Differential Antioxidant Effects of Plumbagin in Rat Tissues

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Abstract

Oxidative stress has been implicated in the etiology of a number of human ailments. Hence, antioxidants, especially derived from natural sources and capable of protecting against damage induced by reactive oxygen species (ROS/RNS) may have potential applications in prevention and/or cure of the disease. Indian medicinal plants provide a rich source of these potentially useful compounds. Plumbago zeylanica (known as ‘chitrak’) and its constituents are credited with potential therapeutic properties including antiatherogenic, cardioprotective, hepatoprotective and neuroprotective properties. Plumbagin (2-methyl-5-hydroxy, 1,4-naphthoquinone), isolated from the root of this plant was considered as the active ingredient. To examine the possible antioxidant activity of plumbagin in relation to its reported beneficial properties, its ability to protect against oxidative damage in mitochondria from rat tissues was studied, by incorporating it in mitochondria during isolation. Mitochondria from rat liver, brain and heart were exposed to ROS generated by ascorbate–Fe²⁺, H₂O₂–Fe²⁺ and the peroxyl radical generator, 2,2′-Azobis(2-methylpropionamide) dihydrochloride (AAPH). The parameters of damage assessed were products of lipid peroxidation, loss of mitochondrial enzymes and protein oxidation. Our results indicate that plumbagin, gave protection to different extents in the tissues examined. This differential ability can be ascribed to the presence of various amounts of substrates for oxidative reactions and antioxidants/prooxidants in these tissue preparations. The possible mechanisms involved were examined by pulse radiolysis. Upon exposure to radiation-derived hydroxyl radical there was formation of plumbagin radical, with absorption at 390 nm. The formation kinetics of this radical reveal a fairly high rate constant of plumbagin with hydroxyl radical in the range of 2 x10³ M⁻¹ s⁻¹. In conclusion, our studies reveal that the membrane protective properties of plumbagin, in mitochondria, may be related to its radical scavenging abilities and that this antioxidant ability, may at least in part, explain its potential therapeutic properties.

Introduction

Enhanced generation of reactive oxygen/nitrogen species (ROS/RNS) and the ensuing phenomenon in the form of oxidative stress have been implicated in the etiology of a large number of human ailments including the major forms such as cardiovascular diseases, neurodegenerative diseases and cancer (Sies, 1996; Thomas and Kalyanaraman, 1997; Payne, Bernstein et al., 1999). Antioxidants due to their ability to neutralize the toxic free radicals or damage induced by them have potential applications in the prevention and/or therapy of human ailments (Sies, 1996; Rao and Agarwal, 2000; Cuzzocrea, Riley et al., 2001). Natural compounds especially derived from dietary sources or medicinal plants and having antioxidant abilities may have the dual benefits of dietary component/therapeutic agent as well as that of neutralizing the reactive species (Mantle, Lennard et al., 2000; McDermott, 2000). India has a rich
heritage of possessing ancient systems of medicine for treating illnesses in the form of ‘Ayurveda’, ‘Siddha’ and ‘Unani’. These have identified a large number of plants with potential therapeutic efficacies (Lele, 1999; Tripathi, 2000; Okamoto and Hino, 2000; Lele, 2001).

Among these medicinal plants, Plumbago zeylanica (Chitrak) has been credited with therapeutic properties to treat several diseases (Kirtikar and Basu, 1984; Thakur, Puri et al., 1989; Sharma, Singh et al., 1991a; Oyedapo, 1996). These include digestive diseases such as dysentry, rubefacient in rheumatism, lumbago, paralytic affections, leucoderma, piles, bronchitis, anemia, liver diseases, obesity, anasarca, dyspepsia and leprosy. The plant preparations are also being used for the treatment of arthritis, epilepsy, hysteria and other diseases connected with the nervous system besides for bacterial, microbial and helminth infections. It has also been useful in increasing appetite. In combination with other plants it also has been used in antihepatotoxic herbal formulations.

The root contains several bioactive chemical constituents which include plumbagin, 3-chloroplibagin, drosorone, chitrane, zeylanone, isozyelenone, plumbazeylone, coumarin, elliptinone, triterpenoids, β-sitosterol, maritinone, 2-methylnaphthazarin and anthroquinones (Gunaherath, Gunatilaka et al., 1988; Dinda and Saha, 1989; Thakur, Puri et al., 1989; Dinda, Hajra et al., 1997; Gupta, Siddiqui et al., 2000). Plumbagin is a naphthoquinone and is a major component constituting about 0.03% of dry weight of the roots and is considered as the active ingredient responsible for therapeutic effects (Gupta, Verma et al., 1993). Besides it has a strong germicidal action. In small doses it stimulates muscular tissue of heart, intestines and uterus. It has a stimulant action on nervous system and possesses cardiotonic, hypolipidaemic, wound healing, antithersclerotic, anticoagulant, antifungal, antibacterial, antitumor, antimutagenic and antifertility properties (Premakumari, Rathinam et al., 1977; Kirtikar, Basu et al., 1984; Bhargava, 1986; Itoigawa, Takeya et al., 1991; Sharma, Gusain et al., 1991b; Durga, Sridhar et al., 1992; Sugie, Okamoto et al., 1998). The mechanisms of action of plumbagin, however, are not fully understood. The detailed studies on its possible antioxidant properties also have not been carried out. The present study attempts to fulfill this lacuna.

Various ROS, generated in vivo by endogenous systems, can significantly alter subcellular components and induce different forms of oxidative damage. Among the subcellular organelles mitochondria are among the crucial ones that are highly susceptible for oxidative damage (Salet and Moreno, 1990; Munday, Sriratana et al., 1996). Both lipids and proteins are susceptible to ROS induced damage. The protection afforded by antioxidants differs in tissues like brain, liver and heart, depending on their composition (Pushpendran, Subramanian et al., 1998). These tissues also have implications in the medicinal properties credited to plumbagin. Hence, in this study we have examined the in vitro oxidative damage to mitochondria isolated from rat liver, brain and heart induced by different endogenous model systems such as ascorbate-Fe³⁺, peroxyl radicals and hydroxyl radical besides the protection afforded by plumbagin. To examine possible mechanisms involved we have studied the reaction of plumbagin with hydroxyl radical by pulse radiolysis.

**Materials and Methods**

**Materials**

1,2,4-Aminonaphtholsulphonic acid (ANSA), adenosine triphosphate, ammonium molybdate, ascorbic acid, dichlorophenol indophenol, dinitrophenyl hydrazine, ethylene diamine tetraacetic acid (EDTA),
ferrous sulphate, guanidine hydrochloride, perchloric acid, phenazine methosulphate, sodium cyanide, succinate, 2-thiobarbituric acid and trichloroacetic acid were purchased from Sigma Chemical Co., U.S.A. 2,2'-Azobis (2-aminopropane) dihydrochloride (AAPH) (=2,2'-Azobis(2-methylpropionamide) dihydrochloride) was from Aldrich Chemical Co., U.S.A. Tetraethoxypropane was used as the standard for estimating malonaldehyde equivalents. Other chemicals used in our studies were of the highest quality commercially available from local suppliers.

Methods

Isolation of plumbagin from roots of *P. zeylanica*

380 gms of fresh roots of *P. zeylanica* were extracted (Soxhlet) with petroleum ether three times at 60 to 80 °C. All the extracts were mixed and evaporated by rotavapor. The concentrate was dissolved in minimum amount of distilled petroleum ether and the crystalline compound with a yield of 0.03% was obtained. The purity of this preparation was checked by thin layer chromatography (TLC) and gas liquid chromatography (GLC).

Isolation of mitochondrial fraction

Three months old female Wistar rats (weighing about 250 ± 20 g) were used for the preparation of mitochondria (Kamat, Boloor et al., 2000). In brief, rat liver, brain and hearts were excised, homogenized in 0.25 M sucrose containing 1 mM 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 3000 x g for 10 min to sediment mitochondria. This pellet was washed thrice with 0.15 M Tris-HCl buffer, pH 7.4, to remove sucrose. Protein was estimated and pellets were suspended in the above buffer at the concentration of 10 mg protein/ml. For incorporating plumbagin, it was dissolved in ethanol at a concentration of 10 mM, added to the mitochondrial pellet before the last washing, homogenized for 1 min at low speed and then the pellet was sedimented by centrifugation. The concentration of plumbagin in mitochondria was calculated by its selective absorption at 267 nm after sonication of mitochondria to release the compound incorporated inside this organelle.

Exposure of mitochondria to agents for inducing oxidative stress

Oxidative damage was induced by ascorbate-Fe²⁺-system as described previously (Kamat and Devasagayam, 1996). The procedure for ascorbate-Fe²⁺ induced lipid peroxidation was that of Hammer and Wills (1978) as modified by Devasagayam (1986). The incubation mixture (0.5 ml) contained: (i) 700 µl of basic medium (0.15 mM Tris-HCl, pH-7.4), (ii) 100 µl of FeSO₄ in 0.1 N HCl (final concentration 50 µM), (iii) 100 µl ascorbic acid (final concentration of 0.4 mM) and (iv) 100 µl of mitochondrial sample. Incubations were carried out at 37 °C in a shaker-water bath. After the incubations, pink coloured thiobarbituric acid reactive substances (TBARS) formed were estimated at 532 nm spectrophotometrically as malonaldehyde equivalents after accounting for appropriate blanks. Malonaldehyde standard was prepared by the acid hydrolysis of tetraethoxypropane. Peroxyl radical induced lipid peroxidation was examined using AAPH by incubation at 37 °C (Kamat & Devasagayam, 1995). Oxidative damage in mitochondria was also induced using hydroxyl radicals, generated by H₂O₂-Fe²⁺ system by using 150 µM H₂O₂ and 150 µM Fe²⁺ (final concentrations) in HEPES buffer, pH 7.4.

Biochemical assays

The total ATPase activity (Quigley and Gotterer, 1969) and succinate dehydrogenase activity (Caplan and Greenwalt, 1968) were estimated by standard methods. Succinate dehydrogenase activity was expressed as units and one unit is defined as one µmole of dichlorophenol indophenol reduced/min/mg protein (Sajan, Satav et al. 1995). Protein oxidation products were estimated as protein carbonyls (Palamanda and Kehrer, 1992). Appropriate sample blanks were taken to
ensure that plumbagin does not interfere with the assay methods. Statistical analysis of the data was done using Student's 't' test.

**Determination of rate constant by pulse radiolysis studies**

To study the reaction of plumbagin with 'OH, pulse radiolysis technique was employed. 7 MeV electrons from linear accelerator of pulse widths 50 ns were used and the transients were detected by kinetic spectrophotometry. Typical maximum doses with 50 ns pulses were 15 Gy (Das and Priyadarsini, 1994). The dosimetry was performed with air saturated 0.01 mol dm$^{-3}$ KSCN solution with a $G_{e}$ (500 nm) value of $2.23 \times 10^{-4}$ M$^{-1}$s$^{-1}$ for (SCN)$^{−}$ transient species. The kinetic spectrophotometric detection system covered the wavelength range from 280 to 800 nm. Cells with optical path length of 1 cm were used for these measurements. For pulse radiolysis measurements the absorbed dose was kept to a minimum to avoid decomposition of the test compound and the samples were changed after every pulse to minimize losses due to sample decomposition. The reactions of plumbagin ($1 \times 10^{-4}$ mol dm$^{-3}$, pH 6.7) with 'OH were carried out using N$_2$O-saturated aqueous solution where $e_{aq}$ is quantitatively converted to 'OH ($N_2O + e_{aq}$ $\rightarrow$ 'OH + OH$^−$ + N$_2$). The bimolecular rate constants were calculated by plotting pseudo first order rate of formation of the transient against the concerned solute concentration. The uncertainty in the measurement in bimolecular rate constant is ±10%.

**Results**

Fig. 1a presents data on the comparative potentials of the three different tissues, brain, liver and heart to ascorbate-Fe$^{2+}$ induced lipid peroxidation. The lipid peroxidation in the brain was much higher than other tissues (almost 3 times than liver and 10 times that of heart, at 10 min of incubation). The increase in lipid peroxidation as a function of time also started very early in the brain. The lipid peroxidation induced by AAPH and H$_2$O$_2$-Fe$^{2+}$ was quite low as compared to that induced by ascorbate-Fe$^{2+}$ (Fig. 1b). Heart showed relatively high potential for peroxidation with AAPH, almost similar to that of brain. With H$_2$O$_2$-Fe$^{2+}$ induced lipid peroxidation also, maximum damage was seen in brain while the extent of damage in liver and heart were
much less and almost similar. The differential susceptibilities of the three tissues can be due to varied composition of substrate for lipid peroxidation as well as the relative amounts of different antioxidants.

The effects of plumbagin, added in ethanol, on lipid peroxidation in rat liver and brain mitochondria are shown in Fig. 2. In liver, addition of plumbagin showed a concentration dependent inhibition of lipid peroxidation (Fig. 2a). The inhibition amounted to 79.44 % with 100 μM, 95.27 % with 500 μM and 69.23 % with 2.5 mM. In brain, the pattern of alteration was different (Fig. 2b). With 100 and 500 μM there were enhancements of lipid peroxidation by 10.43 % and 5.42 % respectively. At a higher concentration of 2.5 mM, on the other hand, it showed inhibition of lipid peroxidation (49.26 %).

The values are ± S.E.M. from 4 experiments. * P < 0.01 and ** P < 0.001, as compared to respective controls.
Fig 3 shows the extent of inhibition observed when plumbagin was incorporated into mitochondria from the three tissues during their isolation. The inhibition observed in rat liver mitochondria (Fig. 3a) with 156 μM was very prominent and amounted to 96.35 % at 5 min (P<0.01), 85.58 % at 30 min (P<0.01), and 91.97 % (P<0.01) at 60 min. The inhibition by plumbagin (138 μM) in brain mitochondria (Fig. 3b) was lower amounting to 56.32 % at 5 min, 64.68 % at 15 min, 54.65 % at 30 min and 53.20 % at 60 min. In heart mitochondria (Fig. 3c) too the incorporated plumbagin showed a significant inhibition (P<0.001), amounting to 68.7 % at 60 min of incubation.

The effects of incorporated plumbagin on lipid peroxidation induced by H_2O_2-Fe^{2+} in different tissues are shown in Fig 4. In both liver and brain (Fig 4a and Fig 4b), plumbagin did not show any significant effect over the entire incubation period of 60 min. However, in rat heart mitochondria, plumbagin incorporation showed significant inhibition of lipid peroxidation. Similar inhibition by plumbagin incorporation was also seen with AAPH-induced lipid peroxidation in heart but not in other tissues (Table 1). In liver, however, there was no significant effect.

Figs. 5 and 6 show the effect of plumbagin on the levels of two mitochondrial enzymes namely ATPase and succinate dehydrogenase (SDH). Plumbagin did not influence the levels of total ATPase altered by both ascrobate-Fe^{2+} and H_2O_2-Fe^{2+} in rat liver mitochondria (Fig 5a & 5b). The effect of plumbagin on AAPH induced oxidative damage of SDH is shown in Fig 6a and Fig 6b. Since ascorbate interfered with SDH assay, we could not ascertain the effect of plumbagin incorporation on ascrobate-Fe^{2+} induced damage to SDH. In both liver and brain mitochondria, plumbagin was able to significantly protect SDH against oxidative damage.
Table 1: Effect of 'incorporated plumbagin' on AAPH-induced lipid peroxidation in rat liver and heart mitochondria.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>nmoles TBARS/mg protein</th>
<th>Control</th>
<th>Plumbagin</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>60 min</td>
<td>0 min</td>
</tr>
<tr>
<td>Liver</td>
<td>0</td>
<td>0.26 ± 0.02</td>
<td>0</td>
</tr>
<tr>
<td>Heart</td>
<td>0</td>
<td>0.43 ± 0.02</td>
<td>0</td>
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</tbody>
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Fig. 5: Effect of incorporated plumbagin on ascorbate-Fe2+ induced (Fig. 5a) and H2O2-Fe3+ induced (Fig. 5b) oxidative damage on ATPase in rat liver mitochondria. The values are ± S.E.M. from 4 experiments. *P< 0.001 as compared to respective controls.

Fig. 6: Effect of incorporated plumbagin on AAPH induced oxidative damage on succinate dehydrogenase in mitochondria of rat liver (Fig. 6a) and brain (Fig. 6b). The values are ± S.E.M. from 4 experiments. # P < 0.05, * P< 0.01 and *P< 0.001 as compared to respective controls.
Effect of plumbagin on protein oxidation induced by ascorbate-Fe$^{2+}$ in liver and brain is shown in Fig. 7. The ascorbate-Fe$^{2+}$ system induced significant protein oxidation in both rat liver and brain mitochondria. Plumbagin showed a significant protection in liver (56.19% at 60 min, P<0.01).

![Fig. 7: Effect of incorporated plumbagin on ascorbate-Fe$^{2+}$ induced oxidative damage on protein oxidation in rat liver mitochondria. The values are ± S.E.M. from 4 experiments. * P< 0.01 as compared to respective controls.](image)

![Fig. 8: Transient absorption spectra obtained from a N$_2$O-saturated aqueous solution of pH 6.7 containing 1 x 10$^{-4}$ mol dm$^{-3}$ plumbagin. Inset: Absorbance versus time plot for (a) formation and (b) decay of the plumbagin radical formed during the reaction with OH radical.](image)

Based on the results, the antioxidative effect of plumbagin, especially after tissue incorporation was evident in various biological test systems. Considering that Fenton systems (both ascorbate-Fe$^{2+}$ and H$_2$O$_2$-Fe$^{2+}$) generally operate via the intermediary of hydroxyl radical, its reaction with plumbagin was also examined using pulse radiolysis technique.

Reaction of plumbagin with OH derived from radiation pulses yielded a plumbagin radical (PbZ radical) (Fig. 8). The time course of such radical formation is shown in Inset of Fig. 8. The radical formation increased rapidly up to 20 μs after which it got stabilized. The decay of PbZ radical started after about 100 μs and continued up to 500 μs to reach approximately half the absorbance (Inset of Fig. 8). The absorption characteristics of the PbZ radical are shown as a transient absorption spectrum (Fig. 8). The peak of absorption is at 390 nm with bleaching region due to depletion of parent at around 450 nm. Such absorbance of PbZ radical plotted as a function of plumbagin gave a relatively high rate constant of 2.03 x 10$^9$ dm$^3$ mol$^{-1}$ s$^{-1}$ (Fig. 9).

![Fig. 9: Plot of pseudo-first order rate constant for the formation of plumbagin radical (measured at 380 nm) for the determination of bimolecular rate constant for the reaction of radical with plumbagin. The bimolecular rate constant as found by this method is 2.03 x 10$^9$ dm$^3$ mol$^{-1}$ s$^{-1}$](image)

**Discussion**

Oxidative stress resulting from enhanced generation of ROS/RNS has been implicated in the etiology of a large number of human ailments. These include cardiovascular ailments, neural disorders and hepatotoxicities induced by various toxicities (Sies, 1996; Thomas and Kalyanaraman, 1997). Mechanisms of damage linking oxidative
stress to disease involve undesirable alterations to lipid and protein components of membranous organelles (Sies, 1996; Cuzzocera, Riley et al., 2001). Among the subcellular organelles, mitochondria are considered as important (major) targets of oxidative damage being responsible for reduced cellular functions in the form of energy generation or responsible for cell death (Raha and Robinson, 2000). Oxidative damage to mitochondria adversely affects specific carriers for succinate, citrate and oxaloacetate, as a result their intracellular levels are reduced (Salet and Moreno, 1990). Membrane lipids, due to the presence of polyunsaturated fatty acids, are highly prone to oxidative damage induced by various oxidants. In our studies we have used mitochondria from rat liver, brain and heart whose damage may be linked to the diseases for which Plumbago zeylanica has been suggested as a cure.

P. zeylanica has been credited with various therapeutic properties. Some of these pertain to diseases of the nervous, cardiovascular and gastrointestinal systems as well as for hepatotoxicities. Plumbagin from P. zeylanica has been suggested as the 'active ingredient' in medications and herbal preparations (Gupta, Verma et al., 1993). To examine possible mechanisms of action, we have studied the ability of plumbagin to prevent oxidative damage in mitochondria isolated from these tissues. The three endogenously relevant systems are ascorbate-Fe⁺ that generates OH radical-like species, H₂O₂- Fe⁺ that yields OH and AAPH that releases peroxy radical (ROO·) on incubation at the physiological temperature of 37 °C (Kamat, Boloor et al., 2000). The above three systems also differ in their mechanisms in inducing lipid peroxidation.

The responses of the tissues used namely brain, liver and heart to the above mentioned oxidant-generating systems significantly differ. Brain is rich in polyunsaturated fatty acids that are substrates for peroxidation. Liver and heart contain lesser amounts of these components. The amounts of endogenous antioxidants like glutathione, vitamins C and E as well as antioxidant enzymes also are different in these tissues (Pushpendran, Subramanian et al, 1998). The differential response of the three tissues to oxidants as well as the extent of inhibitions with plumbagin can be attributed to these factors.

Lipid peroxidation in tissues is controlled by the interaction of several factors. These include (i) availability of substrates for peroxidation in the form of unsaturated fatty acids mainly present in phospholipids, (ii) inducers of peroxidation such as ascorbate, Fe²⁺, compounds which can convert Fe³⁺ to Fe²⁺, oxygen, initiators of free radical reactions and functioning of the electron transport chain which serve as sources of reactive species, (iii) antioxidant defense in the form of glutathione, α-tocopherol, superoxide dismutase, carotenoids, substances chelating Fe²⁺, substances reducing lipid hydroperoxide, a GSH dependent labile factor and glutathione peroxidase system etc., and (iv) the physical properties of the membrane lipid such as fluidity and surface charge and the location of the polyunsaturated fatty acid in the membrane. Our earlier studies showed that the tissues examined (brain, liver and heart) significantly differ from each other in their susceptibility to undergo lipid peroxidation when oxidative damage is induced in vitro (Devasagayam, 1983; Pushpendran, Subramanian et al., 1998). Among these tissues brain is extremely susceptible to oxidative damage due to high levels of unsaturated fatty acids and the increased capacity of oxygen consumption (Kamat and Devasagayam, 1999).

Plumbagin protects against oxidative damage in majority of our experiments. The inhibition is observed with lipid peroxidation, protein oxidation as well as against inactivation of succinate dehydrogenase. The protective effect, however, was not seen with ATPase. Among the three systems used to induce oxidative damage, protection was observed against oxidative damage induced by ascorbate-Fe²⁺ and AAPH, and not against H₂O₂- Fe²⁺. This can probably be attributed to inability of plumbagin to neutralise either H₂O₂ or Fe²⁺ or both in this system.
Incorporation of plumbagin into mitochondria also was more effective than external addition to mitochondria. This may possibly be due to better uptake and presence of plumbagin at the site of ROS generation inside mitochondria. The extent of inhibition observed is relatively less as compared to other antioxidants like α-tocopherol, tocotrienols or curcumin (Nesaretnam, Devasagayam et al., 1993; Kamat and Devasagayam, 1995; Sreejayan, Rao et al., 1997; Devasagayam, Kamat et al., 2001). Hence it may indicate that *P. zeylanica* may also contain other components with better antioxidant activities.

Though in our experiments plumbagin has not been used in whole cell systems, whole organs or animal levels many studies using such systems have indicated the beneficial effects of plumbagin. For instance it has immunomodulatory effects as assessed by macrophage functions in BALB/c mice (Kamal and Rao, 1995), and anticancer effects in different systems such as Ehrlich ascites tumors and sarcoma 180 in mice (Singh and Udupa, 1997; Naresh, Udupa et al., 1996, Devi, Solomon et al., 1999), lymphocytic leukemia in mice (Krishnaswamy and Purushothaman, 1980), Dalton's ascites lymphoma in mice (Kavimani, Ilango et al., 1996), methylocholanthrene induced fibrosarcoma in rats (Krishnaswamy and Purushothaman, 1980) and azoxymethane induced intestinal carcinogenesis in rats (Sugie, Okamoto et al. 1998). Similar in vivo studies for checking the antioxidant activity of plumbagin using various indicators of oxidative damage can yield rich dividends.

In certain systems plumbagin has been observed to have prooxidant effects due to its redox cycling. This is observed mainly in biological systems lacking superoxide dismutase (SOD) as in the case of anaerobic bacteria. This also has been postulated to account for its antimicrobial activity (Archibald and Fridovich, 1981; Diguirosepi and Fridovich, 1982). In aerobic systems, however, such an effect was not observed.

The possible reason for the observed antioxidant effect of plumbagin can be due to radical scavenging as observed by pulse radiolysis. In our studies we have shown that reaction of radiation derived radicals lead to the formation of plumbagin radical that is fairly stable. The radical is probably a phenoxy type of radical. The characteristic absorption peak at 430 nm is buried into the bleaching spectrum due to the depletion of the parent. But the broad absorption around 500 nm due to the said radical is evident. The decay of the radical as measured at 390 nm was found to be of mixed order. The spectral as well as kinetic characteristics of the radical, matches well with the known phenoxy radical. Hence the mechanistic pathway for the free radical scavenging property of plumbagin is via the formation of phenoxy radical.

In conclusion, our studies show that plumbagin, isolated from *P. zeylanica* exhibit antioxidant effects in the form of membrane protective properties in mitochondria from three different rat tissues, liver, brain and heart. The extent of inhibition observed differs in these tissues. The observed antioxidant effects of plumbagin can be explained based on its ability to scavenge OH, as is seen in our pulse radiolysis experiments. The antioxidant ability of plumbagin, considered as the active ingredient of *P. zeylanica*, may at least, in part, explain observed therapeutic properties of this medicinal plant.

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