necrosis factor- α (TNF- α) mRNA expression in cardiac tissue of rat
 RT-PCR was performed as described in material and method section. Representative agarose gel photographs and results of densitometric analyses of at least five independent experiments are shown. Values are means \pm SE. Cont, control group; Ob, obesity, non-treated group; Ob+ pineapple; obese group treated with pineapple juice. * $P = 0.05$ vs. Control group

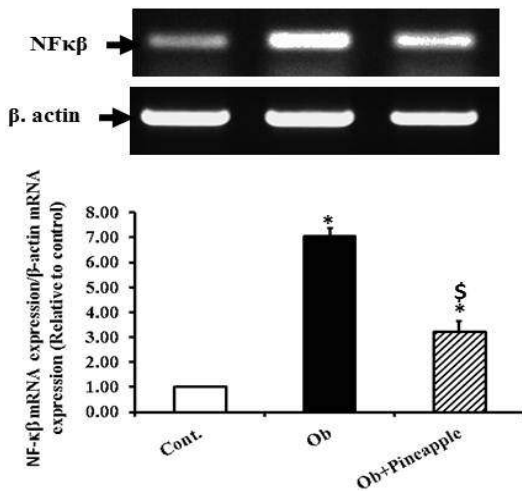


Fig. 3. Effect pineapple juice on nuclear factor kappa beta (NF- κ B) mRNA expression in cardiac tissue of rat
 RT-PCR was performed as described in material and method section. Representative agarose gel photographs and results of densitometric analyses of at least five independent experiments are shown. Values are means \pm SE. Cont, control group; Ob, obesity, non-treated group; Ob+ pineapple; obese group treated with pineapple juice. * $P = 0.05$ vs. Control group. ** $P = 0.05$ vs. Obesity, non-treated group

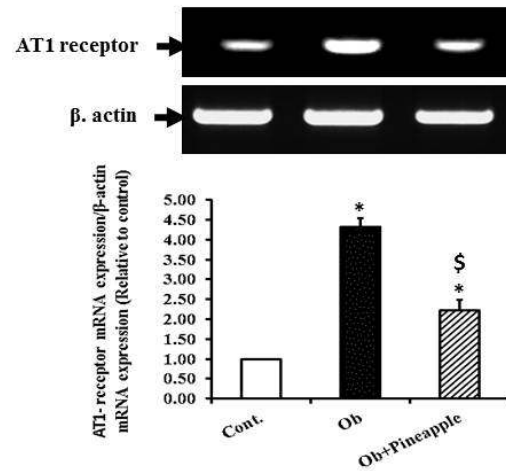


Fig. 4. Effect pineapple juice on: Angiotensin II receptor, type 1 (AT1 receptor) mRNA expression in cardiac tissue of rat
 RT-PCR was performed as described in material and method section. Representative agarose gel photographs and results of densitometric analyses of at least five independent experiments are shown. Values are means \pm SE. Cont, control group; Ob, obesity, non-treated group; Ob+ pineapple; obese group treated with pineapple juice. * $P = 0.05$ vs. Control group. ** $P = 0.05$ vs. Obesity, non-treated group

4. DISCUSSION

The prevalence of obesity is increasing globally. The treatment cost of obesity and its associated diseases is also increasing, and expected to reach 9% of the total global health expenses [31]. Thus, seeking alternative remedies for obesity has gained many interests. Plants and their extracts have become alternative intervention as treatment option for obesity. In the current study, the role of pineapple juice in treatment of obesity and its associated cardiovascular dysfunctions has been tested.

Endothelin-1 is known to be increased in endothelial dysfunction, was suggested as a marker of cardiovascular disorders [32]. Previous studies [33,34] reported that plasma endothelin-1 levels elevate in cases of advanced atherosclerosis and coronary artery disease progression. In the present study, HFD-induced obesity upregulated the mRNA expression of endothelin-1 and this upregulation was significantly inhibited by pineapple juice treatment. This effect was accompanied by correction of the HFD-induced dyslipidemia and significant reduction in body weight by pineapple juice treatment. In other words, pineapple juice

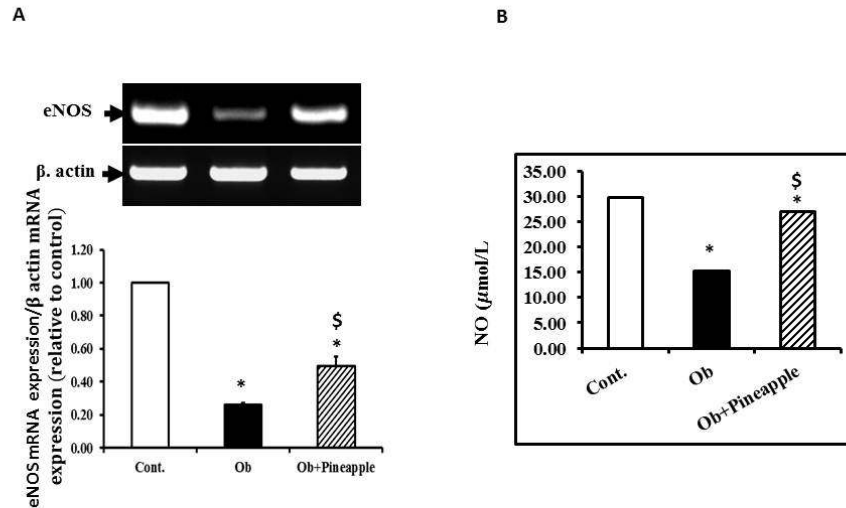


Fig. 5. A: Effect pineapple juice on endothelial constitutive nitric oxide synthase (eNOS) mRNA expression in cardiac tissue of rat. RT-PCR was performed as described in material and method section. Representative agarose gel photographs and results of densitometric analyses of at least five independent experiments are shown. Values are means \pm SE. Cont, control group; Ob, obesity, non-treated group; Ob+ pineapple; obese group treated with pineapple juice. B: Effect pineapple juice on serum level of nitric oxide (NO). Serum was prepared and level of NO was prepared as described in material and method section. $^i P = 0.05$ vs. Control group. $^s P = 0.05$ vs. Obesity, non-treated group

ameliorated cardiovascular dysfunction by downregulation of endothelin-1 (ET-1) in cardiac tissue, and dyslipidemia in HFD-fed rats. The results go in agreement with previous study by Kho et al. [35] on the red ginseng showed that diet -induced obesity stimulated endothelin-1 expression. As Obesity is characterized by abnormal, pathological expansion of the adipose tissue [36], which is usually undergo pathophysiological changes in obesity state [37]. These results in a state of chronic inflammation [38], which is also known as metaflammation [39]. This agrees to and could interpret at least in part the present findings of upregulation of TNF- α and its downstream signaling molecule; NF κ B in HFD-induced obesity, the effect that was counteracted by pineapple juice treatment. These results are consistent with the previously reported anti-inflammatory effect of pineapple [22]. Also the present results agree with those of Picchi et al. [40] who demonstrated that the endothelial dysfunction occurring in the metabolic syndrome results from effects of the inflammatory cytokine, TNF- α and subsequent production of Reactive oxygen species.

Angiotensin II receptor, type 1 (AT1 receptor) has vasopressor effects and regulates aldosterone secretion. Therefore, it plays an

important role in regulating controlling blood pressure. As obesity is a metabolic condition characterized by chronic activation of the renin-angiotensin system [41], the current study evaluated effect of HFD-induced obesity on the AT1 receptor and Angiotensin Converting Enzyme (ACE) mRNA expressions and ability of pineapple juice to counteract this effect. The present results showed upregulation of both AT1 receptor and ACE mRNA expressions and these upregulations were ameliorated by pineapple juice treatment. These results are consistent with the previous findings of Oliveira-Junior et al. [42] who showed that, HFD-induced obesity resulted in an increased activity of ACE, the effect that was reversed by blockade of AT1 receptor. Therefore, amelioration of the HFD-induced ACE mRNA expression by pineapple juice treatment could be due to its ability to inhibit AT1 receptor mRNA expression. Endothelial dysfunction could be due to either a decreased nitric oxide (NO) bioavailability or production, or both [43]. When angiotensin II is increased, bioavailability of NO may be decreased due to activation of NADPH oxidase, which favors reactive oxygen species production, thus increases NO scavenging [44]. Alterations in NO synthase (NOS) isoforms could result in reduced NO levels. Uncoupling of eNOS or upregulation of iNOS decreases NO level by

stimulating ROS formation and promoting oxidative stress, thereby increasing the risk of cardiac damage [45]. In addition, Linz et al. [46] stated that, angiotensin converting enzyme (ACE) inhibitors enhance eNOS expression and NO bioavailability suggests an interaction between RAS and oxidative stress. This could explain the findings of the present study where obesity was associated with an upregulation and a downregulation of iNOS and eNOS mRNA expressions respectively, as well as reduced NO level while pineapple juice treatment reversed these effects of HFD-induced obesity.

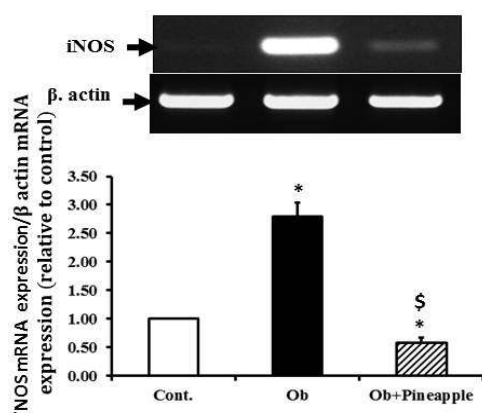


Fig. 6. Effect pineapple juice on inducible nitric oxide synthase (iNOS) mRNA expression in cardiac tissue of rat

RT-PCR was performed as described in material and method section. Representative agarose gel photographs and results of densitometric analyses of at least five independent experiments are shown. Values are means \pm SE. Cont, control group; Ob, obesity, non-treated group; Ob+ pineapple; obese group treated with pineapple juice. * $P = 0.05$ vs. Control group. [§] $P = 0.05$ vs. Obesity, non-treated group



Fig. 7. Effect pineapple juice on Angiotensin Converting Enzyme (ACE) mRNA expression in cardiac tissue of rat

RT-PCR was performed as described in material and method section. Representative agarose gel photographs of at least five independent experiments are shown. Cont, control group; Ob, obesity, non-treated group; Ob+ pineapple; obese group treated with pineapple juice. * $P = 0.05$ vs. Control group. [§] $P = 0.05$ vs. Obesity, non-treated group

5. CONCLUSION

To conclude, the present study clarified the ability pineapple juice to ameliorate HFD-induced obesity and its associated cardiovascular dysfunctions. It inhibited the obesity-induced upregulation of ET-1, TNF- α , NF κ B, AT1 receptor, iNOS and ACE mRNA expression, while it reversed the obesity-induced decrease of eNOS mRNA expression, and NO production. Thus, pineapple juice might be a candidate therapeutic approach for HFD-induced obesity and its associated cardiovascular dysfunctions.

ETHICAL APPROVAL

All procedures of this study were approved by the Institutional Animal Care and Use Committee of Taif University, Saudi Arabia.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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