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Strawberry fruit (*Fragaria x ananassa* Romina) juice attenuates oxidative imbalance with concomitant modulation of metabolic indices linked to male infertility in testicular oxidative injury

Ochuko L. Erukainure[1] | Veronica F. Salau[2] | Ayodeji B. Oyenihi[3] | Ndumiso Mshicileli[4] | Chika I. Chukwuma[5] | Md. Shahidul Islam[2]

[1]Department of Pharmacology, School of Clinical Medicine, Faculty of Health Sciences, University of the Free State, Bloemfontein, South Africa

[2]Department of Biochemistry, School of Life Sciences, University of KwaZulu-Natal, (Westville Campus), Durban, South Africa

[3]Functional Foods Research Unit, Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville, South Africa

[4]AgriFood Technology Station, Faculty of Applied Sciences, Cape Peninsula University of Technology, Bellville, South Africa

[5]Center for Quality of Health and Living, Faculty of Health Sciences, Central University of Technology, Bloemfontein, South Africa

Correspondence
Ochuko L. Erukainure, Department of Pharmacology, School of Clinical Medicine, Faculty of Health Sciences, University of the Free State, Bloemfontein 9300, South Africa.
Emails: loreks@yahoo.co.uk; ErukainureOL@ufs.ac.za

Abstract

This study investigated the protective properties of strawberry fruit on testicular oxidative injury. Oxidative injury was induced in vitro in testicular tissue homogenates by incubation with ferrous sulphate ($FeSO_4$) in the presence and absence of strawberry fruit extract (SFE) for 30 min at 37˚C, with gallic acid serving as the standard antioxidant drug. Induction of oxidative injury significantly reduced glutathione, cholesterol and triglyceride levels; and inhibited SOD, catalase and ENTPDase activities when compared to normal control. It also led to exacerbated nitric oxide, malondialdehyde, LDL-cholesterol levels, acetylcholinesterase, ATPase and lipase activities. These effects were, however, reversed following treatment with SFE when compared to the untreated control, except for cholesterol and triglyceride levels. Additionally, the induction of the oxidative injury led to alterations in testicular lipid metabolites that were accompanied by the activation of α-linolenic acid and linoleic acid metabolic pathways. While SFE treatment had no significant impact on the altered metabolites, it repressed pathways for mitochondrial beta-oxidation of long-chain saturated fatty acids and plasmalogen synthesis. High-performance liquid chromatography analysis of SFE revealed the presence of rutin, caffeic acid, p-coumarin and cinnamic acid. These data imply the protective potentials of strawberry fruits against testicular oxidative injury.

KEYWORDS

antioxidants, metabolomics, strawberry fruits, testicular oxidative injury

1 | INTRODUCTION

Elevated levels of free radicals leading to oxidative stress have been implicated in most cases of infertility especially testicular functions. Oxidative stress ensues when the increased generation of free radicals overrides the intrinsic antioxidant defence system (Asadi et al., 2017; Wu et al., 2020). Owing to the high content of unsaturated fatty acids, coupled with its constant rate of division and high mitochondria oxygen demand in the testis, it is highly exposed to oxidative attack (Asadi et al., 2017; Obode et al., 2015). The testicular activities, therefore, depend on a delicate balance between the generation of free radicals and their metabolism, as failure to detoxify or repair the injurious effects of free radicals may be fatal to the testicular tissues (Asadi et al., 2017).

Iron-mediated testicular toxicity as a result of oxidative injury has been vastly reported and attributed mostly to oxidative stress (Chen et al., 2018; Yesil et al., 2018). Iron toxicity has been linked to male infertility as toxic interaction between the iron and testicular

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macromolecules – lipids, proteins and DNA molecules –, can lead to changes in the architecture of the epididymis, seminiferous tubules and Sertoli as well as cause sperm cells' DNA fragmentation (Anderson et al., 2014; Lucesoli et al., 1999; Yesil et al., 2018).

Fruits are essential in the human diet and their consumption has been correlated with multiple health benefits as they are dense in vitamins, minerals, fibre and many bioactive compounds (Amao, 2018). Their medicinal properties have been linked to their potent antioxidant activities which reduce the risks of short life span, cardiovascular diseases, stroke, obesity, diabetes, neuronal dysfunction and cancer (Boffetta et al., 2010; Oyebode et al., 2014; Rolls et al., 2004). One of such fruits is the strawberry fruit *(Fragaria x ananassa* Romina*)*.

Fragaria x ananassa Romina, popularly called strawberry, is among the common fruits globally consumed with increasing cultivation worldwide. It belongs to the genus *Fragaria* in the *Rosaceae* family (Hancock et al., 2008). The fruit is rich in phytochemicals including anthocyanins and flavonols which are strong antioxidants. It is also highly packed with beneficial minerals such as magnesium, calcium, selenium, manganese, copper, zinc as well as vitamin, which include folic acid, niacin, riboflavin and vitamins A, C, E and K (Aaby et al., 2005; Erukainure et al., 2020; Panico et al., 2009). The biological activities of strawberry fruit include anticancer, anti-inflammatory, cardioprotective, antioxidant and antidiabetic properties (Abdulazeez & Ponnusamy, 2016; Amatori et al., 2016; Erukainure, Salau, Oyenihi, et al., 2020). These pharmacological effects have been often associated with its phytochemical and micronutrient constituents. However, the effects of strawberry fruits on oxidative-mediated testicular dysfunction have been less studied.

This study was, therefore, undertaken to investigate the protective activities of strawberry fruit on oxidative-induced testicular injury by determining its ability to ameliorate oxidative imbalance, its modulatory effect on cholinergic and purinergic dysfunctions, and lipid dysmetabolism in testicular tissues ex vivo.

2 | MATERIALS AND METHODS

2.1 | Collection and extraction of strawberry fruits

Strawberry fruits were procured from a local fruit market in Durban, South Africa. About 200 g of the fruits were rinsed and blended in an electric glass jug blender (Russel Hobbs, Johannesburg, South Africa). The juice puree was centrifuged thereafter for 15 min at 15,000 g at 4°C using 50 ml centrifuge tubes. The supernatants in glass beakers were then concentrated at 50°C in a water bath which yielded 33% concentrated samples. Samples were then preserved at 2°C for subsequent analyses.

2.2 | High-Performance Liquid Chromatography (HPLC)

The fruit extract was subjected to HPLC-diode array detection analysis using an Agilent 1,100 series (Agilent, Waldbronn, Germany) instrument equipped with photodiode array, autosampler, column thermostat and degasser. The working parameters were as follows: **Stationary phase**: A Phenomenex: Luna 5 μm C_{18} (2) (150 × 4.6 mm; 5 μm particle size) column; Mobile phases: Water containing 0.1% of formic acid (A) and acetonitrile (B); **Flow rate**: 1 ml/min; **Gradient elution**: Initial ratio 95% A: 5% B, keeping for 10 min; changed to 90% A: 10% B in 10 min; changed to 70% A: 30% B in 10 min and to 50% A: 50% B in 10 min, maintaining for 0.5 min and back to initial ratio in 0.5 min; and **Temperature:** 30°C. Extract and standards were dissolved in HPLC grade methanol (2 mg/ml) and the injection volume was 20.0 μL. Chromatograms were obtained at 254 nm.

2.3 | Animals

Five (5) male Sprague–Dawley rats with average weights of 180 ± 20 g were procured from the Biomedical Research Unit of the University of KwaZulu-Natal, Durban, South Africa. The animals were fasted overnight before they were sacrificed humanely with insofar. Testes of each rat were excised for in vitro studies.

This experiment was carried out in line with the approved guidelines of the University of KwaZulu-Natal animal ethics committee, South Africa, Durban (protocol approval number: AREC/022/019D).

2.4 | Induction of testicular oxidative injury

Excised testicular tissues were washed in normal saline and homogenised in 50 mM phosphate buffer with 1% Triton X-100 (pH 7.5). The homogenates were centrifuged at 20,000 g for 10 min at 4°C, and the resulting supernatants were transferred into 2 ml Eppendorf tubes. Testicular toxicity was induced using a previously described protocol (Erukainure et al., 2021). A total volume 100 μL of varying concentrations (30–240 μg/mL) of the strawberry extract was incubated with 100 μL of supernatant and 0.1 mM $FeSO_4$ for 30 min in 5% CO_2 incubator at 37°C. Reaction mixture without the strawberry extract served as the negative control (untreated), while normal control consists of the tissue supernatant with no extract and $FeSO_4$. The reaction mixture was then subjected to biochemical analyses as described below.

2.5 | Redox status

The redox status of testes was determined by investigating the reduced glutathione (GSH) level (Ellman, 1959), superoxide dismutase (SOD) and catalase activities (Aebi, 1984; Kakkar et al., 1984) and malondialdehyde (MDA) level (Chowdhury & Soulsby, 2002) in the reaction mixtures.

2.6 | Nitric Oxide (NO) level

The Griess method was employed for determining the NO level of the testicular tissues (Tsikas, 2005).

2.7 | Cholinergic enzymes activities

The Ellman method was utilised for determining the cholinergic activity of the testicular tissues, which covers for acetylcholinesterase activity (Ellman et al., 1961).

2.8 | Purinergic activities

The purinergic activities of testicular tissues were determined by analysing the reaction mixtures for adenosine triphosphatase (ATPase) (Adewoye et al., 2000; Erukainure et al., 2017) and ecto-nucleoside triphosphate diphosphohydrolase (ENTPDase) (Akomolafe, Akinyemi, et al., 2017) activities in the reaction mixture.

2.9 | Lipase activity

The method of Kim et al., (2010) for determining the lipase activity of the testicular tissues as modified by Salau, Erukainure, Ibeji, Olasehinde, et al., (2020).

2.10 | Testicular lipid metabolism

Testicular tissue supernatants were incubated with 0.1 mM $FeSO_4$ and 240 μg/ml of strawberry fruit extract for 12 hr (Erukainure, Salau, Oyenihi, et al., 2020). After incubation, the samples were centrifuged for 10 min at 20,000 g and 4 °C. The supernatants were collected and subjected to lipid metabolite extraction and lipid profiling.

2.11 | Lipid metabolite extraction and profiling

The collected supernatant was subjected to lipid metabolites extraction using a slightly modified method (Ralston-Hooper et al., 2011). Briefly, the supernatant was mixed with cold chloroform in a ratio of 5:1 and vortexed for about 1 min. The reaction mixture was then allowed to stand in ice for 20 min before centrifuging for 10 min at 15,000 rpm and 4℃. Of the two liquid phases obtained, the lower contained the lipid metabolites and other nonpolar metabolites. This was collected with a micropipette into HPLC vials and profiled with GC-MS.

2.12 | Identification of lipid metabolites via GC-MS analysis

The extracted lipid and nonpolar metabolites were subjected to GC-MS profiling (Agilent technologies 6,890 series GC coupled with [an Agilent] 5,973 Mass Selective Detector and driven by Agilent Chemstation software). The operating parameters and conditions were as follows: **Column**: HP-5MS capillary column (30 m × 0.25 mm ID, 0.25 μm film thickness and 5% phenylmethylsiloxane); **Carrier gas**: ultrapure helium; **Flow rate**: 1.0 ml/min and a linear velocity of 37 cm/sec; **Injector temperature**: set at 250℃; **Initial oven temperature**: 60℃, programmed to 280℃ at the rate of 10℃/min; **Injection**: 1 μL made in split mode at a split ratio of 20:1; **Electron ionisation mode**: 70 eV; **Electron multiplier voltage**: at 1859 V; **Ion source temperature**: 230℃; **Quadrupole temperature**: 150℃; **Solvent delay**: 4 min; **Scan range**: 50–70 amu (Erukainure, Salau, Oyenihi, et al., 2020). An inbuilt NIST library was used in identifying the metabolites.

2.13 | Metabolic pathway analysis

The GC-MS identified metabolites were computed into the MetaboAnalyst 5.0 online server and subjected to pathway enrichment analysis to identify the relevant lipid metabolic pathways (Chong et al., 2018).

2.14 | Determination of testicular lipid profiles

The supernatants were subjected to lipid profiling that represents 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 manufacturer's guidelines.

2.15 | Data analysis

Data were evaluated in at least three independent experimental repeats. One-way analysis of variance (ANOVA) used for analysing all data and presented as mean ± *SD*. Significant differences were established at $p < .05$ using the Tukey's HSD multiple-range post-hoc test. Statistical analyses were carried out with the aid of IBM Statistical Package for the Social Sciences (SPSS) for Windows, version 23.0 (IBM Corp., Armonk, NY, USA).

3 | RESULTS

Rutin, caffeic acid, p-coumarin and cinnamic acid were identified in the strawberry fruit extract following the HPLC analysis as shown in Table 1.

As shown in Figure 1a–d, induction of testicular oxidative injury significantly ($p < .05$) suppressed GSH level, SOD and catalase activities, while concomitantly increasing MDA level. These activities and levels were significantly ($p < .05$) reversed following treatment with strawberry fruits when compared to the respective values in untreated controls. Except for catalase activity, the changes observed in the SFE-treated tissues were dose dependent.

As depicted in Figure 2, induction of oxidative injury led to a significant ($p < .05$) elevation of testicular NO level. The level was

TABLE 1 HPLC identified compounds in *F. ananassa* fruit

Compounds (Retention time)	Parameters	*F. ananassa*
Rutin (1.44 min)	RT (min) DFS (min)	1.40 −0.04
Caffeic acid (1.1.52 min)	RT (min) DFS (min)	1.52 0.00
p-Coumarin (1.167 min)	RT (min) DFS (min)	1.66 −0.01
Cinnamic acid (2.05 min)	RT (min) DFS (min)	2.47 0.42

Abbreviations: DFS, difference in retention time; RT, retention time.

significantly reduced in testicular tissues treated with strawberry fruit extract, and compared favourably with the standard antioxidant, gallic acid.

Following the induction of testicular oxidative injury, there was a significant (p <.05) increased acetylcholinesterase activity in untreated testicular tissues as indicated in Figure 3. This activity was reduced significantly (p <.05) after treating with strawberry fruit extract. The reduced activity was slightly dose dependent and compared favourably with gallic acid.

As shown in Figure 4a and b, testicular ATPase activity was significantly (p <.05) elevated, with concomitant suppressed ENTPDase activity on induction of testicular oxidative injury. These activities were significantly (p <.05) reversed on treatment with strawberry fruit extract and compared favourably with gallic acid.

As depicted in Figure 5, testicular lipase activity was significantly (p <.05) elevated following the induction of testicular oxidative injury. The activity was significantly (p <.05) inhibited following treatment with strawberry fruit extract.

As shown in Table 2, induction of testicular oxidative injury led to the depletion of 1-hexadecanol, 3-methylsalicylic acid, 1-tetradecanol, n-hexadecanoic acid, hexadecanoic acid, ethyl ester and stearic acid and 2-(1-octadecenyloxy)ethyl ester, (E)-. It also caused the generation of tetradecanoic acid, 2-thiobarbituric acid, hexadecanoic acid, methyl ester, palmitoleic acid, n-hexadecanoic acid, 9,12-octadecadienoic acid (Z,Z)-, methyl ester, cis-13-octadecenoic acid, methyl ester, methyl stearate, linoelaidic acid, oleic acid, gamolenic acid, 3,4-dihydroxymandelic acid, DL-norepinephrine, epinephrine, glycerol 1-palmitate, erucic acid and squalene. Interestingly, treatment with strawberry fruit extract did not restore the depleted metabolites but led to the depletion of 2-thiobarbituric acid, 9,12-octadecadienoic acid (Z,Z)-, methyl ester, cis-13-octadecenoic acid, methyl ester, gamolenic acid, 3,4-dihydroxymandelic acid, DL-norepinephrine, epinephrine, erucic acid and squalene. It also led to the concomitant generation of 9,12-octadecadienoic acid (Z,Z)-, 6-octadecenoic acid, pentadecanoic acid, arachidonic acid, 2,5-dihydroxybenzaldehyde, cis-13-octadecenoic acid, stearic acid, 2-(1-octadecenyloxy)ethyl ester, (Z)- and 3,4-dihydroxymandelic acid.

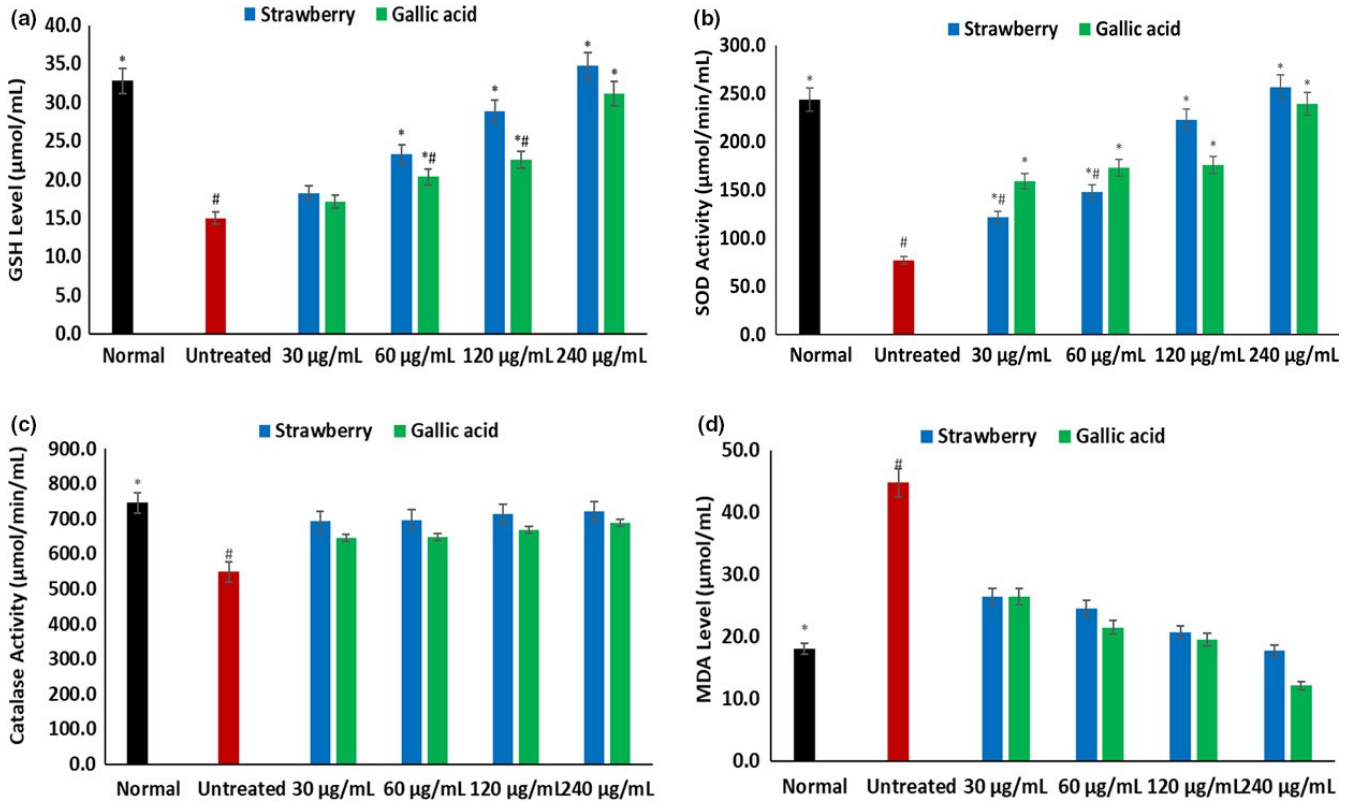


FIGURE 1 Effect of strawberry fruit (*F. x ananassa*) on (a) GSH level, (b) SOD activity, (c) catalase activity and (d) MDA level in oxidative testicular tissues. Value =mean ± *SD*; $n = 3$. *Statistically significant (p <.05) compared to untreated tissue; #statistically significant (p <.05) compared to normal tissue

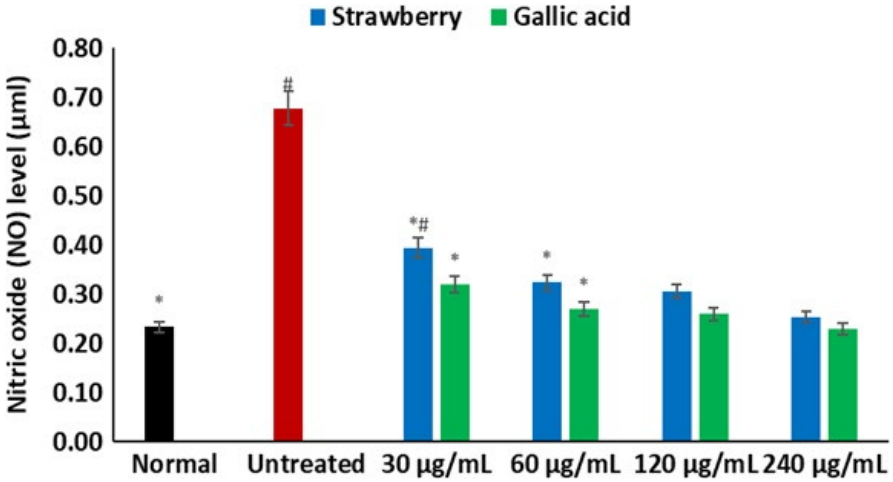


FIGURE 2 Effect of strawberry fruit (*F. x ananassa*) on nitric oxide (NO) level in oxidative testicular injury. Value =mean ± *SD*; *n* = 3. *Statistically significant ($p < .05$) compared to untreated tissue; #statistically significant ($p < .05$) compared to normal tissue

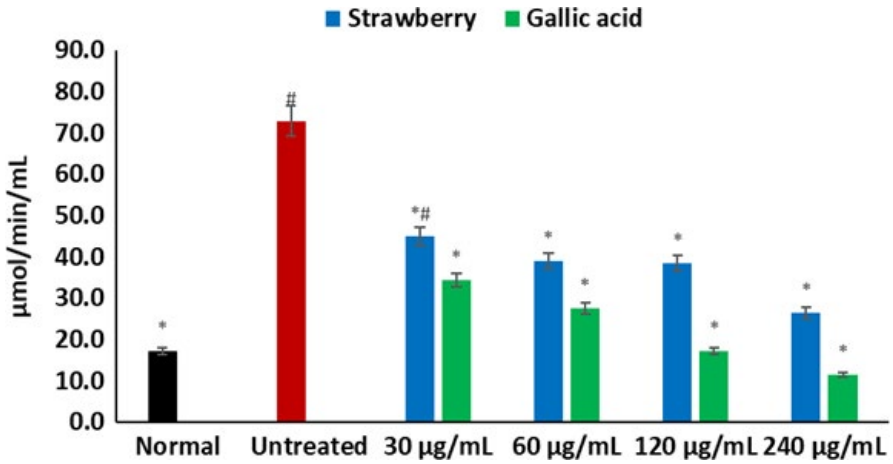


FIGURE 3 Effect of strawberry fruit (*F. x ananassa*) on acetylcholinesterase activity in oxidative testicular injury. Value =mean ± *SD*; *n* = 3. *Statistically significant ($p < .05$) compared to untreated tissue; #statistically significant ($p < .05$) compared to normal tissue

Pathway enrichment analysis, as shown in Figure 6 and Table 3, revealed activation of α-linolenic acid and linoleic acid metabolism pathways following the induction of oxidative injury. Treatment with strawberry fruit extract did not affect these pathways but caused the deactivation of pathways for mitochondrial beta-oxidation of long-chain saturated fatty acids and plasmalogen synthesis.

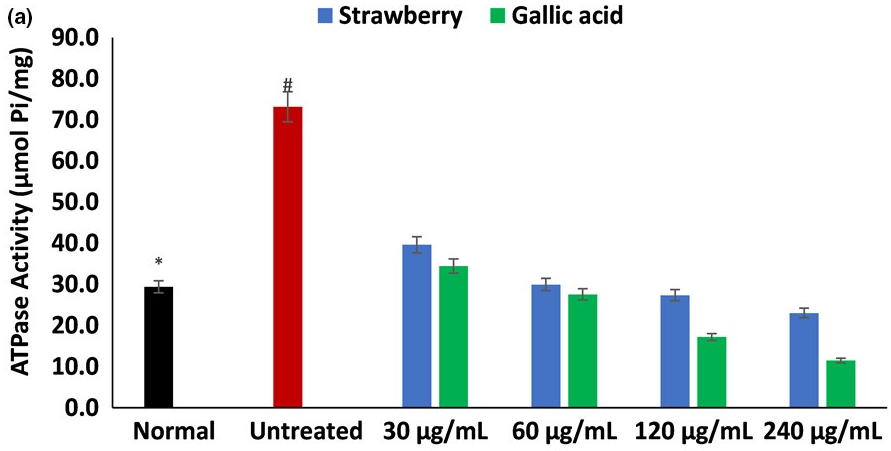
There were significantly ($p < .05$) depleted testicular levels of cholesterol and triglyceride on induction of oxidative testicular injury, while that of LDL cholesterol was elevated as shown in Figure 7. Treatment with strawberry fruit extract had no significant effects on these levels except that of cholesterol which further reduced, this being accompanied by an increased HDL cholesterol level.

4 | DISCUSSION

Oxidative testicular injury has been linked to the pathogenesis of testicular dysfunction, which if not well treated can lead to infertility in the long run (Asadi et al., 2017). There has been increasing interest in the usage of natural products of plant origin for treating and managing male reproductive dysfunctions. Strawberry ranks among the common fruits globally consumed, with reported nutritional and medicinal properties (Erukainure, Salau, Oyenihi, et al., 2020). In the present study, the protective potentials of strawberry fruits against were investigated in Fe^{2+}-induced oxidative injury in testicular tissues ex vivo.

The medicinal properties of fruits, herbs and vegetables have been correlated with their phytoconstituents (Erukainure et al., 2018; Salau et al., 2020). The identified phytocompounds following HPLC analysis (Table 1) of strawberry fruits are well-known phenolics and have been extensively studied for their potent antioxidant properties (Gülçin, 2006; Prasad & Prasad, 2019; Salau et al., 2020). The identification of rutin, caffeic acid and cinnamic acid in the present study corroborates previous reports on their presence in strawberries (Aaby et al., 2012; Kelebek & Selli, 2011; Yang et al., 2016). The presence of these phenolics may synergistically contribute to the reported antioxidant and other medicinal of strawberries (Erukainure, Salau, Oyenihi, et al., 2020; Fortalezas et al., 2010; García-Alonso et al., 2004).

Oxidative stress has been reported in the perturbation of normal testicular physiology leading to its dysfunctions like poor sperm quality, suppressed testosterone levels, fragmented sperm DNA and, in the long term, infertility (Agarwal et al., 2008; Tremellen, 2008). In the present study, there was an occurrence of oxidative stress following the induction of testicular oxidative injury as depicted by the suppressed GSH level, catalase and SOD activities, with concomitant



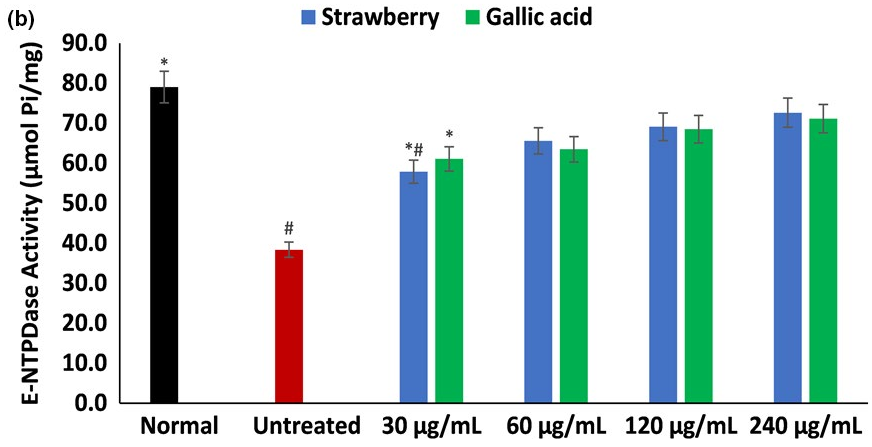


FIGURE 4 Effect of strawberry fruit (*F. x ananassa*) on (a) ATPase and (b) ENTPDase activities in oxidative testicular injury. Values =mean ± *SD*; $n = 3$. *Statistically significant ($p < .05$) compared to untreated tissue; #statistically significant ($p < .05$) compared to normal tissue

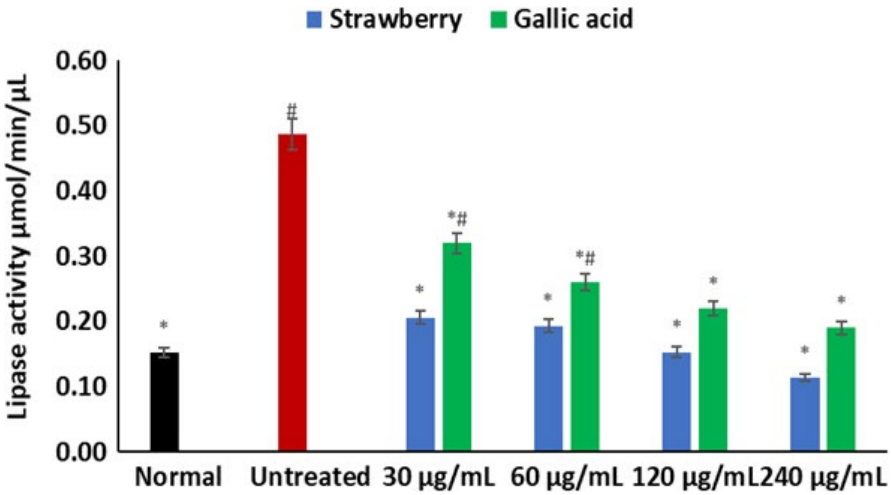


FIGURE 5 Effect of strawberry fruit (*F. x ananassa*) on lipase activity in oxidative testicular injury. Value =mean ± *SD*; $n = 3$. *Statistically significant ($p < .05$) compared to untreated tissue; #statistically significant ($p < .05$) compared to normal tissue

high level of MDA in the untreated tissues (Figure 1). These reduced activities and levels corroborate a previous study that reported on the oxidative stress induced by Fe^{2+} in testicular tissues (Erukainure, Matsabisa, et al., 2021) and may be linked to the oxidation of Fe^{2+} to Fe^{3+} which exacerbates the generation of reactive oxygen species (ROS). Interestingly, the endogenous antioxidant defence system of the testes possesses a high level of SOD in response to high spermatozoa-generated superoxide, O_2^-. The defence system can, however, crumble in a state of chronic ROS production, thereby leading to oxidative stress. The high PUFAs content of the testes increases its susceptibility to peroxidative damage, which is depicted in the present study by the exacerbated MDA indicating an occurrence of lipid peroxidation. Lipid peroxidation has been reported for its links with the altered sperm morphology and sperm DNA fragmentation (Farombi et al., 2013; García-Díaz et al., 2015). The exacerbated testicular level of NO following the induction of oxidative injury (Figure 2) indicates its availability to react with O_2^- to generate peroxynitrite ($ONOO^-$) (Erukainure, Salau, Xiao, et al., 2020;

TABLE 2 LC-MS identified metabolites in experimental testicular tissues

Metabolites	Normal	Untreated	Treated
1-Hexadecanol	X	-	-
3-Methylsalicylic acid	X	-	-
1-Tetradecanol	X	-	-
3,4-Dihydroxyphenylglycol	X	X	-
Norepinephrine, (R)-	X	X	-
n-Hexadecanoic acid	X	-	-
2,5-Dihydroxybenzoic acid	X	X	-
Octadecanoic acid	X	X	-
Hexadecanoic acid, ethyl ester	X	-	-
Epinephrine, (.beta.)-	X	X	X
Stearic acid, 2-(1-octadecenyloxy)ethyl ester, (E)-	X	-	-
Cholesterol	X	X	X
Tetradecanoic acid	-	X	X
2-Thiobarbituric acid	-	X	-
Hexadecanoic acid, methyl ester	-	X	X
Palmitoleic acid	-	X	X
n-Hexadecanoic acid	-	X	X
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	-	X	-
cis−13-Octadecenoic acid, methyl ester	-	X	-
Methyl stearate	-	X	X
Linoelaidic acid	-	X	X
Oleic Acid	-	X	X
Gamolenic acid	-	X	-
3,4-Dihydroxymandelic acid	-	X	-
DL-Norepinephrine	-	X	-
Epinephrine	-	X	-
Glycerol 1-palmitate	-	X	X
Erucic acid	-	X	-
Squalene	-	X	-
9,12-Octadecadienoic acid (Z,Z)-	-	-	X
6-Octadecenoic acid	-	-	X
Pentadecanoic acid	-	-	X
Arachidonic acid	-	-	X
2,5-Dihydroxybenzaldehyde	-	-	X
cis−13-Octadecenoic acid	-	-	X
Stearic acid, 2-(1-octadecenyloxy)ethyl ester, (Z)-	-	-	X
3,4-Dihydroxymandelic acid	-	-	X

Abbreviations: –, not present; Key: X, present.

Salau, Erukainure, Ibeji, Olasehinde, et al., 2020). Thus, portraying an occurrence of proinflammatory. The exacerbated GSH level, SOD and catalase activities, with concomitant suppressed levels of MDA and NO (Figures 1 and 2), therefore, indicate an antioxidative and anti-proinflammatory effect of strawberry fruits. This corroborates with previous reports on the antioxidative and anti-inflammatory activities of strawberries (Erukainure, Salau, Oyenihi, et al., 2020; Giampieri et al., 2014; Rutledge et al., 2019). These activities can be attributed to the HPLC-identified phenolics (Table 1) as they have been well documented for their potent antioxidative and anti-inflammatory activities (Arowoogun et al., 2021; Salau et al., 2021; Salau et al., 2020).

The elevated acetylcholinesterase activity in the untreated testicular tissues (Figure 3) indicates hydrolysis of acetylcholine to acetate and choline on the induction of oxidative injury. Thereby, indicating a suppressed testicular level of acetylcholine which depicts alteration in testicular cholinergic activity. This alteration has been linked to testicular dysfunction (Akomolafe, Oboh et al., 2017), as acetylcholine regulates Leydig cell activities (Mor & Soreq, 2011). It also indicates an inflammatory effect (Das, 2007; Lataro et al., 2015). The suppressed acetylcholinesterase activity in following treatment with strawberry fruits, therefore, indicates an improved cellular availability of acetylcholine for normal testicular physiology. This corroborates reports on the efficacy of acetylcholinesterase inhibitors for treating and managing testicular dysfunctions (Erukainure, Atolani, et al., 2021; Uzun et al., 2009). Strawberries have been reported previously for their ability to inhibit acetylcholinesterase activity (Erukainure, Salau, Oyenihi, et al., 2020; Hwang et al., 2019), and can also be attributed to the HPLC-identified phenolics (Table 1).

The role of ATP and adenosine in testicular physiology and male fertility has been reported (Gorodeski, 2015). They are involved in purinergic co-transmission which controls the reproductive system and are also involved in the modulation of the male genital tract activities such as stimulation of sperm capacitation and its fertilisation potential (Bellezza & Minelli, 2017; Burnstock, 2014; Gorodeski, 2015). The exacerbated activity of ATPase and suppressed E-NTPDase activity in the untreated testicular tissue (Figure 4a and b) depict reduced cellular levels of ATP and adenosine, respectively, following the induction of oxidative injury. Thus, suggesting a reduced availability of these nucleotides to modulate testicular normal physiological activities and depicts a perturbed purinergic activity. Therefore, the suppressed ATPase and increased E-NTPDase activities in tissues treated with strawberry fruits suggest an improved purinergic activity depicted by the availability of ATP and adenosine.

The testis is highly rich in PUFAs and contributes to male fertility as it is involved in spermatozoa – viability, maturity and capacitation, spermatogenesis, fertilisation, steroidogenesis and germ cell membrane remodelling (Pushpendra & Jain, 2015; Shi et al., 2018). Any alteration of its constituents and metabolites may, therefore, lead to testicular dysfunction. The increased lipase activity in the untreated testicular tissues on the induction of oxidative injury (Figure 5) indicates a breakdown of testicular triglyceride to free

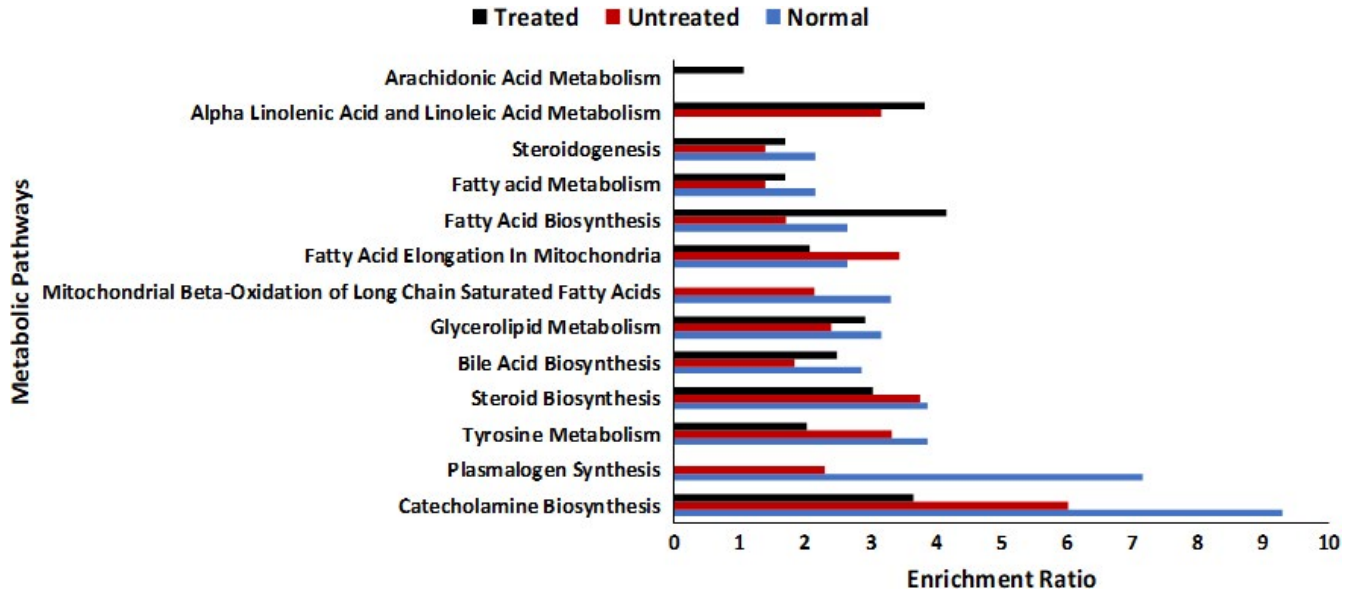


FIGURE 6 Pathway enrichment ratio for identified metabolic pathways in oxidative testicular injury

TABLE 3 Pathway analysis of the most relevant metabolic pathways in testicular tissues

Metabolites	Normal	Untreated	Treated
Catecholamine Biosynthesis	X	X	X
Plasmalogen Synthesis	X	X	-
Tyrosine Metabolism	X	X	X
Steroid Biosynthesis	X	X	X
Bile Acid Biosynthesis	X	X	X
Glycerolipid Metabolism	X	X	X
Mitochondrial Beta-Oxidation of Long-Chain Saturated Fatty Acids	X	X	-
Fatty Acid Elongation in Mitochondria	X	X	X
Fatty Acid Biosynthesis	X	X	X
Fatty acid Metabolism	X	X	X
Steroidogenesis	X	X	X
Alpha Linolenic Acid and Linoleic Acid Metabolism	-	X	X

Abbreviations: –, not present; Key: X, present.

fatty acids (FFAs). Thus, insinuating a perturbation in testicular lipid metabolism which suggests a lipotoxic effect. This is further depicted by the altered lipid metabolites as well as the activation of α-linolenic acid and linoleic acid metabolism pathways in the untreated tissues (Tables 2 and 3). The inability of strawberry fruits to restore the altered metabolites and inactivate the oxidative-activated pathway, despite suppressing the lipase activity, suggests that strawberry fruits may not be protective against lipotoxicity in testicular oxidative injury.

Testicular cholesterol plays an important role in male fertility as it is an important metabolite for steroid biosynthesis, bile acid biosynthesis and steroidogenesis (Eacker et al., 2008; Thompson et al., 2004). The identification of these pathways in all the testicular tissues (Table 3 and Figure 6) can be attributed to the identified cholesterol metabolites in all the tissues (Table 2). The reduced testicular cholesterol and triglyceride levels in the untreated tissues (Figure 7) further depict a lipotoxic effect and dysregulated lipid metabolism on the induction of oxidative injury. These reduced levels corroborate the increased lipase activity in the untreated tissues (Figure 5), as the enzyme catalyses the hydrolysis of triglyceride to FFAs (Chung et al., 2001). The inability of strawberry fruits to restore these levels further depicts the inability of the fruits to protect against lipotoxicity in testicular oxidative injury.

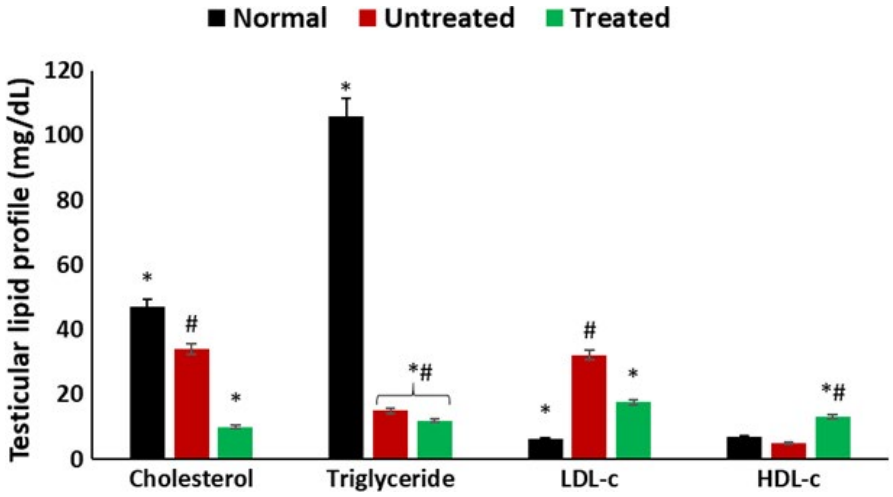


FIGURE 7 Effect of strawberry fruit (*F. x ananassa*) on lipid profile of oxidative testicular injury. Values =mean ± *SD*; *n* = 3. *Statistically significant ($p < .05$) compared to untreated tissue; #statistically significant ($p < .05$) compared to normal tissue

5 | CONCLUSION

Taken together, these data demonstrate the potentials of strawberry fruits in protecting against testicular oxidative injury. This is depicted by the ability of the fruits to improve antioxidant activity, as well as to attenuate cholinergic and purinergic dysfunctions. Although the fruits were able to inhibit testicular lipase activity, it did not have any effect on lipid dysmetabolism. Thus, suggesting that the fruits may not be effective against testicular Fe^{2+}-mediated alterations in lipid metabolism. However, the limitation of the present study is the lack of in vivo models; thus, in vivo studies are recommended to further decipher the molecular mechanisms involved in the protective effect of the fruits.

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CONFLICT OF INTERESTS

None.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

ORCID

Ochuko L. Erukainure https://orcid.org/0000-0003-0489-338X
Ayodeji B. Oyenihi https://orcid.org/0000-0001-9912-1539

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