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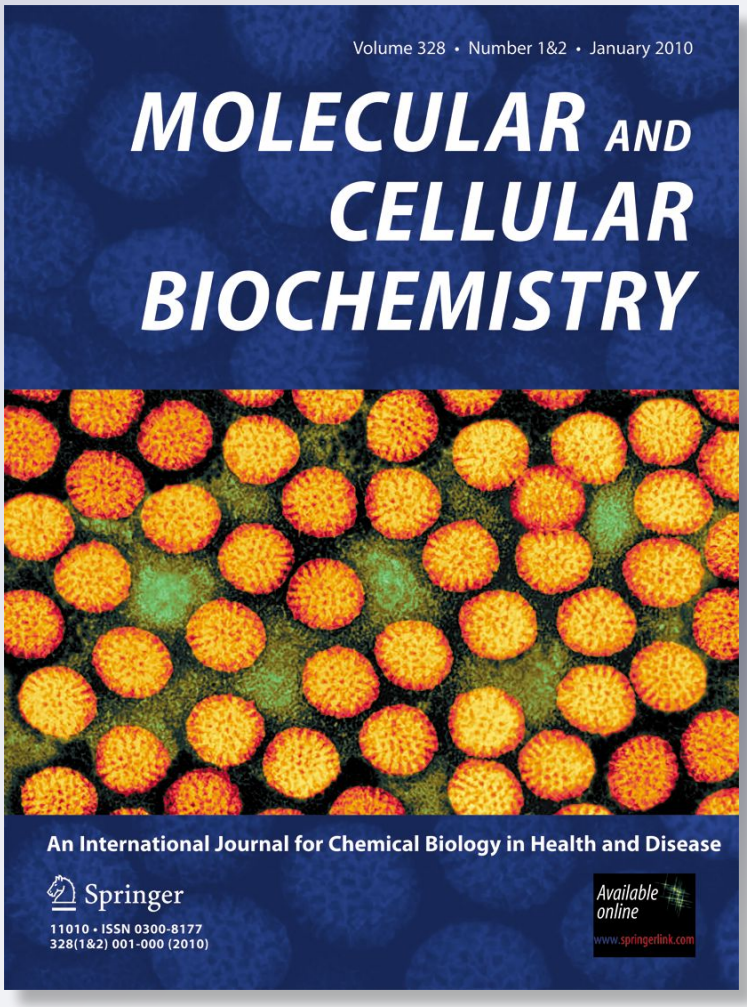

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14-Aminotetradecanoic acid exhibits antioxidant activity and ameliorates xenobiotics-induced cytotoxicity

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Abstract Natural compounds with free-radical scavenging activity have potential role in maintaining human health and preventing diseases. In this study, we report the antioxidant and cytoprotective properties of 14-aminotetradecanoic acid (ATDA) isolated from the *Decalepis hamiltonii* roots. ATDA is a potent scavenger of superoxide ($O_2^{\bullet -}$), hydroxyl ($^{\bullet}OH$), nitric oxide ($^{\bullet}NO$), and lipid peroxide ($LOO^{\bullet}$) physiologically relevant free radicals with IC_{50} values in nM (36–323) range. ATDA also exhibits concentration-dependent secondary antioxidant activities like reducing power, metal-chelating activity, and inhibition of protein carbonylation. Further, ATDA at nM concentration prevented $CuSO_4$-induced human LDL oxidation. ATDA demonstrated cytoprotective activity in primary hepatocytes and Ehrlich ascites tumor cells against oxidative stress inducing xenobiotics apart from the in vitro free-radical scavenging activity. The mechanism of cytoprotective action involved maintaining the intracellular glutathione, scavenging of reactive oxygen species, and inhibition of lipid peroxidation. It is suggested that ATDA is a novel bioactive molecule with potential health implications.

Keywords 14-Aminotetradecanoic acid · *Decalepis hamiltonii* · Antioxidant · ROS · Lipid peroxidation · Glutathione

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Introduction

There are over a thousand phytochemicals found in foods and one serving of a fruit or vegetable may have as many as 100 different phytochemicals [1, 2]. Antioxidant phytochemicals promote human health by exhibiting diversified physiological and pharmacological effects, viz., inactivate cancer-causing substances, stimulate the immune system, protect the heart from free radical attack, and help prevent cataracts in the eye lens [3, 4]. These antioxidant phytochemicals influence multiple signaling pathways, including survival pathways such as those regulated by NF-kB, Akt, and growth factors; cytoprotective pathways dependent on Nrf2; and metastatic and angiogenic pathways. Some of the common types of phytochemicals include flavonoids, indoles, isoflavones, alkaloids, nonprotein aminoacids, isothiocyanate, phytosterols, carotenoids, chlorophyll derivatives, etc. [5]. Currently, there is a great deal of interest in the study of natural compounds with free-radical scavenging capacity and their role in human health.

Tuberous roots of *Decalepis hamiltonii* (Wight and Arn.) (Family: Asclepiadaceae) are consumed in southern India as pickles and juice for its alleged health promoting properties. The roots are also used in folk medicine and ayurvedic preparations as general vitalizer and blood purifier [6]. We have earlier reported that the roots of *D. hamiltonii* possess potent antioxidant properties and

have shown their hepatoprotective and neuroprotective potential [7–10]. We have isolated/characterized the antioxidant constituents of *D. hamiltonii* roots which could be associated with their alleged health benefits [11–13]. In this article, we report the antioxidant properties of 14-aminotetradecanoic acid (ATDA) (Fig. 1) isolated from the aqueous extract of *D. hamiltonii* roots using a battery of free-radical scavenging assays and other additional antioxidant activity assays. Further, we demonstrate the cytoprotective activity of ATDA in primary hepatocytes and Ehrlich Ascites Tumor (EAT) cells against oxidative stress inducing xenobiotics.

Materials and methods

Chemicals

Thiobarbituric acid (TBA), glutathione (GSH), bovine serum albumin (BSA), tetraethoxypropane (TEP), HEPES buffer, 2,2-diphenyl-1-picrylhydrazyl (DPPH), phenzine methosulfate (PMS), cumene hydroperoxide (CHP), sodium nitroprusside (SNP), ethylenediamine tetraacetic acid (EDTA), Griess reagent, trypan blue were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Trichloroacetic acid (TCA), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide reduced (NADH), deoxyribose, ascorbic acid, dimethyl sulphoxide (DMSO), and other chemicals were purchased from Sisco Research Laboratories, Mumbai, India. All the chemicals used were of highest purity grade available.

Isolation of 14-aminotetradecanoic acid

The roots of *D. hamiltonii* were collected from B.R. Hills, Karnataka, India. The roots were washed with water, followed by crushing with a roller to separate the inner woody core from the outer fleshy layer. The fleshy portion was collected, dried at 40°C in a hot air oven and finely powdered in a grinder. This root powder was used for extraction. The aqueous extract was prepared by homogenizing the root powder (200 g) in 1 l of warm water (50°C) using a mixer and allowed to stand for 24 h, and filtering through Whatman No. 1 paper; the filtrate was lyophilized and weighed (34.75 g). The lyophilized aqueous extract was re-extracted with methanol in a total volume of 500 ml and concentrated under reduced pressure (−10 kPa, 37°C); it was subjected to conventional purification techniques (silica gel, 60–120 mesh, column chromatography and preparative thin layer chromatography). The compound isolated was pure by RP-HPLC and TLC. The active compound was characterized as ATDA by classical (UV, IR, LC–MS, and NMR) spectroscopic techniques which we have reported earlier [12].

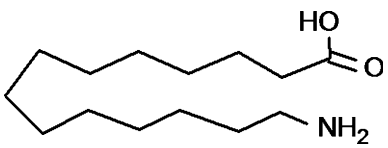

Fig. 1 Structure of ATDA

Antioxidant activity

Superoxide radical-scavenging assay

Superoxide radical-scavenging activity was measured by NBT method [14]. The reaction mixture, PMS (0.1 mM), NADH (1 mM), and NBT (1 mM) in phosphate buffer (0.1 M pH 7.4), was incubated at room temperature for 5 min with/without different concentrations of ATDA and the color developed, due to NBT reduction, was read at 560 nm against a blank (Shimadzu UV–vis spectrophotometer UV-160). The radical scavenging activity was measured as the decrease in the absorbance and was calculated using the following equation:

$$\text{Scavenging effect}\,(\%) = \left[1 - A_{\text{Sample}(560\,\text{nm})}/A_{\text{Control}(560\text{nm})}\right] \times 100$$

Hydroxyl radical-scavenging assay

Reaction mixtures containing different concentrations of ATDA were incubated with deoxyribose (10 mM), H_2O_2 (10 mM), $FeCl_3$ (5 mM), EDTA (1 mM), and ascorbic acid (5 mM) in potassium phosphate buffer (50 mM, pH 7.4) for 60 min at 37°C. The reaction was terminated by adding TCA (5% w/v) and the reaction product was measured by the reaction with TBA (0.2% w/v) in a boiling water bath for 15 min. The absorbance was measured at 535 nm against the reagent blank and inhibition of the oxidation of deoxyribose was calculated against the control [15].

Nitric oxide radical-scavenging assay

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which is measured by the Griess reaction. SNP (5 mM) was mixed with different concentrations of ATDA in phosphate-buffered saline and incubated at 25°C for 150 min followed by the addition of Griess reagent (1% sulphanilamide, 2% H_3PO_4, and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the reaction was read at 546 nm and referred to the absorbance of standard solutions of potassium nitrite, treated in the same way with Griess reagent [16]. The radical scavenging activity was measured, using the equation described above.

Inhibition of microsomal lipid peroxidation

Liver excised from adult male Wistar rats was homogenized (20% w/v) in 0.02 M Tris buffer (pH 7.4). Microsomes were isolated by the calcium aggregation method [17]. Microsomes (0.5 mg protein) were mixed with $FeSO_4$ (1 mM) and ascorbic acid (1 mM) with or without ATDA in a total volume of 1 ml of 0.1 M phosphate buffer (pH 7.4) and incubated at 37°C for 60 min. This was followed by adding 1 ml each of TCA (10%) and TBA (0.67%), boiling in a water bath for 15 min and centrifuging (5,000×*g*, 4°C, 5 min). The absorbance of the supernatant was read at 535 nm and thiobarbituric acid reactive substances (TBARS) value of the supernatant was calculated using tetraethoxy propane as the standard [18]. The TBARS value was taken as a measure of lipid peroxide ($LOO^{\bullet}$) generation.

Reducing power

The reducing power of ATDA was quantified by the method described earlier with modifications [19]. In brief, 1 ml of reaction mixture, containing ATDA in phosphate buffer (0.2 M, pH 6.6), was incubated with potassium ferricyanide (1% w/v) at 50°C for 20 min. The reaction was terminated by adding TCA solution (10% w/v) and centrifuged at 2000×*g* for 10 min. The supernatant was mixed with distilled water and ferric chloride (0.1% w/v) solution and the resulting color was read at 700 nm. The concentration required to get an $OD_{700\ nm}$ of 0.5 was used as measure of reducing power.

Protein carbonyls

Rat liver homogenate (10% w/v) was prepared in 20 mM Tris–HCl buffer (pH 7.4), centrifuged at 10,000×*g* for 10 min at 4°C. 1 ml of the supernatant was incubated with CCl_4 (1 mM) along with ATDA (0.1–2.5 mol/ml) for 1 h and was precipitated with an equal volume of 20% TCA and centrifuged (5,000×*g*, 4°C, 5 min). The pellet was resuspended in 1 ml of DNPH (10 mM in 2 M HCl) and allowed to stand at room temperature for 60 min with occasional vortexing. 0.5 ml of 20% TCA was added to the reaction mixture and centrifuged (5,000×*g*, 4°C, 5 min), the pellet obtained was washed three times with acetone and 1 ml of 2% of SDS (in 20 mM Tris–HCl, 0.1 M NaCl, pH 7.4) was added to solubilise the pellet. The absorbance of the sample was read at 360 nm and the carbonyl content was calculated using a molar extinction coefficient of 22,000 M^{-1} cm^{-1} [20].

Metal ion chelation assay

The Fe^{2+}-chelating ability of ATDA was assayed by measuring the formation of ferrous iron–ferrozine complex. The reaction mixture containing $FeCl_2$ (2 mM) and ferrozine (5 mM) and the isolated compound (0.1–50 mol/ml) was adjusted to a total volume of 0.8 ml with methanol, shaken well, incubated for 10 min at room temperature and the absorbance of the resultant color was read at 562 nm against a blank [21]. EDTA was used as the positive control. The metal-chelating ability of the purified compound was calculated using the equation as described earlier.

Inhibition of human low density lipoprotein oxidation

Human LDL (100 μg protein/ml) was oxidized with 10 μM $CuSO_4$ in the presence or absence of ATDA in 10 mM PBS, followed by the addition of 1 mM EDTA to stop the reaction. After incubation, aliquots of the reaction mixture were used for measuring TBARS formation and relative electrophoretic mobility (REM). TBARS was measured by adding to 0.5 ml of the above aliquots, 1 ml each of 2.5% TCA and 1% TBA, vortexing and boiling for 30 min. After cooling to room temperature, the samples were centrifuged (12,000×*g*, 4°C, 10 min) and the fluorescence of the product formed was measured in a spectrofluorometer (Shimadzu RF-5000U Spectrofluorometer) at 515 nm excitation and 553 nm emission wavelengths [22]. The electrophoretic mobility of the native or oxidized LDL was studied by agarose gel electrophoresis. Aliquots of the LDL reaction mixture were electrophoresed in 0.7% agarose gel at 85 V in the running buffer (40 mM Tris, 40 mM glacial acetic acid and 1 mM EDTA) for 2 h. The lipoprotein bands were stained with Coomassie Brilliant Blue and REM, defined as the ratio of the distances migrated from the origin by oxidized LDL versus native LDL, was calculated [23].

Cell studies

Hepatocyte isolation

Hepatocytes were isolated from male Wistar rats (200–240 g) by collagenase perfusion of the liver [24]. The pre-perfusion and perfusion solutions were buffered at pH 7.4 with 0.01 M HEPES. The total length of the perfusion was approximately 15–20 min. Aliquots of freshly isolated hepatocytes were immediately counted with a hemocytometer in 0.4% trypan blue solution containing 0.9% NaCl. The viability of the cells isolated by this method was always more than 90%.

Ehrlich ascites tumor cells

Ehrlich ascites tumor cells were cultured in the peritoneum of male Swiss albino mice [25]. After harvesting, cells were suspended in Hanks balanced salt solution (HBSS) with 0.1% dextrose and 0.4% BSA. Cells were used for the

experiments within 6 h after harvesting. EAT cells were chosen because they offer a good model to study ROS induction and consequent oxidative stress by xenobiotics.

Cell viability

Cells (10×10^6) suspended in 1 ml of HBSS were treated with the toxicant [for hepatocytes CCl_4 0.4 mM (dissolved in DMSO) or ethanol 1 mM; and for EAT cells CCl_4 0.4 mM or CHP 4 mM or HCH 1.6 mM (dissolved in DMSO)] at LC_{50} concentration with/without ATDA and incubated for 60 min in a shaking water bath at 37°C. At the end of incubation, an aliquot of cells was taken for viability assay by trypan blue exclusion method [26].

Lactate dehydrogenase leakage

After incubation of cells (hepatocytes/EAT cells) in the presence of toxicant [for hepatocytes CCl_4 0.4 mM (dissolved in DMSO) or ethanol 1 mM; and for EAT cells CCl_4 0.4 mM or CHP 4 mM or HCH 1.6 mM (dissolved in DMSO)] with/without ATDA cells were centrifuged ($2{,}000 \times g$, 25°C, 2 min) and the supernatant was assayed for LDH with sodium lactate as the substrate [27]. The reaction mixture consisted of NADH (0.02 M), sodium pyruvate (0.01 M), sodium phosphate buffer (0.1 M, pH 7.4) in a total volume of 3 ml. The changes in the absorbance were recorded at 340 nm at 30 s interval for 3 min.

Lipid peroxidation

After incubation, as above, the cells (hepatocytes/EAT cells) were centrifuged ($2{,}000 \times g$, 25°C, 2 min) and the cell pellet was washed in saline. The cell pellet was boiled in TCA (5.5%) and TBA (0.34%) for 15 min, cooled and centrifuged ($5{,}000 \times g$, 4°C, 5 min). Fluorescence of the supernatant was measured in a fluorescence spectrophotometer at excitation and emission wavelengths of 532 and 553 nm respectively [28]. Lipid peroxidation was quantified by the amount of malondialdehyde (MDA) formed which was calculated using a standard curve prepared with TEP (0–100 nmol/ml).

Reactive oxygen species (superoxide anion)

The cells (hepatocytes/EAT cells) (10×10^6) suspended in 1.0 ml HBSS were incubated with NBT (0.2 mM), toxicant [for hepatocytes CCl_4 0.4 mM (dissolved in DMSO) or ethanol 1 mM; and for EAT cells CCl_4 0.4 mM or CHP 4 mM or HCH 1.6 mM (dissolved in DMSO)], and with/without ATDA in a shaking water bath at 37°C. The generation of ROS by cells (respiratory burst) was measured by the formation of colored formazan due to reduction of NBT [29].

Glutathione

Hepatocytes/EAT cells (10×10^6) suspended in 1 ml HBSS were treated with toxicant [for hepatocytes CCl_4 0.4 mM (dissolved in DMSO) or ethanol 1 mM; and for EAT cells CCl4 0.4 mM or CHP 4 mM or HCH 1.6 mM (dissolved in DMSO)] and with/without ATDA and incubated for 60 min in a shaking water bath at 37°C. At the end of incubation, cells were homogenized in 1 ml of 5% (w/v) TCA, centrifuged at $2{,}000 \times g$ for 5 min and glutathione (GSH) in the deproteinized supernatant was estimated by Ellman's reagent with a standard curve (0–500 nmol/ml) and represented as nmol/mg protein [30].

Protein estimation was done by Lowry's method using BSA as the standard.

Statistical analysis

Data were expressed as mean ± standard error of three separate experiments and the significant difference was determined by the analysis of variance ($P < 0.05$) (Newman Keul's post-hoc test) using Statistica software (Statistica, Version 5.5, 99th ed. Stat-soft Inc., Tulsa, OK, USA).

Results

Free-radical scavenging activity

Free radicals like $LOO^{\bullet}$, superoxide ($O_2^{\bullet -}$), hydroxyl ($^{\bullet}OH$), and nitric oxide ($^{\bullet}NO$) are physiologically relevant and when excess cause oxidative stress which can lead to pathological conditions. We tested the efficacy of ATDA in scavenging these free radicals. ATDA showed concentration-dependent scavenging of free radicals (Fig. 2). The calculated IC_{50} values ($LOO^{\bullet} = 36.78 \pm 2.98$; $O_2^{\bullet -} = 135.89 \pm 12.69$; $^{\bullet}OH = 323.15 \pm 30.8$; $^{\bullet}NO = 159.56 \pm 13.89$ nmol/ml) were in nmol range indicating its high potency.

Additional antioxidant activity

One of the major consequences of oxidative stress is irreversible protein modification such as generation of protein carbonyls which lead to their fragmentation, increased aggregation, and enzyme dysfunction. ATDA in a dose-dependent fashion prevented CCl_4-induced protein carbonyl formation in rat liver tissue (Fig. 3). The metal ion-chelating capacity plays a significant role in the antioxidant mechanism because it prevents oxyradical

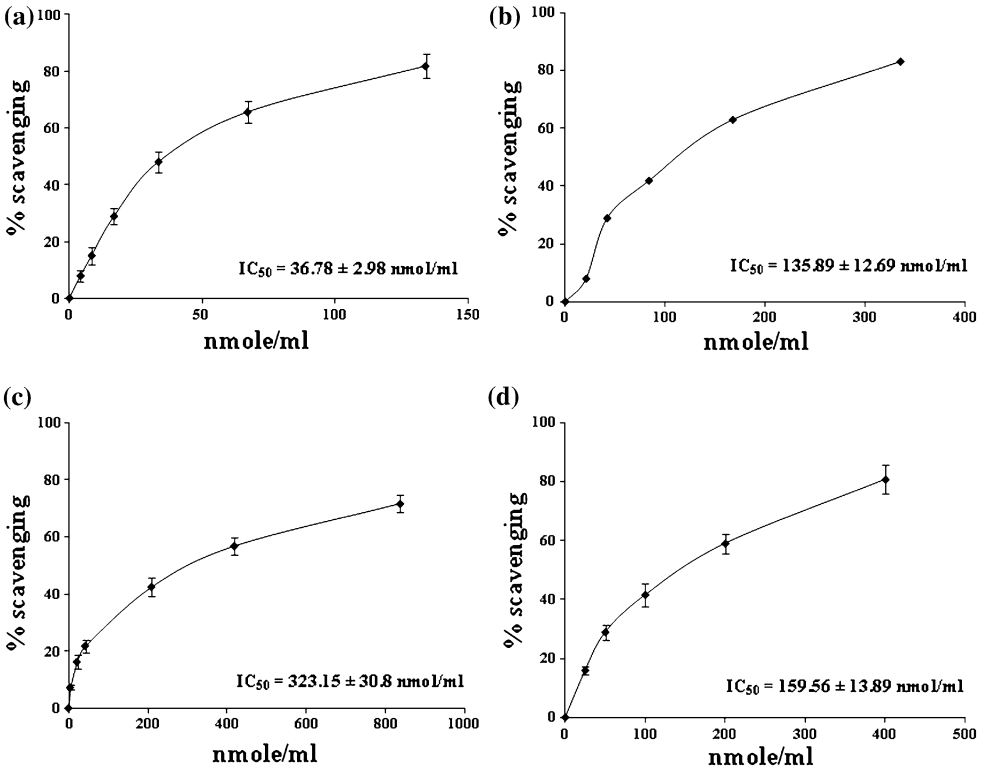

Fig. 2 Free-radical scavenging activity of ATDA. **a** Lipid peroxide ($LOO^{\bullet}$) radical, **b** superoxide ($O_2^{\bullet-}$) radical, **c** hydroxyl ($^{\bullet}OH$) radical, **d** Nitric oxide ($^{\bullet}NO$) radical. *Values* are expressed as means with standard error of mean

generation and the consequent oxidative damage. ATDA showed significant ferrous ion-chelating activity, as a measure of its antioxidant capacity (Fig. 3). Ferric reducing antioxidant power assay is conventionally used to measure the reducing ability of antioxidants. 14.02 ± 0.96 nmol/ml of ATDA was sufficient to get an $OD_{700\ nm}$ of 0.5 compared to 45.72 nmol/ml of quercetin, a well known natural antioxidant.

Inhibition of LDL oxidation

Oxidation of human LDL is considered to be an essential step in the pathogenesis of atherosclerosis. LDL oxidation is characterized by alterations in structure and biological properties of lipids and apolipoprotein B (apo B). ATDA showed dose-dependent protection against copper-induced human LDL oxidation as measured by REM and TBARS formation (Fig. 4).

Cell viability

In vitro studies using cell cultures offer a good model system to understand the mechanism of xenobiotic-induced cell injury/death and its amelioration by phytochemicals. EAT cells offer a good model to study oxidative stress-mediated cytotoxicity involving LPO and ROS induction by xenobiotics. Primary hepatocytes are also a useful in vitro cell model for pharmacological and toxicological studies of xenobiotics. Xenobiotics such as hexachlorocyclohexane (HCH), carbon tetrachloride (CCl_4), ethanol, and CHP are well-known inducers of oxidative stress in cell systems. To assess the cytoprotective effect of ATDA (hepatocytes and EAT cells) were exposed to different xenobiotics at LC_{50} concentration (hepatocytes—CCl_4 and ethanol) (EAT cells—HCH, CHP, and CCl_4). ATDA dose-dependent ameliorated toxicant-induced cytotoxicity as measured by trypan blue method and lactate dehydrogenase leakage. ATDA, by itself, was not toxic to the cells at the highest concentration used (Fig. 5).

Markers of oxidative stress

14-Aminotetradecanoic acid dose-dependent ameliorated xenobiotic-induced oxidative stress in both hepatocytes as well as EAT cells. Oxidative stress was measured by lipid peroxidation (LPO), reactive oxygen species (ROS) production, i.e., "respiratory burst", and depletion of glutathione level (Fig. 6). The toxicants-induced oxidative stress to different degree, in hepatocytes CCl_4 induced more oxidative stress compared to ethanol at LC_{50} concentration. In EAT cells HCH-induced highest production of ROS and CHP caused most LPO at LC_{50} concentration. ATDA decreased ROS either by inhibiting their production or scavenged them leading to decreased LPO and maintained the GSH level. ATDA, per se, did not have any adverse effect on oxidative stress parameters of hepatocytes and EAT cells.

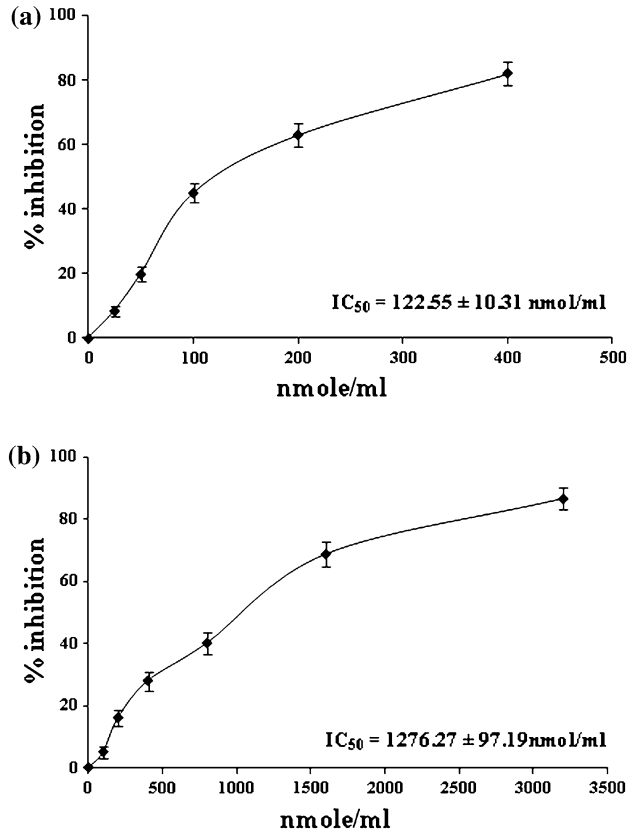

Fig. 3 Additional antioxidant activity of ATDA. **a** Inhibition of protein carbonylation, **b** metal-chelating activity. *Values* are expressed as means with standard error of mean

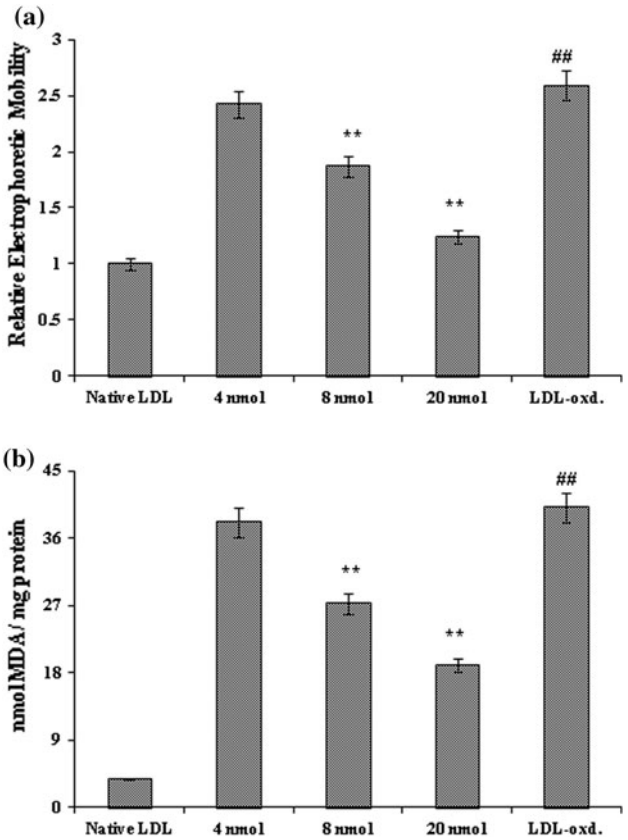

Fig. 4 Inhibition of human LDL oxidation by ATDA. **a** REM, **b** TBARS content. *Values* are expressed as means with standard error of mean. Statistics was done by one way ANOVA. # comparison between control and $CuSO_4$ groups and * comparison between HMMB + $CuSO_4$ treatment groups. # or * statistical significance at $P < 0.05$ and ## or $^{**}P < 0.01$

Discussion

The beneficial health effects of antioxidants have been attributed to their ability to scavenge free radicals [31]. There is overwhelming evidence that phytochemicals could be used as effective antioxidants for improving human health by preventing or delaying degenerative diseases [32]. ATDA, isolated from the roots of *D. hamiltonii*, is a potent scavenger of $O_2^{\bullet -}$, $^{\bullet}OH$, $^{\bullet}NO$, and $LOO^{\bullet}$ physiologically relevant free radicals. Metals are known to generate free radicals through the Fenton and Haber–Weiss reaction [33]. Chelating agents which form r-bonds with a metal are effective as secondary antioxidants as they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion. We demonstrate that ATDA has a high-metal-chelating activity and thereby can reduce the generation of metal catalysed free radicals. It is believed that antioxidant activity and reducing power are related [34]. Reductones inhibit LPO by donating a hydrogen atom and thereby terminating the free radical chain reaction [35]. ATDA showed higher reducing power compared to quercetin, a known antioxidant. Oxidative stress leads to irreversible protein modification, such as generation of carbonyls or loss of thiol residues [36]. These oxidative modifications alter the biological properties of proteins, leading to their fragmentation, increased aggregation and enzyme dysfunction. ATDA dose-dependent prevented protein carbonylation which has beneficial implication in oxidative stress-induced post-translation modification of proteins. By virtue its free-radical scavenging, metal-chelating, high reducing, and protein carbonylation inhibiting properties ATDA can act as a powerful antioxidant in vivo.

Oxidation of low-density lipoprotein (LDL) is considered to be an essential step in the pathogenesis of atherosclerosis [37]. Transition-metal-induced oxidation of LDL is one of the classical models of oxidation employed in research [38]. ATDA showed dose-dependent protection against copper-induced human LDL oxidation. The protection of LDL oxidation by ATDA could be due to: (a) scavenging of various radical species in the aqueous phase, (b) interaction with peroxyl radicals at the LDL surface, (c) partitioning into the LDL particle and terminating chain-reactions of LPO by scavenging lipid

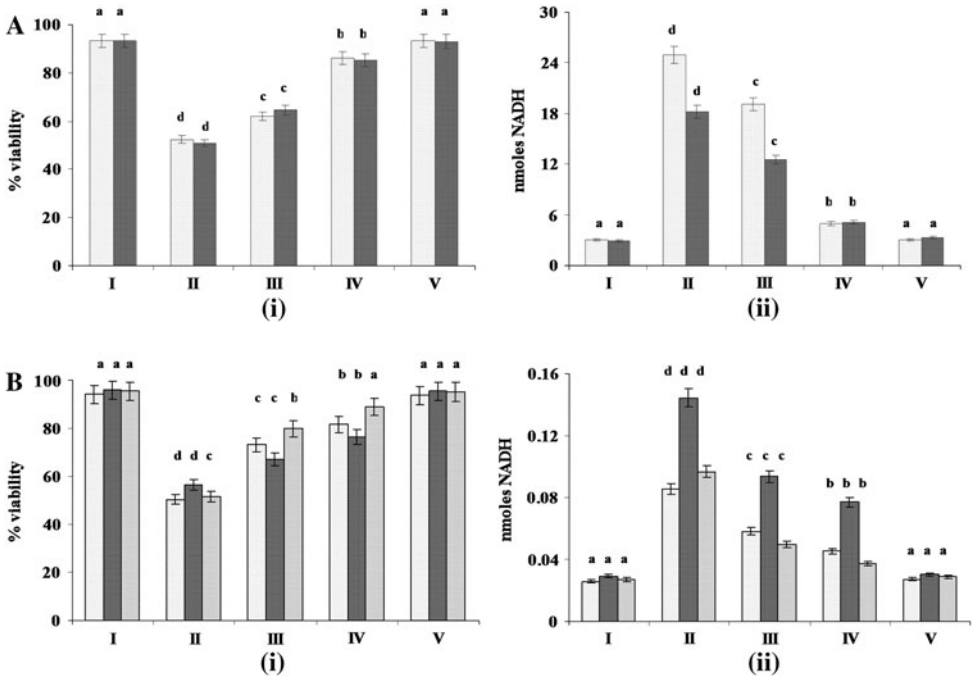

Fig. 5 Cytoprotective effect of ATDA. (*i*) Trypan blue exclusion method, (*ii*) LDH leakage. **a** Rat primary hepatocytes: Groups I: control, II: toxicant [CCl_4 (0.4 mM) or Ethanol (1 mM)], III: toxicant + ATDA (0.041 mM), IV: toxicant + ATDA (0.164 mM), V: ATDA (0.164 mM). **b** EAT cells: Groups I: control, II: toxicant [HCH (1.6 mM) or CHP (4 mM) or CCl_4 (3 mM)], III: toxicant + ATDA (0.20 mM), IV: toxicant + ATDA (0.41 mM), V: ATDA (0.41 mM). *Values* are expressed as means with standard error of mean. Different *letters* indicate statistical significance at $P < 0.05$

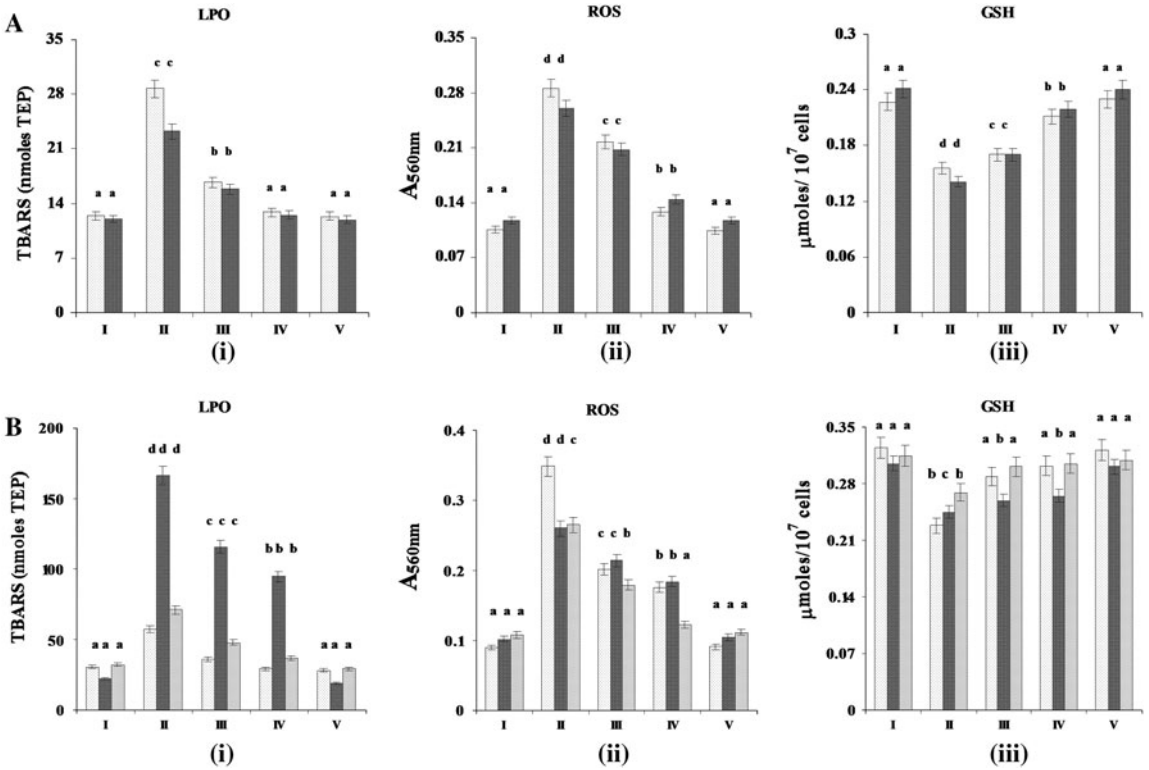

Fig. 6 Amelioration of oxidative stress by ATDA. **a** Inhibition of LPO, **b** ROS scavenging, **c** restoration of glutathione (GSH) levels. **a** Rat primary hepatocytes: Groups I: control, II: toxicant [CCl_4 (0.4 mM) or ethanol (1 mM)], III: toxicant + ATDA (0.041 mM), IV: toxicant + ATDA (0.164 mM), V: ATDA (0.164 mM). **b** EAT cells: Groups I: control, II: toxicant [HCH (1.6 mM) or CHP (4 mM) or CCl_4 (3 mM)], III: toxicant + ATDA (0.20 mM), IV: toxicant + ATDA (0.41 mM), V: ATDA (0.41 mM). *Values* are expressed as means with standard error of mean. Different *letters* indicate statistical significance at $P < 0.05$

radicals, (d) regenerating endogenous α-tocopherol back to its active antioxidant form, and (e) metal ion chelation.

In vitro studies on cell cultures are often used as a model system to study the mechanism of xenobiotic-induced cell injury/death and its amelioration by phytochemicals [39, 40]. Several phytochemicals have been evaluated for their protective activity against xenobiotic-induced toxicity in experimental models in vitro and in vivo conditions [41]. A number of prooxidant drugs and other chemicals (including CHP, HCH, CCl_4, and ethanol) have been implicated in the oxidative stress and cell injury resulting from the intracellular production of injurious ROS [40, 42]. ATDA inhibited xenobiotic-induced LPO in both EAT cells and hepatocytes. Further, ATDA scavenged the ROS produced, which could be responsible for the amelioration of xenobiotic-induced cytotoxic insult. Furthermore, xenobiotic-induced GSH depletion in EAT cells and hepatocytes was restored by ATDA. It is reported that cytotoxic insult caused by xenobiotics is initiated through the generation of ROS which further leads to activation of downstream signaling like mitogen-activated protein kineses (MAPK) and mammalian target of rapamycin (mTOR) pathways [43]. The cytoprotective action of ATDA involved relieving oxidative stress and, at least in part, restoring the cellular redox status. Our results support the view that induction of ROS in cells by xenobiotics is correlated with oxidative stress-mediated cell death and antioxidants can be successfully used to ameliorate such oxidative stress-induced cytotoxicity.

In conclusion, ATDA has potent free-radical quenching activity, exhibits secondary antioxidant properties, and inhibits human LDL oxidation. We have further demonstrated the ability of ATDA to prevent xenobiotic-induced cellular damage in hepatocytes and EAT cells. The mechanism of cytoprotective action appears to involve maintaining the intracellular GSH, scavenging of ROS, and inhibition of LPO. Based on the results it is suggested that ATDA is a novel bioactive molecule with implications in both prevention and amelioration of degenerative diseases as well as in general well being.

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