UNIVERSITI PUTRA MALAYSIA

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

MOHAMMED ABDULRAZZAQ ASSI AL-JUHAISHI

FPV 2017 5

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

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

January 2017

COPYRIGHT

DEDICATION

To Allah, Prophet Muhammad

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

To …my father (Abdul Razzaq) and softhearted 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

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

By

MOHAMMED ABDULRAZZAQ ASSI

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

Pengerusi : Mohd Hezmee Mohd Noor, PhD
Fakulti : Perubatan Veterinar

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 satu 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 menujukkan lesi yang sederhana. Terdapat pengurangan kawasan yang ditanda dengan PAS yang menujukkan 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 bagaimanapun, 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.

ACKNOWLEDGEMENTS

In the name of the God, the first who deserves all thanks and pleasure for granting me health, strength, and help by which this research has been accomplished.

This project would have been impossible for me to undertake without inputs from others, to which much credit is owed. Special appreciation and thanks go to Assoc. Prof. Dr. Mohd Hezmee Mohd Noor (the chairman of the supervisory committee), and the rest of the committee members; Prof. Dr. Abd Wahid Haron, Assoc. Prof. Dr. Md Sabri Mohd Yusoff and finally to Prof. Dr. Mohamed Ali Rajion who was the Chairman before his retirement subsequent handing over to the current supervisor. Their valuable supervision provides me with necessary observation and advice throughout this work. I am deeply indebted and grateful to the Dean and Deputy Dean of the Faculty of Veterinary Medicine, Universiti Putra Malaysia. I would also like to express my gratitude to the Head of the Department of Preclinical Sciences, Assoc. Prof. Dr. Goh Yong Meng and Dr. Hafandi Ahmad, and to all staff of this department for their great support and access to research facilities.

Deep appreciation and sincere thanks are paid to Prof. Dr. Mohamed Ariff Omar, Prof. Dr. Mesha'an Abbas Al-Zuhairi, Prof. Dr. Hussni Omar Mohammed and Dr. Siti Zubaidah Ramanoon for supporting me and helping me in the statistical analysis.

I am also grateful with great thanks to Dr. Mahdi Ibrahimmi. I am grateful with great thanks to Dr. Yusuf Abba for his help in the histopathology aspect of this research. I am also grateful with great thanks to Prof. Dr. Abdulhadi Sallal Mohammed and Prof. Dr. Jawad Kadim Arrak. I wish to express my sincere gratitude to the respective Deans of the Faculty of Veterinary Medicine and Faculty of Medicine, Universiti Putra Malaysia for the use of their facilities and ultimate assistance from their staff during the course of this study. I would like to thank the staff members of the Veterinary Physiology, Theriogenology and Cytogenetics, Histopathology, Hematology and Virology Laboratories, in particular, Mr. Kufli Che Nor, Mrs. Zainab Nasri, Mr. Johari Ripin, Mr. Yap Keng Chee, and Mr. Ganesamurthi Perumal for their technical assistance. I am grateful with great thanks to Dr. Khumran Armiya'u Mada, Dr. Noor Farhana Bachek for helping me in my work for this research.

Mohammad

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Mohd Hezmee Mohd Noor, PhD
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Abd Wahid Haron, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Md Sabri Md Yusof, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

ROBIAH BINTI YUNUS, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by graduate student

I hereby confirm that:

- this thesis is my original work;
- quotations, illustrations and citations have been duly referenced;
- this thesis has not been submitted previously or concurrently for any other degree at any institutions;
- intellectual property from the thesis and copyright of thesis are fully-owned by Universiti Putra Malaysia, as according to the Universiti Putra Malaysia (Research) Rules 2012;
- written permission must be obtained from supervisor and the office of Deputy Vice-Chancellor (Research and innovation) before thesis is published (in the form of written, printed or in electronic form) including books, journals, modules, proceedings, popular writings, seminar papers, manuscripts, posters, reports, lecture notes, learning modules or any other materials as stated in the Universiti Putra Malaysia (Research) Rules 2012;
- there is no plagiarism or data falsification/fabrication in the thesis, and scholarly integrity is upheld as according to the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) and the Universiti Putra Malaysia (Research) Rules 2012. The thesis has undergone plagiarism detection software

Signature: _____ Date: _____

Name and Matric No.: Mohammed Abdulrazzaq Assi / GS40281

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) were adhered to.

Signature: _____
Name of Chairman of Supervisory Committee: Dr. Mohd Hezmee Mohd Noor

Signature: _____
Name of Member of Supervisory Committee: Professor Dr. Abd Wahid Haron

Signature: _____
Name of Member of Supervisory Committee: Associate Professor Dr. Md Sabri Md Yusof

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percent
<	Less than
±	Plus-minus
^{0}C	degrees centigrade
ALA	δ-aminolevulinic acid
ALAD	δ-aminolevulinic acid dehydratase
ALAS	δ-aminolevulinic acid synthetase
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
ATPase	adenylpyrophosphatase
BBB	blood-brain barrier
BW	body weight
Ca^{2+}	calcium
Ca-EDTA	Calcium- Ethylene diamine tetra acetic acid
CAT	catalase
CCl4	Carbon tetrachloride
Cd	cadmium
cm	Centimeter
CNS	Central Nervous System
COX-2	cycloocygenase-2
dl	deciliter
DMSA	Dimercaptosuccinic acid

DNA	Deoxyribonucleic acid
ESR	Electron spin resonance
Fe^{2+}	iron
FSH	Follicle stimulating hormone
g	gram
G6PD	glucose-6-phosphate dehydrogenase
GABA	Gamma-Aminobutyric acid
GFAP	glial fibrillary acidic protein
GIT	Gastrointestinal Tract
GnRH	Gonadotropin-releasing hormone
GPx	glutathione peroxidase
GR	glutathione reductase
GRP	glucose regulated protein
GSH	glutathione
GSSG	oxidized glutathione dismutase
GST	glutathione S-transferase
H2O2	Hydrogen peroxide
Hb	hemoglobin
HDL	High-density lipoprotein
HSC	Hematopoietic Stem Cell
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1B	Interleukin 1B
IL-3	Interleukin 3

iNOS	Inducible nitric oxide synthase
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-sulfosalicyclic acid
nm	nanometer
nmole	nanomole
dil	dilution
vol	volume
Mg^{2+}	magnesium ion
ml	milltlitre
MT-1	metallothionein-1 gene
MT-2	metallothionein-2 gene
Na	Sodium
NADPH	Nicotinamide Adenine Dinucleotide Phosphate

NO	Nitric oxide
Ns	Nigella sativa
Pb	Lead
PBUH	"peace be upon him"
PCV	packed cell volume
pH	pH
ANOVA	analysis of variance
ES	standard error
PPM	parts-per Million
RBC	red blood cells
ROS	Reactive oxygen species
SC	subcutaneous
SH	sulfhydryl
SOD	superoxide dismutase
T3	Triiodothyronine
T4	Free thyroxine
TBARS	thiobarbituric acid reactive substances
TQ	Thymoquinone
TSH	Thyroid-stimulating hormone
TS	Testosterone
UK	United Kingdom
USA	United State of America
WBCs	White Blood Cells
WHO	World Health Organization
ESS	Epididymal semen suspension

BTB	Blood-testis barrier
cAMP	Cyclic adenosine monophosphate
ABP	Androgen binding protein
HPG	Hypothalamic-pituitary-gonadal
ICSH	Interstitial cells Stimulating Hormone
RNA	Ribonucleic acid
DHT	Dihydrotestosterone
GH	Growth hormone
T1	Treatment 1
PC	Positive control
5-LO	5-Lipooxygenase
IACUC	Institutional Animal Care and Use Committee
CO2	Carbon dioxide
TD	Tubular degeneration
HV	Hepatocytes vacuolation
KCP	Kupffer cell proliferation
PH	pyknotic hepatocytes
MOHE	Ministry of Higher Education
TBWR	Testicular Body Weight Ratio
ZPP	zinc protoporphyrin
β	Beta
α	Alpha
δ	Gamma

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 anti-fungal, 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 (Oiffce 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.

REFERENCES

Abba, Y., Hassim, H., Hamzah, H., & Noordin, M.M. (2015). Antioxidant Vitamins, Oxidant Injuries and Diseases. *Pertanika Journal of Scholarly Research Reviews,1*, 58-66.

Abbasnezhad, A., Hayatdavoudi, P., Niazmand, S., & Mahmoudabady, M. (2015). The effects of hydroalcoholic extract of N. sativa seed on oxidative stress in hippocampus of STZ-induced diabetic rats. *Avicenna Journal of Phytomedicine*, *5*(4), 333.

Abdel-Fattah, A. F. M., Matsumoto, K., & Watanabe, H. (2000). Antinociceptive effects of N. sativa oil and its major component, thymoquinone, in mice. *European Journal of Pharmacology*, *400*(1), 89-97.

Abdel-Sater, K. A. (2009). Gastroprotective effects of N. sativa oil on the formation of stress gastritis in hypothyroidal rats. *International Journal of Physiology, Pathophysiology, and Pharmacology, 1*(2), 143-149.

Abdou, H. M., & Hassan, M. A. (2014). Protective role of omega-3 polyunsaturated fatty acid against lead acetate-induced toxicity in liver and kidney of female rats. *BioMed Research International,* 435857-435857.

Abdulelah, H. A. A., & Zainal-Abidin, B. A. H. (2007). In vivo anti-malarial tests of N. sativa (Black Seed) different extracts. *American Journal of Pharmacology and Toxicology*, *2*(2), 46-50.

Adhikari, N., Sinha, N., Narayan, R., & Saxena, D. K. (2001). Lead-induced cell death in testes of young rats. *Journal of Applied Toxicology*, *21*(4), 275-277.

Aftab, A., Asif, H., Mohd, M., Shah, A. K., Abul, K. N., Nasir A.S. & Firoz, A. (2013). A review on therapeutic potential of N. sativa: a miracle herb. *Asian Pacific Journal of Tropical Biomedicine, 3*(5), 337-352.

Aggarwal, B. B., Kunnumakkara, A. B., Harikumar, K. B., Tharakan, S. T., Sung, B., & Anand, P. (2008). Potential of spice-derived phytochemicals for cancer prevention. *Journal of Planta Medica, 74*(13), 1560-1569.

Ahamed, M., & Siddiqui, M. K. J. (2007). Environmental lead toxicity and nutritional factors. *Clinical Nutrition Journal, 26*(4), 400-408.

Ahamed, M., & Siddiqui, M. K. J. (2007). Low level lead exposure and oxidative stress: current opinions. *Clinica Chimica Acta Journal, 383*(1), 57-64.

Ahamed, M., Verma, S., Kumar, A., & Siddiqui, M. K. J. (2005). Environmental exposure to lead and its correlation with biochemical indices in children. *Science of the Total Environment, 346*(1), 48-55.

Ahmad, A., Husain, A., Mujeeb, M., Khan, S. A., Najmi, A. K., Siddique, N. A. & Anwar, F. (2013). A review on therapeutic potential of N. sativa: A miracle herb. *Asian Pacific Journal of Tropical Biomedicine*, *3*(5), 337-352.

Ahmed, W.M., Abdel-Hameed, A.R., & Elbound Moghazy, F.M. (2008). Some reproductive and health aspects of female buffaloes in relation to blood lead concentration. *International Journal of Dairy Science,3*(2), 63-70.

Ahmed, Z. A. (2010). Protective effect of N. sativa oil against CCl4 induced hepatotoxicity in rats. *Al-Mustansiriyah Journal for Pharmaceutical Sciences, 8*(2), 46-55.

Akhtar, M. S., Nasir, Z., & Abid, A. R. (2003). Effect of feeding powdered N. sativa L. seeds on poultry egg production and their suitability for human consumption. *Journal of Veterinarski Arhiv, 73*(3), 181-190.

Al Naimi, R. A., Abdulhadi, D., Zahroon, O. S., & Al-Taae, E. H. (2011). Toxicopathological Study of Lead Acetate Poisoning in Growing Rats and The Protective Effect of Crystien or Calicium. *Al-Anbar Journal of Veterinary Science, (4)*, 26-39.

Al-Asadi, A.H. (2000). *The effect of lectins isolated from N. sativa Linn. In glucose, cholesterol and serum protein levels.* M.Sc. Thesis, Department of Physiology and Pharmacology / College of Veterinary Medicine –University of Baghdad.

Al-Attar, A. M., & Al-Taisan, W. A. A. (2010). Preventive effects of black seed (N. sativa) extract on Sprague-Dawley rats exposed to diazinon. *Australian Journal of Basic and Applied Sciences, 4*(5), 957-968.

Al-Awadi, F. M., & Gumaa, K. A. (1987). Studies on the activity of individual plants of an antidiabetic plant mixture. *Acta Diabetologica Latina, 24*(1), 37-41.

Al-Awadi, F. M., Fatania, H., & Shamte, U. (1991). The effect of a plants mixture extract on liver gluconeogenesis in streptozotocin induced diabetic rats. *Diabetes Research (Edinburgh, Scotland), 18*(4), 163-168.

Al-Awadi, F. M., Khattar, M. A., & Gumaa, K. A. (1985). On the mechanism of the hypoglycaemic effect of a plant extract. *Diabetologica, 28*(7), 432-434.

Al-Duri, S.A. (1998). *Purification and characterization of medically important lectines from N. Sativa L. seeds.* Ph. D. Thesis, Education College – University of Baghdad.

Al-Gaby, A. M. A. (1998). Amino acid composition and biological effects of supplementing broad bean and corn proteins with N. sativa (black cumin) cake protein. *Food/Nahrung Journal, 42*(5), 290-294.

Al-Ghamdi, M. S. (2001). The anti-inflammatory, analgesic and antipyretic activity of N. sativa. *Journal of Ethnopharmacology, 76*(1), 45-48.

Al-Hader, A., Aqel, M., & Hasan, Z. (1993). Hypoglycemic effects of the volatile oil of N. sativa seeds. *Journal of Pharmaceutical Biology, 31*(2), 96-100.

Ali, B. H., & Blunden, G. (2003). Pharmacological and toxicological properties of N. sativa. *Phytotherapy Research, 17*(4), 299-305.

Al-Jishi, S. A. A. (2000). *A study the effect of N. sativa on blood hemoststic functions.* M.Sc. thesis. King Fasial University, Dammam, Saudi Arabia.

Al-Jishi, S. A., & Abuo Hozaifa, B. (2000). A study the effect of N. sativa on blood hemostatic functions in rats. *Journal of Ethnopharmacology, 85*(1), 7-14.

Al-Khalaf, M. I., & Ramadan, K. S. (2013). Antimicrobial and Anti-cancer Activity of N. sativa oil-A Review. *Australian Journal of Basic Applied Science,* 7, 505-514.

Allouche, L., Hamadouche, M., & Touabti, A. (2009). Chronic effects of low lead levels on sperm quality, gonadotropins and testosterone in albino rats. *Experimental and Toxicologic Pathology, 61*(5), 503-510.

Allouche, L., Hamadouche, M., Touabti, A., & Khennouf, S. (2011). Effect of long-term exposure to low or moderate lead concentrations on growth, lipid profile and liver function in albino rats. *Advance in Biological Research*, *5*(6), 339-347.

Al-Masri, S. A. (2015). Effect of pumpkin oil and vitamin E on lead induced testicular toxicity in male rats. *JAPS, Journal of Animal and Plant Sciences*, *25*(1), 72-77.

Al-Mayali, H.K. (2007). *The use of microbial Alefittez black seed in reducing the toxicity of cadmium chloride on the reproductive system in male rat's efficiency eggs.* PhD. Thesis, College of Education - University of Qadisiyah.

Al-Naggar, T. B., Gómez-Serranillos, M. P., Carretero, M. E., & Villar, A. M. (2003). Neuropharmacological activity of N. sativa L. extracts. *Journal of Ethnopharmacology, 88*(1), 63-68.

Al-Okaily, B. N., Mohammed, R. S., Al-Mzain, K. A., & Khudair, K. K. (2012). Effect of Flavonoids Extracted from Black Cumin (N. sativa) and Vitamin E in Ameliorating Hepatic Damage Induced by Sodium Nitrate in adult male rats. *The Iraqi Journal of Veterinary Medicine, 36*(2), 172-181.

Al-Roubaee, F.S. (2006). Effect of *N. sativa, Hibiscus sabdariffa* and *Quercus infectoria* Plant extracts in viability and growth of *Ecchinococcus granulosus* protoscolecws from sheep origin in vitro and in vivo. M. Sc. Thesis, science, biology, College of Education, Mosul University.

Al-Rouby, N. M., & Gawish, S. M. (2013). Histological study on the possible protective effect of nigella sativa oil on experimentally induced hepatotoxicity in albino rats treated with sodium valproate. Glo Adv Res J of Med Med Sci, 2(4), 90-99.

Al-Sa'ady, M.S.M., Kamil, H.Z., & Jasim, S.H. (2011). Effect of Lead Acetate in Some Physiological & Genetic Parameters in White Male Rat Rattus rattus. *Faculty of Education Karbala University,3*(9), 295-301.

Al-Sa'aidi, J. A. A., Al-Khuzai, A. L. D., & Al-Zobaydi, N. F. H. (2009). Effect of alcoholic extract of N. sativa on fertility in male rats. *Iraqi Journal of Veterinary Sciences, 23*: Supplement II: 123-128.

Alsaif, M. A. (2008). Effect of N. sativa oil on metabolic responses to prolonged systemic injury in rats. *Journal of Biological Sciences, 8*(6), 974-983.

Al-Zahrani, S., Mohany, M., Kandeal, S., & Badr, G. (2012). Thymoquinone and vitamin E supplementation improve the reproductive characteristics of heat stressed male mice. *Journal of Medicinal Plants Research*, *6*(3), 493-499.

Al-Zamily, H. A. A. (2008). *The impact of black bean seeds N. sativa L. In some physiological traits and sperm in male sheep Awassi.* Ph.D. Thesis. (College of Veterinary Medicine-Baghdad University, Iraq).

Al-Zubiady, N.F.H. (2007). *Alcoholic extract of the seeds of black bean effect (N. sativa L.) fertility in male rats.* M.Sc. Thesis, (College of Veterinary Medicine-Al- Qadisiyah University, Iraq).

Al-Zuhairy, R. G. M. (2012). The phototherapeutic effect of traditional crude oil of N. sativa on male reproductive system of albino mice treated with low toxic dose of paracetamol. *Iraqi Academic Scientific Journal, 9*(1), 229-237.

Amin, K. A., & Nagy, M. A. (2009). Effect of Carnitine and herbal mixture extract on obesity induced by high fat diet in rats. *Diabetology and Metabolic Syndrome, 1*(1), 1-14.

Anjum, M. R., & Reddy, P. S. (2012). Supplementation of testosterone restores the suppressed fertility in male rats exposed to lead during perinatal period. IOSR *Journal of Pharmacy,2*(6), 49-53.

Anjum, M. R., Sainath, S. B., Suneetha, Y., & Reddy, P. S. (2011). Lead acetate induced reproductive and paternal mediated developmental toxicity in rats. *Ecotoxicology and Environmental Safety, 74*(4), 793-799.

Ansari, A. A., Hassan, S., Kenne, L., & Wehler, T. (1988). Structural studies on a saponin isolated from N. sativa. *Phytochemistry Journal, 27*(12), 3977-3979.

Aqel, M., & Shaheen, R. (1996). Effects of the volatile oil of N. sativa seeds on the uterine smooth muscle of rat and guinea pig. *Journal of Ethnopharmacology, 52*(1): 23-26.

Arrak, J. K., Al-Khafaji, N. J., & Hassan, A. A. (2012). Effect of Date Palm Pollen Suspension on Ovarian Function and Fertility in Adult Female Rats Exposed to Lead Acetate. *Diyala Journal of Medicine, 3*(1), 90-96.

ATSDR, (Agency for Toxic Substances and Disease Registry) (2007). *Toxicological profile for lead. (Draft for Public Comment). Agency for Toxic Substances and Disease Registry,* Public Health Service, United State Department of Health and Human Services, Atlanta, GA. Pp: 43-59.

Atta, M. B. (2003). Some characteristics of Nigella (N. sativa L.) seed cultivated in Egypt and its lipid profile. *Food Chemistry Journal, 83*(1), 63-68.

Atta-Ur-Rahman, A., Malik, S., Hasan, S.S., Chudhary, M.N., & Clardy, J. (1995). Nigellidine-A New Indazole Alkaloid from the Seeds of N. sativa. *Journal of Cheminformatics, 26*(30).

Awadalla, E.A. (2012). Ameliorative effect of the crude oil of the N. sativa on oxidative stress induced in rat testes by cisplatin treatment. *Biomedicine & Preventive Nutrition*, *2*(4), 265-268.

Ayed, A. L., & Talal, Z. (2011). Long-term effects of N. sativa L. oil on some physiological parameters in normal and streptozotocin-induced diabetic rats. *Journal of Diabetes Mellitus,1*, 46-53.

Aziz, F. M., Maulood, I. M., & Chawsheen, M. A. H. (2012). Effects of melatonin, vitamin Cand E alone or in combination on lead-induced injury in liver and kidney organs of rats. IOSR *Journal of Pharmaceutical,2*, 13-18.

Badary, O. A., Abdel-Naim, A. B., Abdel-Wahab, M. H., & Hamada, F. M. (2000). The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats. *Toxicology, 143*(3), 219-226.

Badary, O. A., Nagi, M. N., Al-Shabanah, O. A., Al-Sawaf, H. A., Al-Sohaibani, M. O., & Al-Bekairi, A. M. (1997). Thymoquinone ameliorates the nephrotoxicity induced by cisplatin in rodents and potentiates its antitumor activity. *Canadian Journal of Physiology and Pharmacology, 75*(12), 1356-1361.

Bagchi, D., & Preuss, H. G. (2005). Effects of acute and chronic oval exposure of lead on blood pressure and bone mineral density in rats. *Journal of Inorganic Biochemistry*, *99*(5), 1155-1164.

Bailey, C. J., & Day, C. (1989). Traditional plant medicines as treatments for diabetes. *Diabetes care Journal, 12*(8), 553-564.

Bailey, C., & Kitchen, I. (1985). Ontogenesis of proenkephalin products in rat striatum and the inhibitory effects of low-level lead exposure. *Developmental Brain Research Journal*, *22*(1), 75-79.

Bamosa, A. O., Ali, B. A., & Al-Hawsawi, Z. A. (2002). The effect of thymoquinone on blood lipids in rats. *Indian Journal of Physiology and Pharmacology, 46*(2), 195-201.

Bamosa, A. O., Ali, B. A., & Sowayan, S. A. (1997). Effect of oral ingestion N. sativa seeds on some blood parameters. *Saudi Pharmaceutical Journal, 5*(2-3), 126-129.

Baranowska-Bosiacka, I., Gutowska, I., Rybicka, M., Nowacki, P., & Chlubek, D. (2012). Neurotoxicity of lead. Hypothetical molecular mechanisms of synaptic function disorders. *Neurologia i Neurochirurgia Polska Journal, 46*(6), 569-578.

Bashandy, A.S. (2007). Effect of fixed oil of N. sativa on male fertility in normal and hyperlipidemic rats. *International Journal of Pharmacology,3*(1), 27-33.

Batra, N., Nehru, B., & Bansal, M. P. (2001). Influence of lead and zinc on rat male reproduction at 'biochemical and histopathological levels'. *Journal of Applied Toxicology*, *21*(6), 507-512.

Benoff, S., Jacob, A., & Hurley, I. R. (2000). Male infertility and environmental exposure to lead and cadmium. *Human Reproduction Update*, *6*(2), 107-121.

Bharali, M. R. (2013). Effect of acute lead acetate exposure on liver of mice. *Journal of Global Biosciences, 2*, 121-125.

Birben, E. Sahiner, U. M., Sackesen, C., Erzurum, S., & Kalayci, O. (2012). Oxidative Stress and Antioxidant Defense. Review Article. *World Allergy Organization*, 5, 9–19.

Boskabady, M. H., Mohsenpoor, N., & Takaloo, L. (2010). Antiasthmatic effect of N. sativa in airways of asthmatic patients. *Phytomedicine, 10*(17), 707-713.

Boskabady, M. H., Shafei, M. N., & Parsaee, H. (2005). Effects of aqueous and macerated extracts from N. sativa on guinea pig isolated heart activity. *Die Pharmazie-An International Journal of Pharmaceutical Sciences, 60*(12), 943-948.

Brent, J.A. (2006). Review of: "Medical Toxicology". *Journal of Clinical Toxicology,44*, 355–355.

Bressler, J. P., & Goldstein, G. W. (1991). Mechanisms of lead neurotoxicity. *Biochemical Pharmacology Journal*, *41*(4), 479-484.

Bressler, J., Kim, K. A., Chakraborti, T., & Goldstein, G. (1999). Molecular mechanisms of lead neurotoxicity. *Neurochemical Research*, *24*(4), 595-600.

Burki, T. K. (2012). Nigeria's lead poisoning crisis could leave a long legacy. *Lancet*, *379*(9818), 792.

Canfield, R. L., Henderson Jr, C. R., Cory-Slechta, D. A., Cox, C., Jusko, T. A., & Lanphear, B. P. (2003). Intellectual impairment in children with blood lead concentrations below 10 μg per deciliter. *New England Journal of Medicine*,*348*(16), 1517-1526.

Carmignani, M., Volpe, A. R., Boscolo, P., Qiao, N., Di Gioacchino, M., Grilli, A., & Felaco, M. (2000). Catecholamine and nitric oxide systems as targets of chronic lead exposure in inducing selective functional impairment. *Life Sciences Journal*, *68*(4), 401-415.

Chakravarty, H. L. (1976). Plant wealth of Iraq (a dictionary of economic plants): vol. 1. *Baghdad: Ministry of Agriculture & Agrarian Reform xiv, 506p.-illus., col. illus.(Ara) Icones. Geog*, *2*.

Chakravarty, N. (1993). Inhibition of histamine release from mast cells by Nigellone. *Annals of Allergy, 70*(3), 237-242.

Chowdhury, A. R. (2009). Recent advances in heavy metals induced effect on male reproductive function-A retrospective. *Al Ameen Journal Medical Science*, *2*(2), 37-42.

Chowdhury, A. R., Dewan, A., & Gandhi, D. N. (1983). Toxic effect of lead on the testes of rat. *Biomedica Biochimica Acta Journal*, *43*(1), 95-100.

Cleveland, L. M., Minter, M. L., Cobb, K. A., Scott, A. A., & German, V. F. (2008). Lead Hazards for Pregnant Women and Children: Part 1: Immigrants and the poor shoulder most of the burden of lead exposure in this country. Part 1 of a two-part article details how exposure happens, whom it affects, and the harm it can do. *AJN the American Journal of Nursing, 108*(10), 40-49.

Conterato, G. M., Bulcão, R. P., Sobieski, R., Moro, A. M., Charão, M. F., Freitas, F. A. & Batista, B. L. (2013). Blood thioredoxin reductase activity, oxidative stress and hematological parameters in painters and battery workers: relationship with lead and cadmium levels in blood. *Journal of Applied Toxicology, 33*(2), 142-150.

Corradi, M., Goldoni, M., & Sabbadini, M. (2011). Intossicazione acuta da piombo: un caso singolare di anemia emolitica e colica saturnina/Acute lead poisoning: a singular case of hemolytic anemia and lead colic. *La Medicina Del Lavoro, 102*(3), 243-249.

Daba, M.H., & Abdel-Rahman, M.S. (1998). Hepatoprotective activity of thymoquinone in isolated rat hepatocytes. *Toxicology Letters, 95*(1): 23-29.

Dahri, A. H., Chandiol, A. M., Rahoo, A. A., & Memon, R. A. (2005). Effect of N. sativa (kalonji) on serum cholesterol of albino rats. *Journal of Ayub Medical College Abbottabad*, *17*(2), 72-74.

Dai, S., Yin, Z., Yuan, G., Lu, H., Jia, R., Xu, J. & He, C. (2013). Quantification of metallothionein on the liver and kidney of rats by subchronic lead and cadmium in combination. *Environmental Toxicology and Pharmacology, 36*(3), 1207-1216.

Dalia, M. (2010). Effect of using pectin on lead toxicity. *Journal of American Science,6*, 541-554.

Davidovics, Z., & DiCicco-Bloom, E. (2005). Moderate lead exposure elicits neurotrophic effects in cerebral cortical precursor cells in culture. *Journal of Neuroscience Research*, *80*(6), 817-825.

De-Tommasi, N., Autore, G., Bellino, A., Pinto, A., Pizza, C., Sorrentino, R., & Venturella, P. (2000). Antiproliferative Triterpene saponins from Traversie p almata. *Journal of Natural Products, 63*(3), 308-314.

Dorostghoal, M., Dezfoolian, A., & Sorooshnia, F. (2011). Effects of maternal lead acetate exposure during lactation on postnatal development of testis in offspring Wistar rats. *Iranian Journal of Basic Medical Sciences*, *14*(2), 122-131.

Drozed, A. G., Komissarenko, F. N., & Litvinenko, E. A. (1970). Coumarins of some species of Ranunaulaceae family. *Farm ZH Journal, 25*(4), 57-60.

Dwivedi, S. N. (2003). Ethnobotanical studies and conservational strategies of wild and natural resources of Rewa district of Madhys Pradesh. *Journal of Economic and Taxonomic Botany, 27*(1), 233-234.

El Mezayen, R., El Gazzar, M., Nicolls, M. R., Marecki, J. C., Dreskin, S. C., & Nomiyama, H. (2006). Effect of thymoquinone on cyclooxygenase expression and prostaglandin production in a mouse model of allergic airway inflammation. *Immunology Letters, 106*(1), 72-81.

El-Dakhakhny, M., Barakat, M., El-Halim, M. A., & Aly, S. M. (2000). Effects of N. sativa oil on gastric secretion and ethanol induced ulcer in rats. *Journal of Ethnopharmacology, 72*(1), 299-304.

El-Faham, S. Y. (1994). Comparative studies on chemical composition of N. sativa L. seeds and its cake (defatted meal). *Journal of Agricultural Science, Mansoura University. (Egypt).*

El-Gaafarawy, A. M., Zaki, A. A., El-Sedfy, R., & El-Khenawy, K. H. I. (2003). Effect of feeding N. sativa cake on digestibility, nutritive value and reproductive performance of Frisian Iranian cows and Immuno activity of their offspring. In Proceeding of The 9th Conference on Animal Nutrition, October, *Egyptian Journal of Nutrition Feeds,* 6, 539-549.

Elgawish, R. A. R., & Abdelrazek, H. M. (2014). Effects of lead acetate on testicular function and caspase-3 expression with respect to the protective effect of cinnamon in albino rats. *Toxicology Reports*, *1*, 795-801.

El-Kadi, A., & Kandil, O. (1986, November). Effect of N. sativa (the black seed) on immunity. *In Proceedings of the 4th International Conference on Islamic Medicine, Kuwait. Bulletin Islamic Medicine, 4*, 344-352.

Elkhateeb, A., El Khishin, I., Megahed, O., & Mazen, F. (2015). Effect of N. sativa Linn oil on tramadol-induced hepato-and nephrotoxicity in adult male albino rats. *Toxicology Reports,2*, 512-519.

Ellmer, M., & Andersson, S. (2004). Inbreeding depression in Nigella degenii (Ranunculaceae): fitness components compared with morphological and phenological characters. *International Journal of Plant Sciences*, *165*(6), 1055-1061.

El-Mehi, A. E., & Amin, S. A. (2012). Effect of Lead Acetate on the Thyroid Gland of Adult Male Albino Rats and the Possible Protective Role of Zinc Supplementation: A Biochemical, Histological and Morphometric Study. *Journal of American Science, 8*(7).

El-Naggar, A. M., & El-Deib, A. M. (1992). A study of some biological activities of N. sativa (black seeds)" Habat El Baraka. *Egyptian Society of Pharmacology and Experimental Therapeutics, 11*(2), 781-800.

El-Naggar, T., Gómez-Serranillos, M. P., Palomino, O. M., Arce, C., & Carretero, M. E. (2010). N. sativa L. seed extract modulates the neurotransmitter amino acids release in cultured neurons in vitro. *Journal of Biomedicine and Biotechnology*, 398312-398312.

El-Shabrawy, O. A., & Nada, S. A. (1996). Biological evaluation of multicomponent tea used as hypoglycemic in rats. *Fitoterapial, 67*(2), 99-102.

El-Tahir, K. E. D. H., & Bakeet, D. M. (2006). The black seed N. sativa Linnaeus-A mine for multi cures: a plea for urgent clinical evaluation of its volatile oil. *Journal of Taibah University Medical Sciences, 1*(1), 1-19.

El-Tahir, K. E., Al-Tahir, A. Y., & Ageel, A. M. (1999). Pharmacological Studies on Sesame and N. sativa Fixed Oils: Effect on the Sensitivities of the Adrenoreceptors, Baroreceptors, Platelets and the Uterus of the Rat. *Saudi Pharmaceutical Journal, 7*(4), 205-215.

El-Tantawy, W. H. (2015). Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. *Journal of Traditional Complementary Medicine,* 1-5 (Article in Press).

El-Tohamy, M. M., & El-Nattat, W. S. (2010). Effect of antioxidant on lead-induced oxidative damage and reproductive dysfunction in male rabbits. *Journal of American Science*, *6*(11), 613-622.

Ercal, N., Gurer-Orhan, H., & Aykin-Burns, N. (2001). Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Current Topics in Medicinal Chemistry*, *1*(6), 529-539.

Farrag, A. R., Mahdy, K. A., Abdel, R. G., & Osfor, M. M. (2007). Protective effect of N. sativa seeds against lead-induced hepatorenal damage in male rats. *Pakistan Journal of Biological Sciences: PJBS*, *10*(17), 2809-2816.

Feeser, V. R., & Loria, R. M. (2011). Modulation of traumatic brain injury using progesterone and the role of glial cells on its neuroprotective actions. *Journal of Neuroimmunology*, *237*(1), 4-12.

Finley, J. (2014). Compositions and methods for the prevention and treatment of diseases or conditions associated with oxidative stress, inflammation, and metabolic dysregulation. *U.S. Patent No. 8,652,518*. Washington, DC: U.S. Patent and Trademark Office.

Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: A review with recent updates. *Interdisciplinary Toxicology Journal,5*(2), 47–58.

Flora, S. J. (2011). Arsenic-induced oxidative stress and its reversibility. *Free Radical Biology and Medicine Journal, 51*(2), 257-281.

Flora, S. J. S. (2002). Nutritional components modify metal absorption, toxic response and chelation therapy. *Journal ofNutrition and Environmental. Medicine, 12*(1), 53-67.

Flora, S. J. S., Flora, G., & Saxena, G. (2006). Environmental occurrence, health effects and management of lead poisoning. Lead chemistry, analytical aspects, environmental impacts and health effects. Netherlands: *Elsevier Publication,* 158-228.

Flora, S. J., Saxena, G., & Mehta, A. (2007). Reversal of lead-induced neuronal apoptosis by chelation treatment in rats: role of reactive oxygen species and intracellular Ca2+. *Journal of Pharmacology and Experimental Therapeutics, 322*(1), 108-116.

Foster, W. G., McMahon, A., YoungLai, E. V., Hughes, E. G., & Rice, D. C. (1993). Reproductive endocrine effects of chronic lead exposure in the male cynomolgus monkey. *Journal of Reproductive Toxicology*, *7*(3), 203-209.

Gali-Muhtasib, H., El-Najjar, N., & Schneider-Stock, R. (2006). The medicinal potential of black seed (N. sativa) and its components. *Advances in Phytomedicine*, *2*, 133-153.

García-Lestón, J., Méndez, J., Pásaro, E., & Laffon, B. (2010). Genotoxic effects of lead: an updated review. *Environment International*, *36*(6), 623-636.

Garza, A., Vega, R., & Soto, E. (2006). Cellular mechanisms of lead neurotoxicity. *Medical Science Monitor, 12*(3), RA57-RA65.

Ghai, C. L. (1999). *A Textbook of Practical Physiology,* 5th ed. Jaypee Brothers, New Delhi, Pp: 47–69.

Ghoneim, M. T., El-Gindy, A. R., El-Alami, R., Shoukry, E., & Yaseen, S. (1982, November). Possible effect of some extracts of N. sativa L. seeds on blood coagulation system and fibrinolytic activity. *In Proceeding of 2nd International Conference on Islamic Medicine, Kuwait* (pp. 528-35).

Ghosheh, O.A.; Houdi, A.A., & Crooks, P.A. (1999). High performance liquid chromatography analysis of the pharmacologically active quinines and related compounds in the oil of black seed (N. sativa Linn.). *Journal of Pharmaceutical and Biomedical Analysis, 19*, 757-762.

Gilani, A. H., Aziz, N., Khurram, I. M., Chaudhary, K. S., & Iqbal, A. (2001). Bronchodilator, spasmolytic and calcium antagonist activities of N. sativa seeds (Kalonji): a traditional herbal product with multiple medicinal uses. JPMA. *The Journal of the Pakistan Medical Association, 51*(3), 115-120.

Gilani, A.H., Jabeen, Q., & Asad Ullah Khan, M. (2004). A review of medicinal uses and pharmacological activities of N. sativa. *Pakistan Journal of Biological Sciences, 7*(4), 441-451.

Grant, L. D. (2008). *Lead and compounds. Environmental Toxicants:* Human Exposures and Their Health Effects, Third Edition, 757-809.

Gray, J. D. (2013). Rasullulah is my doctor. PTS Islamika Sdn Bhd: Kuala Lumpur.

Gupta, M., Mazumder, U. K., Kumar, T. S., Gomathi, P., & Kumar, R. S. (2004). Antioxidant and Hepatoprotective effects of Bauhinia racemose against paracetamol and carbon tetrachloride induced liver damage in rats. *Iranian Journal of Pharmacology and Therapeutics, 3*(1), 12-20.

Hailat, N., Al-Kahil, S., Alkofahi, A., Lafi, S., Al-Ani, F., Al-Darraji, A., & Bataineh, Z. (1998). Effects of N. sativa extracts on antibody response of rats vaccinated with Brucella vaccine (Rev-1). *Pharmaceutical Biology Journal, 36*(3), 217-221.

Hajra, N. (2011). N. sativa: the miraculous herb. *Pakistan Journal of Biochemistry & Molecular Biology, 44*(1), 44-48.

Hala, M. A. (2011). Protective effect of N. sativa, linseed and celery oils against testicular toxicity induced by sodium valproate in male rats. *Journal of American Science, 7*(5), 687-693.

Halamova, K., Kokoska, L., Flesar, J., Sklenickova, O., Svobodova, B., & Marsik, P. (2010). In vitro antifungal effect of black cumin seed quinones against dairy spoilage yeasts at different acidity levels. *Journal of Food Protection®, 73*(12), 2291-2295.

Halliwell, B., & Gutteridge, J. C. (1984). Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. *The Lancet Journal, 323*(8391), 1396-1397.

Hamad, Z.M. (2012). Protective effect Ethanol extract of N. sativa. L on hepatic damage induced by naphthalene in male rats. *Journal of Al-Qadisiya for Pure Science, 17*(2), 1-10.

Hammed, M. S., Arrak, J. K., Al-Khafaji, N. J., & Hassan, A. A. (2012). Effect of Date palm pollen suspension on ovarian function and fertility in adult female rats exposed to lead acetate. *Diyala Journal Medicine,3*(1), 90-96.

Haq, A., Abdullatif, M., Lobo, P. I., Khabar, K. S., Sheth, K. V., & Al-Sedairy, S. T. (1995). N. sativa: effect on human lymphocytes and polymorphonuclear leukocyte phagocytic activity. *Immunopharmacology, 30*(2), 147-155.

Haq, A., Lobo, P.I., Al-Tufail, M., Rama, N.R., & Al-Sedairy, S.T. (1999). Immunomodulatory effect of N. sativa proteins fractionated by ion exchange chromatography. *International Journal of Immunopharmacology, 21*(4), 283–295.

Hassan, M. I., Mabrouk, G. M., Shehata, H. H., & Aboelhussein, M. M. (2012). Antineoplastic Effects of Bee Honey and N. sativa on Hepatocellular Carcinoma Cells. *Integrative Cancer Therapies, 11*(4), 354-363.

Hassanin, N. I., & Hassan, F. M. (1996). A preliminary study on the effect of N. sativa L. seeds on hypoglycemia. *Veterinary Medical Journal (Egypt),44*, 966-708.

Hegazy, A. M., & Fouad, U. A. (2014). Evaluation of Lead Hepatotoxicity; Histological, Histochemical and Ultrastructural Study. Forensic Medicine and Anatomy Research, 2(03), 70.

Hosseinzadeh, H., & Parvardeh, S. (2004). Anticonvulsant effects of thymoquinone, the major constituent of N. sativa seeds, in mice. *Phytomedicine, 11*(1), 56-64.

Hosseinzadeh, H., Parvardeh, S., Nassiri-Asl, M., & Mansouri, M. T. (2005). Intracerebroventricular administration of thymoquinone, the major constituent of N. sativa seeds, suppresses epileptic seizures in rats. *Medical Science Monitor Basic Research, 11*(4), 106-110.

Hrapkiewicz, K., & Medina, L. (Eds.). (2013). *Clinical laboratory animal medicine: an introduction*. John Wiley & Sons.

Hsu, P. C., & Guo, Y. L. (2002). Antioxidant nutrients and lead toxicity. *Toxicology*, *180*(1), 33-44.

Hsu, P. C., Hsu, C. C., & Guo, Y. L. (1999). Hydrogen peroxide induces premature acrosome reaction in rat sperm and reduces their penetration of the zona pellucida. *Toxicology*, *139*(1), 93-101.

Hultberg, B., Andersson, A., & Isaksson, A. (2001). Interaction of metals and thiols in cell damage and glutathione distribution: potentiation of mercury toxicity by dithiothreitol. *Toxicology*, *156*(2), 93-100.

Humphreys, D. J. (1991). Effects of exposure to excessive quantities of lead on animals. *British Veterinary Journal*, *147*(1), 18-30.

Hwang, K. Y., Schwartz, B. S., Lee, B. K., Strickland, P. T., Todd, A. C., & Bressler, J. P. (2001). Associations of lead exposure and dose measures with erythrocyte protein kinase C activity in 212 current Korean lead workers. *Toxicological Sciences Journal*, *62*(2), 280-288.

Ibrahim, N. M., Eweis, E. A., El-Beltagi, H. S., & Abdel-Mobdy, Y. E. (2012). Effect of lead acetate toxicity on experimental male albino rat. *Asian Pacific Journal of Tropical Biomedicine, 2*(1), 41-46.

Ilaiyaraja, N., & Khanum, F. (2010). *N. sativa* L.: A review of the therapeutic applications. *Journal of Herbal Medicine and Toxicology, 4*(2), 1-8.

Ishikawa, E. T., Gonzalez-Nieto, D., Ghiaur, G., Dunn, S. K., Ficker, A. M., Murali, B., & Cancelas, J. A. (2012). Connexin-43 prevents hematopoietic stem cell senescence through transfer of reactive oxygen species to bone marrow stromal cells. *Proceedings of the National Academy of Sciences,109*(23), 9071-9076.

Islam, M. H., Ahmad, I. Z., & Salman, M. T. (2013). Antibacterial activity of N. sativa seed in various germination phases on clinical bacterial strains isolated from human patients. *E3 Journal of Biotechnology and Pharmaceutical Research, 4*(1), 8-13.

Jadhav, S.H., Sarkar, S.N., Patil R.D., & Tripathi, H.C. (2007). Effects of subchronic exposure via drinking water to a mixture of eight water contaminating metals: a biochemical and histopathological study in male rats. *Archives of Environmental Contamination and Toxicology*, *53*(4), 667-677.

Jahromy, M. H., Jalili, M., Mohajer, A. J., Poor, F. K., & Dara, S. M. (2014). Effects of *N. sativa* Seed Extract on Perphenzine-Induced Muscle Rigidity in Male Mice. *World Journal of Neuroscience*, *2014*.

Janfaza, S., & Janfaza, E. (2012). The study of pharmacologic and medicinal valuation of thymoquinone of oil of N. sativa in the treatment of diseases. *Annals of Biological Research, 3*(4), 1953-1957.

Jangid, A. P., John, P. J., Yadav, D., Mishra, S., & Sharma, P. (2012). Impact of chronic lead exposure on selected biological markers. *Indian Journal of Clinical Biochemistry, 27*(1), 83-89.

Jassim, H. M., & Hassan, A. A. (2011). Changes in some blood parameters in lactating female rats and their pups exposed to lead: effects of vitamins C and E. *Iraqi Journal of Veterinary Science,25*, 1-7.

Jesse, F.F.A., Abba Y., Tijjani A., Sadiq M.A., Konto M., Adamu L., Wahid A.H., MohdAzmi M.L., Eric L.T.C., & Ab Rahman M.F. (2016). Gonado-hypophyseal lesions and reproductive hormonal changes in brucella melitensis-infected mice and its lipopolysaccharides (lpss). *Comparative Clinical Pathology,25*, 31-36.

Ji, J. S., Schwartz, J., Sparrow, D., Hu, H., & Weisskopf, M. G. (2014). Occupational Determinants of Cumulative Lead Exposure: Analysis of Bone Lead Among Men in the VA Normative Aging Study. *Journal of Occupational and Environmental Medicine /American College Occupational and Environmental Medicine, 56*(4), 435.

Jomova, K., & Valko, M. (2011). Advances in metal-induced oxidative stress and human disease. *Toxicology*, *283*(2), 65-87.

Juma, F. T., & Hayfaa, M. A. (2011). A: The effects of N. sativa oil administration on some physiological and histological values of reproductive aspects of rats. *The Iraqi Journal of Veterinary Medicine, 35,* 52-60.

Kamal, A., Arif, J. M., & Ahmad, I. Z. (2010). Potential of N. sativa L. seed during different phases of germination on inhibition of bacterial growth. *Journal of Biotechnology Pharmaceutical Research, 1*(1), 09-13.

Kaneko, J. J., Harvey, J. W., & Bruss, M. L. (Eds.). (2008). *Clinical Biochemistry of Domestic Animals.* Academic press.

Kasperczyk, A., Machnik, G., Dobrakowski, M., Sypniewski, D., Birkner, E., & Kasperczyk, S. (2012). Gene expression and activity of antioxidant enzymes in the blood cells of workers who were occupationally exposed to lead. *Toxicology*, *301*(1), 79-84.

Kasperczyk, S., Birkner, E., Kasperczyk, A., & Zalejska-Fiolka, J. (2004). Activity of superoxide dismutase and catalase in people protractedly exposed to lead compounds. *Annals of Agricultural and Environmental Medicine*, *11*(2), 291-296.

Katsuyama, M., Matsuno, K., & Yabe-Nishimura, C. (2012). Physiological roles of NOX/NADPH oxidase, the superoxide-generating enzyme. *Journal of Clinical Biochemistry and Nutrition, 50*(1), 9-22.

Keshri, G., Singh, M. M., Lakshmi, V., & Kamboj, V. P. (1995). Post-coital contraceptive efficacy of the seeds of N. sativa in rats. *Indian Journal of Physiology and Pharmacology, 39*, 59-62.

Khaki, A., Bayatmakoo, R., Nouri, M., & Khaki, A. A. (2013). Remedial Effect of Cinnamon zeylanicum on serum anti-oxidants levels in male diabetic Rat. *Life Science Journal, 10*(4s), 103-107.

Khan, M. A. U., Ashfaq, M. K., Zuberi, H. S., Mahmood, M. S., & Gilani, A. H. (2003). The in vivo antifungal activity of the aqueous extract from N. sativa seeds. *Phytotherapy Research Journal, 17*(2), 183-186.

Khanna, T., Zaidi, F. A., & Dandiya, P. C. (1993). CNS and analgesic studies on N. sativa. *Fitoterapia,* 5, 407-410.

Khuder, S.I. (2012). The effect of N. sativa oil and some antibiotics on bacteria isolated from wound infection in hospitals. *College of Basic Education Researches Journal, 12*(1), 707-715.

Kilikdar, D., Mukherjee, D., Dutta, M., Ghosh, A. K., Rudra, S., Chandra, A. M., & Bandyopadhyay, D. (2013). Protective effect of aqueous garlic extract against lead-induced cardiac injury in rats. *Journal of Cell Tissue Research, 13*(3), 3817.

Kilikdar, D., Mukherjee, D., Mitra, E., Ghosh, A. K., Basu, A., Mohan Chandra, A., & Bandyoapdhyay, D. (2011). Protective effect of aqueous garlic extract against lead-induced hepatic injury in rats. *Indian Journal of Experimental Biology, 49*(7), 498.

Kiziler, A. R., Aydemir, B., Onaran, I., Alici, B., Ozkara, H., Gulyasar, T., & Akyolcu, M. C. (2007). High levels of cadmium and lead in seminal fluid and blood of smoking men are associated with high oxidative stress and damage in infertile subjects. *Biological Trace Element Research*, *120*(1-3), 82-91.

Krishnan, N., & Muthukrishnan, S. (2012). Effect of N. sativa seed extract on carbon tetrachloride-induced hepatotoxicity in rats. *Journal of Acute Medicine*, *2*(4), 107-113.

Kumar, A., Prasad, M. N. V., Achary, V. M. M., & Panda, B. B. (2013). Elucidation of lead-induced oxidative stress in Talinum triangulare roots by analysis of antioxidant responses and DNA damage at cellular level. *Environmental Science and Pollution. Research, 20*(7), 4551-4561.

Kumara, S.S., & Huat, B.T. (2001) Extraction, isolation and characterization of anti-tumour principal, alpha-hedrin from the seeds of *N. sativa* L. *Planta Medica, 67*(1), 29-32.

Lakshmi, B. V. S., Sudhakar, M., & Aparna, M. (2013). Protective potential of Black grapes against lead induced oxidative stress in rats. *Environmental Toxicology and Pharmacology, 35*(3), 361-368.

Lee, K. J., Woo, E. R., Choi, C. Y., Shin, D. W., Lee, D. G., You, H. J., & Jeong, H. G. (2004). Protective effect of acteoside on carbon tetrachloride-induced hepatotoxicity. *Life Sciences Journal, 74*(8), 1051-1064.

Lidsky, T. I., & Schneider, J. S. (2003). Lead neurotoxicity in children: basic mechanisms and clinical correlates. *Brain*, *126*(1), 5-19.

Liu, C. M., Ma, J. Q., & Sun, Y. Z. (2012). Puerarin protects the rat liver against oxidative stress-mediated DNA damage and apoptosis induced by lead. *Experimental Toxicology and Pathology, 64*(6), 575-582.

Mahdavi, R., Heshmati, J., & Namazi, N. (2015). Effects of black seeds (N. sativa) on male infertility: A systematic review. *Journal of Herbal Medicine*, *5*(4), 133-139.

Mahmood, M. S., Gilani, A. H., Khwaja, A., Rashid, A., & Ashfaq, M. K. (2003). The in vitro effect of aqueous extract of N. sativa seeds on nitric oxide production. *Phytotherapy Research*, *17*(8), 921-924.

Mahmoud, M. R., El-Abhar, H. S., & Saleh, S. (2002). The effect of N. sativa oil against the liver damage induced by Schistosoma mansoni infection in mice. *Journal of Ethnopharmacology, 79*(1), 1-11.

Makhlouf, M., Eldien, H., Zagloul, D., Abu Dief, E., & AbdElhaliem, N. (2008). The effect of the lead acetateon testicular structure and protective effect of vitamin E in adult albino rat. *Egypt Journal Histology,31*, 406-418.

Mann, T., & Lutwak-Mann, C. (2012). *Male reproductive function and semen: themes and trends in physiology, biochemistry and investigative andrology.* Springer Science & Business Media.

Mansour, M.A., Nagi, M.N., El-Khatib, A.S., & Al-Bekairi, A.M. (2002). Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: a possible mechanism of action. *Cell Biochemistry Function, 20*(2), 143-151.

Marchlewicz, M., Wiszniewska, B., Gonet, B., Baranowska-Bosiacka, I., Safranow, K., Kolasa, A., & Rać, M. E. (2007). Increased lipid peroxidation and ascorbic acid utilization in testis and epididymis of rats chronically exposed to lead. *Biometals*, *20*(1), 13-19.

Markovac, J., & Goldstein, G. W. (1988). Picomolar concentrations of lead stimulate brain protein kinase C. *Nature Journal*, *334*(6177), 71-73.

Mates, J. M. (2000). Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology*, *153*(1), 83-104.

McDowell, L.R. (2003). *Minerals in animal and human nutrition.*2nd Edition. Elsevier Science,361-364.

Meyer, O., Blom, L., & Sondergaard, D. (1982). The influence of minerals and protein on the nephrocalinosis potential for rats of semi synthesis diets. *Laboratory Animals*, *16*(3), 271-273.

Mohamed, A. M., Metwally, N. M., & Mahmoud, S. S. (2005). Sativa seeds against Schistosoma mansoni different stages. *Memórias do Instituto Oswaldo Cruz, 100*(2), 205-211.

Mohammad, M. A., Mohamad, M. M., & Dradka, H. (2009). Effects of black seeds (N. sativa) on spermatogenesis and fertility of male albino rats. *Research Journal Medical Science, 4*(2), 386-390.

Mohammadi, M., Ghaznavi, R., Keyhanmanesh, R., Sadeghipour, H. R., Naderi, R., & Mohammadi, H. (2014). Caloric restriction prevents lead-induced oxidative stress and inflammation in rat liver. *Scientific World Journal,* 821524-821524.

Mohammed, A. K. (2010). Ameliorative effect of black seed (N. sativa L) on the toxicity of aluminum in rabbits. *The Iraqi Journal of Veterinary Medicine, 34*(2), 110-116.

Mohammed, M. J., Mahmood, M. T., & Yaseen, J. M. (2009). Biological effect of saponins isolated from N. sativa (seeds) on growth of some bacteria. *Tikrit Journal Pure Science, 14*(2), 30-33.

Moneim, A. E. A., Dkhil, M. A., & Al-Quraishy, S. (2011). The protective effect of flaxseed oil on lead acetate-induced renal toxicity in rats. *Journal of Hazardous Materials, 194*, 250-255.

Moore, D. M.(2000). Hematology of rabbits. In: B.F.Feldman, J.G. Zinkl and N.C. Jain (eds.) Schalm's Veterinary Hematology. *Lippincott Williams & Wilkins, Philadelphia, PA, United State of America.*

Morikawa, T., Xu, F., Ninomiya, K., Matsuda, H., & Yoshikawa, M. (2004). Nigellamines A3, A4, A5, and C, new dolabellane-type diterpene alkaloids, with lipid metabolism-promoting activities from the Egyptian medicinal food black cumin. *Chemical and Pharmaceutical Bulletin, 52*(4), 494-497.

Mouhajir, F., Pedersen, J. A., Rejdali, M., & Towers, G. H. N. (1999). Antimicrobial thymohydroquinones of Moroccan N. sativa seeds detected by electron spin resonance. *Pharmaceutical Biology Journal, 37*(5), 391-395.

Mtui, E., Gruener, G., & FitzGerald, M. J. T. (2011). *Clinical neuroanatomy and neuroscience*. Elsevier Health Sciences.

Mudipalli, A. (2007). Lead hepatotoxicity & potential health effects. *Indian Journal of Medical Research*, *126*(6), 518.

Mugahi, M. N., Heidari, Z., Sagheb, H. M., & Barbarestani, M. (2003). Effects of chronic lead acetate intoxication on blood indices of male adult rat. *DARU Journal of Pharmaceutical Sciences*, *11*(4), 147-1.

Mujaibel, L. M., & Kilarkaje, N. (2015). Mitogen-activated protein kinase signaling and its association with oxidative stress and apoptosis in lead-exposed hepatocytes. *Environmental Toxicology, 30*(5), 513-529.

Murray, R.K., Granner, D.K., Mayes, P.A., & Rodwell, V.W. (2003). *Harper's Illustrated Biochemistry.* 26th ed. McGraw-Hill, New York. Pp: 190-241.

Murugesan, M., Ragunath, M., Prabu, T., Nadanasabapathi, S., Sakthivel, M., & Manju, V. (2012). Protective role of black cumin (N. sativa) on isoproterenol induced myocardial infarction in rats. *International Journal Pharmacology and Clinical Sciences, 1*, 45-53.

Mustafa, H. N. A. (2016). Does allicin combined with vitamin B-complex have superior potentials than alpha-tocopherol alone in ameliorating lead acetate-induced Purkinje cell alterations in rats? An immunohistochemical and ultrastructural study. *Folia Morphol (Warsz).*, *75*(1), 76-86.

Nair, A. R., DeGheselle, O., Smeets, K., Van Kerkhove, E., & Cuypers, A. (2013). Cadmium-induced pathologies: where is the oxidative balance lost (or not)? *International Journal of Molicular Science, 14*(3), 6116-6143.

Nair, S. C., Salomi, M. J., Panikkae, B., & Panikkar, K. R. (1991). Modulatory effects of Crocus sativus and N. sativa extracts on cisplatin-induced toxicity in mice. *Journal of Ethnopharmacology, 31*(1), 75-83.

Najmi, A., Haque, S. F., Naseeruddin, M., & Khan, R. A. (2008). Effect of N. sativa oil on various clinical and biochemical parameters of metabolic syndrome. *International Journal of Diabetes in Developing Countries, 16*: 85-87.

Namba, T., Tsunezuka, M., Dissanayake, D. M. R. B., Polaptiya, U., Saito, K., Kakiuchi, N., & Hattori, M. (1985). Studies on Dental Caries Prevention by Traditional Medicines (Part VII): Screening of Ayurvedic Medicines for Anti-plaque Action. *Shoyakugaku Zasshi Journal, 39*(2), 146-153.

National Research Council (US). Committee on Biologic Effects of Atmospheric Pollutants. (1972). *Lead; Airborne Lead in Perspective*. National Academy Press.

Navas-Acien, A., Guallar, E., Silbergeld, E. K., & Rothenberg, S. J. (2007). Lead Exposure and Cardiovascular Disease-A Systematic Review. *Environmental Health Perspectives Journal, 115*(3), 472-482.

Nazariah, S. S. N., Juliana, J., & Abdah, M. A. (2013). Interleukin-6 via sputum induction as biomarker of inflammation for indoor particulate matter among primary school children in Klang Valley, Malaysia. *Global Journal of Health Science*, *5*(4), 93.

Needleman, H. (2004). Lead poisoning. Annual Review of Medicine, *55*, 209-222.

Nergiz, C., & Ötleş, S. (1993). Chemical composition of N. sativa L. seeds. *Food Chemistry Journal, 48*(3), 259-261.

Nisar, M. F., Nasir, I., Shaheen, S., Khalid, A., & Tazeen, N. (2014). Chronic Lead Acetate Nephrotoxicity: A Histological Study on Albino Rats. *ANNALS*, *17*(3), 239.

Nriagu, J. O., Blankson, M. L., & Ocran, K. (1996). Childhood lead poisoning in Africa: a growing public health problem. *Science of the Total Environment*,*181*(2), 93-100.

Omobowale, T. O., Oyagbemi, A. A., Akinrinde, A. S., Saba, A. B., Daramola, O. T., Ogunpolu, B. S., & Olopade, J. O. (2014). Failure of recovery from lead induced hepatoxicity and disruption of erythrocyte antioxidant defence system in Wistar rats. *Environmental Toxicology and Pharmacology, 37*(3), 1202-1211.

Othman, A. M., Abba, Y., Jesse, F. F. A., Ilyasu, Y. M., Saharee, A. A., Haron, A. W., & Lila, M. A. M. (2016). Reproductive Pathological Changes Associated with Experimental Subchronic Corynebacterium pseudotuberculosis Infection in Nonpregnant Boer Does. *Journal of Pathogens*.

Oyagbemi, A. A., Omobowale, T. O., Akinrinde, A. S., Saba, A. B., Ogunpolu, B. S., & Daramola, O. (2015). Lack of reversal of oxidative damage in renal tissues of lead acetate-treated rats. *Environmental Toxicology, 30*(11), 1235-1243.

Paarakh, P. M. (2010). N. sativa Linn. –A comprehensive review. *Indian Journal of Natural Products and Resources, 1*(4), 409-429.

Papanikolaou, N. C., Hatzidaki, E. G., Belivanis, S., Tzanakakis, G. N., & Tsatsakis, A. M. (2005). Lead toxicity update. A brief review. *Medical Science Monitor*, *11*(10), RA329-RA336.

Patra, R. C., Rautray, A. K., & Swarup, D. (2011). Oxidative stress in lead and cadmium toxicity and its amelioration. *Journal of Veterinary Internal Medicine,* 457327.

Patrick, L. (2006). Lead toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. *Alternative Medicine Review*, *11*(2), 114.

Patrick, L. (2006). Lead toxicity, a review of the literature. Part 1: Exposure, evaluation, and treatment. *Alternative Medicine Review: a Journal of Clinical Therapeutic, 11*(1), 2-22.

Paul, F. (2009). *Laboratory animal anesthesia.* Academic press is an imprint of Elsevier. Newcastle University, Pp: 208.

Payne, J., & Livesey, C. (2010). Lead poisoning in cattle and sheep. *In Practice*, *32*(2), 64-69.

Perveen, T., Haider, S., Kanwal, S., & Haleem, D. J. (2009). Repeated administration of N. sativa decreases 5-HT turnover and produces anxiolytic effects in rats. *Pakistan Journal of Pharmaceutical Science, 22*(2), 139-144.

Piomelli, S. (2002). Childhood lead poisoning. *Pediatric Clinics of North America Journal, 49*(6), 1285-1304.

Pizzino, G., Bitto, A., Interdonato, M., Galfo, F., Irrera, N., Mecchio, A., & Squadrito, F. (2014). Oxidative stress and DNA repair and detoxification gene expression in adolescents exposed to heavy metals living in the Milazzo-Valle del Mela area (Sicily, Italy). *Redox Biology*, *2*, 686-693.

Potter, J., Topping, D., & Oakenfull, D. (1979). Soya, saponins, and plasma-cholesterol. *The Lancet Journal, 313*(8109), 223.

Qian, Y., Harris, E. D., Zheng, Y., & Tiffany-Castiglioni, E. (2000). Lead targets GRP78, a molecular chaperone, in C6 rat glioma cells. *Toxicology and Applied Pharmacology Journal*, *163*(3), 260-266.

Qidwai, W., Hamza, H. B., Qureshi, R., & Gilani, A. (2009). Effectiveness, safety, and tolerability of powdered N. sativa (Kalonji) seed in capsules on serum lipid levels, blood sugar, blood pressure, and body weight in adults: results of a randomized, double-blind controlled trial. *The Journal of Alternative and Complementary Medicine, 15*(6), 639-644.

Rahman, S., & Sultana, S. (2006). Chemopreventive activity of glycyrrhizin on lead acetate mediated hepatic oxidative stress and its hyperproliferative activity in Wistar rats. *Chemico-Biological Interactions*, *160*(1), 61-69.

Rajsekhar, S., & Kuldeep, B. (2011). Pharmacognosy and pharmacology of *N. sativa*-A review. *International Research Journal of Pharmacy, 2*(11), 36-39.

Randhawa, M. A., & Al-Ghamdi, M. S. (2002). A review of the pharmaco-therapeutic effects of N. sativa. *Pakistan Journal of Medical Research, 41*(2), 1-10.

Randhawa, M. A., & Alghamdi, M. S. (2011). Anticancer activity of N. sativa (black seed)—a review. *The American Journal of Chinese Medicine, 39*(06), 1075-1091.

Rangan, G. K., & Tesch, G. H. (2007). Quantification of renal pathology by image analysis (Methods in Renal Research). *Nephrology*, *12*(6), 553-558.

Rastogi, S. K. (2008). Renal effects of environmental and occupational lead exposure. *Indian Journal of Occupational and Environmental Medicine*, *12*(3), 103.

Renner, R. (2010). Exposure on tap: drinking water as an overlooked source of lead. *Environ Health Perspect,* 118(2), A68-A72.

Riaz. M., Syed, M., & Chaudhary, F.M. (1996). Chemistry of the medicinal Plants of the genus Nigella (family-Ranunculaceae). *Hamdard Medicus Journal, 39*(2), 40-45.

Rice, D.C. (1992). Behavioral impairment produced by developmental lead exposure: evidence from primate research. *Human Lead Exposure*, 138-152.

Rifat-uz-Zaman, M. S. A., & Khan, M. S. (2004). Gastroprotective and anti-secretory effect of N. sativa seed and its extracts in indomethacin-treated rats. *Pakistan Journal of Biological Sciences, 7*(6), 995-1000.

Rifqi, M. Z. (2012). Ibnu qayyim al-jauziah al tibb an nabawiy berubat mengikut nabi s.a.w terapi bagi orang islam. Jasmin Enterprise: Kuala Lumpur.

Rogozhin, E. A., Oshchepkova, Y. I., Odintsova, T. I., Khadeeva, N. V., Veshkurova, O. N., Egorov, T. A., & Salikhov, S. I., (2011). Novel antifungal defensins from N. sativa L. seeds. *Plant Physiology and Biochemistry*, *49*(2), 131-137.

Sagesaka, Y. M., Uemura, T., Suzuki, Y., Sugiura, T., Yoshida, M., Yamaguchi, K., & Kyuki, K. (1996). Antimicrobial and anti-inflammatory actions of tea-leaf saponin. Yakugaku zasshi. *Journal of the Pharmaceutical Society of Japan, 116*(3), 238-243.

Saleem, U., Ahmad, B., Rehman, K., Mahmood, S., Alam, M., & Erum, A. (2012). Nephro-protective effect of vitamin C and N. sativa oil on gentamicin associated nephrotoxicity in rabbits. *Pakistan Journal of Pharmaceutical Sciences, 25*(4), 727-730.

Saleh, H. A., El-Aziz, G. A., El-Fark, M. M., & El-Gohary, M. (2009). Effect of maternal lead exposure on craniofacial ossification in rat fetuses and the role of antioxidant therapy. *Anatomia, Histologia, Embryologia, 38*(5), 392-399.

Salem, M.L. (2005). Immunomodulatory and therapeutic properties of the N. sativa L. seed. *International Immunopharmacology*, *5*(13), 1749-1770.

Salim, M. (2015). Evaluation of Performance of Date Palm Pollen on Urea and Creatinine Levels in Adult Female Rats Exposed to Lead Acetate Intoxication. *International Journal of Biomedical Advance Research, 6*(1), 20-24.

Salińska, A., Włostowski, T., & Zambrzycka, E. (2012). Effect of dietary cadmium and/or lead on histopathological changes in the kidneys and liver of bank voles Myodes glareolus kept in different group densities. *Ecotoxicology*, *21*(8), 2235-2243.

Samantha, S., Nalinie, W., Ira, T., Neelakanthi, R., & Mayuri, G. (2003). Protection against diethylnitrosamine induced hepatocarcinogenesis by an indigenous medicine comprised of N. sativa. *Journal of Carcinogenesis, 2*, 6-18.

Sanders, T., Liu, Y., Buchner, V., & Tchounwou, P. B. (2009). Neurotoxic effects and biomarkers of lead exposure: a review. *Reviews on Environmental Health, 24*(1), 15-46.

Sawsan, A.B., & Somia, M. (1992). Effect of N. sativa extract on experimental giardiasis. *The New Egyptian Journal of Medicine, 7*(1), 1-3.

Sayed, M.D. (1980). Traditional medicine in healthcare. *Journal of Ethanopharmacology, 2*(1), 19-22.

Sharma, N. K., Ahirwar, D., Jhade, D., & Gupta, S. (2009). Medicinal and pharmacological potential of N. sativa: a review. *Ethnobotanical Leaflets,* (7), 11.

Sharma, R., & Garu, U. (2011). Effects of lead toxicity on developing testes in Swiss mice. *Universal Journal of Environmental Research and Technology*, *1*(4), 390-398.

Sharma, S., & Singh, B. (2014). Effects of Acute and Chronic Lead Exposure on Kidney Lipid Peroxidation and Antioxidant Enzyme Activities in BALB-C Mice (Mus Musculus). *International Journal of Science Research*, *3*, 1564-1566.

Sharma, V., & Pandey, D. (2010). Protective role of Tinospora cordifolia against lead-induced hepatotoxicity. *Toxicology International*, *17*(1), 12.

Sharma, V., Sharma, A., & Kansal, L. (2010). The effect of oral administration of Allium sativum extracts on lead nitrate induced toxicity in male mice. *Food and Chemical Toxicology, 48*(3), 928-936.

Shih, I. M., Chiang, H. S., Yang, L. L., & Wang, T. L. (1990). Antimotility effects of Chinese herbal medicines on human sperm. Journal of the Formosan Medical Association, *Taiwan Yi Zhi, 89*(6), 466-469.

Shirlyn, B. S. (2004). *Clinical Laboratory Hematology,* 1st Education, University of Texas Science Center at San Antonio, U.S.A.

Singh, L. W. (2011). Traditional medicinal plants of Manipur as anti-diabetics. *Journal of Medicinal Plants Research, 5*(5), 677-687.

Singh, M. K., Dwivedi, S., Yadav, S. S., Sharma, P., & Khattri, S. (2014). Arsenic-induced hepatic toxicity and its attenuation by fruit extract of Emblica officinalis (amla) in mice. *Indian Journal of Clinical Biochemistry, 29*(1), 29-37.

Spivey, A. (2007). The weight of lead: Effects add up in adults. *Environmental Health Perspectives Journal, 115*(1), A30-36.

Sujatha, K., Srilatha, C. H., Anjaneyulu, Y., & Amaravathi, P. (2011). Lead acetate induced nephrotoxicity in Wistar albino rats, pathological, immunohistochemical and ultra-structural studies. *International Journal of Pharmacy Biological Sciences, 2*(2), B459-B469.

Suresh Kumar, T. V., Negi, P. S., & Udaya Sankar, K. (2010). Antibacterial Activity of N. sativa L. Seed Extracts. *British Journal of Pharmacology and Toxicology, 1*(2), 96-100.

Tasawar, Z., Siraj, Z., Ahmad, N., & Lashari, M. H. (2011). The effects of N. sativa (Kalonji) on lipid profile in patients with stable coronary artery disease in Multan, Pakistan. *Pakistan Journal Nutrition, 10*(2), 162-167.

Tawfeek, F. K., Ahmed, S. M., & Kakel, S. J. (2006). Effect of N. sativa oil treatment on the sex organs and sperm characters in rats exposed to hydrogen peroxide. *Mesopotamia Journal of Agriculture, 34*(1), 2-8.

Telisman, S., Cvitkovic, P., Jurasovic, J., Pizent, A., Gavella, M., & Rocic, B. (2000). Semen quality and reproductive endocrine function in relation to biomarkers of lead, cadmium, zinc, and copper in men. *Environmental Health Perspectives*, *108*(1), 45.

Tisserand, R., & Balacs, T. (1995). *Essential Oil Safety*. Churchill Livingstone Edinburgh, Pp: 108-110.

Uslu, C., Taysi, S., & Bakan, N. (2003). Lipid peroxidation and antioxidant enzyme activities in experimental maxillary sinusitis. *Annals of Clinical and Laboratory Science, 33*(1), 18-22.

Vargas, H., Castillo, C., Posadas, F., & Escalante, B. (2003). Acute lead exposure induces renal haeme oxygenase-1 and decreases urinary Na excretion. *Human and Experimental Toxicology Journal*, *22*(5), 237-244.

Varghese, E. S. V. D. (1996). *Applied ethnobotany, a case study among the Kharias of Central India.* New Delhi: DEEP Publications xix.

Vaziri, N. D., & Khan, M. (2007). Interplay of reactive oxygen species and nitric oxide in the pathogenesis of experimental lead-induced hypertension. *Clinical and Experimental Pharmacology and Physiology*, *34*(9), 920-925.

Vaziri, N. D., & Sica, D. A. (2004). Lead-induced hypertension: role of oxidative stress. *Current Hypertension Reports Journal*, *6*, 314-320.

Velaga, M. K., Daughtry, L. K., Jones, A. C., Yallapragada, P. R., Rajanna, S., & Rajanna, B. (2014). Attenuation of lead-induced oxidative stress in rat brain, liver, kidney and blood of male Wistar rats by Moringa oleifera seed powder. *Journal of Environmental Pathology, Toxicology and Oncology, 33*(4).

Vij, A. G., & Dhundasi, S. A. (2009). Hemopoietic, hemostatic and mutagenic effects of lead and possible prevention by zinc and vitamin C. *Al Ameen Journal of Medical Sciences, 2*(2), 27-36.

Wang, J., Yang, Z., Lin, L., Zhao, Z., Liu, Z., & Liu, X. (2012). Protective effect of naringenin against lead-induced oxidative stress in rats. *Biology Trace Element Research, 146*(3), 354-359.

Wang, X., Wang, M., Dong, W., Li, Y., Zheng, X., Piao, F., & Li, S. (2013). Subchronic exposure to lead acetate inhibits spermatogenesis and downregulates the expression of Ddx3y in testis of mice. *Reproductive Toxicology*, *42*, 242-250.

Weiner, I. D., Mitch, W. E., & Sands, J. M. (2015). Urea and ammonia metabolism and the control of renal nitrogen excretion. *Clinical Journal of American Society Nephrology, 10*(8), 1444-1458.

Weisbroth, S.H., Flatt, R.E., & Kraus, A.L. (Eds). (2013). *The biology of the laboratory rabbit.* Academic Press.

Yaman, İ., & Balikci, E. (2010). Protective effects of N. sativa against gentamicin-induced nephrotoxicity in rats. *Experimental and Toxicologic Pathology*, *62*(2), 183-190.

Yedjou, C. G., & Tchounwou, P. B. (2007). N-acetyl-l-cysteine affords protection against lead-induced cytotoxicity and oxidative stress in human liver carcinoma (HepG2) cells. *International Journal of Environmental Research and Public Health*, *4*(2), 132-137.

Yokoi, K., Uthus, E. O., & Nielsen, F. H. (2003). Nickel deficiency diminishes sperm quantity and movement in rats. *Biological Trace Element Research*, *93*(1-3), 141-153.

Yoruk, O., Gur, F., Uyanik, H., Yasar, M., Mutlu, V., Altas, E., & Taysi, S. (2010). Antioxidant effects of *N. sativa* in the treatment of experimentally induced rhinosinusitis. *Macedonian Journal of Medical Sciences, 3*(2), 132-137.

Yuan, G., Dai, S., Yin, Z., Lu, H., Jia, R., Xu, J., & Chen, Z. (2014). Sub-chronic lead and cadmium co-induce apoptosis protein expression in liver and kidney of rats. *International Journal of Clinical and Experimental Pathology, 7*(6), 2905-14.

Zaher, K. S., Ahmed, W. M., & Zerizer, S. N. (2008). Observations on the biological effects of black cumin seed (N. sativa) and green tea (Camellia sinensis). *Global Veterinaria*, *2*(4), 198-204.

Zahoor, A., Ghafoor, A., & Aslam, M. (2004). *N. sativa* – a potential commodity in crop diversification traditionally used in healthcare. Project on introduction of medicinal herbs and spices as crops. *Ministry of Food. Agriculture and Livestock Education, Islamabad, Pakistan,* Pp: 280-295.

Zaoui, A., Cherrah, Y., Alaoui, K., Mahassine, N., Amarouch, H., & Hassar, M. (2002). Effects of *N. sativa* fixed oil on blood homeostasis in rat. *Journal of Ethnopharmacology, 79*(1), 23-26.

Zohary, M. (1983). The genus Nigella (Ranunculaceae)—a taxonomic revision. *Plant Systematics and Evolution*, *142*(1-2), 71-105.