RESEARCH ARTICLE

The Ameliorating Effects Of Zingiber Zerumbet Linn On Sodium Arsenite-Induced Changes Of Blood Indices In Experimental Mice
The Ameliorating Effects Of Zingiber Zerumbet Linn On Sodium Arsenite-Induced Changes Of Blood Indices In Experimental Mice

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Abstract

The aim of this study was to evaluate the protective effect of Zingiber zerumbet Linn powder on sodium arsenite-induced changes of blood indices in experimental mice. Swiss albino male mice were divided into four groups. The first group was used as control, while the second, third and fourth groups were treated with Z. zerumbet (L.) powder, sodium arsenite and Z. zerumbet (L.) powder plus sodium arsenite, respectively. Animals (third and fourth groups) were exposed to sodium arsenite at a dose of 10 mg/kg body weight/day for 12 weeks. Exposure to sodium arsenite revealed a significant (p < 0.05) increase of serum urea, uric acid, triglyceride (TG), glucose levels and alkaline phosphatase (ALP) activity. Serum butyryl cholinesterase (BChE) activity significantly (p < 0.05) decreased in sodium arsenite-treated group as compared with control group. Interestingly, food supplemented with Z. zerumbet (L.) (50 mg/kg body weight/day) showed protective effect against sodium arsenite-induced increase of serum urea, uric acid and TG levels except serum glucose levels. Moreover, Z. zerumbet (L.) also abrogated the sodium arsenite-induced changes of BChE and ALP activities. Therefore, the ameliorating effects of Z. zerumbet (L.) on sodium arsenite-treated mice suggested the future application of Z. zerumbet (L.) to reduce or prevent arsenic toxicity in human.

Keywords: Arsenic; Zingiber zerumbet Linn.; mice; serum indices.

1. Introduction

Arsenic, a naturally occurring toxicant that is present in food, soil and water. Exposure to higher level than the average level of arsenic occurs either in workplace, for example, in smelting industries, coal fired power plants, cosmetic industries, agriculture or through arsenic-contaminated food and drinking water. The most common forms of arsenic are water-soluble arsenite (the trivalent form, As III) and arsenate (the pentavalent form, As V); trivalent arsenic is more toxic than the pentavalent form and its inorganic forms are more toxic than the
organic forms [1]. Moreover, it is reported that the inorganic As (III) form as $\text{H}_2\text{AsO}_3$ is 40–60 times more toxic than the As (V) form as $\text{H}_2\text{AsO}_4$ [2]. Arsenic has been reported to be associated with multi-site cancers, cardiovascular diseases, diabetes mellitus, dermatitis, immunotoxicity, lymphoproliferative disorders, peripheral neuropathy and many other complications [3–8].

Arsenic toxicity has caused an environmental tragedy in Bangladesh and West Bengal of India where millions of people have been affected because of the drinking of arsenic-contaminated ground water [9–11]. A huge numbers of toxicity cases have been already reported in the north-west region of Bangladesh and it is becoming alarming day by day as the new cases of toxicity are still being found. In Bangladesh, the number of toxicity cases has exceeded over the number of Chernobyl catastrophe [12]. Alarmingly, arsenic has entered the food chain [13,14]. Therefore, exposure to arsenic is unavoidable.

The major metabolic pathway of inorganic arsenic in humans is its methylation in liver, and the methylation of arsenic is proved by the presence of monomethylarsonic acid (MMA) and dimethylarsinic acid in urine and bile [15,16]. Generally, toxicity of arsenic is thought to arise largely by its reaction with free sulfhydryl groups of enzymes and proteins followed by their cross-linking [17,18]. The cross-linking of enzymes or proteins activates the multiple intracellular signalling pathways inside the cells that may be responsible for arsenic-mediated pathogenesis. Moreover, arsenic-induced intracellular signals are largely mediated through redox-linked mechanism because reactive oxygen species (ROS) produced by arsenic act as second messengers [17,19].

Attempts to apply nutritional antioxidant to prevent or treat the diseases caused by oxidative stress have been getting attention in recent years. Many plant products exert their protective effects against oxidative stress-mediated diseases by scavenging free radicals. Although arsenic-induced oxidative stress promotes serious human sufferings but few reports on the beneficial effects of plant products against arsenic toxicity are available. *Zingiber zerumbet* (L.), a flowering plant, in the ginger family, is commonly used as a flavouring agent and is one of the basic ingredients in curry powder. It has also been used as a traditional medicine for the treatment of indigestion, dyspepsia, asthma, leprosy and dysentery [20,21]. Zerumbone is an active ingredient of *Z. zerumbet* (L.), which has been shown to have a wide range of therapeutic effects like antioxidant, anti-inflammatory activity [22–25]. Because of the antioxidant properties of *Z. zerumbet* (L.), it can be postulated that it may have some protective effects against arsenic toxicity. Therefore, in this study, we investigated the efficacy of the rhizome of *Z. zerumbet* (L.) on arsenic-induced changes of blood indices through mice model.

2. Methods

2.1. Animal maintenance

Adult healthy (3 weeks of age) Swiss albino male mice with average body weight (BW) of 20–22 g were purchased from ICDDR, B (International Centre for Diarrhoeal Disease Research, Bangladesh). The animals were randomly selected and housed in polycarbonate cages with steel wire tops and wood-cobe bedding (six mice per cage). After 1 week of acclimatization, animals were divided into four equal groups named control, Z.
zerumbet (L.), sodium arsenite and Z. zerumbet (L.) plus sodium arsenite. They were maintained with 12 hour: 12 hour dark–light cycle with available supply of distilled water and feed. Sodium arsenite was given to the mice with distilled water (10 mg/kg body weight) and Z. zerumbet (L.) powder (50 mg/kg) was added to the normal diet (as a supplement) for 12 weeks.

2.2. Preparation of Zingiber zerumbet (L.) powder
First, rhizomes of Z. zerumbet (L.) plant were collected from the Rajshahi University campus and then cleaned and washed repeatedly. The rhizomes were then sliced and sun-dried. Finally, Z. zerumbet (L.) powder was obtained by grinding the sun-dried slice and kept at 4°C with sealed plastic packet to avoid the microbial contamination.

2.3. Collection of serum from experimental mice
Blood specimens were collected from the thoracic arteries of mice after anaesthetizing with diethyl ether. For coagulation, blood was kept for 30 minutes at room temperature. After centrifugation at 1600g for 15 minutes at 4°C, serum were drawn off and stored at −80°C until the experiments were performed.

2.4. Laboratory examination
The analyser CHEM-5V3 (Erba, Mannheim, Germany) was used for the measurement of serum indices using commercially available kits according to the manufacture’s protocol. Serum urea, uric acid, glucose and TG levels were measured using the kits from Human, Germany. Alkaline phosphatase (ALP) activity was determined using the kit from BioSystems, SA, Spain and serum butyryl cholinesterase (BChE) activity was measured by using butyryl cholinesterase (CHE) kit (RANDOX, UK). All serum samples were analysed in duplicate and then mean values were recorded.

2.5. Statistical analysis
Statistical analyses were performed with SPSS for Windows, version 15.0 (SPSS, Chicago, IL). Data were expressed as mean ± SE. Differences between the serum indices of different groups of mice were analysed by using t-test.

3. Results
In this study, we measured the serum urea levels in all groups of the experimental mice and evaluated the effect of Z. zerumbet on the serum urea level. As presented in Table 1, the serum urea levels (mean ± SE) of the four groups of mice were 50.02 ± 3.36, 34.34 ± 1.85, 64.78 ± 2.45 and 46.52 ± 1.50 mg/dL in control, Z. zerumbet, sodium arsenite and Z. zerumbet plus sodium arsenite, respectively. The results showed that sodium arsenite treatment significantly (p < 0.05) increased serum urea levels as compared with the control group, and food supplemented with Z. zerumbet significantly (p < 0.05) inhibited the sodium arsenite-induced elevation of serum urea levels. Next, we measured serum uric acid levels in all the groups of the experimental
mice to evaluate the effect of *Z. zerumbet* (Table 1). The serum uric acid levels (mean ± SE) of four groups of mice were 4.17 ± 0.07, 4.06 ± 0.47, 5.05 ± 0.18 and 4.41 ± 0.11 mg/dL in control, *Z. zerumbet*, sodium arsenite and *Z. zerumbet* plus sodium arsenite groups of mice, respectively. We found that mice exposed to sodium arsenite show significantly (*p* < 0.05) increased serum uric acid levels compared with the control group and food supplemented with *Z. zerumbet* which significantly inhibited the sodium arsenite-induced elevation of serum uric acid levels (*p* < 0.05). Therefore, these results suggested the protective effect of *Z. zerumbet* on the elevation of sodium arsenite-induced serum urea and uric acid levels.

In 2004, Tseng has reported that arsenic induces diabetes mellitus in the chronically exposed subjects [26]. Therefore, next we investigated the serum glucose levels in four groups of experimental mice. Serum glucose levels (mean ± SE) of the control, *Z. zerumbet*, sodium arsenite and *Z. zerumbet* plus sodium arsenite groups were 150.18 ± 16.04, 150.21 ± 8.30, 178.71 ± 4.41 and 180 ± 19 mg/ dL, respectively. We found that arsenic treatment increased the serum glucose levels; however, *Z. zerumbet* did not provide any protection against arsenic-induced elevation of blood glucose level (Table 1).

**Table 1**: Serum urea, uric acid, TG and glucose levels of the groups of experimental mice.

<table>
<thead>
<tr>
<th>Serum indices (mg/dL)</th>
<th>Experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Urea</td>
<td>50.02 ± 3.36</td>
</tr>
<tr>
<td>Uric acid</td>
<td>4.17 ± 0.07</td>
</tr>
<tr>
<td>Glucose</td>
<td>150.18 ± 16.04</td>
</tr>
<tr>
<td>TG</td>
<td>125.60 ± 11.09</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SE, n = 6 for each group of mice.

\(^a\)Significantly different from control at *p* < 0.05.

\(^b\)Significantly different from the sodium arsenite-treated group at *p* < 0.05.

In this study, we also evaluated the serum TG levels and observed that TG levels were significantly (*p* < 0.05) elevated (160.33 ± 5.61 mg/dL) in mice exposed to sodium arsenite compared with the control group (125.60 ± 11.09 mg/dL). The elevated levels of TG in sodium arsenite-treated mice were diminished to 105.93 ± 3.97 mg/dL by the addition of *Z. zerumbet* powder (*Zingiber zerumbet* plus sodium arsenite group) as a food supplement. *Z. zerumbet* alone also decreased the baseline serum TG level since the level of TG in *Z. zerumbet*-treated mice is lower (94.60 ± 8.39 mg/dL) than the control group (Table 1). Thus, these results indicated that *Z. zerumbet* had protective effect on sodium arsenite-induced elevation of total serum TG levels.
Liver is the primary organ for arsenic intoxication [27] and the elevated activities of liver enzymes in serum represent the liver dysfunction [28]. Hence we measured the activity of serum ALP of the four groups of experimental mice and found that enzyme activities were significantly (p < 0.05) increased in the groups exposed to sodium arsenite (Table 2). *Zingiber zerumbet* was found to inhibit the sodium arsenite-induced elevation of ALP activity significantly (p < 0.05). Previously, it has been demonstrated that exposure to arsenic decreased the cholinesterase activity in rats and humans [29–31]. Therefore, next we investigated whether *Z. zerumbet* could prevent the sodium arsenite-induced decrease of serum BChE activity. Serum BChE activity (mean ± SE) were 13,247 ± 343.92 (U/L), 13,604.67 ± 987.96 (U/L), 11,289.67 ± 417.55 (U/L) and 12,750.33 ± 2354.92 (U/L) in the control, *Z. zerumbet*, sodium arsenite and *Z. zerumbet* plus sodium arsenite groups, respectively (Table 2). The enzyme activity was significantly decreased in the group exposed to sodium arsenite compared with the control group. Intriguingly, we observed that *Z. zerumbet* prevented the sodium arsenite-induced perturbation of cholinesterase activity.

### Table 2: Activities of liver enzymes in serum of the groups of experimental mice.

<table>
<thead>
<tr>
<th>Serum indices (U/L)</th>
<th>Control</th>
<th><em>Zingiber zerumbet</em></th>
<th>Sodium arsenite</th>
<th><em>Zingiber zerumbet</em> + sodium arsenite</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALP</td>
<td>150.03 ± 11.54</td>
<td>147.87 ± 11.60</td>
<td>210.44 ± 5.75^a</td>
<td>165.04 ± 7.62^b</td>
</tr>
<tr>
<td>BChE</td>
<td>13,247 ± 343.92</td>
<td>13,604.67 ± 987.96</td>
<td>11,289.67 ± 417.55^a</td>
<td>12,750.33 ± 2354.92</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SE, n = 6 for each group of mice.

^aSignificantly different from control at p < 0.05.

^bSignificantly different from the sodium arsenite-treated group at p < 0.05.

### 4. Discussion

Arsenicals are the potent environmental pollutants and well-established human carcinogen. The main cause of arsenic toxicity is the drinking of ground water contaminated by arsenic. Although, arsenic exposure is the major threat to the public health in Bangladesh and some other countries in the world but there are very few reports on the therapeutic approaches that can reduce arsenic toxicity. The main purpose of the present study is to investigate the ameliorating effects of *Z. zerumbet* (L.) on the sodium arsenite-induced biochemical alterations in the serum of mice. Arsenic has been reported to be associated with diabetes mellitus, cardiovascular diseases, hepatic and renal dysfunction, neurotoxicity and multi-site cancers [32–37]. Several soluble enzymes and proteins of serum have been considered as indicators of the cardiovascular diseases, hepatic and kidney dysfunctions.
The blood urea becomes raised when the kidney tubules are prevented from removing the urea and other waste products from the blood. Elevated blood urea is correlated with an increased protein catabolism in mammalian body or from more efficient conversion of ammonia to urea as a result of increased synthesis of enzyme involved in urea production in liver. In this study, we found that sodium arsenite treatment increased the serum urea level in mice that might be an indicator of the adverse effects of arsenite on kidney and liver and Z. zerumbet potentially inhibited the sodium arsenite action on serum urea level (Table 1).

Hepatic disorder appears to be a primary cause of arsenic-related mortality [38–40]. Elevated serum ALP activity is used as one of the markers for liver dysfunction. An increase in serum ALP levels is frequently associated with a variety of diseases such as extra-hepatic bile obstruction, intrahepatic cholestasis, and infiltrative liver disease [41]. In this study, we found that treatment with sodium arsenite significantly increased the serum ALP activity. Elevated activity of ALP in arsenite-treated mice was in agreement with the results of previous studies [28, 42]. Protective effect of Z. zerumbet on the elevation of arsenite-induced ALP activity observed in this study suggested that Z. zerumbet has some protective effects on arsenic-induced liver intoxication. Decreased cholinesterase activity is associated with hepatitis, hepatic metastases, heart attack [43–45]. Usually, the measurement of blood and tissue cholinesterase activities is a useful tool for monitoring exposure to organophosphate and carbamate insecticides and diagnosing their poisoning effect [46–48]. BChE present in serum/plasma has a broader range of esterase activity referred to as “pseudo” or “non-specific” cholinesterase, which hydrolyses both acetylcholine (a neurotransmitter) and other aliphatic esters [49]. Very recently, we have reported that BChE activity significantly decreases in human and mice exposed to arsenic [29,31]. Interestingly, in this study, we observed that sodium arsenite decreased the serum BChE activity and Z. zerumbet prevented the decrease of BChE activity. All these results suggested that sodium arsenite administration induced liver dysfunction and neurotoxicity, and Z. zerumbet had protective effects on them.

We found that sodium arsenite treatment significantly increased the serum uric acid level and Z. zerumbet abrogated that elevation (Table 1) suggesting that Z. zerumbet had some protective role on arsenic-induced increased level of serum urea level. Effect of Z. zerumbet on sodium arsenite-induced elevation of serum uric acid levels are interesting since elevated level of uric acid in blood has been reported to be a risk factor for cardiovascular diseases, metabolic syndrome and diabetes mellitus [50,51]. Later, we checked the serum marker related to cardiovascular risk. We measured serum TG levels in all the groups of experimental mice. We found that TG levels were significantly (p < 0.05) higher in sodium arsenite-treated mice (Table 1). Intriguingly, food supplemented with Z. zerumbet powder abrogated the elevation of serum TG levels in the arsenic-treated mice. Epidemiological studies suggested that cardiovascular diseases are the major causes for the chronic arsenic exposure-related mortality and alterations of serum lipid levels have been recognized as the risk for cardiovascular diseases [52,53]. Therefore, the protective effect of Z. zerumbet on the sodium arsenite-induced elevation of serum TG levels is particularly important. Serum total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) along with TG levels can be used to assess the risk of cardiovascular diseases. We did not investigate these parameters (TC, LDL-C

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and HDL-C) in this study. Further research is needed to evaluate the effect of *Z. zerumbet* on the arsenic-induced alteration of TC, LDL and HDL. Sodium arsenite-induced elevation of blood glucose level observed in this study was consistent with the result of Tseng (2004), who showed that chronic arsenic exposure increased blood glucose level in human population [26]. It is reported that arsenic induces oxidative stress [54] and oxidative stress destroys β cells of the pancreatic islets that leads to the insufficient production of insulin required for the utilization of blood glucose [55]. We found no protective effect of *Z. zerumbet* on the arsenite-induced elevation of blood glucose level.

The present study clearly indicated the ameliorating effects of *Z. zerumbet* on the arsenic-induced changes of serum indices. However, we did not clarify in this study how *Z. zerumbet* showed the protection against arsenic action. Further study is needed to explain the mechanism of *Z. zerumbet* for the reduction of arsenic toxicity. One possibility was that zerumbone, an active ingredient of *Z. zerumbet*, might inhibit the arsenic action by the perturbation of the arsenic-mediated signal transduction pathway because of its antioxidant property. It has been well-documented that ROS produced by arsenic acts as a second messenger for transducing intracellular signal [17,19]. Probably zerumbone or other ingredient of the *Z. zerumbet* inhibited ROS production induced by arsenic, which ultimately inhibited the signalling pathways associated with the toxicity of arsenic. Application and usefulness of *Z. zerumbet* powder to reduce the level of arsenic toxicity are practically important as *Z. zerumbet* has already been recognized as safe natural flavouring agents. *Zingiber zerumbet* is being used as food flavouring and traditional medicine by the population of Indian subcontinent and other countries for a long time [56]. Thus, this study provided supportive role of *Z. zerumbet* against arsenic toxicity indicating its usefulness for the bioremediation of arsenic toxicity in future.

5. Conclusion

In conclusion, the results of this study showed that *Z. zerumbet* reduced the arsenic-induced changes of blood indices in mice. Therefore, it is suggested that *Z. zerumbet* could be used to prevent the toxic effect of arsenic in humans in future.

**Abbreviations**

ALP, alkaline phosphatase; BW, body weight; BChE, serum butyryl cholinesterase; CHE, choline esterase; HDL, high density lipoprotein-cholesterol; MMA, monomethylarsonic acid; ROS, reactive oxygen species; TC, total cholesterol.

**Competing Interests**

The authors declare that they have no competing interests.

**Ethical Clearance**

The work involving mice as experimental model was approved by the institutional ethical committee.
Authors’ Contributions

MR and ZAS performed the experiments and prepared the manuscript. EH, KI and MRK performed the experiments. TY, FN and AM critically read the manuscript; KH conceived and prepared the manuscript. All authors read and critically contributed to the work.

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