Original Article

Protective Effect of *Psidium guajava* in Arsenic-induced Oxidative Stress and Cytological Damage in Rats

Neeraj Tandon, Manju Roy, Sushovan Roy, Neelu Gupta

Department of Veterinary Biochemistry, College of Veterinary Science and A.H., Durg, Chhattisgarh, India

ABSTRACT

This study was undertaken to evaluate the protective effect of aqueous extract of *Psidium guajava* leaves against sodium arsenite-induced toxicity in experimental rats. Animals were divided into four groups. Control group received arsenic free distilled water and three treatment groups (II, III, and IV) exposed to the arsenic ($NaAsO_2$) (20 mg/kg b.wt) through drinking water. Group III and IV were administered a daily oral dose of *P. guajava* leaf extract 50 and 100 mg/kg b.wt. ($AEPG_{50}$ and $AEPG_{100}$) for the period of 6 weeks. Blood samples and organs were collected at the end of the experiment. Arsenic exposure resulted in significant rise in lipid peroxidation (LPO) levels in erythrocyte, liver, kidney, and brain. In addition toxin decreased ($P<0.05$) the level of reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities in the studied tissues. Residual effect of arsenic in various tissues was also observed. Histopathological results revealed mild to severe type of necrosis and degenerative changes in kidney and liver of arsenic intoxicated animals. Cytological alteration in brain tissue was also observed. Treatment with $AEPG_{100}$ (aqueous extract of *P. guajava*) @100 mg/kg body weight) significantly restored activities of oxidative stress markers like LPO levels, GSH levels, SOD, and CAT activities but having the limited protective activity of the herbal extract was observed on tissues architecture. It is therefore concluded that prophylactic co-administration of AEPG could provide specific protection from oxidative injury and to some extent on tissue damage.

Key words: Histopahtology, oxidative stress, *Psidium guajava*, rats, sodium arsenite

INTRODUCTION

Arsenic is a naturally occurring element that has been recognized as a human poison since ancient times. Arsenic (As) is a ubiquitous element present in low concentrations in air, soil, and water. Chronic exposure of human and animals to arsenic contaminated environment is indicated by its higher levels in hair nail, hoof, and urine.[1,2] Arsenic affects the mitochondrial enzymes, impairs the cellular respiration, and causes cellular toxicity. It can also substitute phosphate intermediates, which could theoretically slow down the rate of metabolism and interrupt the production of energy.[3] Ramos *et al*.[4] observed a positive correlation between arsenic concentration and lipid peroxidation levels in liver, kidney, and heart. Overproduction or an ineffective elimination of ROS may induce oxidative stress and cause damage to all types of molecules such as proteins, lipids, and nucleic acids Droge.[5] The results of long-term exposure due to elevated levels of inorganic arsenic in drinking water supplies include skin, lung, liver, and bladder cancers as well as malignant neoplasms, diabetes, and vascular diseases.[6] Exposure to arsenic causes malfunctioning of various organs including liver and lungs.[7] Many medicinal plants sources of natural antioxidants are reported to be

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Address for correspondence: Dr. Sushovan Roy, Department of Veterinary Biochemistry, College of Veterinary Science and A.H. Anjora, Durg, Chhattisgarh - 491 001, India, E-mail: dr_sushovan_roy@yahoo.com

useful against arsenic-induced injury of brain.[8] Numerous biological materials against arsenic toxicity have been listed for removal of toxic ions from the aqueous solution over the last two decades. However, only a limited number of studies have investigated the use of adsorbents derived from biological sources. Antioxidant efficacy of *Psidium guajava* was studied by various research workers but this study was aimed to test the efficacy of antioxidant effect of *P. guajava* in induced arsenic toxicity in rats.

MATERIALS AND METHODS

Chemicals

5,5 dithiobis-2-nitrobenzoic acid (DTNB), sodium arsenite (NaAsO2), Sigma Chemical, St Louis, MO, USA), thiobarbituric acid, cacodylic acid, diethylene triamine penta acetic acid, Triton® X100 and all other chemicals were of the analytical grade and were purchased from Merck (Darmstadt, Germany), BDH Chemical (Mumbai, India) Sigma (USA), HIMEDIA (Mumbai, India).

Preparation of extract

Mature leaves of *P. guajava* tree were collected from nearby areas of Durg district, India. The plant was authenticated by Dr. M. Sharma, Professor and head, Science College, Durg, (C.G) India. The leaves were washed thoroughly, shade dried, and blended to form a fine powder. Then, 250 g of dry powder was macerated with 1 L of double distilled water for 72 h at 30±4°C. The extract was filtered to remove particulates and concentrated in water bath at 50°C. Finally, the concentrated crude aqueous extract was subjected to drying to solid mass (yield 16.6%) was obtained. The testing samples were prepared by dissolving the solid mass with double distilled water.

Animals and experimental design

Twenty four Albino Wistar rats (10–12 weeks old) weighing approximately 150–200 g were used for this study were housed in plastic cages in a 12 h dark light cycle with temperature ranging between 25–30°C and were provided with standard laboratory animal feed and water. After 15 days of acclimatization, the rats were randomly divided into four groups of six rats each. Group I served as negative control and rats in Groups II–IV received arsenic as sodium arsenite (NaAsO2) at 20 mg/kg in drinking water adlib for 6 weeks. Group II was considered as positive control as the animals of this group received arsenic exposure alone. Group III and IV along with arsenic exposure also received aqueous extract of *P. guajava* leaves (AEPG 50 and 100 mg/kg body weight, respectively) everyday by stomach tube. The experimental protocol was subjected to scrutiny of Institutional Animal Ethical Committee for experimental clearance (No. 445/IAEC/VCA/2009).

Sample collection

The mice were anesthetized by light chloroform anesthesia after 6 weeks. Blood was collected by cardiac puncture before sacrifice and tissue samples were collected for further analysis. The Liver, kidneys, and brain were removed and in a part were fixed in 10% buffered formalin for histological examination. These were processed, microtomed at 5 μ and stained with hemotoxylin and eosin (H and E) stain. Half portion of the liver and one of the kidneys from each rat were processed immediately for biochemical estimation and the remaining was stored at –20°C before wet acid digestion with concentrated HNO_3 for estimation of arsenic contents.

Biochemical assay

Lipid peroxide level in 10% RBC hemolysate was determined as per Placer *et al*.[9] and was expressed as nmol malondialdehyde (MDA)/mg of hemoglobin (Hb) using 1.56×10^5 as extinction coefficient Utley *et al*.[10] For the preparation of 10% RBC hemolysate, blood samples were centrifuged at 2000 rpm for 10 min and supernatant plasma were separated out. The sedimented cells were washed with sterile 0.85% NaCl solution three times. Washed erythrocytes were hemolyzed with ninefold volume of distilled water to prepare 10% RBC hemolysate. Hemoglobin in the hemolysate was estimated by the cyano-methaemoglobin method Van Kampen and Ziglstra.[11] Lipid peroxides in 10% crude tissue homogenate of liver, kidneys and brain from rats was estimated following Okhawa *et al*.[12] and was expressed in nmol of MDA/mg of protein. Tissues were immediately perfused with cooled buffer that contained 0.9% KCl, pH 7.2. They were homogenized in 9 volume of 1.15% KCl, 125 mM sucrose, pH 7.2. The homogenates were employed for the assays. The protein in 10% tissue homogenate was measured by the method of Lowry *et al*.[13] Superoxide dismutase activity in 10% supernatant tissues and RBC hemolysate was estimated as per Marklund and Marklund[14] with certain modifications suggested by Menami and Yoshikawa.[15] Each unit of the SOD activity is defined as the quantity of enzyme that inhibits auto-oxidation of pyrogallol by 50% under suitable experimental conditions. The catalase activity in tissues and RBC hemolysate was estimated as per Cohen *et al*.[16]

Arsenic analysis

Tissue and blood samples were wet digested,[17] and concentration of arsenic in the digested samples was measured using a hydride generation atomic absorption spectrophotometer (AAS, ECIL-4141, India) at 193.7 nm wave length and 10 mA current and the values were expressed in μg/mL or μg/mg for blood and wet tissues, respectively. Standard references material as used for ensuring the quality control and maximum allowed deviation

Statistical analysis

Data are expressed as means±SEM and were analyzed statistically using analysis of variance to compare the means of different treatment groups with that of the negative and positive control groups.[18]

RESULTS

This study evaluated the effect of aqueous extract of *P. guajava* leaves on arsenic-induced oxidative stress, cytopathological effect, and residual effect of arsenic in tissues such as liver, kidney, and brain.

Effect on LPO

The role of lipid peroxidase was assessed by studying the level of formation of malondialdehyde, an indicator of lipid peroxidation [Table 1]. depicts arsenic exposure for 6 weeks resulted in significantly ($P<0.05$) increased LPO levels in erythrocytes, liver, kidney, and brain tissues as compared to negative control animals. $AEPG_{50}$ and $AEPG_{100}$ treatment significantly reduced ($P<0.05$) lipid peroxidation in the studied tissues. However, LPO values appeared more near to normal with $APEG_{100}$ treatment.

Effect on nonenzymatic antioxidant

Result presented in Table 2 represented the reduced glutathione (GSH) levels of studied tissue in experimental animals. Administration of NaAsO2 caused significant decrease ($P<0.05$) in the GSH level in erythrocyte, liver, kidney, and brain. More decrease level was observed in liver followed by kidney and brain tissues.

Effect on antioxidant enzymes

Table 3 showed decreased SOD and CAT activities in arsenic exposed animals, that is, in positive control animals. Significantly ($P<0.05$) decreased activities of SOD and CAT enzymes were reported in erythrocyte and liver whereas non significant decrease in kidney and brain tissue. The simultaneous administration of $APEG_{50}$ and $APEG_{100}$ significantly elevated ($P<0.05$) SOD and CAT activities. However, nonsignificant increase in studied antioxidant enzymes was observed in kidney and brain.

Arsenic concentration

Blood and tissue arsenic concentrations in the arsenic exposed animals are presented in Table 4. Significantly ($P<0.05$) increased concentration of arsenic was observed in blood, liver, and kidney but nonsignificant increase was reported in brain tissue, suggesting that transport of inorganic arsenic to the brain may be limited by the blood–brain barrier. AEPG treatment was found effective in significant ($P<0.05$) reduction of arsenic levels in blood and liver tissue but nonsignificant decrease was observed in kidney and brain tissue.

Histopahtological alterations

In arsenic toxicity kidney parenchyma revealed extensive hemorrhage and vascular congestion. In addition, there were degenerative changes in the nature of both cloudy swelling and fatty degeneration and swelling of the epithelium of proximal convoluted tubule. There was coagulative necrosis (pyknosis) in the epithelium of Henle's loop and presence of albuminus exudates in the proximal convoluted tubules. Histopahtological slides of liver showed marked fatty degeneration, vacuolated hepatocytes, hydropic degeneration, coagulative necrosis, fatty degeneration, and cloudy swelling. In brain tissue necrosis of neurons and gliosis was observed. Neurons were

Table 1: Protective effect of *Psidium guajava* on LPO values in arsenic exposed and arsenic exposed treated groups

Groups	Erythrocytes (nmol MDA/mg of Hb)	Liver (nmol MDA/ mg of protein)	Kidney (nmol MDA/ mg of protein)	Brain (nmol MDA/ mg of protein)
Negative control	2.62±0.22	2.25±0.31	2.52±0.16	2.16±0.23
Positive control	4.87±0.31*	5.13±0.44*	4.65±0.20*	3.75±0.18*
Arsenic+AEPG 50	3.65±0.24	4.52±0.26	3.57±0.35	3.16±0.27
Arsenic+AEPG100	2.23±0.45[a]	3.78±0.33[a]	2.62±0.28[a]	2.87±0.33

Values are mean±SE; n=6; *P<0.05 compared to normal animals (negative control) and [a]compared with (positive control) arsenic exposed

Table 2: Protective effect of *P. guajava* on GSH (reduced glutathione) values in arsenic exposed and arsenic exposed treated groups

Groups	Erythrocytes µmole/g of Hb	Liver µmole/g tissue	Kidney µmole/g tissue	Brain µmole/g tissue
Negative control	4.66±0.31	4.02±0.25	3.85±0.38	3.27 ±0.29
Positive control	2.87±0.42*	2.51±0.19*	2.13±0.46*	2.63±0.35
Arsenic+AEPG 50	3.42±0.26	2.89±0.43	2.74±0.37	2.55±0.43
Arsenic+AEPG100	4.16±0.33[a]	3.70±0.27[a]	3.38±0.34[a]	2.89±0.21

Values are mean±SE; n=6; *P<0.05 compared to normal animals (Negative control) and [a]P<0.05 compared with (positive control) arsenic exposed animals

Table 3: Protective effect of *P. guajava* on SOD and CAT values in arsenic exposed and arsenic exposed treated groups

Groups	Enzymes	Erythrocytes (nmol MDA/mg of Hb)	Liver (nmol MDA/mg of protein)	Kidney (nmol MDA/mg of protein)	Brain (nmol MDA/mg of protein)
Negative control	SOD	4.62±0.42	3.51±0.20	3.67±0.42	3.42±0.36
	CAT	7.14±0.25	18.37±0.18	8.65±0.28	6.32 ±0.39
Positive control	SOD	2.16±0.31*	2.44±0.34*	2.39±0.36	2.65±0.25
	CAT	4.32±0.33*	15.32±0.33*	7.43±0.44	5.36±0.32
Arsenic+AEPG 50	SOD	3.24±0.40	2.73±0.25	3.21±0.32	2.91±0.27
	CAT	5.58±0.26	16.85±0.28	7.32±0.45	5.65±0.33
Arsenic+AEPG100	SOD	3.82±0.73[a]	3.11±0.32[a]	3.45±0.28	3.12±0.42
	CAT	6.22±0.44[a]	17.76±0.27[a]	8.17±0.41	5.87±0.39

Values are mean±SE; *n*=6; **P*<0.05 compared to normal animals (negative control) and [a]compared with (positive control) arsenic exposed

Table 4: Ameliorative potential of *P. guajava* on arsenic accumulation in arsenic exposed and arsenic exposed treated groups

Groups	Blood µg/mL	Liver µg/mg	Kidney µg/mg	Brain µg/mg
Negative control	0.14±0.12	0.16±0.24	0.13±0.12	0.12±0.31
Positive control	3.86±0.46*	5.38±0.12*	3.65±0.23*	1.87±0.16
Arsenic + AEPG 50	2.64± 0.36	4.62±0.243	3.42±0.25	1.32±0.37
Arsenic +AEPG100	1.12±0.13[a]	3.14±0.31[a]	2.65±0.44	1.13±0.28

Values are mean±SE; *n*=6; **P*<0.05 compared to normal animals (negative control) and [a]compared with (positive control) arsenic exposed

shrunken due to necrosis that indicates the degenerative process.

DISCUSSION

Arsenic exposure produces free radicals that cause damage to lipid, protein, and DNA of the body.[19] Present result do confirm that inorganic arsenic causes lipid peroxidation in target organs, namely liver, kidney, and brain. Increased lipid peroxidation thought to be consequences of oxidative stress which occurs when the dynamic balance between peroxidant and antioxidant mechanism is impaired.[20] Increased lipid peroxidation has been reported even at low doses of arsenic treatment in rats.[21] A marginal decrease in GSH correlated well with an increase in arsenic concentration in the respective tissues. Co-administration of APEG increased GSH levels in dose-dependent manner. Animals treated with APEG100 along with arsenic exposure significantly ($P<0.05$) increase GSH level near to normalcy. Adequate levels of the cellular GSH pool required not only for maintaining the cellular redox status by keeping sulfhydryl groups of cytosolic proteins in their reduced form but also because numerous toxic or potentially toxic compounds, including some metals, are either taken up by or removed from the cells by GSH-mediated pathways Chouchane and Snow.[22] A decrease in cellular GSH concentration has been inversely correlated with lipid peroxidation in the liver;[23,24] therefore, an increased GSH concentration by the APEG treatment given in group III and IV could presumably protect the organ from arsenic induced lipid peroxidation. Antioxidant enzymes are considered to be the first line of cellular defense against oxidative damage. SOD is an antioxidant metalloenzyme that reduces superoxide radicals to water and molecular oxygen.[25] CAT is a haemoprotein, which reduces hydrogen peroxide to molecular oxygen and water Gutteridge.[26] In support to our results observed decreased SOD and CAT enzyme activities in the tissues of experimental animals against arsenic intoxication. In contrast to this experiment, significantly increased SOD and CAT activities with increased LPO levels have been documented in the blood, liver, and kidneys of arsenic treated rats.[27] Arsenic concentration was found to be increased in blood and studied tissues due to arsenic exposure. $APEG_{50}$ and $APEG_{100}$ treatment restore the normal arsenic values in blood and like liver, kidney, and brain tissue. Flora *et al*.[28] also observed increase concentration of arsenic in kidney and liver in rats exposed to arsenic. Ferzand *et al*.[29] observed fatty degeneration and hydropic degeneration in proximal and distal tubules of kidney, as well as coagulative and liquifactive necrosis in the cells of kidney tissue. Santra *et al*.[30] conducted a study on arsenicosis and their results are parallel to this study and they concluded the changes in liver cells were due to oxidative stress in mitochondria that plays an important role in the pathogenesis of arsenic-induced apoptoic cell injury. A decrease in GSH triggers the activation of neuronal 12-lipoxygenase, which leads to the production of peroxides, and, ultimately, cell death Schulz *et al*.[31] Histopathological observation of liver, kidney, and brain of animals receiving sodium arsenite salt exhibited cytological alterations as compared to the normal tissue architecture. Treatment with $AEPG_{50}$ and $AEPG_{100}$ both did not showed much effect in restoration of the normal tissue structure. The findings of this study suggest a therapeutic role of *P. guajava* leaves in protecting from oxidative damage by ROS (reactive oxygen species) and prevent the arsenic-induced alterations in the oxidative stress markers value. Treatment with AEPG might reduce the peroxidation rate and restore the

body's antioxidant capacity. Co-administration of AEPG also reduces the arsenic increased concentration levels in blood and tissues and showed metal chelation property. Quantitative phytochemical investigation revealed the presence of Flavonoids and polyphenols in the alcoholic extract of *P. guajava* leaves.[32] Therefore, it is concluded that the arsenic exposure led to varying degree of changes in antioxidant defense mechanism and tissue architecture and supplementation of AEPG provide protection from damage due to arsenic in rats.

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