Synergistic Effects of Vitamin A and Spirulina on Arsenic Load in Rat Tissues and Blood

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ABSTRACT

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Arsenic (As) is found in contaminated ground water as the source of pollution. In this study, 60 Long-Evans rats were used to assess the levels of As in the blood and organs and to compare the effectiveness of vitamin A and spirulina (Spirulina platensis) in preventing a chronic As accumulation. Twelve rats were assigned to each group of animals. The experimental groups were the control (T0), As (T1), As + spirulina (T2), As + vitamin A (T3), and As + spirulina + vitamin A (T4). The T1, T2, T3, and T4 groups were orally administered with sodium arsenite (NaAsO²⁻) @ 4 mg/kg body weight (BW) for 63 days. In addition to NaAsO₂, the T2 and T4 received 1 g/kg BW spirulina. The T3 and T4 received 2500 IU/kg BW vitamin A for 63 days, respectively. Four rats were euthanized in each group to evaluate the As concentration in the liver, lung, kidney, and blood at an interval of 21 days. Total As concentration was quantified from the organs using Hydride Generation Atomic Absorption Spectrophotometer (HG-AAS). The results revealed that the T0 had no visible clinical symptoms. However, after 63 days of treatment, the T1 (As only administration) accumulated more As compared to other groups. The concentration of As was highest in the blood, then in the kidney, liver, and lung. In this case, spirulina and vitamin A substantially (p<0.01) decreased the concentration of As in the rats' organs and tissues. Spirulina is more effective than vitamin A in reducing As accumulation in rats. In summary, the combination of both spirulina and vitamin A has a positive impact on reducing the accumulation of chronic arsenicosis in rats compared to the individual administration of either spirulina or vitamin A alone.


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INTRODUCTION

Among the most critical environmental concerns in Bangladesh is arsenic poisoning, which is also a significant health hazard in Asia. It gives rise to a significant public health concern in developing nations. Arsenic has the potential to infiltrate the food chain, resulting in its extensive distribution across various plant and animal kingdoms. Arsenic is an abundantly present lustrous grayish non-essential trace element in the natural environment. The toxicity level of arsenic is greatest in its inorganic forms (FAO, 1983, Khatun et al., 2020). Arsenic is one of the most pervasive and highly toxic metalloids found in the environment. On a global scale, millions of individuals are coming into contact with inorganic arsenic via contaminated food and potable water (Silbergeld et al., 2008). Arsenic (As), a metalloid, is prevalent in various forms (organic and inorganic) found in soil and water across the globe, with Bangladesh, India, and several other Southeast Asian countries being particularly susceptible (Bhattacharya et al., 2009). The Bangladeshi government has established a safety limit of 0.05 mg/liter for arsenic in potable water (WHO, 1999). According to Robinson et al., (2003), the World Health Organization (WHO) establishes limits of 0.01 mg/liter for potable water and 2 mg/liter for far foodstuffs when measured by fresh weight. Arsenic has emerged as a significant public health concern in several developing nations (Rahman, 2006), where inorganic arsenic (As) has contaminated potable water. At the present time, chronic arsenic toxicity is a worldwide health concern (Yoshida et al., 2004). Furthermore, it poses a significant health concern in Bangladesh and surrounding regions as well (Khatun et al., 2020).

Inorganic arsenic (As) is found in drinking water in most developing countries including Bangladesh. It is harmful to human health (Ahmad et al., 2023; Noh CH et al., 2023). Serious health issues like cancer, hyperkeratosis, lung, and heart disease can be brought on by it (Khan et al., 2022; Sinha et al., 2020). There is no specific drug that can cure arsenicosis. The mainstay of therapy is the immediate discontinuation of drinking water containing As and switching to As-free drinking water (Pandey et al., 2020). It is recommended to improve the nutritional status and use chelating agents (Björklund et al., 2020; Kalia et al., 2005; Mitra et al., 2004; Milton et al., 2004). However, it cannot be denied that chelating chemicals play a role in chronic As poisoning (Guha majumdar et al., 1998). In 1998 Ahmed studied that As-induced melanosis and keratosis could be improved with vitamin A, C and E diet and As-free water. Spirulina was found to be helpful in reducing chronic As toxicity in goats (Ghosh et al., 2014). Spirulina (Spirulina platensis) is a blue-green alga that has been shown to reduce the accumulation of harmful metals from tissues, including mercury (El-Desoky et al., 2013). It is proved that spirulina alone or in combination with other minerals and/or vitamins can remove As from As-containing tissues of many species, including humans (Khatun et al., 2020; Awal, 2007; Misbahuddin, 2006; Fariduddin, 2001; Khan, 2001; Karim, 1999).

The broad term “vitamin A” is used in several medical contexts. Gene transcription, vision, immunological response, reproduction and fetal development, bone metabolism, hemopoiesis, skin health, reduced risk of heart disease, antioxidant activity and many other processes all depend on vitamin A (O’Connor et al., 2022) Vitamin A, an antioxidant, is very important in the treatment of poisoning (Talukdar, 1999). Arsenicosis can still result from prolonged exposure to water containing arsenic concentrations as low as 0.00017 mg/L (0.17 ppb), according to more recent research (WHO, 2001). It has been observed that the removal of arsenic from arsenic-loaded tissues in numerous species, including humans, is efficacious when utilized alone or in combination with vitamins and/or minerals (Misbahuddin et al., 2006; Awal, 2007). In 1946 Hall and others first demonstrated the therapeutic effect of oral vitamin A supplementation in the management of cutaneous arsenicosis. Ahmad et al., (1998) documented its application as an oral supplement in conjunction with vitamin A (retinol) for the management of cutaneous arsenicosis. Spirulina supplementation is said to offer a defense against arsenic-induced poisoning in goats (Ghosh et al., 2014). When arsenic poisoning occurs in ducks, spirulina helps with toxic symptoms, body weight, and hematological parameters (Islam et al., 2009).

In the context of Bangladesh, comprehensive data on arsenic contamination is primarily available for tube well water. However, there is limited evidence regarding particular interventions aimed at mitigating arsenic poisoning in both human and animal populations. Therefore, it is anticipated that there will be a generation of fresh data regarding the relative effectiveness of vitamin A as well as spirulina in the prevention of arsenicosis, particularly in regards to Bangladesh and other regions globally. Therefore, based on the aforementioned information, this study aimed to conduct a quantitative evaluation of the overall levels As in the lungs, liver, kidney, and blood of rats that have been subjected to As exposure. In addition, the impact of As, vitamin A, and spirulina on alterations in body weight and different organs in rats subjected to As consumption was also investigated.
MATERIALS AND METHODS

Animals and Experimental design

In this experiment, about six months of age 60 Long-Evans rats were used for 63 days. At first all the rats were randomized, divided into 5 groups (N=12) and were identified as T0 for control group and reared with only ad libitum normal feed and water, T1 for As group were treated with sodium arsenite (NaAsO2:197.84g/mol MW, May & Baker Ltd, Dagenham, England) at 4mg/kg body weight (BW) in drinking water daily, T2 for As plus Spirulina group were treated with same doses of As of T1 group daily and also added Spirulina (Spirulina platensis) (Tab. Spirulina®; Life Line International Company, Bangladesh) at a dose of 1 g/kg feed (Khatun et al., 2020), T3 for As plus Vitamin A group were treated with same doses of As of T1 group daily and also added Vitamin A (Capsule Retinol forte; Drug international limited; Tongi Gazipur; Bangladesh) simultaneously at a dose of 2500 IU/kg feed (Hossain et al., 2013) and lastly T4 for As plus Vitamin A and Spirulina group were treated with same doses of T1 group's As with Vitamin A at 2500 IU/kg feed and Spirulina (Spirulina platensis) at 1 g/kg feed. The sodium arsenite doses were chosen based on our preliminary study and also reference article (Hossain et al., 2013). All treatments were given for 63 days (Hossain et al., 2013) because the experimental trial was conducted for 63 days.

Body weight (BW)

After grouping and marking, rats were individually weighed and the results were recorded on day 0 which means the day immediately before starting treatment, day 21, 42 and 63 finally.

Clinical signs

Throughout the whole study period (from Day 1 to Day 63), rats were closely observed three times each day (morning, afternoon, and evening) for any clinical indications that may have appeared. The results were then recorded.

Treatment materials preparation

Solution of sodium arsenite

At the dose of 4 mg/kg BW of NaAsO2 (NaAsO2:197.84g/mol MW, May & Baker Ltd, Dagenham, England) was weighted for a day for each group of rats and was usually mixed with 10 ml of drinking water so that each group of rats consumed the entire dose. Normal drinking water was added as needed after ingestion of NaAsO2 diluted water.

Preparation of spirulina powder mixed feed

A homogenous powder was made from each spirulina tablet (Tab. Spirulina®; Life Line International Company, Bangladesh) containing 500 mg of Spirulina platensis, and the appropriate dosage was determined using an electric balance and then a small amount of distill water was added drop by drop to make it suspension was introduced to the feed for homogenous mixing. After mixing, the feed was dried for 24 hours at 50°C in an electric oven and then stored in an airtight plastic bag.

Vitamin A mixed feed

A mixture of 2 kg dried pellet feed was prepared with 50,000 I.U. vitamin A capsule (Capsule Retinol forte; Drug international limited; Tongi Gazipur; Bangladesh). A little amount of distill water was added drop by drop to the capsule to make it emulsion was introduced to the feed for homogenous mixing. After Mixing, feed was dried and then stored in an airtight plastic container.

Sampling

4 (Four) rats from each group were sacrificed after 21 days interval (Day 21, 42 and 63) and minimum 5ml of blood were taken from the heart of each rat to measure the concentration of blood arsenic level. Total liver, kidney and lungs samples were taken aseptically, cleaned with physiological saline and stored in a zippered polythene bag with pre-marked labels. All tissue and blood samples were preserved at -20°C until testing in order to detect arsenic.
Digestion of organ sample (Lung, liver and kidney)

Concentrated Nitric and Perchloric acid in a ratio of 5:3 (Nitric acid: Perchloric acid) were used for organ digestion (Uddin et al., 2016). Briefly each sample was placed individually in each digestion tube. Added 5 ml of digestion acid mixture and then heated at 120 °C until a clear solution appeared. After digestion, the tubes were allowed to cool and made up to a volume of 50 ml using filter paper (Whatman 42) and stored in polypropylene vials until As is determined.

Digestion of blood sample

For analysis, 5 ml of concentrated nitric acid was added to a blood sample that was stored previously at 4 °C for analysis. To facilitate the digestion of the blood samples, a microwave was employed. To initiate digestion of the samples, 4.0 ml of the sample as well as 10.0 ml of a mixture containing concentrated hydrochloric acid and nitric acid in (5 ml conc. HCl +5 ml conc. HNO3) were put into a 125 ml vial that can withstand pressure. The samples underwent digestion for a duration of 4 minutes at a power level of 300 watts. Once a colorless solution was obtained, digestion was halted and the substance was evaporated to dryness. Deionized water was used to dilute the solution to a volume of 25.0 ml (Sani and Abdullahi, 2017)

Determination of arsenic concentration

The calibration curve was constructed using standard solutions of As in Atomic Absorption Spectrometry (AAS). The As samples were made by diluting a stock solution of 1 g L−1 with distilled water.

Atomization atomic absorption spectrometry analysis

Arsenic was identified utilizing the Hydride Generation Atomic Absorption Spectrophotometer (HG-AAS; PG-990, PG Instruments Ltd. UK). This method operates on the principle that an acidified sample reacts with sodium borohydride (NaBH4) to produce a hydride analyte. By completely separating the analyte from the matrix prior to measurement, matrix interference is significantly diminished. Pre-reduction of standards and samples from an arsenate pentavalent (V) to an arsenite trivalent (III) state constituted this method. A reducing solution comprising 5% (w/v) KI, 5% (w/v) ascorbic acid, and 10% HCl was utilized to accomplish this. Before analysis, the treated samples as well as standards were left undisturbed at room temperature for an estimated duration of 40 minutes. We used a type of EDL lamp with a wavelength of 193.7 nm, a slit width of 0.7 nm, and an atomization temperature of 900 °C (Uddin et al., 2016).

Statistical analysis

Data were designed in CRD and statistically analyzed with the software SPSS 11.5 using one-way ANOVA. Duncan's multiple range test (DMRT) was used to differentiate mean values between treatments (Steele & Torrey, 1980).

RESULTS

Clinical signs

Throughout the whole investigation, trial rats exhibited no clinical symptoms of As toxicity.

Body weight (BW) of the rats

In Table-1, T4 group showed highest BW and T1 group showed lowest on Day 0 but the differences were not significant. On Day 21, 42 and 63 highest BWs were found in control (T0) group and lowest in T1 group rats. But no significant variance was seen among the rats of different groups on Day 21, 42 and 63 in BWs. But in As group (T1) body weight decreased day by day. In T3 and T4 group BWs were decreased up to day 42 from day 0 and then increased on day 63 but in T2 group it fluctuated.
Table 1. Changes of body weight (g) of rats at different days

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day-0</td>
</tr>
<tr>
<td>Control (T0)</td>
<td>210.3±6.0</td>
</tr>
<tr>
<td>Arsenic (T1)</td>
<td>194.3±8.1</td>
</tr>
<tr>
<td>Spirulina+ Arsenic (T2)</td>
<td>200.4±8.1</td>
</tr>
<tr>
<td>Arsenic + Vitamin A (T3)</td>
<td>199.2±8.3</td>
</tr>
<tr>
<td>Arsenic+ Spirulina+Vitamin A (T4)</td>
<td>212.7±5.6</td>
</tr>
</tbody>
</table>

Arsenic load in organs

As concentration in lung: On Day 21, 42 and 63 T1 group showed highest concentration of As in lung and T0 group showed lowest. Compared to control group, As concentration significantly (p<0.01) increased in all treated groups. On Day 21, T1 group was significantly (p<0.01) different when compared to T4 group, i.e., the values of T2 and T3 were in between T1 and T4 groups. On Day 42, As contents were decreased in T2 and T4 group and increased in T1 and T3 group compared to day 21. On day 63, compared to day 42, all treated group showed increasing As concentration except T3 was decreased (Figure 1 and Table 2).

Table 2. Different treatment effect in rats on Arsenic content of lung (ppm)

<table>
<thead>
<tr>
<th>Treatment (Mean±SE)</th>
<th>Arsenic concentration in lung (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day-21</td>
</tr>
<tr>
<td>Control (T0)</td>
<td>0.41±0.05</td>
</tr>
<tr>
<td>Arsenic (T1)</td>
<td>19.70±3.41</td>
</tr>
<tr>
<td>Spirulina+ Arsenic (T2)</td>
<td>16.77±2.47</td>
</tr>
<tr>
<td>Arsenic + Vitamin A (T3)</td>
<td>17.08±0.17</td>
</tr>
<tr>
<td>Arsenic+ Spirulina+ Vitamin A (T4)</td>
<td>15.73±0.81</td>
</tr>
</tbody>
</table>

Significance level: **Highly significant (p<0.01); Figures with similar superscripts mean did not differ significantly among respective figures, but figures with dissimilar superscripts mean differed significantly as per DMRT

**Figure 1. Effect of different treatment on As concentration in lungs (ppm) at different days**
As concentration in liver: On Day 21, As contents were increased in T1 groups up to 63 days. On day 42, T3 group showed increasing As concentration and T2 & T4 group showed decreasing concentration compared to day 21 and 63. On Day 63, T1 group was statistically significant (p<0.01) compared to T2 and T4 group (Figure 2 and Table 3).

Table 3. Different treatment effect in rats on Arsenic content of liver (ppm)

<table>
<thead>
<tr>
<th>Treatment (Mean±SE)</th>
<th>Arsenic concentration in liver (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day-21</td>
</tr>
<tr>
<td>Control (T0)</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Arsenic (T1)</td>
<td>5.84±0.20</td>
</tr>
<tr>
<td>Spirulina+ Arsenic (T2)</td>
<td>4.63±0.61</td>
</tr>
<tr>
<td>Arsenic + Vitamin A (T3)</td>
<td>5.10±1.14</td>
</tr>
<tr>
<td>Arsenic+ Spirulina+ Vitamin A (T4)</td>
<td>4.50±0.16</td>
</tr>
</tbody>
</table>

** Highly significant (p<0.01); Figures with similar superscripts mean did not differ significantly among respective figures, but figures with dissimilar superscripts mean differed significantly as per DMRT

Figure 2. Effect of different treatment on As concentration in liver (ppm) at different days

As concentration in kidneys: On Day 21, T1 and T3 groups showed increasing As concentration and T2 and T4 groups showed decreasing concentration. On day 42, As

As content in kidneys: On Day 21, T1 and T3 groups showed increasing As concentration and T2 and T4 groups showed decreasing concentration. On day 42, As concentration was increased in all treated group compared to day 21. On day 63, T1, T2 and T4 groups showed increasing As concentration and T3 groups showed decreasing concentration compared to day 42 (Figure 3 and Table 4).
Table 4. Different treatment effect in rats on Arsenic content of kidney (ppm)

<table>
<thead>
<tr>
<th>Treatment (Mean±SE)</th>
<th>Arsenic concentration in kidney (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day-21</td>
</tr>
<tr>
<td>Control (T0)</td>
<td>1.06±0.45</td>
</tr>
<tr>
<td>Arsenic (T1)</td>
<td>27.03±5.84</td>
</tr>
<tr>
<td>Spirulina+ Arsenic (T2)</td>
<td>16.71±3.03</td>
</tr>
<tr>
<td>Arsenic + Vitamin A (T3)</td>
<td>26.82±3.37</td>
</tr>
<tr>
<td>Arsenic+ Spirulina+ Vitamin A (T4)</td>
<td>9.36±1.09</td>
</tr>
</tbody>
</table>

Significance level

**Highly significant (p<0.01); Figures with similar superscripts mean did not differ significantly among respective figures, but figures with dissimilar superscripts mean differed significantly as per DMRT.

Figure 3. Effect of different treatment on As concentration in kidney (ppm) at different days

As concentration in Blood: On Day 21, T1 group showed highest As concentration in blood and T0 (control) group showed lowest concentration. On day 42, all treated groups showed increasing concentration compared to day 21. On day 63, T1 group showed increasing As concentration and T2, T3 and T4 group showed decreasing As concentration compared to day 42 (Figure 4 and Table 5).

Table 5. Different treatment effect in rats on Arsenic content of Blood (ppm)

<table>
<thead>
<tr>
<th>Treatment (Mean±SE)</th>
<th>Arsenic concentration in Blood (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day-21</td>
</tr>
<tr>
<td>Control (T0)</td>
<td>2.13±0.64</td>
</tr>
<tr>
<td>Arsenic (T1)</td>
<td>110.82±4.91</td>
</tr>
<tr>
<td>Spirulina+ Arsenic (T2)</td>
<td>97.71±6.22</td>
</tr>
<tr>
<td>Arsenic + Vitamin A (T3)</td>
<td>99.78±5.49</td>
</tr>
<tr>
<td>Arsenic+ Spirulina+ Vitamin A (T4)</td>
<td>92.11±13.64</td>
</tr>
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</table>

Significance level

**Highly significant (p<0.01); Figures with similar superscripts mean did not differ significantly among respective figures, but figures with dissimilar superscripts mean differed significantly as per DMRT.
DISCUSSION

Following the induction of arsenic poisoning, the concentrations of arsenic in the lungs, liver, kidneys, as well as blood of rats were shown to increase. However, it was found that therapy with Spirulina supplemented with vitamin A resulted in a reduction in arsenic accumulation in the organs and blood of the rats.

In the present study, it was observed that none of the rat groups exhibited any clinical signs, symptoms, or lesions during the duration of the examination. However, a majority of the groups did experience a minor elevation in body weight. The group labeled as "T1" exhibited the lowest BW in comparison to the remaining groups, as indicated in Table 1. Nevertheless, the observed alterations were not deemed to be substantial.

The levels of As in the lungs, liver, kidneys, and blood were found to be significantly higher (p<0.01) in the As group (T1) compared to the control group (T0) of rats after being administered NaAsO2 at a dosage of 4 mg/kg body weight. The investigations conducted by Kamaludin and Misbahuddin (2006) as well as Nasir et al. (2002) have indicated that there is a positive correlation between the increase in exposure time and the concentration of As. The researchers provided evidence to support the notion that administering varying doses of As to rats over varied durations results in a notable elevation in As levels.

The results of our investigation indicate that the blood samples had the highest quantity of As when compared to the kidney, lung, and liver samples. The results of this study presented a contradiction to the findings of Marafante (1982), who observed that the spleen had the highest level of As accumulation, followed by the lung, liver, kidneys, skin, and intestine. Therefore, it can be posited that the elevated concentrations of As in the bloodstream, as found in this investigation, are attributable to the direct absorption of As into the circulatory system.

When comparing the group treated with As (T1) to the groups treated with Spirulina alone and Spirulina combined with vitamin A, it was seen that there was a substantial reduction (p<0.01) in the levels of As in the kidney, lung, liver, and blood (Figure 1, 2, 3 and 4 and Table 2, 3, 4 and 5). Ahmed et al. (2019) demonstrated the efficacy of Spirulina in effectively reducing arsenic (As) concentrations in the tissues of rats exposed to high levels of As. In a study conducted by Ghosh et al. (2014), it was found that Spirulina was successful in effectively removing arsenic from the blood of goats with induced arsenicosis. Additionally, our investigation demonstrated that the presence of vitamin A resulted in a reduction of As levels in lung, liver, and kidney tissues. However, its efficacy is comparatively lower than that of Spirulina, and it fails to fully capture the comprehensive data depicted in (Figure 1, 2, 3 and 4 and Table 2, 3, 4 and 5). Further research is required in order to achieve comprehensive findings and conclusive outcomes. Based on the obtained findings, it can be concluded that Spirulina had a higher efficacy in reducing the concentration of As in tissues and blood when compared to vitamin A. Furthermore, the combined administration of Spirulina and vitamin A (referred to as the T4 group) demonstrated
a more pronounced beneficial effect on all tissues in comparison to the other treatment groups. Different dose combinations may have varying outcomes; nevertheless, neither of these combinations has a well-established therapeutic dosage. Hence, the optimization of spirulina and vitamin A dosage is hampered by limitations in time and facility resources.

Previous research has indicated that antioxidants as well as micronutrients have a significant role in the management of chronic arsenic poisoning. Several studies have reported the preventive effects of Vitamin A, Iron, Zinc, Spirulina, Ascorbic acid, lipoic acid, and tocopherol against chronic As poisoning (Ahmad et al., 1998; Saha et al., 2003; Halim et al., 2007; Ramanathan et al., 2003; Rabbani et al., 2003). Spirulina is recognized as a notable provider of vitamin A, minerals, and several micronutrients, all of which exhibit antioxidant properties. Consequently, one could postulate that the integration of minerals, vitamins, antioxidants, and various other micronutrients could potentially serve as a viable therapeutic strategy in mitigating the initial signs of arsenicosis.

CONCLUSION

The findings suggest that combining Spirulina and Vitamin A in a synergistic manner could be beneficial for the treatment of chronic arsenicosis in rats. The current investigation serves as an initial study on the efficacy of a combined treatment involving Spirulina and Vitamin A for the management of arsenicosis in Bangladesh. Nevertheless, the findings of this study will help future researchers by providing guidance for conducting more in-depth research. Spirulina may benefit from further research in this area to provide stronger proof that it may be used as a therapeutic intervention for arsenic toxicity. To reduce arsenicosis in animals, more research is required to measure the amount of arsenic in blood and to identify the ideal dosage of spirulina and vitamin A.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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