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Bioavailability study of arsenic and mercury in traditional Chinese medicines (TCM) using an animal model after a single dose exposure

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INTRODUCTION

Traditional Chinese medicines (TCM) have been used for thousands of years, and because of their many applications in treatment of illnesses, their use also has attracted interest in many countries. However, there have been many reports that the use of TCM can result in adverse health effects (Ernst, 2004; Efferth and Kaina, 2011; Tang et al., 2008). Because the medicines are readily available from many suppliers as alternative remedy for health conditions, this has caused public health concern if these products are not appropriately regulated. In Australia, a nationwide survey indicated an increase in the use of Chinese medicines (MacLennan et al., 2002). These Chinese medicines and their raw products have been reported to contain significantly high levels of toxic heavy metals such as arsenic, mercury and lead, as a result of contamination or intentionally being added with cinnabar (mercuric sulfide) or realgar (arsenic sulfide) during processing (Harris et al., 2011; Lu et al., 2011; Ting et al., 2013). A regular intake of Chinese medicines is a cause of concern, and it has been reported that intakes of traditional Chinese herbs by breast feeding women can result in increase of lead concentrations in breast milk, and this can pose a considerable risk to the breast-fed infants (Chien, et al. 2006). A survey of traditional Chinese medicines in Queensland, Australia has shown to contain high levels of arsenic and mercury in some products (Rutherford et al., 2004; Cooper et al., 2007).

Many countries have provided legislations and guidelines for the control and use of TCM (Shia et al., 2007). Over one hundred of Chinese medicines are prohibited from sale at the USA or European markets because the levels of Hg and As are higher than the permitted levels allowed for food and drugs (Liu et al., 2008; Wu et al., 2011). In Singapore, its government has provided comprehensive legislation and guidelines for levels of chemical constituents and ingredients in TCM. For instance, the legal limits for toxic metals such as arsenic and mercury are 5 and 0.5 mg/kg, respectively (Yee et al., 2005). In Australia, the Standard for the Uniform Scheduling of Drugs and Poisons (SUSDP, 2005) has indicated that a product contains > 1 mg/kg of arsenic or mercury should be classified as a Schedule 4 substance. In food, the permitted maximum level of mercury is 1 mg/kg and 2 mg/kg for arsenic (FSANZ, 2012).

Recently, there has been an interest on the study of toxic metal bioavailability and bioaccessibility from TCM and other herbal medicines. The toxicity and bioavailability of metals depend on their routes of exposure, metal species, solubility and interactions with other dietary components. Others have reported that bioaccessibility of As as realgar and Hg as cinnabar is generally low because of poor solubility of these compounds when in vitro gastrointestinal simulations were used for assessment (Koch et al., 2007; 2011; Jayawardene et al., 2010). It has been reported that mercury can be absorbed and incorporated into tissue proteins as mercury-binding metallothioneins. Huang et al. (2004) have demonstrated that rats given oral doses of mercuric sulfide in the form of cinnabar, a major ingredient of TCM, resulted in an increase of mercury-binding metallothioneins in liver and kidney tissues. In order to assess the potential risk of TCM,

it is important to establish data as a guideline for bioavailability assessment from exposure of As and Hg from these products. The objective of this study was to assess the bioavailability of As and Hg from TCM using an animal model after a single exposure dose of As and Hg compounds.

MATERIALS AND METHODS

Animal Experimentation

Twenty four female Sprague-Dawley rats (approx. 200 g body weight, 6-7 weeks old), obtained from the Laboratory Animal Services, University of Adelaide, were used for this study. The animal study was approved by the Queensland Health Scientific Services Animal Ethics Committee (NRC 03/03/19), and the experiment was conducted in accordance with the guidelines for the care and use of laboratory animals. Each rat was placed in a metabolic cage for the collection of 24h urine and faeces prior to and after dosing. The rats were allowed ad-lib access to water and kept in a room with the temperature (23 ± 2°C) and a twelve-hour light/dark cycle were maintained throughout the experiment with lights on at 0800 hrs. The rats were divided into 6 groups and each group consisted of 4 rats. The two groups (G1 and G3) were used as reference after treatment with pure compounds of sodium arsenite (G1, UNILAB, $NaAsO_2$, 99.0%, NSW, Australia), and mercuric chloride (G3, $HgCl_2$, 99.5%, NSW, Australia). For rats in group G2, they were given arsenic sulfide (As_2S_3) as an orpiment ore, and group G4 were given mercury sulfide (HgS) as cinnabar ore. The mineral ore samples of As2S3 and HgS

were provided by the School of Earth Sciences, the University of Queensland. The other two groups (G5, *Liu Shen Wan* and G6, *Niuhang Jie du Pian*) were given TCM which were obtained and purchased from an over-the-counter medicine shop in Hangzhou, China. These two TCM were selected for the study because they are widely used for treatment of multiple symptoms and found to contain high As and Hg (Cooper et al., 2007; Rutherford et al., 2004). At the beginning of the experiment all rats were given a single dose by oral gavage containing As or Hg at 2-5 mg/kg/body weight. Prior to dosing, the solid tablets were ground to fine powder and suspended in a mixture of water and wheat flour for efficient delivery during oral gavage. One mL of each treatment solution was given to each rat for oral gavage. The dosing regime is shown in Table 1. During this period, the animals were given a commercial rodent diet (NORCO Pty Ltd, Brisbane, Australia), containing fish meal protein and relatively low levels of As (0.71 mg/kg) and Hg (0.32 mg/kg). Samples of urine and faeces were collected every 24 hours for 9 days after dosing. At the end of the experiment, the rats were anaesthetised with a mixture of carbon dioxide and oxygen and samples of liver and kidney were collected for analysis of As and Hg content and histological examinations.

Analysis of As and Hg

The samples of rat kidney, liver and faeces were homogenised, and the subsamples were taken and weighed (0.5 – 1.0 g) into Teflon digestion vessels for analysis. The samples of TCM pellets and tablets were weighed (0.3 – 0.5 g) directly into Teflon digestion vessels. The samples were then digested with nitric acid using microwave digestion system (MDS

2100, CEM, Mathews, USA). An aliquot (0.5 mL) of urine samples was taken for analysis. The Lyphochek Urine Metals Control Levels 1 & 2 (BIO-RAD, Gladesville, Australia) were used for quality control and assurance for urine analysis. The high levels of arsenic and mercury were analysed by ICP-AES (Vista AX, Varian Australia) and for lower levels, and for greater sensitivity the analysis was carried out using ICP-MS (7500a, Agilent, Tokyo, Japan). The standard reference materials of Bovine Liver, (AGAL-4, Australian Government Analytical Laboratories, NSW) and seafood tissues (Laboratory in-house reference materials, QC 180 and FFMO4) were used for quality control, and these reference materials were treated similarly to the samples throughout the study.

Bioavailability Determination

The determination and estimation of bioavailability (BA) was based on the methods described elsewhere (Ng et al., 1998; Roberts et al., 2002; Ng et al., 2015). In summary, the absolute bioavailability (ABA) was calculated based on the excretion of total urinary As or Hg levels collected from 0 to 216 hr (0 – 9 days) at 24 h intervals. The ABA was then calculated according to Equation 1:

$$\% \text{ ABA} = [\text{total urinary As or Hg}]/[\text{tested As or Hg dose}] \times 100 \quad (1)$$

The relative bioavailability (RBA) was calculated as the ratio of ABA of test materials to the ABA of reference materials. The soluble reference materials used were sodium arsenite ($NaAsO_2$) and mercuric chloride ($HgCl_2$). The RBA was then calculated according to Equation 2:

$$\%RBA = [ABA(\text{tested materials})/ABA(\text{reference materials})] \times 100 \quad (2)$$

RESULTS AND DISCUSSION

Method quality control and assurance

The accuracy and precision of the method for analysis of As and Hg were validated by analysing the appropriate standard reference materials, and the results are shown in Table 2. The detection limit of both As and Hg was found at 0.01 mg/kg for ICP-MS analysis. The detection limit was calculated based on 3 times the standard deviation of the blanks from a series of measurements (n=20), for sample weight at 0.5 g and diluted to 40 mL volume. The levels of As and Hg found in urine quality control samples were satisfactory and within the reference values (Lyphocheck Urine Metals Control). The recoveries of As and Hg from SRMs of Bovine Liver and QC 180 and QC FFM04 were satisfactory which ranged from 89 to 109%. The precision of the method was validated by determining the coefficients of variation (C.V.%) of between-batches of in-house reference materials QC

180 and FFM04 over the period of 2 months. The method gave good precision with C.V. values ranged from 5.4% to 10.9% (Table 2). The in-house reference materials of QC 180 and QC FFM04 were validated with certified reference material (CRM) of Mussel Tissue (SRM 2976, NIST). The recoveries of As and Hg from Mussel Tissue SRM were satisfactory which ranged from 96-116% (Table 2). The analysis of Montana Soil SRM also gave good recoveries for As (91%) and Hg (87%) (Table 2). This laboratory has also participated in proficiency trial, organised by the National Institute of Measurement (NMI), Australia, for analysis a range of metals and metalloids including total arsenic and mercury in soil and animal feed for quality control and assurance. Our results for total As (16.0 mg/kg, measurement uncertainty (MU), ± 0.5 mg/kg) and Hg (2.0 mg/kg, MU, ± 0.3 mg/kg) in soil were satisfactory, and within the consensus values (15.1 mg/kg for As and 1.96 mg/kg for Hg) as assigned by NMI (2013). The analysis of As (0.52 mg/kg, MU, ± 0.06 mg/kg) in animal feed was also satisfactory and within the consensus value (0.505 mg/kg, MU, ± 0.094) assigned by NMI (2014).

As and Hg levels in TCM and tissues

The levels of As and Hg found in these two TCM are shown in Table 3. The *Liu Shen Wan* was found to contain relatively high arsenic levels (7.7-9.1%) and there was a considerable variation between batches for mercury levels (1.4-5.0%). The *Niuhang Jie du Pian* was found to contain high As (6.2 - 7.9%) and very low level Hg (<0.001 %). In this study, the levels of As and Hg were not determined in cinnabar ore (HgS) due to insufficient sample size. However, a different batch of cinnabar ore (HgS) was analysed,

and found to contain about 1.2% Hg and low As (42.0 mg/kg). From the mass balance data (Table 4), the earlier batch of cinnabar ore was estimated to contain Hg at about 16.4% and low As (trace). The analysis of orpiment ore (As_2S_3) was found to contain As at 55.7%, which are comparable to estimated mass balance level at 52.1%, and very low Hg (Table 4). Others have also reported high As level (7 ± 1%) in *Niuhang Jie du Pian* (Koch et al., 2007). High levels of As and Hg in these traditional Chinese medicines have caused public health concern as there were reported cases of toxic effects in humans for short-term and long-term exposure (Ernst, 2004; Espinoza et al., 1995).

With the exception of reference groups treated with $NaAsO_2$ (G1) and $HgCl_2$ (G3), the levels of As and Hg in rat liver and kidney tissues were low (Figures 1 and 2). These results indicated that As and Hg as sulfides and from TCM were not readily accumulated in kidney and liver. However, in G4 group, there was unexpected spike of As levels in live and kidney tissues, even though these animals were treated only with mercury sulfide (Figure 1). It was found that the mercury sulfide of cinnabar ore contained considerable amount of arsenic when mass balance was evaluated (Table 4). Similarly, there was unexpected Hg spike in kidney of rats (G1) given only with sodium arsenite (Figure 2). It is likely that the animal diet which was found to contain Hg (0.32 mg/kg) could contribute to increased level in kidney. The level of Hg found in the animal diet is considered low and would have little influence on Hg bioavailability, as indicated by low level in urine (Table 4). However, it is also possible the rats in groups G1 and G4 could have exhibited a higher food intake resulting in greater accumulation of Hg and As in tissues. Others have also reported of low levels of Hg in liver tissue of mice after

exposure to cinnabar and traditional Chinese medicines, but high levels were observed when these mice were treated with methylmercury and $HgCl_2$ (Lu et al., 2011a, 2011b; Shi et al., 2011). Low levels As in liver and kidney tissues of mice have also been observed when treated with higher dose (600 mg/kg) of TCM and realgar when compared with sodium arsenite (36 mg/kg) and arsenate (88 mg/kg) (Miao et al., 2011).

In this present study it was estimated that high levels of As and Hg from TCM were excreted via faeces and only small amount in urine (Table 4). It is possible that the low solubility of As as realgar (As_4S_4) or orpiment (As_2S_3) and Hg as cinnabar present in these TCM may contribute to their poor absorption in gastrointestinal tract and subsequently to be readily excreted via faeces. Cinnabar as HgS has a low solubility (<0.001g/L at 20°C), and it is poorly absorbed (0.2%) from gastrointestinal tract and accumulated in tissues when compared to $HgCl_2$ (7-15%) and methylmercury (>95%) (Liu et al., 2008b; Lu et al., 20011a). It has been observed in hamsters that As as orpiment is poorly absorbed, and over 82% is found in faeces within 3 days after oral dose compared to more soluble sodium arsenate at only 12% excreted in faeces (Marafante and Vahter 1987). In humans, As is also excreted via urine in small amount (<1%) after taking the *Niuhang Jie du Pian* tablets (Koch et al., 2007; Liu et al., 2008a). Koch et al (2007) have also demonstrated that the presence of realgar in *Niuhang Jie du Pian* tablets are readily metabolised in human body to other forms of As species as As III, dimethylarsinic acid (DMA (V)), and monomethyarsonic acid (MMA (V)) and excreted in urine.

The subsequent histopathological examination of liver and kidney tissues in this study (results not shown) did not show toxic effects, and furthermore, exposure to As and Hg compounds did not show any effect on rat body weight gain (Figure 3). There was a trend in weight gain for all the rats in this study, and all animals were in good health when exposure to low level As and Hg (5 mg/kg body weight) during the experiment. A significant difference in weight gain was found for G2, G3 and G4 (Figure 3). Others have also demonstrated that rats exposed to HgS after oral administration over 5 consecutive days at higher dose (1.0g/kg day) showed no effect on body weight and peripheral neurotoxicity (Chuu et al., 2007). However, mild reversible peripheral neurotoxicity was observed in these rats when the treatment was extended to 14 consecutive days. Even though this present study did not show toxic and accumulative effects of Hg from TCM, a higher exposure of Hg as HgS (2.5 g/kg body weight) has been shown to accumulate in liver and caused neurotoxic effect on the vestibulo-ocular system of guinea pigs (Young et al., 2002). In another study, others have also shown that HgS was readily absorbed by gastrointestinal tract and transported to various tissues (brain, kidney and liver) in mice after the animals were given cinnabar (10 mg/kg per day) by oral gavage for 11 consecutive weeks (Huang, et al., 2007).

Bioavailability of As and Hg

Toxicity can be influenced by bioavailability and there has been little study on bioavailability of As and Hg from taking TCM using rats as animal model. There is still a debate on a suitable animal model that can simulate the gastrointestinal tract

characteristics of humans for metabolism of metals or metalloids. Arsenic for instance, has been reported to metabolise differently in animal species (Vahter et al. 1983). The use of larger animals such as dogs and pigs with larger doses of metal exposure for risk assessment studies has also been discussed as a potentially superior model system; however, the methylation of arsenic in pigs is still not well understood (Groen et al., 1994; Rees et al., 2009). Rats have also been widely used for assessing bioavailability of Hg in soil, and the effect of animal species differences in absorption rates, particularly for inorganic mercuric compounds, has not been observed (Paustenbach et al., 1997; Schoof and Nielsen 1997). At present, there is not much evidence to indicate that more complex feeding systems are going to yield more relevant data, and in this experiment, rats were used as they are less expensive, and widely used for evaluation of carcinogenicity and toxicity and risk assessment (IARC 2004; Ruby et al., 1999).

In this study, the values of absolute and relative bioavailability were estimated and evaluated from mass balance data. It should be emphasised the mass balance values were estimated based on levels of As and Hg in liver, kidney, faeces and urine, and by taking into account of kidney, liver and faeces weight, and the volume of urine. The mass balance data did not include the accumulation in other tissues. It is estimated that for the reference group (G1), treated with $NaAsO_2$, about 61.9% of As were recovered, and the rest of As would likely to be accumulated in other tissues and particularly in blood (Table 4). As for the reference group (G3), treated with $HgCl_2$, all of Hg (106%) were recovered from kidney, liver, urine and faeces, indication low accumulation in other tissues after a short exposure (Table 4). The present study showed low relative bioavailability of As

(0.60 – 1.10%) and Hg (<0.001%) in rats treated with As and Hg compounds and TCM (Table 4). This poor bioavailability could be attributed to the presence of insoluble As and Hg compounds in the form of sulfides in these TCM. The sulfide forms of As as realgar (As_4S_4) and orpiment (As_2S_3), and cinnabar as HgS have been identified in some TCM, and they are parts of important ingredients and additives for the preparation of TCM (Koch et al., 2007; Huang et al., 2004; Huang et al., 2007; Chuu et al., 2007). A study by Koch et al (2007) using extraction techniques to simulate stomach extraction showed that up to 4% of As was bioaccessible from realgar, which constituted to about 99% As as realgar in *Niuhang Jie du Pian* pills. This value of 4% is considerably higher than the bioavailability of As found in this present study. However, the low results of As relative bioavailability (0.60 – 1.10%) in this study were comparable to the values (0.17 - 0.6%) reported by others using HCl (0.07 N) and artificial gastric juice extraction to mimic the stomach extraction (Kwan, et al., 2001). Others have also shown that As as realgar (0.41%) and Hg as cinnabar (<0.001%) were poorly leached from Chinese medicinal materials when extracted using artificial stomach fluids (Wu et al., 2002). The wide variation in bioavailability and bioaccessibility results of As and Hg could be attributed to a number of factors and these may include their solubility, oxidation states (speciation), as these may influence their interaction with metallothioneins such as Hg, and the formation of As complex as seleno bis(S-glutathionyl) arsinium ion in erythrocytes, and this will subsequently affect their distribution in tissues and excretion (Huang et al., 2004; Manley et al., 2006; Gailer 2007).

CONCLUSIONS

This study has shown that As and Hg did not readily accumulate in rat liver and kidney tissues after a low single dose of TCM, and no toxic effects were observed during histopathological examination. Most of the As and Hg from TCM were excreted via faeces and only a small amount in urine. The study has also shown that the use of animal model after a single low-dose exposure via oral gavage was found to be efficient and reliable for estimating the bioavailability of high As and Hg levels in TCM. It was found that the bioavailability of As and Hg from these TCM was very low. However, the levels of total elements such as As and Hg in TCM may not be sufficient information for compete assessment of their toxicity and bioavailability without taking into consideration of elemental speciation, which is also important for determining the stability and solubility of the compounds. Because of limited information on the elemental speciation, there has been an increasing interest on the speciation study and the forms of elemental compounds in TCM and other Chinese herbal medicines which can have direct effect on bioavailability and toxicity (Liu et al., 2013; Zhou et al., 2010). It is important that to ensure the safety and wellbeing of the public there is a need for more information and data on As and Hg speciation in TCM including the raw materials and other ingredients that could also be used or added into these medicines during processing and manufacturing. Further information on the speciation of toxic metals and metalloids would be useful for health risk assessment and for policy makers and regulatory bodies to establish guidelines and regulations from exposure to TCM which are increasingly available in market places.

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Table 1. Dosing regime for bioavailability study of As and Hg in rats

Group ID	Treatment	Dose preparation[a]	Equivalent dose/mL
G1	$NaAsO_2$ (reference/control)	8.69 mg/100mL	500 µg (as As)
G2	As_2S_3 (orpiment ore)	16.42 mg/10mL	1642 µg (as As_2S_3)
G3	$HgCl_2$ (reference/control)	135 mg/100mL	1000 µg (as Hg)
G4	HgS (cinnabar ore)	11.60 mg/10mL	1160 µg (as HgS)
G5	Liu Shen Wan (LSW)	113.9 mg/10mL	11390 µg (as LSW)
G6	Niuhang Jie du Pian (NJDP)	147.0 mg/10mL	14700 µg (as NJDP)

[a]A dose of 5 mg of As or Hg/kg/body weight is considered low (oral LD_{50} values for rats are between 15mg/kg – 175mg/kg for As (ATSDR, 2007) and 25.9mg/kg – 77.7 mg/kg for Hg (ATSDR, 1999)).

Table 2. The recovery of arsenic and mercury from standard reference materials (SRM)

SRM	Concentration (mg/kg, mean ± SD)	
	Arsenic	Mercury
AGAL-4 (Bovine Liver SRM)		
This study (n=5)[a]	0.41 ± 0.05	0.35 ± 0.05
Reference values	0.46 ± 0.11	0.35 ± 0.06
Recovery (%)[b]	89	100
QC 180 (Fish tissue in-house SRM)		
This study (n=12)	29.1 ± 1.6	3.6 ± 0.3
Reference values	28.8 ± 1.7	3.3 ± 0.2
Recovery (%)	101	109
Coefficients of variation (C.V. %)[c]	5.4	9.3
QC FFM04 (Fish tissue in-house SRM)		
This study (n=12)	4.1 ± 0.3	0.92 ± 0.10
Reference values	4.2 ± 0.1	1.0 ± 0.1
Recovery (%)	98	92
Coefficients of variation (C.V. %)	6.7	10.9
SRM 2976 (Mussel Tissue, NIST)		
This study (n=10)	13.9 ± 0.6	0.053 ± 0.008
Certified values	13.3 ± 1.8	0.061 ± 0.004
Recovery (%)	96	116
Lyphochek Urine Metals Control Level 1		
This study (n=13, µg/L)	68.0 ± 2.7	30.1 ± 2.0
Reference values (range, µg/L)	56 - 85	28 - 58
Level 2		
This study (n=13, µg/L)	156.4 ± 3.4	92.1 ± 8.1
Reference value range (µg/L)	123 - 185	110 - 165
SRM 2710a (Montana Soil, NIST)		
This study (n=7)	1400 ± 5	8.57 ± 0.70
Certified values	1540 ± 100	9.88 ± 0.21

Recovery (%)	91	87

[a]N = number of determinations

[b]Recovery (%) is calculated as: [Determined mean/reference mean] x 100

[c]C.V. (%) values were determined over a period of 2 months

Table 3. Levels (%) of As and Hg in TCM

TCM	Batch 1 (%)		Batch 2 (%)	
	As	Hg	As	Hg
Niuhang Jie du Pian	6.5 ± 0.3 (n=4)[a] (6.2 - 6.8)[b]	<0.001 -	7.1 ± 0.4 (n=12) (6.6 - 7.9)	<0.001 -
Liu Shen Wan	8.8 ± 0.3 (n=4) (8.5 - 9.1)	1.5 ± 0.1 (1.4 - 1.6)	8.1 ± 0.6 (n=3) (7.7 - 8.8)	4.9 ± 0.1 (4.8 - 5.0)

[a]n=number replicate analyses
[b]values in brackets indicate range of results

Table 4. Estimated mean values of mass balance (µg) and bioavailability of As and Hg based on urine excretion of rat

Group	Treatment	Level in tissue (µg)				Total	Estimated concentration (%)	Absolute Bioavailability (%)	Relative Bioavailability (%)
		Liver	Kidney	Urine	Faeces				
Level of As									
G1 (reference/control)	$NaAsO_2$	51.9	5.0	80.5	196	333	61.9	15.4	-[a]
G2	As_2S_3	3.6	0.3	2.9	872	879	52.1	<0.001	<0.001
G3 (reference/control)	$HgCl_2$	5.6	0.4	3.2	14.8	24	-[b]	-[b]	-[b]
G4	HgS	13.9	1.3	5.3	14.7	35	trace[c]	<0.001	<0.001
G5	LSW	2.8	0.4	4.4	724	732	6.2	0.17	1.10
G6	NJDP	4.5	0.3	3.8	663	671	4.4	0.09	0.60
Level of Hg									
G1 (reference/control)	$NaAsO_2$	0.3	0.4	<0.1	1.5	2.2	-[d]	-[d]	-[d]
G2	As_2S_3	0.2	0.1	<0.1	<0.1	0.3	<0.001	<0.001	<0.001
G3 (reference/control)	$HgCl_2$	1.7	20.3	31.8	1008	1062	106.0	3.2	-[e]
G4	HgS	0.4	0.5	<0.1	192	193	16.4	<0.001	<0.001
G5	LSW	0.2	0.2	<0.1	584	584	5.1	<0.001	<0.001
G6	NJDP	<0.1	<0.1	<0.1	<0.1	<0.1	<0.001	<0.001	<0.001

[a]Relative bioavailability of G1 for As was not determined, as it was used for reference group.

[b]The G3 was used for reference and control for As treatment, and the only source of As came from the animal diet (the estimated values were determined after subtraction from the control group).

[c]Trace level of As was detected, which was close to detection limit of the method.

[d]The G1 was used for reference and control for Hg treatment, and the only source of Hg came from the animal diet (the estimated values were determined after subtraction from the control group).

[e]Relative bioavailability of G1 for Hg was not determined, as it was used for reference group.

Figure captions

Figure 1. The levels (mg/kg, wet weight) of As in liver and kidney tissues of rats from each treated group (G1= sodium arsenite ($NaAsO_2$) treated group; G2 = arsenic sulfide (As_2S_3) treated group; G3 = mercuric chloride ($HgCl_2$) treated group; G4 = mercuric sulfide (HgS) treated group; G5 = Liu Shen Wan treated group; G6 = Niuhuang Jie du Pian treated group).

Figure 2. The levels (mg/kg, wet weight) of Hg in liver and kidney tissues of rats from each treated group (G1= sodium arsenite ($NaAsO_2$) treated group; G2 = arsenic sulfide (As_2S_3) treated group; G3 = mercuric chloride ($HgCl_2$) treated group; G4 = mercuric sulfide (HgS) treated group; G5 = Liu Shen Wan treated group; G6 = Niuhuang Jie du Pian treated group).

Figure 3. The rat weight before and after treatment with As and Hg compounds. An asterisk (*) indicates significant difference in weight gain for groups G2 ($p<0.01$), G3 ($p<0.05$) and G4 ($P<0.01$). (G1= sodium arsenite ($NaAsO_2$) treated group; G2 = arsenic sulfide (As_2S_3) treated group; G3 = mercuric chloride ($HgCl_2$) treated group; G4 = mercuric sulfide (HgS) treated group; G5 = Liu Shen Wan treated group; G6 = Niuhuang Jie du Pian treated group).

Figure 1

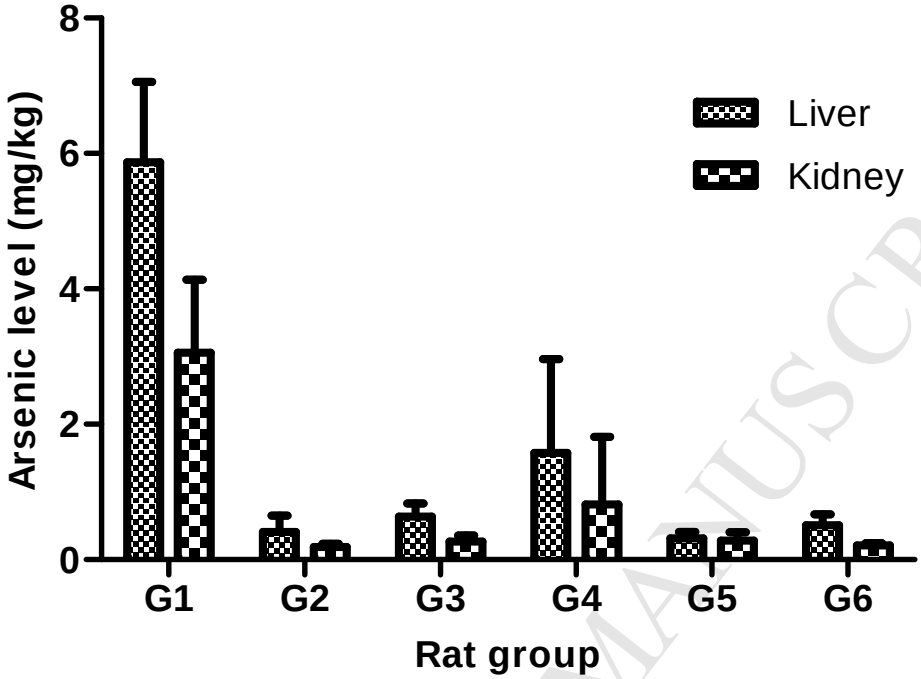


Figure 2

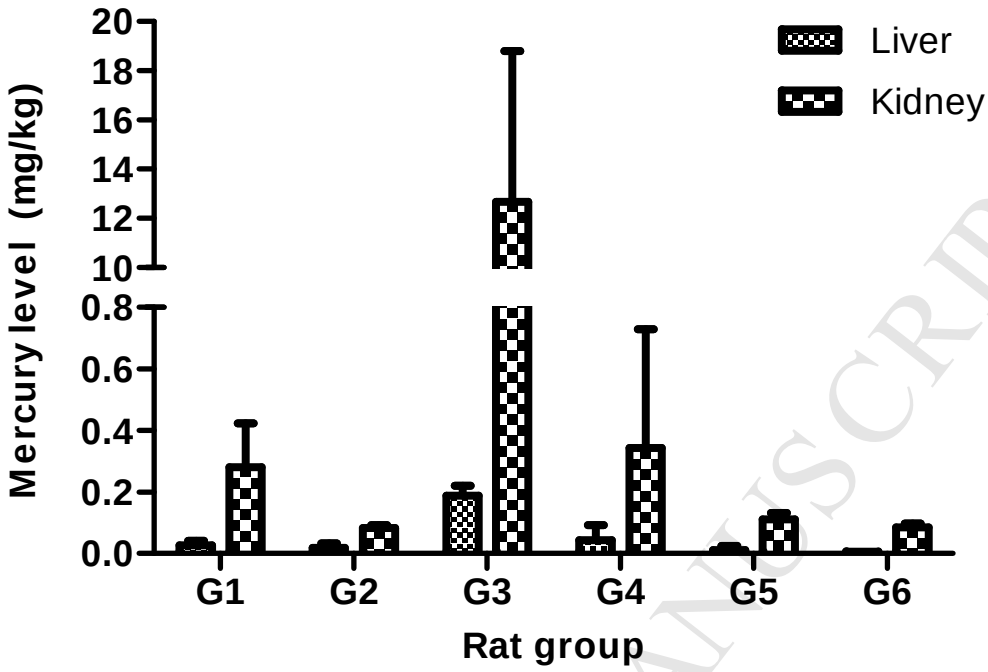
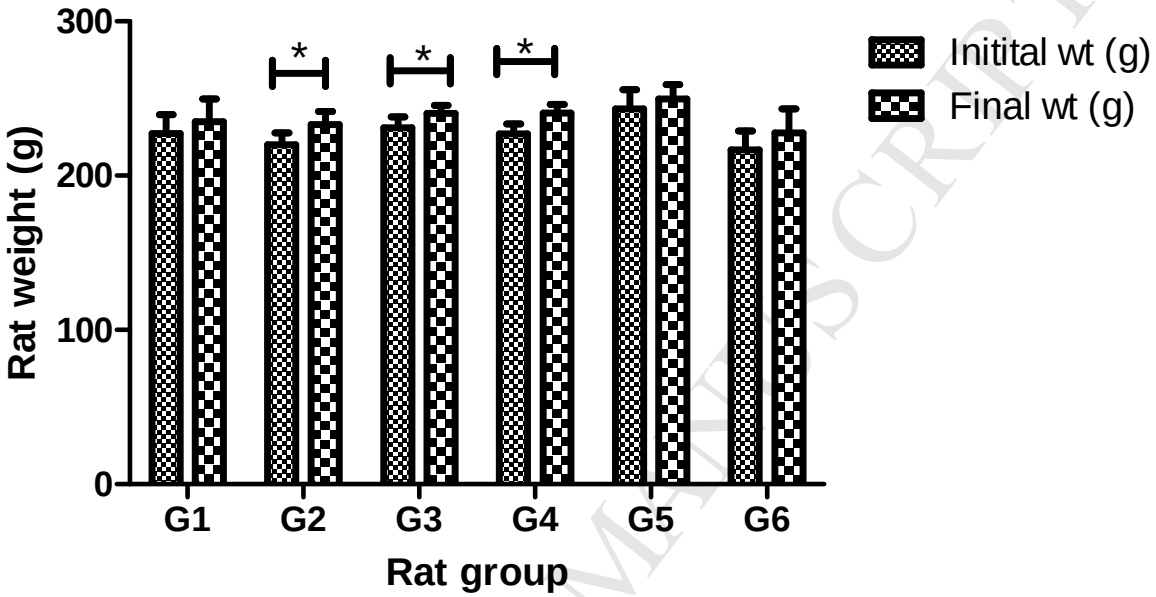


Figure 3



Highlights

- Traditional Chinese Medicines (TCM) were found to contain high Hg and As.
- Relatively low levels of As and Hg were found in rat liver and kidney tissues after TCM exposure.
- The study showed low relative bioavailability of As and Hg from TCM.