

# Gender difference in arsenic biotransformation is an important metabolic basis for arsenic toxicity

**Maihaba Muhetaer**

Public Health College of Xinjiang Medical University

**Mei Yang**

Basic Medical College of Xinjiang Medical University

**Rongxiang Xia**

Center for Disease Control and Prevention of Xinjiang Uygur Autonomous Region

**Jun Wu** (✉ [wuj1997@sohu.com](mailto:wuj1997@sohu.com))

Public Health College of Xinjiang Medical University

---

## Research Article

**Keywords:** Arsenic, Gender difference, MMA, DMA, SAM, ARR, NAD, PNP, PK, MPO, As3MT

**Posted Date:** March 3rd, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-252094/v1>

**License:** © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

---

**Version of Record:** A version of this preprint was published at BMC Pharmacology and Toxicology on February 28th, 2022. See the published version at <https://doi.org/10.1186/s40360-022-00554-w>.

# Abstract

**Background:** There are gender differences in the biotransformation of arsenic. We investigated the effects of gender differences on arsenic metabolism and arsenic toxicity mechanisms in rat liver tissues.

**Methods:** Rats were treated with different amounts of arsenic compounds. Arsenic form MMA and DMA in the liver was determined by high performance liquid chromatography-hydride generation atomic fluorescence spectroscopy. SAM, ARR, NAD, PNP, PK, and MPO in rat liver were determined by enzyme-linked immunoassay. RT-qPCR was used to determine AS3MT in the liver.

**Results:** Compared with male and female animals in the same group, MMA and DMA were statistically significant in the three groups of  $iAs^{3+}$  high,  $iAs^{3+}$  medium and  $iAs^{5+}$  low ( $P < 0.05$ ). The MMA of male rats in  $iAs^{3+}$  high and medium groups was higher than that of female rats, and the DMA of male rats was lower than that of female rats. *As3MT* mRNA in the male  $iAs^{3+}$  high group was higher than that of females. Besides, compared between male and female, only in  $iAs^{3+}$  low dose,  $iAs^{3+}$  medium dose,  $iAs^{5+}$  low dose, and  $iAs^{5+}$  medium dose groups, there was significant difference in SAM level ( $P < 0.05$ ). Compared with male and female animals in the same group, male rats had significantly higher PNP and ARR activities while lower PK activity than female rats ( $P < 0.05$ ). Between the male and female groups, only the  $iAs^{3+}$  high dose and medium dose group had a statistically significant difference ( $P < 0.05$ ). The NAD activity of females in  $iAs^{3+}$  high dose group was higher than that of males.

**Conclusion:** Conclusively, under the same arsenic exposure, there were gender differences between female and male rats, and arsenic metabolism was more cytotoxic to male rats than to females.

## Background

Arsenic exposure is common, which can be from contaminated drinking water and industrial activities [1]. The toxicity of different forms of arsenic is not only related to the environment, but also closely related to the metabolism and detoxification mechanism of the organism [2]. When inorganic arsenic enters the organism, it is converted into an organic form mainly through methylation in the liver, which is then excreted from the body. Generally, inorganic arsenic enters the body in the form of arsenite ( $iAs^{3+}$ ) or arsenate ( $iAs^{5+}$ ). The  $iAs^{5+}$  can be reduced to  $iAs^{3+}$ , and then undergo methylation and other reduction reactions to form monomethyl aracid (MMA) and dimethyl aracid (DMA). Arsenic (+ 3 oxidation state) methyltransferase (AS3MT) uses S-adenosylmethionine (SAM) as a methyl donor to catalyze the methylation of arsenic [3, 4]. Arsenate respiratory reductase (ARR) catalyzes the reduction of arsenate to arsenite [5]. In human liver, purine nucleoside phosphorylase (PNP) reduces  $As^V$  to  $As^{III}$  [6]. In addition, nicotinamide adenine dinucleotide (NAD), as one of the glycolysis substrates, greatly enhances the reduction of  $As^{5+}$  [7]. Arsenic exposure increases the level of pyruvate kinase M2 from week 2 of exposure [8] and promotes a significant increase in serum myeloperoxidase (MPO) activity [9]. The severity of arsenic poisoning is closely related to changes in metabolic enzyme activities. Therefore, exploring

changes in metabolic enzyme activity during arsenic poisoning may help to identify individuals who are particularly vulnerable to arsenic toxicity.

The methylation ability of arsenic varies among species, individuals and populations. Previous study has shown that gender is a major factor influencing arsenic metabolism [10]. Women have better methylation efficiency than men, although women are likely to consume nutritious foods, like meat and fresh vegetables [11]. Another important methyl donor is choline, which could either be derived from the diet or from phosphatidylcholine. The synthesis of phosphatidylcholine was reported to be up regulated by estrogen, partially explaining the better methylation of arsenic among women compared to men [12].

In order to explore the difference in methylation ability between male and female rats, in this study, we measured arsenic metabolites and enzyme activity in the liver. Meanwhile, we studied the effects of arsenic, gender, and exposure level on arsenic metabolism in rats.

## Materials And Methods

### Animals

Four-week-old Wistar rats with body weight between 80-120g (n = 70, including 35 males and 35 females) were from the Experimental Animal Center of Xinjiang Medical University. The animal usage license number was SYXK (new) 2003-0001. All rats were kept in an environment with a relative humidity of 40–60% and room temperature of 18°C to 22°C.

All animal experiments were conducted according to the ethical guidelines of Experimental Animal Center of Xinjiang Medical University.

This study was approved by the Ethics Committee of Xinjiang Medical University.

All efforts were made to minimize animal suffering.

### Arsenic poisoning model establishment and animal grouping

After 1 week of adaptive feeding, the rats were randomly divided into 7 groups, namely the normal control (deionized water) group, the low-dose (1/45LD50, 2.33mg/kg)/medium-dose (1/15LD50, 6.67mg/kg)/high-dose (1/5LD50), 20.00mg/kg)  $iAs^{5+}$  exposure groups, and the low dose (1/45LD50, 2.33mg/kg)/medium dose (1/15LD50, 6.67mg/kg)/high dose (1/5LD50, 20.00mg/kg)  $iAs^{3+}$  groups, with 10 animals in each group, half male and female. Free drinking water was used for the poisoning, and the poisoning was continued for 90 days. The stock solution of  $iAs^{3+}$  and  $iAs^{5+}$  was re-dispensed every two days during the poisoning period. During the poisoning period, the animal's water consumption was recorded daily, and the animal's body weight was measured every 6 days. The Horn method [13] was used to determine that the oral intake of sodium arsenite ( $iAs^{3+}$ ) and sodium hydrogen arsenate ( $iAs^{5+}$ ) in Wistar rats. The food intake was recorded, and the concentration of the arsenic-exposed aqueous

solution was adjusted according to the average body weight and water consumption of each group of animals, that is, appropriate amounts of  $iAs^{3+}$  and  $iAs^{5+}$  were measured.

The sodium bicarbonate stock solution was diluted according to the average weight of the rats and the amount of water they drank on the day to prepare the arsenic-exposed aqueous solutions for each group of rats to ensure the consistency of the arsenic dose.

## Sample collection

After 90 days of exposure, the animals were sacrificed by cervical dislocation. Then the liver was dissected immediately, and rinsed with normal saline at 4°C. After that, the wet weight of the liver was weighed. Then, the liver tissues were then homogenized with an electric homogenizer in a volume of 3mL PBS. The homogenate was centrifuged at 3000 r/min at 4°C for 20 minutes. Then, the supernatant was collected and stored at -80°C until use.

## High performance liquid chromatography-hydride generation atomic fluorescence spectroscopy

The rapid solvent extraction (ASE) method was used for sample pretreatment [14]. The contents of arsenic speciation products ( $iAs^{3+}$ ,  $iAs^{5+}$ , MMA, DMA) in liver tissues were determined by high performance liquid chromatography-hydride generation atomic fluorescence spectroscopy. The detection limit of the method was 6.67–12.03 $\mu$ g/L, RSD < 3%. The average recovery rate of DMA in each  $iAs^{3+}$  group was between 98.9% and 102.9%.

## ELISA

The activities of pyruvate kinase (PK), ARR, MPO, SAM, NAD and PNP were measured with Enzyme-linked immunoassay kits (Shanghai Huole Biological Technology Co, Ltd, Shanghai, China) according to the instructions. PK was expressed as mU/L. The results of ARR, MPO, NAD, and PNP were shown as U/L. The result of SAM was shown as nmol/L.

### Real-time fluorescent quantitative PCR(RT-qPCR)

Total RNA was isolated from liver tissues using the Trizol reagent (Invitrogen, USA). cDNA was synthesized with a high capacity cDNA reverse transcription Kit (Fermantas) from 1 $\mu$ g total RNA. The PCR procedure for AS3MT was: 95°C, 2 min; 95°C, 5 sec, and 58°C, 30 sec, 40 cycles; that for  $\beta$ -actin was : 95°C, 2 min; 95°C, 5 sec, and 55°C, 30 sec, 40 cycles. The primers used were as follows: rat AS3MT: 5-GGG ACA CAT CAC CGG GAT AGA C-3' (Forward) and 5'-AAC ATC TCA ATT TGG CCG TGA AG-3' (Reverse); and rat  $\beta$ -actin: 5'-TCC TGT GGC ATC CAT GAA ACT-3' (Forward) and 5'-GAA GCA TTT GCG GTG CAC GTA-3' (Reverse). Each sample was analyzed in duplicate and expression of the AS3MT mRNA was normalized to that of  $\beta$ -actin.

## Statistical analysis

SPSS 15.0 software was used for data analysis. All experiments were repeated three times, and the results were expressed as mean  $\pm$  standard deviation. For data of normal distribution, one-way variance analysis was used for multi-comparison followed by LSD-t test for pairwise comparison. Dunnett test was used when the data was of non-normal distribution. Pearson correlation method was used for correlation analysis. The  $P < 0.05$  was considered as statistically significant.

## Results

### Comparison of arsenic DMA and MMA in rat liver

The ASE method was used to extract the arsenic metabolites in the liver, and the arsenic metabolites, including MMA and DMA, in the liver were analyzed by high performance liquid chromatography-hydride generation atomic fluorescence spectrometry. The results showed that in each group, DMA and MMA in the liver of male and female rats were significantly higher than those in the normal control group (Fig. 1A and 1B,  $P < 0.05$ ). Compared with the low-dose group, except for the male  $iAs^{5+}$  high-dose group, the differences in MMA and DMA in other groups were all statistically significant ( $P < 0.05$ ). When comparing the effects of different arsenic compounds in the same dose group, the MMA differences between the female  $iAs^{5+}$  low-dose group and the male  $iAs^{5+}$  high-dose and medium-dose groups were statistically significant. The DMA levels were all statistically significant between male and female rats ( $P < 0.05$ ). Compared with male and female animals in the same group, the MMA in groups of  $iAs^{3+}$  high, medium, and  $iAs^{5+}$  low was all statistically significant, and the differences in DMA were statistically significant ( $P < 0.05$ ). However, the MMA of male rats was higher in  $iAs^{3+}$  high and medium dose groups than that of female rats, and the DMA of male rats was lower than that of female rats.

#### Effect of gender on the relative expression of *As3MT* mRNA in rats

RT-qPCR was used to detect the expression of *As3MT* mRNA in the liver. After different doses of  $iAs^{3+}$  or  $iAs^{5+}$  treatment, *As3MT* in both male and female rats in each group was higher than that in the normal control group ( $p < 0.05$ ) (Fig. 2). Compared with the low-dose group, only the same test substance in the female AH group, AM group, male AH group, and BH group had statistical differences ( $P < 0.05$ ). Comparing different test substances in the same dose group, only the female AH group, the female AM group, and the male AH group had statistical differences ( $P < 0.05$ ). Compared with male and female animals in the same group, the expression of *As3MT* mRNA in AH group was higher in males than females ( $P < 0.05$ ).

#### Effects of gender on MPO activity in the liver of rats

The MPO activity in the liver was determined by ELISA. Compared with the control group, the MPO activity of the  $iAs^{3+}$  low-dose group and the  $iAs^{5+}$  high-dose group increased ( $P < 0.05$ ). Besides, the difference between male and female in the same group was statistically significant ( $P < 0.05$ , Fig. 3).

### **Effects of gender on SAM in the liver of rats**

The SAM level in the liver was also determined by ELISA. Compared with the control group, the SAM activity of each  $iAs^{3+}$  or  $iAs^{5+}$  group was significantly increased ( $P < 0.05$ ) (Fig. 4). Compared with the  $iAs^{3+}$  low-dose group, the activity of SAM in the  $iAs^{3+}$  high-dose group was lower ( $P < 0.05$ ). The activity of SAM was lower in  $iAs^{5+}$  high and medium dose groups than that in  $iAs^{5+}$  low dose groups ( $P < 0.05$ ). In the  $iAs^{3+}$  medium/low-dose groups, and  $iAs^{5+}$  medium/low-dose groups, the SAM activity of females was lower than that of males ( $P < 0.05$ , Fig. 4).

### **Effects of gender on ARR activity in the liver of rats**

As shown in Fig. 5, the ARR activity of the  $iAs^{3+}$  low-dose group was higher than that of the normal control group, while the ARR activity of the male  $iAs^{5+}$  high-dose group was higher than that of the normal control group. Additionally, the ARR activity of the  $iAs^{3+}$  high-dose group was lower than that of the  $iAs^{3+}$  low-dose group. The ARR activity of  $iAs^{5+}$  high-dose group was higher than  $iAs^{5+}$  low-dose group. In the same group, compared between male and female, except for the  $iAs^{3+}$  high-dose group, the ARR activity of males in other groups was higher than that of females (Fig. 5).

### **Effect of gender on PNP level in the liver of rats**

The analysis of PNP activity in the liver of each group of rats found that in the same group of animals, the PNP activity of male rats was higher than that of female rats ( $P < 0.05$ , Fig. 6).

### **Effects of gender on the PK activity in the liver of rats**

The PK activity in the liver was determined by ELISA. After exposure to different doses of  $iAs^{3+}$  or  $iAs^{5+}$ , except for the  $iAs^{3+}$  high-dose group, the PK activity in remaining groups was statistically different from that in the control group ( $P < 0.05$ ) (Fig. 7). Compared with the low-dose group, only  $iAs^{3+}$  had statistical difference ( $P < 0.05$ ). In addition, there was a statistical difference between the female  $iAs^{3+}$  high-dose group and the  $iAs^{5+}$  high-dose group ( $P < 0.05$ ). In the same group, comparison between male and female, except for the control group, PK activity in other groups of female rats was higher than that of male rats ( $P < 0.05$ , Fig. 7).

### **Effect of gender on NAD level in the liver of rats**

ELISA was performed to determine the NAD level in the liver. Compared with the control group, there was a statistical difference in NAD of female  $iAs^{3+}$  low dose group, the  $iAs^{5+}$  low/medium dose group and the male  $iAs^{3+}$  low dose group ( $P < 0.05$ ) (Fig. 8). Compared with the low-dose group, the activity of NAD in the  $iAs^{3+}$  high-dose group decreased ( $P < 0.05$ ). In comparison between male and female, the NAD activity of females in  $iAs^{3+}$  high and medium dose groups was higher than that of males ( $P < 0.05$ ).

These results indicate that under the same  $iAs^{3+}$  exposure, arsenic inhibited NAD activity in males, and promoted NAD activity in females.

## Discussion

The results of this study showed that after exposure to  $iAs^{3+}$  or  $iAs^{5+}$ , the content of DMA and MMA in the liver of male rats and female rats in each group was significantly higher than that in the normal control group, reflecting that arsenic exposure may affect DMA and MMA content. The DMA content of male rats was lower than female rats in the same group. This is consistent with the conclusion of a study that women had higher urine excretion levels of DMA than men [15]. Studies have shown MMA is more toxic than DMA [16, 17]. The MMA content of male rats was greater than that of female rats in the same group. This may be the metabolic basis for the gender difference in arsenic poisoning. The results of an animal experiment also showed that arsenic in males was more pathogenic than that in females [18], and the cytotoxic effect of arsenic on males was stronger than that of females. It is inferred that arsenic metabolites may have higher toxic effects on liver tissues of male rats than on those of females. This conclusion is also consistent with the results of population based epidemiological investigations and clinical studies [19, 20].

AS3MT is a cysteine-rich enzyme that can catalyze the biomethylation of arsenic in vivo and in vitro. The liver is the main site of arsenic methylation metabolism. In this study, we explored the effects of different valences of arsenic on the expression of AS3MT in rat liver. The results showed that the expression of AS3MT in the high, medium, and low dose groups of  $iAs^{3+}$  and  $iAs^{5+}$  were higher than those in the control group, indicating that arsenic exposure can increase expression of AS3MT. The expression level of AS3MT in the high and medium dose groups of  $iAs^{3+}$  was higher than that in the low dose group, and the expression level gradually increased as the dose of  $iAs^{3+}$  increased. The expression level of AS3MT in the  $iAs^{5+}$  high-dose group was lower than that in the low-dose group, and the expression level gradually decreased as the  $iAs^{5+}$  dose increased. It shows that there may be a positive correlation and a negative correlation between the exposure of  $iAs^{3+}$  and  $iAs^{5+}$  and the expression of AS3MT, and there may be a certain dose-effect relationship, which was contrary to the study using frogs [21]. The expression of AS3MT in the liver in the  $iAs^{3+}$  high dose group was higher than that of the  $iAs^{5+}$  high-dose group. Thus, it can be inferred that arsenic with different valences has different arsenic methylation patterns in the body. The arsenic methylation and level of arsenic would accelerate the excretion of methylated arsenic through urine [22, 23], but it can also enhance the potential genotoxicity and long-term effects [24], which is consistent with the same metabolic pattern of low-dose  $iAs^{5+}$  liver arsenic. Obviously, the high dose of  $iAs^{5+}$  in the liver inhibited the expression of AS3MT to a certain extent. Compared with male and female animals in the same group, the relative expression of AS3MT mRNA in male rats in the AH group was higher than that in female rats, indicating that arsenic has different arsenic methylation patterns in different sexes. This result reflects the tension or disorder of arsenic methylation and detoxification pathways in males, which is consistent with previous study [25].

In this study, it was found that compared with the control group, both the  $iAs^{3+}$  low-dose group and the  $iAs^{5+}$  high-dose group promoted the increase of MPO activity in rats. MPO is present in the cell and all three subtypes of MPO can form a strong complex with DNA to prevent damage during oxidation and ensure the normal differentiation of cell functions [26]. The increase in MPO content caused by arsenic poisoning may also contribute to lipid peroxidation and oxidative cell damage [27]. In this study, MPO activity of male rats was greater than that of female rats. These results indicate that male rats and female rats may have different lipid peroxidation processes. It has been found that after external environmental stimuli, the activity of MPO decreases and the production of ROS is also reduced, thereby reducing the damage of arsenic to the body [28]. Therefore, the higher MPO activity in males will increase the damage of arsenic to the body.

The results showed that SAM in the liver of rats after exposure to  $iAs^{3+}$  or  $iAs^{5+}$  was significantly higher than that in the control group, indicating that different valences of arsenic affected the activity and level of SAM to change the methylation of DNA and histones [29]. Comparing with the low-dose group, the content of SAM in  $iAs^{3+}$  high-dose group,  $iAs^{5+}$  high- and medium dose groups was lower, and the content gradually decreased with increasing dose. It can be inferred that there is a certain dose-effect relationship between the exposure of  $iAs^{3+}$  and  $iAs^{5+}$  and the content of SAM in the body, that is, the higher the arsenic exposure, the lower the SAM level [30, 31]. Additionally, we observed that compared with different valences,  $iAs^{3+}$  had higher SAM excessive consumption or failure than  $iAs^{5+}$ . Therefore, the arsenic methylation of  $iAs^{5+}$  is relatively sufficient, which will generate more MAs and DMAs [32]. During this process, there will be more active free radicals, resulting in abnormal DNA methylation, leading to stronger genotoxicity and exerting long-term effects such as carcinogenesis [30, 33]. Compared with male and female animals in the same group, the SAM activity of female rats in the  $iAs^{3+}$  medium and low-dose groups and  $iAs^{5+}$  medium and low-dose groups was lower than that of male rats. This indicates that the same arsenic exposure may exert greater acute toxicity, stronger genotoxicity and long-term effects such as carcinogenesis in males.

We also found that the activity of ARR in the  $iAs^{5+}$  high-dose group and  $iAs^{3+}$  low-dose group was higher than that in the control group. The ARR activity of the  $iAs^{3+}$  high-dose group was lower than that of the  $iAs^{3+}$  low-dose group, and the ARR activity of the  $iAs^{5+}$  high-dose group was higher than that of the  $iAs^{5+}$  low-dose group. Therefore, exposure to high doses of arsenic can lead to changes in ARR activity, and the effect of  $iAs^{5+}$  on ARR shows a certain dose relationship. On the other hand, the ARR activity of the  $iAs^{5+}$  high dose/medium dose group was higher than that of the  $iAs^{3+}$  high dose/medium dose group; and that of the  $iAs^{5+}$  low dose group was lower than that of the  $iAs^{3+}$  high dose group. This may be related to the different mechanisms by which arsenic of different valences inhibit ARR activity [34, 35]. Comparison between male and female animals in the same group, except for the  $iAs^{3+}$  high dose group, the ARR activity of males in the other groups was higher than that of females, indicating that the males may compensatively stimulate the body to produce more ARR.

As for PNP, we found that male rats could promote the compensatory increase of PNP activity more than female rats. Li et al found that PNP mRNA expression was significantly positively correlated with MMA% [36]. The increased expression of PNP mRNA will increase the level of MMA in the urine of the population, thus exerting greater long-term effects such as genotoxicity or carcinogenesis and mutagenesis. Therefore, arsenic may exert greater long-term effects such as genotoxicity or carcinogenesis and mutagenesis in males than females [37].

By detecting the activity of PK can help to understand the degree of arsenic methylation and its influencing factors, and to infer the toxicity mechanism of arsenic [38]. The results of this study showed that the activity of PK in the control group was higher than that in the  $iAs^{5+}$  high-dose group, indicating that high-dose arsenic exposure can cause abnormal PK activity. The PK activity of the  $iAs^{3+}$  group was higher than that of the  $iAs^{5+}$  group. It can be inferred that arsenic with different valences may affect PK activity through interfering with the glycolysis process, leading to abnormal cell energy metabolism [8]. Compared with males and females of the same group of animals, the PK activity of females was higher than that of males, suggesting that arsenic may change the glycolysis process by inhibiting the activity of PK in males, which may then affects the body's energy supply and causes greater toxicity.

Furthermore, we found that after exposing to different doses of  $iAs^{3+}$  or  $iAs^{5+}$ , the NAD of male  $iAs^{3+}$  high-dose group was lower than that of control group. It may be caused by the interference of physiological and biochemical process by high-dose  $iAs^{3+}$ , which leads to insufficient cellular ATP synthesis required for metabolism [39, 40]. There was a statistically significant difference between the different doses of  $iAs^{3+}$ , suggesting that lower dose of  $iAs^{3+}$  promoted the activity of NAD, which may be because of the oxidation to  $iAs^{5+}$  by NADH [41]. In addition, the difference between males and females in each group was statistically significant, indicating that arsenic promoted the NAD activity of females and inhibited the NAD activity of males. Moreover, arsenic also inhibited glycolysis and caused abnormal metabolism, thus exerting a toxic effect [39, 40].

## Conclusion

In conclusion, most studies only observed and analyzed the relationship between a single gene and arsenic. This study analyzed the content and activity of multiple arsenic metabolism-related enzymes in the liver of rats of different genders. It is found that arsenic had more toxic effects on male animals than females. In the future, it is necessary to further study the combination of multiple arsenic metabolism-related genes and the interaction between genes and the environment.

## Declarations

The study was carried out in compliance with the ARRIVE guidelines.

## Ethics approval and consent to participate

All animal experiments were conducted according to the ethical guidelines of Experimental Animal Center of Xinjiang Medical University. This study was approved by the Ethics Committee of Xinjiang Medical University.

### **Consent for publication**

Not applicable.

### **Availability of data and materials**

The data used and/or analysed during the current study are available from the corresponding author on reasonable request.

### **Competing interests**

All authors declare no financial competing interests.

All authors declare no non-financial competing interests.

### **Funding**

This work was funded by grants from the National Natural Science Foundation of China (Grant No.81560513), Natural Science Foundation of the Xinjiang Uygur Autonomous Region in China (Grant No. 2018D01C147), and Public Health and Preventive Medicine, a key discipline in the 13th Five-Year Plan of Xinjiang Uygur Autonomous Region.

### **Authors' contributions**

JW designed the study. MM, MY and RX collected the data. MM analyzed and interpreted the data. JW collected the funds. MM wrote the paper. JW revised the paper. All authors have read and approved the final manuscript.

### **Acknowledgements**

Not applicable.

## **References**

1. Tolins M, Ruchirawat M, Landrigan P: **The developmental neurotoxicity of arsenic: cognitive and behavioral consequences of early life exposure.***Ann Glob Health* 2014, **80**:303-314.
2. Bhattacharya P, Welch AH, Stollenwerk KG, McLaughlin MJ, Bundschuh J, Panaullah G: **Arsenic in the environment: Biology and Chemistry.***Sci Total Environ* 2007, **379**:109-120.
3. Lin S, Shi Q, Nix FB, Styblo M, Beck MA, Herbin-Davis KM, Hall LL, Simeonsson JB, Thomas DJ: **A novel S-adenosyl-L-methionine:arsenic(III) methyltransferase from rat liver cytosol.***J Biol Chem* 2002,

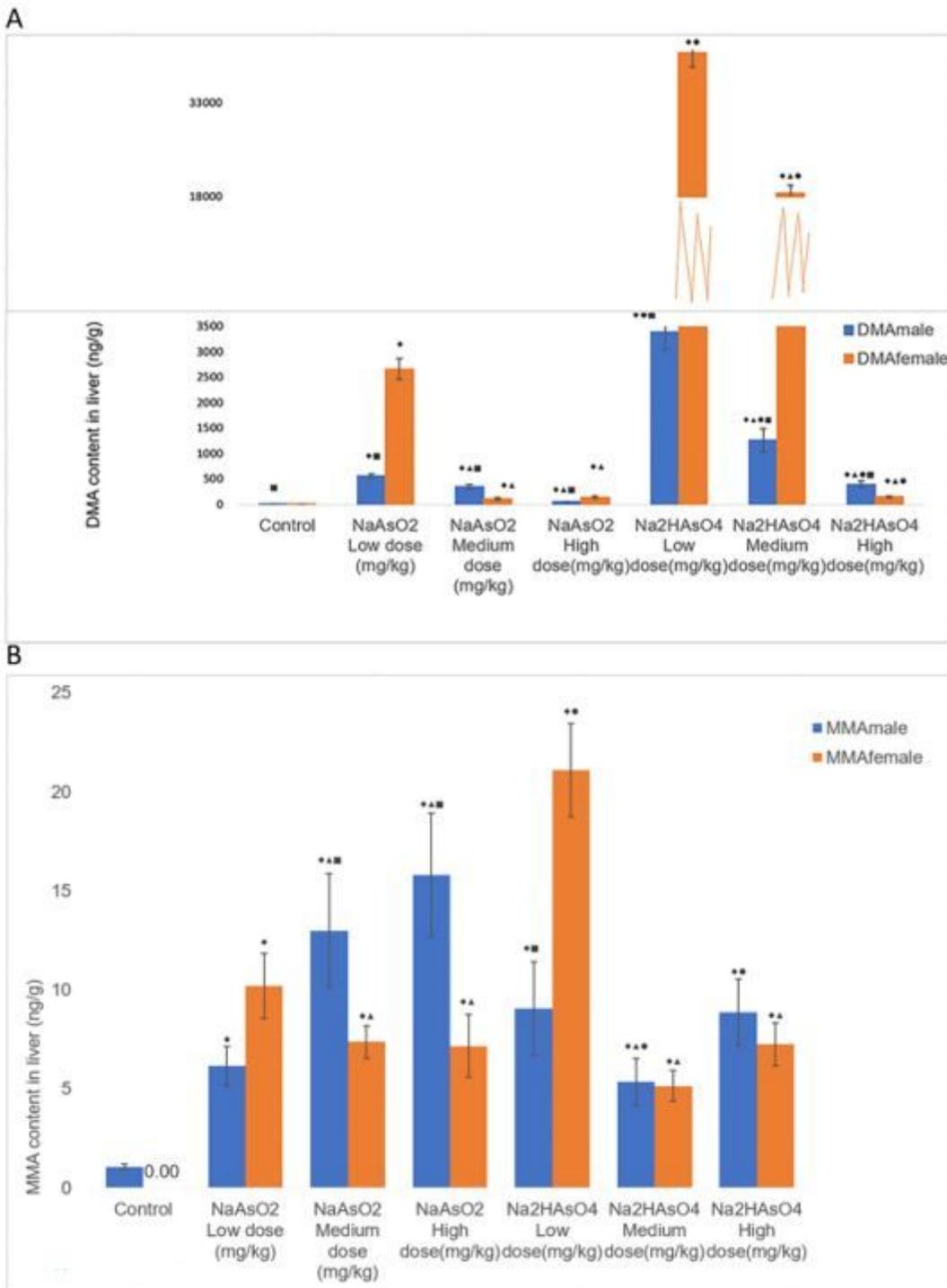
277:10795-10803.

4. Martinez VD, Vucic EA, Becker-Santos DD, Gil L, Lam WL: **Arsenic exposure and the induction of human cancers.***J Toxicol* 2011, **2011**:431287.
5. Perez-Jimenez JR, DeFraia C, Young LY: **Arsenate respiratory reductase gene (arrA) for Desulfosporosinus sp. strain Y5.***Biochem Biophys Res Commun* 2005, **338**:825-829.
6. Gaxiola-Robles R, Labrada-Martagon V, Bitzer-Quintero OK, Zenteno-Savin T, Mendez-Rodriguez LC: **Purine nucleoside phosphorylase and the enzymatic antioxidant defense system in breast milk from women with different levels of arsenic exposure.***Nutr Hosp* 2015, **31**:2289-2296.
7. Nemeti B, Gregus Z: **Reduction of arsenate to arsenite by human erythrocyte lysate and rat liver cytosol - characterization of a glutathione- and NAD-dependent arsenate reduction linked to glycolysis.***Toxicol Sci* 2005, **85**:847-858.
8. He J, Liu W, Ge X, Wang GC, Desai V, Wang S, Mu W, Bhardwaj V, Seifert E, Liu LZ, et al: **Arsenic-induced metabolic shift triggered by the loss of miR-199a-5p through Sp1-dependent DNA methylation.***Toxicol Appl Pharmacol* 2019, **378**:114606.
9. Oyagbemi AA, Omobowale TO, Ola-Davies OE, Adejumobi OA, Asenuga ER, Adeniji FK, Adedapo AA, Yakubu MA: **Protective Effect of Azadirachta indica and Vitamin E Against Arsenic Acid-Induced Genotoxicity and Apoptosis in Rats.***J Diet Suppl* 2018, **15**:251-268.
10. Lindberg AL, Ekstrom EC, Nermell B, Rahman M, Lonnerdal B, Persson LA, Vahter M: **Gender and age differences in the metabolism of inorganic arsenic in a highly exposed population in Bangladesh.***Environ Res* 2008, **106**:110-120.
11. Sudo N, Sekiyama M, Watanabe C, Mozammel Haque Bokul ATM, Ohtsuka R: **Gender differences in food and energy intake among adult villagers in northwestern Bangladesh: a food frequency questionnaire survey.***International Journal of Food Sciences and Nutrition* 2004, **55**:499-509.
12. Fischer L, daCosta K, Kwock L, Stewart P, Lu T, Stabler S, Allen R, Zeisel S: **Sex and menopausal status influence human dietary requirements for the nutrient choline.***Am J Clin Nutr* 2007, **85**:1275-1285.
13. Hayakawa T, Kobayashi Y, Cui X, Hirano S: **A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase Cyt19.***Arch Toxicol* 2005, **79**:183-191.
14. Wu J, Jiang P, Wang H, Wu S, Zheng Y: **Study on the pretreatment method of arsenic speciation analysis in liver tissue of rats exposed to sodium arsenite.***Journal of Xinjiang Medical University* 2010, **33**:325-328.
15. Garcia-Alvarado FJ, Neri-Melendez H, Perez Armendariz L, Rivera Guillen M: **[Polymorphisms of the Arsenite Methyltransferase (As3MT) gene and urinary efficiency of arsenic metabolism in a population in northern Mexico].***Rev Peru Med Exp Salud Publica* 2018, **35**:72-76.
16. Vega L, Styblo M, Patterson R, Cullen W, Wang C, Germolec D: **Differential effects of trivalent and pentavalent arsenicals on cell proliferation and cytokine secretion in normal human epidermal keratinocytes.***Toxicol Appl Pharmacol* 2001, **172**:225-232.

17. Wang QQ, Lan YF, Rehman K, Jiang YH, Maimaitiyiming Y, Zhu DY, Naranmandura H: **Effect of arsenic compounds on the in vitro differentiation of mouse embryonic stem cells into cardiomyocytes.***Chem Res Toxicol* 2015, **28**:351-353.
18. Qu C, Niu Y, Zhong Y: **Effects of subchronic arsenic exposure on glutamate-glutamine cycle in mice brain.***J Environ Health* 2007, **24**:751-754.
19. Zhu B, Sun M, Wang X, Jiang Q, Feng H, Li K, Sun G: **Relationship between Arsenic Exposure from Drinking Water and Children's Intelligence: a Meta-analysis.***JOURNAL OF ENVIRONMENT AND HEALTH* 2010, **27**:1070-1071.
20. Hou Y, Xu L, Zhong Y, Lv X, Jin Y, Zhang X, Xue P, Sun G: **Effects of Laver Intake on Excretion of Urinary Arsenic Metabolites of People.***JOURNAL OF ENVIRONMENT AND HEALTH* 2009, **26**:1039-1040.
21. Koch I, Zhang J, Button M, Gibson LA, Caumette G, Langlois VS, Reimer KJ, Cullen WR: **Arsenic(+3) and DNA methyltransferases, and arsenic speciation in tadpole and frog life stages of western clawed frogs (*Silurana tropicalis*) exposed to arsenate.***Metallomics* 2015, **7**:1274-1284.
22. Engstrom K, Vahter M, Mlakar SJ, Concha G, Nermell B, Raqib R, Cardozo A, Broberg K: **Polymorphisms in arsenic(+III oxidation state) methyltransferase (AS3MT) predict gene expression of AS3MT as well as arsenic metabolism.***Environ Health Perspect* 2011, **119**:182-188.
23. Vahter M: **Methylation of inorganic arsenic in different mammalian species and population groups.***Sci Prog* 1999, **82 ( Pt 1)**:69-88.
24. Cohen SM, Arnold LL, Eldan M, Lewis AS, Beck BD: **Methylated arsenicals: the implications of metabolism and carcinogenicity studies in rodents to human risk assessment.***Crit Rev Toxicol* 2006, **36**:99-133.
25. Vahter M, Akesson A, Liden C, Ceccatelli S, Berglund M: **Gender differences in the disposition and toxicity of metals.***Environ Res* 2007, **104**:85-95.
26. Liu C: **Evaluation of the application of myeloperoxidase index in the diagnosis of acute promyelocytic leukemia.***J Diabetes World* 2020, **17**:156.
27. Adeyemi OS, Meyakno E, Akanji MA: **Inhibition of Kupffer cell functions modulates arsenic intoxication in Wistar rats.***Gen Physiol Biophys* 2017, **36**:219-227.
28. Ahsan H, Chen Y, Kibriya MG, Islam MN, Slavkovich VN, Graziano JH, Santella RM: **Susceptibility to arsenic-induced hyperkeratosis and oxidative stress genes myeloperoxidase and catalase.***Cancer Lett* 2003, **201**:57-65.
29. Allan AM, Hafez AK, Labrecque MT, Solomon ER, Shaikh MN, Zheng X, Ali A: **Sex-Dependent effects of developmental arsenic exposure on methylation capacity and methylation regulation of the glucocorticoid receptor system in the embryonic mouse brain.***Toxicol Rep* 2015, **2**:1376-1390.
30. Reichard JF, Puga A: **Effects of arsenic exposure on DNA methylation and epigenetic gene regulation.***Epigenomics* 2010, **2**:87-104.
31. Ren X, McHale CM, Skibola CF, Smith AH, Smith MT, Zhang L: **An emerging role for epigenetic dysregulation in arsenic toxicity and carcinogenesis.***Environ Health Perspect* 2011, **119**:11-19.

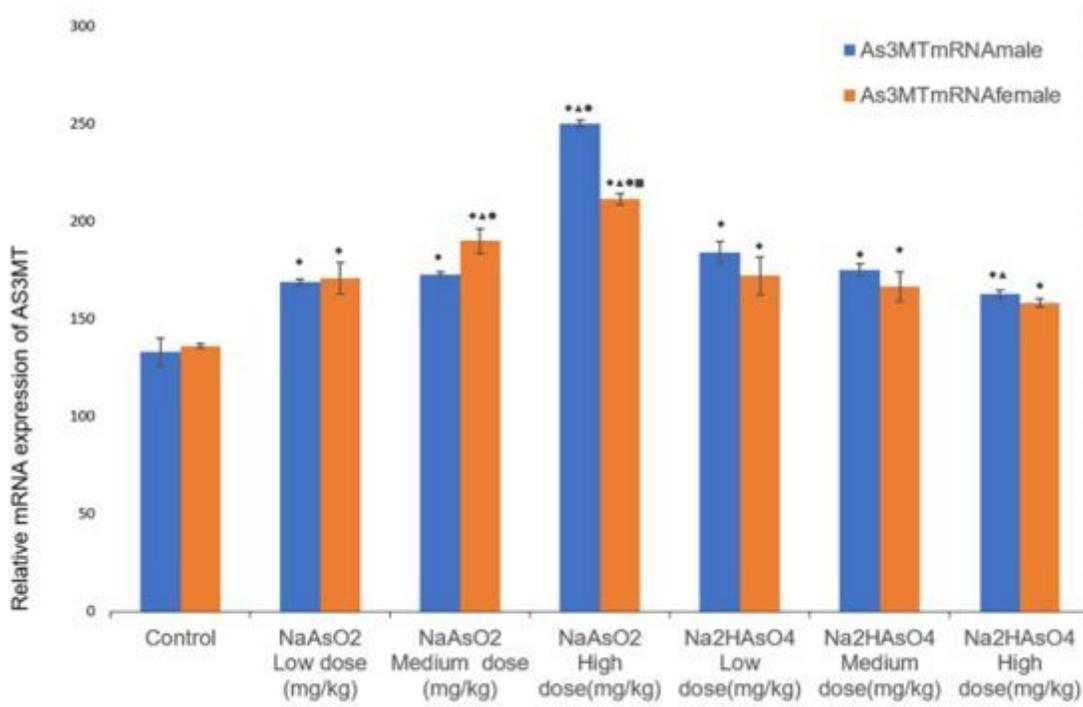
32. Mass MJ, Wang L: **Arsenic alters cytosine methylation patterns of the promoter of the tumor suppressor gene p53 in human lung cells: a model for a mechanism of carcinogenesis.***Mutat Res* 1997, **386**:263-277.
33. Zhang J, Mu X, Wang X, Huang Q, Tian M, Liu L, Shen H: **Adverse effects of arsenic exposure on DNA methylation: a review of recent studies.***Journal of Environment and Health* 269-276, **31**:2014.
34. Duan GL, Wang LH, Chen Y, Xu YX, Meng XY: **Health risk from consumption of rice with elevated arsenic and studies of arsenic metabolism in rice plants.***Journal of Agro-environment Science* 2007, **26**:430-435.
35. Rosen BP, Liu Z: **Transport pathways for arsenic and selenium: a minireview.***Environ Int* 2009, **35**:512-515.
36. Radabaugh TR, Sampayo-Reyes A, Zakharyan RA, Aposhian HV: **Arsenate reductase II. Purine nucleoside phosphorylase in the presence of dihydrolipoic acid is a route for reduction of arsenate to arsenite in mammalian systems.***Chem Res Toxicol* 2002, **15**:692-698.
37. Li S: **Study on the relationship between arsenic metabolism-related enzyme gene polymorphism and mRNA expression, arsenic poisoning susceptibility and arsenic methylation.***Chinese Center for Disease Control and Prevention* 2009:1-131.
38. Yang M, Ai J, Wang N, He S, Yang B: **Effects of Xue Tang An on the activity of pyruvate kinase in type 2 diabetic rats.***JOURNAL OF HARBIN MEDICAL UNIVERSITY* 2004, **38**:16-18.
39. Wang Q, Qin D, Zhang S, Wang L, Li J, Rensing C, McDermott TR, Wang G: **Fate of arsenate following arsenite oxidation in *Agrobacterium tumefaciens* GW4.***Environ Microbiol* 2015, **17**:1926-1940.
40. Knowles F, Benson A: **The biochemistry of arsenic.***Trends Biochem Sci* 1983, **8**:178–180.
41. Wu J, Wu SH, Zhang J, Zheng YJ: **Analysis on the arsenic speciation in urine of rats treated with sodium arsenite and sodium arsenate.** 2010, **29**:23-26.

## Figures



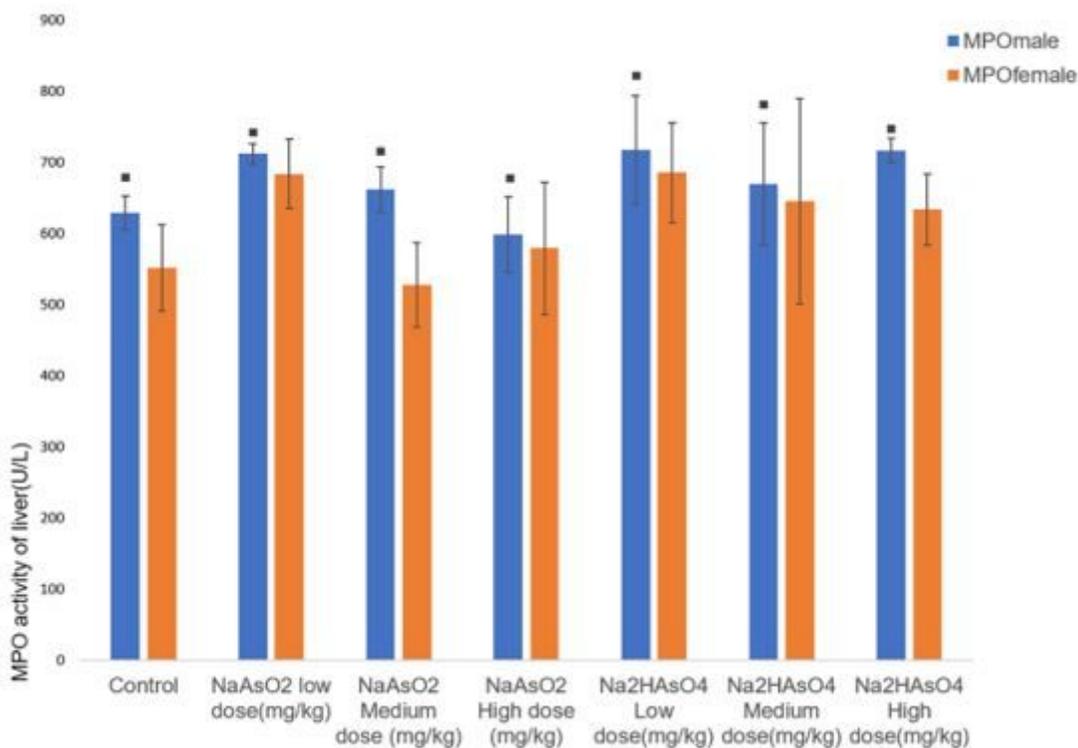
**Figure 1**

Levels of MMA and DMA in liver tissues after arsenic exposure in each dose group. A: MMA content. B: DMA content. □ Compared with normal control group,  $P < 0.05$ ; ▲ Compared with low-dose group,  $P < 0.05$ ; ● Compared with different compounds in the same dose group,  $P < 0.05$ ; ■ Compared with animals in the same dose group,  $P < 0.05$ .



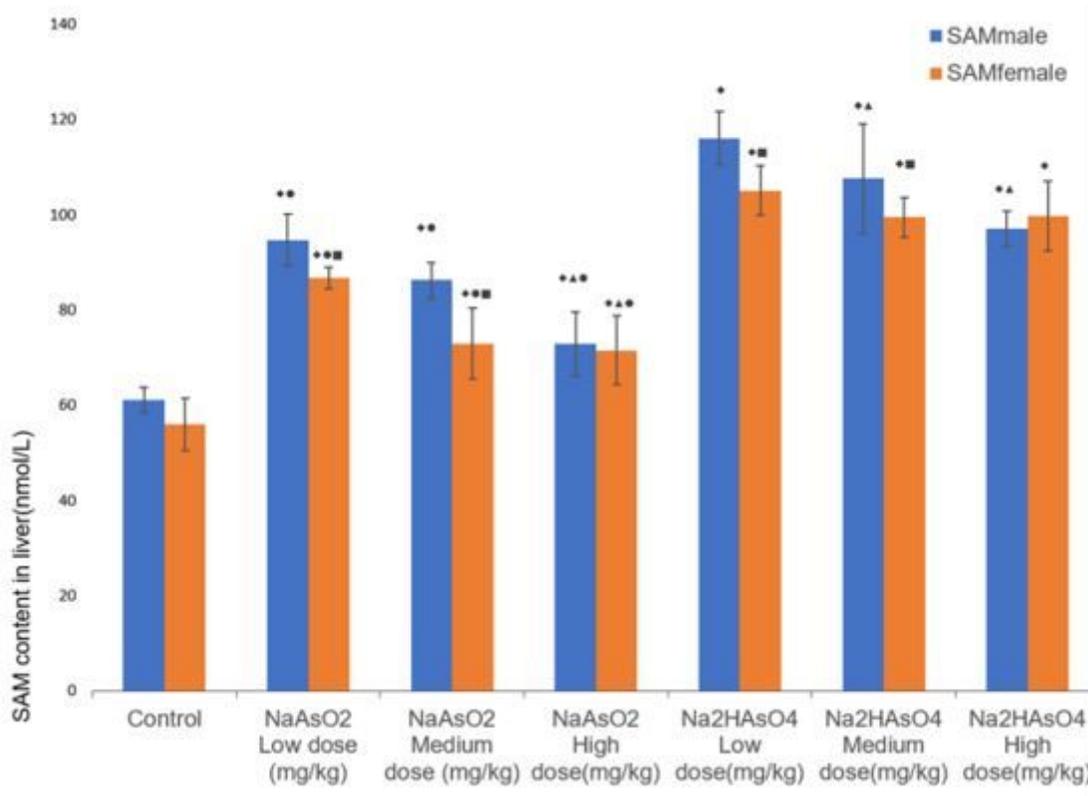
**Figure 2**

Relative mRNA expression of AS3MT in rats exposed to arsenic in each group. RT-qPCR was used to detect mRNA level. □ Compared with the normal control group,  $P < 0.05$ ; ▲ Compared with the low-dose group,  $P < 0.05$ ; ● Compared with the different dose group,  $P < 0.05$ ; ■ Compared with the same group of animals,  $P < 0.05$ .



**Figure 3**

Liver MPO activity in each group. Compared with the normal control group, \* $P < 0.05$ ; comparing the same test substance with the low-dose group,  $\blacktriangle P < 0.05$ ; comparing the male and female animals in the same group,  $\blacksquare P < 0.05$ .



**Figure 4**

The content of SAM in the liver in each group.  $\square$  Compared with normal control group,  $P < 0.05$ ;  $\blacktriangle$  Compared with low-dose group,  $P < 0.05$ ;  $\bullet$  Compared with different subjects in the same dose group,  $P < 0.05$ ;  $\blacksquare$  Compared with animals in the same dose group,  $P < 0.05$ .

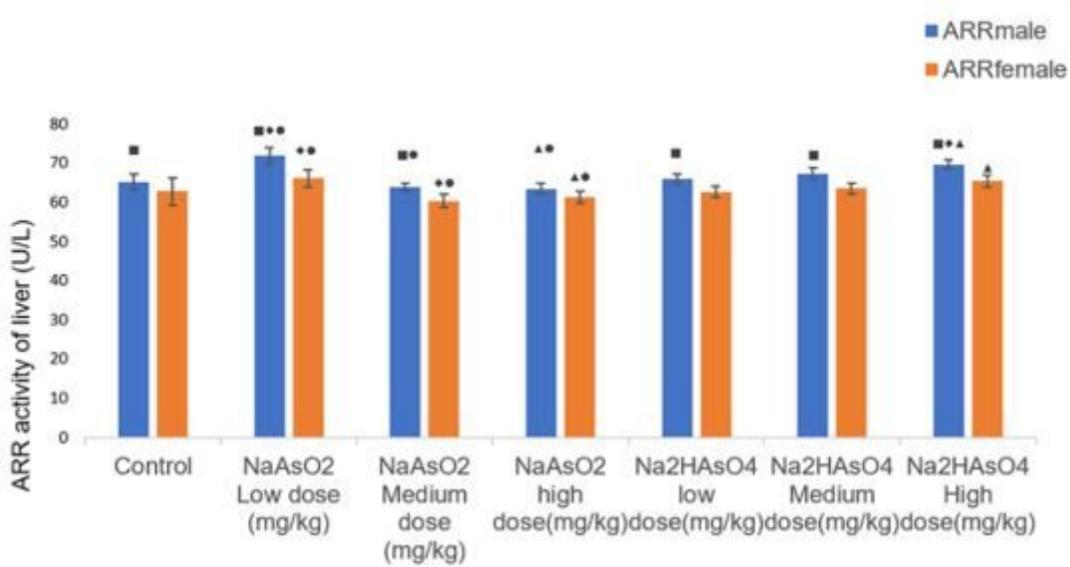


Figure 5

Liver ARR activity in each group. □ Compared with normal control group,  $P < 0.05$ ; ▲ Compared with low-dose group,  $P < 0.05$ ; ● Compared with different subjects in the same dose group,  $P < 0.05$ ; ■ Compared with animals in the same dose group,  $P < 0.05$ .

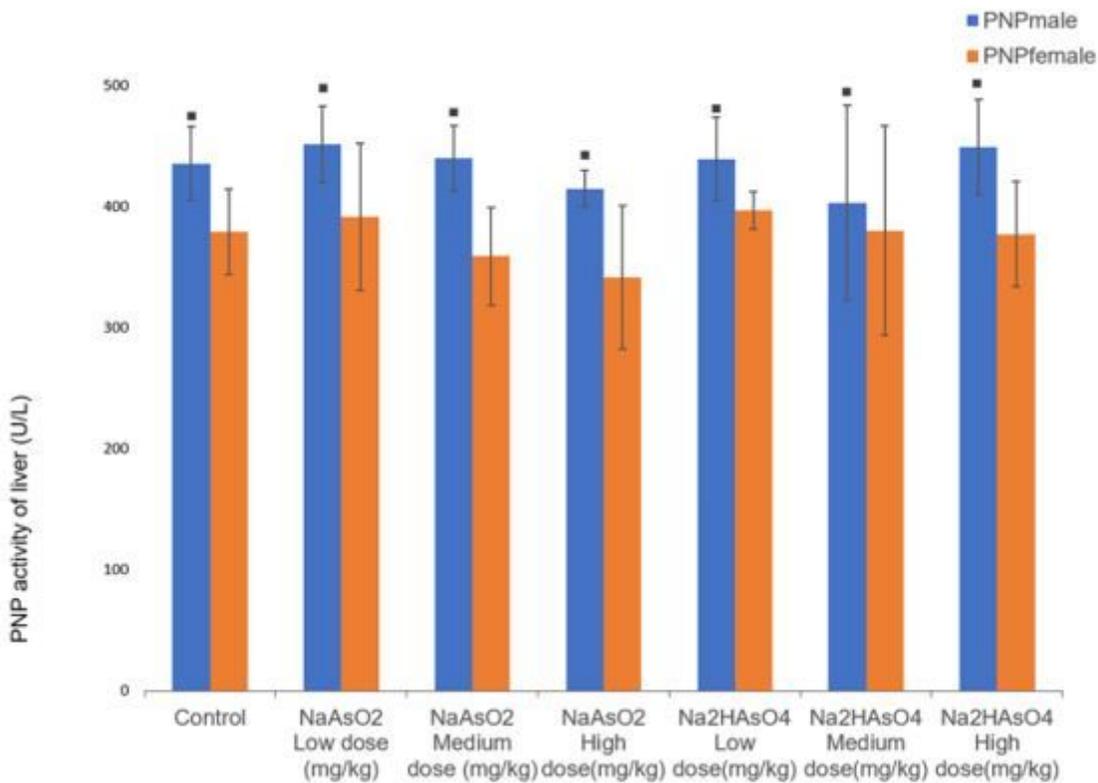
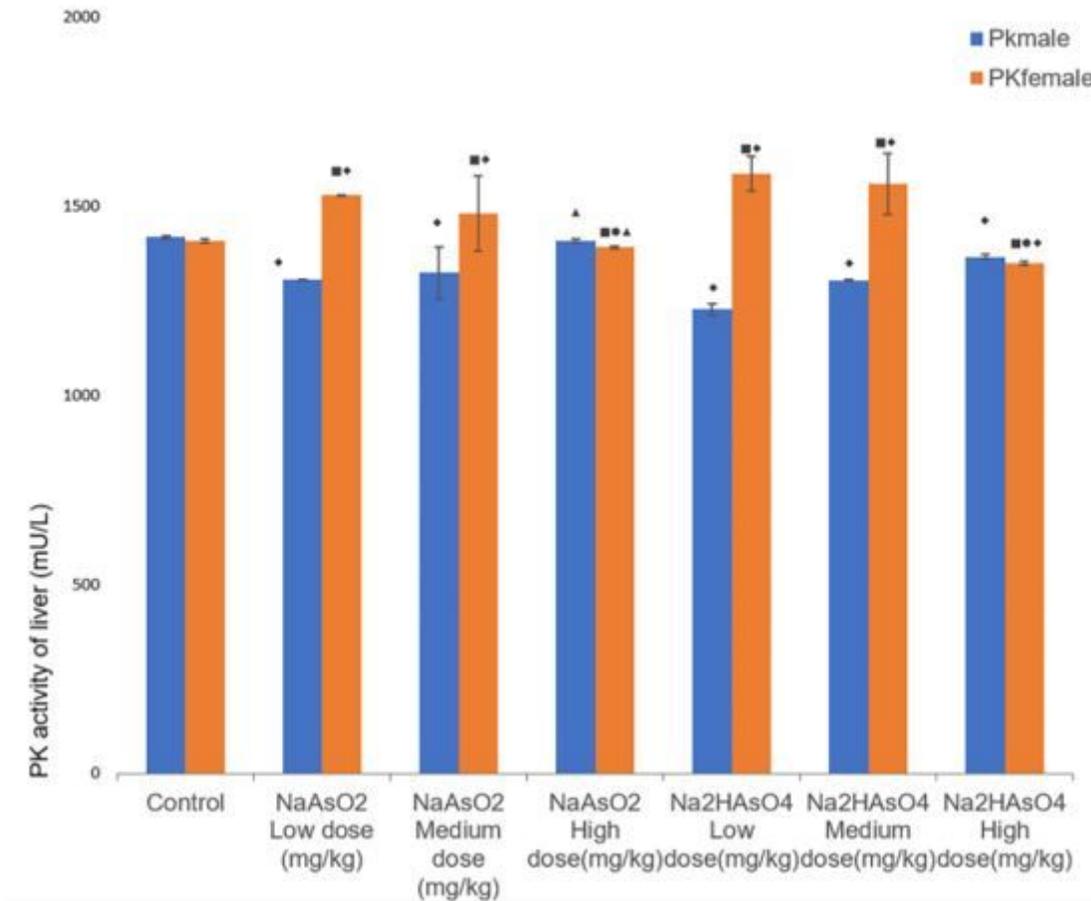


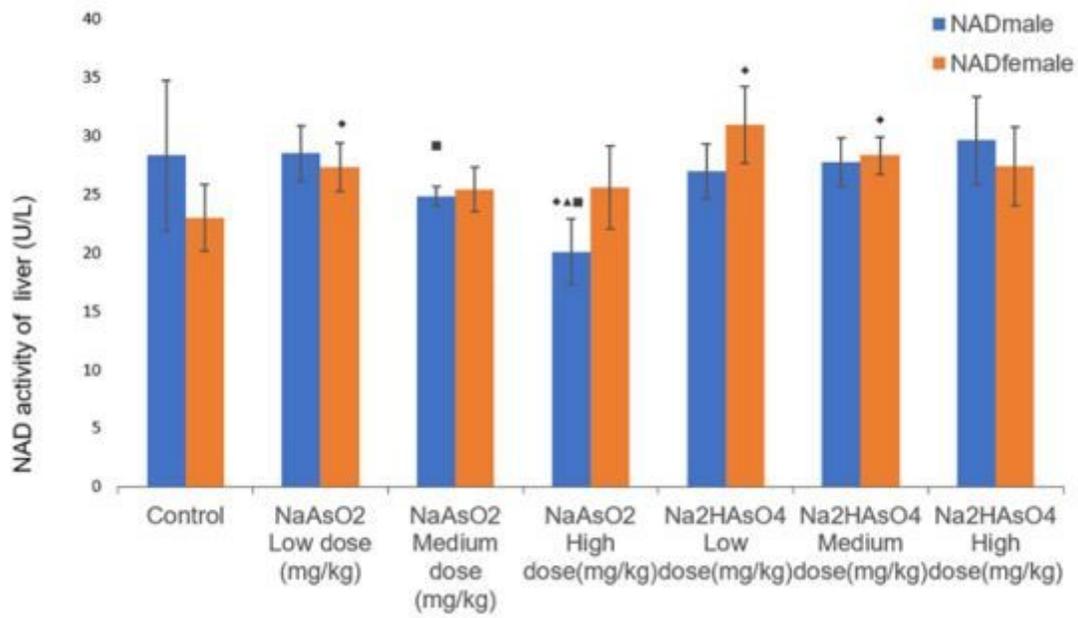
Figure 6

PNP activity in the liver in each group. ☒ Compared with normal control group,  $P < 0.05$ ; ▲ Compared with low-dose group,  $P < 0.05$ ; ● Compared with different subjects in the same dose group,  $P < 0.05$ ; ■ Compared with animals in the same dose group,  $P < 0.05$ .



**Figure 7**

Liver PK activity in each group. ☒ Compared with normal control group,  $P < 0.05$ ; ▲ Compared with low-dose group,  $P < 0.05$ ; ● Compared with different subjects in the same dose group,  $P < 0.05$ ; ■ Compared with animals in the same dose group,  $P < 0.05$ .



**Figure 8**

Liver NAD activity in each group. □ Compared with normal control group,  $P < 0.05$ ; ▲ Compared with low-dose group,  $P < 0.05$ ; ● Compared with different subjects in the same dose group,  $P < 0.05$ ; ■ Compared with animals in the same dose group,  $P < 0.05$ .