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Full Length Research Paper

Hepatoprotective potentials of water extract of *Bauhinia purpurea* bark against alcohol induced toxicity

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Bark of *Bauhinia purpurea* tree was collected and both the water and methanol extracts were prepared. Both extracts were tested for free radical scavenging activities and phenol content. Water extract was then tested for its protective potentials against alcohol induced toxicity. For this experiment, twenty rats were randomly divided into four groups, alcohol control group, normal control and two experimental groups. The rats in alcohol control group received alcohol (5 g/kg), rats in normal control were given water. Rats in two experimental groups were administered two doses of methanol extract (50 and 100 mg/kg respectively) plus alcohol (5 g/kg) for 30 days. At the end of the experiment, rats were killed after ether anesthesia. Liver and blood samples were collected for biochemical measurements. Parameters measured were thiobarbituric acid reactive substances, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, reduced glutathione, glutathione peroxidase, superoxide dismutase, catalase and ascorbic acid. Results showed that both methanol and water extracts scavenged free radicals equivalent to gallic acid scavenging and were found rich in total phenol content. Results of *in vivo* experiments showed that the water extract inhibited lipid peroxidation, protected the experimental animals from hepatic toxicity and maintained the levels of antioxidants in dose dependent manner.

Key words: Lipid peroxidation, anti-oxidants, glutathione, ascorbic acid.

INTRODUCTION

Alcohol abuse is one of the major problems in African continent and is responsible for significant percentage of hospital admission. Ethanol molecule is small and soluble in both water and lipids, It permeates all tissues of the body and affects most vital functions of virtually all organs including liver, kidney, brain, heart and pancreas (Lieber, 1997). Metabolism of alcohol leads to the generation of free radicals and chain reaction of lipid peroxidation that causes damage of liver and other vital organs. Therefore inhibition of free radicals generation is important in providing protection against hepatic damage. For a long period of time, plants have been a valuable source of natural product for maintaining human health and nowadays, many therapeutic studies are devoted to plants. Since plants are natural source of antioxidants and hence reduces oxidative stress. Therefore they can be used in the treatment of many oxidative stress originated diseases like hepatic damage.

Bauhinia purpurea, commonly known as Kachnar in Ayurvedic system of medicines, is used as liver tonic and also for the treatment of thyroid disorders. Modern reports also support the thyrogenic effects of the plant (Panda and Kar, 1999). There are different species of *Bauhinia* and almost all of them have been reported for their biological activities, example, *Bauhinia cheilandra* and *Bauhinia forficata* for hypoglycemic effects (Almeida et al., 2006; da Silva et al., 2000), *Bauhinia microstachya* for antioxidant and free radical scavenging properties (Evandro et al., 2007), *Bauhinia variegate* for hepatoprotective effects (Bodakhe et al., 2007), and *B. purpurea* for its antinoceceptive and anti inflammatory properties (Zakaria et al., 2007).

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B. purpurea is world wide in distribution although it is a native plant of Southeastern Asia ranging from India to China. It is a fast growing small to medium size tree. The leaves are smooth and elliptical in shape. Flowers are purple in color and borne on unbranched auxiliary or terminal corymbs in winter and autumn.

Stem bark of *B. variegate* has been reported for antioxidant and hypolipaemic (Rajani and Purnima, 2009) and hepatoprotective effects against CCl4 induced liver toxicity (Bodakhe et al., 2007). Since, *B. purpurea*, also belongs to same genus and has been reported for antioxidant and hepatoprotective effects, it has been proposed that this species might have hepatoprotective potentials like *B. variegate*. Therefore, the present study aims to investigate the hepatoprotective potentials of WBP (water extract of *B. Purpurea*) against alcohol induced toxicity. Since water extract is usually used by people for herbal treatment, we have tried to evaluate the protective effects using the same.

MATERIALS AND METHODS

Collection of plant material and preparation of extracts

The plant was identified by Dr. M. P. Setshogo of Department of Biological Sciences Herbarium, University of Botswana. The voucher specimen was deposited there. Bark of the tree was collected from the University Campus, washed, chopped into small pieces and sun dried. It was then crushed to powder. Water extract (WBP) was prepared by boiling 200 g of the powder with water (8 times the volume of powder). The extract (WBP) was filtered and water was evaporated in a rotary evaporizer (BUCHI Rotavapor R-200) to get the solid mass. The yield was 29.83% w/w. To prepare the methanol extract, the powder was soaked with 70% methanol for cold extraction for seven days and made methanol free after evaporation under reduced pressure. The yield was 35%.

Experimental animals

Male albino SD rats of approximately 200 g weight were used for all experiments. Initial weights of all the rats were recorded. They were housed in colony cages at an ambient temperature of 25 ± 2°C with 12/12 h cycle of light/darkness. Animals had free access to water *ad libitum* and were fed on commercial diet bought from Nola Food Corporation, South Africa. Experiment was conducted as per internationally accepted principles for laboratory animal care Unit, Department of Biological Sciences, University of Botswana.

Acute toxicity study

Animals were orally administered different doses of WBP between 50 to 500 mg/kg^{-1} and were observed for 24 h for behavioral change like general activity, sedation, convulsion and mortality. Two doses 50mg and 100mg/kg body weight were selected for the study.

Experimental design

Twenty rats were used for this experiment and were divided into four groups of five each. Group NC was normal control group administered distilled water, Group AC was alcohol control group received alcohol (5 g/kg) every day, Group E1 was experimental group, received the plant extract (50 mg/kg) body weight, Group E2 was another experimental group received the plant extract (100 mg/kg). The experiment was run for 30 days. All the administration was oral and done with the help of syringe and tube.

At the end of the experiment, rats were killed by decapitation after ether anesthesia. Blood was collected from brachial artery, plasma was separated from it and frozen (-70°C). Body weights of rats were taken after every weak.

Statistical analysis

Programme used for data analysis was Sigma Stat (3.1 version). Data was subjected to descriptive statistics after that differences among the groups were analyzed using two way ANOVA followed by Turkey's test for comparisons.

Phytochemical measurements

Determination of the free radical scavenging activity by TLC and DPPH staining

Method described by Yeboa and Majinda (2009) has been followed for this estimation. Diluted samples of WBP and MBP were carefully loaded onto aluminium back TLC sheet (10 ×10 cm) and kept for drying for two hours. Drops of WBP and MBP extracts (0.1 to 10 µl) dissolved in water (100 µg/ml) were loaded using a microlitre syringe along the row. Gallic acid was used as positive control. After drying, the sheet bearing the dry spots, it was sprayed with 0.2% DPPH solution. Stained silica layer reveals a light purple back ground with white spots where the radical scavenging activity is present. The intensity of the white color corresponds with the amount of scavenger present.

Determination of total phenol content

Total phenol content of water extract and methanol extract were determined by the method of Yeboa and Majinda (2009) using Folin- Ciocalteau reagent. 500 mg of the extract is mixed with 5 ml of the solvent (methanol extract was mixed with methanol and water extract was mixed with water). 0.5 ml of Folin-Ciocalteau reagent was added to this mixture. After 3 min, 1 ml of saturated sodium carbonate was added to this reaction mixture and was shaked vigorously for 2 min. The mixture was allowed to stand at room temperature for 2 h. The contents were then centrifuged at 2000 g for 5 min. Absorbance of the supernatant was recorded at 725 nm. A standard curve was prepared using different concentrations of gallic acid. Total phenolic content was expressed as gallic acid equivalents (GAE) from the standard curve using the equation derived by linear regression and expressed as mg GAE / g of extract.

Biochemical measurements

Measurement of the thiobarbituric acid reactive substances (TBARS)

TBARS in plasma was measured by the method described by Tripathi et al.(2001).Lipid peroxidation is measured by the formation of thiobarbituric acid reactive substances (TBARS) such as malondialdehyde (MDA). MDA formed from breakdown of fatty acids, serves as a convenient index for determining the extent of peroxidation reaction. MDA has been identified as the product of lipid peroxidation that reacts with thiobarbituric acid to give red

species absorbing at 535 nm. 0.1 ml of plasma was treated with 2 ml of TCA-TBA-HCl (1:1:1) mixture and incubated in boiling water bath for ten minutes ,then the mixture was cooled and 2 ml of freshly prepared 1 N NaOH was added, the absorbance was measured at 535 nm.

Measurement of the reduced glutathione

Reduced glutathione was measured by the method of Ellman (1959). 0.25 ml of plasma was mixed with 0.5 ml of precipitating buffer (5%TCA in 1 mM EDTA), It was centrifuged and the supernatant was collected and was mixed with 2.5 ml of 0.1 M phosphate buffer (pH 8.0).Colour was developed by adding 100 µl DTNB (0.01%)and the absorbance was taken at 412 nm.

Superoxide dimutase

Superoxide dismutase was assayed by the method of Tripathi et al. (2002). The role of superoxide dismutase (SOD) is to accelerate the dismutation of the superoxide radical ($O_2^{\cdot-}$) produced during oxidative energy processes to hydrogen peroxide and molecular oxygen. The assay of SOD activity is based on the principle of inhibitory effects of SOD on reduction of nitroblue tetrazolium dye by superoxide radicals. A single unit of enzyme was expressed as 50% inhibition of NBT (Nitroblue tetrazolium) reduction/min/mg protein. The reaction mixture contained 150 µl EDTA, 600 µl L-methionine, 300 µl NBT and the volume was made up to 2.8 ml by the addition of SOD buffer. 200 µl of the plasma was added to the reaction mixture, except in the control. Finally 200 µl of riboflavin was added to start the reaction. The test tube was kept under a fluorescent lamp and the reaction kinetics measured for four minutes. The absorbance was read at 560 nm for four minutes.

Catalase

Catalase was estimated by the method of Bisswagner (2004). 0.2 ml of plasma was added to 0.98 ml of H_2O_2 – solution (10 mM). The absorption was read at 240 nm and the catalase activity was calculated using the extinction coefficient of H_2O_2 – (0.071) and the activity was expressed as micromoles of H_2O_2 oxidized per minute per milligram protein.

Vitamin C

The method of Roe (1961) was followed for the estimation of ascorbic acid in plasma. Ascorbic acid in the presence of sulphuric acid forms hydrazone with 2, 4 dinitrophenyl hydrazine (DNPH) which gives absorption maxima at 520 nm. The intensity of the colour formed is directly proportional to the concentration of ascorbic acid present in the sample. To 0.4 ml of plasma, 1.6 ml of 10% TCA was added. The contents were mixed well and allowed to stand for 5 min. They were centrifuged at 2000 rpm for 10 min. Blank and standard were also simultaneously processed with distilled water in the blank tube and the respective standard in the standard tube. 0.4 ml of DNPH reagent was added to 1.0 ml of the supernatant. The stopped tubes were incubated at 37°C for three hours. After incubation period the tubes were kept in ice-bath and then 1.6 ml of cold 65% H_2SO_4 was added. The contents were mixed and the colour formed was read at 520 nm after 30 min.

Measurements of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and total proteins

These parameters were diagnosed by commercial kit bought from Agape Diagnostic, India and manufacturer's guidelines were followed.

RESULTS

WBP and MBP free radical scavenging activity and total phenol content

Results of free radical scavenging activity of WBP and MBP are presented in Figures 1 and 2. Strong intensity of white spot appeared quickly at the dose of 10 µl followed by 6 and 5 µl in both the cases. The results were similar to the scavenging activity of gallic acid that was used as positive control. Total phenol contents of methanol extract and water extract were equivalent to 183.1± 4.1 and 151.6±2.7 mg of gallic acid/g of the dried extract respectively.

Acute toxicity

No mortality and gross change in the behaviour was observed for doses up to 500 mg/kg body weight.

Effects on marker parameters

The activities of SGOT and SGPT were significantly enhanced in AC ($p<0.05$) as compared to NC indicating damage to the liver cell in this group (Table 1). The activities of these enzymes were significantly lowered in EX1 and EX2 ($p<0.05$) as compared to Group AC. The levels of both enzymes in EX2 differed significantly ($p<0.05$) from the levels in NC and this indicates towards the normalization of the liver structure in this group. Levels of TBARS in EX1 and EX2 differed significantly ($p<0.05$) from both AC and NC groups while the levels of total proteins differed significantly from AC (Table 1).

Effects on plasma anti-oxidants

Effects of WBP administration on plasma antioxidants against alcohol induced toxicity are presented in Table 2. The results showed that alcohol administration for 30 days had significantly reduced the plasma glutathione levels in AC as compared to NC and EX2. In EX1, the levels differed significantly from NC ($p<0.05$) but the difference was non significant with AC. The levels in EX2 differed significantly ($p<0.05$) from both AC and NC.

Similarly glutathione reductase activities were also increased in both experimental group as compared to AC ($p<0.05$). Activities of plasma catalase were significantly increased in EX1 and EX2 as compared to AC but it had not reached the normal activities as in NC ($p<0.05$).

Activities of plasma SOD showed slightly different pattern. The differences in the levels of SOD were not

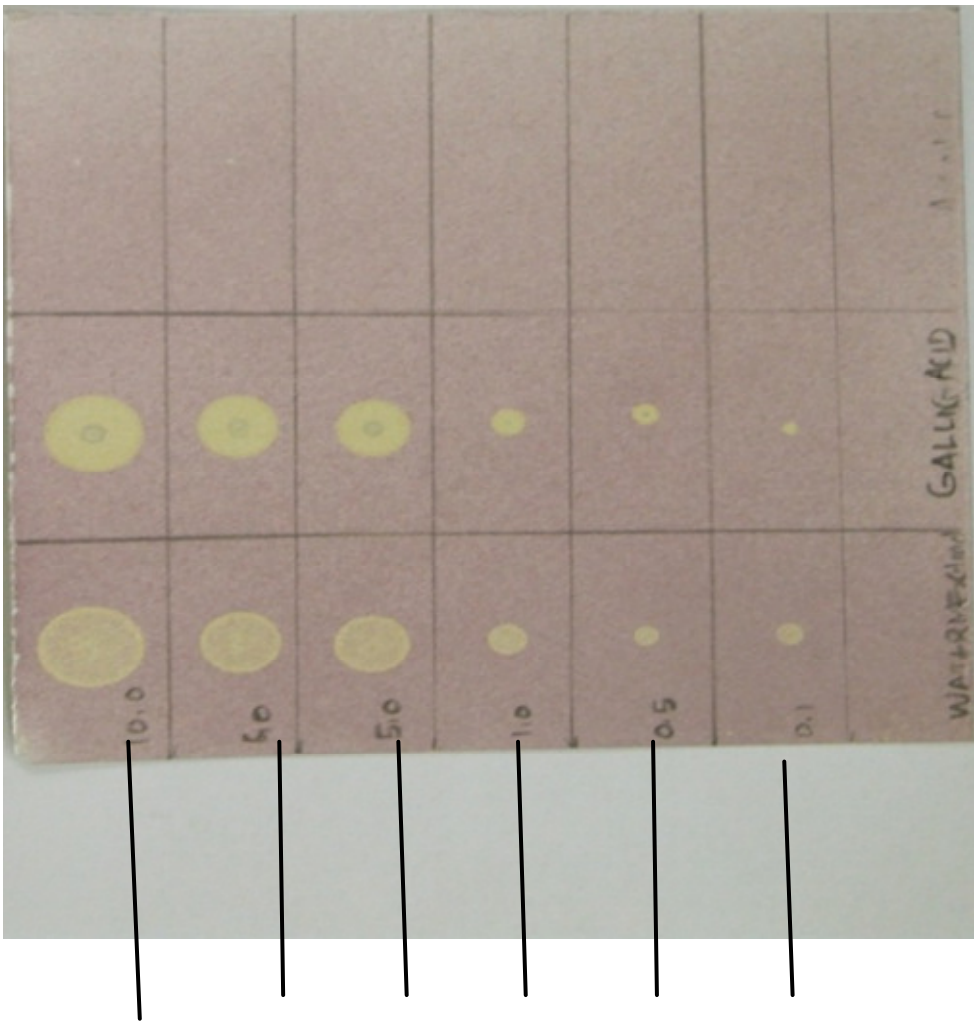

Figure 1. Semi- quantitative TLC -DPPH assay indicating the extent of yellow spot formation of *Bauhinia purpurea* water extract at different amounts (A) 0.1µl, (B)0.5 µl,(C)1.0 µl,(D) 5.0 µl, (E) 6.0 µl, (F)10.0 µl with gallic acid used as standard and the solvent (water) used as blank.

significant in EX1 as compared to AC but the activities in EX2 differed significantly ($p<0.05$) from both NC and AC.

DISCUSSION

In the liver, the ethanol is oxidized to cytotoxic acetaldehyde by alcohol dehydrogenase and then to acetate by aldehyde dehydrogenase or xanthine oxidase (Lieber, 1997). This metabolism is accompanied by generation of free radical like ethyl and hydroxyl ethyl radicals. Ethanol inducible cytochrome CYP2E1 has high rate of NADPH oxidase activity that leads to the production of both super oxide anion and hydrogen peroxide at very high rate (Lieber, 1994). Thus, alcohol induced hepatotoxicity is linked to oxidative stress. Acetaldehyde also reacts with macromolecules of hepatic cells and causes liver damage (Zima et al., 2001).

Thiobarbituric acid reactive substances (TBARS) are one of the diagnostic indices of lipid peroxidation due to oxidative stress. A significant increase in the levels of plasma TBARS in alcohol treated groups (AC) clearly indicates the adverse effect of alcohol metabolism (Table 2). Administration of alcohol together with the WBP extract has significantly reduced the deleterious effects of free radicals generated by alcohol metabolism as indicated by the significantly low levels of these substances in EX1 and EX2. Although the level of

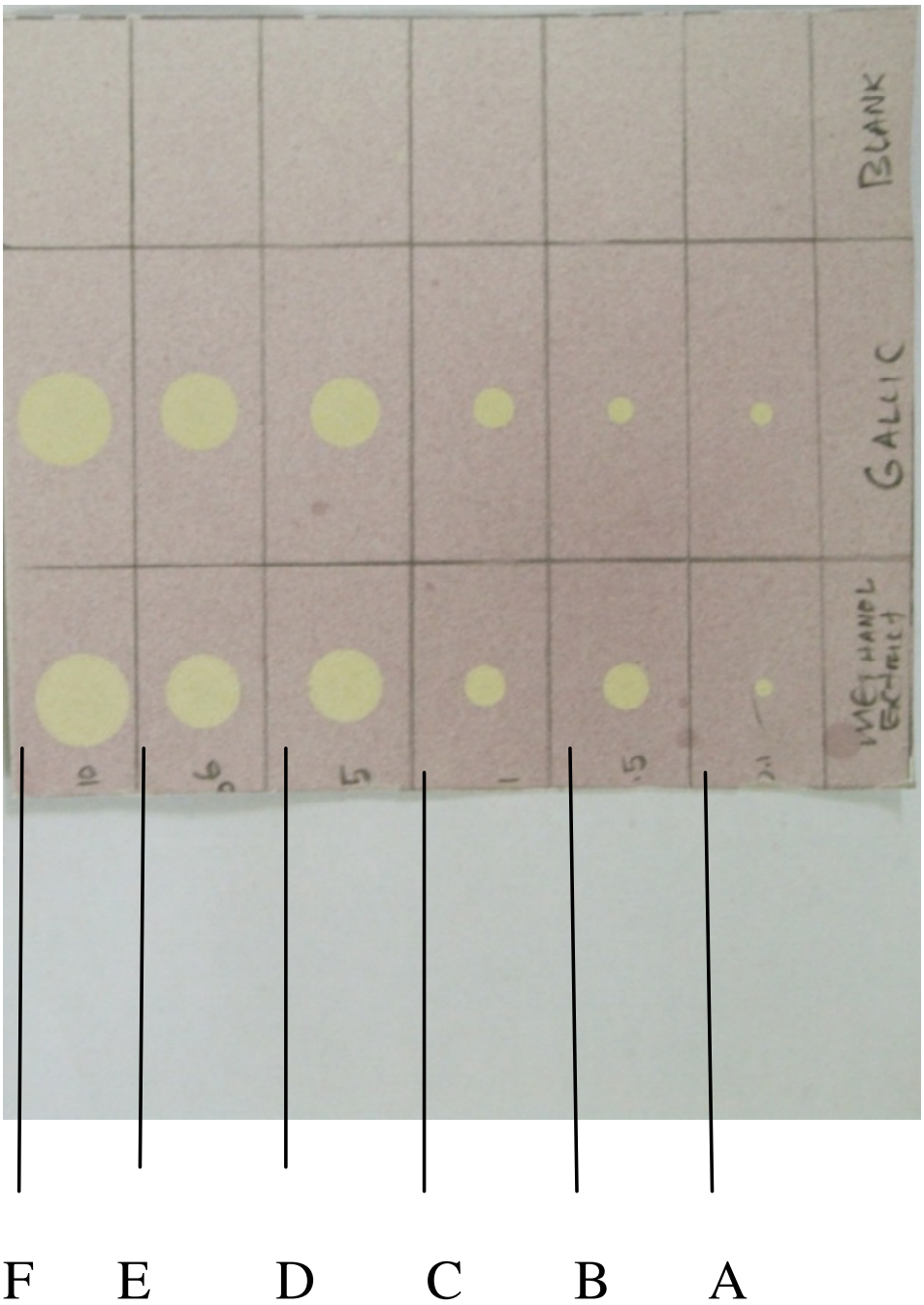

Figure 2. Semi- quantitative TLC -DPPH assay indicating the extent of yellow spot formation of *Bauhinia purpurea* methanol extract at different amounts(A) 0.1µl, (B)0.5 µl,(C)1.0 µl,(D) 5.0 µl, (E) 6.0 µl, (F)10.0 µl with Gallic acid used as standard and the solvent(methanol) used as blank.

TBARS in two experimental groups is lower than AC group, it also differs significantly from NC. This clearly shows that although the rate of lipid peroxidation is reduced but not completely prevented. These findings are further supported by low levels of SGOT and SGPT and high levels of total proteins in experimental groups (Table 2)

Excessive alcohol consumption leads to vitamin deficiency (Gerster, 1995). Thus it appears that low levels of Vitamin C in Group AC is because of continuous alcohol administration that has led to the subsequent reduction in reduced glutathione content of plasma and liver. Levels of Vitamin C and reduced glutathione have

Table 1. Total phenolic content of the extracts of *Bauhinia purpurea*.

Extract	TPC (mg GAE/g)
Water	151.6±2.7
70% methanol/H_2O	183.1±4.1

Values are the average of three trials ± standard deviation.

Table 2. Effects of *Bauhinia purpurea* water extract (WBP) on marker parameters of alcohol induced toxicity in albino rats.

Groups	TBARS (nmol/dl)	SGOT (U/L)	SGPT (U/L)	Total proteins (mg/dl)
AC	328.8±2.61	58.41±2.39	78.8±1.28	3.76±0.12
NC	64.29±3.7	25.69±0.91	45.3±1.87	6.85±0.21
EX1	228.6±7.64^{ab}	37.13±3.63^{ab}	51.49±1.89^{a}	4.98±0.11^{a}
EX2	126.8±5.12^{ab}	35.37±2.71^{a}	42.49±1.28^{a}	5.93±0.17^{a}

AC = Alcohol control group administered alcohol (5 g/kg body weight) every day; NC- Normal control group received distilled water every day; EX1 and EX2 are two experimental groups that received 50 mg and 100 mg/kg body weight respectively for 30 days. Values are expressed as mean ± SEM, n=5 in each group. a = $p<0.05$ when compared with AC; b = $p<0.05$ when compared with NC.

Table 3. Effects of Bauhinia purpurea water extract (WBP) on plasma anti-oxidants against alcohol induced toxicity in albino rats.

Groups	Plasma GSH(mg/dl)	Plasma VIT C(mg/dl)	Plasma GPX (mg/dl)	Plasma CAT(U/L)	Plasma SOD (U/L)
AC	32.36±3.61	1.6±0.09	3.47±0.61	41.66±1.63	16.89±1.52
NC	88.66±4.05	2.88±0.13	14.23±1.6	85.13±3.78	35.17±1.19
EX1	42.9±3.26^{b}	2.19±0.13^{a}	11.91±1.16^{a}	50.73±1.05$^{a\ b}$	25.47±0.96^{b}
EX2	62.04±2.63$^{a\ b}$	2.94±0.16^{a}	15.88±1.77^{a}	66.36±1.79$^{a\ b}$	28.27±1.32$^{a\ b}$

AC = Alcohol control group administered alcohol (5 g/kg body weight) every day; NC- Normal control group received distilled water every day; EX1 and EX2 are two experimental groups that received 50 mg and 100 mg/kg body weight respectively for 30 days. Values are expressed as Mean ± SEM, n = 5 in each group. a = $p<0.05$ when compared with AC; b = $p<0.05$ when compared with NC.

significantly elevated in both experimental groups as compared to the AC group (Table 3). Reduced rate of lipid peroxidation could be related to the elevated levels of Vitamin C and reduced glutathione. Vitamin C has been shown to scavenge free radicals very efficiently before they reach the cellular membrane. It also reduces the oxidized glutathione to reduced form and thus makes availability of reduced glutathione to the antioxidant system for further scavenging action (Miglani et al., 2003; Kaminski and Boal, 1992). Reduced glutathione in its turn also regenerates Vitamin C from dehydro-ascorbic acid (Noctor and Foyer, 1998). In the present study, supplementation of alcohol treatment with WBP inhibited the lipid peroxidation (Table 2), maintains the levels of these two water soluble antioxidants and thus strengthens the first line of antioxidants to fight the generated free radicals as a result of metabolism of alcohol.

The enzymatic and non-enzymatic antioxidant systems are intimately linked to each other in scavenging free radicals from the system. SOD is an intracellular produced enzyme and is present in almost every cell of the body. It eliminates reactive oxygen species by reducing superoxide form to hydrogen peroxide (Culotta, 2000). Catalase is present in all body organs being especially concentrated in the liver and erythrocytes. Glutathione peroxidise is present in most aerobic cells in animal tissues. Both the enzymes are responsible for reducing H_2O_2 to H_2O (Husain and Somani, 1997). The glutathione redox cycle is a central mechanism for reduction of hydro-peroxides to water by oxidizing reduced glutathione to oxidized one which in turn is reduced to reduced glutathione by glutathione reductase. An increase in the production of SOD without a subsequent elevation of catalase or glutathione peroxidase leads to the accumulation of hydrogen peroxide, which leads to the accumulation of hydrogen peroxide and ultimately to the hydroxyl radical. In the present study, activities of catalase and glutathione peroxidise are significantly increased in both the experimental groups (Ex12 and EX2) as compared to Group AC (Table 3). The activities of SOD are enhanced significantly only in Group EX2. In EX1, the activities of SOD do not differ significantly (Table 3) from Group AC.

This indicates that, WBP maintains an intricate balance between these enzymes to avoid over accumulation of H_2O_2 in the system.

The protective effects of WBP could be ascribed to the fact that it is rich in antioxidants, particularly phenols. Results of *in vitro* experiments have demonstrated the significant anti-oxidative properties of WBP. In dot blot and DPPH staining experiment, antioxidant properties are equivalent to anti oxidant capacity of gallic acid. This result is consistent with previous findings of Rajani and Purnima (2009) for *B. variegata*.

Thus it appears that protective effects of WBP are dose dependent. The effects are more significant at higher dose (100 mg/kg) against alcohol induced toxicity. WBP scavenges free radicals and thus boosts the antioxidant capacity of organism.

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