The Effects of Maternal Exposure Riboflavin to Prevent Uterus Arsenic Damage in Offspring Rats

Parichehr Nouri a | Ali Olfati b *

a. Department of Midwifery, School of Nursing and Midwifery, Kermanshah University of Medical Sciences, Kermanshah, Iran. 
b. Researcher, Young Researchers and Elites Club, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran.

*Corresponding author: Researcher, Young Researchers and Elites Club, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran. E-mail address: A.Olfati65@gmail.com

ARTICLE INFO

Article type: Original article

Article history:
Received: 19 October 2021
Revised: 11 December 2021
Accepted: 23 December 2021

© The Author(s)

DOI: 10.52547/jhehp.7.4.182

Keywords:
As2O3
Inflammation
Puberty
Vitamin B2

ABSTRACT

Background: In this study, the efficacy of riboflavin (VB2) in preventing uterus As2O3 damage was investigated for the first time in the literature.

Methods: The rats received 40 μg LHRHa for estrus synchronization. 48 pregnant Wistar rats were included in the study. Four groups were formed with 7 rats in each group: Sham, 1.5 mg arsenic trioxide (As2O3/L) alone or in combination with VB2 (20 and 40 mg/L) in drinking water (for 21 days continuously). Moreover, similar to maternal generation treatment the F1- female generation was arranged (for 35 days continuously until puberty). Similar to maternal generation treatment the F1- female generation was arranged (for 35 days continuously until puberty).

Results: Based on the results, As2O3 reduced body weight and feed intake (P < 0.05). Furthermore, the serum malondialdehyde levels in the As2O3 group were significantly higher than that of the control group (P < 0.05). At the same time, the total antioxidative status and the activities of glutathione peroxidase, superoxide dismutase, and catalase were reduced (P < 0.05). Meanwhile, As2O3 remarkably increased the inflammatory markers production [interleukin 6 and C-reactive protein] (P < 0.05). As2O3 administration induced uterus apoptosis-related genes by upregulating caspase-3, iNOS, and Bax genes and downregulating Bcl-2 gene of pubertal F1-female rats (P < 0.05).

Conclusion: Our observation indicated that VB2 therapy is potentially an effective strategy to modify the detrimental effects of As2O3 in pubertal F1-female rats via suppresses oxidative damages.

1. Introduction

Arsenic trioxide is a common environmental contaminant that is widely distributed all around the world. In the substance priority list issued by the Agency for Toxic Substances and Disease Registry 2019, arsenic occupies the 1st rank in terms of its toxicity. Hence, over the past decade researchers have paid more attention to arsenic and its globalized effects. Several studies exist in the literature on the arsenic-impaired organ systems in human and experimental animals such as brain [1], cardiovascular [2], respiratory [3], reproductive [4], and renal [5] particularly the maternal-fetal interface.

The maternal-fetal interface is the critical target organ of chronic toxic insults. Latest studies have shown that maternal exposure to environmental agents affect the succeeding generation [6,7,8]. Meanwhile, health science has faced major challenges for managing maternal transfer disorders and common therapeutic methods often lead to unsatisfactory results.
Riboflavin, formerly known as vitamin B2 (VB2) with molecular formula C17H20N4O6 and molecular weight 376.36 g/mol serves as the main precursor used by animal cells to form both flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) co-enzymes [9], in which VB2 plays important roles in the enzymatic reactions. Both FMN and FAD co-enzymes in association with their client flavoproteins participate in critical cellular processes that include energy and lipid metabolism, redox signaling, programmed cell death, growth regulation, and biologic rhythms regulation [10, 11]. Despite the VB2 food sources (as an effective antioxidant in the nutrition) such as milk, breads, and fortified cereals only a few nutritional studies have been published about the blunted effect of VB2 on breads, and fortified cereals only a few nutritional studies provide a novel mechanistic approach concerning As2O3 toxicity.

2. Materials and Methods

2.1. Chemicals and ethics

All the reagents were obtained from Merck (Darmstadt, Germany). The kits were purchased from Nanjing Jiancheng Bioengineering Institute, (Nanjing, China) to evaluate reactive oxygen species (ROS), various oxidative stress indices including total antioxidative status (TAS), malondialdehyde (MDA), glutathione peroxidase (GSH), superoxide dismutase (SOD), catalase (CAT), and inflammatory markers including interleukin 6 (IL-6) and C-reactive protein (CRP). The study protocol was carried out in compliance with the guidelines for care and handling of laboratory animals, which was approved by an ethics committee at Islamic Azad University, Kermanshah, Iran (98-02-32-51985).

The current study was conducted using 48 female Wistar rats weighing between 230-280 g obtained from the Pasteur Institute of Iran (Karaj, Iran). During the study, all the animals were kept under standard laboratory conditions (12 h of daylight, 12 h of darkness, ventilation, constant temperature, free access to water and food). The rats received 40 μg LHRHa (Sigma, St Louis, MO) for estrus synchronization.

Pregnant rats were placed in an individual birth plastic cages and all the facilities were prepared for their parturition.

Four groups were formed with 7 rats in each group (for 21 days continuously: a complete gestation cycle in rat): group 1 served as vehicle group which received normal saline, group 2 was treated with 1.5 mg As2O3/L with molecular formula As2O3 and molecular weight 197.841 g/mol, group 3 received 1.5 mg As2O3/L + 20 mg VB2/L [7,8-dimethyl-[N-10-ribityl] isoalloxazine], and group 4 received 1.5 mg As2O3/L + 40 mg VB2/L. As2O3 and VB2 dissolved in deionized water. At birth, the weights of offspring and litter size (pups number/mother) were recorded. Similar to maternal generation treatment, the F1-female generation was also arranged into four groups (n = 7 in each group) as follows: 1.5 mg As2O3/L alone or in combination with VB2 (20 and 40 mg/L/day) for 35 days continuously (until puberty). Mean body weight (BW) and feed intake in each group were recorded weekly.

2.2. Sampling

Intracardiac blood samples were collected in gel biochemistry tubes, centrifuged for 10 min at 3000g, aliquoted, and stored at -80 °C. The rats were administered intraperitoneal (IP) anesthesia (ketamine 20 mg/kg and xylazine 0.64 mg/kg) before the surgical procedure. Tissue samples obtained for biochemical assessment were stored under laboratory conditions at -80 °C (Figure 1).

2.3. Oxidative stress indices determination

The method used to detect the oxidative stress indices level in this study was performed as previously described by Jiang et al. (16). Briefly, the ROS level was assayed using a fluorescence spectrophotometer and 2, 7-dichlorofluorescein dictate based on the assay kits and the manufacturer's instructions. Appropriate amounts of uterus homogenate (200 mg) were pre-incubated for 70 min with DCFH-DA (10 μM) at 37 °C to allow the DCFH-DA to be incorporated into any membrane-bound vesicles. The conversion of DCFH to DCF (green fluorescence) was evaluated using a fluorescence spectrophotometer (λ excitation = 485 nm and λ emission = 525 nm). Obtained tissue samples were tested for levels of MDA, T-AOC, GSH, SOD, and CAT were measured by commercial kits. Supernatant absorbance was measured at 532, 520, 420, 550, and 405 nm, respectively.

The serum IL-6 and CRP concentrations were measured using ELISA (Blue Gene, Shanghai, China: code: IL-6: #HS600B, and CRP: #1000). Standard commercial kits were used for analysis and the procedures were adopted as recommended by the manufacturer of the kits. All the samples were analyzed in duplicates and the mean of the duplicate was used for the statistical analysis.
2.4. Quantitative real-time PCR

Total RNA was isolated from the right testis weighing 25-30 mg using Trizol reagent (Life Technologies, Carlsbad, CA, USA). The 2% agarose gel electrophoresis was used to assess the integrity of the total RNA and the A260/280 ratio was in the range of 1.8–2.0 evaluated by NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). RNA was reverse transcribed using a PrimeScript™ RT Master Mix kit. qRT-PCR was carried out using the QuantStudio 7 Flex qRT-PCR system (Stratagene, USA) and SYBR® Premix Ex Taq™ II kit. Specific primers were designed by Invitrogen, USA (Table 1). β-actin (reference gene) was used to normalize the expression level of target genes. Duplicated Ct values were measured for each sample and the comparative Ct method was used to determine the relative expression level of the target genes.

2.5. Statistical analysis

Statistical analysis was performed using SPSS 13.0 software. The results were expressed as the means and standard deviations (mean ± SD) and performed with one-way analysis of variance (ANOVA) followed by Dunnett’s new multiple range test and values of \( P < 0.05 \) were considered as statistically significant.

Table 1: Primers used for qRT-PCR, sequence, and product size

| Gene name | Product size [bp] | Primer sequence |
|-----------|-------------------|-----------------|
| Caspase 3 | 214               | Forward:-TCTTCATTCAGGCGCTGCCG<br>Reverse:-TGCGCGTACAGTTTCAGCATGG |
| iNOS      | 177               | Forward:-AAATGCAGGAGATGGTCCGCAAG<br>Reverse:-ATGCGCACATCGCCACAAAC |
| Bax       | 111               | Forward:-AGGCCAATTGGCGCCCAATCGCCACAAAC<br>Reverse:-ATGCGCACATCGCCACAAAC |
| Bcl-2     | 240               | Forward:-TGTTGGAACACATCGCTTCTGT<br>Reverse:-TTTGTTTGGCAGGCTTGCTG |

Primer sets designed using free online software Primer3Plus [v. 0.4.0] http://primer3plus.cgi.

3. Results and Discussion

Effects of \( \text{As}_2\text{O}_3 \) and VB2 treatments on the BW and feed intake of animals are presented in Table 2. Results regarding BW and feed intake show a significant decrease in the rats fed a diet supplemented with \( \text{As}_2\text{O}_3 \) as compared to the control group at the end of the experiment (\( P < 0.05 \)). These weights and feed intake recovered with the application of the VB2 as a candidate therapy.

Results shown in Table 3 revealed a significant effect of dietary supplementation of \( \text{As}_2\text{O}_3 \) on oxidative stress biomarkers of the rats.
The MDA contents of the As$_2$O$_3$ group were significantly higher than that of the control group ($P < 0.05$). At the same time, results of fluorescence spectrophotometer assay showed that the TAS and the activities of GSH, SOD, and CAT were reduced in As$_2$O$_3$-treated animals ($P < 0.05$). Meanwhile, As$_2$O$_3$ increased the production of inflammatory markers including IL-6 and CRP levels. All these changes were recovered by VB2 as a candidate therapy.

mRNA expression levels of apoptosis-related genes including caspase-3, iNOS, Bax, and Bcl-2 were examined by qRT-PCR (Figure 2). Compared with the control group, mRNA expression levels of all apoptosis-related genes were significantly up-regulated in 1.5 mg As$_2$O$_3$/L exposed ($P < 0.05$), and Bcl-2 mRNA expression significantly decreased in As$_2$O$_3$ exposure groups ($P < 0.05$). The qRT-PCR analysis in the tissue samples (uterus) shows that VB2 as a candidate therapy markedly reduced the toxicity of As$_2$O$_3$ via down-regulation of the expressions of apoptosis-related genes.

In the era of rising environmental contaminants, researchers need to be more aware of the detrimental effects of these agents on the maternal-fetal system. Supplementing vitamins in the animal model for promoting health is an increasingly more common management tool, is increasing. Likewise, this experiment first reported the potential beneficial health effect of VB2 on As$_2$O$_3$-induced rats with female reproductive dysfunction and explored the possible mechanisms.

Millions of people are exposed to arsenic via drinking water, contaminated soil, air, fish, and other sea organisms rich in methylated arsenic species. Occupational exposure was observed especially in the USA, Bangladesh, Pakistan, India, China, and other Asian countries [17]. Further understanding of environmental contaminants including arsenic and their effects on both mother and her fetus can help improving offspring disorders. To the best of our knowledge, this is the first comprehensive research exploring the mechanistic basis for the functional effects of VB2 in F1 female generation by using As$_2$O$_3$-induced laboratory animal models.

In the present study, the difference in BW, feed intake, inflammatory, and oxidative indices between the control group and treated As$_2$O$_3$ animals is likely attributed to differences in the daily content of drinking water. It can be concluded that As$_2$O$_3$ increases lipolysis through increased production of free radicals, ROS formation, and decreased feed intake. On the other hand, MDA (an end product of lipid peroxidation) is an index for the level of ROS-induced biological damage [18]. Results also showed that As$_2$O$_3$ increased serum MDA concentration of pubertal F1-female rats. In agreement with the results of the current study, previous studies have reported that arsenic enhances free radicals production and ROS formation, thus it increases lipid peroxidation and subsequently MDA levels in blood and tissues [1, 4]. Meanwhile, in VB2 groups the decreased serum ROS and MDA concentrations confirm the antioxidant activity of VB2 administration. This phenomenon can be explained by the antioxidant theory. The antioxidant theory states that increased antioxidant vitamins decrease lipid peroxidation. The VB2 is a vital co-enzymes in lipid metabolism. It is known that VB2 is mainly metabolized in the liver and becomes FAD to regulate metabolism [19, 20].

The MDA contents of the As$_2$O$_3$ group were significantly higher than that of the control group ($P < 0.05$). At the same time, results of fluorescence spectrophotometer assay showed that the TAS and the activities of GSH, SOD, and CAT were reduced in As$_2$O$_3$-treated animals ($P < 0.05$). Meanwhile, As$_2$O$_3$ increased the production of inflammatory markers including IL-6 and CRP levels. All these changes were recovered by VB2 as a candidate therapy.

### Table 2: Riboflavin effect on body weight and feed intake in arsenic-treated female rat offspring

| Group [mg/kg/day] | Body weight [g] | Feed intake [g] |
|------------------|----------------|----------------|
| Vehicle [control] | 21.0±1.11 $^a$ | 2.98±0.67 $^a$ |
| As$_2$O$_3$ 1.5  | 13.8±0.98 $^b$ | 1.87±0.32 $^b$ |
| As$_2$O$_3$+VB2 20 | 15.19±0.78 $^b$ | 2.08±0.58 $^b$ |
| As$_2$O$_3$+VB2 40 | 15.69±1.03 $^b$ | 2.11±0.60 $^b$ |

As$_2$O$_3$: arsenic trioxide; VB2: vitamin B2 or riboflavin

Values are given as means ± SD. The same superscripts (a-b) are not significantly different from each in column ($P < 0.05$).

Riboﬂavin to Protect Uterus Damage in Arsenic Treated-Rat

**Nouri P, et al.**

Journal of Human Environment and Health Promotion. 2021; 7(4): 182-8

185
Table 3: Effects of riboflavin on inflammatory and oxidative stress indices in arsenic-treated female rat offspring

| Group [mg/kg/day] | Vehicle [control] | As$_2$O$_3$ 1.5 | As$_2$O$_3$ + VB2 20 | As$_2$O$_3$ + VB2 40 |
|------------------|-------------------|-----------------|---------------------|---------------------|
| ROS              | 1.18 ± 0.63       | 2.32±0.82       | 1.52 ± 0.61         | 1.43 ± 0.55         |
| MDA              | 1.73 ± 0.31       | 3.33±0.87       | 2.12 ± 0.65         | 2.09 ± 0.71         |
| GSH              | 70.3 ± 5.33       | 35.1±4.02       | 65.2 ± 1.96         | 66.8 ± 2.16         |
| SOD              | 70.3 ± 5.33       | 73.2±6.01       | 84.5 ± 6.28         | 88.3 ± 7.12         |
| CAT              | 532.5 ± 78.9      | 440.1±56.8      | 508 ± 61.7          | 520 ± 70.5          |
| IL-6             | 72.2 ± 13.6       | 88.9±18.5       | 79.1 ± 16.0         | 75.9 ± 15.1         |
| CRP              | 6.36 ± 1.48       | 7.55±0.82       | 6.83 ± 0.93         | 6.71 ± 1.12         |

As$_2$O$_3$: arsenic trioxide; VB2: riboflavin. Values are given as means ± SD [n=7]. The same superscripts (a-e) are not significantly different from each other in each row [P < 0.05].

During gestation, the obtained results revealed that maternal use of VB2 during the sensitive period of fetus development reduced the oxidative stress defects in particular. It can be concluded that As$_2$O$_3$ is linked as a risk factor in a number of both mother and her fetus damages. Generally, it was observed that As$_2$O$_3$ exposure could significantly inhibit the growth and development of rodents. As reported, after treatment with As$_2$O$_3$, HepG2 cells and hippocampus cells underwent significant apoptosis [27, 28].

As$_2$O$_3$-induced uterus cells death was blocked by VB2 as a candidate therapy. In this case, a balance between apoptosis and cell proliferation might have occurred in supplemented groups with VB2 since the tissue was in homeostasis. VB2 exerts protective effects through its antioxidant, anti-fibrosis, and anti-inflammatory properties that consequently restore the structure and functionality of the target organs [29].

In this study, the mRNA expression levels of an anti-apoptotic gene like Bcl-2 were significantly down-regulated and the mRNA expression levels of pro-apoptotic genes including caspase 3 and Bax were significantly up-regulated in uterus tissue. Overexpression of the Bcl-2 gene could increase cell viability and prevent apoptosis in adverse tissue circumstances [30,31,32]. As$_2$O$_3$ administered generates inflammatory-oxidative stress that induces apoptosis of uterus tissue by activating caspase 3 and iNOS gene expressions occurs together with Bax/Bcl-2 imbalance. Our findings indicated that VB2 as a candidate therapy could change the expression of apoptosis-related genes and contribution with genes related to survivalists, which has been an effective strategy to overcome the drawbacks of As$_2$O$_3$ toxicity.

4. Conclusion

In summary, the obtained data demonstrated that VB2 therapy is potentially an effective strategy for correcting the detrimental effects of As$_2$O$_3$ in pubertal F1-female rats via inhibiting oxidative changes and cell apoptosis; however, a full understanding of the mechanism by which these vitamin function is still lacking.
Little work has been done about the effect of B vitamin supplements family on the reproductive system. Thus, the present information obtained in the rat model can pave the way for investigating VB2 function, not only about its effects on female reproduction but also as a potential antioxidant with systemic activity. This can help to develop novel treatments and biotechnological applications both in human medicine and animal production.

Authors’ Contributions

Parichehr Nouri: Formal analysis; Methodology; Writing – review and Editing. Ali Olfati: Conceptualization; Data curation; Investigation; Writing – original draft. All the authors read and approved the final manuscript.

Conflicts of Interest

The Authors declare that there is no conflict of interest.

Acknowledgements

The authors thanks Prof. Dr. Mehrdad Payandeh and The Clinical Research Development Unit of Imam Reza Hospital (Kermanshah, Iran) for providing drugs and facilities (Project No. IR.IAU.REC. 98-02-32-51985).

Abbreviations

As2O3: arsenic trioxide; VB2: riboflavin; FMN: flavin mononucleotide; FAD: flavin adenine dinucleotide; ROS: reactive oxygen species; TAS: total antioxidative status; MDA: malondialdehyde; GSH: glutathione peroxidase; SOD: superoxide dismutase; CAT: catalase; IL-6: interleukin 6; CRP: C-reactive protein; BW: body weight

References

1. Sun X, Li J, Zhao H, Wang Y, Liu J, Shao Y, et al. Synergistic Effect of Copper and Arsenic upon Oxidative Stress, Inflammation and Autophagy Alterations in Brain Tissues of Gallus Gallus. J Inor Biochem. 2018; 178: 54-62.
2. Li SW, Sun X, He Y, Guo Y, Zhao HJ, Hou ZJ, et al. Assessment of Arsenic Trioxide in the Heart of Gallus Gallus: Alterations of Oxidative Damage Parameters, Inflammatory Cytokines, and Cardiac Enzymes. Environ Sci Pollut Res Int. 2017; 24(6): 5781-90.
3. Yang P, He XQ, Peng L, Li AP, Wang XR, Zhou JW, et al. The Role of Oxidative Stress in Hormesis Induced by Sodium Arsenite in Human Embryo Lung Fibroblast (HELF) Cellul ar Proliferationmodel. J Toxicol Environ Health A. 2007; 70(11): 976-83.
4. Shao YZ, Zhao HJ, Wang Y, Liu JJ, Li JL, Luo LY, et al. The Apoptosis in Arsenic-Induced Oxidative Stress is Associated with Autophagy in the Testis Tissues of Chicken. Poult Sci 2018; 97(9): 3248-57.
5. Ogaybemi AA, Omobowale TO, Asenuga ER, Ochigbo GO, Adegunbi AO, Adedapo AA, et al. Sodium Arsenite-Induced Cardiovascular and Renal Dysfunction in Rat Via Oxidative Stress and Protein Kinase B (Akt/PKB) Signaling Pathway. Redox Rep. 2017; 22(6): 467-77.
6. Fiandianese N, Borroneo V, Berrini A, Fischer B, Schaedlich K, Schmidt J, et al. Maternal Exposure to a Mixture of [2-ethylhexyl] Phthalate (DEHP) and Polychlorinated Biphenyls (PCBs) Causes Reproductive Dysfunction in Adult Male Mouse Offspring. Reprod Toxicol 2016; 65: 123-32.
7. Hao Y, Liu J, Feng Y, Yu S, Zhang W, Li L, et al. Molecular Evidence of Offspring Liver Dysfunction after Maternal Exposure to Zinc Oxide Nanoparticles. Toxicol Appl Pharmacol 2017; 329: 318-25.
8. Li X, Sun Z, Manthari RK, Li M, Guo Q, Wang J. Effect of Gestational Exposure to Arsenic on Puberty in Offspring Female Mice. Chemosphere. 2018; 202: 119-26.
9. Pinto JT, Cooper AJL. From Cholesterogenesis to Steriodogenesis: Role of Riboflavin and Flavoenzymes in the Biosynthesis of Vitamin D,12. Adv Nutr. 2014; 5(2): 144-63.
10. Massey V. The Chemical and Biological Versatility of Riboflavin. Biochem Soc Trans. 2000; 28(4): 283-96.
11. Tokutomi S, Matsuoka D, Zikihara K. Molecular Structure and Regulation of Phototropin Kinase by Blue Light. Biochim Biophys Acta. 2008; 1784(1): 133-42.
12. Saedisomeolia A, Ashoori M. New Research and Developments of Water-Soluble Vitamins. Chapter Two-Riboflavin in Human Health: A Review of Current Evidences. Adv Food Nutr Res. 2018; 51: 51-81.
13. Al Harbi NO, Imam F, Nadeem A, Al Harbi MM, Iqbal M, Ahmad SF. Carbon Tetrachloride-Induced Hepatotoxicity in Rat Is Reversed by Treatment with Riboflavin. Int Immunopharmacol. 2014; 21(2): 383-8.
14. Alam MM, Iqbal S, Naseem I. Ameliorative Effect of Riboflavin on Hyperglycemia, Oxidative Stress and DNA Damage in Type-2 Diabetic Mouse: Mechanistic and therapeutic strategies. Arch Biochem Biophys. 2015; 584: 10-9.
15. Peraza AV, Guzmán DC, Brizuela NO, Herrera MO, Olguín HJ, Silva ML, et al. Riboflavin and Pyridoxine Restore Dopamine Levels and Reduce Oxidative Stress in Brain of Rats. BMC Neurosci. 2018; 19(1): 1-8.
16. Jiang YP, Yang JM, Ye RJ, Liu N, Zhang WJ, Ma L, et al. Protective Effects of Betaine on Diabetic Induced Disruption of the Male Mice Blood-Tests Barrier by Regulating Oxidative Stress-Mediated p38 MAPK Pathways. Biomed Pharmacother. 2019; 120: 109474.
17. Zheng Y, Flanagan SV. The Case for Universal Screening of Private Well Water Quality in the U.S. and Testing Requirements to Achieve It: Evidence from Arsenic. Environ Health Perspect. 2017; 125(8): 085002.
18. Popova M, Popov C. Damage to Subcellular Structures Evoked by Lipid Peroxidation. Z Naturforsch C J. 2002; 57(3-4): 361-5.
19. Kumar V, Crilon JE, Ohgi KA, Edwards TA, Rose DW, Escalante CR, et al. Transcription Corepressor CtBP Is an NAD[+] -Regulated Dehydrogenase. Mol Cell. 2002; 10(4): 857-69.
20. Barile M, Giancaspero TA, Leone P, Galluccio M, Indiveri C. Riboflavin Transport and Metabolism in Humans. J Inherit Metab Dis. 2016; 39(4): 545-57.
21. Smedts HP, Verkleij-Hagoort AC, de Vries JH, Ottenkamp J, Steegers EA, et al. Maternal Intake of Fat, Riboflavin and Nicotinamide and the Risk of Having Offspring with Congenital Heart Defects. Eur J Nutr. 2008; 47(1): 357-65.
22. Rivlin RS, Bowman BA, Russell RM, Edwards. Riboflavin. In: Present Knowledge in Nutrition. Washington [DC] : ILSI Press. 2001; 191-8. Available from: URL: https://journals.sagepub.com/doi/10.4081/jhi.2011.e21.
23. Angelini C, Nascimbeni AC, Cenacchi G, Tasca E. Lipolysis and Lipophagy in Lipid Storage Myopathies. Biochim Biophys Acta. 2016; 1862(7): 1367-73.
24. Wang P, Fan F, Li X, Sun X, Ma L, Wu J, et al. Riboflavin Attenuates Myocardial Injury Via LSD1-Mediated Crosstalk between Phospholipid Metabolism and Histone Methylation in Mice with Experimental Myocardial Infarction. *J Mol Cell Cardiol.* 2018; 115: 115-29.

25. Barker DJ. The Developmental Origins of Adult Disease. *J Am Coll Nutr.* 2004; 23(Suppl 6): 588S–95S.

26. Institute of Medicine. Feed and Nutrition Board. Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: National Academy Press. 1998.

27. He J, Xu B, Gao W, Su G, Yu H, Shen Y, et al. Effects of Arsenic Trioxide on Migration, Invasion and Apoptosis of Hepatocellular Carcinoma HepG2 cells. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi.* 2020; 37(1): 105-11.

28. Feng W, Wu X, Mao G, Zhao T, Wang W, et al. Neurological Effects of Subchronic Exposure to Dioctyl Phthalate (DOP), Lead, and Arsenic, Individual and Mixtures, in Immature Mice. *Environ Sci Pollut Res Int.* 2020; 27(9): 9247-260.

29. Bashandy SAE, Ebaid H, Moussa SAA, Alhazza IM, Hassan I, Alaaamer A, et al. Potential Effects of the Combination of Nicotinamide, Vitamin B2 and Vitamin C on Oxidative-Mediated Hepatotoxicity Induced by Thioacetamide. *Lipids Health Dis.* 2018; 17(1): 1-9.

30. Toruner M, Fernandez Sapico M, Sha JJ, Pham L, Urrutia R, Egan L. Antimycobacterial Effect of Nuclear Factor-κB through Upregulated Expression of Osteoprotegerin, Bcl-2 and IAP-1. *J Biol Chem.* 2006; 281(13): 8686-96.

31. Olfati A, Tvrdá E. Riboflavin Recovery of Spermatogenic Dysfunction via a Dual Inhibition of Oxidative Changes and Regulation of the PINK1-Mediated Pathway in Arsenic-Injured Rat Model. *Physiol Res.* 2021; 70(4): 591-603.

32. Olfati A, Moghaddam G, Baradaran B. FSH and Estradiol Benzoate Administration Recover Spermatogenesis and Sexual Hormone Levels in a Busulfan-injured Rat Model. *Comp Clin Path.* 2020; 29(1): 53-59.