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Grape sustains male fertility in lead acetate induced testicular dysfunction more efficiency than ginger

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Abstract

Background: Lead acetate is an environmental toxin that causes male dysfunction while grape and ginger have antioxidant activities. The ability to prevent infertility and optimal male fertilization of ginger and grape in the lead acetate-treated group were evaluated in this study. **Methods**: The study included 36 male albino rats arranged in six equal groups; Control, Grape (75 mg/kg), Ginger (500 mg/kg), Lead acetate (30 mg/kg), Grape (75 mg/kg) prior to lead acetate (30 mg/kg) and Ginger (500 mg/kg) prior to lead acetate (30 mg/kg) groups, respectively. Dehydroepiandrosterone sulfate, luteinizing and follicle-stimulating hormones, serum sex hormone-binding globulin and testosterone were measured in the serum. The testis tests include cholesterol, total protein, 3β-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase were detected. The hypothalamus, testis and sperm antioxidants (superoxide dismutase, glutathione, and malondialdehyde) were measured. Sperm monoclonal proliferating antibody Ki-67 was assessed together with sperm counts, motility, and abnormalities. The testis, sperm and hypothalamus adenosine 5′-triphosphatase (ATPase) and nuclear factor-kappa B activities were examined. **Results**: Administration of lead acetate orally reduced levels of sodium/potassium-ATPase activity, sperm count and motility, dehydroepiandrosterone sulfate, glutathione, serum testosterone and superoxide dismutase, whereas elevated levels of total protein, cholesterol, luteinizing hormone, sex hormone-binding globulin, serum follicle-stimulating hormone, glucose-6-phosphate dehydrogenase, 3β-hydroxysteroid dehydrogenase and nuclear factor kappa B levels. Lead acetate induced abnormal sperm, as well as the proportion of first spermatocyte, second spermatocyte, spermatid and spermatogonia to higher levels. Additionally, every parameter that was previously described came back to be near control levels after the oral treatment with ginger and grape prior to lead acetate-treated group. **Conclusions**: In testicular toxicity caused by lead acetate, grape preserves male fertility and conception more effectively than ginger.

Keywords

Grape; Ginger; Lead acetate; Hypothalamus; Testis; Sperm; Oxidative stress

La uva favorece la fertilidad masculina en casos de disfunción testicular inducida por acetato de plomo con mayor eficacia que el jengibre

Resumen

Antecedentes: El acetato de plomo es una toxina ambiental que causa disfunción masculina, mientras que la uva y el jengibre tienen actividades antioxidantes. En este estudio se evaluó la capacidad de prevenir la infertilidad y la fertilización masculina óptima del jengibre y la uva en el grupo tratado con acetato de plomo. **Métodos**: El estudio incluyó 36 ratas albinas macho dispuestas en seis grupos iguales; Control, Uva (75 mg/kg), Jengibre (500 mg/kg), Acetato de plomo (30 mg/kg), Uva (75 mg/kg) antes del acetato de plomo (30 mg/kg) y Jengibre (500 mg/kg) antes del acetato de plomo (30 mg/kg), respectivamente. Se midieron en el suero el sulfato de dehidroepiandrosterona, las hormonas luteinizante y estimulante del folículo, la globulina transportadora de hormonas sexuales séricas y la testosterona. Las pruebas de testículo incluyen colesterol, proteína total, 3β-hidroxiesteroide deshidrogenasa y glucosa-6-fosfato deshidrogenasa. Se midieron los antioxidantes del hipotálamo, los testículos y los espermatozoides (superóxido dismutasa, glutatión y malondialdehído). Se evaluó el anticuerpo monoclonal proliferante Ki-67 en los espermatozoides, junto con el recuento, la motilidad y las anomalías espermáticas. Se examinaron las actividades de la ATPasa y el factor nuclear kappa B en los testículos, los espermatozoides y el hipotálamo. **Resultados**: La administración oral de acetato de plomo redujo los niveles de actividad de la ATPasa de sodio/potasio, el recuento y la motilidad espermática, el sulfato de dehidroepiandrosterona, el glutatión, la testosterona sérica y la superóxido dismutasa, mientras que los niveles elevados de proteína total, colesterol, hormona luteinizante, globulina transportadora de hormonas sexuales, hormona folículo estimulante sérica, glucosa-6-fosfato deshidrogenasa, 3β-hidroxiesteroide deshidrogenasa y los niveles del factor nuclear kappa B. El acetato de plomo indujo espermatozoides anormales, así como una mayor proporción de primer espermatocito, segundo espermatocito, espermátida y espermatogonias. Además, todos los parámetros descritos previamente se mantuvieron cerca de los niveles control tras el tratamiento oral con jengibre y uva, antes del grupo tratado con acetato de plomo. **Conclusiones**: En la toxicidad testicular causada por acetato de plomo, la uva preserva la fertilidad masculina y la concepción con mayor eficacia que el jengibre.

Palabras Clave

Uva; Jengibre; Acetato de plomo; Hipotálamo; Testículo; Esperma; Estrés oxidativo

1. Introduction

As an environmental toxin, lead accumulates in bones and soft tissues. The use of lead in lead-acid batteries increases the lead production globally [1]. Lead bioaccumulation occurs due to continued exposure to lead and its occurrence in the human body, which average 120 mg in adults [2]. Every organ and function in the human body is affected by the exceptionally toxic exposure of lead [3]. Lead can be absorbed through the human body's skin, ingestion or inhalation; majority of lead inside the body occurs through inhalation; for ingestion, this rate from 20 to 70%, with children absorbing a higher quantity than adults [4]. In addition to causing neurological diseases such as brain damage, lead destroys the nervous system and impairs the general health (it causes cardiovascular, renal, and testicular systems toxicities). Lead causes the percentage of dead sperm to rise and decreases sperm motility, which induces the testicular toxicity [5]. Lead also destroys testicular tissue and it has toxic effects on the testis [6]. Lead increases hydrogen peroxide level but decreases testis weight, serum testosterone levels, and catalase and total antioxidant activities [7].

The grape (*Vitis vinifera*) is one of the fruits that is grown and produced most extensively worldwide [8], which has also used traditionally in Europe [9]. 35% of the dried grape seeds are fiber, 29% are phenolic compounds, 11% are proteins, 7% are water and 3% are minerals [10]. Flavonoids (like epicatechin, quercetin, anthocyanin and catechin), as well as non-flavonoids (like resveratrol and proanthocyanidins) are among the phenolic compounds present in grape [11, 12]. Anti-apoptotic, antinecrotic, cardiovascular, anti-cancer, anti-inflammatory and antioxidant properties are the biological activities that grape extract exhibits [13–16].

Ginger, also known as *Zingiber officinale*, is a cooking spice that has a grayish-white rhizome with pale brown rings through the world. It can be eaten as a raw root or powder [17]. Constipation, fever, rheumatoid arthritis and nausea, sore throat, and male dysfunction are the diseases that ginger treated [18, 19]. Anti-inflammatory, anti-cancer, anti-microbial, anti-diabetic and antioxidant, hypolipidemic, and androgenic activities are all confirmed by ginger [20–23]. Volatile oils, choline, folic acid, inositol, pantothenic acid, vitamin B3 and B6, vitamin C compounds, resins and sesquiterpenes, calcium, magnesium, phosphorus, and potassium are the constituents of ginger [24]. However, ginger's primary bioactive components such as gingerone, zingiberene, gingerdiol, gingerol and shogaols are responsible for ginger lipid peroxidation inhibition and free radical scavenging by its antioxidant activity, and to preserve DNA structure [25].

The humans cannot avoid lead toxicity because lead is widely occurring in food, water and air. Therefore, the goal of this study is to restore the lead-related testicular toxicity by identifying a natural substance that is safe, reasonably priced, and devoid of adverse effects. Consequently, the study's objective is to assess how well ginger and grape preserve male fertility and male sperm quality in rats exposed to lead acetate.

2. Materials and methods

2.1 Preparation of grape and ginger water extracts

In March 2024, the Department of Horticulture, Ministry of Agriculture, Dokki, Giza, Egypt, supplied grape seeds and ginger roots to this study. Prof. L. Boulos works at the National Research Centre in Giza, Egypt, identified the botanical constituents of grape and ginger. Separately, the ginger roots and grape seeds were crushed to be fine powders using the mixer (blender). Grape seeds or ginger roots water extract was made by mixing distilled water with either ginger roots or grape seeds. It was then carefully stored in the refrigerator until it was needed.

2.2 Materials

Authors bought lead acetate (PbAc) from Sigma-Aldrich in the United States (MW: 379.34 g/moL, CAS Number: 6080-56-4, MDL Number: MFCD00150023). The dehydroepiandrosterone sulphate (DHEA-SO_4) (MBS2510102), testosterone (Ts) (MBS580136), luteinizing hormone (LH) (MBS2018978) and follicle-stimulating hormone (FSH) (MBS590027) Eliza kits acquired from BioSource Co., Nivelles, Belgium. The authors purchased kits from IBL Company in Hamburg, Germany, for sex hormone binding globulin (SHBG) (30176808), glucose-6-phosphate dehydrogenase (G6PD) (151187), and 3β-hydroxysteroid dehydrogenase (3βHSD) (DB52031). Additionally, the Randox Company in Crumlin, UK, supplied the test kits for glutathione (GSH) (RS505), malondialdehyde (MDA) (M496), cholesterol (CH8310), total protein (TP8066) and superoxide dismutase (SOD) (SD125). The San Francisco, California-based Zymed Laboratories Incorporation provided the monoclonal proliferative antibody known as Ki-67. The above mentioned chemicals were of analytical grade and they came from local branches of International Companies based in Egypt.

2.3 Animals

Ten-week-old male *Sprague-Dawley* albino rats weighing 140–145 g were obtained from the National Research Center's Animal Research Unit in Egypt. Both rat food and tap water were freely available to them and they arranged in their steel cages. A 12-hour light/dark cycle, a conventional temperature range of 26 °C to 30 °C, and a humidity range of 40% to 70% were all applied to the animals' habitat as part of the study.

The study was approved with approval number 13173916 by the Institutional Animal Ethical Committee (IAEC) of the National Regulations on the Animal Welfare of the National Research Center, Egypt. The study's handling and treatment of laboratory animals were controlled by Document No. 85:23 of the National Institutes of Health (NIH), revised in 1985.

2.4 Research proposal scheme

The doses of lead acetate [26], ginger [27] and grape [28] used in the study came from earlier researches on male rats. Six equal groups contained 36 male animals (each group contained six rats) as follow: (1) Control group: Rats were orally administered once a day for four weeks with one milliliter of distilled water. (2) Grape water extract (0.5 mL, 75 mg/kg)-treated group: Grape seeds water extract (75 mg/kg) was given orally to rats once daily for four weeks. (3) Ginger water extract (1.5 mL, 500 mg/kg)-treated group: For four weeks, rats received oral administration of 500 mg/kg of ginger roots water extract once daily. (4) Lead acetate-treated group: For four weeks, rats were treated with 30 mg/kg of lead acetate dissolved in one milliliter of distilled water orally once a day. (5) Grape water extract (75 mg/kg) prior to lead acetate (30 mg/kg)-treated group: For four weeks, rats were given an oral dose of 75 mg/kg of grape seeds water extract and after an hour they received 30 mg/kg of lead acetate once daily. (6) Ginger water extract (500 mg/kg) before lead acetate (30 mg/kg)-treated group: For four weeks, rats received an oral dose of 500 mg/kg of ginger roots water extract, followed by an oral dose of 30 mg/kg of lead acetate after an hour of ginger administration.

Rat convulsions, hair loss, skin patches, a decrease in normal locomotor activity, and any animal deaths were monitored in all rats over the whole experiment.

2.5 The collecting of the animals blood samples

When the study was finished, animals were decapitated following an overnight fast. Using jugular vein tubes, the blood samples were collected and putted in ice. To obtain blood serum, non-heparinized tubes were used by centrifuging them at 3000 g for 10 minutes at −20 °C. For the biochemical examination, the obtained blood serum was stored at −80 °C.

2.6 Preparing the testis and hypothalamus tissues

Animals have been decapitated and then dissected after the one-month period study. Prior to being stored at −80 °C for the biochemical examination, the testis and hypothalamus were chopped into small pieces for homogenization after being cleaned in saline and dried on two filter papers. In summary; 2.5 mL of Tris buffer solution was used to dissolve 0.5 g of hypothalamic and testicular tissues, which were subsequently homogenized in a homogenizer (speed = 2500 g [29] for 30 minutes) using an ice bath. For five minutes for the hypothalamus and ten minutes for the testis, these tissues were centrifuged at 7000 g. To ascertain the results of the biochemical assays, the hypothalamus and testicular supernatants were separated.

2.7 Sperm examination tests

Sperm were obtained by using the previously mentioned technique [30]. At 37 °C, the epididymis of both testes was cut, removed and preserved in saline. This procedure can be summarized as follows; each epididymis's proximal and distal caudal parts were divided into three pieces. The tissue was removed after five minutes of incubation at 37 °C, and the sperm suspensions were stored while being gently mixed.

2.7.1 Total count of the animal sperm

Using fresh medium, the sperm suspension aliquots were diluted 100 times. The sperm count was performed using the Neubauer chamber [31] method. Instead of using the "Giemsa staining method", the authors of this study used the "Neubauer chamber method" because it minimizes counting error, assesses the uniformity of the border cell distribution, and helps eliminate samples with remarkable distributional differences [32].

2.7.2 Using computer-assisted sperm analysis (CASA) to calculate the motility of the sperm

The number of sperms that proceeded and the total number of sperms are compared by the Boyers *et al*. [33] method, where each spermatozoon has a centroid, and the centroid trajectory is used to assess cell motility.

2.7.3 Analyzing the abnormalities in the sperm

After being fixed with alcohol and stained with Eosin-Y, smears were examined for morphological abnormalities in order to identify any sperm abnormalities.

2.7.4 Calculation of Ki-67 monoclonal antibody proliferation in the animal sperm

Ki-67 monoclonal antibody is a nuclear antigen that develops in the nucleus during mitosis and can be found in both normal and abnormal tissues. The cell cycle is maintained by the proteins. This test was created to assess the activity of the testis spermatogenesis process. Each rat's testis and epididymis were removed as soon as it was scarified, cleansed with saline via a caudal puncture, and then submerged in saline. The epididymis was treated for 30 minutes at 37 °C to increase sperm to exit the epididymal tubules. The sperm suspension was used in aliquots after epididymis sections were removed from the aforementioned solution.

2.8 Investigation of the animal biochemistry tests

Following the kit's instructions, the biochemical tests such as the serum male hormones (SHBG, DHEA-SO_4, FSH, LH and Ts), as well as testicular G6PD, cholesterol, total protein and 3βHSD, besides the antioxidant activities (GSH, MDA and SOD) levels were determined.

2.9 The sperm, testis and hypothalamus's ATPase activity

According to Gamaro *et al*. [34] technique, the ATPase activity was measured. Potassium chloride (KCl) (20 μM), magnesium chloride ($MgCl_2$) (5 μM), sodium chloride (NaCl) (80 μM), Tris hydrochloric acid (HCl) (50 μM, pH 7.4) and adenosine triphosphate (ATP) disodium salt (3 μM) were all present to form a solution. After that, adding 50 μL of the testis, hypothalamus or sperm homogenate to the previously described solution, and it was incubated at 37 °C for 10 minutes. The aforementioned solution was then mixed with 50 μL of trichloroacetic acid solution. Following that, the solution was centrifuged at 3000 rpm for five minutes. 500 μL of trichloroacetic acid, 250 μL of ammonium molybdate and 250 μL of ascorbic acid were adding to 1 mL of the supernatant. At 680 nm, the resulting color was measured with a spectrophotometer.

2.10 Testicular, sperm and hypothalamic nuclear factor kappa B (NF-κB) activities

The Eliza kit was used to identify the testis, hypothalamic and sperm NF-κB. The following is a summary of the procedure: Each enzyme well of 96-Eliza wells coated with a monoclonal antibody and previously coated with rat Monoclonal antibody against NF-κB was filled with sperm, testis, or hypothalamus NF-κB homogenate solution and incubated. After that, biotin-labeled NF-κB antibodies were added, and horseradish peroxidase (HRP)-conjugated streptavidin (streptavidin-HRP) was mixed to create an immunological compound. Subsequent incubation and washing were used to eliminate the uncombined enzyme. The absorbance at 450 nm was measured. NF-κB in the testis, sperm or hypothalamus is directly associated with the color generated [35].

2.11 Statistical analysis

In the study tables, the data were organized as mean $\pm$ standard error mean (SEM). In this study, the Gaussian distribution was used. Analysis of variance (ANOVA) test using a one-way method was performed using the SPSS 13 software (SPSS Incorporation, 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412, USA). In *post-hoc* analysis, p-values of 0.05 were considered significant for each treatment group using the Tukey test.

3. Results

3.1 Results of body weight and different organs

Total body weight, food consumed, water intake, and male organs weights of the seminal vesicle, testes, epididymis, prostate and vasa differentia, besides liver, kidney, and brain weights, urine outflow, and fecal output were significantly lower ($p \leq 0.01$) in male sexual organs (testes, epididymis, seminal vesicle, prostate and vasa differentia) while decreased ($p \leq 0.05$) in total body weight, food consumed, water intake, liver, kidney, and brain weights, urine outflow, and fecal output in lead acetate-treated rats compared to the control group (Table 1). Compared to rats treated with lead acetate, these rats when pretreated with grape or ginger prior to lead exposure had almost control levels in total body weight, consumption of food and water, organs weights, urine outflow and fecal output. The ginger ($p \leq 0.05$) treatment was less active than the grape ($p \leq 0.01$) treatment compared to lead acetate-treated group. None of the groups under investigation showed convulsions, animal deaths, rat hair loss or skin patches throughout the study's experimental period.

TABLE 1. Effect of grape and ginger on body weight and organs of lead acetate-treated rats.

Parameters	Control	Ginger (500 mg/kg)	Grape (75 mg/kg)	Lead acetate (30 mg/kg)	Ginger (500 mg/kg) + Lead acetate (30 mg/kg)	Grape (75 mg/kg) + Lead acetate (30 mg/kg)
Bwt (g)	167 ± 6.32	169 ± 6.24 (1.19%)	166 ± 6.45 (−0.59%)	130 ± 5.76[a] (−22.15%)	147 ± 6.18[c] (−11.97%)	164 ± 6.37[d] (−1.79%)
Fd con. (g/d)	11.6 ±1.2	11.4 ± 1.4 (−1.72%)	11.5 ± 1.6 (−0.86%)	7.2 ± 1.3[a] (−37.93%)	9.3 ±1.6[c] (−19.82%)	11.2 ± 1.5[d] (−3.44%)
W. intake (mL/d)	12.6 ± 1.8	12.8 ± 1.6 (1.58%)	12.5 ± 1.4 (−0.79%)	8.0 ± 1.5[a] (−36.50%)	10.2 ± 1.9[c] (−19.04%)	12.3 ± 1.8[d] (−2.38%)
Ur. vol. (mL/100 g/8 h)	0.96 ± 0.19	0.94 ± 0.16 (−2.08%)	0.97 ± 0.18 (1.04%)	0.64 ± 0.13[a] (−33.33%)	0.79 ± 0.17[c] (−17.70%)	0.95 ± 0.18[d] (−1.04%)
Fe. Pe. Cou.	40 ± 4.05	39 ± 3.84 (−2.50%)	38 ± 4.18 (−5.00%)	27 ± 3.12[a] (−32.50%)	32 ± 4.31[c] (−20.00%)	38 ± 4.24[d] (−5.00%)
Testes (g)	2.13 ± 0.08	2.12 ± 0.06 (−0.46%)	2.14 ± 0.07 (0.46%)	1.24 ± 0.03[b] (−41.78%)	1.68 ± 0.06[c] (−21.12%)	2.11 ± 0.07[d] (−0.93%)
Epidydemis (g)	1.25 ± 0.06	1.24 ± 0.04 (−0.80%)	1.26 ± 0.05 (0.80%)	0.60 ± 0.05[b] (−52.00%)	0.92 ± 0.06[c] (−26.40%)	1.23 ± 0.04[d] (−1.60%)
Sem. Ves. (g)	0.41 ± 0.04	0.40 ± 0.05 (−2.43%)	0.42 ± 0.03 (2.43%)	0.16 ± 0.02[b] (−60.97%)	0.28 ± 0.05[c] (−31.70%)	0.39 ± 0.06[d] (−4.87%)
Prostate (g)	0.35 ± 0.05	0.36 ± 0.07 (2.85%)	0.34 ± 0.06 (−2.85%)	0.20 ± 0.03[b] (−42.85%)	0.26 ± 0.06[c] (−25.71%)	0.32 ± 0.05[d] (−8.57%)
Vas. diff. (g)	0.80 ± 0.07	0.79 ± 0.06 (−1.25%)	0.81 ± 0.04 (1.25%)	0.35 ± 0.05[b] (−56.25%)	0.57 ± 0.04[c] (−28.75%)	0.78 ± 0.07[d] (−2.50%)
Liv. Wt (g/100 g bw)	2.9 ± 0.09	2.8 ± 0.07 (−3.44%)	2.7 ± 0.08 (−6.89%)	2.0 ± 0.07[a] (−31.03%)	2.3 ± 0.07[c] (−20.68%)	2.7 ± 0.09[d] (−6.89%)
Kid. Wt (g/100 g bw)	0.36 ± 0.03	0.37 ± 0.05 (2.77%)	0.35 ± 0.04 (−2.77%)	0.21 ± 0.02[a] (−41.66%)	0.28 ± 0.06[c] (−22.22%)	0.34 ± 0.05[d] (−5.55%)
Br. Wt (g)	0.58 ± 0.06	0.59 ± 0.07 (1.72%)	0.60 ± 0.08 (3.44%)	0.41 ± 0.05[a] (−29.31%)	0.48 ± 0.08[c] (−17.24%)	0.56 ± 0.07[d] (−3.44%)

There were six rats in each group. The data are shown as mean ± SEM. [a]Significant difference ($p \leq 0.05$) from the control. [b]Very significant difference ($p \leq 0.01$) from the control. [c]Significant difference ($p \leq 0.05$) from rats treated with lead acetate. [d]Very significant difference ($p \leq 0.01$) from rats-treated lead acetate. Bwt: Body weight; Fd con.: Food consumption; W. intake: Water intake; Ur. vol.: Urinary volume; Fe. Pe. Cou.: Fecal Pellet count; Sem. Ves.: Seminal vesicle; Vas. diff.: Vasa differentia; Liv. Wt: Liver weight; Kid. Wt: Kidney weight; Br. Wt.: Brain weight. ( ): % of reduction or increase compared to the control.

3.2 Levels of antioxidants in the testis, hypothalamus and sperm

The antioxidant activities of the testes, sperms and hypothalamus in rats given lead acetate are shown to be protected by grape and ginger in Table 2. This table shows that, rats treated with lead acetate compared to the control group, exhibited higher levels of malondialdehyde ($p \leq 0.01$) in their testes, hypothalamus and sperm but lower levels in GSH ($p \leq 0.05$) and SOD ($p \leq 0.01$) levels than that in control group. However, oral administration of grape or ginger restored the antioxidant levels in rats treated with lead acetate to normal values, with grape ($p \leq 0.01$) administration having a more favorable outcome than ginger ($p \leq 0.05$) did compared to lead acetate-treated group. Additionally, during the study, normal rats given only grape or ginger showed no changes ($p \geq 0.05$) in their antioxidant levels in the testes, sperm or hypothalamus.

3.3 Serum male hormone results

The impact of ginger and grape on serum male hormones in rats exposed to lead acetate is shown in Table 3. Based on this information, compared to the control group, rats treated with oral lead acetate alone showed lower serum levels of Ts ($p \leq 0.05$) and DHEA-SO_4 ($p \leq 0.05$), but higher blood levels of LH ($p \leq 0.01$), FSH ($p \leq 0.01$) and SHBG ($p \leq 0.05$). Lead acetate-treated rats' serum levels of male hormones were nearly restored to control levels when either grape or ginger was given to them; however, the grape ($p \leq 0.01$) was more successful than the ginger ($p \leq 0.05$) when compared to the lead acetate-treated group. Moreover, normal rats given ginger or grape orally did not show any change ($p \geq 0.05$) in their levels of serum male hormones during the experiments.

TABLE 2. Effect of grape and ginger on antioxidant levels of hypothalamus, testis and sperm of lead acetate-treated rats.

Parameters	Control	Ginger (500 mg/kg)	Grape (75 mg/kg)	Lead acetate (30 mg/kg)	Ginger (500 mg/kg) + Lead acetate (30 mg/kg)	Grape (75 mg/kg) + Lead acetate (30 mg/kg)
Hypothalamus						
SOD (U/g)	160.2 ± 21.7	159.5 ± 22.3 (−0.43%)	161.4 ± 21.5 (0.74%)	87.6 ± 19.4^{b} (−45.31%)	122.5 ± 21.4^{c} (−23.53%)	158.4 ± 20.6^{d} (−1.12%)
GSH (mg/g)	0.51 ± 0.06	0.50 ± 0.05 (−1.96%)	0.52 ± 0.07 (1.96%)	0.34 ± 0.04^{a} (−33.33%)	0.42 ± 0.06^{c} (−17.64%)	0.49 ± 0.05^{d} (−3.92%)
MDA (nmol/g)	245.2 ± 32.5	246.1 ± 30.8 (0.36%)	244.8 ± 31.7 (−0.16%)	350.6 ± 28.7^{b} (42.98%)	298.5 ± 32.4^{c} (21.73%)	246.7 ± 31.9^{d} (0.61%)
Testis						
SOD (U/g)	139.5 ± 19.6	138.7 ± 18.6 (−0.57%)	139.5 ± 20.3 (0.00%)	78.9 ± 16.5^{b} (−43.44%)	109.4 ± 19.6^{c} (−21.57%)	139.5 ± 19.6^{d} (0.00%)
GSH (mg/g)	161.4 ± 22.5	159.8 ± 21.4 (−0.99%)	162.1 ± 20.7 (0.43%)	135.6 ± 19.5^{a} (−15.98%)	148.5 ± 21.7^{c} (−7.99%)	161.4 ± 22.5^{d} (0.00%)
MDA (nmol/g)	49.8 ± 8.9	48.6 ± 8.5 (−2.40%)	50.1 ± 7.8 (0.60%)	70.9 ± 8.1^{b} (42.36%)	60.4 ± 7.9^{c} (21.28%)	49.8 ± 8.9^{d} (0.00%)
Sperm						
SOD (U/g)	154.6 ± 19.7	155.1 ± 18.7 (0.32%)	153.8 ± 19.2 (−0.51%)	85.7 ± 18.4^{b} (−44.56%)	119.3 ± 19.4^{c} (−22.83%)	152.8 ± 19.7^{d} (−1.16%)
GSH (mg/g)	0.30 ± 0.04	0.29 ± 0.05 (−0.03%)	0.31 ± 0.04 (3.33%)	0.20 ± 0.03^{a} (−33.33%)	0.24 ± 0.04^{c} (−20.00%)	0.28 ± 0.06^{d} (−6.66%)
MDA (nmol/g)	472.5 ± 43.1	471.8 ± 41.8 (−0.14%)	473.2 ± 42.8 (0.14%)	561.4 ± 38.6^{b} (18.81%)	517.5 ± 40.9^{c} (9.52%)	473.6 ± 41.5^{d} (0.23%)

There were six rats in each group. The data are shown as mean ± SEM. [a]Significant difference ($p \leq 0.05$) from the control. [b]Very significant difference ($p \leq 0.01$) from the control. [c]Significant difference ($p \leq 0.05$) from rats treated with lead acetate. [d]Very significant difference ($p \leq 0.01$) from rats-treated with lead acetate. ( ): % of reduction or increase compared to the control. SOD: Superoxide dismutase; GSH: Glutathione; MDA: Malondialdehyde.

TABLE 3. Effect of grape and ginger on serum male hormones of lead acetate-treated rats.

Parameters	Control	Ginger (500 mg/kg)	Grape (75 mg/kg)	Lead acetate (30 mg/kg)	Ginger (500 mg/kg) + Lead acetate (30 mg/kg)	Grape (75 mg/kg) + Lead acetate (30 mg/kg)
Ser. Ts (ng/mL)	5.98 ± 0.42	5.96 ± 0.39 (−0.33%)	5.99 ± 0.49 (0.16%)	3.14 ± 0.49^{a} (−47.49%)	4.55 ± 0.37^{c} (−23.91%)	5.95 ± 0.42^{d} (−0.50%)
Ser. LH (mIU/mL)	18.27 ± 1.85	18.30 ± 1.76 (0.16%)	18.25 ± 1.87 (−0.10%)	36.41 ± 2.21^{b} (99.28%)	25.74 ± 1.69^{c} (40.88%)	18.19 ± 1.85^{d} (−0.43%)
Ser. FSH (mIU/mL)	1.05 ± 0.13	1.07 ± 0.12 (1.90%)	1.06 ± 0.10 (0.95%)	2.32 ± 0.22^{b} (120.95%)	1.68 ± 0.15^{c} (60.00%)	1.03 ± 0.13^{d} (−1.90%)
Ser. DHEA-SO_4 (µg/dL)	197.5 ± 23.2	197.6 ± 24.8 (0.05%)	196.8 ± 21.7 (−0.35%)	164.3 ± 17.6^{a} (−16.81%)	175.1 ± 19.8^{c} (−11.34%)	195.9 ± 23.2^{d} (−0.81%)
Ser. SHBG (nmol/L)	6.65 ± 0.49	6.67 ± 0.58 (0.30%)	6.64 ± 0.51 (−0.15%)	8.54 ± 0.83^{a} (28.42%)	7.83 ± 0.69^{c} (17.74%)	6.59 ± 0.48^{d} (−0.90%)

There were six rats in each group. The data are shown as mean ± SEM. [a]Significant difference ($p \leq 0.05$) from the control. [b]Very significant difference ($p \leq 0.01$) from the control. [c]Significant difference ($p \leq 0.05$) from rats treated with lead acetate. [d]Very significant difference ($p \leq 0.01$) from rats-treated lead acetate. Ser. Ts: Serum testosterone; Ser. LH: Serum luteinizing hormone; Ser. FSH: Serum follicle stimulating hormone; Ser. DHEA-SO_4: Serum dehydroepiandrosterone sulfate; Ser. SHBG: Serum sex hormone binding globulin.

3.4 Findings for 3β-hydroxysteroid dehydrogenase, glucose-6-phosphate dehydrogenase, cholesterol and total protein

Table 4 illustrates how grape and ginger preserve testicular total protein, cholesterol, 3βHSD and G6PD in rats treated with lead acetate. G6PD ($p \leq 0.05$), 3βHSD ($p \leq 0.01$), cholesterol ($p \leq 0.01$) and total protein ($p \leq 0.05$) were all lower in rats given lead acetate alone than in the control group, according to this table. Additionally, when grape or ginger was given orally to rats treated with lead acetate, the previously indicated parameters returned to control levels. Additionally, grape ($p \leq 0.01$) had a beneficial effect than ginger ($p \leq 0.05$) compared to lead acetate treated group. Furthermore, normal rats when treated with ginger and grape orally alone, the aforementioned parameters did not alter ($p \geq 0.05$) during the experimental period of the research.

3.5 Findings from sperm abnormality, motility and count

The preventive effects of grape and ginger on sperm abnormalities, motility and count in rats given lead acetate are shown in Table 5. The data indicates that oral lead acetate administration increased sperm abnormality ($p \leq 0.05$) while decreasing sperm motility ($p \leq 0.05$) and count ($p \leq 0.01$). Furthermore, the abnormality, motility and count of sperms were nearly brought back to control values when lead acetate-treated rats pretreated with grape or ginger orally. Additionally, grape ($p \leq 0.01$) administration was more effective than ginger ($p \leq 0.05$) administration compared to lead acetate-treated group. Furthermore, sperm motility, count, and abnormalities did not alter ($p \geq 0.05$) in normal rats treated with grape or ginger orally.

3.6 Testicular proliferative index findings

Table 6 displays the effect of ginger and grape on the rat testis's proliferative index treated with lead acetate. According to the results in this table, the lead acetate induced higher percentages of first spermatocytes ($p \leq 0.05$), second spermatocytes ($p \leq 0.05$), spermatids and spermatogonia ($p \leq 0.01$) than that in the control group. Furthermore, pretreatment with grape and ginger to lead acetate group returned the percentage of spermatogonia, first spermatocyte, second spermatocyte and spermatid to levels approached to the control values. When compared to the lead acetate-treated group, the administration of grape ($p \leq 0.01$) was more effective than that of ginger ($p \leq 0.05$). Furthermore, normal rats treated with grape or ginger orally alone showed no changes ($p \geq 0.05$) during the experimental period of the research in their spermatogonia, first spermatocyte, second spermatocyte and spermatid.

3.7 The testis, sperm and hypothalamus's NF-κB levels and ATPase activities

The effects of grape and ginger on ATPase activity and NF-κB level in the hypothalamus, sperm and testis in rats treated with lead acetate are shown in Table 7. This table shows that, in comparison to the control group, oral lead acetate treatment significantly reduced ATPase activity while raising NF-κB levels in the sperm ($p \leq 0.05$), testis ($p \leq 0.05$) and hypothalamus ($p \leq 0.01$). In rats given lead acetate, the levels of NF-κB and ATPase activity were returned to levels that were nearly identical to the control values after the oral administration of grape and ginger, but the effect of administration of grape ($p \leq 0.01$) was more effective than that of ginger ($p \leq 0.05$) compared to lead acetate-treated group. Normal rats treated with grape or ginger orally alone during the research without any effect ($p \geq 0.05$) on ATPase activity or NF-κB level.

4. Discussion

The morphology, count and motility of sperm were all significantly reduced by lead acetate. It induced also a decline in the antioxidants levels in the testis, hypothalamus and sperms. This finding is corroborated by earlier studies [36] and implies that rats exposed to environmental toxins such as lead acetate showed higher oxidative stress levels compared to the control group and it increased reactive oxygen species production that attack organs and results in oxidative damage. The environmental pollutants interact with the steroid receptors in the testis and delay the proliferation and differentiation of spermatogenesis cells [37].

According to the current study, oral lead acetate administration increased serum LH, FSH, SHBG, G6PD, 3βHSD, total protein and cholesterol, NF-κB, abnormalities in sperm, as well as the proportion of spermatogonia, first spermatocytes, second spermatocytes and spermatid, and MDA in the testis, while decreasing serum Ts and DHEA-SO_4, number and motility of sperm, ATPase activity and SOD and GSH. Lead acetate significantly decreased the level of blood Ts [7]. Lead acetate's main role occurs through 2 processes; (1) during the enzymatic phases of Ts biosynthesis, cholesterol is converted to pregnenolone, which is then converted to progesterone, respectively, via the 3β-hydroxysteroid dehydrogenase-isomerase processes and cholesterol side chain cleavage and (2) other possible lead acetate action sites include 3β-hydroxysteroid dehydrogenase-isomerase processes and cholesterol side chain cleavage [38]. Extended exposure to lead acetate also affects the biosynthesis of Ts in the testicles, resulting in decreased enzymes and associated proteins, and consequently caused testicular atrophy [6, 7].

Lead acetate also decreased the activity of 3βHSD and elevated amounts of FSH and LH in the testes. Lead acetate's impact on testicular function has been explained by a number of researchers [39]. Precursor and immature Leydig cells' P450c17 activity is directly suppressed by lead acetate, and it suppressed the activity of P450 and/or 3βHSD. Ts, which is secreted by Leydig cells and controls spermatogenesis by binding to the androgen receptors on the germinal epithelium, is reduced in response to high FSH and LH levels in lead acetate exposure. FSH controls spermatogenesis through targeting Sertoli cell receptors which consequently promotes the growth of several Sertoli cell components.

Because lead acetate interferes with the activity of the Leydig cells that regulate spermatogenesis in response to LH, it lowers male fertility [7]. By reducing the amount of testicular

TABLE 4. Effect of grape and ginger on testis G6PD, 3βHSD, cholesterol and total protein in lead acetate-treated rats.

Parameters	Control	Ginger (500 mg/kg)	Grape (75 mg/kg)	Lead acetate (30 mg/kg)	Ginger (500 mg/kg) + Lead acetate (30 mg/kg)	Grape (75 mg/kg) + Lead acetate (30 mg/kg)
Testis G6PD (U/g)	11.92 ± 0.75	11.90 ± 0.81 (−0.16%)	11.94 ± 0.96 (0.16%)	7.64 ± 0.68^a (−35.90%)	9.76 ± 0.92^c (−18.12%)	11.89 ± 0.64^d (−0.25%)
Testis 3βHSD (U/g)	4.46 ± 0.83	4.45 ± 0.75 (−0.22%)	4.47 ± 0.68 (0.22%)	2.15 ± 0.36^b (−51.79%)	3.29 ± 0.58^c (−26.23%)	4.43 ± 0.87^d (−0.67%)
Testis cholesterol (mg/g)	130.7 ± 8.23	131.4 ± 7.51 (0.53%)	132.1 ± 8.14 (1.07%)	75.9 ± 8.46^b (−41.92%)	102.4 ± 7.81^c (−21.65%)	128.9 ± 7.63^d (−1.37%)
Testis total protein (mg/g)	290.6 ± 15.32	292.3 ± 14.72 (0.58%)	291.5 ± 15.29 (0.30%)	256.8 ± 14.84^a (−11.63%)	273.4 ± 15.39^c (−5.91%)	289.7 ± 14.50^d (−0.30%)

There were six rats in each group. The data are shown as mean ± SEM. [a]Significant difference ($p \leq 0.05$) from the control. [b]Very significant difference ($p \leq 0.01$) from the control. [c]Significant difference ($p \leq 0.05$) from rats treated with lead acetate. [d]Very significant difference ($p \leq 0.01$) from rats-treated lead acetate. G6PD: Glucose-6-phosphate dehydrogenase; 3βHSD: 3β-hydroxysteroid dehydrogenase. ( ): % of reduction or increase compared to the control.

TABLE 5. Effect of grape and ginger on sperm count, motility and abnormality in lead acetate-treated rats.

Parameters	Control	Ginger (500 mg/kg)	Grape (75 mg/kg)	Lead acetate (30 mg/kg)	Ginger (500 mg/kg) + Lead acetate (30 mg/kg)	Grape (75 mg/kg) + Lead acetate (30 mg/kg)
Count ($\times 10^6$/mL)	185 ± 19.64	184 ± 18.75 (−0.54%)	186 ± 19.28 (0.54%)	94 ± 18.56^b (−49.18%)	139 ± 18.74^c (−24.86%)	183 ± 19.83^d (−1.08%)
Motility (%)	86 ± 10.54	85 ± 9.63 (−1.16%)	87 ± 10.82 (1.16%)	67 ± 9.71^a (−22.09%)	76 ± 10.58^c (−11.62%)	84 ± 10.23^d (−2.32%)
Abnormality (%)	7.9 ± 0.58	7.8 ± 0.61 (−1.26%)	8.0 ± 0.70 (1.26%)	9.8 ± 0.49^a (24.05%)	8.7 ± 0.63^c (10.12%)	7.6 ± 0.57^d (−3.79%)

There were six rats in each group. The data are shown as mean ± SEM. [a]Significant difference ($p \leq 0.05$) from the control. [b]Very significant difference ($p \leq 0.01$) from the control. [c]Significant difference ($p \leq 0.05$) from rats treated with lead acetate. [d]Very significant difference ($p \leq 0.01$) from rats-treated lead acetate. ( ): % of reduction or increase compared to the control.

TABLE 6. Effect of grape and ginger on proliferative index in the sperm of lead acetate-treated rats.

Parameters	Control	Ginger (500 mg/kg)	Grape (75 mg/kg)	Lead acetate (30 mg/kg)	Ginger (500 mg/kg) + Lead acetate (30 mg/kg)	Grape (75 mg/kg) + Lead acetate (30 mg/kg)
Spermatogonia	6.85 ± 0.72	6.84 ± 0.59 (−0.14%)	6.86 ± 0.61 (0.14%)	14.36 ± 0.59^b (109.63%)	10.59 ± 0.73^c (54.59%)	6.83 ± 0.69^d (−0.29%)
1st spermatocyte	13.7 ± 0.84	13.6 ± 0.79 (−0.72%)	13.8 ± 0.92 (0.72%)	20.6 ± 0.79^a (50.36%)	17.1 ± 0.64^c (24.81%)	13.5 ± 0.58^d (−1.45%)
2nd spermatocyte	17.6 ± 0.92	18.1 ± 0.85 (2.84%)	17.5 ± 0.79 (−0.56%)	26.4 ± 0.68^a (50.00%)	21.9 ± 0.86^c (24.43%)	17.4 ± 0.78^d (−1.13%)
Spermatid	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	5.3 ± 0.06	2.7 ± 0.04^c	0.2 ± 0.01^d

There were six rats in each group. The data are shown as mean ± SEM. [a]Significant difference ($p \leq 0.05$) from the control. [b]Very significant difference ($p \leq 0.01$) from the control. [c]Significant difference ($p \leq 0.05$) from rats treated with lead acetate. [d]Very significant difference ($p \leq 0.01$) from rats-treated lead acetate. ( ): % of reduction or increase compared to the control.

TABLE 7. Effect of grape and ginger on ATP activity and NF-κB level of lead acetate-treated rats.

Parameters	Control	Ginger (500 mg/kg)	Grape (75 mg/kg)	Lead acetate (30 mg/kg)	Ginger (500 mg/kg) + Lead acetate (30 mg/kg)	Grape (75 mg/kg) + Lead acetate (30 mg/kg)
Hypothalamus						
ATPase activity (nmol pi/min/mg)	4600 ± 197	4650 ± 185 (1.08%)	4550 ± 169 (−1.08%)	2900 ± 174[b] (−36.95%)	3750 ± 182[c] (−18.47%)	4500 ± 191[d] (−2.17%)
NF-κB (ng/mg)	0.49 ± 0.08	0.48 ± 0.07 (−2.04%)	0.50 ± 0.09 (2.04%)	1.62 ± 0.05[b] (230.61%)	1.07 ± 0.06[c] (118.36%)	0.52 ± 0.08[d] (6.12%)
Testis						
ATPase activity (nmol pi/min/mg)	6550 ± 249	6600 ± 263 (0.76%)	6500 ± 252 (−0.76%)	5250 ± 226[a] (−19.84%)	5850 ± 276[c] (−10.68%)	6450 ± 258[d] (−1.52%)
NF-κB (ng/mg)	0.34 ± 0.05	0.33 ± 0.03 (−2.94%)	0.35 ± 0.04 (2.94%)	0.58 ± 0.03[a] (70.58%)	0.47 ± 0.05[c] (38.23%)	0.36 ± 0.04[d] (5.88%)
Sperm						
ATPase activity (nmol pi/min/mg)	8200 ± 324	8250 ± 346 (0.60%)	8150 ± 329 (−0.60%)	7150 ± 316[a] (−12.80%)	7600 ± 334[c] (−7.31%)	8100 ± 351[d] (−1.21%)
NF-κB (ng/mg)	0.19 ± 0.02	0.18 ± 0.01 (−5.26%)	0.20 ± 0.03 (5.26%)	0.28 ± 0.01[a] (47.36%)	0.24 ± 0.02[c] (26.31%)	0.21 ± 0.03[d] (10.52%)

There were six rats in each group. The data are shown as mean ± SEM. ATPase activity: Sodium/potassium-ATPase activity; NF-κB: Nuclear factor kappa B. [a]Significant difference ($p \leq 0.05$) from the control. [b]Very significant difference ($p \leq 0.01$) from the control. [c]Significant difference ($p \leq 0.05$) from rats treated with lead acetate. [d]Very significant difference ($p \leq 0.01$) from rats-treated with lead acetate. ( ): % of reduction or increase compared to the control.

protein, lead acetate results in testicular dysfunction [40], additionally, it reduced total protein, cholesterol, 3βHSD, and G6PD levels in the testis and reduced also the number of testicular Leydig cells [41]. Lead acetate reduced the production of steroid hormones [5] and the amount of protein in the testicles due to the reduction of protein biosynthesis occurs after exposure to lead acetate [42]. Carrier proteins, such as SHBG, help move sex hormones through plasma and have an impact on the blood's amount of unbound steroids. Lead acetate significantly raised SHBG while significantly lowering Ts concentration, demonstrating SHBG's strong binding affinity for Ts. SHBG acts as a hormone reservoir and it regulates the drop in steroid hormone concentration to restore the proportions of free and bound steroids in balance [43].

In the testis, exposure to lead acetate increased the percentage of spermatogonia, first spermatocytes, second spermatocytes, and spermatids. Lead acetate exposure increased sperm abnormalities while decreased sperm production and motility. Lead acetate disrupts three processes that cause this effect: (1) steroidogenesis; (2) spermatogenic; and (3) the generation of primary spermatocytes, spherical spermatids, and elongating spermatids. The sperm proliferating index rises and the number of active sperm decreases when exposed to lead acetate. This implies that some spermatogenic cells halted their cycle, which is why there were less sperm. The germ cells were therefore destined to perish since cell arrest resulted in cell death without the capacity to regenerate spermatogenic cells [44].

Lead acetate decreased ATPase function in the testis, sperm, and hypothalamus [6] due to an imbalance in the oxidative state after lead exposure, which produces free radicals that lead to apoptosis and oxidative damage. G6PD and 6-phosphogluconate dehydrogenase are two oxidative phase pathway-related enzymes are inhibited by lead acetate. Consequently, glutathione reductase has less nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) to be utilized in sustaining the regeneration of decreased GSH [7].

The pretreatment with either grape or ginger orally to lead acetate-treated rats returned the aforementioned parameters to levels that were close to the control. Numerous researchers, including Afkhami Fathabad *et al.* [27], reported that ginger treatment improved testicular histopathological results and prevented the toxic effects in sperm parameters (morphology and motility), epididymal tissue and seminiferous tubules in testicular toxicity. Furthermore, ginger lowered MDA levels [45] and raised glutathione reductase levels, Ts in testicular damage [45], glutathione peroxidase [24, 44], and total antioxidant activity such as SOD and catalase (CAT) [22, 23]. Ginger also declines the effect of ethanol-induced male infertility. Additionally, ginger raised the levels of dehydroepiandrosterone sulfate, SHBG and Ts [45]. In cases of reproductive impairment, ginger restored normal testicular morphology, spermatogenesis and sperm parameters (counts, motility, and morphology) [46].

On the other hand, grape increased testicular weight [47], reduced MDA levels [48, 49], and raised total antioxidant status [48]. Grape also increased the antioxidant defense system's enzymes, steroidogenesis-related genes, and Ki-67 expression [49]. The diameter and structure of seminiferous tubules were improved by grape [49]. Treatment with

grape juice concentrate improved testicular tissue architecture [50], sperm production, counts and transit time [51], sperm morphology [50], and restored the weights of the testicles, epididymis, and ventral prostate [50] to approach the normal levels. Furthermore, grape raised Ts and GSH levels and reduced mitochondrial SOD activity [50].

Ginger's mode of action performs through increasing the weight of the seminal vesicles, prostate, testicles, and epididymis, as well as sperm parameters (counts and motility). Additionally, ginger lowered (1) plasma glucose, (2) the amounts of glutathione peroxidase, SOD, and CAT (antioxidant enzymes), (3) the levels of MDA in the testis, (4) Ts, FSH and LH levels in the blood, as well as the levels of metabolic enzymes such as aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and alkaline phosphatase [20, 21].

While grape's mode of action is attributed to improving histological structures of testis, sperm motility and numbers, and a rise in antioxidant enzymes including SOD and glutathione peroxidase [13, 15]. Trans-resveratrol (a naturally occurring antioxidant found in grape) gives grape its action. It increased testicular weight to a control level, decreased the diameter of seminiferous tubules but increased their length, and increased the tubular density compared to the control group. Additionally, it increased the sperm count and the levels of FSH, LH and Ts [15].

As a whole, lead acetate is a well-known environmental toxin that negatively impacts the development and reproduction of both humans and animals. It causes oxidative damage in particular SOD and GSH, and by increasing MDA levels and decreasing antioxidant enzymes. Additionally, it results in changes in sperm rate, a decrease in sperm motility and concentration, and histological damage to the testicles. Additionally, testicular 3βHSD activity and circulatory Ts levels significantly decreased in rats exposed to lead acetate [5–7]. On the other hand, pretreatment with ginger and grape orally to Lead acetate-treated rats preserves male fertility and sperm quality, with grape having a better effect on male fertilization than ginger.

5. Conclusions

Grape and ginger enhanced endogenous antioxidants, especially SOD and GSH, and decreased MDA to shield the hypothalamus, testis and sperm from oxidative stress caused by lead acetate. As a result, normal sperm production motility and morphology, as well as regular germ cell division were preserved. Additionally, it was demonstrated that either grape or ginger may shield the sperm, testes, and hypothalamus from the oxidative damage brought on by lead acetate; additionally, grape was more successful in protecting normal male fertility and characteristics than ginger.

6. The study limitations

This study does not contain histopathological or molecular study because both studies are discussed in detail in a separate article focused on pathological change and immunohistochemistry technique to detect p53 and bcl2 genes in testicular tissue in pretreatment with either grape or ginger in lead acetate-exposed male rats.

ABBREVIATIONS

Ts, Testosterone; LH, Luteinizing hormone; FSH, Follicle stimulating hormone; DHEA-SO_4, Dehydroepiandrosterone sulfate; SHBG, Sex hormone binding globulin; G6PD, Glucose-6-phosphate dehydrogenase; 3βHSD, 3β-Hydroxysteroid dehydrogenase; SOD, Superoxide dismutase; GSH, Glutathione; MDA, Malondialdehyde; NF-κB, Nuclear factor kappa B; ATPase activity, Sodium/potassium-ATPase activity; NADPH, Nicotinamide adenine dinucleotide phosphate hydrogen; PbAc, lead acetate; ANOVA, Analysis of variance; SEM, standard error mean; CASA, Computer-Assisted Sperm Analysis; IAEC, Institutional Animal Ethical Committee; NIH, National Institutes of Health; ATP, Adenosine triphosphate; CAT, Catalase; HRP, horseradish peroxidase; KCl, Potassium chloride; $MgCl_2$, Magnesium chloride; NaCl, Sodium chloride; HCl, Hydrochoric acid; ATPase, adenosine 5′-triphosphatase.

AVAILABILITY OF DATA AND MATERIALS

The data are contained within this article.

AUTHOR CONTRIBUTIONS

KK—designed the research study; analyzed the data; wrote the manuscript. KK and SS—performed the research. Both authors contributed to editorial changes in the manuscript. Both authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study and all experimental methods were authorized by the National Research Centre Institutional animal care and use committee; the approval code was 13173916. This study was conducted at the National Research Centre, Giza, Egypt which adhered to strict guidelines for the housing of animals and all other experimental study procedures, particularly the protocol for the care and use of animals.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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