

# Cairo University Faculty of Veterinary Medicine



# Effect of lead acetate on some organs of adult albino rats with possible protective trials

A thesis submitted by

Noha Ali Elsayed Yasin

(BVSc, Cairo University, 2011; MVSc, Cairo University, 2015)

For the degree of the Ph.D

(Cytology & Histology)

Under Supervision of

Youssef Y. M. Shaheen

Prof. of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

#### Mohamed I. Abdrabou

Assistant Prof. of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

### Ebtihal M. M. Elleithy

Lecturer of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

### **Supervision Sheet**

# Effect of lead acetate on some organs of adult albino rats with possible protective trials

Ph.D. Thesis presented

By

## Noha Ali Elsayed Yasin

(BVSc., Cairo University, 2011)

(MVSc., Cairo University, 2015)

#### **SUPERVISION COMMITTEE**

#### Prof. Dr. Youssef Y. M. Shaheen

Professor of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

### Dr. Mohamed I. Abdrabou

Assistant Professor of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

### Dr. Ebtihal M. M. Elleithy

Lecturer of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

Name of candidate: Noha Ali Elsayed Yasin

**Date of birth** : 1/10/1989

Place of birth : Ash Sharqiyah, Egypt

Nationality : Egyptian

Career : Assistant lecturer of Cytology and Histology, Fac. of Vet. Med.,

**Cairo University** 

Degree : Ph. D

Title of thesis : Effect of lead acetate on some organs of adult albino rats with

possible protective trials

**Supervisors**:

#### Prof. Dr. Youssef Y. M. Shaheen

Professor of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

#### Dr. Mohamed I. Abdrabou

Assistant Professor of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

#### Dr. Ebtihal M. M. Elleithy

Lecturer of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University.

#### **Abstract:**

Lead (Pb) is a ubiquitous environmental and industrial pollutant with worldwide health problems. The present study was designed to investigate the neuronal and reproductive toxicity of Pb in albino rats and to evaluate the ameliorative role of garlic as well as Spirulina maxima against such toxic effects. Forty adult male rats were used in this study (10 rats/group). Group I: served as control, Group II: rats received lead acetate (100 mg/kg), Group III: rats received both lead acetate (100 mg/kg) and garlic (600 mg/kg) and Group IV: rats received both lead acetate (100 mg/kg) and spirulina (500 mg/kg) daily by oral gavage for one month. Exposure to Pb acetate adversely affected the measured acetyl cholinesterase enzyme activity, dopamine level, serum testosterone level, oxidative stress and lipid peroxidation parameters as well as caspase-3 gene expression in both brain and testicular tissues. Light and electron microscopical examination of the cerebrum, cerebellum and testis showed various lesions after exposure to Pb which were confirmed by immunohistochemistry. In addition, it adversely affected sperm concentration, motility and viability. On the other hand, administration of garlic and spirulina concomitantly with lead acetate ameliorated most of the undesirable effects. It could be concluded that, the adverse effects induced by lead acetate, were markedly ameliorated by co-treatment with S. maxima more than garlic.

Keywords: Lead, Garlic, Spirulina, Brain, Testis

## Acknowledgement

Praise to Allah the most gracious and the most merciful, for all his countless graces, guidance and help to accomplish this research and for providing me with such encouraging and supportive supervisors.

It gives me great pleasure to express my deepest gratitude and appreciation to **prof. Or. Youssef Y. M. Shaheen** who made great effort with me in this thesis for devoting much of his precious time, true concern, meticulous supervision and fruitful instructions to achieve this work in the best possible image. It was an honor to be supervised by him.

My sincere thanks are offered to **Dr. Mohamed I. Abdrabou** for his careful supervision, insistence on perfection together with the valuable assistance he devoted in the supervision of this study.

I would like to express my great thanks to **Dr. Ebtihal M. M. Elleithy** for her great assistance and for providing me with the experience, continuous cooperation and close supervision throughout the work. I appreciate her unforgettable support as well as her generous effort in this study.

My appreciation and deep thanks to **Dr. Mona Khamis Galal**, assistant professor of Biochemistry, Faculty of Veterinary Medicine, Cairo University, for her sincere help in the biochemical study in this work, for valuable experience and time and great concern to bring this study to completion.

Sincer thanks to **Dr. Mohamed Fathy Mohamed abd-alla**, lecturer of Theriogenology, Faculty of Veterinary Medicine, Cairo University for his kind help in semen analysis.

I extend my thanks to my senior staff members, my colleagues and all technicians in the department of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University, for their continuous help and kindness during this work.

Finally, I would like to express my appreciation and gratitude to my beloved father "may mercy be upon him", my beloved mother and my whole family for teaching me devotion to work and for their endless love, care, support and motivation.

# **Contents**

Chapter	Page
Chapter (1) Introduction	1 - 14
Chapter (2): Review of literature	15 - 61
Chapter (3): Published papers	
3.1. Modulation Of Caspase-3 Gene	62 - 130
Expression And Protective Effects Of Garlic	
And Spirulina Against CNS Neurotoxicity	
Induced By Lead Exposure In Male Rats	
3.2. Ameliorative Effects of Spirulina maxima and Allium sativum on Lead Acetate-induced Testicular Injury in Male Albino Rats with respect to Caspase-3 Gene Expression	131 - 173
Chapter (4): Discussion	174 - 191
Chapter (5): Conclusion and Recommendation	192 - 193
Chapter (6): Summary	194 - 202
Chapter (7): References	203 - 247
Appendix	248 - 283
الملخص العربي	1 - 5

#### List of Abbreviation

ACh: Acetylcholine

AChE: Acetylcholinesterase

ACP: Acid phosphatase

AKP: Alkaline phosphatase

ATP : Adinosine triphosphate

BAX: BCL2-associated X protein

BBB: Blood Brain Barrier

BCl-2: B- cell lymphoma 2 protein

BTB: Blood Testes Barrier

b.wt. : Body Weight

CAT: Catalase

CCL<sub>4</sub>: Carbon tetrachloride

CNS: Central Nervous System

DAS: Diallyl sulfide

DADS: Diallyl disulfide

DATS: Diallyl trisulfide

DMSA: 2, 3-meso-dimercaptosuccinic Acid

DNA: Deoxy-ribonucleic Acid

FSH : Follicle Stimulating Hormone

GFAP: Glial Fibrillary Acidic Protein

GPx : Glutathione Peroxidase

G6PDH: Glucose -6- phosphate dehydrogenase

GSH: Reduced Glutathione

GST: Glutathione S-Transferases

H & E: Hematoxylin and Eosin

H<sub>2</sub>O<sub>2</sub> : Hydrogen Peroxide

HSP70: anti-heat shock protein 70

IHC: Immunohistochemistry

Kg: kilogram

LH : Luteinizing Hormone

LPO: Lipid Peroxidation

LPP: Lipid Peroxidation Product

MAO: Monoamine Oxidases

mg : Milligram

ml : Milliliter

nmol: Nanomol

NOS: Nitric Oxide Synthase

6-OHDA: 6-hydroxydopamine

Pb: Lead

PbAc: Lead Acetate

rER : Rough Endoplasmic Reticulum

ROS: Reactive Oxygen Species

Se : Selenium

sER : Smooth Endoplasmic Reticulum

SOD: Superoxide Dismutase

TEM: Transsmition Electron Microscope

μm : Micrometer

 $Zn^{2+}$ : Zinc ion

Fe: Iron

# **List of Figures**

No. of	Comment	Page
figure		
	1 <sup>st</sup> Published Paper	
1	Protective influence of garlic or spirulina on the oxidative stress	74
	parameters against lead acetate induced neurotoxicity.	
2	Protective influence of garlic or spirulina on Acetylcholinesterase	75
	enzyme activity against lead acetate induced neurotoxicity.	
3	Graphical representation of mRNA expression of caspase-3 gene	<b>76</b>
	in different experimental groups in both cerebrum and cerebellum	
4	regions estimated by qPCR.	70
4	A photomicrograph showing the cerebral cortex in different groups of rat (control and lead-exposed Gp). (H & E)	<b>78</b>
5	A photomicrograph showing the cerebral cortex in different	80
S	groups of rat. (for garlic and spirulina-exposed Gp). (H & E)	00
6	A photomicrograph showing the cerebellar cortex in different	82
U	groups of rat. (H & E)	02
7	A photomicrograph showing the immunohistochemical staining of	84
	caspase-3 expression of the cerebral cortex in different groups of	
	rat.	
8	A photomicrograph showing the immunohistochemical staining of	85
	caspase-3 expression of the cerebellar cortex in different groups of	
	rat.	
9	A photomicrograph showing the area percentage of caspase-3	86
	immune-reactivity of the cerebral and cerebellar cortex in different	
10	groups of rat.	00
10	Electron micrographs of cerebral sections from different groups of	88
	rats (control and lead-exposed Gp).  (Uranyl acetate and lead citrate)	
11	Electron micrographs of cerebral sections from different groups of	91
11	rats (garlic and spirulina-exposed Gp).	71
	(Uranyl acetate and lead citrate)	
12	Electron micrographs of cerebellar sections from different groups	95
	of rats.	
	2 <sup>nd</sup> Published Paper	
1	Protective influence of garlic or spirulina on serum testosterone	142
	levels and oxidative stress parameters against lead acetate-induced	
	reproductive toxicity	
2	Graphical representation of mRNA expression of the caspase-3	143

_	·	
	gene in different experimental groups in testicular tissue estimated by qPCR.	
3	Protective effects of garlic and spirulina on seminiferous tubule diameter and epithelial height in testicular tissue from rats	144
	intoxicated with lead acetate	
4	Photomicrograph of control rat testes showing normal histological	146
	structure of active, mature, functioning seminiferous tubules	
	associated with complete spermatogenic series. (H & E)	
5	<b>5A.</b> Photomicrograph of a testicular section from lead-exposed rats	147
	depicting loosening and detachment of spermatogenic cells and	
	intercellular vacuolation with diminished epithelial height.	
	<b>5B.</b> Photomicrograph of a testicular section from lead-exposed rats	
	shows shrunken seminiferous tubules, an irregular basement	
	membrane, degenerated and desquamated spermatocytes,	
	vacuolation and desquamated cells within the lumina of	
	seminiferous tubules	
	<b>5C.</b> Photomicrograph of a testicular section from garlic-treated	
	rats depicting nearly normal spermatogenesis but with vacuolation	
	and slight degeneration.	
	<b>5D.</b> Photomicrograph of a testicular section from spirulina-treated	
	rats showing apparently normal stages of spermatogenesis and	
	large numbers of sperm were observed. (H & E)	
6	Electron photomicrograph of rat testis from control and lead-	151
	exposed group. (Uranyl acetate and lead citrate)	
7	Electron photomicrograph of rat testis from garlic- and spirulina-	154
	treated group. (Uranyl acetate and lead citrate)	
	3 <sup>rd</sup> Appendix	
1	Photomicrograph of different sperm abnormalities of lead acetate-	249
	exposed rats. (Eosin Nigrosin stain)	
2	Protective influence of garlic or spirulina on dopamine level	250
	against lead acetate-induced neurotoxicity.	
3	Protective influence of garlic or spirulina on glutathione	251
	peroxidase (GPx) enzyme activity in brain (A) and testicular (B)	
	tissues against lead acetate-induced neurotoxicity.	
4	A photomicrograph showing the area percentage of GFAP	252
	immune-reactivity of the cerebral (A) and cerebellar cortex (B) in	
	different groups of rat.	
5	A photomicrograph showing the immunohistochemical staining of	253
	GFAP of the cerebral (A & B) and cerebellar cortex (C & D) in	
	different groups of rat.	
6	A photomicrograph of a cerebral section from control group	254

	showing normal cytoarchitecture. (Einarson's stain)	
7	A photomicrograph of cerebral sections from lead-exposed group	255
	stained with Einarson's stain and Ameur stain and Mallory's	
	phosphotungestic acid-hematoxylin.	
8	A photomicrograph of cerebral sections from lead-exposed rat	256
	stained with Crystal violet and Mallory's phosphotungestic acid-	
	hematoxylin.	
9	A photomicrograph of a cerebral section from lead-exposed group	257
	stained with Ameur stain.	
10	A photomicrograph of cerebral sections from lead-exposed rat	258
	stained with Crystal violet stain, Einarson's stain and Mallory's	
	phosphotungestic acid-hematoxylin.	
11	A photomicrograph of a cerebral section from garlic co-treated	259
	group showing partial recovery with nearly normal neurons.	
	(A: Crystal violet stain), (B: Einarson's stain)	
12	A photomicrograph of cerebral sections from garlic co-treated	<b>260</b>
	group. (Mallory's phosphotungestic acid-hematoxylin)	
13	A photomicrograph of a white matter of cerebral section from	261
	garlic co-treated group.	
	(Mallory's phosphotungestic acid-hematoxylin)	
14	A photomicrograph of a cerebral section from spirulina co-treated	262
	group revealed nearly normal histological architecture.	
	(A: Crystal violet stain),(B: Einarson's stain)	
15	A photomicrograph of a cerebellar section from control group	263
	showing normal cytoarchitecture.	
	(A & B: Einarson's stain), (C: Crystal violet stain)	
16	A photomicrograph of a cerebellar section from lead-exposed	264
	group showing sever degeneration with meningitis. (H & E)	
17	A photomicrograph of cerebellar sections from lead-exposed	265
	group showing substantial reduction in overall population of	
	Purkinje cells.	
	(A & B: H & E stain), (C: Einarson's stain & D: Ameur stain)	
18	A photomicrograph of cerebellar sections from lead-exposed	266
	group stained with Crystal violet stain and Einarson's stain.	
19	A photomicrograph of cerebellar sections from lead-exposed	267
	group stained with Mallory's phosphotungestic acid-hematoxylin	
20	A photomicrograph of a cerebellar section from lead-exposed	268
	group stained with Ameur stain.	
21	A photomicrograph of cerebellar section from lead-exposed group	269
	stained with Bielschowsky's stain.	
22	A photomicrograph of cerebellar sections from garlic co-treated	270