

# Physiological, Biochemical and Histopathological changes in African catfish (*Clarias gariepinus* ) Exposed to Atrazine Herbicide

# Thesis Submitted in partial Fulfillment for the requirements of the degree of ph.D. of Science in Zoology

#### By Heba Salah Mohamed Hamed

Assistant lecturer of Zoology department, Women's College, Ain Shams university.

#### **Board of Scientific Supervision**

#### **Prof. Dr. Shadia Mohamed Kadry**

Professor and Head of Zoology Department, Women's College for Arts, Science & Education Ain Shams University

#### **Prof. Dr. Mohamed Sayed Marzouk**

Professor of Fish Diseases and Management Department, Faculty of Veterinary Medicine Cairo University

#### **Prof. Dr. Afnan Moustafa Amer**

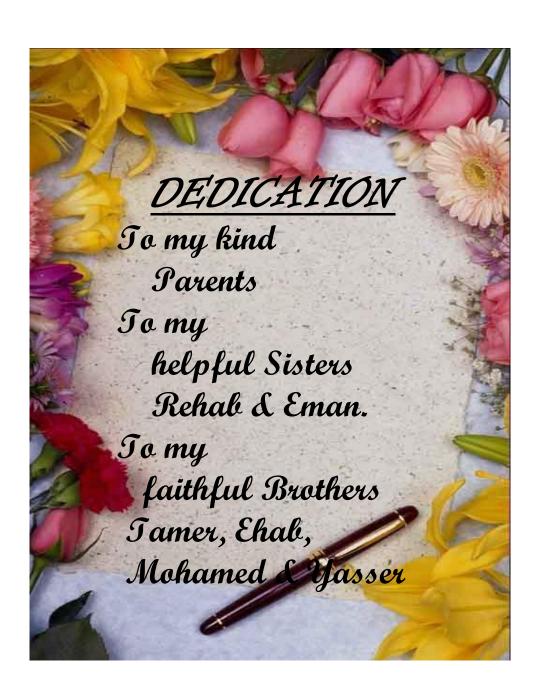
Professor of Physiology , Zoology Department Women's College for Arts, Science & Education Ain Shams University

#### **Dr. Magdy Ibrahim Hanna**

Assistant Professor of Fish Diseases and Management Department, Faculty of Veterinary Medicine Cairo University

#### Dr. Amal Hassan Azmy

Lecturer of Histology, Zoology Department, Women's College for Arts, Science & Education Ain Shams University



## Qualifications

Name : **Heba Salah Mohamed Hamed** 

Scientific Degree : M.Sc.

Department : **Zoology** 

College : Women's College for Arts, Science &

**Education** 

University : Ain Shams University

Graduation year : 2005

### **Courses**

Courses studied by the candidate in partial fulfillment of the requirements for the degree of **M. Sc.** 

- 1- Physiology
- 2- Histology
- 3- Histopathology
- 4- Ecology
- 5- Statistical Analysis
- 6- Scientific English
- 7- Computer



Ain Shams University Women's College for Arts, Science&Education. Zoology Department

#### **APPROVAL SHEET**

This is to approve that the dissertation presented by **Heba Salah Mohamed Hamed** 

to Women's College entitled:

"Physiological, Biochemical and Histopathological changes in African catfish (*Clarias gariepinus*) exposed to atrazine herbicide"

Scinetific Degree: Ph.D. of Science.

### **Board of Scientific Supervisors**

#### Prof. Dr. Shadia Mohamed Kadry

Professor and Head of Zoology Dept., Women's College for Arts, Science &Education, Ain Shams University.

#### Prof. Dr. Afnan Moustafa Ahmed Amer

Professor of Physiology, Zoology Dept., Women's College for Arts, Science &Education, Ain Shams University.

#### Prof. Dr. Mohamed Sayed Mohamed Marzouk

Professor of Fish Diseases and Management,. Faculty of Veterinary Medicine, Cairo University

#### Dr. Magdy Ibrahim Hanna

Assistant Professor of Fish Diseases and Management, Faculty of Veterinary Medicine, Cairo University

#### Dr. Amal Hassan Azmy

Lecturer of Histology, Zoology Dept., Women's College for Arts, Science & Education, Ain Shams University.

Date / / 2012



Praise be to Allah who extend in knowledge whom be wills I am extremely grateful to Allah who gave me the ability to finish this work.

I wish to express my deepest gratitude to **Prof. Dr. Shadia Mohamed Kadry**, Professor and Head of Zoology Department, Women's College for Arts, Science & Education, Ain Shams University, for her careful guidance, supervision, kind encouragement, continuous support and instructive criticism, she suggested the subject, set up the plan and offered brilliant ideas from the start to the end.

I am very thankful to **Prof. Dr. Afnan Moustafa Amer** Professor of Physiology, Zoology Department, Women's College for Arts, Science & Education, Ain Shams University, for her supervision, valuable advice, kindness, endless donation and facilitating all the difficulties while supervising this work.

My grateful thanks and sincere appreciation to **Prof. Dr.**Mohamed Sayed Mohamed Marzouk, Professor of Fish diseases and Management, Faculty of Veterinary Medicine, Cairo University, for his keen supervision, continuous guidance and I must confess that I can not spell success without him.

I am greatly indebted to **Dr. Magdy Ibrahim Hanna**, Assistant Professor of Fish diseases and Management, Faculty of Veterinary Medicine, Cairo University, for his indispensable help, doing his best, guidance, and un failing courtesy which made the completion of the work possible.

My cordial thank for **Dr. Amal Hassan Azmy**, Lecturer of Histology, Zoology Department, Women's College for Arts, Science & Education, Ain Shams University, who helped me in the histopathological investigation of this work.

Finally, special thanks to **My mother & My father** for their love, donation and continuous encouragement.

#### **Abstract**

In recent years, there is wide spread use of atrazine and it is commonly monitored in groundwater and surface water. A study is needed to determine its effects on the aquatic organisms and its potential effects as an environmental contaminant. In this study, the half lethal concentration (LC<sub>50</sub>) of atrazine for female catfish, *Clarias gariepinus* (*C. gariepinus*) was calculated. Atrazine toxicity was evaluated through acute and chronic exposures of fish to 1/2 and 1/10 the calculated LC<sub>50</sub> for 4 days and 6 weeks respectively. As well as, the evaluation of role of vit. E in preventing or alleviating the induced defects. Results showed that the LC<sub>50</sub>/96h was 13.75 mg/l. The effects of both acute and chronic exposures revealed clinical abnormalities which were manifested by loss of appetite, sluggish or restlessness swimming, rapid opercular movements and abnormal skin pigmentation in the form of faded skin. Moreover, the biometric parameters showed highly significant decrease in body weight and gonad weight but the liver weight of atrazine exposed fish was significantly increased. In addition, the results of absolute fecundity showed highly significant decrease in (T.R. Egg No.). The results also revealed a marked decrease in RBCs, Hb content, MCV, MCH, MCHC and WBCs. Where as, significant increase in AST and ALT activities, creatinine, uric acid, glucose and serum cortisol levels were recorded. Furthermore, atrazine exposures were associated with an inhibition of cholinesterase activity. Marked decrease in estradiol, testosterone levels and total protein concentration in serum with a reduction in both albumin and globulin were also recorded. Results also showed a significant induction of oxidative damage in liver tissue as evidenced by increased level of lipid peroxidation (LPO) and reduced glutathione content (GSH). In addition, significant increase in the activities of catalase (CAT) and superoxide dismutase (SOD). The histopathological alterations of different organs of the exposed fish were recorded. Additionally, the supplementation of vit.E can reduce the incidence of growth retardation and can improve the haematological, biochemical and histological abnormalities in female catfish, C. gariepinus exposed to atrazine in relation to fish exposed to atrazine only. This may be related to successive reduction of the oxidative stress, caused by atrazine toxicity.

*Keywords:* Atrazine, Freshwater fish, vit.E, LC<sub>50</sub>, Clinical signs, Biometric parameters, Fish fecundity, Blood parameters, Biochemical parameters, Oxidative stress, and Histopathological changes.

## **List of Contents**

P	age
LIST OF ABBREVIATIONS	Ι
LIST OF TABLES	V
LIST OF FIGURES	IX
1. INTRODUCTION AND AIM OF THE WORK	1
2. REVIEW OF THE LITERATURE	8
(1) Classification of pesticides	8
(2)Determination of half lethal concentration (LC <sub>50</sub> )	10
(3)Clinical signs and behavioural effects	12
(4)Biometric parameters and reproductive performance of	
female Clarias gariepinus fish	15
(5)Blood parameters	21
(6)Liver function	24
(7) kidney function	27
(8)Total proteins	29
(9) Stress indicators	32
(I) Achetylcholinesterase (AchE)	32
(II) Glucose	35
(III)Cortisol hormone	38
(10) Effect of atrazine on sexual hormones activity of	
exposed fish	40
(11) Oxidative stress and antioxidant enzymes	43
(I)Lipid peroxidation (LPO)	43
(II) Glutathione content (GSH)	47
(III)Antioxidant enzymes	49

## **List of Contents (Cont.)**

Page
(12) Histopathological changes54
(13) Vitamin E64
(14) Public health hazard65
3. MATERIALS AND METHODS68
(I)Materials68
(II)Methods74
- Determination of 96 hours half lethal concentration
(LC <sub>50</sub> ) of atrazine74
- Experimental design
- Clinical investigation and post mortem
examination76
- Biometric parameters of female C. gariepinus
fish
- Estimation of fish fecundity77
- Haematological studies79
- Serum biochemical analysis80
- Oxidative stress and antioxidant enzymes84
- Histopathological studies86
- Statistical Analysis86
4. RESULTS87
5. DISCUSSION AND CONCLUSION290
6. SUMMARY324
7. REFERENCES
ARARIC SUMMARY

## **List of Abbreviations**

%	Percent
°C	Degree selezius
μ /Ι	Micro per liter
μg/dl	Microgram per deciliter
μg/kg	Microgram per kilogram
μg/l	Microgram per liter
μm	Micrometer
11-KT	11-Ketotestosterone
2,4- D	2,4- Dichlorophenoxyacetic acid
A/G ratio	Albumin/globulin ratio
AAP	4-aminophenazone
AchE	Acetylcholinestrase
ACTH	Adrenocorticotropic hormone
ALP	Alkaline phosphatase
ALT	Alanine amino transferase
AMT	Aminotriazole
ANTU	A-Naphthyl thiourea
ASDI	Atrazine, simazine, diuron and isoproturon
AST	Aspartate amino transferase
ATR	Atrazine
B.L.	<b>Body length</b>
B.Wt.	Body weight
CAMP	Cyclic adenosine monophosphate
CAT	Catalase
CBZ	Carbamazepine
CF	Condition factor
Cm	centimeter
CMS	Carboxy methyl cellulose
COPECs	Chemicals of potential ecological concern
CRF	Corticotropin releasing factor
DbcAMP	Dibutyryl-Camp
DCA	3,4-Dichloroaniline

## List of Abbreviations (Cont.)

	List of Appleviations (Cont.)
Dep. Eq.	Dependence equation
DFB	Diflubenzuron
DHBS	3,5 Dichloro-2-hydroxy benzene sulfonic acid
DNA	Deoxyribonucleic acid
DTNB	5,5; dithiobis(2-nitrobenzoic acid)
E.P.N.	Ethyl-p- nitrophenyl-thionobenzene, phosphonate
$\mathbf{E_2}$	17-ß estradiol
<b>EDCS</b>	<b>Endocrine disrupting chemicals</b>
EPA	<b>Environment protection Agency</b>
FBL	Fecundityrelated to body length
FBW	Fecundity related to body weight
fl	Femoliter
FOW	Fecundity related to gonadal weight
g	Gram
G.F.R.	Glomerular filtration rate
g/ dl	Gram per deciliter
g/l	Gram per liter
Goal	2,4-D-oxyfluorfen
GSH	Glutathione content
GSI	Gonadosomatic index
GSSG	Oxidized glutathione
GW	Gonad weight
H	Hour
H and E	Hematoxylin and Eosin
$H_2O_2$	Hydrogen per oxide
H <sub>2</sub> O	Water
Hb	Haemoglobin
HRP	Peroxidase
HSI	Hepatosomatic index
Ht	Haematocrite
$I_{G}$	Gonadosomatic index
I <sub>H</sub>	Hepatosomatic index
K	Condition factor
$LC_{50}$	Half lethal concentration

## List of Abbreviations (Cont.)

LPO	Lipid peroxidation
LSI	Liver somatic index
LW	Liver weight
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
MDA	Medical Devices Agency
MDA	Malondialdhyde
mg	Milligramper liter
mg / Kg bw	Milligram per kilogram body weight
mg/dl	Milligram per deciliter
Mg Hg/l	Milligram mercury per liter
mg/g tissue	Milligram per gram tissue
mg/gww	Milligram per gram weight
mg/l	Milligram per liter
Min.	Minute
Ml	Milliliter
Ml /l	Milliliter per liter
Ml/min	Milli per minute
n mol/g tissue	Nano mol per gram tissue
Nacl	Sodium chloride
ng /l	Nanogram per liter
ng /ml	Nanogram per milli
nm	Nanometer
O.W.	Ovarian weight
$O_2$	Oxygen
$O_2$	Superoxide anions
$\mathbf{O_2}$	Oxygen free radical
$O_2$	Oxygen free radical
PCV	Packed cell volume
Pg/cell	Pico gram/cell
PIP	Pesticide Information Profiles

## List of Abbreviations (Cont.)

PM	Postmortem
PPb	Part per billion
PPm	Part per million
PQ	Gramoxone herbicide
RBCs	Red blood cells
R.Egg	Ripened egg
ROS	Reactive oxygen species
rpm	Revolution per minute
RUP	Restricted Use Pesticide
SOD	Superoxide dismutase
Sol.	Solution
ß-carotene	Beta carotene
SW	Spleen weight
T	Testosterone hormone
T.R.Egg No.	Total ripened egg number
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substance
U/g tissue	Micro per gram tissue
Vit. E	Vitamin E
VTG	Vitellogenin
WBCs	White blood cells
W.	Week
$\mathbf{W}_{\mathbf{G}}$	Gonadal weight
$\mathbf{W}_{\mathbf{H}}$	Hepatic weight
WHO	World Health Organization
α- tocopherol	Alpha tocopherol

## List of Tables

1 2 3 4 5 6 7	Title  The composition analysis of 10 Kg of basal diet (32% protein).  Half lethal concentration (LC <sub>50</sub> ) of atrazine in female <i>Clarias gariepinus</i> Comparative reproductive parameters of female <i>C. gariepinus</i> exposed to 1/2 LC <sub>50</sub> of atrazine for 4 days:  Effect of chronic exposure to atrazine and treatment with vit.E on B.L. values (cm) of female <i>C. gariepinus</i> .  Effect of chronic exposure to atrazine and treatment with vit.E on B.Wt. values (g) of female <i>C. gariepinus</i> .  Effect of chronic exposure to atrazine and treatment with vit.E on W <sub>G</sub> values (g) of female <i>C. gariepinus</i> .	87 117 124 126
2 3 4 5	Half lethal concentration (LC <sub>50</sub> ) of atrazine in female <i>Clarias gariepinus</i> Comparative reproductive parameters of female <i>C. gariepinus</i> exposed to 1/2 LC <sub>50</sub> of atrazine for 4 days:  Effect of chronic exposure to atrazine and treatment with vit.E on B.L. values (cm) of female <i>C. gariepinus</i> .  Effect of chronic exposure to atrazine and treatment with vit.E on B.Wt. values (g) of female <i>C. gariepinus</i> .	87 117 124 126
3 4 5	Clarias gariepinus  Comparative reproductive parameters of female <i>C. gariepinus</i> exposed to 1/2 LC <sub>50</sub> of atrazine for 4 days:  Effect of chronic exposure to atrazine and treatment with vit.E on B.L. values (cm) of female <i>C. gariepinus</i> .  Effect of chronic exposure to atrazine and treatment with vit.E on B.Wt. values (g) of female <i>C. gariepinus</i> .  Effect of chronic exposure to atrazine and treatment with	117 124 126
3 4 5	Clarias gariepinus  Comparative reproductive parameters of female <i>C. gariepinus</i> exposed to 1/2 LC <sub>50</sub> of atrazine for 4 days:  Effect of chronic exposure to atrazine and treatment with vit.E on B.L. values (cm) of female <i>C. gariepinus</i> .  Effect of chronic exposure to atrazine and treatment with vit.E on B.Wt. values (g) of female <i>C. gariepinus</i> .  Effect of chronic exposure to atrazine and treatment with	117 124 126
5 6	Effect of chronic exposure to atrazine and treatment with vit.E on B.L. values (cm) of female <i>C. gariepinus</i> .  Effect of chronic exposure to atrazine and treatment with vit.E on B.Wt. values (g) of female <i>C. gariepinus</i> .  Effect of chronic exposure to atrazine and treatment with vit.E on B.Wt. values (g) of female <i>C. gariepinus</i> .	124
5	Effect of chronic exposure to atrazine and treatment with vit.E on B.L. values (cm) of female <i>C. gariepinus</i> .  Effect of chronic exposure to atrazine and treatment with vit.E on B.Wt. values (g) of female <i>C. gariepinus</i> .  Effect of chronic exposure to atrazine and treatment with	126
6	vit.E on B.Wt. values (g) of female <i>C. gariepinus</i> .  Effect of chronic exposure to atrazine and treatment with	
	•	128
7		
	Effect of chronic exposure to atrazine and treatment with vit.E on $W_H$ values (g) of female $\emph{C. gariepinus}$ .	130
8	Effect of chronic exposure to atrazine and treatment with vit.E on morphoanatomical parameter ( $I_G$ ) values of female $\emph{C. gariepinus}$ .	132
9	Effect of chronic exposure to atrazine and treatment with vit.E on morphoanatomical parameter ( $I_H$ ) values of female $\emph{C. gariepinus}$ .	134
10	Effect of chronic exposure to atrazine and treatment with vit.E on morphoanatomical parameter (CF) values of female <i>C. gariepinus</i> .	136
11	Effect of chronic exposure to atrazine and treatment with vit.E on relative fecundity (FBL) values of female <i>C. gariepinus</i> .	138
12	Effect of chronic exposure to atrazine and treatment with vit.E on relative fecundity (FBW) values of female <i>C. gariepinus</i> .	140

## List of Tables(Cont.)

No.	Title	Page
13	Effect of chronic exposure to atrazine and treatment with vit.E on relative fecundity (FOW) values of female <i>C. gariepinus</i> .	142
14	Effect of chronic exposure to atrazine and treatment with vit.E on relative fecundity (Dep.Eq) values of female <i>C. gariepinus</i> .	144
15	Effect of chronic exposure to atrazine and treatment with vit.E on absolute fecundity (T.R.Egg NO.) values of female <i>C. gariepinus</i> .	146
16	Effect of acute exposure to atrazine on WBC <sub>S</sub> $(10^3/\text{mm}^3)$ count of female <i>C. gariepinus</i> .	148
17	Effect of acute exposure to atrazine on RBC <sub>S</sub> count $(10^6/\text{mm}^3)$ , Hb content (g/dl) and Ht (%) values of female <i>C.</i> gariepinus.	150
18	Effect of acute exposure to atrazine on blood indices of female <i>C. gariepinus</i> .	152
19	Effect of chronic exposure to atrazine and treatment with vit.E on WBC <sub>S</sub> count (10 <sup>3</sup> /mm <sup>3</sup> ) of female <i>C. gariepinus</i> .	154
20	Effect of chronic exposure to atrazine and treatment with vit.E on RBC <sub>S</sub> count (10 <sup>6</sup> /mm <sup>3</sup> ) of female <i>C. gariepinus</i> .	156
21	Effect of chronic exposure to atrazine and treatment with vit.E on Hb values (g/dl) of female <i>C. gariepinus</i> .	158
22	Effect of chronic exposure to atrazine and treatment with vit.E on Ht values (%) of female <i>C. gariepinus</i> .	160
23	Effect of chronic exposure to atrazine and treatment with vit.E on MCV values (fl) of female <i>C. gariepinus</i> .	162
24	Effect of chronic exposure to atrazine and treatment with vit.E on MCH values (Pg/cell) of female <i>C. gariepinus</i> .	164
25	Effect of chronic exposure to atrazine and treatment with vit.E on MCHC values (µg/dl) of female <i>C. gariepinus</i> .	166
26	Effect of acute exposure to atrazine on liver function of female <i>C. gariepinus</i> .	168