

Original Article

Effect of an extract of *Bauhinia variegata* leaves on chronic arsenic intoxication in mice (*Mus musculus*): A preliminary study

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ABSTRACT

Ethanol leaf extract of *Bauhinia variegata* has been tested for its possible antioxidant potentials against sodium arsenite induced toxicity in mice. Mice were randomized into two groups of five and fifty mice. Group I consisting of 5 mice without any treatment with food and water ad libitum which served as normal control. Group II mice were fed with sodium arsenite in drinking water at 100 ppm concentration for two monthsthen they were segregated into five groups which were treated differently. Group II a mice received only arsenic as sodium arsenite with drinking water, Group II b were fed chronically 1 : 20 alcohol to distilled water (vehicle), Group II c, d, e mice were orally fed 50 mg/kg, 150 mg/kg and 250 mg/kg of *B. variegata* leaf extract of once daily for 15 and 30 days respectively along with arsenic. Several toxicity marker enzymes such as gamma glutamyl transferase, lactate dehydrogenase, aspartate and alanine aminotransferase, acid and alkaline phosphatase, catalase and superoxide dismutase along with haematological variables such as glucose 6-phosphate dehydrogenase, creatinine, bilirubin, haemoglobin and sugar in different groups of treated and control mice were studied. Results obtained from the in vivo experiment revealed that administration of sodium arsenite caused a significant increase in some enzymes while decrease in some. A similar trend was also observed with haematological variables. In contrast *B. variegata* treatment at 150 mg/kg favourably modulated these alterations and maintained the antioxidant status than other two doses i.e. 50 mg/kg and 250 mg/kg thereby making it a good candidate to be used as supportive palliating measures in arsenic induced toxicity.

Keywords *Bauhinia variegata*, biomarkers, sodium arsenite, free radical, antioxidant

INTRODUCTION

Bauhinia variegata (BV) Linn (Fabaceae) commonly known as camel foot tree is a plant of great interest due to its pharmacological properties. It is widely grown in India as an ornamental plant. It is traditionally used for the treatment of several diseases such as leprosy, bronchitis, tumors (Sahu and Gupta, 2012; Shah and Joshi, 1971). Further it has been reported by several workers that it has chemopreventive properties (Rajkapoor et al., 2006). The stem of the plant displays antibacterial and antiviral properties while the root shows anti-inflammatory activity (Ali et al., 1999; Yadava and Reddy, 2003). Phytochemical studies revealed presence of several flavonoids that have been isolated from seeds, stems and flowers such as 7-dimethoxy-3,4-methylene dioxyflavonone, kämpferitrin (Gupta et al., 1980; Rahman and Begum, 1966; Yadava and Reddy, 2001).

High arsenic (As) groundwaters in deltaic environments are common and wide-spread with documented evidence from many parts of the world (Acharyya et al., 1999; IARC, 1987; Milton, 2005; NRC, 2001; Ratnaik, 2003; Rossman, 2003; Simeonova and Luster, 2000; Wang, 2007). Among the As-

affected deltaic environments, the Bengal Delta Plain is the worst in terms of human exposure (40 million) covering a large geographical area. The groundwater in this region often exceeds WHO permissible limit for arsenic concentration in drinking water i.e. 50 microgram per liter. Arsenic related health problems in the Indo-Gangetic plains have been reported by different research groups (Belon et al., 2007; Chakraborty et al., 2004; Guha Majumdar et al., 1988) but to date there is no remedy. Orthodox medicines such as dimercaptosuccinic acid, diethylenetriamine pentaacetic acid and British Anti Lewisite have been unsuccessful so far to treat these patients, further they have a varying efficacy and harmful side effects, hence there is need for alternative agents which are inexpensive and can easily be procured by common masses. The use of plant and plant materials for curing various ailments have been known to the world since time immemorial and gaining wide acceptance by scientific community, particularly in view of the toxic side effects of most synthetic drugs. Hence the present study is designed to evaluate whether ethanolic leaf extract of *Bauhinia variegata* can modulate sodium arsenite induced-toxicity in mice.

MATERIALS AND METHODS

Animals

The study was conducted on random bred Swiss albino mice (*Mus musculus*) weighing about 23 - 28 g body weight under the supervision of Institutional Animal Ethical Committee (Midnapore College, West Bengal, India, Registration No:

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1617/GO/a/12/CPCSEA, Ministry of Environment and Forest Government of India). Mice were randomized into two groups of five and fifty mice. Group I consisting of 5 mice without any treatment with food and water ad libitum which served as normal control. Group II mice were fed with sodium arsenite in drinking water at 100 ppm concentration for two months then they were segregated into five groups which were treated differently. Group II a mice received only arsenic as sodium arsenite with drinking water, Group II b were fed chronically 1 : 20 alcohol to distilled water (vehicle), Group II c, d, e mice were orally fed 50 mg/kg, 150 mg/kg and 250 mg/kg of *B. variegata* leaf extract once daily for 15 and 30 days respectively along with arsenic.

Preparation of the leaf extract: The *Bauhinia variegata* leaves were collected from Midnapore district, West Bengal, India during the months of June and July 2013 and identified by Dr. Somdatta Ghosh, Assistant Professor and Head, Department of Botany, Midnapore College. The voucher specimen has been submitted in the Botany Department (Voucher No: V-1238/2014). The extraction procedure was carried out as described by Shon et al. (2004). Briefly Sundried grounded leaves (20 g) were extracted in 90% ethanol (the ratio of plant material to solvent was (1 : 10 m/v) with a Soxhlet apparatus. The extraction was carried out at 55°C with constant stirring for 24 h. The extracts obtained were evaporated to dryness and stored at 4°C until required. The yield of the dried leaves were 12.5% which was calculated by the following equation: Yield (g/100g of dry plant material) = $W1 \times 100/W2$, where W1 and W2 were the weight of the extract after evaporation of solvent and the weight of the dry plant material. Henceforth *Bauhinia variegata* will be referred to as BV in rest of the manuscript.

Preliminary phytochemical screening

The presence or absence of phytochemical constituents was analyzed by routine procedures.

Flavonoids (Shinodas test): 100 g of plant material was extracted with 5 ml ethanol and filtered. To 1 (ml) of the filtrate, magnesium ribbon and few drops of concentrated HCl was added. Pinkish red colour indicates presence of flavonoids.

Alkaloids: 25 g of plant material was boiled in 15 ml of 1% concentrated H_2SO_4 in 50% ethanol and filtered. To the filtrate 5 drops of NH_4OH was added followed by 15 ml chloroform and two layers were separated. The chloroform layer was extracted with 15 ml dilute H_2SO_4 . On addition of 5 drops of Mayers reagent to the extract, a creamy red orange, brownish precipitate indicates presence of alkaloids.

Tannins: To 2 ml of the filtrate from the above, 1 ml of ferric chloride was added, a blue to greenish black precipitate indicates presence of tannins.

Carbohydrates: 100 g of *B. variegata* leaves were boiled in 25 ml distilled water and filtered. To 1ml filtrate, 1 ml of Molisch reagent was added followed by 1 ml of concentrated H_2SO_4 . Formation of reddish ring infers presence of carbohydrates.

Reducing sugars: 1 ml of above filtrate was boiled with 2 ml of Fehlings reagent for 3 - 4 min. A brick red precipitate indicates presence of reducing sugars.

Blood collection and tissue isolation

Blood was collected from retro-orbital plexus and serum was obtained from blood without EDTA by centrifugation for determination of creatinine, bilirubin, catalase, gamma-glutamyl transferase and lactate dehydrogenase activity. Blood with EDTA samples was used for determination of G-6PD activity. Liver tissue of sacrificed animal were quickly isolated

and separately processed. Briefly 50 mg of liver tissue was homogenized in 10 ml of phosphate buffer and centrifuged at 7000 g for 15 min in cooling centrifuge (C-24BL, REMI, Instruments). Before carrying out the enzymatic estimations the quantitative estimation of total protein was conducted by the method of Lowry et al. (1951).

Estimation of mean activities of gamma glutamyl transferase, lactate dehydrogenase

GGT activity was assayed by the method of Szasz (1976). Reagent kit was supplied by Reckon Diagnostics P. Ltd. (Code-6LX010, Baroda, India). LDH activity was assayed by the UV-Kinetic method of Gay et al. (1968). Reagent kit was supplied by Reckon Diagnostics P. Ltd., Gorwa, Baroda, India.

Estimation of mean activities of lipid peroxidation (LPO)

The LPO was estimated from the supernatant by the method of Buege and Aust (1984). One milliliter of sample (homogenate containing 0.1 - 0.2 mg of protein) was mixed thoroughly with 2 ml of TCA-TBA-HCl (15% w/v TCA and 0.375% w/v TBA in 0.25-N HCl). The absorbance of the sample was determined at 535 nm in a double beam spectrophotometer (UV-1800, Shimadzu, Japan) against a suitable blank. The malonaldehyde concentration of the sample was calculated by using extinction coefficient of 1.56×10^5 M/cm.

Estimation of mean activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT)

For estimation of AST and ALT in liver tissue samples the methods of Bergmeyer and Brent (1974) was followed with some minor modifications. For AST 0.1 ml of tissue homogenate was made to react with 0.5 ml of the substrate solution L-aspartate and was incubated for 60 min at 37°C. This was followed by addition of 0.5 ml of dinitrophenolhydrazine and then by 5.0 ml 0.4-N NaOH. The absorbance was measured at 510 nm. For the analysis of ALT, 0.1 ml of tissue homogenate was made to react with 0.5 ml of the substrate solution (L-alanine) and incubated for 30 min at 37°C. Rest of the procedure was same as that of ALT and the absorbance was measured at 510 nm.

Estimation of mean activities of acid phosphatase (ACP) and alkaline phosphatase (ALP)

For the study of ACP and ALP, the method of Walter and Schutt (1974) was followed. For ACP, to 2 ml of tissue homogenate 1ml of acid buffer was added, mixed and incubated at 37°C for 30 min. Then 2 ml of 0.1 N NaOH was added and the absorbance was measured at 405 nm against the standard. For ALP activity 0.05 ml of tissue homogenate was mixed with 2 ml of alkaline buffer and incubated at 37°C for 30 min; then 10 ml of 0.05 N NaOH was added and the absorbance was measured at 405 nm against the standard.

Estimation of catalase activity

Catalase activity in the liver tissue was assayed following the procedure of Sinha (1972). Briefly 0.1 ml of 5% liver tissue homogenate was incubated with 0.5 ml of H_2O_2 (0.2 M) at 37°C in presence of 0.01 M phosphate buffer whose pH was 7.4. The reaction was stopped by adding 5% dichromate solution. Then the samples were incubated at 100°C for 15 min in boiling water. The amount of H_2O_2 consumed was determined by spectrophotometer at 570 nm and expressed as μ mol of H_2O_2 consumed/min/mg protein.

Estimation of superoxide dismutase (SOD) activity

Liver tissue SOD was assayed by the method of Kakkar et al.

(1984). Briefly reaction mixture contained 1.2 ml of 0.052 sodium pyrophosphate buffer, 0.1 ml of phenazine methosulfate, 0.3 ml of 300 μ M nitroblue tetrazolium. Reaction was initiated by adding 0.2 ml of NADH (780 μ M) and stopped by adding 1ml of glacial acetic acid. The colour intensity was determined by at 560nm by UV-1800 Shimadzu spectrophotometer and expressed as units/min/mg protein.

Determination of haematological variables

For G-6-PD activity, 500 μ l blood was estimated using diagnostic kit procured from Reckon Diagnostic Pvt Ltd., India. The creatinine assay was performed by Jaffe kinetic method. For estimation of total serum bilirubin reagent kit was supplied by Reckon diagnostics (code 64X014). Haemoglobin content was determined by Sahlis method with help of hemometer (Marienfield, Germany).

Determination of arsenic from various tissues

Arsenic content in various tissues (liver and kidney), urine and blood was determined by a Perkin Elmer Analyst (AA200) (Texas, USA) Atomic Absorption Spectrophotometer adopting the standard AAS protocol (Belon et al., 2006). 100 μ l of each of the urine and blood samples and 1 ml of tissue homogenate was taken separately into 25 ml volumetric flask, to which 5.0 ml of a mixture $\text{HNO}_3/\text{HClO}_4/\text{H}_2\text{SO}_4$ (3 : 1 : 1) was added and kept for pre-digestion for about 2 h. Subsequently, the flasks were heated to 150°C on sand bath. The digestion continued until a colorless liquid 0.5 ml was obtained. All samples were performed in triplicate except blood and urine samples which were performed in duplicate due to less availability of test materials.

Statistical analysis

Statistical comparisons were made between the positive control + Alcohol groups to that of BV fed group. The significance of difference between data of the different groups was calculated by students't-test. ANOVA (SPSS 10.0 software) was used to compare multiple groups and within the groups. All the analyses were conducted observer blinded with respect to the animal belonging to treatment group.

RESULTS

Table 1 reveals preliminary phytochemicals which were present during the investigation in the ethanolic leaf extracts of *Bauhinia variegata*. The presence of alkaloids and flavonoids were more when compared to the presence of tannins, carbohydrate and reducing sugars where the intensity were denoted by +.

The GGT activity in the As 100 ppm + Ethyl alcohol vehicle of the plant extract (Alc) and As 100 ppm were considerably higher than normal control and As 100 ppm + BV at both the fixation intervals also it increased with the duration of As administration. When the data of GGT activity was compared between As 100 ppm + Alc and As 100 ppm + BV(150 mg/kg), it was appreciably low in BV fed series at both the fixation intervals which was statistically significant ($p < 0.001$, Tables 2a and b). However BV at 150 mg/kg appeared to give better protection than 50 mg/kg and 250 mg/kg at both the fixation intervals.

There was a significant fall in the mean LDH activities in the arsenic 100 ppm + BV (150 mg/kg) fed series at both the fixation intervals when compared to As 100 ppm + Alc and As 100 ppm series.

There was enhanced lipid peroxidation activity in both the

fixation intervals in liver tissues of mice fed with As 100 ppm, As 100 ppm + Alc when compared to normal ($p < 0.001$). However lipid peroxidation activity decreased significantly in As 100 ppm + BV (150 mg/kg) treated mice ($p < 0.05$ to $p < 0.001$) at both the fixation intervals when compared to As 100 ppm + BV (50 mg/kg) and As 100 ppm + BV (250 mg/kg) treated series. A similar trend was also observed in the mean activities of AST/ALT (Tables 2a and b).

On analysis of the results of ACP and ALP there was an increase in activity of both the enzymes in liver tissues in As 100 ppm, As100 ppm + Alc when compared to normal controls ($p < 0.001$). There was a decline in activities of ACP in mice fed with As100 ppm + BV (150 mg/kg) at both fixation

Table 1. Preliminary phytochemical screening of *Bauhinia variegata* leaf extracts which shows presence of several bioactive compounds of which flavonoids and alkaloids are predominant when compared to other compounds

| Chemical compounds | Results |
|--------------------|---------|
| Flavonoids | ++ |
| Alkaloids | +++ |
| Tannin | + |
| Carbohydrate | + |
| Reducing sugars | + |

intervals when compared to As100 ppm + Alc which was statistically significant. Although the activity of ALP decreased at both fixation intervals in mice fed with As 100 ppm + BV it was not significant when compared to As100 ppm + Alc (Table 2a and b). Further, ACP and ALP activities were low in mice when they were fed BV extract at 150 mg/kg concentration when compared to 50 mg/kg and 250 mg/kg at both the fixation intervals.

Oral administration of sodium arsenite decreased both catalase and SOD activity in As 100 ppm, As100 ppm + Alc treated mice at both fixation intervals when compared to normal control and mice fed with As100 ppm + BV which was statistically significant ($p < 0.05$ through $p < 0.001$, Tables 2a and b). However there was an increase in activity of catalase and SOD at both fixation intervals in mice fed with As 100 ppm + BV(150 mg/kg) when compared to As100 ppm + Alc and As100 ppm + BV (50 mg/kg and 250 mg/kg) and the increase was statistically significant ($p < 0.001$, Tables 2a and 2b).

Haematological variables

There was a decrease in the mean activities of G6PD in mice fed with As 100 ppm, As100 ppm + Alc at both 15 day and 30 day fixation intervals when compared to normal mice and mice treated with As 100 ppm + BV. The decrease of the activities of G6PD As100 ppm + Alc was statistically significant when compared to As 100 ppm + BV (150 mg/kg) ($p < 0.05$ to $p < 0.001$, Table 3).

In As 100 ppm, As100 ppm + Alc series there was an elevation in serum creatinine, sugar, bilirubin levels and reduction in haemoglobin concentration in As 100 ppm, As100 ppm + Alc when compared to As 100 ppm + BV treated mice ($p < 0.001$, Table 3). When the data was compared between various series it revealed that 150 mg/kg of *B. variegata* extract appeared to offer better protection than the other two doses, i.e. 50 mg/kg and 250 mg/kg.

Amount of arsenic deposited in various tissues has been represented in Table 4. The concentration arsenic in liver, kidney and blood in As 100 ppm, As 100 ppm + Alc treated series was appreciably high when compared to normal control at both the fixation intervals. However liberation of arsenic

Table 2a. Mean activities of different enzymes in liver of different treated and control series at 15 day fixation interval. GGT-gamma glutamyl transferase, LDH-lactate dehydrogenase activities (IU/L) in serum and LPO-lipid peroxidation (nM MDA/gm of tissue), AST-aspartate transaminase, ALT-alanine aminotransaminase (mM/min/mg in different tissues), ACP-acid phosphatase, ALP-alkaline phosphatase (mM phenol liberated/100 mg protein), CAT-catalase (nm of H₂O₂ decomposed/min/mg protein), SOD-superoxide dismutase (n moles of CDNB conjugated/min/mg protein)

| Series | GGT | LDH | LPO | AST | ALT | ACP | ALP | CAT | SOD |
|--------------------------|---------------|----------------|---------------|----------------|--------------|---------------|--------------|---------------|----------------|
| Normal | 14.23 ± 0.76 | 155.29 ± 5.77 | 9.00 ± 0.50 | 0.08 ± 0.01 | 0.03 ± 0.01 | 0.04 ± 0.01 | 0.09 ± 0.03 | 70.50 ± 1.16 | 105.14 ± 0.40 |
| As 100 ppm | 60.23 ± 0.12 | 219.44 ± 5.63 | 34.60 ± 0.10 | 0.34 ± 0.26 | 0.17 ± 0.13 | 0.45 ± 0.12 | 0.58 ± 0.01 | 28.91 ± 1.02 | 60.25 ± 0.26 |
| As 100 ppm + Alc | 73.26 ± 0.13 | 218.59 ± 7.97 | 35.30 ± 0.12 | 0.39 ± 0.04 | 0.18 ± 0.01 | 0.42 ± 0.01 | 0.63 ± 0.02 | 30.60 ± 0.01 | 66.25 ± 0.33 |
| As 100 ppm + BV 50 mg/kg | 50.12 ± 0.21 | 179.63 ± 1.65 | 22.66 ± 1.26 | 0.33 ± 0.05 | 0.18 ± 0.25 | 0.40 ± 0.14 | 0.60 ± 0.07 | 40.61 ± 1.40 | 66.61 ± 0.14 |
| As 100 ppm + BV 150g/kg | 50.27 ± 0.45* | 145.0 ± 1.59** | 15.87 ± 1.01* | 0.15 ± 0.85*** | 0.13 ± 0.51* | 0.25 ± 0.45** | 0.40 ± 0.47n | 71.61 ± 1.20* | 90.27 ± 1.11** |
| As 100 ppm + BV 250 g/kg | 52.16 ± 0.26 | 176.77 ± 0.64 | 32.71 ± 1.15 | 0.25 ± 0.08 | 0.17 ± 0.04 | 0.47 ± 0.06 | 0.55 ± 0.02 | 50.16 ± 2.12 | 60.14 ± 0.01 |

Values are expressed as mean ± SE for 5 mice in each series. SE = Standard error, **p* < 0.05; ***p* < 0.01; ****p* < 0.001; n = non significant; BV: *Bauhinia variegata*, Alc = ethyl alcohol vehicle of the plant extract.

Table 2b. Mean activities of different enzymes in liver of different treated and control series at 30 day fixation interval. GGT-gama glutamyl transferase, LDH-lactate dehydrogenase activities (IU/L) in serum and LPO-lipid peroxidation (nM MDA/gm of tissue), AST-aspartate transaminase, ALT-alanine aminotransaminase (mM/min/mg in different tissues), ACP-acid phosphatase, ALP-alkaline phosphatase (mM phenol liberated/100 mg protein), CAT-catalase (nm of H₂O₂ decomposed/min/mg protein), SOD-superoxide dismutase (n moles of CDNB conjugated/min/mg protein)

| Series | GGT | LDH | LPO | AST | ALT | ACP | ALP | CAT | SOD |
|--------------------------|---------------|-----------------|---------------|---------------|----------------|--------------|---------------|----------------|---------------|
| Normal | 14.65 ± 3.26 | 152.10 ± 7.34 | 9.60 ± 0.75 | 0.09 ± 0.04 | 0.05 ± 0.06 | 0.05 ± 0.06 | 0.10 ± 0.06 | 75.51 ± 0.02 | 100.26 ± 0.31 |
| As 100 ppm | 70.22 ± 3.79 | 209.67 ± 8.66 | 40.33 ± 1.67 | 0.40 ± 0.03 | 0.19 ± 0.12 | 0.57 ± 0.08 | 0.90 ± 0.07 | 40.16 ± 0.10 | 50.55 ± 1.06 |
| As 100 ppm + Alc | 86.45 ± 2.16 | 221.33 ± 10.67 | 48.96 ± 4.11 | 0.43 ± 0.12 | 0.22 ± 0.11 | 0.55 ± 0.27 | 0.99 ± 0.07 | 40.11 ± 0.07 | 55.21 ± 1.01 |
| As 100 ppm + BV 50 mg/kg | 62.58 ± 1.44 | 199.60 ± 1.02 | 39.26 ± 4.08 | 0.30 ± 0.03 | 0.16 ± 0.06 | 0.52 ± 0.06 | 0.89 ± 0.21 | 39.80 ± 0.60 | 55.00 ± 0.38 |
| As 100 ppm + BV 150 g/kg | 52.66 ± 1.25* | 176.00 ± 2.10** | 25.11 ± 1.25* | 0.26 ± 0.04** | 0.13 ± 0.01*** | 0.36 ± 0.21* | 0.60 ± 0.03** | 79.52 ± 0.21** | 96.40 ± 0.06* |
| As 100 ppm + BV 250 g/kg | 65.80 ± 1.64 | 196.30 ± 5.03 | 39.54 ± 1.01 | 0.34 ± 0.25 | 0.16 ± 0.04 | 0.48 ± 0.04 | 0.77 ± 0.52 | 39.66 ± 0.15 | 70.37 ± 0.59 |

Values are expressed as mean ± SE for 5 mice in each series. SE = Standard error, **p* < 0.05; ***p* < 0.01; ****p* < 0.001; n = non significant; BV: *Bauhinia variegata*, Alc = ethyl alcohol vehicle of the plant extract.

from body via urine was statistically significant in As 100 ppm + BV treated mice when compared to As 100 ppm As 100 ppm + Alc (*p* < 0.001).

DISCUSSION

The present investigation demonstrates modulation of sodium arsenite induced stress (post arsenic exposure) in mice by administration of BV leaf extract by taking into consideration of various toxicity biomarkers. An analysis of the results of the toxicity biomarkers in As 100 ppm, As100 ppm + Alc fed series at both 15 day and 30 day reveals increase in activities of GGT, LDH, LPO, AST, ALT, ACP and ALP and decrease in activity of G6PD. In As 100 ppm + BV treated mice this trend was largely altered which indicates a detoxifying effect and reduced oxidative stress. This might be due to presence of considerable

amounts of phenolic compounds and flavonoids present in the ethanolic extracts of BV leaves which could act as a hydrogen donor antioxidant.

Previous studies reveals that leaves of *Bauhinia* contains ascorbic acid (Chaturvedi et al., 2011) which might have some role in countering sodium arsenite induced toxicity as revealed in the present investigation. As ascorbic acid has marked nucleophilic properties it might intercept the reactive metabolites which have arisen due to sodium arsenite intoxication thereby preventing their attack and hence have a regulatory role on protein metabolism and repair activities in the cells. Ethanolic extract of the stem of BV showed chemoprevention against N-nitrosodiethylamine in rats and cancer cell lines (Raj Kapoor et al., 2006). They investigated that oral administration of BV suppressed various toxicity marker enzymes and also have antitumor activity against Daltons ascetic lymphoma (Raj Kapoor et al., 2003). Various other

Table 3. Mean activities of G6PD, serum creatinine content, bilirubin levels (mg/dl), haemoglobin content and blood sugar of mice of different treated and control series at different fixation intervals

| | Series | G6PD | Creatinine | Bilirubin | Hb | Sugar |
|---------------------------------|---------------------------|----------------|----------------|--------------|---------------|---------------|
| 15 days fixation interval | Normal | 15.21 ± 1.45 | 0.36 ± 0.12 | 0.40 ± 0.03 | 12.30 ± 0.12 | 87.21 ± 2.12 |
| | As 100 ppm | 7.21 ± 0.26 | 0.42 ± 0.36 | 0.57 ± 0.13 | 10.33 ± 1.11 | 94.68 ± 2.33 |
| | As 100 ppm + Alc | 6.88 ± 2.99 | 0.41 ± 1.16 | 0.60 ± 0.12 | 11.60 ± 0.04 | 97.54 ± 4.31 |
| | As 100 ppm + BV 50 mg/kg | 8.26 ± 0.24 | 0.48 ± 0.11 | 0.80 ± 0.01 | 10.63 ± 0.11 | 91.01 ± 1.15 |
| | As 100 ppm + BV 150 mg/kg | 13.96 ± 0.89** | 0.31 ± 0.21* | 0.78 ± 0.61 | 12.70 ± 0.21 | 86.14 ± 1.68n |
| | As 100 ppm + BV 250 mg/kg | 9.45 ± 0.14 | 0.41 ± 0.14 | 0.79 ± 0.12 | 10.98 ± 0.15 | 91.75 ± 0.05 |
| 30 days fixation interval | Series | G6PD | Creatinine | Bilirubin | Hb | Sugar |
| | Normal | 17.21 ± 1.89 | 0.36 ± 0.16 | 0.41 ± 0.05 | 12.70 ± 1.11 | 89.45 ± 1.16 |
| | As 100 ppm | 9.63 ± 0.23 | 0.57 ± 0.07 | 0.62 ± 0.11 | 10.66 ± 0.89 | 76.26 ± 6.79 |
| | As 100 ppm + Alc | 10.66 ± 0.26 | 0.54 ± 0.07 | 0.65 ± 0.09 | 10.05 ± 2.88 | 106.39 ± 4.99 |
| | As 100 ppm + BV 50 mg/kg | 10.25 ± 0.15 | 0.51 ± 0.04 | 0.60 ± 0.102 | 9.08 ± 0.12 | 92.06 ± 0.14 |
| | As 100 ppm + BV 150 mg/kg | 12.00 ± 0.25* | 0.48 ± 0.03*** | 0.54 ± 0.13 | 11.22 ± 0.02* | 88.46 ± 0.25n |
| | As 100 ppm + BV 250 mg/kg | 10.61 ± 1.02 | 0.52 ± 0.15 | 0.59 ± 0.02 | 10.06 ± 1.04 | 95.05 ± 2.03 |

Values are expressed as mean ± SE. SE = Standard error, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n = non significant; BV : *Bauhinia variegata*, Alc = ethyl alcohol vehicle of the plant extract.

Table 4. Mean concentrations of arsenic in liver, kidney, blood and urine in different series of mice at 15 and 30 day fixation interval (n = 5) in ppb. (ND-non detected)

| Series (15 days) | Liver | Kidney | Blood | Urine |
|---------------------------|-----------------|----------------|----------------|-----------------|
| Normal | ND | ND | ND | ND |
| As 100 ppm | 125.00 ± 0.02 | 80.60 ± 0.06 | 92.60 ± 0.10 | 130.60 ± 0.12 |
| As 100 ppm + Alc | 130.10 ± 0.11 | 78.70 ± 0.01 | 96.80 ± 0.17 | 135.80 ± 0.20 |
| As 100 ppm + BV 50 mg/kg | 102.06 ± 2.08 | 70.52 ± 2.01 | 91.25 ± 1.02 | 135.20 ± 0.25 |
| As 100 ppm + BV 150 mg/kg | 90.60 ± 0.02*** | 65.60 ± 0.15** | 90.40 ± 0.02* | 144.20 ± 0.10** |
| As 100 ppm + BV 250 mg/kg | 104.58 ± 1.22 | 71.87 ± 4.08 | 93.26 ± 1.02 | 140.26 ± 0.14 |
| Series (30 days) | Liver | Kidney | Blood | Urine |
| Normal | ND | ND | ND | ND |
| As 100 ppm | 130.00 ± 0.21 | 92.20 ± 0.09 | 100.60 ± 0.01 | 160.50 ± 0.15 |
| As 100 ppm + Alc | 130.10 ± 0.01 | 95.30 ± 0.15 | 102.80 ± 0.21 | 188.40 ± 0.18 |
| As 100 ppm + BV 50 mg/kg | 90.06 ± 2.11 | 91.69 ± 4.07 | 95.68 ± 0.26 | 143.02 ± 2.01 |
| As 100 ppm + BV 150 mg/kg | 84.70 ± 0.04** | 79.80 ± 0.08* | 80.20 ± 0.05** | 115.30 ± 0.02** |
| As 100 ppm + BV 250 mg/kg | 102.33 ± 1.78 | 90.20 ± 1.09 | 96.87 ± 0.87 | 120.03 ± 0.05 |

Values are expressed as mean ± SE for 5 mice in each series. SE = Standard error, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n = non significant; BV: *Bauhinia variegata*, Alc = ethyl alcohol vehicle of the plant extract.

pharmacological studies were conducted with regard to nephroprotective ability of whole stem ethanolic extracts of BV against cisplatin, gentamicin induced nephrotoxicity (Pani et al., 2011; Sharma, 2011).

Chronic feeding of sodium arsenite has been reported to have various toxic effects by generating reactive oxygen species which are directly involved in oxidative damage to DNA, proteins and lipids which ultimately leads to cell death (Garcia-Chavez et al., 2003; Nandi et al., 2005a,b; Sinha et al., 2010). In animals orally administration of inorganic arsenic is methylated to several metabolites mainly dimethylarsinic acid which are excreted from urine and stool (Cohen et al., 2002; Rossman, 2003; Vahter, 1983, Vahter and Concha, 2001; Waalkes, 2007). It is evident in the present study that post arsenic exposure treatment with *Bauhinia variegata* leaves extract at 150 mg/kg significantly protected the animals from toxic effects of arsenic and removal of arsenic via urine when

compared to other two doses i.e. 50 and 250 mg/kg. Further, flavonoids are naturally occurring substances which are present in *B. variegata* as revealed in the present study which acts as scavengers of most oxidizing molecules including free radicals and singlet oxygen. To the best of our knowledge this is the first study that demonstrates protective ability of *B. variegata* leaves against arsenic induced oxidative stress. Further, the process of lipid peroxidation is initiated by the attack of free radical on unsaturated lipids and the resulting chain reaction is terminated by the production of lipid breakdown products such as aldehydes, malonaldehyde. Quantification of malonaldehyde thus reflects the state of hepatotoxicity (Biswas et al., 2008; Biswas and Khuda-Bukhsh, 2005). This was evident in present investigation too as there was abnormal activity of biomarkers of toxicity as a result of arsenic intoxication. However treatment with BV extracts significantly suppressed lipid peroxidation thereby rendering a hepatoprotective action.

Bodakhe and Ram (2007) obtained similar results against CCl_4 induced hepatotoxicity on Sprague-Dawley rats.

However, further in-depth studies are warranted to understand some other aspects of the mechanism of action of the plant extract in showing positive amelioration of sodium arsenite induced toxicity. The doses in the present investigation were selected on the basis of previous studies conducted by us and where we have found that feeding of only *B. variegata* leaf extract does not show any cytotoxicity or genotoxicity (Biswas et al., 2013). The effect of BV supplementation on the activity of cellular antioxidant enzymes catalase and SOD were analyzed in liver tissue. Oral administration of sodium arsenite decreased both catalase and SOD activity in As 100 ppm, As100 ppm + Alc treated mice at both fixation intervals when compared to As100 ppm + BV fed series which was statistically significant. SOD and catalase are most important enzymes against toxic effects of oxygen metabolism. SOD accelerates the dismutation of superoxide to H_2O_2 which prevents generation of free radicals while catalase catalyzes removal of H_2O_2 formed during the reaction catalyzed by SOD. In the present experiment a decrease in SOD can be attributed to more production of superoxide during arsenic intoxication which in turn suppresses the activity of catalase. Serum creatinine was significantly reduced in the BV treated series. The ethanolic extract of BV showed a significant reduction which clearly indicates that the leaf extract protected the mice against sodium arsenite induced injury. The mechanism underlying the ameliorative potential of leaves extract and its active principles is not clear; the plausible explanation may be due to either individual or combined effects of its constituents. Glucose 6-phosphate dehydrogenase is a cytosolic enzyme in the pentose phosphate pathway which supplies reducing energy to cells such as erythrocytes by maintaining the level of NADPH. NADPH in turn maintains the level of glutathione in RBC thereby protects RBC against oxidative damage. Decrease in the activity of G6PD in the present investigation in both As 100 ppm, As 100 ppm + Alc treated mice at both fixation intervals indicates that arsenic might have a profound impact on red blood cells and simultaneously there was a reduction in Hb concentration at both these fixation intervals though it was not statistically significant. However in the BV treated series there was a significant increase in activity of G6PD and increase in Hb concentration which clearly indicates that BV might have the ameliorating effect against arsenic induced toxicity.

In conclusion this study suggest that administration of an ethanolic extract of *Bauhinia variegata* offers significant protection against sodium arsenite induced oxidative stress in mice with regard to biomarkers of toxicity. Identification of such a plant is essential since it can be given as dietary supplement to human population exposed to ground water arsenic intoxication and would protect population coming from low socioeconomic status without being appreciably harmful itself. The mechanism of protective effect is attributed mainly to the antioxidant property of the plant extract. It is important that such studies are replicated independently in various animal models particularly in the light of recent reports of beneficial use of plant extracts in modulation of arsenic toxicity.

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CONFLICT OF INTEREST

No authors/co-authors have any competing interest to declare.

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