

Cairo University Faculty of Veterinary Medicine



Pathological and Immunohistochemical Studies on the Protective Effect of Spirulina Platensis Against Reproductive Arsenic Toxicity in Rats

A thesis submitted by

Khaled Said Ahmed Hassan

B.V.Sc (2002), Faculty of Veterinary Medicine Tripoli University M.V.Sc (2012), Faculty of Veterinary Medicine Cairo University

For the Degree of PhD Pathology (General, Special and Postmortem)

Under Supervision of

Prof. Dr. Kawkab Abd El Aziz Ahmed

Prof. of Pathology Faculty of Veterinary Medicine Cairo University

Dr. Reda Mohammed Sayed

Assistant Prof. of Pathology
Faculty of Veterinary Medicine
Cairo University.

Dr. Hanaa Ali El Halawany

Chief Researcher of Reproductive Pathology Animal Reproduction Research Institute



Cairo University
Faculty of Veterinary Medicine
Department of Pathology

Supervision Sheet

Prof. Dr. Kawkab Abd El Aziz Ahmed

Professor of Pathology, Faculty of Veterinary Medicine Cairo University

Dr. Reda Mohammed Sayed

Assistant Professor of Pathology Faculty of Veterinary Medicine Cairo University

Dr. Hanaa Ali El Halawany

Chief Researcher of Reproductive Pathology Animal Reproduction Research Institute



Faculty of Veterinary Medicine Department of Pathology

Name: Khaled Said Ahmed Hassan

Date of birth: 16/03/1978 **Nationality:** Egyptian

Degree: Ph.D. of Vet. Med. Sciences

Specialization: Pathology (General, Special and Postmortem)

Title: Pathological and Immunohistochemical Studies on the Protective Effect of Spirulina

Platensis Against Reproductive Arsenic Toxicity in Rats

Supervisors:

Prof. Dr. Kawkab Abd El Aziz Ahmed Professor of Pathology, Faculty of Veterinary

Medicine, Cairo University

Dr. Reda Mohammed Sayed Assistant Professor of Pathology, Faculty of

Veterinary Medicine, Cairo University

Dr. Hanaa Ali El HalawanyChief Researcher of Reproductive Pathology

Animal Reproduction Research Institute

Abstract

Arsenic (As) is currently one of the most important environmental global contaminants and toxicants, particularly in the developing countries. The aim of this study was to investigate the effect of arsenic (As) on the male and female reproductive organs, liver, kidneys, lungs, heart and brain and to assess the effect of *Spirulina platensis* (Sp) as an ameliorating agent in the arsenic-induced toxicity and exploring its pathway in protection. 120 male and female albino rats were divided into four equal groups, 30 rats in each group (15 males and 15 females): Control group and three treatment groups that daily received As, Sp and Sp+As respectively, Ten rats (5 males and 5 females) from each group were euthanized monthly during the experimental period (3 months). Results showed that the body weight in male and female rats was significantly reduced in As-treated groups compared to the control one, while the cotreatment with Sp significantly recovered the body weight in comparison with As-treated

groups. Arsenic significantly increased serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, malondialdehyde (MDA) and significantly reduced serum glutathione (GSH) activities, testosterone and estradiol in comparison with the control groups. Sp co-treatment significantly recovered AST, ALT, GSH, testosterone and estradiol levels and significantly reduced MDA in comparison to As-treated groups. Histopathologically, testes, uterus, ovaries, liver, kidneys, lungs, heart and brain of As-treated groups showed various degenerative and inflammatory changes which were relieved with the Sp co-treatment. Immunohistochemistry revealed increased apoptosis and explore the role of Nrf2 in the arsenic-induced toxicity. In conclusion, As-induced toxicity in rats could be ameliorated by *Spirulina platensis* co-administration, this with the expression of nrf2 and pro-apoptotic caspase 3 which indicating that *Spirulina platensis* is combating the generated oxidative stress in As-induced toxicity in albino rats.

Key words: Arsenic, *Spirulina platensis*, Pathology, Immunohistochemistry, Rats.

Dedication

To souls of my father and mother,
to my wife and my sons Maryam, Sama & Malek
for their constant support and unconditional love.
I love you all dearly.

Acknowledgment

Foremost, all thanks to the most merciful ALLAH who gave me all the graces to accomplish this work then I would like to express my sincere gratitude to my supervisor Prof. Kawkab Abd El-Aziz Ahmed for the continuous support of my Ph.D. study and related research, for her patience, motivation, and immense knowledge. Her guidance helped me in all the time of research and writing of this thesis. I could not have imagined having a better advisor and mentor for my Ph.D. study.

Besides my advisor, I would like to thank the rest of my thesis supervisors: Dr. Reda Mohammed Sayed for her insightful comments, assistance and encouragement, but also for the hard question which incented me to widen my research from various perspectives. And Dr. Hanaa Ali El Halawany for her guidance, assistance and sharing of information and researches, her continuous encouragement was always incentive for me. Their role in my thesis cannot be forgotten.

My sincere thanks also go to the Department of Pathology at the veterinary faculty and My Department of Reproductive Pathology at my institute which gave access to the laboratory and research facilities. Without their precious support it would not be possible to conduct this research.

I am grateful to Department of Immunity at my institute for their help and assistance.

Last but not least, I would like to express my deepest gratitude to my family. This dissertation would not have been possible without their warm love, continued patience, and endless support.

List of Contents

- Supervision Sheet	I
- Abstract	II
- Dedication	IV
- Acknowledgment	V
- Contents	VI
- List of Tables	VII
- List of Figures	VIII
- List of Abbreviations	XII
- Chapter (1): Introduction	1
- Chapter (2): Review of Literature	5
- Chapter (3): published papers	26
- Chapter (4): Discussion	98
- Chapter (5): Conclusion and Recommendations	108
- Chapter (6): Summary	109
- Chapter (7): References	113
- Appendix	138
- Arabic Summary	
- Arabic Abstract	

List of Tables

Table	Content of table	Page
1	Grading of histopathological lesions in testes, liver and kidneys in all groups	36
2	Effect of sodium arsenate and <i>Spirulina</i> co-administration on size of endometrial glands, thickness of endometrium and myometrium in female albino rats	60
3	Effect of sodium arsenate and <i>Spirulina</i> co-administration on the number of ovarian follicles	60
4	Scoring of histopathological lesions in different organs of all experimental groups	88
5	Mean values \pm SD of serum GSH (mmol/l), serum MDA (nmol/ml) among different experimental male groups after 3^{rd} months of the study.	138
6	Mean values \pm SD of serum AST (unit/ml), serum ALT (units/ml) and serum creatinine (mg/dl) among different experimental groups after 3^{rd} months of the study.	139

List of Figures

Figure	Content of figure	Page
1	Effect of arsenic and <i>Spirulina platensis</i> on the body weight among different experimental groups	34
2	Mean values of Testosterone \pm SD among different experimental groups.	35
3	Effect of arsenic and <i>Spirulina platensis</i> on serum GSH and MDA ± SD among different experimental groups	35
4	Effect of arsenic and <i>Spirulina platensis</i> on serum ALT, AST and creatinine ± SD among different experimental groups	36
5	Testis of rat from a) control, untreated group showing the normal histological structure of seminiferous tubules with normal spermatogoneal cells and complete spermatogenesis with sperm production, b) As-treated group showing marked degeneration and necrosis of spermatogoneal cells lining seminiferous tubules, the affected seminiferous tubules became void of cellularity with reduced diameter of seminiferous tubules (short arrow) and interstitial edema (long arrow), c) As-treated group showing marked necrosis, exfoliation and absence of germ cells (short arrow) with formation of spermatid giant cells in the lumen of some seminiferous tubules (long arrow), d) Sp+As co- treated group showing an evidence of maintaining the normal seminiferous tubules with no histopathological alterations and complete spermatogenesis.(H & E scale bar 50 um).	37
6	Liver of rat from a) control group showing the normal histological structure of hepatic lobules, b) arsenic treated group showing Kupffer cell activation and marked cytoplasmic vacuolization of hepatocytes (arrows), c) arsenic treated group showing focal necrosis of hepatocytes associated with mononuclear cell infiltration as well as apoptosis of hepatocytes, d) Sp+As co- treated group showing slight hydropic degeneration of hepatocytes (short arrow) and slight congestion of hepatic sinusoids (long arrow) (H & E scale bar 25 um	38
7	Kidney of rat from a) control group showing the normal histological structure of renal parenchyma, b) arsenic treated group showing congestion of renal blood vessels (short arrow), focal necrosis of epithelial lining renal tubules associated with mononuclear cells infiltration (long arrow) as well as cystic dilatation of some renal tubules (arrow head), c) arsenic treated group showing congestion of renal blood vessel (short arrow) and interstitial peritubular mononuclear inflammatory cells infiltration (long arrow), d) As+Sp co-administered group showing slight congestion of glomerular tufts (arrows). (H & E scale bar 25 um (a, c & d) and 50 um (b).	39
8	Immunostaining of caspase-3 in testes, liver and kidney of, a) control rat showing no caspase-3 immune-reactive cells, b) Sp-treated rat showing no caspase-3 immune-reactive cells. c) arsenic-treated rat showing strong positive strong positive immune expression. d) Sp + As co-treated group showing weak positive caspase-3 expression. e) immunostaining area % of caspase-3 expression. Data shown as mean±SE; error bars show the variations of determinations in terms of standard error. One—way analysis	40

	of variance was used for data analysis (n=5), mean values with unlike	
9	superscript letters were significantly different ($p \le 0.05$). Female average body weight (g) among different experimental groups.	59
10	Serum GSH (mmol/l) and serum MDA (nmol/ml) among different experimental groups.	59
11	Micrograph, uterus, female rat. Control untreated group showing the normal histological structure of the uterus with normal invagination of uterine lumen (white arrow) and normal uterine glands (black arrow) (a). In arsenic treated group the uterus appeared with decreased size and invaginations of the uterine lumen with decreased endometrial glands number (arrow) (b). Recovery was noted in sections of uterus coadministered with <i>Spirulina</i> as the number of uterine glands showed noticeable increase (arrows) (c). (H & E X100).	62
12	Micrograph, ovary, female rat. Control untreated group showing considerable number of ovarian healthy follicles (arrows) (a). In arsenic treated group the ovary showing dramatic decrease in the ovarian follicles (arrow) (b). In groups co-administered with <i>Spirulina</i> , the ovary restored its function with increased healthy ovarian follicles number (arrows) (c). (H & E X40).	63
13	Immunostaining of Nrf2. Arsenic treated group showing strong positive cytoplasmic reaction in stromal cells of uterus (a) and ovary (b). In <i>Spirulina</i> co-treated group, the immunostaining reaction is weak in both uterus (c) and ovary (d). (H & E X400).	64
14	Mean values of Estradiol (E) \pm SD among different experimental groups.	78
15	Mean values of female serum MDA and GSH \pm SD among different experimental groups.	79
16	Mean values of female serum ALT and AST \pm SD among different experimental groups.	80
17	Micrograph, uterus, female rat. Control untreated group showing the normal histological structure of the uterus with normal endometrial lining epithelium (arrow head) and normal uterine glands (arrow) (a). Arsenic treated rat showing decreased invaginations of the uterine lumen with decreased endometrial glands number (b). Co-administration with spirulina showing noticeable increase in the number of uterine glands (c). (H&E X100).	82
18	Micrograph, liver, female rat. Control untreated group showing the normal histological structure of hepatocytes (H), portal blood vessel (BV) and bile ducts (arrow) (a) (H&E X400). Arsenic treated group showing severe sinusoidal dilatation (arrow), sinusoidal leukocytosis and karyomegaly (arrow head) (b) (H&E X400), this group also showing vacuolar degeneration of hepatocytes (c) (H&E X400), portal area showing portal fibrosis with infiltration of mononuclear cells (arrow) and congestion of portal blood vessels (BV) (d) (H&E X200), higher magnification of the previous photo showing hyperplasia of bile ducts (BD) (e) (H&E X400). Spirulina co-treated group showing slight vacuolation of hepatocytes with slight congestion of portal blood vessels and few mononuclear cells infiltration in portal area (f) (H&E X400).	83

10	MTC-stained liver sections. Control group showing no deposition of	0.4
19	collagen in portal area (a) (MTCX400). Arsenic treated group showing	84
	extensive deposition of collagen in portal area which appearing blue in	
	color (arrow) (b) (MTCX400). Spirulina co-treated group showing reduced	
	collagen deposition in portal area (c) (MTCX400)	
10	Micrograph, myocardium, female rat. Control untreated group showing the	05
19	normal histological structure of myocytes (M) and their nuclei (N) (a)	85
	(H&E X600). Arsenic treated group showing myositis with infiltration of	
	mononuclear cell (black arrow), Zenker's necrosis of focal myocytes (white	
	arrow) and congestion of intermyocardial blood vessels (B) (b) (H & E	
	X600). Co-administration with spirulina showing normal structure of	
	myocytes (c) (H&E X400).	
20	Micrograph, lungs, female rat. Control untreated group showing the normal	86
20	histological structure of lung alveoli (A) and bronchiole (B) (a). Arsenic	80
	treated group showing thickening of alveolar wall by mononuclear	
	inflammatory cells infiltration and dilated perialveolar blood capillaries	
	(arrows), there is peribronchiolar mononuclear inflammatory cells	
	infiltration (b). Co-administration with spirulina showing deceased	
	thickness of alveolar wall with few number of mononuclear cells	
	infiltration (c) (H&E X200).	
21	Micrograph, brain, female rat. Control untreated group showing normal	87
21	cerebellar structure with normal Purkinje cells (PC) and granular layer (GL)	07
	(a). Arsenic treated group showing reduction in granular layer (GL) and	
	density of Purkinje cells with vascular congestion in white matter (WM)	
	(b). Co-administration of spirulina with arsenic is showing an improvement	
	in the structure of cerebellum structure (c) (H&E X400). Cerebral cortex of	
	control rat showing clusters of normal nerve cells (d) (H&E X200). Arsenic	
	treated rat showing neurofibrillary tangles in degenerated neurons (arrows)	
	(e) (H&E X200), glial cells proliferation (arrows) (f) (H&E X400). Co-	
	administration of spirulina with arsenic showing an improvement in the	
	structure of cerebral cortex with degeneration of sporadic neurons (g)	
	(H&E X400)	
22	Testis of arsenic intoxicated rat (3 months) showing marked degeneration	141
	and necrosis of spermatogoneal cells lining seminiferous tubules associated	
	with intraluminal spermatid giant cells. Note the affected seminiferous	
	tubules became void of cellularity with reduced diameter of seminiferous	
	tubules. (H & E X 200).	
23	Testis of arsenic intoxicated rat (3 months) showing marked degeneration	141
	and necrosis of spermatogoneal cells lining seminiferous tubules associated	
	with intraluminal spermatid giant cells. (H & E X 400).	
24	Testis of rat co-treated with spiurlina and arsenic (3 months) showing	142
	congestion of interstitial blood vessels. (H & E X 200).	
25	Liver of arsenic intoxicated rat (3 months) showing cytoplasmic	142
	vacuolization of hepatocytes and focal hepatocellular necrosis of	
	centrilobular hepatocytes associated with mononuclear cells infiltration (H	
	& E X 400).	