RADIOPROTECTIVE AND ANTIOXIDANT PROPERTIES OF INDIAN MEDICINAL PLANT, Terminalia arjuna

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Abstract

Using rat liver mitochondria as model systems, we examined the radioprotective and antioxidant effects of the Indian medicinal plant Terminalia arjuna. Various soxhlet fractions of the bark and the medicinal formulation arjunsal besides an active ingredient, baicalein, were examined for their ability to protect both rat liver mitochondria and cardiac homogenate against radiation and oxidative stress apart from their ability to scavenge radicals and for ferric reducing/antioxidant power. Among the various extracts from arjunsal the ones from organic solvents were found to be the most effective in DPPH, ABTS and FRAP assays. In T. arjuna bark, the methanolic extract showed the highest antioxidant activity. Radioprotection studies also showed that the methanolic extract was the most effective. Baicalein showed antioxidant as well as radioprotective activity. To look at the possible mechanisms for the observed antioxidant/radioprotective effects pulse radiolysis was performed to examine the reactions of baicalein with various radiation-related and biologically relevant reactive species such as hydroxyl radical (•OH), azide radical (N3•), lipid peroxy radical (LOO•), trichloro methyl peroxy radical (CCl3O2•) and thiyl (RS•) radical. Baicalein reacts with these radicals at almost diffusion controlled rates. The bimolecular rate constants for the reaction of these radicals were in the order of 10^9 dm^3 mol^-1 s^-1. The above results indicate that various preparations from T. arjuna and its component baicalein have significant radioprotective and antioxidant activities and the ability to react with radiation-derived or radiation-related reactive species may be the factor responsible.

Introduction

In an aerobic environment, all animals and plants require oxygen and hence reactive oxygen species (ROS) are ubiquitous. It is established that excess generation of ROS is involved in structural alterations of cellular molecules leading to cytotoxicity and cell death. This eventually results in a variety of biological phenomena such as mutation, carcinogenesis, ageing, radiation or UV exposure, inflammation, ischemia-reperfusion injury, atherosclerosis, diabetes mellitus and neurodegenerative disorders (Yoshikawa et al, 2000).

Exposure to physical and chemical agents including ionizing radiation can result in excess ROS generation. Low LET (low energy transfer) radiation such as γ-rays can cause damage through ROS generation. The living systems comprised of aqueous media are prone to impairment due to radiolysis of water yielding hydroxyl radical (•OH), hydrated electrons (e^-aq), hydrogen peroxide (H2O2), etc. In presence of
oxygen, other reactive species such as superoxide radical \( (\text{O}_2^-) \), singlet oxygen \( (\text{O}_2) \) are also produced. In biological systems, cellular membranes are made up of polyunsaturated fatty acids (PUFA). They are highly prone to damage by ROS by process known as lipid peroxidation, which drastically alters the biomembranes structurally and functionally. It also generates highly toxic byproducts which can act at places away from their site of generation. Another important type of damage to cellular membranes can be induced by peroxyl radicals (ROO\(^{\cdot}\)). This reactive species is predominant in LOOH-dependent lipid peroxidation. 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) on thermal decomposition in presence of oxygen gives rise to peroxyl radicals which can initiate the chain of lipid peroxidation.

Among the subcellular organelles, mitochondria are crucial sites for energy generation and ATP synthesis by tetravalent reduction of oxygen by mitochondrial cytochrome oxidase. There are also important sites of ROS generation (such as \( \text{O}_2^- \)). Besides affecting ATP synthesis, it can compromise energy transduction by faultily synthesized proteins or lead to DNA fragmentation etc. (Yoshikawa et al, 2000). The oxidative damage to mitochondria can also lead to membrane permeability transition, cytochrome c release and dysfunction of mitochondria associated with decrease in membrane potential, respiratory control, apoptosis etc. (Yoshikawa et al, 2000). Hence the mechanistic study of membrane damage induced by ROS in relation to various human ailments and its prevention by antioxidants from natural dietary sources is very important.

There are several Indian medicinal plants known for their beneficial therapeutic effects which also might have antioxidant properties (Tilak et al, in press). *Terminalia arjuna* is one of these plants credited for its cardiotonic and cardioprotective properties. The bark of the tree is used in ‘Ayurvedic’ system of medicines for over three centuries, primarily as cardiac tonic besides cure for haemorrhages, fractures, diarrhoea, ulcers and acne (Kirtikar and Basu, 1984). It has also been known to possess antimutagenic, anti-ischemic, hypcholesterolemic, cardioprotective, and antioxidant abilities. In the present study, the radical scavenging activities and membrane protective abilities of *T. arjuna* extracts, as well as baicalein, its active ingredient, were examined.

### Materials And Methods

#### Materials

Ascorbic acid, aluminum chloride, 2,2'-azobis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS) diammonium salt, \( \beta \)-phycoerythrin, 1,1'-diphenyl-2-picrylhydrazyl (DPPH), ethylene diamine tetra acetic acid (EDTA), ferric chloride, Folin-Ciocalteu reagent, hydrogen peroxide, methylene blue, myoglobin, potassium ferricyanide, potassium phosphate (monobasic and dibasic), sodium carbonate, \( \alpha \)-tocopherol, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 1,1,3,3-tetraethoxypropane, 2,4,6-tripyridyl-s-triazine (TPTZ), 2-thiobarbituric acid and trichloroacetic acid were purchased from Sigma Chemical Co., U.S.A. 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH) was from Aldrich Chemical Co., U.S.A. Other chemicals used in our studies were of the highest quality commercially available from local suppliers.

#### Methods

*Preparation of the Arjunsal extracts:* ‘Arjunsal’ is an herbal preparation of *T. arjuna*, known for its cardiotonic properties which is commercially available. In this study we have used two different extracts of arjunsal, using aqueous and mixture of organic solvents such as acetone, chloroform, methanol, isopropyl alcohol and water. Both the extracts were prepared by stirring the total extract of arjunsal in the respective solvents at room temperature for 2 hrs.
Preparation of T. arjuna bark extracts: The Soxhlet extracts of T. arjuna were prepared by taking the bark free of dust and other impurities. It was sun-dried and finely powdered. Depending on the increasing order of polarity of the solvents, sequential extraction was done using benzene, chloroform, acetone, methanol and methanol-HCl using Soxhlet apparatus. The bark powder was extracted with each solvent for 8-10 hours to remove the soluble matter. The aqueous extract was prepared by stirring the bark powder in distilled water for 4 hours and then filtering it.

Isolation of mitochondrial fraction from rat liver: Three months old female Wistar rats (weighing about 250 ± 20 g) were used for the preparation of mitochondria (Devasagayam et al., 1986). In brief, rat livers were homogenized in 0.25 M sucrose containing 1 x 10^{-3} mol dm^{-3} EDTA. The homogenate was centrifuged at 3000 x g for 10 min to remove cell debris and the nuclear fraction. The resultant supernatant was centrifuged at 10,000 x g for 10 min to sediment mitochondria. This pellet was washed thrice with 5 mM potassium phosphate buffer, pH7.4, to remove sucrose. Protein was estimated and pellets were suspended in the above buffer at the concentration of 10 mg protein/ml.

Exposure of rat liver mitochondria to radiation and agents for inducing oxidative stress: Oxidative damage was induced by exposure to γ-rays from a {sup 60}Co source (dose rate 65 Gy/min, BARC, Mumbai). The mitochondria (final concentration 2 mg/ml) were suspended in 5 mM phosphate buffer (pH 7.4) and exposed to radiation with and without extracts or baicalein. The dose selected was 450 Gy at which optimum damage was obtained. The unexposed samples served as controls.

Peroxyl radical-induced lipid peroxidation was observed using azobis-amidinopropane hydrochloride. In the system for treating mitochondria to AAPH, mitochondria (final concentration 0.2 mg/ml) were incubated with AAPH (final conc. 10 mM) at 37°C for 30 min in a shaker-water bath with continuous bubbling of oxygen.

Biochemical assays

After exposure of mitochondria to oxidative stress, the products of lipid peroxidation were measured as lipid hydroperoxides (LOOH) and thiobarbituric acid reactive substances (TBARS). The lipid hydroperoxides were measured by using FOX (Ferrous Oxidation in Xylenol orange) II method. FOX II reagent contains 90% (v/v) methanol, which facilitates lipids to solubilize. Concentration of LOOH is then calculated with the help of standard graph using H_2O_2. FOX II gives ε for hydroperoxides as 4.46 x 10^4 M^{-1}cm^{-1}. TBARS were measured immediately after the treatment (Devasagayam, 1986). On addition of TBA reagent comprising of 0.5% thiobarbituric acid, 10% trichloroacetic acid, 2 mM EDTA, 0.63 M hydrochloric acid and the samples were boiled for 20 min. The pink coloured TBARS formed were estimated spectrofluorometrically using excitation at 532 nm and emission at 553 nm, after accounting for appropriate blanks. Malonaldehyde standard was prepared by the acid hydrolysis of tetramethoxypropane. The superoxide dismutase (SOD) activity was also estimated. Data were presented as mean ± SE. Significance of inter-group differences was determined by Student’s t test (two-tailed). A p value of p < 0.05 was considered statistically significant.

Ferric Reducing Antioxidant Power (FRAP) assay

The ferric complexes reducing ability of the extracts by FRAP assay were measured at low pH. The stock solutions of 10 x 10^{-3} mol dm^{-3} TPTZ in 40 x 10^{-3} mol dm^{-3} HCl, 20 x 10^{-3} mol dm^{-3} FeCl_3, 6H_2O and 0.3 mol dm^{-3} acetate buffer (pH 3.6) were prepared. The FRAP reagent contained 2.5 ml TPTZ solution, 2.5 ml ferric chloride solution and 25 ml acetate buffer. It was prepared freshly and warmed to 37°C.
Then, 900 µl of FRAP reagent was mixed with 90 µl of D/W and 30 µl of test sample/methanol/DW/standard solutions. The reaction mixture was then incubated at 37°C for 30 min and absorbance was recorded at 595 nm. The concentration of FeSO$_4$ was in turn plotted against concentrations of the standard antioxidants (L-ascorbic acid and Trolox).

**DPPH radical scavenging assay**

Determination of the scavenging effect on DPPH was carried out with different extracts. In this method a commercially available and stable free radical DPPH, which is soluble in methanol, was used. In its radical form, DPPH has an absorption band at 515 nm, which disappears on reduction by an antioxidant compound. The calibration curve was plotted with % DPPH$^+$SCAENGED versus concentration of the standard antioxidants (L-ascorbic acid and Trolox).

**ABTS radical scavenging assay**

In the spectrophotometric assay, the inhibition of radical formation by the extracts was determined by using the ferrylmyoglobin/ABTS$^+$ protocol. The calibration curve was plotted with lag time in seconds versus concentration of the standard antioxidants (L-ascorbic acid and Trolox).

**Pulse radiolysis studies**

The pulse radiolysis system using 7 MeV electrons has been described earlier (Mukharjee, 1997). The dosimetry was carried out using an air-saturated aqueous solution containing 5x10$^{-2}$ mol dm$^{-3}$ KSCN ($\varepsilon = 23,889$ dm$^3$ mol$^{-1}$ cm$^{-1}$ per 100 eV at 500 nm). The kinetic spectrophotometric detection system covered the wavelength range from 250 to 800 nm. The optical path length of the cell was 1.0 cm. The width of the electron pulse was 50 ns and the dose was 16 Gy per pulse. Alkaline pH was obtained by adding NaOH only. High purity (> 99.9 %) N$_2$O, from BOC India Pvt. Ltd. was used.

**Results**

Fig. 1 presents data on the antioxidant properties of arjunsal extracts as assessed by standard assays for measurement of inhibition of radical formation and radical scavenging. The aqueous extracts were compared to water-soluble antioxidant, ascorbic acid (AEAC), while the solvent extracts were expressed in terms of Trolox (TEAC), an ethanol soluble standard antioxidant, equivalent.

![Fig. 1: Antioxidant abilities of arjunsal extracts with a) FRAP, b) DPPH scavenging and c) ferrylmyoglobin /ABTS assays](image-url)
The results of FRAP assay were presented in Fig. 1a. The aqueous and organic solvent extracts of arjunsal were used with the concentrations ranging from 0.05% to 0.5%. Both the extracts show concentration dependent increase in their ferric reducing capacities. The organic solvent extract was more potent (TEAC-0.24) than aqueous one (TEAC-0.04). Fig 1b presented the data on DPPH radical scavenging abilities, while Fig.1c showed the data on inhibition of formation of ABTS radical measured by ferrylmyoglobin/ABTS assay. Among the arjunsal extracts, the organic solvent extracts were superior to the aqueous ones.

The data on antioxidant properties of T. arjuna extracts were presented in Fig.2. Three different concentrations of T. arjuna were used i.e. 0.05, 0.1 and 1% and concentration dependent effect of the extracts in terms of their antioxidant potential was observed. Fig. 2a showed the data on ferric reducing activities as assessed by FRAP assay. In this, methanolic extract possessed the highest ferric reducing property (TEAC-0.37) followed by methanolic-HCl, acetone, aqueous and chloroform. The results of DPPH radical scavenging were presented in Fig. 2b, showing methanolic-HCl extract to be the most potent scavenger (TEAC-0.7) followed by methanolic, aqueous, acetone and chloroform. Fig.2c showed the data on ferrylmyoglobin/ABTS assay, in which methanolic extract was highly significant (TEAC-0.34) in inhibiting formation of ABTS radical.

Aqueous, methanolic-HCl, acetone and chloroform extracts also showed significant antioxidant activities in the decreasing order.

The data on radiation- and AAPH-induced lipid peroxidation products and their inhibition by T. arjuna (final concentration 0.1 %) extracts were shown in Table 1. A dose of 450 Gy resulting in a significant increase in peroxidation was selected for the studies. Mitochondria were exposed to 450 Gy with and without T. arjuna extracts. Methanolic extract was observed to be the most potent in protection against lipid peroxidation by inhibiting TBARS formation by 100 % and LOOH formation by 90 %. This was followed by methanolic-HCl, acetone and aqueous extracts of T. arjuna. In case of AAPH-induced TBARS and LOOH formation again methanolic one was found to be the most significant protector with 82 and 72 % protection respectively, followed by methanolic-HCl, aqueous and acetone extracts of T. arjuna.
The data on the effect of baicalein on radiation- and AAPH-induced oxidative damage in terms of LOOH formation in rat liver mitochondria were shown in Table 2. When mitochondria were exposed to γ-rays at 450 Gy with and without baicalein, there was a significant enhancement in LOOH formation at the dose of 450 Gy. Baicalein at concentrations of 5 and 10 µM, was observed to possess a significant ability to inhibit LOOH formation induced by both radiation and AAPH. When exposed to radiation, the inhibition by baicalein of 5 and 10 µM were 48 % and 68 % respectively. The enhanced formation of LOOH owing to radiation exposure at 450 Gy was effectively reduced by baicalein even at 5 µM. Lowering of LOOH was also observed with baicalein at concentration of 5 and 10 µM on AAPH treatment in rat liver mitochondria.

Table 1: Effect of T. arjuna extracts on radiation and AAPH-induced formation of TBARS and LOOH in rat liver mitochondria.

<table>
<thead>
<tr>
<th>TBARS (nmoles/mg protein)</th>
<th>LOOH (nmoles/mg protein)</th>
<th>TBARS (nmoles/mg protein)</th>
<th>LOOH (nmoles/mg protein)</th>
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<tbody>
<tr>
<td>Control</td>
<td>0.550 ± 0.134</td>
<td>2.98 ± 0.12</td>
<td>0.888 ± 0.134</td>
</tr>
<tr>
<td>Treatment</td>
<td>3.217 ± 0.374</td>
<td>18.66 ± 3.54</td>
<td>4.163 ± 0.222</td>
</tr>
<tr>
<td>Treatment + Aqueous</td>
<td>0.834 ± 0.123</td>
<td>10.96 ± 1.82</td>
<td>1.284 ± 0.295</td>
</tr>
<tr>
<td>Treatment + Methanolic-HCl</td>
<td>0.417 ± 0.177</td>
<td>3.22 ± 3.974</td>
<td>0.400 ± 0.144</td>
</tr>
<tr>
<td>Treatment + Methanolic</td>
<td>0.167 ± 0.286</td>
<td>4.48 ± 1.93</td>
<td>1.450 ± 0.117</td>
</tr>
<tr>
<td>Treatment + Acetone</td>
<td>0.934 ± 0.112</td>
<td>4.09 ± 1.04</td>
<td>0.917 ± 0.057</td>
</tr>
<tr>
<td>Treatment + Chloroform</td>
<td>1.022 ± 0.543</td>
<td>3.51 ± 1.25</td>
<td>1.300 ± 0.148</td>
</tr>
<tr>
<td>Treatment + Benzene</td>
<td>0.700 ± 0.215</td>
<td>5.94 ± 2.25</td>
<td>2.817 ± 0.247</td>
</tr>
</tbody>
</table>

TBARS- thiobarbituric acid reactive substances; LOOH- lipid hydroperoxides

Values are mean ± SE from 4 different experiments.

* p < 0.001 compared with radiation treatment.

Table 2: Effect of baicalein on radiation and AAPH-induced formation of LOOH in rat liver mitochondria

<table>
<thead>
<tr>
<th>LOOH (nmoles/mg protein)</th>
<th>γ-radiation exposure</th>
<th>AAPH treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.73 ± 0.32</td>
<td>4.58 ± 0.43</td>
</tr>
<tr>
<td>Treatment</td>
<td>25.52 ± 1.72</td>
<td>16.49 ± 1.82</td>
</tr>
<tr>
<td>Treatment + 5 µM baicalein</td>
<td>15.08 ± 0.81</td>
<td>10.44 ± 0.49</td>
</tr>
<tr>
<td>Treatment + 10 µM baicalein</td>
<td>10.70 ± 1.44</td>
<td>9.63 ± 1.07</td>
</tr>
</tbody>
</table>
Table 3: Effect of baicalein on radiation and AAPH-induced formation of superoxide dismutase (SOD) activity in rat liver mitochondria

<table>
<thead>
<tr>
<th></th>
<th>SOD (Units/mg protein)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>γ-radiation exposure</td>
</tr>
<tr>
<td>Control</td>
<td>2.27 ± 0.15</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.24 ± 0.22</td>
</tr>
<tr>
<td>Treatment + 1 µM baicalein</td>
<td>1.30 ± 0.36</td>
</tr>
<tr>
<td>Treatment + 5 µM baicalein</td>
<td>1.56 ± 0.21</td>
</tr>
<tr>
<td>Treatment + 10 µM baicalein</td>
<td>1.88 ± 0.25</td>
</tr>
<tr>
<td>Treatment + 50 µM baicalein</td>
<td>2.05 ± 0.45</td>
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</table>

SOD- superoxide dismutase

Values are mean ± SE from 4 different experiments.

* p < 0.001 compared with respective radiation treatment.

Superoxide dismutase activity was significantly reduced by radiation exposure as well as AAPH treatment as shown in Table 3. As with lipid peroxidation baicalein caused significant inhibition of damage. It also significantly protected the enzyme against oxidative damage in a concentration dependent manner. At concentrations of 1, 5, 10 and 50 µM, the protection against damage was 52, 65, 80 and 89% respectively. With AAPH treatment the percentage protection by baicalein were 61, 79, 81 and 87 % for 1, 5, 10 and 50 µM concentrations. Thus increasing protection was observed with increasing concentrations of baicalein.

The ability of baicalein to scavenge ROS generated by radiolysis of water was demonstrated using pulse radiolysis. Baicalein has significant abilities to react with OH, with a bimolecular rate constant of \(3.7 \times 10^9\) dm\(^3\) mol\(^{-1}\) s\(^{-1}\). The studies also reveal that with azide radical it gives the rate constant of \(1.34 \times 10^9\) dm\(^3\) mol\(^{-1}\) s\(^{-1}\). Azide radical (N\(_3\)) is highly selective and reacts with phenolic compounds by univalent oxidation giving rise to aroxyl (phenoxy) radical. Since the transient absorption spectra for OH and N\(_3\) radicals were similar, it is evident that baicalein reacts with these radicals by single electron transfer reactions. Our study also shows that baicalein also has a fairly high rate constant with a model peroxyl radical i.e. trichloromethylperoxyl radical (\(8.5 \times 10^7\) dm\(^3\) mol\(^{-1}\) s\(^{-1}\)). Linoleic acid peroxyl radical is very relevant in biological systems and baicalein forms transient with LOO\(^{\cdot}\) at 100 µs at 450 nm.

**Discussion**

Many plants are known to have beneficial therapeutic effects as noted in the traditional Indian system of medicine, *Ayuveda*. However, they have received little attention for their radioprotective as well as antioxidant activities. Medicinal plants can protect against harmful effects of ionizing radiation. Natural plant extracts or pure compounds are safe ingredients, which do not have any toxic effects. *T. arjuna* is a well-known cardiotonic, cardio protective plant and used in India for centuries. It is used as a cure for various heart disorders such as congestive heart failure, coronary artery disease, myocardial necrosis, angina, atherosclerosis and ischemia-reperfusion injury besides having hypolipidaemic activity. It was shown that 500 mg capsule of bark extract is a safe and effective remedy for patients suffering from refractory congestive heart failure. Because of its high
calcium content it is given in all types of hemorrhages. When given with honey, it is known to promote union of fractures. The other beneficial properties include anti-pyretic, anti-allergic, antimutagenic, antigenotoxic, antitumor, chemo preventive and antioxidant effects. Therefore the aim of the present study was to evaluate radioprotective and antioxidant effects of *T. arjuna* and its active ingredient baicalein.

In biological systems, oxidative stress *in vitro* can be generated using various physical/chemical agents. Among them, ionizing radiation such as γ-rays is an important source. The exposure of biological systems to radiation results in radiolytic cleavage of water yielding \( \text{OH} \), \( \text{H} \), \( e_{aq} \) etc. In presence of oxygen even \( \text{O}_2^- \), \( \text{H}_2\text{O}_2 \), \( 1\text{O}_2 \) are also produced. Thermal decomposition of an azo-initiator (AAPH) in presence of oxygen gives rise to a constant source of peroxy radicals. These free radicals can initiate lipid peroxidation. In this paper, we have demonstrated that ROS induce significant lipid peroxidation in the model system i.e. rat liver mitochondria as measured by LOOH, an unstable intermediate, which further breaks down to stable aldehydes and react with thiobarbituric acid (TBA) to form TBARS, the final stable end product (Girotti, 1990). Apart from enhancing lipid damage, radiation and AAPH treatments lead to inactivation of antioxidant enzymes such as superoxide dismutase (SOD).

Our results have shown that methanolic and aqueous extracts of *T. arjuna* as well as baicalein, when present during radiation exposure, can prevent the damage to the cell membranes. At a concentration of 1 mg/ml, methanolic Soxhlet extract of *T. arjuna* could prevent against lipid damage in terms of TBARS and LOOH formation. Whereas, at 1 mg/ml, methanolic-HCl Soxhlet extract of *T. arjuna* significantly inhibited the formation of TBARS and LOOH when present during the AAPH-induced damage.

Plants are complex mixtures of compounds and no single compound can provide the observed activity. Plant extracts can be characterized by polyvalent formulations and interpreted as additive, or, in some cases, potentiating (Kulkarni, 1997). The exact mechanism of action of *T. arjuna* is not known. However, it can scavenge free radicals produced by γ-radiation and other sources of oxidative stress. Thus it reduces the radiation-induced damage to cellular biomolecules including genome. Our studies with the bark of *T. arjuna* show that the observed cardioprotective effects may be due to the antioxidant properties seen at different levels.

Majority of flavonoids in *T. arjuna* including baicalein are soluble in methanol, and are capable of protecting against oxidative damage by scavenging free radicals. Hence free radical scavenging capacities were measured by standard assays like DPPH and ferrylmyoglobin/ABTS assays. The ferric reducing capacities were also determined using FRAP assay. The trolox/ascorbic acid equivalent antioxidant capacities were calculated and *T. arjuna* as well as arjunsal extracts possessed fairly high T/AEAC scores. If LDL oxidation plays a key role in pathogenesis of atherosclerosis, then its inhibition by antioxidants should prevent atherosclerosis (Halliwell and Gutteridge, 1997; Yoshikawa et al, 2000). Considering the activities of free radicals and concentrations of substrates, the phenolic compounds from natural sources are promising candidates for drugs for atherosclerosis, depending on their reactivity towards free radicals, localization, mobility in lipoprotein and fate of its radicals. Baicalein is a naturally occurring flavone from the medicinal plant *T. arjuna*. Our results show that baicalein possess significant antioxidant properties in terms of radical scavenging and ferric reducing activities efficiently protects mitochondrial membrane against radiation damage. These
observations suggest that baicalein is a potent radioprotector in biological systems.

The significant Trolox equivalent antioxidant capacity (TEAC) values of the extracts in ferric reducing and radical scavenging assays might be responsible for inhibition of lipid peroxidation. Thus it can protect against radiation induced cell death. *T. arjuna* has been reported to possess antioxidant, anti-lipoperoxidative and anti-radical properties. *T. arjuna*’s active constituents include tannins, triterpenoid saponins, gallic acid, ellagic acid, oligomeric proanthocyanidines, phytosterols, Ca, Mg, Zn, Cu besides flavonoids such as arjunone, arjunolone, luteolin, baicalein etc. (Anonymous, 1999). Most of these compounds have been reported to possess antioxidant activities (Ratty and Das, 1988). Further baicalein has also been reported to have antioxidant properties (Gao et al., 1996; Shieh et al., 2000). Therefore antioxidant properties of *arjunsal*, *T. arjuna* extracts and baicalein observed earlier and in the present study might have contributed to the observed radioprotective action in the present study.

**References**


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Dr S. Adhikari joined BARC Training School in 1990 (34th batch). After successful completion of the one-year orientation course, he joined the then Chemistry Division in 1991. Since then he has been involved in studying radiation chemistry biologically important molecules in microheterogeneous media. He is the recipient of the prestigious IUPAC (International Union of Pure and Applied Chemistry) Prize for Young Chemists. His recent research interest is the free radicals induced damage in bio-systems and the mechanistic aspect of the antioxidant activity of natural antioxidants.