Aerobic exercise and octopamine protects against deep-frying oil-induced cardiomyocyte apoptosis through modulation of caspase and pro-caspase 3

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Abstract

Background : Deep-frying is a common cooking method in which cooking is accompanied by carcinogenic byproducts such as acrolein. Acrolein is a toxic byproduct of lipid peroxidation, which has been implicated in pulmonary, cardiac, and neurodegenerative diseases. This study aimed to explore the effect of aerobic exercise and octopamine on caspase 3 and pro-caspase 3 expression levels in the heart tissue of rat exposed deep-frying oil.

Methods : 30 male Wistar rats were divided into 5 groups (n=6 in each) including 1) control (CO), 2) deep-frying oil (DFO), 3) deep-frying oil+exercise (DFO+EXE), 4) deep-frying oil+octopamine (DFO+OCT), and 5) deep-frying oil+exercise+octopamine (DFO+EXE+OCT). The apoptotic effects of DFO in heart tissue were examined by TUNEL assay. Masson's trichrome stain used to study cardiomyocytes' fibers. Moreover, caspase 3 and pro-caspase 3 genes and proteins expression in all groups were evaluated using quantitative real-time PCR and western blotting method, respectively.

Results : Data showed a significant increase in apoptotic cells in the DFO-treated group ($P < 0.05$). Masson's trichrome stain analysis demonstrated that the number of cardiomyocytes' fibers are decreased, and collagen deposition is increased in the DFO group. In comparison, the collagen percentage was significantly reduced in exercise, OCT, and exercise+OCT groups. Also, the expression level of caspase 3 and pro-caspase 3 was significantly decreased in deep-frying oil+exercise+OCT group ($P < 0.05$).

Conclusions : The results of our study show that DFO lead to programmed cell death via the activation of caspase in heart tissue. However, it seems that aerobic exercise with octopamine supplementation improves heart tissue by significantly decrease the expression of caspase 3 and pro-caspase 3 that inhibit apoptosis.

Background

Cardiomyocytes are the muscle cells that make up the cardiac muscle [1]. Each myocardial cell contains myofibrils, which are specialized organelles consisting of long chains of sarcomeres, the basic contractile units of muscle cells [2]. Cardiovascular disease (CVD) is a resulting cause of death globally that the death of cardiomyocytes is a primary contributing factor to these diseases. Cell death in conditions such as heart defeat and myocardial infarction is associated with apoptosis [3]. Cell death has become apparent as a basic biological phenomenon substantial for the regulation of tissue homeostasis whose change has an important concept in pathology [4]. Autophagy is an intracellular degradation system that renders cytoplasmic constituents to the lysosome. It has shown that autophagy plays a wide diversity of pathophysiological roles [5]. Cardiomyocyte apoptosis does occur in several cardiac diseases such as ischemic heart disease, heart failure, and aging [6].

A direct relationship has reported between CVD risk and consumption of cooking oil polar compounds [7]. Deep-frying is a cooking way for food to be submerged in hot fat, most generally oil[8]. Deep-frying oil

produces desirable or undesirable flavor compounds and alterations the flavor stability and quality of the oil by hydrolysis, oxidation, and polymerization [9]. It has demonstrated that the heating of dietary oils and fats results in lipid oxidation and apoptotic induction. Caspases are a family of end proteases that furnish critical links in cell regulatory networks controlling inflammation and cell death [10]. The activation of Caspases represents a critical step in the pathways resulting in the biochemical and morphological alterations that underlie apoptosis [11]. Among Caspases, *Caspase 3* is a frequently activated death protease, catalyzing the special cleavage of plenty of key cellular proteins. However, the particular requirements of *Caspase 3* in apoptosis are still unknown [12]. Activation of *pro-Caspase 3* is a central event in the execution phase of apoptosis and appears to serve as the convergence point of several apoptotic signaling pathways [13].

Octopamine is an endogenous biogenic amine closely related to norepinephrine, and affects adrenergic and dopaminergic systems. It is naturally present in many plants such as oranges and is derived from the synthesis of D- (-) - enantiomer octopamine by β -hydroxylation of tyramine and by the enzyme dopamine β -hydroxylase and is marketed under the trade names Epirenor, Norden and Norfen (14). Octopamine leads to an increase in the amplitude and frequency of the heartbeat. It has proposed that octopamine is not the transmitter of cardio-accelerator neurons [14]. Octopamine was studied as a therapeutic agent to treat hypotensive disorders, with doses of 450–600 mg day⁻¹ resulting in mild increases in blood pressure without the presence of adverse effects [15]. However, studies about the supplementation of octopamine on heart tissue and cardiac apoptosis in cardiovascular disease are limit. Ross et al. (2017) show that acute supplementation with a low dose of octopamine did not influence endurance cycle performance, substrate oxidation or circulating hormonal concentrations, which could be due to the low serum octopamine concentrations observed [16]. Exercise-based cardiac rehabilitation aims to restore people with coronary heart disease to health through either regular exercise alone or a combination of exercise with education and psychological support [17]. Sujkowski et al (2014) show that octopamine drives endurance exercise adaptations in drosophila [18]. There are limit study on effects of exercise with octopamine in cardiomyocyte. Looking for the answer to the question of whether aerobic exercise and octopamine supplement affect cardiomyocytes viability, the present study was designed. Thus, the aim of this study was to investigate the impact of aerobic exercise and octopamine supplement affects cardiomyocytes histopathology and also changes the expression of the *Caspase 3* and *pro-Caspase 3* in heart tissue of DFO-treated.

Materials And Methods

Animals

In this experimental study, overall 30 healthy adult male Wistar rats (*Rattus norvegicus*), eight weeks old and weighing approximately between 200 to 250 g, were obtained from Pasteur Institute of Iran (Tehran, Iran). The rats studied were exclusively housed in plastic cages with sawdust bedding. All rats were fed a standard diet and urban water. The

temperature and humidity used for cages were 20 ± 2 ° C and about 60%, respectively. The lighting period was held at 12h light/12h dark cycle. All the procedures were approved and monitored by the ethics committee of laboratory animals of Islamic Azad University (Tehran, Iran). The procedures followed the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978) (Ethical code: IR.MUK.REC.1398.242). In this study, the animals were randomly assigned to 5 groups (n=6). Groups were as follows: 1) control (CO), 2) deep-frying oil (DFO), 3) deep-frying oil + exercise (DFO+EXE), 4) deep-frying oil + octopamine (DFO+OCT), and 5) deep-frying oil + exercise + octopamine (DFO+EXE+OCT).

Chemicals

DFO

Deep-frying oil was made by frying 5 kg catfish three times in 2.5 L cooking palm oil at 200°C (measured with a cooking thermometer) for 15 min [19-21]. After each frying the oil was left to cool for 5 hours at room temperature [22]. The 3-times heated DFO preparation will subsequently be referred to as DFO. DFO was gavaged five days per week (10 mL/kg) for four weeks.

Octopamine

OCT (Sigma, UK) was dissolved in distilled water and then diluted with a Krebs-bicarbonate solution. OCT (100 µL in each injection) was injected intraperitoneally five days per week (10 mL/kg) for four weeks.

Aerobic exercise protocol

Exercised rats were introduced to treadmill running for a habituation period, during which each animal exercised on a motorized rodent treadmill at 9 m/min for 20 min per day (including 10 min at a prescribed speed, a 5-min warm-up, and 5-min cool-down). After the habituation period, rats were subjected to run at moderate exercise intensity for five days per week over four weeks (total duration of the exercise protocol and OCT supplementation). On the first day of exercise, the training began with the rats exercising at

11 m/min for 10 min per day. The speed gradually accelerated from 11 to 20 m/min over the duration of the experiment. The exercise time was also increased by 2 min per day over the same period until it reached 26 min/day at the end of the second week.

Animal tissue sampling

Forty-eight hours after the last training session, all rats were sacrificed, and heart tissue was removed immediately and washed in saline normal 0.9%. The tissues (ventricle) were divided into two parts for freezing (-80 °C) and fixing (10% neutralized formalin). Bodyweight and heart weight also measure at the end of the protocol (Table 1).

Terminal transferase dUTP nick end labeling (TUNEL) assay

The apoptotic effects of aerobic exercise and octopamine on heart tissue samples in DFO treated rats were determined using the transferase-mediated deoxyuridine triphosphate (dUTP)-digoxigenin nick end labeling assay. The control and experimental groups were fixed with 4 % (w/v) paraformaldehyde and processed using a commercial kit (In Situ Cell Death Detection Kit; Roche, Germany) in accordance with the manufacturer's instructions. Cells were stained with Hoechst dye and imaged in a fluorescent microscope. The data were expressed as a percentage of the area of terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling (TUNEL)-positive cells in ten random fields. The number of TUNEL positive cells was measured carefully in 3 sections of the heart tissue per animal. The percentage of positively stained cells against total nuclei in the area was analyzed with Image J software v1.8 (NIH, USA).

Histopathological examination: H&E and Masson's trichrome staining

Histopathological examination of cardiac muscle tissue after treatment with DFO in the experimental groups was done using hematoxylin and eosin (H&E) staining. To perfusion fixation, fresh heart tissues were placed into 10 % Formalin. Following this, they were dehydrated using alcohol. Samples were embedded in paraffin, sectioned, and stained with H&E (Sigma-Aldrich, UK). H&E staining was performed according to previously described methods [23]. Myofibers were counted in 10 fields of muscle sections for each rat.

Masson's trichrome stain (Histogenotech. Co., Tehran, Iran) were performed according to kit directions except as follows: Aniline Blue-Solution I-90 min incubation. The prolonged incubation is a standard procedure for trichrome stains of muscle. Briefly, heart tissue slides were placed in staining jar and deparaffinized by submerging into three series of absolute xylene for 4 min. After that, the slides were washed with running tap water for 2 min. Then, slides were treated with the phosphomolybdic acid solution for another 10 min as a mordant, and immediately slides were submerged into methyl blue (Merck, Germany). Next, slides were washed in running water for 2 min and lastly treated with 1% acetic acid solution for 1 min.

RNA and protein extraction

RNA and protein were extracted from the heart tissue using TRizol reagent (Invitrogen, USA) according to the manufacturer's instruction. The quality of the extracted RNA and protein was determined according to the 260/280 absorbance ratio, measured by the NanoDrop spectrometer (Thermo Scientific, USA).

cDNA synthesis and quantitative RT-PCR analysis (qRT-PCR)

cDNA synthesis for *Caspase 3* and *pro-Caspase 3* was carried out using a universal cDNA synthesis kit (Exiqon, Denmark) according to manufacturer's instructions. Specific primers for *Caspase* and *GAPDH* (as housekeeping gene) genes were designed using Primer Premier 5 software (Premier Bio-Soft International, Palo Alto, CA, USA) and summarized in Table 2. Real-time quantitative PCR reactions were carried out in triplicate by using standard protocols with an ABI step one system (ABI, USA). Briefly, in a total volume of 10 μ l, 20 ng/ μ l of cDNAs were added to a master mix comprising 10 pmol/ μ l of each primer (*Caspase 3*) and 5 mL of SYBR premix ExTaq II (Thermo Scientific, USA). The program for the run was set as follows: 95 °C for 15 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. The PCR reaction was followed by a melting curve program (60–95 °C with a temperature transition rate of 1 °C s⁻¹ and a continuous fluorescence reading). *GAPDH* was quantified as the reference to normalize differences in total RNA levels. The calculation was performed as follows:

$$\text{Ratio} = \frac{2^{\Delta c_T, \text{target (calibrator-test)}}}{2^{\Delta c_T, \text{ref (calibrator-test)}}}$$

Western blotting analysis

Heart tissue was lysed in lysis buffer (RIPA, Beyotime Institute of Biotechnology) supplemented with protease inhibitors (PMSF, Aladdin). The equal amounts of protein were separated by SDS-PAGE with 5%–12% Tris-Glycine gel (Invitrogen) and subjected to standard western blotting analysis. Western blotting was performed using *Caspase 3* and pro-*Caspase 3* antibodies (1:1000 dilution; Abcam), which was then reacted with the corresponding secondary HRP-conjugated antibodies (1:2000 dilution; Abcam, USA). Finally, blots were developed using the ECL western blotting detection system (Amersham Life Sciences Inc., Arlington Heights, IL). The results were subjected to densitometry analysis using the ImageJ software to assist with the blind analysis of image data. To ensure equal amounts of protein were loaded, the GAPDH protein was employed as an internal control.

Statistical analysis

The statistical analysis of the obtained data was performed using the Graph Pad Prism statistical software version 5.01 (GraphPad, San Diego, CA, USA). The normality of the data was evaluated using the Kolmogorov-Smirnov test, two-way ANOVA, and Tukey post-Hoc test was used to analyze the data from different groups. Data are expressed as the mean $\pm$ SD. The level of significance was set at $P < 0.05$.

Results

Histopathological assessment and fibrosis assessment by Masson's trichrome stain

According to the results in the histopathological part, it was found that muscle tissue was cohesive in control and DFO+exercise+octopamine groups compared to other experimental groups. On the other hand, the myofibrils were closely spaced near to each other. Besides, the number of myofibrils in control and DFO+exercise+octopamine groups had a significant difference ($P \leq 0.01$) compared to DFO, DFO+exercise, and DFO+octopamine treatment groups (Figure 1). Also, the results showed that the

diameter of these myofibrils in the DFO+exercise+octopamine group was higher compared to the DFO group significantly ($P = 0.031$).

The results showed that in the DFO group, the amount of collagen fiber increased significantly ($P = 0.003$) compared to the control group. In consideration of treatment intervention the results show that, aerobic exercise significantly reduced collagen percentage ($F = 38.10$, $P = 0.001$, $\eta^2 = 0.826$). Also, octopamine treatment was able to significantly reduce collagen levels in heart tissue ($F = 27.62$, $P = 0.001$, $\eta^2 = 0.775$). The effect of aerobic exercise and octopamine treatment simultaneously was able to significantly reduce the number of collagen fibers ($F = 16.48$, $P = 0.004$, $\eta^2 = 0.673$) (Figure 2).

Apoptosis

As a result of receiving DFO, the number of apoptotic cells in the tissue increased significantly ($P = 0.001$). Although, aerobic exercise significantly reduced the number of apoptotic cells ($F = 38.10$, $P = 0.001$, $\eta^2 = 0.826$). On the other hand, octopamine treatment significantly reduced the number of apoptotic cells ($F = 14.24$, $P = 0.005$, $\eta^2 = 0.640$) in cardiac tissue. The effect of aerobic exercise and simultaneous octopamine treatment was able to significantly reduce the number of apoptotic cells ($F = 0.547$, $P = 0.481$, $\eta^2 = 0.064$) (Figure 3 A&B).

Caspase 3 gene expression

As a result of receiving DFO, the expression of *Caspase-3* gene ($P = 0.001$) increased significantly in the heart tissue. Although, aerobic exercise significantly reduced the expression of *Caspase 3* gene ($F = 9.16$, $P = 0.007$, $\eta^2 = 0.375$). On the other hand, octopamine treatment was able to significantly reduce the expression of *Caspase 3* gene ($F = 16.39$, $P = 0.001$, $\eta^2 = 0.506$) in cardiac tissue. The effect of aerobic exercise and octopamine treatment simultaneously was able to significantly reduce the expression of *Caspase 3* gene ($F = 0.003$, $P = 0.954$, $\eta^2 = 0.003$) (Figure 4).

Caspase 3 and pro-*Caspase 3* proteins expression

Western blot results are depicted in Figure 5A. As a result of receiving DFO, the expression of Pro-Caspase 3 protein increased significantly in the heart tissue ($P = 0.001$). It should be noted that the expression ratio of Caspase 3 protein to Pro-Caspase 3 ($P = 0.053$) did not change significantly ($P = 0.221$). Aerobic exercise did not have a significant effect on the expression of Pro-Caspase 3 protein ($F = 0.903$, $P = 0.370$, $\eta^2 = 0.101$). On the other hand, octopamine reduced the expression of Pro-Caspase 3 protein ($F = 14.19$, $P = 0.005$, $\eta^2 = 0.651$). However, it did not have a significant effect on the ratio of Caspase 3 to Pro-Caspase 3 ($F = 0.143$, $P = 0.715$, $\eta^2 = 0.018$). In the exercise and supplement group, Caspase-3 protein ($F = 19.32$, $P = 0.002$, $\eta^2 = 0.707$) and the ratio of Caspase 3 to Pro-Caspase 3 protein expression decreased significantly ($F = 14.32$, $P = 0.005$, $\eta^2 = 0.641$) (Figure 5 B and C).

Discussion

Due to the importance of cooking and the taste of food, various techniques have been proposed for cooking food around the world [24]. One of the welcomed methods in some countries is frying food at high temperatures with heated oils, which gives a special taste to the food, and it makes the food crispy and delicious [25]. However, apart from the good taste of this type of food, it can be hazardous for human health. One of the body's organs that is affected by diet is the cardiovascular system. As well as high fat intake is a high-risk factor for cardiovascular disease, increasing the use of DFO due to the disruption of their fat structure can leave cardiovascular risks [26]. Among the therapeutic modalities for improving heart disease due to bad diet, is improving lifestyle by doing exercise or using herbal and pharmacological supplements[27]. Therefore, this study aimed to investigate the effect of exercise training with octopamine supplementation on apoptosis of cardiomyocytes rats exposed to DFO.

This study shows that exposure to DFO for four weeks can disrupt cardiomyocytes' regularity and increase collagen deposition in heart tissue. In considering apoptosis, the result of tunnel assay shows that apoptotic cells significantly increased with DFO in animal models. This result also consists of the upregulation of caspase 3 and pro-caspase 3. It seems that one of the reasons for these destructions caused by DFO is related to its changes in structural oil with boiling and frying [25, 28]. Various chemical reactions occur during the frying process, such as thermal oxidation, hydrolysis, and polymerization, due to the exposure of the oil to high temperatures in the presence of air and moisture. All of these changes have a risk factor for CVD. It is stated that the repeated heating of oil at high temperatures (180°C) results in the thermal oxidation of the oil, which causes the configuration of the fatty acid to change from the cis isomer to the Trans isomer [29]. However, in this study, use DFO with 200 °C. This configuration change causes the polyunsaturated fatty acids to acquire undesirable properties associated with saturated fatty acids, such as their correlation with increased serum cholesterol levels and higher low-density lipoprotein (LDL) cholesterol in particular [30]. Also, during the frying process, the oil is aged and becomes more oxidized when the natural antioxidants are depleted; thus, after repeated heating, the antioxidants can no longer prevent the oxidation of the fatty acids in the oil [31, 32]. All of this agent can cause increase the reactive oxygen species (ROS) in cardiomyocytes. An increase in ROS at the cellular level is the start point in beginning apoptosis [33]. In this study although the oxidative stress marker was not measured, however apoptosis marker (caspase 3 and pro-caspase 3) measured and increased with DFO for four weeks. Gupta et al. (2014) consider the effect of different deep fried vegetable oil on the cardiovascular system in rats model and show that deep-fried vegetable oil cause increase plasma nitric oxide level and vascular reactivity on blood pressure and aortic structure change [34]. However, in this study, the type of oil and the cellular marker were different. The results of this study in the adverse effects of DFO in heart tissue show that the diameter of muscle fibers in this group (DFO) is significantly less than that of another group. However, treatment with exercise and OCT improve this distraction.

The results showed that four weeks of aerobic exercise and octopamine supplementation improve heart tissue integration and downregulate caspase and pro-caspase 3 in the cardiac cell. Regular aerobic exercise can increase the antioxidant enzymes in various tissues, especially the heart [35, 36]. Regular aerobic exercise has been shown to increase antioxidant defense and reduce fat and protein peroxidation. Reinforcing antioxidant defenses improves the neutralization of free radicals. Therefore,

oxidative stress is reduced after aerobic exercise [37, 38]. In this study, although the antioxidant capacity of cardiac tissue was not measured, declining changes in apoptotic cells could indicate the control of apoptotic markers by reducing oxidative stress. Apart from this mechanism, it has been shown that cellular adaptation to exercise can be associated with both endogenous and exogenous factors [39]. Exercise induces cardiac growth via hypertrophy and renewal of cardiomyocyte [40]. Also, it is stated that exercise induces endothelial progenitor cells to proliferate, migrate and differentiate into mature endothelial cells, giving rise to endothelial regeneration and angiogenesis in heart tissue [41], that all of this agent show cardioprotective effects of exercise, and seems all of this mechanism improve apoptosis in heart. In this study, we also use OCT with exercise. It appears that part of the better effects of combined groups of this study attribute to OCT.

The results of this study show that a combination of aerobic exercise and octopamine may have a negative effect on both genes studied (*Caspase and pro-Caspase 3*). These factors can also overcome the cytotoxicity of DFO (reduce apoptosis induced by DFO). Studies demonstrated the ability of octopamine to activate B3 adrenoreceptors and stimulate lipolysis [42], suggesting octopamine could influence fat metabolism[43]. Therefore OCT supplements with lipolysis-stimulating activity, have potential to act as an efficient [anti-obesity](#). Thus, it seems that OCT administration could potentially rescue the heart from obesity related loss of cardiomyocyte. OCT with controlling harmful fats caused by DFO has a role in reducing the cellular damage caused by apoptosis, this positive effect is doubled along with exercise.

Clinical evidence suggests inflammation and oxidative stress linked to the overgeneration of ROS [44]. In our previous study on octopamine, we showed that OCT supplementation significantly reduced NF- κ B and TNF- α gene expression levels in the heart tissue of rats fed DFO [28]. Therefore, it seems that one of the possible mechanisms of action of octopamine in heart improvement is the reduction of the ROS, which leads to a reduction in inflammation and oxidative stress and, ultimately, apoptosis.

Strengths and limitations

This study provided a detailed base data regarding myocardial injury and apoptosis markers at protein and gene levels. However, there were several potential limitations in the present study. First, do not use the characteristic analysis of DFO. Also, we did not measure lipid peroxidation acid value, peroxide value, smoke point, and ultraviolet absorbance at 232 nm. Another limitations of this study is a lack of presentation and investigation of the mechanism of the protective effects of exercise and octopamine against apoptosis in the heart and whether the protective effect of these two treatments can be related to the regulation of stress proteins such as MnSOD, ERK, IGF-1 / Akt, HSPs and NF- κ B or not. Also, we did not track the weight gain (especially fat mass) throughout the intervention. Due to the effect of weight gain on cardiovascular problems, it remains possible that the impact of the intervention (s) was the result of differences in weight gain.

Conclusion

Based on the results of this study, aerobic exercise training for four weeks with octopamine can inhibit apoptosis factors in rats exposed to DFO. It seems that performing exercise training with OCT supplementation can improve cardiomyocyte homeostatic disturbances due to apoptosis-induced by DFO. However, more studies (*in-vitro* and *in-vivo*) are needed, especially on the human sample.

Abbreviations

DFO: Deep-frying oil, CVD: cardio vascular disease.

Declarations

Ethics approval and consent to participate

The protocol was approved by the Ethics Committee of Islamic Azad University, Tehran, Iran (Code: IR.MUK.REC.1398.242).

Consent for publication

‘Not applicable’.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

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Tables

Table 1. Bodyweight and heart weight in different groups of study (mean $\pm$ SD)

Heart weight (g)	Body weight (g)	Groups
1.05 ± 0.03	256 ± 7	<i>Co</i>
1.03 ± 0.05	265 ± 4	<i>DFO</i>
1.11 ± 0.07	249 ± 8	<i>DFO+EXE</i>
0.99 ± 0.03	261 ± 3	<i>DFO+OCT</i>
1.08 ± 0.07	260 ± 5	<i>DFO+EXE+OCT</i>

CO: control, DFO: deep-frying oil, DFO+EXE: deep-frying oil+exercise, DFO+OCT: deep-frying oil+octopamine, DFO+EXE+OCT: deep-frying oil+exercise+octopamine.

Table 2. Primer sequences for real time PCR assessment

Product length (bp)	TM ()	Primer sequences	Gene name
121	81.1	F: 5'- CAT ACT CAG CAC CAG CAT CAC C -3' R: 5'- CATACTCAGCACCAGCATCACC -3'	<i>GAPDH</i>
82	80.5	F:5'- GCACAAAGTGACTGGATGAAC-3' R:5'- TGTATTCTTACTCTACCGCAC-3'	<i>Caspase 3</i>

Figures

Figure 1

Histopathological examination of cardiac muscle tissue after treatment with DFO in the experimental groups using H&E staining. Images of longitudinal sections show tissue integrity, and the images of transverse sections show the diameter and number of muscle fibers. a: significant differences with the control group at $P \leq 0.01$, b: significant differences with the DFO group at $P \leq 0.01$. The results represent the mean $\pm$ SD with five repeats.

Figure 2

Fibrotic tissue and collage deposition in cardiac muscle tissue after treatment with DFO in the experimental groups (Masson's trichrome staining). a: significantly different with the control group (CO) at $P \leq 0.0032$, b: significantly different with the DFO at $P \leq 0.005$. CO: control, DFO: deep-frying oil, DFO+EXE: deep-frying oil + exercise, DFO+OCT: deep-frying oil+octopamine, DFO+EXE+OCT: deep-frying oil + exercise + octopamine. The results represent the mean $\pm$ SD with five repeats.

B)

Figure 3

A) Apoptotic effects of DFO were recognized by the TUNEL assay. B) Semi-quantitative analysis of TUNEL assay. a: significantly different from the control group (CO) at $P \leq 0.001$, b: significantly different from the DFO at $P \leq 0.0042$. CO: control, DFO: deep-frying oil, DFO+EXE: deep-frying oil+exercise, DFO+OCT: deep-frying oil+octopamine, DFO+EXE+OCT: deep-frying oil+exercise+octopamine. The results represent the mean $\pm$ SD with five repeats.

Figure 4

Average relative Caspase 3 expression in the group studied compared to GAPDH. a: significantly different from the control group (CO) at $P \leq 0.002$, b: significantly different from the DFO at $P \leq 0.01$. CO: control, DFO: deep-frying oil, DFO+EXE: deep-frying oil+exercise, DFO+OCT: deep-frying oil+octopamine, DFO+EXE+OCT: deep-frying oil+exercise+ octopamine. The results represent the mean $\pm$ SD with five repeats.

Figure 5

A) Western blotting analysis for Caspase 3 and pro-Caspase 3 proteins expression in different groups. GAPDH was used to confirm the normalization of the protein load. In western blotting bands, the groups are arranged in order of expression (low to high). B) Semi-quantitative analysis of western blot for different proteins. C) The ratio of activated caspase 3 to procaspase 3. a: significantly different with Co group at $P \leq 0.01$, b: significantly different with the DFO group at $P \leq 0.01$, CO: control, DFO: deep-frying oil, DFO+EXE: deep-frying oil+exercise, DFO+OCT: deep-frying oil+octopamine, DFO+EXE+OCT: deep-frying oil+exercise+octopamine. The results represent mean $\pm$ SD with five repeats.