

**EFFECTS OF *Cnidoscolus aconitifolius* LEAF EXTRACTS ON
MONOSODIUM GLUTAMATE (MSG)-INDUCED OXIDATIVE
STRESS IN RATS**

EVBUOMWAN, SARAH ADESUWA

(19PCP02017)

SEPTEMBER, 2021

**EFFECTS OF *Cnidioscolus aconitifolius* LEAF EXTRACTS ON
MONOSODIUM GLUTAMATE (MSG)-INDUCED OXIDATIVE
STRESS IN RATS**

BY

**EVBUOMWAN, SARAH ADESUWA
(19PCP02017)
B.Sc Biochemistry, Caleb University, Lagos**

**A DISSERTATION SUBMITTED TO THE SCHOOL OF POST
GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE AWARD OF THE DEGREE OF
MASTERS OF SCIENCE (M.Sc) IN BIOCHEMISTRY IN THE
DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF SCIENCE AND
TECHNOLOGY, COVENANT UNIVERSITY.**

SEPTEMBER, 2021

ACCEPTANCE

This is to attest that this dissertation is accepted in partial fulfilment of the requirement for the award of the degree of Master of Science in Biochemistry in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria.

Mr. John A. Philip

(Secretary, School of Postgraduate Studies)

Signature & Date

Prof. Akan B. Williams

(Dean, School of Postgraduate Studies)

Signature & Date

DECLARATION

I, EVBUOMWAN, SARAH ADESUWA (19PCP02017) declares that this research was carried out by me under the supervision of Dr. Omolola E. Omotosho of the Department of Biochemistry, Covenant University, Ota, Ogun State, Nigeria. I attest that the thesis has not been presented either wholly or partly for the award of any degree elsewhere. All the sources of data and scholarly information used in this dissertation are duly acknowledged.

EVBUOMWAN, SARAH ADESUWA

Signature & Date

CERTIFICATION

We certify that this dissertation titled **“EFFECTS OF *Cnidoscolus aconitifolius* LEAF EXTRACTS ON MONOSODIUM GLUTAMATE (MSG)-INDUCED OXIDATIVE STRESS IN RATS”** is an original research carried out by **EVBUOMWAN, SARAH ADESUWA (19PCP02017)** in the Department of Biochemistry, College of Science and Technology, Covenant University, Ota, Ogun State, Nigeria, under the supervision of **Dr. Omolola E. Omotosho**. We have examined and found the work acceptable as part of the requirements for the award of a degree of Master of Science in Biochemistry.

Dr. Omolola E. Omotosho

(Supervisor)

Signature & Date

Prof. Israel S. Afolabi

(Head, Department of Biochemistry)

Signature & Date

Prof. Joshua O. Ajele

(External Examiner)

Signature & Date

Prof. Akan B. Williams

(Dean, School of Postgraduate Studies)

Signature & Date

DEDICATION

This research work is greatly dedicated to the Lord God Almighty the giver of life and source of all inspiration for His grace, love, favour, strength and protection during the course of and after this research.

ACKNOWLEDGEMENTS

I greatly appreciate God for everything He did during the course of this research. I acknowledge the Chancellor, Vice Chancellor and the entire Management team of Covenant University for their commitment towards raising a new generation of World leaders. I appreciate my Supervisor, Dr. Omotosho Omolola for her time, efforts and tutelage during the course of this research. I also appreciate the Faculty members of the Department of Biochemistry for their corrections and helpful remarks. I am highly indebted to all the members of the Department of Biochemistry, Covenant University, Ota who helped me all through the progress of this research especially Mr Adeyemi, Mrs Afolabi, Miss Bose Adegboye, Mrs Akinlabu and Mr Micheal Ezekiel for their assistance. I also acknowledge the efforts and guidance of Dr S.O Rotimi throughout the period of the research work. My final and profound gratitude goes to my parents Elder and Elder (Mrs) Michael and Helen Evbuomwan for their physical, spiritual, moral, emotional and financial support. My siblings Mr Osazuwa Evbuomwan and Dr Ehimwenma Evbuomwan are not left out, I really appreciate all your efforts, assistance and encouragements. Finally to my son, Oluwademiladeogo who motivates me to do better, I love you so much and I am proud of you. Thank you.

TABLE OF CONTENT

CONTENTS	PAGES
COVER PAGE	i
TITLE PAGE	ii
ACCEPTANCE	iii
DECLARATION	iv
CERTIFICATION	v
DEDICATION	vi
ACKNOWLEDGEMENTS	vii
TABLE OF CONTENT	viii
LIST OF FIGURES	xii
LIST OF TABLES	xiii
LIST OF ABBREVIATIONS	xiv
ABSTRACT	xiv
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background of the Study	1
1.2 Statement of Research Problem	3
1.3 Justification of the Study	3
1.4 Aims and Objectives	4
CHAPTER TWO	5
LITERATURE REVIEW	5
2.1 Monosodium Glutamate (MSG)	5
2.1.1 History	6
2.1.2 Production of MSG	7
2.1.3 Toxic Effects of Monosodium Glutamate (MSG)	8
2.1.3.1 Obesity and Diabetes	8
2.1.3.2 Oxidative stress and Hepatotoxicity	8
2.1.3.3 Neurotoxicity	9
2.1.3.4 Genotoxicity	10
2.1.3.5 Reproductive toxicity	10
2.1.3.6 Monosodium Glutamate Poisoning	11
2.2 Free Radicals	11

2.2.1 Free Radical Formation in the human body	11
2.2.2 Physiological roles of Free Radicals	12
2.3.1 Detrimental Effects of Oxidative Stress to Human Health	13
2.3.1.2 CardiovascularDiseases	14
2.3.1.3 Neurological Diseases	14
2.3.1.4 Respiratory Diseases	15
2.3.1.5 Rheumatoid Arthritis	15
2.3.1.6 Kidney Disease.....	15
2.3.1.7 Delayed Sexual Maturation/Puberty.....	15
2.3.1.8 Fetal Growth Restriction.....	15
2.4 Antioxidants and Free Radicals	16
2.4.1 Levels of Action of Antioxidants	16
2.4.1.1 First line defense antioxidants.....	16
2.4.1.2 Second line defense antioxidants	16
2.4.1.3 Third line defense antioxidants	17
2.4.1.4 Fourth line defense antioxidants	17
2.5 Antioxidant classification	17
2.5.1 Endogenous Enzymatic Antioxidants	17
2.5.1.1 Superoxide dismutase (SOD, EC 1.15.1.1)	17
2.5.1.2 Catalase (CAT, EC 1.11.1.6)	18
2.5.1.3 Glutathione peroxidases (GPx, EC 1.11.1.9).....	19
2.5.2 Endogenous Non-enzymatic antioxidants.....	20
2.5.2.1 Glutathione.....	20
2.5.2.2 Coenzyme Q10.....	20
2.5.2.3 Melatonin	20
2.5.2.4 Uric acid.....	21
2.5.3 Dietary Antioxidants.....	21
2.5.4 Other Dietary Antioxidants.....	22
2.5.4.1 Beta-carotene:.....	22
2.5.4.2 Lycopene	22
2.5.4.3 Flavonoids	22
2.5.4.4 Omega-3 and omega-6 fatty acids:	22
2.6 Medicinal Plants.....	23
2.6.1 Phytochemicals	23
2.6.2 Cniduscolus Aconitifolius	25

CHAPTER THREE	28
MATERIALS AND METHODS	28
3.1 MATERIALS	28
3.1.1 Plants	28
3.1.2 Chemical/Reagents/Equipment	28
3.2 Methods	28
3.2.1 Sample Preparation and Extraction.....	28
3.2.2 Quantitative Phytochemical Analysis	28
3.2.2.1 Total Phenol Content	29
3.2.2.2 Total Flavonoid Content	29
3.2.2.3 Total Tannin Content.....	30
3.2.2.4 Total Alkaloid Content	30
3.2.2.5 Total Saponin Content	31
3.2.3 Animal Studies	31
3.2.4 MSG and Extract preparation and Administration	31
3.2.5 Experimental Design.....	32
3.2.6 Sacrifice/Blood and Organ Collection	33
3.2.7 Preparation of Sample	33
3.2.8 Preparation of Tissue Homogenates	33
3.3 Assays.....	34
3.3.1 Assessment of Liver and Kidney Function	34
3.3.1.1 ALT	34
3.3.1.2 AST.....	34
3.3.1.3 Urea	35
3.3.1.4 Creatinine.....	35
3.3.1.5 Protein Determination.....	36
3.3.2 Determination Of Malondialdehyde (MDA) Concentration.....	37
3.3.3 Catalase Activity.....	38
3.3.5 Histopathological Examination	38
3.3.6 Gene Expression Profiling	39
3.3.6.1 RNA Extraction.....	39
3.3.6.2 Determination of RNA purity and concentration.....	39
3.3.6.3 Dilution of Samples	39
3.3.6.4 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)	40
3.4 Statistical Analysis	41

CHAPTER FOUR	42
RESULTS	42
CHAPTER FIVE	74
DISCUSSION	74
CHAPTER SIX	78
CONCLUSION	78
6.1 Conclusion and Recommendations	78
6.2 Contribution to Knowledge	79
REFERENCES	80
APPENDICES	98

LIST OF FIGURES

FIGURES	TITLE OF FIGURES	PAGES
2.0:	Monosodium Glutamate	6
2.1:	Structure of Monosodium Glutamate (MSG)	7
2.2:	<i>Cnidoscopus aconitifolius</i> plants	25
4.0:	Plot showing the results of the phytochemical analysis of the plant extract.	42
4.1:	Plot showing the changes in body weight	43
4.2:	Plot showing the activity of Serum Alanine aminotransferase (ALT) enzyme..	44
4.3:	Plot showing the activity of Serum Aspartate aminotransferase (AST) enzyme.....	45
4.4:	Plot showing the Serum Urea concentration.	46
4.5:	Plot showing the Serum Creatinine concentration	47
4.6:	Plot showing the Serum Total protein concentration	48
4.7:	Plot showing the results of the Lipid peroxidation assay in the liver.	49
4.8:	Plot showing the results of the Lipid peroxidation assay in the kidney..	50
4.9:	Plot showing the results of the Lipid peroxidation assay in the brain.....	51
4.10:	Plot showing the results of the Lipid peroxidation assay in the testis.....	52
4.11:	Plot showing the results of the Catalase activity in the Liver.....	53
4.12:	Plot showing the results of the Catalase activity in the kidney.....	54
4.13:	Plot showing the results of the Catalase activity assay in the brain.	55
4.14:	Plot showing the results of the Catalase activity assay in the Testis.....	56
4.15:	Plot showing the expression levels of SOD1 Gene in the Liver.....	57
4.16:	Plot showing the expression levels of SOD1 Gene in the Kidney.	58
4.17:	Plot showing the expression levels of SOD1 Gene in the Testis.	59
4.18:	Plot showing the expression levels of CAT Gene in the Liver.....	60
4.19:	Plot showing the expression levels of CAT Gene in the Kidney.....	61
4.20:	Plot showing the expression levels of CAT Gene in the Testis.	62
4.21:	Plot showing the expression levels of GPX1 Gene in the Liver.	63
4.22:	Plot showing the expression levels of GPX1 Gene in the Kidney.	64
4.23:	Plot showing the expression levels of GPX1 Gene in the Testis.	65
4.24:	Liver Histology.	67
4.25:	Kidney Histology	69
4.26:	Brain Histology	71
4.27:	Testis Histology	73

LIST OF TABLES

TABLES	TITLE OF TABLES	PAGES
3.0:	Experimental Design.....	32
3.1:	Protocol for protein estimation.....	36
3.2:	List of primers and their sequences.....	41

LIST OF ABBREVIATIONS

MSG	Monosodium glutamate
SOD	Superoxide dismutase
CAT	Catalase
GPX	Glutathione peroxidase
MDA	Malondialdehyde
EXT	Extract
ROS	Reactive Oxygen Species
RNS	Reactive Nitrogen Species
B.W	Body Weight
LPO	Lipid Peroxidation
NO	Nitric Oxide
Cd	Cadmium
Hg	Mercury
Pb	Lead
As	Arsenic
Fe	Iron
Cu	Copper
Co	Cobalt
Cr	Chromium
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
<i>C. aconitifolius</i>	<i>Cnidioscolus aconitifolius</i>

ABSTRACT

Monosodium glutamate (MSG) is found naturally in some foods. MSG is used in cooking as a flavour enhancer with an umami taste that intensifies the flavour of food. MSG has been linked with several diseases such as metabolic disorders, Chinese restaurant syndrome, neurotoxic effects, oxidative stress and reproductive toxicity. The aim of this research was to assess the effect of *Cnidoscolus aconitifolius* leaf extracts on the oxidative stress intake of MSG using antioxidant enzyme activity, assessment of liver and kidney functions, histopathological parameters and gene expression analysis. Forty five animals were used for this study and were assigned into nine (9) experimental groups of 5 animals each. Group 1 was given 0.9% normal saline, groups 2 and 3 were given only MSG and extract respectively, while groups 4 and 5 were pre- and post-treated with the extract before and after the intake of MSG for four (4) weeks. The MSG doses given were 2.4g/kg body weight while 200mg/kg and 400mg/kg of extracts were administered respectively. All treatments were carried out for a period of eight (8) weeks (56 days). The animals were sacrificed after the stated period of administration with collection of blood and organs for assays. Assays such as assessment of Liver and Kidney function, antioxidant enzyme activity and gene expression analysis were carried out. Results showed that there was a significant increase ($P<0.05$) in the body weights, Aspartate amino transferase (AST) activity and creatinine concentration of the rats given only MSG compared with the controls. Alanine amino transferase (ALT) activity and urea concentration was also increased in the MSG group. The content of the phytochemicals in the showed high value in saponin ($0.28\pm 0.002\text{mg/ml}$), followed by flavonoids ($0.17\pm 0.007\text{mg/ml}$) which might give the plant the effect to modulate the negative effects of the MSG. The extracts also improved the histologic structure of the rat organs compared to the groups given MSG which had severe necrosis and degeneration of their cells. Administration of MSG downregulated the expression of Superoxide dismutase (SOD) and Catalase (CAT) genes in the liver and kidneys when compared to the control group. It can therefore be concluded that *Cnidoscolus aconitifolius* has some bioactive properties with antioxidant potentials which need to be fully explored.

Keywords: Monosodium glutamate, *Cnidoscolus aconitifolius*, Antioxidants, Oxidative stress, Gene expression