Phytochemical analysis

The following phytochemical analysis was carried out on leaves of *V. amygdalina* using the procedure of[16] as outlined below:

Test for flavonoids

0.5g of plant sample was suspended in 5ml of water and 2.5ml of methanol was added to it and filtered. 1ml of NaOH 10% was added to 1ml of the filtrate. The presence of a yellow precipitate indicated the presence of flavonoids.

Test for tannins

7.5ml of water was added to plant extract (1g) and heated in a water bath. It was then filtered upon cooling. Few drops of iron III chloride ($FeCl_3$) 0.5% were added to 2ml of the filtrate. The appearance of a green or dark-blue precipitate indicated the presence of tannins.

Test for alkaloids

2g of plant sample was heated in a test tube containing 25ml of HCl (1%) for 15 min in a boiling water bath. The suspension was then filtered and 5 drops of Meyer's reagent (potassium tetra iodomecurate) were added into the filtrate (1ml). The formation of a precipitate indicated the presence of alkaloids.

Test for saponins

0.5g of plant extract was introduced into a test tube containing 7.5ml of distilled water and the mixture heated for 5 min in a boiling water bath. The solution was then filtered and cooled to room temperature. Five millilitres of the filtrate was introduced into a test tube and agitated for 10s. The formation of persistent foam indicated the presence of saponins.

Test for triterpenes and steroids

0.5g of plant sample was dissolved in chloroform (3ml) and a few drops of acetic anhydride and concentrated H_2SO_4 were added. A purple coloration indicated the presence of triterpenes while bluish-green coloration indicated the presence of steroids. The formation of two layers upon addition of H_2SO_4 is characteristic of the presence of both triterpenes and steroids.

Test for coumarins

One milligram of moistened sample was placed in a test tube and the test tube was covered with a filter paper moistened with 10% NaOH solution. After exposition of the paper to UV light for a few minutes, yellow green fluorescence was indicative of the presence of coumarins

Test for cardiac glycosides

One milligram of plant sample was suspended in 5ml of glacial acetic acid containing one drop of ferric chloride solution. Then 1ml of concentrated sulphuric acid was added gradually along the wall of the test tube. The formation of a brown ring at the interface indicated the presence of a deoxysugar, characteristics of cardenolides.

Statistical analysis

The data were analysed using statistical software (Jandel Sigmastat, for windows version 2.03). The results are expressed as mean ± SEM for each group. The statistical significance was determined by one-way analysis of variance (ANOVA), followed by the Tukey test. The level of statistical significance was set at $p < 0.05$ and < 0.01.

RESULTS

Effects of *V. amygdalina* leave extract on the sodium arsenite induced micronuclei

The protective effects of *V. amygdalina* leave extract on bone marrow micronuclei after seven days pre-treatment are shown in Tables 1. Sodium arsenite at a dose of 2mg/kg induced significant MN over the negative control [Table 1]. Pre-treatment with 50mg/kg *V. amygdalina* leave extract significantly reduced sodium arsenite induced MN in bone marrow by 70%, compared with the frequency of MN induced in the bone marrow of rat treated with sodium arsenite alone. Similarly, pre-treatment with 100mg/kg *V. amygdalina* leave extract significantly reduced sodium arsenite induced MN in bone marrow by 74%, compared with the frequency of MN induced in the bone marrow of sodium arsenite alone

Scavenging effects of the *V. amgdalina* on DPPH radical

The DPPH radical scavenging activity of *V. amygdalina* extract (IC_{50} value of 4.0 ± 0.1μg/ml) is comparable to the IC_{50} value gallic acid (3.8 ± 0.2μg/ml), which is a well-known antioxidant [Table 2]. Scavenging of DPPH radical was found to rise with increasing concentration of the extracts [Table 2].

Histology

The photomicrography of the liver reveals that the treatment of rats with sodium arsenite produced altered normal architecture of the parenchyma [Figure 1a]; group treatment with sodium arsenite plus *V. amygdalina* reduced the necrotic areas [Figures 1c and 1d] in comparison with negative control group [Figure 1b].

Phytochemical constituents of crude extracts of *V. amygdalina*

The test for the secondary metabolite showed the presence of flavonoids and saponin. Tripeternes and cardiac glycosides were also present in appreciable trace while tannins and alkaloids were present in low quantity. Coumarin is absent [Table 3].

Table 2: Scavenging effects of the *V. amgdalina* on DPPH radical

Sample	% Inhibition at concentration (µg/ml)								
	500.0	250.0	125.0	62.5	31.3	15.6	7.8	3.9	IC_{50}
V. amgdalina extract	93.3 ± 42	93.1 ± 311	92.6 ± 30	92.6±25	91.0 ± 20	91.3 ± 30	90.2 ± 32	89.1 ± 16	4.0 ± 01
Gallic acid	95.5 ± 30	95.8 ± 36	94.5 ± 4.0	94.7±46	93.1 ± 4.2	92.0 ± 3.9	91.5 ± 3.0	90.0 ± 1.8	3.8 ± 0.2

Values represent mean ± S.E.M., n = 3

Table 3: Phytochemical constituents of crude extracts of *V. amygdalina*

Plant extract	Flavonoids	Tannins	Alkaloids	Saponins	Triterpenes	Steroids	Coumarins	Cardiac glycosides
Vernonia amygdalina	+++	+	+	+++	++	++	–	++

+ = Presence of constituent; – = Absence of constituent, +++ detected in high quantity, ++detected in medium quatity, +detected in trace amount

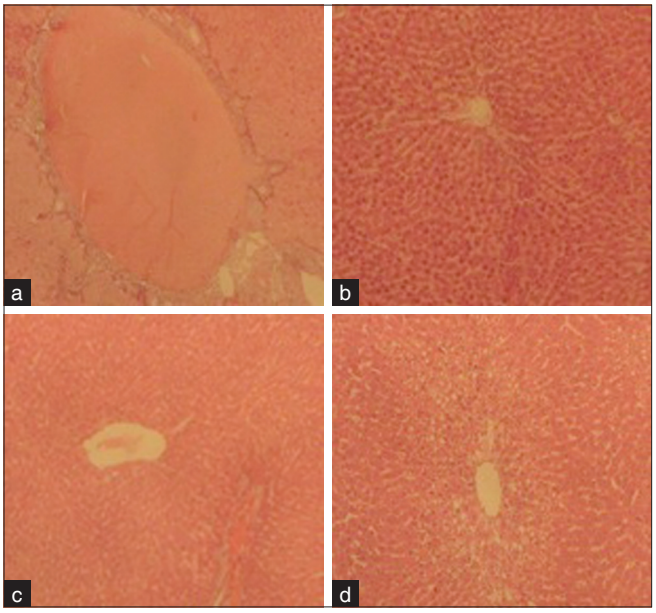

Figure 1: Representative of liver sections obtained from rats treated with sodium arsenite alone (a), distilled water alone (b), *V. amgdalina* (50 µg/ml) plus sodium arsenite-treated rats (c) and *V. amgdalina* (100 µg/ml) plus sodium arsenite-treated rats (d)

DISCUSSION

The genotoxicity of arsenic is well known as it induces chromosomal damage in different test systems.[17,18] The present results confirmed the genotoxic potential of sodium arsenite as evident from the significant induction of MN in rat bone marrow. Pre-and co-treatment with *V. amygdalina* at doses of 50 and 100mg/kg, b.w. for 7 days significantly reduced the sodium arsenite induced MN frequencies in rat bone marrow.

GGT and ALP are found in many tissues, the most notable one being the liver, and has significance in medicine as a diagnostic marker. GGT plays a key role in drug and xenobiotic detoxification. Oxidative injury and lipid peroxidation can be monitored by measuring liver or blood GGT and ALP concentration.[19] Consequently, the effect of pre-treatment of *V. amgdalina* on concentration of ALP and GGT was evaluated in this study. Similarly, with the same treatment *V. amygdalina* significantly reduced the concentration of ALP and GGT medium in serum of the rat. The increase of ALP and GGT concentration with sodium arsenite group (positive control) was marginal over the saline treated groups (negative control). However, with the co-treatment of sodium arsenite with *V. amygdalina* the concentration of the enzymes was decreased. The effects of *V. amgdalina* leave extract discussed until here are important mediators to have beneficial response to prevent liver fibrosis induced by sodium arsenite administration and may have an impact in the prevention of diseases.[20] As the *V. amgdalina* treated group avoided the liver fibrosis the main markers of cholestatic damage, GGT and ALP were normal at the end of the treatment. This study shows the beneficial response to the treatment with *V. amgdalina* leave extract in the experimental model of rat liver fibrosis induced by administration of sodium arsenite.

An antioxidant dietary supplement can reduce the level of DNA oxidative damage and protect normal cells against the adverse side-effects of some carcinogens.[21] In this study *V. amygdalina* leave extract exhibited strong radical scavenging property and previous reports demonstrated that *V. amygdalina* extract scavenges hydroxyl radicals and exhibit chain breaking antioxidant activity against metal ion induced lipid peroxidation.[22] Furthermore, it has been determined that the antioxidant effect of plant products is mainly due to radicals scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes.[23] In this study, some of these phytochemicals were found to be essential components of *V. amygdalina* leave extract. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important

role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.[24] Therefore, the reduction in the toxicities and clastogenicity exhibited in this study might be due to counteracting effect of antioxidant components of *V. amygdalina* on reactive species generated by sodium arsenite in rat. This further suggests that *V. amygdalina* has a potent anticlastogenic effect against sodium arsenite induced genotoxicity. The present report suggests the antigenotoxic effect of *V. amygdalina* and the suppression of genotoxicity may be either due to the free radical scavenging mechanisms or different DNA reparative processes against sodium arsenite. Further investigations are needed to reveal the exact mechanistic basis of this type of chemoprotective effect exhibited by *V. amygdalina*. Additional work in terms of different genotoxic end-points with specific fractions and components of *V. amygdalina* is in progress to strengthen the chemoprotective potential of *V. amygdalina* against the toxicity induced by different clastogens.

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