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Research Article

Effect of *Terminalia Arjuna* against Arsenic-Induced Renal Toxicity in Mice

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Abstract

Arsenic is an established toxin to both renal and neuronal system. It induces oxidative stress through free radical formation within the renal system. Clinical effects of arsenic toxicity depend on chronicity. Acute renal damages are very common patho-physiological disturbances that are caused by arsenic. The present study is conducted to evaluate the protective role of the aqueous extract of *Termnalia arjuna* bark. *T. arjuna* is an important plant of Indian origin that is widely used against kidney disorders (renal failures). Antioxidant level in the kidney is estimated by the activity of antioxidative enzymes like superoxide dismutase (SOD), glutathione-s-transferase (GST), glutathione reductase (GR) reduced glutathione (GSH) and thiobarbutaric acid reactive substances (TBARS). Mice were first treated with the aqueous extract of *T. arjuna* (100 mg/kg body weight) for two week and then subjected to arsenic trioxide toxicity (5 mg/kg body weight). The results shows a remarkable rise in TBARS level along with the significant diminution of GSH, SOD, GST, GR levels in the kidney tissues. The results were further analyzed for the antioxidant activity. The aqueous extract of *T. arjuna* possesses a strong free radical scavenging activity. The results suggested that the *T. arjuna* extracts can protect kidney tissues against arsenic-induced oxidative stress probably by increasing antioxidative defense activities.

Introduction

Arsenic, a highly poisonous metalloid, is one of the natural constituents of the earth's crust [1]. It is found in various concentrations in all ecosystems [2]. Its extensive applications in mining, smelting and refining of some ores have dispersed it into the atmosphere [3]. Burning of coal has also contributed the dispersion of arsenic in the environment [4]. High levels of inorganic arsenic in ground water due to the geochemical processes are posing serious health risks to humans in many regions of the world [5-7]. Arsenic exposure in humans has been reported through traditional medicines and by consuming contaminated sea food [8,9]. Exposure to arsenic in humans also occurs through air, soil and occupational settings [10-12]. Both the organic and inorganic forms of arsenic exist in nature, while humans are mainly exposed to the inorganic form through drinking water and job-related sources [13-15].Arsenic is considered to be more toxic for its ability to bind with the sulfhydryl group of proteins and disrupting the enzyme activity [16-18]. Arsenic and its inorganic compounds have long been known as neurotoxic as well as nephrotoxic [19]. Its exposure induces oxidative stress causing brain and kidney damages by free radicals formation [20]. Health problems that are associated with the chronic arsenic exposure are hypertension [21], developmental abnormalities [22], cardiovascular diseases[23], diabetes [24], hearing loss [25], fibrosis of liver and lungs [26], hematological disorders[27], neurological and kidney problems [28], and cancer [29].

Kidney failure is one of the major global issues prevailing in present situation. Kidney is the most sensitive organ for more toxic inorganic arsenic that includes arsenite [As (III)] and arsenate [As (V)] [30]. Trivalent arsenic decreases cellular ATP production by citric acid cycle disruption by inhibiting numerous enzymes like glutathione and pyruvate dehydrogenase [17]. At a more specific level, the pentavalent arsenic mimics the inorganic phosphate and replaces it to form ADP-arsenate instead of ATP causing the uncoupling of oxidative phosphorylation.

Arsenic easily crosses the blood brain barrier leading to neurobehavioral abnormalities [31]. Uremia is mainly constituted by the Arsenic toxicity producing a potentially fatal condition that demands immediate treatment [32]. Treatment option for uremia includes kidney transplantation and dialysis, which is expensive with many side effects. Many scientists have tried to find out different phytomedicines to manage uremia. Arsenic in oxidative

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stress is defined as an imbalance between formation of reactive oxygen species (ROS) and anti-oxidative defense mechanisms.

In recent years numerous clinical and experimental studies have been focused on detection of signs of oxidative stress in renal patients. Several evidences indicate that the uremia is associated with enhanced oxidative stress, deficiency of antioxidant activity and vitamin E deficiency [33]. Interstitial inflammation and oxidative stress may participate jointly in the development and reduction of the number of nephron units, which thereby limits sodium filtration. Low density lipoprotein (LDL) from arsenic toxicity patients presents an elevated susceptibility to oxidation [34]. Arsenic induced oxidative stress is characterized from a biochemical point of view as a state of reactive aldehyde and oxidized thiol group accumulation, together with depletion of reduced thiol groups, which are particularly important as part of antioxidant defense [35]. As a consequence of diminish adrenal catabolism and function, uremic oxidant mediators accumulate, favoring vascular cell dysfunction and progression to kidney failure giving road to many other diseases. To combat against arsenic-induced oxidative insult, initial aim of this particular study was to find out a suitable antagonist of arsenic poisoning. Herbal formulations could be a solution to this problem, because they are readily available often at low cost [36-43]. Many herbal compounds are rich sources of antioxidants Medicinal uses of many plants have been reported in the literature One of them is *T. arjuna*.

The present study has been designed to investigate the ameliorating effect of *T.arjuna* against arsenic-induced oxidative stress in kidney. The free-radical-scavenging activity and the in vivo antioxidant power of *T. arjuna* were determined by the levels of lipid peroxidation end products, cellular metabolites such as reduced glutathione (GSH), activities of intracellular antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione-*S*-transferase (GST), glutathione reductase (GR), and glutathione peroxidase (GP*x*).

Materials and Methods

Chemicals

Oxidized glutathione(GSSG), reduced glutathione (GSH), glutathione reductase(GR), Superoxide dismutase(SOD) Nicotinamide adenine dinucleotide phosphate (NADPH), 1-chloro-2, 4-dinitrobenzene (CDNB), (−)epinephrine, thiobarbituricacid (TBA), trichloroacetic acid (TCA), Bradford reagent, bovine serum albumin (BSA), 5, 5'-dithiobis(2-nitrobenzoic acid 5, 5'-dithiobis-2-nitrobenzoic acid(DTNB) and ethylene diaminetetraacetic acid (EDTA), Arsenic trioxide (As_2O_3) were purchased from Sigma-Aldrich Chemicals Pvt. Ltd, India. Disodium phosphate (Na_2HPO_4), Monosodium phosphate (NaH_2PO_4), glacial acetic acid, sulfosalicylic acid, hydrogen peroxide (H_2O_2) was obtained from Merck, India.

Plant extracts

Extract was prepared from bark of the plant *Terminali aarjuna* (TA) obtained from Saiba Industries Mumbai, India (Batch No. U/1558).Initially the bark of TA was cut into pieces and was dried at 40°C in incubator and then it was crushed using a grinder and the resulting powder was then separated. 20 gm of the fine powder was dissolved in 200 mL of distilled water and kept in an airtight glass jar. This mixture was incubated in a Soxhlet extraction apparatus for 72 h at 37°C [44]. The obtained deep reddish brown extract of TA was collected and centrifuged at 12,000 g for 30 min in order to remove unwanted impurities. Then this extract was dried in vacuum desiccators to obtain a dry mass and stored in a refrigerator at 4°C till use.

Animals

Twenty-four male Swiss albino mice (10–12 weeks old) weighing around (25–30 gm) were obtained from Central Animal House Facility available in Jamia Hamdard, New Delhi, India. The animals were kept in propylene cages under standard condition of illumination with a 12-h light –dark cycle at 25±2°C with humidity 45–50%. They were provided free access of tap water and balanced diet. The experiments were performed according to international guidelines and approved by the Animal Ethics Committee of Jamia Hamdard, New Delhi, India.

Experimental design

Mice were allocated to four groups on the basis of their matching weight. Group I (Control) represented as a saline treated control group (daily received saline orally for 15 days followed by single injection of saline on 15th day, subcutaneous). Group II (AS) daily received saline orally for 15 days and followed by single injection of freshly prepared Arsenic trioxide (5 mg/kg body weight on 15th day; subcutaneous).The dose and the route of administration were selected on the basis of the pilot study and previous published reports [45,46]. Group III daily received TA (100mg/kg body weight) for 15 days followed by single injection of TA (5 mg/kg

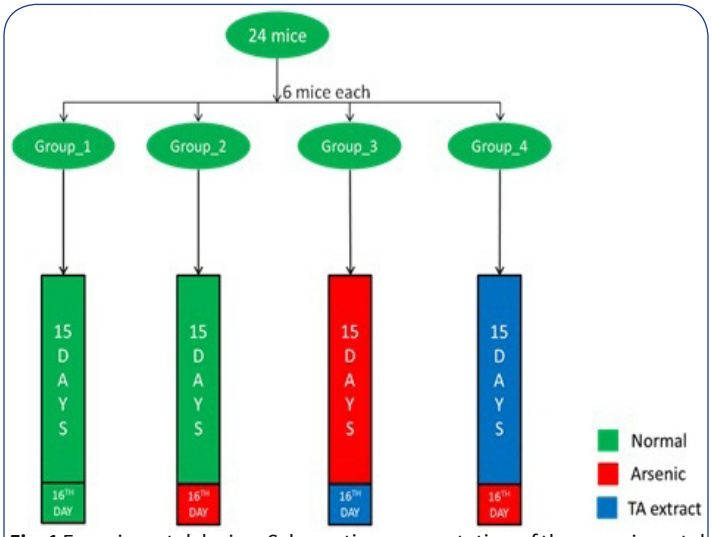

Fig. 1 Experimental design: Schematic representation of the experimental design. AS, arsenic trioxide; TA, Terminalia arjuna bark extract

body weight) on 15th day, and group IV (Pretreated) received TA (100 mg/kg body weight) daily for 15 days followed by freshly prepared saline injection on 15th day (figure 1). After 24 h of the administration of Arsenic trioxide, sacrificed them to collect organ (kidney) on ice and stored at −80°C till use. Each group was evaluated for oxidative stress. For, enzymatic and non-enzymatic studies, organ was homogenized in ice cold phosphate buffer (0.01 M; pH 7.4) to give homogenate and centrifuged at 500×g for 10 min at 4°C to remove cell debris. The supernatant thus obtained, was used for lipid peroxidation (LPO) and remaining homogenates were further centrifuged at 10,500×g for 20 min at 4°C to obtain post mitochondrial supernatant (PMS) which was further used for the estimation of GSH, GR, GST ,SOD, and antioxidant enzymes.

TBARS

The homogenate 0.25 ml was taken in a glass test tube and incubated at 37°C in a shaker (120strokes/min) for one hour. Similarly, 0.25 ml of the same homogenate sample was taken in another glass tube and incubated at 0°C. After 1 h of incubation, 0.25 ml of chilled 5% trichloroacetic acid and 0.5 ml of 0.67% thiobarbituric acid were added in each test tube and mixed properly. The aliquot from each test tube was transferred to new centrifuge tube and centrifuged at 3000×g for 15 min. The resulting supernatant was transferred to another test tube and incubated in boiling water bath for 10 min. Thereafter, the test tubes were cooled, and the absorbance of each aliquot was measured at 535 nm [47].The rate of lipid peroxidation was expressed as nmoles of thiobarbituric acid reactive substances (TBARS) formed/h/mg of protein using a molar extinction coefficient of 10^5 $M^{-1}cm^{-1}$.

Reduced Glutathione (GSH)

PMS was precipitated with sulfosalicylic acid (4%) in the ratio of 1:1. The samples were kept at 4°C for 1 h and centrifuged at 1200×g for 15 min at 4°C. The assay mixture contained 0.1 ml of supernatant, 1.0 mM DTNB and 0.1 M phosphate buffer (pH 7.4) in a total volume of 1.0 ml. The optical density of reaction product was read immediately at 412 nm [48]. The GSH content was calculated as μ moles GSH/mg protein using a molar extinction coefficient of 13.6×10^3 $M^{-1}cm^{-1}$.

Superoxide dismutase

Superoxide dismutase (SOD) activity was assayed by monitoring the auto oxidation of (−) epinephrine at pH 10.4 for 3 min at 480 nm [49]. The enzyme activity was calculated in terms of n moles (−) epinephrine protected from oxidation/min/mg protein using molar extinction coefficient $4.02\times10^3M^{-1}cm^{-1}$. Protein was determined by the Lowry methods using bovine serum albumin (BSA) as standard.

Glutathione S-transferase

GST activity was assayed spectrophotometrically at 23°C using a UV maxmicroplate reader (Molecular Devices, Wokingham, UK), to measure the rate of conjugation of GSH to CDNB. Final substrate concentrations were 1 mM (CDNB) and 5 mM (GSH) in 0.1 M sodium phosphate buffer, pH 6.0. Reagents were prepared fresh prior to use. Three replicate 1.2-ml reaction mixtures (1mMCDNB and 5mMGSH in 0.1 M sodium phosphate buffer, pH 6.0) were placed in glass vials. Insect homogenate (60 ml) or purified enzyme was added to the reaction mixture. Three blanks were prepared for each experiment with 60 ml distilled water and 1.2 ml of reaction mixture. Aliquots (210 ml) from each of the above reactions were placed in a microtiter plate and the reaction rates were measured at 340 nm for 5 min. The GST activity per individual was calculated in mmol CDNB conjugated/min/well using the published extinction coefficient (9.6 $mM^{-1}cm^{-1}$) [50]. Specific activity was calculated by correcting for protein content.

Glutathione Reductase

The assay mixture consisted of phosphate buffer (0.1 M, pH 7.6), NADPH (0.1 mM), EDTA (0.5 mM) and oxidized glutathione (1 mM) and 0.05 ml of PMS in total volume of 1 ml. The enzyme activity was determined at room temperature by measuring the disappearance of NADPH at 340 nm [51] and activity was calculated as n moles NADPH oxidized/min/mg protein using molar extinction coefficient of 6.22×10^3 $M^{-1}cm^{-1}$.

Statistical analysis

Experimental results were expressed as mean ± standard deviation (SD) by using three set of observations with the help of origin 8.5, and the statistical significance was examined with ANOVA test. The value of $p<0.05$ was considered as significant results.

Results and Discussion

Effect of TA on enzymatic and non-enzymatic markers level due to Arsenic-induced oxidative stress. As expected, As_2O_3 treatment resulted in significant nephrotoxicity in mice, and pre-treatment with TA attenuated or prevented the oxidative stress, thereby mitigating the subsequent renal damage. These data indicated that TA could markedly renew the activities of those antioxidant enzymes in the kidneys of As_2O_3-treated mice and attenuates oxidative stress by increasing the lipid peroxide level as well as decreasing SOD, GST, GSH and GR levels in As_2O_3-treated mice kidney, thereby mitigate the defense capacity of mice.

TBARS

The concentrations of TBARS in Kidney tissue homogenate was significantly increased in the Arsenic-treated group by 119.6% ($p<0.05$) when compared with the control (Group1). However, TA (100 mg/kg) caused a significant reduction in the level of TBARS (37.5%) as shown in figure 2. The toxicity of TA was also monitored in group IV which was found to be increase the level by 10%.

Reduced Glutathione

The GSH contents in Arsenic trioxide (AS) intoxicated mice were found to be decreased by 50.2%, however significant elevation in the content of GSH (20%) was observed after treatment with TA in comparison to Arsenic trioxide administered group as stated in figure 3.The toxicity of TA was monitored and found to be decrease in the level of GSH by only 3.41 % and the result was statistically significant.

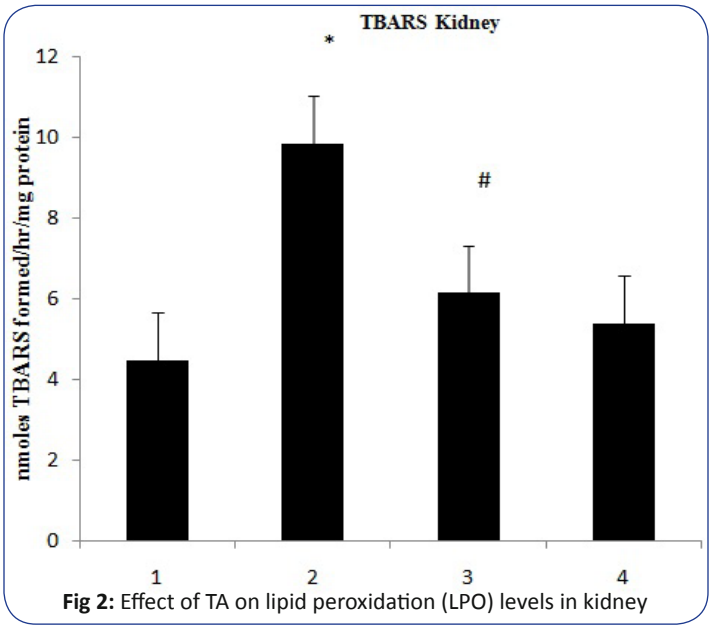

Fig 2: Effect of TA on lipid peroxidation (LPO) levels in kidney

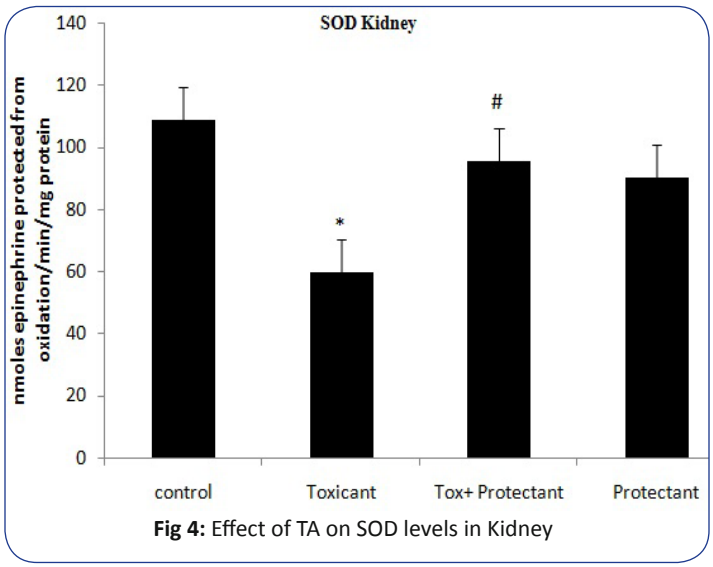

Fig 4: Effect of TA on SOD levels in Kidney

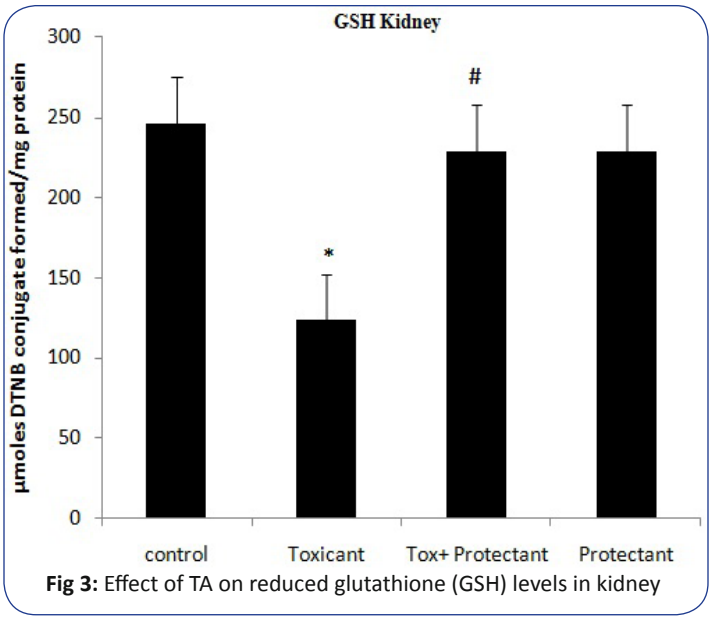

Fig 3: Effect of TA on reduced glutathione (GSH) levels in kidney

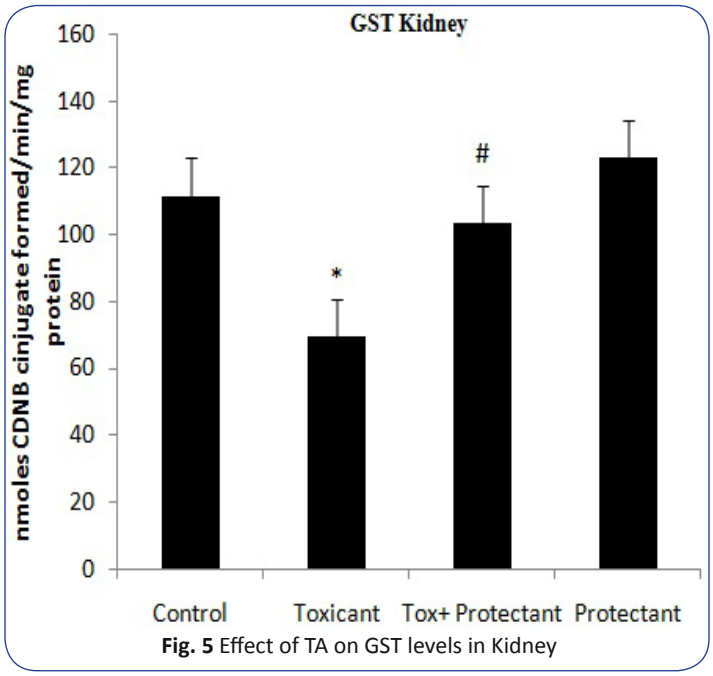

Fig. 5 Effect of TA on GST levels in Kidney

Superoxide dismutase

We also determined the activities of antioxidant enzyme SOD. Conspicuously this enzymes was significantly down regulated by Arsenic trioxide 54.98% ($p<0.05$), and the decrease in this enzyme was largely prevented by TA treatment 23.5% as shown in figure 4. The toxicity of TA was monitored and found to be decrease in the level of SOD by 5.78%.

Glutathione S-transferase

The administered dose of TA (100 mg/kg) caused the significant improvement in the level of GST in comparison to Arsenic trioxide administered mice as shown in figure 5. The GST level was reduced by 62.12% in arsenic trioxide treated group as compared to control (group1); however TA caused the significant increase up to 46.25% as shown in table 1. The toxicity of TA was monitored and found to be decrease in the level of GST by 2.8% only.

Glutathione Reductase

The concentration of GR was significantly decreased in the arsenic trioxide treated group in comparison to control group. Elevation in the level of GR was observed after treatment with TA which was found to be 40.2% as shown in figure 6. The toxicity of TA was monitored and found to be decreased in the level of GR by only 1.5% .The result was statistically significant at $p< 0.05$.

Table 1: Effect of Arsenic Trioxide on antioxidant status of the Kidney.

Parameters	Control	Arsenic Trioxide(AS)	Arsenic trioxide+TA	TA
LPO (nmoles of TBARS formed/h/mg protein)	4.48±0.4	9.84±1.62(119.6)	6.15±0.70(-37.5)	4.032±0.71(-10)
GSH (µmoles of GSH/min/mg protein)	246.82±14.61	124.20±21.56(-50.2)	149.04±1.8(20)	238.39±24.41(-3.41)
SOD (nmoles of (–) epinephrine protected/min/mg protein)	108.93±7.341	59.90±4.49(-54.98)	73.67±2.8(23.5)	102.63±4.49(-5.78)
GST(mmol CDNB conjugated/min/well)	111.46±5.21	69.25±3.6(-62.12)	101.27±8.1(46.25)	108.32±3.6(-2.8)
GR (nmoles of NADPH utilized/min/mg protein)	1118.48±70.52	585.14±25(-52.31)	820.36±12.18(40.2)	1101.7±17.43(-1.5)

LPO lipid peroxidation, GSH glutathione, SOD superoxide dismutase,GST Glutathione S reductase, GR glutathione reductase, were assessed in different groups in kidney tissue.

Values are expressed as mean ± SEM. Values in parentheses show the percentage increase or decrease with respect to their corresponding control.

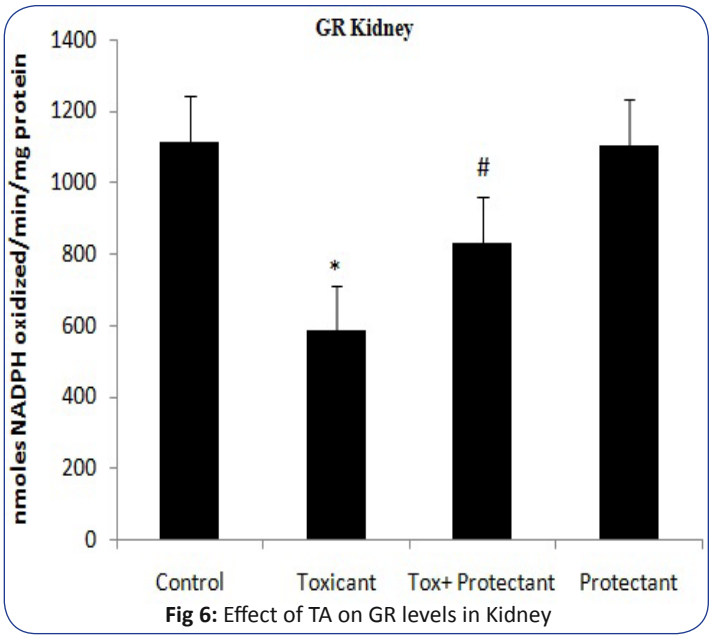

Fig 6: Effect of TA on GR levels in Kidney

Discussion

Arsenic intoxication by polluted food, water or air is the major health concern of modern world. Exposure of arsenic can affect a number of vital organs like liver, kidney and brain etc. Increasing number of evidences showed that arsenic exposure leads to altered function of kidney [52,53]. Arsenic toxicity is associated with its reactivity and generation of ROS and sulfur containing compounds [54]. In Agreement, TA has been recently reported to protect against carbon tetrachloride induced toxicity against hepatic and renal toxicity while hepatoprotective role of arjunolic acid against arsenic-induced oxidative damages in murine liver Since, TA has been reported to have a good antioxidant effect [55-57]. Therefore, we further led to investigate its role in inhibiting the arsenic induced ROS generation and oxidative stress in kidney. Herein, in the present study, we observed an elevated level of lipid peroxidation and decreased level of GSH, SOD, GST and GR in kidney which were found to be in line with previous studies [58,59]. However, administration of TA successfully corrected levels of TBARS, GSH, SOD, GST and GR in kidney and these corrected activities of antioxidant enzymes treated group mice were observed near normal values of the control group. Our results are in line with previous study showing a potent antioxidant property of TA [60,61].

Conclusion

The present study revealed that AS-induction in mice remarkably increased the level of lipid peroxides and decrease in level of GSH, SOD, GST and GR. The change in their markers level after treatment with TA leads to the attenuation of arsenic induced renal toxicity. It was also noticed that TA was almost non toxic to the enzymatic and non enzymatic markers of the kidney.

References

1. Chung JY, Yu SD, Hong YS (2014) Environmental source of arsenic exposure. J Prev Med Public Health 47(5): 253- 257.
2. Garelick H (2008) Arsenic pollution sources. Rev Environ Contam Toxicol 197: 17-60.
3. Abernathy CO (1999) Arsenic: health effects, mechanisms of actions, and research issues. Environ Health Perspect 107(7): 593-597.
4. Li D (2006) Current status and prevention strategy for coal-arsenic poisoning in Guizhou, China. J Health Popul Nutr 24(3): 273-276.
5. Jurgens BC, Bexfield LM, Eberts SM (2014) A ternary age-mixing model to explain contaminant occurrence in a deep supply well. Ground Water 52 Suppl 1: 25-39.
6. Jiang JQ (2013) Arsenic contaminated groundwater and its treatment options in Bangladesh. Int J Environ Res Public Health 10(1): 18-46.
7. George CM (2013) Approaches to increase arsenic awareness in Bangladesh: an evaluation of an arsenic education program. Health Educ Behav 40(3): 331-338.
8. Rahbar MH (2012) The role of drinking water sources, consumption of vegetables and seafood in relation to blood arsenic concentrations of Jamaican children with and without Autism Spectrum Disorders. Sci Total Environ 433: 362-370.
9. Navas-Acien A (2011) Seafood intake and urine concentrations of total arsenic, dimethylarsinate and arsenobetaine in the US population. Environ Res 111(1): 110-8.

10. Mc Carty KM, Hanh HT, Kim KW (2011) Arsenic geochemistry and human health in South East Asia. Rev Environ Health 26(1): 71-78.

11. Cooper RG, Harrison AP (2009) The exposure to and health effects of antimony. Indian J Occup Environ Med 13(1): 3-10.

12. Binomar M (2015) Screening of Different Soil Sources of New York City for Antimicrobial Activity against Methicillin-Resistant Staphylococcus aureus (MRSA). Journal of Pure and Applied Microbiology 9: 77-86.

13. Hajalilou B (2011) Effects of abandoned arsenic mine on water resources pollution in north west of iran. Health Promot Perspect 1(1): 62-70.

14. Heaney CD (2013) Public infrastructure disparities and the microbiological and chemical safety of drinking and surface water supplies in a community bordering a landfill. J Environ Health 75(10): 24-36.

15. Krewski D (2007) Human health risk assessment for aluminium, aluminium oxide, and aluminium hydroxide. J Toxicol Environ Health B Crit Rev10 Suppl 1: 1-269.

16. Shen S (2013) Arsenic binding to proteins. Chem Rev 113(10): 7769-7792.

17. Finnegan PM, Chen W (2012) Arsenic toxicity: the effects on plant metabolism. Front Physiol 3: 182.

18. Tchounwou PB (2012) Heavy metal toxicity and the environment. EXS 101: 133-164.

19. Singh AP, Goel RK, Kaur T (2011) Mechanisms pertaining to arsenic toxicity. Toxicol Int 18(2): 87-93.

20. Reyes JL (2013) Tight junction proteins and oxidative stress in heavy metals-induced nephrotoxicity. Biomed Res Int 2013: 730-789.

21. Islam M R (2012) Association between hypertension and chronic arsenic exposure in drinking water: a cross- sectional study in Bangladesh. Int J Environ Res Public Health 9(12): 4522-4536.

22. Tchounwou P B, Patlolla A K, Centeno J A (2003) Carcinogenic and systemic health effects associated with arsenic exposure--a critical review. Toxicol Pathol 31(6): 575-588.

23. Moon K, Guallar E, Navas-Acien A (2012) Arsenic exposure and cardiovascular disease: an updated systematic review. Curr Atheroscler Rep 14(6): 542-555.

24. Gribble MO (2012) Arsenic exposure, diabetes prevalence, and diabetes control in the Strong Heart Study. Am J Epidemiol 176(10): 865-874.

25. Bencko V, Symon K (1977) Test of environmental exposure to arsenic and hearing changes in exposed children. Environ Health Perspect 19: 95-101.

26. Liu J, Waalkes M P (2008) Liver is a target of arsenic carcinogenesis. Toxicol Sci 105(1): 24-32.

27. Rahbar MH (2014) Role of metabolic genes in blood arsenic concentrations of Jamaican children with and without autism spectrum disorder. Int J Environ Res Public Health 11(8): 7874-7895.

28. Peters BA (2014) Creatinine, arsenic metabolism, and renal function in an arsenic-exposed population in Bangladesh. PLoS One 9(12): e113760.

29. Chen Y, Ahsan H (2004) Cancer burden from arsenic in drinking water in Bangladesh. Am J Public Health 94(5): 741-744.

30. Tokar E J (2013) Chronic exposure of renal stem cells to inorganic arsenic induces a cancer phenotype. Chem Res Toxicol 26(1): 96-105.

31. Jiang S (2014) Fluoride and arsenic exposure impairs learning and memory and decreases mGluR5 expression in the hippocampus and cortex in rats. PLoS One 9(4): e96041.

32. Correia N (2009) Haemolytic anaemia secondary to arsenic poisoning: a case report. Cases J 2: 7768.

33. Mittal M, Flora S J (2007) Vitamin E supplementation protects oxidative stress during arsenic and fluoride antagonism in male mice. Drug Chem Toxicol 30(3): 263-281.

34. Karim MR (2013) Increases in oxidized low-density lipoprotein and other inflammatory and adhesion molecules with a concomitant decrease in high-density lipoprotein in the individuals exposed to arsenic in Bangladesh. Toxicol Sci 135(1): 17-25.

35. Birben E (2012) Oxidative stress and antioxidant defense. World Allergy Organ J 5(1): 9-19.

36. Anwer K (2015) Role of N-terminal residues on folding and stability of C-phycoerythrin: simulation and urea-induced denaturation studies. J Biomol Struct Dyn 33(1): 121-33.

37. Gramany V (2015) Cloning, expression and molecular dynamics simulations of a xylosidase obtained from Thermomyces lanuginosus. J Biomol Struct Dyn 19: 1-34.

38. Khan FI (2015) Chitinase from Thermomyces lanuginosus SSBP and its biotechnological applications. Extremophiles 1-12.

39. Khan FI (2015) Thermostable chitinase II from Thermomyces lanuginosus SSBP: Cloning, structure prediction and molecular dynamics simulations. J Theor Biol 374: 107-114.

40. Khan FI (2015) Large scale analysis of the mutational landscape in beta-glucuronidase: A major player of mucopolysaccharidosis type VII. Gene.

41. Stephens DE, Khan FI, Singh P, Bisetty K, Singh S, et al. (2014) Creation of thermostable and alkaline stable xylanase variants by DNA shuffling. J Biotechnol 187: 139-146.

42. Khan FI, Bisetty K, Singh S, Permaul K, Hassan MI, et al. (2015) Chitinase from Thermomyces lanuginosus SSBP and its biotechnological applications.Extremophiles.

43. Anwer R, Al Qumaizi KI, Khan FI,Al Shaqha WM (2015) From Pancreatic to Non-Pancreatic Insulin: A Miraculous Journey. International Journal of Biological Chemistry 9(6): 302-317.

44. Mandal S, Patra A, Samanta A, Roy S, Mandal A, et al. (2013) Analysis of phytochemical profile of Terminalia arjuna bark extract with antioxidative and antimicrobial properties. Asian Pac J Trop Biomed 3(12): 960-966.

45. Dabrowska Bouta B, Struzynska L, Rafalowska U (1996) Does lead provoke the peroxidation process in rat brain synaptosomes? Mol Chem Neuropathol 29(2-3): 127-39.

46. Nowak P, Szczerbak G, Nitka D, Kostrzewa RM, Sitkiewicz T, et al. (2008) Effect of prenatal lead exposure on nigrostriatal neurotransmission and hydroxyl radical formation in rat neostriatum: dopaminergic-nitrergic interaction. Toxicology 246(1): 83-89.

47. Islam F, Zia S, Sayeed I, Zafar KS, Ahmad AS, et al. (2002) Selenium-induced alteration of lipids, lipid peroxidation, and thiol group in circadian rhythm centers of rat. Biol Trace Elem Res 90(1-3): 203-214.

48. Vaibhav K, Shrivastava P, Javed H, Khan A, Ahmed ME, et al. (2012) Piperine suppresses cerebral ischemia-reperfusion-induced inflammation through the repression of COX-2, NOS-2, and NF-kappaB in middle cerebral artery occlusion rat model. Mol Cell Biochem 367(1-2): 73-84.

49. Stevens MJ, Obrosova I, Cao X, Van Huysen C, Greene DA (2000) Effects of DL-alpha-lipoic acid on peripheral nerve conduction, blood flow, energy metabolism, and oxidative stress in experimental diabetic neuropathy. Diabetes 49(6): 1006-1015.

50. Roy VK, Chenkual L, Gurusubramanian G (2015) Protection of testis through antioxidant action of Mallotus roxburghianus in alloxan-induced diabetic rat model. J Ethnopharmacol 176: 268-280.

51. Lateef A, Khan AQ, Tahir M, Khan R, Rehman MU, et al. (2013) Androgen deprivation by flutamide modulates uPAR, MMP-9 expressions, lipid profile, and oxidative stress: amelioration by daidzein. Mol Cell Biochem 374(1-2): 49-59.

52. Singh S, Mulchandani A, Chen W (2008) Highly selective and rapid arsenic removal by metabolically engineered Escherichia coli cells expressing Fucus vesiculosus metallothionein. Appl Environ Microbiol 74(9): 2924-2927.

53. Wang G, Li C, He L, Lei K, Wang F, et al. (2014) Design, synthesis and biological evaluation of a series of pyrano chalcone derivatives containing indole moiety as novel anti-tubulin agents. Bioorg Med Chem 22(7): 2060-2079.

54. Hughes MF, Beck BD, Chen Y, Lewis AS, Thomas DJ (2011) Arsenic exposure and toxicology: a historical perspective. Toxicol Sci 123(2): 305-332.

55. Manna P, Sinha M, and Sil PC (2008) Arsenic-induced oxidative myocardial injury: protective role of arjunolic acid. Arch Toxicol 82(3): 137-49.

56. Manna P, Sinha M, Sil PC (2007) Protection of arsenic-induced hepatic disorder by arjunolic acid. Basic Clin Pharmacol Toxicol 101(5): 333-338.

57. Sinha M, Manna P, Sil PC (2008) Arjunolic acid attenuates arsenic-induced nephrotoxicity. Pathophysiology 15(3): 147-156.

58. Ribarov SR., Benov LC, Benchev IC (1981) The effect of lead on hemoglobin-catalyzed lipid peroxidation. Biochim Biophys Acta 664(3): 453-459.

59. Ye XB, Fu H, Zhu JL, Ni WM, Lu YW, et al. (1999) A study on oxidative stress in lead-exposed workers. J Toxicol Environ Health A 57(3): 161-172.

60. Biswas M, Kar B, Bhattacharya S, Kumar RB, Ghosh AK, et al. (2011) Antihyperglycemic activity and antioxidant role of Terminalia arjuna leaf in streptozotocin-induced diabetic rats. Pharm Biol 49(4): 335-340.

61. Shivananjappa MM., Mhasavade D, Joshi MK (2013) Aqueous extract of Terminalia arjuna attenuates tert-butyl hydroperoxide-induced oxidative stress in HepG2 cell model. Cell Biochem Funct 31(2): 129-135.