IJFANS
International Journal of Food And Nutritional Sciences

Volume 4, Issue 1, Jan-Mar 2015, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

IJFANS International Journal of Food And Nutritional Sciences

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

e-ISSN 2320 –7876 www.ijfans.com
Vol.4, Iss.1, Jan-Mar 2015
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Research Paper *Open Access*

PROTECTIVE ROLE OF *Aloe Vera* WINE AGAINST OXIDATIVE STRESS INDUCED BY *salmonella* INFECTION IN A MURINE MODEL

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Received on: 19th January, 2015 **Accepted on: 17th March, 2015**

ABSTRACT

In this study, we have used *Aloe vera* gel supplemented with cane sugar as a substrate for the production of functional fermented *Aloe vera* based herbal wine. We evaluated the protective role of *Aloe vera* wine against *Salmonella* induced oxidative stress in a murine model. Oral administration of *Aloe vera* wine exhibited antibacterial activity against *S*. Typhimurium leading to its significant clearance from liver, spleen and intestine of the infected group. Restoration of liver-function enzyme alanine aminotranferase (ALT), aspartate aminotransferase (AST) levels and improved hepatic histoarchitecture in the infected wine–fed group indicated the protective efficacy of *Aloe vera* wine. Furthermore, the *Aloe vera* wine administration reduced hepatic lipid peroxidation, increased the superoxide dismutase activity and reduced glutathione levels indicating improved resistance to oxidative stress. In parallel, infected mice administered with *Aloe vera* wine exhibited a 41.1% increase in the ferric reducing antioxidant power (FRAP) plasma levels compared to infected counterpart which is suggestive of an improved antioxidant status in the wine fed mice. Morphological changes in the cells of *S*. Typhimurium in the presence of *Aloe vera* wine was observed by scanning electron microscopy (SEM) which provided qualitative information of the change in its morphology when treated with *Aloe vera* wine. These observations suggest that *Aloe vera* wine has the potential to modulate the changes induced by *Salmonella* infection and exerts protective effect against generated oxidative stress; however, further investigations are required to explore its complete mechanism of action.

Keywords: *Aloe vera* wine, Oxidative stress, Alanine aminotransferase, Lipid peroxidation, Superoxide dismutase, reduced glutathione.

INTRODUCTION

Beverages with bioactive compounds are consumed for their food value, thirst quenching ability and the health benefit they confer to consumers (Awe et. al., 2013). Wine, one of the examples of functional fermented beverage, is a complex mixture of hundreds of compounds, many of which contribute significantly to the colour, mouth feel or aromatic properties of this beverage (Sumby et. al., 2010). An essential biological effect of wine is its potent antimicrobial activity which has been verified under various experimental conditions (Liu et. al., 2006; Fernandes et. al., 2007; Boban et. al., 2010). Numerous significant health benefits related with modest consumption of wine have been credited to the phenolic content of wine (Villiers et. al., 2012). Until early in this century, wine was universally used as a base for medicinal preparations compounded with a range of herbs tailored to definite ailments (George et. al., 1997). Many wines are made from herbs with perceived medicinal value and such wines have many additional benefits. In this context *Aloe vera*, a well known herbal plant has the potential to be the focal point for the yield of a functional beverage since it is known to offer protection from oxidative stress (El-Shemy et. al., 2010). Pharmacologically active ingredients of *Aloe vera* plant are concentrated in both the gel and the rind of leaves as revealed by clinical evaluations (Heggers et. al., 1996). *Aloe vera* juice available in the market is used to treat liver and spleen infection as well as to enhance immune response against various diseases (Neall, 2004).

Salmonella is responsible for a variety of infections which may range from gastroenteritis caused by *Salmonella typhimurium* to severe systemic disease caused by *Salmonella typhi* (Monack et. al., 2004). *Salmonella* induced infection, results into lipid peroxidation and finally to tissue damage due to excessive production of ROS (Rishi et. al., 2006). Enzymatic and non-enzymatic systems of the body preserve the oxidant/antioxidant status, but they are overwhelmed during oxidative stress. Therefore, it is expected that the naturally occurring nutritional sources of antioxidants, such as fruits, vegetables, tea or wine, would attenuate the damage caused by oxidative challenges. *Aloe vera* is known for its

curative and therapeutic properties, thus it is used as a source of functional food (Habeeb et. al., 2007). Accordingly, the polyphenolic compounds of wine, implicate in enhancing the antioxidant system, since they behave as ROS scavengers, metal chelators and enzyme modulators (McDonald et. al., 1998; Pietta et. al., 1998). On the basis of this information, the present study was designed to use *Aloe vera* gel as a substrate for the production of functional fermented *Aloe vera* based herbal wine and evaluation of the protective role of *Aloe vera* wine against *Salmonella* induced oxidative stress in a murine model.

MATERIAL AND METHODS

MICROORGANISMS

Saccharomyces cerevisiae (MTCC 786) procured from Institute of Microbial Technology (IMTECH), Chandigarh, India was used to carry out the fermentation. *Salmonella enteric*a serovar Typhimurium (Virulent strain NCTC 74) (*S.* Typhimurium) was procured from the Central Research Institute (CRI), Kasauli (India).

WINE SAMPLE

The *Aloe vera* wine used in the present work was prepared as per the method reported by Trivedi el. al., (2012). Briefly, *Aloe vera* gel was extracted from the leaves which were then blended in a mixer after supplementing with cane sugar to adjust the TSS to 20°B, pH 4.5. This was then subjected to batch fermentation by inoculating 1 L of medium, dispensed into a 5 L Erlenmeyer flask with 10% (v/v) inoculum made with an overnight grown culture of *Saccharomyces cerevisiae* having a viable count of 1×10^8 cells/mL, supplemented with 100 ppm sodium metabisulphite and incubated in a stationary state at 28 ± 2°C. The contents of the flask were mixed 2-3 times a day and the progress of fermentation was noted at regular intervals of 24 h by analyzing total soluble solids (TSS). After completion of fermentation, the wine was clarified by repeated siphoning, which was carried out 3-4 times with a sedimentation period of 3 days between each siphoning. Ageing of *Aloe vera* wine was done in an oak wood barrel (2.5 L), procured from M/S Jagatjit Industries Limited, Hamira, Punjab (India). The containers were filled to the brim and analyzed for various components after one year of ageing.

PHYSICO-CHEMICAL ANALYSIS OF WINE

ESTIMATION OF TOTAL SOLUBLE SOLIDS (^{O}BRIX)

Brix (^{o}B) reading of the wine samples was determined using ERMA hand refractometer having a range of 0-32°Brix.

ESTIMATION OF pH

pH of the samples were recorded by using the pH meter (ME-962-P), Max Electronics, India. Standard buffer solutions of pH 4.0, 7.0 and 9.0 were used as reference to calibrate.

ESTIMATION OF TOTAL SUGARS

The sugar content was detected by the method of Fehling, (1849). Glucose was used as a standard and titration was done as per the Fehling's standard method to know the amount of glucose required to completely reduce 10 mL of Fehling solution.

ESTIMATION OF ETHANOL

The ethanol was estimated by colorimetric method as described by Caputi *et. al.,* (1968). 1 mL of representative samples of wine was transferred to 250 mL round bottom distillation flask connected to the condenser and was diluted with 30 mL distilled water. The sample was distilled at 80-90°C. The distillate was collected in 25 mL of $K_2Cr_2O_7$ reagent, which was kept at receiving end. The distillate containing alcohol was collected till total volume of 45 mL was obtained. Similarly standards (1-10% ethanol) were mixed with 25 mL of $K_2Cr_2O_7$ separately. The distillate of samples and standards were heated in water bath at 70°C for 20 min and cooled. The volume was made up to 50 mL with distilled water and the optical density was measured at 600 nm using UV 1900 spectrophotometer.

ESTIMATION OF TITRATABLE ACIDITY

It was expressed as percent acidity and analyzed using method of Amerine *et al.,* (1980). Titratable acidity was determined by titrating known amount of wine sample (10 mL) against 0.2 N NaOH using a few drops of 1% phenolphthalein solution as indicator. The end point was appearance of pink/purple colour which should persist for 15-20 sec.

ESTIMATION OF TOTAL PHENOLIC CONTENT (TPC)

Total phenolic content (TPC) was determined using the Folin-Ciocalteu reaction method by Waterhouse, (2002) with gallic acid as the standard. To 1.0 mL of appropriately diluted wine sample, 1.0 mL of Folin - Ciocalteau reagent was added and the content was mixed thoroughly. After 3 min, 3 mL of 2% Na_2CO_3 was added then the mixture was allowed to stand for 2h with intermittent shaking. The absorbance was measured at 765 nm using spectrophotometer and expressed as mg gallic acid equivalents per litre of sample (mg GAE/L).

EVALUATION OF ANTIOXIDANT ACTIVITY

The ferric reducing antioxidant potential (FRAP) assay was conducted according to Benzie and Strain, (1996). The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2$. $3H_2O$ and 16 mL $C_2H_4O_2$) pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution. Optical density of the colored product (ferrous tripyridyltriazine complex) was then taken at 593 nm. Results were compared with a standard curve prepared with different concentrations of $FeSO_4.7H_2O$ (0.01-1µmol). Ferric reducing power of the sample was expressed in µmol Fe^{2+}/L.

IN VIVO STUDY

ANIMALS

Male Balb/c mice (20 to 25 g body weight, 4 - 6 weeks of age) were procured from the Central Animal House of Panjab University, Chandigarh, India. The animals were maintained in a controlled environment under standard condition of temperature and humidity with alternating light dark cycle (12 h light/12 h dark 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.

EXPERIMENTAL GROUPS AND PROTOCOL

To investigate the protective role of wine administration through oral route mice were divided into following four groups, each comprising of 8 mice.

1. **Control group:** In this group mice were administered normal saline orally for ten consecutive days.
2. **Wine *per se* group:** Each mouse in this group was orally fed with the standardized dose of 0.3 mL of wine, once in a day for ten consecutive days.
3. ***Salmonella*-Infected group:** 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 (Rishi et. al., 2007).
4. **Infected-wine fed group:** Each mouse in the group 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.

At the end of experimental period, blood samples were collected from mice's retro-orbital plexus before being sacrificed by cervical dislocation. Livers were removed, rinsed in cold phosphate buffered saline (PBS) (0.05 M, pH 7.4) and stored at -62°C till further use.

BACTERIAL TRANSLOCATION IN THE LIVER, SPLEEN AND INTESTINE

Liver, spleen and intestine of *Salmonella*-infected group (group 3) and infected-wine fed group (group 4) were taken out aseptically, rinsed in 0.05 M PBS (pH 7.4) and weighed. A 10% percent (w/v) tissue homogenate was prepared in 0.05 M phosphate buffer (pH 7.4) using a potter Elvehjem homogenizer. Serially diluted homogenates (0.1 mL) were plated separately on bismuth sulphite agar (BSA). Colonies were counted after incubation at 37°C for 24 h.

ASSESSMENT OF LIVER FUNCTION

The serum obtained was used for the estimation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities using ERBA test kits (ERBA Diagnostics, Mannheim, Germany).

LIVER HISTOLOGY

Liver tissues removed aseptically from the animals were cut into small pieces and fixed in 10% buffered formalin. Samples were processed, stained with hematoxylin-eosin and examined under the light microscope. Histological interpretation was done by Dr. B. N. Datta, Ex-Professor of Pathology, Post Graduate Institute of Medical Education and Research, Chandigarh (India).

MECHANISTIC STUDIES

Livers removed aseptically from all the above mentioned animal groups were rinsed in 0.05 M PBS, pH 7.4 and weighed. For every animal, a 10% (w/v) tissue homogenate was prepared in PBS (0.05 M, pH 7.4) using a Potter-Elvehjem homogenizer. An aliquot of the liver homogenate was used for the estimation of lipid peroxidation. For the estimation of superoxide dismutase and reduced glutathione levels, post mitochondrial preparation was prepared. For this, the liver homogenates were centrifuged at 10000 rpm for 20 min at 4°C. The supernatants thus obtained were called post mitochondrial supernatants (PMS).

ASSESSMENT OF OXIDATIVE STRESS

EXTENT OF PEROXIDATIVE LIVER DAMAGE

The quantitative measurement of lipid peroxidation in liver was performed according to the method of Wills, (1966). The results were expressed as nanomoles of malondialdehyde (MDA) per milligram of protein, using the molar extinction coefficient of chromophore (1.56×10^5 M^{-1} cm^{-1}). The protein content of tissue homogenates was calculated according to the method of Lowry et. al., (1951).

EVALUATION OF HEPATIC SUPEROXIDE DISMUTASE (SOD) ACTIVITY

SOD activity was assayed according to the method of Kono, (1978) 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%.

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.,* (1974). 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}).

EVALUATION OF ANTIOXIDANT ACTIVITY IN BLOOD PLASMA

The effect of *Aloe vera* wine administration on FRAP levels in plasma was characterized by taking100 µL of plasma as a sample from all the four experimental groups. FRAP assay was conducted according to Benzie and Strain, (1996).

MORPHOLOGICAL CHANGES IN THE CELLS OF *S*. TYPHIMURIUM IN THE PRESENCE OF *Aloe vera* WINE

The ultrastructural changes if any, induced by the treatment with *Aloe vera* wine were examined by scanning electron microscopy (SEM) according to the method of Singh *et. al.,* (2013) with modifications.

STATISTICAL ANALYSIS

The data were expressed as mean ± standard deviation (SD). The bacterial count was expressed as $\log_{10}$. Statistical analysis was done by Student's unpaired *t* test and one-way analysis of variance (ANOVA), followed by pairwise comparison procedures (Tukey test), using Jandel Sigma Stat statistical software, version 2.0. In all data analysis, p-values of 0.05 or less ($p< 0.05$) were considered significant.

RESULTS AND DISCUSSION

Nutritional studies on examining the foods for their protective and disease preventing potential have established that apart from red wine, other fruit wines and herbal wines are equal or sometimes better sources of flavonoids and phenolics (Liu *et. al.,* 2006; Dey *et. al.,* 2009; Sumby et. al., 2010; Awe *et. al.,* 2013; Negi *et. al.,* 2013). These facts have given a new dimension to the non grape wines or herbal wines. Wine being an important source of dietary antioxidants and *Aloe vera* juice known for its immuno-stimulating properties constitute the highly acceptable classes of beverages throughout the world (Vahedi et. al., 2011; Kumar et. al., 2012). Therefore, the above knowledge formed the basis of our study to use *Aloe vera* gel as a substrate for the production of herbal wine and to explore its potential in combating oxidative stress against induced Salmonellosis.

PHYSICO-CHEMICAL PROPERTIES OF *Aloe vera* WINE

Sugar is known as the most important raw material during fermentation process, which dictates the final concentration of ethanol in wine. In the present study, the ethanol content reached 10.04 ± 0.03 % (v/v). The content of sugar (0.18 ± 0.03 g/100 mL) indicated that the final product of *Aloe vera* was of semi-dry type with a pH of 3.7 ± 0.02 because as per Conde et. al., (2007) technically dry wines have sugar concentration of <400 mg/100 mL. TSS of the medium reduced from 20°B to 4.1^{o}B. Acids are essentially accountable for the physicochemical and microbial stability of wines and affect the taste and mouth-feel of wine (Waterhouse and Ebeler, 1998). The titratable acidity, in terms of tartaric acid, in the present study was found to be 0.89 ± 0.07 %. The process developed in the present study for *Aloe vera* wine resulted in a table wine, as the alcohol contents of such wines are usually 7–16% (Joshi, 1997).

TOTAL PHENOLIC CONTENT (TPC) AND ANTIOXIDANT ACTIVITY OF *Aloe vera* WINE (*IN VITRO* STUDY)

In regard to human health the phenolic constituents of wine are judged as a “protective factor” (Ghiselli et. al., 2000). These are important, as they add to sensory characteristics, principally astringency, colour, and bitterness and as they are also involved in pharmacological effects, including anti-microbial, anti-carcinogenic and antioxidant properties (Lesschaeve and Noble, 2005; Boselli et. al., 2009). Phenolic compounds in wine act as antioxidants, with mechanisms involving both metal chelation and free-radical scavenging (Li et. al., 2009). The *Aloe vera* wine prepared in the present study was found to have the total phenolic compounds at a level of 1065 ± 10.11mg GAE/L. These results are in agreement with the available literature as Lopez-Velez *et. al.,* (2003) reported the average total phenolic content for commercial wines as determined by Folin-Ciocalteu method, varied from 1848 to 2315 mg GAE/L and these values were found to be in conformity with those reported by Simonetti, (1997) and Minnusi *et. al.,* (2003). The TPC value of *Aloe vera* wine is indicative of potential health advantages as phenolics have several pharmacological activities including anti-inflammatory, antioxidative, cardiac arrest protection, and hepatoprotection (Janbaz *et. al.,* 2002; Lopez-Revuelta et. al., 2006). Based on the hypothesis that the high contents of total phenolic display potential antioxidant activity, the *in vitro* antioxidant activity of *Aloe vera* wine was computed by FRAP assay. FRAP value for the analyzed *Aloe vera* wine was found to be 2950 ± 18.48 µmol/L signifying a substantial antioxidant capacity of this wine. The *in vitro* antioxidant potential of the lab made *Aloe vera* wine was on a par with the values reported for the commercial red wines (Cabernet Shiraz and Beaujolais) by Negi et. al., (2013). Our results confirm previous studies which have indicated a positive relationship between phenolic compound concentration and ferric reducing capacities (Rupasinghe and Clegg, 2007; Dharmalingam and Nazni, 2013 and Nazni and Dharmalingam, 2013; Johnson and de. Mejia, 2012; Negi *et. al.,* 2013).

BACTERIAL TRANSLOCATION IN THE LIVER, SPLEEN AND INTESTINE OF THE MICE CHALLENGED WITH *S.* Typhimurium

The bactericidal effect of ingested wine on *S.* Typhimurium was studied in a murine model. The presence of *S.* Typhimurium in the infected mice was confirmed by plating the serially diluted homogenates of the same on BSA agar and simultaneously performing biochemical test in accordance with the earlier report on *Salmonella* (Rishi et. al., 2006; 2009). A decrease in the bacterial load in liver, spleen and small intestine of the infected-wine fed animals was observed *in vivo* suggesting the therapeutic activity of wine against *S.* Typhimurium infection. *Aloe vera* wine resulted in a 2.0 log unit decrease in cfu from liver with respect to infected control and a 1.9 log unit decrease in cfu from spleen and small intestine ($p<0.001$) (Fig. 1).

Liver and spleen becomes the primary sites for the multiplication of *Salmonella* indicative of infection becoming systemic (Nnalue et. al., 1992; Gerstel and Romling, 2001). In the present study, the recovery of bacteria from the liver, spleen and intestine of infected mice confirmed the spreading of the organism into the respective tissues. A decreased bacterial load in liver, spleen and small intestine of the infected-wine fed animals indicate the antibacterial effect of wine in Salmonellosis. The therapeutic activity of wine can be attributed to various known antibacterial components present in it however a comprehensive mechanism behind the displayed antibacterial effect is not clearly understood. Antibacterial effect of wine during *E. coli* O157:H7, *S.* Typhimurium (Just and Daeschel, 2003), *L. innocua* (Fernandes et. al., 2007), *C. jejuni* (Carneiro et. al., 2008) infection has been studied using synthetic stomach systems which simulate a wine consumption like situation during a meal. Carneiro et. al., (2008) using artificial stomach model system suggested that wine consumption along with meal can reduce the load of *C. jejuni* persisting further in the alimentary tract resulting in lowered infection risk. This observation is in agreement to the study of Fernandes et al., (2007) where wine was found to reduce the initial titer of *L. innocua* by 4-5 folds in acidic conditions which suggest that consumption of wine during a meal may reduce the risk of *Listeria* infection in alimentary canal. The word wine signifies the fermented product from grapes that contains moderately high ethyl alcohol content in addition to phenolics, acids responsible for the antibacterial action. Moreover, *Aloe vera* is known to cause immune modulation of the host thus increasing the resistance against pathogens. Therefore, the decreased translocation of bacteria in the infected-wine fed group may be due to the synergistic contribution of the wine components and the bioactive components of *Aloe vera* gel, as the antibacterial activity of wine could be enhanced because of this herb.

EFFECT OF *Aloe vera* WINE ON LIVER DAMAGE CAUSED DUE TO SALMONELLOSIS

Hepatic damage is a notable feature of *Salmonella* infection (Rishi et. al., 2009). The histological analysis of the *S*. Typhimurium infected group revealed marked morphological disruption such as kupffer cell hyperplasia, necrosis, multiple small abscesses distributed randomly in the liver and a very marked liver cell nucleus enlargement (Fig. 2B) in comparison to the control group (Fig. 2A). In disparity, the histological examination of tissue sections from the infected-wine fed group showed an improvement of liver morphology as compared with the damage in the non-wine fed infected mice except for mild kupffer cell hyperplasia, necrotic cells were nearly absent, liver cells were mostly normal (Fig. 2C). Liver morphology of *per se* group was found to be normal (Fig. 2D). These observations provide histological support to the protection conferred by *Aloe vera* wine. Ricardo da Silva et. al., (1991) and Frankel et. al., (1993) had reported that the components in wine counteract the oxidizing processes leading to hepatic damage. The protection conferred by wine may be attributed to the phenolic compounds present in it as they are known to possess anti-inflammatory, immuno-stimulatory, antioxidative, antiproliferative activities functioning in favor of host tissue. The presence of superoxide dismutase, catalase, β-carotene, α-tocopherol and other antioxidants may be responsible for the hepatoprotective action attributed to *Aloe vera* gel (Chandan et. al., 2007; Hamman, 2008; Iji et. al., 2010). The results are in consonance with earlier report by Ramachandraiahgari et. al., (2012) where regenerative liver and kidney histological changes were observed in the *Aloe vera* extract treated diabetic rats. Kant et. al., (2013) investigated the hepatoprotective effect of aqueous extract of *Aloe vera* against carbontetrachloride induced hepatotoxicity in rats and results revealed that the aqueous extract of *Aloe vera* exhibited dose dependant hepatoprotective activity, both biochemically and histologically. The diverse pharmacological and therapeutic activities observed for *Aloe* gel may be attributed to its heterogeneous composition.

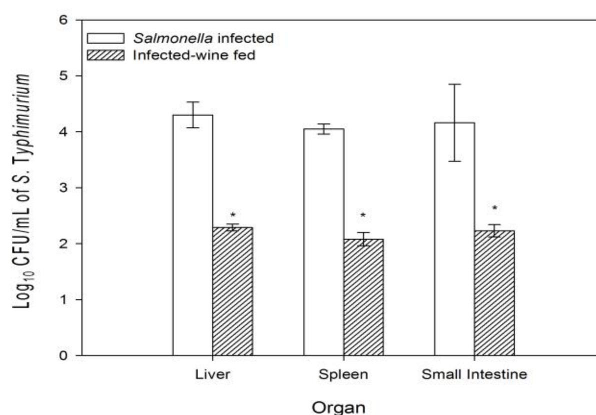

Fig. 1 Effect of *Aloe vera* wine administration on the bacterial load. Values are expressed as mean ± S.D. of five different observations. *p<0.001 vs. *Salmonella* infected group.

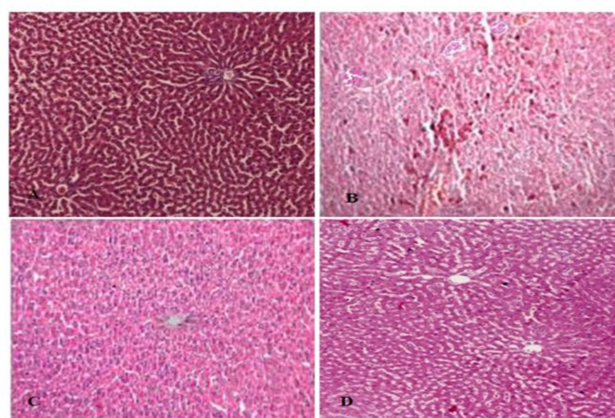

Fig. 2 Representative photomicrographs of hematoylin-eosin stained mice liver sections (100X). A)Normal/control mouse liver; B)*Salmonella* Infected liver section; C) *Aloe vera* wine-fed infected liver section; D) Liver section from *per se* group.

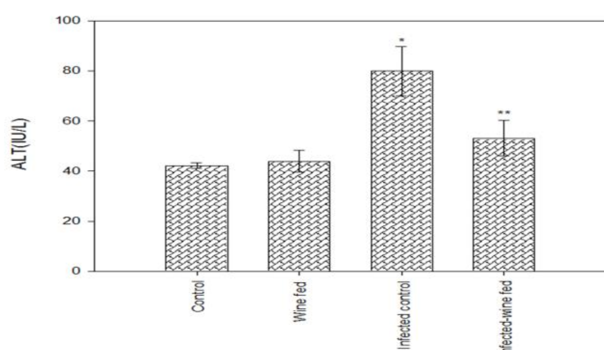

Fig. 3 Effect of *Aloe vera* wine administration on ALT levels. Values are expressed as mean ± S.D. of five different observations. *p<0.001 vs. control and wine-fed groups; **p<0.001 vs. *Salmonella* infected group.

EVALUATION OF LIVER FUNCTION FOLLOWING WINE ADMINISTRATION

Liver function in all the experimental groups was monitored by analyzing the levels of serum ALT and AST which is commonly measured as a part of a diagnostic evaluation of hepatocellular injury, to determine liver health.

In the present study, marked elevation was seen in both ALT and AST activities in infected mice as compared to their respective control group (Fig. 3 and Fig. 4) which provided evidence in support of damage to the liver. This was also confirmed by histological studies as shown earlier in the micrographs (Fig. 2). Similarly, a significant rise in liver-derived enzymes and marked histological changes in starved *Salmonella* infected mice have also been reported by Nakonezna and Hsu et. al., (1980); Rishi et. al., (2006). Challenge with *S.* Typhimurium caused nearly 1.8 fold increase in the serum levels of ALT (79.8 IU/L vs 42.1 IU/L) (p<0.001) and 1.9 fold increase in the levels of AST (242.1 IU/L vs 124.5 IU/L) (p<0.001) in comparison to their respective control groups. However, administration of *Aloe vera* wine to the infected group in the present study prevented the leakage of these enzymes as is evident from the restored activity of these enzymes in the serum of infected-wine fed group. The level of ALT got significantly reduced to 53.1 IU/L, (p<0.001) in the animals fed with *Aloe vera* wine relative to infected group. Also the levels of AST enzyme got significantly reduced to 155.9 IU/L, (p<0.001) in the animals fed with *Aloe vera* wine in comparison to infected group. No negative effects were observed on the marker enzymes of hepatic damage in the *per se* group with respect to control. In support of our findings, Arola et. al., (1997) reported that the consumption of wine did not cause hepatic lesion and thus maintained the level of liver enzymes. This suggests that all the components in wine act in synergy to counteract the toxic effect of ethanol. *Aloe vera* extract has been extensively reviewed for its antioxidant potential and has been found to be beneficial against liver injury (Can et. al., 2004; Loots et. al., 2007). Prolonged administration of *Aloe vera* gel was found to improve liver enzyme function and was found to have no negative effect on hepatic damage markers (Gupta and Flora, 2005; Iji et. al., 2010). Hepatic damage induced by carbon tetrachloride in mice model was found to be reduced significantly with the administration of aqueous extract of dried aerial parts of *Aloe vera*. It was also found to normalize the levels of other tested biochemical parameters (Chandan et. al., 2007). Similar results were recorded by Kant et. al., (2013) who have reported significantly low values of alkaline phosphatase, alanine aminotransferase and billirubin in mice group receiving aqueous extract of *Aloe vera* relative to carbontetrachloride challenged group. It is suggested that leakage of liver enzyme ALT and AST from the hepatocellular membrane into the blood stream due to the systemic infection caused by *Salmonella* may be decreased by the membrane stabilizing action of phenolic components of *Aloe vera* wine. Some still unexplained mechanisms of *Aloe vera* may be assumed to be involved in protecting liver from injury. *Aloe vera* aqueous extract has been reported as a potent hepatoprotective agent (Kant et. al., 2013). *Aloe vera* possess a range of bioactive compounds, the best studied being acemannan, a polysaccharide which modulates the biological activities of all the other components contained in it to act synergistically (Eshun and He, 2004; Boudreau and Beland, 2006). *Aloe vera* could have afforded protection to hepatic cells from toxic damages by improving the reduced glutathione status in cells. *Aloe vera* might also act as antioxidant and free radical scavenger contributing to its hepatoprotective properties.

ANTIOXIDATIVE POTENTIAL OF *Aloe vera* WINE

Tissue injury results when the equilibrium between the generation of toxic radicals and their counterbalance using various antioxidative mechanisms is disturbed (Windrow et. al., 1993; Rishi et. al., 2006). It is a well established fact that oxidative stress is generated by *Salmonella* upon infection in the host. Thus, the same was assessed in liver tissue homogenates of infected mice model and the proposed antioxidative potential of the prepared wine was estimated by measuring malondialdehyde (MDA) levels, superoxide dismutase (SOD) levels and glutathione (GSH) content.

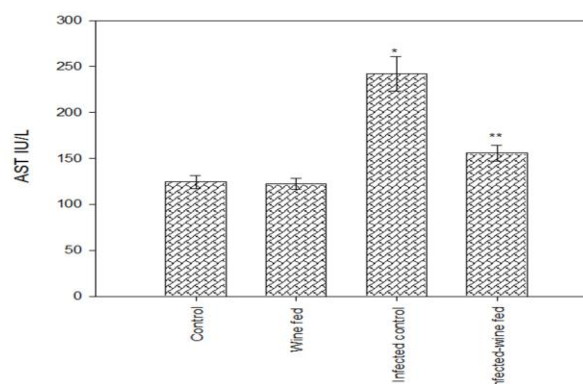

Fig. 4 Effect of *Aloe vera* wine administration on AST levels. Values are expressed as mean ± S.D. of five different observations. *p<0.001 vs. control and wine-fed groups; **p<0.001 vs. *Salmonella* infected group.

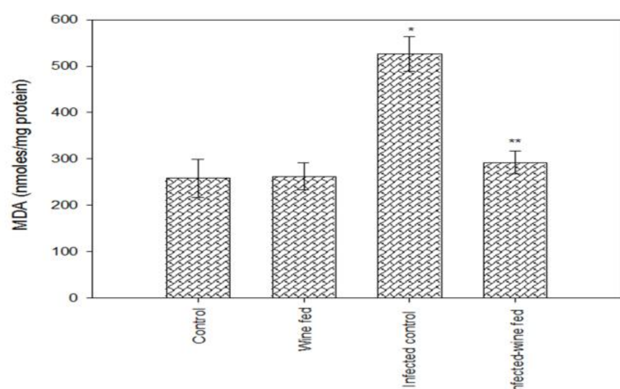

Fig. 5 Effect of *Aloe vera* wine administration on liver MDA levels.Values are expressed as mean ± S.D. of five different observations. *p<0.001 vs. control and wine-fed groups; **p<0.001 vs. *Salmonella* infected group, #p<0.05 vs. control.

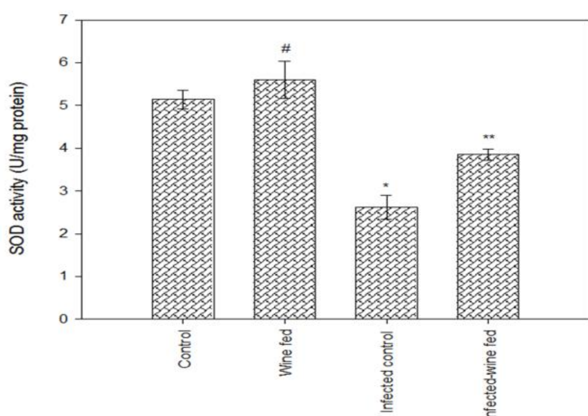

Fig. 6 Effect of *Aloe vera* wine administration on liver SOD levels.Values are expressed as mean ± S.D. of five different observations. *p<0.001 vs. control and wine-fed groups; **p<0.001 vs. *Salmonella* infected group, #p<0.05 vs. control.

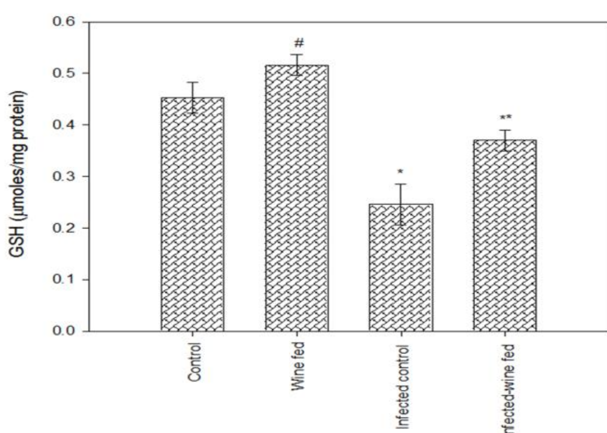

Fig. 7 Effect of *Aloe vera* wine administration on liver GSH content.Values are expressed as mean ± S.D. of five different observations. *p<0.001 vs. control and wine-fed groups; **p<0.001 vs. *Salmonella* infected group, #p<0.05 vs. control.

HEPATIC MDA LEVELS

It was observed that *Salmonella* infection produces oxidative stress as the levels of MDA increased significantly (p<0.001) in the mice challenged with *S.* Typhimurium as compared to the control group indicating an increase of (50.84%). The rise in the oxidative stress in terms of increased MDA levels in the liver upon *Salmonella* infection has been previously reported (Rishi et. al., 2006; 2008). Wine administration had a healing influence on the generated oxidative stress as infected-wine fed group displayed reduced lipid peroxidation (292.30 vs 526.04 nmoles MDA/mg protein) as compared to the infected counterpart (Fig. 5). To the best of our knowledge, there have been no studies related to antioxidative effects of *Aloe vera* wine. However, our results are comparable with *in vivo* study conducted by Negi et. al., (2013) where administration of sea buckthorn wine significantly decreased and reverted the MDA levels resulting in a protection against lipid peroxidation. Roig et. al., (1999) also reported that administration of red wine results in lower hepatic malondialdehyde in wine-fed rats. Corroborating with the present data Yan cui et. al., (2014) reported that *Aloe vera* gel diminished the oxidative stress partly through a decrease in MDA levels signifying the hepatoprotective efficacy of *Aloe vera*. A related observation has been made by Werawatganon et. al., (2014) who reported that *Aloe vera* gel restored the MDA levels in mice with acetaminophen-induced hepatitis. The phenolic composition of the prepared *Aloe vera* wine has an important role in controlling lipid peroxidation which is associated with its antioxidant activity. Furthermore, the anti-lipid peroxidative efficacy associated with *Aloe vera* gel may be a sum of activity of multiple bioactive components present in the gel which is responsible for protection of liver against the *Salmonella* induced oxidative stress.

EFFECT OF *Aloe vera* WINE ON SUPEROXIDE DISMUTASE (SOD) ACTIVITY

Infection with *S.* Typhimurium (group 3) decreased the activity of hepatic SOD (2.6±0.28 U/mg protein) compared with the control group (5.1±0.22 U/mg protein) (Fig 6). SOD catalyzes the conversion of superoxide anions into oxygen and hydrogen peroxide thus acting as a potent antioxidant enzyme to protect the cell from superoxide radical damage (Salin and McCord, 1974; Williams et. al., 1998; Chen et. al., 2005). Decreased activity of SOD enzyme may results in the elevated levels of lipid peroxides contributing in the succession of liver injury (Dhaunsi et. al., 1992; Cederbaum, 2001). Under these intracellular conditions when *Aloe vera* wine was administered to the infected group (group 4), SOD activity was enhanced. *Aloe vera* wine showed an increase of 31.9% in SOD activity in infected wine-fed group as compared to infected control. Moreover, wine administration resulted in higher hepatic superoxide dismutase activity in *per se* group (p<0.05) as compared to the control.

The antioxidant enzyme SOD is involved in neutralization of superoxide anions thus preventing the

formation of hydroxyl radicals. However, the reduced SOD activity would increase the production of hydroxyl radicals which may, in turn, enhance the peroxidation of lipid membranes to a higher extent. The increased extent of lipid peroxidation observed previously in the infected group of mice (Fig. 5) might be attributed to the same phenomenon. These observations are in concordance with the results of the earlier study by Rishi et. al., (2006) where they reported a decrease in SOD activity and increased MDA levels in starved infected mice. This is very well supported by our histopathological studies as well where there is a clear damage to the liver tissue (Fig. 2).

Negi et. al., (2013) had earlier shown an increase in liver superoxide dismutase and glutathione peroxidase levels after treatment with sea buckthorn wine for a period of 21 days. Red wine administration resulted in a twofold enhanced SOD activity in diabetic rat model (Montilla et. al., 2004). Furthermore, *Aloe vera* has been reported to contain SOD enzymes which are involved in neutralizing the superoxide radicals (Sabeh, 1995). Rishi et. al., (2008) reported that *Aloe vera* modulated the inflammatory response when applied either topically, administered intraperitoneally or in combination. It has been revealed that the reported modulation could be due to the potential of *Aloe vera* to decrease the levels of lipid peroxides. The presence of SOD in *Aloe vera* itself might be responsible for enhancing the enzyme levels (Rishi et. al., 2008). Animal studies have provided supporting data 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 (Lim et al., 2003). The increase in the levels of SOD following consumption of *Aloe vera* wine in the present study is thus fully justified.

EFFECT OF *Aloe vera* WINE ON REDUCED GLUTATHIONE (GSH) LEVELS

GSH, the most important endogenous antioxidant reduces the oxidative stress by protecting the cells from lipid peroxidation or by protecting the protein sulfhydryl groups from the action of reactive oxygen species (Greenberg and Demple, 1989; Haest et. al., 1997). GSH has also been shown to soothe liver injury due to its property to trap reactive oxygen species generated by kupffer cells and neutrophils in the vasculature (Koul et. al., 2003).

Challenge with *S.* Typhimurium resulted in a (46.6 %) decrease in the hepatic GSH concentration relative to control group mice. The administration of *Aloe vera* wine was seen to restore back the GSH levels in the infected-wine fed group which were found to have been significantly reduced upon infection ($p<0.001$, Fig. 7). According to present study, it is evident that the prepared *Aloe vera* wine has antioxidant potential which was demonstrated by the fact that reduced glutathione levels were increased by the administration of *Aloe vera* wine by 29.9% in the infected-wine fed group as compared to the infected control. *Per se* group did show fairly significant change in the levels of GSH ($p<0.05$) when compared to control.

There have been many reports of increase in GSH/GSSG ratios upon administration of wine and a variety of herbal extracts including *Aloe vera* (Koul et. al., 2003; Gupta and Flora, 2005; Negi et. al., 2013). Negi et. al., (2013) reported the restoration of glutathione levels and the redox ratio with sea buckthorn wine administration. Rodrigo et. al.; (2002) have reported an increase of 140% in GSH/GSSG ratio in the renal cortex of mice fed with red wine in comparison to those fed with water. Montilla et. al., (2004) demonstrated the restoration of reduced glutathione levels with red wine administration which had previously been diminished by 50% upon injection of Streptozotocin in male wistar rat model. Khan et al., (2013) reported glutathione peroxidase, superoxide dismutase enzyme to be present in *Aloe vera* gel which might be responsible for the exhibited antioxidant effects.

Aloe vera wine is endowed with strong *in vitro* and *in vivo* antioxidant properties which might have contributed in fighting the oxidative stress generated by *Salmonella* infection. The mechanism of protection against oxidative stress can either be the decreased production of free radical derivatives or due to the antioxidant activity of phenolic component present in this herbal substrate or a cumulative effect of phenolic component, tocopherol and other vitamins, along with the components which are responsible for increasing the bioavailability of the vitamins. There are studies that have shown an inverse relation between lipid peroxidation (LPO) concentration and GSH levels (Koul et. al., 2006; Rishi et. al., 2008). In our study, wine administration might also have decreased the levels of lipid peroxidation by increasing the levels of antioxidants such as GSH and SOD therefore, neutralizing the oxidative stress, resulting in improved hepatic tissue.

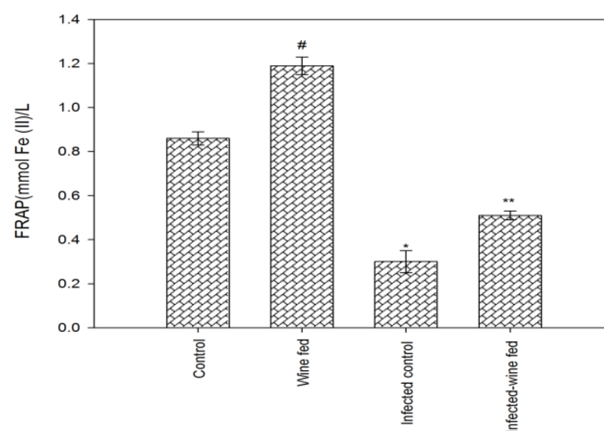

Fig. 8 Effect of oral administration of *Aloe vera* wine on plasma FRAP levels. Values are expressed as mean ± S.D. of five different observations. *p<0.001 vs. control and wine fed groups; **p<0.001 vs. *Salmonella* infected group; #p<0.001 vs. control.

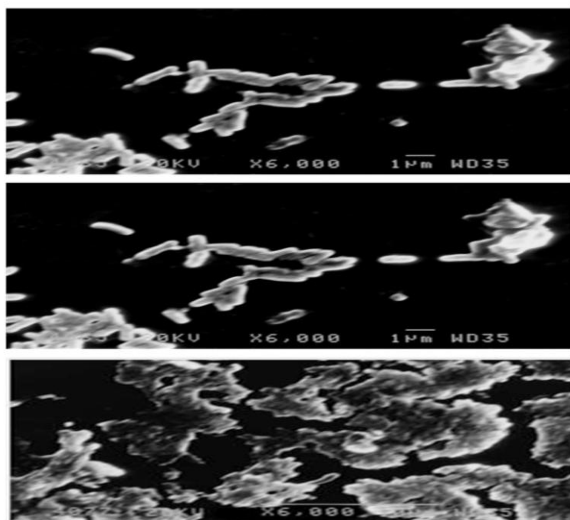

Fig. 9 SEM images of *S*. Typhimurium (A) Control, (B) Treated with *Aloe vera* wine for 6h, (C) Treated with *Aloe vera* wine for 24 h.

EFFECT OF *Aloe vera* WINE ON FERRIC REDUCING ANTIOXIDANT POWER (FRAP) LEVELS IN BLOOD PLASMA

Plasma antioxidant capacity has been projected as a good indicator of antioxidant status (Ghiselli et. al., 2000). Fig. 8 shows the levels of plasma antioxidant capacity, assessed by FRAP for the experimental groups. As compared with the control group, FRAP values were markedly decreased in infected control, but increased by the administration of *Aloe vera* wine by 41.1% in the infected wine-fed group reaching significance between both groups (p<0.001). Moreover, there was a significant increase of an average 30.0% in the FRAP values in *per se* group as compared to control (p<0.001) which is suggestive of an improved antioxidant status in the wine fed mice. The antioxidant defense system appears to be enhanced in the plasma, by increased FRAP levels (Fig. 8). The significantly increased levels of FRAP in wine-fed group could be attributed to the absorbed polyphenols present in wines. This observation is based on the known reducing power, capacities to quench lipid peroxidation and scavenging action of these compounds.

Our results showed a marked increase in the plasma FRAP levels in wine *per se* group which is in concurrence with the data reported for human subjects under acute exposure to red wine (Whitehead et. al., 1995). Rodrigo et al., (2002) also reported increased FRAP values in the rats fed with red wine for a period of ten weeks. Moreover, red wine when supplemented with regular diet has been shown to increase the total antioxidant capacity in plasma and reduces oxidative damage as reported in a study carried out in the health and wine field (Fernandez-Mar et. al., 2012). Moniruzzaman et. al., (2012) assessed the hypolipidemic and antioxidant activity of *Aloe vera* extract and the extracts were found to give high FRAP values, indicating the potential of this plant to be used as an antioxidant. In our study, the increased FRAP levels after consumption of *Aloe vera* wine strongly suggest that upon wine consumption, antioxidant status improves significantly. Thus it may be implied that the wine provides general oxidative protection in circulation *via* the increase in antioxidant status and expands the treatment choices for the management of infections.

MORPHOLOGICAL CHANGES IN THE CELLS OF *S*. Typhimurium IN THE PRESENCE OF *Aloe vera* WINE

The possible bactericidal mechanism of the effect of *Aloe vera* wine on *S.* Typhimurium was studied by scanning electron microscopy (SEM). SEM provided qualitative conformation of the change in the morphology of *S.* Typhimurium treated with *Aloe vera* wine. SEM of untreated and wine treated (6 h, 24 h) *S.* Typhimurium cells are shown in Fig. 9 (A, B, C). SEM images of *S.* Typhimurium grown in BHI broth revealed the morphology of cells as medium to long slender shaped rods, with smooth cell surfaces (Fig. 9A). In contrast to control sample, marked changes were evident on the outer membranes of *S.* Typhimurium treated with wine. SEM observation of *S.* Typhimurium treated with 50% *Aloe vera* wine for 6 h were much shorter and the outer membrane of the cells were rough and deformed (Fig. 9 B) as compared to the normal morphology of cells. SEM examination of *S.* Typhimurium treated with 50% *Aloe vera* wine for 24 h revealed membrane disruption that appeared to have caused leakage of intracellular contents (Fig. 9C). Similar study was previously reported by Bish, (2011) for *S.* Typhimuirum treated with peach and cherry wines. SEM observation of *S.* Typhimurium treated with 60% peach wine for 24 h showed that the cells were all medium length with no long cells present. The outer membranes of the cells were very rough and deformed, with some holes visible, and intracellular contents appearing to have leaked out. *S.* Typhimurium treated with 60% cherry wine for 24 h were also much shorter than the control grown in TSB (Tryptic Soy broth). Material from the cherry wine adhered to the cell's outer membrane, and it appeared that cells were torn open, or deformation at the ends of the cells occurred during cell division. The outer membrane was rough, and loss of intracellular content was apparent in some cells whereas SEM pictures of *S.* Typhimurium grown in TSB for 24 h were medium to very long slender shaped rods, with smooth cell surfaces.

CONCLUSION

The lab made herbal *Aloe vera* wine was assessed for its protective efficacy against oxidative stress induced by *Salmonella* infection in a murine model. Our results are of great practical importance as *Aloe vera* wine besides being a tasteful addition to food might also prove to be a health drink with antibacterial potential against a common food-borne pathogen as well as antioxidant potential capable of combating oxidative stress generated in the body under various pathological conditions. It was observed that the tested wine conferred potential benefits in a *Salmonella* infected murine model. Furthermore, assessment of its protective potential may be extended to other infections as well.

ACKNOWLEDGMENTS

The authors express their gratitude to **Dr. B. N. Datta, ex-Professor of Pathology, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh (India),** for providing help in the histological analysis of the samples.

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