3-*O*-*β*-D-Galactopyranoside of Quercetin as an Active Principle from High Altitude *Podophyllum hexandrum* and Evaluation of its Radioprotective Properties

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The aqueous-ethanolic extract (AEE) of high altitude *Podophyllum hexandrum* has earlier been reported to render a radioprotective effect against lethal gamma radiation in *in vitro* model. AEE has also been reported to possess metal chelating and DNA protecting properties. The present study was undertaken to isolate and characterize the bioactive principle present in AEE and investigate its role in radiation protection. A novel molecule was found to be present in AEE and was assigned as 3-*O*-*β*-D-galactoside of quercetin by acid hydrolysis, LC-MS, LC-APCI-MS/MS and ^{13}C NMR spectra. Various biological activities were investigated at *in vitro* level. The antioxidant potential of AEE in lipid and aqueous phase was determined against numerous stresses. AEE was found to be significantly ($p < 0.05$) protective, *i.e.*, against Fe^{2+} and Cu^{2+}-induced linoleic acid degradation, respectively. Radiation-induced lipid oxidation studies revealed that AEE maximally works at a [lignan]/0.25 kGy ratio 400 (ratio of concentration of AEE divided by the radiation dose, *i.e.*, 0.25 kGy) and no drug-induced lipid oxidation at all concentrations tested was found. In a time-dependent study, total antioxidant activity was maximally exhibited at 1 mg/ml. The site-specific and non-site-specific deoxyribose degradation assay exhibited a dose-dependant hydroxyl scavenging potential of AEE (0.05–500 μg/ml). The anti-lipid peroxidation ability of AEE against radiation (0.25 kGy)-induced lipid peroxidation was higher in case of neural tissue homogenate as compared to kidney homogenate [activity ratio: 0.039 (brain) < 0.24 (kidney)]. The protein protection study using bovine serum albumin was also done for two time intervals (2 h and 4 h) and significant ($p < 0.05$) protection was observed at 500 μg/ml (> 97%). This study implies that 3-*O*-*β*-D-galactoside present in AEE renders radioprotection by protecting lipids, proteins in renal and neural model system against supra-lethal (0.25 kGy) gamma radiation.

Key words: Radioprotection, Quercetin-3-*O*-*β*-D-galactopyranoside, Lipid Oxidation

Introduction

Natural plant products, owing to the presence of plethora of compounds with a diverse array of properties, have been considered more effective than molecular drugs. Recent reports have established the usefulness of plant products as radioprotective and radiorecovery agents (for review: Arora *et al.*, 2005). *Podophyllum hexandrum* Royale syn. *P. emodi* Wall. (Himalayan Mayapple, family: Berberidaceae) is a perennial rhizomatous herb, and is usually found in the high altitude regions of India and China. The roots and rhizome of the plant have been extensively used in the traditional Indian as well as Chinese systems of medicine for treatment of a number of ailments afflicting humans, *e.g.*, cold, constipation, biliary fever, septic wounds, burning sensation, erysipelas, insect bites, mental disorders, rheumatism and plague (Chatterjee and Pakrashi, 1995; Singh and Shah, 1994). The whole plant, especially the root, is chol-

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agogue, cytostatic, cathartic and purgative. The plant finds mention as 'Aindri' – a divine drug in the traditional Indian system of medicine, the Ayurveda. Amongst the plant products, the genus *Podophyllum* has received a lot of attention in the area of natural products chemistry and pharmacology ever since the isolation of podophyllotoxin from its rhizomes, which have extensively been used to design and synthesize potential anticancer agents and antiarthritic compounds (Lerndal and Svensson, 2000). The crude plant extract as such and the various bioactive constituents present in the plant have been successfully used in both traditional and modern medicine for the treatment of a variety of ailments including metabolic disorders, lymphomas, leukemia, rheumatoid arthritis, genital warts, Kaposi's sarcoma and several forms of cancer (Chatterjee and Pakrashi, 1995; Singh and Shah, 1994; Wong *et al.*, 2000). The radioprotective properties of the whole extract of *Podophyllum* have been extensively studied by our group in rendering protection against lethal doses of gamma radiation (Gupta *et al.*, 2003; Arora *et al.*, 2005; Chawla *et al.*, 2005). However, the compounds responsible for radioprotection have not been identified and reported so far. Bioactivity-guided fractionation was attempted in the present study with a view to identifying and characterizing the bioactive molecule(s) responsible for radioprotection. We hereby report a novel active principle (3-*O*-*β*-D-galactopyranoside of quercetin, Fig. 1) from the aqueous-ethanolic fraction of high altitude *Podophyllum hexandrum* on the basis of bioactivity-guided fractionation. The compound identified using LC-APCI-MS/MS, has been thoroughly characterized using ^{1}H NMR and ^{13}C NMR (being communicated separately) and the bioactivity data of the fraction having relevance to free radical-mediated ailments, including radioprotection, was investigated.

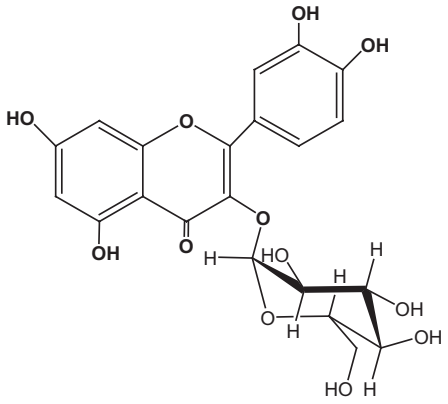

Fig. 1. Structure of quercetin-3-*O*-*β*-D-galactopyranoside.

Materials and Methods

Chemicals

Chemicals and solvents were of HPLC or analytical grade. Water was passed through a Milli Q water purification system (Millipore, Bedford, MA, USA). Ferric chloride, ferrous chloride, sodium hydroxide, ferrous sulphate, copper sulphate, potassium di-hydrogenorthophosphate, di-potassium hydrogen orthophosphate, Tween-20, trichloroacetic acid (TCA), di-sodium ethylene diamine tetra acetic acid (EDTA), ascorbate, thiobarbituric acid (TBA) were purchased from Sigma Chemicals (St. Louis, MO, USA). Eppendorf, micro-centrifuge tubes, and other plastic wares like pipette tips and sterilized 35 mm petri dishes were obtained from Tarsons Company (Kolkata, India). Dimethylsulfoxide and hydrochloric acid were obtained from BDH Chemical Co. (Toronto, Ontario, Canada). The rest of the chemicals including linoleic acid, deoxy-D-ribose, ammonium thiocyanate were of analytical reagent (AR) grade and were obtained from reputed local suppliers in India. The HPLC column was purchased from Merck.

Collection, authentication and processing of plant material

The rhizome of *Podophyllum hexandrum* Royale (syn. *Podophyllum emodi* Wall.) was collected from the high altitude regions (> 3000 m) of Leh and Ladakh region, Jammu and Kashmir, India. The plant material was identified and authenticated on the basis of botanical characteristics. Voucher specimens have been deposited in the repositories, both at Institute of Nuclear Medicine and Allied Sciences, Delhi and Regional Research Laboratory, Jammu (Voucher No.: INM/HAPH/2004). Adequate precaution was taken to ensure that the plant material was devoid of foreign materials like soil, dust, insects and other extrinsic contamination. Standardization of herbal material was carried out as per FAO/UNDP/WHO norms on the basis of physio-chemical characteristics (Chawla *et al.*, 2005).

Preparation of aqueous-ethanolic extract

The plant material was successively extracted in a Soxhlet apparatus with 50% ethanol at 55 ± 2 °C for a minimum of six times using 10 times solvent on w/v basis each time over the course of 6–8 d and the filtrate of each consecutive extraction was collected and later pooled, filtered, and concentrated in a rotary evaporator (Buchi, Switzerland) and subsequently dried in a lyophilizer. The yield on w/w basis was 14.29%. The dried fraction was pulverized through a micro-pulverizer and passed through a number 40 sieve and was stored at 4 °C until use. The fractionated extract was designated as AEE.

Isolation, identification and characterization of active principle

The aqueous-ethanolic fraction was analyzed by HPLC on a Shimadzu (CLASS-VP V6.12 SP4) liquid chromatography system with a photo diode array (PDA) detector set at 290 nm. The sample was injected *via* an auto-sampler and resolved on a RP-18 (5 μm; 4.0 × 250 mm; Merck) column. The temperature of the column was set at 30 °C and the flow rate for the separation was 0.6 ml/min. The mobile phase was initially set as methanol/water (65:35) for 0 to 5 min and the composition was changed to methanol/water (35:65) over a period of 60 min. The HPLC profile showed two peaks at RT 25.6 min and 29.02 min. The fraction was subjected to preparative HPLC when the compound at 10.57 min with *m/z* 464.32 appeared. The novel compound was isolated and assigned as 3-*O*-*β*-galactopyranoside of quercetin (Fig. 1) by acid and enzymatic hydrolysis, LC-MS, LC-APCI-MS/MS, ^{1}H NMR and ^{13}C NMR spectra.

Radiation stress in the presence of metal ions

The antioxidant activity of AEE was evaluated following the ammonium thiocyanate assay (Asamari *et al.*, 1996). Varied concentrations of AEE (500 μl) were mixed with 5 ml of a pre-emulsion of linoleic acid [prepared by mixing 3 volumes of linoleic acid with an equal volume of Tween-20 in 200 volumes of 30% (v/v) ethanol] containing 500 μl of copper sulphate solution (10 μM) or ferrous sulphate solution (100 μM). The total assay volume mixture was adjusted to 10 ml with deionized distilled water and incubated at 37 °C for 30 min. Aliquots were taken for assaying the end products of peroxidation using the ammonium thiocyanate assay. The assay mixture contained 2.5 ml of 75% ethanol, 50 μl of ferrous chloride (0.1% w/v) along with 50 μl of aliquot of the respective sample. Color development was measured at 500 nm against ethanol in a reference cell.

Radiation stress in the absence of metal ions

A similar study was performed to evaluate the inhibition of radiation-induced lipid peroxidation by AEE without using any metal ion. Copper sulphate solution was replaced with a similar volume of deionized distilled water in a total volume of 10 ml of assay mixture and stress (0.25 kGy) with one-hour incubation at 37 °C. The reaction was monitored for lipid peroxidation using the thiocyanate method.

Total antioxidant activity

A time-dependent study was conducted to evaluate the inhibition of lipid peroxidation by AEE without using any metal ion. Metal sulphate solution was replaced with a similar volume of deionized distilled water in a total volume of 10 ml of assay mixture, and the reaction was monitored for lipid peroxidation using the thiocyanate method for a period of 70 h.

Hydroxyl ion scavenging potential

The non-site-specific hydroxyl ion scavenging potential of AEE was measured using the deoxyribose degradation assay (Halliwell *et al.*, 1987). Different concentrations of AEE (500 μl) were mixed with 100 μM each of ferric chloride solution, EDTA, and L-ascorbic acid along with H_2O_2 (1 mM), deoxyribose (3.6 mM) in potassium phosphate buffer (pH 7.4) in a total assay volume of 1 ml. This was followed by incubation for 1 h at room temperature. 1 ml each of TCA (10% w/v) and TBA (0.5% w/v, TBA in 0.025 M NaOH) were added to each sample and re-incubated in a hot water bath (Yorco Instruments, India) at 55 °C for 15 min. The tubes were cooled to room temperature and the absorbance recorded at 532 nm against a blank containing phosphate buffer, while the control contained no drug. The decrease in absorbance at a particular concentration indicated higher hydroxyl ion scavenging potential with respect to control. The percentage of inhibition of degradation of deoxyribose or hydroxyl ion scavenging potential was evaluated as follows:

$$\% \text{ Inhibition} = (\text{O.D.}_{\text{control}} - \text{O.D.}_{\text{sample}}/\text{O.D.}_{\text{control}}) \times 100\%$$

Hydroxyl ion scavenging potential

The procedure for evaluating the site-specific hydroxyl ion scavenging potential was similar to the above-mentioned assay with a small change that in lieu of using EDTA, a similar volume of buffer was used in a 1 ml reaction mixture.

Experimental animals

Adult (6–8 week old) male Swiss albino strain 'A' mice (25 ± 2 g), bred locally in the animal house of the Institute of Nuclear Medicine and Allied Sciences (INMAS), Delhi (India), were used for the study. The animals were maintained under standard laboratory conditions, *i.e.* controlled temperature (25 ± 2 °C; 12 h alternating dark and light cycle) and humidity (RH ± %) in polypropylene cages. Standard food pellets (M/s Amrut Feeds Pvt. Ltd., Kolkata, India) and drinking water were provided *ad libitum* to the experimental animals. Permission for use of animals was taken from the Institutional Animals Ethics Committee (IAEC) of INMAS, and all experiments were carried out in strict compliance with the guidelines laid down by the Indian National Science Academy (INSA) for the care and use of laboratory animals for research purposes.

Irradiation

Radiation (0.25 kGy) was delivered to mice brain/kidney homogenate from a ^{60}Co gamma chamber (Gamma Cell 5000, Bhabha Radiation Isotope Technology, Bombay) at a dose rate of 3.40 kGy/h. Dosimetry was carried out using Baldwin Farmer's secondary dosimeter and Fricke's chemical dosimetry method.

Preparation of mice brain and kidney homogenate

Lipid peroxidation in terms of thiobarbituric acid like reactive substances (TBARS) was evaluated by standard methodology (Srour *et al.*, 2000). Mice were randomly selected and sacrificed by cervical dislocation and dissected. The whole brain was dissected out and visible blood clots were carefully and maximally removed and 10% homogenate was prepared in cold-buffered saline (pH 7.4). Further it was filtered to get a clear homogenate. Similarly, kidneys were also carefully removed from mice and a 10% homogenate was prepared.

Estimation of radiation-induced lipid peroxidation activity

Brain and kidney homogenate (2 ml) were taken separately in a series of 35-mm petri dishes to which different concentrations of AEE were added, and mixed gently to form a homogenous solution. Lipid peroxidation was initiated by exposure to high dose radiation (0.25 kGy), followed by incubation of the solutions at 37 °C for 30 min. 1 ml of homogenate was drawn out for estimating lipid peroxidation levels in terms of TBARS. Absorbance was recorded at 535 nm. Lipid peroxidation values are expressed as nanomoles of TBARS formed per hour per gram of tissue.

Inhibition of autooxidation of bovine serum albumin (BSA) by AEE

A modified spectroscopic evaluation method as described elsewhere (Kitts *et al.*, 2000) was employed. The standard solution of BSA was prepared and a standard protein estimation curve was obtained by using the Bradford method. Briefly, to an aliquot of 500 μl of BSA (1 μg/μl), different concentrations of AEE were added (0.05 μg/ml to 2000 μg/ml) containing peroxidizing agents [Fe^{3+} (0.1 mM) and H_2O_2 (1 mM)] and the total volume was made up to 3 ml using phosphate buffer (50 mM, pH 7.4). The samples were incubated at 37 °C and the protein content was estimated at 2 h and 4 h interval using the Bradford method. The test samples were compared with untreated control (containing BSA only), oxidative stress control (containing BSA and peroxidizing agent) and drug control (containing BSA and varied concentrations of drug). The percentage protection of BSA was evaluated considering the untreated control as 100% protein, while oxidative stress was considered as negative control on the protection scale. The increased values on the protection scale with respect to negative control indicated the percent protection at a particular concentration.

Statistics

All data are expressed as mean ± SE or as percentage. The difference between groups was assessed using Student's *t*-test. $p < 0.05$ was considered as significant difference.

Results and Discussion

Ammonium thiocyanate assay (in the presence of Fe^{2+} and Cu^{2+})

With a view to understand the antioxidant action of AEE, lipid oxidation studies were performed in the presence of Fe^{2+} or Cu^{2+} separately and in the presence of radiation (0.25 kGy). $Fe^{2+}$and $Cu^{2+}$are maximally present in hemoglobin (> 60% of total iron content of our body) and ceruloplasmin, respectively. Irradiation causes increased hemolysis resulting in free availability of these amplifiers that further enhance the free radical-mediated oxidative stress. Previous reports on plant products have revealed the fact that chelation of such metal ions decreases the risk of amplification of radiation-induced oxidative stress (Arora *et al.*, 2005). In the present study, Fe^{2+}, Cu^{2+} or their combination with radiation augmented hydroxyl ion generation, thereby accelerating lipid oxidation. The results (Figs. 2A and 2B) indicated that AEE effectively mitigated the peroxidation caused by Fe^{2+} or radiation (250 Gy) + Fe^{2+} maximally at a lignan:metal ratio of 1000, while a slight prooxidant activity was observed at a lignan:metal ratio of 1 (10 μg/ml), with 100 μM concentration of Fe^{2+}. Such a transient increase in absorbance is a signature mark of its ability to exhibit a biphasic mechanism (antioxidant-prooxidant), possibly due to its bioactive constituents, which interact with free Fe^{2+}. The present results could be corroborated with the metal chelating ability in aqueous media of fractionated extracts of *P. hexandrum* reported earlier by our group (Chawla *et al.*, 2005). The bioactivity can be attributed to the presence of a novel galactoside of quercetin present in AEE. Quercetin is a well-known metal chelator (Ferrali *et al.*, 1997). A similar pattern was observed in case of combined stress [Fe^{2+} + radiation (0.25 kGy)] in the presence of AEE. Chelation of Fe^{2+} is an important mode of achieving radioprotection (Arora *et al.*, 2005). It has been reported that radiation-induced ROS-mediated damage including lipid peroxidation gets further amplified in the presence of metal ions (Morel *et al.*, 1993; Alfanas'ev *et al.*, 1989). In case of Cu^{2+}-induced lipid oxidation, a dose-dependent inhibitory activity of AEE was observed indicating its immense ability to render protection against oxidative stress by reducing ·OH ion generation. AEE effectively mitigated the peroxidation caused by Cu^{2+} or radiation (0.25 kGy) + Cu^{2+} maximally at a lignan:metal ratio of 100.

Ammonium thiocyanate assay (in the presence of supra-lethal radiation)

In order to elucidate the inhibitory ability of AEE against radiation-induced lipid oxidation, the ammonium thiocyanate assay was performed. In this method, the ability of AEE to restrict the peroxidation of a linoleic acid emulsion induced by radiation (0.25 kGy) was evaluated and AEE was found to significantly ($p < 0.05$) inhibit linoleic acid degradation in a dose-dependent manner at all concentrations tested. The drug control showed no significant change at different ratios indicating no toxic manifestations associated with AEE in lipid media. The maximum decrease in absorbance was observed at ratios of 200 and 400, respectively, with 0.25 kGy of radiation dose (with a dose rate of 3.40 kGy/h; Fig. 2C), as compared to radiation control ($p < 0.05$). The most effective ratio, considering the drug control, was observed to be 400 with least induction of linoleic acid degradation as compared to other concentrations. Taken together, these findings suggest that AEE decreased the lipid oxidation induced by varied stresses over a short time span. The antioxidant activity pattern of AEE gives positive indication towards the ability of AEE to tackle radiation-induced stress. Numerous workers have suggested a relationship between the antioxidant property and radiation protection by plant flavonoids (Shimoi *et al.*, 1994; Emerit *et al.*, 1997; Aruouma *et al.*, 1987). These findings suggested that the antioxidant ability of AEE could be attributed to the presence of 3-*O*-β-D-galactopyranoside of quercetin. The total antioxidant potential also depends upon the auto-degradative ability of drug, which needs monitoring over a period of time. The total antioxidant ability of AEE to restrict the peroxidation of a linoleic acid emulsion over an extended period of incubation at 37 °C was tested using metal free emulsion (Fig. 3). The concentration-dependent inhibition of lipid oxidation by AEE over an incubation period of 72 h at 37 °C indicated the high affinity of AEE to scavenge peroxyl radicals. Moderate suppression of lipid oxidation at lower concentrations (0.01 and 0.1 mg/ml) by AEE was observed first for 48 h, while at higher concentration (1 mg/ml), both initiation and propagation of lipid oxidation are effectively taken care of. Significant ($p < 0.01$)

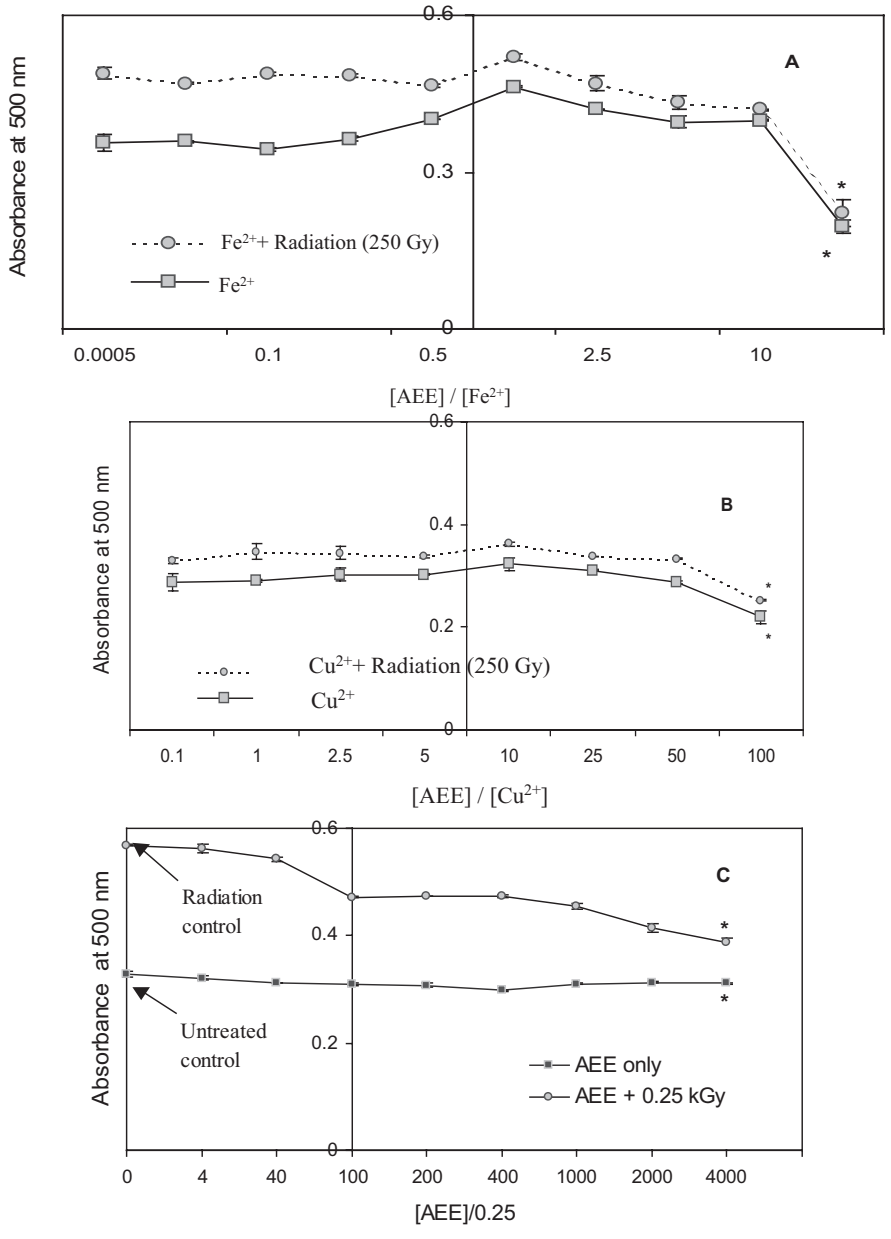

Fig. 2. Evaluation of antioxidant potential in lipid media against: (A) Fe^{2+} and radiation (250 Gy)-induced linoleic acid degradation (lipid oxidation assay); (B) Cu^{2+} and radiation (250 Gy)-induced linoleic acid degradation (lipid oxidation assay); (C) radiation (0.25 kGy)-induced linoleic acid degradation (lipid oxidation assay). Values ([lignan]/[metal] ratio) are expressed as mean ± S.D. * ($p < 0.05$) with respect to control.

peroxy-radical scavenging activities were observed for AEE at 1 mg/ml for all time periods (Fig. 3). AEE (0.01, 0.1 and 10 mg/ml) was not able to exhibit strong inhibitory activity against lipid peroxidation. These studies indicated that AEE is able to maintain homeostatic balance under stressed states in the lipid phase and could provide protection to membranes. An earlier report of higher antioxidant potential of different fractions of *P. hexandrum*, as compared to butylated hydroxyl

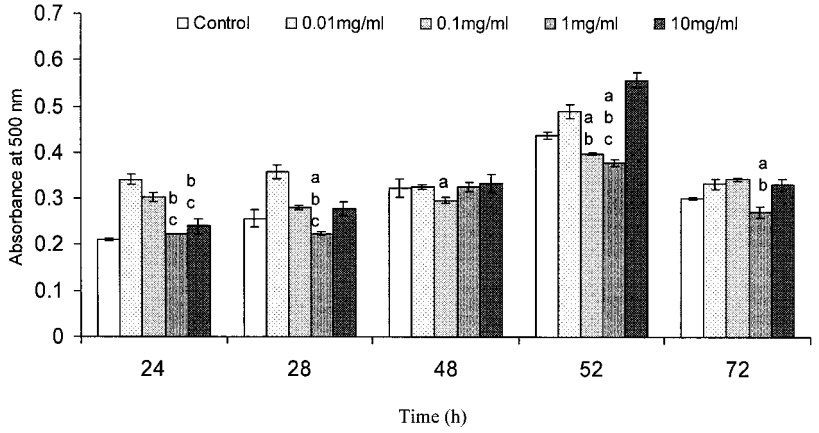

Fig. 3. A time-dependent evaluation of antioxidant potential of AEE by the ammonium thiocyanate assay. All the values are expressed as mean ± S.D. One way ANOVA, followed by Tukey's HSD test, was performed to evaluate the inter-group differences (a–d) and $p < 0.05$ was considered significant.

toluene (Chawla *et al.*, 2005), further supports the antioxidant ability of AEE in the emulsion system.

Hydroxyl ion scavenging potential (site-specific and non-site-specific)

Since the antioxidant ability of AEE in lipid media alone could not explain the overall ability of AEE to restrict amplification of radiation-induced oxidative stress, the deoxyribose assay was also used to study the non-site-specific [Fe^{2+} + H_2O_2 + EDTA] as well as site-specific [Fe^{2+} + H_2O_2] hydroxyl ion scavenging activity in the aqueous system. In a site-specific assay, Fe^{2+} induces hydroxyl generation but maximal attack results directly to deoxyribose prior to hydroxyl generation (Aruouma *et al.*, 1987). The hydroxyl ion scavenging potential was found to increase concomitantly with increase in concentration (0.05–500 μg/ml) of AEE (Fig. 4). Maximum hydroxyl ion scavenging potential was evaluated as percentage inhibition of deoxyribose degradation estimated using the TBA method. The maximum percentage inhibition of AEE and α-tocopherol (used as standard) was 85.56% (500 μg/ml) and 63.32% (500 μg/ml), respectively. All the values were found to be signifi-

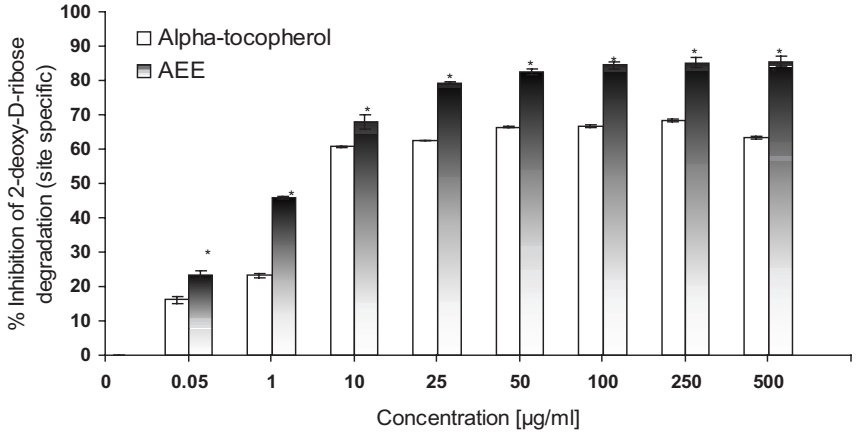

Fig. 4. Evaluation of site-specific ·OH ion scavenging activity of AEE in terms of inhibition of deoxy-D-ribose degradation in %. All the experiments were done in triplicate and repeated thrice. Control samples (0% inhibition) contained no drug or standard. All the values are expressed as mean ± S.D. * ($p < 0.05$) with respect to control (0% inhibition).

cant ($p < 0.05$) vis-a-vis control (0% inhibition). Other workers have also reported that numerous phyto-constituents like silymarin, catechin and luteolin chelate metal ions, thereby rendering radioprotection (Duthie *et al.*, 2000; Gebhardt, 2002; Korina and Alfanas'ev, 1997; Morel *et al.*, 1993). These results corroborated with the ability of AEE to reduce metal-induced linoleic acid degradation in lipid media. On the other hand, in the non-site-specific assay, the presence of EDTA makes Fe^{2+} non-available for attacking deoxyribose directly and therefore, hydroxyl generation predominates (Kitts *et al.*, 2000). In case of the non-site-specific assay, the hydroxyl ion scavenging potential of AEE was found to increase in a dose-dependant manner (0.05–500 μg/ml) (Fig. 5). The maximum percentage inhibition of AEE and α-tocopherol (used as standard) was 78.48% (500 μg/ml) and 80.87% (500 μg/ml), respectively. All the values were found to be significant ($p < 0.05$) vis-a-vis control (0% inhibition). Some workers have reported that quercetin exhibits potent antioxidant activity in both the aqueous and lipid phase (Nakagawa *et al.*, 2000), and thereby suppresses apoptosis (glucose-oxidase mediated) in mouse thymocytes (Lee *et al.*, 2003). Other studies on the protection against H_2O_2-induced DNA damage by quercetin could be attributed to its ability to facilitate electron delocalization because of the presence of the *O*-dihydroxy structure of B-ring of 3,5,7,3′,4′-pentahydroxy flavone (Horvathova *et al.*, 2004).

Anti-lipid peroxidation activity

One of the cardinal biomolecules, which is directly targeted by the hydroxyl ion, is lipid and its degradation leads to membrane damage of the cell. The anti-lipid peroxidation ability of AEE was therefore evaluated in mouse kidney and brain. The radiation-induced TBARS formation in both brain and kidney homogenate was found to decrease in a dose-dependent manner and maximum inhibition, *i.e.*, 81.96% (1 mg/ml; brain homogenate) and 30.25% (1 mg/ml; kidney homogenate), respectively, was observed (Fig. 6). All the values were found to be significant ($p < 0.05$) with respect to control (0% inhibition). The ratio of decrease in lipid peroxidation activity (nmol of TBARS formed/h/g of tissue) at 0.01 mg/ml to 1 mg/ml was 0.039 and 0.24 for brain and kidney homogenate, respectively. The percentage inhibition of AEE in case of brain homogenate was significantly higher from 0.1 mg/ml to 1 mg/ml, as compared to inhibition of AEE (in %) in case of kidney homogenate indicating the ability of AEE to protect brain tissue against radiation-induced oxidative stress. Conversely, in the lower range of concentrations (0.01–0.05 mg/ml), AEE effectively mitigated radiation-induced lipid peroxidation more prominently in kidney homogenate as compared to the brain. A lower ratio is indicative of effectiveness of the drug even at lower concentration indicating higher protective potential of AEE against radiation-induced stress. In both

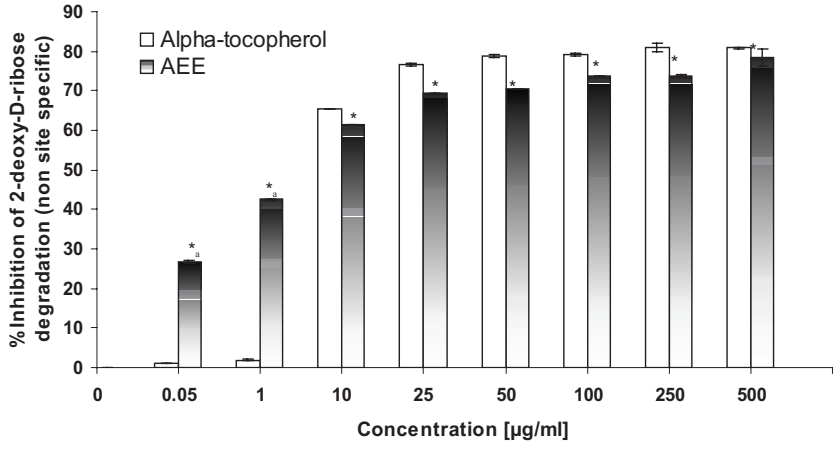

Fig. 5. Evaluation of non-site-specific ·OH ion scavenging activity of AEE in terms of inhibition of deoxy-D-ribose degradation in %. All the experiments were done in triplicate and repeated thrice. Control samples (0% inhibition) contained no drug or standard. All the values are expressed as mean ± S.D. * ($p < 0.05$) with respect to control (0% inhibition).

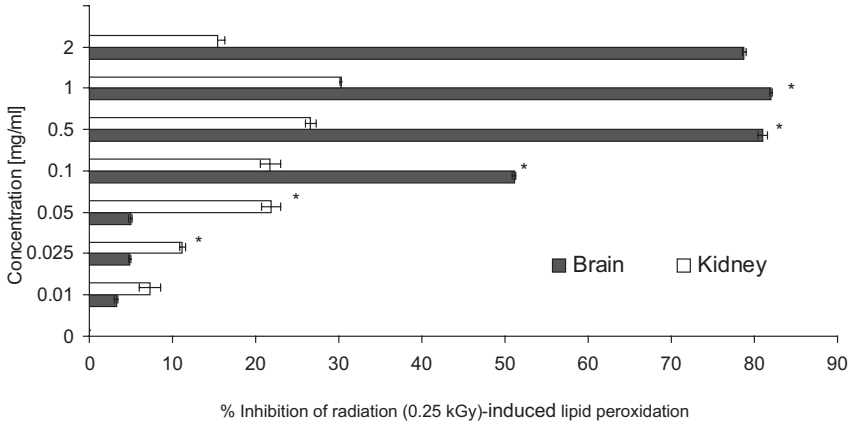

Fig. 6. Evaluation of anti-lipid peroxidation activity of AEE in kidney and liver homogenate against radiation (0.25 kGy) + 3 h estimated in terms of inhibition of induction of formation of TBARS in %. All the values are expressed as mean ± S.D. * ($p < 0.05$) with respect to control (0% inhibition).

cases, beyond 1 mg/ml, the efficacy of AEE was found to be decreasing, indicating that drug-induced lipid peroxidation predominates at 2 mg/ml and above. The results corroborated with the earlier findings on antioxidant activity in lipid media. A comparative evaluation revealed that at lower concentrations, AEE exhibited more renal protection while at higher concentrations, prominent neural protection was observed against radiation-induced oxidative stress. These results could be further corroborated with the ability of AEE to scavenge peroxyl as well as hydroxyl radicals. It indicated that AEE was very effective over a wide range of concentrations. However, AEE was found more protective to neural tissue than kidney tissue [0.039 (brain) < 0.24 (kidney)]. Earlier studies by our group have shown that *Podophyllum hexandrum* is protective against radiation-induced degradation of developing neural tissue.

Inhibition of autooxidation of proteins

The principle of generation of hydroxyl ions from reduced Fe^{2+} (Fenton reagent-induced oxidative stress) results in the subsequent degradation of soluble proteins (Kitts *et al.*, 2000) and was exploited to investigate the protein protection ability of AEE spectroscopically. The protection-potential of AEE was evaluated using bovine serum albumin, a standard protein used world-wide for evaluation against chemical peroxidative stress (Fe^{3+}/H_2O_2). The protective potential was evaluated in terms of inhibition of BSA degradation (in %) (Kitts *et al.*, 2000) and was found to increase in a concentration-dependent manner (0.05–500 μg/ml). The protein degradation in stress control (in %) with respect to untreated control (100% protection) was 54.83% in the first 2–4 h, which further decreased to 22.68% at 4th h with respect to the respective control (Fig. 7). Maximum protection in the first 2 h was observed at 500 μg/ml (97.19%) as well as maximal protection in the corresponding drug control (98.87%) and continued till 4th h interval with 99.15% in test and 99.95% protection in the drug control, respectively. At 4th h, a 2 mg/ml test sample was found to induce slightly the protein degradation on its own, indicating its increased toxicity. The ratio of efficacy at 0.05 to 500 μg/ml (0.872 and 0.98 at 2nd and 4th h, respectively) indicated the effectiveness of drug over a wide range of concentrations. These findings could be corroborated to its ability to scavenge free radicals in aqueous media. Few authors have suggested that blocking the hydroxyl group in position 3 can reduce the pro-oxidant activity of quercetin, thereby preventing its autooxidation (Kessler and Jung, 2003). It is very rare to find the 3-galactoside of quercetin, although the 3- or 7-glucoside are very common in the ratio of 1:1000 in nature and the galactose derivative at position 3 needs an extra step in degradation to generate quercetin. This results in its longer stay inside the body, and hence possibly exhibits enhanced bioac-

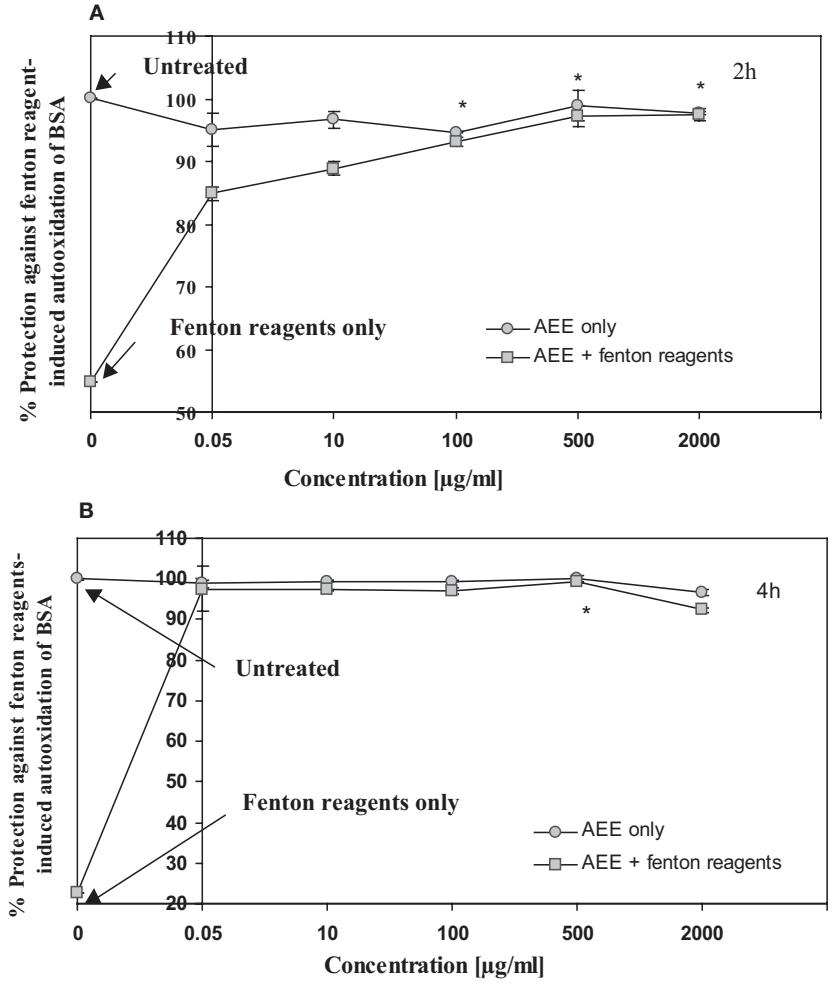

Fig. 7. Evaluation of Fenton reagent-induced autooxidation of bovine serum albumin at (A) 2 h and (B) 4 h. Values are expressed as mean ± S.D. All the values of drug + stress were found to be significant ($p < 0.05$).

tivity. In the present study, AEE exhibited higher antioxidative potential in the aqueous phase, which could be attributed to the extra stability of quercetin-3-*O*-*β*-D-galactoside due to blockage of position 3. The above findings suggest that this novel plant flavonoid, present in the aqueous-ethanolic extract of *Podophyllum hexandrum*, plays a cardinal role in enhancing the bioactivity specifically against radiation-induced oxidative stress.

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