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Biochemical Profiling of Arsenic Trioxide-Induced Impaired Carbohydrate Metabolism and its Therapeutic Intervention via Modulation of Metabolic Pathways

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MSHA administered and supervised the project and planned methodology. KR and MSHA presented the concept. KR, MSHA and SR wrote the manuscript. SK, SR and HS did literature search. HS performed data curation, experimental analysis and validation. KR was responsible for investigation. SK helped in data validation and editing the final draft.

Key words
HbA1c, α-amylase, Resveratrol, Hexokinase, Glucose-6-phosphatase

ABSTRACT

We aimed to investigate the impact of arsenic trioxide (ATO) in impairing carbohydrate metabolism and therapeutic potential of resveratrol (RSV) to treat ATO-induced metabolic disorder. Twenty Wistar rats were used for this study; one group was normal control (NC), second group was exposed to ATO only while the other two groups received metformin (MF) and RSV separately along with ATO. We measured serum levels of glycemic index biomarkers and carbohydrate metabolizing enzymes. Simultaneously, we also measured arsenic concentration in liver using HG-AAS technique. ATO exposed rats showed a significant elevation in serum glucose, reduction in serum insulin, rise in insulin resistance and alteration of carbohydrate metabolizing biomarkers when compared with unexposed rats. Arsenic concentration was found to be significantly high in liver tissues of ATO exposed rats. Contrarily, RSV was found to be effective in regulating normal glycemic level, insulin tolerance, and metabolic biomarkers. Hence, it was found that ATO exposure is correlated with onset of impaired metabolism and RSV can be used as therapeutic intervention for arsenic-induced impaired metabolism.

INTRODUCTION

Heavy metals are metallic elements entering into our environment by several natural and anthropogenic sources, such as, natural weathering of earth crust, soil erosion, industrial discharge, sewage effluents, mining, urban runoff, use of pesticides and many other sources (Matta and Gjyli, 2016; Rehman *et al*., 2018a). The main source of exposure of heavy metals are usually through diet and water consumption. These heavy metals in wastewater are responsible for several adverse health outcomes and environmental intoxication (Hutton, 1987; Matta and Gjyli, 2016; Sabir *et al*., 2019). Arsenic is one of these environmental toxicants which is the 20th abundantly present element in the earth crust, commonly present in drinking water, food, soil and air which results into challenging health impacts and outcomes upon exposure both in humans and animals. Arsenic accumulates in the liver where it can affect a number of metabolic pathways (Hutton, 1987; Irshad *et al*., 2021; Kulshrestha, 2014; Mandal and Suzuki, 2002; Ng, 2005; Tariang *et al*., 2019). Trivalent arsenicals are more toxic as compared to pentavalent arsenicals (Duker *et al*., 2005; Liebl *et al*., 1995). Ground water contains both forms of arsenic and hence is associated with chronic arsenicosis in many developing countries such as India, China, Pakistan, Bangladesh, Iran, Nepal, Taiwan and Chile (Ng, 2005; Sabir *et al*., 2019; Saha *et al*., 1999). Drinking water contaminated with inorganic arsenicals is the major exposure route of arsenic in developing countries where arsenic concentration in drinking water is found to be more than the maximum permissible value i.e., 10 ppb (Kulshrestha *et al*., 2014). The toxicity of trivalent arsenicals is due to their binding with thiol group of various biologically active proteins which can inhibit a number of enzymes that are involved in carbohydrate metabolism and other metabolic pathways

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(Liebl *et al*., 1995).

Type 2 diabetes mellitus (T2DM) is widely spread metabolic ailment, accounting for 90-95% of all diabetic cases. T2DM results in disrupted carbohydrate and lipid metabolism which is caused by inappropriate insulin secretion and improper adipocyte functioning. Symptomatically, T2DM is related to insulin resistance particularly in body's peripheral tissues, hyperglycemia, and varied insulin secretory action of pancreatic β-cells. Risk factors associated with T2DM are older age, physical inactivity, life stress, obesity, genetic predisposition, central adiposity, family history, medications, some viral or bacterial infections and environmental toxicants. Various epidemiological studies have shown that the chronic exposure of iAs can also cause T2DM (Kulshrestha *et al*., 2014; Longnecker and Daniels, 2001; Navas-Acien *et al*., 2006; Sharif *et al*., 2021; Tseng *et al*., 2002). According to American Diabetes Association, arsenic-induced DM is classified under the other specific types of diabetes but based on clinical manifestations, the pathophysiology of arsenic-induced DM is similar to that of T2DM (Association, 2004; Paul *et al*., 2007). Association of arsenic exposure and onset of DM is comparatively a novel finding. This relationship has been observed in human beings drinking contaminated water with arsenic in Bangladesh, Taiwan, and other developing countries and among the peoples working in art glass industry and copper smelters in Sweden (Chen *et al*., 2007; Rahman and Axelson, 1995; Rahman *et al*., 1996, 1998; Tseng, 2004). Arsenic can disrupt the normal metabolic pathway of carbohydrate by impairing various enzymes involved in its metabolism. The activity of hexokinase, an important enzyme in glycolysis, is found to be inhibited with arsenic exposure (Zhang *et al*., 2015). iAs^{III} can also significantly inhibit many other enzymes involved in glucose metabolism (Kulshrestha *et al*., 2014; Mukherjee *et al*., 2004; Santra *et al*., 2000). ROS generated by arsenic in body may induce the apoptosis in pancreatic β-cells which not only impairs the release of insulin from β-cells into the blood circulation but also causes the destruction of islet cells leading to further aggravation of diabetic conditions. Hence, we can correlate these two facts that consumption of drinking water with arsenic may be one of the risk factors of increased diabetic cases in developing countries (Sabir *et al*., 2020; Tseng, 2004).

Various preventive and therapeutic measures are available which are recommended to treat DM. Metformin (MF), a biguanide, is an oral antidiabetic drug that is widely prescribed for the treatment of T2DM, especially for obese patients (Meng *et al*., 2017; Rena *et al*., 2017; Zhang *et al*., 2017). It is used as a standard medication in experimental diabetic models. MF is usually associated with hypoglycemic effects due to decrease in basal and postprandial blood glucose level. Synthetic antidiabetic drugs usually exhibit several unpreventable side effects; thereby several studies are being conducted to analyze the use of natural remedies and drugs for DM therapy (Duarte-Vázquez *et al*., 2016; Rehman *et al*., 2018). Resveratrol (trans-3, 4, 5-trihydroxystilbene), a polyphenolic compound naturally present in grapes, peanuts, wine and in some berries, is one of such compounds which can scavenge intracellular free radicals effectively and also some oxidants in many cell types (de la Lastra and Villegas, 2005; Kulshrestha *et al*., 2014; Zhang *et al*., 2013). Resveratrol (RSV) is the most widely studied stilbene which possesses potential health benefits to human beings including anti-cancer, neuro-protective, anti-viral, anti-inflammatory, anti-aging and life prolonging effects and also found to be efficacious in treating DM and obesity as well. Based on our previous studies, it has been found that RSV effectively increases insulin sensitivity in both diabetic patients and diabetic rats. Thereby, RSV can be employed as an inexpensive therapy for T2DM (Kalantari and Das, 2010; Rehman *et al*., 2018; Sharif *et al*., 2020; Zhu *et al*., 2017). RSV improves hyperglycemia, increases glucose tolerance, protects pancreatic β-cells from necrosis and improves diabetic cardiomyopathy (Palsamy and Subramanian, 2008; Tsai *et al*., 2017). Due to antioxidant activity, RSV is also proved to be effective in preventing renal injuries which include diabetic nephropathy. Despite of these various beneficial effects of RSV, it is still not approved drug for human use and drug needs further research and investigation to explore its usage and benefits in various therapies (Duarte-Vázquez *et al*., 2016; Sovak, 2001).

In order to understand the arsenic-induced impaired metabolism, we hypothesized that arsenic exposure alters the normal metabolic reactions of carbohydrate via affecting some enzymes involved in its metabolism. To address this hypothesis, the impact of arsenic trioxide (As_2O_3) was observed on glycemic control biomarkers (serum glucose, HbA1c, insulin and insulin resistance) and biomarkers of carbohydrate metabolism (α-amylase, α-glucosidase, hexokinase, and glucose-6-phosphatase catalytic) along with detection of arsenic concentration in liver samples of Wistar rats. We also investigated the therapeutic potentials of RSV in arsenic-induced impaired metabolism taking MF as a standard drug.

MATERIALS AND METHODS

Chemicals required

Arsenic trioxide (catalog number; 80206) and resveratrol (catalog number; 32060) were purchased from

Bristol Mayer Biotech Pakistan. Metformin (MF) was purchased from Care Pharmacy Faisalabad, Pakistan. 0.9% normal saline (NS) was prepared using laboratory chemicals (NaCl and deionized water). The rest of the chemicals used during the research were of analytical grade.

Study design

Twenty adult Wistar rats (weighing about 120-160 g), purchased from the animal house unit of University of Agriculture Faisalabad (UAF), Pakistan, were used for this study after approval from Ethical Committee (ERC 2191, study no. 19791) of Government College University, Faisalabad (GCUF) Pakistan. After acclimatization, the rats were divided into four groups, each of 5 rats. Group 1 normal control (NC) received only 0.9% normal saline intraperitoneally (i.p). Group 2 (ATO group) ATO group received 2.5 mg ATO/kg body weight/day i.p for 15 days. The group 3 (MF group) received 500 mg metformin/kg body weight/day, one hour before the administration of 2.5 mg ATO/kg body weight/day for 15 days. Group 4 (RSV group) received 8 mg RSV/kg body weight/day, one hour before the administration of 2.5 mg ATO/kg body weight/day for 15 days. The rats were daily monitored for the water and diet intake. The weights of all rats were also monitored at the beginning, after one week and at the termination of study.

On 15th day of experiment, animals were sacrificed through cervical dislocation and their whole blood was collected in EDTA tubes. After that, the abdomen was dissected and liver were taken out and preserved for arsenic detection. Serum was separated from the blood samples after centrifugation at 3000 × g for 30 min at 4 °C and used for the estimation of serum insulin level and HbA1c using ELISA kit (Catalog Number; INS5275, Elabscience®) and HbA1c kit (Catalog Number; SG10984, Elabscience®).

HOMA-IR (homeostatic model assessment for insulin resistance) was used for predicting the level of insulin resistance in body's peripheral tissues.

HOMA IR = Fasting insulin (μU) X Fasting glucose (mM)/22.5

ELISA kits of α-amylase (Catalog Number; E-EL-R2544, Elabscience®), α-glucosidase (Catalog Number; E-EL-R1083, Elabscience®), hexokinase (Catalog Number; E-EL-RR0502, Elabscience®), and G6PC (Catalog Number; E-EL-M1362, Elabscience®) were used to determine the afore-mentioned carbohydrate metabolizing enzymes.

Measurement of arsenic concentration in liver

The liver samples were homogenized in the presence of lysis buffer solution and 0.1 M phosphate buffer solution (PBS). Then homogenized tissue samples were collected in the falcon tubes already having 0.1 M PBS and fluid was collected after centrifugation at 3000 × g at 4℃ for 15 minutes. The fluid was predigested at room temperature for 24 h and then was digested using 37% hydrochloric acid, 65% nitric acid and 30% hydrogen peroxide followed by the procedure as described previously (Mohammed *et al*., 2017). Then after the dilution of five times, these samples were used for measuring the arsenic concentration by using hydride generation-atomic absorption spectroscopy (HG-AAS).

Statistical analysis

The values of all biochemical parameters were presented as mean ± standard deviation (SD). One-way ANOVA and two-way ANOVA tests were performed for statistical analysis using Graph pad prism version 5.01. The level of significance was $P<0.05$. All the values were compared with the values of control group for better understanding.

RESULTS

Effect of ATO and treatment on glucose insulin

Hyperglycemic effect was significantly high in the ATO, MF and RSV groups ($P<0.001$) when it was compared with the NC group. On the other hand, the level of significance was $P<0.01$ in case of both MF and RSV group when compared with ATO group, which means that both metformin and resvetrol reduce blood glucose level. While the comparison of hypoglycemic effects of MF and RSV remained non-significant showing that RSV and MF both show similar hypoglycemic effect (Fig. 1).

We estimated the HOMA-IR value to predict the insulin resistance in experimental rats. We found that in ATO-exposed rats, the value of HOMA-IR was significantly high as compared to that of control group whereas RSV ameliorated the insulin resistance as evidenced from the decreased value of HOMA-IR in RSV-treated group when compared with that of ATO-exposed experimental rats (Fig. 1). ATO exhibited a significant elevation in blood glucose level except the NC group (Fig. 1). We found a non-significant ($P>0.05$) difference between the blood glucose level of MF and RSV groups which showed that both of these interventions are efficacious in normalizing the glucose level by ameliorating the toxic effects ATO. We also estimated the value of HbA1c (%) and found that ATO has significantly increased the percent value of HbA1c when compared with that of NC group whereas RSV improved the percent value of HbA1c by ameliorating the toxic effects of ATO (Fig. 1).

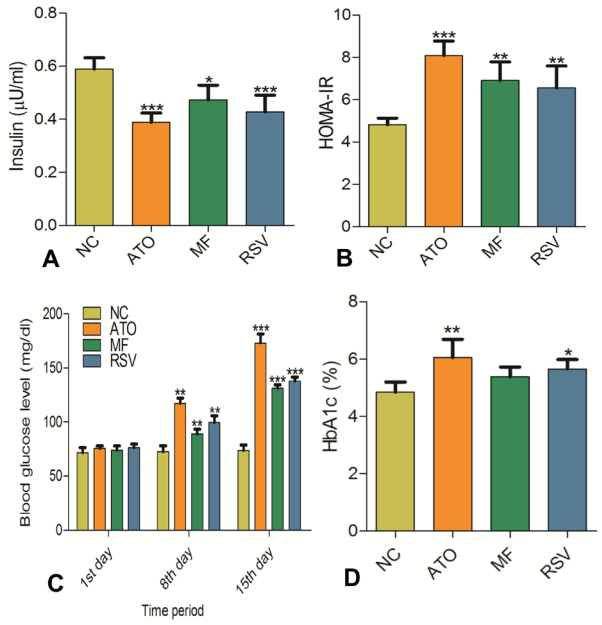

Fig. 1. Effect of ATO on serum insulin (A), insulin resistance (B), bloood glucose level (C) and HbA1c level (D). Significance was estimated by Bonferroni post-test using one way ANOVA. * represents P < 0.05, ** represents P < 0.01 and ns represents non-significant when compared with NC group. ATO, arsenic trioxide group; HbA1c, hemoglobin A1c; MF, metformin group; NC, normal control group; RSV, resveratrol group.

Effect of ATO on carbohydrate metabolizing enzymes

The values of α-amylase were significantly high in ATO group as compared to NC group. The results of RSV group and MF group remained significant when both of these groups were compared with the ATO group while the effect of arsenic on the serum level of α-glucosidase remained non-significant among all groups which showed that arsenic did not impose any effect on this enzyme (Fig. 2). Similarly, the serum level of hexokinase was surprisingly reduced in ATO group as compared to NC group (P<0.001). The hexokinase level was non-significant in case of MF group in comparison to NC group (P>0.05), while RSV group showed less decline in hexokinase level when it was compared with NC group (P<0.01). On the other hand, when we compared the values of ATO group with MF group, the level of significance seen was P<0.001, and when ATO group and RSV group were compared then level of significance was P<0.01. Furthermore, the hexokinase level of MF and RSV group was non-significant (Fig. 2). In ATO group, the serum level of G6PC was significantly declined when compared with NC group. When we compared the results of ATO group with MF group, values of G6PC was found to be high (P<0.001), while in comparison with RSV group, the level of significance was found to be P<0.01. The comparison of graphical peaks of MF and RSV remained non-significant (Fig. 2).

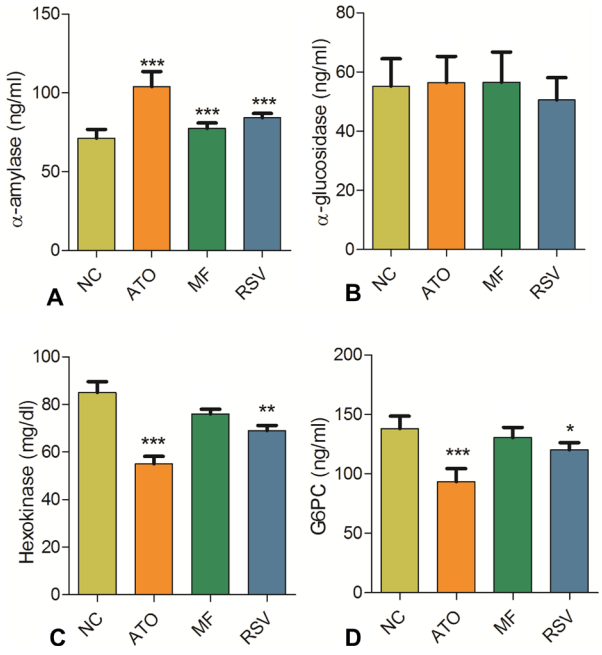

Fig. 2. Effect of ATO on activities of α-amylase (A), α-glucosidase (B), hexokinase (C) and G6PC (D). For statistical details and abbreviations, see Figure 1.

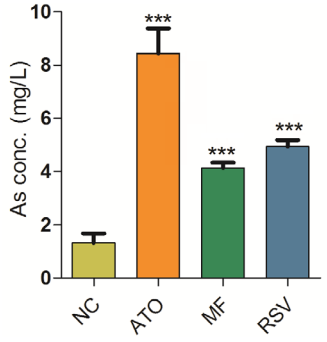

Fig. 3. Arsenic concentration in the liver sample is detected by HG-AAS. For statistical details and abbreviations, see Figure 1. *** represents P < 0.001 when compared with NC group. As, arsenic; HG-AAS, hydride generation atomic absorption spectroscopy.

Arsenic detection in liver samples

Detection of arsenic concentration was evaluated surprisingly high in ATO group (P<0.001) in comparison to NC group. Similarly, arsenic concentration was significantly high in case of both RSV and MF group when

compared with NC group (P<0.001). The comparison of RSV and MF group with ATO group was also significant (P<0.001), while in case of comparison of RSV group with MF group, the level of significance was P<0.01 as shown in Figure 3.

DISCUSSION

Arsenic exposure causes multiple diseases, and it affects millions of people throughout the world. Inorganic form of arsenic (iAs) usually tends to be more toxic as compared to organic form. Arsenic causes toxicity to the majority of body organs and its poisoning extent depends on multiple factors such as arsenic dose, age of the individual and its susceptibility (Douillet *et al*., 2013; Jomova *et al*., 2011; Ngu and Stillman, 2006). DM is a global concern whose rate is elevating in a fluttering way throughout the world, according to an estimate it would be the leading cause of morbidity and mortality in near future (Huang *et al*., 2011; Yadav *et al*., 2013). DM actually is a group of metabolic disorders that are majorly characterized by hyperglycemia due to altered carbohydrate metabolism and/or defective β-cells or altered functioning of pancreatic β-cells (Lu *et al*., 2011). Arsenic is the endocrine disrupting chemical (EDC), ubiquitously present both in organic and inorganic form in our environment (Fu *et al*., 2010). iAs exposure imposes an alarming threat to human health and an unrecognized contributor to the onset and aggravation of DM (Andrew *et al*., 2018). As proved by several epidemiological studies, exposure of arsenic via drinking water can induce DM (Huang *et al*., 2011). Based on various studies, drinking water contaminated with arsenic is a potent predictor of poor dietary conditions which leads to increased prevalence of DM. Thereby, environmental contaminants can play a potential role in the prevalence of DM (Haq *et al*., 2020; Tanvi *et al*., 2018; Wang *et al*., 2009). For the treatment of DM, various synthetic drugs are used which are associated with multiple side effects, particularly weight gain and acute hypoglycemia (Rehman *et al*., 2018). To avoid such untoward effects, naturally present bioactive chemicals are now preferred for treating DM, RSV, is one of these compounds (Kalantari and Das, 2010). RSV is a stilbene having diverse pharmacological properties and can be used to treat DM (Akash *et al*., 2014; Tsai *et al*., 2017).

In this study, we aimed to investigate the impact of arsenic exposure in inducing DM in rats and compare the therapeutic potential of RSV and standard anti-diabetic drug MF for the treatment of arsenic-induced DM. Keeping in view of widespread arsenic toxicity, the present study was undertaken to analyze the adverse effects of ATO on biomarkers of carbohydrate metabolism. Furthermore, the concentration of arsenic in liver was also computed out to analyze the accumulation of arsenic in liver tissues.

Effects on glucose, insulin and insulin resistance

ATO administration has significantly increased the glucose level and decreased the serum insulin level along with a significant elevation in insulin resistance. The limitation of this study is the insignificant level of HbA1c in ATO group as it was only two weeks study and for the HbA1c, represents the average glucose level in past three months. The serum level of insulin in ATO group was also significantly reduced when it was compared with NC group; while serum level of insulin in case of MF group was slightly reduced than NC group. On the other hand, the insulin level in RSV group was significantly reduced when compared with NC group. MF and RSV both groups showed significant hypoglycemic activity when compared with ATO group. This showed that arsenic is also involved in reducing the insulin release from the pancreas that is may be due to the accumulation of ATO in islets cells which ultimately leads towards their destruction because of oxidative stress.

ATO group showed a high peak of insulin resistance in comparison to NC group (P<0.001), while RSV and MF groups showed similar decline in insulin resistance than NC group. After comparing the values of insulin resistance between standard group and treatment group, RSV found to be effective in lowering the insulin resistance as compared to MF. These results showed the impact of arsenic on insulin intolerance. Insulin resistance is the major causative factor which further aggravates the diabetic complications. Insulin resistance is responsible for hyperglycemia, reduces glucose utilization by muscles, elevates glycogenolysis and impairs insulin secretion from pancreatic β-cells (Rehman *et al*., 2018b). Arsenic induces insulin resistance due to the impairment of normal insulin secretion in body via multiple mechanisms of action. One of such mechanisms is the formation of ADP-arsenate instead of ATP that results into down regulation of ATP-dependent insulin release. Arsenate possesses the similar biochemical properties as that of phosphate and thus can replace phosphate group in energy transfer phosphorylation reactions forming ADP-arsenate. Moreover, arsenic also possesses the potential to make covalent bonds with the disulfide bridges of insulin molecules and with its receptors, thus impairing normal insulin functioning. More adversely, arsenic can induce the superoxide production and it's over expression in pancreas causes the destruction of pancreatic β-cells which further reduces the insulin secretion (Sabir *et al*., 2019; Tseng, 2004). Insulin resistance due to arsenic exposure is usually due to enhanced expression of NF-κB, IL-6 and TNF-α and decreased expression of PPARγ (Tseng, 2004).

iAs^{III} and methylated compounds of arsenic remarkably interfere with the major signal transduction pathways in the body cells which then result into the obstruction of the expression or activation of protein kinase B (PKB, also known as AKT), a key component of the insulin stimulated signal transduction pathway. In this way, iAs remarkably elevates the glycemic level in body through the inhibition of insulin-dependent signal transduction at the PKB level (Kohn *et al*., 1996; Kulshrestha *et al*., 2014).

Effects on carbohydrates metabolizing enzymes

The serum level of α-amylase of ATO group was found to be surprisingly high as compared to the NC group in this study (P<0.001). On the other hand, the serum level of α-amylase of RSV group was slightly higher than NC group (P<0.01). Whereas, our study found insignificant arsenic impact on α-glycosidase level, which explains that arsenic does not impair the enzymatic activity of this enzyme. Various tissues secrete α-amylase among which pancreas secretes the major proportion of this enzyme. The normal range of α-amylase is 100 IU/L to 300 IU/L (Rompianesi *et al*., 2017). α-amylase and α-glucosidase are the important digestive enzymes which play critical role in carbohydrate metabolism through the breakdown of polysaccharides (starch) into α-limit destrin, maltose and maltotriose, thus facilitating the absorption of carbohydrates from intestines into the blood (Haq *et al*., 2020; Tanvi *et al*., 2018). As evidenced through several studies, arsenic can elevate the serum α-amylase level. But there are lot of controversies, based on various studies arsenic down regulates the release of various endocellular enzymes including α-amylase (Sabir *et al*., 2019; Xue *et al*., 2007). Mukherjee *et al*. (2004) observed the serum elevation of α-amylase and β-cells destruction in rabbits which were exposed to arsenic for 30 consecutive day and thus he claimed this possible mechanism of action of arsenic in inducing DM.

We found the reduction in serum level of hexokinase in ATO group as compared to NC group. Hexokinases belong to the family of intracellular enzymes which are the initial enzymes in the process of glycolysis and play significant role in carbohydrate metabolism in the glycolytic pathway by catalyzing the phosphorylation of glucose, fructose and mannose to their corresponding hexose-6-phosphates (Van Schaftingen, 2020). Based on evidence, higher exposure of arsenic can inhibit the hexokinase activity both in *in vivo* and *in vitro* (Sabir *et al*., 2019; Zhang *et al*., 2015). Glycolytic pathway is thus inhibited due to down regulation of hexokinase (Tariang *et al*., 2019). Zhang *et al*. (2015) analyzed the effect of arsenic in decreasing the enzymatic activity of hexokinase-2 due to ATO exposure.

In our study, ATO exposure reduced G6PC level while the comparison of graphical peaks of MF and RSV remained non-significant which showed the equal potency of both of these drugs in regulating the serum level of G6PC. G6PC is an important enzyme in gluconeogenesis, decline in the G6PC level might be due to the down regulation of MDH activity as oxaloacetate formalate would not be available for the process of gluconeogenesis (Shahid *et al*., 2014; van Schaftingen and Gerin, 2002).

Arsenic accumulation in liver

We detected arsenic in liver using HGAAS. The concentration of arsenic was significantly high in the liver of ATO exposed rats while RSV was found to be effective in decreasing the arsenic concentration in the liver. RSV possesses antioxidant activity, and it has been found that RSV has potential to excrete out the ATO from body. Liver is the major organ where metabolism of arsenic takes place. Arsenic can be accumulated in different body organs, including liver, kidney and pancreas. In the pancreas, arsenic can increase the ROS production which ultimately leads to pancreatitis due to β-cells destruction (Sabir *et al*., 2019). Lu *et al*. (2011) explored the role of reactive oxygen species (ROS) in ATO induced pancreatic β-cells apoptosis. He investigated the *in vitro* role of ATO and observed altered insulin secretion, impaired blood glucose tolerance, enhanced lipid peroxidation and apoptosis of islet cells. Due to the destruction of pancreatic β-cells, insulin secretion is decreased and insulin resistance in peripheral tissues is increased which aggravates the hyperglycemic conditions (Rehman *et al*., 2019). Based on the investigation of Connelly *et al*. (2011) chronic exposure of arsenic leads towards pancreatitis (Connelly *et al*., 2011). RSV has promising anti-diabetic properties in the mice models of arsenic-induced DM, in future clinical trials on RSV will bring revolutionary changes in the treatment interventions of DM. We have found that ATO has associated with impaired metabolism and metabolic disorders while RSV possesses therapeutic effcicacy against metabolic disorders especially arsenic induced diabetes as RSV regulates insulin tolerance, glycemic level and metabolic biochemical parameters. Further studies on RSV are required to elaborate it's therapeutic efficacy against metabolic impairments specifically arsenic induced diabetes mellitus.

CONCLUSION

The possible association of arsenic exposure and onset of DM, particularly in developing countries, is the concern of research and public health. Hundreds of thousands of people are exposed to arsenic via drinking water and food ingestion throughout the world which

exceeds the safety limits of the WHO. As DM is the major health concern in this era, particularly in developing countries, for better understanding the risk factors of arsenic exposure associated with DM, further research is needed to explore the effect of arsenic on metabolic impairments and molecular pathways through which arsenic disrupts the islet cells functioning. Such insights would be significant for developing strategies to alleviate the detrimental impacts of environmental pollutant and diabetic risk factor. Such investigations will surely help to accurately appraise and explore the associations that may exist between arsenic exposure and development and/or progression of DM giving new insights into the targets for therapeutic interventions. Therefore, experimental and/or research studies that involve the arsenic concentrations relevant to human exposures, and high-quality prospective epidemiologic studies that utilize relevant means of exposure evaluation in addition to conscientious criteria to define arsenic exposure consequences should be the research priorities in this era. Moreover, resveratrol can be employed as one of the treatment strategies for arsenic-induced DM, thereby, this compound needs further investigation to explore its multiple benefits.

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Compliance with ethical standards

This study was ethically approved from the Institutional Review Board (IRB-GCUF-19791) of Government College University Faisalabad.

Statement of conflict of interest

The authors have declared no conflict of interest.

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