Catechin Protects Against Dyslipidemia and Nephro-Hepatototoxicity Induced in Rats Exposed to Arsenic

Olusegun K. Afolabi[1,*], Regina N. Ugbaja[2], Abimbola Arinola[1], Olubukola S. Olorunnisola[1], Gbadebo E. Adeleke[1].

[1]*Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria*

[2]*Department of Biochemistry, Federal University of Agriculture, Abeokuta, Nigeria*

Abstract

Arsenic poisoning is a major environmental event affecting millions worldwide and its treatment with chelating agents has met with limited success. While arsenic toxicity affects multiple systems in the human body, its mode of action has not been fully elucidated. The present study therefore, investigated the possible protective effects of catechin against hepatorenal damage and dyslipidemia induced by arsenic exposure. Rats were exposed to arsenic (100 ppm) through their drinking water and were treated with catechin (40 mg/kg and 80 mg/kg, body weight) for 30 days. Arsenic exposure resulted in liver dysfunction obvious with increased activities of the hepatic enzymes alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST). This was accompanied with significant elevation of kidney function markers urea and creatinine ($p < 0.05$). Furthermore, arsenic caused the distortion of lipid metabolism resulting in hypercholesterolemia, hypertriglyceridemia and increased plasma phospholipid in the animals. Co-treatment with catechin effectively protected against arsenic-mediated hepatotoxicity, prevented renal damage and restored lipid homeostasis in the rats. The present data indicate the ability of catechin to potentially prevent arsenic-induced nephro-hepatotoxicity and dyslipidemia in rats.

Keywords: Arsenic, Catechin, Hepatotoxic, Nephrotoxic, Dyslipidemia.

1. Introduction

Environmental pollution by heavy metals has become a major global concern, most especially in the developing countries. Arsenic (As) because of its highly toxic nature is one of the most important of these metals (ATSDR, 2007). It is a carcinogen and is considered to be one of the most hazardous chemicals. It is widely distributed in nature and is anthropogenically released into the environment through industrial processes and agricultural usage (Flora et al., 1995; ATSDR, 2007). Studies have shown that arsenic exposure could lead to a variety of biochemical and physiological dysfunctions in humans and experimental animals (Lena et al., 2014). Toxicities induced by arsenic have been associated with different forms of cancer, apoptosis, genetic damage, hematological disorders, and cardiovascular diseases, among many others (Prakash et al., 2014). Environmental exposures to arsenic majorly occur through the consumption of drinking water from contaminated groundwater sources (Murcut, 2012). In some of these groundwater, arsenic concentrations range far above the current maximum permissible limit of 10 µg/l recommended by World Health Organization (WHO, 2011).

Traditionally, the treatment of arsenic poisoning is carried out by administering a chelating agent such as 2, 3-dimercapto-1-propanol (BAL). More orally effective and less toxic dithiol chelating agents, sodium 2,3-dimercaptosuccinic acid (DMSA) and sodium 2,3-dimercaptopropane 1-sulphonate (DMPS), are also in use (Flora et al., 1995; Aposhian and Aposhian., 1989). However, chelation therapy as a form of arsenic poisoning treatment is rife with side effects and is ineffective against some forms of arsenic toxicity (Gupta and Flora, 2005). Oxidative stress has been suggested to be one of the mechanisms by which arsenic effects its toxicity (Kumar et al., 2014). Generally, inorganic arsenic increases reactive oxygen species (ROS) by binding vicinal thiols or sulfur-containing groups (Majhi et al., 2011; Patra et al., 2012). It is, therefore, reasonable to hypothesize that an antioxidant agent could protect against some of the effects induced by arsenic exposure.

In the present study, we test the hypothesis that catechin, a widely distributed flavonoid present in

---

* Corresponding author. e-mail: okafolabi@lautech.edu.ng.

plant foods and drinks, possessing strong antioxidant activity can protect against arsenic-induced hepatotoxicity, nephrotoxicity and dyslipidemia in rats. The demonstration of this may be an important finding for developing new treatment for arsenic poisoning.

2. Materials and Method

2.1. Chemicals

Sodium arsenite and catechin were obtained from Sigma-Aldrich, Germany. All other chemicals used were of analytical grade.

2.2. Animals and Treatment

Thirty five male Wistar rats (bred in the Animal House, Faculty of Basic Medical Sciences, LAUTECH) weighing an average of 120g were used for the present study. The animals were housed in an animal room with controlled temperature (22±2˚C) and a regular 12h light-dark cycle (06:00-18:00h). They were allowed to acclimatize for 14 days before used for the experiment. They were allowed free access to rat chow and drinking water. The experimental protocol was approved by the Biochemistry Department Ethic Committee on Research of LAUTECH.

Animals were randomly divided into 5 groups of 7 rats each and were treated as below for a period of 30 days: Group I, normal animals; Group II, arsenic (in the form of sodium arsenite), 100 ppm in drinking water; Group III, Catechin at 40 mg/kg body weight/day, orally; Group IV, arsenic (as in Group II) plus Catechin at 40 mg/kg body weight/day, orally; Group V, arsenic (as in Group II) plus Catechin at 80 mg/kg body weight/day, orally.

The doses for arsenic and catechin were selected based on previously published studies (Afolabi et al., 2015; Chopra et al., 2004). At the end of the treatment period, blood was collected from the animals into heparinized tubes by cardiac puncture under light ether anesthesia after an overnight fast. The blood was centrifuged at 3000g for 10 min to separate the plasma. Samples were stored at -20oC for subsequent biochemical analyses.

2.3. Biochemical Analyses

Plasma concentrations of total cholesterol, triglyceride and phospholipids were determined with standard diagnostic kits (Chemelex®, Barcelona, Spain). HDL cholesterol was analyzed with the same diagnostic kit for total cholesterol after very low density lipoproteins (VLDL) and low density lipoproteins (LDL) were precipitated with heparin-MnCl2 solution as described by Gidez et al. (1982). LDL cholesterol was estimated using Friedewald formula (1972). Plasma urea and creatinine were determined spectrophotometrically by the method of Patton and Crouch (1977) and Henry et al.'s method (1974), respectively. The activities of plasma hepatic marker enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined by the method of Reitman and Frankel (1957), while alkaline phosphatase (ALP) was assayed using the method of Bessey et al. (1946).

3. Results

Figure 1 shows the levels of AST, ALT and ALP in the plasma of rats. In the group exposed to arsenic only, ALT level was significantly increased when compared with the control group (38.16 ± 3.63 and 26.14 ± 2.59 U/L, respectively, $p < 0.05$). Treatment of arsenic exposure with either high or low dose catechin lowered ALT level significantly when compared with arsenic exposure only (19.18 ± 2.13 and 26.64 ± 2.03 UL, respectively, $p < 0.05$). There was no significance difference between control and catechin only groups ($p > 0.05$). Similarly, arsenic caused drastic elevation of both ALP and AST in the rats, compared to the control. Arsenic increased both activities from 26.66 ± 3.79 and 77.20 ± 4.05 to 48.66 ± 3.07 and 93.60 ± 4.84 U/L, respectively. High and low dose catechin however, reversed this trend and significantly lowered ALP to 26.26 ± 1.83 and 33.51 ± 2.83 U/L, and AST to 87.70 ± 2.65 and 70.60 ± 3.69 U/L respectively.
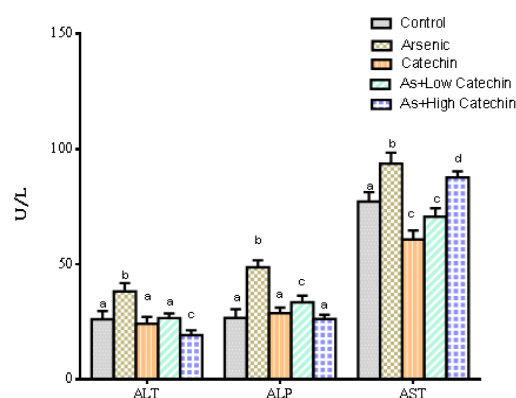

Figure 1. Effects of catechin on the levels of ALT, ALP and AST in the plasma of rats exposed to arsenic. Values are mean ± SD. Bars bearing different alphabets are significantly different from each other at $p < 0.05$.

Arsenic exposure resulted in a marked impairment of kidney function as demonstrated by the high levels of urea and creatinine shown in Figure 2. In arsenic only group, urea level was significantly increased when compared to the control and catechin only groups (45.81 ± 1.24, 18.23 ± 2.04 and 24.67 ± 1.32 mg/dl, respectively, $p < 0.05$). Treatment with either low or high dose catechin reduced high urea level induced by arsenic exposure (32.74 ± 1.58, and 35.41 ± 2.74 mg/dl, respectively, $p < 0.05$). Creatinine was also significantly elevated in arsenic only group compared with control, and catechin only groups (1.82 ± 0.10, 0.94 ± 0.03 and 0.89 ± 0.04 mg/dl, respectively, $p < 0.05$). Creatinine was lowered by treatment with both low and high doses of catechin (1.15 ±0.06 and 1.05 ± 0.05 mg/dl, respectively, $p < 0.05$).
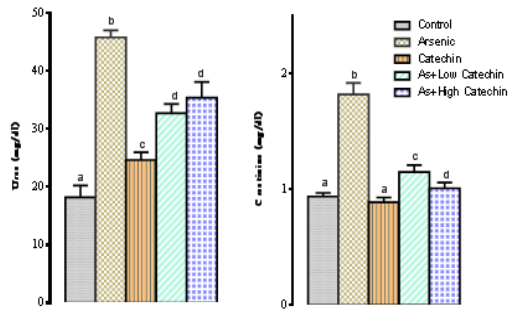
Figure 2. Effects of catechin on plasma urea and creatinine levels in rats exposed to arsenic. Values are mean ± SD. Bars bearing different alphabets are significantly different from each other at $p < 0.05$.

The present study demonstrates that the arsenic exposure induced dyslipidemia in rats. Estimated values of plasma cholesterol, triglyceride and phospholipid levels are depicted in Figure 3. There were no statistically significant changes in plasma cholesterol, triglyceride and phospholipid between control and catechin only groups ($p > 0.05$). However, arsenic exposure caused significant elevation of these lipids compared with the control (90.11 ± 4.59, 48.91 ± 3.81 and 127.36 ± 7.25 mg/dl vs. 50.71 ± 6.01, 40.83 ± 3.75 and 96.69 ± 6.50 mg/dl, respectively, $p < 0.05$). Both high and low dose catechin treatments restored plasma triglyceride and phospholipid concentrations back to control levels ($p > 0.05$). In addition, low and high catechin treatments significantly lowered cholesterol level when compared with arsenic only group (68.09 ± 5.09 and 58.47 ± 3.43 mg/dl, respectively, $p < 0.05$), but the concentrations were not comparable to the basal level.
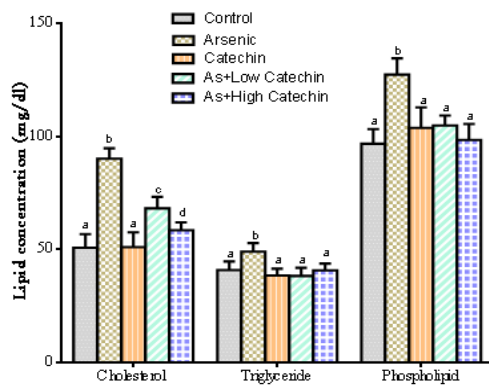

Figure 3. Effects of catechin on the plasma cholesterol, triglyceride and phospholipid concentrations in rats exposed to arsenic. Values are mean ± SD. Bars bearing different alphabets are significantly different from each other at $p < 0.05$.

Arsenic caused the lowering of the HDL concentration while increasing the LDL levels in the rats (Fig. 4). The metal induced a significant 63% reduction in HDL level in rat which was subsequently reversed, to different degrees by catechin treatments. Low dose catechin raised the HDL concentration from 7.89 ± 0.57 mg/dl in arsenic alone group to 25.67 ± 1.96 mg/dl. The high

dose treatment also increased the lipoprotein level (14.43 ± 1.07 mg/dl), albeit the increment was not comparable to that induced by the low dose catechin treatment. LDL concentration was significantly elevated by arsenic from 8.67 ± 0.79 in control to 9.78 ± 0.76 mg/dl ($p < 0.05$). Catechin only group had their LDL concentration lowered significantly ($p < 0.05$), compared with the control group (7.67 ± 0.99 mg/dl). The treatment of arsenic group with low and high doses of catechin also brought about significant reduction of LDL levels (7.63 ± 0.86 and 8.12 ± 0.69 mg/dl, respectively, $p < 0.05$).
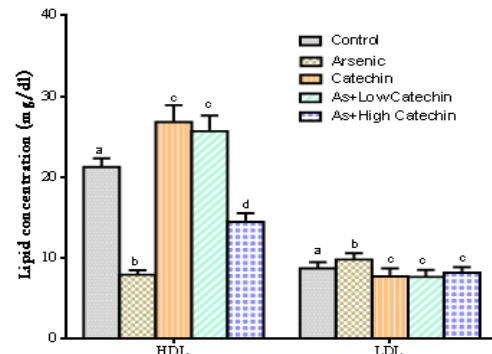

Figure 4. Effects of catechin on HDL and LDL concentrations in rats exposed to arsenic. Values are mean ± SD. Bars bearing different alphabets are significantly different from each other at $p < 0.05$.

4. Discussion

In the present study, it was demonstrated that arsenic exposure could cause dyslipidemia by disrupting lipid homeostasis. Additionally, the findings indicated that arsenic induces liver and kidney injury in rats. However, catechin was able to prevent these toxicities caused by arsenic.

Plasma aminotransferase activities are sensitive indicators of liver damage. This damage may alter transport function and membrane permeability of hepatocytes, resulting in enzymes leakage from the cell. The efflux of these enzymes from the hepatocytes causes a depletion in AST, ALP and ALT levels with concomitant elevation of their activities in the plasma. In the present study, the plasma activities of these enzymes were significantly elevated by arsenic exposure, suggestive of hepatic damage induced by the metalloid. Similar report of liver damage induced by arsenic exposure was given by Miltonprabu and Sumedha (2014). The liver is the major organ involved in xenobiotic metabolism and is thus, susceptible to oxidative damage caused by increased Reactive Oxygen Species (ROS). Arsenic has been demonstrated to generate oxidative stress (Nandi *et al.*, 2006), and the involvement of ROS has been linked with arsenic pathogenicity. Experimental studies suggest that lipid peroxidation, one of the determinants of oxidative stress induced by ROS, contributes to arsenic-induced oxidative damage of membrane (Flora *et al.*, 2008). Arsenic affinity for –SH group of proteins enables it to conjugate and

form covalent attachment with intracellular GSH and inhibit glutathione reductase activity, in addition to glutathione synthesis (Quig, 1988). This leads to depletion of intracellular thiols, escalating oxidative damage to several biological macromolecules (Hansen *et al.*, 2006). The elevated level of the liver enzymes in the plasma may thus be attributed to this process. Catechin treatment on the other hand, was able to reverse the damage induced by arsenic. It was able to restore the enzymes' activities to the basal levels, especially the high dose treatment. In the case of AST, however, treatment with catechin at high dose even though lowered the enzyme level below arsenic-induced activity, was unable to restore it to normal as did the low dose treatment. The ability of catechin to protect against liver injury may be predicated upon its antioxidant activity, which could have prevented the escalation of lipid peroxidation induced by arsenic (Mishra and Flora, 2008; Nandi *et al.*, 2006), leading to membrane damage in the organ. Studies have already demonstrated catechin's protective effect against ROS generation, as well as, the ability of polyphenols to induce antioxidant enzymes (Zhang *et al.*, 2014; Du *et al.*, 2007). Catechin also possesses chelating property and might have chelated with arsenic, removing the metal from circulation thereby, preventing its damaging effects (Sugihara *et al.*, 2001).

The protective property of catechin, through its antioxidant ability, against the subsequent membrane damage induced by ROS generation seemed to be at play in its protection of the kidney against arsenic toxicity. Renal dysfunction was demonstrated by arsenic exposure in the present study as exhibited by the increase in plasma urea and creatinine levels. Several studies have demonstrated arsenic toxicity of in the kidney (Eom *et al.*, 2011; Chen *et al.*, 2011) and the major mechanism of kidney damage by this metalloid has been suggested to be oxidative stress (Jomova *et al.*, 2011). The increased generation of ROS by arsenic has been implicated in the stimulation of proinflammatory and profibrogenic cytokines (Brunati *et al.*, 2010), which are significant causal factors in nephrotoxicity. Oxidative stress may have stimulated lipid peroxidation, damaging cell membranes and organelles, releasing reactive aldehydes with potent proinflammatory properties (Zhang *et al.*, 2014). Catechin as was observed in the liver, markedly reversed the damage induced by arsenic in the kidney. Although a total restoration of renal function was not obtained, plasma urea and creatinine levels in the treatment groups were significantly lowered by catechin, compared with the arsenic intoxicated group.

Although there are differences in the status of individual lipids reported, studies have implied that arsenic exposure can alter lipid metabolism, resulting in cardiovascular disorder (Afolabi *et al.*, 2015; Muthumani and Prabu, 2014). In the present study, exposure to this toxicant induced hypercholesterolemia and hypertriglyceridemia in addition to causing an elevation in phospholipid and LDL levels in rats. On the other hand, HDL was drastically reduced in the animals by the metal. Co-treatment of arsenic with catechin however, significantly reduced levels of cholesterol, triglyceride, phospholipids and LDL compared with the group treated with arsenic alone. Arsenic has already been demonstrated to stimulate HMG CoA reductase, the rate limiting enzyme in cholesterol biosynthesis (Afolabi *et al.*, 2015), resulting in the elevation of the lipid in the plasma. The reduction in plasma cholesterol concentration could result from the inhibition of intestinal absorption of the lipid as suggested by Ikeda (2005). Alternatively, catechin might have prevented the accumulation of the lipid by stimulating the activity of LCAT and other enzymes involved in cholesterol catabolism.

The hydrolysis of triglycerides leads to an efflux of Free Fatty Acid (FFA) from the adipose tissue into the circulation. Physiological stress, such as induced by arsenic exposure, can increase the mobilization of this lipid from the adipose tissue (Newsholme and Start, 1981) and arsenic exposure has been linked with elevated plasma free fatty acid (FFA) concentration (Afolabi *et al.*, 2015). High circulating FFA inhibits the activity *of* lipoprotein lipase (Saxena *et al.*, 1989) which can disturb lipid homeostasis with the resultant hypertriglyceridemia, and decreased HDL cholesterol level as observed in this study (Afolabi *et al.*, 2015). Catechin's ability to restore the distorted lipid homeostasis could be explained by it having a stabilizing effect on lipoprotein lipase, probably through its free radical scavenging properties, thereby preventing the enzyme's inhibition by the arsenic. Similar action of catechin against diabetes-induced hypertriglyceridemia in rats was reported by Mostafa (2013). Catechin may also possess the ability to inhibit pancreatic lipase, causing a delay in fat absorption that may be responsible for the lowering of the plasma triglyceride concentration observed.

5. Conclusion

On the basis of the results obtained from the present study, it is further demonstrated that arsenic exposure can result in dyslipidemia, nephrotoxicity and hepatotoxicity. But more significantly, the data suggest that the flavonoid, catechin possesses the potential to ameliorate hepatic and renal injury, as well as, modify the disruption to lipid metabolism induced by arsenic exposure through drinking water.

References

Afolabi OK, Wusu AD, Ogunrinola OO, Abam E, Babayemi DO, Dosumu OA, Onunkwor OO, Balogun EA, Odukoya OO and Ademuyiwa O. 2015. Arsenic-induced dyslipidemia in male albino rats: comparison between trivalent and pentavalent inorganic arsenic in drinking water. *BMC Pharmacol Toxicol,* **16**:15.

Agency for Toxic Substances and Disease Registry. 2007. Toxicological Profile for Arsenic. Agency for Toxic Substances and Disease Registry.

Aposhian HV and Aposhian MM. 1989. Newer developments in arsenic toxicity. J Amer Coll Toxicol, 8: 1297–1305.

Bessey OH, Lowry MD and Brock A. 1946. A method for the rapid determination of alkaline phosphatase with 5 mm^3 of serum. *J Biol Chem*, 164:321–329.

Brunati AM, Pagano MA, Bindoli A and Rigobello MP. 2010. Thiol redox systems and protein kinases in hepatic stellate cell regulatory processes. *Free Radic Res,* **44**:363‑378.

Chen JW, Chen HY, Li WF, Liou SH, Chen CJ, Wu JH and Wang SL. 2011. The association between total urinary arsenic concentration and renal dysfunction in a community‑based population from central Taiwan. *Chemosphere,* **84**: 17‑24.

Chopra K, Singh D and Chander V. 2004. Nephrotoxicity and its prevention by catechin in ferric nitrilotriacetate promoted oxidative stress in rats. *Hum Exp Toxicol*, **23**:137-143.

Du Y, Guo H and Lou H. 2007. Grape seed polyphenols protect cardiac cells from apoptosis via induction of endogenous antioxidant enzymes. *J. Agric. Food Chem*, **55**: 1695-1701.

Eom SY, Lee YC, Yim DH, Lee CH, Kim YD, Choi BS, Park CH, Yu SD, Kim DS, Park JD and Kim H. 2011. Effects of low‑level arsenic exposure on urinary N‑acetyl‑β‑D‑glucosaminidase activity. *Hum Exp Toxicol*, **30**:1885‑1891.

Flora SJ, Dube SN, Arora U, Kannan GM, Shukla MK, et al. 1995. Therapeuticpotential of meso 2,3-dimercaptosuccinic acid or 2,3-dimercaptopropane1-sulfonate in chronic arsenic intoxication in rats. *Biometals,* **8**: 111-116.

Friedewald WT, Levy RI and Fredrickson DS. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*, **18**:499-502.

Gidez LT, Miller GH, Burnstein M, Slagle S and Eder HA. 1982. Separation and quantitation of subclasses of human plasma high density lipoproteins by a simple precipitation procedure. J Lipid Res, 23:1206–1223.

Gupta R and Flora SJ. 2005. Protective value of Aloe vera against some toxic effects of arsenic in rats. *Phytother Res*, **19**(1): 23-28.

Henry RJ, Cannon DC and Winkelman W. 1974. **Clinical Chemistry Principals and Techniques**, eleventh ed. Happer and Row Publishers.

Ikeda I, Tsuda K, Suzuki Y, Kobayashi M, Unno T, Tomoyori H, Goto H, Kawata Y, Imaizumi K, Nozawa A and Kakuda T. 2005. Tea catechins with a galloyl moiety suppress postprandial hypertriacylglycerolemia by delaying lymphatic transport of dietary fat in rats. *J Nutr*, **135**:155-159.

Jomova K, Jenisova Z, Feszterova M, Baros S, Liska J, Hudecova D, Rhodes CJ and Valko M. 2011. Arsenic: toxicity, oxidative stress and human disease. *J Appl Toxicol,* **31**:95‑107.

Kumar S, Yedjou CG and Tchounwou PB. 2014. Arsenic trioxide induces oxidative stress, DNA damage, and mitochondrial pathway of apoptosis in human leukemia (HL–60) cells. *J Exp Clin Cancer Res, 33*:1 – 12.

Lena QM, Hong-Jie S, Bala R, Bing W, Jun L and Li-Ping P. 2014. Arsenic and selenium toxicity and their interactive effects in humans. *Environ Inter*, **69**:148–158.

Majhi CR, Khan S, Leo MD, Manimaran A, Sankar P and Sarkr SN. 2011. Effects of acetaminophen on reactive oxygen species and nitric oxide redox signaling in kidney of arsenic-exposed rats. *Food Chem Toxicol*, **49**:974–982.

Miltonprabu S and Sumedhaa NC. 2014. Arsenic-induced hepatic mitochondrial toxicity in rats and its amelioration by diallyl trisulfide. *Toxicol Mech Meth*, **4**:124-135.

Mishra D and Flora SJ. 2008. Differential oxidative stress and DNA damage in rat brain regions and blood following chronic arsenic exposure. *Toxicol Ind Health,* **24**: 247‑256.

Mostafa UE. 2013. Effect of Green Tea and Green Tea Rich with Catechin on Blood Glucose Levels, Serum Lipid Profile and Liver and Kidney Functions in Diabetic Rats. *Jordan J Biol Sci*, **7**:7-12.

Murcott S. 2012. **Arsenic contamination in the world: An international sourcebook**. IWA Publishing, London, UK.

Muthumani M and Prabu SM. 2014. Silibinin potentially attenuates arsenic-induced oxidative stress mediated cardiotoxicity and dyslipidemia in rats. *Cardiovasc Toxicol*, **14**:83–97.

Nandi D, Patra RC and Swarup D. 2006. Oxidative stress indices and plasma biochemical parameters during oral exposure to arsenic in rats. *Food Chem Toxicol,* **44**: 1579‑1584.

Newsholme EA and Start C. 1981. **Regulation in Metabolism**. John Wiley and Sons. Chichester, UK.

Patra PH, Bandyopadhyay S, Kumar R, Datta BK, Maji C, Biswas S, et al. 2012. Quantitative imaging of arsenic and its species in goat following long term oral exposure. *Food Chem Toxicol*, **50:**1946–1950.

Prakash A, Khan S, Kumar D, Telang AG and Malik JK. 2014. Concurrent administration of curcumin mitigates arsenic – and chlorpyrifos – induced apoptosis in rat thymocytes. *Adv Anim Vet Sci*, **2**: 407 – 413.

Reitman S and Frankel S. 1957. A colorimetric method for the determination of serum glutamate–oxaloacetate and pyruvate transaminases. *Am J Clin Pathol*, **28**:56–63.

Saxena U, Witte LD and Goldberg IJ. 1989. Release of endothelial cell lipoprotein lipase by plasma lipoproteins and free fatty acids. *J Biol Chem*, **264**:4349–4355.

Sugihara N, Ohnishi M, Imamura M and Furuno K. 2001. Differences in antioxidative efficiency of catechins in various metal-induced lipid peroxidations in cultured hepatocytes. *J Health Sci*, **47**: 99-106.

US Environmental Protection Agency. 2001**. National Primary Drinking Water Regulations: Arsenic and Clarifications to Compliance and New Source Contaminants Monitoring**. Federal Register **40**:41–142.

World Health Organisation. 2011. **Guidelines for drinking–water quality**. Fourth edition. Edited by WHO. ISBN: 978 92 4 154815 1.

Zhang J, Pan X, Li N, Li X, Wang Y, Liu X, Yin X and Yu Z. 2014. Grape seed extract attenuates arsenic-induced nephrotoxicity in rats. *Exper Therap Med*, **7**: 260-266.