Dietary Compounds to Reduce *In Vivo* Inorganic Arsenic Bioavailability

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ABSTRACT

It is estimated that approximately 200 million people are exposed to arsenic levels above the WHO provisional guideline value, and various agencies have indicated the need to reduce this exposure. In view of the difficulty of removing arsenic from water and food, one alternative is to reduce its bioavailability (the amount that reaches the systemic circulation after ingestion). In this study, dietary components [glutathione, tannic acid and Fe(III)] were used to achieve this goal. As(III) or As(V) (1 mg/kg body weight) was administered daily to BALB/c mice, along with the dietary components, for 15 days. The results confirm the efficacy of Fe(III) and glutathione as reducers of arsenic bioavailability and tissue accumulation. Also, these treatments did not result in reductions of Ca, K, P and Fe contents in the liver. These data suggest that use of these two compounds could be part of valid strategies for reducing inorganic arsenic exposure in chronically exposed populations.

Keywords: Inorganic arsenic; Bioavailability; Tissue accumulation; Faecal excretion; Glutathione; Iron salts; Tannic acid.

INTRODUCTION

The toxicity of arsenic (As) varies depending on the chemical form in which it is found. Thus far, inorganic As is the most toxic species found, in water and food. It is considered by the International Agency for Research on Cancer (IARC) as a carcinogen for humans (Group 1A).[1] Currently, approximately 200 million people are exposed to levels of inorganic As greater than the WHO provisional guideline value (0.01 mg/L) through drinking water.[2] Even in areas where there is no water contamination, there are populations with recorded intakes of inorganic As that are not completely without risk.[3] This situation has led international health agencies to recommend reducing exposure to this toxic element.

Decreasing the levels of As from the sources of exposure is not an easy task. Coagulation with aluminium sulfate or ferric chloride and flocculation with iron salts are the most used techniques for the elimination of inorganic As from water. However, these techniques are not always applicable on a small scale, and therefore, in rural populations where well water contaminated with inorganic As is consumed, exposure to high As concentrations continues to be a problem. Low-cost decontamination options that can be applied in these rural areas have been proposed,[4] but, for the technology to be successful, it must fit into the daily routine and be accepted by the exposed population.[5]

Eliminating the As present in food or avoiding the contamination of these matrices is an even more complex objective, and research has mostly focused on rice. After characterising the mechanisms of uptake and transport of the metalloid from soils and water to the plant,[6] strategies have been designed to reduce As uptake[7] through changes in the type of cultivation or by the creation of genetically modified plants, although these strategies are difficult to implement. The problem has also been addressed at the stage of processing and/or culinary treatment. It has been proved that washing the rice grain and increasing the water/rice ratio during cooking favours the reduction of inorganic As content.[8] Recently, a procedure

proposed to reduce the inorganic As content by cooking rice by percolation in a continuous stream of water close to boiling point showed reductions of up to 96%.[9] This alternative cooking method was successfully applied on a domestic level,[10] but it did also produce a decrease of mineral content and water-soluble nutrients.

Reducing consumption of the foods that make the greatest contribution to inorganic As dietary exposure is practically impossible. Rice is a staple food and is the main source of energy in many regions with chronic endemic arsenicism. Moreover, modification of eating habits in many of the exposed populations is difficult to achieve, especially if one considers that in many cases they are populations with limited economic resources. Of all the problematic matrices as far as inorganic As is concerned, consumption recommendations have only been issued for the alga *Hizikia fusiforme*. Food safety agencies in various countries have recommended avoiding consumption of this brown alga.[11,12]

Another way of reducing exposure is to modulate the bioavailability (entry into the bloodstream after ingestion), excretion and/or metabolism of the element. In this regard, it has been shown that the toxicokinetics of inorganic As can be modulated through the use of certain compounds, some of them of food origin. *In vitro* studies have identified compounds that could be effective in reducing the oral bioavailability of inorganic As, either by their ability to reduce its bioaccessibility (amount of As solubilised during gastrointestinal digestion) or by their effect on its transport through the epithelial monolayer of intestinal cells. Clemente et al.[13] proved the efficacy of iron (Fe) salts, tannic acid, lignin and some celluloses as reducers of the bioaccessibility of the inorganic As present in water and food. It has also been shown that compounds with thiol groups [cysteine (Cys), glutathione (GSH)], curcumin, epigallocatechin and quercetin reduce the amount of As transported by intestinal cells.[14] The effects of these compounds that are observed *in vitro* may not occur in animal models, where factors not present in the *in vitro* models are involved. Therefore, in order to

confirm the suitability of these compounds as strategies for future population-level interventions, an *in vivo* evaluation is necessary.

The objective of this work is to evaluate the efficacy of some dietary compounds [GSH, tannic acid, Fe(III)] to reduce the bioavailability and accumulation of inorganic As in BALB/c mice dosed by gavage. A further objective is to determine whether any of these compounds have a negative effect on the bioavailability of essential elements by evaluating their contents in the liver.

MATERIALS AND METHODS

Animals. Female BALB/c mice were used, obtained from Charles River (n=56). They were acquired at the age of 6 weeks and with a weight that varied between 16 and 20 g. During the study, the animals were kept in controlled environmental conditions (cycles of 12 hours of light and dark, room temperature of 22 °C and humidity of 75%), and they were fed ad libitum with standard rodent maintenance feed. After two weeks of acclimatisation, bioavailability, accumulation and excretion tests were initiated.

The protocols applied to the animals were designed in conformity with the regulations for the use of experimental animals,[15] and were approved by the Ethical Committee for Use of Laboratory Animals of the University of Valencia (Spain) and the Agriculture, Fisheries, and Food Council of the Generalitat Valenciana (Spain).

Arsenic Standard Solutions. The standard solution of As(V) (1000 mg/L, As_2O_5) was obtained from Merck. The standard of As(III) (1000 mg/L) was prepared by dissolving 1.32 g of As_2O_3 (Riedel de Haën) in 25 mL of 20% (m/v) KOH (Panreac), neutralising with 20% (v/v) H_2SO_4 (Merck), and diluting to 1 litre with 1% (v/v) H_2SO_4.

Evaluation of As Plasma Concentration as a Function of the Dose Used. Three animals were used per group to establish the concentrations of As(III) and As(V) suitable for the bioavailability study. The solutions of the As species were prepared in phosphate buffered saline (PBS, Hyclone), and an intragastric tube was used to administer 3 doses [0.1, 0.5 and 2.0 mg/kg body weight (bw)] to the mice. The blood was obtained by a puncture in the submandibular venous sinus (≈ 0.1 mL) at different time points (1, 3, 6 and 24 h). The samples were collected in heparin tubes (Microvette, Sarstedt) and centrifuged at 2000 rpm for 5 min to obtain the plasma. The samples of plasma were treated for the determination of As, following the protocol described in the Determination of Arsenic section.

Treatment with Dietary Compounds. The compounds selected on the basis of the previous studies were ferric sulfate [6 mg Fe(III)/kg bw, Merck], tannic acid (30 mg/kg bw, Merck) and GSH (4 mmol/kg bw, Sigma). They were co-administered daily with As(III) or As(V) (both at 1 mg/kg bw), and gavage was performed for 15 consecutive days. The dietary compounds/As solutions in PBS were prepared immediately before administration to avoid possible precipitations. The animals were housed in collective cages grouped by treatment (7 animals/treatment).

Calculation of Toxicokinetics Parameters. The relative bioavailability (F) was determined after the first administration of the treatment. Plasma was obtained as described in section 2.3. and the As content was analysed. The area under the curve (AUC) of each treatment was calculated from the representation of the plasmatic concentration of As as a function of time, using the SigmaPlot program (version 13). The relative bioavailability was calculated by applying Equation 1:

$$F = \frac{AUC_{a}}{AUC_{reference}} \times 100 \qquad \text{(Equation 1)}$$

where AUC*a* indicates the area under the curve in animals co-exposed to inorganic As and food compound, and AUC*reference* is the area under the curve in animals exposed only to inorganic As.

Additionally, stool samples were collected before the sacrifice of the animals to determine the influence of the food components on the magnitude of faecal excretion. The As contents were analysed according to the protocol described in the Determination of Arsenic section.

Sacrifice and Removal of Organs. After anaesthesia by inhalation of isoflurane, euthanasia was performed by cervical dislocation. Subsequently, the organs of interest (liver, small intestine and lungs) were extracted. The tissue samples were cleaned with saline solution and were then frozen with liquid nitrogen and stored at –80 °C. The As content was determined following the protocol described in the Determination of Arsenic section. Additionally, the contents of Ca, K, Fe and P in the liver were determined, following the protocol described in the Determination of Minerals section.

Determination of Arsenic. The determination of total As concentrations in plasma, stool and tissues was performed by flow injection-hydride generation-atomic absorption spectrometry (FI-HG-AAS) after a dry ashing step.[16]

Samples were treated with an ashing aid suspension (20% m/v $MgNO_3$ + 2% m/v MgO, Merck) and HNO_3 (7 mol/L, Merck), evaporated to dryness, and mineralised at 450 °C (12 h) with a gradual increase in temperature in a muffle furnace. The process was repeated until white ash was obtained, and the ash was then dissolved in 6 mol/L HCl (Merck) and

prereduced (5% m/v ascorbic acid + 5% m/v KI, Merck). The samples were then filtered through Whatman No. 1 paper and made up to final volume with 6 mol/L HCl.

Arsenic quantification was performed with an AAS (model 3300, Perkin-Elmer, Spain) equipped with an autosampler (AS-90, Perkin-Elmer), an FI-HG system (FIAS-400, Perkin-Elmer), and an electrothermally heated quartz cell. The experimental conditions used were the following: loop sample, 0.5 mL; reducing agent, 0.2% (m/v) $NaBH_4$ in 0.05% (m/v) NaOH, 5 mL/min flow rate; HCl solution 10% (v/v), 10 mL/min flow rate; carrier gas argon, 100 mL/min flow rate; wavelength, 193.7 nm; spectral band-pass, 0.7 nm; electrodeless discharge lamp system 2; lamp current setting, 400 mA; and cell temperature, 900 °C.

The analytical characteristics of the methodology were: limit of detection = 0.008 ng/g; limit of quantification = 0.026 ng/g; precision 2%. Throughout the experiment, the quality assurance/quality control of the methodology was checked by analysing a water sample (certified As content = 30.2 ± 0.293 μg/L; RTC QCI-049-1, LGC Standards) and a rice flour sample (certified As content = 0.29 ± 0.03 μg/g; SRM1568a, National Institute of Standards and Technology, NIST) with each batch of samples.

Determination of Minerals. The Ca, K, Fe and P contents were determined by microwave-assisted acid digestion (MARS, CEM, Vertex) and subsequent detection by inductively coupled plasma-optical emission spectrometry (ICP-OES, model 4300D, Perkin Elmer). The samples were weighed in Teflon reactors and treated with 4 mL of HNO_3 (14 mol/L, Merck) and 1 mL of H_2O_2 (30% v/v, Prolabo). The reactors were irradiated (180 °C, 15 min), and the digests were made up to volume with deionised water. The instrumental conditions were the following: radio frequency power, 1300 W; nebulisation gas flow rate, 0.8 L/min; auxiliary argon flow rate, 0.2 L/min; argon flow rate, 15 L/min; sample flow rate, 2 mL/min;

wavelengths (nm), Ca 317.933, Mg 285.213, K 766.490, Fe 238.204, P 213.617. The Ca, Mg, K, Fe and P standards were obtained from Merck.

Statistical Analysis. The statistical analysis was performed by applying the t-student test or analysis of variance with a single factor (ANOVA) with multiple post hoc comparisons, using the Tukey HSD test (SigmaPlot, version 13). The differences were considered significant when $p < 0.05$. The determination of the sample size was carried out using the GPower 3.1 program, with $\alpha = 0.05$ and statistical power (1-β) of 0.8.

RESULTS

Preliminary Studies for the Selection of the Dose to be Tested. Initially, 3 doses were tested: 0.1, 0.5 and 2 mg/kg bw. The selection of these doses was based on previous studies that indicated that, in this range of doses, there were no adverse effects at exposures of less than 15 days.[17] The results obtained for both inorganic arsenic species are shown in Figures 1 [As(III)] and 2 [As(V)]. At doses ≤ 0.5 mg/kg bw of both arsenical forms, the plasmatic As values at some time points are close to the limit of detection. Under these conditions, it would not be easy to find effects on the bioavailability of As due to the co-exposure with dietary compounds. For this reason, it was decided to carry out the remaining trials with a dose of 1 mg/kg bw.

Effect of Food Components on the Bioavailability of Inorganic Arsenic. The AUC of As obtained after dosing animals with As(III) (1 mg/kg bw) or As(V) (1 mg/kg bw), combined with the three selected food components [tannic acid, Fe(III) and GSH], is shown in Figures 3 and 4 respectively. The data indicated that animals treated with GSH had significantly lower plasmatic As concentrations for both inorganic arsenic forms. The treatments with tannic acid

198 and Fe(III) did not produce statistically significant reductions of As in the plasma after As(III)
199 exposure, but they did lead to significant reductions after As(V) exposure.

200 The relative bioavailability calculated by comparing the AUC of the co-exposures with
201 those of the controls treated only with As (Equation 1) are shown in Table 1. All treatments
202 significantly reduced the bioavailability of As(V) [tannic acid = 31 ± 8%; Fe(III) = 34 ± 8%;
203 GSH = 51 ± 10%]. For As(III)-treated animals, however, a significant reduction of oral
204 bioavailability (75%) was only observed when GSH was co-administrated.

205

206 **Effect of Food Components on Faecal Excretion of Inorganic Arsenic.** Table 2 shows the
207 faecal As excretion of mice exposed to As(III) and As(V) in the presence and absence of the
208 food components after 15 days of exposure. For animals orally given As(V), differences
209 between treatments were not statistically significant. However, considerable differences can
210 be seen between the medians of the control and co-exposures with tannic acid and Fe(III),
211 showing that with these treatments there were a significant number of animals that had a
212 greater faecal excretion than the control. For As(III), there were significant increases in faecal
213 excretion. Specifically, for animals treated with Fe(III) and GSH (2–3 times) with respect to
214 animals treated only with As(III).

215

216 **Effect of the Food Components on the Accumulation of Inorganic Arsenic in Tissues.**
217 Figure 5 shows the accumulation of As in liver (Figure 5A), lungs (Figure 5B) and small
218 intestine (Figure 5C) after 15 days of exposure to As(III) and As(V) in the presence and
219 absence of the dietary components tested. Hepatic and intestinal accumulation was similar for
220 the two species, which contradicts some previous studies that showed greater tissue
221 accumulation in animals dosed with As(III).[18] The accumulation in lungs followed the trend
222 of previous studies, as there was greater accumulation in animals dosed with As(III).

The modifications seen in hepatic accumulation (Figure 5A) coincided with those seen in plasma, that is, all the treatments that led to a reduction of the relative bioavailability (Table 1) produced a decrease in the hepatic levels of the toxic element. The reductions in the liver were of greater magnitude for the animals orally given As(V) [tannic acid, 39 ± 14%; Fe(III), 65 ± 9%; GSH, 88 ± 4%] than for those exposed to As(III) (GSH: 26 ± 7%).

The reductions in pulmonary (Figure 5B) and intestinal (Figure 5C) accumulation were not completely coincident with the relative bioavailability data. In animals gavaged with As(III) there were significant reductions in lung contents with all dietary compounds [tannic acid, 79 ± 22%; Fe(III), 82 ± 7%; GSH, 86 ± 4%], whereas, for the exposure to As(V), reductions were observed only in the presence of Fe(III) (33 ± 8%) and GSH (47 ± 11%). In the intestine, reductions were observed with GSH (55 ± 7%) for animals exposed to As(III), and with Fe(III) (38 ± 7%) and GSH (78 ± 3%) for animals exposed to As(V).

In general, it can be said that there was a reduction of accumulation in the organs with most of the treatments tested. In the case of liver and lungs, both considered as target organs of inorganic As, this lesser accumulation may have been due to a reduction of the entry of the metalloid into the bloodstream. For the intestine, the reduction may have been caused by the formation of complexes in the lumen which were transported and accumulated, to a lesser extent, in the intestinal epithelium.

Effect of Food Components on the Tissular Accumulation of Essential Elements. Table 3 shows the concentrations of Ca, K, P and Fe in liver samples of animals gavaged only with inorganic As, and of those co-exposed with Fe(III) and GSH, the two most effective strategies for reducing the bioavailability and tissue accumulation of As(V). In none of the animals treated with these components was a statistically significant change observed in the hepatic levels of these minerals with respect to animals exposed only to inorganic As. Consequently,

Fe(III) and GSH are strategies that do not involve changes in the body contents of the minerals studied and therefore, in relation to this aspect, can be considered safe strategies.

DISCUSSION

The possibility of reducing exposure to toxic substances by using food components or dietary supplements is a strategy that has several advantages. In addition to being compounds that, at the usual concentrations in the diet, are not harmful, many of them have functional properties. These bioactive compounds can normally contribute to improvement of the health problems derived from continuous exposures to the toxic substances.

Chronic exposure to inorganic As through drinking water is possibly one of the problems of exposure to toxic trace elements that causes most concern to international organisations. Numerous epidemiological studies have reported adverse health effects, with the number of people affected being high. In populations exposed to inorganic As, biomarkers show that a considerable part of the ingested element is absorbed and subsequently eliminated after undergoing a metabolic process.[19] It is considered that, once in the systemic circulation, the conversion of inorganic As into the dimethylated form (DMA) facilitates excretion and reduces its accumulation. For this reason, some studies have tested compounds to improve the methylation percentage of inorganic As in exposed populations.[20]

One of the objectives of the present study was to modify the toxicokinetics of As, acting at the intestinal absorption level, to reduce entry of the toxic element into the systemic circulation. This type of intervention is more protective than acting on the metabolism after the metalloid has reached the bloodstream. The results obtained were promising for treatments with GSH and ferric salts, which, in addition to reducing the bioavailability of inorganic As and its tissular accumulation, did not reduce the hepatic contents of essential elements. However, arsenate oxyanion is chemically similar to phosphate, an essential

element involved in many biological functions, such as cell signalling, energy metabolism and bone mineralisation. When using a strategy to reduce the entry of As into the bloodstream, it must be ensured that it does not have the same effect on the levels of phosphate or any other essential mineral.

The lower bioavailability observed in animals gavaged with As(V) and Fe(III) may have been because, after co-ingestion of these elements, salts with reduced solubility that could not be absorbed through the intestinal wall were formed during the passage of these elements through the lumen. The formation of insoluble As(V)-Fe complexes has been shown in environmental samples.[21,22] Clemente et al.[13] and Yu et al.[23] also showed how ferric salts could reduce the solubility of As(V) in the conditions of gastrointestinal digestion, and even how they hindered transport in cellular models of intestinal epithelium.[14] Treatment with Fe(III) does not reduce the bioavailability of As(III), although it does affect tissue accumulation and faecal excretion. This difference, however, is not evident in *in vitro* studies, where Fe(III) reduces both the bioaccessibility and the intestinal transport of both inorganic As species.[13,14,23] In addition to the formation of insoluble salts, the effect of Fe(III) on the toxicokinetics of inorganic As could also have been due to its influence on the metabolism of the intestinal microbiota. Yu et al.[23] showed that the presence of Fe(III) increased the methylation of As(III) in an *in vitro* system.

The interaction of GSH and As can also occur at different levels. GSH binds inorganic As species through the cysteinyl moiety, forming soluble complexes[24] whose transport through the intestinal wall, entry into the systemic circulation and excretion may be different from those of the inorganic salts of As. In fact, a recent study showed a reduction of the transport of As(III) and As(V) through intestinal epithelial monolayers in the presence of GSH.[14] In addition, GSH participates as a reducing agent during the conversion of pentavalent inorganic As into trivalent As,[25] which is the substrate of the enzyme responsible for the metabolic

process that gives rise to DMA. Consequently, GSH favours the metabolism of inorganic As and therefore its excretion. Furthermore, Kala et al.[26] indicated that the multidrug resistance transporter MRP2 and GSH are essential for the biliary excretion of inorganic As and its metabolites, and therefore GSH could also facilitate the excretion of As. On the basis of these previous data, the reductions in the plasma and tissue observed with the GSH treatment could have been a result of a combination of all these effects. On the other hand, some studies have shown that, after ingestion, GSH partially transforms into cysteine (Cys) in the gastrointestinal lumen.[27] If so, Cys may also have been partly responsible for the effects observed in the present study in animals treated with GSH. In fact, in *in vitro* studies performed with cellular models, this amino acid has been shown to be as effective as, or more effective than, GSH in reducing inorganic As transport.[14]

Previous studies have shown that these compounds may have a protective role against the toxicity of inorganic As, beyond their ability to reduce its entry into the systemic circulation, and therefore they can be considered strategies that have an added value. Thus, Liu et al.[28] revealed, after orally exposing mice simultaneously to As (3 mg/L) and $FeCl_3$ (5 mg/L) for 90 days, that Fe reduced the changes in hepatic transcriptome profiles as well as in serum and urinary metabolic profiles caused by inorganic As. On the other hand, GSH plays a fundamental role in the protection of cells against oxidative damage and the toxicity of xenobiotics.[29]

The results obtained in the present study showed the possibilities of GSH and ferric salts as strategies for the reduction of exposure to inorganic As. It is necessary to extend the trials to other compounds, such as Cys and some polyphenols, and also to lactic bacteria and yeast strains, whose ability to chelate inorganic As during digestion or to prevent its cellular transport has been confirmed in previous *in vitro* studies.[13,14] It is also possible that combinations of strategies could be used to increase the effectiveness of the treatments.

Furthermore, it should not be forgotten that *in vivo* studies have only been carried out by dosing aqueous standards, but food, especially food cooked with contaminated water, also makes an important contribution to inorganic As in the diet. There is also a need, therefore, to find strategies that are successful in reducing the absorption and accumulation of As ingested with food. Previous *in vit*ro studies have shown that it is not always possible to extrapolate the data obtained from aqueous solutions to food.[30]

Finally, and notably, the use of dietary components can be considered a practical strategy, since in most cases there are dietary supplements and plant extracts on the market with a high content of these components, which can be taken by the consumer without adverse effects. However, the effectiveness of these supplements or extracts must also be tested *in vivo*, as they have a composition different from those of pure compounds.

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425

FIGURE CAPTIONS

Figure 1. Plasma concentration of As as a function of the dose of As(III) administered. Data obtained after the administration of a dose of As(III) (0.1, 0.5, 2 mg/kg bw) by gavage. Values expressed as ng As (mean ± SD, n = 3).

Figure 2. Plasma concentration of As as a function of the dose of As(V) administered. Data obtained after the administration of a dose of As(V) (0.1, 0.5, 2 mg/kg bw) by gavage. Values expressed as ng As (mean ± SD, n = 3).

Figure 3. Area under the curve (AUC) of As of mice orally given As(III) and food components. Data obtained after a single dose (1 mg/kg bw) of As(III) by gavage in the absence and presence of food components. Values expressed as ng As/mL/min (mean ± SD, n = 7). Asterisks show significant reductions with respect to animals exposed only to As ($p < 0.05$).

Figure 4. Area under the curve (AUC) of As of mice orally given As(V) and food components. Data obtained after a single dose (1 mg/kg bw) of As(V) by gavage in the absence or presence of food components. Values expressed as ng As/mL/min (mean ± SD, n = 7). Asterisks show significant reductions with respect to animals exposed only to As ($p < 0.05$).

Figure 5. Tissue accumulation of As in mice exposed to As(III) or As(V) and food components. Concentration of As in liver (5A), lungs (5B) and small intestine (5C) of animals orally given As(III) or As(V) (1 mg/kg bw) in the absence (control) or presence of food components. Values expressed as ng As/g of tissue (mean ± SD, n = 7). Asterisks show significant reductions with respect to the control animals ($p < 0.05$).

Table 1. Relative Bioavailability (F) of As(III) and As(V) in Animals Co-exposed to Inorganic As and Food Components. Data obtained after a single dose (1 mg/kg bw) of inorganic As by gavage in the absence or presence of food components. Values expressed as mean ± SD (n = 7). Asterisks show significant reductions with respect to animals exposed only to As ($p < 0.05$).

	F	
	As(III)	**As(V)**
Tannic acid	93 ± 19	69 ± 8*
Fe(III)	75 ± 9	66 ± 8*
GSH	25 ± 8*	49 ± 10*

Table 2. Faecal Excretion of Arsenic in Mice Exposed to As(III) or As(V) and Food Components. Concentration of As in faeces of animals treated with As(III) or As(V) (1 mg/kg bw) by gavage in the absence or presence of food components for 15 days. Values expressed as ng As/g of faeces [range (median), n = 7]. Asterisks show significant reductions with respect to animals exposed only to As ($p < 0.05$).

	Treatment	Faecal excretion
As(III)	As(III)	172-377 (236)
	As(III) + Tannic acid	105-269 (199)
	As(III) + Fe(III)	199-1176 (649)*
	As(III) + GSH	385-537 (488)*
As(V)	As(V)	235-564 (403)
	As(V) + Tannic acid	309-2302 (896)
	As(V) + Fe(III)	469-2377 (911)
	As(V) + GSH	279-1763 (541)

Table 3. Effect of Food Components on Hepatic Mineral Contents. Concentrations of Ca, K, Fe and P in the liver of animals gavaged with As(V) (1 mg/kg bw) in the absence or presence of food components. Values expressed as ng/g of tissue (mean ± SD, (median), n = 7).

Treatments	Ca	K	Fe	P
As	183 ± 123 (138)	2570 ± 1292 (2278)	104 ± 28 (97)	3397 ± 881 (3100)
As + Fe(III)	214 ± 82 (169)	2009 ± 696 (1794)	135 ± 51 (115)	4168 ± 1716 (3375)
As + GSH	141± 23 (131)	1519 ± 446 (1470)	94 ± 17 (92)	3056 ± 477 (2889)

Figure 1.

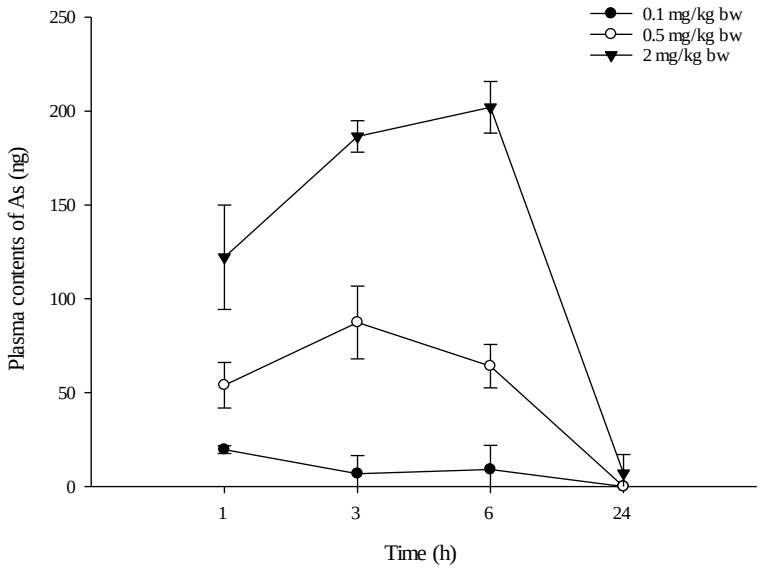

0.1 mg/kg bw
0.5 mg/kg bw
2 mg/kg bw
Plasma contents of As (ng)
250
200
150
100
50
0
1
3
6
24
Time (h)

Figure 2.

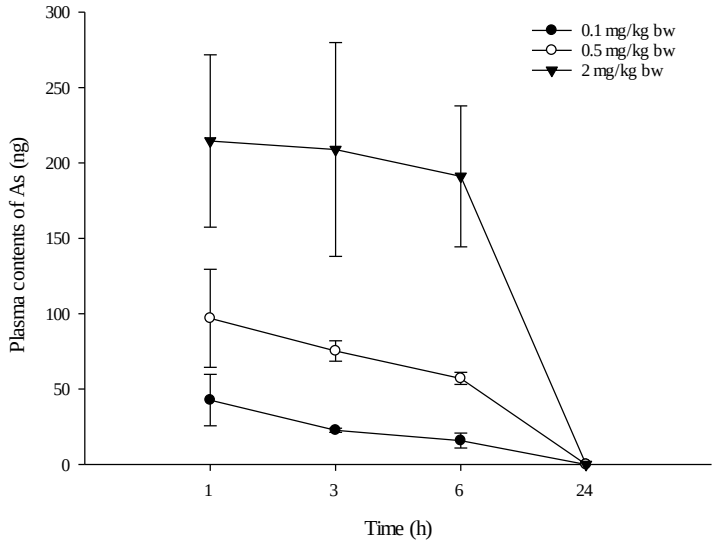

0.1 mg/kg bw
0.5 mg/kg bw
2 mg/kg bw
Plasma contents of As (ng)
300
250
200
150
100
50
0
1
3
6
24
Time (h)

Figure 3.

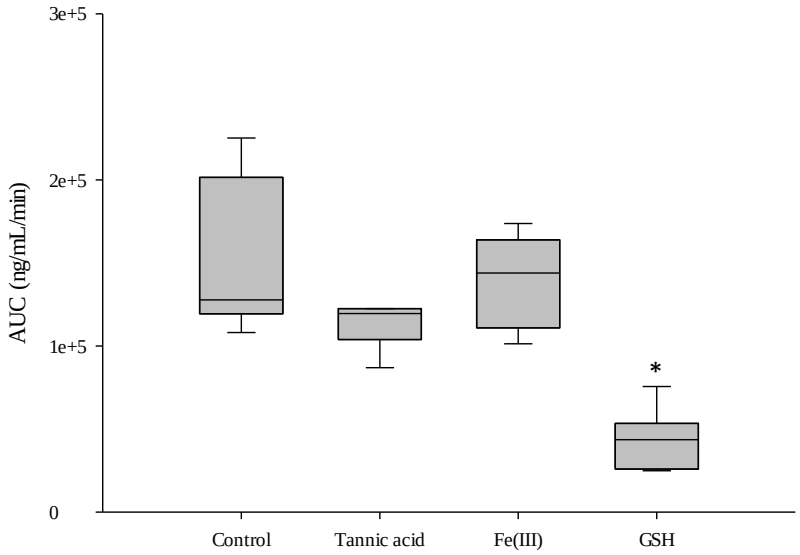


Figure 4.

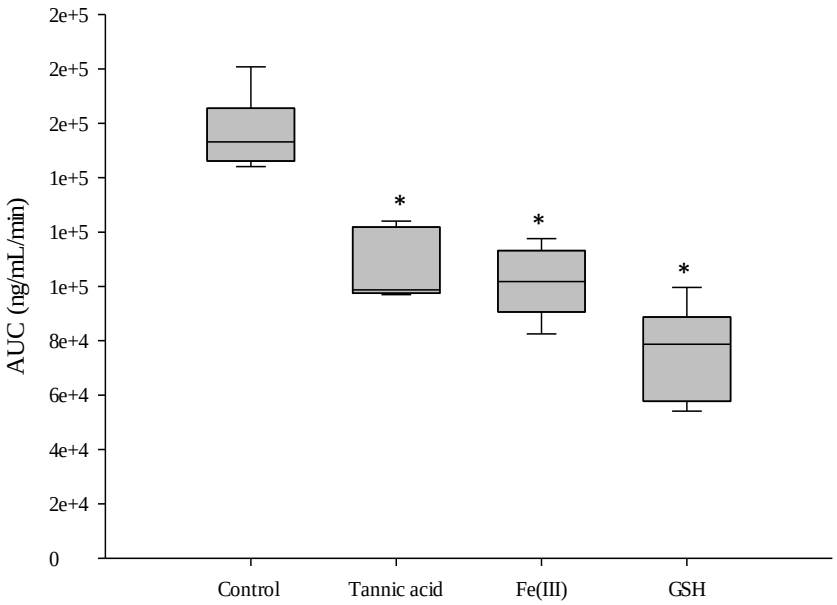
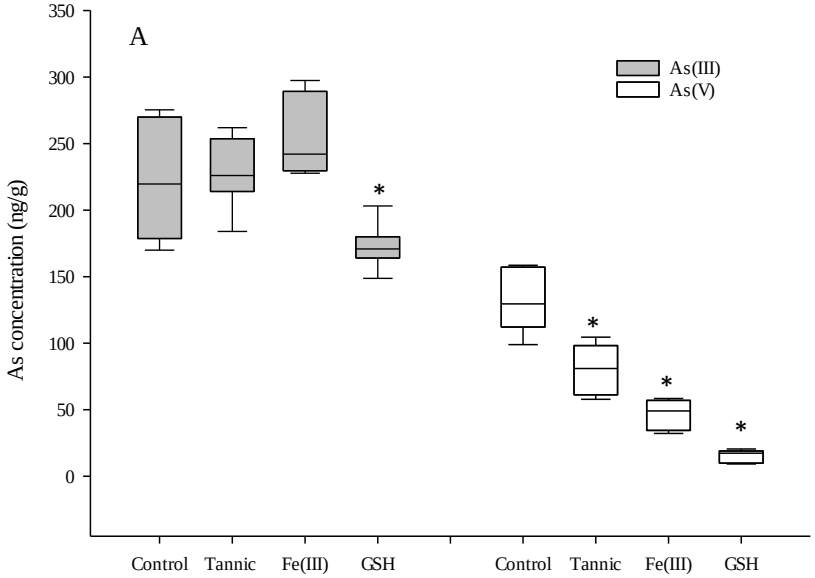
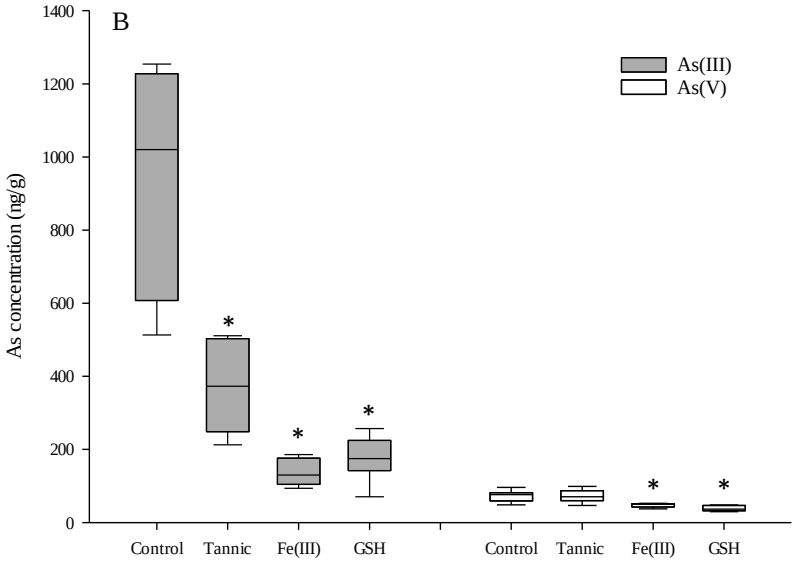
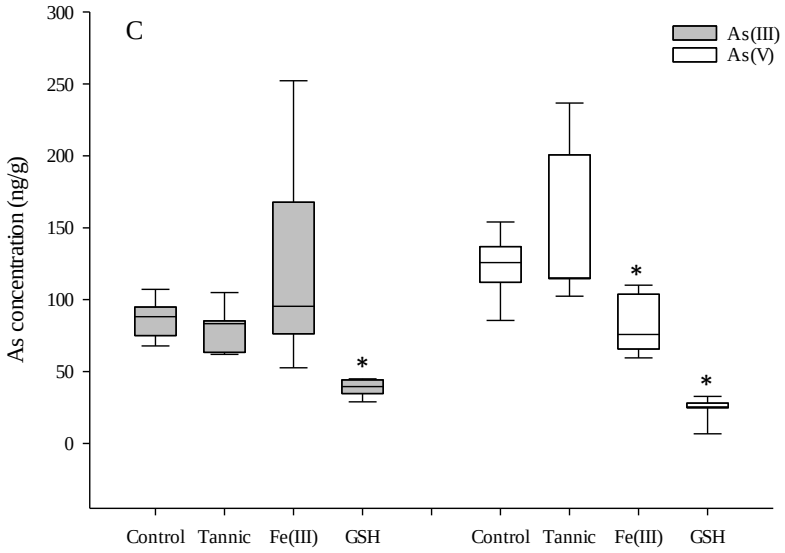

AUC (ng/mL/min)
2e+5
2e+5
2e+5
1e+5
1e+5
1e+5
8e+4
6e+4
4e+4
2e+4
0
*
*
*
Control
Tannic acid
Fe(III)
GSH

Figure 5.


A
As(III)
As(V)
As concentration (ng/g)
350
300
250
200
150
100
50
0
Control
Tannic
Fe(III)
GSH
Control
Tannic
Fe(III)
GSH


B
As(III)
As(V)
As concentration (ng/g)
1400
1200
1000
800
600
400
200
0
Control
Tannic
Fe(III)
GSH
Control
Tannic
Fe(III)
GSH


C
As(III)
As(V)
As concentration (ng/g)
300
250
200
150
100
50
0
*
*
*
Control
Tannic
Fe(III)
GSH
Control
Tannic
Fe(III)
GSH

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