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STUDIES ON PROTECTIVE EFFECTS OF *ALOE VERA* L. LEAF EXTRACTS ON OXIDANT INDUCED DAMAGE TO LIPID PEROXIDATION (LPO) IN DIFFERENT BIOMEMBRANE MODELS

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ABSTRACT

There are many medicinal plants using traditionally as the source of antioxidants or protective agents against oxidative stress induced tissue or cell damage by lipid peroxidation. Oxidative stress is one of the major contributory factors that stimulate numerous intracellular pathways leading to the increased free radical generation causing oxidative damage or lipid peroxidation. Since ancient time *Aloe vera* L. are using in many herbal preparations as antioxidants or as protective agents against oxidant induced damage to cellular biomolecules such as membrane lipids. *Aloe vera* L. possesses an important role in absorbing and neutralizing oxidant induced free radicals. In present study the protective effects of *in vivo* grown and *in vitro* propagated *Aloe vera* L. whole leaves and only gel extracts against oxidant induced lipid peroxidation were examined in three different biological membrane models including goat RBC ghost, goat liver homogenate and goat liver slices. Results were showing that among the different extracts (prepared with the solvents of different polarities such as aqueous, hexane, methanolic, ethyl acetate and chloroform) of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaves and inner gel, the extracts with *in vitro* regenerated *Aloe vera* L. leaves were showing higher degree of protection against oxidant induced lipid peroxidation. While among all different extracts of *in vitro* propagated *Aloe vera* L. leaves, the methanolic extract were showing the maximum protection against lipid peroxidation.

KEYWORDS: *Aloe vera* L., membrane models, oxidant induced damage, lipid peroxidation, antioxidants and protective effects.

INTRODUCTION

Currently, there is a renewed global interest in the study and use of plants because such investigations provide important new lead on novel and active molecules of therapeutic importance and providing protective effects against free radical damages including lipid peroxidation. *Aloe vera* L. has been used for medicinal purposes in several cultures for millennia: Greece, Egypt, India, Mexico, Japan, and China.[1]

Oxygen derived free radicals and their products are known to play an important role in the pathogenesis of chronic inflammatory disorders. These activated, oxygen intermediates, together with secondarily formed radicals, like the hydroxyl radicals ($OH^{\bullet}$) are able to destroy membrane lipids, proteins, DNA, hyaluronic acid and cartilage.[2]

Lipid peroxidation (LPO) is a free radical mediated chain reaction that can inactivate cellular components. This process is purportedly associated with various chronic disorders including carcinogenesis.[3] Therefore, lipid peroxidation mainly refers to the reaction of oxidative deterioration of polyunsaturated lipids. Peroxidation involves the direct reaction of oxygen and lipid to form radical intermediates and to produce semi stable peroxides, which, in turn, damage the enzymes, nucleic acids, membranes and proteins. Malondialdehyde (MDA), which is a major end product and an index of LPO, cross-links protein and nucleotides on the same and opposite strands. MDA is mutagenic in mammalian systems, and readily reacts with deoxynucleotides to produce adducts and cause DNA damage.[4]

An antioxidant is a molecule capable of protecting cells against the damaging effects of reactive oxygen species otherwise called, free radicals such as singlet oxygen,

super oxide, peroxyl radicals, hydroxyl radicals and peroxynite which results in oxidative stress leading to cellular damage.[5] Natural antioxidants play a key role in health maintenance and prevention of the chronic and degenerative diseases, such as atherosclerosis, cardiac and cerebral ischemia, carcinogenesis, neurodegenerative disorders, diabetic pregnancy, rheumatic disorder, DNA damage, lipid peroxidation and ageing.[6,7]

Thus, antioxidants are often added to foods to prevent the radical chain reactions of oxidation, and they act by inhibiting the initiation and propagation step leading to the termination of the reaction and delay the oxidation process. Due to safety concerns of synthetic compounds, food industries have focused on finding natural antioxidants to replace synthetic compounds. In addition, there is growing trend in consumer preferences for natural antioxidants, all of which has given more impetus to explore natural sources of antioxidants.

Membrane lipids are very susceptible biomolecules to oxidative stress in the biological system, that's why in the present study we have investigated protective effects of several extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and only gel against oxidant induced Lipid peroxidation in different bio-membrane models including goat RBC ghosts (plasma membrane lipids), goat liver homogenate (plasma membrane and intracellular lipids) and goat liver slices (intact cells).

MATERIALS AND METHODS

Preparation of crude extract

Leaves of the *Aloe vera* L. were collected from the already *in vitro* propagated and properly acclimatized 9-12 months old plants. *In vitro* propagation was the previous phase of our study to produce quality plant material to meet industrial requirement. Simultaneously leaves from 9-12 months old *in vivo* grown *Aloe vera* L. plants were also collected. Freshly collected *Aloe vera* L. leaves were washed with distilled water, followed by disinfecting with ethanol 70%. Later, in case of whole leaf crude extract preparation, leaves were chopped into the small pieces and were exposed to 50ºC for 3 days to get dried. After complete drying, leaf parts were powdered using electric grinder, simultaneously in case of only gel crude extract preparation, upper green skin/rind of leaves was removed and latex was cut into small pieces and both types of leaf materials were homogenized separately. The homogenized materials were extracted with ethanol (95%). The ethanol from the extracted leaf materials was evaporated at 65°C temperature in water bath. The solvent was completely removed and dried to get powder. All the powdered plant materials including whole leaf and only gel were used for the preparation of aqueous and solvent extracts.

Aqueous extract

Extracts were prepared using the modified method of Case.[8] 1:3 (w/v) ratios were used for the powdered leaf material and distilled water for extract preparation. The pulverized leaf material was used to prepare an infusion in hot (95ºC) distilled water. The infusion was left overnight under refrigeration (4ºC) to prevent any possible contamination. After 24 h the extracts were kept in rotary shaker at 100 rpm for 1 h and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at – 47.5ºC. The frozen extract was then freeze dried to a powder, weighed, transferred into separate vial and preserved at 4ºC for future analysis.

Solvent extracts

As in case of aqueous extract here also 1:3 (w/v) ratios were used for the powdered leaf material and different solvents for extract preparation. The pulverized leaves material was mixed with sufficient quantity of solvents viz., hexane, ethyl acetate, methanol and chloroform. It was kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at – 47.5ºC. The dried extracts thus obtained was weighed, transferred into separate vials and preserved at 4ºC for future analysis.

Further examinations were performed with different extracts of whole leaf and inner gel of *in vivo* and *in vitro* regenerated *Aloe vera* L., to check their protective effects against oxidative damages on biomolecule lipid.

Evaluation of the antioxidant effects of *Aloe vera* L. leaf extracts on oxidant induced damage to lipids/lipid peroxidation (LPO)

An attempt was made to study the extent of oxidative damage to cellular biomolecule like membrane lipids. The effects of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and inner gel extracts on different types of membrane models subjected to oxidative stress was studied. Lipid peroxidation (LPO), a well-established mechanism of cellular injury, is used as an indicator of oxidative stress.[9]

In present investigation, the extent of lipid peroxidation was assessed in three different membrane models, namely goat RBC ghost (membrane model I), goat liver homogenate (membrane model II) and goat liver slices (membrane model III) and the extent of formation of thiobarbituric acid reactive substances (TBARS) from the damaged lipids by oxidizing agents was used as a measure of damage to membrane lipids.

Membrane Model I (Goat RBC Ghost)

The reagents used were Isotonic KCl (1.15%), Hypotonic KCl (0.5%), Tris buffered saline (TBS) (10mM Tris, 0.15M NaCl, pH 7.4), Ferrous sulphate (10µM), Thiobarbituric acid (TBA) (1%), Ascorbic acid (0.06mM), Ethanol (70%) and Acetone.

Goat blood (50ml) was collected fresh in a sterile container. The blood was immediately defibrinated using acid-washed stones. The defibrinated blood was transferred to another sterile container and diluted 1:1

with sterile isotonic KCl and transferred to the laboratory on ice. The RBCs were pelleted by centrifuging at 3000 x g for 10 minutes at 4°C. The supernatant was discarded and the pellet was washed thrice with isotonic KCl. The cells were then lysed at 37°C for one hour in hypotonic (0.5%) KCl. After lysis, the lysate was centrifuged at 5000 x g for 10 minutes at 4°C. The pellet obtained was washed repeatedly with hypotonic KCl until most of the haemoglobin was washed off and a pale pink pellet was obtained. The pellet was suspended in 1.5ml of TBS and 50µl aliquots were used for the assay, as described by Dodge *et al.*[10]

Assay

The reaction mixture contained 50µl of RBC ghosts, 50µl of *Aloe vera* L. leaf extracts, 50µl $FeSO_4$ and 100µl of ascorbate in a total volume of 500µl, which was made up with TBS. A blank was prepared without the plant extract and lipid source, but containing only $FeSO_4$, ascorbate and TBS in a final volume of 0.5ml. An assay medium corresponding to 100% oxidation was prepared, which contained all the other constituents except the plant extract. The experimental medium corresponding to auto-oxidation contained only RBC ghosts. All the tubes were incubated at 37°C for one hour. After incubation, the reaction was arrested by adding 0.5ml of 70% alcohol to all the tubes. Then 1.0ml of TBA was added to all the tubes and heated in a boiling water bath for 20 minutes. After cooling to room temperature, the tubes were centrifuged and 0.5ml of acetone was added to the supernatant. The pink colour developed was measured at 535nm in a spectrophotometer.

Membrane Model II (Goat Liver Homogenate)

Goat liver was procured fresh from the slaughter house and washed free of blood using Tris-HCl buffer (40mM, pH 7.0). A 20% liver homogenate was prepared in the same buffer using a motorized Teflon homogenizer. The homogenate was clarified to remove debris and used as the membrane source for assessing LPO as per the method of Okhawa *et al.*[11]

All the reagents used were KCl (30mM), $FeSO_4$ (0.16mM), Ascorbate (0.06mM), TBA (1%), Acetic acid and n-propanol: pyridine (15:1 v/v).

Assay

The reaction mixture containing 0.1ml of liver homogenate, 0.1 ml of KCl, 0.1ml of $FeSO_4$ and 0.1ml of ascorbate was incubated at 37°C for one hour in the presence (0.1ml corresponding to 50mg) and the absence (0.1ml of KCl) of extracts of *Aloe vera* L. leaves. To 0.4ml of the reaction mixture, 1.5 ml each of TBA and acetic acid were added and mixed well. The contents were heated in a boiling water bath for 20 minutes. After cooling, 1.0ml of distilled water and 5.0ml of n-propanol and pyridine mixture was added. After centrifugation, the pink coloured chromophore obtained was measured at 532nm in a spectrophotometer. The percentage inhibition of LPO was determined by comparing the results of the control and the test samples.

Membrane Model III (goat liver slices)

The extent of LPO in goat liver slices was estimated by the method described by Nichans and Samuelson.[12]

The reagents used were Phosphate buffered saline (PBS), H_2O_2 and TBA-TCA-HCl reagent (0.375% TBA, 15% TCA, 0.25N HCl).

The goat liver was collected fresh from a slaughter house, plunged into cold sterile PBS and maintained at 4°C till use. Thin slices of 1mm thickness were cut using a sterile scalpel.

Assay

One gram of goat liver slice was taken in 4.0ml of sterile PBS in a broad flatbottomed vessel. The oxidizing agent H_2O_2 (500µM) and/or the *Aloe vera* L. leaf extract (500mg/kg tissue) were added and incubated at 37ºC with mild shaking for one hour. Appropriate controls were also set up.

After incubation, the goat liver slices were homogenized in the incubation medium using a Teflon homogenizer and the homogenate was used for the assay. The reaction was terminated by mixing 1.0ml of homogenate and 2.0ml of TBA-TCA-HCl reagent. The contents were incubated in a boiling water bath for 15 minutes and the pink colour developed was estimated at 535nm against a reagent blank, in a spectrophotometer.

Statistical analysis

All the analysis were carried out in triplicates and expressed as mean ± SD. Analysis of variance (ANOVA) were performed using the one-way analysis of variance. Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

RESULTS

Evaluation of biomolecular protective effects of *Aloe vera* L. whole leaf and inner gel was accomplished in current study. Different extracts such as *n*-hexane, chloroform, ethyl acetate, methanol and aqueous of all four samples prepared by *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and only gel were tested for their protective effects on lipid biomolecule against lipid peroxidation (LPO). So that impact of all four different types of leaf samples including Sample I - *in vivo* grown *Aloe vera* L. whole leaf samples, Sample II - *in vivo* grown *Aloe vera* L. only gel samples, Sample III - *in vitro* regenerated *Aloe vera* L. whole leaf samples, Sample IV - *in vitro* regenerated *Aloe vera* L. only gel samples, were assessed for all the three membrane preparations included goat RBC ghosts (Membrane Preparation I), goat liver homogenate (Membrane Preparation II), goat liver slices (Membrane Preparation III).

The effect of leaf extracts against the oxidant (H_2O_2) induced damage were evaluate in various membrane preparations. Three different membrane preparations were taken to study the extent of lipid peroxidation inhibition by the different extracts of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and inner gel. The different membrane models used for the study included goat RBC ghost i.e. Membrane Preparation I (plasma membrane devoid of intracellular membranes), goat liver homogenate i.e. Membrane Preparation II (a mixture of plasma membrane and internal membranes) and goat liver slices i.e. Membrane Preparation III (intact cells). These models differ from each other in their lipid composition and architecture.

The extant of *in vitro* lipid peroxidation in different membrane preparations by different extracts of *in vitro* and *in vivo* grown *Aloe vera* L. whole leaf and inner gel is represented in the Table 1-3. The methanol extract possessed the greater protection as it showed the maximum inhibition of lipid peroxidation in all the three lipid preparations compared to the other extracts. Chloroform extracts were showing comparatively lower lipid peroxidation inhibition followed by ethyl acetate, aqueous and then *n*-hexane extract.

Among the different samples tested, sample IV in every membrane preparation were quite higher in the inhibition of the lipid peroxidation compared to other sample including sample III, sample II and then sample I respectively (Table 1-3).

Protective effects of *Aloe vera* L. against lipid peroxidation (LPO) in goat RBC ghost (membrane preparation I)

As shown in the Table 1, for sample I (*in vivo* grown *Aloe vera* L. whole leaf samples), *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts were showing 33.07 ± 1.92, 50.24 ± 2.47, 47.85 ± 3.23, 54.18 ± 1.91 and 42.33 ± 2.28 percent inhibition of lipid peroxidation respectively in membrane preparation I.

While different extracts of the sample II (*in vivo* grown *Aloe vera* L. only gel samples) were showing the 40.21 ± 3.57, 62.28 ± 1.06, 55.20 ± 3.80, 69.83 ± 2.62 and 50.15 ± 3.32 percent inhibition of lipid peroxidation in membrane preparation I for *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts respectively (Table 1).

Simultaneously in the sample III (*in vitro* regenerated *Aloe vera* L. whole leaf samples) there were 45.77 ± 2.06, 68.20 ± 2.39, 58.16 ± 1.84, 74.42 ± 4.57 and 54.28 ± 3.17 percent inhibition of lipid peroxidation occurred for *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts respectively in membrane preparation I (Table 1).

At the same time protective effects of sample IV (*in vitro* regenerated *Aloe vera* L. only gel samples) against lipid peroxidation in membrane preparation I were 57.18 ± 2.38, 76.24 ± 3.38, 70.62 ± 1.58, 87.50 ± 3.42 and 64.39 ± 2.28 percent for *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts respectively (Table 1).

Protective effects of *Aloe vera* L. against lipid peroxidation (LPO) in goat liver homogenate (membrane preparation II)

As results depicted in the Table 2, for the sample I (*in vivo* grown *Aloe vera* L. whole leaf samples), percent inhibition of lipid peroxidation in membrane preparation II were 40.26 ± 4.83, 54.66 ± 1.16, 46.02 ± 3.54, 62.28 ± 2.39 and 44.19 ± 1.36 for *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts respectively.

For the sample II (*in vivo* grown *Aloe vera* L. only gel samples), the *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts were showing 51.84 ± 2.24, 68.02 ± 4.27, 60.42 ± 1.19, 77.53 ± 3.16 and 54.06 ± 2.04 percent inhibition respectively against lipid peroxidation in membrane preparation II (Table 2).

Likewise, with the sample III (*in vitro* regenerated *Aloe vera* L. whole leaf samples), the percent inhibition of lipid peroxidation in membrane preparation II were 53.39 ± 2.18, 75.45 ± 1.21, 66.57 ± 3.28, 83.24 ± 1.07 and 56.62 ± 2.34 for *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts respectively (Table 2).

Consequently, for the sample IV (*in vitro* regenerated *Aloe vera* L. only gel samples), the percent inhibition of lipid peroxidation in membrane preparation II were 65.04 ± 2.62, 87.33 ± 1.53, 78.39 ± 1.84, 93.30 ± 3.53 and 69.86 ± 1.54 for *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts respectively (Table 2).

Protective effects of *Aloe vera* L. against lipid peroxidation (LPO) in goat liver slices (membrane preparation III)

As shown in the Table 3, for the sample I (*in vivo* grown *Aloe vera* L. whole leaf samples), the *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts were showing 36.24 ± 2.24, 50.39 ± 4.78, 47.10 ± 3.12, 59.52 ± 2.18 and 37.36 ± 1.04 percent respectively, protection against lipid peroxidation in membrane preparation III.

Accordingly, for the sample II (*in vivo* grown *Aloe vera* L. only gel samples), the percent inhibition of lipid peroxidation in membrane preparation III were 48.32 ± 1.19, 62.36 ± 3.57, 57.14 ± 1.20, 74.57 ± 2.34 and 54.42 ± 3.28 with *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts respectively (Table 3).

Similarly, for the sample III (*in vitro* regenerated *Aloe vera* L. whole leaf samples), was showing the percent inhibition of lipid peroxidation in membrane preparation III ranging from 55.16 ± 2.62, 71.53 ± 1.43, 62.80 ± 2.68, 77.38 ± 3.33 and 58.09 ± 1.72 for *n*-hexane,

chloroform, ethyl acetate, methanol and aqueous extracts respectively (Table 3).

While at the same time for sample IV (*in vitro* regenerated *Aloe vera* L. only gel samples), the protective effects against lipid peroxidation by *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts were 62.03 ± 3.47, 83.28 ± 2.19, 76.57 ± 4.16, 89.91 ± 1.82 and 67.83 ± 2.36 percent respectively in the membrane preparation III (Table 3).

Among all the extracts including *n*-hexane, chloroform, ethyl acetate, methanol and aqueous extracts tested for their percent inhibition against lipid peroxidation in all the membrane preparations including membrane preparation I (goat RBC ghost), membrane preparation II (goat liver homogenate), membrane preparation III (goat liver slices), the methanol extract of all the samples including Sample I (*in vivo* grown *Aloe vera* L. whole leaf samples), Sample II (*in vivo* grown *Aloe vera* L. only gel samples), Sample III (*in vitro* regenerated *Aloe vera* L. whole leaf samples), Sample IV (*in vitro* regenerated *Aloe vera* L. only gel samples), were showing the maximum percent inhibition of lipid peroxidation followed by chloroform, ethyl acetate, aqueous and then *n*-hexane extract. Whereas, when compared among all the four samples then Sample IV was found to have greatest potential to inhibit the lipid peroxidation (LPO) in all the three membrane preparations, followed by Sample III, Sample II and then Sample I (Table 1-3).

Therefore, all the outcomes found in the current investigation indicated that the lipid components of both plasma membrane as well as intracellular membranes can be protected from lipid peroxidation by *Aloe vera* L. whole leaf and inner gel extracts. It implies that the leaf extract rendered the more or less equal extent of inhibition of lipid peroxidation in all the three membrane lipid systems. All the three systems exhibited substantial reduction in the peroxidation activity, though it was well evident in the liver homogenate followed by liver slices and then RBC ghosts. So, it was observed that the extent of protection was much better in the liver homogenate than in the other two membrane models. So that in the present study after determining the protective effects of *Aloe vera* L. against lipid peroxidation in various membrane preparations, it can be concluded that *Aloe vera* L. has a significant potential of protecting against oxidant induced peroxidation in lipid biomolecule (Table 1-3).

Table 1: Effect of different extracts of *Aloe vera* L. leaf samples on lipid peroxidation (LPO) in RBC ghosts (Membrane preparation I).

S. No.	Leaf Samples	% Inhibition of LPO				
		n-Hexane	Chloroform	Ethyl Acetate	Methanol	Aqueous
1	Sample I	$33.07 \pm 1.92^{a*}$	50.24 ± 2.47^{c}	47.85 ± 3.23^{b}	54.18 ± 1.91^{c}	42.33 ± 2.28^{b}
2	Sample II	40.21 ± 3.57^{b}	62.28 ± 1.06^{d}	55.20 ± 3.80^{c}	69.83 ± 2.62^{d}	50.15 ± 3.32^{c}
3	Sample III	45.77 ± 2.06^{b}	68.20 ± 2.39^{d}	58.16 ± 1.84^{c}	74.42 ± 4.57^{e}	54.28 ± 3.17^{c}
4	Sample IV	57.18 ± 2.38^{c}	76.24 ± 3.38^{e}	70.62 ± 1.58^{e}	87.50 ± 3.42^{f}	64.39 ± 2.28^{d}

The values are given as Mean ± SD of triplicates
*Values having different letters in superscript in each column are significantly different from each other (P≤0.05) according to Duncan's Multiple Range Test (DNMRT).
Sample I – *In vivo* grown *Aloe vera* L. whole leaf samples
Sample II – *In vivo* grown *Aloe vera* L. only gel samples
Sample III – *In vitro* regenerated *Aloe vera* L. whole leaf samples
Sample IV – *In vitro* regenerated *Aloe vera* L. only gel samples

Table 2: Effect of different extracts of *Aloe vera* L. leaf samples on lipid peroxidation (LPO) in goat liver homogenate (Membrane preparation II).

S. No.	Leaf Samples	% Inhibition of LPO				
		n-Hexane	Chloroform	Ethyl Acetate	Methanol	Aqueous
1	Sample I	40.26 ± 4.83^{a}	54.66 ± 1.16^{b}	46.02 ± 3.54^{a}	62.28 ± 2.39^{c}	44.19 ± 1.36^{a}
2	Sample II	51.84 ± 2.24^{b}	68.02 ± 4.27^{c}	60.42 ± 1.19^{c}	77.53 ± 3.16^{d}	54.06 ± 2.04^{b}
3	Sample III	53.39 ± 2.18^{b}	75.45 ± 1.21^{d}	66.57 ± 3.28^{c}	83.24 ± 1.07^{e}	56.62 ± 2.34^{b}
4	Sample IV	65.04 ± 2.62^{c}	87.33 ± 1.53^{e}	78.39 ± 1.84^{d}	93.30 ± 3.53^{f}	69.86 ± 1.54^{c}

The values are given as Mean ± SD of triplicates
*Values having different letters in superscript in each column are significantly different from each other (P≤0.05) according to Duncan's Multiple Range Test (DNMRT).
Sample I – *In vivo* grown *Aloe vera* L. whole leaf samples
Sample II – *In vivo* grown *Aloe vera* L. only gel samples
Sample III – *In vitro* regenerated *Aloe vera* L. whole leaf samples
Sample IV – *In vitro* regenerated *Aloe vera* L. only gel samples

Table 3: Effect of different extracts of *Aloe vera* L. leaf samples on lipid peroxidation (LPO) in goat liver slices (Membrane preparation III).

S. No.	Leaf Samples	% Inhibition of LPO				
		n-Hexane	Chloroform	Ethyl Acetate	Methanol	Aqueous
1	Sample I	36.24 ± 2.24[a]	50.39 ± 4.78[c]	47.10 ± 3.12[b]	59.52 ± 2.18[c]	37.36 ± 1.04[a]
2	Sample II	48.32 ± 1.19[b]	62.36 ± 3.57[d]	57.14 ± 1.20[c]	74.57 ± 2.34[e]	54.42 ± 3.28[c]
3	Sample III	58.16 ± 2.62[c]	71.53 ± 1.43[e]	62.80 ± 2.68[d]	77.38 ± 3.33[e]	58.09 ± 1.72[c]
4	Sample IV	62.03 ± 3.47[d]	83.28 ± 2.19[f]	76.57 ± 4.16[e]	89.91 ± 1.82[f]	67.83 ± 2.36[d]

The values are given as Mean ± SD of triplicates
*Values having different letters in superscript in each column are significantly different from each other (P≤0.05) according to Duncan's Multiple Range Test (DNMRT).
Sample I – *In vivo* grown *Aloe vera* L. whole leaf samples
Sample II – *In vivo* grown *Aloe vera* L. only gel samples
Sample III – *In vitro* regenerated *Aloe vera* L. whole leaf samples
Sample IV – *In vitro* regenerated *Aloe vera* L. only gel samples

DISCUSSION

Several plants have been shown to inhibit lipid peroxidation in various systems. There is very rich literature available about the studies and reports on the lipid protection ability of herbs and plant or plant parts in various lipid sources which strongly supports our findings in the present study. There are many investigators who have worked on the extraction of valuable constituents of medicinal use from the plants or herbal resources, because these are traditionally trusted, natural, having lesser side effects, cheaper and more accessible. Therefore, plant extracts and their isolated constituents have always been an important part of various therapeutic systems.[13] Reactive Oxygen Species (ROS) has the ability to degrade macromolecules such as lipid, nucleic acid and proteins, which finally leads to cell death. In the biological system, lipids are the immediate targets of oxidative moieties.[14]

Membrane lipids present in subcellular organelles are highly susceptible to free radical damage and cause peroxidation of polyunsaturated fatty acids in the membranes.[15] When reactive oxygen species attack polyunsaturated fatty acids on the cell membrane of living organisms in the presence of molecular oxygen, a chemical cascade is triggered. This eventually leads to the disintegration of fatty acids and the formation of malondialdehyde (MDA) which is called as lipid peroxidation (LPO).[16] MDA, the end product of lipid peroxidation, has also been demonstrated to be a mutagenic and genotoxic agent that can contribute to the development of human cancers.[17] The damage caused by LPO is highly detrimental to the functioning of the cell.[18] A similar finding was reported and postulated the involvement of an endogenous factor that acts in conjugation with the components of *Whithanis somnifera* to render a better protection to the intracellular membrane lipids.[19]

The ability of the herbal components to inhibit LPO, as a reflection of their protective antioxidant capacity has been a routine investigation in determining the antioxidant effects. Assessment of *Majorana hortensis* leaves against lipid peroxidation was done to determine their protective effects, also supporting our current investigation. Three solvent extracts including methanol, chloroform and aqueous were prepared for *Majorana hortensis* plant leaves and subjected to test the extent of inhibition of *in vitro* lipid peroxidation by using different lipid membrane preparations, namely RBC ghosts, goat liver homogenate and goat liver slices. All the three extracts caused a substantial decline in the extent of LPO in all the three membrane preparations. The decrease in LPO was more prominent in the liver homogenate. Among the three extracts used, the methanolic extract evinced a better protection in all three lipid preparations compared to the aqueous and chloroform extracts.[20]

The methanol extracts of *Cocculus hirsutus*,[21] *Rhus coriaria* L. fruits,[22] and *Triumfetta rhomboidei*.[23] showed a great protection against LPO *in vitro*. Whereas, the ethanol extract of the leaves of *Leucas cephalotes* decreased LPO in diabetic rats,[24] The methanolic extract of the plant *Hedyotis corymbosa* significantly reduced the accumulation of lipid peroxides *in vitro* in a dose-dependent manner in rat liver homogenate,[25] *Moringa oleifera* leaf extract was found to inhibit the amount of MDA generated (and thus LPO) in liver homogenate.[26]

The methanol and chloroform extracts of *Lysichiton camtschatcense* (L.) were potent inhibitors of LPO induced by Fe^{2+} and ascorbate in rat kidney and brain homogenate.[27]

It was found that Amifostine, triphosphate free oxygen scavenger showed significant inhibition of LPO in spinal cord homogenate.[28] While, the application of methanol extracts of the lichen species *Peltigera rufescans* (weis.) Humb, diclofenac and indomethacin were showing a decrease in the LPO in paw tissues in acute and chronic inflammation models.[29] Embelin (from *Embelia ribes*), a component of herbal drugs was found to inhibit LPO and restore impaired Mn-superoxide dismutase in rat liver mitochondria.[30] Gulcin.[31] reported that resveratrol was strongly inhibiting the LPO of linoleic acid emulsion. The flowers of the plant *Dendrobium nobile*

inhibited the *in vitro* LPO significantly in both liver homogenate and RBC ghosts.[32]

Quercitrin, a glycoside form of quercetin, was able to prevent the formation of TBARS induced by pro-oxidant agents.[33] The methanolic extract of *Aphanes arvensis* extract significantly reduced TBARS formation, indicating significant anti-lipid peroxidation in a concentration dependent manner and their inhibitory effects were comparable to the reference compound, Trolox.[34] Ascorbic acid showed a dose-dependent decrease in H_2O_2-induced LPO, which was assayed by measuring the TBARS in cultured peripheral human blood lymphocytes.[35] Polyphenols present in *Cinnamomum zeylanicum* and *Acacia catechu* were suggested to be predominantly responsible for the LPO inhibitory action and the antioxidant activity of the plants.[36]

In another study the aqueous and methanolic extracts of the tuberous roots of *Decalepis hamiltonii* was able to inhibited microsomal LPO.[37] A high degree of inhibition of LPO was shown by the polar and non-polar extracts of *Cyanthillium cinereum* (Less.) H. Rob.[38]

Lou *et al*.[39] reported that the burdock leaves fraction in combination with tertiary butylhydroquinone exhibited high LPO inhibitory activity. Several plant extracts have been shown to inhibit LPO as measured by the levels of TBARS, similarly, the aqueous extracts of *Clitoria ternatea*, *Solanum nigrum* and *Aloe vera* leaves were reported to inhibit LPO in goat liver slices.[40] The aqueous and methanolic extracts of *Phyllanthus niruri* were potent inhibitors of microsomal lipid peroxidation induced by Fe_2^+ and ascorbate *in vitro*.[41]

A water extract of *Selaginella* inhibited lipid peroxidation in rat liver. Polyphenols have also been shown to render protection against LPO in ethanol treated rats.[42] The methanolic extract of *Hedyotis corimbosa* significantly reduced the accumulation of lipid peroxides *in vitro* in a dose-dependent manner in rat liver homogenate.[43] A methanolic extract of Bay leaf showed that the inhibition of lipid peroxidation could be attributed to the presence of antioxidant phytochemicals.[2] Related work also done by some other workers on three different sources of membrane lipids including RBC ghosts (plasma membrane preparations), liver homogenate (mixture of plasma membrane and internal membranes) and liver slices (intact cells) were challenged with an oxidant assault and the effect of the three different extracts including methanolic, aqueous and chloroform extract of *Zea mays* leaf were studied on the damage inflicted to the lipid preparations. The *Zea mays* leaf extracts rendered strong protection to all the membrane model systems used. Methanolic and aqueous extracts inhibited LPO more effectively than the chloroform extract.[44]

Ethanol extract of *Pyrrosia petiolosa* exhibited strong antioxidant activity as reflected by their effectiveness in inhibiting lipid peroxidation.[45] Phenyl propanoid glycosides have been shown to inhibit lipid peroxidation and LDL oxidation.[46] *Achyrocline satureoides* extracts inhibit human LDL oxidation in three different systems (copper, peroxynitrite and lipoxygenase).[47] The extract of *Adhatoda vasica, Amaranthus paniculatus, Brassica compestris, Mentha piperita and Spirulina fusiformis* inhibited lipid peroxidation in liver.[48] Leaves of perilla [*Perilla frutescens* (L.) Britt. var. japonica (Hassk.) Hara] reversed the t-BHP-induced lipid peroxidation in rat livers.[49] A methanol extract of rhizomes of *Curculigo orchioides Gaertn* showed potent inhibition of lipid peroxidation induced by iron / ADP / ascorbate complex in rat liver homogenate.[50] Fenugreek (*Trigonella foenum-graecum* L.) extract inhibited LPO caused by cyclophosphamide and L-buthionine-SR-sulfoximine in the urinary bladder of mice.[51] The alcoholic bark extract of *Butea monosperma* possessed antioxidant properties, as reflected by its ability to reduce lipid peroxidation.[52]

The sea weeds were potent in suppressing TBARS formation by H_2O_2 induced lipid peroxidation in RBC.[53] The essential oil from the crushed fruits of *Chaerophyllum libanoticum* inhibited LPO assayed using ß-carotene bleaching and haemoglobin induced linoleic acid peroxidation.[54] The aqueous extracts of stem and flower of *Dendrobium nobile* showed a better *in vitro* lipid peroxidation activity by the measurement of thiobarbituric acid reactive substances (TBARS) in both liver homogenate and RBC ghosts.[32] Biomolecule-protective activity of the bacoside fraction from *Bacopa monnieri* in three different membrane systems including goat liver slices, goat liver homogenate and RBC ghosts were studied and the results of study showed that the bacoside fraction of *Bacopa monnieri* rendered a better protection to lipid molecules against oxidant-induced *in vitro* lipid peroxidation.[55]

The above discussed reports strongly support our findings, where in the leaf extracts were very effective in inhibiting lipid peroxidation (LPO) in the different membrane systems. Here all the three systems taken for the study are comprised of plasma membrane lipids. According to our observation, a better protection against LPO occurs in homogenate than in plasma membrane and intact cell, which make it clear that some endogenous component in the cells is involved. It implies that an endogenous factor in the cells, interacts synergistically with the leaf components to render better protection against lipid damage.

CONCLUSION

Aloe vera L. was found to show significant antioxidant potentials. Subsequently, evaluation of biomolecular protective effects of *Aloe vera* L. whole leaf and inner gel was accomplished in current study. All the outcomes found in the current investigation indicated that the lipid

components of both plasma membrane as well as intracellular membranes can be protected from lipid peroxidation by *Aloe vera* L. whole leaf and inner gel extracts. It implies that the leaf extract rendered the more or less equal extent of inhibition of lipid peroxidation in all the three membrane lipid systems. All the three systems exhibited substantial reduction in the peroxidation activity, though it was well evident in the liver homogenate followed by liver slices and then RBC ghosts. So, it was observed that the extent of protection was much better in the liver homogenate than in the other two membrane models. So that in the present investigation, after determining the protective effects of *Aloe vera* L. against lipid peroxidation in various membrane preparations, it can be concluded that *Aloe vera* L. has a significant potential of protecting against oxidant induced peroxidation in lipid biomolecule.

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