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Research Article

ANTILIPIDEMIC AND CARDIOPROTECTIVE EFFECTS OF VITAMIN B_{12} AND FOLIC ACID AGAINST ARSENIC TOXICITY

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ABSTRACT

The present work elucidates anti-lipidemic and cardioprotective effects of vitamin B12 and folic acid against arsenic-induced cardiotoxicity and the possible mechanisms involved therein. Swiss albino mice were treated intraperitoneally with sodium arsenite at a dose of 7.2 mg/kg b.w./day for a period of 30 days. Oral administration of vitamin B_{12} at a dose of 1 µg/kg/day and folic acid at a dose of 50µg/kg/day was used separately and also in combination in mice for the last 14 days of arsenic treatment. Arsenic treatment significantly altered serum lipid profile as indicated by enhanced level of triglycerides, cholesterol, LDL with decrease in HDL content of serum. The activities of GPT, GOT, γ-GT, CK and uric acid level in serum were elevated markedly, indicating adverse effects of arsenic on cardiac tissue. GSH and total thiol contents were decreased whereas NO, LPO, protein carbonyl content, free .OH radical production increased in arsenic-treated animals. Serum GPT and GOT activities and tissue NADPH oxidase activity increased significantly, whereas GR, GST, GPx, SOD and catalase activities of cardiac tissue decreased after arsenic treatment. In addition, arsenic treatment alters cardiac ultrastructure. Treatment with vitamin B_{12} and folic acid appreciably restored the altered lipid profile and other associated metabolic changes in cardiac tissue to their respective control level, which indicates potential beneficial effects of vitamin B_{12} and folic acid against arsenic-induced cardiotoxicity.

Keywords: Arsenic, cardiotoxicity, vitamin B_{12}, folic acid, cardioprotection.

INTRODUCTION

Arsenic trioxide is highly effective and very commonly used for the treatment of acute promyelocytic leukemia. Various evidences suggest that use of this drug for long time is associated with the formation of cardiotoxicity, mediated through alteration of different lipid and oxidative stress parameters of serum and tissue.[1] Arsenic exposure is associated with significant cardiovascular dysfunctions including atherosclerosis, cardiac conduction disturbance, QT interval prolongation, coronary heart disease, apoptosis in cardiomyocytes, myocardial infarction, hypertension and ischemic heart disease[2-4].

Antioxidants are used to prevent arsenic- induced toxicity. Vitamin B_{12} and folic acid are well known antioxidants. They are widely used to reduce various toxicological effects in different tissues. However, the effectiveness of these antioxidants to reduce arsenic-cardiotoxicity has not yet been evaluated. Folic acid reduces oxidative effects of chronic ethanol consumption in kidney and cardiac tissue.[5] Arsenic-induced oxidative stress is reduced in pancreatic tissue of rat by the use of vitamin B_{12} alone or with folic acid combination.[6] Folate and vitamin B_{12} deficiency also increases oxidative stress in type-2 diabetic patients.[7] But their effect in the protection of arsenic induced cardiotoxicity is not well established. So, the

present study was conducted to investigate the possible influence of arsenic on cardiac antioxidant defense system, serum parameters and histological changes by which it brings about cardiac damage and also to assess the effects of selective nutritional supplements like folic acid, vitamin B_{12} individually or in combination on those parameters related to oxidative stress markers.

MATERIALS AND METHODS

Chemicals and Drugs

Folic acid, vitamin B_{12}, vitamin C, 2-thiobarbituric acid, sodium arsenite ($NaAsO_2$), toluene, butanol, dimethyl sulphoxide, fast blue BB salt, acetic acid, BSA, 5,5-Dithio-bis-2-nitrobenzoic acid, NADPH, GSH, TCA, EDTA, and other chemicals used in the study were of analytical grade and purchased from the Sigma Aldrich, MERCK and SRL. Biochemical Kits such as cholesterol, triglycerides, HDL and uric acid were purchased from Coral clinical systems.

Experimental Design

42 Swiss albino mice weighing 30gm to 40gm were purchased from authorized animal supplier of CPCSEA for this study. They were then divided into six different groups of average body weight and kept in separate cages. They were supplied with standard protein diet and sufficient drinking water and were labelled namely Group A, Group B, Group C, Group D, Group E and Group F. Animals were acclimatized under laboratory conditions for two weeks before starting the experiment in the animal house (1667/GO/a/12/CPCSEA) of Tripura University.

Treatment schedule of the study

The mice were randomly divided into six groups in which one of the mice was taken after the end of treatment schedule from each group for histological study. The treatment schedule for the present study is given below:-

Group A: 0.9% NaCl/day (i.p.) was given to this group for 30 days and was regarded as control group.

Group B: 7.2mg of $NaAsO_2$ /kg/day (i.p.) was given to this group for a period of 30 days and was considered as arsenic-treated.

Group C: The animals of this group were treated with 7.2mg of $NaAsO_2$ /kg/day (i.p.) for 30 days followed by co-administration with vitamin B_{12} at a dose of 1 µg/kg/dayfor last 14 days of arsenic treatment.

Group D: This group was treated with 7.2mg of $NaAsO_2$ /kg/day (i.p.) for 30 days followed by co-administration with folic acid at a dose of 50µg/kg/day for last 14 days of arsenic treatment.

Group E: The animals of this group was treated with 7.2mg of $NaAsO_2$ /kg/day (i.p.) for 30 days followed by co-administration with vitamin B_{12}+folic acid at a dose of their respective doses for last 14 days of arsenic treatment.

Group F: 7.2mg of $NaAsO_2$ /kg/day (i.p.) was given to this group for 30 days followed by co-administration with vitamin C at a dose of 100 mg /kg/day for last 14 days of arsenic treatment.

Animal Sacrifice

Animals were sacrificed after the end of treatment period by cervical dislocation following ether anesthesia. The sacrifice was done according to the guidelines proposed by the Institutional Animal Ethical Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India.

Collection of serum

Blood was collected from all experimental animals from all groups. The samples were collected by puncturing of mice hearts and kept overnight to clot. Serum samples were obtained by centrifuging at 3000g for 10 min.

Collection of heart tissue

Hearts from the experimental animals were quickly excised and washed in ice-cold saline, blotted dry and kept in -40^{0}C until analysis. One heart from each group was kept separately for histological study and other six animals from each group were used for biochemical analysis.

Tissue homogenate preparation

A 5% tissue homogenate of cardiac tissue was prepared in 0.1 M phosphate buffer (pH 7.4) using all glass homogenizer and kept at -40°C until biochemical analysis was performed.

Biochemical Studies

Assessment of serum markers

Collected serum samples were used to determine different serum markers. Creatine kinase activity of serum was assayed by the method of Jacobus and Lehninger.[8] Serum transaminase activities (SGPT & SGOT) associated with cardiac tissue damage was assayed according to the method of Reitman & Frankel.[9] Serum γ -glutamyltranspeptidase activity was determined

according to Sasz.[10] The other serum markers such as cholesterol, triglycerides, HDL and uric acid related to cardiac dysfunction were also evaluated by using standard kits. The LDL cholesterol was determined by using Freidewald's formula,

LDL cholesterol = {total cholesterol – (triglycerides/5) – HDL cholesterol}

Measurement of primary parameter of oxidative stress and endogenous free hydroxyl radical

Protein carbonyl content of cardiac tissue was estimated by using tissue homogenate according to the method of Stadtman and Levine.[11] Reduced glutathione content was measured by the method of Davila *et al*.[12] Lipid peroxidation level in heart was measured by the method of Buege and Aust.[13] Nitric oxide level in heart was measured by the method of Raso *et al.*[14] A study of the free hydroxyl radical formation in the cardiac tissue was carried out according to the method of Babbs and Steiner[15] by using tissue homogenate.

Determination of antioxidant enzyme activities

Tissue NADPH oxidase, a superoxide producing enzyme of heart was studied according to the method of Chen *et al*.[16] Xanthine oxidase activity was assayed according to the method of Bergmeyer *et al.*[17] Glutathione peroxidase (GPX) activity of tissues was assayed by the method as used by Maiti and Chatterjee.[18] Glutathione S-transferase (GST) activity was assayed by the method of Warholm *et al*.[19] SOD activity of heart was determined according to the method of Martin *et al*.[20] Cardiac catalase activity was measured by the method of Aebi.[21] Glutathione reductase activity of the cardiac tissue was measured by the method of Carlberg and Mannervik.[22]

Assay of total thiols

The total thiol (total sulfhydryl groups) content was measured according to the method of Sedlak and Lindsay[23] with some modifications. About 50 µl of the sample was mixed with 0.6 ml of Tris–EDTA buffer, 40 µl of 10 mM DTNB in methanol. The final volume was made up to 1 ml by adding methanol. The reaction mixture was incubated at room temperature for 20 min and the absorbance was measured at 412 nm. The content of total thiols was calculated using molar extinction coefficient of 13,600/M/cm.

Tissue protein content

Proteins were estimated by the method of Lowry *et al*.[24] using bovine serum albumin as the standard protein.

Statistical Analysis

The values were expressed as mean ± SEM. The data were statistically analyzed by using one way ANOVA followed by multiple comparison T test to determine the significance of differences between the two related groups. The $p < 0.05$ was considered statistically significant.

Histopathological Studies

Before doing histopathology, the size, shape and weight of hearts from each of the groups were noted. Then tissues were preserved in 4% buffered formal solution for 24 hours. After proper dehydration in graded alcohol, clearing, impregnation and embedding, tissue sections were prepared by rotary microtome. These sections obtained from the respective six groups were stained using hematoxylin-eosin and examined under high power microscope (40x) and photomicrographs were taken.

RESULTS AND DISCUSSION

Table 1 represents significant ($p^a<0.001$) increase in the triglyceride, cholesterol, LDL, uric acid, CK activity levels, γ –glutamyltranspeptidase (γ-GT) activity and decrease in the HDL level of serum due to arsenic intoxication. Vitamin B_{12}, folic acid separately caused partial restoration of above mentioned parameters and their combination showed better protection in restoring serum triglyceride, cholesterol, HDL, CK and γ-GT activities.

Table 2 represents that GSH and total thiol (TT) contents were decreased whereas NO, LPO, protein carbonyl content (PCC), free .OH radical formation of cardiac tissue were increased in arsenic treated animals. Additive beneficial effects of vitamin B_{12} and folic acid were observed in restoration of these changes caused by arsenic.

Serum GPT and GOT activities and tissue NADPH oxidase activity increased significantly, whereas GR, GST and GPx activities of cardiac tissue decreased due to arsenic treatment (Table 3). All the above mentioned parameters were appreciably restored to their respective control values by combined supplementation of vitamin B_{12} and folic acid.

Figure 1 showed a decrease in SOD activity in arsenic-treated mice. Combined supplementation

of these nutrients showed better protective effect in restoration of cardiac SOD activity.

The catalase activity of cardiac tissue decreased significantly after arsenic exposure, which was restored to its control level by vitamin B_{12} and folic acid supplementation (Fig.2).

The figure 3 represents histopathological changes in cardiac tissue after arsenic toxicity. The nucleus of the cardiac tissue became globular in shape with disorientation of the tissue architecture after arsenic treatment. Vitamin B_{12} and folic acid separately exhibited partial counteractive effects to restore tissue architecture, whereas their combined supplementation restored much better the tissue organization as control heart.

Sub-acute exposure of arsenic altered the lipid profile as reflected by enhanced production of triglycerides, cholesterol, LDL with the decrease in the level of HDL of serum. Lipids play an important role in development of cardiovascular diseases. Changes in lipid metabolism alter the cardiac functions by changing the structure, composition and stability of cellular membranes leading to formation of diseases like hypercholesterolemia, hypertriglyceridaemia, ischemia etc.[25] In the present study, significant alteration in the serum levels of lipids occur which may be correlated with alteration in cardiovascular metabolic homeostasis in arsenic intoxicated animals. Treatment with vitamin B_{12} and folic acid appreciably restored most of the lipid profile to their respective control level, which indicates protection against arsenic-induced metabolic stress to cardiac tissue. The activities of GPT, GOT, γ-GT, CK and uric acid level in serum were elevated markedly, indicating cardiac tissue damage after arsenic treatment. Insufficient glucose or oxygen supply causes rupture of cardiac tissue and liberation of GPT, GOT and CK in blood leading to myocardial damage.[26] The serum γ-GT one of the stress markers is associated with the formation of atherosclerosis, arterial stiffness and cardiovascular mortality.[27] Increased uric acid formation in arsenic-treated mice potentiates the cardiovascular mortality. This may be due to enhanced activity of xanthine oxidase that is associated with the formation of superoxide radical (O2.-) by the oxidation of hypoxanthine/xanthine to uric acid.[28] Vitamin B_{12} and folic acid normalize those enzyme activities and thereby protects cardiac tissue from oxidative damage.

The production of free $^{\bullet}$OH radicals, PCC, NO and LPO levels and activities of enzymes like XO and NADPH oxidase were elevated after arsenic treatment. Increase generation of .OH causes cellular oxidative damage. NADPH oxidase is also associated with the generation of ROS leading to cardiac tissue damage.[29] Other stress parameters such as total thiol, GSH and activities of antioxidant enzymes like GR, GST, GPx, catalase and SOD of cardiac tissue decreased due to arsenic intoxication. Such alterations of these parameters are associated with the formation of oxidative stress mediated myocardial injury.[30-31] Supplementation of vitamin B_{12} or folic acid in combination shows appreciable protective effects in restoring all of these parameters. Histopathological studies also revealed that arsenic causes abnormal ultra structural changes in cardiac tissues, which can be restored by combined supplementation of vitamin B_{12} and folic acid.

Table 1: Changes in Triglycerides, Cholesterol, HDL, LDL, UA (uric acid) level, creatinine kinase (CK) and γ –GT (γ –glutamyltranspeptidase) activity in serum of mice due to exposure of arsenic with vitamin B_{12}, folic acid and their combination.

Groups	Triglycerides (mg/dl)	Cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	UA (mg/dl)	CK (U/L)	γ –GT (mU/ml)
Control (6)	212.62±16.4	158.77±5.45	71.34±2.27	44.90±7.03	8.66±0.09	323.30±12.58	46.30±4.89
As (6)	399.61±15.30 p^{a***}	288.52±9.28 p^{a***}	39.25±1.54 p^{a***}	169.36±7.53 p^{a***}	11.26±0.13 p^{a***}	555.5±24.3 p^{a***}	88.89±4.44 p^{a***}
As +B_{12} (6)	332.35±14.09 $p^{a***}p^{b*}$	203.14±7.57 $p^{a**}p^{b***}$	57.17±1.68 $p^{a**}p^{b*}$	79.50±8.88 $p^{a*}p^{b***}$	7.12±0.13 $p^{a\#}$ p^{b***}	383.3±11.74 $p^{a*}p^{b***}$	65.15±3.79 $p^{a\#}$ p^{b**}
As +FA (6)	214.79±8.99 $p^{a\#}p^{b***}$	226.30±4.57 $p^{a***}p^{b***}$	55.84±2.13 $p^{a**}p^{b*}$	127.5±7.07 $p^{a***}p^{b***}$	8.63±0.08 $p^{a\#}$ p^{b***}	397.18±18.89 $p^{a*}p^{b***}$	68.69±3.72 p^{a*} p^{b**}
As + B_{12} +FA (6)	153.85±8.95 $p^{a*}p^{b***}$	183.23±5.76 $p^{a\#}p^{b***}$	68.22±1.58 $p^{a\#}p^{b**}$	84.23±8.14 $p^{a**}p^{b***}$	9.01±0.13 $p^{a\#}$ p^{b***}	291.08±16.13 $p^{a\#}p^{b***}$	55.56±4.44 $p^{a\#}$ p^{b***}
As +Vit. C (6)	222.2±12.6 $p^{a\#}p^{b***}$	177.81±7.11 $p^{a\#}$ p^{b***}	66.67±2.71 $p^{a\#}$ p^{b***}	66.69±5.84 $p^{a*}p^{b***}$	8.65±0.17 $p^{a\#}$ p^{b***}	347.74±9.87 $p^{a\#}p^{b***}$	59.27±4.06 $p^{a\#}$ P^{b***}

Values are Means±S.E.M. p^a compared with control group.
p^b compared with arsenic-treated group. *** indicates $p<0.001$, ** indicates $p<0.01$, * indicates $p<0.05$ and # indicates $p>0.05$.

Table 2: Changes in GSH (reduced glutathione), TT (total thiol), LPO (lipid peroxidation) level, PCC (protein carbonyl content), NO (nitric oxide) content, ·OH radical in cardiac tissue due to exposure of arsenic with vitamin B_{12}, folic acid and their combination.

Groups	GSH (µmol/mg protein)	TT (nmol/mg protein)	LPO (nmol/mg protein)	PCC (nmol/mg prtein)	NO (µM/mg protin)	·OH radical (nM/mg tissue)
Control (6)	29.11±1.71	171.87±9.31	1.85±0.07	9.80±0.42	1.16±0.04	4.45±0.28
As (6)	12.12±0.74 p^{a***}	94.24±6.19 p^{a***}	7.39±0.16 p^{a***}	20.72±0.32 p^{a***}	2.38±0.05 p^{a***}	13.08±0.47 p^{a***}
As +B_{12} (6)	21.28±0.53 p^{a**} p^{b***}	102.75±6.59 p^{a***} $p^{b\#}$	3.96±0.22 p^{a***} p^{b***}	14.66±0.79 p^{a**} p^{b***}	1.88±0.02 p^{a**} p^{b***}	9.71±0.35 p^{a***} p^{b**}
As +FA (6)	25.05±0.41 p^{a*} p^{b***}	104.38±3.42 p^{a***} $p^{b\#}$	3.10±0.06 p^{a**} p^{b***}	14.40±0.45 p^{a**} p^{b***}	1.79±0.07 p^{a***} p^{b***}	7.98±0.56 p^{a***} p^{b**}
As + B_{12} +FA (6)	29.09±1.17 $p^{a\#}$ p^{b***}	142.71±8.8 p^{a***} p^{b***}	1.77±0.07 $p^{a\#}$ p^{b***}	11.43±0.29 p^{a*} p^{b***}	1.39±0.05 p^{a**} p^{b***}	5.67±0.45 $p^{a\#}$ p^{b***}
As +Vit. C (6)	29.92±0.91 $p^{a\#}$ p^{b***}	124.94±3.77 p^{a***} p^{b***}	1.94±0.11 $p^{a\#}$ p^{b***}	11.17±0.63 p^{a*} p^{b***}	1.61±0.03 p^{a***} p^{b***}	4.60±0.27 $p^{a\#}$ p^{b***}

Values are Means±S.E.M. p^a compared with control group.
p^b compared with arsenic-treated group. *** indicates $p<0.001$, ** indicates $p<0.01$, * indicates $p<0.05$ and # indicates $p>0.05$.

Table 3: Effects of vitamin B_{12}, folic acid and their combination on arsenic-induced alteration of GPT, GOT of serum and XO (xanthine oxidase), NADPH(O), GR (glutathione reductase), GST (glutathione S-transferase), GPX (glutathione peroxidase) activities of cardiac tissue

Groups	GPT (U/dl)	GOT (U/dl)	XO (µmol/min/mg protein)	NADPH(O) activity (nmole/min/mg protein)	GR (nmol/min/mg protein)	GST (nmol/min/mg protein)	GPX (nmol/min/mg protein)
Control (6)	35.60±1.07	26.48±1.56	0.540±0.02	630.68±47.19	94.46±1.95	2.74±0.18	183.65±4.86
As (6)	68.83±2.36 p^{a***}	64.73±2.8 p^{a***}	0.767±0.03 p^{a***}	1897.62±113.74 p^{a***}	36.73±1.78 p^{a***}	1.32±0.10 p^{a***}	58.86±1.16 p^{a***}
As +B_{12} (6)	53.73±2.85 p^{a***} p^{b**}	49.48±1.20	0.55±0.01 p^{a*} p^{b***}	1191.91±52.64 p^{a***} p^{b***}	61.26±1.31 p^{a**} p^{b***}	2.32±0.15 $p^{a\#}$ p^{b***}	132.69±3.09 p^{a***} p^{b***}
As +FA (6)	44.38±2.99 p^{a**} p^{b***}	49.62±0.87 p^{a**} p^{b***}	0.51±0.04 p^{a*} p^{b***}	1015.55±66.62 p^{a**} p^{b***}	70.26±2.23 p^{a**} p^{b***}	2.28±0.12 $p^{a\#}$ p^{b***}	149.39±2.93 p^{a***} p^{b***}
As + B_{12} +FA (6)	34.22±2.24 p^{a**} p^{b***}	39.55±1.5 p^{a***} p^{b***}	0.42±0.02 $p^{a\#}$ p^{b***}	696.43±17.98 $p^{a\#}$ p^{b***}	82.58±2.27 p^{a**} p^{b***}	2.48±0.12 $p^{a\#}$ p^{b***}	178.72±3.1 $p^{a\#}$ p^{b***}
As +Vit. C (6)	39.35±1.93 $p^{a\#}p^{b***}$	32.23±2.55 $p^{a\#}p^{b***}$	0.330±0.01 p^{a**} p^{b***}	687.28±28.09 $p^{a\#}$ p^{b***}	82.49±1.56 p^{a**} p^{b***}	2.45±0.13 $p^{a\#}$ p^{b***}	173.79±3.76 $p^{a\#}p^{b***}$

Values are Means±S.E.M. p^a compared with control group.
p^b compared with arsenic-treated group. *** indicates $p<0.001$, ** indicates $p<0.01$, * indicates $p<0.05$ and # indicates $p>0.05$.

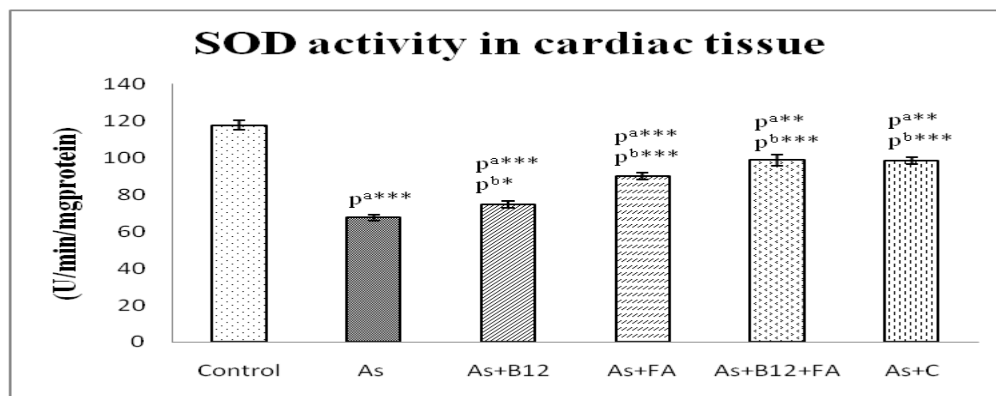

Fig. 1: Effects of co-administration of vitamin B_{12} and folic acid in SOD activity in arsenic-exposed animals

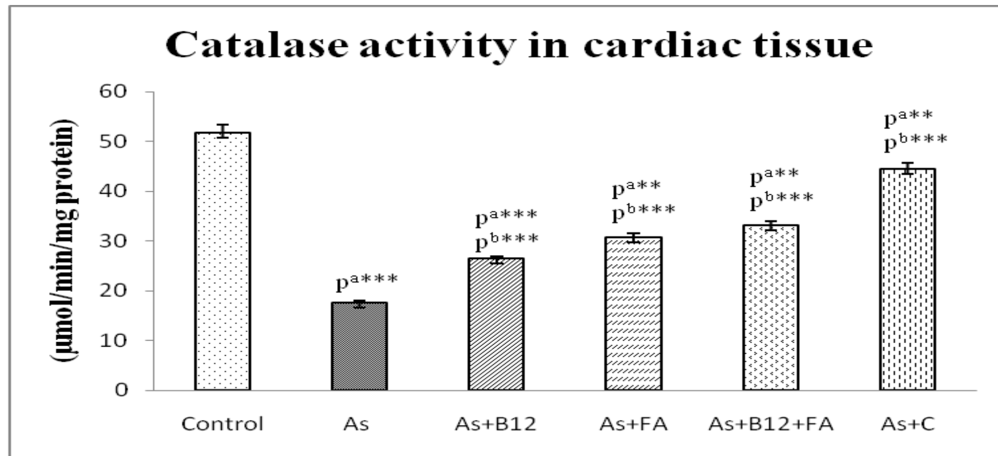

Fig. 2: Catalase activity in arsenic-exposed animals and the effects of co-administration of vitamin B12 and folic acid

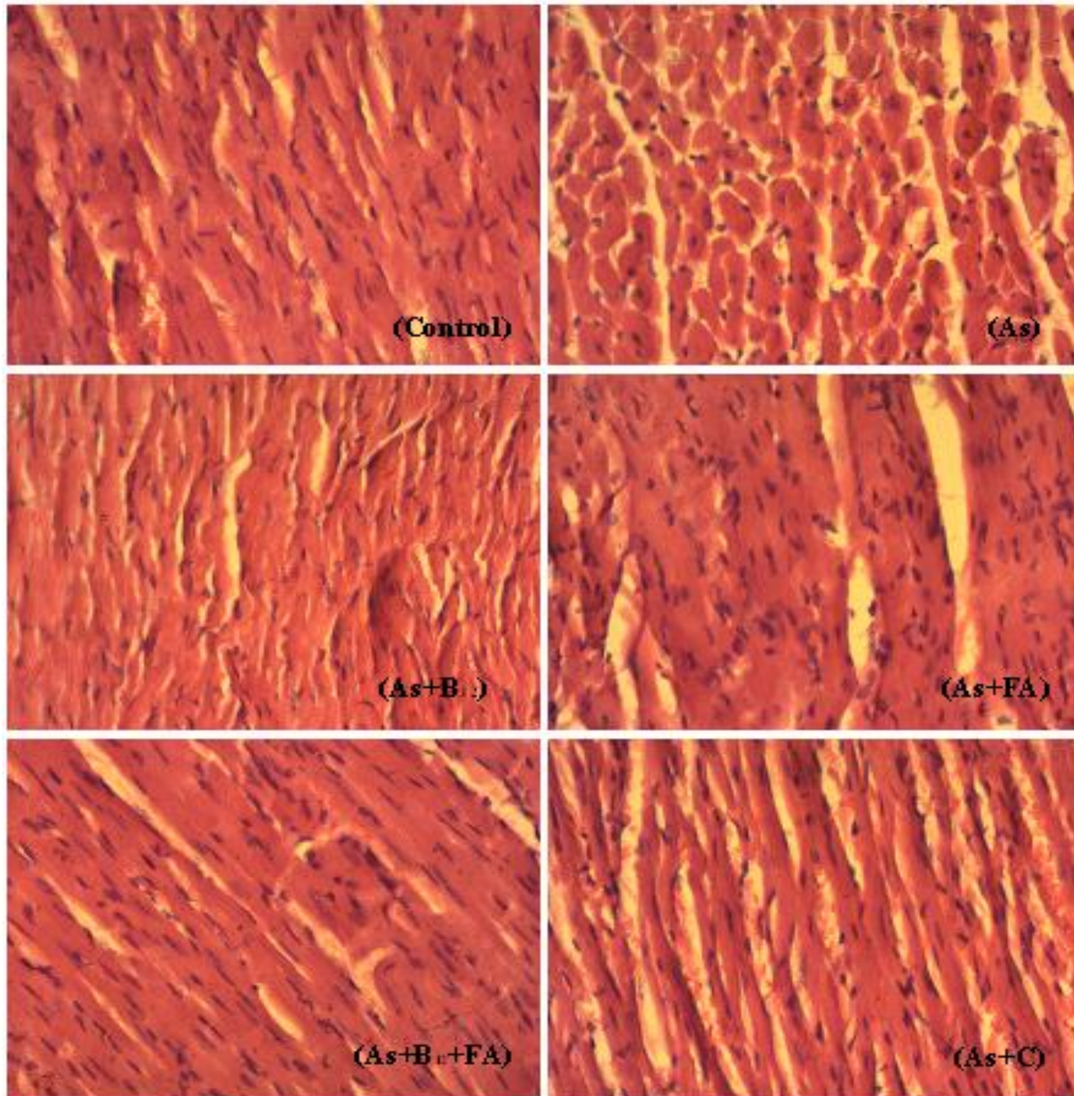

Fig. 3: Representation of the photomicrographs of the histological changes in cardiac tissues of mice has done by staining of the sectioned cardiac tissues with hematoxylin and eosin (x40)

CONCLUSION

From these observations it is suggested that vitamin B_{12} and folic acid, when given in combination exhibits better beneficial effects in restoration of serum lipid profile and associated stress parameters of cardiovascular diseases to protect cardiac tissues from oxidative and metabolic stress mediated dysfunctions of heart. Histopathological findings also support the additive beneficial effects of these two neutraceuticals in protecting cardiac tissue from arsenic-induced cardiotoxicity.

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