# Biochemical studies of some plant extracts on glutathione and its related enzymes in the desert locust (Schistocerca gregaria)

# Thesis Submitted for the award of the Ph.D. Degree in Biochemistry

Presented By

## **Ghada Soliman Ahmed Abdel Karim**

M.Sc. in Biochemistry (2010)

### Supervised by

#### **Dr. Tahany Mahmoud Maharem**

Professor of Biochemistry Biochemistry Department Faculty of Science Ain-Shams University

#### Dr. Manal Asem Emam

Assistant Professor of Biochemistry Biochemistry Department Faculty of Science Ain-Shams University

#### Dr. Ragaa Reda Hamed

Professor of Biochemistry Molecular Biology Department Genetic Engineering and Biotechnology Division National Research Centre

#### Dr. Rasha Awni Guneidy

Assistant Professor of Biochemistry
Molecular Biology Department
Genetic Engineering and
Biotechnology Division
National Research Centre

To Biochemistry Department Faculty of Science Ain-Shams University 2017

# Biochemical studies of some plant extracts on glutathione and its related enzymes in the desert locust (Schistocerca gregaria)

## **Board of Scientific Supervision**

## Dr. Tahany Mahmoud Maharem

Professor of Biochemistry, Biochemistry Department Faculty of Science, Ain-Shams University

## Dr. Ragaa Reda Hamed

Professor of Biochemistry, Molecular Biology Department Genetic Engineering and Biotechnology Division, National Research Centre

### Dr. Manal Asem Emam

Assistant Professor of Biochemistry, Biochemistry Department Faculty of Science, Ain-Shams University

# Dr. Rasha Awni Guneidy

Assistant Professor of Biochemistry, Molecular Biology Department Genetic Engineering and Biotechnology Division, National Research Centre

# Acknowledgement

I would like to express my sincere gratitude to, **Prof. Dr.**Tahany Mahmoud Maharem, Professor of Biochemistry,

Biochemistry Department, Faculty of Science, Ain-Shams

University for her limitless help, close supervision, continuous guidance, constructive criticism and significant contribution to the manuscript writing

I would like to express my sincere and cordial appreciation to **Prof. Or. Ragaa Reda Hamed**, Professor of Biochemistry, Molecular Biology Department, National Research Centre, for her close supervision, kind co-operation, support and encouragement. Her helpful and unlimited support and for all the valuable things I learned from her. It was a great honor to work under her supervision.

I am greatly indebted to **Dr. Rasha Awny Gunidi**, Assistant Professor of Biochemistry, Molecular Biology Department, National Research Centre, for her valuable assistance in the practical part, and her cooperation throughout this work.

Many thanks go in particular to **Dr. Manal Asem Emam**, Assistant Professor of Biochemistry, Biochemistry Department, Faculty of Science, Ain-Shams University for her support and guidance throughout the work.

I would also like to thank all my colleagues in Molecular Biology Department, National Research Centre, for their help and support during my work.

Deepest gratitude is indebted to my family for their help and continuous encouragement. I wouldn't be here, if I didn't have them beside

# **List of Contents**

	Page
Abstract	I
List of Figures	III
List of Tables	VII
List of abbreviations	X
Aim of work	XII
Introduction	1
1- Grasshopper life cycle	3
2- Control of grasshopper	4
3- Detoxification system	7
4- Antioxidant enzymes	8
5- Phenolic compounds	17
5.1- Flavonoids	18
5.2- Stilbenes	26
5.3- Coumarins	26
5.4- Tannins	27
Materials and Methods	30
Materials	30
Chemicals	30
Buffers	30
Plant material	31
Insects	31
Methods	31
1- Preparation of plant extracts	31
2- Preparation of insect GST cytosolic fraction	32

3-Analytical methods	33
1- Protein determination	33
2- Glutathione determination	35
3- Determination of total phenolic content	37
4- Determination of the total flavonoid content	39
5- Determination of total anthocyanin	41
6- Determination of the antioxidant capacity	43
7- Determination of the hydrolysable tannins	45
8- Determination of total condensed tannins	47
9- High performance liquid chromatography	
analysis	49
4- Enzyme assays	53
1- Glutathione S-transferase	53
2- Glutathione S- transferase assay using other	
substrates	54
3- Glutathione peroxidase	56
4- Glutathione reductase	57
5- Catalase	58
5- Column chromatography	59
1- Preparation of GSH-Sepharose affinity matrix	59
2- GSH-Sepharose column chromatography	60
3- DEAE-Sepharose column chromatography	60
6- Gel electrophoresis	61
1- Native polyacrylamide gel electrophoresis.	61
2-Sodium dodecyl sulfate polyacrylamide gel	
electrophoresis	63

7- Biochemical characterization of GSTs purified	
from S. gregaria ovary and fat body	67
1- Determination of Michaelis constant and	
Maximal velocity	67
2- Inhibition studies	67
8- Statistical analysis	68
Results	70
1-Glutathione and its related enzymes in the	
developed instars and different organs of S.	
gregaria	70
2-Effect of some Umbelliferae, Leguminosae and	
Malvacceae plant extracts on GST activity of	
different S. gregaria organs crude homogenates	77
3-Bioactive compounds of the most effective plant	
extracts on GST activity of S. gregaria organs	85
4-Purification of GST enzymes from S. gregaria fat	
body	94
5-Glutathione and its related enzymes in the adult <i>S</i> .	
gregaria ovary on different development stages	121
6-Purification of GST from <i>S. gregaria</i> ovary	123
Discussion	142
Summary	173
References	182
Arabic Summary	
Arabic Abstract	

### **Abstract**

Ghada Soliman Ahmed, Biochemical studies of some plant extracts on glutathione and its related enzymes in the desert locust (*Schistocerca gregaria*)

Ph. D. Thesis: Biochemistry Department, Faculty of Science, Ain-Shams University.

The present study has focused on the investigation of the efficiency of the naturally occurring phenolic compounds extracted from some plants (Family: Umbellifera, Leguminosae and Malvacceae) on the *Schistocerca gregaria* glutathione Stransferase (GST) as a selective GST inhibitors. The second section of this study has focused on the purification and characterization of the major GST from *S. gregaria* fat body and ovary.

Comparing all the plant extracts for their IC<sub>50</sub> values, ethanolic and aqueous extracts of *Glycyrrhiza glabra* and *Hibiscus sabdriffa* were the most effective inhibitors for *S. gregaria* fat body and ovary GSTs. Simple reproducible procedure for the purification of *S. gregaria* fat body GST was established using GSH-Sepharose and DEAE-Sepharose columns. While for *S. gregaria* ovary, one step purification was carried out using GSH-Sepharose column. The purified enzymes proved to be homogenous as judged by polyacrylamide gel

electrophoresis and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, where one band could be detected.

Kinetic studies for both fat body and ovary GSTs display a typical Michaelis-behavior with respect to CDNB and GSH as substrates. Characterization of both fat body and ovary GSTs including optimum pH, substrate specificity and inhibitors effect were carried out. The effect of ethanolic and aqueous extracts of *G. glabra* and *H. sabdriffa* on purified fat body and ovary GSTs activity was carried out. Also the effect and the type of inhibition of quercetin and delphinidine chloride (the major component of *G. glabra* and *H. sabdriffa*, respectively) were carried out.

**Key words:** Schistocerca gregaria, phenolic compounds, glutathione S-transferase, fat body, ovary, delphinidine chloride, quercetin.

# **List of Figures**

Fig.	(No.)	P	
Fig.	(1):	Grasshopper life cycle	5
Fig.	(2):	Antioxidant enzymes and GSH redox system	10
Fig.	(3):	Schematic classification of phenolic compounds, including phenolic acids and polyphenols	19
Fig.	(4):	Structural diversity of flavonoids	22
Fig.	(5):	Common anthocyanin structure and corresponding anthocyanidins	25
Fig.	(6):	Chemical structure of tannic acid, type of tannins	28
Fig.	<b>(7):</b>	Standard curve of protein	34
Fig.	(8):	Standard curve of glutathione	36
Fig.	(9):	Standard curve of gallic acid	38
Fig.	(10):	Standard curve of rutin	40
Fig.	(11):	Standard curve of tannic acid.	46
Fig.	(12):	Standard curve of catechin.	48
Fig.	(13):	HPLC profile of the standard mixture of phenolic compounds	51

Fig. (14):	HPLC profile of (a) cyanidin 3-O-glucoside chloride	52
	and (b) delphinidine HCl as standard	
Fig. (15):	HPLC profile of 18 α-glycyrrhetinic acid as standard	52
Fig. (16):	HPLC profile of phenolic compounds in ethanolic	96
	extract of <i>H. sabdariffa</i>	
Fig. (17):	HPLC profile of phenolic compounds in aqueous	97
	extract of <i>H. sabdariffa</i>	
Fig. (18):	HPLC profile of anthocyanin compounds in ethanolic	98
	extract of <i>H. sabdariffa</i>	
Fig. (19):	HPLC profile of anthocyanin compounds in aqueous	98
	extract of <i>H. sabdariffa</i>	
Fig. (20):	HPLC profile of phenolic compounds in ethanolic	99
	extract of G. glabra	
Fig. (21):	HPLC profile of phenolic compounds in aqueous	100
	extract of G. glabra	
Fig. (22):	HPLC profile of ethanolic extract of <i>G. glabra</i> at 254 nm	101
Fig. (23):	HPLC profile of aqueous extract of G. glabra at 254 nm	101
Fig. (24):	Typical elution profile of S. gregaria fat body crude	104
	homogenate on GSH-Sepharose column	
Fig. (25):	Typical elution profile of the GSH- Sepharose affinity	105
	pooled fractions of S. gregaria fat body GSTs on	
	DEAE Sepharose column chromatography	

Fig. (26):	Native-PAGE (7%) for <i>S. gregaria</i> fat body GST	106
Fig. (27):	SDS-PAGE (12%) for <i>S. gregaria</i> fat body GST	107
Fig. (28):	Effect of pH on the purified fat body GST1	108
Fig. (29):	(a) Michaelis -Menten plot, (b) Lineweaver-Burk plot	110
	of the purified fat body GST1 with GSH as substrate	
Fig. (30):	(a) Michaelis -Menten plot, (b) Lineweaver-Burk plot	111
	of the purified fat body GST1 with CDNB as substrate	
Fig. (31):	Determination of IC <sub>50</sub> for bromosulphophthalein (a),	114
	ethacrynic acid (b) and cibacron blue (c) as inhibitors	
Fig. (32):	Effect of different concentrations from quercetin (a),	117
	delphinidine chloride (b) and rutin (c) on GST1 activity	
Fig. (33):	Lineweaver-Burk plot of the purified GST1 with	118
	respect to (a) GSH and (b) CDNB as substrates in the	
	presence of 2.23 μM quercetin as inhibitor	
Fig. (34):	Lineweaver-Burk plot of the purified GST1 with	119
	respect to (a) GSH and (b) CDNB as substrates in the	
	presence of 3.88 μM delphinidine chloride as inhibitor	
Fig. (35):	Typical elution profile for the affinity chromatography	124
	of S. gregaria ovary GST on GSH-Sepharose column	
Fig. (36):	Native-PAGE (7%) for S. gregaria ovary GST, (a)	126
	stained for protein and (b) stained for GST activity	
		_

Fig. (37):	SDS-PAGE (12%) for <i>S. gregaria</i> ovary GST	127
Fig. (38):	Effect of pH on the affinity purified ovary GST	128
Fig. (39):	(a) Michaelis -Menten plot, (b) Lineweaver-Burk plot	130
	of the purified ovary GST with GSH as substrate	
Fig. (40):	(a) Michaelis -Menten plot, (b) Lineweaver-Burk plot	131
	of the purified ovary GST with GSH as substrate	
Fig. (41):	Inhibition of purified ovary GST as a function of the	134
	concentration of (a) hematin and (b) cibacron blue	
Fig. (42):	Inhibition of purified ovary GST as a function of the	135
	concentration of (a) bromosulphophthalein and (b)	
	ethacrynic acid	
Fig. (43):	Inhibition of GST as a function of the concentration of	138
	quercetin	
Fig. (44):	Inhibition of GST as a function of the concentration of	138
	delphinidine HCl	
Fig. (45):	Lineweaver-Burk plot of affinity purified ovary GST	139
	with respect to (a) GSH and (b) CDNB as substrates in	
	the presence of quercetin	
Fig. (46):	Lineweaver-Burk plot of affinity purified ovary GST	140
	with respect to (a) GSH and (b) CDNB as substrates in	
	the presence of delphinidine HCl	

# **List of Tables**

Table (No.)	Table (No.)	
<b>Table (1):</b>	Anti-insect effects of some flavonoid	23
<b>Table (2):</b>	Conditions for the spectrophotometric assays of	55
	GST activity using various aromatic substrates	
<b>Table (3):</b>	Preparation of SDS-PAGE gels	66
<b>T</b> 11 (4)		
<b>Table (4):</b>	Glutathione concentration and the catalytic	71
	activity of its related enzymes in different S.	
	gregaria developed instars	
<b>Table (5):</b>	Glutathione concentration and the catalytic	75
	activity of its related enzymes in adult S. gregaria	
	organs	
<b>Table (6):</b>	GST Substrates specificity of different S. gregaria	76
	organ homogenates	
<b>Table (7):</b>	Total phenolic and flavonoid contents of some	79
	Umbelliferae, leguminosae and Malvacceae plants	
<b>Table (8):</b>	Effect of different plant extracts on GST activity	81
	of S. gregaria ovary crude homogenate	
<b>Table (9):</b>	Effect of different plant extracts on GST activity	82
	of S. gregaria fat body crude homogenate	
<b>Table (10):</b>	Effect of different plant extracts on GST activity	83
	of S. gregaria mid gut crude homogenate	

<b>Table (11):</b>	Effect of different plant extracts on GST activity	84
	of S. gregaria hind gut crude homogenate	
<b>Table (12):</b>	Bioactive compounds of the aqueous extract for	90
	the four plants	
<b>Table (13):</b>	Bioactive compounds of the ethanolic extract for	91
	the four plants	
<b>Table (14):</b>	Characterization of phenolic compound extracted	95
	from <i>H. sabdariffa</i> and <i>G. glabra</i>	
<b>Table (15):</b>	Summary of the purification of GST from S.	103
	gregaria fat body crude homogenate	
<b>Table (16):</b>	Substrate specificity of the purified GST1 from	112
	S. gregaria fat body	
<b>Table (17):</b>	The IC <sub>50</sub> of some inhibitors on the activity of the	113
	purified GST1 from S. gregaria fat body	
<b>Table (18):</b>	Effect of G. glabra and H. sabdriffa extracts on	115
	the purified GST1 activity of <i>S. gregaria</i> fat body	
<b>Table (19):</b>	$K_{\text{m}}$ and $V_{\text{max}}$ for the purified GST1 with GSH and	120
	CDNB as a substrate, in presence and absence of	
	inhibitors	
<b>Table (20):</b>	Gonad somatic index of adult S. gregaria females	122
	during development	
<b>Table (21):</b>	Glutathione and its related enzymes in ovary of	122
	adult S. gregaria during development	
<b>Table (22):</b>	Summary of the purification of GST from	123
	S. gregaria ovary on GSH-Sepharose affinity	
	column	