UNIVERSITI PUTRA MALAYSIA

ALLEVIATION OF LEAD ACETATE-INDUCED TESTICULAR DYSFUNCTION AND RELATED TOXICITY CHANGES IN RATS USING Nigella sativa L. SEEDS

MOHAMMED ABDULRAZZAQ ASSI

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By

MOHAMMED ABDULRAZZAQ ASSI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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DEDICATION

To Allah, Prophet Muhammad

(May the blessings and the peace of Allah be upon him)

To ... my father (Abdul Razzaq) and soft-hearted mother (Amel)

Who dedicate their lives to illuminate my steps in this world

To ... my heart (Mustafa), my spirit (Eham), my brother (Ali) and sister (Khadhra'a)

Who have been beside me in their hearts

With Respect ... 

Mohammad
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Doctor of Philosophy

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January 2017

Chairman : Mohd Hezmee Mohd Noor, PhD
Faculty : Veterinary Medicine

Lead acetate (LA) toxicity can occur either through ingestion or inhalation from contaminated surfaces or from the environment. N. Sativa is a natural product with immense pharmacological properties, which include antioxidant, antibacterial, anti-anemia and reproductive enhancement properties. Several studies have reported that LA can alter hematological, biochemical, reproductive and oxidative stress in short-term. In this study, the prophylactic and therapeutic effects of N. Sativa on chronic and sub-chronic lead acetate induced hematological-biochemical changes, histopathology and influence on male reproductive hormonal levels were evaluated. In the first experiment (therapeutic study), a total of 75 male Sprague-Dawley rats were divided into three groups with 25 rats in each group and in this single group, it was further sub-divided into five groups of 5 rats each. Group 1 acted as the negative control and were given distilled water, group 2 acted as the positive control (PC) and were given 10 mg/kg of lead acetate (LA) orally/daily, group 3 (T1), 4 (T2) and 5 (T3) were each given LA 10 mg/kg and graded concentrations (100 mg/kg, 150 mg/kg and 200 mg/kg) of N. Sativa orally, respectively. Twenty-five rats were euthanized at day 30, 60 and 90, respectively, for the collection of whole blood, serum, and organs. In the second experiment (prophylactic study), a total of 20 male Sprague-Dawley rats were divided into four groups of five mice each. Group 1 (NC) was the negative control, group 2 was the positive control (PC) and was administered 10 mg/kg/per day of lead acetate (LA) per OS, group 3 (T1) was administered 200 mg/kg/daily of N. Sativa per OS for one month and Group 4 (T2) was pre-treated with 200 mg/kg/daily of N. Sativa per OS for one month, followed by administration of 10 mg/kg/daily of lead acetate (LA) alone per OS for another month. At the end of the experiment, whole blood, serum and tissue were collected to evaluate the complete blood profile, serum biochemistry, hormonal concentration, spermiogram and histopathological changes. For the determination of the spermiogram, the right epididymal segment was collected and homogenized in phosphate-buffered saline, and the homogenates were used for the aforementioned purpose.
The haemogram from the therapeutic study showed a lower ($p<0.05$) red blood cell count, packed cell volume (PCV) and mean corpuscular hemoglobin concentration (MCHC) in the PC and T1 groups, while T2 and T3 have normal haemograms. Biochemical analysis revealed an elevated ($p<0.05$) aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine levels in the PC and T1 groups on day 90 for AST and day 30 for ALT and creatinine respectively. The level of alkaline phosphatase (ALP) was higher ($p<0.05$) in the PC at 30 and 60 days of sampling. Other parameters such as WBCs, prothrombin, urea and cholesterol were not significantly altered in all groups. Histopathological lesions in the liver and kidneys were more severe in the PC and T1 groups, while the T2 and T3 groups showed mild lesions. There was a decrease ($p<0.05$) in the total PAS stained area signifying glycogen depletion in the PC, T1 and T2 groups at 60 days, and a higher distribution of PAS stained areas ($p<0.05$) in the T3 group. At 90 days, the PC group had a lower ($p<0.05$) distribution of PAS stained areas in comparison to the other groups. There was reduced spermatogenesis and epididymal sperm reserves in the PC group in comparison to the treatment groups. The level of testosterone concentration was lower ($p<0.05$) in the PC group at 90 days, while FSH was lower ($p<0.05$) in T3 at 30 days and LH was higher ($p<0.05$) in T1 at 90 days. Estradiol concentration was higher ($p<0.05$) and comparable between the control and T3 at all sampling points. There was a decreased level of superoxide dismutase (SOD) and total glutathione (GSH) in the PC group, and an increased GSH level in the T3 group at all sampling points. The spermiogram showed an increase in sperm concentration, viability, and motility in the treated group as compared to the PC. Furthermore, the PC had a higher ($p<0.05$) incidence of sperm abnormalities.

The haemogram from the prophylactic study showed lower ($p<0.05$) level of hemoglobin, PCV, and prothrombin in the PC, while WBC, band neutrophil, segmented neutrophil, lymphocyte and monocyte counts were higher ($p<0.05$) in the PC than the treatment groups. However, eosinophil count was higher in T2, while no changes were observed in RBC and MCV values. Both AST and ALT were higher on the PC when compared to other groups. Similarly, the levels of ALP, cholesterol, urea, and creatinine were all higher ($p<0.05$) in the PC group and comparable ($p>0.05$) in the control, T1, and T2 groups. The sperm concentration, general and individual motilities were higher ($p<0.05$) in the NC and T1 animals, while the T2 had intermediate and the PC had lower ($p<0.05$) values of each parameter. Percentage of sperm viability was higher ($p<0.05$) in the T1 and lower ($p<0.05$) in the PC group. However, percentage abnormality was lower in T1, comparable in NC and T2, and higher ($p<0.05$) in PC. Spermatogenic cell population and epididymal sperm reserve (ESR) were optimal in control and pre-treated animals, while the PC had lower spermatids and ESR. The concentration of estradiol was lower ($p<0.05$) in the PC and T2, while LH concentration was lower ($p<0.05$) in the PC, and comparable ($p>0.05$) between control and T2. The concentration of FSH was comparable ($p>0.05$) in all groups, while TSH concentration was lower ($p<0.05$) in the PC and higher in the control and T1 groups. The level of SOD and GSH were lower ($p<0.05$) in the PC and T2 groups. In summary, this study has shown the prophylactic and therapeutic potentials of N. Sativa seed extract in both sub-chronic and chronic lead acetate induced toxicity in the male reproductive system in rats.

**Keywords**: N. sativa, lead acetate, male reproductive system, histopathological changes, toxicity.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

**PENGURANGAN KESAN DISFUNGSI TESTIKULAR DAN PERUBAHAN KETOKSIKAN YANG BERKAITAN AKIBAT KESAN TERARUH PLUMBUM ASETAT MENGGUNAKAN BIJI BENIH Nigella sativa L. DI DALAM TIKUS**

Oleh

**MOHAMMED ABDULRAZZAQ ASSI**

Januari 2017

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Keracunan plumbum asetat (PA) boleh terjadi samada melalui pemakanan atau sedutan dari permukaan atau persikitaran yang tercemar dengan plumbum asetat. *N. sativa* adalah satu produk asli yang mempunyai kesan farmakologikal yang tinggi seperti mempunyai kesan anti-pengoksidaan, anti-bakteria, anti-anemia dan perangsang reproduksi. Beberapa kajian telah melaporkan bahawa PA boleh mengubah dalam jangka masa pendek tekanan hematologikal, biokimia, pembiakan dan pengoksidaan. Di dalam kajian ini, kesan pencegahan dan terapeutik *N. sativa* terhadap kesan negatif PA ke atas perubahan hematologi dan biokimia, histopatologikal dan tahap hormon jantan telah dikaji. Di dalam eksperimen yang pertama (kajian terapeutik), sejumlah 75 ekor tikus jantan baka Sprague Dawley dibahagikan kepada 3 kumpulan besar mengandungi 25 ekor setiap kumpulan dan ianya terus dibahagikan lagi kepada 5 kumpulan kecil yang mengandungi 5 ekor tikus jantan. Kumpulan 1 bertindak sebagai kumpulan kawalan negatif dan diberikan air suling, Kumpulan 2 bertindak sebagai kumpulan kawalan positif dan diberikan secara oral 10 mg/kg PA setiap hari, Kumpulan 3 (T1), 4 (T2) dan 5 (T3) diberikan setiap PA sebanyak 10 mg/kg dan larutan bergraduasi (100 mg/kg, 150 mg/kg dan 200 mg/kg) *N. sativa* secara oral. 25 ekor tikus dikorbankan pada hari ke 30, 60 dan 90 untuk tujuan pengambilan darah, sera dan organ. Di dalam eksperimen kedua (kajian pencegahan), sebanyak 20 ekor tikus jantan baka Sprague Dawley dibahagikan kepada 4 kumpulan dengan 5 ekor tikus untuk setiap kumpulan yang dimaksudkan. Kumpulan 1 adalah kumpulan kawalan negatif (KN), kumpulan 2 adalah kumpulan kawalan positif (KP) dan telah diberikan sebanyak 10 mg/kg/hari PA secara oral, kumpulan 3 (T1) diberikan sebanyak 200 mg/kg/hari *N. sativa* untuk tempoh sebulan dan kumpulan 4 (T2) telah diberikan secara pra-rawatan sebanyak 200 mg/kg/hari *N. sativa* secara oral selama sebulan dan diikuti dengan pemberian sebanyak 10 mg/kg/hari PA sahaja secara oral selama sebulan. Pada akhir eksperimen, sampel darah, sera dan tisu telah dikumpulkan untuk menilai profil lengkap darah, biokimia sera, kandungan hormone, spermiogram dan perubahan
Histopatologikal. Untuk penentuan spermiogram, segmen epididimis kanan telah
diasingkan dan dihomogenasikan di dalam larutan garam penimbal fosfat dan larutan
homogenasi tersebut telah digunakan untuk tujuan yang dimaksudkan di atas.

Hemogram dari kajian terapeutik menunjukkan kiraan sel darah merah, volum sel
terpadat (PCV) dan purata kepekatan korpuskular hemoglobin (MCHC) yang rendah
($p<0.05$) untuk kumpulan KP dan T1 manakala T2 dan T3 mempunyai hemogram
normal. Analisa biokimia menunjukkan peningkatan aspartate aminotransferase
(AST), alanine amino transferase (ALT) dan creatinine ($p<0.05$) untuk kumpulan
KP dan T1 pada hari ke 90 untuk AST dan hari ke-30 untuk ALT dan creatinine.
Paras alkaline phosphatase (ALP) adalah tinggi ($p<0.05$) untuk kumpulan KP pada
hari ke-30 dan ke 60 pengambilan sampel. Parameter-parameter yang lain seperti sel
darah putih, prothrombin, urea dan kolesterol adalah tidak signifikan dalam kesemua
kumpulan. Lesi histopatologikal pada buah pinggang dan hati adalah lebih teruk di
dalam kumpulan KP dan T1 manakala kumpulan T2 dan T3 hanya menunjukkan lesi
yang sederhana. Terdapat pengurangan kawasan yang ditanda dengan PAS yang
menunjukkan kemerosotan glikogen pada kumpulan kawalan positif T1 dan T2 untuk
hari ke 60 pasca ketoksikan dan taburan yang tinggi untuk kawasan ditandai PAS
($p<0.05$) pada kumpulan T3. Pada hari ke 90 pasca ketoksikan, kumpulan kawalan
positif (PC) mempunyai taburan kwasan yang ditandai PAS yang lebih rendah
($p<0.05$) jika dibandingkan dengan kumpulan-kumpulan lain. distribution of PAS
stained areas in comparison to the other groups. Terdapat pengurangan proses
spermatogenesis dan simpanan sperma di dalam epididimis pada kumpulan KP jika
dibandingkan dengan keseluruhan kumpulan rawatan. Paras kandungan testosteron
adalah rendah ($p<0.05$) untuk kumpulan KP pada hari ke 90, manakala paras
hormon perangsang folikel (FSH) adalah rendah ($p<0.05$) di Kumpulan 3 pada hari
ke 30 dan paras hormon LH adalah lebih tinggi di dalam Kumpulan T1 pada hari ke
90. Paras kandungan estradiol adalah tinggi ($p<0.05$) dan adalah sebanding di antara
kumpulan kawalan dan T3 pada kesemua titik pengambilan sampel. Terdapat
pengurangan paras superoxide dismutase (SOD) dan jumlah keseluruhan glutathione
(GSH) di dalam kumpulan KP dan peningkatan paras GSH di dalam kumpulan T3 di
kesemua titik pengambilan sampel. Spermiogram pula menunjukkan peningkatan
kandungan sperma, kedapatan dan pergerakan di dalam kumpulan terawat jika
dibandingkan dengan kumpulan KP dan sebagai tambahan, kumpulan KP
mempunyai insiden kecacatan sperma yang lebih tinggi ($p<0.05$).

Hemogram dari kajian pencegahan menujukkan paras hemoglobin, volum sel
terpadat (PCV) dan phrothrombin yang rendah ($p<0.05$) manakala jumlah sel darah
putih, neutrofil bersegmen, limfosit dan monosit adalah lebih tinggi ($p<0.05$) di
dalam Kumpulan KP jika dibandingkan dengan kumpulan terawat. Walau
bagoaimanapun, kiraan eosinofil adalah tinggi di dalam T2, manakala tiada perubahan
direkodkan untuk nilai sel darah merah dan volum purata korpuskular (MCV).
Kedua-dua paras AST and ALT adalah lebih tinggi di dalam KP apabila
dibandingkan dengan kumpulan-kumpulan lain. Pada masa yang sama, paras ALP,
kolesterol, urea dan creatinine adalah tinggi ($p<0.05$) di dalam kumpulan KP dan
setara jika dibandingkan dengan kumpulan kawalan, T1 dan T2. Kepekatan sperma,
pergerakan sperma individu dan umum adalah lebih tinggi ($p<0.05$) di dalam
kumpulan KN dan T1 manakala untuk T2 adalah sederhana dan kumpulan KP
mempunyai nilai yang rendah untuk setiap parameter ($p<0.05$). Peratusan sperma
hidup adalah tinggi dalam T1 ($p<0.05$) dan rendah ($p<0.05$) dalam kumpulan KP.
Walau bagaimanapun peratusan kecacatan sperma adalah rendah di dalam T1 jika dibandingkan dengan kumpulan KN dan T2 dan lebih tinggi ($p<0.05$) di dalam KP. Populasi sel spermatogenia dan simpanan sperma epididimis (ESR) adalah optimum di dalam kumpulan kawalan dan haiwan pra-rawatan manakala kumpulan KP mempunyai populasi sperma dan ESR yang rendah. Kepekatan estradiol adalah rendah ($p<0.05$) di dalam kumpulan KP dan T2 manakala kepekatan LH adalah rendah ($p<0.05$) di dalam kumpulan KP dan jika dibandingkan di antara kawalan dan T2. Kepekatan FSH adalah setara ($p<0.05$) pada kesemua kumpulan manakala kepekatan TSH adalah rendah ($p<0.05$) di dalam kumpulan KP dan tinggi di dalam kumpulan kawalan dan T1. Paras SOD dan GSH adalah rendah ($p<0.05$) di dalam kumpulan KP dan T2. Kesimpulannya, kajian ini telah menunjukkan potensi pencegahan dan terapeutik ekstrak biji benih N. sativa di dalam keracunan plumbum asetat terhadap organ pembiakan tikus jantan pada kedua-dua keadaan kronik dan separa-kronik.

**Kata Kunci:** N. sativa, plumbum asetat, sistem pembiakan jantan, perubahan histopatologikal, keracunan.
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Mohammad
I certify that a Thesis Examination Committee has met on 17 January 2017 to conduct the final examination of Mohammed Abdulrazzaq Assi Al-Juhaishi on his thesis entitled "Alleviation of Lead Acetate-Induced Testicular Dysfunction and Related Toxicity Changes in Rats Using Nigella sativa L. Seeds" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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<th>Description</th>
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<tbody>
<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>&lt;</td>
<td>Less than</td>
</tr>
<tr>
<td>±</td>
<td>Plus-minus</td>
</tr>
<tr>
<td>°C</td>
<td>degrees centigrade</td>
</tr>
<tr>
<td>ALA</td>
<td>δ-aminolevulinic acid</td>
</tr>
<tr>
<td>ALAD</td>
<td>δ-aminolevulinic acid dehydratase</td>
</tr>
<tr>
<td>ALAS</td>
<td>δ-aminolevulinic acid synthetase</td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline phosphatase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine transaminase</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate transaminase</td>
</tr>
<tr>
<td>ATPase</td>
<td>adenylpyrophosphatase</td>
</tr>
<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
</tr>
<tr>
<td>BW</td>
<td>body weight</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>calcium</td>
</tr>
<tr>
<td>Ca-EDTA</td>
<td>Calcium- Ethylene diamine tetra acetic acid</td>
</tr>
<tr>
<td>CAT</td>
<td>catalase</td>
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<tr>
<td>CCl₄</td>
<td>Carbon tetrachloride</td>
</tr>
<tr>
<td>Cd</td>
<td>cadmium</td>
</tr>
<tr>
<td>cm</td>
<td>Centimeter</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>COX-2</td>
<td>cycloxygenase-2</td>
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<td>dl</td>
<td>deciliter</td>
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<tr>
<td>DMSA</td>
<td>Dimercaptosuccinic acid</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>ESR</td>
<td>Electron spin resonance</td>
</tr>
<tr>
<td>Fe$^{2+}$</td>
<td>iron</td>
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<tr>
<td>FSH</td>
<td>Follicle stimulating hormone</td>
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<tr>
<td>g</td>
<td>gram</td>
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<td>G6PD</td>
<td>glucose-6-phosphate dehydrogenase</td>
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<td>GABA</td>
<td>Gamma-Aminobutyric acid</td>
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<tr>
<td>GFAP</td>
<td>glial fibrillary acidic protein</td>
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<td>GIT</td>
<td>Gastrointestinal Tract</td>
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<tr>
<td>GnRH</td>
<td>Gonadotropin-releasing hormone</td>
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<tr>
<td>GPx</td>
<td>glutathione peroxidase</td>
</tr>
<tr>
<td>GR</td>
<td>glutathione reductase</td>
</tr>
<tr>
<td>GRP</td>
<td>glucose regulated protein</td>
</tr>
<tr>
<td>GSH</td>
<td>glutathione</td>
</tr>
<tr>
<td>GSSG</td>
<td>oxidized glutathione dismutase</td>
</tr>
<tr>
<td>GST</td>
<td>glutathione S-transferase</td>
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<tr>
<td>H2O2</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>Hb</td>
<td>hemoglobin</td>
</tr>
<tr>
<td>HDL</td>
<td>High-density lipoprotein</td>
</tr>
<tr>
<td>HSC</td>
<td>Hematopoietic Stem Cell</td>
</tr>
<tr>
<td>IgA</td>
<td>Immunoglobulin A</td>
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<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
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<td>IgM</td>
<td>Immunoglobulin M</td>
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<tr>
<td>IL-1B</td>
<td>Interleukin 1B</td>
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<tr>
<td>IL-3</td>
<td>Interleukin 3</td>
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<td>Description</td>
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iNOS        | Inducible nitric oxide synthase |
<p>|IP          | intraperitoneal |
|IV          | intravenous |
|K           | Potassium |
|kg          | kilogram |
|LDH         | Lactate dehydrogenase |
|LDL         | Low-density lipoprotein |
|LH          | Luteinizing hormone |
|MCHC        | mean corpuscular hemoglobin concentration |
|MCV         | Mean corpuscular volume |
|MDA         | Malondialdehyde |
|mg          | milligram |
|mg          | microgram |
|min         | minutes |
|SSA         | 5-sulfosalicylic acid |
|nm          | nanometer |
|nmole       | nanomole |
|dil         | dilution |
|vol         | volume |
|Mg²⁺        | magnesium ion |
|ml          | milliliter |
|MT-1        | metallothionein-1 gene |
|MT-2        | metallothionein-2 gene |
|Na          | Sodium |
|NADPH       | Nicotinamide Adenine Dinucleotide Phosphate |</p>
<table>
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<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>Ns</td>
<td>Nigella sativa</td>
</tr>
<tr>
<td>Pb</td>
<td>Lead</td>
</tr>
<tr>
<td>PBUH</td>
<td>&quot;peace be upon him&quot;</td>
</tr>
<tr>
<td>PCV</td>
<td>packed cell volume</td>
</tr>
<tr>
<td>pH</td>
<td>pH</td>
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<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ES</td>
<td>standard error</td>
</tr>
<tr>
<td>PPM</td>
<td>parts-per Million</td>
</tr>
<tr>
<td>RBC</td>
<td>red blood cells</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
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<tr>
<td>SC</td>
<td>subcutaneous</td>
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<td>SH</td>
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<td>SOD</td>
<td>superoxide dismutase</td>
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<td>T3</td>
<td>Triiodothyronine</td>
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<td>T4</td>
<td>Free thyroxine</td>
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<td>thiobarbituric acid reactive substances</td>
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<td>TQ</td>
<td>Thymoquinone</td>
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<td>Thyroid-stimulating hormone</td>
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<td>TS</td>
<td>Testosterone</td>
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<td>UK</td>
<td>United Kingdom</td>
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<td>United State of America</td>
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<td>WBCs</td>
<td>White Blood Cells</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<td>ESS</td>
<td>Epididymal semen suspension</td>
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<td>Full Form</td>
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</tr>
<tr>
<td>BTB</td>
<td>Blood-testis barrier</td>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
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<td>ABP</td>
<td>Androgen binding protein</td>
</tr>
<tr>
<td>HPG</td>
<td>Hypothalamic-pituitary-gonadal</td>
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<td>ICSH</td>
<td>Interstitial cells Stimulating Hormone</td>
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<td>RNA</td>
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<td>Dihydrotestosterone</td>
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<td>5-Lipoxygenase</td>
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<tr>
<td>IACUC</td>
<td>Institutional Animal Care and Use Committee</td>
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<tr>
<td>CO2</td>
<td>Carbon dioxide</td>
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<tr>
<td>TD</td>
<td>Tubular degeneration</td>
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<tr>
<td>HV</td>
<td>Hepatocytes vacuolation</td>
</tr>
<tr>
<td>KCP</td>
<td>Kupffer cell proliferation</td>
</tr>
<tr>
<td>PH</td>
<td>pyknotic hepatocytes</td>
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<tr>
<td>MOHE</td>
<td>Ministry of Higher Education</td>
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<tr>
<td>TBWR</td>
<td>Testicular Body Weight Ratio</td>
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<tr>
<td>ZPP</td>
<td>zinc protoporphyrin</td>
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<tr>
<td>β</td>
<td>Beta</td>
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<tr>
<td>α</td>
<td>Alpha</td>
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<tr>
<td>δ</td>
<td>Gamma</td>
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CHAPTER 1

INTRODUCTION

Prior to the invention of orthodox medicine, people would seek the use of traditional medicine as therapy for multiple diseases. Hence, the use of plants with medicinal properties has been warranted. Such medicinal plants are still being used for the aforementioned purposes (Kamal et al., 2010) until today. However, ignored is the fact that most persons using these plants have no prior knowledge of their toxic effects. At the same time, synthetic drugs for therapeutic uses possess well-documented adverse side effects that are proven scientifically and therefore, increase the interest towards medicinal plants and their active ingredients for medical purposes (Rifqi, 2012; Gray, 2013).

The oriental medicinal spice plant, *N. Sativa* can be found in most parts of the world and has been used as a natural remedy for a variety of acute as well as chronic conditions (Gilani et al., 2004). For centuries, seeds of *N. Sativa* plant have been used against diseases and various illnesses in the Middle East and Southeast Asia. It is referred to as Kalonji in the Southeast Asia and Al-habbah Al-Sawda or black cumin by the Arabs and English respectively (Zahoor et al., 2004; Gray, 2013).

Asian countries, and especially the Arabian region frequently use *N. Sativa* seeds in folk medicine to promote good health and treat many ailments which include fever, common cold, headache, asthma, rheumatic diseases, and some infections and also used as de-wormer for gastrointestinal parasites. Black cumin seeds are used against scorpion and spider stings and snake, cat, and dog bites as well. In addition, it is used as a flavoring additive to bread and pickles (El-Kadi and Kandil, 1986; Al-Jishi, 2000; Zahoor et al., 2004; Singh, 2011).

Thymoquinone is the pharmacologically active ingredient in *N. Sativa* seeds. It has several properties that include pain and inflammatory relieve actions (Abdel-Fattah et al., 2000). Daba and Abdel-Rahman (1998) and Mansour et al., (2002) reported that oxidative injury was prevented by thymoquinone in *in-vitro* and *in vivo* studies that were conducted in rats while Mansour et al., (2002) concludes that thymoquinone may have antioxidant properties and prevents tissues from lipid peroxidation.

The *N. Sativa* seeds have huge medicinal benefits, more especially in Greco-Arab/Unani-Tibb and Ayurveda systems of medicine (Salem, 2005; Paarakh, 2010). Various studies reported its numerous pharmacological properties such as antifungal, anti-helminths (Rogozhin et al., 2011), anti-malarial (Abdulelah and Zainal-Abidin, 2007), anti-allergic (Dahri et al., 2005), anti-bacterial (Halamova et al., 2010), anti-viral (Zaher et al., 2008). Mahmood et al. (2003) and Haq et al. (1999)
have also shown that *N. Sativa* seeds have anti-inflammatory activities, as well as an ability to enhance the immune response that is mediated by T cells.

Lead is considered as one of the most hazardous and cumulative environmental pollutants that affect all biological systems through exposure from air, water and food sources. The main sources of contamination of feed by lead are from industrial pollution, soil, feed processing and agricultural technology. Consumption of lead could result in poor performance, poisoning and death in animals (Patra *et al.*, 2011; Burki, 2012). Exposure to lead produced clinical, pathological changes through to kidney and endocrine system toxicities (Jadhav *et al.*, 2007). Lead is a naturally heavy metal occurring in the Earth’s crust. It is found to be toxic on many systems of the body, especially in the developing nervous system, hematological and cardiovascular systems as well as kidney (Agency for Toxic Substances and Disease Registry, 2007). Many studies have shown that reproductive toxicity is an important feature of lead toxicity (Adhikari *et al.*, 2001; Batra *et al.*, 2001). Exposure to lead has been found detrimental, and its associated toxicity leads to abnormal behavior, deficits in hearing, neuromuscular weakness and impaired cognitive functions in humans and experimental animals (Ahamed and Siddiqui, 2007a, b; Flora *et al.*, 2012). The toxicity of lead that is relevant to public health-protective level in drinking water, and propose any necessary changes in the previous risk assessment were based on the new findings (Office of Environmental Health Hazard Assessment, 1997a; OEHHA, 2007). Considering the impact of lead toxicity, many countries have removed it from gasoline. This action brought down the level of public exposure in those countries. However, high industrious use of lead due to its properties (such as low melting point, malleability and resistance to corrosion) makes it higher in certain areas (Wang *et al.*, 2012). Marchlewicz *et al.*, (2007) reported an elevation of lipid peroxide concentration in the reproductive organs of rats that are chronically exposed to lead in areas where lead is persistently used.

This study aimed to investigate the protective role of *N. Sativa* seeds in prevention and/or reduction of the side effects of lead acetate on the reproductive system of matured male rats. The current study has been carried out in order to investigate the preventive role of *N. Sativa* seeds suspension to prevent or suppress the lead acetate toxicity on some aspects of reproductive performance in adult male rats, by studying the following parameters: body weight, testicular weight to body weight ratio, hematology (CBP), biochemistry, hormonal assay (Testosterone, Estradiol, FSH and LH), antioxidant enzymes concentration (superoxide dismutase and glutathione), spermiogram (sperm concentration, viability, individual motility, general motility, and abnormality), histopathological analysis of male reproductive organs (testes, epididymis, vas deferens, prostate gland, and seminal vesicle), and histopathological analysis of liver and kidney.

**Problem Statement**

Lead acetate poisoning commonly occurs in animals and humans. The common and accepted treatment of its toxicity mostly involves chemically defined drugs that usually produce side effects. However, *N. Sativa* has been proven to provide
beneficial protective effects on multiple physiological disorder in both humans and animals.

**Hypothesis**

The administration of lead acetate concurrently with graded doses of *N. Sativa* extract for a period of 90 days will ameliorate the adverse effects of LA by restoring hemato-biochemical, reproductive hormones and histopathological changes and functions. Furthermore, this study also hypothesized that pre-treatment of rats with *N. Sativa* prior to LA administration will reduce the adverse effects of LA in the blood and tissues.

**Objective of this study**

**General objective**

To determine the protective effects of *N. Sativa* on the changes in male reproductive system, kidneys and liver of rats induced with lead acetate.

**Specific objectives**

- To evaluate the protective effect on the hemato-biochemical and histopathological changes associated with concurrent *Nigella sativa* administration in chronic lead acetate toxicity.
- To evaluate the effect of chronic lead acetate and *Nigella sativa* administration on gonadal pathology and reproductive hormonal levels.
- To evaluate the effect of subchronic lead acetate administration and *Nigella sativa* pre-treatment on the hemato-biochemical and histopathology of rats.
- To evaluate the effect of subchronic lead acetate administration and *Nigella sativa* pre-treatment on the spermiogram and gonadal pathology of rats.
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