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Synergistic effect of folic acid and vitamin B_{12} in ameliorating arsenic-induced oxidative damage in pancreatic tissue of rat

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Abstract

The efficacies of two nutritional factors, folic acid and vitamin B_{12}, were assessed in this study against arsenic-induced islet cellular toxicity. Rats were divided into four groups consisting of five rats in each group: Group A, control; Group B, arsenic-treated; Group C, arsenic+folic acid; and Group D, arsenic+folic acid+vitamin B_{12}. The dose of arsenic, folic acid and vitamin B_{12}, respectively, was 3 mg, 36 μg and 0.63 μg kg^{-1} body weight day^{-1} for 30 days. Results showed that, compared to control group, there was a significant increase in the levels of nitric oxide (NO), malondialdehyde (MDA) and hydroxyl radical (OH^-) formation in the pancreatic tissue of arsenic-treated rats, while the activity of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), and cellular content of antioxidant glutathione (GSH) were low in these animals. The serum level of tumor necrosis factor-α (TNF-α) and IL-6 was significantly high in these animals. Light microscopic examination showed a marked fall in the number of islet cells. Concomitant administration of either folic acid or folic acid and vitamin B_{12} with arsenic significantly restored all these parameters. Although folic acid alone could not restore the normal level of TNF-α and IL-6, combined folic acid and vitamin B_{12} could restore it. Folic acid and vitamin B_{12} combined also could recover islet cell count. These results suggest that folic acid+vitamin B_{12} are capable of reducing arsenic-induced cellular oxidative and inflammatory toxic changes. Thus, supplement with vitamin B_{12}+folic acid may be predicted as a possible nutritional management strategy against arsenic-induced toxicity.

Keywords: Arsenic trioxide; Pancreatic oxidative stress; Islet cell atrophy; Folic acid+vitamin B_{12}

1. Introduction

Arsenic contamination has reached an alarming proportion in various districts of West Bengal, an eastern state of India, and in Bangladesh, so much so that it has been earmarked as "the biggest arsenic calamity in the world" [1–4]. A large number of people from this area are endemically exposed to arsenic-contaminated groundwater due to drinking of tube well water containing arsenic level above World Health Organization's maximum permissible limit of 50 μg/L [5].

Exposure to arsenic compounds is associated with several human diseases, including Blackfoot disease, hypertension and cancers of the skin, lung, bladder and liver [6]. According to several survey reports, exposure to inorganic arsenic compounds may be associated with development of diabetes mellitus [7–10]. A recent report from this laboratory has confirmed this observation in experimental studies with rabbit [11]. Oxidative stress has been implicated in arsenic-induced cytotoxicity and genotoxicity [6], and also in a wide variety of human diseases and syndromes including diabetes mellitus [12,13]. Besides, a positive correlation between arsenic-induced nitric oxide (NO) production and oxidative stress has been reported earlier [6]. Further, it has been reported that generation of reactive oxidants during arsenic metabolism can play an important role in arsenic-induced injury. These studies suggest involvement of oxidative stress in the pathogenic effects of arsenic exposure [14–16].

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 arsinic acid (MMA) and dimethyl arsonic acid (DMA) has been believed by many to be the major mechanism for detoxifying inorganic arsenic,

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although there exist considerable differences between species and individuals [17]. Arsenic metabolism involves methylation, so nutritional factors with methylation capacity might influence the toxicity of arsenic [18].

The biochemical role of folate in DNA synthesis, repair and methylation is well established [19]. Folate is an essential cofactor in the generation of endogenous methionine [20], which is the source of methyl group in the methylation process. It has also been reported that vitamin B_{12} acts as a methionine synthase enzyme to add methyl group in the methylation process [21].

In view of the rapid spread of various diseases arising out of arsenic contamination (e.g., arsenical dermatitis, keratosis, oedema, gangrin, etc.), in different areas of West Bengal and also in adjoining Bangladesh [3,4,22,23], some effort has been made to provide arsenic-free drinking water (purified through chemical plants) to people living in the arsenic-contaminated zones, but this has proved to be grossly inadequate so far [24]. From the vastness of the problem and new reports of arsenic contamination still emerging from other parts of Southeast Asia [25–27], Slovakia [28], Mexico [29], a disastrous situation seems to loom large unless a tremendous all-out effort is urgently made to bring the situation under control. The problem became more complex because of the poor health and hygiene conditions and the relatively low affordability of the greater majority of population living in these zones and for the lack of awareness of the possible consequences of arsenic intoxication in most of them. In addition, malnutrition, poor socioeconomic conditions and illiteracy have also been implicated in arsenic-induced toxicity [30].

Considering the ever-increasing public health problems associated with oral exposure of inorganic arsenic, we could recognize the need to examine whether nutritional intervention may be an effective strategy of detoxification to help prevent disorders and pancreatic islet cell damaging effects on exposure to arsenic.

2. Materials and methods

2.1. Animal model

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, 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.

2.2. Experimental design

For experiments, rats were randomly selected into four groups consisting of five rats in each: Group A, control; Group B, arsenic-treated; Group C, arsenic+folic acid; Group D, arsenic+folic acid+vitamin B_{12} supplemented. The animals of all groups were provided with a control diet composed of 71% carbohydrate, 18% protein, 7% fat and 4% salt mixture [31]. For chronic oral exposure to arsenic, a dose was selected, 3 mg kg^{-1} body weight day^{-1}, which is within the range of LD_{50} of a 70-kg body weight human (1–4 mg kg^{-1}) and lesser than one-thirteenth of LD_{50} value of rats (40 mg kg^{-1}) [32]. Animals of Group B were orally treated with arsenic trioxide (As_2O_3; 3 mg kg^{-1} body weight day^{-1} for 30 days), and animals of Groups C and D, respectively, were orally treated simultaneously one after another with arsenic and folic acid (36 μg kg^{-1} body weight day^{-1} for 30 days), and arsenic, folic acid and vitamin B_{12} (0.63 μg kg^{-1} body weight day^{-1} for 30 days) [33]. To overcome the impact of any altered food intake, animals of Group A were pair-fed with experimental groups B, C and D.

2.3. Serum preparation

After the treatment period was over (30 days), the animals of all groups were anaesthetized and sacrificed by cervical dislocation, which is one of the recommended physical methods of euthanasia by the IAEC. Blood was drawn from the heart, and serum was separated for the assay of TNF-α and IL-6.

2.4. Preparation of enzyme extracts

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. [34]; the resulting supernatant of the first homogenate was retained as the source of enzyme.

For catalase (CAT) estimation, the tissue was homogenized in ice-cold isotonic phosphate buffer. The homogenate was processed according to the method of Cohen et al. [35]. 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 CAT estimation. For estimation of hydroxyl radical, the tissue was homogenized in phosphate buffer saline, pH 7.4 [36].

2.5. Measurements

The role of nitric oxide synthase (NOS) was indirectly assessed by estimating the amount of NO production. Nitric oxide decomposes rapidly in aerated solutions to form stable nitrite/nitrate products. In our study, nitrite accumulation was estimated by Griess reaction [37] and was used as an index of NO production. The amount of nitrite in the sample (micromolar 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 [38]. The amount of MDA formed was quantitated with TBA and used as an index of lipid peroxidation. The results were expressed as nanomoles of MDA per milligram of protein using molar extinction coefficient (1.56×10^5 cm^2 $mmol^{-1}$).

Hydroxyl radical was assayed by monitoring the hydroxylation of salicylate by Fe^{3+}–ascorbate–H_2O_2 system [36]. Absorbance at 510 nm was measured in a UV-Double Beam Spectrophotometer (Shimadzu 160A). The amount of hydroxyl radical in the sample (micromolar unit) was calculated from a standard curve using 2,3-dihydroxybenzoate (2,3-DHB), a hydroxylation product of salicylate and an indicator of hydroxyl radical formation.

Superoxide dismutase was assayed according to the method of Misra and Fridovich [39]. The change in absorbance due to the conversion 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). The unit of enzyme activity is defined as the enzyme required giving 50% inhibition of autooxidation of epinephrine. Catalase was assayed by the method of Cohen et al. [35]. The enzyme-catalyzed decomposition of H_2O_2 was measured at 480 nm in a UV-Double Beam Spectrophotometer (Shimadzu 160A). Glutathione (GSH) was estimated according to the method of Ellman [40].

The protein content of homogenates used for the study was determined essentially by following the method described by Lowry et al. [41].

Serum TNF-α level was estimated by using the CYTELISA rat TNF-α kit obtained from Cytimmune Sciences (Maryland, USA). All samples were assayed in duplicate. The intra-assay variation was 6.7%. To avoid interassay variation, all samples were run at one time.

Serum IL-6 level was estimated by using the ELISA Quantikine rat IL-6 immunoassay kit obtained from R&D Systems (Minneapolis, USA). All samples were assayed in duplicate. The intra-assay variation was 5.5%. To avoid interassay variation, all samples were run at one time.

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- to 5-μm-thin sections were cut with a high-precision microtome (IEC Minotome, USA), and routine microscopic slides were prepared. Haematoxylin and eosin-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 a stage micrometer to give a comparative value for diameter of islets under observation (40× magnification). The mean diameter of islet represents the mean of maximum diameter and a diameter at right angle to it [11,42]. For counting the number of cells present per islet in different groups of animals, only equal size islets were considered.

2.6. Statistics

Data were expressed as mean±S.E. Kruskal–Wallis nonparametric ANOVA test was performed to find whether or not scores of different groups differ significantly. To test intergroup significant difference, Mann–Whitney *U* multiple comparison test was performed. SPSS-10 was used for statistical analysis. Differences were considered significant if $P<.05$.

3. Results

The increased production of NO by chronic oral exposure to As_2O_3 and its recovery by either folic acid or folic acid and vitamin B_{12} combined supplementation are shown in Table 1. Results indicate that, as compared to control, nitrite accumulation, an indicator of NO production, was increased significantly ($P<.01$) in arsenic-treated animals by 94%. Folic acid supplementation in these animals could significantly ($P<.05$) reduce production of nitrite by 42%, while folic acid and vitamin B_{12}

Table 1
Effects of folic acid (36 μg kg^{-1} body weight day^{-1} for 30 days) and folic acid+vitamin B_{12} (0.63 μg kg^{-1} body weight day^{-1} for 30 days) combined supplementation on As_2O_3 (3 mg kg^{-1} body weight day^{-1} for 30 days)-induced changes in the production of NO, MDA and OH^- in pancreatic tissue of rat

Parameter studied	Control (A)	As-treated (B)	Percent increase	As+folic acid (C)	Percent restored	As+folic acid+vitamin B_{12} (D)	Percent restored	Significance level[a]	Significance level[b]			
									A vs. B	B vs. C	B vs. D	C vs. D
NO (μmol^{-1} mg protein)	1.95±0.15	3.78±0.31	94	3.02±0.29	42	2.35±0.08	78	$P<.05$	$P<.01$	$P<.05$	$P<.01$	$P<.05$
MDA ($nmol^{-1}$ mg protein)	2.29±0.12	4.22±0.17	82	3.02±0.11	62	2.50±0.11	89	$P<.05$	$P<.01$	$P<.01$	$P<.01$	$P<.01$
OH^- (2,3-DHB) (μmol^{-1} mg protein)	65.0±2.23	148.0±6.05	128	126.0±11.69	27	90.0±6.84	70	$P<.01$	$P<.01$	$P<.05$	$P<.01$	$P<.01$

Values are expressed as mean±S.E. ($n=5$).
[a] Significance based on Kruskal–Wallis test.
[b] Significance based on Mann–Whitney *U* multiple comparison test.

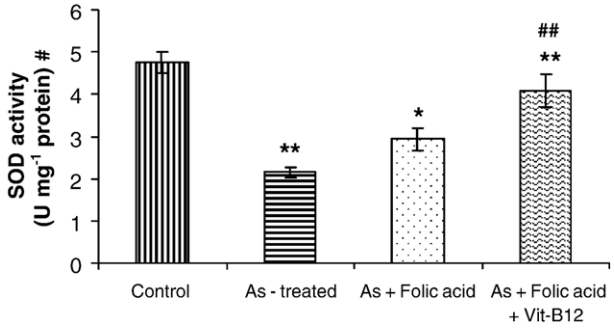

Fig. 1. Effects of folic acid (36 μg kg^{-1} body weight day^{-1} for 30 days) and folic acid+vitamin B_{12} (0.63 μg kg^{-1} body weight day^{-1} for 30 days) combined supplementation on As_2O_3 (3 mg kg^{-1} body weight day^{-1} for 30 days)-induced change in the activity of SOD in pancreatic tissue of rat. Error bar represents mean±S.E. ($n=5$). # Significance based on Kruskal–Wallis test ($P<.01$). Significance based on Mann–Whitney U multiple comparison test: **control vs. arsenic ($P<.01$); *arsenic vs. arsenic+folic acid ($P<.05$); **arsenic vs. arsenic+folic acid+vitamin B_{12} ($P<.01$); ## arsenic+folic acid vs. arsenic+folic acid+vitamin B_{12} ($P<.05$).

combined could produce much better recovery by 78% ($P<.01$) (Table 1).

The increased production of MDA by chronic oral exposure to As_2O_3 and its recovery by either folic acid or folic acid and vitamin B_{12} combined supplementation are shown in Table 1. Results indicate that MDA production, an indicator of lipid peroxidation, was excessive ($P<.01$) in arsenic-treated animals and was increased by 82% as compared to control. Folic acid supplementation in these animals could significantly reduce the production of MDA by 62% ($P<.01$), while folic acid and vitamin B_{12} combined could produce further recovery by 89% ($P<.01$) (Table 1).

The increased production of hydroxyl radical by chronic oral exposure to As_2O_3 and its recovery by either folic acid or folic acid and vitamin B_{12} combined supplementation are

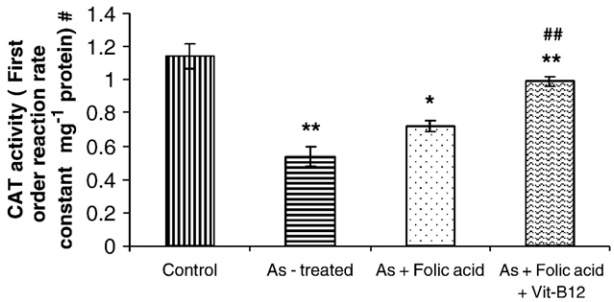

Fig. 2. Effects of folic acid (36 μg kg^{-1} body weight day^{-1} for 30 days) and folic acid+vitamin B_{12} (0.63 μg kg^{-1} body weight day^{-1} for 30 days) combined supplementation on As_2O_3 (3 mg kg^{-1} body weight day^{-1} for 30 days)-induced change in the activity of CAT in pancreatic tissue of rat. Error bar represents mean±S.E. ($n=5$). # Significance based on Kruskal–Wallis test ($P<.01$). Significance based on Mann–Whitney U multiple comparison test: **control vs. arsenic ($P<.01$); *arsenic vs. arsenic+folic acid ($P<.05$); **arsenic vs. arsenic+folic acid+vitamin B_{12} ($P<.01$); ## arsenic+folic acid vs. arsenic+folic acid+vitamin B_{12} ($P<.01$).

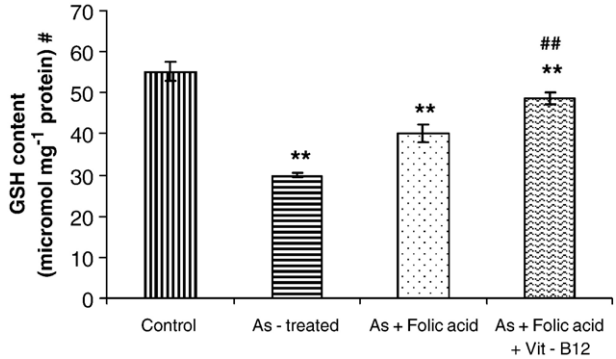

Fig. 3. Effects of folic acid (36 μg kg^{-1} body weight day^{-1} for 30 days) and folic acid+vitamin B_{12} (0.63 μg kg^{-1} body weight day^{-1} for 30 days) combined supplementation on As_2O_3 (3 mg kg^{-1} body weight day^{-1} for 30 days)-induced change in the level of GSH in pancreatic tissue of rat. Error bar represents mean±S.E. ($n=5$). # Significance based on Kruskal–Wallis test ($P<.01$). Significance based on Mann–Whitney U multiple comparison test: **control vs. arsenic ($P<.01$); **arsenic vs. arsenic+folic acid ($P<.01$); **arsenic vs. arsenic+folic acid+vitamin B_{12} ($P<.01$); ## arsenic+folic acid vs. arsenic+folic acid+vitamin B_{12} ($P<.01$).

shown in Table 1. Results indicate that, as compared to control, 2,3-DHB formation, an indicator of hydroxyl radical formation, was increased significantly ($P<.01$) in arsenic-treated animals by 128%. Folic acid supplementation in these animals could significantly ($P<.05$) reduce production of 2,3-DHB by 27%, while folic acid and vitamin B_{12} combined could produce much better recovery by 70% ($P<.01$) (Table 1).

Fig. 1 shows the effects of either folic acid or folic acid and vitamin B_{12} combined supplementation on SOD activity of chronic oral arsenic-exposed rats. Results indicate that, as compared to control, SOD activity was decreased significantly ($P<.01$) in arsenic-treated animals by 55%, while folic acid supplementation in these animals significantly ($P<.05$) recovered the activity of SOD by 30%. However,

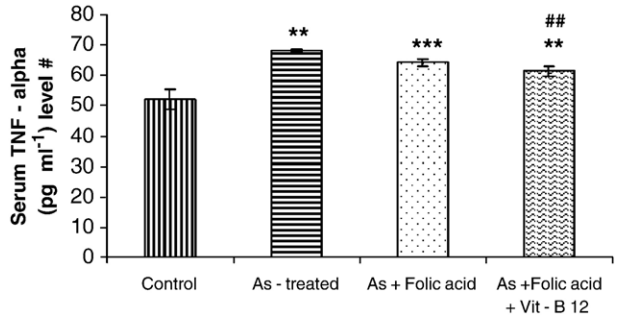

Fig. 4. Effects of folic acid (36 μg kg^{-1} body weight day^{-1} for 30 days) and folic acid+vitamin B_{12} (0.63 μg kg^{-1} body weight day^{-1} for 30 days) combined supplementation on As_2O_3 (3 mg kg^{-1} body weight day^{-1} for 30 days)-induced change in the serum level of TNF-α in rat. Error bar represents mean±S.E. ($n=5$). # Significance based on Kruskal–Wallis test ($P<.01$). Significance based on Mann–Whitney U multiple comparison test: **control vs. arsenic ($P<.01$); ***arsenic vs. arsenic+folic acid (not significant); **arsenic vs. arsenic+folic acid+vitamin B_{12} ($P<.01$); ## arsenic+folic acid vs. arsenic+folic acid+vitamin B_{12} (not significant).

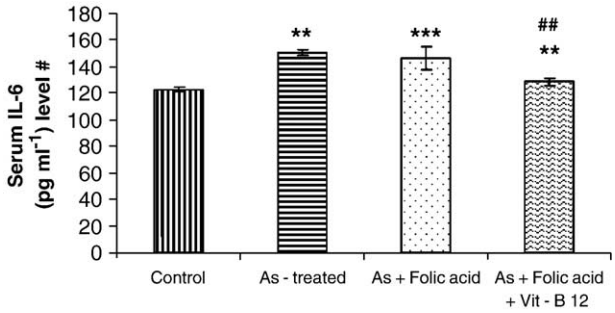

Fig. 5. Effects of folic acid (36 μg kg^{-1} body weight day^{-1} for 30 days) and folic acid+vitamin B_{12} (0.63 μg kg^{-1} body weight day^{-1} for 30 days) combined supplementation on As_2O_3 (3 mg kg^{-1} body weight day^{-1} for 30 days)-induced change in the serum level of IL-6 in rat. Error bar represents mean±S.E. (n=5). # Significance based on Kruskal–Wallis test (P<.01). Significance based on Mann–Whitney U multiple comparison test: **control vs. arsenic (P<.01); ***arsenic vs. arsenic+folic acid (not significant); **arsenic vs. arsenic+folic acid+vitamin B_{12} (P<.01); ## arsenic+folic acid vs. arsenic+folic acid+vitamin B_{12} (P<.05).

folic acid and vitamin B_{12} combined could produce further recovery by 73% in SOD activity (P<.01) (Fig. 1).

Fig. 2 shows the effects of either folic acid or folic acid and vitamin B_{12} combined supplementation on CAT activity of chronic oral arsenic-exposed rats . Results indicate that CAT activity was decreased significantly (P<.01) in arsenic-treated animals by 53% as compared to control. Folic acid supplementation in these animals significantly recovered the activity (P<.05) of CAT by 30%, while folic acid and vitamin B_{12} combined could produce further recovery by 75% (P<.01) (Fig. 2).

Fig. 3 shows the effects of either folic acid or folic acid and vitamin B_{12} combined supplementation on GSH level of chronic oral arsenic-exposed rats. The GSH level was found to be decreased significantly (P<.01) in the arsenic-treated animal by 46% as compared to control. Folic acid supplementation in these animals could significantly restore the level of GSH by 40% (P<.01), while folic acid and vitamin B_{12} combined could produce further recovery by 75% (P<.01) (Fig. 3).

Fig. 4 shows the effects of either folic acid or folic acid and vitamin B_{12} combined supplementation on TNF-α level of chronic oral arsenic-exposed rats. Compared to control, serum TNF-α level was elevated by 31% (P<.01) in arsenic-exposed rats. Folic acid supplementation alone produced statistically insignificant reduction (24%) of TNF-α level in these animals, but folic acid and vitamin B_{12} combined could reduce TNF-α level by 43% (P<.01).

Fig. 5 shows the effects of either folic acid or folic acid and vitamin B_{12} combined supplementation on serum IL-6 level of chronic oral arsenic-exposed rats. Serum IL-6 level was elevated by 23% (P<.01) in arsenic-exposed rats, as compared to control. In arsenic-exposed rats treated with folic acid, there was a reduction in the serum IL-6 level (by 16%), which is not statistically significant, while folic acid and vitamin B_{12} combined could significantly reduce the serum IL-6 level by 79% (P<.01).

Table 2 shows the effects of either folic acid or folic acid and vitamin B_{12} combined supplementation on oral As_2O_3-induced change in the population of islet cells of rat pancreas. Results show that compared to control (Fig. 6A), in similar-sized (μm) pancreatic islets of rats, the number of cells in arsenic-exposed group was markedly low. Percent reduction in cell counts in the islets of arsenic-exposed rats, compared to control, respectively, was 43, 50, 36, 35, 38 and 38 (Fig. 6B). Such reduction in islet cell counts was markedly prevented by supplementation with folic acid, and the percent restored was 47, 33, 42, 64, 52 and 33, respectively (Fig. 6C). In an identical way, combined supplementation with folic acid and vitamin B_{12} showed much better recovery of cell counts and the percent restored, respectively, was 87, 73, 81, 85, 81 and 74 (Fig. 6D).

4. Discussion

Results of the present study show that chronic oral exposure of male rats to As_2O_3 enhanced the production of NO, MDA, OH^-, TNF-α and IL-6, with the concomitant decrease in the activity of SOD and CAT, and level of GSH in the pancreatic tissue of rat. Such enhanced production of reactive oxidants and decrease in the activity of antioxidative enzymes and GSH level were always marked with significant reduction in the total population of islet cells (Fig. 6 and Table 2). It is well documented that pancreatic islet cells are highly susceptible to the toxic effects of

Table 2
Effect of folic acid (36 μg kg^{-1} body weight day^{-1} for 30 days) and folic acid+vitamin B_{12} (0.63 μg kg^{-1} body weight day^{-1} for 30 days) combined supplementation on As_2O_3 (3 mg kg^{-1} body weight day^{-1} for 30 days)-induced reduction in islet cell population of rat pancreas

No. of cells/islet		Percentile decrease	No. of cells/islet	Percent restored against arsenic response	No. of cells/islet	Percent restored against arsenic response
Control	As-treated		As+folic acid		As+folic acid+vitamin B_{12}	
35	20	43	27	47	33	87
60	30	50	40	33	52	73
87	56	36	69	42	79	81
94	61	35	82	64	89	85
128	80	38	105	52	119	81
167	106	38	126	33	151	74

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.

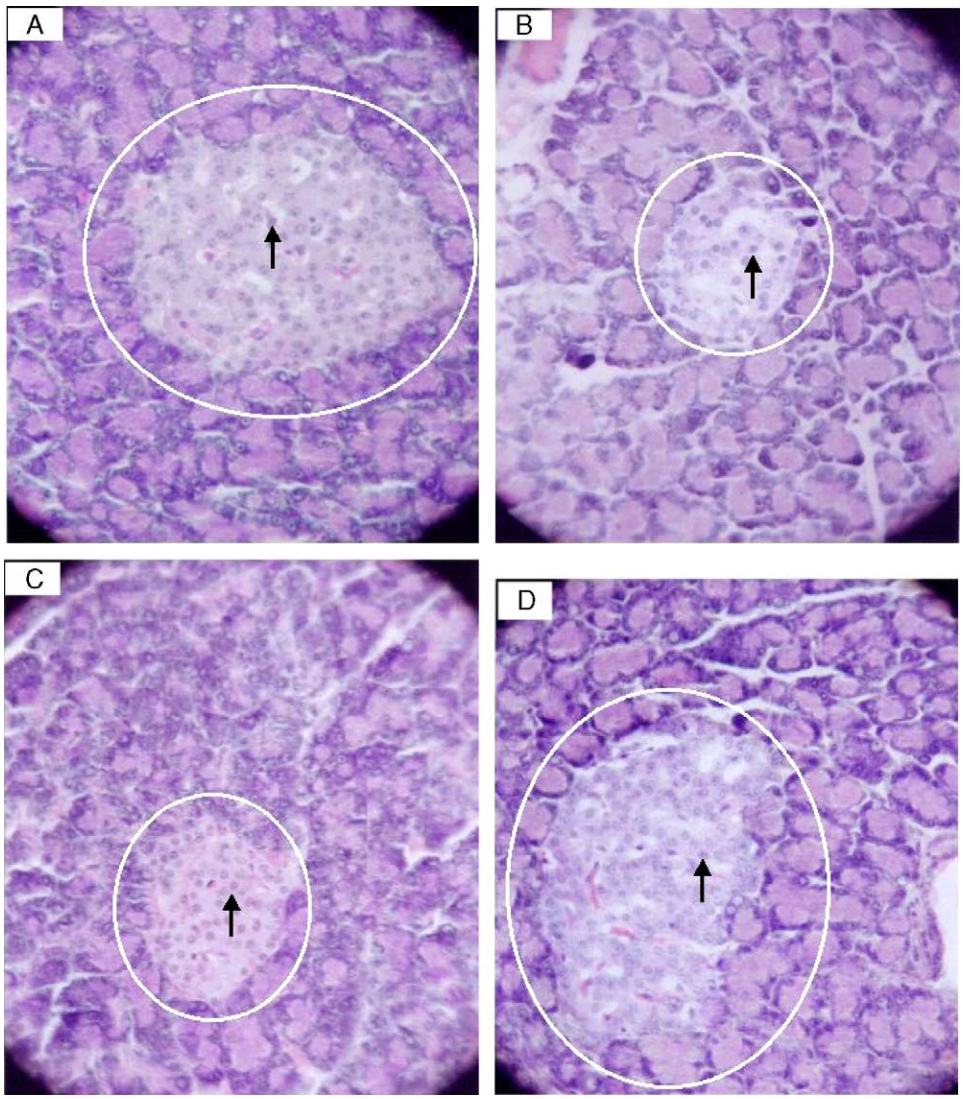

Fig. 6. Representative photomicrographs of haematoxylin and eosin-stained section (×40) showing morphology and population of cells in pancreatic islet of (A) control rat; (B) arsenic-treated rat (3 mg kg^{-1} body weight day^{-1} for 30 days); (C) arsenic+folic acid (36 μg kg^{-1} body weight day^{-1} for 30 days)-supplemented rat; (D) arsenic+folic acid+vitamin B_{12} (0.63 μg kg^{-1} body weight day^{-1} for 30 days)-supplemented rat. In (A), no pathological changes were seen. Normal architecture of pancreatic islet and surrounding acini was vivid. Islet size and cell population were normal. (B) Arsenic treated: typical characteristics of islet cell injury were present. Islet was shrunken (marked by white circle); cell numbers were reduced (marked by black arrows). (C, D) Marked recovery of these cell injury changes respectively by folic acid only and folic acid and vitamin B_{12} (denoted by white circle and black arrows).

reactive oxygen intermediates and NO [43]. In our study, compared to control animals, NO production was increased significantly by 94% in arsenic-treated animals, which is in good correlation with the earlier observation that oxidative stress induced by arsenic may be due to the generation of NO [6]. Compared to control, OH^- in arsenic-treated animals was increased significantly ($P<.01$) by 128%. This result indicates the development of oxidative stress by arsenic treatment as OH^- causes oxidative damage to the adjacent cells and can attack almost every molecule in its vicinity [44]. It is also reported that the extent of damage to the cells by O_2^- and H_2O_2 increases in the presence of the more powerful OH^- [44]. Lipid peroxidation has been identified as one of the basic reactions involved in oxygen free radical-induced cellular damages and used as a marker for free radical-mediated damages [45]. In the present study, MDA formation was also increased significantly by 82% ($P<.01$) in arsenic-treated group. These results suggest that the oral dose of arsenic used in our study was potentially effective for enhanced formation of highly reactive intermediates to cause oxidative stress and to decrease the population of islet cells by oxidative damage as evidenced from our histological observations where the total population of islet cells was decreased in arsenic-treated animals (Fig. 6B). Peripheral blood level of TNF-α and IL-6 was increased significantly ($P<.01$) by 31% and 23%, respectively, in arsenic-treated animals, compared to control. Marked elevation of TNF-α and IL-6 levels is possibly

related to the severity and poor prognosis of arsenic-induced islet cell damage [46,47].

Antioxidant enzymes like CAT, SOD and glutathione peroxidase (GPx) form the first line of defense against ROS, and the decrease in their activities contributes to the oxidative insult on the tissue [48]. Activities of SOD and CAT were also decreased by 55% ($P<.01$) and 53% ($P<.01$), respectively, in arsenic-treated animals as compared to control group. Decrease in the activities of antioxidant enzymes in the present study corroborates well with our earlier study [11], which evidenced that an increased oxidative stress is always accompanied with depleted antioxidative enzymes in the pancreatic islets of arsenic-treated animals.

Nonprotein thiols like GSH are one of the important primary defences that counteract oxidative stress [49]. Earlier [50] it was suggested that reactive intermediates can react with GSH either by a direct chemical reaction or by a glutathione transferase-mediated reaction. If excessive, these reactions can deplete the cellular glutathione. Also, reactive metabolites can oxidize glutathione and other thiol groups, such as those in proteins, and thereby cause a damage in thiol status. We observed a significant reduction in GSH content by 46% ($P<.01$) in the pancreatic tissue of arsenic-exposed rat. The observed decrease may be due to utilization of nonprotein thiols by increased oxygen radicals produced by arsenic exposure. This result is in good agreement with an earlier finding that while exposure of cells occurs to As_2O_3 and causes oxidative stress, glutathione readily conjugates to reduce the cellular GSH level [24]. Therefore, the role of glutathione in cellular protection means that if glutathione level is depleted, the cell is more vulnerable to toxic compounds like As_2O_3. Arsenic is also known to exert at least some of its toxic effects through interaction with sulfhydryl group, and nonprotein sulfhydryl glutathione appears to play an important role in the detoxification of arsenic [51].

As summarized earlier, methylation of arsenic to MMA and DMA has been suggested to be the major mechanism for detoxifying inorganic arsenic [17]. It is an enzymatic process, which is catalysed by methyltransferases that use S-adenosyl methionine (SAM) as a methyl group donor [52]. However, methionine, the source of methyl group in the methylation cycle, is capable of transferring methyl group only after being activated with ATP to form SAM [53]. Deficiencies of folic acid and methionine, two of the major components of methyl metabolism, cause a reduction in SAM. An earlier report reveals that arsenic causes a reduction in SAM level, and this is believed to alter some methylation process [54]. Folate is an essential cofactor in the generation of endogenous methionine [20], which is the source of methyl group in the methylation process. It has also been reported that vitamin B_{12} acts as a methionine synthase enzyme to add methyl group in the methylation process [21]. Folic acid is a free radical scavenger. If present in physiological concentration, it can protect bioconstituents from free radical damage at least by competition, which otherwise can lead to oxidative stress [19]. In spite of being a water-soluble molecule, folic acid can inhibit lipid peroxidation also. The scavenging and repair of thiyl radicals by folic acid make it a potential vitamin to be considered as an antioxidant [19].

Arsenic metabolism involves methylation, so nutritional factors that might influence the toxicity of arsenic include methyl group donors [18]. To verify this possibility, arsenic-exposed rats were supplemented with either folic acid or folic acid and vitamin B_{12} combined . Treatment of arsenic-exposed rats with folic acid or folic acid and vitamin B_{12} combined caused a significant decrease in the production of NO, MDA and OH^- (Table 1). The activities of SOD and CAT were found to increase significantly in these rats (Figs. 1 and 2). We also observed a significant increase in GSH content in arsenic-exposed rats treated with either folic acid or folic acid and vitamin B_{12} (Fig. 3). An insignificant but 24% decrease in TNF-α level (Fig. 4) was observed in arsenic-exposed rats treated with folic acid only. But folic acid and vitamin B_{12} combined supplementation in arsenic-exposed rats, compared to control, showed significant reduction of TNF-α by 43% ($P<.01$) (Fig. 4). Similarly, an insignificant but 16% decrease in IL-6 level (Fig. 5) was observed in arsenic-exposed rats treated with folic acid only. But folic acid and vitamin B_{12} combined supplementation in arsenic-exposed rats, compared to control, showed significant reduction of IL-6 by 79% ($P<.01$) (Fig. 5). Results suggest that either folic acid or folic acid and vitamin B_{12} have recovery influence to help prevent the arsenic-induced toxic effects on pancreatic islets tissue, because the population of islet cells was significantly higher (Table 2; Fig. 6C and D) in these groups of animals compared to arsenic-exposed animals. Taken together, these data suggest that folic acid, as a source for the generation of endogenous methionine, and vitamin B_{12}, acting as methionine synthase enzyme to add methyl group in the methylation process, possibly were the crucial factors for the detoxification of inorganic arsenic in our study and thus helped to prevent disorders involving excessive islet cell damage and also to develop an antioxidant defence system, including a rise in the activity of antioxidant enzymes. Apart from this, the antioxidant and free radical scavenging property of folic acid [19] was possibly responsible, at least in part, for preventing arsenic-induced toxicity in pancreatic islet cells of rat.

In conclusion, the results of the present study convincingly demonstrate that folic acid and vitamin B_{12} combined have promise as nutritional supplements to help prevent disorders involving arsenic-induced damaging effects on pancreatic islets in rats. As supplying arsenic-free drinking water cannot totally rule out chances of arsenic contamination from other sources, the problem of eradicating arsenic-related diseases cannot be addressed through such effort alone. Therefore, folic acid and vitamin B_{12} may be strong candidates for effective use in alleviating arsenic toxicity in

the contaminated areas, at least till better facilities can be made available to the risk-exposed people to combat this problem more effectively by some other tested means.

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