Bacopa monniera Linn. as an antioxidant: Mechanism of action

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Bacopa monniera, Linn. (Brahmi: Scrophulariaceae) an Ayurvedic medicine is clinically used for memory enhancing, epilepsy, insomnia and as mild sedative. For the first time the effect of alcohol and hexane fraction of Brahmi has been studied on FeSO₄ and cumene hydroperoxide induced lipid peroxidation. Alcohol fraction showed greater protection with both inducers. Results were compared with known antioxidants tris, EDTA and a natural-antioxidant vitamin E. The effect of Brahmi was also examined on hepatic glutathione content. The mechanism of action could be through metal chelation at the initiation level and also as chain breaker. The results suggested that Brahmi is a potent antioxidant. The response of Brahmi was dose dependent. Tris, an hydroxyl trapped did not show any protection in comparison to Brahmi where as EDTA and vitamin E did protect against FeSO₄. In experimental conditions 100 μg Brahmi extract (alcoholic) was equivalent to 247 μg of EDTA (0.66 mM) and 58 μg of vitamin E. Interestingly Brahmi only slightly protected the autooxidation and FeSO₄ induced oxidation of reduced glutathione on lower doses 100 μg/ml and below, but on higher concentrations it enhanced the rate of oxidation.

Bacopa monniera, Linn. (Scrophulariaceae) commonly known as ‘Brahmi’ or Jalanima is a component of several popular drugs of the Ayurvedic system of medicine. It belongs to a special branch of Ayurvedic medicine known as Medhya rasayana. Traditionally, various preparations of Brahmi were used for memory enhancing, epilepsy, insomnia and as mild sedative. In the Ayurvedic literature it is also recommended as anti-inflammatory, analgesic, anti-pyretic, mehaghna, vishaghna, apasmaarahara etc. It has been reported that Brahmi prevents the rate of depletion of blood acetylcholine level in aged human population. Phytochemical investigations have shown the presence of alkaloids, saponins, D-mannitol, betulinic acid, β-sitosterol, stigmasterols etc from the whole plant of Brahmi.

Different class of phytochemicals have been shown to have antioxidant property e.g. alkaloids: strychnine, eugenol, withaperuvin, norberginine, benzylisoquinoline, flavonoids: sylbin, anthraquinones: rubiadin, emodin, steroids etc. are a few examples of natural product antioxidants. All the above reports and presence of some of these phytochemicals in this plant tempted us to investigate the antioxidant property and mechanism of action of Brahmi extract, because in most of the diseases, described above free radicals play an important role.

In this report we have investigated its antioxidant property in albino rats, in vitro. The effect of alcoholic extract of Brahmi on lipid peroxidation, induced by cumene hydroperoxide and ferrous sulphate in liver homogenate has been studied. These effects have been compared with hexane fraction of Brahmi (a non-polar fraction). The efforts have been made to explain the mechanism of action whether it acts at the initiation or at termination level or as a chain breaker in free radical chain reaction. Its kinetic study on the protection of reduced glutathione content in liver homogenate has also been studied.

Materials and Methods

Chemicals—Thiobarbituric acid (TBA), trichloroacetic acid (TCA), ferrous sulphate and acetic acid were purchased from Central Drug House (Pvt) Ltd. Cumene hydroperoxide (CHP), 1,1,3,3-tetra ethoxy propane (TEP), reduced glutathione (GSH), 5,5'-Dithio-bis(2-Nitrobenzoic acid) (DTNB) were obtained from Sigma Chemical Co., St. Louis Mo. USA. All other reagents were of analytical grade.

Experimental Design—Normal albino rats (100-150g body wt) of Charles Foster strain were randomly selected and kept on fast overnight. Next day animals were anaesthetized by pentobarbitone (3 mg/100g body wt, ip). Liver was exposed and perfused with phosphate buffer saline, pH 7.4 (20 ml, through hepatic portal vein). Blood free liver was taken out and homogenized in a glass teflon homogenizer (10%, w/v).

Preparation of the extract and drug doses—Fresh Brahmi/Jalanimb was collected from a pond and dried in shade. It was compared with the standard
sample preserved in the University Department of Dravyagun, Institute of Medical Sciences. It was powdered and extracted with hexane and ethanol separately. Extracts were evaporated under low pressure by using Buchi type rotary evaporator. The concentrated extract was kept in vacuum desiccator till the constant weight of solvent free extract was attained. The extracts were mixed with tween-80 and diluted with distilled water to get the final ratio of water : tween 80 as 9:1. The same solution was used as drug vehicle in control plates.

**Effect on Lipid Peroxidation**—Two sets of experiments were performed. In one set lipid peroxidation was induced by adding cumene hydroperoxide (1.5mM)\(^1\) and in other set peroxidation was induced by adding 0.5 mM FeSO\(_4\) in water\(^2\). 3 ml of 5% liver homogenate was taken to each 35 mm petridish. In the control dishes only inducers were added to see the extent of maximum induction and in the experimental dishes Brahmi extract (both alcoholic and hexane fractions) and other pharmacological tools (Tris, EDTA, vitamin E) were added with the inducers to evaluate their protective effect. The plates were incubated at 37\(^\circ\)C for 20 min in the case of cumene hydroperoxide and for 30 min with FeSO\(_4\) to obtain the optimum induction of lipid peroxidation. At the end, 100 \(\mu\)l of incubation mixture was taken out from each plate to estimate the concentration of TBARS (thiobarbituric acid reactive substances) by using the standard method of Yagi et al.\(^3\) as described earlier\(^4\).

**Estimation of glutathione content (GSH)**—10% liver homogenate (3 ml) was used. In the experimental plates, different concentrations of Brahmi extract and 3 mM FeSO\(_4\) were simultaneously added and in the control plates only FeSO\(_4\) was added. At different time intervals, 250 \(\mu\)l aliquots were taken out of each plate to estimate GSH content as described earlier\(^5\) by using DTNB (0.01%) as per Ellman’s method\(^6\). Absorbance was recorded at 412 nm in Jasco Spectrophotometer.

**Statistical evaluation**—The results given here are the mean±SD of 6 separate experiments. Level of significance has been evaluated by using Student ‘t’ test.

**Results**

**Effect of Brahmi on lipid peroxidation**—Both fractions of Brahmi showed the reduction in lipid peroxidation induced by cumene hydroperoxide and ferrous sulphate. The degree of depletion was more with the alcoholic extract (ED\(_{50} 100 \mu\)g, 177 \(\mu\)g) as compared with hexane fraction (ED\(_{50} 290 \mu\)g, 400 \(\mu\)g) on ferrous sulphate and cumene hydroperoxide induced lipid peroxidation respectively (Table 1).

**Comparison of protective effect of Brahmi extract (alcoholic) with EDTA, Tris and vitamin E**—EDTA, a strong metal chelator\(^7\) inhibited the FeSO\(_4\) (0.5 mM) induced lipid peroxidation in the dose dependent manner (ED\(_{50} 247 \mu\)g) (Fig. 1A). In similar conditions tris (pH, 6.8), an established hydroxyl trap\(^8\) failed to inhibit FeSO\(_4\) induced peroxidation (Fig. 1B). Addition of vitamin E, a natural antioxidant in increasing concentration inhibited lipid peroxidation (ED\(_{50}\), 58 \(\mu\)g) (Fig. 1C). The alcoholic extract of Brahmi also inhibited the peroxidation in similar conditions in the dose dependent manner (ED\(_{50} 100 \mu\)g) (Fig. 1D). This shows that Brahmi extract possesses such phytochemicals which might have metal chelating property also.

**Effect of the alcoholic extract of Brahmi on reduced glutathione content**—In the lower concentration range, Brahmi extract had marginal inhibitory effect on the rate of aerial oxidation as well as FeSO\(_4\) induced oxidation of reduced glutathione content whereas at higher concentration there was marked enhancement in the rate of depletion in the reduced glutathione content (Table 2).

**Discussion**

In the living system lipid peroxidation is induced by free radicals and reactive oxygen species\(^9\). These moieties are continuously generated in the normal physiological processes as well as under the influence of external factors. It is an outcome of a chain reaction. The primary radicals are the superoxides which are further converted to hydrogen peroxide. If these products are not scavenged or converted to less reactive forms, then hydroxyl, peroxy, singlet oxygen etc. are produced which attack the unsaturated bond of the macromolecules and ultimately damage the cell\(^10\). Transition metals are also involved in initiating

<table>
<thead>
<tr>
<th>Dose ((\mu)g/ml)</th>
<th>FeSO(_4) (0.5 mM)</th>
<th>CHP (1.5 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alcoholic</td>
<td>Hexane</td>
</tr>
<tr>
<td>0</td>
<td>446.54±8.19</td>
<td>446.54±8.19</td>
</tr>
<tr>
<td>25</td>
<td>307.17±6.97*</td>
<td>389.65±7.82*</td>
</tr>
<tr>
<td>50</td>
<td>253.13±5.64*</td>
<td>341.30±5.69*</td>
</tr>
<tr>
<td>100</td>
<td>224.67±7.13*</td>
<td>318.55±6.01*</td>
</tr>
<tr>
<td>200</td>
<td>190.56±7.22*</td>
<td>247.44±6.59*</td>
</tr>
<tr>
<td>400</td>
<td>150.74±5.31*</td>
<td>187.71±5.63*</td>
</tr>
<tr>
<td>800</td>
<td>110.92±6.42*</td>
<td>125.14±5.32*</td>
</tr>
<tr>
<td>1200</td>
<td>065.41±5.11*</td>
<td>088.17±5.73*</td>
</tr>
<tr>
<td>ED(_{50}) 100 (\mu)g</td>
<td>290 (\mu)g</td>
<td>177 (\mu)g</td>
</tr>
</tbody>
</table>

\(\text{P}<0.001\)
lipid peroxidation\textsuperscript{24}. Broadly, lipid peroxidation can be enzymatic and non-enzymatic\textsuperscript{25}.

Antioxidants are specific to the species of reactive oxygen\textsuperscript{26}. Some of them enhance the endogenous defense enzymes involved in metabolizing these moieties, some directly interact with superoxides, singlet oxygen, hydrogen peroxides and hydroxyl radicals such as carotene, \(\alpha\)-tocopheral, tris, mannitol\textsuperscript{27}. In addition, there are many agents which chelate the transition metals and inhibit the formation of free radicals at the initiation level itself\textsuperscript{28}.

From the present data it is clear that hexane and alcoholic extract of \textit{Brahmi} reduce lipid peroxidation induced by \(\text{FeSO}_4\) and cumene hydroperoxide which have different mechanisms. This indicated that

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Time} (min) & \textbf{FeSO}_4 (3.0 mM) & \textbf{Brahmi} (25 \(\mu\)g) & \textbf{Brahmi} (100 \(\mu\)g) & \textbf{Brahmi} (800 \(\mu\)g) \\
\hline
5 & 0.548 ± 0.010 & 0.624 ± 0.013\textsuperscript{a} & 0.583 ± 0.009\textsuperscript{a} & 0.477 ± 0.013\textsuperscript{a} \\
10 & 0.456 ± 0.010 & 0.506 ± 0.015\textsuperscript{b} & 0.473 ± 0.005\textsuperscript{a} & 0.262 ± 0.011\textsuperscript{a} \\
20 & 0.306 ± 0.015 & 0.339 ± 0.016\textsuperscript{b} & 0.328 ± 0.016\textsuperscript{b} & 0.166 ± 0.009\textsuperscript{a} \\
30 & 0.219 ± 0.009 & 0.262 ± 0.004\textsuperscript{b} & 0.233 ± 0.013\textsuperscript{a} & 0.131 ± 0.013\textsuperscript{a} \\
40 & 0.144 ± 0.008 & 0.184 ± 0.012\textsuperscript{b} & 0.148 ± 0.012\textsuperscript{a} & 0.102 ± 0.001\textsuperscript{b} \\
50 & 0.091 ± 0.008 & 0.133 ± 0.009\textsuperscript{b} & 0.115 ± 0.013\textsuperscript{b} & 0.085 ± 0.012\textsuperscript{b} \\
60 & 0.079 ± 0.012 & 0.108 ± 0.006\textsuperscript{b} & 0.095 ± 0.003\textsuperscript{d} & 0.075 ± 0.009\textsuperscript{d} \\
\hline
\end{tabular}
\caption{Effect of \textit{Brahmi}-alcoholic extract on hepatic glutathione content in presence of \textit{FeSO}_4. [Values, expressed as mg/g of tissue, are mean ± SD of 6 animals in each group.]

\textit{P} values: \textsuperscript{a} < 0.001; \textsuperscript{b} < 0.01; \textsuperscript{c} < 0.02; \textsuperscript{d} < 0.05; \textsuperscript{eNS}}
\end{table}

Fig. 1—Effect of (A) EDTA, (B) Tris, (C) Vitamin E and (D) \textit{Brahmi} on lipid peroxidation in rat liver homogenate. The compounds are added at the concentrations indicated. The control value was 446.54 ± 8.19 nmol TBARS/100 mg protein. Results are mean ± SD, \(n = 6\) rats.
Brahmi extract acted at the initiation level by chelating Fe$^{++}$ ion, not through hydroxyl radicals as in the case of Haber Weiss/Fenton reaction$^{29}$. This conclusion is further supported by the results on tris which failed to inhibit FeSO$_4$ induced lipid peroxidation. Under similar conditions, EDTA inhibited lipid peroxidation by chelating ferrous ion. From the dose response curve it appears that 0.66 mM of EDTA (247 µg) was equivalent to 100 µg of the alcoholic extract of Brahmi in our experimental conditions. The results obtained from Brahmi has been compared with Vitamin E, a well known natural chain breaking antioxidant$^{30}$. It converts free radical to a non-radical product by donating its hydroperoxide and reduces the TBARS release. From the present data, it is difficult to say whether Brahmi also acts as a chain breaker but one aspect seems to be clear that it does not act like tris which is only a hydroxyl trap.

The effect of Brahmi on glutathione content showed an interesting result. Unlike other medicinal plants$^{6-31-33}$, it failed to maintain the level of reduced glutathione both in the case of aerial oxidation and in the presence of FeSO$_4$. At lower doses it showed slight protection of GSH but at higher doses it enhanced the rate of oxidation. Thus long term treatment, divided in low doses may be recommended for clinical use rather than single high dose. Similar observations have been made by Dubey et al$^4$, in their clinical trial report. Thus it might be concluded that the anti-lipid peroxidative property of Brahmi is not through the regulation of glutathione content, as there is depletion in glutathione level at higher doses of drug without affecting lipid peroxidation.

Brahmi, a known drug for memory enhancing and for sedation also possesses anti-lipid peroxidative property in general. It might serve as a medicine for aging and several nervous disorders because free radical are involved in these pathologies$^{34}$.

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