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FULL ARTICLE

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Strawberry fruit (*Fragaria x ananassa* cv. Romina) extenuates iron-induced cardiac oxidative injury via effects on redox balance, angiotensin-converting enzyme, purinergic activities, and metabolic pathways

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Abstract

The potential cardioprotective properties of strawberry fruit (*Fragaria x ananassa*) (SF) were investigated in cardiac tissues ex vivo. Oxidative injury was induced by incubating freshly harvested cardiac tissue homogenates from healthy Sprague Dawley male rats with 0.1 mM $FeSO_4$ for 30 min at 37°C. The induction of oxidative injury resulted in depleted levels of glutathione, superoxide dismutase, catalase, E-NTPDase activities, and HDL-c, while elevating the levels of malondialdehyde, angiotensin-converting enzyme, acetylcholinesterase, ATPase, lipase activities, cholesterol, triglyceride, and LDL-c. Co-incubation with SF significantly reversed these levels and activities with concomitant depletion of oxidative-induced metabolites and reactivation of oxidative-inactivated pathways, while limiting beta-oxidation of very long chain fatty acids and mitochondrial beta-oxidation of medium-chain saturated fatty acids pathways. These data portray the potential cardioprotective effects of strawberry fruits against oxidative-induced cardiopathy via the attenuation of oxidative stress, inhibition of ACE and acetylcholinesterase activities, and modulation of lipid dysmetabolism.

Practical applications

Fruits and other fruit-based products have been enjoying wide acceptability among consumers due to their immense medicinal benefits particularly, on cardiovascular health. Strawberries are among the common fruits in the world. Over the years, cardiovascular diseases have been known to contribute greatly to global mortality irrespective of age. This study reports the potentials of strawberry fruits to protect against oxidative mediated cardiovascular dysfunctions. Thus, the fruits can be utilized as a cheap alternative for the development of nutraceuticals for maintaining cardiac health.

KEYWORDS

cardioprotection, lipid dysmetabolism, oxidative injury, strawberry fruit

1 | INTRODUCTION

Over the years, there have been increased cases of cardiopathies; and the cardiac energy metabolism defects leading to cardiomyopathy were reported as major mechanisms (Antozzi & Zeviani, 1997; Erukainure et al., 2020). These defects include mitochondrial oxidative phosphorylation and lipid metabolism notably the β-oxidation pathway (Erukainure et al., 2020). Initiation of oxidative stress has associated with cardiac energy defects and has been related to the pathogenesis of cardiopathy (Gammella, Recalcati, Rybinska, Buratti, & Cairo, 2015). This arises from the exacerbated production of reactive oxygen species (ROS) which outweigh the cellular endogenous antioxidant defenses. Oxidative stress has been linked with increased activities of cardiopathy and cardiovascular dysfunction markers such as cardiac lipase, cardiac lipid dysmetabolism, angiotensin-converting enzyme (ACE), and acetylcholinesterase (De Blasio et al., 2015; Erukainure et al., 2020; Huynh et al., 2013). Studies have linked the modulation of these markers to antioxidant therapies (Erukainure et al., 2020; Wojciechowska et al., 2014).

Fruit consumption has been linked with good health and has been part of human diets from time immemorial. They have been acknowledged as a major source of nutrients particularly minerals and vitamins and are often parts of therapeutic diets (Karasawa & Mohan, 2018; Shibumon & Benny, 2010). Fruits have also been reported for their medicinal properties. This is evidenced by several epidemiological studies which associate high intake of fruits with low risks of chronic diseases including cardiovascular diseases, cancer, diabetes, and neuronal dysfunctions (Karasawa & Mohan, 2018; Pérez, del Castillo, Gil, Blanch, & Flores, 2014). These properties have been associated with their phytochemical constituents particularly polyphenols (Zhao et al., 2017). An example of these fruits is the *Fragaria x ananassa*.

Commonly known as strawberry, *F. x ananassa* is among the most popular fruits globally. It belongs to the *Fragaria* genus and the Rosaceae family. It is an important part of the Mediterranean diet and has been reported for its nutritional and medicinal properties (Giampieri et al., 2012). The fruits are rich in calcium, magnesium, phosphorus, zinc, copper, manganese, selenium, vitamins A, C, E, and K, folate, thiamin, riboflavin, niacin, and polyphenols, with anthocyanin being the most prominent (Aaby, Skrede, & Wrolstad, 2005; Giampieri et al., 2012). Other phenolics present are the hydrolyzable tannins, flavonols, flavanols, and phenolic acids (Aaby et al., 2005; Giampieri et al., 2012). The presence of these micronutrients and phenolics has been correlated with the antioxidant, antidiabetic, anticancer, and anti-inflammatory activities including other medicinal properties of strawberries (Afrin et al., 2016; Fortalezas et al., 2010; Giampieri et al., 2012).

The cardioprotective activities of phenolics are well documented. This has been attributed to their ability to mop free radicals as well as improve cardiac antioxidant defense system (Céspedes, El-Hafidi, Pavon, & Alarcon, 2008; Wu et al., 2001). Amongst these phenolics is gallic acid. Gallic acid and its derivatives are widely distributed in fruits and vegetables and like other phenolics have been reported for their potent antioxidant properties (Choubey, Varughese, Kumar, & Beniwal, 2015). These antioxidant properties including iron chelation have been utilized in the treatment and management of various ailments including diabetes, cardiovascular diseases, and cancer (Choubey et al., 2015; Perron & Brumaghim, 2009). Their ability to modulate the antioxidant endogenous defense system and mitigate peroxidative activities as well as the restoration of myocardial cells in cardiopathy have also been reported (Kahkeshani et al., 2019; Kulkarni & Swamy, 2015; Patel & Goyal, 2011).

This present study aims at investigating the cardioprotective properties of strawberry fruits on oxidative cardiopathy by investigating its ameliorative effect on oxidative imbalance, and its ability to modulate ACE, cholinergic and purinergic activities, and cardiac lipid metabolism using gallic acid as the reference antioxidant standard.

2 | MATERIALS AND METHODS

2.1 | Chemicals

Chemicals were purchased from Sigma-Aldrich (Johannesburg, South Africa). Ferrous Sulphate ($FeSO_4$), trichloroacetic acid (TCA), 5,5′-dithio-bis-[2-nitrobenzoic acid] (DTNB), 6-hydroxydopamine (6-HD), diethylenetriaminepentaacetic acid (DETAPAC), thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), acetic acid, malondialdehyde (MDA), dichromate solution, hydrogen peroxide, disodium hydrogen sulphate dodecahydrate, sodium phosphate monobasic dihydrate, N-[3-(2-Furyl) acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG), acetylcholine iodide, potassium chloride, ascorbic acid, ammonium molybdate, Tris-HCl, adenosine triphosphate (ATP), calcium chloride ($CaCl_2$), sucrose, glucose, ethylenediaminetetraacetic acid (EDTA), p-nitrophenyl butyrate in dimethylformamide (p-NPB), gallic acid, and chloroform.

2.2 | Preparation of the strawberry fruit extract

Strawberry fruits were purchased from an organic food mall in Durban, South Africa. The fruits (200 g) were rinsed in tap water and blended with a glass jug blender with stainless steel blades (Russel Hobbs, Johannesburg, South Africa). The resulting fruit paste was transferred to 50 ml centrifuge tubes and centrifuged for 15 min at 10,000 rpm and 4°C. After centrifuging, the supernatants were transferred into glass beakers and concentrated at 50°C in a water bath. The percentage yield after concentrating was 33%. Concentrated samples were then stored in glass vials at 2°C.

2.3 | Animals

Four male albino rats of Sprague Dawley strain weighing about 200–250 g were obtained from the Biomedical Research Unit (BRU), University of KwaZulu-Natal, Durban, South Africa. The

rats were collected a day to the experiment and fasted overnight (12 hr). After fasting, animals were euthanized by an overdose of isofluorane (Isofor, Safeline Pharmaceuticals, South Africa) in an anesthetic chamber and hearts were immediately harvested and placed in 0.9% NaCl solution to rinse off blood stains. The hearts were chopped into pieces with a stainless-steel scissors before homogenizing in 50 mM sodium phosphate buffer (pH 7.5; containing 1% Triton X-100). The homogenized tissue samples were centrifuged for 10 min at 15,000 rpm and 4°C. The supernatants were collected into Eppendorf tubes and used immediately for ex vivo studies.

The present study was performed under the approved guidelines of the Animal Ethics Committee of the University of KwaZulu-Natal, Durban, South Africa (Protocol approval number: AREC/020/017D).

2.4 | Induction of oxidative cardiac injury ex vivo

An aliquot of 100 µl of the cardiac tissue supernatant was incubated with varying concentrations (30, 60, 120, and 240 µg/ml) of the fruit extracts and 30 µl of 0.1 mM $FeSO_4$ (pro-oxidant; final concentration: 0.02 mM) for 30 min at 37°C (Erukainure et al., 2020). Negative control (untreated) was a reaction mixture without extracts, while normal control was a reaction mixture without the extract and pro-oxidant.

2.5 | Activities

2.5.1 | Determination of the reduced glutathione (GSH) level

The GSH level of the experimental cardiac tissues was determined using the Ellman's method (Ellman, 1959). Briefly, the tissues were deproteinized with 10% TCA and centrifuged for 5 min at 3,500 rpm. An aliquot of 200 µl of the supernatants was collected into a 96 well plate, and 50 µl of Ellman reagent was thereafter added. Absorbance was measured at 415 nm and the GSH level of the tissues was extrapolated from a standard curve of plotted GSH concentrations.

2.5.2 | Determination of superoxide dismutase (SOD) activity

An established protocol based on the oxidation of 6-hydroxydopamine (6-HD) by H_2O_2 from SOD catalyzed dismutation of $O_2^{\cdot-}$ (Gee & Davison, 1989). Briefly, 15 µl of the tissue samples was mixed with 170 µl of 0.1 mM diethylenetriaminepentaacetic acid (DETAPAC) in a 96-well plate. Thereafter, 15 µl of 1.6 mM 6-HD was added to the reaction mixture and the absorbance was measured at 492 nm at 1 min interval for 5 min.

2.5.3 | Determination of catalase activity

The catalase activity of the experimental cardiac tissue was determined using a previously described protocol (Aebi, 1984). Briefly, an aliquot of about 10 µl of the tissue samples was mixed with 340 µl of 50 mM sodium phosphate buffer (pH 7.0), and then incubated with 150 µl of 2M H_2O_2 for 5 min. Absorbance was read at 240 nm at 1 min interval for 3 min.

2.5.4 | Determination of lipid peroxidation levels

The lipid peroxidation levels of the experimental cardiac tissues were determined by analyzing the thiobarbituric acid reactive substances (TBARS) which is expressed as the malondialdehyde (MDA) level (Chowdhury & Soulsby, 2002). Briefly, 100 µl of the supernatants was mixed with 375 µl of 20% acetic acid, 100 µl of 8.1% SDS solution, and 1 ml of 0.25% thiobarbituric acid (TBA). The reaction mixture was boiled for 1 hr. About 200 µl of the boiled mixture was transferred into 96-well plate, and absorbance measured at 532 nm. The MDA level of the tissues was then extrapolated from a standard curve of plotted MDA concentrations.

2.6 | Determination of ACE activity

The ACE activity of the experimental cardiac tissue was determined spectrophotometrically using N-[3-(2-Furyl) acryloyl]-L-phenylalanyl-glycyl-glycine (FAPGG) as a substrate (Holmquist, Bünning, & Riordan, 1979) with slight modifications (Erukainure et al., 2020). Briefly, 20 µl of the tissue samples was mixed with 100 µl of 0.5 mM FAPGG and incubated at 37°C for 10 min. Absorbance was read at 2 min intervals at 345 nm. The activity was measured as the rate of reaction (ΔA/min) as expressed below:

$$\text{ACE activity} = \frac{(\text{AI} - \text{AF})\ \text{Sample} - (\text{AI} - \text{AF})\ \text{Blank}}{\text{Time interval (min)}}$$

where AI = initial absorbance; AF = final absorbance.

2.7 | Acetylcholinesterase activity

Acetylcholinesterase activity of the experimental cardiac tissues was determined using the Ellman's method (Ellman, Courtney, Andres, & Featherstone, 1961). Briefly, 20 µl of the tissue samples was incubated with 10 µl of 3.3 mM Ellman's reagent (pH 7.0) and 50 µl of 0.1 M phosphate buffer (pH 8) for 20 min at 25°C. About 10 µl of 0.05 M acetylcholine iodide was added to the reaction mixture to stop the reaction. Absorbance was thereafter read at 412 nm at 3 min intervals.

2.8 | Purinergic enzymes activities

2.8.1 | Determination of ATPase activity

The ATPase activities of the experimental cardiac tissues were determined according to the previously described method (Adewoye, Bolarinwa, & Olorunsogo, 2000; Erukainure, Mopuri, Oyebode, Koorbanally, & Islam, 2017). Briefly, 200 µl of the tissue samples was mixed with 200 µl of 5 mM KCl, 1,300 µl of 0.1 M Tris-HCl buffer, and 40 µl of 50 mM ATP. The reaction mixture was incubated in a shaker for 30 min at 37°C. To stop the reaction, 1 ml of distilled water and 1.25% ammonium molybdate were added to the mixture. Thereafter, 1 ml of freshly prepared 9% ascorbic acid was added to the mixture and allowed to stand for 30 min. Absorbance was read at 660 nm.

2.8.2 | Determination of E-NTPDase activity

The E-NTPDase activity of the experimental cardiac tissues was determined according to the modified previously reported methods (Ademiluyi, Ogunsuyi, & Oboh, 2016; Schetinger, Morsch, Bonan, & Wyse, 2007). Briefly, 20 µl of the tissue samples was incubated with 200 µl of the reaction buffer (1.5 mM $CaCl_2$, 5 mM KCl, 0.1 mM EDTA, 10 mM glucose, 225 mM sucrose and 45 mM Tris-HCl) for 10 min at 37°C. 20 µl of 50 mM ATP was added to the reaction mixture and incubated at 37°C for 20 min. About 200 µl of 10% TCA was added to the mixture to stop the reaction. The mixture was incubated in ice for 10 min and absorbance measured at 600 nm.

2.9 | Lipase activity

The lipase activity of the experimental cardiac tissues was determined using a slightly modified previous protocol (Erukainure et al., 2020; Kim et al., 2010). Briefly, 100 µl of the tissue samples was mixed with 169 µl of Tris buffer (100 mM Tris–HC1 and 5 mM $CaCl_2$, pH 7.0) and incubated at 37°C for 15 min. About 5 µl of 10 mM p-NPB (p-nitrophenyl butyrate in dimethylformamide) was then added to the reaction mixture and again incubated for 15 min at 37°C. The absorbance was measured at 1 min interval at 405 nm. Lipase activity was expressed as the rate of reaction (ΔA/min).

2.10 | Cardiac lipid profile

The lipid profile of the cardiac tissues was determined by incubating the tissue supernatants with 0.1 mM $FeSO_4$ and 240 µg/ml of the extract or gallic acid as described previously but overnight (12 hr) (Erukainure et al., 2020). The highest dose (240 µg/ml) of the fruit extract was chosen as it gave the best activities compared to the other doses. The incubated samples were centrifuged at 15,000 rpm for 10 min at 4°C. The supernatants were decanted and used immediately to assay for lipid profile levels which covers for total cholesterol, triglycerides, and HDL-cholesterol using an Automated Chemistry Analyzer (Labmax Plenno, Labtest Co. Ltd., Lagoa Santa, Brazil) with commercial assay kits according to the manufacturer's manual.

2.11 | Extraction and profiling of cardiac lipid metabolite

A reaction mixture was prepared as explained above. After incubation, the cardiac lipid metabolites were extracted from the tissue samples and profiled with GC-MS (Erukainure et al., 2020; Ralston-Hooper, Jannasch, Adamec, & Sepúlveda, 2011). Briefly, the incubated samples were mixed with cold chloroform at ratio 5:1 and vortexed for 1 min. The mixture was then centrifuged at 15,000 rpm at 4°C for 10 min to yield two liquid phases, after allowing to stand on ice for 20 min. The lower phase (chloroform) layer which contains non-polar metabolites including lipids was collected and subjected to GC-MS profiling.

2.12 | GC-MS analysis of extracted metabolites

The extracted non-polar metabolites were profiled with GC-MS (Agilent technologies 6890 series GC coupled with (an Agilent) 5973 Mass Selective Detector and driven by the Agilent Chemstation software). The operating parameters were as follows: **Column**: HP-5MS capillary column (30 m × 0.25 mm ID, 0.25 µm film thickness, 5% phenylmethylsiloxane); **Carrier gas**: ultra-pure helium; **Flow rate**: 1.0 ml/min and a linear velocity of 37 cm/sec; **Injector temperature**: set at 250°C. Initial oven temperature: 60°C, programmed to 280°C at the rate of 10°C/min. **Injection**: 1 µl made in split mode at a split ratio of 20:1; **Electron ionization mode**: 70 eV; **Electron multiplier voltage**: at 1,859 V; **Ion source temperature**: 230°C; **Quadrupole temperature**: 150°C; **Solvent delay**: 4 min; **Scan range**: 50–70 amu. Lipid metabolites were identified using an inbuilt NIST library.

2.13 | Metabolic pathway analysis

The most relevant pathways involved in lipid metabolism in the experimental cardiac tissues were determined by subjecting the identified metabolites to pathway enrichment analysis using the MetaboAnalyst 4.0 online server (Chong et al., 2018).

2.14 | GC-MS analysis of strawberry fruits

The extracted fruit sample was subjected to GC-MS profiling to identify its phytochemical constituents that are likely responsible for the studied biological activities. The operating conditions were the same as above.

2.15 | Statistical analysis

Significant differences between means were measured at $p < .05$ with the use of the Tukey's HSD-multiple range post hoc test and data presented as mean ± *SD* after subjection to one-way analysis of variance (ANOVA). Statistical analyses were carried out with IBM Statistical Package for the Social Sciences (SPSS) for Windows, version 23.0 (IBM Corp., Armonk, NY, USA).

3 | RESULTS AND DISCUSSION

Consumption of fruits has been correlated with decreased cases of cardiovascular dysfunctions and diseases, with their phytochemical and nutritional constituents playing synergistic roles in bringing about these cardioprotective effects (Karasawa & Mohan, 2018; Pérez et al., 2014; Zhao et al., 2017). In this study, the cardioprotective effect of strawberry fruits was investigated on iron-induced oxidative cardiopathy ex vivo.

Figure 1 shows a significant ($p < .05$) depletion in the GSH level, SOD and catalase activities as well as elevated MDA level on induction of oxidative injury in the experimental cardiac tissues. These changes in the levels and activities of oxidative biomarker indicate a redox imbalance which portrays an oxidative state and can be linked to the iron-mediated generation of ROS by Fenton's and Haber Weiss reactions (Latunde-Dada, 2017). Oxidative stress has been reported in cardiopathies (Huynh, Bernardo, McMullen, & Ritchie, 2014). Its implication in DNA fragmentation, altered signal transduction, activation of apoptotic, and necrotic pathways in myocardial cells have been reported (Cai & Kang, 2001). On treatment with strawberry fruits, there was a significant ($p < .05$) elevation of GSH level, SOD and catalase activities, with concomitant depletion of MDA level. These reversions portray an antioxidative effect of the fruit and corroborate previous reports on its antioxidant activities (Barros, Carvalho, Morais, & Ferreira, 2010; Szajdek & Borowska, 2008). They also corroborate earlier reports on the therapeutic effects of antioxidants in the treatment and management of cardiovascular dysfunctions (Cai & Kang, 2001; Huynh et al., 2013).

The ACE activity of the cardiac tissues significantly ($p < .05$) increased on the induction of oxidative injury as depicted in Figure 2. Elevated ACE activity in cardiac tissues has been linked to the vasoconstrictive effect of angiotensin II (ANG II) (Agunloye et al., 2019), with oxidative stress acting as a trigger (Landmesser & Drexler, 2003; Usui et al., 1999). The increased activity of ACE in the experimental cardiac tissues was significantly ($p < .05$) depleted on treatment with strawberry fruits. The depleted activity corroborates previous reports on strawberry fruits as potent ACE-inhibitors (Cheplick, Kwon, Bhowmik, & Shetty, 2010; da Silva Pinto, de Carvalho, Lajolo, Genovese, & Shetty, 2010). Inhibition of ACE activity has been demonstrated to be beneficial in the treatment and management of cardiovascular dysfunctions (Flather et al., 2000; Nikolaidis, Doverspike, Huerbin, Hentosz, & Shannon, 2002).

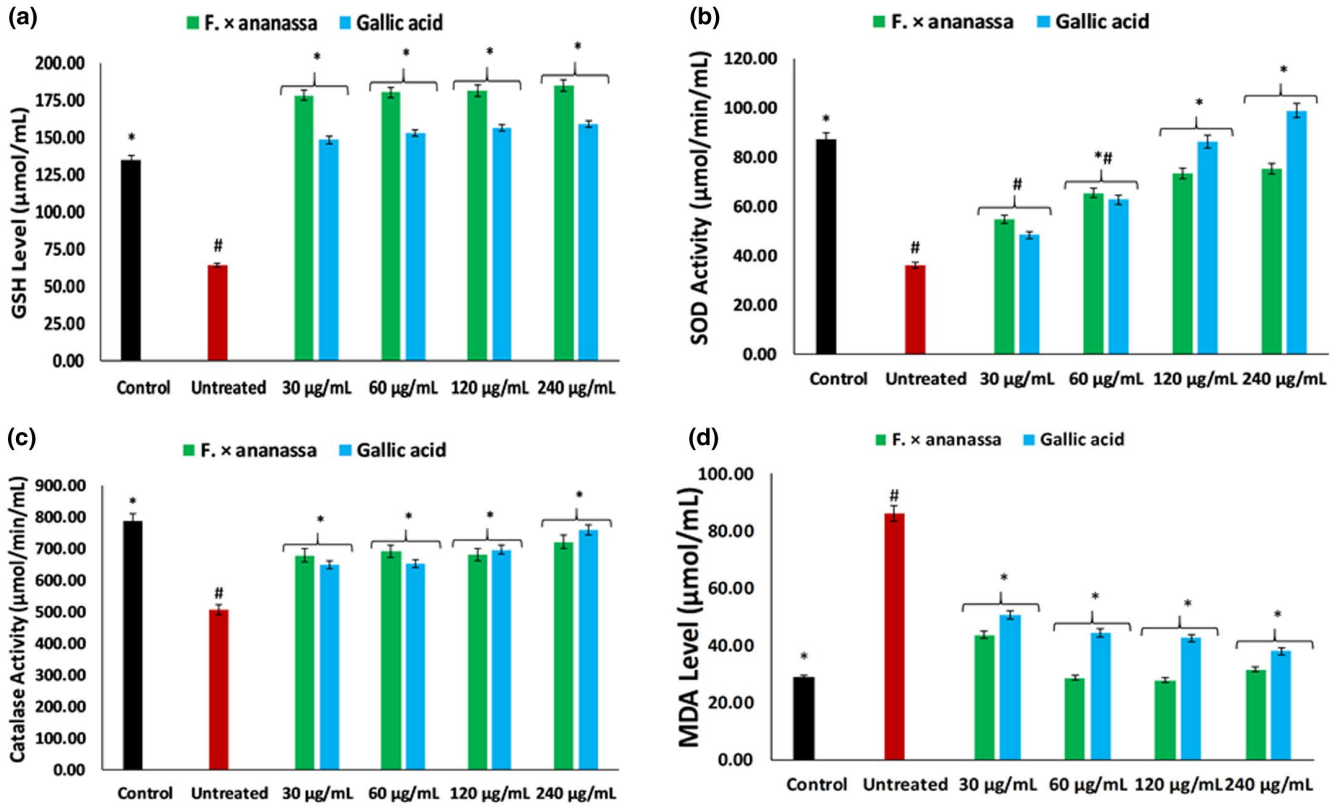

FIGURE 1 Effect of strawberry fruit (*F. x ananassa*) on (a) GSH level, (b) SOD, (c) catalase and (d) MDA level in oxidative cardiopathy. Data = mean ± *SD*; $n = 3$. *Statistically significant compared to untreated tissue; #statistically significant compared to normal tissue

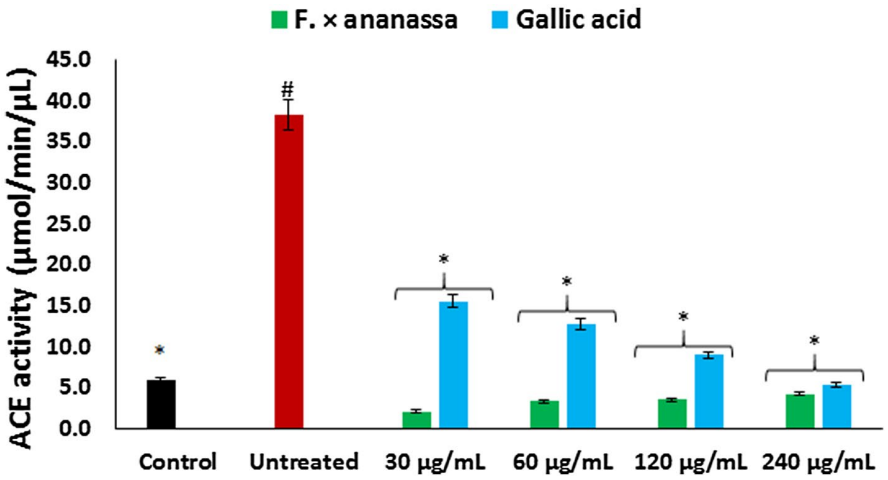

FIGURE 2 Effect strawberry fruit (*F. x ananassa*) on ACE activity in oxidative cardiopathy. Data = mean ± *SD*; *n* = 3. *Statistically significant compared to untreated tissue; #statistically significant compared to normal tissue

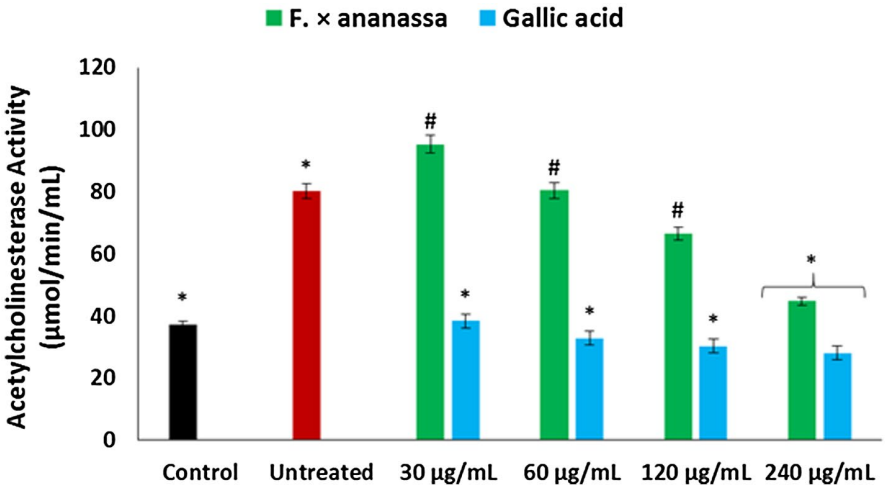

FIGURE 3 Effect of strawberry fruit (*F. x ananassa*) on acetylcholinesterase activity in oxidative cardiopathy. Data = mean ± *SD*; *n* = 3. *Statistically significant compared to untreated tissue; #statistically significant compared to normal tissue

As depicted in Figure 3, induction of oxidative injury led to significant ($p < .05$) elevation of acetylcholinesterase activity which depicts an occurrence of cholinergic dysfunction in the experimental cardiac tissues. This supports earlier reports on exacerbated acetylcholinesterase activity in oxidative stress (Agunloye et al., 2019; Erukainure et al., 2020). Cholinergic dysfunction characterized by exacerbated acetylcholinesterase activity has been associated with the pathology of cardiopathies and other cardiovascular dysfunctions owing to the catalytic role of acetylcholinesterase on the hydrolysis of acetylcholine (Agunloye et al., 2019). Acetylcholine has been reported for its vasodilatory effect via the activation of the muscarinic receptors (Erukainure et al., 2020; Kellogg, Zhao, Coey, & Green, 2005). There was a dose-dependent depleted acetylcholinesterase activity in the experimental cardiac tissues on treatment with strawberry fruits (Figure 3). Although not significant except at the highest dose (240 µg/ml), the reversed activity insinuates an acetylcholinesterase inhibitory effect by the fruits which has also been validated in earlier studies (Subash et al., 2014; Szwajgier, Borowiec, & Zapp, 2019). It also indicates an improved cholinergic function thus portraying a therapeutic effect. This corroborates reports on the therapeutic effects of acetylcholinesterase-inhibitors on cardiovascular dysfunction (Gavioli et al., 2014; Roy, Guatimosim, Prado, Gros, & Prado, 2014).

Following the induction of oxidative injury, there was a significant ($p < .05$) elevation of ATPase activity, while concomitantly depleting ENTPDase activity in the experimental cardiac tissues as shown in Figure 4, indicating decreased ATP and adenosine levels, respectively. The role of these endogenous signaling nucleotides in cardiovascular functions has been reported. Adenosine is a strong dilator of the coronary vessels, while ATP has been reported for its vasodilatory effect (Burnstock, 2017; Burnstock & Ralevic, 2014). Treatment with strawberry fruits significantly ($p < .05$) depleted the activity of ATPase, with concomitant dose-dependent elevation

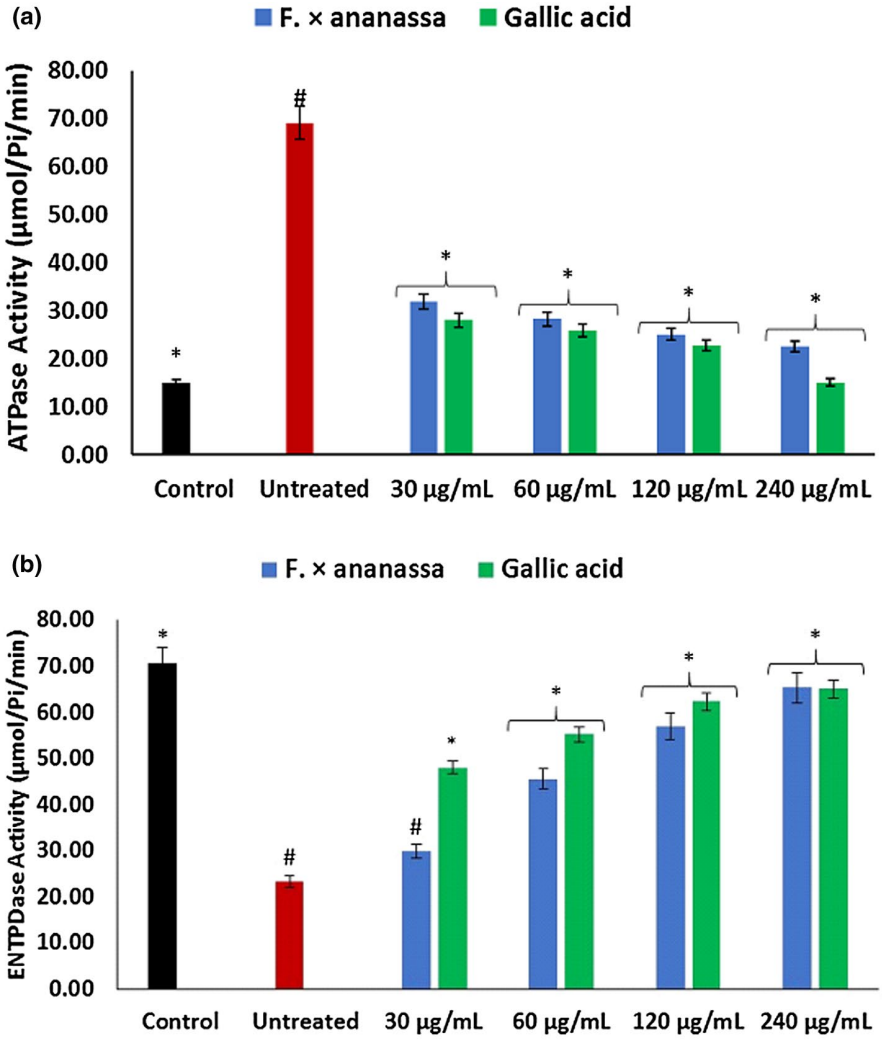

FIGURE 4 Effect of strawberry fruit (*F. x ananassa*) on ATPase and ENTPDase activities in oxidative cardiopathy. Data = mean ± *SD*; *n* = 3. *Statistically significant compared to untreated tissue; #statistically significant compared to normal tissue

of ENTPDase activity (Figure 4), there by insinuating increased ATP and adenosine levels, respectively. Thus, demonstrating a therapeutic effect. This activity may be responsible for the reported anti-vasoconstrictive effect of strawberry fruits (Mudnic et al., 2009).

Induction of oxidative injury significantly increased lipase activity in the experimental cardiac tissues as depicted in Figure 5. Exacerbated cardiac lipase activity has been correlated with elevated cardiac levels of free fatty acids (FFAs) (Lee et al., 2004; Pulinilkunnil & Rodrigues, 2006). In normal physiology, cardiac lipase catalyzes the breakdown of cardiac triglyceride to fatty acids (FA) as the heart cannot synthesize FA (Erukainure et al., 2020; Pulinilkunnil & Rodrigues, 2006). High cardiac levels of FA particularly saturated FA have been linked to cardiovascular dysfunction (Sun, Pan, Tan, & Yu, 2012). Lipase activity was significantly depleted in a dose-dependent manner on treatment with strawberry fruits. Thus, demonstrating the ability of the fruit to protect against FFA accumulation in oxidative cardiopathy.

As depicted in Figure 6, induction of oxidative injury significantly ($p < .05$) increased the cardiac levels of cholesterol, TG and LDL-c, as well as depleted HDL-c. There by insinuating a disrupted cardiac lipid profile. Elevated cardiac cholesterol, TG, and LDL-c levels have been linked with cardiac dysfunctions as they lead to increased vasoconstriction (Kopkan, Khan, Lis, Awayda, & Majid, 2009; Rosendorff, 2002). Treatment with strawberry fruits caused a significant ($p < .05$) depletion in the cardiac levels of cholesterol and LDL-c, while concomitantly elevating triglyceride and HDL-c levels. These reversed levels further insinuate the fruits' potential to protect against oxidative cardiac lipid accumulation. The elevated TG level on treatment with the fruit may, however, insinuate a hypertriglyceridemic effect and thus will require further studies particularly in in vivo models.

GC-MS analysis of the experimental cardiac tissue showed that the induction of oxidative injury caused a total depletion of tetradecane, phosphonoacetic acid, 5-methylsalicylic acid, (-)-epinephrine, 1,4-androstadien-17.beta.-ol-3-one, glutaric acid,

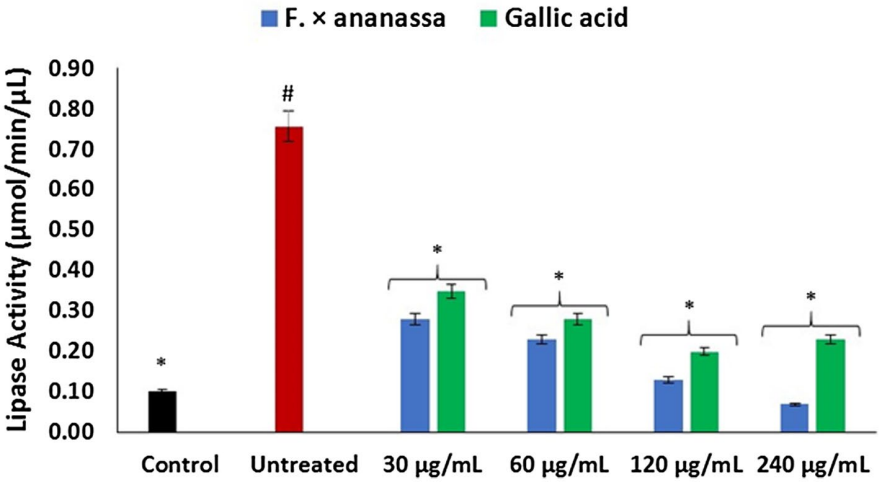

FIGURE 5 Effect of strawberry fruit (*F. x ananassa*) on lipase activity in oxidative cardiopathy. Data = mean ± *SD*; *n* = 3. *Statistically significant compared to untreated tissue; #statistically significant compared to normal tissue

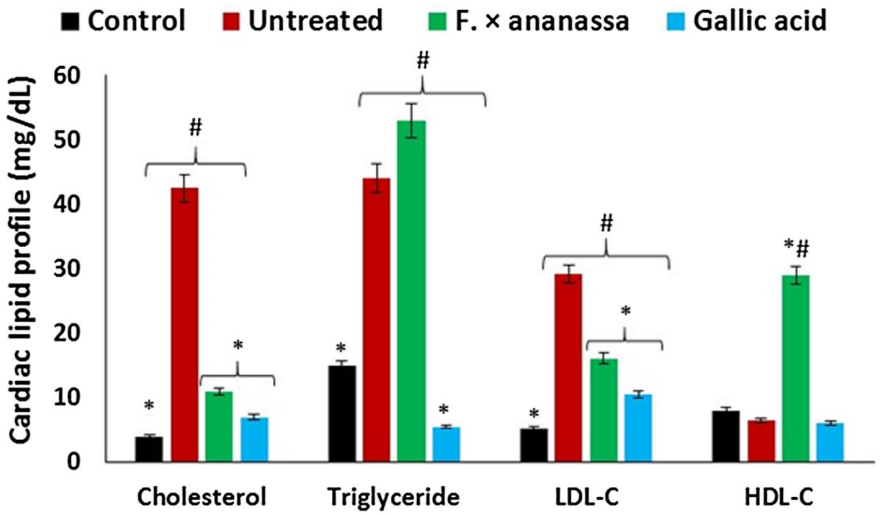

FIGURE 6 Effect of strawberry fruit (*F. x ananassa*) on lipid profile in oxidative cardiopathy. Data = mean ± *SD*; *n* = 3. *Statistically significant compared to untreated tissue; #statistically significant compared to normal tissue

di(3-methylphenyl) ester, n-hexadecanoic acid, 1-octadecanol, 3,4-dihydroxymandelic acid, DL-norepinephrine, 5,16-pregnadien-3-ol-20-one, 3-acetate-20-oxime-, nonadecanoic acid, and cholesterol as depicted in Table 1. Induction of oxidative injury also led to the concomitant generation of 2-pyrrolidinone; pentanoic acid, 2,4-dimethyl-4-nitro-, methyl ester; tetradecanedioic acid; 3,4-dihydroxyphenylglycol; heptacosane; alpha-ketostearic acid; dodecanoic acid; 2-pentadecanol; 1-heptadecanamine; 2-hexadecanol and stearic acid, 2-(1-octadecenyloxy)ethyl ester, (E)- (Table 2). These alterations insinuate disturbances in cardiac metabolites particularly lipids, which corroborates reports on the elevated cardiac concentration of lipids in cardiovascular dysfunctions (Erukainure et al., 2020; Glatz et al., 1988). The oxidative-generated metabolites except 2-pyrrolidinone and 2-pentadecanol were completely depleted on treatment with strawberry fruits, with concomitant generation of 1-decanol; succinic acid, 2-methylpent-3-yl tetrahydrofurfuryl ester; 2,4,6-trimethylheptane; sulfurous acid, nonyl pentyl ester; 3-methylsalicylic acid; tetradecanoic acid; norepinephrine, (R); benzo[h]quinoline and 1-methoxy-1,9,12-octadecatriene (Table 1). Thus, portraying the ability of the fruits to protect against lipid accumulation in oxidative cardiopathy. This correlates with the decreased cardiac levels of cholesterol, LDL-c, and lipase activity (Figures 5 and 6).

On induction of oxidative injury, there was an inactivation of pathways for steroid biosynthesis, bile acid biosynthesis, glycerolipid metabolism, plasmalogen synthesis, fatty acid elongation in mitochondria, fatty acid metabolism, and steroidogenesis, while concomitantly activating the beta-oxidation of very long chain fatty acids and mitochondrial beta-oxidation of medium-chain saturated fatty acids as shown in Figure 7 and Table 2. Deactivation

TABLE 1 GC-MS identified metabolites in experimental cardiac tissues

Metabolites	Control	Untreated	Treated
Tetradecane	X	–	X
Phosphonoacetic acid	X	–	X
5-Methylsalicylic acid	X	–	–
(-)-Epinephrine	X	–	–
Eicosane	X	X	X
1,4-Androstadien-17. beta.-ol-3-one	X	–	–
Glutaric acid, di(3-methylphenyl) ester	X	–	–
n-Hexadecanoic acid	X	–	X
1-Octadecanol	X	–	–
3,4-Dihydroxymandelic acid	X	–	X
DL-Norepinephrine	X	–	–
Epinephrine, (.beta.)-	X	X	–
5,16-Pregnadien-3-ol-20-one, 3-acetate-20-oxime-	X	–	–
Nonadecanoic acid	X	–	–
Cholesterol	X	–	X
2-Pyrrolidinone	–	X	X
Pentanoic acid, 2,4-dimethyl-4-nitro-, methyl ester	–	X	–
Tetradecanedioic acid	–	X	–
3,4-Dihydroxyphenylglycol	–	X	–
Heptacosane	–	X	–
Alpha-Ketostearic acid	–	X	–
Dodecanoic acid	–	X	–
2-Pentadecanol	–	X	X
1-Heptadecanamine	–	X	–
2-Hexadecanol	–	X	–
Stearic acid, 2-(1-octadecenyloxy)ethyl ester, (E)-	–	X	–
1-Decanol	–	–	X
Succinic acid, 2-methylpent-3-yl tetrahydrofurfuryl ester	–	–	X
2,4,6-Trimethylheptane	–	–	X
Sulfurous acid, nonyl pentyl ester	–	–	X
3-Methylsalicylic acid	–	–	X
Tetradecanoic acid	–	–	X
Norepinephrine, (R)-	–	–	X
Benzo[h]quinoline	–	–	X
1-Methoxy-1,9,12-octadecatriene	–	–	X

Note: X = present; – = not present.

TABLE 2 Metabolic pathways in experimental cardiac tissues

Pathways	Control	Untreated	Treated
Catecholamine biosynthesis	X	X	X
Tyrosine metabolism	X	X	X
Steroid biosynthesis	X	–	X
Bile acid biosynthesis	X	–	X
Glycerolipid metabolism	X	–	X
Plasmalogen synthesis	X	–	–
Fatty acid elongation in mitochondria	X	–	X
Fatty acid biosynthesis	X	X	X
Fatty acid metabolism	X	–	X
Steroidogenesis	X	–	X
Beta-oxidation of very long chain fatty acids	–	X	–
Mitochondrial beta-oxidation of medium-chain saturated fatty acids	–	X	–

Note: X = present; – = not present.

of these pathways denotes a defect in cardiac lipid metabolism and may insinuate alterations in energy metabolism which has been reported as a major mechanism of cardiovascular dysfunctions (Antozzi & Zeviani, 1997; Erukainure et al., 2020). The activation of beta-oxidation of very long chain fatty acids and mitochondrial beta-oxidation of medium-chain saturated fatty acids insinuates increased generation of the electron donors NADH and $FADH_2$, which when channeled into the respiratory chains for ATP production will give rise a high mitochondrial membrane potential (Brownlee, 2001; Du et al., 2001). This, in turn, will lead to the reduction of oxygen to (O_2) to superoxide ($O_2^{\cdot-}$) which in the presence of low SOD activity will lead to oxidative stress (Brownlee, 2001). Treatment with strawberry fruits led to the restoration of the oxidative-inactivated pathways, with concomitant deactivation of the oxidative-generated pathways (beta-oxidation of very long chain fatty acids and mitochondrial beta-oxidation of medium-chain saturated fatty acids). Thus, insinuating a reduction in the generation of $O_2^{\cdot-}$.

These cardioprotective activities of strawberry fruits may be linked to the synergistic activity of its phytochemical constituents. The GC-MS analysis revealed the presence of benzyl 2,6-dihydroxybenzoate, mesitylene, tetradecane, phosphonoacetic acid, 3-methylsalicylic acid, and thymol in strawberry fruits as depicted in Figure 8. Benzyl 2,6-dihydroxybenzoate is a derivative of hydroxybenzoic acids. Hydroxybenzoic acid and its derivatives have been reported for their cardioprotective activities (Juurlink, Azouz, Aldalati, AlTinawi, & Ganguly, 2014). Mesitylene has been reported as a constituent of most essential oil and may be a contributor to the fruit's flavor (Miyazawa

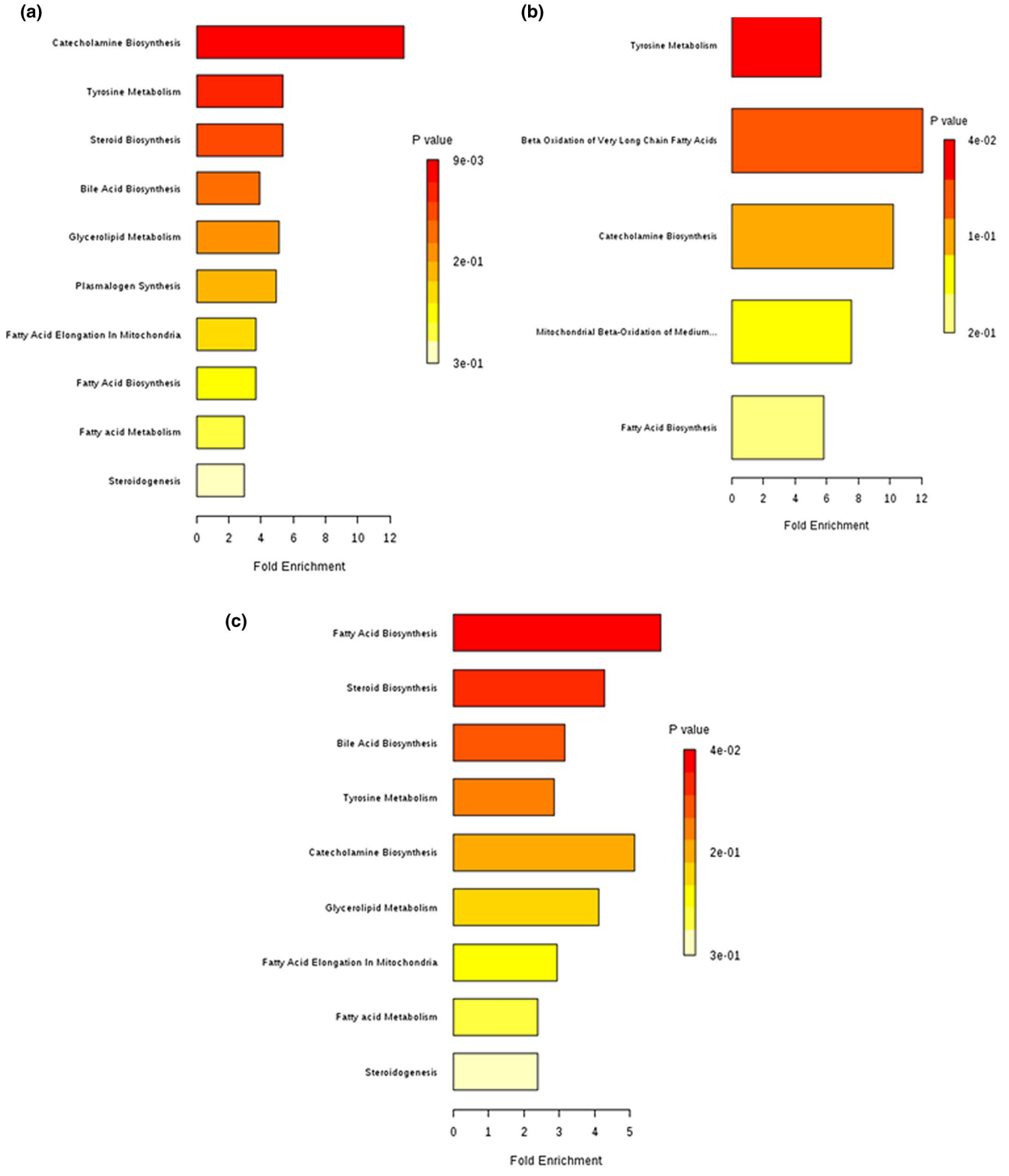

FIGURE 7 Fold enrichment of identified metabolic pathways of (a) control; (b) untreated; and (c) strawberry fruit (*F. x ananassa*)—treated oxidative cardiopathy

et al., 2015; Salvador et al., 2011). The medicinal properties of salicylic acids and its esters are well reported and are attributed to their antioxidant potentials (Aresta & Zambonin, 2016). Thymol has been reported for its antioxidant potentials (Aeschbach et al., 1994; Mastelic et al., 2008) and cardioprotective properties (Meeran, Jagadeesh, & Selvaraj, 2015, 2016).

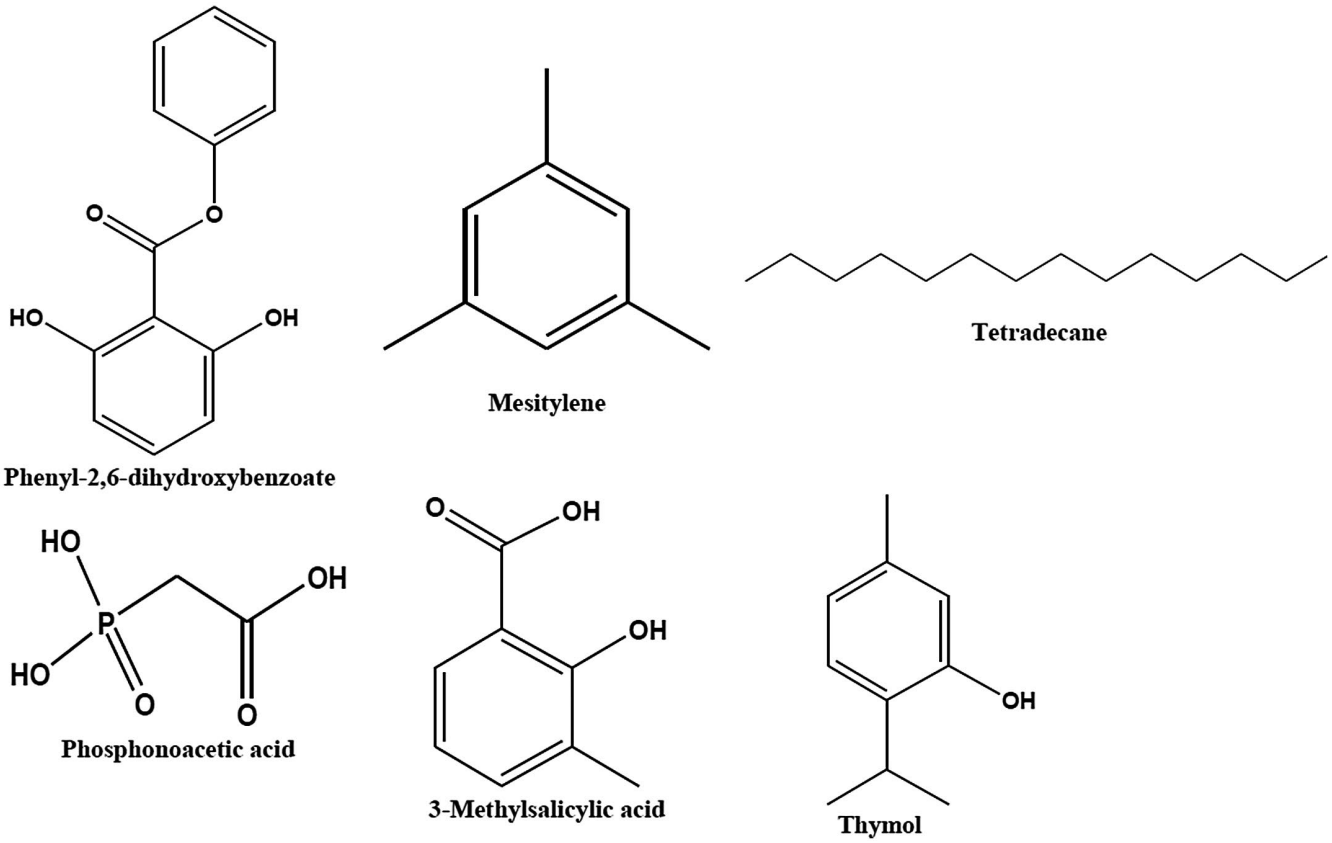

FIGURE 8 GC-MS identified compounds in strawberry fruit (*F. x ananassa*)

4 | CONCLUSION

Taken together, these data thus portray the cardioprotective effect of strawberry fruits against oxidative cardiopathy as indicated by the ability of the fruit to (1) attenuate oxidative stress, (2) inhibit ACE and acetylcholinesterase activities, and (3) modulate lipid dysmetabolism. These activities may be attributed to the synergistic effects of the identified phytochemicals. However, in vivo studies are recommended to further explain the cardioprotective mechanism of the fruit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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