CARDIOPROTECTIVE EFFECT OF PERSEA AMERICANA MILL FRUIT EXTRACT IN EXPERIMENTAL MYOCARDIAL INFARCTION

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ABSTRACT
We aimed to investigate the protective effect of avocado fruit extract (AVOE) and elucidate its mechanisms on isoproterenol induced myocardial infarction in rats. The biochemical markers for myocardial infarction, antioxidant status on heart tissue, the histopathological examination of heart tissue and the genes expressions levels of nitric oxide synthase (NOS), nuclear factor-kappa B (NF-kB) and tumor necrosis factor-alpha (TNF-α) were all evaluated. Pretreatment with avocado followed by isoproterenol injection significantly prevented almost all the parameters of isoproterenol induced myocardial infarction. Results were confirmed by the histopathological examination. No significant change was observed in the baseline group. Avocado pretreatment upregulated the expression of endothelial nitric oxide synthase, whereas expressions of nuclear factor-kappa B and tumor necrosis factor-alpha were downregulated in isoproterenol-treated rats. This suggests that the protective effects of avocado fruit extract may be mediated through upregulation of nitric oxide production, antioxidant mechanisms, and its ability to inhibit TNF-α and NF-kB.

KEYWORDS: Myocardial infarction, Avocado, tumor necrosis factor-alpha, nuclear factor-kappa B.

INTRODUCTION
Myocardial infarction (MI) is the major form of ischemic heart diseases (IHD) and is characterized by an imbalance of coronary blood supply and myocardial demand which results in ischemia and myocardial death. Experimental and clinical studies have shown that there is an increase of free radical generation, and increased migration of neutrophils to the ischemic tissue and/or interrupted production of endogenous antioxidant enzymes which play...
an important role in the pathophysiology of ischemic myocardial injury in heart diseases (Sahna et al., 2008).

Isoproterenol (ISO), a β-adrenergic agonist causes oxidative stress in the myocardium resulting in gross and microscopic infarct in heart muscles of rats. One of the possible mechanisms of ISO-induced cardiac injury is high production of free radicals and lipid peroxides by the way of auto-oxidation of catecholamines (Rajadurai and Prince, 2007). Several studies have demonstrated that nuclear factor-kappa B (NF-kB), an ubiquitous transcription factor, activated by various stimuli such as reactive oxygen species (ROS), hypoxia, and inflammatory cytokines such as tumor necrosis factor α (TNF-α), is substantially involved in the progression of cardiac remodeling (Frantz et al., 2006). Evidence suggests that reduced nitric oxid (NO) availability may play an important role in the pathophysiology both after experimental myocardial ischemia and in patients with MI (Drexler, 1998).

HO-1-mediated cytoprotection has been shown to be critical for tissues that are vulnerable to oxidative stress (Perrella and Yet, 2003). The cytoprotective action of HO-1 derives mostly from decreased intracellular prooxidant levels, increased bilirubin levels, and elevated carbon monoxide (CO) production. Bilirubin, as an antioxidant, provides cellular protection against free radical mediated cell injury. CO exerts strong antiapoptotic and anti-inflammatory effects through the induction of soluble guanylyl cyclase (Durante, 2002).

Persia americana fruit is commonly referred to as avocado pear or alligator pear. Avocado fruit (100gm) is a nutrient and phytochemical dense food consisting of the following: dietary fiber (6.8 g), total sugar (0.3 g), potassium (507 mg), sodium (8 mg), magnesium (29 mg), vitamin A (7 μg RAE), vitamin C (8.8 mg), vitamin E (1.97 mg), vitamin K (21 μg), folate (89 mg), vitamin B-6 (0.29 mg), niacin (1.91 mg), pantothentic acid (1.46 mg), riboflavin (0.14 mg), choline (14.2 mg), lutein/zeaxanthin (271 μg), cryptoxanthin (27μg), phytosterols (83 mg; stigmasterol (5mg), campesterol (5mg) and beta-sitosterol (76mg)) Flavonoids (4.25mg), Phenols (2.94 mg) and high-monounsaturated fatty acids (9.8 g), which may support a wide range of potential health effects (Moreno et al., 2003; Shaw et al., 1980; Duester, 2001). These bioactive substances in this fruit and in its extract or individual components have been shown to have antioxidative and radical suppressing activities (Duester, 2001).
Therefore, the aim of the present study was to investigate the role of avocado fruit extract in modulating the antioxidant status and expressions of nitric oxide synthases (NOS), TNF-α, and NF-kB against ISO-induced acute MI.

MATERIALS AND METHODS

Chemicals

Isoproterenol hydrochloride, was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

Plant material and extraction

Fresh fruits of P. Americana, hass type were purchased from local market in Cairo, Egypt. The plant name has been checked with www.theplantlist.org, and the type of avocado fruit was identified by botany department in faculty of science, Ain shams university. The seed was removed and the edible part was chopped into small pieces, dried at 50-60 °C and ground into powder. One hundred grams of powder was extracted at room temperature by the process of maceration in an aspirator using 95% ethanol. The resulting extract was concentrated under reduced pressure by rotary evaporator to until a crude solid extract was obtained and stored at 4 °C. The yield was approximately 10% of fresh fruit. Working concentrations of the extract were made in distilled water before use in the experiment. Frantz et al., 2003

Experimental animals

Adult male albino rats of Wistar strain weighing 150–180 g obtained from animal house of faculty of medicine were used for the present study. The animals were housed at 27±2 °C in temperature, 55% in humidity, and a 12 h-light/12 h-dark cycle. The animals were allowed a standard diet and water ad libitum. All experiments were carried out according to the guidelines of Institutional Animal Ethics Committee of the Ain Shams University.

Experimental design

Rats were divided into four equal groups, comprising 10 rats each:

- Control group: rats received a standard diet for a period of 30 days and were injected with physiological saline alone for 2 days at 29th and 30th day only
- Avocado (AVOE) group: rats were orally administered with avocado extract, (300 mg /kg body weight/day) (Nayak et al., 2008) by intragastric intubation for a period of 30 days.
- Isoproterenol (ISO) group: rats were injected with isoproterenol (120 mg/kg bw/day), i.p. for 2 days at 29th and 30th day only (Ribeiro et al., 2009).
AVOE + ISO group: rats were pretreated with avocado extract for 30 days along with isoproterenol administration on 29th and 30th.

At the end of the experiment animals were sacrificed 48 hours after the first dose of isoproterenol, blood samples were collected from overnight fasted rats by cardiac puncture under mild ether anesthesia, and serum was separated by centrifugation. Heart was excised and divided into parts, where one part was fixed in 10% formalin for histopathological examination while the rest was frozen at -80°C for RNA isolation, and enzyme activity assay.

Biochemical estimations
Cardiac markers including creatinine kinase-MB (CK-MB), lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT) as well as uric acid were measured in the serum using standard commercial kits (Biodiagnostic, Cairo, Egypt). The lipid profile including total cholesterol (TC), HDL cholesterol (HDL-C) and triglycerides (TGs) were measured in serum using standard commercial kits (Biodiagnostic, Cairo, Egypt). Oxidative stress markers including nitrite level (Kumar et al., 2009), malondialdehyde (MDA) content (Draper and Hadley, 1990), non-enzymatic antioxidants including vitamin C (Omaye et al., 1979) and Vitamin E (Baker et al., 1980) were measured in serum, while antioxidant enzymes including catalase (CAT) (Aebi, 1984), glutathione-S-transferase (GST) (Habig and Jakoby, 1981), glutathione reductase (GR) (Goldberg and Spooner, 1983) as well as heme oxygenase-1 (HO-1) (Maines, 1996) were estimated in cardiac tissue.

RNA isolation and real-time quantitative polymerase chain reaction (qRT-PCR)
Total RNA was isolated from cardiac tissue samples using the Thermo Scientific GeneJET RNA purification kit according to the manufacturer’s instructions. RNA was quantified by optical density measurement at 260 nm using a spectrophotometer. One microgram of total RNA was used for the cDNA synthesis using the Thermo Scientific RevertAid™ First Strand cDNA synthesis kit. The relative expression levels of mRNA were measured using the Thermo Scientific Maxima SYBR Green/ROX qPCR Master Mix, according to manufacturer’s protocol, and the results were computerized using Stratagene (Mx3000P™) machine. Primer sequences were: 5'-ATG GCG AAG CGT GTG AAG-3' (sense) and 5'-ATT GTG GCT CGG GTG GAT-3' (anti-sense) for NOS (Gen Bank accession no. NM 021838. 2); 5'-CCT AGC TTT CTC TGA ACT GCA AA-3' (sense) and 5'- GGG TCA GAG GCC AAT AGA GA-3' (anti-sense) for NF-kB (GenBank accession no.U83656.1) and 5'- AGT CTT CCA GCT GGA AAG GG-3' (sense) and 5'- GCC ACT ACT TCA GCA TCT
CG-3’ (anti-sense) for TNF-α (Gen Bank accession no. NM 012675.3) and 5’-GTCAGGTCATCACTATCGGCAAT-3’ (sense) and 5’- AGAGGTCTTTACGGATGTCAACGT-3’ (anti-sense) for β-actin (Gen Bank accession no. NM 031144.3). The expression level of these genes was normalized to β-actin and presented as fold change relative to untreated control.

Histopathological examinations
Tissue specimens were cleared in xylene and embedded in paraffin at 56ºC in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained by hematoxylin and eosin (H&E) stains (Banchroft et al., 1996) for histopathological examination using the electric light microscope.

Statistical analysis
Data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 17. All the values are expressed as mean ± S.E, statistical significance between more than two groups was tested using one-way ANOVA. Differences were considered to be statistically significant when P < 0.05.

RESULTS AND DISCUSSION
Effect of AVOE on body weight and heart weight
The mean body weight of rats at the end of experiment period in all experimental groups had no significant change (Table 1). The heart weight and the ratio of heart weight to body weight were increased significantly (P<0.001) in ISO-administered groups when compared with normal control groups. Rats pre-treated with AVOE before ISO administration showed a significant reduction in the heart weight and the ratio (P<0.01&P<0.001, respectively) as compared to ISO treated groups.

Table 1: Effect of AVOE pretreatment on heart weight and body weight in isoproterenol induced myocardial infarction in rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ISO</th>
<th>AVOE</th>
<th>AVOE+ISO</th>
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<tbody>
<tr>
<td>Heart weight (g)</td>
<td>0.44 ± 0.01</td>
<td>0.85 ± 0.03a</td>
<td>0.45 ± 0.02</td>
<td>0.58 ± 0.03b</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>157.5 ± 5.8</td>
<td>150.7 ± 6.1</td>
<td>158.0 ± 4.5</td>
<td>156.3 ± 2.3</td>
</tr>
<tr>
<td>Heart weight/ body</td>
<td>0.280±0.006</td>
<td>0.610±0.02a</td>
<td>0.290±0.004</td>
<td>0.410±0.017b</td>
</tr>
<tr>
<td>Body weight ratio</td>
<td></td>
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</tbody>
</table>
Values are presented as mean ± SE for 10 animals in each group. a: Significant difference at p< 0.05 compared to the normal control group. b: Significant difference at p< 0.05 compared to the ISO group.

Effect of AVOE on biochemical parameters

The activities of cardiac marker enzymes including CK-MB, LDH, AST and ALT as well as uric acid were increased significantly (P <0.01) in ISO-treated rats as compared to normal control group rats (Table 2), whereas AVOE pretreatment significantly reversed these elevated levels (P<0.01).

Table 2: Effect of AVOE pretreatment on isoproterenol-induced changes in the activities of serum CK-MB, LDH, ALT, AST and uric acid.

<table>
<thead>
<tr>
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<th>Control</th>
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<th>AVOE</th>
<th>AVOE+ISO</th>
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<tbody>
<tr>
<td>CK-MB (IU/L)</td>
<td>428.45 ± 45.83</td>
<td>617.67 ± 14.99</td>
<td>416 ± 20</td>
<td>465 ± 15</td>
</tr>
<tr>
<td>LDH (IU/L)</td>
<td>636.63 ± 54.79</td>
<td>1100 ± 62.98a</td>
<td>672.5 ± 15.48</td>
<td>791 ± 39.08a</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>137.67± 15.33</td>
<td>267.32±12.58a</td>
<td>136.5±7.39</td>
<td>157.5±8.18b</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>37±2.09</td>
<td>61.3±5.99a</td>
<td>26.67±6.68</td>
<td>44.17±1.91b</td>
</tr>
<tr>
<td>Uric (mg/dl)</td>
<td>3.83±0.32</td>
<td>9.76±0.95a</td>
<td>4.08±0.41</td>
<td>7.76±0.64b</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SE for 10 animals in each group. a: Significant difference at p< 0.05 compared to the normal control group. b: Significant difference at p< 0.05 compared to the ISO group.

The effects of AVOE pre-treatment on serum and tissue lipids (total cholesterol, triglycerides and HDL- cholesterol) of normal and ISO-treated animals are listed in Table 3. Treated rats with isoproterenol alone showed a significant increase (P<0.001) in TC and TGs, with a significant decrease (P<0.001) in the level of HDL cholesterol. AVOE pretreatment in isoproterenol intoxicated rats showed a significant reduction in serum TC (P<0.001) and TGs (P<0.05) levels, while, a significant increase (P<0.001) in serum HDL-C level was observed compared to ISO-treated rats.

Table 3: The effect of AVOE on serum and cardiac TC, HDL-C and TGs levels in ISO induced myocardial infarction in rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>ISO</th>
<th>AVOE</th>
<th>AVOE+ISO</th>
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<tbody>
<tr>
<td>TC (serum) (mg/dl)</td>
<td>109.70 ± 16.3</td>
<td>140.40 ± 13.9a</td>
<td>98.14 ± 8.5</td>
<td>117.80 ± 15.9b</td>
</tr>
<tr>
<td>TC (mg/g wet tissue)</td>
<td>5.90 ± 0.7</td>
<td>8.84 ± 1.2a</td>
<td>5.82 ± 1.1</td>
<td>6.98 ± 0.5b</td>
</tr>
<tr>
<td>HDL-C (serum mg/dl)</td>
<td>29.12 ± 3.6</td>
<td>13.47 ± 0.8a</td>
<td>35.91 ± 4.8b</td>
<td>24.15 ± 2.4b</td>
</tr>
<tr>
<td>HDL-C (mg/g wet tissue)</td>
<td>1.53 ± 0.60</td>
<td>0.79 ± 0.04a</td>
<td>1.80 ± 0.06</td>
<td>5.09 ± 0.07b</td>
</tr>
<tr>
<td>TGs (serum) (mg/dl)</td>
<td>101.40 ± 29.00</td>
<td>151.00 ± 37.50a</td>
<td>99.40 ± 11.04</td>
<td>114.65 ± 21.90b</td>
</tr>
<tr>
<td>TGs (mg/g wet tissue)</td>
<td>4.50 ± 0.9</td>
<td>6.17 ± 0.5a</td>
<td>3.60 ± 0.7</td>
<td>4.92 ± 1.0b</td>
</tr>
</tbody>
</table>
Values are presented as mean ± SE for 10 animals in each group. a: Significant difference at p< 0.05 compared to the normal control group. b: Significant difference at p< 0.05 compared to the ISO group.

The antioxidant system including enzymatic antioxidants (CAT, GST, GR & HO-1) and non-enzymatic antioxidants (vitamins C and E) are shown in Table 4. The cardiac antioxidative enzymes activities of CAT, GST, GR & HO-1 and the serum vitamins C & E were significantly decreased (P<0.01) in ISO-treated rats as compared to normal control rats. Normal rats administrated only AVOE did not show any significant changes, indicating that AVOE not exert any adverse effects. AVOE pretreatment along with ISO treatment on 29th and 30th day showed significant increases in CAT, GST, GR & HO-1 activities (P < 0.001) as well as vitamins C and E compared to ISO group.

Table 4: Effect of AVOE on catalase, glutathione-s-transferase (GST) and glutathione reductase (GR) activity in the heart of ISO induced myocardial infarction in rats.

<table>
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<th>AVOE</th>
<th>AVOE+ISO</th>
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<tbody>
<tr>
<td>Catalase (U/mg protein)</td>
<td>9.6±1.19</td>
<td>5.2±0.84</td>
<td>9.8±1.07</td>
<td>8.74±0.48</td>
</tr>
<tr>
<td>GST (U/mg protein)</td>
<td>968.7±54.79</td>
<td>677±11.78</td>
<td>979±17.66</td>
<td>858±18.21</td>
</tr>
<tr>
<td>GR (U/mg protein)</td>
<td>35.6±3.38</td>
<td>21.3±1.92</td>
<td>44.8±3.8</td>
<td>31.6±4.5</td>
</tr>
<tr>
<td>HO-1 activity (nmol bilirubin/mg protein)</td>
<td>0.56±0.05</td>
<td>0.25±0.06</td>
<td>0.54±0.03</td>
<td>1.59±0.1</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SE for 10 animals in each group. a: Significant difference at p< 0.05 compared to the normal control group. b: Significant difference at p< 0.05 compared to the ISO group.

As shown in Table 5, the MDA level was significantly increased (P < 0.001) among rats of the ISO group compared to those of the normal control group. Concurrently, a significant decrease (p < 0.001) in the level of nitrite was observed among ISO treated rats compared to the normal control group. Notably, AVOE pretreatment before ISO significantly suppressed the ISO-induced elevation of serum MDA and restored near control levels of nitrite, as compared to the ISO group. In contrast, no significant change in the serum levels of MDA and nitrite was observed among rats receiving AVOE alone when compared to normal control group.
Table 5: Effect of AVOE on serum vitamins C & E, serum malondialdehyde (MDA) and nitrite levels in ISO induced myocardial infarction in rats.

<table>
<thead>
<tr>
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<th>AVOE+ISO</th>
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</thead>
<tbody>
<tr>
<td>Vit. C (mg/dl)</td>
<td>2.46±0.41</td>
<td>0.88±0.09</td>
<td>2.93±0.45</td>
<td>1.96±0.25</td>
</tr>
<tr>
<td>Vit. E (mg/dl)</td>
<td>1.82±0.07</td>
<td>0.94±0.06</td>
<td>1.91±0.04</td>
<td>1.36±0.1</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>3.83±0.32</td>
<td>9.76±0.95</td>
<td>4.08±0.41</td>
<td>7.67±0.61</td>
</tr>
<tr>
<td>Nitrite (μmol/L)</td>
<td>96.77±1.29</td>
<td>64.7±8.18</td>
<td>97.52±1.39</td>
<td>87.9±6.56</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SE for 10 animals in each group. a: Significant difference at p< 0.05 compared to the normal control group. b: Significant difference at p< 0.05 compared to the ISO group.

Effect of avocado on NOS, TNF-α and NF-kB genes expressions

NF-kB and TNF-α expressions were increased while NOS expression was significantly decreased in ISO control rats compared to normal rats. Pretreatment of rats with AVOE before ISO administration, showed a decrease in expressions of these inflammatory genes (NF-kB and TNF-α), while increase in NOS expression level as compared to ISO control rats. (Table 6)

Table 6: Effect of AVOE on NOS, TNF-α and NF-kB, gene expressions on the heart tissue of normal and experimental rats

<table>
<thead>
<tr>
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<th>AVOE+ISO</th>
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<tbody>
<tr>
<td>fold changes of NOS</td>
<td>0.96 ± 0.15</td>
<td>0.54 ± 0.08</td>
<td>1.02 ± 0.2</td>
<td>0.87 ± 0.04</td>
</tr>
<tr>
<td>fold changes of TNF-α</td>
<td>1 ± 0.25</td>
<td>4.8 ± 0.14</td>
<td>0.84 ± 0.18</td>
<td>1.26 ± 0.45</td>
</tr>
<tr>
<td>fold changes of NF-kB</td>
<td>1.2 ± 0.36</td>
<td>6.5 ± 0.64</td>
<td>0.95 ± 0.25</td>
<td>1.6 ± 0.85</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SE for 10 animals in each group. a: Significant difference at p< 0.05 compared to the normal control group. b: Significant difference at p< 0.05 compared to the ISO group.

Effect of avocado on histopathological findings

The protective effect of AVOE on ISO-induced myocardial infarction was observed from histological examination (Figure 1). Histopathological findings of the ISO-group showed infarcted zone with edema, inflammatory cells and separation of muscles fibers (Figure 1b). Pretreatment with AVOE showed mild edema and the myocardial fibers were within normal limits (Figure 1d). AVOE administration to normal rats did not have any histopathological changes in the myocardium (Figure 1c). Figure 1a shows the normal architecture of the rat myocardium.
Medicinal plants are of great importance for both individual and community health. It is well known that medicinal and aromatic plants production is challenging and involves a wide variety of issues, including agricultural, commercial, ecological, pharmacological, as well as social (Vinha et al., 2012).

Following ISO administration, the heart weight increased significantly, with relatively unchanged body weight resulting in the increase of the heart weight to bodyweight ratio. Increase in heart weight might be attributed to increased water content, edematous intramuscular space (Salter and White, 1996). Pre-treatment of AVOE brings down the heart weight to body weight ratio indicative of its protection of myocardium against infiltration and it also could be due to the decrease in water content of the myocardium.

Myocardium contains plentiful concentrations of diagnostic markers of myocardial infarction such as CK-MB, LDH, AST and ALT and once metabolically damaged, it releases its
contents into the extracellular fluid (Prince et al., 2008). In the present study ISO injected rats showed significant elevation in the levels of these marker enzymes in serum, which were in line with the previous reports (Lim et al., 2013; Mehdizadeh et al., 2013) and indication of ISO induced necrotic damage of the myocardium and leakiness of the plasma membrane. AVOE pre-treatment resulted in the lowered activity of the marker enzymes in serum. It demonstrated that AVOE could maintain membrane integrity thereby restricting the leakage of these enzymes.

A significant increase in serum uric acid level was observed in ISO injected rats, which is in line with the previous report (Al-Yahya et al., 2013). Large cohort studies have shown that uric acid is an important independent risk factor for cardiovascular mortality and in the development of MI. During hypoxic condition tissues are disturbed, the enzyme xanthine dehydrogenase is converted to xanthine oxidase by the oxidation of essential-SH-groups. Xanthine oxidase catalyzes the conversion of hypoxanthine to xanthine, uric acid and superoxide (Nivethetha et al., 2009). This could be one of the reasons for the elevated levels of serum uric acid in the present study.

Administration of AVOE can significantly decrease the level of uric acid, this might be the antioxidant property of AVOE which prevents the SH group of enzyme from oxidation and thereby elevated uric acid level.

ISO induced MI is associated with increased levels of lipids in the serum. In this study, we observed increased levels of TC, and TGs in the serum and myocardium of myocardial infarcted rats. Hypercholesterolemia is a risk factor for the development of MI. Increased levels of blood cholesterol and their accumulations in the heart are well associated with myocardial damage (Gokkusu and Mostafazadeh, 2003). Strong evidence suggests that hypercholesterolemia induces oxidative stress by causing a reduction in the enzymatic antioxidant defense potential of tissues and generation of oxygen free radicals like superoxide anions. As a result of these metabolic events peroxidation reactions are accelerated leading to cellular injury (Gokkusu and Mostafazadeh, 2003).

Anandan et al. (2007) have reported that the increase in the myocardial cholesterol content in ISO treated rats is due to increased uptake of low density lipoprotein cholesterol from the blood by myocardial membranes. Accumulation of TGs is one of the risk factors of CVD. The mechanism of observed increase in TGs after MI may be due to elevated flux of fatty acids and impaired removal of very low density lipoprotein (VLDL) from the serum.
Pretreatment with AVOE decreased the levels of TC and TGs in myocardial infarcted rats. These results were in agreement with the previous results, which reported that the hypolipidemic effect of avocado was due to its high contents of monounsaturated fatty acids and phytosterols (Pieterse et al., 2005).

The equilibrium between antioxidants (such as CAT, GST, GR, ascorbic acid and \( \alpha \)-tocopherol) and free radicals is an important process for the effective removal of oxidative stress in intracellular organelles. However, in pathological conditions like myocardial infarction, the generation of reactive oxygen species can dramatically disturb this balance with an increased demand of the antioxidant defense system (Prince et al., 2008).

In the present study, we demonstrated that the decrease in enzymatic and non-enzymatic antioxidant levels in the ISO alone group were significantly returned to near normal level by AVOE pretreatment as compared to normal control group. These results suggested that AVOE could bolster the myocardial antioxidative defense system against oxidative stress which may be attributed to the free radical scavenging activities of the extract of avocado fruits.

HO-1 is recognized as an important target of a number of chemopreventive and cytoprotective agents (Prawan et al., 2005). In the present study, increased activity of HO-1 by AVOE pretreatment was a novel pleiotropic effect of AVOE on heart protection to resist oxidant injury by ISO.

MDA is a major lipid peroxidation end product; increased MDA content may contribute to increased generation of free radicals and/or decreased activities of antioxidant defense system (Liu et al., 2012). In the present study isoproterenol administration resulted in marked elevation in MDA content, which is in line with the previous report (Liu et al., 2012). Oral administration of AVOE to ISO-intoxicated rats tends to bring MDA to near control levels, which could be a result of improved antioxidant status.

Histopathological examination of myocardial tissue in control and AVO-treated rats depicted clear integrity of the myocardial cell membrane. In the ISO-administered group, increased hyalinization, fragmentation of muscle fibers, and myocardial necrosis were observed. Pretreatment with AVOE demonstrated reversal of focal lesions, fragmentation of muscle fibers, and retrogressive lesions with hyaline necrosis in the ISO-treated group,
confirming further the cardioprotective activity of AVOE, these results are in consistent with a previous study (Al-Yahya et al., 2013).

Activation of NF-kB appears to play a significant role in the pathophysiology of endothelial dysfunction, unstable angina pectoris, acute MI, and heart failure (Frantz et al., 2003). Thus, suppression of NF-kB activity can be a potential mechanism for regulating inflammatory responses (Morishita et al., 1997). Upregulation of TNF-α is also observed during ISO-induced MI (Prabhu et al., 2009). Many studies have reported that ISO administration stimulates the proinflammatory cytokine TNF-α (Kumar et al., 2009). In the present study the level of nitrite in blood was significantly increased after AVOE pretreatment to ISO intoxicated rats which may be due to an increased production of NO, as evident from the increased activity of NOS. NO is known to inhibit ROS-mediated reactions, and it has been suggested that the protective effects found in a variety of conditions are due to the ability of NO to detoxify ROS such as superoxid, hydroxyl radical, and/or ferryl hemoprotein (Wink et al., 2001).

Our study showed that AVOE pre-treatment downregulates expressions of NOS, NF-kB and TNF-α in ISO-induced rats, which may be due to a decrease in oxidative stress stimulus via no-mediated protection. This observation suggests that AVOE is effective in preventing inflammatory responses that are triggered by ISO-induced MI.

**CONCLUSION**

In conclusion, our study shows that AVOE exhibits cardioprotective effect in ISO-induced MI rats by decreasing the lipid peroxide products and improving the antioxidant status. This protective effect of AVOE seems to be mediated through its ability to modulate the nitric oxide pathway, and inhibit mRNA expressions of NF-kB and TNF-α due to the antioxidant effect of AVOE. Therefore, AVOE seem to be promising tool to explore therapeutic alternatives in cardiovascular diseases, and a diet containing avocado could prove beneficial to the heart.

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Conflict of Interest Statement
The Authors declare that there is no conflict of interest.

REFERENCES


