Possible cardio-protective effect of ginger and lipoic acid on normal senile female rats

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Cardiovascular disease and oxidative stress are involved in aging. Aging causes many alterations in myochardial functions and metabolism. The aim of this study was to investigate the effect of aging on heart functions, total antioxidant capacity and membrane lipid composition and the effects of oral administration of ginger and lipoic acid for 30 days. The levels of serum lactate dehydrogenase, creatine kinase and heart antioxidant capacity exhibited marked alterations in normal senile female rats. The treatment of ginger and lipoic acid for 30 days improved these alterations. Furthermore, the relationship between membrane lipid composition and aging was detected. There were significant increases in lauric and myristic acids and significant decrease in arachidic acid in senile rats. On the other hand, the unsaturated fatty acids (olic, linoleic, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were significantly decreased. Ginger treatment showed significant decreases in saturated fatty acids. In lipoic acid treated group, there was a significant improvement in the level of unsaturated fatty acids (EPA and DHA) as compared with normal senile female rats. In conclusion, ginger and lipoic acid may have ameliorative effect of heart functions, total antioxidant capacity and membrane free fatty acids in old female rats.

Key words: Ginger, lipoic acid, senile, heart, antioxidant, free fatty acids.

INTRODUCTION

Aging is a major factor for the development of oxidative damage and heart disease (Gao et al., 2013) and associated with fibrosis and inflammation that leads to cellular senescence (Li et al., 2014). Shih et al. (2011) stated that myocardial infarction and chronic heart failure increased with age. Heart is provided with antioxidant systems to scavenge excess reactive oxygen species (ROS), repair oxidative stress and maintain sulphhydril homeostasis (Meyer et al., 2009). Nageswari et al. (1999) demonstrated that myochardial antioxidant status decreased and lipid peroxidation increased with age. Antioxidant reduced the reactive oxygen species which causes carcinogenesis, DNA damage, heart disease and other health problems revealed to advancing age (Yeh et al., 2014).

Ginger is a traditional medicine which processes antiinflammatory, anticancer, antioxidant, antipyretic and antibacterial properties (Kabuto et al., 2005; Chung et al., 2009; Kim et al., 2010; Durak et al., 2015; Hosseini and Mirazi, 2014; Lee et al., 2014; Yeh et al., 2014 and Yudthavorasit et al., 2014). Also, ginger acts as a hypolipidimic factor in which it stimulates the conversion
of cholesterol to bile acids leading to the excretion of cholesterol from the body (Afshari et al., 2007). The biological activities of ginger come from its active chemical component such as gingerols and gingerol related compounds (Chang et al., 2011; Lee et al., 2012; Jafarzadeh et al., 2014; Yudthavorasit et al., 2014). On the other hand, lipoic acid is a natural dithiol compound with an antioxidant activity in which it raises the level of sulfane sulfur and rodanese activity in the tissues (Dudek et al., 2008, 2014). In addition, it plays a role in lipid and carbohydrate metabolism by enhancing glucose transport in muscle cells (Paker et al., 2001; De-Oliveira et al., 2011).

The present study aimed to evaluate the age-related changes on heart tissue in normal female senile rats and the effect of oral administration of two antioxidants ginger and lipoic acid administered for 30 days on the heart functions, antioxidant activity and membrane free fatty acids.

MATERIAL AND METHODS

Experimental animals

Adult female albino rats weighing approximately 130 to 150 g (3 to 4 months old) and senile (24 months old) weighing 280 to 300 g were used. Rats were maintained in iron mesh cages, each cage contained six rats and housed for 10 days prior to the initiation of the experiments, for adaptation to laboratory conditions. Animals were fed with commercial standard rat-pellet and tap water provided ad libitum. Handling and usage of animals agreed strictly with the regulations and guidelines set by the research Ethics Committee of the Ain Shams University authorities and followed Egyptian rules for animal protection, which was performed according to the UK Animals (Scientific Procedures) Act, 1986.

Chemicals

Ginger was purchased from Arab Company for Pharmaceuticals and Medicinal Plants, Egypt (MEPACO) and alpha lipoic acid was purchased from EVA Company, Egypt. All other chemicals and solvents used were of the high performance liquid chromatography (HPLC) and analytical grade. Saturated fatty acids (SFAs), lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0) and arachidic (C20:0). Unsaturated fatty acids (USFAs), oleic (C18:1 ω–9), linoleic (C18: 2 ω–6), cis-9,12-14,17-eicosapentaenoic (C20:5 ω–3) (EPA) and cis-4,7,10,13,16,19-docosahexaenoic acid (DHA) (22:6 ω–3). All FAs purchased from Sigma-Aldrich Co. (St. Louis, USA). All other chemicals and solvents used were of the HPLC and analytical grade.

Experimental design

The animals were divided into four groups each containing six rats as follows: the first group was the control adult rats and received orally 0.1 ml/100 g b.wt. carboxy-methyl cellulose sodium salt (0.5% CMC). The second group was the senile rats and received the same amount of CMC. The third group, senile rats administrated ginger at a dose of 250 mg/kg body weight and dissolved in CMC vehicle. The fourth group was senile rats administrated alpha-lipoic acid (ALA) at a dose of 65 mg/kg body weight and dissolved in CMC. All groups received treatments for 30 days. Doses were calculated in relation to the human therapeutic dose according to Reagan-Shaw et al. (2008).

Biochemical assay

At the end of the experiments, the rats were sacrificed after 12 h from the last dose by rapid decapitation. Heart was excised for the determination of free fatty acids by GC according to the method of Firlag et al. (2013). Serum was collected for determination of creatine kinase MB (CKMB) according to the method of Young (1997), lactate dehydrogenase (LDH) according to the method of Van der heiden et al. (1994), and total antioxidant capacity according to the method of Koracevic et al. (2001).

Statistical analysis

Reported values represent means ± standard error (SE). Statistical analysis was evaluated by one-way analysis of variance (ANOVA). Once a significant F-test was obtained, least significant difference (LSD) comparisons was performed to assess the significance of differences among various treatment groups. Statistical processor system support “SPSS” for Windows software, Release 12.0 (SPSS, Chicago,IL) was used.

RESULTS

Table 1 shows the effects of ginger and lipoic acid administration on serum lactate dehydrogenase, serum creatine kinase-MB, total antioxidant capacity, saturated fatty acids and unsaturated fatty acids levels in heart tissue of adult and senile female rat groups. The data demonstrated significant decrease in serum lactate dehydrogenase and heart tissue total antioxidant activity in senile female rats compared with that of the adult group. These levels increased significantly in ginger and lipoic acid treated senile group in comparison with senile rats. Significant increases was observed in serum creatine kinase-MB level in senile groups and this increase diminished after the treatment with ginger or lipoic acid as compared with adult control and ginger treated groups.

The results of saturated fatty acids (lauric acid, myristic acid, stearic acid and arachidic acid) and unsaturated fatty acids (palmitic, olic, linoleic, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) represented in Table 1 showed significant increase in lauric and myristic acids and a significant decrease reported in arachidic acid in senile group. While in ginger treated rats, there were significant decrease in lauric acid and arachidic acid as compared with normal senile group. Generally, there were a significant decrease in all saturated fatty acids in ginger treated rats as compared with adult control, senile and lipoic acid treated groups.

After 4 weeks of treatment, unsaturated fatty acids (olic, linoleic, EPA and DHA acids) and total unsaturated free fatty acids decreased significantly in senile group. The content of all unsaturated fatty acids
Table 1. Effect of ginger and lipoic acid on lactate dehydrogenase (LDH U/L), creatine kinase-MB (CKMB U/L), total antioxidant activity (AOA mMol/L), polyunsaturated fatty acids and saturated fatty acids content (mg/g wet heart tissue) in all groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Aged</th>
<th>Ginger</th>
<th>Lipoic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH</td>
<td>1471.59±58.91</td>
<td>1150.14±60.78a</td>
<td>1552.48±43.80b</td>
<td>1493.14±26.36b</td>
</tr>
<tr>
<td>CKMB</td>
<td>904.35±38.36</td>
<td>1111.83±39.63a</td>
<td>936.01±37.37ab</td>
<td>960.74±47.97ab</td>
</tr>
<tr>
<td>AOA</td>
<td>2.9795±0.0879</td>
<td>1.9035±0.0519a</td>
<td>2.4298±0.0906b</td>
<td>2.7047±0.1159bc</td>
</tr>
<tr>
<td>(C12:0) lauric acid</td>
<td>0.165±0.1422</td>
<td>0.3492±0.1975a</td>
<td>0.22±0.0213b</td>
<td>0.340±0.523ac</td>
</tr>
<tr>
<td>(C14:0) myristic acid</td>
<td>0.202±0.246</td>
<td>0.322±0.0338a</td>
<td>0.273±0.0166</td>
<td>0.017±0.0319abc</td>
</tr>
<tr>
<td>(C18:0) stearic acid</td>
<td>0.561±0.043</td>
<td>0.456±0.0149a</td>
<td>0.516±0.0410</td>
<td>1.0197±0.0615abc</td>
</tr>
<tr>
<td>(C16:0) palmitic acid</td>
<td>8.491±8.491</td>
<td>11.479±0.0149a</td>
<td>5.798±0.442ab</td>
<td>11.280±0.323abc</td>
</tr>
<tr>
<td>(C20:0) arachidic acid-mg/g</td>
<td>1.106±0.223</td>
<td>0.384±0.0423a</td>
<td>0.996±0.0114a</td>
<td>0.679±0.0394abc</td>
</tr>
<tr>
<td>Saturated fatty acids</td>
<td>10.526±0.727</td>
<td>12.989±0.715a</td>
<td>6.908±0.475ab</td>
<td>13.828±0.407ac</td>
</tr>
<tr>
<td>(C18:1 ω–9) olic acid</td>
<td>9.618±1.187</td>
<td>2.373±0.360a</td>
<td>0.948±0.064a</td>
<td>2.438±0.282a</td>
</tr>
<tr>
<td>(C18: 2 ω–6) linoleic acid</td>
<td>15.548±0.257</td>
<td>12.084±0.347a</td>
<td>8.066±0.699ab</td>
<td>12.784±0.432ac</td>
</tr>
<tr>
<td>(C20:5 ω–3) eicosapentanoic acid</td>
<td>15.096±0.536</td>
<td>9.393±0.303a</td>
<td>11.441±0.454ac</td>
<td>17.896±0.723bc</td>
</tr>
<tr>
<td>Polyunsaturated fatty acids</td>
<td>294.448±10.766</td>
<td>237.167±10.748a</td>
<td>245.413±10.748a</td>
<td>299.535±10.738bc</td>
</tr>
</tbody>
</table>

Values are means of 6 rats± SE. a = significant change from control, b = significant change from senile rats and c = significant change from senile rats treated with ginger at p ≤ 0.05.

decreased after the treatment with ginger except in EPA and DHA which increased insignificantly as compared with adult control group. In lipoic acid treated group, there were significant improvements in the levels of EPA and DHA as compared to senile and ginger treated group and the results reached to the values of the control group.

DISCUSSION

Aging is a major risk factor of heart failure that is associated with an increment myocardial rigidity, impaired ventricular filling, coronary artery disease and oxidative dysfunction (Sample et al., 2006). Lactate dehydrogenase (LDH) and creatine kinase (CK) are important enzymes participating in transferring the lactic acid to pyruvate and formation of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) in anaerobic systems and they are also known as indicators of oxidative stress (Nikbakht et al., 2014). The experimental results recorded a significant decline in serum lactate dehydrogenase and a significant increase in creatine kinase-MB (CK-MB) levels in normal senile female rats. Sample et al. (2006) investigated the effects of aging on the profile of myocardial substrate utilization and cardiac function and stated that there was a marked decline in cardiac function and efficiency. Also, Anitha and Asha Devi (1996) and Prathima and Asha Devi (1999) reported significant decreases in total myocardial LDH activity and LDH isoenzyme profile in aged rats. Aging heart is thought to have many features in terms of remodeling and adaptation such as changes in the myocardial energy substrate profile, a decline in fatty acid oxidation and an increase in carbohydrate metabolism, oxidative function disturbance, metabolic reconstruction, mitochondrial dysfunction, oxidative damage and alteration in the plasma membrane integrity (Chuffa and Seiva, 2013; Sample et al., 2006; Cordero-Reyes et al., 2014; Chitra et al., 2013).

Oxidative stress is associated with several pathological conditions including aging (Bonnefont-Rousselot and Collin, 2010; Guney et al., 2013). Also, the increment of mitochondrial reactive oxygen species (ROS) in aged heart has been reported by Dröge (2002) and Turrens (2003) and the endogenous mitochondrial antioxidant defenses may be diminished with age. Current study recorded a significant decrease in total antioxidant activity in normal senile female rats. Our result is in agreement with Youdim and Deans (1999a) and Asha-Devi et al. (2003). Youdim and Deans (1999b) indicated that total myocardial antioxidant status decreased significantly in old rats. Free radicals induced lipid peroxidation proposed as an etiologic factor in cell membrane damage, atherosclerosis, cancer and aging (Nageswari et al., 1999). Aging results in changes related to molecular and functional alteration in the properties of biological membranes (Vazquez-Memije et al., 2005). Esterbauer et al. (1991) found that the release of polyunsaturated fatty acids from membrane phospholipids undergo lipid peroxidation by reacting with ROS to produce various aldehydes, alkenals and alkenes. The present study represents the relationship between membrane lipid composition and aging.

Data analysis reveals a distinct age dependent decrease in the total polyunsaturated fatty acids...
(especially EPA and DHA) accompanied by an increase in saturated fatty acids content in senile female group. The increment in the level of saturated fatty acids may be due to increased lipolysis (Nageswari et al., 1999). Herrero et al. (2001) showed that long lived animals have lower fatty acid double bond content in their mitochondrial membranes than short-lived ones. This is due to a decrease in the process of unstauration. The degree of in vitro lipid peroxidation increases as the process of unstauration of fatty acid substrates increase (Bondy and Marwah, 1995). Therefore, we can say that, the decrease in fatty acid double bond content of the mitochondria of long-lived animals will protect them against lipid peroxidation (Herrero et al., 2001). Linoleic acid levels were inversely associated with age and decreasing about 3% per year up until age 70 (Harris et al., 2013). The n-3 class EPA and DHA accumulated in the phospholipids in our membranes especially in brain, heart and testes (Leaf et al., 2003).

Harris et al. (2013) mentioned that, n-3 fatty acids EPA and DHA decreases in red blood cell (RBC) membranes have been associated with cardiovascular disease, neuropsychiatric diseases and cellular aging. The reduction in unsaturated fatty acids may be attributed to a reduction in the number of myocytes, reduced efficiency in mechanisms to detoxify ROS, alteration of metabolic, ionic and electrical properties of myocytes which characterize aged myocardium (Anversa and Sonnenbikk, 1990; Olivetti et al., 1991; Lakatta, 1992; Lakatta, 1993; Walker et al., 1993). Since oxidative stress in myocardium is a factor that contributes to aging, antioxidant treatment is considered to be a potential strategy for prevention of heart aging. Ginger plant and its single constituent such as 6-gingerol, 6-paradol and zingerone plays an effective role against lipid peroxidation (Ippoushi et al., 2003; Chrubasik et al., 2005). Our results revealed that ginger administration for 30 days have an amelioration effect on heart functions, total antioxidant activity and free fatty acids regarding to aged control group. Ahmed et al. (2000) and Liu et al. (2003) reported that rats which received a diet with ginger showed an increase in glutathione and decrease in plasma lipid peroxide levels. Also, mice fed ginger oil by gavage for 14 days showed significant elevation in glutathione s-transferase and aryl hydrocarbon hydroxyylase (Chrubasik et al., 2005).

Moreover, many studies showed that ginger causes an increase in plasma antioxidant level and decrease lipid peroxidation (Afshari et al., 2007; Nicoll and Henein, 2007). Free radicals and oxidative stress causes oxidation of polyunsaturated fatty acids (PUFA) which embedded in the cell membrane (Afshari et al., 2007). Oxidized arachidonic acid metabolites such as the cyclooxygenase and lipoxygenase products cause arterial inflammation and heart disease. Ginger constitutes inhibit the production of arachidonate -5-lipoxygenase, prostaglandins and leukotrienes from the cyclooxygenase and lipoxygenase, respectively (Grzanna et al., 2005; Nicoll and Henein, 2007; Mozaffari-Khosravi et al., 2014). Also, ginger plant and its constituents protects against linoleic acid peroxidation and reduced atherosclerotic lesions (Chrubasik et al., 2005; Nicoll and Henein, 2007).

The present study evidenced that the lipoic acid treatment improved heart functions, total antioxidant activity and unsaturated fatty acids (EPA and DHA) as compared to normal senile female rats. Many studies have reported the antioxidant and cardioprotective properties of lipoic acid (Smith et al., 2004; Ghibu et al., 2009). Sokolowska et al. (2014) returned the antioxidant activity of lipoic acid to its normalizing effect on the antioxidant status in cardiomyocytes, restoration of normal catalase activity, increase the level of cysteine, cystathionase, mercaptopyrovate sulfurtransferase activation and activation of aldehyde dehydrogenase 2. Moreover, Dudek et al. (2014) reported that lipoic acid elevated the level of sulfane sulfur which played an important role in the release of hydrogen sulfide. Hydrogen sulfide is an endogenous signaling molecule which activates potassium ATP-sensitive channels of cardiovascular system. It can affect blood pressure, vasodialation, protects heart against ischemia-reperfusion injury and has antioxidant activity (Dudek et al., 2014; Ji et al., 2008; Bian et al., 2006; Szabo et al., 2011).

Conclusion

From the present results, ginger and lipoic acid showed ameliorative effect in ageing that induced significant changes in heart functions and total antioxidant deficiency in heart tissues of female aged rats through decreasing oxidation of membrane free fatty acids and reduction of oxidative stress.

Conflict of interest

The authors have declared that there is no conflict of interest.

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