

THE EFFECTS OF CHRONIC LOW DOSE ORGANIC
ARSENIC EXPOSURE ON THE KIDNEY: MECHANISM
OF INJURY AND MICROSCOPIC CHANGES

BY

WAN MUHAMAD SALAHUDIN BIN WAN SALLEH

A thesis submitted in of the requirement for the degree of
Doctor of Philosophy in Medical Sciences

Kulliyyah of Medicine
International Islamic University Malaysia

MARCH 2021

ABSTRACT

Chronic exposure to inorganic arsenic has been linked with multiple medical conditions, which shifted the use of inorganic to the organic-based herbicide, monosodium methyl arsenate (MSMA). However, with increasing numbers of chronic kidney disease of unknown causes (CKDu), chronic exposure to herbicide is believed to be one of the potential explanation. To date, studies on the effects of organic arsenic exposure on the kidney are limited. Therefore, this study aimed to investigate the effect of chronic oral organic arsenic exposure on the rat's kidney. Thirty-six Sprague Dawley rats (N=36) were randomly divided into MSMA exposed, and its corresponding control groups for 2-, 4- and 6-month, each with six animals per group. The exposed groups were given oral MSMA at 63.20 mg/kg body weight, while control groups received distilled water. At the end of each duration, the serum was collected for the creatinine level. The kidney tissues were harvested for arsenic level measurement, histopathological, immunohistochemistry, real-time PCR analysis and ultrastructural analysis. Gene expressions were done for kidney injury marker gene (*KIM-1*), oxidative stress genes (*Catalase*, *GSR*, *NOS1*), apoptosis genes (*Tp53*, *Caspase-3* and *Caspase-9*) and inflammatory genes (*Interleukin-6* and *Interleukin-8*). Serum creatinine was not significantly different between exposed and control groups. Tissue arsenic level was significantly higher in exposed groups as compared to that of the control group. All gene expression markers were downregulated at 2-month and upregulated at 4-month except for *Catalase* which remained downregulated. At 6-month, only *KIM-1*, *GSR* and *Caspase-3* remained upregulated. Histological, immunohistochemistry and ultrastructural findings showed chronological changes in the glomeruli and proximal tubules with increased expressions of malondialdehyde (MDA) staining, *Caspase-3* and TUNEL staining with the duration of exposure. Therefore, chronic oral exposure to low dose organic arsenic has demonstrated evidence of kidney injury in rats possibly due to oxidative stress.

خلاصة البحث

ارتبط التعرض المزمن للزرنيخ غير العضوي بالعديد من الحالات الطبية، مما أدى إلى التوجه لاستخدام مبيد الأعشاب العضوي، أرسونات ميثيل أحادي الصوديوم، بدلا من المبيدات غير العضوية، ومع تزايد المصابين بمرض الكلى المزمن مجهول الأسباب، فإنه يُعتقد أن التعرض المزمن لمبيدات الأعشاب هو أحد التفسيرات المحتملة. الدراسات المتعلقة بآثار التعرض للزرنيخ العضوي على الكلى محدودة إلى الآن. ولذلك فقد هدفت هذه الدراسة إلى التحقيق في آثار التعرض المزمن للزرنيخ العضوي المعطى عن طريق الفم على كلى الفئران. تم تقسيم 36 من جرذان السبراق داوولي بشكل عشوائي إلى مجموعات معرضة لأرسونات ميثيل أحادي الصوديوم ومجموعات ضابطة مقابلة لها، لمدة 2 شهرين، و 4 أشهر، و 6 أشهر، وفي كل مجموعة ست جرذان. تم إعطاء أرسونات ميثيل أحادي الصوديوم للمجموعات المختبرة عن طريق الفم بجرعة 63.20 مجم/كجم من وزن الجسم، بينما أعطيت المجموعات الضابطة الماء المقطر. في نهاية كل فترة تم جمع أمصال الدم لقياس مستوى الكرياتينين. تم حصاد أنسجة الكلى لقياس مستوى الزرنيخ، ولتحليل الهيستوباثولوجي، والتحليل الكيميائي النسيجي المناعي، وتحليل تفاعل البوليميراز المتسلسل اللحظي، وتحليل التركيب الدقيق. تم القيام بالتعبير الجيني للجين المشير لإصابة الكلى (KIM-1)، وجينات الإجهاد التأكسدي (الكاتالاز، و GSR، و NOS1)، وجينات موت الخلايا المبرمج (Tp53، و Caspase-3 و Caspase-9) وجينات الالتهاب (Interleukin-6 و Interleukin-3)، وكان مستوى الزرنيخ في الدم مختلفا بشكل كبير في المجموعات المعرضة مقارنة بالمجموعة الضابطة. وكان مستوى الزرنيخ في الأنسجة أعلى بشكل ملحوظ في المجموعات المعرضة مقارنة بمستويات المجموعات الضابطة. تم ملاحظة التنظيم التخفيضي في جميع مؤشرات التعبير الجيني في مجموعة الشهرين، والتنظيم الرفعي في مجموعة الـ 4 أشهر باستثناء جين الكاتالاز الذي نظم تخفيضيا. في مجموعة الـ 6 أشهر، تم التنظيم الرفعي فقط في جينات KIM-1، و GSR، و Caspase-3. أظهرت نتائج التحليل الهيستوباثولوجي والتحليل الكيميائي النسيجي المناعي وتحليل التركيب الدقيق تغيرات كرونولوجية في الكبيبات والأنابيب القريبة مع زيادة تعبير تصبغ المالونديالدهيد وتصبغ Caspase-3 و TUNEL تناسبيا مع مدة التعرض. وبهذا فإن التعرض المزمن عن طريق الفم لجرعات منخفضة من الزرنيخ العضوي قد أظهر علامات على الإصابة الكلوية في الجرذان وذلك بسبب الإجهاد التأكسدي على الأرجح.

APPROVAL PAGE

The thesis of Wan Muhamad Salahudin Wan Salleh has been approved by the following:

Zunariah Buyong
Supervisor

Norlelawati A.Talib
Co-Supervisor

Nor Zamzila Abdullah
Co-Supervisor

Norsidah Ku Zaifah
Internal Examiner

Husni Ahmed Abdullah Al-Goshae
External Examiner

Marina Kapitonova
External Examiner

Mohd Said Nurumal
Chairman

DECLARATION

I hereby declare that this thesis is the result of my own investigation, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Wan Muhamad Salahudin Wan Salleh

Signature..... Date

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

**DECLARATION OF COPYRIGHT AND AFFIRMATION OF
FAIR USE OF UNPUBLISHED RESEARCH**

**THE EFFECTS OF CHRONIC LOW DOSE ORGANIC ARSENIC
EXPOSURE ON THE KIDNEY: MECHANISM OF INJURY AND
MICROSCOPIC CHANGES**

I declare that the copyright holder of this thesis is International Islamic University
Malaysia.

Copyright ©2021 by International Islamic University Malaysia. All rights reserved.

No part of this unpublished research may be reproduced, stored in a retrieval system, or
transmitted, in any form or by any means, electronic, mechanical, photocopying,
recording or otherwise without prior written permission of the copyright holder except
as provided below.

1. Any material contained in or derived from this unpublished research may
be used by others in their writing with due acknowledgement.
2. IIUM or its library will have the right to make and transmit copies (print or
electronic) for institutional and academic purposes.
3. The IIUM library will have the right to make, store in a retrieval system and
supply copies of this unpublished research if requested by other universities
and research libraries.

By signing this form, I acknowledged that I have read and understand the IIUM
Intellectual Property Right and Commercialization policy

Wan Muhamad Salahudin Wan Salleh

.....
Signature

.....
Date

ACKNOWLEDGEMENT

Alhamdulillah all praise to Allah for His blessings and help throughout this long PhD journey. Words could not describe enough my gratitude to Allah SWT for giving this chance, the inner strength, the perseverance, the courage to go through all the challenges that I had faced during this study period.

I wish to express my special thanks and deep appreciation to Asst. Prof Dr Zunariah Buyong, my supervisor, for her invaluable advice, help and guidance throughout my research. I am also indebted to my co-supervisor Assoc Prof. Dr Norlelawati A. Talib for her support and assistance throughout this study. I also would like to acknowledge and thank you for my co-supervisor Assoc. Prof Dr Nor Zamzila Abdullah, Asst. Prof Dr Sanda Aung & Asst. Prof. Dr Asmah Hanim for their support.

I am also indebted to the Kulliyyah of Medicine, IIUM administration for the trust, support and encouragement. The first and foremost goes to Prof. Dr Azmi, the Dean of the Kulliyyah of Medicine. My thanks also go to Prof. Dr Jamalludin, the Deputy Dean of Research and Postgraduate Studies. My utmost appreciation also goes to the head of the Department of Basic Medical Sciences; Mohammed Imad A. Mustafa Mahmud for the support. Special thanks to Sr. Zainab and Sr. Asma for the technical support.

Certainly, my research would not have been completed without the technical laboratory support from these individuals; Br. Hanif and Sr. Nur Nadia, who facilitated me in most laboratory procedures.

I am grateful for having such tremendous families and friends support who always give encouragement and help me get through some tough time.

Finally, I would like to thank the Ministry of Higher Education, Malaysia, for providing the FRGS grant for the study.

TABLE OF CONTENTS

Abstract	ii
Abstract in Arabic	iii
Approval Page.....	iv
Declaration	v
Copyright	vi
Acknowledgement	vii
List of Tables	xii
List of Figures	xiii
List of Abbreviations	xv
CHAPTER ONE: INTRODUCTION	1
1.1 Background and Justification	1
1.2 General Objective	3
1.3 Specific Objectives	3
1.4 Research Hypothesis.....	4
CHAPTER TWO: REVIEW OF LITERATURE	5
2.1 Epidemiology of Human Arsenic Exposure	5
2.2 Brief Information about Arsenic.....	8
2.2.1 Chemical Properties, Classification, Speciation and Toxicity of Arsenic	8
2.2.2 History and Application of Arsenic	9
2.2.3 Toxicity of Organic and Inorganic Arsenic	10
2.2.4 Sources and Cycle of Arsenic	10
2.3 Monosodium methyl arsenate (MSMA).....	13
2.4 Health complications of arsenic.....	13
2.5 Chronic Kidney Disease	15
2.5.1 Definition and Epidemiology	15
2.5.2 Chronic Kidney Disease Unknown Etiology (CKDu).....	16
2.5.3 Controversial Relationship Between Arsenic and Chronic Kidney Disease Unknown Etiology (CKDu)	18
2.6 Animal Study of Arsenic and Its Effects on Kidney	19
2.7 Inorganic Arsenic Metabolism and Mechanism of Injury to The cell.....	24
2.7.1 Arsenic Absorption	24
2.7.2 Metabolism of Arsenic	25
2.7.2.1 Oxidation and Reduction.....	25
2.7.2.2 Methylation of Arsenic.....	26
2.7.3 Arsenic Excretion.....	29
2.8 The Postulated Mechanism of Toxicity to The Kidney Cell	30
2.8.1 Arsenic and Oxidative Stress	30
2.8.2 Arsenic and Inflammation.....	31
2.8.3 Arsenic and DNA Damage.....	31
2.8.4 Activation of <i>Caspase-3</i> and <i>Caspase-9</i> Cascade System.....	32
2.8.5 Apoptosis	32
2.8.6 Cell Necrosis	33

2.9 Kidney Biomarkers	33
2.9.1 Creatinine	35
2.9.2 Kidney Injury Molecule - 1 (<i>KIM-1</i>)	35
2.10 Conceptual Framework.....	37
CHAPTER THREE: MATERIALS AND METHODOLOGY.....	38
3.1 Materials	38
3.2 Ethical Approval.....	38
3.3 Period of Study	38
3.4 Animal Care and Handling	38
3.5 Sample Size Calculation	41
3.6 Dosage Justification and MSMA Preparation	41
3.7 Specimen Collection and Processing.....	42
3.7.1 Serum Creatinine.....	42
3.7.2 Kidney Tissue Collection.....	42
3.8 Determination of Arsenic Compound in Kidney Tissue	43
3.9 Serum Creatinine	43
3.10 Gene Expression Analysis	43
3.10.1 RNA Purification	43
3.10.2 RNA Integrity Analysis	45
3.10.3 RNA Quantification and Dilution	46
3.10.4 cDNA Synthesis	47
3.10.5 Selection of Target and Housekeeping Genes	48
3.10.6 Gene Expression Analysis based on RT ² Profiler PCR Arrays	50
3.11 Histological Analysis.....	55
3.11.1 Tissue Staining.....	57
3.11.1.1 Haematoxylin and Eosin (H&E) Staining.....	57
3.11.1.2 Periodic Acid Schiff (PAS) Staining.....	58
3.11.1.3 Immunohistochemistry (IHC) Staining	59
3.11.1.3.1 IHC of MDA, p53 and Caspase 3	59
3.11.1.3.2 Terminal Deoxynucleotidyl Transferase dUTP Nick End Labelling (TUNEL) staining.....	61
3.11.2 Tissue / Slides Capture using Slidescanner.....	64
3.11.3 Analysis of The Microscopic Morphological View.....	64
3.12 Electron Microscopy.....	65
3.12.1 Scanning Electron Microscopy (SEM)	65
3.12.2 Transmission Electron Microscopy (TEM)	65
3.13 Statistical Analysis.....	67
CHAPTER FOUR: RESULTS	68
4.1 General Characteristic of The Animal	68
4.2 Body Weight and Organ Weight	68
4.2.1 Body Weight	68
4.2.2 Relative Kidney Weight.....	69
4.3 Serum Creatinine	70
4.4 Arsenic Level In the Kidney Tissue	71
4.5 Histological Analysis.....	72
4.5.1 Microscopic Appearance of the Control	72
4.5.2 Analysis of Two-Month Exposed Group.....	74

4.5.3 Analysis of Four-Month Exposed Group.....	76
4.5.4 Analysis of Six-Month Exposed Group.....	78
4.6 Gene Expression.....	81
4.6.1 RNA Purity and Integrity Analysis.....	81
4.6.2 Gene Expression Analysis.....	82
4.6.2.1 Kidney Injury Biomarker Genes.....	83
4.6.2.2 Oxidative Stress Genes.....	84
4.6.2.3 Inflammatory Genes Expression IL-6 and IL-8.....	85
4.6.2.4 Apoptosis Genes.....	86
4.6.2.5 Combination of expression of all genes.....	87
4.7 Immunohistochemistry Staining Analysis.....	89
4.7.1 Malondialdehyde (MDA) Staining.....	89
4.7.2 <i>Tp53</i> Staining.....	91
4.7.3 <i>Caspase-3</i> Staining.....	93
4.7.4 TUNEL staining.....	95
4.8 Electron Microscopy Analysis.....	97
4.8.1 Scanning Electron Microscopy.....	97
4.8.1.1 Comparison of Glomerular Structures in Control and Exposed groups.....	97
4.8.1.2 Comparison of Tubular Structures in the Control and Exposed Groups.....	99
4.8.2 Transmission Electron Microscopy.....	101
4.8.2.1 Comparison of Glomerular Structures in the Control and Exposed groups.....	101
4.8.2.2 Comparison of Tubular Structures in the Control and Exposed groups.....	103
CHAPTER FIVE: DISCUSSION.....	105
5.1 Study Animals.....	105
5.1.1 Body Weight.....	105
5.1.2 Relative Kidney Weight.....	105
5.1.3 Serum Creatinine.....	106
5.1.4 Arsenic Level in The Kidney Tissue.....	106
5.2 Histological Analysis.....	107
5.3 Potential Mechanisms of Arsenic Induced Kidney Injury: The Gene Expression and Immunohistochemistry Findings.....	109
5.3.1 Kidney Injury Biomarker Genes.....	109
5.3.2 Oxidative Stress Mechanisms.....	110
5.3.3 Inflammatory Genes.....	112
5.3.4 Apoptosis.....	114
5.3.4.1 P53 Gene and Staining.....	114
5.3.4.2 Caspases Mechanisms.....	115
5.4 Ultrastructural Findings Of Arsenic Induced Injury of the Kidney.....	116
5.4.1 Glomeruli Changes in Kidney Injury.....	116
5.4.2 Tubular Changes in Kidney Injury.....	119
CHAPTER SIX: CONCLUSION.....	120
6.1 Limitations and Recommendations.....	120

REFERENCES.....	122
APPENDIX I: CHEMICAL REAGENTS AND DISPOSABLE MATERIALS	143
APPENDIX II: EQUIPMENT AND INSTRUMENTS.....	144
APPENDIX III: ANIMAL ETHICS APPROVAL.....	145
APPENDIX IV: DOSAGE CALCULATION	146
APPENDIX V: PUBLICATION, ACHIEVEMENTS AND AWARDS	147

LIST OF TABLES

Table 2.1	Global Distribution of Groundwater Arsenic Contamination, Concentration and Their Sources of Arsenic	7
Table 2.2	Previous Animal Studies Related to Arsenic and Direct Relations With Kidney Organs.	21
Table 2.3	Kidney Biomarkers In Relation With The Pathophysiological Process	34
Table 3.1	Purelink® RNA Mini Kit (ThermoFisher, USA) Contents	44
Table 3.2	Genomic DNA Elimination Mix	47
Table 3.3	Reverse-Transcription Mix	47
Table 3.4	Selected Target and Housekeeping Genes	50
Table 3.5	Contents of The RT ² SYBR Green qPCR Mastermix	51
Table 3.6	Cycling Condition for Bio-Rad CFX 96 Cycler	51
Table 3.7	Tissue Processing Protocols	56
Table 3.8	The Content of The Rabbit Specific HRP/DAB (ABC) Detection IHC Kit	59
Table 3.9	The Content of The TUNEL, In Situ Apoptosis Detection Kit	61
Table 4.1	Mean Relative Kidney/Body Weight Ratio Between The Control and The Exposed Groups	69
Table 4.2	Comparison of The Serum Creatinine Between The Control and The Exposed Groups	70

LIST OF FIGURES

Figure 2.1	Arsenic Sources and Cycles in The Environment in Relation to Groundwater Contamination.	12
Figure 2.2	Summary of The Effects of Chronic Toxicity of Inorganic Arsenic in Various Organs or Systems.	14
Figure 2.3	Oxidation and Reduction Pathways Involved in Methylation of Arsenic.	26
Figure 2.4	Methylation of Arsenic Through the Enzymatic Detoxification Process and By Forming the Conjugation Complex	27
Figure 2.5	Proposed Pathways for Arsenic Methylation Via GSH-Conjugated Arsenic.	29
Figure 2.6	Conceptual Framework	37
Figure 3.1	Flowchart of The Study	40
Figure 3.2	Experimental Flowchart for Gene Expression Study	53
Figure 3.3	The Experimental Flow for Histological Study	55
Figure 3.4	The Experimental Flowchart for Ultrastructure Electron Microscopy	66
Figure 4.1	Rate of Body Weight Changes (%) in the Control and The Exposed Groups Over 6 Months Duration.	69
Figure 4.2	The Arsenic Level in The Control Group and The Exposed Group were Compared Using Independent Sample t-Test	71
Figure 4.3	Histopathological Appearance of Representative from The Control Groups	73
Figure 4.4	Histopathological Appearance of Representative From 2-Month Exposed Group	75
Figure 4.5	Histopathological Appearance of Representative From 4-Month Exposed Group	77
Figure 4.6	Histopathological Appearance of Representative From 6-Month Exposed Group	79
Figure 4.7	Inflammatory Cell Infiltration in The Exposed Groups in Comparison with Control Groups	80

Figure 4.8	A Representative of Denaturing Agarose Electrophoresis of The Isolated RNA Using Qiaxcel	81
Figure 4.9	Gene Expression of <i>Havcr1</i> (<i>KIM-1</i>) In the Exposed Groups for the 2-, 4- And 6-Month Groups	83
Figure 4.10	Gene Expression of <i>Catalase</i> , <i>GSR</i> And <i>NOS1</i> In the Exposed Groups of the 2-, 4- And 6-Month Groups	84
Figure 4.11	Gene Expression Of <i>IL-6</i> And <i>IL-8</i> In The Exposed Groups Of The 2-, 4- And 6- Month Groups Duration	85
Figure 4.12	Gene Expressions of <i>Tp53</i> , <i>Caspase 9</i> And <i>Caspase 3</i> In The 2-, 4- and 6- Month Exposed Groups	86
Figure 4.13	Summary of All Genes in This Study	88
Figure 4.14	MDA Staining of The Representative of The Rat's Kidney	90
Figure 4.15	<i>Tp53</i> Staining of The Representative of The Rat's Kidney	92
Figure 4.16	<i>Caspase-3</i> Staining of The Rat's Kidney	94
Figure 4.17	TUNEL Staining in The Representative of The Rat's Kidney	96
Figure 4.18	Scanning Electron Photomicrographs of The Chronological Changes in The Glomerular Structures on Scanning Electron Microscopy	98
Figure 4.19	Scanning Electron Photomicrographs of The Chronological Changes in The Tubular Structures on Scanning Electron Microscopy	100
Figure 4.20	Transmission Electron Photomicrographs of Chronological Changes in The Glomerular Structures	102
Figure 4.21	Transmission Electron Photomicrographs of The Chronological Changes in Tubular Structures	104

LIST OF ABBREVIATIONS

As ₂ O ₃	Arsenic Trioxide
CKD	Chronic Kidney Disease
CKDu	Chronic Kidney Disease of Unknown Causes
DMA	Dimethylarsinic Acid
GR	Glutathione reductase
GSH	Glutathione
GSR	Glutathione-Disulfide Reductase
H&E	Haematoxylin and Eosin
ICP-MS	Inductively coupled plasma mass spectrometry
MDA	Malondialdehyde
MMA	Methylarsonic Acid
MSMA	Monosodium Methyl Arsenate
PAS	Periodic Acid-Schiff
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND AND JUSTIFICATION

Human exposure to environmental arsenic remains a significant public health challenge. The World Health Organization (WHO) in 2018 had reported that 140 million people from 50 countries were exposed to arsenic above the recommended level of 10 $\mu\text{g/L}$ (WHO, 2018). The primary sources of arsenic contamination were from anthropogenic activities such as agricultural, industrial and domestic activities. These activities include the usage of arsenic-based fertilizers, pesticides and herbicides, mining and refining, and efflux of industrial wastes into the water system (Smedley & Kinniburgh, 2002). Arsenic toxicity was suggested to be acquired by oral ingestion of drinking water from contaminated groundwater such as a tube or shallow wells (Mar Wai et al., 2019).

Chronic exposure to inorganic arsenic has been known to cause many medical conditions such as diabetes, hypertension, peripheral artery disease and various tumour formation in skin, lungs, bladder liver and kidneys (Robles-Osorio et al., 2015). Due to the adverse effects of inorganic arsenic on health, organic arsenical herbicides were introduced in the 1950s. Monosodium methyl arsenate (MSMA) is one of the most popular among organic arsenical herbicide due to its effectiveness, lower price and lower toxicity as compared to inorganic arsenic. Currently, MSMA is widely used for weed control in cotton and on turf and lawn (Matteson et al., 2014). After years of its usage, MSMA which was earlier thought to have less toxic chemical properties, was reported to be as toxic as inorganic arsenic in later studies. For example, the methylated arsenical, methylarsonous acid, $\text{MMA}^{\text{(III)}}$, is more toxic than inorganic arsenic species

(Stybło et al., 2000). Oral exposure of MSMA may produce more toxic metabolites such as dimethylarsinic acid (DMA) species in vivo including MMA^(III) and DMA radicals (Albert et al., 2008a) and dimethylarsinic acid (DMA) may enhance prostate carcinogenesis (Suzuki et al., 2019).

In agricultural producing countries (i.e. Bangladesh, India), the reported cases of Chronic Kidney Disease of Unknown Causes (CKDu) is increasing (Ranasinghe et al., 2019). Many factors have been postulated as the potential causes such as physical exertion, heat stress, water quality and exposure to agrochemicals, (Weaver et al., 2015). Though no definite cause has been confirmed, the exposure to agrochemical such as MSMA has been identified to be one of the risk factors (Wimalawansa SJ, 2015).

To date, human studies to establish the association between chronic toxicity of arsenic and its effects on the kidneys are limited (Zheng et al., 2014). Meanwhile, animal studies were mostly focused on the outcome of inorganic arsenic over the acute and sub-acute duration. Only limited studies were found on chronic organic arsenic exposure particularly its effects on the rat's kidney (Albert et al., 2008b; An et al., 2020; Cohen et al., 2001; Perry et al., 2019; Rivas-Santiago et al., 2019; Zhang et al., 2019).

Therefore, this study was carried out to investigate the effect of organic arsenical (MSMA) exposure on rat's kidney. The research provides a platform to describe the possible mechanism of chronic organic arsenic toxicity in the development of chronic kidney injury. Although epidemiological and experimental studies have linked acute inorganic arsenic toxicity to kidney injury, to the best of our knowledge, no study has investigated the possible link between chronic low dose organic arsenic exposure with chronic kidney injury. Thus, the understanding of the mechanisms involved in arsenic-induced kidney injury provides useful information on the management and prevention of the complications.

1.2 GENERAL OBJECTIVE

To study the effects of low dose chronic organic arsenic exposure on the rat's kidney.

1.3 SPECIFIC OBJECTIVES

- i. To assess the renal function (serum creatinine) of the chronic low dose organic arsenic exposed rats.
- ii. To assess the arsenic level in the kidney tissue of the chronic low dose organic arsenic exposed rats.
- iii. To assess the histopathological changes of the chronic low dose organic arsenic exposed rats.
- iv. To assess the tissue protein expression of selective immunomarkers representing kidney injury (*caspase-3*, *Tp53* and TUNEL staining) and oxidative stress (Malondialdehyde staining) of the chronic low dose organic arsenic exposed rats.
- v. To assess the possible mechanism of kidney injury by investigating the expression of selected genes representing kidney injury (*KIM-1*, *caspase-3* and *caspase-9*), oxidative stress (*catalase*, *GSR* and *NOS1*) and inflammation (*interleukin-6* and *interleukin-8*) of the chronic low dose organic arsenic exposed rats.
- vi. To assess the ultrastructural changes of the kidney of the chronic low dose organic arsenic exposed rats.

1.4 RESEARCH HYPOTHESIS

- i. There are significant differences in the renal function in the rats exposed to chronic low dose organic arsenic groups.
- ii. There are significant differences arsenic level in the kidney tissue of the rats exposed to chronic low dose organic arsenic groups.
- iii. There are histopathological changes of the kidney in rats exposed to chronic low dose organic arsenic groups.
- iv. There are changes in immunomarkers of the kidney in rats exposed to chronic low dose organic arsenic groups.
- v. There are significant differences in genes expression of the kidney in rats exposed to chronic low dose organic arsenic groups.
- vi. There are ultrastructural changes of the kidney in rats exposed to chronic low dose organic arsenic groups.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 EPIDEMIOLOGY OF HUMAN ARSENIC EXPOSURE

Exposure to environmental arsenic remains a significant public health challenge. World Health Organization (WHO) in 2018 had reported that 140 millions of people from 50 countries are exposed to arsenic above the recommended level of 10 $\mu\text{g/L}$ (WHO, 2018). Majority of people exposed to arsenic-contaminated groundwater are from South Asian countries (Parvez et al., 2006).

Before the year 2000, there were four significant areas of arsenic groundwater contamination identified in Asia, which were West Bengal, Bangladesh, India, and few sites in China. However, between 2000 and 2015, arsenic-contaminated groundwater problem has increased in various Asian countries, including Mongolia, Cambodia, Nepal, Afghanistan, Myanmar, Western Iran, Korea, Vietnam and Pakistan. Recently, several new sites of arsenic-contaminated groundwater have been reported worldwide, especially in Asian countries (Shahid et al., 2018). In Malaysia, it was reported that the level of arsenic in 19% of well water samples from Rosob village in Sabah exceeded the WHO health-based guidelines (Kato et al., 2010). There was also a report on increase arsenic level in soil samples, vegetables and fish samples in Malaysia (Ong et al., 2013). However, the palm oil plantation soil was uncontaminated by the arsenic (Ab Manan et al., 2018).

The primary source of human arsenic exposure was from the groundwater contamination either from tube wells or shallow wells, and the arsenic toxicity was suggested to be acquired through oral ingestion (Wai et al., 2019). The global

distribution of groundwater arsenic contamination, their concentration and source of arsenic are summarized in Table 2.1.

Table 2.1 Global Distribution of Groundwater Arsenic Contamination, Concentration and Their Sources of Arsenic

Country / Region	Estimated exposed population	Arsenic Speciation	Concentration (mg/L)	Source
Asian				
Bangladesh	36 000 000	NA	0.001- 2.5	Natural
Chile	130 000 to 400 000	iAs (III) and iAs (v)	0.1 - 0.8	Natural
China	5 600 000	NA	0.22 - 4.40	Natural
India (west Bengal and Northern India)	6 000 000	NA	0.22 - 3.2	Natural
Indonesia	NA	iAs (III)	0.000008 - 2.0	Natural and Industrial
Japan	NA	iAs (V)	0.01 - 0.400	Natural and Industrial
Malaysia	NA	NA	0.22	Natural
Philippines	NA	NA	0.1	Industrial
Taiwan (South – West Coast)	100 000 to 200 000	iAs (III) and iAs (V)	0.01 - 1.82	Natural
Thailand	14 085	NA	0.001 - 5.00	Industrial
Vietnam	> 1 000 000	NA	0.001 - 3.05	Natural
Africa				
Ghana	< 100 000	NA	0.01 - 0.17	Natural and Industrial
Americas				
Argentina	2 000 000	NA	0.05 - 9.9	Natural
Canada	NA	iAs (V)	0.10 - 3.00	Natural
Mexico	400 000	iAs (III) and iAs (V)	0.008 - 0.624	Natural
USA	13 000 000	iAs (III) and iAs (V)	0.05 - 1.7	Natural
Europe				
Hungary and Romania	400 000	NA	0.06 - 4.00	Natural
Poland	NA	NA	NA	NA
Spain	> 50 000	NA	0.001 - 0.1	Natural
Oceania				
New Zealand	5 600 000	iAs (III)	8.5	Natural

Source from (Adekunle, 2016) * N/A - not available, iAs - inorganic arsenic.

2.2 BRIEF INFORMATION ABOUT ARSENIC

2.2.1 Chemical Properties, Classification, Speciation and Toxicity of Arsenic

Arsenic is classified as metalloid due to its both metal and non-metal properties. Arsenic exists in both reduced and oxidized states. Arsenic has an atomic number of 33, an atomic mass of 75 with a density of 5.72 g.cm⁻³. It occupies group V of the periodic table. Its most common commercial form is arsenic trioxide (As₂O₃) (Mana et al., 2017).

Arsenic compounds are classified into three major groups; inorganic arsenic compounds, organic arsenic compounds and arsine gas. Inorganic arsenic compounds consist of trivalent and pentavalent state. The most common inorganic trivalent compounds are arsenic trioxide, sodium arsenite and arsenic trichloride. Pentavalent inorganic compounds are arsenic pentoxide, arsenic acid and arsenate. Common organic arsenic compounds are arsanilic acid, methylarsonic acid, dimethylarsinic acid (cacodylic acid) and arsenobetaine (NRC, 1999).

Speciation referred to the existence of an element in different chemical forms, oxidation states and mineral phases. It may represents element toxicity and bioavailability in the soil (Abbas et al., 2018). The arsenic speciation (based on the oxidation state) and arsenic forms (organic or inorganic) in relation to the toxicity properties are also extensively studied to date. The most common studied arsenic species are the (+3) and (+5) arsenicals. It was reported that (+3) arsenicals are more toxic than the (+5) (Braeuer et al., 2020). The order of the toxicity for arsenicals based on previous findings began with arsenite, followed by monomethylarsine oxide, dimethylarsinic acid, dimethylarsonate, monomethylarsonate and arsenate (Vega et al., 2001).

The inorganic form of arsenic has attracted researchers' attention more than organic arsenic. Therefore, limited studies on organic arsenic as it was assumed to be

less toxic than inorganic arsenic (Cohen et al., 2006; Hughes et al., 2011). However, a few recent pieces of evidence showed that organic arsenicals are as toxic as inorganic arsenicals. For instance, the methylated arsenical, methylarsonous acid MMA(III) is as toxic as inorganic arsenic species and potentially induced oxidative DNA damage (Braeuer et al., 2020; Tokar et al., 2014). Oral exposure of MSMA also may produce more toxic metabolites such as dimethyl arsenic acid (DMA) and monomethyl arsenic acid (MMA) (Albert et al., 2008b).

2.2.2 History and Application of Arsenic

Historically, arsenic was previously used as a therapeutic agent in 400 BC, known as Fowler's solution, for the treatment of chronic bronchial asthma, leukaemia, psoriasis, spirochaetal and protozoal diseases. However, Fowler's solution has been banned in most of the countries due to its toxicity. Arsenic trioxide was also reported being used in the treatment of acute promyelocytic leukaemia due to its anticancer effects by inducing autophagy, thus apoptosis in cancer cells. It also could alter gene regulation patterns, which decreased cell growth in human liver cancer, bladder cancer and breast cancer cells. Other than that, arsenic has been widely used in agricultural industries for pesticides, herbicides (e.g. monosodium arsenate, cacodylic acid), wood preservative (e.g. copper chromium arsenate), and cotton desiccants (e.g. arsenic acid). Arsenic is also used as a decolouring agent in the glass industries. Elemental arsenic is used as an additive in the alloy's productions. In the livestock industry, organic arsenic is added to swine and poultry feed as an antimicrobial medication. Nowadays, arsenic has also been used in semiconductors and solar cells components (Nordberg et al., 2014).