Applied Biological Research 25(4): 485-493; (2023)
DOI: 10.5958/0974-4517.2023.00051.4

EFFECT OF ARSENIC ON PANCREAS WITH SPECIAL EMPHASIS OF GLUCOSE METABOLISM

Joydeep Das

Department of Zoology, Dinabandhu Andrews College (affiliated to the University of Calcutta), Garia, Kolkata – 700 084, West Bengal (India)
*****e-mail: *joydeepdas914@gmail.com*

(Received 19 August, 2023; accepted 2 November, 2023)

ABSTRACT

The increasing human interventions have significantly contributed to the environmental pollution, especially due to heavy metal and metalloid exposure including toxic non-essential elements like arsenic. Arsenic is a potent endocrine disruptor and may adversely affect human health. This study was aimed to assess the detrimental effects of arsenic using Swiss albino mice as a model and postulate the mechanisms behind the harmful effects of arsenic on the structural integrity of pancreas, hormone levels, and downstream metabolic parameters. The effects of arsenic on the structural and hormonal (insulin and glucagon) status of pancreas in mice was determined by observing changes through histological, SEM, and ELISA methods. Alterations in glucose metabolism, with special emphasis on blood glucose and liver glycogen levels, was observed by biochemical estimation and also by examining the role of arsenic in oxidative stress by anti-oxidant enzyme assay. The results revealed that arsenic caused disintegration and degeneration of exocrine and endocrine parts of pancreas. Arsenic decreased insulin and amylase levels and increased glucagon levels. It decreased anti-oxidant enzymes, which may cause an increase in blood glucose with simultaneous decrease in liver glycogen. It is concluded that arsenic induces structural and hormonal alterations in pancreas, which increases blood glucose and alters glucose homeostasis which leads to stress in the animal physiology.

Keywords: Anti-oxidant, ELISA, endocrine disruptor, insulin, stress

INTRODUCTION

Heavy metals are persistent pollutants that bio-accumulate within cells. Their toxicity is a significant global issue due to their adverse biological impacts (Tchounwou *et al.,* 2014). Worldwide increasing ecological and public health concerns about environmental contamination by heavy metals has recently drawn significant attention. The negative effects of heavy metals are sneaky dangers that have adverse and irreversible consequences (Zhou *et al.,* 2017). Although groundwater is considered safe, but the presence of high concentration of some heavy metals like arsenic pose potential human health concern (Shaji *et al.,* 2021). Arsenic is ubiquitously found across the globe, and is one of the most hazardous elements causing a wide range of health hazards like skin lesions, cancer, cardiovascular problem etc. (Kile *et al*., 2011; Sabir *et al.,* 2019). Arsenic poisoning poses serious threat to the people in Southeast Asian nations including India, Bangladesh, and Taiwan, where it causes a number of ailments, disorders and death (Suk *et al.*, 2003).

As per WHO, the permissible limit for arsenic in drinking water is 10 ppb (or 0.01 ppm) (Prozialeck *et al.,* 2008; Walker and Fosbury, 2009). The effects of arsenic on skin, GI tract,

reproduction, immuno-modulation and cardiac function have previously been studied (Guha Mazumdar, 2005; Ahsan *et al.,* 2006). Arsenic is considered as one of the most hazardous endocrine disruptor (ED) [Meakin *et al.,* 2019]. ED induced by various metal pollutants has attracted the attention of scientists and public over several decades (Buha *et al.,* 2018). EDs can negatively impact human health and environment by altering the physiological mechanisms. Evidences suggest that arsenic exposure have a disruptive effect on hypothalamo-pituitary-adrenal axis (Sun *et al.,* 2016) which is involved in controlling stress-related and metabolic physiology. The adrenal glands are directly affected by stress and are responsible for suppressing stressful situations (McGaugh and Roozendaal, 2002). However, the mechanisms involving the malfunctioning of pancreas as well as the respective hormones need to be discerned. Pancreatic hormones are essential in human body as they deal with the problems related to long-term stress and carbohydrate metabolism. However, there is no report elaborating the alterations of pancreas in relation to arsenic exposure. Hence, it is imperative to study the effects of arsenic on pancreas and stress induction so that therapeutic measures may be designed to overcome the problem. The present study was, therefore, aimed to evaluate the adverse impact of arsenic on pancreas in mice, with special reference to the changes in histological architecture, hormone level and glucose metabolic parameters.

MATERIALS AND METHODS

The chemicals such as natrium metaarsenite ($NaAsO_2$), cederwood oil, anthrone reagent, hematoxylin, eosin, ethanol, xylene and D.P.X. mountant were purchased from Merck (Germany). The paraplast paraffin pellets were procured from Leica Biosystems (Netherlands). Reduced glutathione, protease inhibitor, and glutaraldehyde were obtained from Sigma Aldrich (USA).

Animal treatment

Male Swiss albino mice of 3 to 4 weeks age, weighing 25-30 g, were maintained in a controlled environment (25±2°C temperature, 15% relative humidity, and photoperiod of 12 h dark/12 h light) during the experimental period. The animals were maintained in compliance with the Guidelines for the Care and Handling of Laboratory Animals (NIH Publication No 85-23, amended in 1985). The work was conducted after approval from the Institutional Animal Ethical Committee (No. 886/ac/06/ CPCSEA), and in conformity with the Indian Laws of Animal Protection. The animals were acclimatized for 7 days prior to the commencement of experiments. The animals were provided food pellets and water *ad libitum*.

Dose selection

Sodium arsenite was dissolved in sterile water. Mice were randomly divided into two groups, each comprising of five animals (n = 5). The group I (control group) received arsenic-free water while group II received 5 ppm arsenic in drinking water. Arsenic was administered to animals for 7 days. The sodium arsenite concentrations were chosen based on either environmental pertinence or previous reports on groundwater arsenic concentrations in different countries (Bashir *et al.,* 2006; Waalkes *et al.*, 2006; Shen *et al.*, 2007).

Animal necropsy, tissue and blood collection

Sodium barbital was injected intra-peritoneally and tissues were harvested from the experimental animals after 7 days of treatment. The pancreas, and liver were rinsed in 0.9% (w/v) cold normal saline, pat dried, and weighed on an electronic monopan balance. For histological study, tissue slices were preserved in Bouin's fixative and for scanning electron microscopy tissue were preserved in 2.5% glutaraldehyde in 0.2M phosphate buffer (pH 7.4) overnight at 4ºC. Serum was extracted for

ELISA assays using ELISA reader (Biorad-PR4100). Tissues were collected for antioxidant enzyme test, western blot analyses and stored at -20°C till further use.

Histological studies

Pancreas was dissected out, weighed and fixed in freshly prepared Bouin's fixative. Progressive dehydration (70, 90, and 100% alcohol) was performed and tissues were placed in cederwood oil. After tissues were embedded in paraffin wax, then 5 μm thin paraffin sections were stained using the conventional hematoxylin-eosin double staining process (Alturkistani *et al.*, 2015). The pancreas were observed at 40X under a light microscope (Zeiss, USA) and histological alterations noticed. The Dewinter Calliper Pro software (Dewinter Optical Inc., India) was used to assess differential morphometric parameters (like width, size, and shape of tissues) in the processed sections.

Scanning electron microscopy

The pancreas were dissected out, cut into pieces (1 mm x 1 mm) and incubated in 2.5% glutaraldehyde with 0.2 M phosphate buffer (pH, 7.4) overnight at 4°C. The tissues were dehydrated in graded ethanol before their placement in cold acetone. The tissues were allowed to air dry overnight and then rinsed in 0.2 M phosphate buffer, coated in a gold sputter coater (Quorum Q150TES) after critical point drying and finally examined by using scanning electron microscope (Zeiss USA).

Analysis of serum hormonal level

The serum was separated from freshly collected blood from each treatment and the levels of circulating insulin and glucagon were measured using ELISA kits (Ray Biotech, USA), based on competitive enzyme immunoassay as per the instructions of the manufacturers.

Analysis of biochemical parameters

Calorimetric estimation of liver glycogen content was performed as per Hassid and Abraham (1957). The tissue (1 g) was placed in 3 mL 30% KOH and submerged for 20-30 min in a boiling water bath. Glycogen was precipitated after the tissue had disintegrated by adding 5 ML 95% ethanol. The resulting precipitate was mixed with 1 mL distilled water, re-precipitated with 95% ethanol and then centrifuged at 3000 rpm for 10 min. The amount of glycogen was calculated from the solution made by dissolving glycogen precipitate in distilled water. Then 5 mL anthrone reagent was added to 0.1 mL aliquot and mixed well. The tubes were heated in boiling water for 10 min and the absorbance measured at 590 mm spectrophotmetrically (UV-VIS double beam spectrophotometer, Lasany). The findings were compared to those of typical glycogen. Serum glucose content was measured by glucose oxidase-peroxidase (GOD-POD) enzymatic method (Trinder, 1969). Glucose was oxidized by glucose oxidase to gluconic acid and hydrogen peroxide. In subsequent peroxide catalysed reaction, the oxygen liberated was accepted by the chromogen system to form a red coloured quinoneimine compound. The absorbance was measured at 505 nm spectrophotometerically. The amylase assay kit (Abcam UK) was used to measure the serum pancreatic amylase level. kit detects the activity of α-amylase through a two-step reaction. In protocol, α-amylase cleaved the substrate ethylidene–pNP-G7 to produce smaller fragments that were eventually modified by α-glucosidase which led to the release of a chromophore. Serum from control and treated mice were mixed with reaction buffer and the absorbance measured immediately at OD_{405} in a kinetic mode, every 3 min, for 30 min.

Estimation of ROS scavenging enzymes

The catalase (CAT) activity in pancreatic tissue was assayed by measuring the decomposition of H_2O_2 to H_2O and O_2 by CAT present in cell lysates at a wavelength of 240 nm and expressed as μM of H_2O_2 U mg^{-1} protein (Claiborne, 1985). Superoxide dismutase (SOD) activity was evaluated as per Marklund and Marklund (1974) based on self-oxidation rate of pyrogallol at 420 nm and inhibition of same by SOD. One unit enzyme activity corresponded to the 50% inhibition, expressed by units mg^{-1} protein. Reduced glutathione assay was based on the protocol of Jollow *et al.* (1974). The method was based on the reduction of 5,5'-dithio-bis-(2-nitrobenzoic acid) at 412 nm. The reduced glutathione was assessed from a standard curve and expressed in μM mg^{-1} protein.

Statistical analysis

All experiments were conducted in a completely randomized design with each treatment replicated thrice and the mean ± SD worked out by using a statistical software package (Graphpad Prism, Version 6.0). Each arsenic-treated group was compared with control and the contrasts between the group mean values were evaluated by Student's *t*-test (Sander *et al.,* 2016) as all the data sets were normally distributed. Statistical significance levels were set at *p*-values < 0.05.

RESULTS AND DISCUSSION

Effect of arsenic on the structural components of pancreas

A comparative analysis was carried out to assess the histological alterations in the pancreas of experimental and control mice. The pancreas of control mice revealed normal histo-architecture and was divided into two parts: the exocrine pancreas and the endocrine portion. Histopathological examination of pancreas of normal mice showed regular lobular architecture of pancreas. The pancreas had abundant islets of Langerhans interspersed between the pancreatic exocrine acini. The islets appeared lightly stained as compared to the surrounding acinar cells, with intact interlobular connective tissue and interlobular duct. The islet boundaries were clear and the profiles of islet cells were visible. The overall architecture was found healthy in control sections (Fig. 1a). In contrast to control sections, pancreatic acinar cell boundaries of mice exposed to 5 ppm arsenic for 7 days were indistinct and the islets showed signs of disintegration and severe architectural disarray (Fig. 1b).

The scanning electron microscopy revealed that the control pancreas exhibited normal structure and shape of cells. Normal compactness of cells and ducts were found, and no gaps were observed between cells in control pancreas. The acinar cells showed no structural changes (Fig. 1c). Normal compactness of acinar cells was observed in control group. The pancreas of mice treated with 5 ppm arsenic showed significant structural changes and a slight loss of compactness (Fig. 1d). Our results are in agreement with the previous reports wherein the histological studies of pancreatic tissues in heavy metal-treated diabetic rat groups have shown severe degeneration, necrosis, degranulation, and

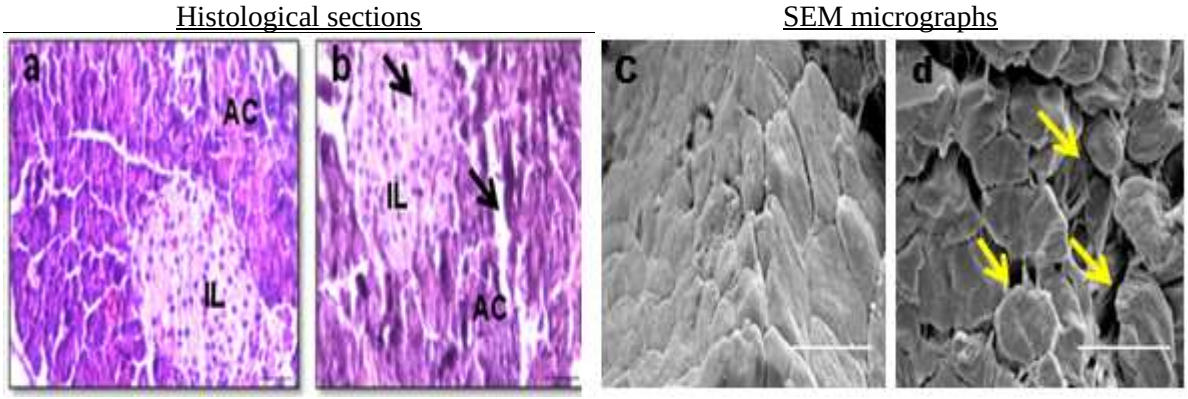

Fig. 1: Structural changes in pancreas of mice due to arsenic; Histological study shows a) control section depicting no structural alterations, and b) arsenic-treated section showing changes in acinar cells and disruption in islets of Langerhans in comparison to control. Arrows indicate structural damages. AC, acinar cells; IL, islets of Langerhans (magnification 40X, scale bar 100 µm). c) SEM micrograph of control tissue delineated normal structure and shape without any structural abnormalities d) Treated group revealed structural changes in pancreas over control. Loss of compactness, disintegration and degeneration were observed in treated group as compared to the control. All the figures represent the ultra-structural images of pancreas by scanning electron microscopy (n = 5) at a magnification of 500X, scale bar 20 µm.

shrinkage in the islets of Langerhans (Muhammad *et al.*, 2020). Histo-architectural studies revealed that disintegration and degeneration of Islets of Langerhans and pancreatic acinar cells increased following arsenic treatment in a dose-dependent manner. All studies indicate that arsenic exposure causes cellular disorganization, as evident from histology and SEM analysis.

Effect of arsenic on hormonal and pancreatic amylase level in pancreas

Arsenic treatment led to a remarkable decline in serum insulin levels in comparison to the control mice (Fig. 2a). Serum insulin levels reduced from 0.05786 to 0.02435 µUL mL^{-1} in 5 ppm arsenic-exposed groups. After arsenic treatment a significant decline in insulin level occurred in arsenic-exposed group as compared to the control group. Sodium arsenite treated mice demonstrated a significant increase in serum glucagon levels as compared to the control mice (Fig. 2b). The glucagon levels increased from 184.66 pg mL^{-1} in control and to 293 pg mL^{-1} in 5 ppm treatment. Concomitantly, sodium arsenite treatment significantly increased serum glucagon levels after 7 days of treatment. In addition, pancreatic amylase activity decreased significantly (1.5 fold) in arsenic-exposed group (Fig. 2c). Heavy metals have been shown endocrine disruptors in both human and animals, altering the hormone homeostasis and resulting in endocrine imbalance (Dongling *et al*., 2023).

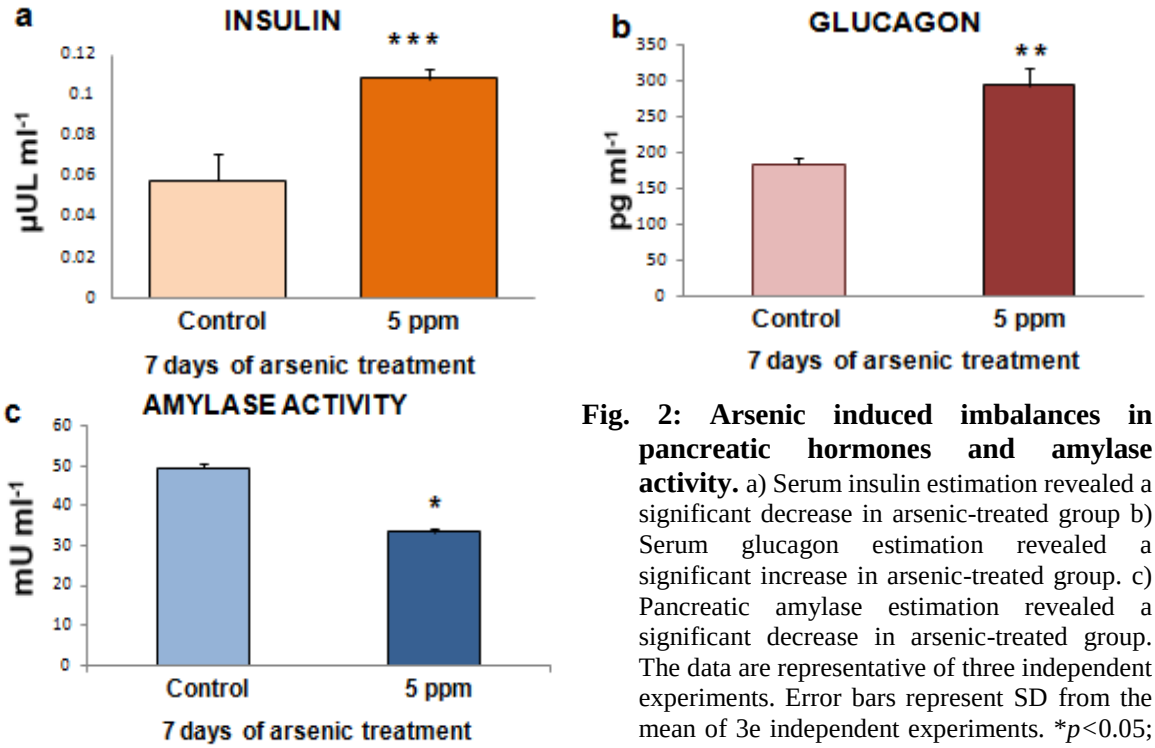

Fig. 2: Arsenic induced imbalances in pancreatic hormones and amylase activity. a) Serum insulin estimation revealed a significant decrease in arsenic-treated group b) Serum glucagon estimation revealed a significant increase in arsenic-treated group. c) Pancreatic amylase estimation revealed a significant decrease in arsenic-treated group. The data are representative of three independent experiments. Error bars represent SD from the mean of 3e independent experiments. *p<0.05; **p<0.01; ***p<0.001 between control and arsenic-treated groups.

ELISA results revealed that after arsenic exposure there was decrease in insulin level and increase in glucagon level, as well as cellular disruption in pancreas. Predictably, in addition to structural disruption of exocrine and endocrine pancreas, insulin levels decreased and glucagon levels increased significantly, leading to a state of hyperglucagonemia. Diagnosis of an acute pancreatic inflammatory condition is commonly achieved by analyses of serum amylases (Oh *et al*., 2017). The present findings reiterated that serum amylase levels reduced in line with the remaining functional capacity of pancreas as its secretory capacity decreased throughout pancreatitis (Oh *et al*., 2017). In addition, as a result of arsenic-induced disintegration of exocrine pancreas the pancreatic amylase levels reduced in a dose-dependent manner, a condition indicative of pancreatitis. The results demonstrated that serum amylase

levels reduced in line with the remaining functional capacity of pancreas as its secretary capacity decreased throughout pancreatitis.

Effect of arsenic on glucose parameter

The glucose levels of arsenic-exposed group increased significantly as compared to the control group. The basal concentration of glucose in control group was 86.25 mg dL^{-1} and it was further elevated to 151.34 mg dL^{-1} in 5 ppm treatment (Fig. 3a). Liver glycogen levels of arsenic-exposed mice were drastically altered in comparison to control animals. Liver glycogen level was reduced to 660.923 µg g^{-1} in 5 ppm dose as compared to the control (1337 µg g^{-1}) as observed in liver of unexposed mice (Fig. 3b). The numerous detrimental consequences of arsenic exposure on pancreas have previously been studied using *in vitro* and animal models (Diaz *et al*., 2007). Arsenic inhibited insulin secretion in *in vitro* study using cell model systems (Fu *et al*., 2010). Rodents exposed to arsenic through drinking water reportedly alter glucose homeostasis in animal models on exposure (Paul *et al*., 2007).

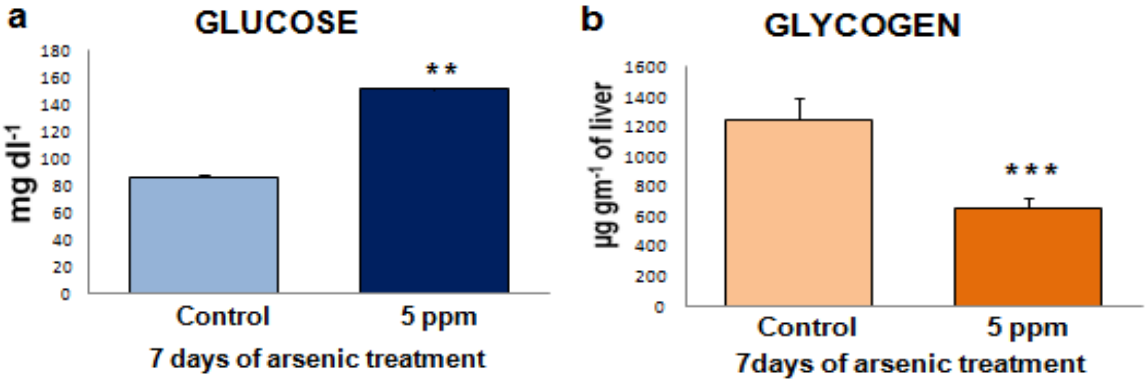

Fig. 3: Arsenic induced glucose homeostasis; a) Glucose level elevated in the group receiving arsenic dose as compared to the control. b) Liver glycogen showing a significant decrease in arsenic-treated group as compared to control. The data are representative of 3 independent experiments. Error bars represent S.D from the mean of 3 independent experiments. $^{**}p<0.01$; $^{***}p<0.001$ between control and arsenic-treated groups.

Decrease in insulin level and increase in glucagon level, as well as cellular disruption led to hormonal imbalance, including dose-dependent increase in blood glucose, which led to abnormalities in liver glycogen metabolism. After exposure to arsenic, the absence of the restraining influence of insulin on glucagon production led to hyperglucagonemia, along with increase in blood glucose levels. Increase in blood glucose and relative hyperglucagonemia, in turn, perturbed liver glycogen metabolism and eventually depleted liver glycogen levels in our experimental animals.

Arsenic-induced oxidative stress in pancreas

Cellular antioxidant enzymes protect biological macromolecules from oxidative damage. The primary defence mechanism against hydrogen peroxide (H_2O_2) is enzyme catalase. Catalases are hemoglobin-containing proteins that catalyse the conversion of H_2O_2 to water and molecular oxygen, thereby protect the cells from toxic effects of H_2O_2. Arsenic also reduced catalase activity in pancreas in a dose-dependent manner 1.8-fold in 5 ppm treatment as compared to the control group (Fig. 4a). The dismutation of superoxide ion by SOD results in H_2O_2 production, which, in turn, may produce hydroxyl radicals on reaction with Fe^{2+}. Arsenic intoxication led to a significant dose-dependent depletion in the activity of superoxide dismutase in pancreas 1.5-fold in 5 ppm exposed mice as compared to the control group (Fig. 4b). GSH depletion reflects the intracellular oxidation and is indicative of the accumulation of ROS in cells. The results showed that arsenic aggravated oxidative stress by reducing intracellular GSH in arsenic treated group in pancreas as compared to control group

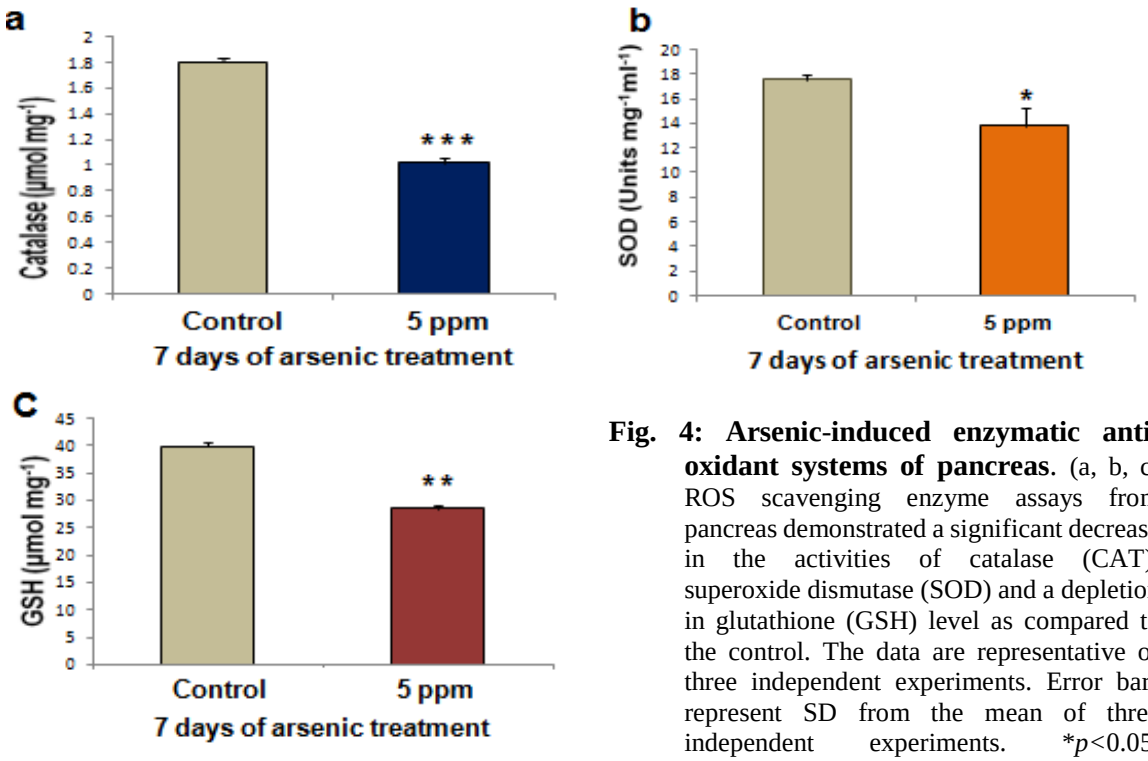

Fig. 4: Arsenic-induced enzymatic anti-oxidant systems of pancreas. (a, b, c) ROS scavenging enzyme assays from pancreas demonstrated a significant decrease in the activities of catalase (CAT), superoxide dismutase (SOD) and a depletion in glutathione (GSH) level as compared to the control. The data are representative of three independent experiments. Error bars represent SD from the mean of three independent experiments. *p<0.05; **p<0.01; ***p<0.001 bet

(Fig. 4c). In this study, the levels of CAT, SOD and GSH were significantly reduced in arsenic-treated cells of pancreas in mice, leading to elevated ROS.

Arsenic toxicity has been implicated mostly in ROS production. A high amount of oxidative stress results from imbalance in ROS producing systems and ROS-scavenging enzymes (Marrocco *et al*., 2017). Antioxidant enzymes like CAT and SOD, which are known to shield biological components from oxidative stress, have been demonstrated to drastically reduce in response to arsenic treatment in mice (Singh *et al*., 2014). This part of the study demonstrated that arsenic had an immense impact on the ROS-scavenging system. ROS-scavenging enzyme assays from the pancreas showed a significant decrease in the activities of catalase and superoxide dismutase and a depletion in the level of glutathione compared to controls. So, hormonal imbalance and high glucose levels were accompanied by ROS generation in pancreas of mice receiving dose of arsenic.

Conclusion: Arsenic causes structural and functional alterations, and decreases antioxidant enzymes of pancreas, as indicated by decrease in insulin level, elevation in glucagon and reduction in pancreatic amylase level, leading to increased blood glucose, which in turn results in abnormalities of liver glycogen metabolism, glucose homeostasis and induces stress. All results indicate the possibility of acquiring severe physiological disorders, alter glucose homeostasis and complete non-functioning of organs (the pancreas) at a higher dose of arsenic.

Acknowledgements: The first author thanks all the faculty and staff of Department of Zoology, Dinabandhu Andrews College for supporting to complete this research work. There was no funding for this research. A special thanks to University of Calcutta for facilitating ELISA reader and scanning electron microscope.

Ethical Statement: All the animals were maintained in compliance with the guidelines for the care and handling of laboratory animals (NIH Publication No 85-23, amended in 1985) and the study was undertaken with approval from the Institutional Animal Ethical Committee (vide No. 886/ac/06/CPCSEA), and in conformity with Indian Laws of Animal Protection (ILAP).

REFERENCES

Ahsan, H., Chen, Y., Parvez, F., Zablotska, L., Hussain, I., Momotaj, H., Levy, D. and Cheng, Z. 2006. Arsenic exposure from drinking water and risk of premalignant skin lesions in Bangladesh: Baseline results from the health effects of arsenic longitudinal study. *Americal Journal of Epidemiology*, **163**(12): 1138-1148.

Alturkistani, H.A., Tashkandi, F.M. and Mohammedsaleh Z.M. 2015. Histological stains: A literature review and case study. *Global Journal of Health Science*, **8**(3): 72-79.

Bashir, S., Sharma, Y., Irshad, M., Nag, T.C., Tiwari, M., Kabra, M. and Dogra, T.D. 2006. Arsenic induced apoptosis in rat liver following repeated 60 days exposure. *Toxicology*, **217**: 63-70.

Buha, A., Matovic, V., Antonijevic, B., Bulat, Z., Curcic, M., Renieri, E.A., Aristidis, M.T., Amie, S. and David, W. 2018. Overview of cadmium thyroid disrupting effects and mechanisms. *International Journal of Molecular Sciences*, **19**(5): 1501. [*doi: 10.3390/ijms19051501*].

Claiborne, A. 1985. Catalase activity. pp. 283-284. **In**: *CRC Handbook of Methods for Oxygen Radical Research* (ed. R.A. Greenwald). CRC Press, Boca Raton, USA.

Díaz-Villaseñor, A., Burns, A.L., Hiriart, M., Cebrián, M.E. and Ostrosky-Wegman, P. 2007. Arsenic-induced alteration in the expression of genes related to type 2 diabetes mellitus. *Toxicology and Applied Pharmacology,* **225**(2): 123-133.

Dongling, L.,Qianhan, S.,Cuiqing, L., Qinghua, S. and Xiang, Z. 2023. Effects of endocrine-disrupting heavy metals on human health. *Toxics*, **11**(4): 322. [*doiI: 10.3390/toxics11040322*].

Fu, J., Woods, C.G., Yehuda-Shnaidman, E., Zhang, Q., Wong, V., Collins, S., Sun, G., Andersen, M.E. and Pi, J. 2010. Low-level arsenic impairs glucose-stimulated insulin secretion in pancreatic beta cells: Involvement of cellular adaptive response to oxidative stress. *Environmental Health Perspective*, **118**(6): 864-870.

Guha Mazumdar, D.N. 2005. Effect of chronic intake of arsenic-contaminated water on liver. *Toxicology and Applied Pharmacology*, **206**(2): 169-175.

Hassid, W.Z. and Abraham, S. 1957. Chemical procedures for analysis of polysaccharides: Determination of glycogen with anthrone reagent. pp. 34-36. **In**: *Methods in Enzymology, volume **3*** (eds. S.P. Colowick and N.O. Kaplan). Academic Press, New York, USA.

Kile, M.L., Hoffman, E., Rodrigues, E.G., Breton, C.V., Quamruzzaman, Q., Rahman, M., Mahiuddin, G., Hsueh., Y.M. and Christiani, D.C. 2011. A pathway-based analysis of urinary arsenic metabolites and skin lesions. *American Journal of Epidemiology,* **173**(7): 778-786.

Marrocco, I., Altieri, F. and Peluso, I. 2017. Measurement and clinical significance of biomarkers of oxidative stress in humans. *Oxidative Medicine and Cellular Longevity*, **2017**: 6501046. [*doi:10.1155/2017/6501046. Epub 2017*].

Marklund, S. and Marklund, G. 1974. Involvement of superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *European Journal of Biochemistry,* **47**(3): 469-474.

McGaugh, J.L. and Roozendaal, B. 2002. Role of adrenal stress hormones in forming lasting memories in brain. *Current Opinion of Neurobiology*, **12**(2): 205-210.

Meakin, C.J., Martin, E.M., Szilagyi, J.T., Nylandar-French. and Fry, R.C. 2019. Inorganic arsenic as an endocrine disruptor: Modulation of the glucocorticoid receptor pathway in placental cells via CpG methylation. *Chemical Research in Toxicology,* **32**(3): 493-499.

Muhammad, A.R., Zaib, U.N., Muhammad, S.A., Hira, B., Azra, Mehmood., Ayesha, R. and Amtul, B.T.A. 2020. Assessment of metals induced histopathological and gene expression changes in different organs of non-diabetic and diabetic rats**.** *Scientific Reports*, **10**(1): 5897. [*doi: 10.1038/s41598-020-62807-0*].

Oh, H.C., Kwon, C.I., El, Hajj II, Easler, J.J., Watkins, J., Fogel, E.L., McHenry, L., Sherman, S., Zimmerman, M.K. and Lehman G.A. 2017. Low serum pancreatic amylase and lipase values are simple and useful predictors to diagnose chronic pancreatitis. *Gut Liver,* **11**(6): 878-883.

Paul, D.S., Harmon, A.W., Devesa, V., Thomas, D.J. and Stýblo, M. 2007. Molecular mechanisms of the diabetogenic effects of arsenic: Inhibition of insulin signaling by arsenite and methylarsonous acid. *Environmental Health Perspective,* **115**(5): 734-742.

Prozialeck, W.C., Edwards, J.R., Nebert, D.W., Woods, J.M. and Barchowsky, A. 2008. Atc1hison WD: The vascular system as a target of metal toxicity. *Toxicological Science,* **102**(2): 207-218.

Sabir, S., Akash, M.S.H., Fiayyaz, F., Saleem, U., Mehmood, MH. and Rehman, K. 2019. Role of cadmium and arsenic as endocrine disruptors in the metabolism of carbohydrates: Inserting the association into perspectives. *Biomedicine and Pharmacotherary*, **114**: 108802. [*doi: 10.1016/j.biopha.2019.108802*].

Sander, G., Stephen, J.S., Kenneth, J.R., John, B.C., Charles, P., Steven, N.G. and Douglas, G.A. 2016. Statistical tests, *P* values, confidence intervals, and power: A guide to misinterpretations. *European Journal of Epidemiology*, **31**: 337-350.

Shaji, E., Santosh, BC., Sarath, K.V., Prakash, P., Deepchand, V. and Divya, B.V. 2021. Arsenic contamination of groundwater: A global synopsis with focus on the Indian Peninsula. *Geoscience Frontiers*, **12** (3): 109079. [*doi: 10.1016/j.gsf.2020.08.015*].

Shen, J., Liu, J., Xie, Y., Diwan, B.A. and Waalkes, M.P. 2007. Fetal onset of aberrant gene expression relevant to pulmonary carcinogenesis in lung adenocarcinoma development induced by in utero arsenic exposure. *Toxicological Sciences*, **95**(2): 313-320.

Singh, M.K., Yadav, S.S., Yadav, R.S., Singh, U.S., Shukla, Y., Pant, K.K. and Khattri, S. 2014. Efficacy of crude extract of *Emblica officinalis* (amla) in arsenic-induced oxidative damage and apoptosis in splenocytes of mice. *Toxicology International*, **21**(1): 8-17.

Suk, W.A., Ruchirawat, K.M., Balakrishnan, K., Berge,r M., Carpenter, D., Damstra, T., de Garbino, J.P., Koh, D., Landrigan, P.J., Makalinao, I., Sly, P.D., Xu, Y. and Zheng, B.S. 2003. Environmental threats to health in Southeast Asia and the Western Pacific. *Environmental Health Perspective*, **111**: 1340-1347.

Sun, H.J., Xiang, P., Luo, J., Hong, H., Lin, H., Li, H.B. and Ma, L.Q. 2016. Mechanisms of arsenic disruption on gonadal, adrenal and thyroid endocrine systems in humans: A review. *Environment International*, **95**: 61-68.

Tchounwou, G.G., Yedjou, A.K., Paltolla, D.J. and Sutton, D.J. 2014. Heavy metal toxicity and environment. *Journal of Experimental Zoology*, **101**: 133-164.

Trinder, P. 1969. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *Journal of Clinical Pathology,* **22**(1): 158-161.

Walker, M. and Fosbury, D. 2009. Arsenic, As (III), and tungsten in Nevada County's private water supplies. *Journal of Water and Health*, **7**(2): 293-301.

Zhou, Q., Gu, Y., Yue, X., Mao, G., Wang Y. and Su, H. 2017. Combined toxicity and underlying mechanism of a mixture of eight heavy metal. *Molecular Medicine Reports,* **15**(2): 859-866.