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Salvia officinalis L. Methanolic Extract Reduces Lead and Nicotine-Induced Sperm Quality Degeneration in Male Rats

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Most heavy metals and industrial chemicals such as nicotine and lead cause harm to the reproduction process through a decrease in sperm motility, fertilization process, and sperm binding to the oocyte. *Salvia officinalis* L. (sage) has been reported to enhance serum testosterone levels and other certain biochemical enzymes. Thus, the current study is aimed at evaluating the potential health benefits of *S. officinalis* L. methanol extract on lead and nicotine hydrogen tartrate-induced sperm quality degeneration in male rats and also identifying some of the non-polar volatile bioactive compounds that might be attributed to the bioactivity of *S. officinalis* extract using gas chromatography-mass spectrometry (GC/MS). In the study, fifty-four mature male albino rats of about 220–250 g [were divided randomly and equally into 9 groups ($n=6$)]. Sperm quality degeneration was induced through the oral administration of 1.5 g/L of lead acetate in drinking water or peritoneal injection of 0.50 mg/kg (animal weight) nicotine hydrogen tartrate for sixty days. Two doses (200 & 400 mg/kg b.w.) of *S. officinalis* L. were used. The rats were anesthetized after the experimental period and then sacrificed. Blood samples were collected while the epididymis, testicle, and accessory sex organs (prostates and seminal vesical) were taken for histopathological studies. Twelve major compounds were identified through the GC/MS analysis of *S. officinalis* L. methanol extract. Lead and nicotine toxicity had a great effect on the rats' sperm quality causing a significant ($p<0.05$) decrease in the quantity of sperm and sperm motility as well as an upsurge in the abnormalities of the sperm and a reduction in the length & diameter of seminiferous tubules and size & weight of sexual organs (accessory sex glands, epididymis, and testis). The administration of *S. officinalis* L. methanol extract, however, had a positive impact on the sexual organ weights, semen quality & quantity, and rats' fertility, thus, ameliorating the adversative effects of both lead and nicotine. Further evaluation and isolation of the bioactive components are recommended as potential drug leads.

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Introduction

Fertility is the natural capability of humans or animals to produce offspring. Thus, improving fertility increases production efficiency, economic feasibility, and animal health. On the other hand, Infertility is defined by the World Health Organization (WHO) as the lack of conception in the face of unprotected and regular intercourse for 12 months or longer as a result of impairment to the reproduction capacity individually or with one spouse.[1,2] Multiple factors could affect the process of pregnancy. Infertility and sub-fertility, for instance, could result from untreated and undiagnosed thyroid disease-causing high prolactin levels, anovulatory cycles, sex hormone imbalances, and luteal phase defects, thus, having important economic, psychological, and medical implications in society.[3] Male infertility is responsible for about 40–50% of infertility cases due to endocrine disorders immune system disorders, testicular and prostate pathology, and urogenital infections.[4] Semen quality, the first step in assessing male infertility, is usually evaluated by counting sperm concentration, morphology, and motility using semen analyzing procedures.[5,6] Semen motility is the sperm's capability to move properly toward an oocyte. Improper or insufficient motility is the main cause of subfertility or infertility. The fertilization capability of sperm depends on viability, sperm DNA fragmentation, and motility.[1,7] Many industrial chemicals such as nicotine, lead, and other heavy and trace metals harm the reproduction process.[8–10] Lead (Pb) is a widespread heavy metal toxic to humans and wildlife. It causes male subfertility through damage to the structural integrity of the testis, inhibition of spermatogenesis, and hormonal feed-back circuits' disruption at the level of the hypothalamus-pituitary-testicular (HPT) axis.[11] Exposure to Pb occurs in both humans and animals causing health problems and poisonings that can result in brain damage and the entire central nervous system causing coma, convulsions, and even death. Exposure to

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Pb is mostly through Pb particles inhalation from Pb-containing burning materials such as during recycling, smelting, stripping of leaded paint, use of leaded aviation fuel or leaded gasoline; consumption of contaminated water (from leaded pipes); ingestion of lead-contaminated dust, and food (from lead-soldered or lead-glazed containers). Similarly, Pb poisoning cases occur in animals during the search for alternative feed sources by hungry animals. Though cattle succumb more likely to acute lead poisoning, sheep can also be affected. Other common Pb sources on the farm include burnt lead batteries, caulking, grease & oil filters, linoleum, painted surfaces (car bodies, machinery, sheds, yards) paint tins, sump oil, and putty.[12,13] The risk is generally believed to be directly associated with both duration of exposure and increasing concentration. Several studies have shown the adverse effect of lead acetate on male fertility; thus, it has become a common experimental model to induce infertility in males.[8,14] The review of the literature has also shown nicotine hydrogen tartrate's adverse effect on male fertility and its impacts on cardiovascular, endocrine, and reproductive systems.[15,16] Piri and his colleagues linked the nicotine hydrogen tartrate's adverse effect to the regulation of neuronal nitric oxide synthase (nNOS) activity and the release of acetylcholine and nitric oxide in the nervous system. Both nNOS and nicotinic acetylcholine receptors are co-expressed abundantly in the hippocampal neurons and alter anxiety-like behaviors.[17] The effects of nicotine toxicity have also been linked to the cumulative effects of oxygen radicals and alteration in glutathione-linked enzymes, lactate dehydrogenase, apoptosis indicator, and serum creatinine.[10] Exposure to nicotine also occurs through direct exposure to a chemical released into the indoor air as a liquid spray (aerosol) or a fine powder that can contaminate food, water, and agricultural land. One of the most popular methods of exposure to nicotine is smoking tobacco, as the cigarette contains nicotine and smoking electronic cigarettes and hookahs.[18] Nicotine exposure has shown a decrease in sperm motility, fertilization process, and sperm binding to the oocyte, in addition to Leydig cells functions' disturbances, which decreases the production of testosterone.[19] The testosterone hormone has a central role in the maintenance and regulation of spermatogenesis.[20] Exposure to nicotine reportedly causes a decrease in the testis weight and genital atrophy including secondary glands, epididymis, and seminal vesical as well as an increase in sperm abnormalities.[11]

Salvia officinalis L. (sage) is a medicinal plant with strong antioxidant properties attributed to its richness in phenolic compounds (majorly rosmarinic acid). The administration of *S. officinalis* L. extracts caused an increase in the serum testosterone level and certain biochemical enzymes[21,22] *S. officinalis* L. is an evergreen shrub growing 50–70 cm in the family Labiatae/Lamiaceae. Salvia has been reported to be the largest genus within the family and has about 900 species. *S. officinalis* L. is native to the Middle East and Mediterranean areas and it grows now all over the world. *S. officinalis* L. is extensively used in traditional medicine for treating disorders and ulcers, gout, seizure, inflammation, rheumatism, dizziness, tremor, diarrhea, paralysis, and hyperglycemia. In many areas such as Asia and South America, it has also been used as a flavoring for food and drinks.[23,24]

In recent years, the extracts of many plants have been examined for their antioxidant and hypoglycemic activities.[9,25–29] The antioxidant effects of sage are primarily due to the phenolic compounds of the plant.[30] *S. officinalis* L. has a wider range of biological and pharmacological activities, such as antibacterial, antioxidant, hypoglycemic, and anti-inflammatory properties. Furthermore, some studies have found that sage has positive effects on the physiology of the heart, liver, kidney, and testes.[31] The common constituents found in sage include rosmarinic acid, carnosic compounds, phenolic acids, and flavonoids cineol, borneol, flavonoids, pinene, glycoside, saponin, resin, vitamin C and E, and tannin. These bioactive compounds have antibacterial, anti-inflammatory, anti-oxidant, anti-fungal, and anti-hypoglycemic properties.[32,33] The sage oil extract has a potentially positive effect on male reproductive functions.[34,35] It has been screened as effective against brain and nervous disorders, cardiovascular diseases, and various infections (such as dental abscesses, throat infections, and mouth ulcers) and is claimed to be beneficial to diabetic patients.[36] The sage oil extract reportedly contains different terpenoids, for instance, 12-O-methylcarnosic, 20-hydroxyferruginol, α-linolenic acid, oleanolic acid, and viridiflorol. Both α-linolenic and methyl carnosic acid considerably reduce obesity status, improve fertility, and increase sperm numbers.[34] There is not enough data on the specific bioactive volatile non-polar compounds that could be attributed to the enhancing characteristics of *S. officinalis* on sperm qualities. Therefore, this study is aimed at investigating the effects of *S. officinalis* L. methanol extracts on Pb and nicotine-induced sperm quality degeneration in rats and also identifying the volatile non-polar bioactive compounds that might be attributed to the bioactivity of *S. officinalis* L. extract using gas chromatography-mass spectrometry (GC/MS).

Results

Effect of *S. officinalis* L., lead acetate and nicotine hydrogen tartrate on organs' weights

The intraperitoneal injection of 0.50 mg/kg BW nicotine hydrogen tartrate and oral administration of 1.5 g/L lead acetate for two months induced a significant ($p < 0.05$) decrease in the weights of the accessory glands, epididymis, and testes (Table 1) compared to the normal control and negative control groups. However, the co-administration of lead acetate and nicotine hydrogen tartrate with *S. officinalis* L. extracts ameliorated both lead acetate and nicotine hydrogen tartrate's adverse effects on the weights of the various organs as compared to normal vehicle controls. Though the improvement in the accessory gland weight was smaller than the normal values.

Table 1. Effect of *S. officinalis* L., Lead, and Nicotine on Organs' Weights.

Animal Groups	Organs' Weight (g/100 g b.wt) (N = 6)		
	Testis	Epididymis	Accessory gland
Control normal	0.99 ± 0.02^{A}	0.48 ± 0.01^{A}	0.76 ± 0.03^{A}
Control vehicle	0.97 ± 0.01^{A}	0.44 ± 0.03^{A}	0.76 ± 0.04^{A}
Sage 400 mg/kg/day	1.00 ± 0.03^{A}	0.46 ± 0.01^{A}	0.75 ± 0.05^{A}
Lead	0.79 ± 0.04^{B}	0.36 ± 0.01^{B}	0.64 ± 0.02^{C}
Sage200 mg/kg/day + lead	0.91 ± 0.02^{A}	0.46 ± 0.01^{A}	0.68 ± 0.03^{B}
Sage400 mg/kg/day + lead	0.92 ± 0.05^{A}	0.45 ± 0.02^{A}	0.69 ± 0.04^{B}
Nicotine	0.73 ± 0.05^{C}	0.32 ± 0.01^{B}	0.65 ± 0.03^{C}
Sage200 mg/kg/day + nicotine	0.95 ± 0.04^{AB}	0.42 ± 0.01^{A}	0.72 ± 0.03^{B}
Sage400 mg/kg/day + nicotine	0.96 ± 0.02^{AB}	0.44 ± 0.02^{A}	0.72 ± 0.02^{B}

Values are means ± S.E. (n = 6). ANOVA was used for the analysis of the results. Values not sharing a common superscript letter (A, B, C, etc.) within each column are significantly different ($p < 0.05$) with Duncan's new multiple range post hoc tests.

Sperm characteristics

The results showed that lead acetate administration and nicotine hydrogen tartrate injection induced a significant ($p < 0.05$) increase in sperm abnormalities as well as a decrease in sperm number and sperm motility (Table 2) compared to the negative control and normal control groups. However, there was a significant ($p < 0.05$) dose-dependent alleviation of the adversative effect of both lead acetate and nicotine hydrogen tartrate following a co-administration with *S. officinalis* L. extract.

Hormones level, spermatogenic cell layer, and seminiferous tubules

The oral administration of lead acetate and intraperitoneal injection of nicotine hydrogen tartrate for 60 days induced a significant ($p < 0.05$) decrease in the hormones (LH and testosterone) level, the spermatogenic cell layer length, and the diameter of seminiferous tubules (Table 3) compared to the negative control and normal control groups. There was, however, a significant ($p < 0.05$) alleviation in the decrease in the hormone levels, the length of the spermatogenic cell layer,

Table 2. Effect of *S. officinalis* L., Lead, and Nicotine on sperm characteristics.

Animal Groups	Sperm characteristics (N = 6)		
	Sperm number ($\times 10^6$/mL)	Sperm abnormalities (%)	Sperm motility (%)
Control normal	25.00 ± 2.25^{A}	5.21 ± 0.33^{C}	70.00 ± 2.58^{A}
Control vehicle	24.00 ± 2.56^{AB}	5.32 ± 0.31^{C}	69.17 ± 2.39^{A}
Sage 400 mg/kg/day	25.67 ± 0.92^{A}	5.11 ± 0.37^{C}	70.83 ± 3.00^{A}
Lead	16.33 ± 2.23^{D}	8.21 ± 0.48^{A}	15.00 ± 4.08^{D}
Sage200 mg/kg/day + lead	20.50 ± 3.79^{C}	6.11 ± 0.99^{B}	49.17 ± 3.75^{C}
Sage400 mg/kg/day + lead	22.00 ± 3.68^{B}	5.92 ± 0.71^{B}	68.33 ± 2.79^{B}
Nicotine	13.33 ± 1.54^{D}	11.31 ± 0.48^{A}	18.33 ± 2.47^{D}
Sage200 mg/kg/day + nicotine	20.83 ± 2.55^{C}	7.10 ± 0.68^{A}	70.00 ± 3.42^{A}
Sage400 mg/kg/day + nicotine	21.83 ± 3.74^{C}	6.30 ± 0.40^{B}	70.00 ± 2.24^{A}

Values are means ± S.E. (n = 6). ANOVA was used for the analysis of the results. Values not sharing a common superscript letter (A, B, C, etc.) within each column are significantly different ($p < 0.05$) with Duncan's new multiple range post hoc tests.

Table 3. Effect of *S. officinalis* L., lead, and nicotine on hormones level, spermatogenic cell layer, and seminiferous tubules.

Animal Groups	Hormones level		Seminiferous tubules and spermatogenic cell layer	
	Luteinizing hormone (IU/mL)	Testosterone (ng/mL)	Seminiferous tubules (μm)	Spermatogenic cell layer (μm)
Control normal	3.97 ± 0.18^{A}	4.80 ± 0.15^{B}	147.75 ± 2.80^{A}	48.94 ± 4.20^{A}
Control vehicle	3.60 ± 0.21^{C}	4.75 ± 0.15^{C}	145.08 ± 5.15^{A}	46.20 ± 3.17^{B}
Sage 400 mg/kg/day	3.78 ± 0.19^{B}	4.93 ± 0.09^{A}	145.08 ± 5.15^{A}	46.20 ± 3.17^{B}
Lead	2.72 ± 0.24^{F}	1.90 ± 0.07^{G}	102.59 ± 6.50^{D}	21.36 ± 2.52^{E}
Sage200 mg/kg/day + lead	3.13 ± 0.30^{E}	3.70 ± 0.07^{E}	137.94 ± 6.16^{B}	34.21 ± 1.88^{D}
Sage400 mg/kg/day + lead	3.33 ± 0.22^{D}	3.97 ± 0.46^{D}	135.76 ± 3.84^{B}	36.88 ± 2.29^{C}
Nicotine	2.55 ± 0.23^{F}	2.55 ± 0.23^{G}	87.21 ± 3.27^{E}	10.20 ± 0.86^{F}
Sage200 mg/kg/day + nicotine	3.40 ± 0.21^{D}	3.40 ± 0.21^{F}	96.94 ± 6.97^{D}	23.39 ± 2.93^{E}
Sage400 mg/kg/day + nicotine	3.67 ± 0.23^{C}	3.67 ± 0.23^{E}	121.74 ± 7.09^{C}	29.52 ± 4.05^{D}

Values are means ± S.E. (n = 6). One-way analysis of variance (ANOVA) was used for results analysis. Values not sharing a common superscript letter (A, B, C, etc.) within each column are significantly different ($p < 0.05$) with Duncan's new multiple range post hoc tests.

and the seminiferous tubules' diameter following the co-administration of the *S. officinalis* L. extract with both lead acetate and nicotine hydrogen tartrate. There was a marked dose effect of *S. officinalis* L. extract.

Histopathological examination

The normal control group (G1) and the negative control group (G2) given normal drinking water and vehicle, respectively showed no histopathological changes in the examined organs. The testes also presented normal seminiferous tubules demonstrating accumulation of lumen spermatozoa and active spermatogenesis (Figure 1a&b). The histological appearance of the epididymis tubules was normal. The acini structure was also normal with the presence of eosinophilic secretion in the lumen of the prostate gland and seminal vesicles. The testes of the experimental group (G3) administered only *S. officinalis* extract (400 mg/kg) revealed testicular blood vessel congestion and mild interstitial edema despite the normal appearance of most of the seminiferous tubules (Figure 1c). Normal epididymis tubules, prostate gland, and seminal vesicles were observed.

The testes of the experimental group (G4) co-administered a high dose of *S. officinalis* extract (400 mg/kg) and lead acetate, however, revealed testicular blood vessels congestion and marked degeneration of the lining epithelial cells of small numbers of seminiferous tubules, characterized by vacuolated Sertoli and germ cells, with a near absence of spermatozoa in the lumen and markedly reduced spermatogenesis (Figure 1d). There was an undulant basement membrane in the degenerated tubules despite the normal epididymis tubules and seminal vesicles (Figure 2a). There was also mild interstitial edema of the prostate gland with a normal structure of the acini (data not shown).

The testis of the experimental group (G5) with *S. officinalis* L. extract (200 mg/kg) and lead acetate presented blood vessel congestion and testicular degeneration of few to moderate amounts of seminiferous tubules (Figure 1e). The degenerated tubules were characterized by either marked vacuolization of germinal epithelium or loss of almost entire germ cells. There were pyknosis and karyorrhexis of spermatogonia and spermatocytes. Multifocally, there was marked expansion in the homogenous eosinophilic material (edema) of the testicular interstitium in addition to blood vessel congestion of the epididymis and interstitial edema. The epididymal tubules lumen showed necrotic debris admixed with spermatogenic cells, degenerated spermatozoa, and a little inflammatory cell infiltration (Figure 2b). The prostate gland showed interstitial

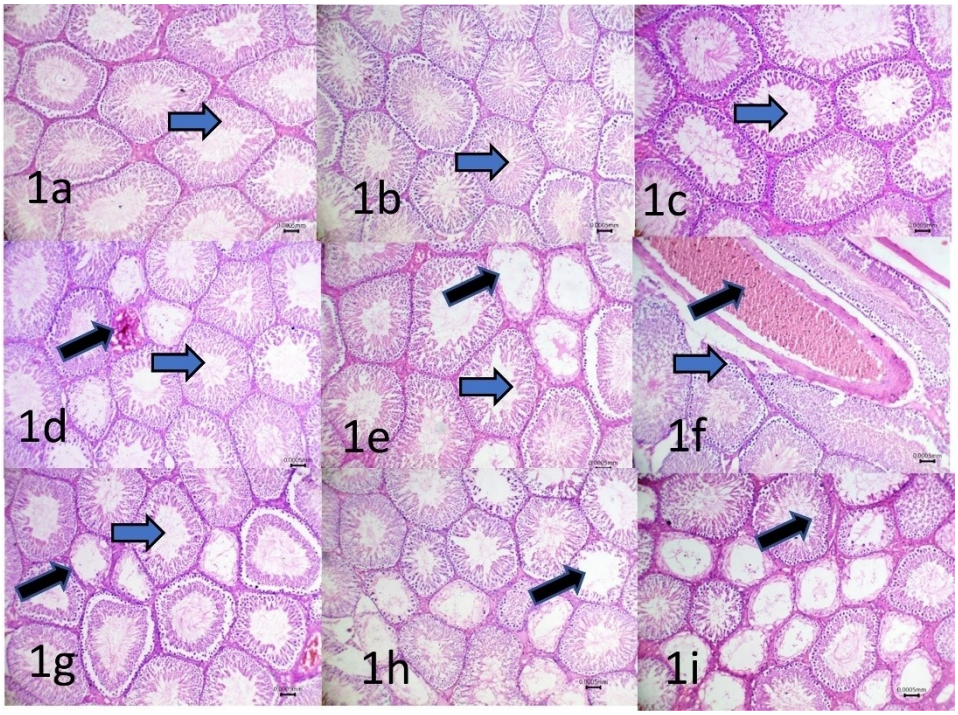

Figure 1. Histopathological examination of the testes of the various rat groups. (a) G1 (with normal drinking water). Testes showed active spermatogenesis and normal seminiferous tubules. (b) G2 (given normal saline). Testes showed active spermatogenesis, normal seminiferous tubules, and accumulation of spermatozoa in the lumen. (c) G3 (Sage 400 mg/kg/day). Testes showed normal seminiferous tubules. (d) G4 (Sage 400 mg/kg/day + lead). Testes showing testicular blood vessel congestion and marked degeneration of the lining epithelial cells of small numbers of seminiferous tubules, characterized by vacuolated germ cells and an absence of spermatozoa with reduced spermatogenesis. (e) G5 (Sage 200 mg/kg/day + lead). Testes of rats showed congested blood vessels and testicular degeneration of a few seminiferous tubules. (f) G6 (Sage 400 mg/kg/day + nicotine). Testes showed marked congestion of the testicular blood vessel. (g) G7 (Sage 200 mg/kg/day + nicotine). Testes showing blood vessel congestion and mild interstitial edema; with testicular degeneration of some seminiferous tubules. (h) G8 (lead acetate only): testes showed the majority of the seminiferous tubules having marked testicular degeneration. (i) G9 (nicotine hydrogen tartrate only); testes showing large numbers of seminiferous tubules with marked testicular degeneration. The black arrow shows the depletion of cells in seminiferous tubules and degenerative changes while the blue arrow shows active spermatogenesis in the seminiferous tubules.

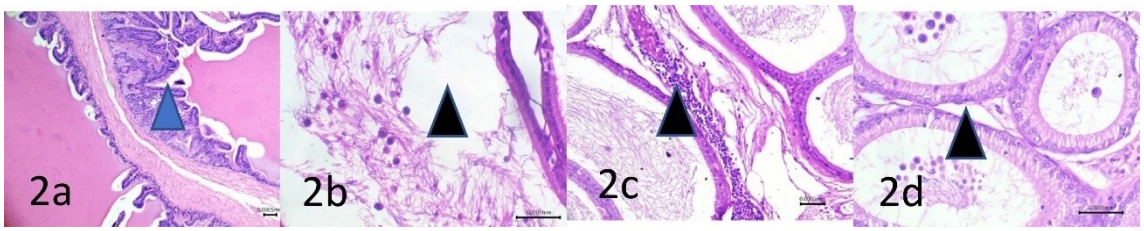

Figure 2. Histopathological examination of the epididymis of the various rat groups. (a) G4 (Sage 400 mg/kg/day + lead). The seminal vesicle of rats shows a normal appearance of the muscular wall glands. (b) and G5 (Sage 200 mg/kg/day + lead); the epididymis showed blood vessel congestion with interstitial edema and necrotic debris with spermatogenic cells, small inflammatory cells infiltration in the epididymal tubules' lumen, and degenerated spermatozoa. (c) G8 (lead acetate only); the epididymis showing blood vessel congestion with interstitial edema and lymphocyte aggregates. (d) G9 (nicotine hydrogen tartrate only); the epididymis showing blood vessel congestion and interstitial edema with lymphocyte aggregates. The black triangle shows degenerative changes including interstitial edema (necrotic debris) and congested blood vessels while the blue triangle shows normal epididymis tubules.

edema and congested blood vessels. The ectatic acini were lined by attenuated epithelium (not shown).

The testes of the experimental groups (G6&G7) given *S. officinalis* extract (400 mg/kg) & nicotine hydrogen tartrate and *S. officinalis* extract (200 mg/kg) & nicotine hydrogen tartrate, respectively, similarly, revealed marked testicular blood vessels congestion (Figure 1f&g) and interstitial edema. Multifocally, there was testicular degeneration of a few seminiferous tubules. The small degenerated tubules were lined by a few germ cells, with cytoplasmic vacuolization; accompanied by the spermatozoa's absence in the lumen and reduced spermatogenesis. A normal epididymis tubule was observed. The seminal vesicles revealed eosinophilic lumen secretion of the glands admixed with moderate numbers of degenerate neutrophils, lymphocytes, macrophages, and plasma cells. The ectatic prostatic acini were lined by attenuated epithelium (data not shown).

A large portion of seminiferous tubules in the two positive control groups (G8&G9) given only lead acetate and only nicotine hydrogen tartrate, respectively (Figure 1h&i) showed marked testicular degenerations. The germ cells of the degenerated tubules exhibited marked cytoplasmic vacuolization. The germinal epithelium had only one or two layers with the spermatozoa's absence in the lumen and reduced spermatogenesis. There was exfoliated epithelium in the lumen of the degenerated tubules admixed with several necrotic debris. There was focal homogenous eosinophilic material (edema) expansion of the testicular interstitium admixed with few lymphocytes. There were blood vessel congestions of the epididymis and interstitial edema mixed with lymphocyte aggregates (Figure 2c&d). There was also homogenous eosinophilic (edema) material expansion of the seminal vesicle interstitium admixed with an inflammatory infiltrate composed of lymphocytes, neutrophils, and macrophages. There was interstitial edema of the prostate gland admixed (data not shown) with similar inflammatory infiltrate and ectatic acini lined by attenuated epithelium.

Analysis of *S. officinalis* L. with GC/MS

The analysis of *S. officinalis* L. methanol extract with GC/MS showed that 12 compounds (Table 4) were present. The major compounds are 2,3,5-trimethylphenol (22.24), megastigmatrienone (12.11), (4bS-trans)-4b,5,6,7, 8,8a,9,10-octahydro-4b,8,8-trimethyl-1-(1-methylethyl)-2-phenanthrenol (10.11), (17β)-17-methoxyandrost-4-en-3-one 3-methoxime (6.78), (1S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (6.06), (all-E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene didehydro deriv. (5.47), isoaromadendrene epoxide (4.96), 1-ethyl-9a,11a-dimethylhexadecahydrocyclopenta[a]phenanthren-7-yl-acetic acid (4.54), 4,4,8-trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol (4.24), [1S-[1α(R*),4aβ,8aα]]-α-ethenyldecahydro-α,5,5,8a-tetramethyl-2-methylene-1-naphthalenepropanol (4.13), and the minor compounds are retinol (3.01), 4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol (2.35). The chromato-

Table 4. Major compounds obtained from the analysis of *S. officinalis* L. methanol extract using GC/MS.

RT (min)	Peak Area (%)	Name	Molecular formula	MW
3.881	6.06	(1S)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-one	$C_{10}H_{16}O$	152
7.573	22.24	2,3,5-trimethylphenol	$C_9H_{12}O$	136
9.879	12.11	Megastigmatrienone	$C_{13}H_{18}O$	190
10.249	4.96	Isoaromadendrene epoxide	$C_{15}H_{24}O$	220
11.080	2.35	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180
12.786	4.24	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	$C_{15}H_{26}O_2$	238
15.046	4.13	[1S-[1α(R*),4aβ,8aα]]-α-ethenyldecahydro-α,5,5,8a-tetramethyl-2-methylene-1-naphthalenepropanol	$C_{20}H_{34}O$	290
19.663	3.01	Retinol	$C_{20}H_{30}O$	286
19.846	4.54	1-ethyl-9a,11a-dimethylhexadecahydrocyclopenta[a]phenanthren-7-ylacetic acid	$C_{23}H_{38}O_2$	346
20.075	6.78	(17β)-17-methoxyandrost-4-en-3-one 3-methoxime	$C_{21}H_{33}NO_2$	331
20.408	10.11	(4bS-trans)-4b,5,6,7, 8,8a,9,10-octahydro-4b,8,8-trimethyl-1-(1-methylethyl)-2-phenanthrenol	$C_{20}H_{30}O$	286
20.979	5.47	(all-E)-2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene didehydro deriv.	$C_{30}H_{48}$	408

gram also showed the presence of other minor compounds but unidentified ones (Figure 3).

Discussion

The present study has demonstrated a profound and remarkable restoration potential of *S. officinalis* L. extracts of the adverse reproductive effects of Pb and nicotine on male reproductive competence. The results showed the effect of lead acetate or nicotine hydrogen tartrate on the sexual organs' weights such as the accessory sex glands, epididymis, and testis.[9] However, the co-administration of *S. officinalis* L. extracts with lead acetate or nicotine hydrogen tartrate resulted in significant ($p < 0.05$) alleviation of the adversative effects of both lead acetate and nicotine hydrogen tartrate. The improvements in the experimental groups were highly similar to the negative and normal groups. Our results align with the literature wherein the negative effects of nicotine on the infertility potential in albino rats were reported.[9,37] Moreover, Graça and colleagues[38] reported common macroscopic changes in the experimental animals such as a decrease in the epididymis, testis, seminal vesicles, and ventral prostate weights. Exposure of rats to intoxication from lead-acetate for about fifty-five days reportedly led to a reduction in the weight of the epididymis, prostate gland, seminal vesicle, testis, and vas deferens.[39]

The co-administration of *S. officinalis* L. extracts which led to an improvement in the sexual organs' weights might be attributed to the presence of bioactive components and antioxidant potentials.[9,27,28,34] Androgens strictly control the size, weight, and secretory function of the accessory sex organs, epididymis, testis, and vasa differentia.[40] The correlation between male fertility and the testis weight is expressed as an increase in sperm production, daily sperm output, and Sertoli cell numbers all of which are associated with large testis weight. On the other hand, diminished spermatogenesis (sperm production), decreased reproductive activity, and a reduction in the seminiferous tubules' length are related to poor fertility and small testis size.[41] The extracts dose-dependently and significantly alleviated the sperm abnormalities found in the experimental groups compared to the positive groups administered lead acetate or nicotine hydrogen tartrate alone. The decrease in the sperm characters (lower sperm number, lower motility, and higher sperm abnormalities) in the nicotine and lead-treated rats might be attributed to the effects of nicotine and lead on androgen level and degeneration of testicular structure. The administration of lead acetate or nicotine hydrogen tartrate alone, in our study, also showed a significant decrease in testosterone and LH levels while their co-administration with the plant extracts resulted in significant alleviation in these

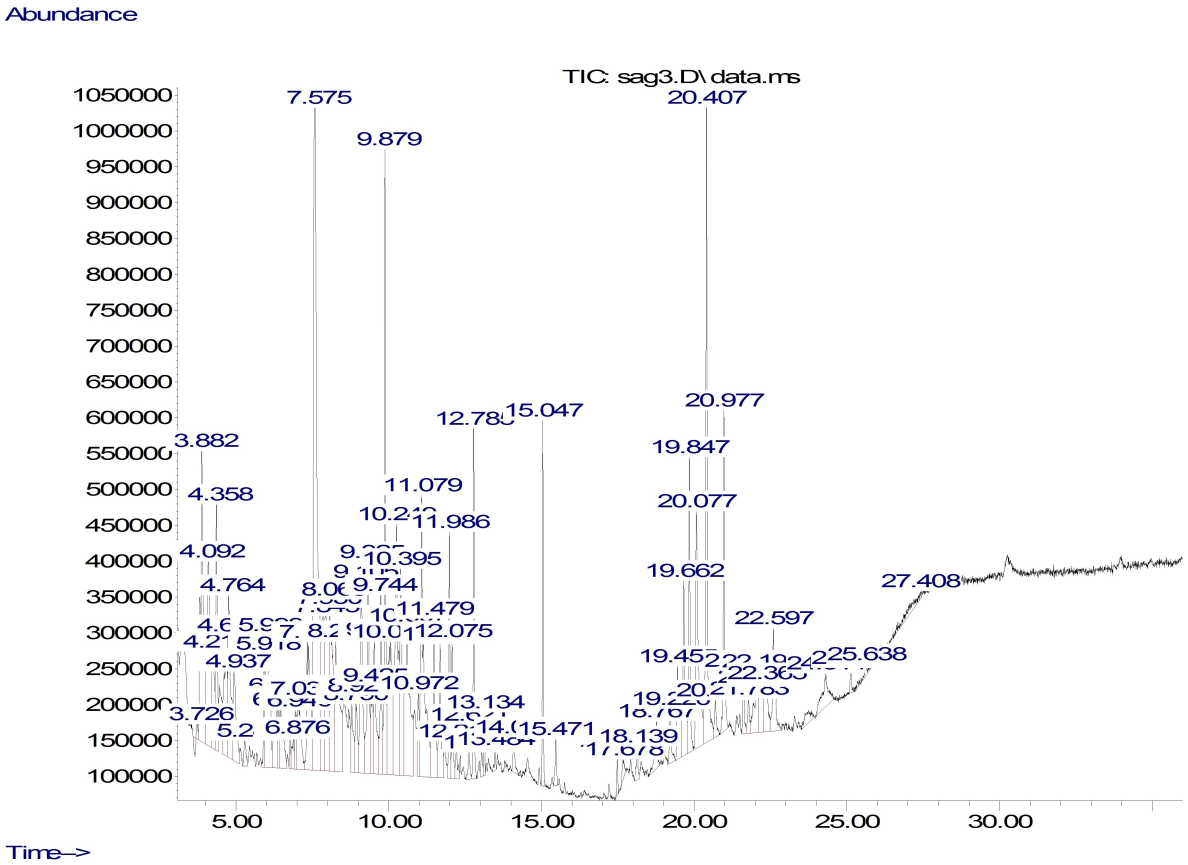

Figure 3. GC/MS Chromatogram from the analysis of methanol extract of *S. officinalis* L.

hormones. The negative and normal control groups showed the highest testosterone and LH hormone levels, followed by the G3 group. The lower testosterone and LH hormone levels in the positive control groups (G8&G9) could be credited to the apoptosis of Leydig cells thus inhibiting the biosynthesis of androgen in the Leydig cells of the rats. The results are in agreement with the work of Zhao et al.[42] who reported that nicotine might impair the hormone system of male reproductives through the induction of Leydig cell apoptosis and inhibition of androgen biosynthesis. Though the mean prolactin level and LH improved in the high-dose group in comparison to the control group there was, however, a decrease in the follicle-stimulating hormone (FSH) level. The histological analysis of the spermatogenesis and sperm characters of the sperm showed marked degeneration in most of the seminiferous tubules in the positive groups orally administered lead acetate or injected nicotine hydrogen tartrate only. There was also a noticeable vacuolization of cytoplasm in the germ cells with reduced sperm production, only a few germinal epithelium layers, and spermatozoa absence in the lumen. The findings in the degenerated tubules, testicular interstitium, epididymis, the interstitium of seminal vesicles, and the prostate gland are in alignment with the reports of Condorelli et al.[43] who stated that nicotine inhibited spermatogenesis and caused a decreased steroidogenesis in males. Nonetheless, the adverse effects could depend on individual sensitivity or susceptibility. Our results also agreed with the report of Jeng et al.[44] who stated that the arrest of spermatogenesis could occur because of a reduction in serum testosterone which is essential for the meiotic division completion, during spermatogenesis. Also, nicotine showed the potential to affect cell division negatively. The recovery group demonstrated a remarkable increase in the epididymal sperm count of the animals, thus, implying that the nicotine effect on sperm count can be ameliorated by the administration of beneficial plant extracts with a nicotine cessation effect. In addition, it has been reported that the most common morphological abnormalities observed in rats exposed to nicotine were rudimentary and curved tails and curved midpiece forms. Usually, these secondary abnormalities occur during the maturation and storage of sperm.

Vigeh et al.[8] reported the negative effects of lead on sperm function and the levels of serum testosterone. Furthermore, it produces an early onset of capacitation through the activation of ROS generation pathways. The ability of Pb to reduce the levels of serum testosterone could also contribute to the reduction in fertility and spermatogenesis following lead exposure. These results agreed with the reports of Ali et al.[45] who considered Pb as a toxic material for testicular tissue and functions, thereby significantly reducing the number of spermatozoa within the epididymis in lead acetate-exposed mice. Ezzatabadipour et al.[46] also reported that a 0.5 mg/kg dose of nicotine decreased sperm count and motility, thus, causing an increase in the percentage of sperm abnormality in rats. Lead has also been reported to significantly cause a decrease in sperm count, motility, epididymis weights, testes, serum testosterone level, spermatogenesis score, decrease in superoxide dismutase, and glutathione levels as well as elevate the levels of malondialdehyde and nitric oxide.[47] Lead-induced reproductive toxicity and altered spermatogenesis are responsible for the increase in pathological sperm alterations and testicular degeneration.[48]

The histological characteristics of rats treated with a high extract dose (sage 400 mg/kg body weight) showed testicular blood vessel congestion but mild interstitial edema and a normal appearance of most of the seminiferous tubules. Few tubules showed marked degeneration with germ cell vacuolization and reduction in spermatogenesis. A higher length of spermatogenic cell layer was also observed in the vehicle group, the control group and the experimental groups co-administered sage at 400 mg/kg while a lower level was observed in the groups treated with lead acetate or nicotine hydrogen tartrate. Nicotine is responsible for many degenerative changes in the seminiferous tubules. It also causes a decrease in the thickness of the spermatogenic cell masses, vacuolation of Sertoli cells, and thickening of the basal lamina. It is also thought to be involved in the reproductive hormone system dysfunction and oxidative stress in the gonads.[37] Our results are in agreement with the work of Vigeh et al.[8] who reported that the degeneration of Leydig cells leads to a reduction in testosterone levels that is responsible for increasing the length and diameter of the seminiferous tubules. The plant extract, however, greatly improved the seminiferous tubules' weight and length in the group treated with the plant extract and influenced spermatogenesis. The results of the GC/MS analysis showed the presence of many bioactive compounds. The novelty of this study includes the identification of the important volatile bioactive compounds that may be responsible for the extracts' ameliorative effects. The antioxidant and different terpenoid compounds contained in the *S. officinalis* L. methanolic extract could be responsible for the sperm quality enhancement effect. Methyl carnosic acid and α-linolenic acid, in particular, have been reported to play an important role.[34] One of the major volatile compounds from the *S. officinalis* L. methanolic extract is 2,3,5-trimethylphenol which is commonly used to synthesize 2,3,5-trimethylhydroquinone, an intermediate of vitamin E.[49,50] Furthermore, megastigmatrienone is a known key aroma compound with a sweet tobacco aroma as well as peppery and spicy notes.[51]

Conclusions

Lead and nicotine toxicity had a great effect on the rats' sperm quality as evident in the reduction in motility, sperm count, and an upsurge in the sperm abnormalities level including the reduction in the size and weight of the sexual organs, decrease in the diameter and length of the seminiferous tubules as well as other negative changes in the reproductive organs. However, the co-administration of *S. officinalis* L. methanol extract not only ameliorate the lead and nicotine adverse effects but also improved the sperm quality of the rats. The presence of several important active constituents which may be responsible for the improvement in semen quality and quantity was also revealed by the analysis of the plant extracts using GC/MS.

Experimental Section

Methodology

Nicotine hydrogen tartrate ($C_{14}H_{20}N_2O_6$: 2,3-Dihydroxybutanedioic acid; 3-(1-methylpyrrolidin-2-yl)nicotine hydrogen tartrate salt, sodium bicarbonate, B-Lead acetate ($Pb(CH_3COO)_2$), and pyridine were from Sigma-Aldrich (Germany) and Merck (Germany). The solvents were either chromatographic or analytical grade.

Animals

The approval for the research was obtained under "5726-CAVM-2019-2-2-I" project at the 1444-1443(1/2) ethical committee meeting. The study was conducted following the European Community guidelines (86/609/EEC). Mature albino male rats (n = 54) were used and supplied by King Saud University (Faculty of Pharmacy), Riyadh, Kingdom of Saudi Arabia. The animals weighed 220–250 g and were kept in cages. The animals were provided with water *ad libitum* and fed on normal animal pellets [with crude protein (20.0 %), ash (6.00 %), crude fiber (3.50 %), calcium (1.00 %), crude fat (4.00 %), phosphorus (0.60 %), salt (0.50 %), vitamin A (20.00 μg), vitamin E (70.00 IU), vitamin D (2.20 μg), energy (ME kcal kg 2850.00)] for 2-weeks before the commencement of the experiment. Only healthy animals were used in the study.

Plants collection

S. officinalis L. whole plant (seeds, flowers, and sticks) were obtained in dried and clean form from the local market (Al-Tamimi and Danube) in the Kingdom of Saudi Arabia and it was imported from the State of Palestine packed in sealed bags and bearing the name Meramia (the Arabic name for the plant). The plant was deposited in the herbarium, Qassim University [voucher specimen no. Sp. Pl:23 (1753)] following authentication by Prof Abdulrahman Alsugair from Agriculture & Veterinary Medicine College, Qassim University, Saudi Arabia.

Preparation of extract

Samples of *S. officinalis* L. were properly washed under running tap water. The plants were, then, air-dried while the various plant parts (flowers, leaves, roots, and sticks) were ground to a very fine powder using a grinding machine. Two hundred grams (200 g) of the powder was, then, extracted using about 2 L of 99.9 % methanol (for 72 h) with mild agitation. The choice of selecting methanol for the extraction was due to the literature.[52,53] Then, the crude extract underwent filtration while the solvent was removed under a rotary evaporator. Then, the crude extract was stored at −12 °C before use.[26]

Chemical preparations and extract doses

Nine (9) randomly divided and equal groups (n = 6) of rats were treated appropriately (Table 5). The oral administration of *S. officinalis* L. extracts and drugs was done daily for 60 days to cover the entire animal spermatogenic cycle period.[54] According to the literature, the acute dermal LD_{50} of sage leaf extract is > 2000 mg/kg. Two doses (200 & 400 mg/kg b. w. of rat) of *S. officinalis* L. were used following the methods of Ommati et al.[55] with slight modification. The extract was dissolved in a gum solution (1 % w/v) for easy oral administration by gavage. Fertility abnormality was induced by peritoneal injection (i. p.) of 0.50 mg/kg BW nicotine hydrogen tartrate prepared in 0.9 % normal saline solution.[56] Similarly, drinking water was used for the preparation of 1.5 g/L lead acetate.[57]

Samples processing

The rats were anesthetized following the expiration of the experimental period with light ether anesthesia inside the anesthetic box. Animal weights were recorded. Then, the rats were sacrificed following blood sample collection. Animals were dissected. The epididymis, testicle, seminal vesicle, and prostates (accessory sex organs) were extracted. Then, they were accurately weighed after a gross examination. Then, sperm samples were obtained from the epididymis. With a drop of normal saline, the sperm samples were then mixed on a warm (clean) slide. To evaluate sperm movement, the samples were examined under a microscope. To preserve the sperm's life, the process was carried out as quickly as possible in ideal conditions). Nigrosin and Eosin stains were used in the histological analysis for the evaluation of sperm abnormality.

Serum preparation

The blood samples collected were allowed to clot at room temperature. Samples were then centrifuged for about 15 min at 3000 r.p.m. to acquire clear serum. The serum was then stored until before other analyses in a deep freezer at −20 °C.

Hormonal analysis

The level serum of testosterone was evaluated using the ELISA testosterone kit (DRG, Germany) following the kit instructions from

Table 5. Description of animal grouping.

Normal control group	G1	Drinking water and rat pellet
Negative control group (vehicle)	G2	Normal saline
Experimental groups	G3	*S. officinalis* L. extract (400 mg/kg/day) by gavage
	G4	*S. officinalis* L. (400 mg/kg/day) + 1.5 % of lead acetate in drinking water
	G5	*S. officinalis* L. (200 mg/kg/day) + 1.5 % of lead acetate in drinking water
	G6	*S. officinalis* L. (400 mg/kg/day) + 0.50 mg/kg BW of nicotine hydrogen tartrate (i.p)
	G7	*S. officinalis* L. (200 mg/kg/day) + 0.50 mg/kg BW of nicotine hydrogen tartrate (i.p.)
Positive group	G8	1.5g/L of lead acetate in drinking water
	G9	0.50 mg/kg BW of nicotine hydrogen tartrate

the manufacturer. Genzyme (Cambridge, USA) supplied the ELISA kits used for serum luteinizing hormone (LH).

Epididymal sperm counting and progressive motility

The epididymal sperm counting and progressive motility were carried out as previously reported.[9]

Sperm cell abnormalities percentage and histopathological examination

The evaluation of the sperm cell abnormalities percentage and the histopathological examination was carried out as previously reported.[9]

Gas chromatography-mass spectrometry analysis

The Gas chromatography-mass spectrometry (GC/MS) analysis was done as previously reported.[9] The total running time was about 36 min. The relative percentages of the various components were evaluated by comparing the average peak areas to the total areas using Turbo Mass Ver 5.2. software.[58]

Statistical analysis

All the analyses and calculations were carried out using SAS (2004). Data are presented as the average of each group (n = 6). The mean differences were compared among the various rat groups using a one-way analysis of variance (ANOVA). Duncan's new multiple-range tests were employed for the post hoc analysis.

Author Contributions

Wael Ammar A. Aldaddou, Ibrahim M. El-Ashmawy, Naser A. Al-Wabel, and Abdullah S. M. Aljohani were involved in the manuscript conceptualization. Wael Ammar A. Aldaddou, Abdullah S. M. Aljohani, and Idris A. Ahmed wrote the first draft of the manuscript and all authors were jointly involved in the first review and subsequent completion of the review. All authors were responsible for the critical review of the manuscript and its further enhancement. All authors were then involved in the final review and editing.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: lead · nicotine · *Salvia officinalis* · sperm quality · toxicity

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