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The Potential Effect of Aqueous Extract of Capsaicin in Improving Testicular Characteristics in Male Albino Rats Pre-Treated with Lead Acetate

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Abstract | The current study was conducted to investigate the effect of an aqueous extract of capsaicin, extracted from chilli peppers, on specific physiological parameters in male albino rats treated with lead acetate. The animals were divided into three groups, each group having ten rats. The first group was considered the control and received only water and the diet, while the second group received lead acetate treatment (90 mg/kg). The third group was injected with lead acetate at a dose of 90 mg/kg and capsaicin at a concentration of 20 mg/kg. The animals were dosed orally for 35 days, after which blood was drawn at the end of the experiment to obtain serum, which was then measured. Each of FSH, LH, testosterone, TSH.T4, T3, MDA, CAT, GPX, SOD and Characterization of sperm (total count, motility, viability, and abnormality. The results demonstrated a substantial reduction in total sperm count and motility, alongside a notable increase in the percentage of dead and abnormal sperm in the lead acetate-treated group compared to the control group and the group treated with both lead acetate and capsaicin. The results also showed a significant decrease in the levels of FSH and LH. In addition, the results showed a substantial increase in the levels of malondialdehyde and the enzyme glutathione peroxidase and a decrease in the levels of the enzymes CAT and SOD. Additionally, the results decrease the levels of thyroid hormones T4 and T3. The TSH in the group treated with lead acetate was compared with the control group and the group treated with lead acetate and capsaicin. The study concludes that capsaicin extracted from chili peppers at a concentration of 20 mg/kg helps mitigate the damage caused by lead acetate and improves specific testicular characteristics and physiological parameters.

Keywords | Capsaicin, Lead acetate, Sperm, Antioxidant

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INTRODUCTION

Infertility is a medical condition characterized by the inability to conceive after 12 months or more of regular unprotected sexual intercourse. Infertility represents a significant global concern, an increasing prevalence impacting 8–12% of couples (Vander and Wyns, 2018). Infertility in 40–50% of couples is linked to male factors (Kumar and Singh, 2015). Research demonstrates that multiple lifestyle factors influence fertility by inducing

oxidative stress, which leads to damage in testicular tissue and sperm DNA, consequently impairing semen quality (Sermondade *et al*., 2013; Joo *et al*., 2012).

A series of research studies with more than 1700 couples found that 40% of patients had a primary cause of infertility, and 20% had a cause of severe infertility (Punab *et al*., 2017). The impact of environmental factors on sperm quality is increasingly recognized. Employment contact with endocrine-disrupting chemicals, such as pesticides and heavy metals, is associated with adverse effects on the male reproductive system, as evidenced by reduced semen parameters and fertility (Giulioni *et al*., 2022; Cannarella *et al*., 2023). Observations of their impact on the male reproductive system have been documented in populations residing in industrial and agricultural regions characterized by pollutant emissions, highlighting a notable global health issue (Jasim and Ali, 2021). The detrimental effects of heavy metals can be attributed to several mechanisms, including disruption of cellular signaling pathways, alterations in gene expression, oxidative stress, and programmed cell death (Anyanwu and Orisakwe, 2020). Lead is frequently a trace element within geological layers and natural sources. Human exposure to lead (Pb) primarily results from anthropogenic activities, including mining and smelting, industrial processes, agricultural practices, pharmaceutical use, smoking, and dietary sources, particularly seafood. Water and air pollution significantly contribute to this exposure, especially in areas with elevated traffic and gasoline emissions. Lead accumulates within the tissues of humans over time, progressively impacting cellular organelles and components, such as mitochondria, lysosomes, and the endoplasmic reticulum, as well as essential metabolic and cell division enzymes. This accumulation leads to DNA damage and alterations in nuclear conformation (Wang and Shi, 2001). Lead exposure adversely affects the male reproductive organs. The mechanism responsible for genotoxic impacts on the male reproductive system continues to be a subject of active debate. The primary mechanism involves detrimental effects on the testis and the hypothalamic-pituitary-gonadal axis. Lead exposure can cause damage by affecting GnRH release, inducing degenerative alterations in pituitary gonadotropic cells, and impairing Sertoli cell activity, ultimately impacting testicular luteinizing hormone receptor levels and steroidogenesis (Gandhi *et al*., 2017). No systematic reviews currently evaluate the impact of lead exposure on male semen quality (Sun *et al*., 2017; Omran *et al*., 2024).

Extended exposure to lead can lead to dysfunction in various organ systems. Chronic lead exposure is every day in many developing countries. The decrease is attributed to improved safety standards, which have resulted in a decline in chronic lead exposure rates. Instances of exposure persist (Richard and Ladou, 2004). Lead negatively affects the reproductive system, as well as other organs. Studies on the reproductive toxicity of lead in male workers demonstrate a decrease in sperm count and motility, along with the occurrence of phenotypically abnormal sperm (Gidlow, 2004). Research on sperm morphology, motility, and reproductive hormones has indicated notable changes in hormonal levels. Studies demonstrate a reduction in androgens and luteinizing hormone (LH) following lead exposure (Ibrahim *et al*., 2023; Abdula *et al*., 2024).

Capsaicin exhibits multiple pharmacological properties, including antimutagenic, anticarcinogenic, and antioxidant activities (Sanchez *et al*., 2007; Galano and Martinez, 2012). Protection of DNA from strand breaks and chromosomal aberrations; safeguarding tissues from free radical-mediated damage induced by exogenous chemicals; suppressing reactive oxygen species generation; and promoting apoptosis (Ito *et al*., 2004). Multiple studies employing chemical, in vitro, and animal models illustrate its antiradical activities. Capsaicin exhibits a peroxyl radical-scavenging capacity that exceeds that of melatonin and caffeine (Luqman and Rizvi, 2006).

The compound demonstrated antioxidant activity comparable to that of polyphenols and α-tocopherol. Capsaicin is thought to interact with xenobiotic-metabolizing peptides. Capsaicin is believed to be converted into a phenoxyl radical intermediate via the catalytic function of hepatic cytochrome P450 2E1. The capsaicin phenoxyl radical represents a reactive species capable of covalently binding to the active sites of enzymes and other biologically active macromolecules within cells. Inhibition of microsomal cytochrome P450 may impede the metabolism of chemical cancer-causing agents, contaminants, and toxic xenobiotic. Research suggests capsaicin may possess chemo preventive effects against certain chemical carcinogens and mutagens. Free radicals are generated during the metabolic processes of aerobic cells and may also originate from external factors such as chemicals, ionizing radiation, and gamma radiation (Surh and Lee, 1995).

MATERIALS AND METHODS

The experimental design

The study was conducted to determine the effect of capsaicin extracted from hot peppers on reducing the harmful effects of lead acetate exposure on certain testicular functions in rats. This study included 30 albino male rats, weighing 165-175 g and 4-6 months old, divided into three groups. The first group was designated as the control group and received only water and food. The second group was administered lead acetate at a

concentration of 90 mg/kg. The third group was given lead acetate at 90 mg/kg and capsaicin at 20 mg/kg. All animals were administered the substances orally for 25 days at a dose of 1 milliliter per substance. The experiments were terminated after 35 days. At the end of the experiment, blood was drawn from the heart after anesthetizing the animals with ketamine at a concentration of 10%. A centrifuge was used to separate the blood at a speed of 3000 rpm for 10 minutes. The serum was kept in the refrigerator at -20 degrees until the biochemical analyzes were performed, which included FSH, LH, and T., TSH.T4, T3, MDA, CAT, GPX, SOD will done. Several criteria were measured for the sperm, including total count, viability, motility, and abnormal morphology.

Characterization of sperm

The total count of sperm was counted, sperm were counted in five random fields, and the average was multiplied by 10^6. To maintain the activity of the viable sperm, all tools, containers, and surfaces used in the experiments were kept at a temperature of 37°C.

Sperm motility and count

To evaluate sperm motility, 100 mg of the caudal epididymis was minced in 1 ml of RPMI-1640 medium. A small amount of the mixed specimen was placed on a slide beneath a cover slip, and the motility of sperm was determined by counting motile and immotile sperm cells per unit area, expressed as an index. The epididymal counts were conducted using standard procedures and reported as millions per milliliter of suspension.

Sperm viability

To assess sperm vitality, 40 µL of freshly liquefied semen was mixed with 10 µL of eosin-nigrosine (Merck, Germany). A drop of this mixture was placed on a clean slide, and under oil immersion at a magnification of ×100 (Olympus Japan), 100 sperm were counted. Sperms that exhibited pink or red staining were classified as dead, while those that remained unstained were deemed viable (Salah *et al*., 2020).

Diagnostic kits

Measurement of follicle stimulating hormone (FSH) enzyme-linked immunosorbent assay kit for follicle stimulating hormone cloud

FSH is based on immunoassay system using antigen antibody interaction and fluorescence technology if sample and detection buffer is mixed completely and then loaded onto a sample well. On a cartridge, the complex of antibody fluorescence is formed on the membrane of the cartridge. The quantity of the FSH is directly proportional to complexes accumulated on the membrane.

Measurement of luteinizing hormone (LH) enzyme-linked immunosorbent assay kit for luteinizing hormone (LH)

Serum luteinizing hormone: The test uses a sandwich immune detection method (LH Kit-Bioteah, UK) such that the detector antibody in buffer binds to LH in blood sample and antigen-antibody complexes are captured to antibody that has been immobilized on test strip. As sample mixture migrates through the nitrocellulose matrix, more the LH antigen in blood higher the antigen antibody complexes accumulated on test strip. The signal intensity of fluorescence on detector antibody reflects amount of antigen captured and is processed from I-chroma reader to show LH concentration in specimen.

Measurement of testosterone (T) enzyme immunoassay for the quantitative determination of testosterone diagnostic automation

Enzyme–linked immunosorbent assay (ELISA) by using testosterone kit (which is based on the competition principle an unknown amount of antigen presents in the sample, and a fixed amount of enzyme labeled antigen compete for the binding site of the antibodies coated onto the well. After incubation the wells were washed to stop the competition reaction. The intensity of the developed color is inversely proportional to the amount of the antigen in the sample.

Antioxidant enzymes

Measurement of malondialdehyde (MDA) was conducted using My BioSource/USA. The measurement of glutathione (GSH) was conducted using My BioSource, USA. For the measurement of catalase (CAT), human catalase (CAT), ELISA Kit were used from My BioSource, USA.

Comet assay

DNA damage related to the cultured lymphocytes was evaluated for four groups using the single-cell electrophoresis (Comet assay) approach. In addition, an aliquot (20 µl) of the cultured cells is added to 250 µl of low-melting agarose at 37 °C and thoroughly mixed. After that, 100 µl of the mixture is applied to Comet slide that is put in dark place at 4°C for 10 mins, while the slide was immersed in the lysis solution for 24 hour, succeeded via immersion in the alkaline solution at 25 °C for 20 mins or in dark place at 4°C for 1 hour. Alkaline electrophoresis solution is added to the tank tray to perform Comet assay electrophoresis; the slide is then placed in the tray. Additionally, electrophoresis is run for 30 minutes at 25 volts. Following electrophoresis, the slide is immersed 2 times in distilled and deionized water for 5 mins, and 1 time in ethanol (70%) for 5 mins. Also, the slide is kept dry for 10-15 minutes at 37 °C. After that, the Comet slide was stained with SYBR Green (100 µl) for 30 minutes in the dark at 25 °C. Lastly, the excess SYBR solution is removed,

and the slide is rinsed with distilled water. At 37°C, the slide was left to dry completely and then analyzed at 40X magnification using a fluorescent microscope. Furthermore, cells are randomly selected for analysis in each sample, and Comet scoring is performed, with four scores indicated based on the degree of DNA damage (no damage, low, medium, and high damage).

RESULTS

The results, shown in Table 1, indicated a notable reduction in the total sperm count in the group administered lead acetate at a concentration of 90 mg/kg body weight (14.33 ± 1.45) sperm/106, in contrast to the control group (28.00 ± 2.64) sperm/106 and the group receiving both lead acetate and capsaicin at 10 mg/kg (22.33 ± 1.85) sperm/106. The results indicated a significant reduction in sperm motility in the group administered lead acetate at a concentration of 90 mg/kg body weight (74.33 ± 4.70%) compared to the control group (95.00 ± 0.58%) and the group receiving both lead acetate and capsaicin (87.67 ± 1.85%). The results indicated a notable increase in the percentage of dead sperm in the group treated with lead acetate at a concentration of 90 mg/kg body weight (26.00 ± 3.05)% when compared to the control group (8.33 ± 0.88)% and the group treated with both lead acetate and capsaicin (13.33 ± 1.45)%. The findings indicated a notable rise in the proportion of abnormal sperm in the lead acetate-treated group (28.00 ± 3.05%) relative to the control group (5.67 ± 1.20%) and the group receiving both lead acetate and capsaicin (15.00 ± 1.00%).

The statistical analysis indicated a significant reduction in hormone levels in the lead acetate-treated group (2.27 ± 1.36) relative to the group receiving both lead acetate and capsaicin (2.00 ± 0.07). The hormone exhibited similar results, with a notable reduction in the lead acetate-treated group (0.846 ± 0.04) compared to the group receiving both lead acetate and capsaicin (2.13 ± 0.18). The results indicated a significant reduction in hormone concentration in the lead acetate-treated group (2.01 ± 0.44) compared to the control group.

The findings indicated a notable elevation in malondialdehyde levels in the lead acetate-treated group (5.13 ± 0.24) relative to the control group (1.72 ± 0.24) and the group receiving both lead acetate and capsaicin (2.66 ± 0.31). The catalase enzyme exhibited similar results, with a notable increase in its level in the lead acetate-treated group (2.91 ± 0.07) compared to the control group (0.933 ± 0.07) and the group receiving both lead acetate and capsaicin (1.233 ± 0.02). The findings indicated a notable reduction in glutathione peroxidase levels in the lead acetate-treated group (2.28 ± 0.29) relative to the control group (4.87 ± 0.12) and the group receiving both lead acetate and capsaicin (3.18 ± 0.23). The enzyme superoxide dismutase exhibited a significant increase in the lead acetate-treated group (4.79 ± 0.24) when compared to the control group (2.11 ± 0.14) and the group receiving both lead acetate and capsaicin (2.64 ± 0.31).

Table 1: Comparison between different groups in total count sperm, motility, dead, live, and abnormality.

Group	Mean ±SE			
	Total count sperm\1^{06}	Motility (%)	Dead and alive (%)	Abnormality (%)
Control	28.00 ±2.64 a	95.00 ±0.58 a	8.33 ±0.88 b	5.67 ±1.20 c
Pb 90 mg\kg	14.33 ±1.45 b	74.33 ±4.70 a	26.00 ±3.05 a	28.00 ±3.05 a
Pb 90 mg\kg +Cap 20mg\kg	22.33 ±1.85 a	87.67 ±1.85 b	13.33 ±1.45 b	15.00 ±1.00 b
L.S.D.	7.079 **	10.166 **	6.984 **	6.856 **
P-value	0.0095	0.0069	0.0021	0.0006

Means having the different letters in the same column differed significantly. ** (P≤0.01).

Table 2: Comparison between different groups in FSH, LH, and Testosterone hormones.

Group	Mean ±SE		
	FSH (mIU/ l)	LH) mIU/ l)	Testosterone (ng/ml)
Control	3.05 ±0.04	2.100 ±0.25 a	4.86 ±0.26 a
Pb90 mg\kg	2.27 ±1.36	0.846 ±0.04 b	2.01 ±0.44 b
Pb90 mg\kg + Cap20mg\kg	2.00 ±0.07	2.13 ±0.18 a	3.67 ±0.34 a
L.S.D.	0.731 NS	0.628 **	1.242 *
P-value	0.769	0.0038	0.0280

Means having the different letters in the same column differed significantly. * (P≤0.05), ** (P≤0.01).

Table 3: Comparison between difference groups in MDA Antioxidants CAT, GSH and SOD.

Group	Mean ±SE			
	MDA (uml\L)	CAT (IU\l)	Glutathione (uml\l)	SOD (IU\l)
Control	1.72 ±0.24 c	0.933 ±0.07 c	4.87 ±0.12 a	2.11 ±0.14 b
Pb90 mg\kg	5.13 ±0.24 a	2.91 ±0.07 a	2.28 ±0.29 a	4.79 ±0.24 a
Pb90 mg\kg +Cap20mg\kg	2.66 ±0.31 b	1.233 ±0.02 b	3.18 ±0.23 a	2.64 ±0.31 b
L.S.D.	0.936 **	0.218 **	0.796 **	0.833 **
P-value	0.0003	0.0001	0.0006	0.0005

Means having the different letters in the same column differed significantly. ** (P≤0.01).

Table 4: Comparison between different groups in TSH, T4, and T3 hormones.

Group	Mean ±SE		
	TSH (uIU\ml)	T4) ug\ml)	T3 (ng/ml)
Control	3.31 ±0.28 a	6.48 ±0.39 a	79.67 ±2.33 a
Pb90 mg\kg	1.47 ±0.23 c	3.73 ±0.52 b	56.00 ±2.64 c
Pb90 mg\kg +Cap20mg\kg	2.34 ±0.17 b	4.57 ±0.31 b	70.67 ±1.20 b
L.S.D.	0.816 **	1.443 **	7.445 **
P-value	0.0046	0.0090	0.0007

Means having the different letters in the same column differed significantly ** (P ≤ 0.01).

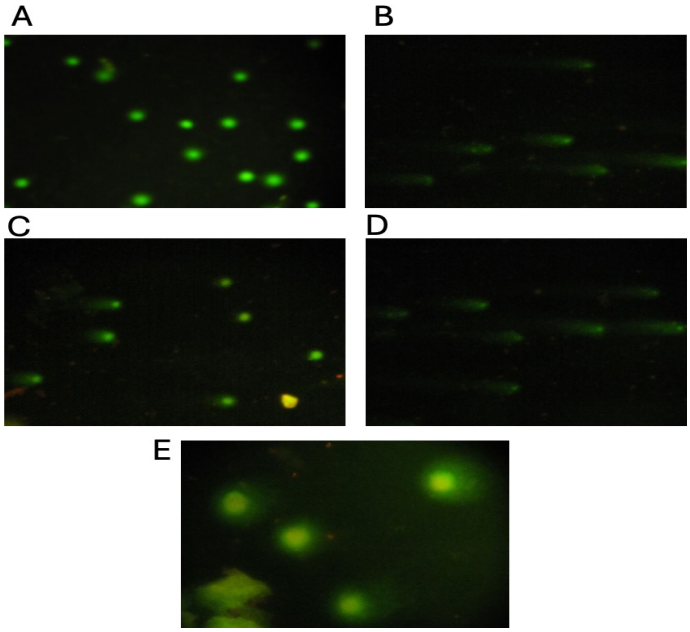

Figure 1: (A) shows the typical appearance of white blood cells in the negative control group. (B, C) shows the DNA fragmentation in white blood cells in the lead acetate group. (D, E) shows the typical appearance of most white blood cells, with some cells exhibiting DNA fragmentation in lead acetate and the aqueous extract of the capsaicin group.

The results showed a significant decrease in hormone level in the group treated with lead acetate (1.47 ± 0.23), compared to the control group (3.31 ± 0.28) and the group treated with lead acetate and capsaicin (2.34 ± 0.17). At the same time, there was a significant decrease in the hormone level in the group treated with lead acetate (3.73±0.52) compared to the control group (6.48±0.39) and the group treated with lead acetate and capsaicin. Finally, the results showed a significant decrease in the hormone level in the group treated with lead acetate (56.00 ± 2.64) compared to the control group (79.67 ± 2.33) and the group treated with lead acetate and capsaicin (70.67 ± 1.20) (Figure 1A-E).

DISCUSSION

The study's primary objective was to assess the impact of lead toxicity on thyroid gland function. This study measured thyroid gland hormones to elucidate the mechanism of action of Pb, despite numerous reports on its effects on the thyroid gland. The thyroid is an endocrine gland responsible for the synthesis and secretion of key regulatory hormones, namely triiodothyronine (T3) and tetraiodothyronine (T4). Iodothyronine (T3, T4), produced in follicular cells, is essential for normal growth and development (Zoeller *et al*., 2002). Through the secretion of calcitonin, parafollicular cells play a crucial role in regulating calcium and phosphate metabolism (Sakai *et al*., 2000). Serum concentrations of thyroid hormones, such as T3, T4, and TSH, are reliable indicators of thyroid function in humans and experimental animals. Variations in serum hormone concentrations may indicate disruptions in glandular synthesis and secretion, as well as abnormalities in extra-thyroidal peripheral metabolism (Kelly, 2000). Evaluating thyroid gland function involves measuring serum levels of thyroid hormones, specifically T3 and T4, as well as pituitary TSH levels. The cellular structure of the thyroid gland can be observed using a light microscope. Recent studies indicate that certain exogenous environmental pollutants disrupt the human endocrine system (Jun *et al*., 2005).

Lead is a common environmental toxin that can cause acute and chronic diseases impacting the circulatory,

neurological, hematological, gastrointestinal, reproductive, and immunological systems. Lead poisoning primarily results from ingesting food or water contaminated with lead. The unintentional consumption of contaminated soil, dust, or lead-based paint may result in poisoning (Harms Children, 2021). Lead-induced oxidative stress has a significant effect on cellular antioxidant defense systems. Lead exhibits a considerable affinity for sulfhydryl (SH) groups. It may compromise antioxidant functions by obstructing functional SH groups in various enzymes, including SOD, CAT, and GPx. It results in heightened free radical generation. The presence of endogenous antioxidant reserves, including glutathione, glutathione peroxidase, superoxide dismutase, and catalase, is diminished, impacting the scavenging of reactive oxygen species (ROS) in individuals exposed to lead. Additionally, it impedes enzyme activation and competitively inhibits the absorption of trace minerals. It interacts with sulfhydryl proteins, interferes with structural protein synthesis, modifies calcium homeostasis, increases lipid peroxidation, decreases the concentration of saturated fatty acids, and raises the concentration of fatty acids in the cell membrane (Bergeson, 2008).

An epidemiological investigation of the male reproductive system has demonstrated positive correlations between seminal plasma lead levels and reactive oxygen species in spermatozoa (Kiziler *et al*., 2007). Enhanced superoxide dismutase (SOD) activity has been observed in individuals with prolonged lead exposure, suggesting a potential mechanism for the increased generation of reactive oxygen species (ROS) associated with lead exposure (Kasperczyk *et al*., 2004). This may lead to oxidative stress within reproductive tissues, which is related to the presence of reactive oxygen species (ROS). Studies show that rat sperm subjected to reactive oxygen species (ROS) *in vitro* showed a diminished penetration rate in zona-intact ova and an early acrosome reaction. Lead-induced oxidative stress elicits diverse responses at various target sites, including sperm, depending on the dose, ranging from low to high levels (Hsu and Guo, 2002). Chronic exposure to lead in animals was associated with increased lipid peroxide concentration in the reproductive organs (Marchlewicz *et al*., 2007). Some studies suggest that elevated reactive oxygen species (ROS) production caused by lead is a crucial molecular mechanism contributing to male reproductive disorders during spermatogenesis or hormonal phases. Reactive oxygen species (ROS) are by-products of various degenerative reactions in multiple tissues. This molecule contains one or more unpaired electrons, making it highly reactive with other molecules, which disrupts normal metabolism and damages cellular components. Reactive oxygen species (ROS) can compromise the structural integrity of carbohydrates, nucleic acids, lipids, and proteins, disrupt their functions, and ultimately lead to cell death. Oxidative stress refers to the imbalance between oxidants and antioxidants, resulting in an excess of oxidants (Birben *et al*., 2012). The research investigated the effects of the working environment on the male reproductive system. Sheiner *et al*. (2002) found that employees in diverse industrial sectors demonstrated increased infertility rates. This study's analysis identified varicocele, sperm anomalies, and hormonal imbalance as sources of infertility (Sheiner *et al*., 2002). Furthermore, motility and sperm head morphological abnormalities constitute additional factors that may be associated with BLL (Telisman *et al*., 2000). The injection molding workers exhibited hypogonadism and diminished testosterone levels, associated with inorganic lead exposure (Cullen *et al*., 1984). A distinct study revealed a relationship between length of exposure and testicular failure (Rodamilans *et al*., 1988).

Animal studies have similarly demonstrated that lead exposure negatively impacts the male reproductive system, consistent with findings in humans. Research on monkeys has shown that lead exposure decreased LH levels and inhibited the FSH ratio (Foster *et al*., 1993). Chronic lead exposure in monkeys induces dysfunction in Sertoli cells (McGregor and Mason, 1990) who have performed a study with monkeys (Foster *et al*., 1998). Deformations in Sertoli cells, basal lamina, seminiferous tubule epithelium, and sperm cells were detected via electron microscopy. Bizzaro *et al*. (2003) have presented present evidence for this result through their research, demonstrating a time-dependent increase in the proportion of damaged mitochondria in the Sertoli cells of mice periodically administered Pb acetate over four weeks (Bizarro *et al*., 2003). Phenolic compounds represent a significant category of secondary metabolites produced by plants, which facilitates their adaptation to biotic and abiotic stress conditions (Shetty, 2004; Pitchersky and Gang, 2000). Phenolic compounds in food and pharmaceuticals are acknowledged for their potent antioxidant properties, often derived from natural sources. These compounds protect various biological components in humans from oxidative damage by neutralizing highly reactive free radicals and inhibiting the propagation of oxidative chain reactions. Aerobic cellular metabolism involves the regulated production of free radicals, which are effectively eliminated by the antioxidant defense system, including enzymes and antioxidants. Unregulated formation due to prolonged exposure to ionizing radiation, chemicals, and pathological conditions such as inflammation and cancer can provoke oxidative stress, resulting in cellular damage and eventual cell death (Bors *et al*., 1996). Synthetic chemicals are recognized for their varying capacities to inhibit oxidative damage; however, they are also linked to

adverse side effects. Consequently, examining free radical-scavenging properties of antioxidant compounds derived from natural food sources has become crucial, owing to their non-toxic characteristics, regular consumption through diet, and beneficial interactions with diverse free radicals produced from both external sources and internal metabolic processes (Thomas and Kalyanaraman, 1999). Oxidative stress and free radical generation are critical factors in the initiation and progression of various diseases (Sosa *et al*., 2013). Free radicals can influence multiple cellular processes, including growth and proliferation, through different metabolic pathways, significantly contributing to the onset and progression of cancer and other diseases (Halliwell, 2007). The decline in the body's antioxidant defense system is considered a significant factor in the progression and development of cancer (Klaunig *et al*., 2010). The human body's anti-inflammatory system, composed of enzyme-dependent antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase, as well as non-enzymatic antioxidants like vitamins E, C, A, flavonoids, metal ion chelators, and polyphenolic compounds, reduces the impact of free radicals (Reuter *et al*., 2010).

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NOVELTY STATEMENT

The novelty statement in the new manuscript is the use of pure active compounds extracted from some plants in treating some pathological cases related to fertility due to exposure to heavy elements and reducing the impact of the negative effects resulting from the release of free radicals from these heavy elements in addition to the simple cost of obtaining these active compounds and the absence of negative side effects.

AUTHOR'S CONTRIBUTION

All three authors participated in all theoretical and practical steps of the research.

Generative AI or AI-assisted Technology Statement

The author(s) declare that no Genrative AI was used in the creation of this manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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