

## Inhibitory effect of arsenic on aerobic gut flora in rat

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### Abstract

An *in vivo* study was carried on rats to see the influence of arsenic on aerobic gut flora. A significant inhibition of gut flora was observed after 2 weeks of administration of arsenic (1 mg/L) *ad libitum* with a decrease in stool arsenic level and increase in liver arsenic level. However, this inhibitory effect of arsenic on gut flora was not observed in presence of vitamin E (1 mg/day) or selenium (0.4 µg/day). Pretreatment with streptomycin (500 mg twice daily) showed similar results. Rats that received folic acid (200 µg/day) showed inhibition of gut floral count but there were decreased liver arsenic level.

### Introduction

Arsenic is a cause of great health concern throughout the world, as it is believed to be related to cardiovascular<sup>1</sup>, gastrointestinal<sup>2</sup>, respiratory<sup>3</sup>, skin<sup>4</sup>, endocrine<sup>5</sup> disorders and carcinogenesis<sup>6</sup>. It has been classified by WHO as Group I carcinogen<sup>7</sup>. Ingested arsenic must undergo detoxification, in order that it is removed from the body, a process brought about by the liver, gut flora also plays a role in arsenic detoxification<sup>8</sup>. Although little work have been carried out to see the interaction between gut flora and arsenic<sup>9</sup>. Recent data suggest that hepatic methylation of arsenic leads to the formation of DMA-III and MMA-III which increases toxicity<sup>10</sup>, so more and more stress is being given on bacterial detoxification of arsenic which is considered to be non-toxic<sup>11</sup>.

Some 300-500 different species of bacteria live and grow in intestine as symbionts and act as body's first line of defense against ingested xenobiotics<sup>12</sup>. Colonization of gut by bacteria starts soon after birth and environmental and nutritional factor play an important role in determining the type and extent of colonization<sup>13</sup>. Nutritional status also facilitates the first and second stage methylation and excretion of inorganic arsenic<sup>14</sup>, some nutrients such as vitamin E<sup>15</sup>, folic acid<sup>16</sup>, selenium<sup>17</sup>, B<sub>6</sub>, B<sub>12</sub>, zinc<sup>18</sup> play an important role in this aspect. Human beings are being chronically exposed to inorganic arsenic through food and drinking water, a major cause of disease and disability. This study was carried out to assess the important role of aerobic gut flora in elimination of arsenic from the body.

### Materials and Methods

**Animals:** Healthy young adult male rats of Long Evans Norwegian Strains, weighing 160-180 g and 3-4 months old were taken for the purpose of study, they were kept in stainless steel cages in animal house. Saw dust was used as bedding and changed every alternate day, a 12 hours light/12 hours dark cycle was maintained. They were fed standard pellet diets and allowed to drink *ad libitum*.

A total of 108 rats were used in this study and divided into three major. Rats in Group B (n=54) were orally pretreated with streptomycin (500 mg) twice daily and complete inhibition of gut flora was ensured by culture in MacConkeys agar. Rats in Group C (n=6) received streptomycin throughout the study period along with arsenic in drinking water 1 mg/L, rats in Group A (n=48) did not receive any streptomycin.

Rats in Group A and Group B were divided into smaller groups of 6 and they were treated with vitamin E (1 mg/day), selenium (0.4 µg/day) and folic acid (200 µg/day), orally through ryles tube. Each half of the rats from both these groups received arsenic (1 mg/L) *ad libitum* in drinking water.

**Stool specimen collection, dilution and culture:** Fecal pellets were collected in clean, sterile glass container as soon as they were passed by the animal. A portion of fresh stool specimen was taken in sterile labeled test tubes containing 1 mL of normal saline, it was vortex and centrifuged at 1600 g, the supernatant was decanted and serial

dilution (1-5) was carried out in sterile test-tubes containing 1 mL of normal saline. From the fifth test-tube 10 µL of specimen was taken and cultured in MacConkey's agar at 37°C for 24 hours for colony count. Stool culture was performed on day 0, day 7 and day 14 of administering drugs.

*Estimation of arsenic in stool and liver:* Stool arsenic estimation was performed simultaneously on remaining portion of fecal pellets. Animals were sacrificed under light chloroform anesthesia on day 14. Liver was taken out and the amount of total arsenic was estimated. Both stool and liver arsenic estimation was done by silver diethyl dithiocarbamate method (SDDC).

## Results

The mean gut aerobic bacterial count in streptomycin untreated and pretreated control rats were 6.91 to 7.26x10<sup>8</sup> cfu/g dry weight of stool (Table I). In case of streptomycin untreated rats

receiving arsenic for 2 weeks, the mean gut aerobic bacterial count decreased to 3.51x10<sup>8</sup> cfu/g (48.9% inhibition). This inhibitory effect of arsenic on gut flora was not observed when co-administered with either vitamin E or selenium. Folic acid alone or with arsenic decreased the bacterial count significantly.

Pretreatment of rats with streptomycin (1 g/day) for three days caused reduction of aerobic gut bacteria to not countable level. Subsequently withdraw of streptomycin lead to increased bacterial count to normal level within the next two days. But if streptomycin administration was continued for the whole experimental period, the number of bacterial count was not detectable.

The inhibitory effect of arsenic on bacterial count in streptomycin pretreated rat was apparent on day 14. Vitamin E and selenium reverses the inhibitory effect of arsenic. Folic acid did not play role like vitamin E or selenium. Instead, it enhanced the inhibitory effect of arsenic.

**Table I:** Effect of arsenic, vitamin E, selenium and folic acid on gut bacterial count in rats

	Bacterial count (cfu/g dry weight of stool)		
	Day 0	Day 7	Day 14
<i>Streptomycin untreated group</i>			
Control	6.91 × 10 <sup>8</sup> ± 0.45 × 10 <sup>8</sup>	6.94 × 10 <sup>8</sup> ± 0.52 × 10 <sup>8</sup>	7.17 × 10 <sup>8</sup> ± 0.34 × 10 <sup>8</sup>
Arсенic (1 mg/L)	6.82 × 10 <sup>8</sup> ± 0.50 × 10 <sup>8</sup>	7.25 × 10 <sup>8</sup> ± 0.83 × 10 <sup>8</sup>	3.51 × 10 <sup>8</sup> ± 1.77 × 10 <sup>8</sup>
Arсенic (1 mg/L) + Vitamin E (1 mg/day)	6.96 × 10 <sup>8</sup> ± 0.40 × 10 <sup>8</sup>	7.11 × 10 <sup>8</sup> ± 0.41 × 10 <sup>8</sup>	6.74 × 10 <sup>8</sup> ± 0.47 × 10 <sup>8</sup>
Arсенic (1 mg/L) + Folic acid (200 µg/day)	7.22 × 10 <sup>8</sup> ± 0.50 × 10 <sup>8</sup>	4.35 × 10 <sup>8</sup> ± 0.60 × 10 <sup>8</sup>	2.57 × 10 <sup>8</sup> ± 0.84 × 10 <sup>8</sup>
Arсенic (1 mg/L) + Selenium (0.4 µg/day)	6.73 × 10 <sup>8</sup> ± 0.58 × 10 <sup>8</sup>	7.52 × 10 <sup>8</sup> ± 1.23 × 10 <sup>8</sup>	7.25 × 10 <sup>8</sup> ± 0.93 × 10 <sup>8</sup>
Vitamin E (1 mg/day)	6.92 × 10 <sup>8</sup> ± 0.48 × 10 <sup>8</sup>	7.47 × 10 <sup>8</sup> ± 0.62 × 10 <sup>8</sup>	4.41 × 10 <sup>8</sup> ± 1.87 × 10 <sup>8</sup>
Folic acid (200 µg/day)	6.91 × 10 <sup>8</sup> ± 0.46 × 10 <sup>8</sup>	5.26 × 10 <sup>8</sup> ± 0.97 × 10 <sup>8</sup>	3.85 × 10 <sup>8</sup> ± 1.46 × 10 <sup>8</sup>
Selenium (0.4 µg/day)	6.79 × 10 <sup>8</sup> ± 0.57 × 10 <sup>8</sup>	6.06 × 10 <sup>8</sup> ± 0.95 × 10 <sup>8</sup>	5.85 × 10 <sup>8</sup> ± 0.79 × 10 <sup>8</sup>
<i>Streptomycin pretreated group</i>			
Control (streptomycin 1 g/day for first 3 days)	7.13 × 10 <sup>8</sup> ± 0.49 × 10 <sup>8</sup>	7.19 × 10 <sup>8</sup> ± 1.06 × 10 <sup>8</sup>	7.26 × 10 <sup>8</sup> ± 0.62 × 10 <sup>8</sup>
Streptomycin (1 g/day for 14 days)	7.08 × 10 <sup>8</sup> ± 0.54 × 10 <sup>8</sup>	Not countable	Not countable
Arсенic (1 mg/L)	7.01 × 10 <sup>8</sup> ± 0.56 × 10 <sup>8</sup>	7.74 × 10 <sup>8</sup> ± 1.33 × 10 <sup>8</sup>	2.83 × 10 <sup>8</sup> ± 3.13 × 10 <sup>8</sup>
Arсенic (1 mg/L) + Streptomycin (1 g/day for 14 days)	6.60 × 10 <sup>8</sup> ± 0.31 × 10 <sup>8</sup>	Not countable	Not countable
Arсенic (1 mg/L) + Vitamin E (1 mg/day) + Streptomycin 1 g/day for first 3 days	6.69 × 10 <sup>8</sup> ± 0.64 × 10 <sup>8</sup>	7.72 × 10 <sup>8</sup> ± 1.12 × 10 <sup>8</sup>	8.96 × 10 <sup>8</sup> ± 1.36 × 10 <sup>8</sup>
Arсенic (1 mg/L) + Folic acid (200 µg/day) + Streptomycin 1 g/day for first 3 days	7.13 × 10 <sup>8</sup> ± 0.62 × 10 <sup>8</sup>	4.03 × 10 <sup>8</sup> ± 1.02 × 10 <sup>8</sup>	1.98 × 10 <sup>8</sup> ± 0.77 × 10 <sup>8</sup>
Arсенic (1 mg/L) + Selenium (0.4 µg/day) + Streptomycin 1 g/day for first 3 days	6.69 × 10 <sup>8</sup> ± 0.41 × 10 <sup>8</sup>	6.94 × 10 <sup>8</sup> ± 0.28 × 10 <sup>8</sup>	7.35 × 10 <sup>8</sup> ± 0.33 × 10 <sup>8</sup>
Vitamin E (1 mg/day) + Streptomycin 1 g/day for first 3 days	6.90 × 10 <sup>8</sup> ± 0.59 × 10 <sup>8</sup>	6.92 × 10 <sup>8</sup> ± 1.07 × 10 <sup>8</sup>	3.48 × 10 <sup>8</sup> ± 1.37 × 10 <sup>8</sup>
Folic acid (200 µg/day) + Streptomycin 1 g/day for first 3 days	6.70 × 10 <sup>8</sup> ± 0.33 × 10 <sup>8</sup>	5.12 × 10 <sup>8</sup> ± 1.20 × 10 <sup>8</sup>	2.79 × 10 <sup>8</sup> ± 0.89 × 10 <sup>8</sup>
Selenium (0.4 µg/day) + Streptomycin 1 g/day for first 3 days	6.53 × 10 <sup>8</sup> ± 0.39 × 10 <sup>8</sup>	6.13 × 10 <sup>8</sup> ± 0.69 × 10 <sup>8</sup>	6.21 × 10 <sup>8</sup> ± 0.74 × 10 <sup>8</sup>

Control group received standard diet and drinking water *ad libitum*. Arsenic was administered to different group in drinking water *ad libitum*. Ryles tube feeding was given for streptomycin, vitamin E, folic acid and selenium. Streptomycin was given in divided dose (500 mg) twice daily. All the groups were treated for 14 days. Each group had six rats. Stool culture was done in Mac Conkey's agar. Values are mean ± SD.

The mean stool arsenic level in control rats (streptomycin untreated and pretreated) ranged from 3.55 and 3.63 mg/g dry weight of stool (Table II). On day 14, it decreased significantly in both the group of rats that received only arsenic. In streptomycin untreated group stool arsenic level decreased to 2.79 mg/g dry weight of stool, decreased by 26.14% and in streptomycin pretreated group it decreased to 2.39 mg/g dry weight of stool. Stool arsenic level did not change in streptomycin untreated group, in rats that received arsenic along with vitamin E 2.84 mg/g dry weight of stool, but it increased considerably in streptomycin pretreated group of rats that received arsenic along with vitamin E 5.61 mg/g dry weight of stool, increased by 57.32%. An increase in stool arsenic level was observed in both groups on day 7

and 14 in rats that received arsenic along with selenium. In streptomycin untreated group on day 7 it was 4.49 mg/g dry weight of stool and on day 14 it was 4.58 mg/g dry weight of stool, both increased by 1.96%. In streptomycin pretreated group on day 7 it was 4.01 mg/g dry weight of stool, increased by 18.63% and on day 14 it was 4.04 mg/g dry weight of stool, increased by 19.70%. Decreased stool arsenic level was observed in both the groups in rats that received arsenic along with folic acid. In streptomycin untreated group it was 2.67 mg/g dry weight of stool, decreased by 24.61% and in streptomycin pretreated group it was 2.53 mg/g dry weight of stool, decreased by 22.01%.

Liver arsenic level in control group of rats in streptomycin untreated group was  $2.84 \pm 0.22$  mg/g of liver tissue and in streptomycin pretreated group

**Table II:** Stool and liver arsenic concentration following administration of arsenic, vitamin E, selenium and folic acid

	Amount of arsenic			
	Stool mg/g dry weight			Liver tissue mg/g
	Day 0	Day 7	Day 14	Day 14
<i>Streptomycin untreated group</i>				
Control	3.55 ± 0.34	3.25 ± 0.52	3.30 ± 0.32	2.84 ± 0.22
Arsenic (1 mg/L)	3.88 ± 0.48	3.55 ± 0.80	2.79 ± 0.54	3.57 ± 0.46
Arsenic (1 mg/L) + Vitamin E (1 mg/day)	3.80 ± 0.73	3.59 ± 0.86	2.84 ± 0.57	2.66 ± 0.51
Arsenic (1 mg/L) + Folic acid (200 µg/day)	3.55 ± 0.37	3.54 ± 0.35	2.67 ± 0.29	2.20 ± 0.33
Arsenic (1 mg/L) + Selenium (0.4 µg/day)	3.86 ± 0.65	4.49 ± 1.29	4.58 ± 1.29	2.54 ± 0.34
Vitamin E (1 mg/day)	3.40 ± 0.81	3.96 ± 0.91	3.40 ± 0.41	2.52 ± 0.43
Folic acid (200 µg/day)	3.42 ± 0.39	2.93 ± 0.47	2.15 ± 0.17	2.08 ± 0.47
Selenium (0.4 µg/day)	3.53 ± 0.37	3.48 ± 0.34	4.22 ± 0.46	2.54 ± 0.27
<i>Streptomycin pretreated group</i>				
Control (streptomycin 1 g/day for first 3 days)	3.38 ± 0.63	3.74 ± 0.67	3.63 ± 0.79	3.33 ± 0.92
Streptomycin (1 g/day for 14 days)	3.56 ± 0.34	3.31 ± 0.52	3.72 ± 0.67	3.36 ± 0.21
Arsenic (1 mg/L)	3.33 ± 0.68	3.31 ± 0.72	2.39 ± 0.27	5.40 ± 0.71
Arsenic (1 mg/L) + Streptomycin (1 g/day for 14 days)	3.43 ± 0.36	2.58 ± 0.24	2.52 ± 0.37	5.78 ± 0.46
Arsenic (1 mg/L) + Vitamin E (1 mg/day) + (streptomycin 1 g/day for first 3 days)	3.58 ± 0.51	4.01 ± 0.65	5.61 ± 0.85	4.10 ± 0.84
Arsenic (1 mg/L) + Folic acid (200 µg/day) + (streptomycin 1 g/day for first 3 days)	3.25 ± 0.26	3.09 ± 0.34	2.53 ± 0.34	2.10 ± 0.59
Arsenic (1 mg/L) + Selenium (0.4 µg/day) + (streptomycin 1 g/day for first 3 days)	3.38 ± 0.21	4.01 ± 0.51	4.04 ± 0.51	3.45 ± 0.34
Vitamin E (1 mg/day) + (streptomycin 1 g/day for first 3 days)	3.59 ± 0.50	3.61 ± 0.48	3.75 ± 0.32	4.14 ± 0.48
Folic acid (200 µg/day) + (streptomycin 1 g/day for first 3 days)	3.35 ± 0.40	1.76 ± 0.46	2.10 ± 0.40	2.27 ± 0.21
Selenium (0.4 µg/day) + (streptomycin 1 g/day for first 3 days)	3.10 ± 0.45	3.08 ± 0.46	4.30 ± 1.14	3.28 ± 0.73

Control group received standard diet and drinking water *ad libitum*. Arsenic was administered with drinking water *ad libitum*. Streptomycin, vitamin E, folic acid and selenium was given orally through ryles tube. Streptomycin was given in a divided dose (500 mg) twice daily. Arsenic was administered to different group in drinking water. All the groups were treated for 14 days. Each group had six rats. Stool and liver arsenic estimation was done by SDDC method. Values are mean ± SD.

it was  $3.33 \pm 0.92$  mg/g of liver tissue (Table II). An increase in liver tissue arsenic level was observed in both the groups in rats that received only arsenic. In streptomycin untreated group liver tissue arsenic level increased to  $3.57 \pm 0.46$  mg/g of liver tissue, increased by 27.50% and in streptomycin pretreated group it increased to  $5.40 \pm 0.71$  mg/g, increased by 62.16%. No change in liver tissue arsenic level was observed in rats that received either vitamin or selenium along with arsenic in both groups. However a significant decrease in liver tissue arsenic level was observed in both the groups in rats that received arsenic along with folic acid. In streptomycin untreated group, rats that received arsenic with folic acid liver tissue arsenic level was  $2.20 \pm 0.33$  mg/g of liver tissue, decreased by 22.54% and in streptomycin pretreated group it was  $2.10 \pm 0.40$  mg/g of liver tissue, decreased by 36.94%.

## Discussion

The rats that were treated with only arsenic throughout the study period showed a significant inhibition of gut bacterial count on day 14 in both streptomycin untreated and pretreated groups. Gut bacteria play an important role in metabolic, tropic and protective functions and play a vital role in bodies immunity<sup>12</sup>. Studies carried out suggest that bacteria play a role in arsenic detoxification<sup>24</sup>. Rats that received only arsenic a decrease in stool and an increase in liver arsenic level was also observed, this might be attributed to inhibition of gut bacterial count resulting in decreased bacterial detoxification of arsenic by gut bacteria and its increased deposition in liver. Microbes have been shown to reduce a wide range of toxic metals through detoxification and elimination<sup>13</sup>.

Inhibition of gut bacterial count was also observed in rats that received folic acid along with arsenic. However the nature of inhibition was different. Here inhibition was observed on both day 7 and day 14 and the extent of inhibition was more profound. This might be the result of combined inhibitory effect of arsenic and folic acid on gut flora, as studies show that folic acid also exerts an inhibitory effect on enteric flora<sup>26</sup>. Both liver and stool arsenic level decreased in this group. Rats that received only arsenic decrease in gut bacterial count was associated with an increase in liver arsenic level, decrease in liver arsenic level in this group of rats might be due to folic acid supplementation. Folic acid plays a role in hepatic methylation of arsenic. Several studies both animal and human trials have confirmed the involvement of folic acid in hepatic methylation of arsenic<sup>21,22</sup>.

Increased bacterial multiplication is associated with increased bacterial activity, in addition intestinal bacteria contain high amount of glutathione<sup>13</sup> which efficiently reduces toxic substances. Streptomycin pretreated group of rats that received vitamin E along with arsenic a marked increase in gut bacterial count was observed and there was also an increase in stool level, suggestive of increased bacterial activity and arsenic detoxification.

Nutrition plays a role in bacterial growth and multiplication<sup>27</sup> and observation in laboratory animals deprived of one or more dietary elements have confirmed the crucial role of vitamin E and selenium in arsenic metabolism<sup>28</sup>. In this study when vitamin E and selenium were used along with arsenic, inhibitory effect of arsenic on aerobic gut flora was not observed, instead in one of vitamin E treated group an increase in aerobic gut bacterial count was seen. So, it can be assumed that vitamin E and selenium are able to overcome the inhibitory effect of arsenic on aerobic gut flora.

The rats that received selenium along with arsenic an increase in stool arsenic level was observed on both day 7 and day 14. Increase in stool arsenic in this group of rat might either be due to gut bacteria, arsenic and selenium being chemically similar ions, compete for sites of reduction in bacteria leading to increased arsenic in stool<sup>8</sup> or alternately increased arsenic metabolism by selenium and hepatobiliary excretion<sup>8</sup>.

In this study isolation and identification of organisms, or any change in morphology or bacterial secretions in bacteria multiplying in arsenic stressed environment was not carried out, so more extensive future studies are needed.

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