Original Article

Ameliorative Potential of *Psidium guajava* on Hemato-biochemical Alterations in Arsenic-exposed Wistar Rats

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ABSTRACT

The present study attempts to investigate the effects of *Psidium guajava* (*P. guajava*) when administered in combination with sodium arsenite @ 20 ppm in drinking water with the aim of achieving normalization of altered biochemical, hematological parameters suggestive of hepatic damage and depletion of inorganic arsenic following chronic arsenic exposure. Thirty adult Wistar rats were given 20 ppm arsenic for eight weeks along with hydro alcoholic leaf extract of *P. guajava* at a dose of 100 mg/kg body weight wt. (orally) (once daily for eight weeks). Arsenic exposure led to significant depletion of hemoglobin, red blood cells (RBC) and packed cell volume (PCV) but elevated leucocyte count (TLC). There was a significant increase ($P<0.01$/$P<0.05$) in serum aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphotase (ALP), acid phosphotase (ACP) and blood glucose whereas decrease in total protein level in arsenic-exposed untreated animals. The changes were accompanied by a significant elevation in blood and soft-tissue arsenic concentration. Co-administration of *P. guajava* was most effective not only in reducing arsenic-induced hematological and biochemical alterations but also in depleting arsenic from blood and soft tissues following arsenic exposure. We thus recommend combined leaf extract of *P. guajava* for achieving optimum effects of chelation therapy.

Key words: Hemato-biochemical, rats, sodium arsenite, toxicity

INTRODUCTION

Arsenic is a common environmental contaminant distributed around the world. Arsenic causes severe ill health effects due to the slow dosing of arsenic present in nature, mainly in drinking water (Cebrian *et al*.).[1] Amelioration of toxic effect of arsenic by using some herbal agents is a recent concept. Numerous plant products contain antioxidants, vitamins, flavonoids and polyphenolic compounds that have been demonstrated as the scavengers of free radicals and inhibitors of lipid peroxidation (Flora, *et al*.).[2] It has been observed that most of the vitamins and antioxidants were used after the induction of oxidative toxicity. However their prophylactic efficacy during sub acute toxicity has not been evaluated. In the present study, a herb *Psidium guajava* (*P. guajava*) is used prophylactically due to its local availability and is proved to be antioxidant in induced arsenic toxicity.

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MATERIALS AND METHODS

Matured leaves of *P. guajava* procured from near by areas of Durg district were shade dried and grinded finely to prepare hydro-alcoholic extract.

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Experimental design

Thirty rats were selected and divided into three groups. Rats of control group were healthy animals. They were housed in propylene cages under standard laboratory conditions. After acclimatization of laboratory conditions, all rats were divided into three groups viz. control, I and II. Groups I II rats received sodium arsenite @ 10 ppm in drinking water. Animals of group II along with arsenic also received leaf extract of *P. guajava* @ 50 mg/kg b.wt. All the rats were given the respective diets along with potable drinking water *ad libitum* throughout the experimental period. The animal treatment protocol employed in the study received prior approval of Institutional Ethics Committee following the standards laid down by Government of India.

Sample collection and analysis

Blood samples were collected from each group on 0, and 45th, day from orbital vein. 2 ml blood was transferred into vials for separation of serum for analysis of biochemical parameters. In another vial, around 1 ml of blood was collected for estimation of hematological parameters.

Blood and tissue samples were digested in concentrated nitric acid and 30% hydrogen peroxide. Digestion was carried out in Teflon bomb at 1000C for 6 h in a hot air oven. Dilution to known concentration was finally made with triple distilled water. Arsenic determination was made by using hydride generation atomic absorption spectrophotometer (HG-AAS, Chemito-201).

Hematological parameters

Hemoglobin (Hb), packed cell volume (PCV) and total erythrocyte (TEC) and leucocyte count (TLC) were determined immediately by using a hematology analyzer MS9.

Biochemical parameters

Blood glucose, total protein, albumin, and activities of ALT (alanine amino transferase), AST (aspartate amino transferase), ALP (alkaline phosphotase), ACP (acid phosphotase) were estimated in serum within 24 h on a semiautomatic biochemistry analyzer (Robonik, 300) by using commercial diagnostic kits as per the manufacturers recommendation procedures.

Arsenic analysis

To determine arsenic concentration in blood and tissue, samples were digested immediately after collection by adding concentrated nitric acid and 30% hydrogen peroxide. Digestion was carried out in Teflon bomb at 100°C for 6 h in hot air oven. Dilution to known concentration was finally made with triple distilled water and arsenic concentration was estimated with the help of atomic absorption spectrophotometer.

Statistical analysis

The data obtained on hemato-biochemical ad arsenic levels were analyzed employing analysis of variance described by (Snedecor and Cochran, 1994)).[3]

RESULTS

Hematological assay

The result of hemoglobin concentration presented in Table 1 shows significant decrease in hemoglobin in group I. At the end of the experiment that is on 45th day, significantly decreased values of PCV were observed in arsenic-exposed group. Group II animals showed restoration of hemoglobin and PCV values near to normal. There was significant decreasing trend ($P<0.05$) in total erythrocyte count in arsenic-exposed group. Prophylactic treatment showed slight increase in TEC values in arsenic-exposed treated groups. Total leukocyte value showed nonsignificantly increasing values in arsenic-exposed groups. TLC values were found to be normal in arsenic-exposed treated group (group II).

Biochemical assay

The results presented in Table 2 shows that the mean blood glucose level increased significantly ($P<0.01$) in group I in comparison to control. Serum proteins showed highly significant decline following arsenic treatment. An insignificant decrease was observed in serum albumin levels. There was statistical difference ($P<0.01$) in ALT activity and maximum ALT activity in arsenic treated groups was observed on 45th day in group I than that of control. Though the mean value of ALT in group II treated with leaf extract decreased, it remained higher than that of the control group. Significantly ($P<0.05$) increased AST values were observed in group I. In arsenic-exposed group I, the ALP activity was significantly higher to its 0 day values. Results presented in Table 2 show that the treatment was found effective to restore the normal activity of ALP enzyme. Significant ($P<0.05$) increase was also noted in serum ACP activities on 45th day in group I**.** Restored ACP values were observed in group II. i.e. arsenic-exposed treated group.

Residual effect of arsenic

Table 3 presents the blood and tissue arsenic concentration in different groups. Blood arsenic level increased significantly ($P<0.01$) in arsenic-exposed group and peak value was observed at the end of the study. Leaf extract of *P. guajava* showed reduction in the level of arsenic in blood in group II as compared to mean level estimated in group I.

The data on concentration of arsenic in different tissues, healthy and arsenic-exposed treated and untreated rats are given in Table 3. At the end of the experiment (45th day),

significant ($P<0.01$) higher concentration of arsenic in comparison to control group was found in the liver and kidney of group I rats. Brain and heart tissue also showed elevated levels of arsenic in group I. Leaf extract treatment was found effective to reduce the level of arsenic in blood but not found much effective to decrease arsenic level in tissues.

DISCUSSION

The anemic condition of arsenic-exposed animals was possibly due to the depression of bone marrow activity. This finding is similar to the findings of Ferzand *et al*.[4] who concluded that arsenic toxicity produces decreased hemoglobin values in mice model. Padmaja *et al*.,[5] also revealed significant decrease in PCV in arsenic toxicity. The decreased level of TEC was possibly due to suppression of bone marrow. Information in connection with the report of TEC in arsenic toxicity in animal is scare in the literature. Increased TLC values observed in group I might be due to slight increase in immune response of the body due to arsenic exposure. In contrast to this study, Mittal and Flora,[6] found decreased WBC count in blood. Biochemical alterations include the rise of blood glucose, might be due to stress condition, which is responsible for the secretion of glucocorticoids from adrenal cortex, which then increases the rate of gluconeogenesis by increasing the delivery of amino acids and fats, the gluconeogenic substrates (Kaneko).[7] Our results support the findings of Tseng *et al*.,[8] who also observed diabetogenic effect of arsenic in chronic arsenic toxicity. The reduction in protein level in group I might be due to renal excretion, impaired protein synthesis or liver damage. Decrease in total serum protein was also observed by Biswas *et al*.,[9] in chronic arsenic toxicity in goats. Decrease of serum albumin is due to impaired synthesis of albumin in severe liver damage as liver is the main site of albumin formation. Increased ALT values revealed the hepatotoxic effect of arsenic (Roy).[10] The present findings are in agreement with the results of Flora *et al*., (1998). Santra *et al*.,[11] stated that continued arsenic feeding resulted in fatty liver with serum aspartate aminotransferase and alanine aminotransferase and hepatic fibrosis. The present observation of increased AST activity supports the findings of Biswas.[9] Increased ALP level was recorded due to liver damage due to arsenic toxicity (Kaneko, 1990). Banerjee *et al*.,[12] observed that vitamin C can combat arsenic toxicity and restore the value of some biochemical parameters like AST, ALT, ACP and ALP. Increased ACP values observed in arsenic-exposed rats might be due to liver infection. Verma *et al*.,[13] also observed significantly decreased acid phosphatase activity in the liver of arsenic-treated animals. The result revealed metal enrichment in blood and tissues. As liver and kidney are the tissues mainly responsible for the metabolism and excretion of arsenic, increased concentration of arsenic was observed in these tissues (Golub *et al*.).[14] Heart and brain tissues also showed elevated arsenic concentration. Kamaluddin and Misbahuddin,[15] also found increased concentration of arsenic in liver, kidney and brain tissue in rats.

Table 1: Changes in hematological parameters in arsenic-induced and arsenic-exposed treated groups (*n*=10)

Groups	Hemoglobin (g/dl)	PCV (%)	TEC ($-\times10^6$/µl)	TLC (No./µl)
Control	13.59 ± 0.16	44.69 ± 0.33	7.25 ± 0.22	9721 ± 4.71
Group I	11.56 ± 0.21*	41.63 ± 0.59*	5.72 ± 0.31*	10245 ± 3.19
Group II	13.12 ± 0.17	42.62 ± 0.56	6.82 ± 0.34	9732 ± 4.02

*Statistically significant difference compared with control ($P<0.05$)

CONCLUSION

Toxicity was evaluated on the basis of hematological and biochemical changes and possible causes of alterations in different parameters were explored. Significant gradual

Table 2: Changes in biochemical parameters in arsenic-induced and arsenic-exposed treated groups (*n*=10)

Groups	Blood glucose	Total protein	Serum albumin	Serum ALT	Serum AST	Serum ALP	Serum ACP
Control	84.32 ± 0.31	6.16 ± 0.40	3.75 ± 0.26	31.23 ± 1.41	43.69 ± 3.07	150.67 ± 0.31	35.76 ± 1.73
Group I	128.16** ± 0.25	4.676* ± 0.30	2.86 ± 0.34	53.11** ± 1.23	52.33* ± 2.16	158.59* ± 0.38	39.66* ± 1.85
Group II	91.33 ± 0.47	5.97 ± 0.25	3.28 ± 0.21	43.69 ± 3.07	45.44 ± 3.19	152.25 ± 0.34	36.31 ± 2.83

*Statistically significant difference compared with control ($P<0.05$). **Statistically significant difference compared with control ($P<0.01$)

Table 3: Status of arsenic accumulation in different tissues in arsenic-induced and arsenic-exposed treated animals (*n*=10)

Group	Blood	Liver	Kidney	Heart	Brain
Control	0.17 ± 0.04	0.22 ± 0.18	0.24 ± 0.19	0.16 ± 0.16	0.19 ± 0.17
Group I	3.45 ± 0.01**	5.58 ± 0.42**	4.82 ± 0.19**	2.43 ± 0.21*	1.85 ± 0.05*
Group II	1.12 ± 0.04	4.05 ± 0.16	3.75 ± 0.20	2.26 ± 0.13	1.16 ± 0.05

*Statistically significant difference compared with control ($P<0.05$). **Statistically significant difference compared with control ($P<0.01$)

decrease in hemoglobin, packed cell volume and total erythrocyte count was observed in arsenic-exposed groups as compared to the control animals. Increased blood glucose was observed in arsenic-exposed group. Significant ($P<0.05$) increased total protein, AST, ALP and ACP values were observed in arsenic-exposed groups as compared to control groups whereas non significant elevated levels of serum albumin was observed in group I. Significant ($P<0.01$) increased levels of arsenic concentration in blood was observed in group II rats, but prophylactic treatment was found effective to reduce the concentration of arsenic in blood. Accumulation of arsenic in tissues was observed in arsenic-exposed rats and chelating effect of the leaf extract in group II animals was not observed. The study proves that mature *P. guajava* leaf extract was found effective to bring values of different parameters to normalcy in group II as compared to group I. This showed that the treatment was effective with arsenic @ 10 ppm in drinking water.

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