Dietary intervention affects arsenic-generated nitric oxide and reactive oxygen intermediate toxicity in islet cells of rats

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This study has revealed that pancreatic tissue of arsenic-treated (3 mg/kg body wt/day for 30 days) rats shows excessive production of nitric oxide (NO) and malondialdehyde (MDA), while the activity of antioxidant enzymes superoxide dismutase and catalase, is significantly low in these animals. Light microscopic examination of sections of pancreas from such animals shows that a larger number of islets are shrunken, containing lower number of islet cells. Nicotinamide (NAM) supplement could reduce excessive production of both NO and MDA as well as toxicity in islet cells. NAM also could enhance the activities of antioxidant enzymes. Islet morphology, size and cell counts were seen to recover close to normal with NAM. Like NAM, pea or pea and casein supplement in diet could reduce excessive production of NO, MDA and toxicity in islet cells, but increase the activities of antioxidant enzymes. Our findings suggest that NAM, pea or pea and casein affect oxygen radical and NO toxicity in islet cells of rats and have promise as dietary supplements to prevent disorders involving damage of islet cells.

According to several survey reports, exposure to inorganic arsenic compounds may be associated with development of diabetes mellitus\textsuperscript{2-6}. In recent years, oxidative stress has been implicated in arsenic-induced cytotoxicity and genotoxicity\textsuperscript{7}, and also in a wide variety of human diseases and syndromes, including diabetes mellitus\textsuperscript{8,9}. Besides, a positive correlation among arsenite-induced nitric oxide (NO) production, oxidative stress, DNA damage, activation of poly[ADP-ribose] polymerase (PARP) has been suggested\textsuperscript{7}. Earlier, PARP activation, consumption of nicotinamide adenine dinucleotide (NAD) and a consequent depletion of ATP had been suggested\textsuperscript{7}. According to several survey reports, exposure to inorganic arsenic compounds may be associated with development of diabetes mellitus\textsuperscript{2-6}. In recent years, oxidative stress has been implicated in arsenic-induced cytotoxicity and genotoxicity\textsuperscript{7}, and also in a wide variety of human diseases and syndromes, including diabetes mellitus\textsuperscript{8,9}. Besides, a positive correlation among arsenite-induced nitric oxide (NO) production, oxidative stress, DNA damage, activation of poly[ADP-ribose] polymerase (PARP) has been suggested\textsuperscript{7}. Earlier, PARP activation, consumption of nicotinamide adenine dinucleotide (NAD) and a consequent depletion of ATP had been suggested\textsuperscript{7}. Arsenite treatment also has been reported to cause ATP depletion\textsuperscript{12}. Nicotinamide (NAM), a PARP inhibitor, has been reported to increase intracellular NAD content\textsuperscript{11} and administration of NAM to 90% depancreatized rats induces regeneration of pancreatic islets, thereby ameliorating surgical diabetes\textsuperscript{14}. Furthermore, the antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD), were shown to effectively reduce the frequency of arsenite-induced sister-chromatid exchanges in human peripheral lymphocytes and X-ray-sensitive cells\textsuperscript{15}.

With the ever-increasing public-health problems associated with oral exposure of inorganic arsenic, there has been a renewed interest in understanding the metabolism and toxicity of the compounds of this metalloid. Methylation of arsenic to monomethyl arsanic acid (MMA) and dimethyl arsanic acid (DMA) has been believed by many to be the major mechanism for detoxifying inorganic arsenic, although there exist considerable differences between species and individuals\textsuperscript{15}. Such methylation of inorganic arsenic to mono-, di- and trimethylated metabolites is an enzymatic process which is catalysed by methyltransferases that use S-adenosylmethionine (SAM) as a methyl-group donor\textsuperscript{17}. Methionine is the source of methyl group in the methylation process, which can be transferred only after being activated with ATP to form SAM\textsuperscript{18}. It has also been reported that specific nutritional factors, viz, methionine, folic acid and vitamin B\textsubscript{12} influence methylation capacity, and may consequently influence arsenic-induced health effects\textsuperscript{19}. Pea (\textit{Pisum sativum}), which contains 22.9 g% protein\textsuperscript{20}, has been re-

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ported as a potent methyl-group transferring agent in the methylation process, and extracts of seedlings of pea were reported to catalyse the synthesis of SAM\textsuperscript{21}.

The present study was designed to test the hypothesis of a causal relationship between arsenic-generated oxidative stress and islet cell damage in rats in situ, and further, to examine whether dietary intervention may be an effective strategy of detoxification to help prevent disorders and pancreatic islet cell damaging effects on exposure to arsenic.

Materials and methods

Animals

All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Culture, Government of India.

Male albino rats weighing 110–125 g were used in all the experiments. Animals were maintained in an environmentally controlled animal house (temperature 24 ± 3°C) and in a 12 h light/dark schedule with free access to water supply. For experiments, rats were randomly selected into four groups consisting five rats each: group A, control; group B, arsenic-treated; group C, arsenic + pea supplemented and group D, arsenic + pea + casein-diet supplemented.

The animals of groups A and B were provided with a control diet composed of 71% carbohydrate, 18% protein, 7% fat and 4% salt mixture\textsuperscript{22} and vitamins were supplied according to Chatterjee et al.\textsuperscript{23}. For chronic oral exposure to arsenic, a dose was selected (3 mg/kg body wt/day), which is within the range of LD\textsubscript{50} of a 70-kg body wt human (1–4 mg/kg) and lesser than one-thirteenth of LD\textsubscript{50} value of rats (40 mg/kg)\textsuperscript{24}. Accordingly, animals of groups B, C and D were orally treated with aqueous solution of arsenic trioxide, 3 mg/kg body wt/day for 30 days. The animals of group C, in addition, were supplemented with pea (37 g/100 g of diet), which contributed 8.5% protein\textsuperscript{20}. The animals of group D, in addition to pea, were further supplemented with casein (9 g/100 g of diet) which contributed additional 9% protein in the formulation of a high protein (27%) diet. To overcome the impact of any altered food intake, control (group A) animals were pair-fed with other experimental groups B, C and D.

For studies with NAM supplement, animals were randomly selected to three separate groups, each containing 5 rats, viz. group E, control; group F, arsenic-treated and group G, arsenic with NAM supplement. Animals of group F were orally treated with similar dose of arsenic trioxide (3 mg/kg body wt/day for 30 days) and those of group G were simultaneously treated with both arsenic (3 mg/kg body wt/day for 30 days) and NAM (0.5 g/kg body wt/day for 30 days)\textsuperscript{4}, orally in two divided doses\textsuperscript{25}. Animals of all the three groups were supplied with control laboratory diet as was given to animals of groups A and B of protein-supplemented studies. To overcome the impact of any altered food intake, animals of group E (control) were pair-fed with experimental groups F and G.

Preparation of enzyme extracts

After the treatment period was over (30 days), the animals were sacrificed by cervical dislocation which is one of the recommended physical methods of euthanasia by the IAEC. The abdomen was opened and a small portion of the pancreas from the gastro-splenic part was quickly removed and placed in a beaker containing ice-cold Tris-HCl buffer (pH 7.4). It was cut into small pieces with the help of a scissors, homogenized immediately in a glass-homogenizing tube equipped with a Teflon pestle. The homogenate was processed according to the method of Koyama et al.\textsuperscript{26}, the resulting supernatant of the first homogenate was retained as the source of enzyme.

For catalase estimation, the tissue was homogenized in ice-cold isotonic phosphate buffer. The homogenate was processed according to the method of Cohen et al.\textsuperscript{27}. To an aliquot of the supernatant fluid, ethanol was added to a final concentration of 0.17 M and samples were incubated for 30 min in an ice-water bath. After 30 min, 10% Triton X-100 was added to a final concentration of 1% and the aliquot was used for catalase estimation\textsuperscript{27}.

Estimation of NO and malondialdehyde

The role of nitric oxide synthase (NOS) was indirectly assessed by estimating the amount of NO production. NO decomposes rapidly in aerated solutions to form stable nitrite/nitrate products. In our study, nitrite accumulation was estimated by Griess reaction\textsuperscript{28} and was used as an index of NO production. The amount of nitrite in the sample (µmolar unit) was calculated from a sodium nitrite standard curve.

The role of lipid peroxidase was assessed by studying the level of formation of malondialdehyde (MDA), an indicator of lipid peroxidation. Quantitative measurement of lipid peroxidation was performed following the thiobarbituric acid (TBA) test\textsuperscript{29}. The amount of MDA formed was quantitated with TBA and used as an index of lipid peroxidation. The results were expressed as nmol MDA/mg protein using molar extinction coefficient (1.56 × 10\textsuperscript{5} cm\textsuperscript{2}/mmol).

Estimation of SOD and CAT

SOD was assayed according to the method of Mishra and Fridovich\textsuperscript{30}. The change in absorbance due to the conver-
sion of epinephrine to adrenochrome can be markedly inhibited by the presence of SOD. The reaction was initiated by addition of epinephrine and the increase in absorbance at 480 nm was measured in a UV-Double Beam Spectrophotometer (Shimadzu 160A).

CAT was assayed by the method of Cohen et al. The enzyme-catalysed decomposition of H$_2$O$_2$ was measured at 480 nm in a UV-Double Beam Spectrophotometer (Shimadzu 160A).

Estimation of protein

The protein content of homogenates used for the study was determined essentially by following the method described by Lowry et al.

Preparation of permanent slides for islet cell studies

Pancreatic tissue from all groups of animals was selectively taken from the gastro-splenic portion and was Bouin’s-fixed. Paraffin blocks were prepared, and 4–5 µm thin sections were cut with a high precision microtome (IEC Minotome, USA) and routine microscopic slides were prepared. For staining, chrome-alum haematoxyline phloxine procedure of Gomori was followed. Stained slides were light-microscopically examined for size and total number of cells present per islet. The islet width and length were measured with an ocular ruler fixed in the eyepiece, and the eyepiece graticule was compared with stage micrometer to give a comparative value for diameter of islets under observation (50 times magnification). Mean diameter of islet represents the mean of maximum diameter and a diameter at right angle to it. For counting the number of cells present per islet in different groups of animals, only equal size islets were considered.

Statistics

Data were expressed as mean ± SE. Kruskal–Wallis non-parametric ANOVA test was performed to find whether or not scores of different groups differ significantly. To test inter-group significant difference, Mann–Whitney ‘U’ multiple comparison test was performed. PSI-PLOT Version 2.0 (Poly Software International; 1992,1993) was used for statistical analysis. Differences were considered significant, if $P < 0.05$.

Results

Oral arsenic trioxide-induced changes in NO and MDA production: effect of PARP inhibitor

At the outset, we investigated whether or not there exists any possible causal relationship between activity of nuclear enzyme PARP and production of DNA-damaging compounds, NO and MDA, in pancreatic tissue of arsenic-treated rats. Table 1 shows that compared to control, rats on exposure to arsenic increased NO production by 89% ($P < 0.01$) and MDA by 91% ($P < 0.01$). Simultaneous supplementation of NAM, a PARP inhibitor, with arsenic had shown to effectively reduce such arsenic-generated increase in NO production by 70% ($P < 0.01$) and MDA by 67% ($P < 0.01$).

Oral arsenic trioxide-induced changes in activity of antioxidant enzymes SOD and CAT: effect of PARP inhibitor

We also analysed whether or not there exists any possible relationship between PARP and the activity of cellular antioxidant enzymes, SOD and CAT, in arsenic-treated animals. Table 1 shows that compared to control, SOD activity was significantly decreased by 44% ($P < 0.01$) and CAT activity by 47% ($P < 0.01$) in arsenic-treated animals. However, NAM, when simultaneously supplemented with arsenic, could significantly affect arsenic-induced inhibition of SOD by 58% ($P < 0.01$) and CAT by 56% ($P < 0.05$).

Oral arsenic trioxide-induced reduction of islet cell population: effect of PARP inhibitor

Results of islet cell studies are shown in Table 2. Histological sections of pancreas of arsenic-treated rats show

<table>
<thead>
<tr>
<th>Parameter studied</th>
<th>Control (A)</th>
<th>As-treated (B)</th>
<th>Per cent increase$^c$</th>
<th>As + NAM (C)</th>
<th>Percent restored</th>
<th>Significance level$^*$</th>
<th>Significance level**</th>
<th>Significance level**</th>
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<tr>
<td>NO ($\mu$mol/mg protein)</td>
<td>3.75 ± 0.65</td>
<td>7.1 ± 0.27</td>
<td>89$^a$</td>
<td>4.75 ± 0.48</td>
<td>70</td>
<td>$P &lt; 0.05$</td>
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<tr>
<td>MDA (nmol/mg protein)</td>
<td>2.24 ± 0.25</td>
<td>4.28 ± 0.22</td>
<td>91$^a$</td>
<td>2.92 ± 0.30</td>
<td>67</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
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<td>SOD (µg/mg protein)</td>
<td>4.3 ± 0.55</td>
<td>2.4 ± 0.06</td>
<td>44$^a$</td>
<td>3.5 ± 0.45</td>
<td>58</td>
<td>$P &lt; 0.05$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
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<tr>
<td>CAT (first-order reaction rate constant/mg protein)</td>
<td>0.95 ± 0.075</td>
<td>0.50 ± 0.075</td>
<td>47$^a$</td>
<td>0.75 ± 0.025</td>
<td>56</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
<td>$P &lt; 0.01$</td>
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</table>

Values are expressed as mean ± SE; $n = 5$. $^*$Significance based on Kruskal–Wallis test; $^**$Significance based on Mann–Whitney ‘U’ multiple comparison test.
that compared to control, a larger number of islets were shrunken with lower number of islet cells (Figure 1a and b). Even in similar sized (µm) pancreatic islets, the number of cells in arsenic-exposed group was markedly low. Per cent reduction in cell counts in the islets of arsenic-exposed rats compared to control was 36, 41, 49, 42, 39, 44 and 42, respectively. However, such reduction in islet cell counts was markedly prevented by NAM supplementation and the per cent restored was 79, 65, 79, 94, 85, 79 and 79. NAM also could restore the normal morphology of islets (Figure 1c).

**Effect of pea or pea and casein supplementation on oral arsenic trioxide-induced changes in NO production**

The increased production of NO by chronic oral exposure to arsenic trioxide and its recovery by either pea or pea and casein diet supplementation are shown in Table 3. Results indicate that compared to control, nitrite accumulation, an indicator of NO production, was increased significantly ($P < 0.01$) in arsenic-treated animals by 63%. Pea supplementation in these animals could significantly ($P < 0.01$) reduce production of nitrite by 75%, while pea and casein diet could produce much better recovery by 103% ($P < 0.01$; Table 3).

**Effect of pea or pea and casein supplementation on oral arsenic trioxide-induced changes in MDA production**

The increased production of MDA by chronic oral exposure to arsenic trioxide and its recovery by either pea or pea and casein diet supplementation are shown in Table 3. Results indicate that MDA production, an indicator of lipid peroxidation, was excessive ($P < 0.01$) in arsenic-treated animals and was increased by 110% compared to control. Pea supplementation in these animals could significantly ($P < 0.01$) reduce the production of MDA by 76% ($P < 0.01$), while pea and casein diet could produce further recovery by 101% ($P < 0.01$; Table 3).

**Effect of pea or pea and casein supplementation on oral arsenic trioxide-induced changes in CAT activity**

Table 3 also describes the effect of either pea or pea and casein supplementation on CAT activity of chronic oral arsenic-exposed rats. Results indicate that CAT activity was decreased significantly ($P < 0.01$) in arsenic-treated animals by 53% compared to control. Pea supplementation in these animals significantly recovered the activity ($P < 0.01$) of CAT by 70%, while pea and casein diet could produce further recovery by 104% ($P < 0.01$; Table 3).

**Effect of pea or pea and casein supplementation on oral arsenic trioxide-induced changes in population of islet cells of rat pancreas**

Table 4 shows the effect of either pea or pea and casein supplementation on oral arsenic trioxide-induced changes in population of islet cells of rat pancreas. Results show that compared to control, in similar sized (µm) pancreatic islets of rats, the number of cells in the arsenic-exposed group was markedly low. Per cent reduction in cell counts in the islets of arsenic-exposed rats compared to control was 33, 41, 33, 35, 32, 36 and 36, respectively. Such reduction in islet cell counts was markedly pre-

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**Table 2.** Effect of PARP inhibitor on arsenic trioxide-induced reduction in islet cell population of rat pancreas

<table>
<thead>
<tr>
<th>No. of cells/islet</th>
<th>Control</th>
<th>As-treated</th>
<th>Percentile decrease</th>
<th>No. of cells/islet</th>
<th>Per cent restored against arsenic response</th>
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Serial sections of pancreas from five different animals were used in all groups. For counting of number of cells present per islet of different groups of animals, only equal-size islets were considered.
Figure 1. Chrome-alum haematoxyline phloxine-stained section showing morphology and population of cells in pancreatic islet of a, Control rat; b, Arsenic-treated rat; c, Arsenic + NAM-treated rat; d, Arsenic + pea diet-supplemented rat, and e, Arsenic + pea and casein diet-supplemented rat. (a–e) × 50.

Discussion

The principal finding of this study is that NAM, pea or pea and casein have promise as dietary supplements to help prevent disorders involving arsenic-induced damaging effects of pancreatic islets in rats. In the present study we provided the experimental demonstration that in rats, chronic oral exposure to arsenic trioxide in situ causes significant decrease in population of pancreatic islet cells, a typical histological feature which may be compared with development of Type 1B insulin-dependent diabetes mellitus (IDDM) caused by beta-cell injury from specific toxic substances bypassing an autoimmune requirement. Results of fasting plasma glucose concentration, oral glucose tolerance tests (OGTTs), glycated haemoglobin (HbA1c), serum amylase and acute decompenstation changes like diabetic ketoacidosis and reduction in islet cell population in our studies with rabbits on exposure to oral arsenic, provide further evidence for an arsenic-induced experimental development of diabetes mellitus (unpublished data).

Arsenic compounds are widely-distributed natural toxicants. According to several survey reports, exposure to inorganic arsenic compounds is associated with development of diabetes mellitus. Several other human diseases, viz. Black foot disease, hypertension and cancers of skin, lung, bladder and liver also have been linked with arsenic compounds. Studies also suggest that oxidants may be involved in arsenic toxicity. Moreover, antioxidants have been shown to reduce sister-chromatid exchanges, micronuclei, apoptosis and cytotoxicity.

The present results show that chronic oral exposure of male rats to arsenic trioxide enhanced production of NO and MDA, concomitant with a decrease in the activity of SOD and CAT in the pancreatic tissue of rat (Table 1). Such enhanced production of NO and MDA, and reduction in activity of antioxidant enzymes were always marked with significant reduction in the total population of islet cells (Figure 1 b; Table 2). It is well-documented that pancreatic islet cells are highly susceptible to the toxic effects of reactive oxygen intermediates (ROI) and NO. There is also evidence that these reactive mediators are released during prediabetic islet inflammation and thus may contribute to beta-cell loss. In our study, compared to control animals, production of NO and
Table 3. Effect of pea or pea + casein supplemented diet on arsenic trioxide-induced changes in the production of NO and MDA, and on the activity of SOD and CAT in pancreatic tissue of rat

<table>
<thead>
<tr>
<th>Parameter studied</th>
<th>Control (A)</th>
<th>As-treated (B)</th>
<th>Per cent increase/ decrease</th>
<th>As + pea (C)</th>
<th>As + pea + casein (D)</th>
<th>Per cent restored</th>
<th>Significance level*</th>
<th>Significance level**</th>
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<td>NO (µmol/mg protein)</td>
<td>3.32 ± 0.56</td>
<td>5.40 ± 0.43</td>
<td>63%</td>
<td>3.84 ± 0.16</td>
<td>75</td>
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<td>MDA (nmol/2.71 mg protein)</td>
<td>2.60 ± 0.24</td>
<td>5.46 ± 0.61</td>
<td>110%</td>
<td>3.29 ± 0.22</td>
<td>76</td>
<td>2.57 ± 0.14</td>
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<td>SOD (µg/mg protein)</td>
<td>2.71 ± 0.13</td>
<td>0.968 ± 0.07</td>
<td>64%</td>
<td>1.45 ± 0.05</td>
<td>28</td>
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<td>CAT (first-order reaction rate constant/mg protein)</td>
<td>0.95 ± 0.07</td>
<td>0.450 ± 0.07</td>
<td>53%</td>
<td>0.80 ± 0.04</td>
<td>70</td>
<td>0.97 ± 0.04</td>
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<td>P &lt; 0.02</td>
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Values are expressed as mean ± SE; n = 5; *Significance based on Kruskal–Wallis test; **Significance based on Mann–Whitney ‘U’ multiple comparison test; NS, Not significant.

Table 4. Effect of pea or pea + casein-supplemented diet on arsenic trioxide-induced reduction in islet cell population of rat pancreas

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<th>No. of cells/islet</th>
<th>Control</th>
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<th>Percentile decrease</th>
<th>No. of cells/islet</th>
<th>Per cent restored against arsenic response</th>
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Serial sections of pancreas from five different animals were used in all groups. For counting the number of cells present per islet of different groups of animals, only equal-size islets were considered.

MDA in arsenic-treated group was 89 and 91%, respectively (Table 1) and activities of SOD and CAT were 44 and 47%. These results suggest that the oral dose of arsenic used in our chronic study was potentially effective for enhanced formation of highly reactive oxygen intermed-iates to cause oxidative stress and to decrease the population of islet cells by oxidative damage. A simultaneous decrease in the activities of antioxidant enzymes in our study corroborates well with earlier evidence of increased oxidative stress with depleted antioxidant enzymes in both type 1 and type 2 diabetes mellitus42.

Evidence suggests that excessive poly-(ADP-ribose) formation by PARP causes the depletion of cellular NAD+ pools and induces the death of several cell types, including the loss of insulin-producing islet cells in type 1 diabetes43. Also, DNA damage, PARP activation and NAD+ depletion are seen in islets exposed to ROI41. The role of arsenic in generation of NO to induce DNA strand-breaks and then to activate PARP also has been speculated7. In addition, the assumption of a causal relationship between PARP activation and cell death is based on the observation that PARP inhibitors, such as NAM, partially prevent ROI or NO toxicity41. To verify whether a similar causal relationship exists in our study between arsenic-generated increased cellular NO and ROI production, PARP activation and reduction in islet cell population, experiments were performed with NAM which, apart from PARP inhibition, also has oxygen-radical-scavenging property44. The conclusions of our study relate to the causal relationship because NAM could afford protection from NO toxicity and significantly revive islet cell population (Figure 1c; Table 2). NAM supplementation also significantly reduced arsenic-generated production of NO and MDA (Table 1). This reduction using NAM may be either due to direct oxygen-radical-scavenging property45 or by decreasing the constitutive levels of both activated NF-κB, that respond to arsenic-generated oxidative stress46. Also, activities of both SOD and CAT were found to be high after NAM supplementation, suggesting that NAM possibly affected certain changes in the equilibrium of ROI and antioxidant enzymes that leads to induction of the antioxidant defense system, including a rise in the activities of the antioxidant enzymes (Table 1). Thus, inactivation of PARP by NAM possibly affected ROI and NO toxicity in islet cells.
As summarized earlier, methylation of arsenic to MMA and DMA has been suggested to be the major mechanism for detoxifying inorganic arsenic\(^1\). It is an enzymatic process which is catalysed by methyltransferases that use SAM as a methyl-group donor\(^2\). However, methionine, the source of methyl-group in the methylation cycle, is capable of transferring methyl groups only after being activated with ATP to form SAM\(^3\). Recently, nutrition intervention strategy has been suggested for arsenic-exposed populations\(^4\). An earlier report indicates that extracts of seedlings of pea catalyse synthesis of SAM from ATP and methionine, and pea itself is a potent methyl-group transferring agent in the methylation process\(^5\). Thus, as a ready source of methionine, arsenic-exposed rats were supplemented either with a diet containing pea whose protein content is 22.9 g% (ref. 20) or pea and casein. Results suggest that either pea or pea and casein both have recovery influence to help prevent the arsenic-induced toxic effects on pancreatic islet tissue, because the population of islet cells was significantly higher (Figure 1 d and e; Table 4) in these groups of animals compared to arsenic-exposed animals fed only with normal laboratory diet. In favour of such a mechanism are the findings that either pea or pea and casein supplementation is effective to reduce the production of NO and MDA, but it markedly increases the activity of antioxidant enzymes (Table 3). Taken together, these data suggest that pea, as a potent methyl-group transferring agent in the methylation process\(^6\), possibly was the crucial factor for detoxification of inorganic arsenic in our study and thus to help prevent disorders involving excessive islet cell damage and also to develop an antioxidant defence system, including a rise in the activity of antioxidant enzymes.

**Conclusion**

In conclusion, the data prove the causal relationship between oxidative stress-induced PARP activation and subsequent islet cell loss and demonstrate that NAM, pea or pea and casein have promise as dietary supplements to help prevent disorders involving damage and loss of islet cells. This study suggests a new direction for physiological management of arsenic toxicity.

RESEARCH ARTICLES


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