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Research Article

Plant Extracts with Antioxidant Properties Ameliorate Lead Toxicity in Rats' Testes

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Abstract:

Lead acetate (LA) as one of lead form with high rate of production and distribution causing sever oxidative hazardous effects. Evaluation the role of plant extracts in amelioration the oxidative burden on human health is a global scientific interest. Aqueous extracts of *Sonchus oleraceus* and *Malva parviflora* plants were examined for their protection role against lead toxicity. The animals were divided into four groups, the first group (control) received distilled water; group 2 received LA (60 mg.kg^{-1} body weight); group 3 received LA with *Sonchus oleraceus* extract (500 mg kg^{-1}day^{-1}); group 4 received LA with *Malva parviflora* extract (500 mg kg^{-1}day^{-1}). Exposure to LA led to significant reduction in sperm properties as sperm count, mobility, viability and increasing in sperm abnormalities. Histological analysis of testes tissues revealed that rats treated with LA showed several deleterious effects associated with LA exposure. Adding of *Sonchus oleraceus* and *Malva Parviflora* extracts to treated rats with LA caused a significant improvement in sperm properties as well as recovery of histological feature of testes tissue to normal form. Obtained results indicate to the antioxidant capability of *Sonchus oleraceus* and *Malva parviflora* extracts to ameliorate the oxidative effects of LA on male sperm proprieties and tests tissue of male rats.

1. Introduction

Pollution with heavy metals has become a main global environmental concern (Gaspéri et al., 2018) where it threatens human health and safety (Wang et al., 2020). Potential hazardous effects of different pollutants in our environment are the main interest of several studies recently (Toppari et al., 1996; Haouas et al., 2015). Harmful effects of environmental pollutants such as tobacco, pesticides and heavy metals on sexual function have been reported (Tuormaa et al., 1995; Issam et al., 2011; Gorbel et al., 2002; Haouas et al., 2015).

Lead (Pb) is the second most hazardous heavy metal because of its non-degradable nature, large distribution (Kumar et al., 2000; Haouas et al., 2015). It accumulates rapidly nowadays due to increased anthropogenic activities such as electroplating, mining, automotive exhausts, coal burning, wastewater irrigation, inorganic fertilizers and steel industry (Saxena et al., 2020). Lead is toxic for human and plant (Sharaf-Eldin et al., 2023; Tchounwou et al., 2012). It affects blood vessels and could lead to immediate heart attack and death (Debnath et al., 2019). Lead could disturb the balance of oxidant-antioxidant system in respiratory and nervous systems where it causes a reduction in antioxidant content (GSH, SOD and CAT). Significant increase in MDA and NO production in various tissues like liver and kidney after lead exposure were observed (Boskabady et al., 2018). Acute Pb toxicity leads to dysfunction of the kidney, reproductive system, and brain while chronic damages are caused to the central nervous system. Lead also inhibits the synthesis of hemoglobin. Pregnant women with low calcium, iron or zinc levels are prone to the effects of lead accumulation (Collin et al., 2022)

Due to Pb accumulation in the seminal plasma (Giulioni et al., 2023), researchers have encouraged to explore its potential impacts on the reproductive function. Testicular atrophy, distraction of spermatogenesis as well as cellular degeneration were documented in humans and animals due to lead exposure (Haouas et al., 2015; Giulioni et al., 2023). It was reported that Pb could cross the blood-testis barrier and affects the germ cells at any of their differentiation stage (Haouas et al., 2015). It has been well documented that Pb impairs the reproductive function of experimental animals through endocrine disruption and depletion of antioxidant reserves (Wahab et al., 2019). Also, it was associated with sperm abnormalities and reduction in sperm count, volume and motility (Apostoli et al., 1998; Haouas et al., 2015). Changes in seminal parameters as well as density, sperm count, chromosomal damage, viability and altered spermatogenesis was detected with Pb exposure (Wu et al., 2012; Carocci et al., 2016; Offor et al., 2019). The testis of Pb treated rats shown notable degeneration and atrophied seminiferous tubules with lack of regular differentiated stages of germ cells (Anjum et al., 2017; Offor et al., 2019).

Antioxidant activity inhibits the generation of free radicals and plays an essential role in guarding against testicular toxicity (Elmoslemany et al., 2024; Sudjarwo and Sudjarwo, 2017; Adibmoradi et al., 2015; Agarwal

et al., 2010). Defending agents, such as antioxidants, against reactive oxygen species (ROS) could be a useful therapeutic for hazardous effects of heavy metal in testis (Sudjarwo and Sudjarwo, 2017). Using of different plant extracts as a source of antioxidants to ameliorate oxidative stress associated with heavy metals exposure was reported (Giulioni et al., 2023; Sudjarwo and Sudjarwo, 2017). *Sonchus oleraceus*, family of Asteraceae, and *Malva parviflora* L, family Malvaceae, are traditional medicinal plants with antitumor, antibacterial, anti-inflammatory, antidiabetic effects and antioxidant properties (Dugani et al., 2016; Alothman et al., 2018; Abd El-Salam et al., 2019; Chen et al., 2020; Munir et al., 2021). For authors knowledge, using of *Malva parviflora* in ameliorating toxicity effects of lead is not documented.

The present study aimed to investigate the capability of aqueous extracts of *Sonchus oleraceus* (So) and *Malva parviflora* (Mp) leaves in reducing hazardous effects of lead in testes tissue and sperm properties of male rats.

2. Materials and Methods

2.1. Plant material and extraction preparation

Sonchus oleraceus and *Malva parviflora* were purchased from the local market at Tanta, Gharbia Governorate, Egypt. aqueous extracts were prepared from leaves and stems with distilled water (1:1) w/v. The crude extract at -20 ^{o}C until used. Doses of used extracts were 1/10 LD_{50} *for Sonchus oleraceus* extract (Aissani et al., 2022) and the same for *M. parviflora* extracts to ease the comparing between the two extract.

2.2. Experimental design

The experiment was conducted at the laboratory of Genetic department, Faculty of Agriculture, Tanta University and at the animal house of the Department of Biological and Environmental Science, Home Economic, Al-Azhar University. Thirty-two male albino rats (*Sprague Dawley*) rats (150±10g and ten to twelve weeks old) were kept in cages under standard conditions (12/12 light/dark cycle, 23 ± 2°C, and 60% humidity) and they had free access to rodent pellet diet and water. The rats took a week to adapt and were then randomly distributed into four groups (8 animals /each). First group (control) were given distilled water (ml/kg); group 2 (LA) received lead acetate (was purchased from Oxford LAB (Fiwe CHEM LIP), Egypt) administrated by stomach tube (60 mg kg^{-1} body weight (Al-Otaibi et al., 2015)); group 3 (LA+So) administrated lead acetate (60mg.kg^{-1}) + *Sonchus oleraceus* extract (500 mg $kg^{-1}day^{-1}$) by stomach tube and finally, group 4 (LA+Mp) administrated lead acetate (60 mg.kg^{-1}) + *Malva Parviflora* extract (500 mg $kg^{-1}day^{-1}$) by stomach tube. All treatments were repeated daily for eight weeks.

2.3. Semen analysis

The samples of semen were collected at the end of experiment by cutting of cauda epididymis using blades and pressed on the clean watch glass. The collected semen was diluted ten times with solution of sodium citrate, $Na_3C_6H_5O_7$ (2.9 %), and quickly scanned to determine sperm cell count, percentage of sperm progress and motility as described by (Bearden and Fluquary, 1980). Sperm viability and abnormalities were determined microscopically in one drop of semen suspension stained by Nigrosin and Eosin (N&E) stain on a glass slide and calculating according to the following equation.

Sperm viability= alive/dead × 100

2.4. Histopathology analysis for testis.

Samples of the testes from rats were fixed in 10% neutral formalin and paraffin-embedded. Sections (5 µm thickness) were stained with hematoxylin and eosin (H & E) for the histological examination (Elmoslemany et al., 2023).

2.5. Ethical approval

Ethical approval ($AY_{2019-2020}$/Session 6/2020.01.13) was established for this experiment by the faculty of agriculture at Tanta University.

2.6. Statistical analysis

Statistical analysis was performed on obtained results using SPSS 20 software (IMB SPSS Statistics Software, Armonk, New York) with analysis of variance (ANOVA). The means of the treatments were compared using Duncan's multiple range tests. Variability in the data was expressed as the standard deviation, and $P \leq 0.05$ was considered to be statistically significant.

3. Results

3.1. Effects of lead acetate, *S. oleraceus* and *M. parviflora* extracts on sperm parameters

Changes in sperm count, mobility, viability and abnormality was detected to investigate associated changes with lead exposure as well as effect of used plant extracts.

3.1.1. Sperm count

Obtained results showed a significant decrease in sperm count from 23x106/ml in control treatment to 15x106/ml in rats treated with LA (Figure 1). This reduction appears as un crowded field, in microscopic screening, in LA treatment (Figure 2 B) comparing with control treatment (Figure 2 A). Treatments with either *S. oleraceus* (LA+So) or *M. parviflora* (LA+Mp) caused a significant increase in sperm count comparing with LA or control groups (Figure1). The highest increase induced with *M. parviflora* (LA+Mp) treatment where it increased from 15x106 at LA group to $27x10^6$ at LA+Mp group. Thus appears as increasing crowded fields in microscopic screening (Figure 2; C, D).

3.1.2. Sperm motility and viability

Sperm motility and viability showed a significant reduction at LA treatment compared with control and the other treatments. Adding plant extracts to LA treatment (LA+So and LA +Mp groups) induced a significant recovery for both of sperm mobility and viability (Table 1).

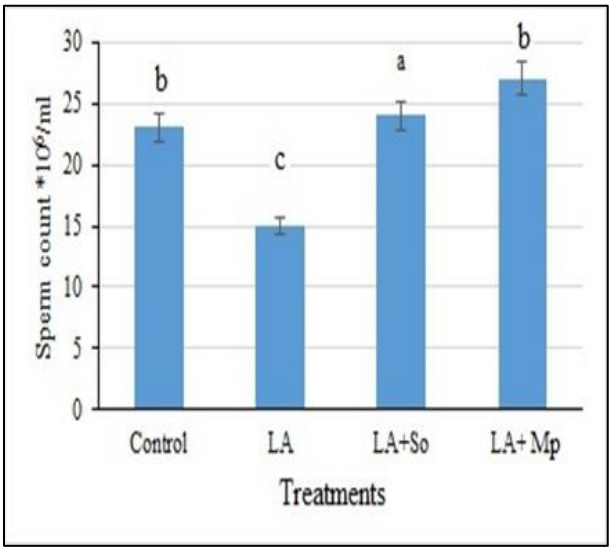

Figure 1. Changes in sperm count under control, Lead acetate treatment (60mg.kg-1) (LA), Lead acetate + *Sonchus oleraceus* extract (LA+So), lead acetate + *Malva parviflora* extract (LA+Mp). Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$.

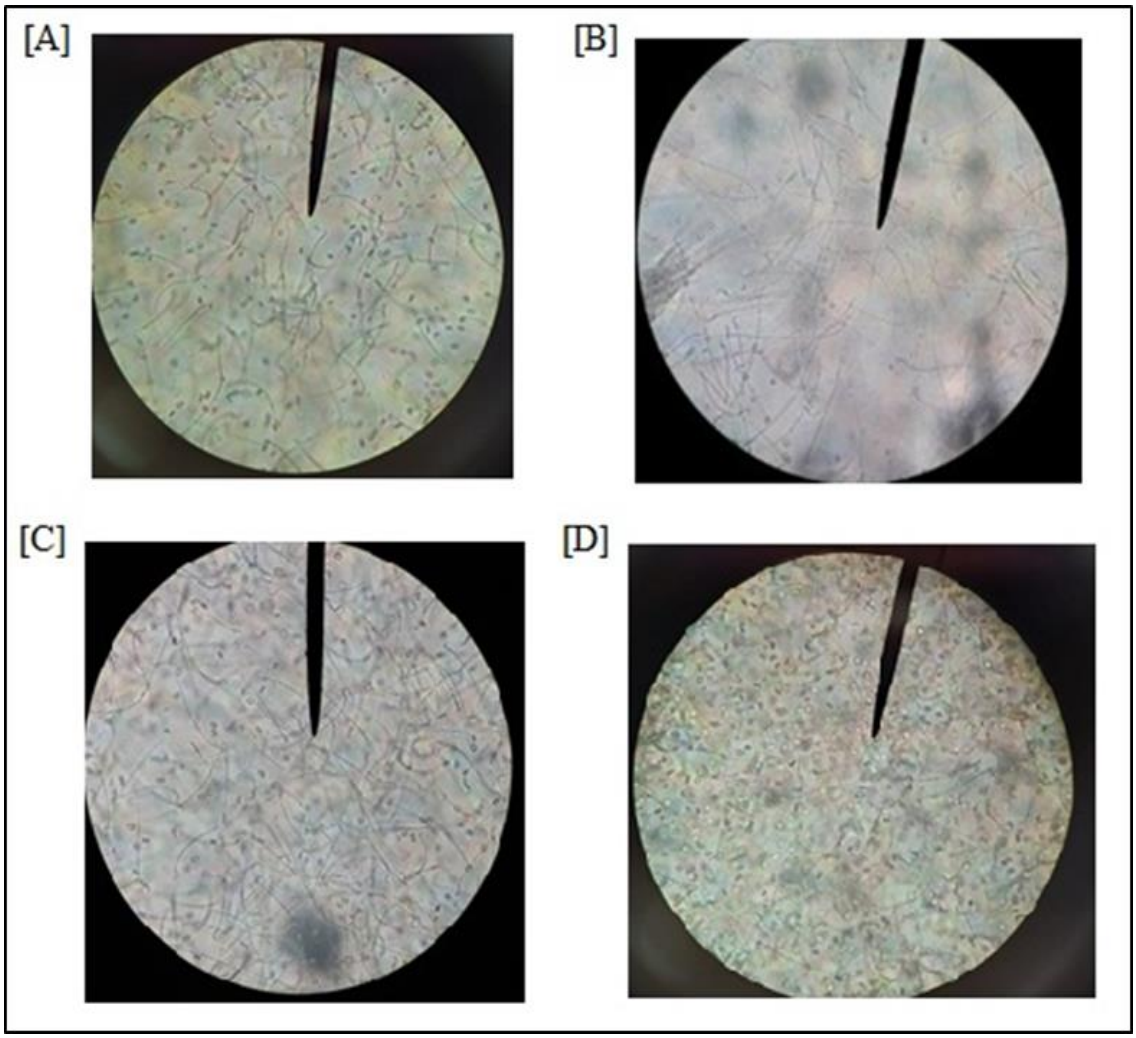

Figure 2. Changes in sperm count as a sperm crowdie under microscopic fields for control [A]; Lead acetate treat ment (60mg.kg-1) [B] Lead acetate + *S. oleraceus* extract [C] and lead acetate + *M. Parviflora* extract [D].

Table 1. Effects of *S. oleraceus* and *M. parviflora* extracts on sperm mobility and viability in male rats exposed to lead acetate.

Treatments	Sperm motility (%)	Sperm viability (%)
Control	71.33±3.21[a]	74.33±1.15[a]
LA	49.66±0.57[b]	64.66±0.57[b]
LA+ So	78.33±7.63[a]	80.00±5.00[a]
LA+ Mp	70.00±15.00[a]	76.66±7.63[a]
Sig.	0.017	0.018

LA: Lead acetate treatment (60mg.kg-1), LA+So: Lead acetate+ *S. oleraceus* extract, LA+Mp: lead acetate + *M. parviflora* extract. Duncan's multiple range test indicates significant values with distinct letters at $P \leq 0.05$.

3.1.3. Sperm abnormality

Obtained data showed that LA has a negative effect on sperm formation where it caused a significant increase in sperm abnormality comparing with control group (Figure 3). Adding of tested extracts of *S. oleraceus* (LA+So) and *M. parviflora* (LA+ Mp) inhibit the toxic effect of LA where they induced a reduction in sperm abnormality comparing with LA group. Thus pointed to the protective effect of tested extracts of *S. oleraceus* and *M. parviflora*.

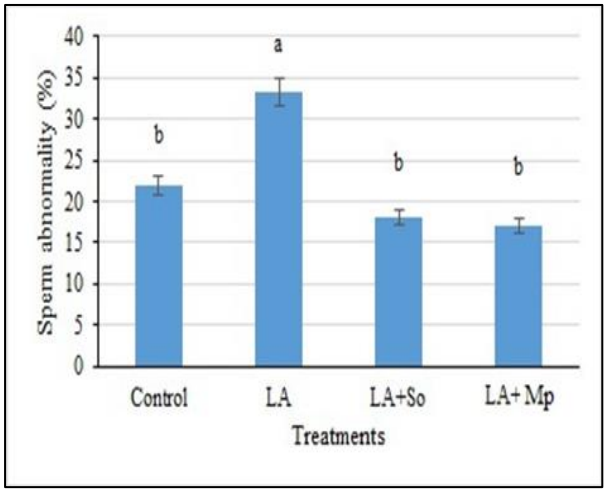

Figure 3. Effects of LA and aqueous extracts of *S. oleraceus* (LA+So) and *M. parviflora* (LA+ Mp) on sperm structure of tested rats. Duncan's multiple range test indicates significant columns with distinct letters at $P \leq 0.05$

3.2. Histological analysis of testes tissue.

Microscopic pictures of testes sections from rats at Control group showed normal appearance of spermatogonia, primary spermatocyte, secondary spermatocyte, and spermatozoa with normal interstitial tissues with Leydig cells (Figure 4A). Histological Screening of testes tissue of rats treated with LA showed high degeneration (yellow arrow) spermatogenic cells with absence of spermatozoa (red arrows), high congestion of interstitial tissues with vacuoles (black arrow) (Figure 4B). Also, disarrangement of spermatogenic cells in tubules (d) were detected with thickness congestion and dilation of blood vessels in interstitial tissues (arrowhead) (Figure 4C). On the other hand, there were recovery for the normal histological appearance and increase in the number of cells (black arrow) and spermatozoa in (yellow arrow) and sertoli cells (arrowhead), normal appearance of interstitial cells after treatment with *S. oleraceus* (LA+So) group (Figure 4 D). In LA with *M. parviflora* (LA+Mp) group, there were normal appearance and increase in fertility (black arrow) with congestion of interstitial tissues (arrowhead) (Figure 4E).

4. Discussion

The present study showed many negative effects and imbalances caused by LA on the spermatic characteristics of male rats. It was reported that lead exposure affects seminal characteristics as sperm count, motility and viability as well as lack of sperm in male rats (Ileriturk et al., 2021). This pointed to the toxic effects of LA on testicular structure. ROS generation as an associated event with Lead toxicity (Owolabi et al., 2012; Haouas et al., 2015) inhibits the production of sulfhydryl antioxidants, damage nuclei, inhibit enzyme reactions and initiate lipid peroxidation in cell membranes, that subsequently alters the integrity and the fluidity of cellular membrane structures because of lipid oxidation (Wathes et al., 2007). These associated changes could be the reason of decreasing the sperm motility (Wathes et al., 2007; Al-Omair et al., 2017). Cell membrane is essential feature for sperm motility, structural integrity, and ultimately for sperm viability (Omair et al., 2017) and required to give the plasma membrane the fluidity essential for sperm motility (Sanocka and Kurpisz, 2004; Wahab et al., 2019). Toxicant induced oxidative stress disrupting the antioxidant and ROS balance which causing the abnormalities of spermatogenesis and male infertility (La Maestra et al., 2015; Othman et al., 2014).

It was reported that oxidative stress in testicular tissues is the key feature in male infertility (Neto et al., 2016; Offor et al., 2019). Lead acetate brought severe testicular toxicity causing splitting of cells from basal region as shown in Figure (4, C). Histological changes in testicular as necrosis in seminiferous tubules, degenerative changes and edema in interstitial tissue reported with lead exposure (Bas et al., 2016; Offor et al., 2019). Changes in seminiferous tubules and decreasing number of spermatogenic cells were associated with marked changes in sperm biochemical parameters (Offor et al., 2019).

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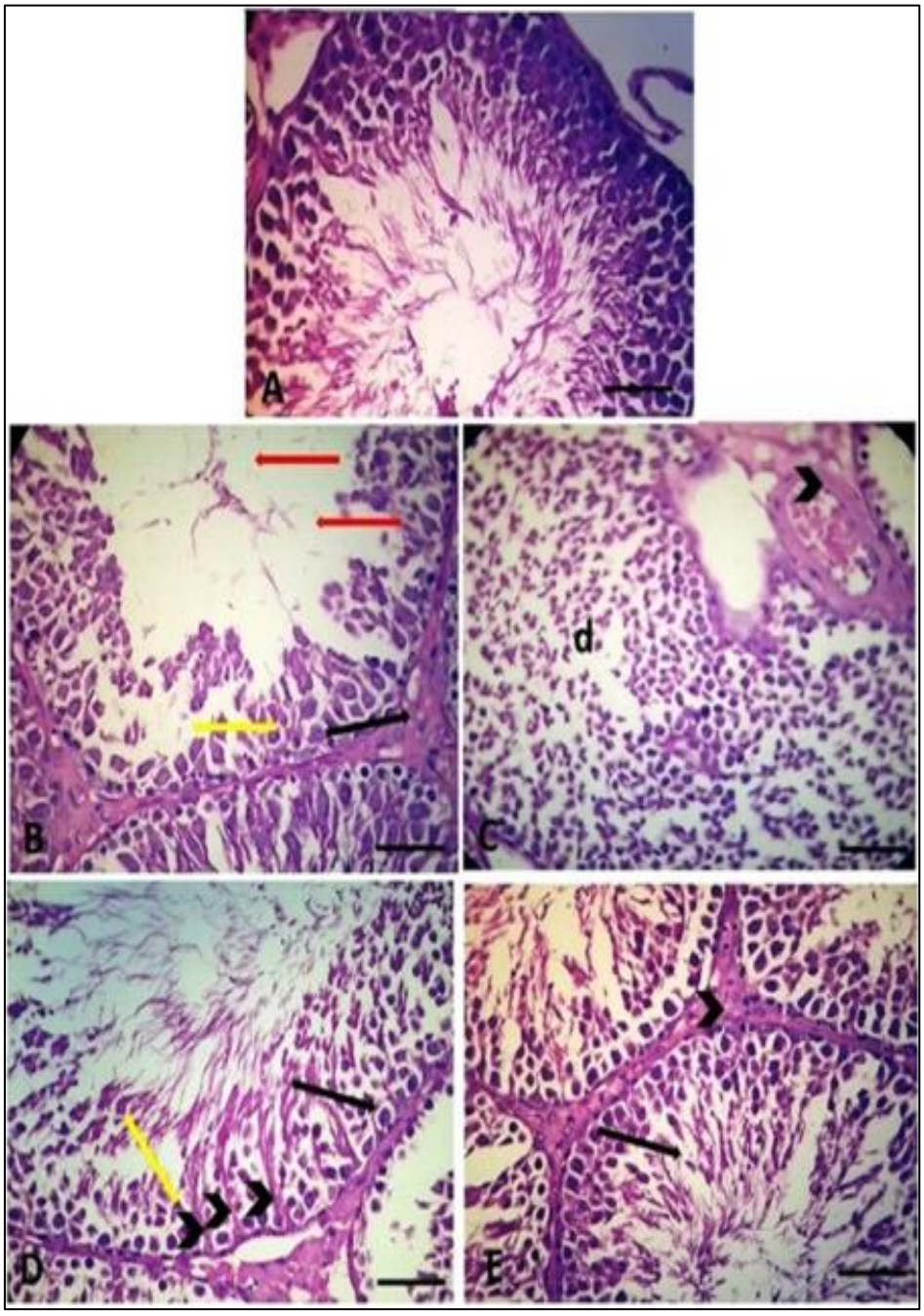

Figure 4. Microscopic pictures of testis sections showing normal appearance of spermatogonia, primary spermatocyte, secondary spermatocyte, and spermatozoa with normal interstitial tissues with Leydig cells in (A) control group. (B) and (C) the group of rat teste sections from the control + group (Pb) showing high degeneration (yellow arrow) spermatogenic cells with absence of spermatozoa (red arrows), high congestion of interstitial tissues with vacuoles (black arrow) (B). In (C) there were disarrangement of spermatogenic cells in tubules (d) with thickness congestion and dilation of blood vessels in interstitial tissues (arrowhead). In the group of Pb with *S oleraceus* (D) there were recovery in testes section with normal appearance and increase in the number of cells (black arrow) and spermatozoa in (yellow arrow) and sertoli cells (arrowhead), normal appearance of interstitial cells. In the group of Pb and *M parviflora* (E) there were normal appearance and increase in fertility (black arrow) with congestion of interstitial tissues. (40x) Scale bar: 40µm.

Usage of antioxidant component to eliminate toxic effects of free radicals could improve the oxidative state of the cell and inhibit lipid peroxidation. Thus maintaining the antioxidant balance of the cells (Arabi, 2004; Jalili et al., 2019). The antioxidant properties of *S. oleraceus* and *M. parviflora* plants were explained due to their content of flavonoids and phenolic compounds which enables them to scavenge free radicals (Shale et al., 2005; Gasparetto et al., 2012). Flavonoids can also cause upregulation of antioxidant defense and can also inhibit reactive oxygen species (ROS) generation enzymes (Ciumărnean et al., 2020; Nwonuma et al., 2022). The biochemical pathways by which the plant extracts mitigate oxidative damage include the function of the non-enzymatic system that trap the free radicals and avoid the radical initiation reaction. Besides the

role of flavonoids as an antioxidant (Nwonuma et al., 2022) in preventing or slowing damage to cells caused by the free radicals. It was reported that some natural products that contain the flavonoids have direct interactions with the ROS to generate stable or less reactive complex compounds, whereas other flavonoids can act as a co-substrate in the catalysis process of some enzymes (Akbari et al., 2022)

Antioxidant protection of used extracts So and MP to sperm against ROS could be related the induced improvement in sperm quality as detected in sperm count Figure1,2) and sperm mobility and viability (Table 1) as well as sperm abnormality (Figure 3). Insufficient sperm motility has been reported to be one of the most important parameters used to assess subfertility or infertility (Remya et al., 2009; Nwonuma et al., 2022). The recovery effects of plant extracts are considerable improvement in fertility state of LA treated rats, this in turn resulted in improved sperm motility and viability as well as the decrease in abnormality % (Jamalan et al., 2016; Nwonuma et al., 2022).

5. Conclusions

Present study showed that exposure to LA was associated with increasing in lipid peroxidation level in sperm and significant reduction in sperm properties as sperm count, mobility, and viability as well as increasing in sperm abnormalities. Histological analysis of testes tissues revealed that rats treated with LA treatment showed several of deleterious effects were associated with LA exposure. Addition of aqueous extracts of *Sonchus oleraceus* (So) and *Malva Parviflora* (Mp) plants to dietary regime of rats treated with Lead acetate caused a significant improvement in sperm properties as well as recovery of histological feature of testes tissue to normal form. Obtained results indicate to the anti-oxidant capability of So and Mp extracts to ameliorate the oxidative effect of lead pollution on male reproductive system of rats. For further investigation, we recommend testing higher dosages than those used in this study. Additionally, we suggest incorporating these plants into diets to reduce the toxicity of other heavy metals that are widespread.

No Acknowledge limitations in this study

Author Contributions:

"Conceptualization, S.O.; A.M.Z.; methodology, S.O; A.A.Z. and A.M.Z software, S.O; A.A.Z. and A.M.Z; validation, S.O; A.A.Z. and A.M.Z; formal analysis, S.O.; A.A.Z. and A.M.Z; investigation, S.O; A.A.Z. and A.M.Z.; data curation, S.O; A.A.Z. and A.M.Z; writing—original draft preparation, S.O; A.A.Z. and A.M.Z; writing—review and editing, S.O. and A.M.Z.; supervision, S.O.; A.M.Z and M.E. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: "The authors declare no conflict of interest."

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