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# BIOMOLECULAR PROTECTIVE EFFECTS OF *BACOPA MONNIERI* (L.) PENNELL LEAF EXTRACTS AGAINST OXIDATIVE DAMAGE

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**Abstract**

In living systems, free radicals are constantly generated and they can cause extensive damage to tissues and biomolecules, leading to various disease conditions, especially degenerative diseases, and extensive lysis.The most effective way to eliminate free radicals which cause oxidative stress is with the help of antioxidants. Antioxidants prevent free radical-induced tissue damage by preventing the formation of radicals by scavenging them, or by promoting their decomposition. In the present study, the biomolecular protective effects of *B. monnieri* leaf extracts against oxidative stress induced damage to lipids and DNA in cell free systems and intact cells were analyzed. Results showed that the leaf extracts of *B. monnieri* rendered protection against oxidative stress induced damage in both cell-free systems and intact cells.

## Introduction

Oxidative stress is commonly defined as a disturbance in the prooxidant and antioxidant balance. It can result either from low levels of antioxidants and/or from increased production of reactive species. Oxidative stress significantly impacts multiple cellular pathways that can lead to the initiation and progression of varied disorders throughout the body (Maiese *et al*. 2010). Oxidative stress is considered as a major etiological and/or pathogenic agent of most degenerative diseases such as cancer, alzheimer's disease, diabetes and aging (Jang *et al*. 2010).

Antioxidants are naturally occurring chemicals present in the medicinal plants that can serve as a defense against free radicals, are used to help the human body in reducing oxidative damage by free radicals and active oxygen. Currently, research interest has been focussed on the role of antioxidants as well as antioxidant enzymes, in the treatment and prevention of many diseases (Raja and Pugalendi 2010). Antioxidants may guard against reactive oxygen species (ROS) toxicities by the prevention of ROS construction, by disruption of ROS attack, by scavenging reactive metabolites and converting them to less reactive molecules or by enhancing the resistance of sensitive biological target to ROS attack (Siddique *et al*. 2010). Recently, natural foods and derived antioxidants such as vitamins and phenol phytochemicals have received growing attention. This is because they are known to function as chemopreventive agents against oxidative damage (Oviasogie *et al*. 2009). Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, anti-inflammatory, antiallergic, antithrombic and vasodilatory activities. Antioxidant activity gives rise to anticarcinogenicity, antiimmunogenicity and antiaging activity (Gulcin *et al*. 2010). Hence, compounds, especially from natural sources, capable of protecting against ROS mediated damage may have potential application in the prevention and/or curing of diseases. The phytoconstituents present in different plant extracts are effective as radical scavengers and also act as inhibitors of oxidative stress induced biomolecular damage. Recent researches mainly focus on the identification of such plants with antioxidant and biomolecular protective effect.

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*Bacopa monnieri* (L.) Pennell, commonly known as "Brahmi" belonging to Scrophulariaceae (Anonymous 1997) has been used for centuries in folklore and traditional systems of medicine as a memory enhancer, antiinflammatory, analgesic, antipyretic, nerve tonic, cardiotonic, sedative and anti-epileptic agent (Russo and Borrelli 2005). The present study was formulated to study the protective effect of *B. monnieri* leaf extracts against oxidative stress induced damage to biomolecules such as membrane lipids and DNA.

**Materials and Methods**

Fresh leaves of *B. monnieri* were collected and 1g of them was homogenized in 10 ml of the solvents of varying polarity such as water, methanol, and chloroform. The organic extracts dried at 60°C were protected from light. The residue was weighed and dissolved in dimethyl sulfoxide (DMSO) to obtain the desired concentration. Fresh aqueous extracts were prepared and investigated.

The biomolecules (membrane preparations and DNA) were subjected to oxidative stress in the presence of the oxidant ($H_2O_2$), and the effect of the leaf extracts against this oxidant-induced damage was studied. The more susceptible biomolecule to oxidative stress is the lipid and the products of lipid peroxidation (LPO) are potential biomarkers for oxidative stress status *in vivo* and its related diseases. Hence, the biomolecule-protective effects of *B. monnieri* leaf extracts against lipid peroxidation were investigated. The extent to which the leaf extracts inhibited the process of lipid peroxidation was studied in three different membrane preparations. The different membrane models used were goat RBC ghosts (plasma membrane devoid of intracellular membranes), goat liver homogenate (a mixture of plasma membrane and internal membranes) and goat liver slices (intact cells). These models differed in their architecture and lipid composition. Oxidative damage of lipids by reactive species can be measured from the extent of formation of thiobarbituric acid reactive substance (TBARS) from the damaged lipids. Erythrocyte ghost membranes were prepared by osmotic lysis using the method of Dodge *et al*. (1963).

Goat liver procured fresh from the slaughter house was 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 (Okhawa *et al*. 1979). The goat liver collected fresh from the slaughter house was plunged into cold sterile PBS and maintained at 4ºC till use. Thin slices of 1mm thickness were cut using a sterile scalpel. The extent of inhibition of LPO in goat liver slices was estimated by the method proposed by Nichans and Samuelson (1968).

DNA damage caused due to oxidative stress plays a critical role in carcinogenesis. During oxidative stress *in vivo* or when ROS react with DNA *in vitro*, several types of DNA damages occur, including strand breaks and base lesions (Staruchova *et al*. 2008). The effect of the aqueous, methanol and chloroform extracts of *B. monnieri* leaves on the oxidant induced DNA damage was assessed in the present study both in cell-free systems and in intact cells.The extent of DNA damage was assessed *in vitro* in commercially available DNA preparations from different hierarchies and in intact cells. The commercially available preparations included λ DNA (linear phage DNA), pUC18 DNA (circular plasmid DNA), herring sperm DNA (genomic haploid DNA) and calf thymus DNA (diploid eukaryotic DNA). The DNA from intact cells was from human peripheral blood cells.The method described by Chang *et al*. (2002) was used to assess the DNA damage in λ DNA and pUC18 DNA.The extent of DNA damage in herring sperm and calf thymus DNA and the effects of *B. monnieri* leaf extracts were studied according to the method proposed by Aeschlach *et al*. (1994). The extent of DNA damage within single cells was determined by the method of Singh *et al*. (1988). The cells used were human peripheral blood cells. Whole blood

was used as the source of lymphocytes.All the parameters studied were analysed statistically using SigmaStat statistical package (Version 3.1). One-way analysis of variance was used to analyse the statistical difference.

**Results and Discussion**

The percent inhibition of *in vitro* lipid peroxidation by the leaf extracts in all the three membrane models namely, goat RBC ghosts (plasma membrane lipids), goat liver homogenate (plasma membrane and intracellular lipids) and liver slices (intact cells) is presented in Fig. 1. The results showed that all the three extracts caused a considerable decrease in the extent of LPO in all the membrane systems. The methanolic extract of the leaves showed a greater extent of protection to the different membrane models compared to the other two extracts. The inhibition was found to be higher in the goat liver homogenate followed by the RBC ghosts and liver slices. Since all the three systems comprise of plasma membrane lipids, the observation that a better protection occurs in homogenate than in plasma membrane and intact cell, 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. Thus, our results clearly demonstrate that the extracts of *B. monnieri* leaves are very effective in protecting membrane lipids against oxidative damage.

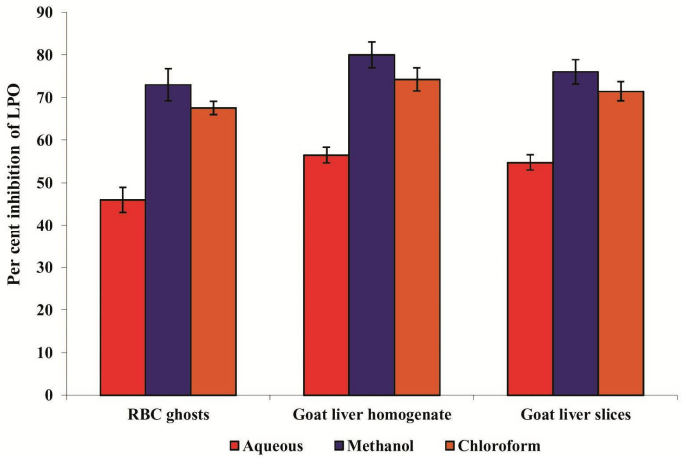


Fig. 1. Effect of *B. monnieri* leaf extracts on lipid peroxidation in different membrane preparations.

Effects of leaf extracts in inhibiting LPO in the different membrane systems were reported in many studies. Sreelatha and Padma (2009) reported that *Moringa oleifera* leaf extract inhibited the amount of MDA generated (and thus LPO) in liver homogenate. A high degree of inhibition of LPO was shown by the polar and non-polar extracts *of Cyanthillium cinereum* (Less.) H. Rob (Guha *et al.* 2009). Bavarva and Narasimhacharya (2010) reported that the ethanol extract of the leaves of *Leucas cephalotes* decreased LPO in diabetic rats.

The extent of damage to λ and pUC18 DNA induced by $H_2O_2$, both in the presence and the absence of the leaf extracts, was studied by viewing the migration pattern of the DNA in agarose gels. The results are presented in Plate 1 and the integrated density values of the bands are presented in Tables 1 and 2. It is clear from the picture that $H_2O_2$ caused a significant damage to both λ and pUC18 DNA as visualized by the absence of specific bands (Lane 2). This damage was

reverted by the *B. monnieri* leaf extracts, as indicated by the intact bands (Lanes 4, 6 and 8). The leaf extracts, by themselves, did not cause any DNA damage (Lanes 3, 5 and 7). The methanolic extract rendered the maximum protection.

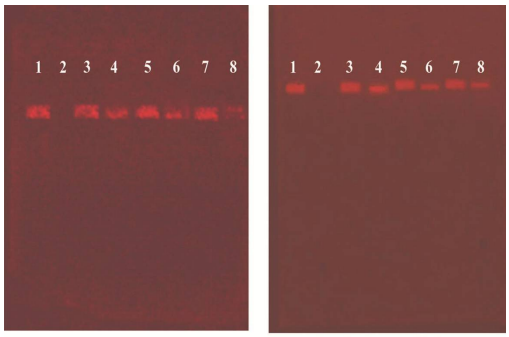


Plate 1. Migration pattern of λ DNA and pUC18 DNA treated with $H_2O_2$ with and without *Bacopa monnieri* leaf extracts.

**Table 1. Integrated Density Values (IDV) of the bands in the agarose gel of DNA fragmentation in λ DNA.**

| Sample | IDV of the bands | |
|---|---|---|
| | Control | $H_2O_2$ treated |
| No extract | 30240 | 3024 |
| Aqueous extract | 28054 | 26026 |
| Methanol extract | 29068 | 27972 |
| Chloroform extract | 28392 | 23184 |

**Table 2. Integrated Density Values (IDV) of the bands in the agarose gel of DNA fragmentation in pUC18 DNA.**

| Sample | IDV of the bands | |
|---|---|---|
| | Control | $H_2O_2$ treated |
| No extract | 24310 | 2156 |
| Aqueous extract | 21296 | 14560 |
| Methanol extract | 22825 | 18018 |
| Chloroform extract | 22440 | 17940 |

The extent of damage induced by $H_2O_2$ to herring sperm and calf thymus DNA is depicted in Fig. 2 and 3, respectively. The values of the $H_2O_2$-treated groups were fixed as 100 per cent and the relative values in percentage were calculated for the other groups. $H_2O_2$ exposure caused an

increase in TBARS production, which is indicative of DNA damage. On treatment with the leaf extracts, the damage was significantly reduced. The maximum reduction was rendered by the methanolic extract followed by the chloroform and aqueous extracts, in both the DNA samples tested. Hamad *et al*. (2010) showed that the *Aphanes arvensis* aqueous and methanolic extracts showed inhibitory effect on DNA oxidation. Guha *et al*. (2009) demonstrated that the aqueous extract of *Cyanthillium cinereum* (Less.) H. Rob. had a protective effect on pBR322 plasmid DNA against oxidative breakdown. Bitter cumin extract offered complete protection to DNA damage induced in calf thymus DNA and reduced uncoiling or open circular form of pUC18 DNA (Ani *et al*. 2006).

The extracts of *B. monnieri* leaves were found to offer very significant protection against oxidative damage in purified DNA samples under physiological conditions. Following this, the extent of DNA damage in the presence and the absence of the leaf extracts was followed in intact cells using the comet assay. Among the methods to measure oxidative damage to DNA, the comet assay has been shown to be the most accurate method for measuring DNA oxidation (Stoyanova *et al*. 2010). Untransformed cells (peripheral blood lymphocytes) were used to assess the oxidant-induced damage in intact cells using the comet assay. The results are presented in Table 3. On treatment with $H_2O_2$, the number of comet bearing cells was found to increase significantly indicating the oxidative damage manifested by the oxidant. All the three extracts were found to revert the damage to the basal (control) levels. The damage caused by the oxidant was completely nullified on exposure to the extracts. The methanolic extract was found to be better in protection when compared to the other two extracts. The results obtained with the purified DNA preparations showed that the damage could not be completely reverted by the leaf extracts. The fact that the same dose of leaf extracts could completely revert the damage to basal levels in the intact cells suggests the possible involvement of some endogenous cellular component that works in conjunction with the leaf components to protect DNA. A similar indication has also been obtained from the results with the inhibition of LPO in different membrane preparations.

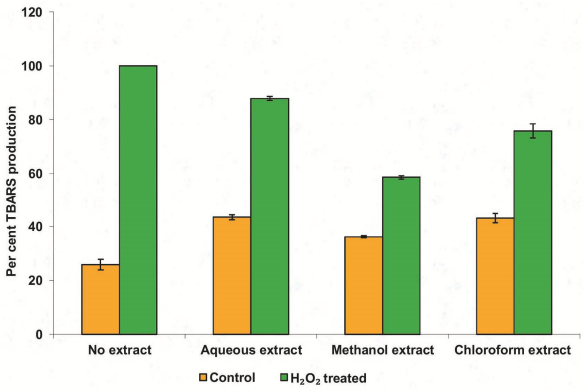


Fig. 2. Effect of *B. monnieri* leaf extracts on oxidative damage induced in herring sperm DNA. The values of the positive control ($H_2O_2$ treated) group were fixed as 100% damage and the per cent damage in the other groups were calculated relative to this.

Jang *et al*. (2010) have shown that the solvents (acetone, ethanol and methanol) extracts of calyx and seed of *Diospyros kaki* exhibited the greatest protective effect on $H_2O_2$ induced DNA damage in human leukocytes. *Acorus calamus* extract effectively protected DNA from radiation induced strand breaks and enhanced the DNA repair process in murine cells and human peripheral blood leukocytes (Sandeep and Nair 2010). Garlic extracts efficiently enhanced the ability of

normal human leukocytes to resist hydrogen peroxide and 4-hydroxynonenal induced oxidative damage (Park *et al*. 2009).

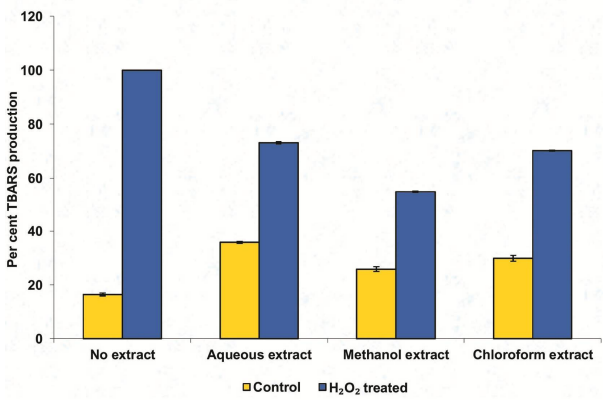


Fig. 3. Effect of *B. monnieri* leaf extracts on oxidative damage induced in calf thymus DNA
The values of the positive control ($H_2O_2$ treated) group were fixed as 100% damage and the per cent damage in the other groups were calculated relative to this.

**Table 3. Effect of *B. monnieri* leaf extracts on oxidative DNA damage induced in human peripheral blood cells.**

| Sample | No. of comet bearing cells / 100 cells | |
|---|---|---|
| | Control | $H_2O_2$ treated |
| No extract | 7 ± 1 | 33 ± 3[a] |
| Aqueous extract | 8 ± 1 | 7 ± 1[b] |
| Methanol extract | 7 ± 2 | 4 ± 1[abc] |
| Chloroform extract | 8 ± 2 | 9 ± 1[b] |

Values are mean ± SD of triplicates, LSD (5%) = 2.548, a - Statistically significant ($p<0.05$) compared to untreated control, b - Statistically significant ($P<0.05$) compared to $H_2O_2$ alone treated group, c - Statistically significant ($p<0.05$) compared to respective plant extract treated group

The results of the assays probing the extent of protection rendered by *B. monnieri* leaf extracts to lipids and DNA strongly suggest the synergistic involvement of some, as yet unidentified, endogenous factor in the target cells that acts in conjunction with the plant components to greatly reduce the extent of oxidative damage. Thus, the study showed that *B. monnieri* leaves are an excellent source of naturally occurring antioxidant compounds with potent lipid and DNA damage inhibiting potential. Among the three different extracts tested for their protective effects, the methanolic extract rendered the maximum protection in cell-free system and intact cells.

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