Free radical induced damages to rat liver subcellular organelles: Inhibition by
Andrographis paniculata extract

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Aqueous extract of Andrographis paniculata was examined for antioxidant activity using rat liver subcellular organelles as model systems. The study deals with two important biological oxidative agents, ascorbate-Fe\(^{2+}\) and AAPH generating hydroxyl and peroxyl radical, respectively. Oxidative damage was examined against the inhibition of membrane peroxidation, protein oxidation and restoration in decreased SOD and catalase activity. The antimutagenic activity of Ap was examined following inhibition in AAPH induced strand breaks in plasmid pBR322 DNA. Extract was a potent scavenger of DPPH, ABTS radicals, exemplified by ESR signals, O\(^{-}\), \(\cdot\)OH and \(\cdot\)H\(_2\)O\(_2\), displayed excellent reducing power, FRAP potentials to reduce Fe\(\text{(III)}\)→Fe\(\text{(II)}\) and had considerable amount of phenolics/ flavonoids contents, an effective antioxidant index. The observed antioxidant effect might be primarily due to its high scavenging ability for ROS. Effect was confirmed \textit{ex vivo} following inhibition in peroxidation, restoration in SOD enzyme, SOD band intensity and protein degradation in Ap fed liver homogenate. Based on these results, it was concluded that the aqueous extract of Andrographis paniculata might emerge as a potent antiradical agent against various pathophysiological oxidants.

Keywords: Antioxidant, Andrographis paniculata, Herbal extract, Membrane damage, ROS scavenging

Oxygen is essential for survival however, its univalent reduction generates several harmful reactive oxygen species (ROS), inevitable to living cells and highly associated with wide range of pathogenesis such as diabetes, liver damage, inflammation, aging, neurological disorders and cancer\(^1\).\(^2\). In spite of comprehensive network of cellular defensive antioxidants, many ROS still escape this surveillance inflicting serious anomalies favouring such diseases states\(^3\). Though synthetic antioxidants, BHT, BHA and radioprotector, Warfarin are being used widely, however, due to their potential health hazards, they are under strict regulation\(^4\).\(^5\). Antioxidant principles from natural resources are multifaceted in their multitude/magnitude of activities and provide enormous scope in correcting the imbalance through regular intake of proper diet. Therefore, in the recent years, the interest is centered on antioxidants derived from herbal medicine in view of their medicinal benefits\(^6\)-\(^10\). Phytoantioxidants, commonly available, less toxic, serving food and medicinal components have been suggested to reduce threat of wide range of ROS\(^8\)-\(^10\). WHO estimation showed 80% of the earth’s inhabitants rely on traditional herbal medicine for their primary health care.

In view of this and the present understanding about ROS-induced multiple diseases, we have selected one such important popular ayurvedic herb, Andrographis paniculata (Ap) (Acanthaceae) commonly known as Kalmegh (Sanskrit) for examination of its antioxidant/antiradical status. This herb is found in many Asian countries, widely acceptable to man and credited with diverse medicinal properties. It is an antihyperglycemic agent\(^11\). Andrographolide, the major active constituent has been isolated from Ap and reported to exhibit anticancer and anti-inflammatory effects\(^12\)-\(^14\). Andrographolide has been shown to inhibit NF-kappaB that reduces expression of proinflammatory proteins, COX-2\(^14\). As it is well established that cancer, diabetes etc. are associated with low levels of antioxidants\(^3\),\(^8\),\(^10\), and this herb possesses anticancer and hypoglycemic activity, it may have good antioxidant property. Therefore, in the present study, using two biologically relevant ROS initiators, AAPH and ascorbate Fe\(^{2+}\) which generates hydroxyl and peroxyl radical,\(^15\),\(^16\) respectively, study

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was carried out to prevent oxidative damage with Ap in liver sub-cellular organelles of rats and mice. Based on several biochemical analysis, mechanistic data and ex vivo observations, it is concluded that aqueous extract of Ap demonstrated promising results for antioxidant effects against the oxidative damage induced by path physiologically relevant ROS. This paper discusses about antiradical activity of Ap in vitro and ex vivo and also highlights the contributions of possible mechanisms involved in observed antioxidant effects.

Materials and Methods

Thiobarbituric acid, AAPH, 1,1-diphenyl-2-picrylhydrazyl, xylene orange, 2,2′azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid), gallic acid, mannitol, deoxyribose, horseradish peroxidase, catalase, NADH, phenazine methosulphate, quercetin, tripyridyl-s-triazin (TPTZ), guanidine hydrochloride, Epinephrine, DNPH, 1,1-diphenyl-2-picrylhydrazyl (DPPH), plasmid pBR322, SOD, α-tocopherol, nitroblue tetrazolium, H₂O₂ and tetraethoxypropane was purchased from Sigma Chemical Co (St Louis, Mo, USA).

Plant Extract—Aqueous extract of Andrographis paniculata (Ap) is obtained as a gift from Zandoo Research Laboratory, Mumbai.

Animals—The animals were bred in the BARC Laboratory Animal House Facility and procured after obtaining clearance from the BARC Animal Ethics Committee. All the experiments were conducted with strict adherence to the ethical guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) constituted by the Animal Welfare Division of Government of India on the use of animals in scientific research. In the present study, three months old female Wistar rats (weight 200 ± 20 g), and Swiss female mice (weight 25 ± 4 g) were maintained, under controlled laboratory conditions (25°C ± 2°C; RH 60 ± 5%; 12 h photoperiod), fed standard animal food, tap water ad libitum were used for antioxidative studies.

Preparation of subcellular organelles—After fasting overnight, mice were killed by cervical dislocation, livers were excised, homogenized in Tris HCL (0.15M; pH 7.6). Isolation of mitochondria from rat liver was carried out. Briefly, rat livers were homogenized in 0.25 M sucrose containing EDTA (1 mM). The homogenate was centrifuged at 3000 × g for 10 min. The resulting supernatant was centrifuged at 10,000 × g for 10 min. to sediment mitochondria in a Sorvall RC5C centrifuge. The mitochondrial pellets, thus, obtained were washed thrice with potassium phosphate buffer (50 mM; pH 7.4) and suspended in the same buffer at 10 mg protein/ml.

Ex vivo experiment to monitor antioxidant potential of Ap extract—Mice administered with aqueous extract of Ap (200 mg/kg body weight) for three days, were killed 24 hr after the last injection of Ap. The liver homogenate was prepared as described above for Ap fed and unfed mice and exposed to ascorbate Fe²⁺ and AAPH system for ex vivo experiments. The oxidative damage was monitored in terms of TBARDS, and status of SOD activity.

Exposure to oxidatives stressors—Liver subcellular organelles (1 mg protein/ml) were exposed to AAPH (for peroxyl radical) and ascorbate Fe²⁺ (hydroxyl radical) in vitro, ex vivo and oxidative damage was monitored using various biochemical parameters.

Assessment of oxidative damage in subcellular membrane—Lipid hydroperoxide (LOOH) was measured by FOX II reagent, where hydroperoxide oxidizes ferrous to ferric ions and detected by ferric sensitive dyes at 560 nm for measurement of LOOH concentration. TBARS were isolated by boiling mitochondrial lipid for 15 min at 100°C with TBA reagent (0.5% 2-thiobarbituric acid/10% trichloroacetic acid/0.63 M HCL) and absorbance was measured at 532 nm. SOD activity was examined by measuring adrenochrome formed by autoxidation of epinephrine at 320 nm. Catalase activity was monitored by decomposition of H₂O₂ at 240 nm. Protein carbonyl was measured by derivatization of mitochondrial proteins with DNPH and elimination of underivatized DNPH by ethanol:ethyl acetate (1:1). Derivatized DNPH was dissolved in 6N guanidine hydrochloride and read at 370nm. Appropriate sample blanks were taken to ensure noninterference of Ap extract with the assays.

Protein degradation, activity staining and DNA strand breaks—Degradation of mitochondrial proteins was monitored by SDS-PAGE. Electrophoresis was performed with 10% protein gel and SOD was located by soaking gel with nitroblue tetrazolium followed by riboflavin illumination. Strand breaks in plasmid pBR322 DNA were measured with and without Ap, exposed to AAPH at 37°C and the resultant supercoiled (Form I) and open circular (Form II) forms were separated by electrophoresis.
Radical scavenging assays—Detection of \( \cdot \text{OH} \) with and without Ap extract was performed by deoxyribose degradation method by studying the competition between deoxyribose and Ap extract for \( \cdot \text{OH} \) generated by Fenton reaction. The damage due to \( \cdot \text{OH} \) was estimated by measuring TBATS\textsuperscript{17}. Superoxide anion generated in a PMS / NADH system reduces the nitroblue tetrazolium (NBT) to blue colored formazan that was measured at 560 nm with and without Ap extract (10-100 \( \mu \)g/ml)\textsuperscript{18}. Ascorbic acid was used as a positive control. \( \text{H}_2\text{O}_2 \) measurement is based on the horseradish peroxidase (HRP)–mediated oxidation of phenol red by \( \text{H}_2\text{O}_2 \), resulting in a chromogenic compound having absorbance at 600 nm\textsuperscript{23}. Scavenging of \( \text{H}_2\text{O}_2 \) by Ap (5-200 \( \mu \)g/ml) was carried out. Scavenging of DPPH was carried out\textsuperscript{24}. Extract donates hydrogen atom or electron and reduces DPPH radical to hydrazine. One ml of 500 x 10\(^{-3}\) mol dm\(^{-3}\) methanolic DPPH was mixed with equal volume of Ap (5-200 \( \mu \)g/ml), mixture was kept in dark for 20 min and absorbance was monitored at 517nm\textsuperscript{25}. Radical scavenging of Ap (5-200 \( \mu \)g/ml) was also performed with ABTS\textsuperscript{2} by ferrylmyoglobin/ABTS\textsuperscript{+}at 720 nm\textsuperscript{25}. Gallic acid, vitamin C and GSH were used as positive controls.

Statistical analysis—The results were expressed as mean ± SEM. Statistical comparison between the groups, ascorbate Fe\textsuperscript{2+} or AAPH alone group vs. Ap with ascorbate Fe\textsuperscript{2+} or AAPH combined group was performed with a Student’s \( t \) test.

Results

DPPH and ABTS radical scavenging ability of Ap—Figure 1 demonstrates scavenging ability of Ap (5-200 \( \mu \)g/ml) against DPPH and ABTS radicals. It has good antioxidant ability for both the radicals. Ap 25, 50 and 100 \( \mu \)g/ml showed 31, 55 and 70% scavenging of DPPH, respectively. IC\textsubscript{50} value of DPPH radical for Ap was found to be as 38 \( \mu \)g /ml and comparable with positive controls, vitamin C, GSH and gallic acid having IC\textsubscript{50} as 49.2, 30.7 and 41\( \mu \)g /ml, respectively. The results of ABTS with Ap 25, 50 and 100 \( \mu \)g/ml also demonstrated 23, 49 and 89% scavenging of ABTS,\textsuperscript{+} respectively. IC\textsubscript{50} for Ap, vitamin C and gallic acid were found as 27, 35 and 29

![Fig. 1—ABTS and DPPH scavenging by Ap- scavenging ability of Ap extract was examined for ABTS and DPPH radicals with various concentrations of Ap extract (5-200 \( \mu \)g/ml).](image-url)
μg/ml, respectively. Scavenging ability of Ap relatively appeared to be more with ABTS’ than DPPH at higher concentration. Preventive effect of Ap against lipid peroxidation—Ap extract showed good protection at 25 and 50μg/ml. Therefore, for further studies same concentrations were used to prevent hydroxyl and peroxyl radical-induced damage in rat liver mitochondria. The results demonstrated significant increase in TBARS (Fig. 2a) in ascorbate Fe²⁺ exposure. However, Ap extract reduced TBARS by 40 and 56%, respectively at 25 and 50 μg/ml. GSH demonstrated 77% inhibition. Ap also significantly prevented the formation of LOOH. As less as Ap25 μg/ml reduced LOOH by 50%. GSH, (50 μg/ml) did not show any protection against these products. However, it required 6 times more concentration (1mM) than Ap to protect these products. A marked increase in both the products (P<0.001) was also demonstrated following AAPH exposure. TBARS and LOOH were inhibited by 36, 63% by Ap at 25 μg/ml and 53 and 63% by Ap at 50 μg/ml concentration. α tocopherol, a positive control (100 μM= 43μg/ml) demonstrated 72% inhibition in LOOH, comparable with Ap.

Prevention of protein damage by Ap—Our results demonstrated an increase in protein carbonyls (the measuring index of protein oxidation) following ascorbate-Fe²⁺ (9 folds) and AAPH exposure (4.5 folds) (Fig. 3). Ap 50 μg/ml showed significant prevention in its formation. Inhibition was more with AAPH than ascorbate-Fe²⁺ system.

Ex vivo studies with Ap—Both the oxidants, AAPH and ascorbate-Fe⁺₂ depleted SOD and catalase activity significantly and Ap showed significant prevention of protein carbonyls and (b) antioxidant enzymes. [All legends are same as given in Fig. 2. After exposing, damage was measured against protein carbonyls and antioxidant enzymes. (Values are mean ± SEM from four experiments. Significant at P<0.01 and P<0.001 compared to ascorbate Fe⁺ and AAPH exposure)].

Fig. 2—Preventive effect of Ap against lipid peroxidation in rat liver mitochondria Liver mitochondria (1mg/ml) were exposed to (a) ascorbate-Fe²⁺ and (b) AAPH with and without Ap at 37°C for 15 min and TBARS and LOOH was measured. [(A) -- No exposure, (B) -- mitochondria exposed to ascorbate-Fe²⁺ (a) and AAPH (b) + Ap (25 μg/ml), (D) -- mitochondria exposed to ascorbate-Fe²⁺ (a) and AAPH (b) + Ap (50 μg/ml). (E) -- mitochondria exposed to ascorbate-Fe²⁺ + GSH- (1mM) and (F) -- mitochondria exposed to AAPH (b) + tocopherol (100 μM). (Values are mean ± SE from 4 experiments. Significant at P<0.01 and P<0.001 compared to ascorbate Fe⁺ and AAPH exposure)].
prevention in decline, more in AAPH than ascorbate-Fe$^{2+}$.

Since the extract has excellent antioxidant ability, ex vivo examination was carried out in liver homogenate. Liver homogenate from Ap fed mice exposed, to ascorbate-Fe$^{2+}$ and AAPH systems showed more inhibition in TBARS ex vivo than in vitro experiments with Ap, 50 μg/ml (Fig 4a). Both the oxidants also inhibited SOD activity as against unexposed ones. However, Ap fed homogenate showed more restoration in SOD activity than in vitro showing less potency for oxidation due to Ap as antioxidants thus, confirming the possible antioxidant role of Ap.

Ex vivo restoration of protein degradation and SOD activity by Ap—Extract protected protein carbonyls by peroxyl radical, an important oxidant constantly generated in the cells. Therefore, examination of protein profiles and detection of SOD enzyme in Ap fed mice was performed (Fig.5a). A drastic reduction in the total proteins was observed with AAPH exposure showing diminished band intensities. However, Ap fed homogenate ex vivo and in vitro Ap added homogenate showed effective reversal in the protein bands (SDS-PAGE; Fig 5a). Activity staining experiments also demonstrated low intensity of SOD enzyme in the homogenate exposed to AAPH. Ap fed homogenate could effectively restore SOD activity as seen by its increased band intensity than in vitro experiments (Fig.5b).

Antioxidant role of Ap was also examined in plasmid pBR322DNA and data on agarose gel electrophoresis (Fig. 5c) in absence or presence of Ap (50 μg/ml) showed that compared to unexposed DNA (control), AAPH exposed DNA led to the extensive conversion of supercoiled form to the open circular form (Damage, D) and presence of Ap reduced the intensity of the band due to the open circular form (Ap50). The results were compared with tocopherol.

Possible mechanism against antioxidant effects

ROS scavenging—One of the possible reasons for antioxidant effect of Ap may be due to scavenging of ROS. The present investigations showed potent scavenging responses with Ap. Even Ap (25 μg/ml) could show 49 and 65% scavenging of O$_2^-$ and OH, respectively. Ap (25 μg/ml) showed only 20% scavenging of H$_2$O$_2$. Scavenging of these radicals increased in Ap treated groups. Mannitol was effective only at 1mM (182 μg/ml) of concentration showing 58% scavenging of OH. Vitamin C requires 175 μg/ml (1mM) to get 67% scavenging of O$_2^-$. Vitamin C lesser than this concentration did not show any scavenging demonstrating a marked potential of Ap compared to established antioxidants. FRAP analysis of Ap (5-100 μg/ml) also demonstrated a concentration dependent increase in FRAP (Table 1).

**Fig. 4**—Ex vivo restoration effects of Ap in mouse liver homogenate (a) TBARS and (b) SOD activity. Liver homogenate from Ap fed mice (1mg/ml) was exposed to above oxidants and damage was measured as given in Figs 2 and 3. [Values are mean ± SEM from four experiments. Significant at *p*<0.01 and **p**<0.001 compared to ascorbate Fe$^{2+}$ and AAPH exposure].

**Fig. 5**—(a) Protein profile by SDS PAGE; (b) Detection of SOD enzyme by activity staining ex vivo. [C – No exposure (control), D –exposed to AAPH (100 mM) for 1 h, Ap50- exposed with Ap 50 μg/ml, Apfed—liver homogenate from Apfed mice, exposed to AAPH]. (c) Gel electrophoresis pattern of plasmid pBR322 DNA [C-No exposure (control), D-DNA-exposed to AAPH (100 mM), Ap 50- DNA exposed to AAPH with Ap50μg/ml, Toc- DNA exposed to AAPH with tocopherol (100μM)].

ESR studies with DPPH radical — A typical ESR spectrum of DPPH radical (Fig. 6a) and % ESR signal intensity of Ap (Fig. 6b) was demonstrated. It increased as a function of Ap concentration. The effect was pronounced only at higher concentrations i.e 50, 70 and 100 μg/ml showing 32, 50 and 65% scavenging. IC_{50} value for Ap was found as 70 μg/ml.

Reducing power, total phenolics/flavonoid contents — Ap (5-100 μg/ml) exhibited increase in the reduction of Fe (III) → Fe (II) as shown increased absorbance (Fig. 7a). We have observed increased phenolics and flavonoids as function of Ap (Fig. 7b).

### Discussion

Profound adverse effects of ROS induced oxidative stress on cell constituents and its wide implications in human pathogenesis is well documented. Antioxidants are known to offer resistance against such events by various mechanisms. Among the several mechanisms involved in redox imbalance, oxidative stress has been considered as the critical ones. Ascorbate–Fe, an in vitro pathological oxidant is known to generate OH, which indiscriminately reacts with cell components and induces several deleterious effects. Lipid peroxidation in vivo is known to generate wide range of carbon center...
species and hence, peroxyl radicals with molecular oxygen\(^{10}\). In general, excess generation of these radicals can cause damage to tissues. Variety of plant products are known to prevent such events through antioxidant mechanisms\(^{3,6,10,29,30}\). However, overexpression of ROS by various means generates constant siege of oxidative stress in cells. Hence, it is always needed to have an efficient antioxidant system. Our study was mainly concerned with protective role of Ap extract against these oxidants.

DPPH and ABTS are stable synthetic free radicals. These compounds, donating \(\text{H} \) atom/ (an electron), reduces DPPH and ABTS activity showing decrease in their absorption. We have examined radical scavenging effects of Ap against DPPH and ABTS radicals. A concentration dependent scavenging effect of Ap was demonstrated against both the radicals showing ability to convert unpaired electrons to paired ones (Fig.1).

The results demonstrated significant inhibition against endogenous oxidants, ascorbate \(\text{Fe}^{2+}\) and AAPH induced lipid peroxidation showing much potent effect compared to standard antioxidant, GSH \((175 \ \mu \text{g/ml})\) that requires six times higher concentration than Ap \((50 \ \mu \text{g/ml})\) for expressing same antioxidant potentials as Ap.

As discussed earlier, living cells are constantly exposed to oxidative stress which are associated with several undesirable changes in membrane proteins leading to protein oxidation, cleaving polypeptide chain/ amino acids and eventually resulting in proteolytic degradation. Protein oxidation plays a key role in aging forming increased oxidized proteins with animal age, ROS generation, decreases in antioxidants and degradation of oxidized proteins\(^{31}\). In this respect, our observed results showing inhibition in protein oxidation by Ap may have therapeutic usefulness (Fig. 3). Wide ranges of physiological reactions are known to generate electrons that instantly react with molecular oxygen and forms \(\text{O}_2^-\), an important pathologies mediator. Though \(\text{O}_2^-\) itself is not very reactive, but can generate a potent oxidizing and nitrating species, peroxyxinitrate\(^\text{32}\) and \(\text{OH}^{-}\) \(\text{via a superoxide-driven Fenton process}\). In this regards, SOD plays an important role as an early cellular defense by catalyzing dismutation of \(\text{O}_2^-\). \(\text{H}_2\text{O}_2\) generated during dismutation of \(\text{O}_2^-\) is further detoxified by catalase. Therefore, cooperative activity of these two enzymes is a crucial step in the maintenance of redox homeostasis\(^{33}\). Present results demonstrating increased activity of these enzymes by Ap indicated activation of the enzymes (Fig. 3b) possibly, scavenging \(\text{O}_2^-\), \(\text{H}_2\text{O}_2\) and \(\text{OH}^{-}\). Hence, mechanism of antioxidant action was studied by radical scavenging assays. Our \textit{ex vivo} data further confirmed the antioxidant role of Ap. Since, \(\text{OH}^{-}\) abstracts hydrogen atom and forms a carbon-centered radical at \(\alpha\) position of amino acid residues\(^{34}\), presumably, AAPH-derived peroxyl radicals may also attack the \(\alpha\)-position of amino acid forming protein radicals that inactivate mitochondrial proteins and also SOD activity. \textit{Ex vivo} feeding data demonstrated excellent restoration in mitochondrial protein degradation and SOD enzyme, as monitored by activity staining experiments (Fig. 4). Interestingly, Ap also could show effective prevention against strand breaks in plasmid DNA, induced by peroxyl radical. Positive control, \(\alpha\) tocopherol effectively reduces the single strand breaks in DNA. Since, AAPH generates \(\text{OH}^{\text{55}}\), the present data demonstrated the scavenging ability of Ap also for \(\text{OH}^{-}\).

Possible antioxidant mechanism was studied by examining scavenging effect of Ap with various radicals. Ap demonstrated promising results showing potent scavenging, though its scavenging ability varies from each ROS. The effect was highly pronounced against \(\text{OH}^{-}\), the most aggressive radical, formed by Fenton’s reaction by interaction of ferrous salt with \(\text{H}_2\text{O}_2\). Scavenging effect of Ap for \(\text{OH}^{-}\) was also seen from ascorbate \(\text{Fe}^{2+}\) \textit{in vitro} and \textit{ex vivo} data (Fig 2a). Variation in the scavenging capacity of Ap for different radicals (Table 1, Fig. 1) can be explained. Like stable radicals, DPPH and ABTS react stoichiometrically with antioxidants which are good hydrogen donors\(^{36,37}\). Antioxidants which are effective metal chelators of transition metal ions may react differently\(^2,15\) Altogether, these results showed excellent radical scavenging activity for \(\text{OH}^{-}\). The effect was as follows; \(\text{OH}^{-}\text{>O}_2^-\text{>H}_2\text{O}_2\). The ESR data for Ap against DPPH is comparable with spectrophotometrical analysis (Fig.1) showing about 45-50% scavenging by Ap \((70 \ \mu \text{g/ml})\).

Currently, the antioxidant research is mostly focused on the phenolics, the major groups of antioxidants present in diet, rich in vegetables and fruits. The bioactivity of phenolics may be due to multifunctional beneficiary roles including antioxidant behaviour, attributed to their reducing, metal chelating, hydrogen donating capacity, and...
scavenging of ROS. Flavonoids with hydroxylized B ring and/or unsaturated C ring are recently suggested as natural proteasome inhibitors and apoptosis inducers, thus providing a molecular basis for the clinically observed cancer-preventive effects of fruits and vegetables. This is also supported by some studies that demonstrated presence of twelve flavonoids, namely 5,7, tetramethoxy flavone and 5-hydroxy-7, trimethoxy flavone in the Ap extract. Our data with total phenolics and flavonoids also indicated the presence of these components, required for antioxidant effects.

The extract contains several active components, andrographolide, 14-deoxy-11-o xoandrographolide, 14-deoxyandrographolide and neoandrographolide. Among these, andrographolide has major contribution in the cellular defense. Andrographolide has been reported to exhibit beneficial uses such as antihepatotoxic, immunomodulatory and anticancer etc. Anticancer effect has been suggested to be triggered by apoptosis via activation of caspase, with a series of sequential events. Its anti-inflammatory effect is expressed through inhibition of NF-kappaB that reduces the expression of proinflammatory proteins, COX-2. Structural perspective chain-breaking antioxidants are hydrogen donors and/or possess an extensive system of conjugated double bonds that stabilizes reaction transients by resonance. The above active component does not have such structural features. Some reports have demonstrated scavenging of O2- by the extract. Our present results also showed significant scavenging of O2- and OH by Ap. Some xanthones have been isolated from Andrographis paniculata extract and hydroxyl group at 2 position of xanthones has been shown as a powerful life enhancing factor with wide range of pharmaceutical properties including inhibition of lipid peroxidation at the initial steps. Present study demonstrated significant inhibition in lipid peroxidation by Ap thus explaining possible antioxidant effect. Ap had good potential to reduce Fe (III)→Fe (II) as indicated by its reductive ability and FRAP analysis data (Fig. 7a, Table 1). The present data showed considerable amount of phenolic/ flavonoid contents, high degree of reducing potential and FRAP, the established indexes for antioxidant action, repairing capacity for DNA damage, scavenging ability with ABTS, DPPH, O2-, ‘OH, H2O2 and ESR signals of DPPH as well as ex vivo experiments significantly justify the antioxidative ability of the Ap extract. Animal studies with Ap have demonstrated wide tissue/organ distribution with a relatively short half-life of approximately 2 h and fair excretion capacity by body, showing its medicinal usefulness.

In conclusion, aqueous extract of Andrographis paniculata significantly prevented the oxidative damage induced by pathophysiological oxidants, inevitable to living cells. Based on earlier studies showing anticancer, antidiabetes and immunomodulating effects of Andrographolide and neoandrographolide, it is possible that these compounds may be responsible for such effects involving scavenging of ROS and exerting observed antioxidant effects. This property may have clinical implications in radiation and chemotherapy that induces several ROS. So far no ideal radioprotectors have been reported except warfarin, expensive drug with several drawbacks. Hence, Andrographis paniculata, a natural herb may have potential antioxidant effects against several oxidants/ROS.

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References