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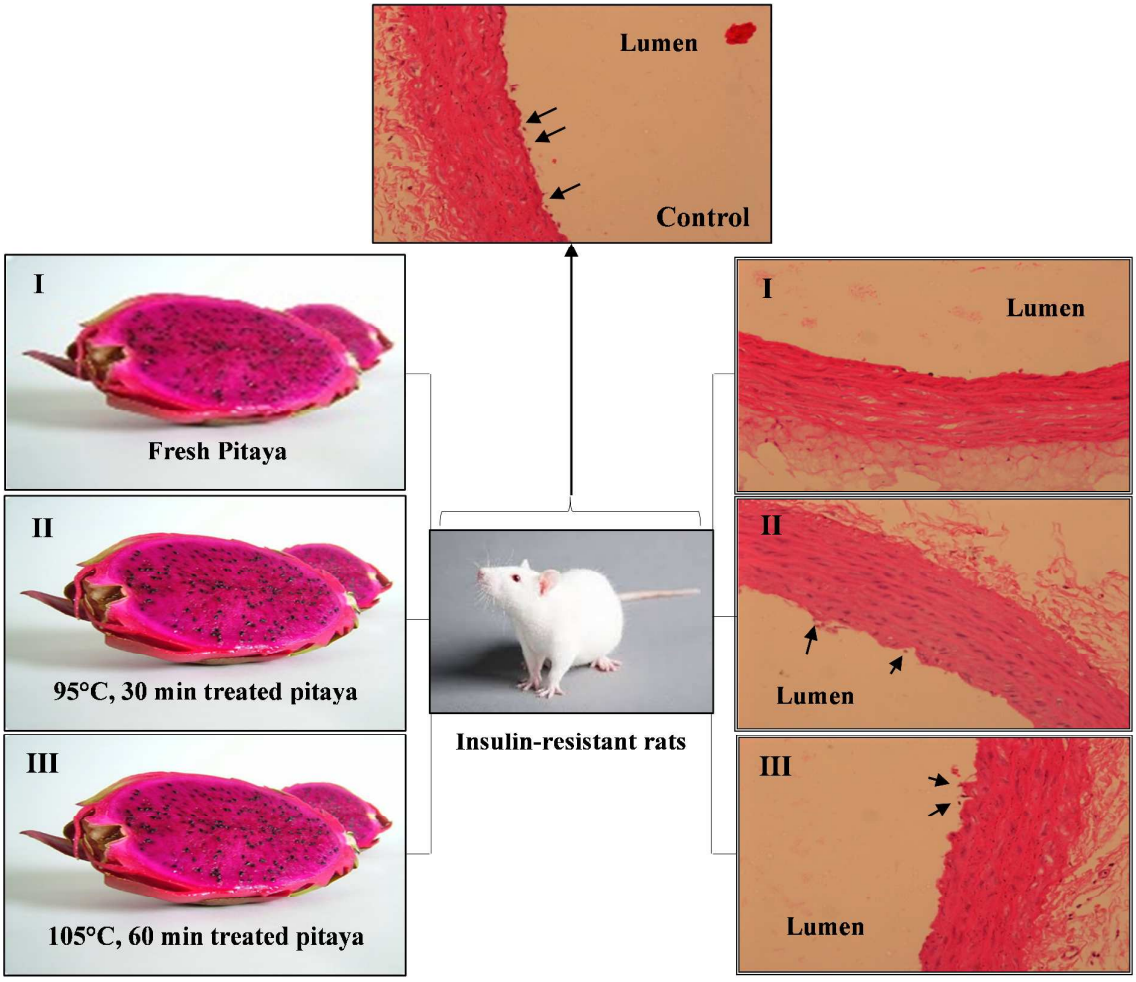

Anti-diabetic activity of red Pitaya (*Hylocereus polyrhizus*) fruit

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Abstract

This study investigated the anti-insulin resistant activity of red pitaya (*Hylocereus polyrhizus*) fruit. Fresh pitaya along with two heat-processed pitaya samples with different proportions of phenolic contents, scavenging activities, and soluble dietary fibers were tested in insulin resistant rats over 6 weeks of treatment in a cause-effect study to investigate the anti-insulin resistant components of red pitaya. The results showed that fresh pitaya significantly ($p < 0.05$) attenuated the insulin resistance, hypertriglyceridemia and atherosclerotic changes induced by fructose supplement in rats. Thermally-treated pitaya at 95°C for 30 min (containing low antioxidant content) significantly ($p < 0.05$) improved hyperinsulinemia. Thermally-treated pitaya at 105˚C for 60 min (contained low antioxidant and soluble dietary fiber content) feed to rats had no significant effect on insulin resistance, dyslipidemia and atherogenesis. Based on this study, the anti-insulin resistant effect of red pitaya can be attributed to its antioxidant and soluble dietary fiber contents.

Keywords: red pitaya, phenolic content, scavenging activity, soluble dietary fiber, insulin-resistant rat, atherosclerotic changes

Abbreviations: DPPH (2,2-Diphenyl-1-picryl-hydrazyl); FRAP (ferric reducing ability of plasma); GAE (gallic acid equivalents); GTT (glucose tolerance test); HFD (high fructose diet); IDF (insoluble dietary fiber); IR (insulin resistant); LDL-C (low density lipoprotein cholesterol); OGTT (oral glucose tolerance test); SA (scavenging activity); SDF (soluble dietary fiber); TC (total cholesterol); TDF (total dietary fiber); TG (triglyceride); TPC (total phenolic content); TPTZ (2,4,6-tri-pyridyl-s-triazine).

Introduction

Although the specific nature of the active constituents and the biologic mechanisms whereby fruits and vegetables exert their antidiabetic effects is not completely clear, antioxidants, fibers, and minerals have all been independently or jointly proposed as potential candidate antidiabetic compounds.[1] High amounts of biologically active compounds are found in tropical and subtropical fruits;[2] and some of these compounds have demonstrated anti-hyperglycemic effects in various model systems.[3] Red pitaya (*Hylocereus polyrhizus*) fruit is cultivated on a large scale in Malaysia. This tropical fruit is a rich source of nutrients as it contains substantial amount of polyphenols, betacyanins,[4] dietary fibers, ascorbic acid, vitamin A, vitamin E, and lycopene.[5]

Phenolic compounds are a large class of plant secondary metabolites consist of a large number of compounds ranging from the simple structure compounds such as phenolic acids to the more complex polyphenols such as flavonoids. These compounds possess several biological activities and have been reported to contribute to the health benefits associated to dietary consumption of fruits and vegetables.[6] The highest the flavonoid contents of red pitaya flesh were myricetin (7.23± 0.86 μg/100 g) and quercetin (6.81± 0.76 μg/100 g). Other flavonoids available at lower concentrations include: kaempferol (3.09 ±0.26 μg/ 100 g), apigenin (2.01± 0.18 μg/ 100 g), luteolin (1.06± 0.11 μg/ 100 g), and rutin (1.03± 0.09 μg/ 100 g).[7] Betacyanins, the pigments found in *Hylocereus* cacti, also contributed to the total phenolics of red pitaya, due to a phenol structure in the molecule. They were 10.3± 0.22 mg betanin equivalent per 100 g of red pitaya flesh.[4]

We have previously showed that red pitaya consumption attenuated the insulin resistance and dyslipidemia caused by high fructose diet (HFD) in rats.[8] While the consumption of fresh pitaya is the ideal option, several processed products such as juice, powder, jam and jelly are commercially available to utilize the large scale production of the fruit and to encourage to

the consumption of fruit-based products.[9] The nutritional value of red pitaya, like other fruits, can be affected by thermal processing methods used to prepare and preserve the products. Previous observations in our laboratory showed that heating of red pitaya (95ºC for 30 min or at 105ºC for 60 min) significantly reduced antioxidant activities (radical scavenging activity and total antioxidant activity) and decreased the total phenolic content (TPC) compared to fresh fruits. On the other hand, drum drying and spray drying were the best methods for the preservation of the antioxidant activity of the fruit.[9]

A functionality-guided approach was used in a subsequent study to identify the best thermal processing options that preserve the biologically active compounds available in the fruit. The results from that study identified that polyphenols were the compounds associated to the cardioprotective activity of red pitaya in rats and that soluble dietary fiber of red pitaya could not independently reduce the serum cholesterol level in rats.[10]

We supposed that the separate fractions and isolated nutrients of red pitaya cannot completely show their anti-insulin resistant effects. So, the study was designed to identify the contribution of total antioxidant activity and fibers of this fruit to its health benefits. The objective of this study was to investigate the involvement of the total antioxidant activity and fibers of red pitaya in the observed anti-insulin resistant. To achieve this goal, a cause-effect study was designed to manipulate the phenolic contents and the antioxidant activity of red pitaya through thermal treatment and examine the fruits in a model in which insulin resistance was induced to rats by feeding high fructose diet (HFD), which subsequently cause the rats to develop hyperinsulinemia and dyslipidemia. Consumption of high levels of fructose in humans and animals leads to insulin resistance, obesity, hypertension and lipid abnormalities.[11] The exposure of the liver to high amount of fructose leads to rapid stimulation of lipogenesis and triglyceride accumulation, which in turn contributes to reduce insulin sensitivity and hepatic insulin resistance and glucose intolerance.[12] Our hypothesis

was that polyphenols and their antioxidant activity contribute to the overall anti-insulin resistant effect of the red pitaya by reducing hyperinsulinemia and dyslipidemia in insulin-resistant (IR) rats. To our knowledge, this is the first study to assess the anti-insulin resistant components of red pitaya.

Materials and methods

Chemicals

Folin-Ciocalteu reagent, sodium acetate trihydrate ($C_2H_3NaO_2.3H_2O$) and ascorbic acid were obtained from Merck Co. (Darmstadt, Germany). Sodium carbonate and fructose of analytic grade came from Fisher Scientific (Leicestershire, UK). Ferrous sulfate ($FeSO_4.\ 6H_2O$) was purchased from BDH Chemicals (USA). Gallic acid, DPPH (2,2-Diphenyl-1-picryl-hydrazyl), TPTZ (2,4,6-tri-pyridyl-s-triazine), ferric chloride ($FeCl_3\ .\ 6H_2O$), heat-stable α-amylase solution, protease, amyloglucosidase, 2-(N-Morpholino) ethanesulfonic acid (MES), and Tris (hydroxymethyl) aminomethane were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Metformin came from Hovid Bhd, Malaysia.

Preparation of red pitaya

Red pitaya (*Hylocereus polyrhizus*) was obtained from Chekap Harvest Sdn. Bhd, Ayer Keroh Melaka, Malaysia. Twenty kilograms of fruits was washed under running tap water, skinned, chopped into small pieces, completely blended and then separated into three portions. The first portion was stored at -20°C as the fresh sample. Two other portions were separately thermally treated as follows; one portion was heated in an oven at 95°C for 30 min, and the other was heated at 105°C for 60 min. All the portions of pitaya (fresh and thermally-treated) were bottled and stored in the dark at -20°C.

Preparation of extract

Fifty grams of fruit was blended with 200 grams of chilled 80% acetone solution in a blender for 10 min. The slurry was filtered through Whatman No. 1 filter paper in a Buchner funnel under vacuum. The solids were scraped into 150 grams of 80% acetone and homogenized again for 3 min before filtration. The combined mixture was evaporated using a rotatory evaporator (BÜCHI Rotavapor R-200, Germany) at 45°C to reach less than 10% of the initial volume. The extract was made up to 50 mL with distilled water and frozen at -80 °C until analysis.[13]

Determination of total phenolic content (TPC) of extracts

Firstly, 125 µL of extract was added to 500 µL of Folin-Ciocalteu reagent in the cuvette. After vortex for 15 seconds, it was allowed to stand for 6 min at 20°C. Then, 1250 µL of 7% sodium carbonate solution was added to the cuvette, and the mixture was diluted to 3 mL with 1125 µL deionized water. After 90 min, absorbance was measured using a UV-Vis spectrophotometer (SECOMAN, France) at 760 nm. A standard curve was constructed using serial dilution of 1 mg gallic acid and the results of the extracts were expressed as mg gallic acid equivalents (GAE) per gram of dried extract.[13]

Determination of scavenging activity (SA) of extracts

Briefly, 100 µL of extract or ascorbic acid was added to 3900 µL of 80% ethanolic solution of 0.6 mM DPPH in the cuvette, and vortexed for 15 seconds. The absorbance was read at 515 nm after 180 min. The reaction time for vitamin C was less than 1 min. Ethanol (80%) was employed as the blank, and DPPH solution without test samples (3.9 mL of DPPH + 0.1 mL of 80% ethanol) was used as the control.[14] The results of SA were expressed as µM ascorbic acid equivalent per gram dried extract.

145 **Dietary fiber analysis of extracts**

146 Using gravimetrical approach,[15] the duplicate test portions of dried pitaya (fresh and
147 thermally-treated) were gelatinized with heat-stable α-amylase and then enzymatically
148 digested with protease and amyloglucosidase to remove protein and starch. Then, the enzyme
149 digestate was filtered using Fibertec system, and residue (insoluble dietary fiber, IDF) was
150 washed with warm water, dried and weighed. For soluble dietary fiber (SDF) determination,
151 combined filtrates and washes were precipitated with ethanol. Afterwards, they were filtered,
152 dried and weighed. Finally, IDF and SDF values were corrected for protein, ash, and blank.
153 Total dietary fiber (TDF) was calculated from summation of IDF and SDF. The results were
154 expressed as g/100 g of flesh weight.

155

156 **Animals and diet**

157 Thirty-six 8-week-old male Sprague-Dawley rats (190 ± 8 g) were obtained from Institute for
158 Medical Research (IMR), Kuala Lumpur, and kept two per cage in polycarbonate cages with
159 stainless steel covers in an air-conditioned room at 25 ± 2°C with lighting from 7 a.m. to 7
160 p.m. Rats were allowed free access to normal rat chow and tap water. The experimental study
161 was approved by the Animal Care and Use Committee (ACUC) of the Faculty of Medicine
162 and Health Sciences, Universiti Putra Malaysia. The normal rat chow (380 kcal per 100
163 grams of diet) consisted of 72 g of carbohydrate, 14 g of protein, 4 g of fat, 5 g of fiber, 5 g of
164 mixture of minerals and vitamins.

165

166 **Experimental design**

167 After a week of adaptation in which the rats (n = 36) were fed with standard basal diet, the
168 rats were randomly separated into two groups. First group (n = 6) was feed the basal diet and
169 water ad libitum throughout the study period as normal control (CON). The second group (n

= 30) was provided with basal diet plus fructose in drinking water at a concentration of 10%, as HFD.[16] After five weeks of induction of insulin resistance by HFD (indicated by the hyperinsulinemia, insulin/glucose ratio increase, glucose intolerance, and hypertriglyceridemia), this group was equally and randomly divided into five subgroups (n = 6 for each group) as follows; 1) IR rats + HFD (FRU); 2) IR rats + HFD + 10% daily energy intake fresh pitaya (FRU+Pit); 3) IR rats + HFD + 10% daily energy intake thermally-treated pitaya at 95˚C for 30 min (FRU+Pit95); 4) IR rats + HFD + 10% daily energy intake thermally-treated pitaya at 105˚C for 60 min (FRU+Pit105); and 5) IR rats + HFD + 200 mg of metformin (FRU+Met). Metformin was given in the last group as the standard treatment of insulin resistance, the main abnormality of type 2 diabetes.[17] Food consumption, water consumption, and energy intake were recorded every 2 days. The weight and length of the rats were measured and recorded every week. Due to the rapid changes in weight, the required supplementations of fruit were adjusted weekly. Food and water were replenished at 10 a.m. every day. Pitaya and metformin were fed by nasogastric (NG) tube. The rats underwent blood sampling four times during the study; at baseline, the end of the induction period (5^{th} week), mid treatment (8^{th} week), and at the end of the experiment (11^{th} week).

Biochemical parameters

After 12 hours of fasting, rats were anaesthetized with diethyl ether, and 3 mL of blood was collected from intracardiac cavity and placed into two plain collecting tubes. Then, the samples were centrifuged (Rotofix 32, Hettich Zentrifugen, Germany) for 10 min at 3000 rpm at 4˚C, and the sera were collected. Serum lipid and glucose levels were measured using the Chemistry Analyser (Hitachi 902 Automatic Analyser, Japan).

Insulin enzyme immunoassay

Serum insulin levels were measured by radioimmunoassay using a commercial kit (Mercodia rat insulin ELISA kit, Sweden). Briefly, 50 µL of enzyme conjugate solution was added to 25 µL of calibrators or serum samples. After 2 hours of incubation on a shaker, 350 µL of wash buffer was added to each well, and then aspirated completely which was repeated 5 times. After final wash, the plate was inverted and tapped against absorbent paper. Then, 200 µL of tetramethylbenzidine (TMB) substrate was added to each well, incubated for 15 min and the reaction was stopped with the addition of 0.5 M H_2SO_4. After 5 seconds of shaking, the absorbance of the plate was measured at 450 nm. The values were quantified using linear regression curve of the calibrators.

Glucose tolerance test (GTT)

Oral glucose tolerance test (OGTT) was performed twice during the experiment; at the end of insulin resistance induction (5^{th} week) and at the end of the experiment (11^{th} week). The rats were fasted for 12 hours prior to OGTT. A fasting blood sample was taken by cutting the tail tip. Glucose solution (2 g/mL/kg body weight) was fed orally. Blood samples were taken and collected into plain collecting tubes separately at 30, 60, 90, and 120 min.[18] The samples were centrifuged for 10 min at 3000 rpm at 4˚C, and the sera were collected. Serum glucose responses to oral glucose load were measured and expressed in terms of the area under the curve (AUC) from 0 to 120 min postload blood sampling, using the trapezoidal rule.[19]

Determination of antioxidant status

Using ferric reducing ability of plasma (FRAP) assay,[20] 300 mM acetate buffer (pH 3.6), 10 mM TPTZ and 20 mM $FeCl_3$ were mixed together in the ratio of 10:1:1 (v/v/v), respectively, to give the working FRAP reagent. To the 1.5 mL of freshly prepared FRAP reagent, 50 µL

of serum samples was added along with 150 µL of distilled water. The absorbance of tripyridyltriazine complex formed with the reduced ferrous ions was measured spectrophotometrically at 593 nm. Values were quantified using the linear regression curve of Fe^{2+} standards.

Histological evaluation

At sacrifice the abdominal aortas were dissected and stored in 10% fixative of formaldehyde. The thickness of the post-mortem aortic sections were 3-4 mm (n = 4, per aorta). The tissues were prepared for histology by a series of dehydration and clearing processes in the automatic histoprocessor (Leica, Germany) for 22 hours. After embedding in paraffin, a serial 3 µm-thick section of tissues were obtained from the paraffin blocks using steel knifes on a microtome (Jung Multicut, Germany) in the tissue embedding centre (Reichert-Jung, Germany) and placed on glass slides for further staining. Hematoxylin-eosin (HE) staining was performed on every section. Briefly, after deparaffinization and rehydration of sections by xylene, different concentrations of ethanol, and water, the slides were submerged into hematoxylin. The tissues then were stained by eosin, dehydrated, and dipped into xylene. Finally, they were mounted with coverslip using dissolved polystyrene in xylene (DPX) mounting. Histological sections were analysed and photographed on an Olympus microscope (Olympus, U-CMAD3, Japan) by Soft Imaging System (analySIS LS). The thickness of the intima-media, and also the integrity of the endothelial lining around the aortic intima were determined from hematoxylin-eosin stained thin sections (3 µm, n = 4 per section).

Statistical analysis

All the data were normally distributed and expressed as mean ± SEM. The data was analyzed using repeated measurements analysis and the results from the various analyses were

subjected to Analysis of variances (ANOVA). Significant differences among mean values were determined by Tukey's test and defined at $p < 0.05$. The statistical analyses were conducted using the SPSS software (SPSS 15.0 for Windows, Chicago, Illinois).

Results

Effect of thermal treatment of red pitaya on total phenolics, radical scavenging activity and fiber content

TPCs of fresh and thermally-treated pitayas are shown in Fig. 1a. TPC of fresh pitaya was 21.41 ± 0.15 mg GAE per gram of dried extract. The concentration of TPC was significantly decreased by heating ($p < 0.05$). The highest loss of TPC was seen in pitaya heated at 105°C for 60 min where a reduction of 8.49 ± 0.43 mg GAE per gram was found. The reduction of TPC in pitaya heated at 95°C for 30 min was 7.98 ± 0.37 mg GAE per gram.

Fig. 1b shows the scavenging activity (SA) of fresh and thermally-treated pitaya extracts. Fresh pitaya, with 226.51 ± 3.99 μM vitamin C equivalents per gram dried material had the highest SA and thermal processing caused significant decrease in the SA of treated pitayas ($p < 0.05$). Thermally-treated pitaya at 105°C for 60 min had the highest reduction (151.39 ± 4.15 μM vitamin C equivalents per gram dried material) of SA. The reduction of SA of thermally-treated pitaya at 95°C for 30 min was 135.15 ± 3.15 μM vitamin C equivalents per gram dried material.

Fig. 1c shows the values of the TDF, IDF and SDF contents of fresh and thermally-treated pitayas. The TDF, IDF, and SDF of fresh red pitaya were 3.13 ± 0.01, 2.18 ± 0.02 and 0.95 ± 0.02 g/100 g of flesh (edible portion), respectively. The TDF and SDF contents were decreased by heating ($p < 0.05$). There was no significant difference in TDF among red pitaya samples. The concentration of SDF was significantly decreased in the thermally-treated pitaya at 105°C for 60 min with a decrease of 0.55 ± 0.02 g/100 g (58%, $p < 0.05$).

IDF of red pitaya behaved quite differently under thermal stress. IDF was intact after 30 minutes of heating at 95°C, and significantly increased in pitaya heated at 105°C for 60 min ($p < 0.05$), compared to fresh pitaya sample. The results above provide a platform for the generation of three dried red pitaya samples with different levels of antioxidants and dietary fibers. A summary of the level of change and differences among the 3 samples of dried pitaya is shown in Table 1. These samples were tested in the animal model study using IR rats to investigate the contribution of TPC and fiber content on the anti-insulin resistant of red pitaya.

The effects of pitaya supplements on insulin-resistant rats

There was no significant difference in the body weight among groups at the end of the experiment. Food, water, and energy intake of different groups after insulin resistance induction (initial), and after treatment (final) were shown in Table 2. Due to differences in the weight of rats in the various groups, pitaya, food, water and energy intake were adjusted by weight of rat (kg) per day. The ranges of food consumption of normal and IR groups were 62-63 and 37- 42 g/kg/day, respectively. Also, the ranges of energy intake by food of normal and IR groups were 236-239 and 141-160 kcal/kg/day, respectively. Induction of insulin resistance resulted in significant decrease in food intake and energy intake by food ($p < 0.05$), compared to the normal rats. Conversely, IR rats consumed significantly higher amount of high fructose water ($p < 0.05$); and therefore, their total energy intake was comparable with normal rats. The results showed that the FRU group exhibited significant increase ($p < 0.05$) in water consumption, and total energy intake in the treatment period; whereas no significant differences in these parameters were found in the pitaya and metformin treated groups.

The effect of different treatments on serum insulin, glucose, insulin/glucose ratio, and glucose tolerance test in rats was shown in Table 2. The initial values of insulin, glucose,

insulin/glucose ratio, and glucose tolerance test were significantly higher in IR rats than CON group ($p < 0.05$). Treatments with pitaya and metformin decreased serum insulin, insulin/glucose ratio, and glucose tolerance test, significantly ($p < 0.05$); with the exception of FRU+Pit105 that the reductions were not significant. Furthermore, significant decreases in serum glucose level were noted in the FRU+Pit, and FRU+Pit95 as compared to the initial values ($p < 0.05$).

The effect of pitaya extracts on lipid profiles of rats was shown in Table 2. The initial value of triglyceride (TG) was significantly higher in IR rats than normal control ($p < 0.05$). There was a significant reduction in serum TG level in the FRU+Pit, and FRU+Met groups compared to the initial values ($p < 0.05$). The serum level of total cholesterol (TC) was significantly decreased in the FRU+Pit, and FRU+Met groups ($p < 0.05$) at the end of experiment, whereas the low density lipoprotein cholesterol (LDL-C) was only attenuated by metformin intake, significantly ($p < 0.05$).

Fig. 2 shows the effect of different treatments on serum antioxidant power in rats. Fresh pitaya and metformin treatments had higher serum total antioxidant power ($p < 0.05$) compared to the remaining treatments. Changes of the antioxidant power of sera were not significant in the other pitaya-treated groups.

Table 3 shows the thickness of intima-media of the abdominal aorta of rats in different groups. The minimum of the intima-media thickness of the abdominal aorta was seen in the FRU+Pit group with 93.65 ± 4.22 μm; whereas the maximum thickness (128.16 ± 2.94 μm) was in the FRU group. The intima-media thickness was significantly higher in the FRU group as compared to the CON group ($p < 0.05$). Also, the thickness was significantly lower in the FRU+Pit and FRU+Met as compared to the FRU group ($p < 0.05$). The mean of intima-media thickness of the abdominal aorta in the groups were in the order of FRU+Pit < FRU+Met < CON < FRU+Pit95 < FRU+Pit105 < FRU.

Fig. 3 demonstrates histological sections of abdominal aortic segments from rats. It shows an endothelial denudation and cell detachment of the abdominal aorta of rats in the FRU (b), FRU+Pit95 (d), FRU+Pit105 (e), and FRU+Met (f) groups. The endothelial structure in the CON group (a) appears continuous and intact. Also, the FRU+Pit group (c) revealed an intact endothelial structure and similar to the CON group.

Discussion

This study examined the hypothesis that the differences in the TPC, fiber content and the antioxidant activity of red pitaya fruit in three samples (fresh pitaya: high in TPC, SA, SDF; thermally-treated pitaya at 95˚C for 30 min: high in SDF; thermally-treated pitaya at 105˚C for 60 min: low in all the measured bioactive compounds) can affect insulin-resistant rats differently. By examining the changes in serum insulin, glucose, glucose tolerance, and lipid profiles in different experimental groups, we could elucidate the role of a single or the combination of anti-insulin resistant compounds presented in red pitaya.

The development of insulin resistance in high fructose-fed rats was indicated from the hyperinsulinemia, insulin/glucose ratio increase, glucose intolerance, and hypertriglyceridemia. Fructose feeding increased the insulin level of serum (73-300%, $p < 0.05$), glucose (17-25%, $p < 0.05$) and insulin/glucose ratio (66-260%, $p < 0.05$). Also, fructose feeding resulted in significant glucose intolerance and hypertriglyceridemia ($p < 0.05$) among IR models (data were not shown). These biochemical changes in high fructose-fed rats were reported in previous observations.[18,19,21] All these variables were remained unchanged in the CON group. In the present study, HFD increased the serum levels of TC, and LDL-C to 2-49 and 11-37%, respectively, but this increase was not significant (data were not shown). Huang et al.[18] reported significant increases in TC and LDL-C in sera of high fructose-fed rats; whereas, the present study was in agreement with the result of

Anithanandhini et al.,[21] who observed no significant alterations in TC and LDL-C in experimental rats. With the continuation of HFD, the FRU group increased its total energy intake by high fructose water instead of basal diet consumption. Hexosamine hypothesis explains how chronic fructose overfeeding can result in higher intake and storage of energy. With overexpression of glutamine: fructose-6-phosphate amidotransferase, the liver produces excess fatty acids, skeletal muscles then become IR, and result in hyperinsulinemia. This pathway of excess hexosamine flux causes long-term storage of energy, and eventually obesity and type 2 diabetes.[22]

In the current study, the histological examination of abdominal aorta of the insulin resistant rats revealed intima-media thickening; and the maximum thickness was observed in the FRU group. This thickening may be due to increase in collagen content of this wall.[23] Arterial medial proliferation is one of the major problems in diabetic patients. This thickening is mediated by multiple causes such as hyperglycemia and/or hyperinsulinemia in diabetes mellitus. This is the presentation of atherosclerosis formation in the NIDDM animal models.[24] It is consistent with the results of the present experiment that both maximum level of serum insulin and thickness of the intima-media of abdominal aorta were seen in the FRU group. Also, endothelial denudation and cell detachment of the abdominal aorta were observed in rats of the FRU group. These endothelial dysfunctions and oxidative stress were also reported in the insulin resistant Wistar rats fed a fructose-enriched diet.[25]

It is well known that lowering endogenous insulin level is a key step to successful therapy of insulin resistance-related diseases.[26] Fresh pitaya significantly ($p < 0.05$) improved the serum level of insulin, glucose, insulin/glucose ratio, glucose intolerance, TG, and TC. The intima-media thickness of the abdominal aorta was significantly lower in fresh pitaya-fed rats than the FRU group ($p < 0.05$), and there were no noticeable changes seen on their endothelial layer. This indicated the influence of fresh pitaya supplementation to improve

368 atherosclerotic changes. These health benefits can be associated with high content of
369 antioxidants, dietary fibers and micronutrients of red pitaya. The significant increase in the
370 serum total antioxidant power in the FRU+Pit group during the treatment period underpinned
371 the importance of antioxidant role to reverse the insulin resistance and its side effects.

372 The thermally-treated pitaya at 95˚C for 30 min that contained high level of SDF
373 attenuated the serum insulin, insulin/glucose ratio, glucose, and glucose intolerance,
374 significantly ($p < 0.05$). The results were in agreement with the findings of some animal
375 experiments[27] and intervention studies[28] that reported the improvement of glucose tolerance,
376 plasma glucose, and insulin response in insulin resistance and type 2 diabetes by high dietary
377 fiber intake. However, the supplement of pitaya, which had significant low antioxidant
378 content, had no obvious effect on dyslipidemia and atherosclerotic changes. The intima-
379 media thickness of the abdominal aorta in this group of rats was comparable with the FRU
380 group, and there was evidence of endothelial denudation and cell detachment in their
381 endothelial layer.

382 It can be assumed that the lack of significant effect of thermally-treated pitaya at 95˚C for
383 30 min on dyslipidemia and atherosclerotic changes was partly related to low antioxidant
384 content of this supplement. This product could not significantly increase the antioxidant
385 power of serum in the FRU+Pit95 group as well. The most probable reason for its low
386 antioxidant activity is the thermal degradation of its betacyanin and flavonoids. Larrauri et al.
387 observed 50% reduction in the betacyanin content of red pitaya when it exposed to 90˚C
388 within 22.6 minutes; and incubation at that temperature for 60 minutes caused 90% loss of
389 betacyanin content in beetroot.[29] Boiling, frying, and cooking can result in the loss of
390 flavonoids in foods.[30] For instance, frying and boiling resulted in significant losses in
391 kaempferol in broccoli, 45% and 85%, respectively. Similarly, the quercetin content of
392 broccoli dramatically decreased after frying and boiling treatments leading to the loss of 72%

and 88% respectively of the initial content in fresh broccoli.[31] Since oxidative stress and a deficient antioxidant system have been shown in the pathology of fructose feeding insulin resistance, the availability of sufficient antioxidant appears necessary to reverse the side effects of insulin resistance such as elevation of cholesterol and TG levels in rats,[32] endothelial dysfunction and development of vascular damage in diabetes mellitus.[33]

The impact of thermally-treated pitaya at 105˚C for 60 min, having low TPC, SA, and SDF, was not significant on serum insulin level, insulin/glucose ratio, lipid parameters, glucose profile, and glucose intolerance. Also, the intima-media thickness of the abdominal aorta in this group was comparable with the FRU group, and endothelial denudation and cell detachment in their endothelial layer were observed. The results supported the contention that the anti-insulin resistant components of red pitaya on IR rats were antioxidants and SDF.

The major limitation of this study was that we did not measure some other biologically active compounds of this fruit. Moreover, the specific antioxidant and/or SDF that exerted antidiabetic benefits did not become clear in the present study. Further studies will be looking at the profile of the phenolics to identify the major phenolics involved in the observed effects, which will support extension of the studies to human trials.

Conclusions

Results from the study supported the hypothesis that polyphenols, antioxidant content, and soluble dietary fiber may be involved in the anti-insulin resistant activity of red pitaya. Moreover, the findings showed that the antioxidant content of red pitaya is essential to improve dyslipidemia and atherogenesis in insulin resistant rats, and the soluble dietary fiber of red pitaya alone could not independently reverse the side effects of hyperinsulinemia in this experimental model. Thermal treatment can affect the bioactivity of tropical fruits and the processing regime need to be established in relation to function. It is concluded that to

process the tropical fruits such as red pitaya, choosing the appropriate methods of processing is extremely important in order to maximize the preservation of biologically active compounds and maximize the health benefits to the consumers of processed products.

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References

1 H. C. Hung, K. J. Joshipura, R. Jiang, F. B. Hu, D. Hunter, S. A. Smith-Warner, G. A. Colditz, B. Rosner, D. Spiegelman and W. C. Willett, *J. Natl. Cancer Institute*, 2004, **96**, 1577-1584.

2 S. Gorinstein, E. Bartnikowska, G. Kulasek, M. Zemser and S. Trakhtenberg, *J. Nutr*., 1998, **128**, 2023-2027.

3 H. M. Mukhtar, S. H. Ansari, Z. A. Bhat, T. Naved and P. Singh, *Pharmazie*, 2006, **61**, 725-727.

4 L. C. Wu, H. W. Hsu, Y. C. Chen, C. C. Chiu, Y. I. Lin and J. A. Ho, *Food Chem*., 2006, **95**, 319-327.

5 K. R. Mohd Adzim, A. H. Norhayati, M. Y. Rokiah, R. Asmah, M. T. Mohd Nasir and M. Siti Muskinah, *J. Trop. Agric. Food Sc.*, 2006, **34**, 269-275.

6 V. Cheynier, *Phytochem. Rev.*, 2012, **11**, 153-177.

7 Rohin, M, Master Thesis, 2006, University Putra Malaysia, Malaysia.

8 Omidizadeh, A, Master Thesis, 2009, University Putra Malaysia, Malaysia.

9 A. Omidizadeh, M. Y. Rokiah, I. Amin, S. Roohinejad, L. Nateghi and A. B. Mohd Zuki, *Adv. J. Food Sc. Tech.*, 2011, **3**, 203-210.

10 A. Omidizadeh, M. Y. Rokiah, I. Amin, S. Roohinejad, L. Nateghi and A. B. Mohd Zuki, *J. Food Agric. Environ*., 2011, **9**, 152-156.

11 H. Basciano, L. Federico and K. Adeli, *Nutr. & Metab*., 2005, **2**, 5.

12 M. C. Moore, A. D. Cherrington, S. L. Mann and S. N. Davis, *J. Clin. Endocrinol. Metab*., 2000, **85**, 4515-4519.

13 K. Wolfe, X. Wu and R. H. Liu, *J. Agric. Food Chem*., 2003, **51**, 609-614.

14 Y. Cai, M. Sun and H. Corke, *J. Agric. Food Chem*., 2003, **51**, 2288-2294.

15 AOAC, Official Methods of Analysis, 2000, Total, soluble, and insoluble dietary fiber in foods: enzymatic-gravimetric method, MES-Tris buffer-final action: Method 991.43, Washington, DC.

16 T. Dimo, S. V. Rakotonirina, P. V. Tan, J. Azay, E. Dongo and G. Cros, *J. Ethnopharmacol*., 2002, **83**, 183-191.

17 P. Faure, D. Barclay, M. Joyeux-Faure and S. Halimi, *J. Trace Elem. Med. Bio*., 2007, **21**, 113-119.

18 Y. J. Huang, V. S. Fang, C. C. Juan, Y. C. Chou, C. E. Kwok and L. T. Ho, *Metabolism*, 1997, **46**, 1252-1258.

19 A. Psyrogiannis, V. Kyriazopoulou, A. Symeonidis, M. Leotsinidis and A. G. Vagenakis, *Hormones (Athens)*, 2003, **2**, 161-168.

20 I. F. F. Benzie and J. J. Strain, *Anal. Biochem*., 1996, **239**, 70-76.

21 A. T. Anithanandhini, S. D. Balakrishnan and C. V. Anuradha, *Nutr. Res.*, 2002, **22**, 343-354.

22 D. A. McClain, J. *Diabetes Complications*, 2002, **16**, 72-80.

23 T. Noma, K. Mizushige, L. Yao, Y. Yu, H. Kiyomoto, N. Hosomi, S. Kimura, Y. Abe, K. Ohmori and H. Matsuo, *Jpn Circ J*, 1999, **63**, 988-993.

24 N. Hosomi, T. Noma, H. Ohyama, T. Takahashi and M. Kohno, *Atherosclerosis*, 2002, **162**, 69-76.

25 D. Behr-Roussel, A. Oudot, S. Caisey, O. L. E. Coz, D. Gorny, J. Bernabé, C. Wayman, L. Alexandre and F. A. Giuliano, *European Urology*, 2008, **53**, 1272-1281.

26 B. J. Goldstein, *Am. J. Cardiol*., 2002, **90**, 3G-10G.

27 J. Li, T. Kaneko, L. Qin, J. Wang, Y. Wang and A. Sato, *Metabolism*, 2003, **52**, 1206-1210.

28 A. Jimenez-Cruz, W. H. Turnbull, M. Bacardi-Gascin and P. Rosales-Garay, *Nutr. Res*., 2004, **24**, 19-27.

29 J. A. Larrauri, *Trends in Food Sci. Technol.*, 1999, **10**, 3-8.

30 A. Crozier, M. E. J. Lean, M. S. McDonald and C. Black, *J. Agric. Food Chem.*, 1997, **45**, 590-595.

31 C. Miglio, E. Chiavaro, A. Visconti, V. Fogliano and N. Pellegrini, *J. Agric. Food Chem.*, 2008, **56***,* 139-147

32 V. Thirunavukkarasu, A. T. Anithanandhini and C. V. Anuradha, *Exp. Diabetes Res*., 2004, **5**, 195-200.

33 P. Wenzel, A. Daiber, M. Oelze, M. Brandt, E. Closs, J. Xu, T. Thum, J. Bauersachs, G. Ertl, M. H. Zou, U. Förstermann and T. Münzel, *Atherosclerosis*, 2008, **198**, 65-76.

Figure captions

Fig. 1 Total phenolic compound (a), Scavenging activity (b), and total, insoluble and soluble dietary fiber content (c) of fresh and thermally-treated pitaya extracts. Data are means (± S.E.M) of three determinations. Asterisk (*) indicates significant difference at the level of $p < 0.05$ between fresh and thermally-treated samples of pitaya. Cross (†) indicates significant difference at the level of $p < 0.05$ between SDF of fresh and thermally-treated samples.

Fig. 2 Effect of different treatments on total antioxidant power of serum in rats. The 6 groups are illustrated by their respective diets.
CON: Normal rats + normal diet; FRU: IR rats + HFD; FRU+Pit: IR rats + HFD + 10% daily energy intake fresh pitaya; FRU+Pit95: IR rats + HFD + 10% daily energy intake thermally-treated pitaya at 95˚C for 30 min; FRU+Pit105: IR rats + HFD + 10% daily energy intake thermally-treated pitaya at 105˚C for 60 min; FRU+Met: IR rats + HFD + 200 mg of metformin per day.
* Significant difference at the level of $p < 0.05$ between initial (at the end of insulin resistance induction) and final (at the end of treatment) values.

Fig. 3 Hematoxylin-eosin-stained histological sections of abdominal aortic segments from rats
E, indicate endothelium/intima; M, media; and A, adventitia.
(a) CON: Normal rats + normal diet; the endothelium/intima appears continuous and intact.
(b) FRU: Insulin resistant rats + high fructose diet (HFD); the evidence of endothelial denudation and cell detachment were seen. Arrows point the separating endothelial cells.
(c) FRU+Pit: Insulin resistant rats + HFD + 10% of daily energy intake fresh pitaya; the endothelium/intima appears continuous and intact and similar to CON.
(d) FRU+Pit95: Insulin resistant rats + HFD + 10% of daily energy intake heated pitaya at 95˚C 30 min; the evidence of endothelial denudation and cell detachment were seen. Arrows point the separating endothelial cells.
(e) FRU+Pit105: Insulin resistant rats + HFD + 10% of daily energy intake heated pitaya at 105˚C 60 min; the evidence of endothelial denudation and cell detachment were seen. Arrows point the separating endothelial cells.
(f) FRU+Met: Insulin resistant rats + HFD + 200 mg (per day) metformin; the evidence of endothelial denudation and cell detachment is seen. Arrows point the separating endothelial cells.

Table 1 Effect of thermal treatment on the reduction in total phenolics content, scavenging activity and soluble dietary fiber of red pitaya used for animal studies

Supplement	Reduction of scavenging activity (%)	Reduction of total phenolic content (%)	Reduction of soluble dietary fiber (%)
Fresh pitaya	0	0	0
Thermally-treated pitaya at 95°C for 30 min	68*	38*	8
Thermally-treated pitaya at 105°C for 60 min	76*	40*	58*

* The mean value for thermally treated sample is significantly different from control ($p < 0.05$)

Table 2 Physical and biochemical characteristics of the rats in CON, FRU, FRU+Pit, FRU+Pit95, FRU+Pit105, FRU+Met groups during the experiment

	CON		FRU		FRU + Pit		FRU + Pit95		FRU + Pit105		FRU + Met		
	Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**Initial**	**Final**	**SEM**
Food consumption (g/kg/day)	63^{a}	62^{a}	39^{b}	38^{b}	42^{b}	38^{b}	38^{b}	38^{b}	40^{b}	37^{b}	41^{b}	42^{b}	2
Water consumption (ml/kg/day)	150^{f}	165^{e}	193^{d}	333^{a}	232^{c}	227^{c}	233^{c}	264^{b}	255^{b}	255^{b}	171^{e}	190^{d}	3.4
Energy intake by food (kcal/kg/day)	239^{a}	236^{a}	148^{bcd}	144^{cd}	160^{b}	144^{cd}	144^{cd}	144^{cd}	152^{bcd}	141^{d}	156^{bc}	160^{b}	3.0
Total energy intake (kcal/kg/day)	239^{de}	236^{ef}	225^{ef}	278^{a}	252^{cd}	259^{b}c	238^{def}	275^{a}	254^{bc}	267^{ab}	224^{f}	236^{ef}	3.0
Insulin (pmol/L)	35.6^{e}	43.9^{de}	85.0^{ab}	107.0^{a}	60.2^{cd}	30.9^{e}	74.5^{bc}	43.1^{de}	75.8^{bc}	63.2^{bcd}	64.9^{bcd}	49.5^{de}	4.7
Glucose (mmol/L)	5.8^{b}	7.0^{ab}	8.4^{a}	7.0^{ab}	8.2^{a}	5.6^{b}	7.9^{a}	5.9^{b}	8.4^{a}	7.2^{ab}	7.7^{a}	7.1^{ab}	0.3
Insulin/glucose ratio	6.2^{ef}	5.9^{ef}	10.1^{b}	17.8^{a}	7.9^{cde}	5.5^{f}	10.2^{b}	7.3^{cdef}	9.1^{bc}	8.8^{bcd}	8.4^{bcd}	7.0^{def}	0.4
Glucose tolerance test (AUC) (mmol/L×h)	18.3^{f}	20.1^{ef}	31.3^{a}	29.7^{ab}	30.5^{a}	22.4^{de}	27.1^{bc}	24.1^{cd}	31.3^{a}	28.9^{ab}	28.6^{ab}	20.4^{ef}	0.7
Triglyceride (mmol/L)	0.5^{e}	0.7^{cde}	0.9^{bcd}	1.1^{b}	1.4^{a}	0.5^{e}	1.1^{ab}	0.7^{cde}	0.8^{bcde}	0.6^{cde}	0.9^{bc}	0.6^{de}	0.1
Total cholesterol (mmol/L)	1.3^{d}	1.3^{d}	1.7^{abc}	1.7^{ab}	1.9^{a}	1.3^{d}	1.5^{bcd}	1.6^{bcd}	1.4^{cd}	1.6^{abcd}	1.5^{bcd}	1.3^{d}	0.07
LDL-cholesterol (mmol/L)	0.29^{ab}	0.32^{ab}	0.25^{ab}	0.31^{ab}	0.34^{ab}	0.30^{ab}	0.33^{ab}	0.36^{ab}	0.35^{ab}	0.38^{a}	0.38^{a}	0.23^{b}	0.03

Data are the mean of six samples (n = 6 in each group. The 6 groups are illustrated by their respective diets

CON: Normal rats + normal diet; FRU: Insulin resistant (IR) rats + High fructose diet (HFD); FRU + Pit: IR rats + HFD + 10% daily energy intake fresh pitaya; FRU + Pit95: IR rats + HFD + 10% daily energy intake thermally-treated pitaya at 95˚C for 30 min; FRU + Pit105: IR rats + HFD + 10% daily energy intake thermally-treated pitaya at 105˚C for 60 min; FRU + Met: IR rats + HFD + 200 mg of metformin per day.

$^{a-f}$ Means with different superscript within each raw are significantly different at $p < 0.05$.

Table 3 The thickness of intima-media of the abdominal aorta in rats

Groups	Intima-media thickness (μm)
CON	97.9^{cd}
FRU	128.2^{a}
FRU+Pit	93.7^{d}
FRU+Pit95	108.5^{bc}
FRU+Pit105	118.2^{ab}
FRU+Met	94.2^{d}
SEM	2.6

Data are shown as mean of six samples in each group and 4 measurements per aorta. The 6 groups are illustrated by their respective diets. SEM = standard error of the means.
CON: Normal rats + normal diet; FRU: Insulin resistant (IR) rats + high fructose diet (HFD); FRU+Pit: IR rats + HFD + 10% of daily energy intake fresh pitaya; FRU+Pit95: IR rats + HFD + 10% of daily energy intake heated pitaya at 95˚C 30 min; FRU+Pit105: IR rats + HFD + 10% of daily energy intake heated pitaya at 105˚C 60 min; FRU+Met: IR rats + HFD + 200 mg per day metformin.
[a] Indicates a significant difference at the level of $p < 0.05$ between insulin resistant groups and CON group.
[b] Indicates a significant difference at the level of $p < 0.05$ between treated insulin resistant groups and FRU group.

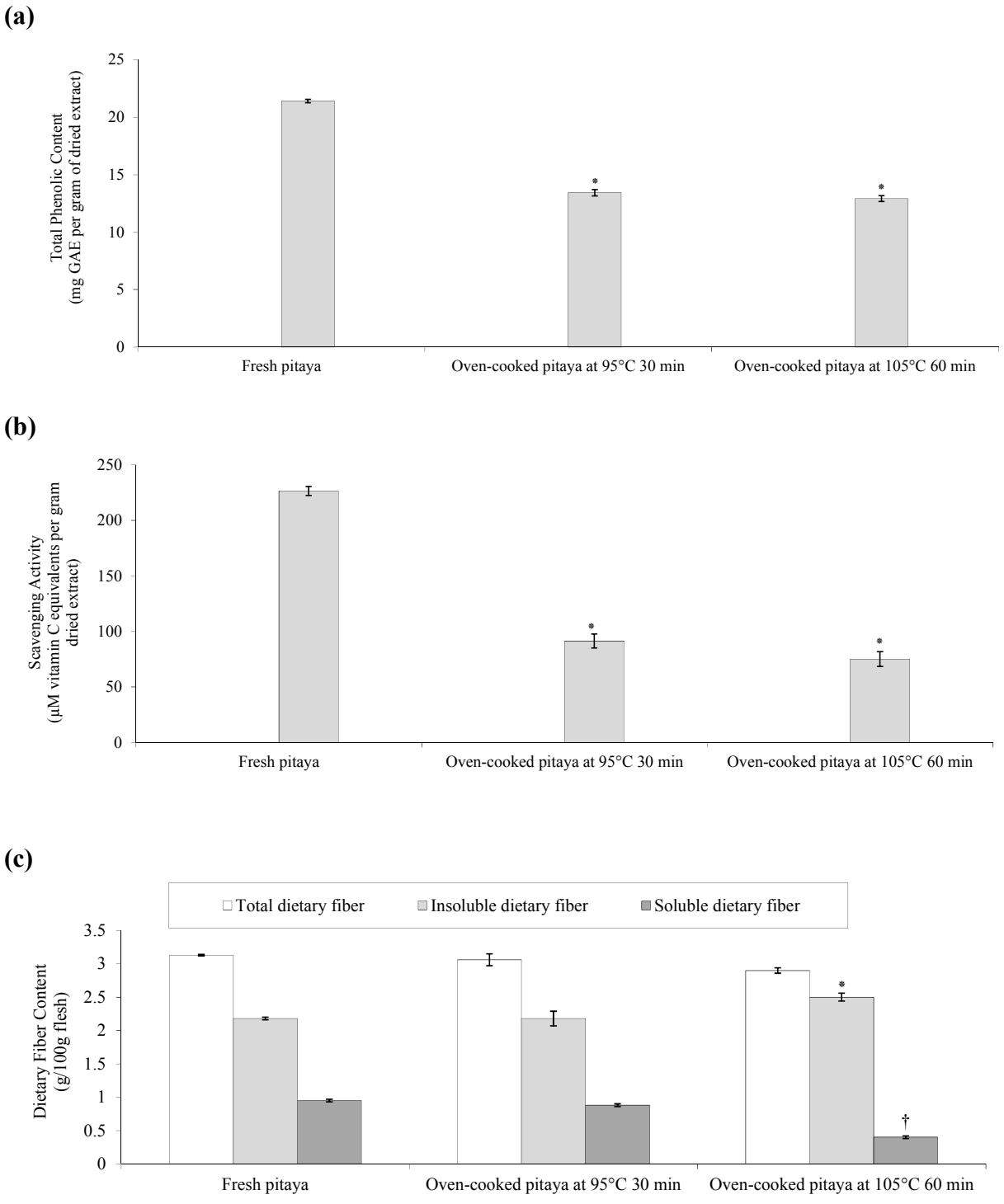

Fig 1.

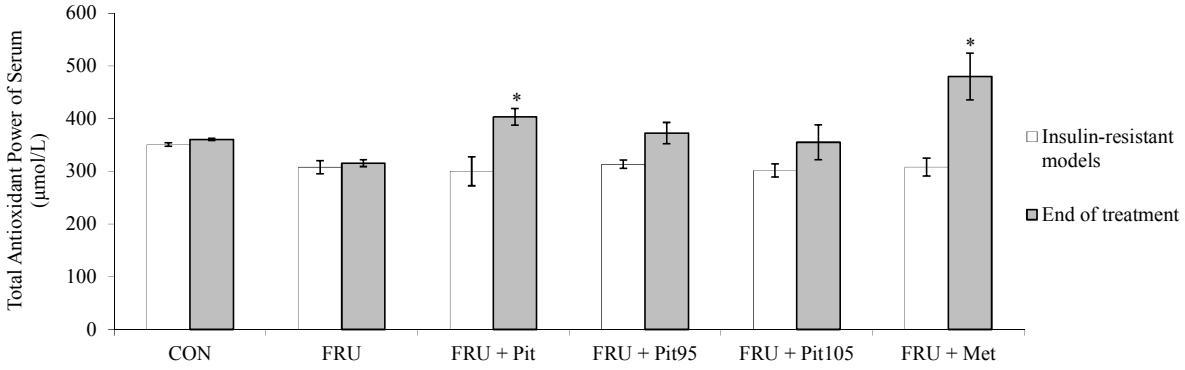

Fig 2.

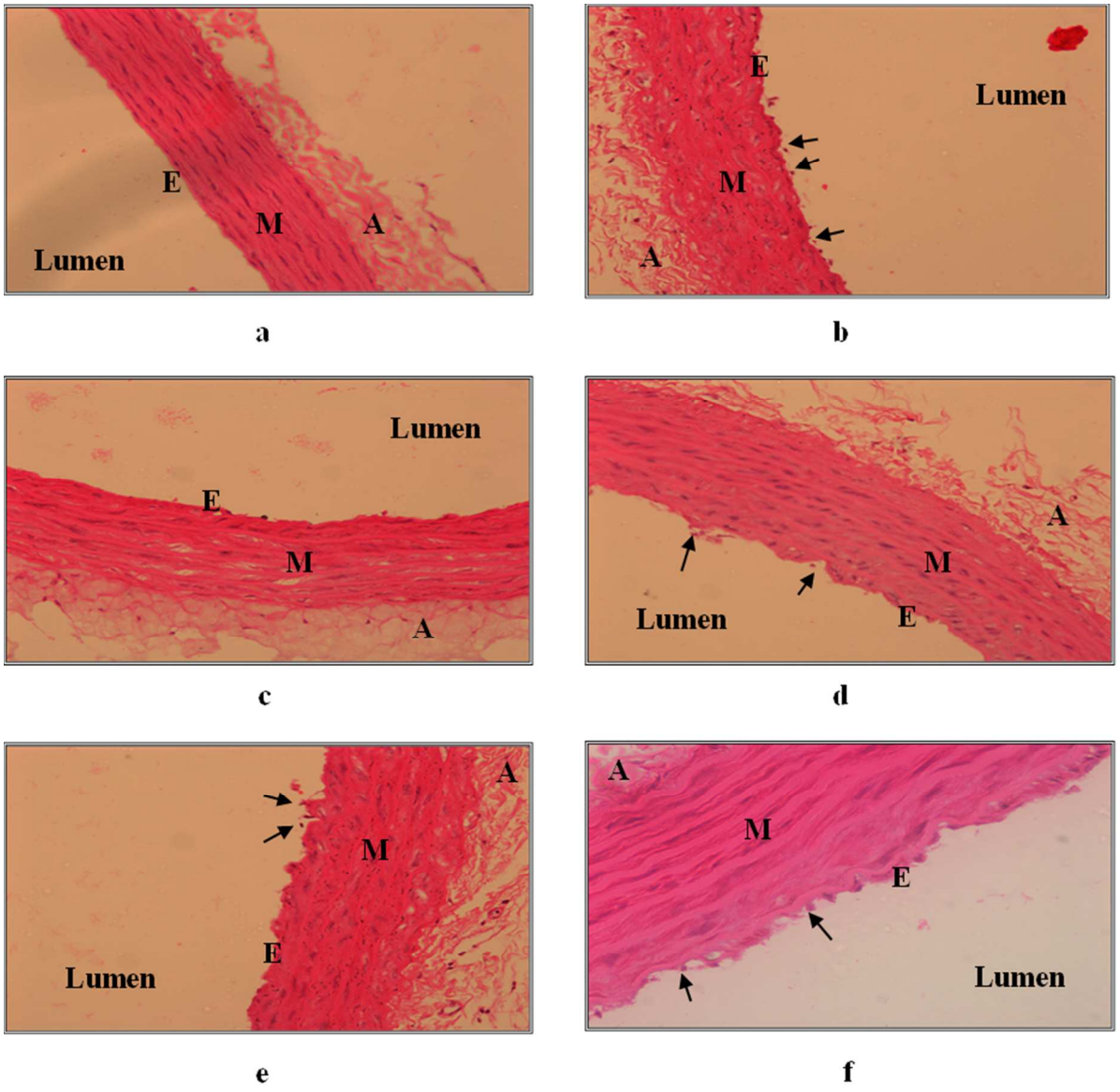

Fig 3.