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Protective efficacy of Aloe-Amla wine against oxidative stress

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Abstract
The objective of the study was to evaluate the protective efficacy of laboratory prepared Aloe-amla wine against oxidative stress caused by the infection in a murine model. Aloe vera gel and hot water extract of amla, ameliorated with sugar made it a good medium for the growth of *Saccharomyces cerevisiae* which fermented the sugars into ethanol to make wine with 10% v/v alcohol. The wine was found similar to any other wine in terms of its composition. The following parameters were analyzed to evaluate the protective efficacy, comparing control, wine fed, infected and infected-wine fed mice: lipid peroxidation, superoxide dismutase and reduced glutathione levels. The data indicated that wine ingestion resulted in decreased hepatic malondialdehyde levels, increased levels of superoxide dismutase and reduced glutathione emphasizing the protective efficacy of prepared functional beverage.

Keywords: Aloe - Amla wine, *Salmonella* Typhimurium, Lipid peroxidation, Superoxide dismutase, Reduced glutathione.

1. Introduction
Production and nutritive value of different fruit wines have been well reported. [1-5] Wine has been considered as safe and healthy drink, besides an important adjunct to the diet.[6] The recent years have observed several reports on the consumption of wine in moderation and positive effect on the cardiovascular system as well as the general well-being of the consumers.[7] In wines, alcohol is a macro nutrient which is an energy source, capable of providing calories for all essential biological activities of the human cells.[8] Wine consists of water, alcohol, acids, esters, carbohydrates, minerals, vitamins, pigments and tannins with perceived therapeutic value.[9] Fruit wines are produced and consumed in large quantities in all advanced countries in the world. A few industries in our country produce wines but fruit wine production at this time is insignificant in spite of tremendous increase in the fruit production.[6] Herbalism is a traditional folk medicine practice based on the use of plants and plant extracts. Herbs are staging a comeback and herbal 'resurgence' is ensuing all over the world. The herbal products today represent safety as these are compatible with human normal physiology. Natural products, obtained from dietary sources provide a huge number of antioxidants.[10] Bioactive constituents commonly found in herbs, and other plants have been shown to have possible health benefits with anti-oxidative, anti-carcinogenic, anti-hypertensive, anti-mutagenic properties.[11-15] Wine being one of the rich sources of antioxidants can be considered as an effective nutraceutical.[16] Many wines are also made from herbs with perceived medicinal value and such wines have additional health benefits. In addition, anti-oxidants of herbal origins have been proved to be beneficial in reversing the hepatotoxicity and oxidative stress [17, 18]. According to World Health Organization [19], the medicinal plants would be the best source to obtain a variety of drugs. The use of plant extracts, with recognized antimicrobial properties, can be of great importance in the treatment of various microbial infections [20]. After observing the trend of increasing interest towards herbal beverages that can deliver health benefits an attempt was made to prepare a new variety of Aloe vera wine by blending it with hot water extract of amla known for its therapeutic potential. The laboratory prepared Aloe-amla wine was found antibacterial against common food-borne pathogens. Further we aimed to investigate the protective efficacy of Aloe-amla wine that was developed in our laboratory.

2. Material and methods

2.1 Wine Sample

Aloe-amla wine used in the present work came from our laboratory (Dept. of Microbiology, Panjab University, Chandigarh). Quantitative analyses were done according to standard methods [21-25]. The composition of wine is shown in Table 1.

Constituents	*Aloe*-amla wine
Colour	Yellowish orange
TSS (°B)	4.6±0.2
Total acids (g/100mL)	0.65±0.02
pH	3.7±0.01
Total sugar (g/100 mL)	0.61±0.08
Ethanol % (v/v)	10.0±1.12
Phenolics (mgGAE/L)	1797.05±32.88
Antioxidant activity (µmol/L)	4550.0±14.84

Values are expressed as mean±SD of the observations (in triplicate) from three independent experiments.

2.2 Animals

Male Balb/c mice (25 to 30 g body weight, 8-10 weeks of age) were procured from the Central Animal House of Panjab University, Chandigarh, India. The animals were kept in plastic cages under hygienic conditions in the animal room of the department with a 12/12 h dark-light cycle. The mice were fed *ad libitum* with standard pellet diet (Hindustan Lever Products, Kolkata, India) and water. Necessary approvals (Reg. No. 45/1999/CPCSEA) for animal studies were obtained from the Institutional Ethics Committee, Panjab University, Chandigarh, India. The treatment protocol was for 10 days.

2.3 Treatment protocol for oxidative stress induced by *Salmonella* infection

Twenty four male Balb/c mice were randomly divided into the following four groups of six mice in each group: group-1 control, group-2 wine fed, group-3 *Salmonella* infected, group-4 infected and wine treated. An aliquot of 0.3mL (per 25 g body weight of animal) of Aloe-amla wine was given to group-2 animals, once in a day for ten consecutive days. Mice were challenged with a single oral dose of 0.2 mL of *S.* Typhimurium suspension with a viable count of 2.5×10^7cfu/mL in group-3.[26] In group-4 each mouse was given a single challenge dose of 0.2 mL of *S.* Typhimurium (2.5×10^7cfu/mL) and then orally fed with the standardized dose of 0.3 mL of wine, once in a day for ten consecutive days. Control group animals received only 0.9% saline.

At the end of experimental period, mice were fasted overnight (12 h) before they were euthanized by cervical dislocation under mild anesthetic ether exposure and livers were rapidly harvested. To obtain post-mitochondrial supernatant (PMS) 10% homogenate was prepared in an ice cold 20 mM Tris-HCl buffer (pH 7.4) and centrifuged at 10000g for 30 min at 4^OC. Further assays were carried out in fresh PMF.

2.4 Extent of Lipid peroxidation

Lipid peroxidation was determined by the method described by Wills, [27] in which the development of a pink colour due to a TBA-MDA (thiobarbituric acid-malondialdehyde) chromophore was taken as a index of lipid peroxidation.

2.5 Evaluation of hepatic superoxide dismutase (SOD) activity

SOD activity was assayed according to the method of Kono, [28] and was expressed as units of SOD per milligram of protein where one unit of activity was defined as the amount of SOD required to inhibit the rate of reduction of NBT by 50%.

2.6 Estimation of hepatic reduced glutathione (GSH) levels

Reduced glutathione (GSH) levels in the liver homogenates were estimated according to the method of Jollow *et. al.*[29] The results were expressed as micromoles of GSH per milligram of protein, using the molar extinction coefficient of 5'-thiobis 2-nitrobenzoic acid (13600 M^{-1} cm^{-1}).

2.7 Statistical Analysis

Statistical analysis was done by Student's unpaired t test and one way analysis of variance (ANOVA) followed by pair wise comparison procedures (Tukey test) using Sigma Stat Statistical Software, version 11.0. In all cases, statistical significance was defined as p value of atleast <0.05.

3. Results and Discussion

3.1 Effect of Aloe-amla wine on oxidative stress induced by *Salmonella* infection

The extent of tissue destruction in experimental mice was assessed on the basis of MDA estimation. Malondialdehyde (MDA) is an endogenous genotoxic product of enzymatic and oxygen radical-induced lipid peroxidation which is a reactive oxygen species (ROS), and as such is also assayed *in vivo* as a bio-marker of oxidative stress.[30] Following infection, maximum damage was observed in challenged group, as MDA levels were the highest in this group (526.04±36.87 nmoles/mg protein), significantly higher than that in control group (258.57±41.22 nmoles/mg protein). MDA levels were statistically similar in the control and wine fed groups which exhibited 258.57±41.22 and 250.67±23.27 nmoles/mg protein respectively. However, a significant decrease was observed in challenged groups fed with wine (231.38±14.00 nmoles/mg protein) as compared to the level in the infected group (526.04±36.87 nmoles/mg protein) (Fig. 1).

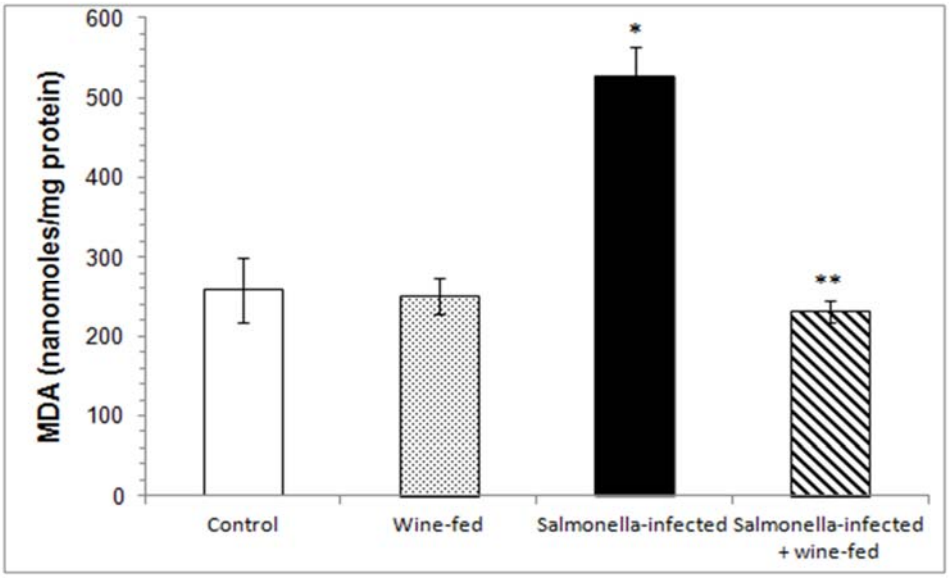

Fig. 1 Effect of Aloe vera - Amla wine on lipid peroxides levels.

MDA levels, an index of lipid peroxidation were measured in liver homogenates of different experimental groups. (Values are expressed as mean ± S.D. of five individual observations).*p<0.01 vs. control and wine-fed groups; **p<0.001 vs. *Salmonella*-infected group.

However, for comparison, *in vivo* studies in relation to Aloe-amla wine feeding are not available but reports on feeding of red wine and other herbal products are available. This approach is supported by the observation made by Roig *et al*.[31] who had reported that wine ingestion results in lower hepatic malondialdehyde in wine fed rats. Studies have shown that the fruit extract of amla reduced the level of lipid peroxidation in the rat brain [32]. Multiple studies have shown that amla possesses inhibitory effects on lipid peroxidation induced by various inducers including ethanol [33]. *In-vitro* studies have shown that amla prevents radiation induced lipid peroxidation and this effect also extends to animal studies. [34, 35, 36] Aloe vera is known for its antioxidant potential. It is likely that the bioactive components of both amla and Aloe vera present in the resulting herbal wine may have contributed in protecting the biomolecules from *Salmonella* induced oxidative damage.

The administration of Aloe-amla wine strongly ameliorated the capacity of liver to metabolize ROS, as shown by increased SOD and GSH levels. Superoxide dismutases are enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. Thus, they are an important antioxidant defense in nearly all cells exposed to oxygen. *S.* Typhimurium challenge reduced the liver SOD activity to 2.62±0.28 U/mg protein as compared to that in the control group which revealed SOD levels of 5.14±0.22 U/mg protein (Fig. 2). The levels were statistically similar in the control (5.14±0.22 IU/mg protein) and wine fed mice (5.97±0.24 U/mg protein). However, there was an increase in the SOD levels to 4.35±0.13 U/mg protein in the mice group fed with wine following *S.* Typhimurium challenge as compared to infected group (Fig. 2).

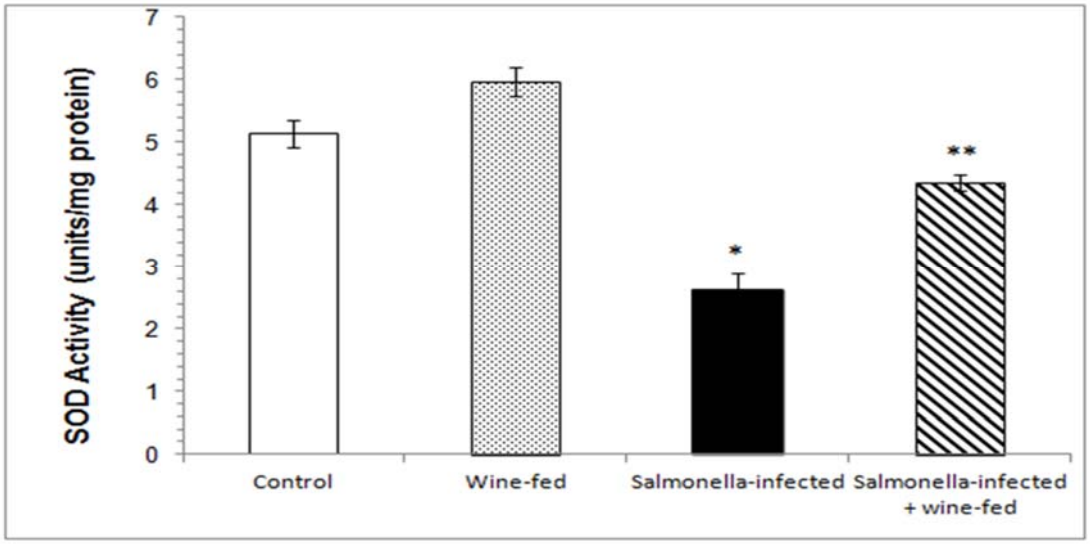

Fig. 2. Effect of Aloe-amla wine on the activity of SOD.

The activity of SOD was monitored in liver homogenates of different experimental groups. (Values are expressed as mean ± S.D. of five individual observations). *p<0.001 vs. control and wine-fed groups; **p<0.001 vs. *Salmonella*-infected group.

GSH, the most important cellular antioxidant, functions either by protecting cells from lipid peroxidation or by protecting the protein sulphydryl group from being oxidized by these radicals. [37] In the present study, challenge with *S.* Typhimurium caused a significant decrease in hepatic GSH levels to 0.24±0.04 µmoles/mg protein against 0.45±0.03 µmoles/mg protein in the control group (Fig. 3). The levels of GSH were statistically similar in the control and wine fed mice groups which revealed the levels of 0.45±0.03 µmoles and 0.50±0.03 µmoles/mg protein respectively. However, oral consumption of wine after infection increased the levels of GSH to 0.42±0.05 µmoles/mg protein in comparison to the infected group not fed with wine (Fig. 3).

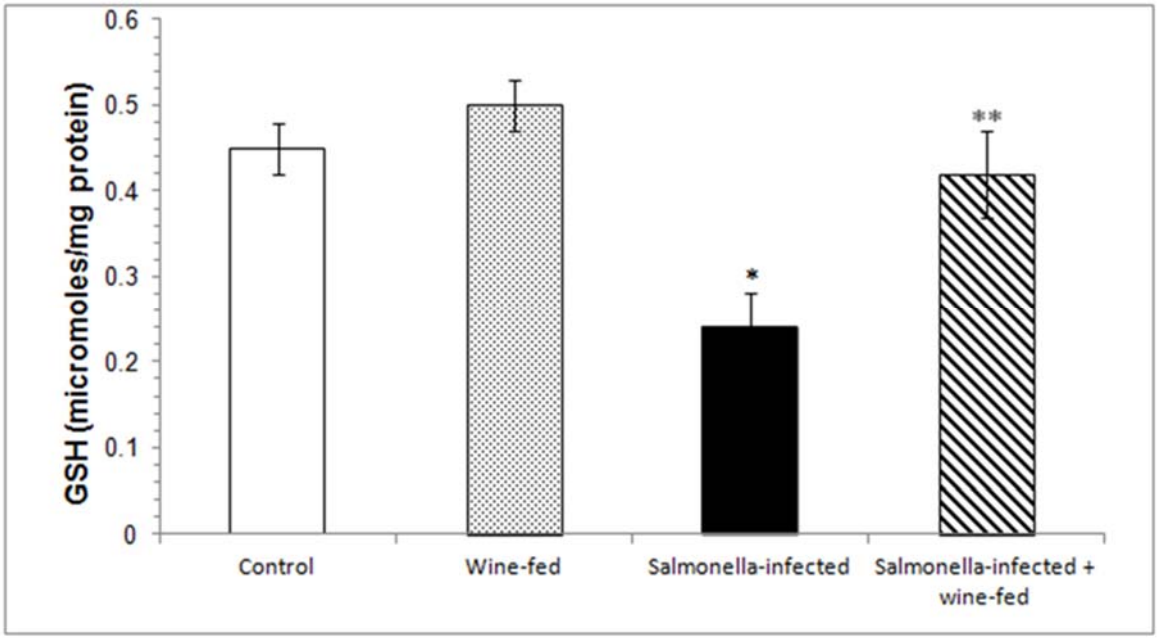

Fig. 3. Effect of Aloe vera - Amla wine on GSH content.

The content of GSH was monitored in liver homogenates of different experimental groups. (Values are expressed as mean ± S.D. of five individual observations). $^{*}p<0.001$ vs. control and wine-fed groups. $^{**}p<0.05$ vs. *Salmonella*-infected group.

These results corroborate the earlier report of Roig et al. [31] who revealed enhanced hepatic superoxide dismutase and glutathione peroxidase activities after 45 days of wine treatment. The up regulation of these enzyme activities is due to activation of transcription of glutathione-S-transferase and the NAD (P)H:quinone reductase genes through an antioxidant responsive element [38] probably via specific phenolic receptors by phenolic compounds. In addition, there are reports that suggest that the fruit extract of amla increases the cortical and striatal concentration of the anti-oxidant enzymes SOD, Catalase, GPx significantly in the rat brain [32] and prevents ethanol induced toxic effects.[39] It has also been reported that *Aloe vera* contains antioxidant enzymes such as SOD which are involved in scavenging reduced oxygen species [40] Studies have shown *Aloe vera* may stimulate the body's antioxidant defenses and increase the bioavailability of antioxidant supplements. Animal studies have also provided supporting evidence for the antioxidant effects of *Aloe vera* where the researchers noted the enhanced superoxide dismutase and catalase activity in the aloe groups compared to control animals [41].

4. Conclusion

The present data provide evidence that Aloe-amla wine has a clear protective effect as shown by the diminished lipid peroxidation and enhanced levels of SOD and GSH in wine administered infected experimental groups. Thus, Aloe-amla wine may be included as a value added product in our daily diet to overcome bacterial infections and the oxidative stresses. Nonetheless, further research is needed to determine the possible mechanisms involved in these phenomenon.

5. Acknowledgements

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