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# Vitamin E and quercetin attenuated the reproductive toxicity mediated by lead acetate in male Wistar

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## Abstract

**Background:** Heavy metals are well documented to induce reproductive toxicity. This study was designed to investigate the role of vitamin E and quercetin on reproductive toxicity mediated by lead acetate in male Wistar rats. Thirty male adult Wistar rats were grouped into six ( $n = 5$  per group) as follows: Group 1 (Control); Group 2 and 3 were administered with 100 mg/kg vitamin E and quercetin, respectively; Group 4 was administered with 30 mg/kg lead acetate; Groups 5 and 6 received lead acetate with vitamin E and lead acetate with quercetin, respectively.

**Results:** Lead acetate significantly increased ( $p < 0.05$ ) testicular malondialdehyde, nitric oxide, lead ion and abnormal sperm morphology, while testicular catalase, superoxide dismutase activities, calcium ion, zinc ion, serum follicle stimulating hormone, luteinizing hormone, testosterone, sperm count, motility, average path, curvilinear velocity, and sperm viability were significantly reduced ( $p < 0.05$ ). The co-administration of lead acetate with vitamin E and quercetin significantly reversed ( $p < 0.05$ ) the testicular level of malondialdehyde, nitric oxide, lead ion, abnormal sperm morphology, catalase superoxide dismutase activities, calcium ion, zinc ion, follicle stimulating hormone, luteinizing hormone, testosterone, sperm count, motility, average path velocity and sperm viability.

**Conclusions:** Vitamin E and quercetin attenuated the reproductive toxicity induced by lead acetate in the male Wistar rats, and this suggests that vitamin E and quercetin may serve as possible therapeutic agents in improving male reproductive functions in heavy metal toxicity.

**Keywords:** Lead acetate, Antioxidant, Vitamin E, Quercetin, Reproductive toxicity

## Background

The lethal health effects of heavy metals on the environment are now a cause for serious concerns across the world. Lead ( $Pb^{2+}$ ) is one of the heavy metals commonly found naturally in the environment (Patra et al. 2011). Human exposure to lead is reported to have effects on

the general body system, such as the kidney (Abdel-Daim et al. 2020), cardiovascular system (Nosratola 2008), lung (Katarzyna et al. 2011), nervous system (Kumawat et al. 2014) and gastrointestinal system (Xiao et al. 2016). Exposure to high concentration of  $Pb^{2+}$  has been linked to reductions in sperm count, concentration, motility, volume and reduces spermatozoa activity (Oyeyemi et al. 2019, 2020). The mechanisms through which  $Pb^{2+}$  causes reproductive damages are: oxidative stress, decrease in testicular antioxidants, direct toxic effects on sperm cells and alteration in the hypothalamic–pituitary–testicular axis (Pandya et al. 2012; Oyeyemi et al. 2019, 2020).

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Alpha tocopherol (Vitamin E; Vit E) is a fat-soluble vitamin with characteristic antioxidant activities (Traber et al. 2006; Verhagen et al. 2006). Vegetable oils, eggs, butter, wholemeal cereals, fruits, green leafy vegetables and seafood are good sources of Vit E. The vitamin is involved in cell signaling, expression of gene control, immune regulation and other metabolic processes (Traber et al. 2006). It has also been reported to protect genetic materials in the spermatozoa, as well as improved fertility (Kalthur et al. 2011; Oyeyemi et al. 2015).

Quercetin is a polyphenolic compound belonging to the class of flavanol, which is found in fruit (red apples), vegetables such as red onions and tomatoes (Smith et al. 2003; Mitchell et al. 2007), plants (sweet potato and tea) and grains (green beans). It is usually used as an ingredient in food supplements and beverages (Justesen and Knuthsen 2001). It is known to improve sperm quality and consequently increase men's fertility rate (Muralidhara 2007). Quercetin has been reported to reduce lead-induced hepatotoxicity, lead contents in blood and liver through its antioxidant properties (Williams et al. 2004; Liu et al. 2013). Despite its antioxidant properties, quercetin is reported to increase testicular reactive oxygen species, lipid peroxidation and decrease in sperm count and motility (Ranawat et al. 2013).

We recently reported the stimulatory action of clomiphene citrate in Pb-induced reproductive toxicity, which might be temporary due to the observed decrease in sperm count, endogenous antioxidants and increased lipid peroxidation (Oyeyemi et al. 2019). We suggest that antioxidant supplementation may be essential to prevent infertility caused by exposure to heavy metals (Oyeyemi et al. 2019). Hence, this study aims at evaluating the role of vitamin E and quercetin in reproductive toxicity induced by lead acetate in male Wistar rats.

## Methods

### Chemicals

For the purpose of this study, the lead acetate and quercetin used are products of Sigma-Aldrich Chemical Corporation (St. Louis, MO, USA), while the Vitamin E is a product of Glenmark pharmaceuticals (Mumbai, India). All other chemicals and reagents used were of analytical grade.

### Animals

This study used thirty male Wistar rats of average age and weight of  $13 \pm 2$  weeks and  $180 \pm 10$  g, respectively. The animals were gotten from the Igbinedion University Animal House and Experimental Research Unit. Each well-ventilated plastic cage housed five animals under normal laboratory setting of 12 h light/12 h of darkness cycle and a temperature of  $21 \pm 4$  °C. They were fed on pelletized

mash and had unlimited access to drinking water. All the rats were acclimatized for two weeks before the commencement of experimental procedures.

### Experimental design

The animals were grouped equally into 6 ( $n=5$  per group) and treated as follows: Group 1 (Control) received 3 mL/kg distilled water; Group 2 and 3 were treated with 100 mg/kg Vit E and 100 mg/kg Quercetin, respectively; Group 4 was treated with 30 mg/kg lead acetate (Pb); Groups 5 and 6 received Pb + Vit E and Pb + quercetin, respectively. The doses of lead acetate, Vit E and quercetin were according to the method used by previous studies (Chander et al. 2014; Elgawish and Abdelrazek 2014; Oyeyemi et al. 2015).

The oral administration of the vehicle, chemicals and vitamin were done for sixty-one days consecutively. Institution for Laboratory Animals Research (ILAR 2011) guide for the care and use of laboratory animals were strictly followed.

All the animals were anaesthetized with 50 mg/kg sodium thiopental via intra-peritoneal injection at the termination of administrations. A cardiac puncture was used to collect blood samples, and testes with epididymis were harvested. The animals were killed by cervical dislocation, and the carcasses were disposed by incineration.

### Testicular oxidant and antioxidant activity

The testicular oxidant and antioxidant status were evaluated as previously reported in our studies (Oyeyemi et al. 2019, 2020). The testes were homogenized in a cold 50 mM Tris-potassium chloride buffer (pH 7.4), and cold centrifuge was used to spin the homogenate at 4000 resolution per minute for 15 min at 4 °C. Malondialdehyde (MDA), catalase and superoxide dismutase (SOD) activities were estimated using the obtained supernatant.

### Testicular nitric oxide analysis

Testicular nitric oxide (NO) level was evaluated with a colorimetric assay kit. The assay procedures were followed as prescribed in the manual (Oxford Biomedical Research, USA).

### Testicular lead, calcium and zinc ions levels

Testicular  $Pb^{2+}$ , calcium ( $Ca^{2+}$ ) and zinc ( $Zn^{2+}$ ) levels were estimated by PerkinElmer atomic absorption spectrometer with winLab 32 software. About 0.2 g of the left testis was digested in 6 mL concentrated nitric acid and diluted to 20 mL with deionizing water. The absorbance of  $Pb^{2+}$ ,  $Ca^{2+}$  and  $Zn^{2+}$  was read at 283.3 nm, 422.7 nm and 213.9 nm, respectively, and the concentration was evaluated and expressed as mg/mL (Ayinde et al. 2012).

### Hormonal assays

Serum was obtained from the blood samples, and enzyme-linked immunosorbent assay (Calbiotech kit, El Cajon, California) method was used for the estimation of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone concentrations.

### Sperm analysis

The right epididymis was incised and put in a petri dish with 2 mL of phosphate buffer saline solution (pH 7.4) that had been pre-warmed (37 °C). The spermatozoa were released by piercing the cauda epididymis into the buffer solution. About 9 $\mu$ L of the spermatozoa suspension was placed on a counting chamber (Oyeyemi et al. 2020). The sperm count and kinetics were evaluated with the computer-aided Sperm Analyzer (CASA; JH-6004 Sperm Quality Analyzer).

Spermatozoa viability and abnormal morphology were assessed using the methods reported by previous study (Oyeyemi et al. 2019). Part of the sperm suspension (10  $\mu$ L) obtained from the right epididymis was mixed with eosin-nigrosin staining solution in an equal volume. A coverslip was mounted over the stained sperm suspension on a microscope slide. The stained slides were air-dried and evaluated with a light microscope ( $\times 1000$ ). The result of unstained spermatozoa (viable) was expressed in percentage.

A thin smear was made from the spermatozoa suspension on a microscope slide. Air-dried spermatozoa smear was stained with nigrosin-eosin and examined with a microscope ( $\times 1000$ ). The abnormal spermatozoa morphology was expressed in percentage.

### Statistical analysis

GraphPad Prism version 8.0.1 was used for the data analysis. One-way Analysis of Variance (ANOVA) was used to determine the difference between means among the groups and followed by Tukey's post hoc test. The results were expressed as mean  $\pm$  SEM. Differences between means were considered significant at  $p < 0.05$ .

## Results

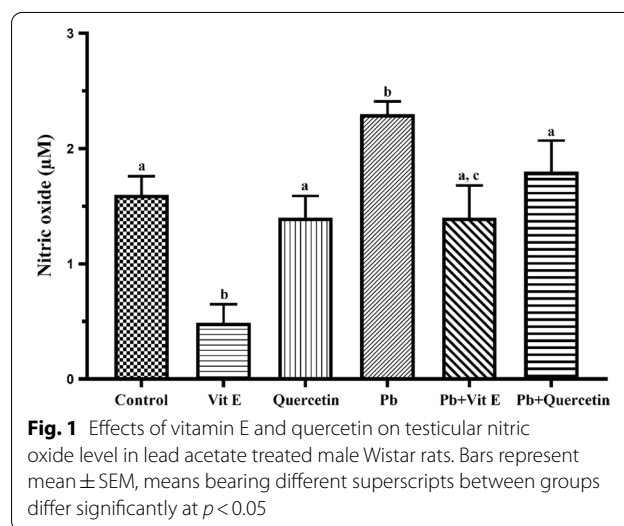
### Testicular oxidant and antioxidant enzymes

Table 1 shows that the testicular level of MDA was increased significantly, while the catalase and SOD activities were significantly reduced in the Pb group when compared with the control group. The co-administration of Pb with Vit E and quercetin significantly decreased the testicular MDA level compared with the Pb group. There was a significant increase in the testicular activities of catalase and SOD in the groups treated with Pb plus Vit E and Pb plus quercetin

**Table 1** Effect of Vitamin E and quercetin on testicular oxidant and antioxidant enzymes in lead acetate treated male Wistar rats (Values represent mean  $\pm$  SEM)

Groups	Malondialdehyde (mmol/mg testis)	Catalase (Unit/min/mg testis)	Superoxide dismutase (Unit/mg testis)
Control	1.3 $\pm$ 0.35 <sup>a</sup>	9.5 $\pm$ 0.88 <sup>a</sup>	15.7 $\pm$ 2.67 <sup>a</sup>
Vit E	1.1 $\pm$ 0.19 <sup>a</sup>	14.1 $\pm$ 0.77 <sup>b</sup>	12.9 $\pm$ 0.74 <sup>a</sup>
Quercetin	1.0 $\pm$ 0.23 <sup>a</sup>	12.3 $\pm$ 0.65 <sup>a</sup>	13.2 $\pm$ 1.33 <sup>a</sup>
Pb	2.8 $\pm$ 0.30 <sup>b</sup>	6.5 $\pm$ 0.81 <sup>b</sup>	8.4 $\pm$ 0.77 <sup>b</sup>
Pb + Vit E	1.5 $\pm$ 0.24 <sup>a, c</sup>	12.5 $\pm$ 1.02 <sup>a, c</sup>	11.4 $\pm$ 0.60 <sup>a, c</sup>
Pb + Quercetin	1.4 $\pm$ 0.17 <sup>a, c</sup>	10.8 $\pm$ 0.75 <sup>a, c</sup>	12.3 $\pm$ 0.65 <sup>a, c</sup>

Means bearing different superscripts between groups differ significantly at  $p < 0.05$



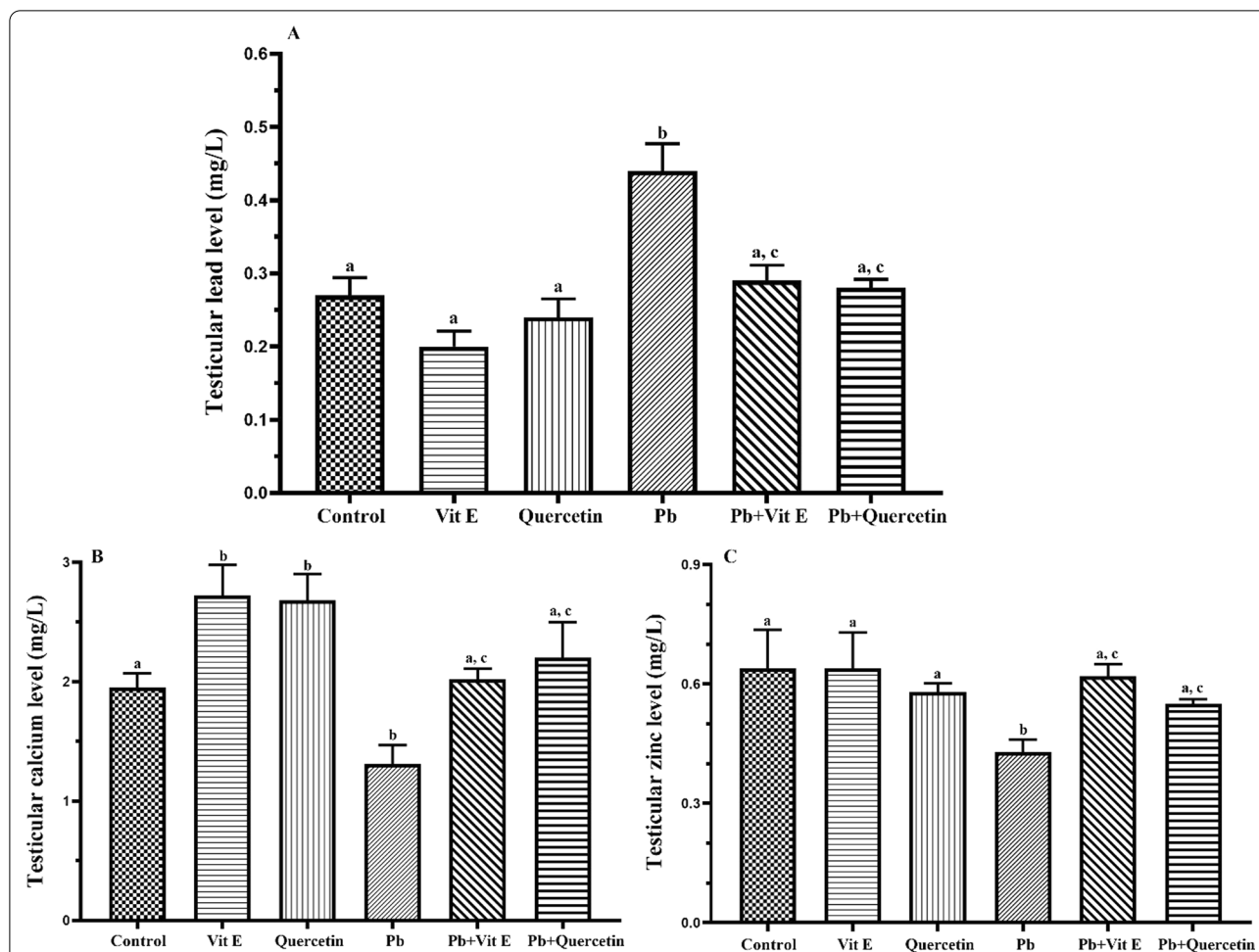
compared with the Pb group. Compare Vit E + Pb with the control.

### Testicular nitric oxide

Figure 1 shows that the testicular NO level was significantly reduced and increased in the Vit E and Pb groups, respectively, when compared with the control. There was a significant decrease in the testicular NO levels of the animals co-treated with Pb and Vit E.

### Testicular lead, calcium and zinc ions levels

Figure 2a shows that the administration of Pb significantly increased the testicular Pb<sup>2+</sup> level when compared with the control group. The co-administration of Pb with Vit E and quercetin significantly reduced the testicular Pb<sup>2+</sup> level compared with the Pb group. The testicular Ca<sup>2+</sup> level was significantly increased in the Vit E and quercetin groups, while significant reduction



**Fig. 2** Effect of vitamin E and quercetin on testicular accumulation of micro-elements in lead acetate treated male Wistar rats. Bars represent mean  $\pm$  SEM, means bearing different superscripts between groups differ significantly at  $p < 0.05$

was observed in the Pb group when compared with the control (Fig. 2b). There was a significant increase in the testicular  $Ca^{2+}$  level of the groups administered with Pb plus vit E and Pb plus quercetin when compared with the control group (Fig. 2b).

Figure 2c shows a significant reduction in the testicular  $Zn^{2+}$  level of Pb-treated group when compared with the control group. There was a significant increase in the testicular  $Zn^{2+}$  level when Vit E and quercetin, respectively, co-treated with Pb when compared with the Pb group.

**Hormones**

Table 2 shows that the serum levels of FSH, LH and testosterone were significantly reduced in the Pb treated group compared with the control group. The FSH, LH and testosterone levels were significantly increased in the

Pb co-treated with Vit E and quercetin treated groups compared with the Pb group.

**Sperm count, motility/kinetics, viability and morphology**

**Sperm count**

Sperm count was increased significantly in the quercetin group and significantly reduced in the Pb treated group when compared to the control group. The co-administration of Vit E and quercetin with Pb significantly increased the sperm count compared with the Pb group (Fig. 3).

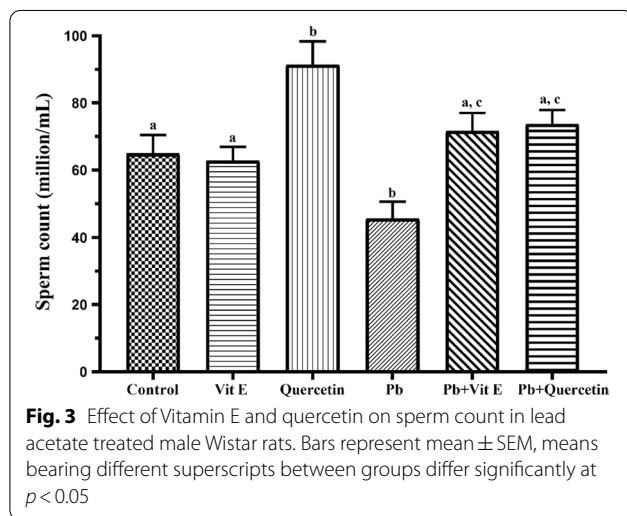
**Sperm motility**

Figure 4a, b shows a significant reduction in the sperm motility and progressive motility of the Pb-treated group when compared with the control. Sperm motility and progressive motility significantly increased in the Pb plus Vit E and Pb plus quercetin groups compared with the Pb group. The non-progressive motility (Fig. 4c) and

**Table 2** Effect of Vitamin E and quercetin on follicle-stimulating hormone, luteinizing hormone and testosterone in lead acetate treated male Wistar rats (Values represent mean  $\pm$  SEM)

Groups	Follicle stimulating hormone (mIU/ml)	Luteinizing hormone (mIU/ml)	Testosterone (ng/ml)
Control	4.37 $\pm$ 0.39 <sup>a</sup>	16.68 $\pm$ 0.41 <sup>a</sup>	3.2 $\pm$ 0.41 <sup>a</sup>
Vit E	4.01 $\pm$ 0.43 <sup>a</sup>	18.20 $\pm$ 1.24 <sup>a</sup>	2.8 $\pm$ 0.17 <sup>a</sup>
Quercetin	4.60 $\pm$ 0.38 <sup>a</sup>	20.45 $\pm$ 1.03 <sup>b</sup>	2.8 $\pm$ 0.24 <sup>a</sup>
Pb	2.63 $\pm$ 0.29 <sup>b</sup>	13.47 $\pm$ 0.64 <sup>b</sup>	1.14 $\pm$ 0.12 <sup>b</sup>
Pb + Vit E	3.75 $\pm$ 0.52 <sup>a, c</sup>	17.82 $\pm$ 1.59 <sup>a, c</sup>	2.7 $\pm$ 0.14 <sup>a, c</sup>
Pb + Quercetin	4.18 $\pm$ 0.39 <sup>a, c</sup>	18.91 $\pm$ 1.72 <sup>a, c</sup>	2.2 $\pm$ 0.19 <sup>a, c</sup>

Means bearing different superscripts between groups differ significantly at  $p < 0.05$



immotile sperm (Fig. 4d) were significantly increased in the Pb group when compared with the control. A significant decrease was observed in the immotile sperm of the Pb plus Vit E and Pb plus quercetin groups compared with the Pb group.

#### Sperm kinetics

Table 3 shows that the average path velocity and curvilinear velocity were significantly decreased in the Pb group compared with the control group. The average path velocity was significantly increased in the Pb groups co-treated with Vit E and quercetin, respectively. Curvilinear velocity significantly increased in the group co-administered with Pb and quercetin when compared with the Pb group.

#### Sperm viability and abnormal sperm morphology

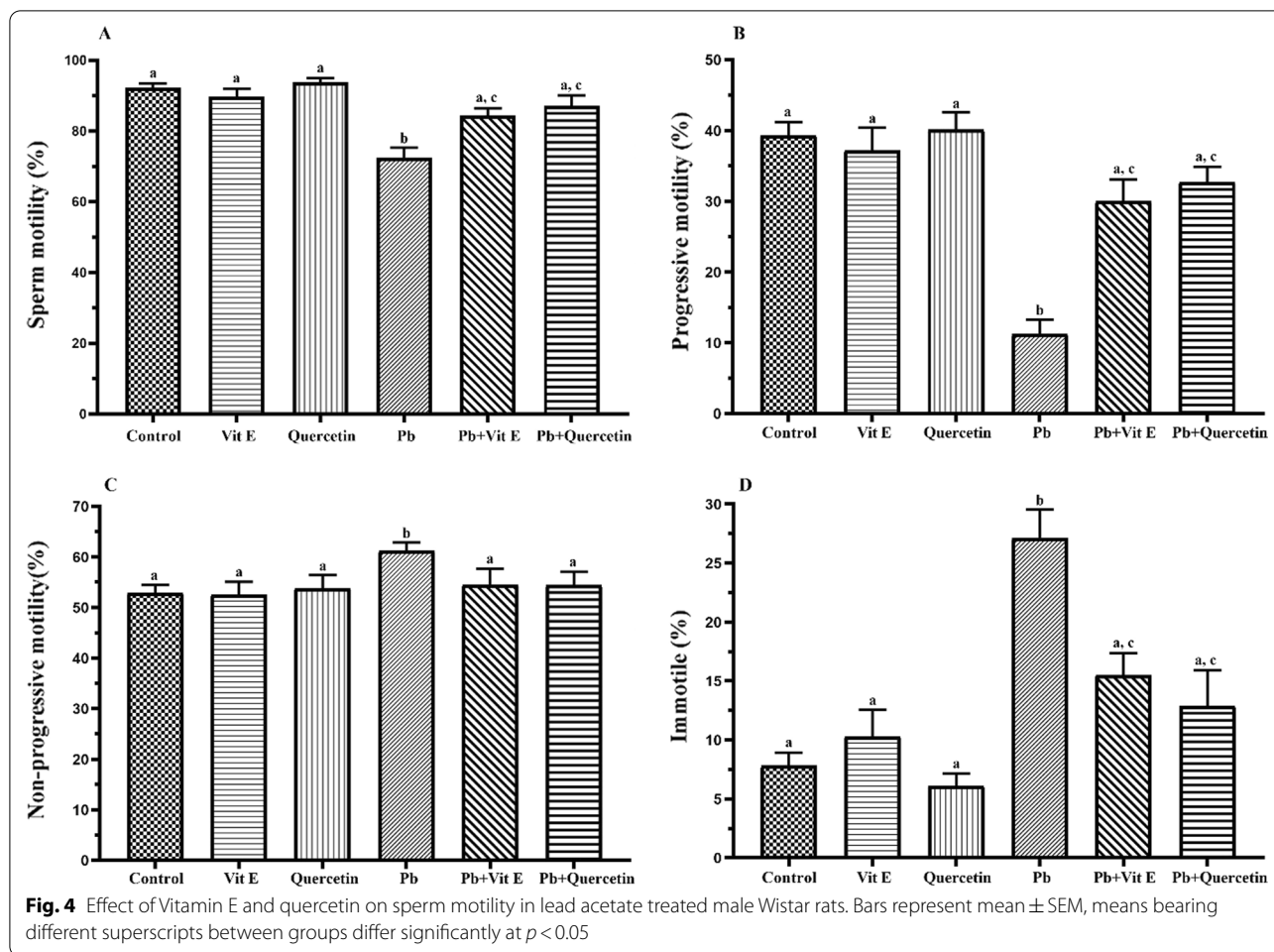
Figure 5a shows that sperm viability was significantly reduced in the Pb treated group compared with the control group. The separate administration of Vit E and quercetin with Pb significantly increased the percentage sperm viability compared with the Pb group. The percentage of abnormal sperm morphology was significantly

increased in the Pb treated group compared with the control group. The observed increase in the percentage of abnormal sperm morphology in the Pb group was significantly reduced with the separate co-administration of Vit E and quercetin (Fig. 5b).

#### Discussion

Several experimental studies have reported that heavy metals caused testicular oxidative stress, which has been linked to infertility. For some years, malondialdehyde has been used as a marker for lipid peroxidation and oxidative stress (Giera et al. 2012). In this study, lead acetate increased testicular MDA concentration, and this could be due to the accumulation of Pb<sup>2+</sup> in the testes as observed in this study. The observed increase in MDA is in agreement with previous study (Oyeyemi et al. 2020). Both Vit E and quercetin decreased testicular MDA level when co-administered with Pb; these could be due to their protective antioxidant properties. Catalase and SOD are well-known endogenous antioxidant enzymes that help cells combat oxidative stress by converting hydrogen peroxide to oxygen and water (Chelikani et al. 2004; Halliwell and Gutteridge 2007). In this study, a reduction in testicular catalase and SOD activities was observed in Pb treated animals. This observation was similar to our previous reports (Oyeyemi et al. 2019, 2020). The increase in MDA concentration and accumulation of Pb<sup>2+</sup> in the testicles of Pb treated rats in this study may be accountable for reducing the catalase and SOD activities in the Pb group. Testicular catalase activity was increased when Pb was co-administered with Vit E and quercetin, respectively. This observation may be attributed to the antioxidant properties of Vit E and quercetin, which include free radical scavenging activities, inhibition of oxidative stress and enhancement of antioxidant (Annapurna et al. 2013; Etsuko 2014).

This study shows an increase in testicular NO level in animals treated with Pb. The increase in NO may be one explanation for the current study's observed increase in MDA concentration. This observation could be due to



**Table 3** Effect of Vitamin E and quercetin on sperm kinetics in lead acetate treated male Wistar rats (Values represent mean  $\pm$  SEM)

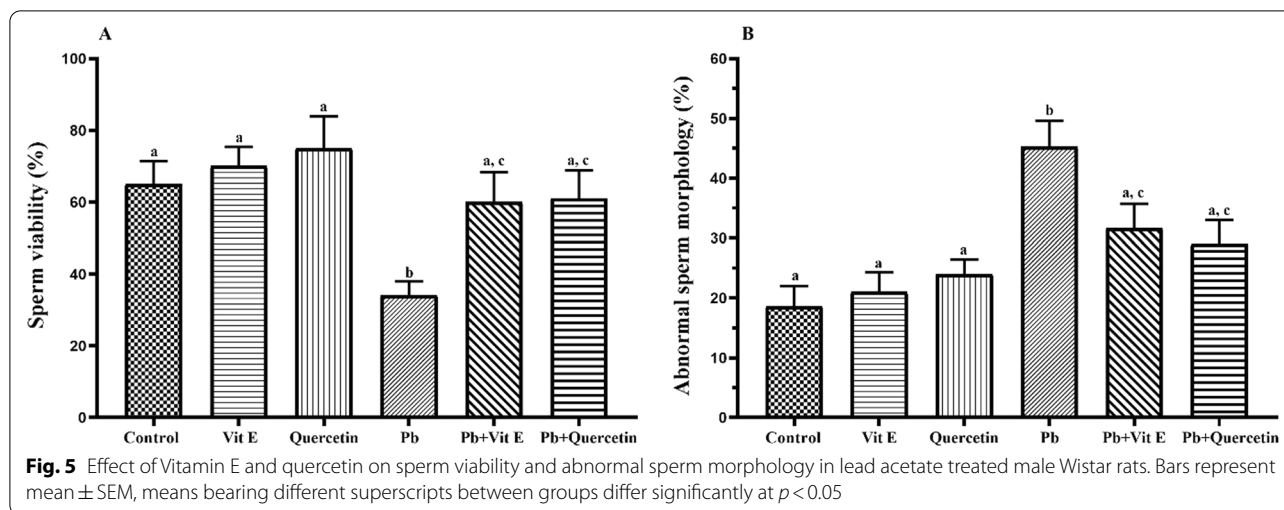
Sperm kinetics	Control	Vit E	Quercetin	Pb	Pb + Vit E	Pb + Quercetin
Average path velocity ( $\mu\text{m/s}$ )	14.0 $\pm$ 0.41 <sup>a</sup>	14.9 $\pm$ 0.31 <sup>a</sup>	14.6 $\pm$ 0.46 <sup>a</sup>	11.9 $\pm$ 0.79 <sup>b</sup>	13.4 $\pm$ 1.07 <sup>a, c</sup>	13.7 $\pm$ 0.79 <sup>a, c</sup>
Curvilinear velocity ( $\mu\text{m/s}$ )	18.3 $\pm$ 0.51 <sup>a</sup>	19.6 $\pm$ 0.75 <sup>a</sup>	18.8 $\pm$ 0.66 <sup>a</sup>	15.0 $\pm$ 1.64 <sup>b</sup>	17.3 $\pm$ 1.81 <sup>a</sup>	18.5 $\pm$ 1.63 <sup>a, c</sup>
Straight-line velocity ( $\mu\text{m/s}$ )	7.4 $\pm$ 0.20 <sup>a</sup>	7.6 $\pm$ 0.19 <sup>a</sup>	7.5 $\pm$ 0.29 <sup>a</sup>	6.6 $\pm$ 0.37 <sup>a</sup>	6.8 $\pm$ 0.28 <sup>a</sup>	7.3 $\pm$ 0.42 <sup>a</sup>
Amplitude lateral head ( $\mu\text{m}$ )	0.63 $\pm$ 0.014 <sup>a</sup>	0.68 $\pm$ 0.019 <sup>a</sup>	0.66 $\pm$ 0.019 <sup>a</sup>	0.54 $\pm$ 0.043 <sup>a</sup>	0.61 $\pm$ 0.054 <sup>a</sup>	0.64 $\pm$ 0.039 <sup>a</sup>
Beat cross frequency (Hz)	2.6 $\pm$ 0.08 <sup>a</sup>	2.8 $\pm$ 0.15 <sup>a</sup>	2.7 $\pm$ 0.13 <sup>a</sup>	2.0 $\pm$ 0.25 <sup>a</sup>	2.3 $\pm$ 0.29 <sup>a</sup>	2.7 $\pm$ 0.25 <sup>a</sup>
Line moving (%)	36.7 $\pm$ 2.07 <sup>a</sup>	40.0 $\pm$ 2.68 <sup>a</sup>	37.0 $\pm$ 1.59 <sup>a</sup>	28.0 $\pm$ 3.24 <sup>a</sup>	31.0 $\pm$ 2.73 <sup>a</sup>	32.7 $\pm$ 2.73 <sup>a</sup>
Wobble (%)	78.7 $\pm$ 0.94 <sup>a</sup>	76.2 $\pm$ 2.67 <sup>a</sup>	77.7 $\pm$ 1.50 <sup>a</sup>	81.4 $\pm$ 3.57 <sup>a</sup>	78.0 $\pm$ 2.72 <sup>a</sup>	75.2 $\pm$ 2.15 <sup>a</sup>
Mean Move angle ( $^\circ$ )	4.8 $\pm$ 0.21 <sup>a</sup>	5.5 $\pm$ 0.36 <sup>a</sup>	5.2 $\pm$ 0.17 <sup>a</sup>	4.2 $\pm$ 0.48 <sup>a</sup>	4.8 $\pm$ 0.64 <sup>a</sup>	5.5 $\pm$ 0.61 <sup>a</sup>

Means bearing different superscripts between groups differ significantly at  $p < 0.05$

increased synthesis of inducible NO as a result of testicular  $\text{Pb}^{2+}$  accumulation. The current observation is consistent with previous studies, which reported that  $\text{Pb}^{2+}$  increases NO biosynthesis (Matovic et al. 2015). Nitrosative stress is caused by excessive NO synthesis, which can react with oxygen to cause fragmentation of DNA and lipid peroxidation (Valko et al. 2007). The Vit

E administration with Pb reduced testicular NO level, and this could be due to its antioxidant property in scavenging free radicals (Etsuko 2014). The quercetin administration was unable to reduce the observed increase testicular NO in the Pb-treated rats.

The observed results showed an increase in testicular  $\text{Pb}^{2+}$  level in the Pb treated group. This observation is in



line with previous studies, which showed that exposure to Pb causes an increase in serum  $Pb^{2+}$  level and accumulation in the testes (Batra et al. 2001). The observed increase in testicular  $Pb^{2+}$  level may be responsible for the increased MDA in Pb treated rats. Since  $Pb^{2+}$  accumulation in organs directly correlates with high MDA level (El-khdragy et al. 2020). The observed increase in testicular  $Pb^{2+}$  level was reversed when Vit E and quercetin were co-administered. This might due to their antioxidant properties and chelating effects of both materials as they help reduce toxic metals in the body (Etsuto 2014; Ademosun et al. 2016).

Calcium ion regulates spermatogonium division, growth, apoptosis in spermatogonium and spermatoocyte during spermatogenesis (Golpour et al. 2016, 2017). Vitamin E and quercetin increased testicular  $Ca^{2+}$  level. This present observation is in line with previous investigations, which reported that quercetin increases the  $Ca^{2+}$  level (Cheng and Li 2012). The Pb exposed group showed a decrease in the testicular  $Ca^{2+}$ ; this ability may be due to an increase in testicular  $Pb^{2+}$  accumulation, which may displace the  $Ca^{2+}$ . The administration of Vit E and quercetin with Pb significantly increased the testicular  $Ca^{2+}$  level. Vitamin E and quercetin could prevent testicular  $Pb^{2+}$  accumulation in this study due to their antioxidant and chelating properties, avoiding the displacement of  $Ca^{2+}$  by  $Pb^{2+}$  and responsible for the observed increase in the testicular  $Ca^{2+}$  level.

Furthermore,  $Zn^{2+}$  is an antioxidant element that is available in body tissues and fluids. It is essential for cell division, growth, immunity and wound healing. Zinc is a vital trace element for spermatogenesis (Yamaguchi et al. 2009). We observed a decrease in the testicular  $Zn^{2+}$  level in the Pb treated rats. The observed decrease in the testicular  $Zn^{2+}$  may be due to  $Pb^{2+}$  competing for

the binding site of  $Zn^{2+}$  in enzymatic reactions and could replace  $Zn^{2+}$  in such reaction (Hari et al. 2016). Lead ion may influence the absorption/distribution of  $Zn^{2+}$  in the testes by inhibiting the trafficking system of  $Zn^{2+}$ , hence may be responsible for the observed decrease in testicular  $Zn^{2+}$  level in this study. In this study, the testicular  $Zn^{2+}$  level increased significantly when Vit E and quercetin were co-treated with Pb. This observation may be ascribed to the action of Vit E and quercetin ability to prevent  $Pb^{2+}$  accumulation in the testes.

In this study, the Pb treated rats showed a decline in the serum FSH and this result is similar to previous reports (El-Sayed and El-Neweshy 2010; Oyeyemi et al. 2020). The decrease in the serum FSH level may be attributed to the deleterious action of Pb on the pituitary-testis axis, causing hormonal disruption and the modulation of Sertoli cell function such as the depletion of Sertoli cell numbers or disruption of the blood-testis-barrier (Siu et al. 2009), which in turn may disrupt spermatogenesis (Wang et al. 2013). However, the decline observed in serum FSH of animals treated with Pb was reversed when co-treated with Vit E and quercetin. Recent results supporting this observation suggest that Vit E has a protective effect on FSH level by obstructing the free radical chain reactions generated by  $Pb^{2+}$ . While quercetin, via its potent antioxidant properties (Galati et al. 2002), is capable of mitigating the Pb-induced oxidative damages in the brain (Chander et al. 2014) and testicular level (Abd El-Latief 2015) in turn protecting the serum FSH level.

Our previous studies recorded a decreased LH and testosterone in Pb treated rats (Oyeyemi et al. 2019, 2020). These observations concur with the results of the present study, which show a reduction in LH and testosterone concentrations in the Pb group. Studies suggests that the reduced LH level is due to Pb-induced disorders in the

hypothalamic control of pituitary hormone secretions, which depicts a disruption in the gonadotropin-releasing hormone (GnRH) secretion from the hypothalamus and disrupting LH release (Sokol et al. 2002). Vitamin E and quercetin are effective exogenous antioxidants (Galati et al. 2002; Valko et al. 2006). We observed a surge in the LH and testosterone concentrations of Pb animals co-treated with Vit E and quercetin, respectively. Previous studies showed that Vit E thwarted the harmful property of Pb through prevention of oxidative stress (Sajitha et al. 2010). Previous studies revealed the protective property of quercetin against oxidative stress (Uzun et al. 2010; Farombi et al. 2012). The free radical scavenging, oxidative stress prevention and chelating activities of Vit E and quercetin may be mechanisms, which ameliorates Pb-induced reproductive toxicity, thereby improving LH and testosterone levels.

This study shows that the administration of Pb reduced sperm count and viability while abnormal sperm morphology was increased, which is in support of previous studies (Hernandez-Ochoa et al. 2005; Oyeyemi et al. 2019, 2020). These observations may be a result of decreased testicular antioxidant levels, increased lipid peroxidation, testicular  $Pb^{2+}$  accumulation, decline in  $Zn^{2+}$ , FSH, LH and testosterone levels in this study. An increase in reactive oxygen species (ROS) and interruption of the hypothalamic-pituitary axis secretion by Pb has also been reported to be involved in causing the sperm count, viability and normal sperm morphology to decrease (Graca et al. 2004; Kasperczyk et al. 2004). The Vit E and quercetin separately reversed the observed effects of Pb on the sperm count, sperm viability and normal sperm morphology. Vitamin E and quercetin's antioxidant properties may be accountable for the observed increase in sperm count, viability and reduced abnormal sperm morphology by counteracting the deleterious effect of Pb-induced ROS (Galati et al. 2002; Sajitha et al. 2010). The results of this study show that Vit E and quercetin prevent testicular lead-induced lipid peroxidation, lead accumulation, and increased testicular  $Ca^{2+}$ ,  $Zn^{2+}$  and antioxidant level. These observations may be the mechanism through which Vit E and quercetin avert Pb toxic effects on spermatozoa.

Sperm motility, especially progressive motility and sperm kinetics, is essential indices for spermatozoa's efficient fertilizing capacity *in vivo* (Winn and Whitaker 2018). This study shows that Pb administration reduced sperm motility, progressive motility, increased immotile and non-progressive motility, similar to previous study (Hernandez-Ochoa et al. 2005). The observed reduction in sperm motility may result from a reduction in the antioxidant level and increased ROS (Kasperczyk et al. 2004). The alteration in the hypothalamic-pituitary axis

secretion (Oyeyemi et al. 2020), testicular accumulation of  $Pb^{2+}$  and reduction in  $Ca^{2+}$  could be responsible for the observed reduction in sperm motility of animals exposed to Pb. In this study, Pb co-administered with Vit E and quercetin increased sperm motility, progressive motility, decreased immotile and non-progressive motility. This may be due to Vit E and quercetin's antioxidative properties that counteract the toxic action of Pb through the scavenging free radicals and ROS (Galati et al. 2002; Sajitha et al. 2010) and improve the testicular  $Ca^{2+}$  level that is essential for sperm motility.

Administration of Pb in this study inhibits average path velocity and curvilinear velocity. Most sperm kinetics such as straight-line velocity, amplitude lateral velocity, beat cross frequency, line moving, wobble and mean move angle were not significantly affected with the Pb administration, as well as Pb co-treated with Vit E and quercetin. Average path velocity and curvilinear velocity were significantly increased in Pb co-treated with quercetin, which concurs with previous studies (Seifi-Jamadi et al. 2016). The observed increase in average path velocity and curvilinear in the Pb co-treated with quercetin may be connected to the ability of the quercetin to enhance the  $Ca^{2+}$ -ATPase, an important enzyme that modulates sperm motility (Seifi-Jamadi et al. 2016). Vitamin E and quercetin elevated testicular  $Ca^{2+}$  level in the Pb treated rats. The elevation of the testicular  $Ca^{2+}$  level may be responsible for the observed increase in sperm path velocity and curvilinear (Williams and Ford 2003).

## Conclusions

Vitamin E and quercetin attenuated the reproductive toxicity induced by lead acetate in the male Wistar rats, and this suggests that vitamin E and quercetin may serve as possible therapeutic agents in improving male reproductive functions in heavy metal toxicity.

## Abbreviations

$Ca^{2+}$ : Calcium; CASA: Computer-aided sperm analyzer; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; MDA: Malondialdehyde; NO: Nitric oxide; Pb: Lead acetate;  $Pb^{2+}$ : Lead; ROS: Reactive oxygen species; SOD: Superoxide dismutase; Vit E: Vitamin E;  $Zn^{2+}$ : Zinc.

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## Authors' contributions

WAO contributed to conception and designed the study, data analysis, interpretation. AOA collected and assayed the samples and performed review. OOD collected and assayed the samples and performed review. IA contributed to writing the manuscript and performed review. OTD contributed to care for animals, data acquisition, and assayed of samples. POA contributed to care for animals, data acquisition, and assayed of samples. IOO contributed to care for animals, data acquisition, and assayed of samples. TDO contributed to data analysis and performed review. All authors read and approved the final manuscript.



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**Availability of data and materials**

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

**Declarations****Ethical approval and consent to participate**

This study was carried out according to the directive 2010/63/EU of the European parliament and of the council 2010 on the protection of animals used for scientific purposes.

**Consent for publication**

Not applicable.

**Competing interests**

No competing interests.

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