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Role of hepatic and pancreatic oxidative stress in arsenic induced diabetic condition in Wistar rats

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Abstract

Arsenic, a potent environmental toxicant has been reported to induce diabetes mellitus, but its potential biological mechanism(s) has not been much investigated. The present study was designed to correlate pancreatic and hepatic oxidative stress with arsenic induced diabetes mellitus in experimental animals. Male albino Wistar rats were administered with low (1.5 mg kg^{-1} b.wt.) and high (5.0 mg kg^{-1} b.wt.) sodium arsenite orally for 4 week. Hyperglycemic condition was observed in arsenic exposed groups as indicated by increased (P<0.001) fasting plasma glucose, glycosylated hemoglobin (HbA1c) and impaired glucose tolerance (IGT), which were accompanied by an increase in the level of lipid peroxidation (P<0.001), protein oxidation (P<0.05 at low dose and P<0.001 at high dose) and nitric oxide (NO) (P<0.001) in hepatic and pancreatic tissue compared to control. Furthermore, superoxide dismutase (SOD) (P<0.001), catalase (CAT) (P<0.001) and glutathione-*S*-transferase (GST) (P<0.05 at low dose and P<0.001 at high dose) activities were elevated, while glutathione peroxidase (GPx) (P<0.05 at low dose and P<0.001 at high dose) and GSH level showed significant (P<0.001) depletion in both studied tissue of arsenic exposed rats compared to control. Arsenic induced hepatotoxicity was manifested by an increase (P<0.001) in serum ALT, AST and ALP. Arsenic exposure leads to accumulation of arsenic (P<0.05) and significant (P<0.05) depletion of copper and zinc level in hepatic and pancreatic tissue as compared to control. Our data suggests that sub-chronic arsenic exposure induces diabetic condition which may be mediated due to increased oxidative stress in hepatic and pancreatic tissue.

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Introduction

Arsenic is one of the most important naturally occurring environmental toxicant and is ubiquitously present in the earth's crust. Millions of people worldwide are suspected to be exposed to arsenic through contaminated drinking water, air and food (Liu *et al.*, 2002). Arsenicals have been accumulated and metabolized in organs like pancreas, liver, kidney, skeletal muscles, adipose tissue and red blood cells (Ademuyiwa and Elsenhans, 2000; Paul *et al.*, 2008). Chronic exposure to arsenic has been mainly related to bladder, kidney, liver, skin and lung cancer (Kitchin, 2001). Clinical manifestation of chronic arsenic poisoning includes non-carcinogenic diseases like skin lesion, hyper Pigmentation, keratosis, non-cirrhotic portal fibrosis, peripheral neuropathy, hypertension, cardiovascular disorder (Guha Mazumder, 2008) and diabetes mellitus (Tseng, 2004; Navas-Acien *et al.*, 2006). The different studies carried out in different ethnic groups during different study periods have shown an association between arsenic exposure and the occurrence of diabetes mellitus (Chen *et al.*, 2007; Navas-Acien *et al*., 2008). The potential biological mechanism of arsenic induced diabetes is not well understood and needs to be explored. Increased oxidative stress in insulin sensitive tissue on arsenic exposure has been associated with impaired glucose homeostasis (Patel and Kalia, 2010). The production of reactive oxygen and nitrogen species by arsenicals are directly involved in oxidative damage to proteins, lipids, DNA and ability to interact with thiol group of enzymes as well as proteins which can lead to cellular toxicity and death (Flora *et al*., 2008).

Diabetes mellitus has been characterized by elevated level of blood glucose, impaired insulin secretion from

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pancreas or impaired insulin action on peripheral tissue as result of insulin resistance which is accompanied by several morphological and biochemical change in liver, pancreas and other tissues. Some non-physiological changes such as oxidative stress particularly in liver and pancreas play an important role in etiology of this metabolic disorder (Kakkar *et al*., 1998).The present study was therefore undertaken to investigate the occurrence of oxidative stress in the pancreatic and hepatic tissues on arsenic exposure by measuring the extent of oxidative damage and the status of the antioxidant defense system which may be implicated with arsenic induced diabetes mellitus.

Materials and Methods

Experimental design : Thirty six male albino rats of Wistar strain (170 ± 25 g) were obtained from animal house of Sardar Patel University, Vallabh Vidya Nagar. They were housed in polypropylene cages under standard laboratory condition of temperature 25 ± 5ºC, relative humidity 50 ± 15% and dark/light photoperiod of 12: 12 hr. The animals were fed with standard food pellets (Amrut feed, Pranav Agro Industries, Pune) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Sardar Patel University. Rats were divided in three groups, comprising twelve animals in each group.

Group-I received 1.0 ml sterile distilled water orally daily and served as control group. Rats of group–II and III were orally administered with arsenic as sodium arsenite at low dose (1.5 mg kg^{-1} b.wt.) and high dose (5.0 mg kg^{-1} b.wt.) daily for a period of 4 week The low and high dose of arsenic were approximately lesser than one-twenty-seventh and one-eighth of LD_{50} (40 mg kg^{-1} b.wt.) value for rats, respectively

Serum, plasma and tissue preparation : At the end of the experimental period of 4 weeks, all animals were sacrificed under light ether anesthesia after overnight fasting. Blood was collected by cardiac puncture with and without EDTA tubes separately. Serum were separated by centrifugation at 600 x g for 15 min. Liver and pancreas of six animals were removed immediately , rinsed with cold saline and blotted with filter paper, stored at -20 ºC for metal (arsenic, zinc and copper) estimation. The pancreatic and liver tissues of remaining 6 animals were homogenized in ice cold 50 mM phosphate buffers (pH 7.4) and centrifuged at 12,000 rpm for 15 min at 4º C. The clear supernant was used for various biochemical analysis. The protein was determined by the method of Lowry *et al*. (1951).

Oral glucose tolerance test (OGTT) : The OGTT was performed in rats after overnight fast, a day before the termination of experiment as describe by Yasuda *et al.* (2002). In brief, rats were administered with glucose (2.0 g kg^{-1} b.wt.) orally with gavage. Blood was drawn from tail vein at 0, 30, 60, 90, 120 and 150 min time interval. The blood glucose level was measured by glucose oxidase/peroxidase enzymatic kit. (Span Diagnostics Ltd., India)

Plasma and serum biochemical parameters: The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities were estimated according to the procedures recommended in commercially available kit. (Span Diagnostics Ltd., India) The plasma glucose was measured by enzymatic GOD/POD kit method and glycosylated hemoglobin was estimated as described previously (Chandalia *et al.*, 1980).

Biochemical analysis in liver and pancreas: Thiobarbituric acid reactive substances (TBARS) was measured according to method describe by Ohkawa *et al*. (1979). The protein carbonyl (PCO) content and advanced oxidation protein products (AOPP), markers of protein oxidation were measured according to method of Reznick and Packer (1994) and Kayali *et al*. (2006) respectively. The stable metabolites of nitric oxide (NO), nitrate (NO_3^-) and nitrites (NO_2^-) were measured with Griess reagent as an index of NO production (Titheradge, 1998). Nitrate was first converted to nitrite by NADPH in the presence of nitrate reductase which was then reacted with Griess reagent containing sulfanilamide and naphthyl-ethylenediamine dihydrochloride (NED) to give a red-diazo dye was measured at 550 nm. The concentration of NO metabolites in sample was determined by comparison with sodium nitrite standard curve and expressed in µmol mg^{-1} of protein. Superoxide dismutase (SOD) activity was measured based on inhibition of NADH-PMS-NBT system according to method described previously (Kakkar *et al.*, 1984). The catalase (CAT) activity was assayed by the method of Sinha (1972). Glutathione peroxidase (GPx) activity was measured according to the method of Rotruck *et al*. (1984). The glutathione-*s*-transferase (GST) activity was determined by the method of Habig *et al.* (1974). The reduced glutathione (GSH) was determined by the method of Ellman with modification of Jollow *et al*. (1974) and expressed as mg of GSH per 100 g tissue using reduced GSH as standard.

Tissue zinc, copper and arsenic estimation: Liver and pancreatic tissue were pooled and digested with acid mixture (H_2SO_4: HNO_3: $HClO_4$ ratio 1:6:1) using a microwave pressure digestion unit. (Berghof speed wave MWS -3+, Germany) The arsenic, copper and zinc content of digested products were determined using flame ionization atomic absorbance spectrophotometer (WinASS novAA400, Analytik Jena AG, Germany) and results were expressed as µg g^{-1} tissue.

Statistical analysis: Data comparison between the different treatment groups and control was carried out by one way analysis of variance (ANOVA) followed by least significant

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difference (LSD) test. Data were expressed as mean ± SE for the six animals in each group. Data were statistically evaluated with SPSS/10 software. Significance at $P < 0.001$ and $P < 0.05$ has been given respective symbols in the tables.

Results and Discussion

The increase in body weight of control and arsenic exposed rats was observed after 4 wk of arsenic exposure, where both arsenic exposed groups showed significantly ($P < 0.001$) less pronounced gain in body mass compared to the control group (Table 1). The FBG and HbA1c were increased significantly ($P < 0.001$) by 33.8 and 54.0% in low dose and 43.8 and 62.0% in high dose of arsenic exposed groups respectively compared to control (Table 1). In addition, increased blood glucose level in arsenic exposed animals indicates hyperglycemic condition has been reported previously (Izquierdo-Vega *et al.*, 2006) could be due to the impaired insulin secretion from ß-cell of pancreas or impaired glucose metabolism in liver. The increased glycosylated hemoglobin in arsenic exposed rats may be due to persistence of high blood glucose which is relatively more precise long term marker of diabetes (Lorenzo *et al.*, 2010). It has been reported that glycosylated hemoglobin (HbA1c) level was increased in blood of workers occupationally exposed to arsenic (Jensen and Hansen, 1998). Result indicate that after oral glucose load, there was a significant difference in peak blood glucose level between control (144.9 mg dl^{-1}), low dose arsenic exposed (192.3 mg dl^{-1}) and high dose arsenic exposed (204.7 mg dl^{-1}) group. The blood glucose level reached the highest at 30 min in control group and at 60 min in both the arsenic exposed groups which was followed by distinct disposal phase (Fig. 1). Before oral glucose load, it was noted that the fasting blood glucose

Table 1 : Body weight, fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c) in rats exposed to arsenic for 4 weeks

Parameters	Group-I (Control)	Group-II (Low dose)	Group-III (High dose)
Initial body weight (g)	190.5 ± 5.1	178.0 ± 4.5	178.8 ± 6.5
Final body weight (g)	241.5 ± 4.2	192.8 ± 3.2**	189.2 ± 3.1**
Body weight gain (%)	26.9	8.1**	6.2**
FBG (mg dl^{-1})			
Before treatment	90.2 ± 1.2	95.7 ± 2.9	89.3 ± 2.8
After treatment	94.1 ± 1.7	125.9 ± 2.4**	135.3 ± 1.6**
HbA1c (%)	4.1 ± 0.2	6.2 ± 0.1**	6.5 ± 0.1**

Value are mean of six replicates ± SE. HD=High dose; Value are statistically significant at **$P < 0.001$ compared to control

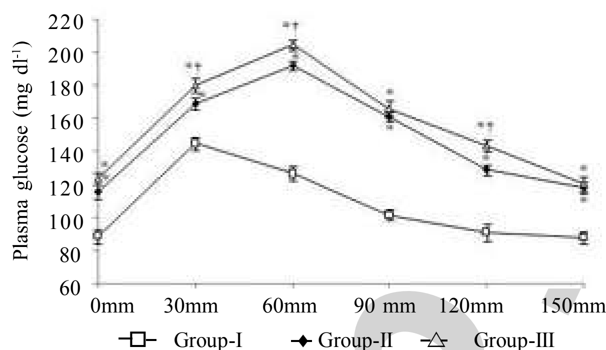

Fig. 1 : Represent the features of oral glucose tolerance test (OGTT) in control (Group-I [□]) and arsenic exposed rats (Group-II [♦]: 1.5 mg kg^{-1} body weight and Group-III [△]: 5.0 mg kg^{-1} body weight). Data have shown mean ± SE (n=12), Value are statistically significant at $^{*}P < 0.05$ compared to control and $^{\dagger}P < 0.05$ compared to low dose group

level was significantly higher in both arsenic exposed groups compared to control. The present study revealed that sub-chronic arsenic exposure induced diabetogenic effect manifested by delayed the disposal of blood glucose and altered normal features of OGTT to impaired glucose tolerance (IGT) consisted with diabetes mellitus which might be mediated due to increased oxidative stress in hepatic and pancreatic tissue. High arsenic levels were used in the present study than commonly found in environmental sources because animal studies require higher arsenic exposure level than humans to produce the diabetogenic effect. Arsenic promotes IGT and changed the normal features of intraperitoneal glucose tolerance test (IPGTT) to a diabetic form (Paul *et al.*, 2007). The observed result could be due to the decreased insulin secretion and/or receptor mediated intracellular transport of glucose, which leads to impaired glucose homeostasis (Yen *et al.*, 2007).

The hepatotoxicity was observed in arsenic exposed animals by an increase in serum AST (31 and 39.64 vs. 22.98), ALT (26.87 and 34.12 vs 17.67) and ALP (175.84 and 191.75 vs. 191.75) activities at low and high dose respectively verses control (Table 2) due to persistence of arsenic exposure, is in agreement with earlier investigations (Gupta and Flora, 2005).

Table 2 : Changes in activities of serum alanine amino transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in rats exposed to arsenic for 4 weeks

Parameters	Group-I (Control)	Group-II (Low dose)	Group-III (High dose)
AST (U l^{-1})	22.98 ± 0.74	31.29 ± 1.29**	39.64 ± 1.23**
ALT (U l^{-1})	17.67 ± 0.74	26.87 ± 0.35**	34.12 ± 0.45**
ALP (U l^{-1})	152.68 ± 2.56	175.84 ± 3.58**	191.75 ± 3.45**

Value are mean of six replicates ± SE. ** Value are statistically significant at $P < 0.001$ compared to controls

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Table 3 : Concentration of thiobarbituric acid reactive substances (TBARS), advanced oxidation protein products (AOPP), protein carbonyl (PCO) and nitric oxide (NO) in pancreatic and hepatic tissue rats exposed to arsenic for 4 weeks

Parameters	Group-I (Control)	Group-II (Low dose)	Group-III (High dose)
TBARS (nmol mg^{-1} of protein)			
Pancreas	0.35 ± 0.01	0.51 ± 0.02**	0.66 ± 0.02**
Liver	0.55 ± 0.02	0.98 ± 0.04**	1.34 ± 0.09**
AOPP (nmol mg^{-1} of protein)			
Pancreas	0.16 ± 0.02	0.21 ± 0.01*	0.25 ± 0.02**
Liver	0.27 ± 0.01	0.31 ± 0.01*	0.48 ± 0.01**
PCO (nmol mg^{-1} of protein)			
Pancreas	1.75 ± 0.13	2.44 ± 0.16*	3.10 ± 0.14**
Liver	1.62 ± 0.02	2.36 ± 0.14*	2.95 ± 0.04**
NO (µmol mg^{-1} protein)			
Pancreas	2.56 ± 0.58	4.86 ± 0.46**	7.21 ± 0.85**
Liver	5.23 ± 0.62	8.04 ± 0.89**	10.71 ± 0.68**

Values are mean of six replictes ± SE. **(P<0.001) and *(P<0.05) significantly different values from the control rats

Table 4 : Reduced glutathione (GSH) level and activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-*S*-transferase (GST) in hepatic and pancreatic tissue rats exposed to arsenic for 4 weeks

Parameters	Group-I (Control)	Group-II (Low dose)	Group-III (High dose)
SOD (U mg^{-1} protein)			
Pancreas	0.46 ± 0.05	0.74 ± 0.07**	0.94 ± 0.05**
Liver	1.08 ± 0.07	1.72 ± 0.05**	2.22 ± 0.07**
CAT (U mg^{-1} protein)			
Pancreas	31.37 ± 2.04	42.55 ± 3.73**	50.71 ± 5.65**
Liver	54.05 ± 3.22	72.39 ± 2.52**	85.53 ± 4.82**
GPx (U mg^{-1} protein)			
Pancreas	6.75 ± 0.24	5.72 ± 0.15*	4.61 ± 0.32**
Liver	11.61 ± 0.23	9.57 ± 0.24*	7.27 ± 0.58**
GSH (mg $100g^{-1}$ tissue)			
Pancreas	22.25 ± 0.92	17.64 ± 0.67**	14.09 ± 0.36**
Liver	33.96 ± 0.67	27.03 ± 0.41**	23.22 ± 0.57**
GST (U mg^{-1} protein)			
Pancreas	0.22 ± 0.03	0.32 ± 0.02*	0.38 ± 0.03**
Liver	0.63 ± 0.02	0.82 ± 0.04*	0.94 ± 0.05**

Value are mean of six replicates ±SE. **(P<0.001) and *(P<0.05) significantly different values from the control rats

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Table 3 shows the levels of TBARS, PCO and AOPP and NO in pancreatic and hepatic tissue of control and arsenic exposed groups. Lipid peroxidation was enhanced in hepatic and pancreatic tissue of arsenic exposed rats at both dosages, as evidenced by significant increase in production of TBARS. In arsenic exposed rats, AOPP and PCO were increased significantly ($P < 0.05$ at low and $P < 0.001$ at high dose) in both studied tissue compared to control. The observed increased level of TBARS in arsenic

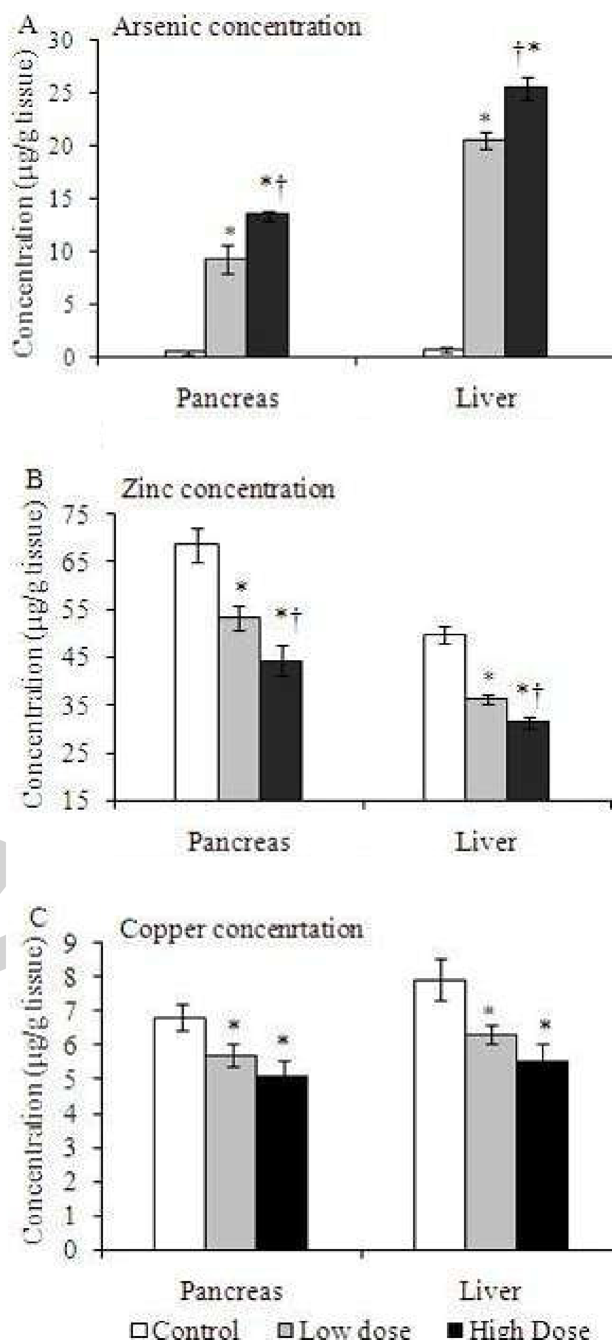

Fig. 2 : Represents (A) arsenic accumulation (B) zinc and (C) copper concentration in pancreatic and liver tissue of control and arsenic exposed rats. Value were shown as mean ± SD. Value are statistically significant at *$P < 0.05$ compared to control and †$P < 0.05$ compared to low dose group

exposed rats may play a role in pancreatic and hepatic damage, which are in agreement with previous observation that arsenic exposed rats have shown marked increase lipid peroxidation in liver (Gupta and Flora, 2005) and pancreas (Izquierdo-Vega *et al.*, 2006). Increased accumulation of PCO and AOPP in both studied tissue of arsenic exposed rats leads to oxidative protein damage which may be involved in

ß-cell damages and development of insulin resistance (Yazdanparast *et al*., 2007). It is well recognized that increased lipid peroxidation and protein oxidation have been implicated in development of diabetes mellitus (Maritim *et al*., 2003). In both arsenic exposed groups, total NO production was observed to be (*P* <.0.001) higher in both of the studied tissue in comparison to control (Table 3). Arsenic induced increased NO production in studied tissue might reduces total population of islet cells pancreas (Mukherjee *et al.,* 2003) and decrease glycogen synthesis and increase glycogen phosphorylase activity in hepatocytes (Sprangers *et al*., 1998) implicated in development of arsenic induced diabetes mellitus.

SOD and CAT activities in hepatic and pancreatic tissue were elevated significantly ($P < 0.001$) in both arsenic exposed groups compared to control (Table 4). The increased SOD and CAT activities in pancreatic and liver tissue of arsenic exposed rats might be to overcome raised oxidative stress by arsenic. Arsenic induced oxidative stress lead to increased endogenous antioxidants enzymes which may disturbs glucose stimulated ROS signals and inhibits glucose stimulated insulin secretion (GSIS) in pancreatic β-cells (Fu *et al*., 2010). Hepatic and pancreatic GSH level was significantly ($P < 0.001$) reduced on arsenic administration compared to control. It has been observed that pancreatic tissue showed greater (36%) decline in GSH level than hepatic tissue (32%) at high dose of arsenic. Arsenic administration significantly decreased GPx activity in both studied tissue ($P < 0.01$ at low dose and $P < 0.001$ at high dose) compared to control suggesting hepatic and pancreatic oxidative stress (Table 4). Additionally, pancreatic and hepatic GST activity increased significantly ($P < 0.05$ at low dose and $P < 0.001$ at high dose of arsenic) at both dosages of arsenic compared to control. The observed decrease GSH level in both studied tissue may be due to utilization of non-protein thiols by increased ROS under arsenic induced oxidative stress. Arsenic is known to bind with sulfhydryl groups of protein and nonprotein sulfhydryl glutathione (GSH) which play an important role in antioxidant defense and in metabolic detoxification (Hultberg *et al.*, 2001). It is well documented that GSH is essential for insulin action. Depletion of GSH could affect the insulin action on hepatic cell lead to insulin resistance (Guarino *et al.,* 2003). Decrease pancreatic and hepatic GPx activity may be due to reduction in GSH level and increase in level of lipid peroxides. The lower antioxidant enzyme activities in pancreas compared with liver found in present study is consistent with previously published reports (Kajimoto and Kaneto, 2004; Kakker *et al.*, 1998) suggest increased susceptibility of pancreatic tissue to arsenic induced oxidative damage and may lead to decreased insulin secretion. The results of our study demonstrate the occurrence of oxidative damage in pancreas and liver by disturbing prooxidant/antioxidant status as suggested by altered level of different antioxidant indices on arsenic exposure. Arsenic induced oxidative stress by disturbing the pro/antioxidant balance play prominent roles in manifestation of diabetes and other disorder (Flora *et al*. 2011). The earlier reports suggested that arsenic generates ROS and free radicals results in oxidative damage (Liu *et al.*, 2001) which might be an important contributory factor in development of arsenic induced diabetes mellitus. Experimental and clinical studies suggest that increased oxidative stress is a widely established contributor in the development and progression of diabetes (Maritim *et al*., 2003).

Arsenic accumulation was more pronounced in liver than pancreatic tissue (Fig 2A). A significant depletion of zinc (Fig. 2B) and copper (Fig. 2C) level were observed in both studied tissues of arsenic exposed both group compared to control. Animals exposed to higher concentration of arsenic exhibits significantly more pronounced arsenic accumulation and zinc depletion than arsenic exposed to low dose group. Zinc plays a very important role in insulin production and consequent action of insulin on metabolism. Zinc deprivation is important symptoms associated with diabetes and also involved in antioxidant mechanism (Taylor, 2005). Zinc concentration seems to be declined in both liver and pancreas by arsenic exposure in present study may aggravate hyperglycemic condition as the lower zinc concentration exacerbates the reduced ability of diabetic mice to utilize glucose and increased rate of gluconeogenesis in liver (Kechird and Bouzerna, 2004). Arsenic exposure has shown a decrease in hepatic and pancreatic copper level compared to control. It was demonstrated that copper deprivation in pancreas resulted in an impairment of glucose tolerance and GSIS (Cohen and Miller, 1986).

The results of the present study demonstrate the occurrence of oxidative damage to pancreatic and hepatic tissue of arsenic exposed animals. It is suggested that sub-chronic arsenic exposure produces hyperglycemic condition and diabetogenic effect in laboratory animals, which may be the cumulative effect of oxidative stress induced damage to both pancreatic and hepatic tissue leads to decreased insulin production by ß-cell and impaired glucose homeostasis in liver, need further investigation.

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References

Ademuyiwa, O. and B. Elsenhans: Time course of arsenite-induced copper accumulation in rat kidney. *Biol. Trace Elem. Res.,* **74**, 81-92 (2000).

Chandalia, H.B., S. Sadiket, D.K. Bhargava and P.R. Krisnaswamy: Estimation of glycosylatedhemoglobin by a simple chemical

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method. *J. Assoc. Phys. India,* **28**, 285-286 (1980).

Chen, C.H., S.L. Wang, J.M. Chiou, C.H. Tseng, H.Y. Chiou, Y.M. Hsueh, S.Y. Chen, M.M. Wu and M.S. Lai: Arsenic and diabetes and hypertension in human populations: A review. *Toxicol. Appl. Pharmacol.,* **222**, 298–304 (2007).

Cohen, A.M. and E. Miller: Effect of copper on insulin release by the intact rat pancreas and the perfused rat pancreas. *Pancreas,* **1**, 309-16 (1986).

Flora, S.J.S.: Arsenic-induced oxidative stress and its reversibility. *Free Radic. Biol. Med.*, **51**, 257-281 (2011).

Flora, S.J.S., M. Mittal and A. Mehta: Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian J. Med. Res.,* **128**, 501-523 (2008).

Fu, J., C.G. Woods, E. Yehuda-Shnaidman, Q. Zhang, V. Wong and S. Collins: Low-level arsenic impairs glucose-stimulated insulin secretion in pancreatic beta cells: Involvement of cellular adaptive response to oxidative stress. *Environ. Hlth. Perspect.,* **118**, 864-870 (2010).

Guarino, M.P., R.A. Afonso, N. Raimundo, J.F. Raposo and M.P. Macedo: Hepatic glutathione and nitric oxide are critical for hepatic insulin-sensitizing substance action. *Am. J. Physiol. Gastrointest. Liver Physiol.,* **284**, 588–594 (2003).

Guha Mazumder, D.N.: Chronic arsenic toxicity and human health. *Indian J. Med. Res.*, **128,** 436-447 (2008).

Gupta, R. and S.J.S. Flora: Protective value of *Aloe vera* against some toxic effects of arsenic in rats. *Phytother. Res.,* **19**, 23-18 (2005).

Habig, W.H., M.J. Pabst and W.B. Jakoby: Glutathione-*s*-transferase: The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.,* **249**, 7130-7139 (1974).

Hultberg, B., A. Andersson and A. Isaksson: Interactions of metals and thiols in cell damage and glutathione distribution: Potential of mercury toxicity by dithiothreitol. *Toxicol.,* **56**, 93–100 (2001).

Izquierdo-Vega, J.A., C.A. Soto, L.C. Sanchez-Tena, A.D. Vizcaya-Ruiz and L.M. Del Razo: Diabetogenic effects and pancreatic oxidative damage in rats sub chronically exposed to arsenite. *Toxicol. Lett.,* **160**, 135–142 (2006).

Jensen, G.E. and M.L. Hansen: Occupational arsenic exposure and glycosylated haemoglobin. *Analyst*, **123**, 77–80 (1998).

Jollow, D.J., J.R. Mitchell, Z. Zamppaglione and J.R. Gillette: Bromobenzene induced liver necrosis, protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolites. *Pharmacol.,* **11**, 151 (1974)

Kajimoto, Y. and H. Kaneto: Role of oxidative stress in pancreatic ß-cell dysfunction. *Ann. NY. Acad. Sci.,* **1011**, 168–176 (2004).

Kakkar, P., B. Das and P.N. Viswanathan: A modified spectro-photometeric assay of superoxide dismutase. *Indian J. Bio chem. Biophys.,* **21**, 130-132 (1984).

Kakker, R., S.V. Mantha, J. Radhi, K. Prasad and J. Kalra: Increased oxidative stress in liver and pancreas during progression of streptozotocin-induced diabetes. *Clin. Sci.,* **94**, 623-632 (1998).

Kayali, R., U. Cakatay, T. Akcay and T. Altug: Effect of alpha-lipoic acid supplementation on markers of protein oxidation in post-mitotic tissues of ageing rat. *Cell Biochem. Funct.,* **24**, 79–85 (2006).

Kechird, Z. and N. Bouzerna: Effect of zinc deficiency on zinc and carbohydrate metabolism in genetically diabetic (C57BL/Ksj Db+/Db+) and non-diabetic original strain (C57BL/Ksj) mice. *Turk. J. Med. Sci.,* **34**, 367-373 (2004).

Kitchin, K.T.: Recent advances in arsenic carcinogenesis: Modes of action, animal model systems, and methylated arsenic metabolites. *Toxicol. Appl. Pharmacol.,* **172**, 249–261 (2001).

Liu, J., B. Zheng, H.V. Aposhian, Y. Zhou, M.L. Chen, A. Zhang and M.P. Waalkes: Chronic arsenic poisoning from burning high arsenic containing coal in Guizhou, China. *Environ. Hlt. Pers.,* **110**, 199-122 (2002).

Liu, S.X., M. Athar, I. Lippai, C. Waldren and T.K. Hei: Induction of oxyradicals by arsenic: Implication for mechanism of genotoxicity. *Proc. Natl. Acad. Sci. USA,* **98**, 1643–1648 (2001).

Lorenzo, C., L.E. Wagenknecht, A.J. Hanley, M.J. Rewers, A.J. Karter and S.M. Haffner: A1C between 5.7 and 6.4% as a marker for identifying pre-diabetes, insulin sensitivity and secretion, and cardiovascular risk factors: the insulin resistance atherosclerosis Study (IRAS). *Diabetes Care*, **33**, 2104-2109 (2010).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall: Protein measurement with the folin phenol reagent. *J. Biochem.,* **193**, 265–275 (1951)

Maritim, A.C., R.A. Sanders and J.B. Watkins III: Diabetes, oxidative stress, and antioxidants: A review. *J. Biochem. Mol. Toxicol.,* **17**, 24-38 (2003).

Mukherjee, S., D. Das, S. Darbar and C. Mitra: Dietary intervention affects arsenic-generated nitric oxide and reactive oxygen intermediate toxicity in islet cells of rats. *Curr. Sci.,* **85**, 786-793 (2003).

Navas-Acien, A., E.K. Silbergeld, R.A. Streeter, J.M. Clark, T.A. Burke and E. Guallar: Arsenic exposure and type-diabetes: A systematic review of the experimental and epidemiological evidence. *Environ. Hlt. Perspect.,* **114**, 641-648 (2006).

Navas-Acien, A., E.K. Silbergeld, R. Pastor-Barriuso and E. Guallar.: Arsenic exposure and prevalence of type 2 diabetes in US adults. *J. Amer. Medi. Associ.,* **300**, 814-822 (2008).

Ohkawa, H., N. Ohishi and K. Yagi: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.,* **95**, 351-358 (1979).

Patel, H.V. and K. Kalia: Sub-chronic arsenic exposure induces oxidative stress in skeletal muscle and epididymal fat pad: Possible role in development of arsenic induced diabetes mellitus. *J. Cell Tissue Res.,* **10**, 2397-2403 (2010).

Paul, D.S., A. Hernández-Zavala, F.S. Walton, B.M. Adair, J. Dìdina, T. Matoušek and M. Stýblo: Examination of the effects of arsenic on glucose homeostasis in cell culture and animal studies: Development of a mouse model for arsenic-induced diabetes. *Toxicol. Appl. Pharmacol.,* **222**, 305-314 (2007).

Paul, D.S., V. Devesa, A. Hernandez-Zavala, B.M. Adair, F.S. Walton, Z. Drobnâ, D.J. Thomas and M. Styblo: Environmental arsenic as a disruptor of insulin signaling. *Met. Ions Biol. Med.,* **10**, 1–7 (2008).

Reznick, A.Z. and L. Packer: Oxidative damage of protein: Spectrophtometric method for carbonyl assay. *Methods of Enzymology.* Academic Press, New York, 223, 357-363 (1994).

Rotruck, J.T., A.L. Pope, H.E. Ganther and A.B. Swanson: Selenium: Biochemical roles as a component of glutathione peroxidase. *Science*, **179**, 588-590 (1984).

Sinha, A.K.: Colorimetric assay of catalase. *Anal. Biochem.,* **47**, 389-394 (1972).

Sprangers, F., H.P. Sauerwein, J.A. Romijn, G.M. van Woerkom and A.J. Meijer: Nitric oxide inhibits glycogen synthesis in isolated rat hepatocytes. *Biochem. J.,* **330**, 1045-1049 (1998).

Taylor, C.G.: Zinc, the pancreas, and diabetes: Insights from rodent studies and future directions. *Biometals,* **18**, 305–312 (2005).

Titheradge, M.A.: The enzymatic measurement of nitrate and nitrite. *Methods Mol. Biol.,* **100**, 83–91 (1998).

Tseng, C.H.: The potential biological mechanisms of arsenic-induced diabetes mellitus. *Toxicol. Appl. Pharmacol.,* **197**, 67-83 (2004).

Yasuda, N., T. Nagakura, K. Yamazaki, T. Inoue and I. Tanaka: Improvement of high fat-diet-induced insulin resistance in dipeptidyl peptidase IV-deficient Fischer rats. *Life Sci.,* **71**, 227–238 (2002).

Yazdanparast, R., A. Ardestani and S. Jamshidi: Experimental diabetes treated with *Achillea santolina*: Effect on pancreatic oxidative parameters. *J. Ethnopharmacol.,* **112,** 13–18 (2007).

Yen, C.C., F.J. Lub, C.F. Huangc, W.K. Chen, S.H. Liu, S.Y. Lin-Shiau: The diabetogenic effects of the combination of humic acid and arsenic: *In-vitro* and *in-vivo* studies. *Toxicol. Lett.,* **172**, 91–105 (2007).

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