International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 1(F); January 2018; Page No. 9159-9163
DOI: http://dx.doi.org/10.24327/ijcar.2018.9163.1502

Research Article

ARSENIC INDUCED HEPATOTOXICITY AND DAMAGE OF PANCREATIC ISLETS IN ALBINO RAT: A POSSIBLE ROLE OF DIABETES MELLITUS

Srikanta Guria*

Department of Zoology, Barasat Govt. College, Kolkata-700124, West Bengal

A R T I C L E I N F O

Article History:

Received 23rd October, 2017
Received in revised form 4th November, 2017
Accepted 16th December, 2017
Published online 28th January, 2018

Key words:

Liver, Pancreas, Islets of Langerhans, Diabetes Mellitus, Arsenic

A B S T R A C T

Arsenic as a potential risk factor for diabetes has been received attention recently. However, the roles of arsenic on development of diabetes are unclear. The aims and objectives of this study were to investigate the arsenic induced diabetic condition by examining histology of liver and pancreas. The pancreatic sections of the treated group showed marked morphological alterations. Photomicrograph of histology of treated pancreas exhibited the disruption of islets, disorientation of cells and disrupted connective tissue septa. Treated liver revealed central vein congestion and dilatation of sinusoidal spaces. Treated liver showed lower PAS response. The changes of pancreatic islets may reveal an inhibition in insulin synthesis. In this study, changes in the architecture of pancreatic islets as well as liver may be the reason behind diabetes, but further experiments needs to be performed.

INTRODUCTION

Diabetes mellitus characterized by hyperglycemia is an increasing worldwide health problem (Inceoglu *et al*., 2012). It is largely classified into insulin-dependent diabetes mellitus (type 1 diabetes) and noninsulin-dependent diabetes mellitus (type 2 diabetes) (Liu *et al*., 2014). The type 2 diabetes (T2D) makes up more than 90% of total diabetes cases (Zimmet *et al*., 2001). There is considerable interest in understanding the contribution of non-traditional risk factors to the diabetes epidemic, including environmental pollutants (Maull *et al*., 2012; Hectors *et al*., 2011). Among these environmental pollutants, arsenic (As) exposure has been paid much attention (Liu *et al*., 2014). Arsenic is a ubiquitous toxic metalloid in the environment. Epidemiological studies carried out in different countries have shown a strong diabetogenic effect of As in human populations mainly through As-contaminated drinking water (Islam *et al*., 2012).

Arsenic contamination of drinking water is a global problem. It is the severe problem in West Bengal, India and Bangladesh. According to some report, arsenic contamination of drinking water is more severe in Bangladesh than in West Bengal (Breslin, 2000; Mukherjee *et al*., 2003), and researchers have observed that concentrations in excess of 300 mg/l are associated with arsenical lesions, although lesions also occur at lower concentrations when nutrition is poor, volume of water consumed is high or contaminated water is consumed for an extended period (Breslin, 2000; Mukherjee *et al*., 2003). According to several survey reports, exposure to inorganic arsenic compounds may be associated with development of diabetes mellitus (Chen *et al*., 1992; Lai *et al*., 1994; Rahman and Axelson, 1995; Mukherjee *et al*., 2003). In recent years, oxidative stress has been implicated in arsenic-induced cytotoxicity and genotoxicity (Lynn *et al*., 1998; Mukherjee *et al*., 2003). Besides, a positive correlation among arsenite-induced nitric oxide (NO) production, oxidative stress, DNA damage, activation of poly [ADP-ribose] polymerase (PARP) has been suggested (Lynn *et al*., 1998) to implicate in the pathogenesis of oxidant induced cell death (Schraufstatter *et al*., 1986; Mukherjee *et al*., 2003).

Uncontrolled industrialization has resulted in human population being exposed to metals that have the potential to cause or exacerbate diseases (Liu *et al*., 2014). Thus, more attention is needed to investigate and prevent the possible factors which may induce diabetes. Several studies have indicated that the deficiency and efficiency of some essential trace metals may play a role in the islet function and development of diabetes mellitus (Marx, 2002).In vitro studies using insulinoma cell lines implicate several pathways by which inorganic arsenic (iAs) can affect pancreatic b-cell function to inhibit insulin expression and/or secretion (Liu *et al*., 2014; Lu *et al*., 2011). From previous findings, chronic exposure to arsenic is an important risk factor for induction of diabetes mellitus in an arsenic-contaminated environment. Arsenic might be impairing glucose metabolism (Diaz-Villasenor *et al*., 2013) however, only few studies have

Corresponding author:* **Srikanta Guria
Department of Zoology, Barasat Govt. College, Kolkata-700124, West Bengal

evaluated that the impairment of insulin secretion in beta-cells associated with environmental arsenic exposure in mammals (Diaz-Villasenor *et al*., 2013). A PI3K-dependent signaling pathway has been demonstrated to exist in β-cells and that it might function to restrain glucose-induced insulin secretion from β-cells. Increased PI3K-mediated PKB/Akt phosphorylation has been reported in β-cells exposed to high dose of arsenic (Souza, 2001). The aims and objectives of this study were to analyse the arsenic induced diabetic condition by examining histology of liver and pancreas.

MATERIALS AND METHODS

The study was carried out on albino rats weighing between 70 to 80 g. The animals were housed in clean plastic cages under natural light and dark cycles at room temperature. Animals in all groups were fed *ad libitum* and allowed free access to water. All animals received human care. Rats were divided into control and their respective treated group. After 5 days of acclimation, the animals were divided into two equal groups (n=6/group) as follow

- Group I (Control group): Untreated animals.
- Group II (Treated group): rats were injected with single dose of As

A dose of 3 mg/ml/kg body weight /day of arsenic tri oxide was given daily for 21 days to experimental group of the animals (after Das, 2013).

Pancreatic tissues and liver were dissected out and fixed in Bouin's fluid for 24 hours and processed via paraffin wax embedding method (Parakkal, 1961).
Paraffin-embedded sections were cut at 5 µm and stained with haematoxylin and eosin (H&E) for light microscopic examination. Liver was also stained with periodic acid Schiff (PAS) method.

For glucose tolerance test (GTT) blood was collected first from the tail veins of control and arsenic treated rats after 18 hrs of fasting followed by challenge with glucose (25 mg glucose/100 g body weight) and at the following time point after glucose infusion: 1.5, 2.5 and 24 hr. blood glucose was measured using a blood glucose monitoring system (glucometer) (Chakrabarti *et al*., 2007; Guria *et al*., 2012 and 2014; Guria, 2017).

The superoxide anion production in hepatic cells was evaluated using NBT reduction test.

RESULTS

Blood glucose level

In the control rats the blood glucose level returned to the normal level after 24 hr of glucose feeding. Like control rats, in experimental rats glucose level increased after 1.5 hr of glucose challenge but the elevated glucose didn't return to control level even after 24 hr of glucose challenge (Table 1 and Fig 1).

Table 1

Group	0hr	1.5hr	2.5hr	24hr
Control	37.18±1.63	69.22±1.69	54.39±2.03	38.67±1.99
Arsenic treated rats	36.69±2.04	71.31±1.47	55.22±1.29	54.03±1.86

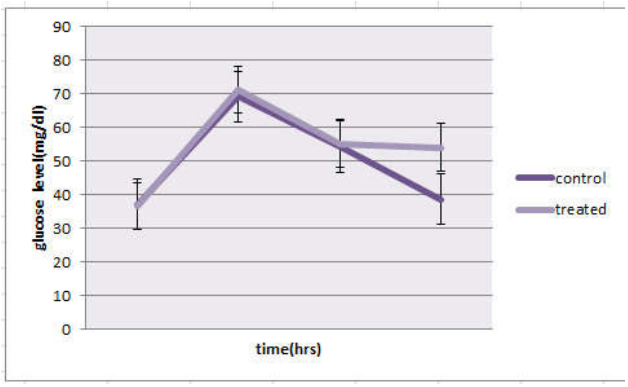

Fig 1

***Table1 and Figure 1*:** Blood glucose level (mg/dl) during glucose tolerance test in control and arsenic treated rats. Values are the blood sugar level (mg/dl) expressed as mean ± SE. P-value < 0.05 is considered to be statistically significant.

Histopathological findings and analysis of pancreas

Histopathology of islets of Langerhan's of pancreas of control animal revealed normal architecture with compact arrangement of cells throughout the study. The islets appeared lightly stained than the surrounding acinar cells, with intact interlobular connective tissue and interlobular duct (Fig 2a and 3a). The pancreatic sections of the treated group showed marked morphological changes. Blood vessels were seen congested and dilated. Some islets cells showed pyknosis. A significant number of islets cells were found to be reduced in number. Photomicrograph of histology of treated pancreas exhibited the disruption of islets, disorientation of cells and disrupted connective tissue septa (Fig 2b and 3b).

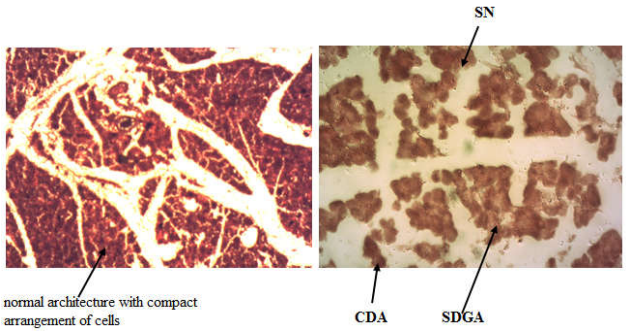

Fig 2a **Fig 2b**

Figure 2 Pancreas under treatment showed cellular disarray (CDA), Severe Degeneration in acini (SDGA), Severe Necrosis (SN) (Fig 2b). (X

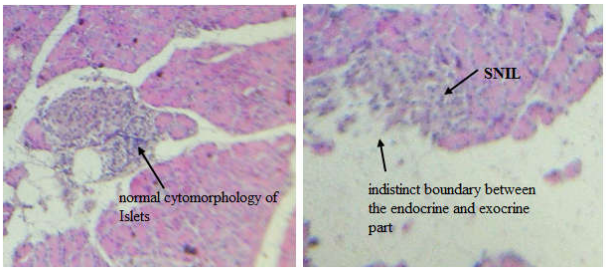

Fig 3a **Fig 3b**

Figure 3 H-E stained section of normal (Fig3a) and treated (Fig3b) rat pancreatic islets (X 400). Photomicrograph of pancreatic tissue of the treated group showed the indistinct boundary between the endocrine and exocrine part. Pancreas under experimental condition showed severe necrosis In Ilet of Langerhans (SNIL) (Fig 3b). (X 400)

Histopathological findings and analysis of liver

Treated rat exhibited marked changes in the general cytomorphology of liver as evident by the enlargement of central hepatic venule and disorientation of hepatocytes. The histological examination of the H-E stained control liver tissues showed normal cytoarchitecture of liver with visible central veins with radiating cords of hepatocytes (Fig 4a). Treated liver revealed central vein congestion with significant dilatation of sinusoidal spaces (Fig 4b).

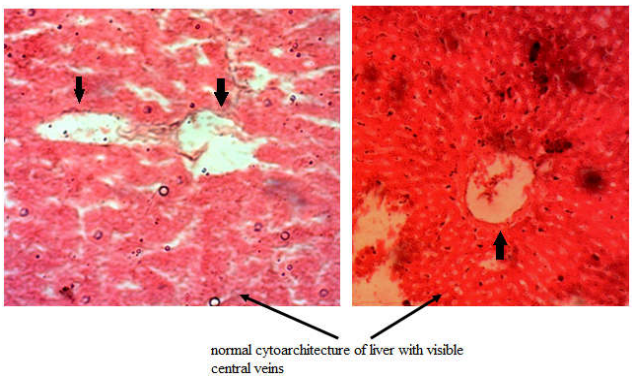

Fig 4a

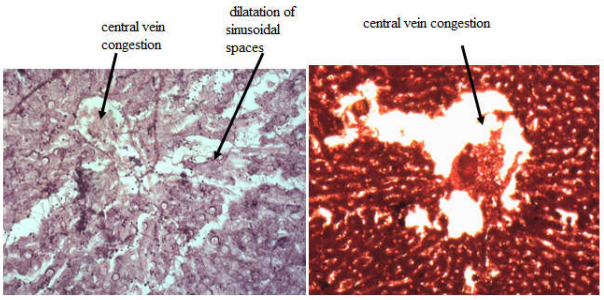

Fig 4b

Figure 4 H-E stained sections of rat liver (X 400). Photomicrograph of liver tissue of the control group showed normal cytoarchitecture of liver with visible central veins (Fig 4a). Treated liver revealed central vein congestion with significant dilatation of sinusoidal spaces (Fig 4b). (X 400)

PAS analysis of liver

Liver section of treated group showed vacuolisation in the liver parenchyma, with significant dilatation of sinusoidal spaces, congestion of blood vessel and increasing amounts of connective tissue in the portal area (Fig 5b). Treated liver showed lower PAS response (Fig 5b).

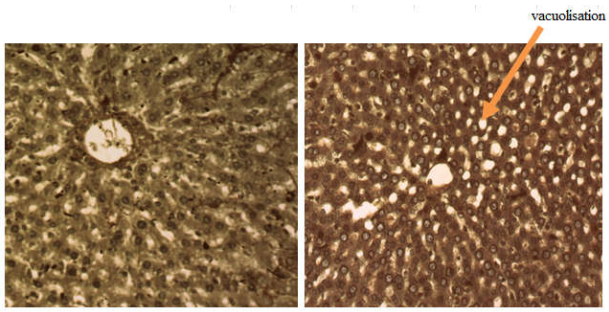

Fig 5a **Fig 5b**

Figure 5 PAS stained section of rat liver (X 100). Photomicrograph of liver tissue showedchanges in glycogen deposits in liver tissue in the control (a) and As treated groups (b). Periodic acid-Schiff. Scale bars: 20µm.

NBT (Nitroblue Tetrazolium) staining of liver cells

Field of treated liver has significant number of NBT positive cells (Fig 6).

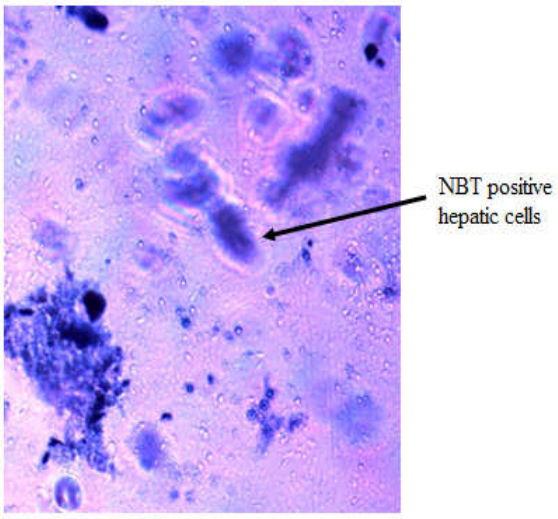

Fig 6

Figure 6 Field of NBT stained treated liver

DISCUSSION

In the recent years, industrial development and agricultural process have resulted in the increased levels of toxic metals in the environment, although relatively high concentrations can also occur naturally (Lopez Alonso *et al*., 2002). Heavy metals have been recognized as strong biological poisons because of their present nature, toxicity, tendency to accumulate in organisms and undergo food chain amplification (Dinodia *et al*., 2002). In the environment arsenic is found in organic and inorganic forms and in different valence or oxidation states. Chronic exposure to arsenic causes increase in blood pressure, diabetes, cardiovascular diseases and cancer (Vijaya Kumar *et al*., 2014). The present study revealed that histopathology of islets of Langerhan's of pancreas of control animal has normal architecture. The pancreatic sections of the treated group showed marked morphological changes. Treated rat exhibited marked changes in the general cytomorphology of liver. Similar histopathological changes were reported in the liver of diabetic rats by Evelson *et al*., 2005. Significant number of treated liver cells showed greater NBT positive response. Increased production of ROS (reactive oxygen species) may occur in treated condition. Accumulation of ROS is damaging to various cellular components and macromolecules including plasma membrane, nucleic acids, and proteins and eventually leads to cell death. NBT reacts with O_2^- (superoxides) to form a dark blue colour whereas the superoxide negative cells did not retain the stain.

In arsenic treated rat increments of blood glucose levels were observed after GTT and the hyperglycemia persisted even 24 h after glucose load. The histochemical PAS staining showed that arsenic induced diabetes resulted in approximately moderate depletion of hepatic glycogen in comparison to the control group. Liver and pancreas tissues both act as glucose sensors, and damage to these tissues plays an important role in the onset of diabetes. The pancreatic β-cell possesses the ability to respond to a minor increase in the blood glucose level, thereby maintaining that level (Gulle *et al*., 2014). The

liver plays a major role in maintaining glucose homeostasis by regulating glucose absorption, accumulation and catabolism through mediation of various metabolic signals (Ferre *et al*., 1996). Thus, the effect of toxicants on tissues such as the liver and pancreas that regulate glucose metabolism is an interesting area to explore. The result of present study also corroborated a previous study (Guria *et al*., 2016). The result of present study may affect glucose homeostasis. Therefore metal induced alteration of pancreas and liver may persuade the condition of diabetes mellitus, but further experiments needs to be performed.

CONCLUSION

The changes in glucose homeostasis, reduced insulin sensitivity and b-cell function are the core pathophysiological defects in T2D (Mertz *et al*., 1975). Present result indicated that iAs exposure could pose higher risk for diabetic individuals and need to be paid more attention. Arsenic as a non-traditional risk factor to the T2D has been given more and more attentions. Although there are many epidemiological researches between iAs and diabetes, the roles of iAs in development of diabetes are still unclear. More experimental data at environmentally relevant concentrations are needed. Previous studies showed that mice might be less susceptible than human to arsenic toxicity, partly due to a faster metabolism and clearance of arsenic (Mazumder, 2005). Therefore, it is necessary to use higher exposure concentration of iAs than the environmentally relevant concentrations in mouse experiment.

Acknowledgement

The author is thankful to the Head, Post Graduate Department of Zoology, Barasat Govt. College for providing infrastructure for conducting the work.

References

1. Breslin, K (2000). Arsenic in Asia: water at its worst. *Environ. Health Perspect*, 108: 393-397.
2. Chakrabarti, S., Guria, S., Samanta, I and Das, M (2007).Thyroid dysfunction modulates glucoregulatory mechanism in Rat. *Ind. J Exp. Biol,* 45 (6): 549-553.
3. Chen, C. J., Lai, M and Hsu, M (1992). Dose–response relationship between ingested inorganic arsenic and diabetes mellitus. 4th Annual Meeting of International Society of Environmental Epidemiology, Abstr., Cuernavaca, Morelos.
4. Das, S.K (2013). The protective role of high dietary protein on arsenic induced hepatotoxicity in albino rat model; a possible role of antioxidants. *IOSR Journal of Pharmacy and Biological Sciences*, 6(5): 20-25.
5. Diaz-Villasenor, A., Cruz, L., Cebrian, A., U, Raul., Ramı´rez, H., Hiriart, M., Garcı´a-Vargas, G., Bassol, S., Sordo, M., Gandolfi, A. J., Klimecki , W. T., Lizbeth L. C., Cebrian , M. E., Ostrosky-Wegman, P (2013). Arsenic exposure and Calpain-10 polymorphisms impair the function of pancreatic beta-cells in humans: A pilot study of risk factors for T2DM. *PLoS One*, 8: e51642.
6. Dinodia, G.S., Gupta, R.K and Jain, K.L (2002). Effect of cadmium toxicity on liver glycogen in some fresh water fish. *Proc. XI Natl, Symp. Environ*, 236 - 238.
7. Evelson, P., Susemihl, C., Villarreal, I., Llesuy, S., Rodríguez, R., Peredo, H., Lemberg, A., Perazzo, J and Filinger, E (2005). Hepatic morphological changes and oxidative stress in chronicstreptozotocin-diabetic rats. *Ann. Hepatol*, 4: 115-120.
8. Ferre, T., Riu, E., Bosch, F and Valera, A (1996). Evidence from transgenic mice that glucokinase is rate limiting for glucose utilization in the liver. *FASEB J*, 10: 1213-1218.
9. Gulle, K., Ceri, N.G., Akpolat, M., Arasli M and Demirci, B (2014). The effects of dexpanthenol in streptozotocin-induced diabetic rats: Histological, histochemical and immunological evidences.*Histol Histopathol*, 29(10): 1305-1313
10. Guria, S (2017). Alteration of testicular cytomorphology in albino rats in alloxan induced diabetes. A histological study. *J. Atoms and Molecules*, 7(5): 1133-1139.
11. Guria, S., Chakraborty, B and Banerjee, M (2016). Chromium (VI) induced histological changes of pancreatic islets and liver: a preliminary study of metal induced diabetes mellitus. *The Experiment*, 35(1): 2171-2181.
12. Guria, S., Ghosh, S and Das, M (2014). Diabetogenic Action Of Alloxan On Liver Histopathology. *the experiment,*28(2): 1906-1912.
13. Guria, S., Chhetri, S., Saha, S., Chetri, N., Singh, G., Saha, P.B., Sarkar, B.S and Das, M (2012). Study of cytomorphology of pancreatic islets and peritoneal macrophage in alloxan induced diabetic rat: a mechanistic insight. *Animal Biology Journal,* 3(3): 101-110.
14. Hectors, T.L., Vanparys, C., van der Ven, K., Martens, G.A., Jorens, P.G., Van Gaal, L.F., Covaci, A., De Coen, W., Blust, R (2011). Environmental pollutants and type 2 diabetes: a review of mechanisms that can disrupt beta cell function. *Diabetologia*, 54(6): 1273–1290.
15. Inceoglu, B., Wagner, K. M., Yang, J., Bettaieb, A., Schebb, N. H., Hwang, S. H., Morisseau, C., Haj, F. G and Hammock, B. D (2012). Acute augmentation of epoxygenated fatty acid levels rapidly reduces pain-related behavior in a rat model of type I diabetes. *Proc Natl Acad Sci*, 109: 11390-11395.
16. Islam, M. R., Khan, I., Hassan, S.N., McEvoy, M., D'Este, C., Attia, J., Peel, R., Sultana, M., Akter, S., Milton, A.H (2012). Association between type 2 diabetes and chronic arsenic exposure in drinking water: Across sectional study in Bangladesh. *Environ Health*, 11: 38 (doi: 10.1186/1476-069X-11-38).
17. Lai, M. S., Hsueh, Y.M., Chen, C.J., Shyu, M.P., Chen, S.Y., Kuo, T.L., Wu, M.M., Tai, T.Y (1994). Ingested inorganic arsenic and prevalence of diabetes mellitus. *Am. J. Epidemiol*, 139: 484-492.
18. Liu, S., Guo, X., Wu, B., Yu, H., Zhang, X and Li, M (2014). Arsenic induces diabetic effects through beta-cell dysfunction and increased gluconeogenesis in mice. *Scientific Reports*, 4: 6894 DOI: 10.1038/srep06894
19. Lopez Alonso, M., Benedito, J. L., Miranda, M., Castillo, C., Hernandez, J and Shore, R.F (2002). Interaction between toxic and essential trace metals in cattle from a region with low levels of pollution. *Arch. Environ. Contam, Toxicol*, 42: 165 -172.

20. Lu, T.H., Su, C.C., Chen, Y.W., Yang, C.Y., Wu, C.C., Hung, D.Z., Chen, C.H., Cheng, P.W., Liu, S.H., Huang, C.F (2011). Arsenic induces pancreatic beta-cell apoptosis via the oxidative stress-regulated mitochondria-dependent and endoplasmic reticulum stress triggered signaling pathways. *Toxicol Lett*, 201(1): 15-26.
21. Lynn, S., Shiung, J. N., Gurr, J. R. and Jan, K. Y (1998). Arsenite stimulates poly (ADP-ribosylation) by generation of nitric oxide. *Free Radic. Biol. Med*, 24: 442-449.
22. Marx, J (2002). Unraveling the causes of diabetes. *Science*, 296: 686-689
23. Maull, E.A., Ahsan, H., Edwards, J., Longnecker, M.P., Navas-Acien, A., Pi, J., Silbergeld, E.K., Styblo, M., Tseng, C.H., Thayer, K.A., Loomis, D (2012). Evaluation of the association between arsenic and diabetes: A national toxicology program workshop review. *Environ Health Perspect*, 120: 1658–1670.
24. Mazumder, D. N (2005). Effect of chronic intake of arsenic-contaminated water on liver.*Toxicol Appl Pharmacol*, 206: 169-175.
25. Mertz, M (1975). Effects and metabolism of glucose tolerance factor. *Nutr. Rev*, 33: 129-135.
26. Mukherjee, S., Das, D., Darbar, S and Mitra, C (2003). Dietary intervention affects arsenic-generated nitric oxide and reactive oxygen intermediate toxicity in islet cells of rats. *Current Science*, 85(6): 786-793.
27. Parakkal, P.F (1961). Mordanting fixation as a means of facilitating the staining of pancreatic cells of mouse. *Stain Technol*, 36: 33-34.
28. Rahman, M and Axelson, O (1995). Diabetes mellitus and arsenic exposure: a second look at case–control data from a Swedish copper smelter. *Occup. Environ. Med*, 52: 773-774.
29. Schraufstatter, I. U., Hyslop, P. A., Hinshaw, D. B., Spragg, R. G., Sklar, L. A and Cochrane, C. G (1986). Hydrogen-peroxide induced injury of cells and its prevention by inhibitors of poly (ADP-ribose) polymerase. *Proc. Natl. Acad. Sci*, 83: 4908-4912.
30. Souza, K., Maddock, D.A., Zhang, Q., Chen, J., Chiu, C., Mehta, S., Wan, Y (2001). Arsenite activation of P13K/AKT cell survival pathway is mediated by p38 in cultured human keratinocytes. *Mol Med*, 7: 767-772.
31. Vijaya Kumar, J., Cynthia Sailaja, M., Praveena, M., Jayantha Rao, K (2014). Impact of sodium arsenate on pancreas, blood glucose and tissues glycogen levels in albino rat. *Indian Journal Of Applied Research*, 4 (11): 467-469.
32. Zimmet, P., Alberti, K. G and Shaw, J (2001). Global and societal implications of the diabetes epidemic. *Nature*, 414: 782-787.

How to cite this article:

Srikanta Guria (2018) 'Arsenic Induced Hepatotoxicity and Damage of Pancreatic Islets In Albino Rat: A Possible Role of Diabetes Mellitus ', *International Journal of Current Advanced Research,* 07(1), pp. 9159-9163.
DOI: http://dx.doi.org/10.24327/ijcar.2018.9163.1502

****