

# Journal of Physiology and Pathophysiology

Volume 4 Number 5 December 2013

ISSN 2141-260X



*Academic  
Journals*

## ABOUT JCPFM

**Journal of Physiology and Pathophysiology (JPAP)** is published monthly (one volume per year) by Academic Journals.

**Journal of Physiology and Pathophysiology (JPAP)** is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as effect of different sitting posture on pulmonary function, study of anti-plasmodial and toxicological effects of methanolic bark extract of *Chrysophyllum albidum*, serum testosterone and morphology of the testes in wistar rats following chronic garlic feeding etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JPAP are peer-reviewed.

### Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: [jpap@academicjournals.org](mailto:jpap@academicjournals.org), [jpap.journal@gmail.com](mailto:jpap.journal@gmail.com). A manuscript number will be mailed to the corresponding author shortly after submission.

For all other correspondence that cannot be sent by e-mail, please contact the editorial office at : [jpap@academicjournals.org](mailto:jpap@academicjournals.org).

Journal of Physiology and Pathophysiology will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

## Editor

### **Dr. Kazi Mirajul Hoque**

*Scientist and Ramalingaswami Fellow,  
Molecular Pathophysiology Division,  
National Institute of Cholera and Enteric Diseases,  
P-33, CIT Road, Scheme-XM, Beliaghata,  
Kolkata-700010,  
INDIA...*

## Editorial Board Members

### **Dr. Yan-Qiu YU**

*Department of Pathophysiology  
Sri Ramachandra Deemed University,  
College of Basic Medical Sciences,  
China Medical University  
92.Beier street, Heping District,  
Shenyang, 110001,  
P. R. China.*

### **Dr. Wanzala Wycliffe**

*P.O. Box 11177-Tom Mboya-00400,  
Nairobi,  
Kenya...*

### **Venkateswara Rao Gogineni**

*Research Scientist  
University of Illinois College of Medicine at Peoria  
Department of Cancer Biology and Pharmacology  
One Illini Drive  
Peoria, IL 61605  
USA*

...

## Editorial Team

*Dr. Ajibola, Abdulwahid  
Olabisi Onabanjo University, Ago-Iwoye,  
Nigeria.*

### **Dr. Mostafa Azimzadeh**

*Dep. of Life Science Engineering,  
Faculty of New Sciences and Technologies,  
University of Tehran, Tehran,  
Iran..*

### **Dr. Birendra Nath Mallick**

*School of Life Sciences, Jawaharlal Nehru University  
New Delhi - 110 067,  
India.*

### **Dr. Ben-zhi CAI**

*Department of Pharmacology, Harbin medical university,  
Harbin, 150081,  
China.*

**Dr. Linda R. Chambliss.**

*4440 North Dromedary Rd  
Panjab University,  
Phoenix, Arizona 85018,  
USA.*

**Dr. Rajesh Chandra Sharma**

*301 Sai Mahal Co- Hsg. Society,  
Sector -3, plot no. 40 New Panvel, Navi  
Mumbai, Maharashtra-410205,  
India.*

**Dr. De-Yu Chen,**

*Shantou University Medical College (SUMC),  
22 Xinling Road, Shantou, Guangdong, P. R. China,  
515041,  
China.*

**Dr. Ayman El-Meghawry El-Kenawy Ali,**

*22 El-Galaa St., El-Mansoura, Egypt.*

**Dr. Karunakaran Ganesh Kumar,**

*Department of Metabolism and Aging, The Scripps  
Research Institute,  
130 Scripps Way, Jupiter, FL-33458,  
USA.*

**Dr. Farouk M. F. El-Sabban,**

*Department of Family Sciences, College for Women,  
Kuwait University, P.O. Box 5969, Safat 13060  
State of Kuwait.*

**Dr. Majid Hassanpour-Ezatti,**

*Dept. of Biology, Science School, Shahed Univ. Tehran,  
Iran.*

**Dr. Hedef Dhafir El-Yassin,**

*College of Medicine, University of Baghdad,  
Bab-Almuadum, Baghdad,  
Iraq.*

**Dr. Irawan Satriotomo,**

*Dept. Comparative Bioscience, University of Wisconsin  
2015 Linden Dr., Madison, WI 53706,  
USA.*

**Dr. Jingdong Li,**

*Institute of Cardiovascular Disease, Union Hospital,  
Tongji Medical College, Huazhong University of Science  
and Technology  
Wuhan, Hubei Province, 430022,  
P.R.of China.*

**Dr. Kazi Mirajul Hoque**

*3501 St. Paul Street, APT# 819, Baltimore, MD-21218,  
USA.*

**Dr. Tao Lianyuan**

*RM S209, QingNianGongYu, XinKai Road 80#,Dong Dan,  
Dong Cheng  
District, Beijing 100005,  
China.*

# Instructions for Author

**Electronic submission** of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

## Article Types

Three types of manuscripts may be submitted:

**Regular articles:** These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

**Short Communications:** A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

**Reviews:** Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

## Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.

Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the JCPFM to publish manuscripts within weeks after submission.

## Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

**The Title** should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

**The Abstract** should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

**The Introduction** should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

**Materials and methods** should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

**Results** should be presented with clarity and precision.

The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

**The Discussion** should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

**The Acknowledgments** of people, grants, funds, etc should be brief.

**Tables** should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

**Figure legends** should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

**References:** In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author's name should be mentioned, followed by 'et al'. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like 'a' and 'b' after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998;

1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. *Afr. J. Biotechnol.* 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant *Staphylococcus aureus* in community-acquired skin infections. *Emerg. Infect. Dis.* 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing *Escherichia coli* in the Calgary Health Region: emergence of CTX-M-15-producing isolates. *Antimicrob. Agents Chemother.* 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). *Microbiology: Concepts and Applications.* McGraw-Hill Inc., New York, pp. 591-603.

### Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

**Proofs and Reprints:** Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

**Fees and Charges:** Authors are required to pay a \$550 handling fee. Journal of Physiology and Pathophysiology not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

**Copyright: © 2013, Academic Journals.**

All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

**Disclaimer of Warranties**

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the JPAP, whether or not advised of the possibility of damage, and on any theory of liability.

This publication is provided "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication.

While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.

## ARTICLES

### Research Articles

**Neurotoxicity of arsenite (As III): In vitro electrophysiological approach to studying neural activity in rat brain slices** 64

Ajonijebu Duyilemi Chris, Ogundele Olalekan Michael, Omoaghe Adams Olalekan, Fabiyi Temitope Deborah and Ojo Abiodun Ayodele

**Cardio protective effects of Nigella sativa oil on lead induced cardio toxicity: Anti inflammatory and antioxidant mechanism** 72

Marwa A. Ahmed and Khaled M. A. Hassanein



Full Length Research Paper

## Neurotoxicity of arsenite (As III): *In vitro* electrophysiological approach to studying neural activity in rat brain slices

Ajonijebu Duyilemi Chris<sup>\*1</sup>, Ogundele Olalekan Michael<sup>2</sup>, Omoaghe Adams Olalekan<sup>1</sup>, Fabiyi Temitope Deborah<sup>1</sup> and Ojo Abiodun Ayodele<sup>3</sup>

<sup>1</sup>Department of Physiology, College of Medicine and Health Sciences., Afe Babalola University, Ado-Ekiti, Nigeria.

<sup>2</sup>Department of Anatomy, College of Medicine and Health Sciences., Afe Babalola University, Ado-Ekiti, Nigeria.

<sup>3</sup>Department of Chemical Sciences, College of Sciences., Afe Babalola University, Ado-Ekiti, Nigeria.

Accepted 1 November, 2013

**Arsenite (Arsenic III) is ubiquitous and a widely distributed environmental toxin implicated in the aetiology of several neurological disorders. It is found in sea food, chemicals and pesticides. As(III) is also a mitochondria poison that induces reactive oxygen species (ROS) formation and membrane distortion. This study is aimed at proposing an *in vitro* method to detecting neural activity in rat's brain treated with As(III). Neurotoxic effects of As(III) on brain slices in rat model and description of its varying cytotoxic pathways using electrophysiological approach shall constitute another focus of the study. Brain slices were sub-cultured in Artificial Cerebro Spinal Fluid (ACSF) and treated with 50 µl of 0.1 M arsenite for 30 min. A superficial pin electrode was used to record the electrical activity following each stimulation. The spike train was analyzed to obtain the frequencies in db and this was recorded for 0.00 to 0.16 ms. As(III) induced changes in the electrical activities of the brain to a varying extent and such changes vary with the cytotoxic pathways for such regions. In the parietal cortex and upper brainstem, As(III) reduced the firing rate when compared with increased neuronal firing rates in the lower brainstem as depicted by spike peaks.**

**Key words:** Arsenic, neuron, peaks, frequency, toxicity, action potential.

### INTRODUCTION

Arsenic (As) is a widespread environmental metalloid toxin released from several anthropogenic sources such as agrochemicals, herbicides, rodenticides, wood preservatives, minerals and industrial waste. It occurs naturally in earth's crust and widely distributed in soil, water and foods (Zhang et al., 2013). The presence of this environmental toxin in human foods (Calatayud et al., 2011), though in small amounts, is traceable to its ubiquitous nature and processes inherent to human activities.

It exhibits several oxidation states such as elemental (0), trivalent (-3, +3) and pentavalent (+5) (Dopp et al., 2004). The trivalent species (for example, arsenite) are known to exhibit greater acute toxicity than the pentavalent species (for example, arsenate) which are most frequently found naturally (Zhang et al., 2013).

It has been reported that human arsenic exposure causes several health problems such as cancer, liver damage, dermatosis and nervous system disturbances

\*Corresponding author. E-mail: [aduyilemi@yahoo.com](mailto:aduyilemi@yahoo.com). Tel: +2347030580324.

such as polyneuropathy, electroencephalography (EEG) abnormalities and in extreme cases, hallucinations, disorientation and agitation (Rodríguez et al., 2002). Although, previous studies showed that liver is one of the most important targets for As(III) toxicity (Chen et al., 2013a, b). It is also evident that arsenic exposure has a toxic effect on the nervous system (Rodríguez et al., 2003). It has been previously reported that the content of catecholamines in striatum, hippocampus and other cerebral regions changes in mice and rats exposed to arsenic (Rodríguez et al., 2001). Studies by Kalimuthu et al. (2013) revealed that As(III) induces both apoptosis and necrosis when administered *in vitro* in different cellular systems through failure of mitochondrial electron transport system, induction of mitochondrial permeability transition and subsequent release of reactive oxygen species (ROS).

Oxidation of As(III) to As(V) is evident in liver metabolism as well as the release of iron from ferritin by trivalent arsenic species (Wu et al., 2012). In biologic cells, both As (V) and As (III) are methylated to yield monomethylarsonic (MMA) and dimethylarsinic acids (DMA), forms which are more rapidly excreted in urine. But a recent proposal by Kitchin and Wallace (2005) submitted a new metabolic pathway for As(III) that it does not involve oxidative methylation but rather is mediated by As-glutathione complexes, S-adenosylmethionine (SAM) and human arsenic methyltransferase Cyt19 to monomethyl-(MADG) and dimethyl-(DMAG) conjugates which are hydrolyzed to MMA and DMA, respectively. Although, pentavalent methylated metabolites are also known to result from oxidation of their trivalent forms rather than the reverse (Lau et al., 2004).

This study examined the effect of As(III) on patterns of neural activity generated by freshly isolated rat brainstem and cortical tissues to propose an *in vitro* electrophysiological approach to studying neural functions. We hope to describe cytotoxic pathways (if possible) associated with arsenite induced neural dysfunction.

## MATERIALS AND METHODS

Adult male Wistar rat was used for this study. The animal was procured and kept in the animal holding facility of Afe Babalola University and allowed to acclimatize. All procedures in the animal handling were in accordance with the guidelines of Animal use Ethical Committee of the Afe Babalola University. The animal was sacrificed by cervical dislocation and the brain removed using bone forceps. The brain tissue was perfused with cold dextrose saline before transfer into cold ACSF. The ACSF composition was as follows: 18 mM glucose, 119 mM NaCl, 2.5 mM KCl, 1.3 mM MgSO<sub>4</sub>, 2.5 mM CaCl<sub>2</sub>, 26.2 mM NaHCO<sub>3</sub> and 1 mM NaH<sub>2</sub>PO<sub>4</sub>. The solution was bubbled with 95% oxygen and 5% carbon dioxide. The tissue perfusion set up was maintained for 30 min both for the parietal cortex, upper and lower brainstems. For each type of set up, we had 2 different groups: the test group treated with 50 µl of

0.1 M arsenic III and the other is the control without any treatment.

## Electrophysiology

The study utilizes the Spiker Box from Backyard Brains ([www.backyardbrains.com](http://www.backyardbrains.com)). The device was connected to a computer interface (Audacity). Stimulation was at 1.5 mV and the action potential generated was recorded as spikes on the Audacity software. This was done for all the four setups.

- A: Upper Brain stem in ACSF
- B: Upper Brain Stem in ACSF + As(III)
- C: Parietal Lobe in ACSF
- D: Parietal lobe in ACSF + AS(III)

## Spectrum analysis

The recorded spikes were processed using the menu on the audacity software (pc version). The project rate was set at 44100 Hz and the spike regions were trimmed out and zoomed to an interval of 0.010 s. The peak patterns were selected at regular intervals and analyzed by spectrum plotting to generate an algorithm in enhanced autocorrelation through which the frequency in db at 0.00 to 0.16 ms at an interval of 0.02 ms were obtained. The spectrum plot was later depicted in the Hanning window to show peak patterns at the recorded intervals.

## Measure of the root mean square (RMS)

This is also known as the quadratic mean. It is the statistical measure of average magnitudes at a rate that account for both heights and peaks in a positive and negative direction. For this purpose we have employed the use of software SigView Version 2.6. By working on the signal tools, noise was eliminated below 350 Hz using the filter function and following the methods of Dagda et al. (2013). Other statistically determined parameters include the mean, max, standard deviation and summation.

## RESULTS

In this study, the trend of action potential (AP) generated is described in different phases. It is also imperative that we distinguish the peaks (positive and negative deflections) within the spike trains. At 0.02 ms, the frequency of the AP (peaks) is highest with a value of 3158 db for the control with a decline observed in the As(III) treated group where the peak occurred at 1862 db. This decline for the treatment was observed for the 3 consecutive peaks. Although at  $P < 0.05$ , the difference between the frequency of the control and treatment was insignificant for the peaks observed at 0.14 ms (Figure 4).

In general, a reduction in neuronal firing was observed in the treated groups when compared with the control (Figures 4 and 5). But duration of AP and spike train potentiation was prolonged in the treated upper brainstem (Figure 5). The spike train for the upper brainstem shows that at 0.02 and 0.06 ms, the control recorded higher peaks

**Table 1.** Statistical analysis.

Parameter	Max	Mean	Standard deviation	Sum	RMS
Cortex control	32019	-193.086	11906.2	1.87E007	11906.2
Cortex-arsenic	32767	-209.601	12951.9	2.07E007	12951.9
Brain stem control	30697	-49.6714	9177.53	1.24E007	9177.53
Brain stem arsenic	31426	90.006	12085.8	1.942E007	12085.8

with value of 4553 and 2459 db which is higher than the treatment when compared at less than  $P < 0.05$  (95% confidence interval) (Figure 5). The result shows that at 0.12 and 0.14 ms, the frequencies of the treatment were higher than the control. As depicted by the tracings (Figures 2A to 2D), premature potentials staggered with full term spiking were also observed in the treated groups. Inconsistency (also disorganized) in spiking was more in the treated upper brainstem than the treated parietal cortex.

Table 1 show the summary of data extrapolated from analyzing the spike trains and peaks-cortex (Figure 1A to D) and brain stem (Figure 2A to D). The RMS shows that for the arsenic treated cortex *in vitro*, an increase (12,951.9) was observed when compared to the control (11,906.2). In the brain stem, a larger increase was recorded; from 9177.3 in the control to 12085.8 in the treatment. Examining the spike train for the cortex also showed a negative mean value for the peaks (-193.086 for the control and -209.601 for the arsenic treatment) which further confirms that arsenic affects the activity and firing on these neural tissues. However in the brain stem, the control recorded a negative value [(-49.67) versus a positive value in the test (90.066)].

## DISCUSSION

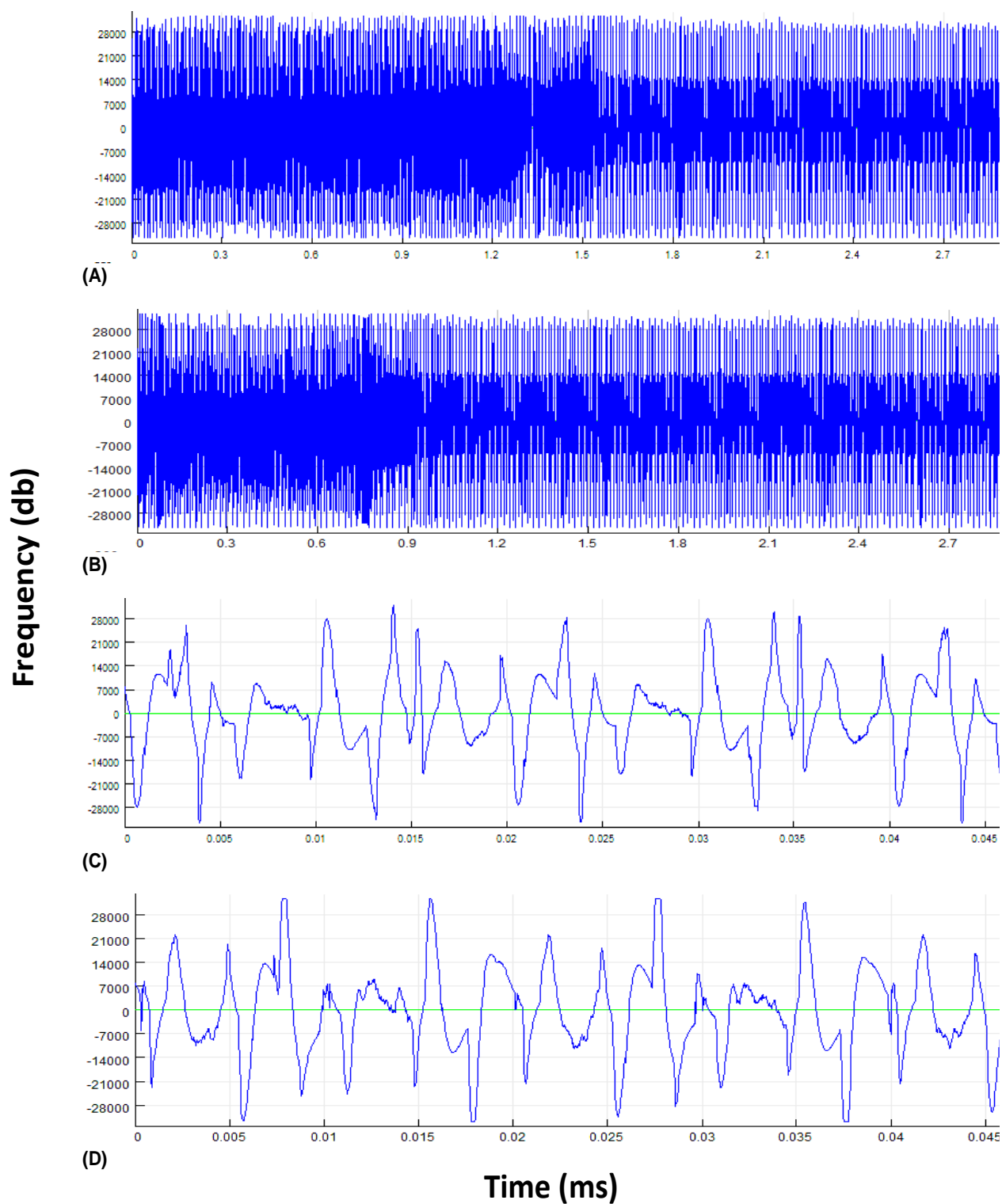
This study investigated the effect of As(III) treatment and its associated changes on the electrical activity of the parietal cortex and upper brainstem to describe an electrophysiological approach to studying neural functions. The characteristic nature of neuronal integration and spike generation is a function of imminent impulse transmission and temporal coding of information in excitable tissues. Most experiments in sensory neurophysiology have widely recorded arrival time of AP generated in nerve cells, temporal coding of information in the patterns of spikes and most importantly spike timing which plays a major role in encoding various aspects of the stimulus. Mostly neglected is the neurological analysis of spike peaks as well as the contributory role of neuronal membrane in impulse transmission induced by changes in conductance and ion fluxes.

The results of this study showed that there was a general

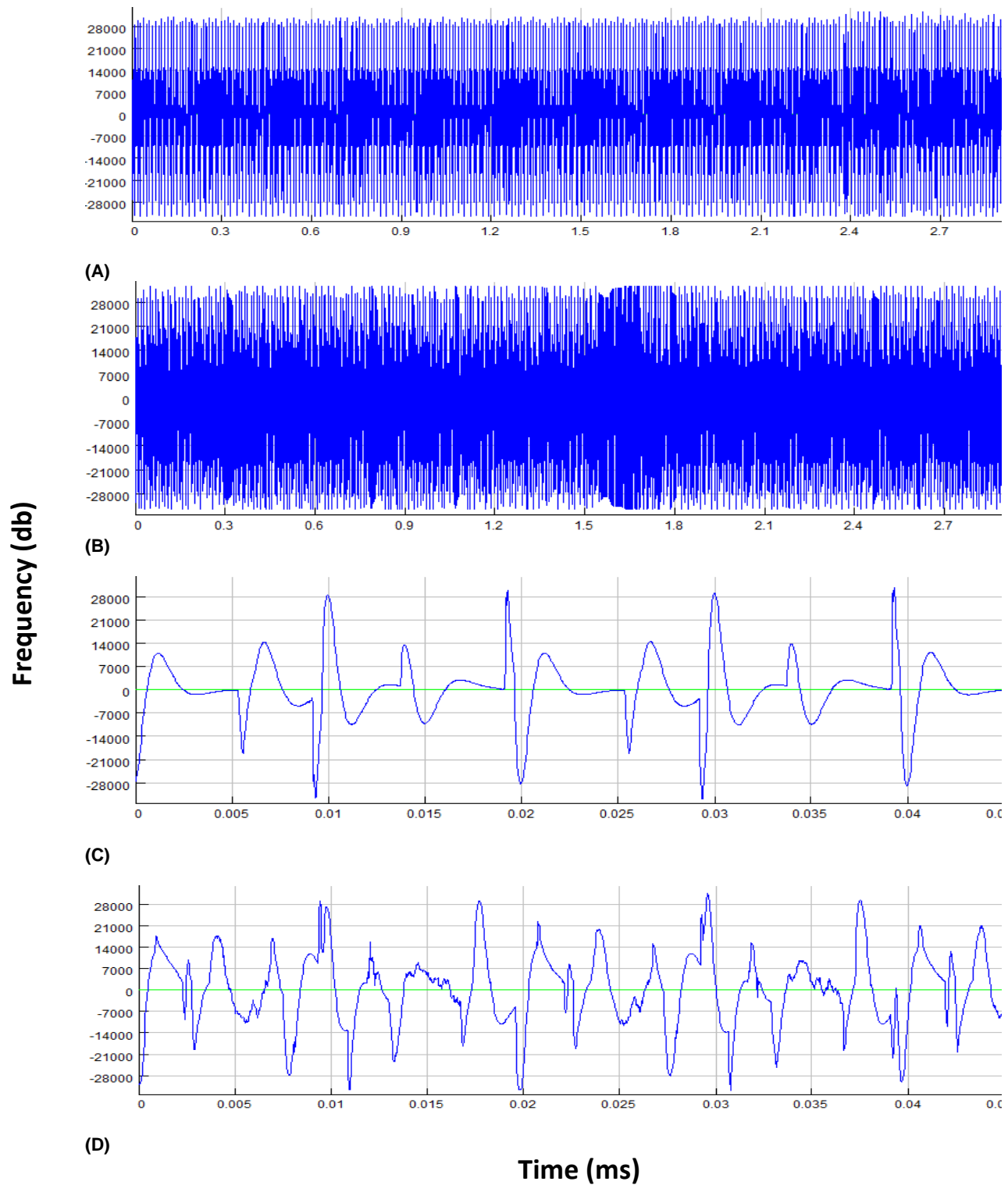
decline in neuronal firing rates in the As(III) treated groups when compared with the control. This is an indication of altered nerve function within the selected regions of the brain which may possibly result from changes in membrane permeability due to induced oxidative damage on membrane lipids and proteins, as previously reported by Jin et al. (2010). As(III) like any other metal toxins, such as Lead (Pb), Cadmium and Mercury, can affect mitochondrial oxidative enzymes. It is possible that this toxin interferes with energy coupling process by altering the redox states of cytochrome C enzyme. The resultant ROS formed will in turn induce peroxidation of membranes and loss of its ion channels.

Prolonged AP observed in the treated upper brainstem may likely be due to a sustained electrolyte leakage resulting from gradual loss of membrane integrity. Such damage could result from various mechanisms including oxidation and inhibition of key membrane proteins such as  $H^+$ -ATPase or changes in the composition and fluidity of membrane lipids (Meharg, 1993; Demidchik et al., 1997). It is also thought to be due to suppression of efflux mechanism similar to inhibiting  $K^+$  pump or down regulation or inhibition of  $Na^+$  pump. Although the mechanism of As(III) toxicity is still not clearly understood but affinity of As(III) for Na ion and how sodium arsenic oxide (a potent toxin) induced processes such as oxidative stress, genotoxicity, cytotoxicity and cell cycle arrest have been previously described by Jiang et al. (2013). In normal physiologic conditions, the permeability of the membrane to potassium (PK) is much greater than the permeability to sodium (PNa) because there are many more leakage (non-gated) channels in the membrane for  $K^+$  than in the membrane for  $Na^+$ . Hence,  $K^+$  leakage is considered a natural phenomenon which is responsible for repolarization and after hyperpolarization.

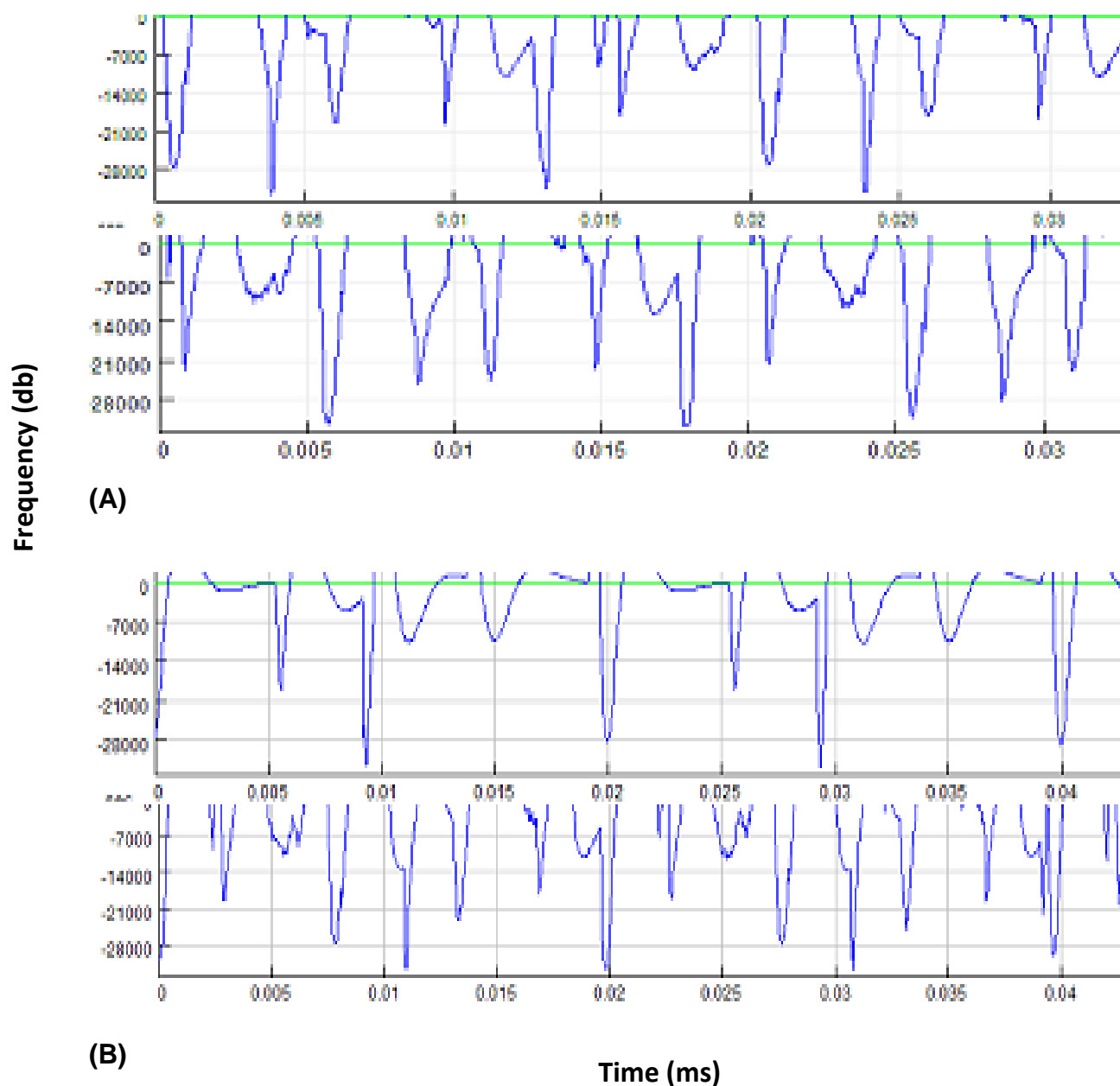
Since heavy metals have been widely reported to interfere with efflux of  $K^+$  from plant cells (Meharg, 1993), we suggest that the observed arsenite-induced alteration in firing rate (Prolonged AP) of nerve cells in the treated upper brainstem employs recruitment of efflux mechanisms which is also similar to the findings by Namgung et al. (2003). Other studies also show that the sodium and potassium channels are either depressed or down regulated in this toxicity process (Shaya et al., 2013). This function can also be taken down to the organelle level where



**Figure 1.** Tracing of spike train and peak patterns representing electrode placements and recordings for the control (1A and C) and the Arsenic III treated parietal cortex (1B and D). The control cortex; RMS 11906.2 and treated cortex RMS is 12951.9 at 0.02 s. The maximum peak recorded for control is 32019, while for the treated group an increase was observed up to 32767.



**Figure 2.** Tracing for spike train and peak patterns representing electrode placements and recordings for the control (2A and C) and the Arsenic III treated brain stem (at the level of upper pons) (2B and D). The control brain stem; RMS 9177.53 and Treated brain stem RMS is 12085.8 at 0.02 s. The maximum peak recorded for control is 32019 while for the treated group an increase was observed up to 32767.



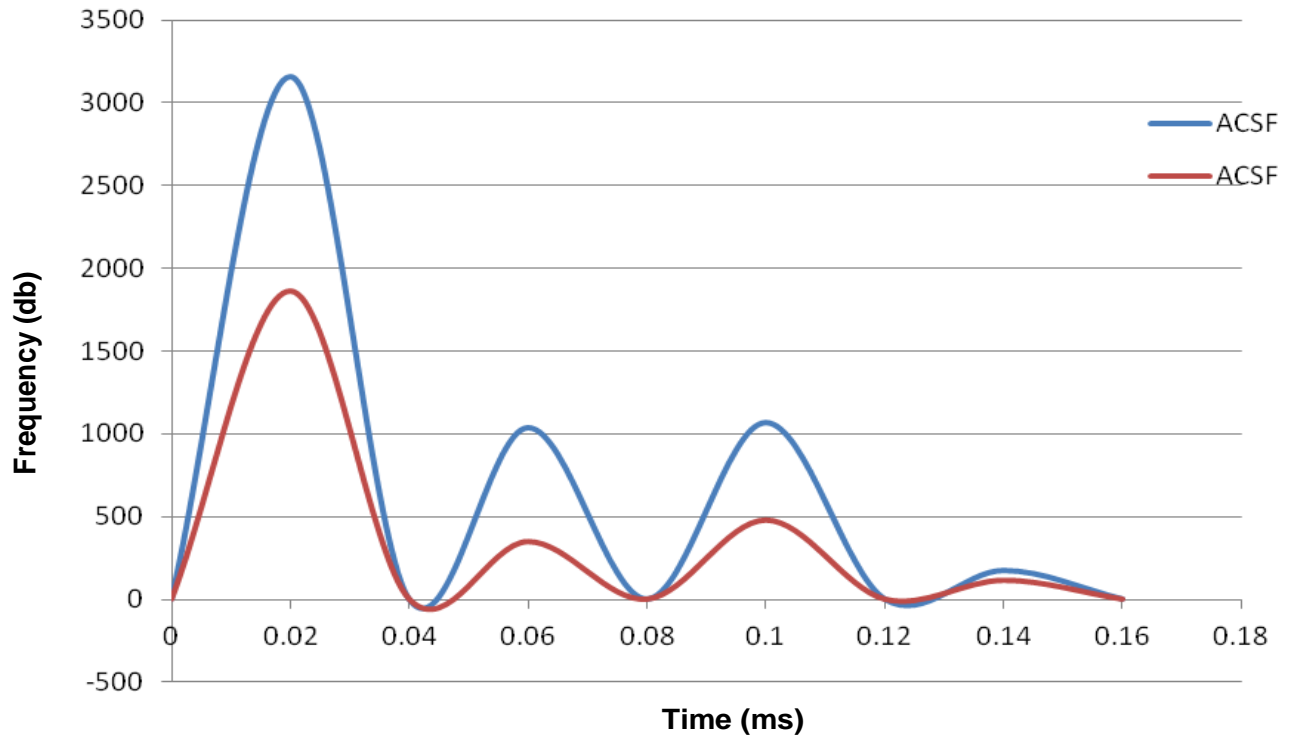
**Figure 3.** Tracing for lower peak comparative analysis; (3A) for the control and arsenic III treated parietal cortex. This shows an increase in the time required for the lower peak to be reached. (3B) In the brainstem a similar effect was achieved although at 0.04 and 0.02 second. A balance was achieved between the spike trains for the treatment and control.

the mitochondria have been found to be rich in  $H^+$  pumps that will in essence affect the summation of electro-positive ions. Calcium imbalance has also been observed following arsenic exposure (Pachauri et al., 2013). Thus As(III) induced changes in the electrical activities of the brain to a varying extent and such changes vary with the

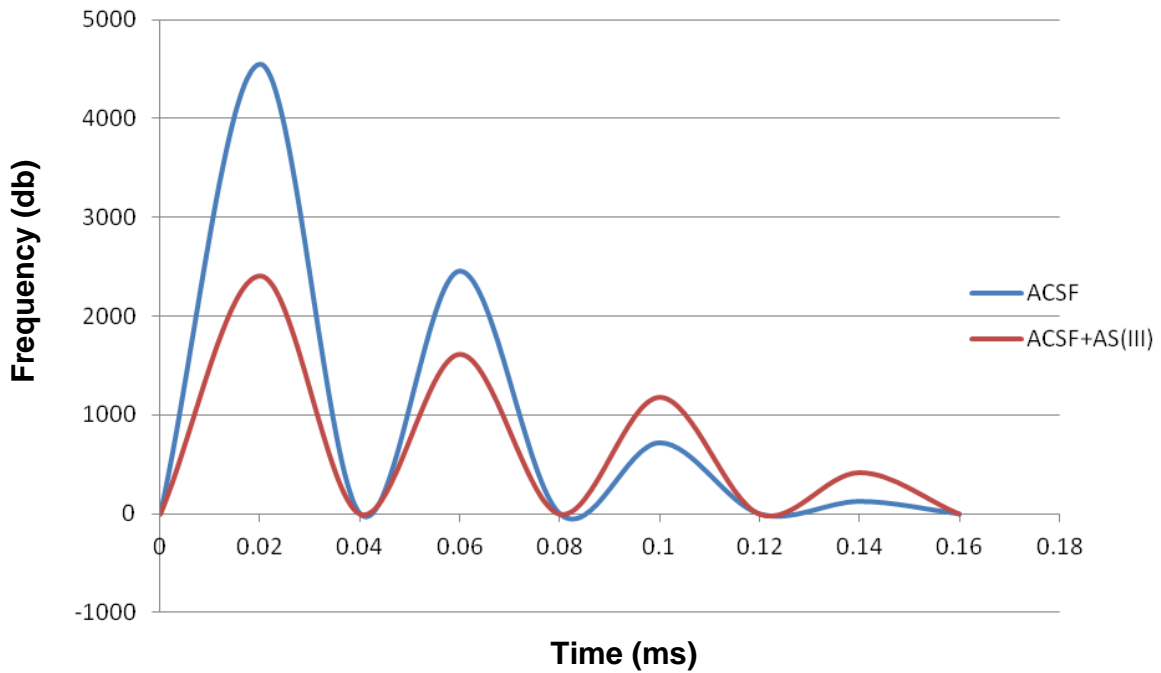
cytotoxic pathways for such regions.

### Conclusion

Neuronal membrane may also play an important role in



**Figure 4.** A curve showing the action potential (at an interval of 0.02 ms) for the control parietal cortex (in ACSF) and the parietal cortex treated with As (III) in ACSF. The result shows reduced firing rate at these intervals.



**Figure 5.** A curve showing the action potential (at an interval of 0.02 ms) for the control upper brain stem (in ACSF) and the upper brain stem treated with As (III) in ACSF. The result also shows reduced firing rates in the treated upper brainstem at these intervals, but prolonged AP when compared with the control.

neurotoxicity through alteration of its electrical potentials either through inhibition or limiting entry of ions into cells or through efflux mechanisms. We present this methodological template as a revised approach to studying neural functions.

## ACKNOWLEDGEMENT

The contributions of Mrs. Olaiya, Mr. Soji Alade and Mrs. Adewumi are acknowledged for their assistance in the instrument set up and animal handling.

## REFERENCES

- Calatayud M, Devesa V, Montoro R, Vélez D (2011). In vitro study of intestinal transport of arsenite, monomethylarsonous acid, and dimethylarsinous acid by Caco-2 cell line. *Toxicol. Lett.* 204(2-3):127-33.
- Chen B, Lu X, Shen S, Arnold LL, Cohen SM, Le XC (2013). Arsenic speciation in the blood of arsenite-treated f344 rats. *Chem. Res. Toxicol.* 26(6):952-62.
- Chen C, Jiang X, Hu Y, Zhang Z (2013). The Protective Role of Resveratrol in the Sodium Arsenite-Induced Oxidative Damage via Modulation of Intracellular GSH Homeostasis. *Biol. Trace Elem. Res.*
- Dagda RK, Thalhauser RM, Dagda R, Marzullo TC, Gage GJ (2013). Using Crickets to Introduce Neurophysiology to Early Undergraduate Students. *J. Undergrad. Neurosci. Educ.* 12(1):A66-A74.
- Demidchik V, Sokolik A, Yurin V (1997). The effect of  $\text{Cu}^{2+}$  on ion transport systems of the plant cell plasmalemma. *Plant Physiol.* 114:1313-1325.
- Dopp E, Hartmann LM, Florea AM, von Recklinghausen U, Pieper R, Shokouhi B, Rettenmeier AW, Hirner AV, Obe G (2004). Uptake of inorganic and organic derivatives of arsenic associated with induced cytotoxic and genotoxic effects in Chinese hamster ovary (CHO) cells. *Toxicol. Appl. Pharmacol.* 201(2):156-65.
- Jiang Xi, Chen C, Zhao W, Zhang Z (2013): Sodium arsenite and arsenic trioxide differently affects the oxidative stress, genotoxicity and apoptosis in A549 cells: An implication for paradoxical mechanism. *Environ. Toxicol. Pharmacol.* 14; 36(2):891-902.
- Kalimuthu P, Heath MD, Santini JM, Kappler U, Bernhardt PV (2013). Electrochemically Driven Catalysis of *Rhizobium* sp. NT-26 Arsenite Oxidase with its Native Electron Acceptor Cytochrome c552. *Biochim. Biophys. Acta.* 1837(1):112-120.
- Kitchin KT, Wallace K (2008). Evidence against the nuclear in situ binding of arsenicals--oxidative stress theory of arsenic carcinogenesis. *Toxicol. Appl. Pharmacol.* 232(2):252-7.
- Lau AT, He QY, Chiu JF (2004). A proteome analysis of the arsenite response in cultured lung cells: evidence for in vitro oxidative stress-induced apoptosis. *Biochem. J.* 382(Pt 2):641-50.
- Meharg AA (1993). The role of the plasmalemma in metal tolerance in angiosperms. *Physiol. Plant.* 88:191-198.
- Namgung U, Kim DH, Lim SR, Xia Z (2003). Blockade of calcium entry accelerates arsenite-mediated apoptosis in rat cerebellar granule cells. *Mol. Cells* 15(2):256-61.
- Pachauri V, Mehta A, Mishra D, Flora SJ (2013): Arsenic induced neuronal apoptosis in guinea pigs is  $\text{Ca}^{2+}$  dependent and abrogated by chelating therapy: Role of voltage gated calcium channels. *Neurotoxicology* 35:137-45.
- Rodríguez VM, Carrizales L, Jiménez-Capdeville ME, Dufour L, Giordano M (2001). The effects of sodium arsenite exposure on behavioral parameters in the rat. *Brain Res. Bull.* 55(2):301-8.
- Rodríguez VM, Carrizales L, Mendoza MS, Fajardo OR, Giordano M (2002). Effects of sodium arsenite exposure on development and behavior in the rat. *Neurotoxicol Teratol.* 24(6):743-50.
- Shaya D, Kreir M, Robbins RA, Wong S, Hammon J, Brüggemann A, Minor DL Jr (2011). Voltage-gated sodium channel (NaV) protein dissection creates a set of functional pore-only proteins. *Proc. Natl. Acad. Sci. USA* 108(30):12313-8.
- Wu J, Sowinska A, Huang X, Klein CB, Pelle E, Frenkel K (2012). Impairment of antioxidant defenses as a contributor to arsenite-induced cell transformation. *Biometals* 25(5):927-37.
- Zhang SY, Sun GX, Yin XX, Rensing C, Zhu YG (2013). Biomethylation and volatilization of arsenic by the marine microalgae *Ostreococcus tauri*. *Chemosphere* 93(1):47-53



## Full Length Research Paper

# Cardio protective effects of *Nigella sativa* oil on lead induced cardio toxicity: Anti inflammatory and antioxidant mechanism

Marwa A. Ahmed<sup>1</sup> and Khaled M. A. Hassanein<sup>2</sup>

<sup>1</sup>Medical Physiology Department, Faculty of Medicine, Assiut University, Assiut 71526, Egypt.

<sup>2</sup>Pathology and Clinical Pathology Department, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt.

Accepted 13 November, 2013

The present study aimed to evaluate cardio-protective effect of *Nigella sativa* oil (NSO) on lead induced cardio toxicity. Forty five albino adult rats were randomly divided into 3 groups: control lead (Pb) group that received lead acetate (20 mg/kg/day) 3 times weekly for 8 weeks and PB + NSO group (rats pretreated with *Nigella sativa* oil (4 ml/kg) orally for 1 h before administration of lead acetate (given as in Pb group). Myocardial injury was assessed by laboratory and pathological studies, and heart rate was recorded in all animals. Lead intake resulted in significant increases in cardiac high-sensitivity C-reactive protein (hs-CRP), interleukin-6 (IL-6), E-selectin, troponin I, malondialdehyde (MDA) and serum creatine kinase-MB (CK-MB). The cardiac apelin, superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione (GSH) levels significantly decreased in Pb group compared to the control. Currently, heart rate and ST segment increased significantly after lead intake. Heart lesions as a result of lead treatment were in the form of hemorrhage, myocardial necrosis, mononuclear cell infiltration and fibrosis. Immuno histochemical results of the heart revealed positive cyclooxygenase-2 (Cox-2) expressions in Pb-treated group. NSO administration produced significant normalization of the physiological parameters as well as restored the histological structure and decreased the COX-2 expression of the heart compared to Pb group. In conclusion, NSO intake has cardio protective potential through its ability to decrease pro inflammatory cytokines, oxidative stress and cardiac tissue damage in lead-induced cardio toxicity.

**Key words:** *Nigella sativa* oil, lead acetate, cardio toxicity, inflammation.

## INTRODUCTION

Cardiovascular diseases (CVD) have become the most frequent cause of death worldwide and their incidence continually rises. There is an association between CVD, inflammation and oxidative stress (Roshan et al., 2011). Lead (Pb) is a non-essential toxic heavy metal widely distributed in the environment; various physiological, biochemical and behavioral dysfunctions are induced by chronic exposure to low levels of pb (Ahamed and Siddiqui,

2007; Alghazal et al., 2008).

A link between ambient air pollutants and health has been reported (Makri and Stilianakis, 2008). Previous studies have reported that exposure to low level Pb has been associated with several disease outcomes such as cardiovascular disease and hypertension. It has been proposed that one possible mechanism of Pb toxicity is generate inflammation (Boris et al., 2008) and the disturbance

of prooxidant and antioxidant balance by generation of reactive oxygen species (ROS) (Alghazal et al., 2008; Roshan et al., 2012). Adipokines such as apelin, C-reactive protein (CRP), tumor necrosis factor TNF- $\alpha$  and E-selectin represent a family of proteins released by adipocytes that affect various biological processes including metabolism, satiety, inflammation and cardiovascular function (Gaeini et al., 2008).

*Nigella sativa* (NS) Linn. is an annual herbaceous plant of the Ranunculaceae family and grows in countries bordering the Mediterranean Sea, Pakistan, India and Iran (Ali and Blunden, 2003). *N. sativa* seed contains more than 30% fixed oil and 0.4 to 0.45% volatile oil. The fixed oil is composed mainly of unsaturated fatty acids. Thymoquinone (TQ) is the major active ingredient of the volatile oil (Worthen et al., 1998). Thymoquinone has a strong antioxidant potential due to its scavenging activity towards free radicals (Kanter et al., 2005). There are many reports on its biological activities including anti-hypertensive, anti diabetic, anti-bacterial, anti-tumour and immunomodulator (Kökdil et al., 2009). Therefore, this study was initiated to investigate the possible cardio protective effects of *N. sativa* oil on lead -induced cardiotoxicity.

## MATERIALS AND METHODS

### Chemicals

Lead acetate was purchased from Sigma Aldrich (St. Louis, MO, USA). *N. sativa* seeds were obtained from local market in Assiut, Egypt. They were authenticated by Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Egypt. Blackseed essential oil was prepared according to the procedure described by Burits and Bucar (2000); 75 g of blackseed was crushed and extraction was done using about 220 ml of light petroleum ether in a Soxhlet apparatus. The extraction continued for four hours and was repeated until sufficient oil was collected. The oil collected was analyzed for thymoquinone by high performance liquid chromatography (HPLC) according to the procedure described by Ghosheh et al. (1999). The column was Reprosil Gold 120 C18 type (250  $\times$  4.6 mm, 5  $\mu$ m particle size). The isocratic mobile phase consisted of H<sub>2</sub>O: methanol: 2-propanol in the ratio of 10: 9: 1 by volume. Column temperature and the flow rate were 28°C and 1 ml/min, respectively. The detector was a DAD (254.4 nm) and the injection volume was 5  $\mu$ l. The oil was kept in deep freezer at -20°C until it was used.

### Animals

The experimental protocol was approved by the Institutional Animal Research Committee of the Faculty of Medicine, Assiut University, Egypt, and the published guidelines and regulations were followed. Forty five adult male Wistar albino rats, 8 weeks of age, weighing about 180 to 200 g were obtained and maintained in The Assiut University Animal Nutrition and Care House. The animals were caged in metabolic cages and kept under standard conventional laboratory conditions at a temperature of 22  $\pm$  2°C, with a relative humidity of 50  $\pm$  5% and a 12-h/12-h light/dark cycle. They had unlimited access to drinking water and rat chow. The rats were

randomly divided into three experimental groups of 15 rats each:

1. Control group: rats given only standard rat chow and water for 8 weeks.
2. Pb group: The rats that received lead acetate from Sigma (St. Louis, MO), at a dose of 20 mg/kg in the form of a saline solution (for intraperitoneal [ip] injection), 3 days weekly for 8 weeks (Ghosheh et al., 1999).
3. Pb + NSO group: Rats pretreated with *N. sativa* oil (4 ml/kg) orally for 1 h before administration of lead acetate which is given at the same dose and for the same duration as in Pb group.

### Electrocardiography

Recording of electrocardiogram (ECG) was done at the end of the treatment. It was recorded by needle electrodes which were inserted under the skin of the four limbs of the animals under anesthesia with urethane (1 g/kg, intraperitoneal injection) in lead II position. The needle was connected to an ECG recorder (ECG Cardiofax Nih Onkohn Kohden, Kogyo Co. Ltd, Kogyo, Japan). The QRS, ST and P-R intervals were recorded. The heart rate (HR) was calculated from the P-R intervals by counting them. At the end of the treatment period, the body weights of the animals of each group were measured and recorded. Blood samples were collected from each rat via retro orbital vein. Then, all animals were decapitated under anesthesia with urethane. Blood samples were initially centrifuged at 3000 rpm for 15 min. Serum was kept at -20°C until analysis of Creatine Kinase-MB (CK-MB) levels. The body cavities were then opened and the heart was quickly excised from the aortic root. Heart tissues were weighed.

### Tissue preparation

Heart tissues were homogenized in ice-cold 10 mmol/L Tris-HCl, pH 8.2, containing 0.25 mol/L sucrose, 2 mmol/L 2-mercaptoethanol, 10 mmol/L sodium azide and 0.1 mmol/L phenylmethylsulfonyl fluoride with a polytron (4 vol/wt), and centrifuged at 50,000 g (20 min, 4°C). The supernatants were lyophilized and stored at -20°C.

### Assay of the cardiac levels of pro-inflammatory cytokines

Enzyme-linked immunosorbent assays (ELISA) were performed for measuring concentrations of Apelin (ELISA kit, Phoenix Pharmaceuticals, Inc.), E-selectin (rat E-selectin ELISA Kit), high sensitive C reactive protein (rat C-reactive protein (CRP) ELISA Kit, e-Bioscience, Inc), interleukin-6 IL-6 (rat ELISA; BioSource, Camarillo, CA); they were assayed in total cell extracts prepared from heart tissues.

### Estimation of cardiac biochemical markers and lead levels

CK-MB levels in serum were determined using a commercial kit supplied by Agappe Diagnostics, Kerala, India and Cardiac troponin I in cardiac homogenate was measured by ELISA. The estimation of cardiac levels of lead was done by an atomic absorption spectrophotometer (Perkin-Elmer, 2380) according to the method of Slater and Sawyer (1971).

### Assay of cardiac lipid peroxidation

Malondialdehyde (MDA), which formed as an end product of the

**Table 1.** Effect of *Nigella sativa* oil on the body weight, heart weight and heart weight/body weight ratio of the experimental animals.

Parameter	Control	Pb	Pb +NSO
Initial body weight (g)	186±6.27	187±6.16	186±6.27
Final body weight (g)	306.9±16.3	290.3±8.11 <sup>****</sup>	306±16.16 <sup>NS</sup>
Heart weight (g)	1.07±0.1	1.18 ±0.12 <sup>*+</sup>	1.1±0.08 <sup>NS</sup>
Heart weight/body weight ratio (%)	0.35±0.03	0.4±0.03 <sup>****</sup>	0.36±0.03 <sup>NS</sup>

All values are presented as mean ±SD. for fifteen rats in each group.\* p < 0.05, \*\* p < 0.01 as compared to control group.+ p < 0.05, ++ p < 0.01 as compared NSO group. NS : non significant as compared to control group.

spectrophotometer, as described previously (Haque, 2005). The MDA level was expressed as n moles/mg protein.

#### Assay of endogenous antioxidants

Glutathione (GSH) was assayed in heart tissue homogenates using the Ellmen's reagent (DTNB) method (Känter et al., 2005). The absorbance was read at 412 nm and results were expressed as μmol of GSH/gm of wet tissue. Glutathione peroxidase (GPx) assay was carried out by the method used by Rotruck et al. (1973) in 10% (w/v) homogenates of heart spectrophotometrically at 420 nm. Superoxide dismutase (SOD) activity was assayed in the tissue homogenates (Lowry et al., 1951) spectrophotometrically at 320 nm. One unit of SOD activity is defined as the enzyme concentration required to inhibit the rate of auto-oxidation of adrenaline by 50% in 1 min at pH 10. The total protein content was determined in heart homogenates (10% w/v) of experimental animals (Lowry et al., 1951).

#### Light microscopic examination

Immediately after euthanasia, heart specimens were fixed in 10% buffered formalin, embedded in paraffin, prepared as 4 μm thick sections and stained with hematoxylin and eosin (HE) (Makri and Stilianakis, 2008). Stained sections were examined under light microscope (Olympus CX31, Japan) and photographed using digital camera (Olympus, Camedia C-5060, Japan). The most significant histopathological lesions were listed and their incidence (present/not present) was recorded.

#### Immunohistochemistry (IHC)

Sections of 5 mm thickness were placed on positive charged slides. Briefly, the sections were de-paraffinised and endogenous peroxidase activity was blocked with 3% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in PBS for 30 min. Antigen retrieval was performed by microwaving the sections in 0.01 M sodium citrate buffer (pH 6.0). The slides were then rinsed in PBS, blocked with normal goat serum and incubated, respectively with the primary antibodies COX2 (diluted 1:200 in PBS, Thermo Fisher Scientific, USA) over night at 4°C.

#### Statistical analysis

Data were expressed as mean ± standard deviation (SD) for all parameters. The data were analyzed using GraphPad Prism data

analysis program (GraphPad Software, Inc., San Diego, CA, USA). For comparison of statistical significance between different groups, Student Newman-Keuls t-test for paired data were used. For multiple comparisons, one-way analysis of variance [(one-way-analysis of variance (ANOVA)] test was done followed by the least significant difference (LSD). Correlations were assessed using Spearman's non-parametric correlation coefficient as described by Knapp and Miller (1992). A value of P ≤ 0.05 was considered statistically significant.

## RESULTS

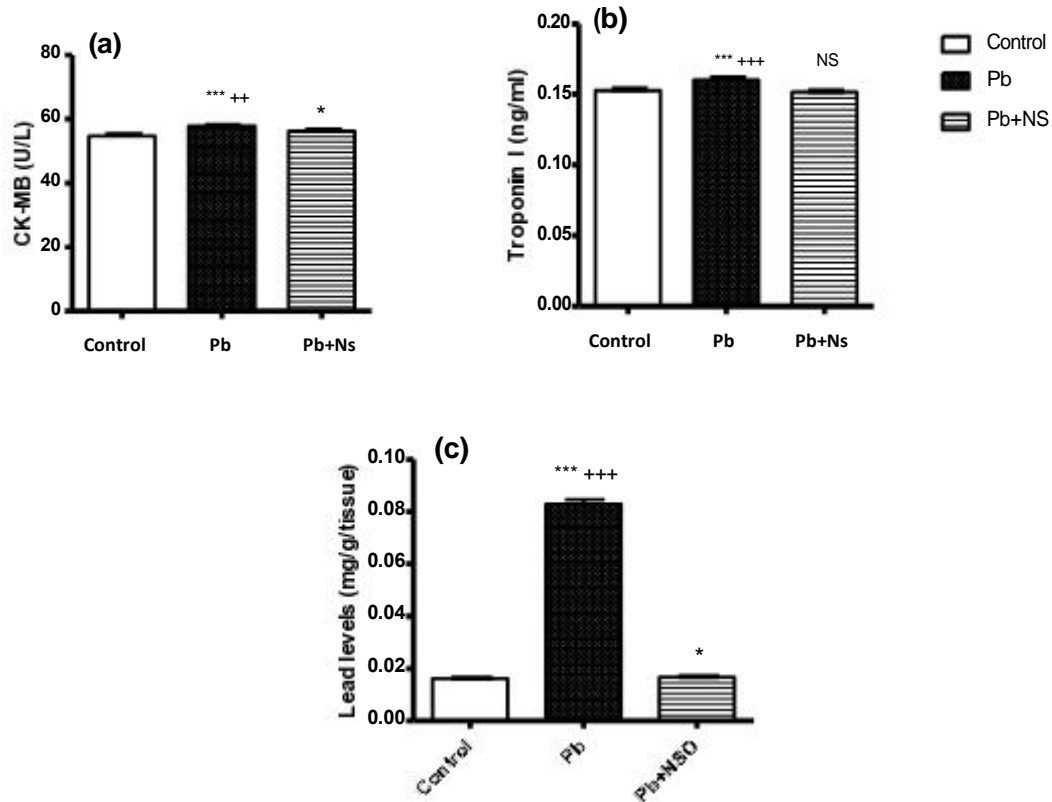
No mortality was observed in animals of any group exposed to lead acetate alone or in combination with *N. sativa* oil during the treatment period of 8 weeks.

#### Body, heart weight and heart weight/ body weight ratio

Table 1 reveals a significant decrease in the final body weight of Pb group compared to control (p < 0.01). However, treatment of rats with NSO significantly attenuated the decrease of the final body weight compared to Pb group (p < 0.01). There was no significant difference in body weights between Pb + NSO and control group. The heart weights of Pb group were significantly higher than those of Pb + NSO and control (p < 0.05, respectively). Pretreatment with NSO for a period of 8 weeks decreased significantly the weights of hearts as they did not differ from those of the control group. The heart weight/body weight ratio of Pb group was significantly increased than those of control and Pb + NSO group (p > 0.01). However, this ratio decreased significantly after NSO treatment, but was still higher than those of control (p > 0.05).

#### Status of tissue leads content

In this study, lead levels in the rat cardiac tissue of Pb group were significantly higher compared to the control (p



**Figure 1.** Cardiac biochemical markers and lead levels. a. Serum CK-MB levels (U/L). (b) Cardiac levels of troponin I (ng/ml). (c) Cardiac levels of lead (mg/g tissues). Data are presented as the mean  $\pm$  SD (15 rats for each group), \*  $p < 0.01$  and \*\*\* $p < 0.001$  versus the control group. ++  $p < 0.01$  and +++  $p < 0.001$  versus Pb+NSO group.

$< 0.001$ ). However, when the rats were pre-treated with NSO, the cardiac tissue lead content was found to be reduced significantly compared to Pb ( $p < 0.001$ ) and ( $p < 0.05$ ) control groups (Figure 1).

#### The effect of *N. sativa* oil on changes of heart rate and electrocardiogram patterns induced by lead acetate

The mean values of heart rate in the rats of Pb group increased significantly than those of the control ( $p < 0.01$ ) and Pb + NSO groups ( $p < 0.01$ ). The treatment with NSO (Pb + NSO group) prevented the increment in heart rate and there were no significant differences of heart rate values between Pb + NSO and those of the control rats. Electrocardiogram patterns of normal and experimental animals are shown in Table 2. Lead acetate induced rats showed a significant ( $p < 0.01$ ) increase in ST-segment compared to the control group. NSO treated group showed decreased ST-segment significantly but still higher than those of control rats ( $p < 0.05$ ). The

differences in the QRS interval were not significant in any group compared to the controls.

#### The effect of *N. sativa* oil on the pro-inflammatory cytokines of cardiac tissues

The protective effect of *N. sativa* oil on the levels of the inflammatory cytokines in chronic exposure to pb at the concentration of 20 mg/kg 3 times per week for 8 weeks was assessed in male Wistar rats. As shown in Table 2 the values of apelin were significantly lower ( $p < 0.01$ ) while HS-CRP, E-selectin and IL-6 were significantly higher ( $p < 0.001$ ) in the Pb group than controls. Furthermore, data in this study indicated that after 8 weeks of treatment with NSO, the values of E-selectin, HS-CRP and IL-6 reduced ( $p < 0.01$ ,  $p < 0.05$ ,  $p < 0.05$ , respectively), while the values of apelin increased in Pb + NSO group significantly than those of Pb group ( $p < 0.05$ ); but they were still significantly different from those of the control group ( $p < 0.05$ ) (Table 3).

**Table 2.** Effect of NSO treatment on electrocardiographic parameters in lead induced cardio toxicity in rats.

Parameter	ECG parameter		
	Heart rate (beat/min)	QRS duration (ms)	T interval (ms)
Control	371.5±4.29	4.59±0.09	10.16±0.11
Pb	379.9±8.98 <sup>***</sup>	4.56±0.1 <sup>NS</sup>	10.39±0.37 <sup>***+</sup>
Pb+ NSO	372.1± 3.84 <sup>NS</sup>	4.58±0.09 <sup>NS</sup>	10.39±0.37*

Data are presented as mean ±SD.\*\*\*p< 0.001,\*\* p< 0.01 and \* p<0.05 versus the control group.\*\*p< 0.01 and +p<0.05 versus Pb+NSO group. NS: non significant compared to control group.

**Table 3.** The levels of pro-inflammatory markers in cardiac tissues of different groups at the 8th week.

Parameter	Control	Pb	Pb+NSO
E-selectin (ng/ml)	71.11±3.53	79.13±2.72 <sup>*** ++</sup>	74.86±5.08*
HS-CRP (mg/ml)	0.054±0.003	0.089±0.035 <sup>***++</sup>	0.06±0.0067*
Apelin (pg/dl)	3.65±0.44	2.74±0.56	3.25±0.49
IL-6 (pg/mg)	633.9±5.15	641.4±4.48	637.5±4.3

Data are presented as the mean± SD (15 rats for each group), HS-CRP: high sensitive c-reactive protein,IL6: interleukin -6. \* p< 0.01 and \*\*\*p< 0.001 versus the control group.+ p< 0.05 and ++ p<0.01 versus Pb+NSO group.

**Table 4.** Effect of *Nigella sativa* oil on cardiac tissues levels of MDA, SOD, GSH and GPx in lead acetate induced cardiac toxicity in rats.

Parameter	Control	Pb	Pb+NSO
MDA(nmol/mg protein)	1.31±0.06	2.54±1.32 <sup>***++</sup>	1.59±0.41*
SOD (U/mg protein)	7.38±0.39	6.35±0.54 <sup>***++</sup>	6.96±0.45*
GSH (µm/mg protein)	4.22±0.26	3.02±0.49 <sup>***++</sup>	3.85±0.54*
GPx (µg of GSH utilized/min/mg)	0.433±0.046	0.298±0.041 <sup>***++</sup>	0.378±0.076*

Data are presented as the mean± SD (15 rats for each group), One U of GPx = µg of GSH utilized/min/mg protein. \* p< 0.01 and \*\*\*p< 0.001 versus the control group.+ p< 0.05, ++ p< 0.01 and +++ p<0.01 versus Pb+NSO group.

### The effects of *N. sativa* oil on cardiac tissue damage markers

In the present study, lead acetate administration induced damage to the myocardium. Changes in cardiac tissue troponin I and serum CK-MB levels are presented in Figure 1. It was found that cardiac damage marker (CK-MB and troponin I) levels increased in Pb group significantly (p < 0.001 and p < 0.01, respectively) compared to the control rats; whereas *N. sativa* oil treatment significantly reversed the elevated CK-MB levels, as they became insignificant to those of control group. On the other hand, cardiac troponin I levels after NSO treatment decreased significantly but they are still higher than those of the control rats (Figure 1).

### The effects of *N. sativa* oil on cardiac lipid peroxidation and endogenous antioxidants

Table 4 shows that lead intake for 8 weeks caused a significant (p < 0.001) increase in levels of lipid peroxidation as measured by the levels of MDA, as well as a significant decrease (p < 0.001) in the levels of the enzymatic antioxidant SOD, GSH and GPx compared to the controls (p < 0.001). The treatment with NSO significantly inhibited the increase in the levels of MDA (p < 0.01) and caused a significant increase in the levels of the endogenous antioxidants (p < 0.01), respectively than those of Pb group. But the levels of MDA and endogenous antioxidants of Pb + NSO group were still significantly different from those of the control group (p < 0.05).

**Table 5.** Incidence of heart lesions in treated rats.

Parameter	Control	Pb-treated	Pb+NSO-treated
Hemorrhage	None	++++	+
Myocardial necrosis	None	+++	+
Mononuclear cell infiltration	None	++++	+
Fibrosis	None	++++	None

+ Lesion observed in 1 to 3 rats; ++ Lesion observed in 4 to 7 rats; +++ Lesion observed in 8-11 rats and ++++ Lesion observed in 12 to 15 rats.

### Histopathological results

The histopathological examination of HE-stained heart sections revealed that most of the significant pathological lesions were found in Pb-treated rats. On the other hand, co-administration of NS with lead markedly improved the incidence and severity of these reported pathological lesions (Table 5).

Heart lesions as a result of lead treatment were in the form of hemorrhage, myocardial necrosis, mononuclear cell infiltration and fibrosis. Interstitial hemorrhage was observed in most of Pb-treated rats (Figure 2a). The myocardial muscles revealed signs of myocardial necrosis in the form of coagulative necrosis, hypereosinophilia of the myocyte, loss of striation and karyopyknosis of the nuclei (Figure 2b). Fibrosis in the interstitium with infiltration of mononuclear cells such as lymphocytes was seen (Figure 2c to e). The heart sections of Pb + NSO treated group showed improvement of the myocardial muscle which resembles the normal myocardial muscles in most cases (Figure 2f).

### Immunohistochemistry results

Immunohistochemical results of Cox-2 in the heart revealed positive Cox-2 expression in Pb-treated group, weak Cox-2 expression in Pb + NSO treated group and negative expression in control group (Figure 3).

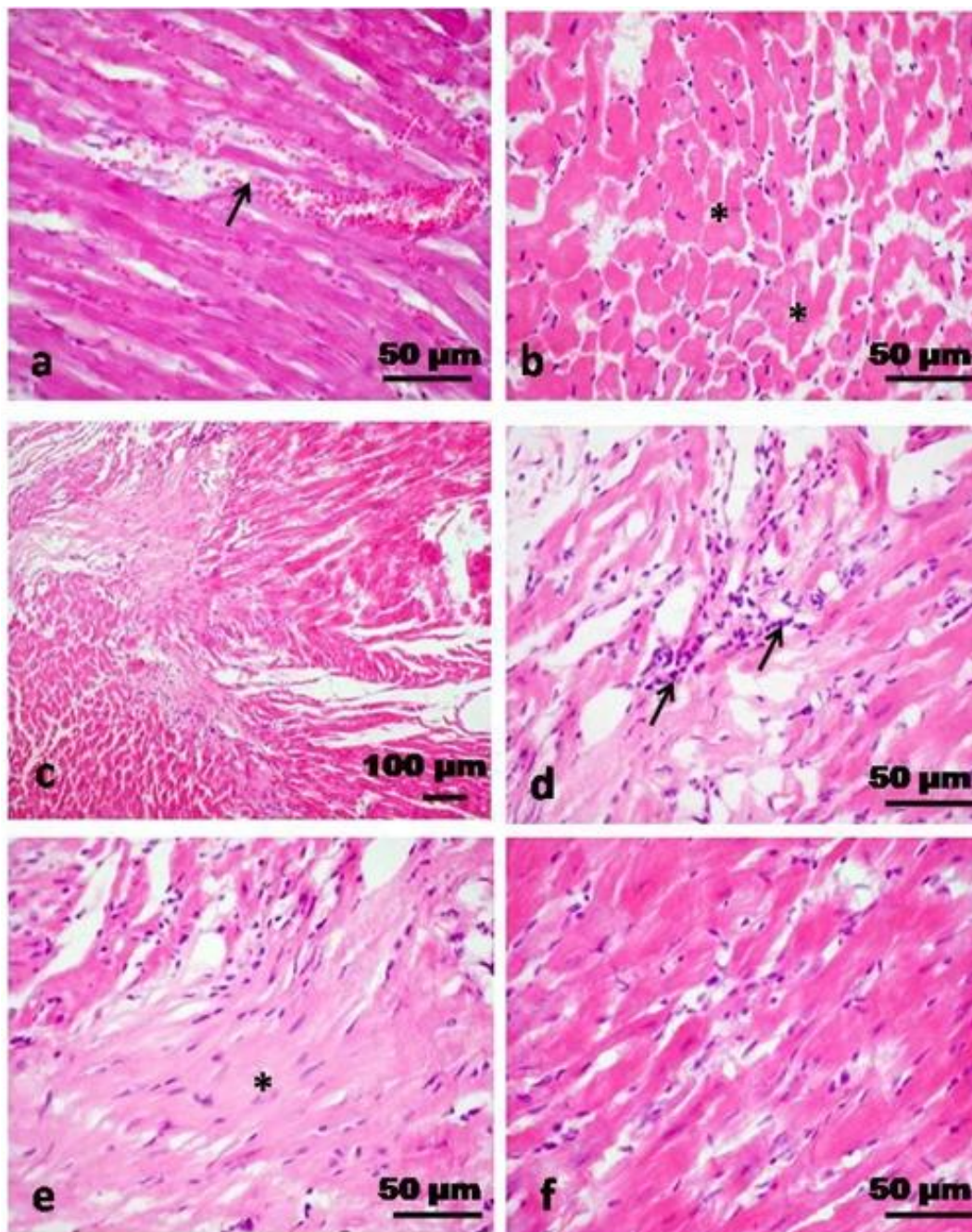
### DISCUSSION

This study was carried out to observe the cardioprotective effects of *N. sativa* oil on lead induced cardiotoxicity in rats. The reduction of weights observed due to lead toxicity in Pb group was almost similar to the findings of some other researchers such as Haque (2005) who reported reduced weight gain after intoxication with lead acetate. Seddik et al. (2010) found that lead intake caused decrease in growth rate of rats which may be due to the imbalance of metabolism produced by impairing zinc status in zinc dependent enzymes which are necessary for many metabolic processes.

In this study, the detected elevation in the heart weight and body weight (HW/BW) ratio of Pb group was thought to be due to necrosis which was attributed to accumulation of lipid (Badalzadeh et al., 2008). This is consistent with our pathological finding as lead acetate intake caused myocardial necrosis, mononuclear cell infiltration and interstitial hemorrhage of pb group. Our study revealed a highly significant content of Pb in the cardiac tissue of the experimental rats following treatment with lead acetate; and this is consistent with the findings of Patra et al. (2011). The high amount of lead in the tissues might have brought about oxidative stress-induced damages. Pre-treatment of rats with NSO reduced the concentration of lead significantly. This may indicate that NSO could remove lead from the organ either by chelating lead or by increasing the clearance of the heavy metal from organs by mechanism(s) not yet clearly known.

The present results showed that lead acetate administration increased the heart rate and caused prolonged ST segment without causing any significant alternation of QRS duration. These results are consistent with the findings of Badalzahed et al. (2008). The increased heart rate in Pb group was probably due to observed central sympathetic nervous system hyperactivity, reduced baroreflex sensitivity and vagal hypotonia in rats treated chronically with lead (Carmignani et al., 2000), while NSO treatment decreased the heart rate to control level and reduced the ST segment significantly; this indicates that NSO has heart rate-reducing effect, which may occur by activating cholinergic mechanisms (Hamed et al., 2010).

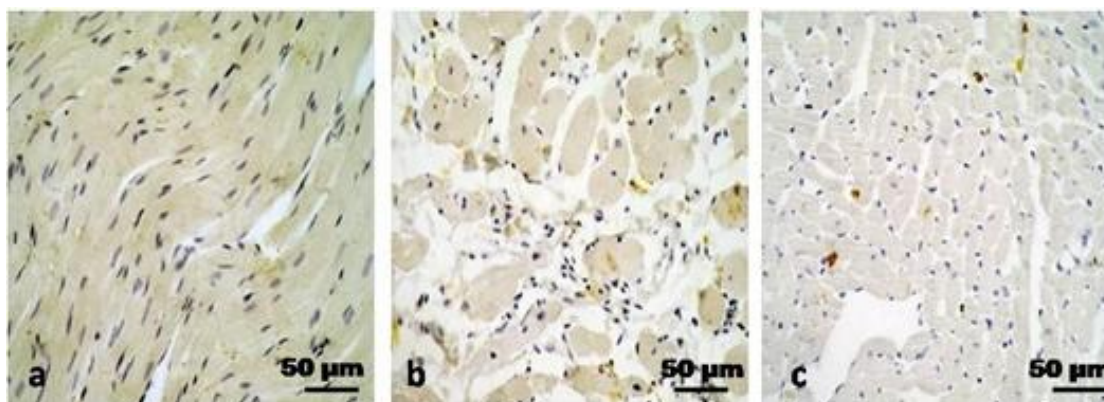
In the current study, chronic exposure of Pb significantly increased the cardiac HS-CRP, IL-6, E-selectin, troponin I and serum CK-MB and significantly decreased apelin levels in the pb group compared to the control and Pb + NSO groups. These cytokines and markers were assayed as indicators of inflammation and tissue damage in heart degenerative cells and serum of rats treated with lead. Various mechanisms were suggested to explain these effects: inhibition of the calcium-pump or a transport protein, disturbances in mineral metabolism, inactivation of several enzymes, etc. (Patrick, 2006). Pathogenesis of Pb poisoning is mainly attributed to lead-induced oxidative stress (Yazdanshenas et al., 2012).



**Figure 2.** Representative micrograph of the heart from Pb and Pb+NS treated rats. (a) Interstitial hemorrhage (arrow). (b) Myocardial necrosis (asterisk). (c) Myocardial fibrosis. (d,e) Higher magnification showing mononuclear infiltration (arrow) fibrous tissues (asterisk). Section in myocardial muscle in Pb + NSO treated group showing normal appearance HE stain.

Lead is capable of inducing oxidative damage to brain, heart, kidneys and reproductive organs (Daniel et al., 2004; Gholamhosseini et al., 2009; Wang et al., 2012) and the results of this study support the hypothesis; since there were a significant increase in the level of MDA and a significant decrease in the endogenous antioxidants in

the cardiac tissue following intake of lead acetate. This was explained by the high affinity of lead for SH group in several enzymes such as SOD, GSH and GPx; thus it can alter antioxidant activities by inhibiting functional SH groups in these enzymes as these enzymes are potential targets of lead toxicity (Marchlewicz et al., 2007). The



**Figure 3.** Immunohistochemistry representative micrograph of the heart from Pb, Pb+NSO treated rats and control rats stained Cox-2. (a) Control rat showing negative expression of Cox-2, (b) Pb-treated rats showing positive expression of Cox-2, (c) Pb+NSO treated rats showing weak expression of Cox-2.

present results support the antioxidant activity of NSO. When it was administered, NSO effectively lowered the levels of MDA and caused a significant increase of the endogenous antioxidants in the cardiac tissue.

Our pathological findings confirmed the biochemical data of the present study and showed that lead acetate intake caused cardiac damage in the form of myocardial necrosis, interstitial hemorrhage, mononuclear cell infiltration and fibrosis. Lead acetate increased the cardiac tissues COX -2 expression. However, administration of NSO reduced the cardiac histo pathologic lesions, preserved its structure and decreased the COX-2 expression in Pb + NSO group.

## Conclusion

Data from this study suggest that NSO supplementation attenuates lead-induced cardio toxicity by mechanisms related, at least in part, to its ability to decrease the pro inflammatory cytokines, oxidative stress and cardiac tissue damage and preserve the activity of antioxidant enzymes. *N. sativa* could serve as a true functional food and may positively affect health promotion via reducing cardiovascular risk.

## REFERENCES

- Ahamed M, Siddiqui MK (2007) Low level lead exposure and oxidative stress: Current opinions, Clin. Chim. Acta. 383:57–64.
- Alghazal MA, Lenártová V, Holovská K, Sobeková A, Falis M, Legáth J (2008) Activities of Antioxidant and Detoxifying Enzymes in Rats after Lead Exposure. Acta. Vet. Brno 77:347–354.
- Ali BH, Blunden G (2003) Pharmacological and toxicological properties of *Nigella sativa*. Phytother. Res. 17:299–305.
- Badalzadeh R, Norouzzadeh A, Heydari A, Asgari A, Khoshbaten A (2008) Effect of low -lead exposure on blood pressure and function of the rat isolated heart. Indian J. Pharmacol. 40:69-72.
- Boris Z, Simkhovich, Michael T, Kleinman, Robert A, Kloner (2008). Air pollution and cardiovascular injury: Epidemiology, Toxicology, and Mechanisms: J. Am. Coll. Cardiol. 52:719-726.
- Burits M, Bucar F (2000) Antioxidant activity of *Nigella sativa* essential oil. Phytother. Res. 14:323–328.
- Carmignani M, Volpe AR, Boscolo P, Qiao N, Gioacchino M (2000). Catcholamine and nitric oxide systems as targets of chronic lead exposure in inducing selective functional impairment. Life Sci. 68:401–415.
- Daniel S, Limson JL, Dairam A, Watkins GM, Daya S (2004). Through mental binding, curcumin protects against lead- and cadmium induced lipid peroxidation in rat brain homogenates and against lead-induced tissue damage in rat brain. J. Inorg. Biochem. 98(2):266-275.
- Gaeini A, Rahnama N, Dabidiroshan V (2008). The effect of continuous training on C- reactive protein of wistar 14848 rats. Gazz. Med. Ital. Arch. Sci. Med. 167:221-229.
- Gholamhosseini B, Khaki A, Khaki AA, Kachabi H, Radsaeed F (2009). Ultra structure study of lead acetate cytotoxic effects on heart tissue in rabbit. Ofogh-e-Danesh. GMUHS J. 14(4):5-12. (in Persian)
- Ghosheh OA, Houdi AA, Crooks PA (1999). High performance liquid chromatographic analysis of the pharmacologically active quinines and related compounds in the oil of the black seed (*Nigella sativa* L). J. Pharm. Biomed. Anal. 19:757–762.
- Hamed EA, Meki AR, Abd El-Mottaleb NA (2010). Protective effect of green tea on lead-induced oxidative damage in rat's blood and brain tissue homogenates. J. Physiol. Biochem. 66(2):143-151.
- Haque MM (2005). Effects of calcium carbonate, potassium iodide and zinc sulfate in lead induced toxicities in mice. MS Thesis, Department of Pharmacology, BAU, Mymensingh.
- Kanter M, Coskun O, Budancamanak M (2005). Hepatoprotective effects of *Nigella sativa* L and *Urtica dioica* L on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. World J. Gastroenterol. 11:6684–6688.
- Knapp GR, Miller MC (1992). Tests of statistical significance: Regression and Correlation. In: Clinical Epidemiology and Biostatistics 1st Ed. Williams and Wilkins, Baltimore, Maryland. pp 255-74.
- Kökdil G, Tamer L, Ercan B, Ilcim A, Aras N, Atik U (2009). Effects of *Nigella unguicularis* fixed oil on blood biochemistry and oxidant/antioxidant balance in rats. J. Ethnopharmacol. 99:131–135.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ(1951). Protein estimation with the Folin- phenol reagent. J. Biol. Chem. 193:265–275.
- Makri A, Stilianakis NI (2008). Vulnerability to air pollution health effects. Int. J. Hyg. Environ. Health 211:326-36.
- Marchlewicz M, Wiszniewska B, Gonet B, Baranowska BI, Safranow K, Kolasa A, Glabowski W, Kurzawa R, Jakubowska K, Rac ME (2007). Increased lipid peroxidation and ascorbic acid utilization in testis and



- epididymis of mice chronically exposed to lead. *Biometals* 20:13-19.
- Patra RC, Rautray AK, Swarup D (2011). Oxidative Stress in Lead and Cadmium Toxicity and Its Amelioration. *Vet. Med. Int.* 1:9-15.
- Patrick L (2006). Lead Toxicity part II: the role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. *Altern Med. Rev.* 11(2):114-127.
- Roshan VD, Assali M, Moghaddam AH, Hosseinzadeh M, Myers J (2011). Exercise training and antioxidants: Effects on rat heart tissue exposed to lead acetate. *Int. J. Toxicol.* Mar 30 (2): 190-196.
- Roshan VD, Ranjbar S, Hosseinzadeh M, Myers J (2012). Left ventricular oxidant and antioxidant markers induced by lifestyle modification in rats exposed to lead acetate. *Eur. J. Sport Sci.* 12 (6) 485- 490.
- Rotruck JT, Pope AL, Ganther HE, Hofeman DG, Hoeksta WG (1973). Biochemical role as a component of glutathione peroxidase. *Science* 179:588–590.
- Seddik L, Bah TM, Aoues A, Brnderdour M, Silmani M (2010). Dried leaf extract protects against lead-induced neurotoxicity in Wistar rats. *Eur. J. Sci. Res.* 42 (1):139-151.
- Slater TF, Sawyer BC (1971). The stimulatory effect of carbon tetrachloride and other halogenalkane or peroxidative reaction in the rat liver function *in vitro*. *Biochem. J.* 123:805–815.
- Wang J, Yang Z, Lin L, Zhanqin ZZ, Liu Z, Liu X (2012). Protective effect of naringenin against lead-induced oxidative stress in rats. *Biol. Trace Elem. Res.* 146:354–359.
- Worthen DR, Ghosheh OA, Crooks PA (1998). The *in vitro* antitumor activity of some crude and purified components of black seed, *Nigella sativa*. *Anticancer Res.* 18: 1527–32.
- Yazdanshenas A, Roshan VD, Ashrafi J (2012). Regular physical activity and diferuloyl methane supplement reverses pro-inflammatory cytokines in heart. *J. Res. Environ. Sci. Toxicol.* 5:107-114.

*UPCOMING CONFERENCES*

93rd Annual Meeting of the German Physiological Society, Mainz, Germany  
*13 Mar 14*

**93<sup>rd</sup> Annual Meeting** March 13-15, 2014  
of the German Physiological Society Mainz, Germany

1st PanAmerican Congress of Physiological Sciences, Iguassu Falls, Brazil  
*02 Aug 14*



## Conferences and Advert

### **December 2013**


20th World congress on Parkinson's Disease and Related Disorders, Geneva, Switzerland, 8 Dec 2013

### **January 2014**

International Conference on Biological, Health and Environmental Sciences, London, UK, 19 Jan 2014

### **February 2014**

International Conference on Developmental Origins of Adiposity and Long-Term Health, Munich, Germany, 13 Mar 2014



# Journal of Physiology and Pathophysiology

Related Journals Published by Academic Journals

- Journal of Physiotherapy and Occupational Therapy
- International Journal of Medicine and Medical Sciences
- Clinical Reviews and Opinions
- Journal of Clinical Pathology and Forensic Medicine
- Journal of Infectious Diseases and Immunity
- International Journal of Nutrition and Metabolism
- Journal of Dentistry and Oral Hygiene

**academicJournals**