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Original article

Protective effect of leaf extract of *Abutilon indicum* on DNA damage and peripheral blood lymphocytes in combating the oxidative stress

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ABSTRACT

The current research explores *in vitro* antioxidant characteristics, radiation-induced DNA damage protection and quenching effects of the oxidative stress by the ethanolic leaf extract of *Abutilon indicum* (EEAI) on human peripheral blood lymphocytes (PBLs). PBLs were incubated with various concentrations of EEAI accompanied by pre- and post-treatment with hydrogen peroxide. Cell viability was investigated by MTT assay. In addition, quenching of free radicals were measured *in vitro* using DPPH, superoxide anion, hydrogen peroxide, reducing power and nitric oxide radical scavenging assays. These activities were compared with ascorbic acid as standard antioxidants. Furthermore, inhibition of UV radiation-induced strand break formation in plasmid pBR322 DNA and anti-Fenton reactions in calf thymus DNA was evaluated. Cytotoxic effects of hydrogen peroxide on PBLs were significantly reduced with EEAI pretreatment compared to post-treatment in a dose-dependent manner comparable with similar cytoprotective effects of ascorbic acid ($p > 0.05$). EEAI has shown strong antioxidant effects in the scavenging of DPPH, superoxide anion, hydrogen peroxide, and nitric oxide. EEAI also has a strong protective effect of UV-induced plasmid pBR322 DNA cleavage and Fenton-induced DNA damage. Overall, the results revealed that *Abutilon indicum* has a cytoprotective, potent antioxidant and DNA protective effect that provide pharmacological credence to justify its overall biological activity. Furthermore, future studies to identifying bioactive molecules and its molecular mechanisms responsible for promising therapeutic applications in the rescue of disease-induced cellular oxidative damage.

1. Introduction

The alterations in DNA, protein, and bio-membranes of human peripheral blood lymphocytes (PBLs) generate oxidative stress that may lead to the fate of cell injury and death. It causes the progression of various disorders and diseases such as cancer, diabetes, Alzheimer's, atherosclerosis, aging, cataracts, and cirrhosis (Weng, 2020; Aslam et al., 2015). Early reports indicated that hydrogen peroxide (H_2O_2) generates oxidative stress in multiple organs including peripheral mononuclear cells, liver, retina, neurons, oocytes, and testes (Zagmutt et al., 2016; Jurica et al., 2018). Under the cytotoxic effects, free radicals act mainly by mobilizing arachidonic acid from cell membrane phospholipids. The consequences lead to enhancement of the intracellular malondialdehyde (MDA) formation, arachidonic acid concentration, and modification in glutathione levels, and intracellular calcium concentration resulting in oxidative damage (Singh et al., 2016; Xie et al., 2013). Phytochemicals are capable of reducing the effects of free radicals or protecting the integrity of cell membranes as being the major focus in combating cellular damage induced by oxidative stress (Tabolacci et al., 2019; Zhang et al., 2016). So, natural bioactive phytochemicals such as flavonoids, polyphenols, micronutrients, anthocyanins, and vitamins may exert several biological effects on cells providing possible therapeutic molecules against various diseases (Zhang et al., 2016; Gismondi et al., 2017). Bioactive polyphenols might modify the cell membrane permeability, reduce the effects of free radicals and chelate the free metals thereby protecting from free radical-mediated oxidative stress and cellular damage (Trigo et al., 2019; Zhang et al., 2016).

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Abutilon indicum (L.) Sweet (Malvaceae) is widely recognized to have medicinal properties used in traditional medicine (Mahalik et al., 2019). The plant has been found useful in the treatment of urinary disease, rheumatism, leprosy, ulcer, high fever, jaundice, pulmonary tuberculosis, gonorrhea, aphrodisiac, bronchitis, mumps, lack of urination and some nervous and ear problems have been reported (Abdul Rahuman et al., 2008; Lim, 2014). In addition, early reports on leaf extracts indicated antibacterial, hepatoprotective, larvicidal, carminative, hypoglycemic, antipyretic, hyper-lipidemic, laxative, blood tonic, diuretic, anti-cough, anti-inflammatory properties (Lim, 2014; Rajeshwari and Sevarkodiyone, 2018; Porchezhian and Ansari, 2005; Tian et al., 2018). Phytochemical screening of *Abutilon indicum* revealed that it contained polyphenols, flavonoids (gallic acid, β-sitosterol, geraniol caryophylline, and two sesquiterpene lactones) (Radhakrishnan et al., 2017; Thakor et al., 2016). Also, alkaloids, saponins, tannins, and glycosides (Abdul Rahuman et al., 2008; Krisanapun et al., 2009) were well characterized. Adding to the increasing information on the significant health benefits of natural antioxidants, a variety of research groups have investigated plants as novel major sources of potential antioxidants. Bioactivity of *A. indicum* described in this investigation has a strong therapeutic effect on animal and human health due to its wide-ranging antioxidant efficacy. This plant is native to the area where the research was conducted and is also a part of natural diet in the community. So, this study investigates the antioxidant and anticarcinogenic properties of this plant.

The present study aimed to investigate the antioxidant and cytoprotective effects of ethanolic leaf extract of *Abutilon indicum* against (EEAI) oxidative stress induced by H_2O_2 in human peripheral lymphocytes (PBLs). The experimental model was selected as lymphocytes that are primary cells from a healthy genome, widely used for cytotoxicity research in many contemporary studies. Further, we also evaluated the toxicity effects in PBLs to confirm the safety of this plant extract and investigate the inhibition of UV induced mutation and Fenton reaction in DNA. We are reporting here for the first time its cytoprotective and anticarcinogenic properties of this plant. Furthermore, future investigations need to focus on identifying bioactive molecules present in these extracts and its *in vivo* molecular mechanisms underlying the cytotoxic and antioxidant functions.

2. Materials and methods

2.1. Plant material collection and extraction

The fresh leaves of *Abutilon indicum* were collected in Tamil Nadu, in January 2016. The specimens were identified and characterized at the Madras Herbarium (MH) (Herbarium accession number 41215/2016), Botanical Survey of India, Coimbatore, India. The fresh leaves were clearly washed and dried at room temperature (shades) (25 °C) and powdered. To minimize the degradation of thermolabile compounds, extraction of 25 g of leaf powder was marshaled by maceration in ethanol over 24 h under 25 °C with constant stirring according to Rouamba et al. (2018). The ethanolic leaf extract was filtered and dried in a vacuum evaporator and stored at 4 °C until further investigation.

2.2. Chemicals and reagents

2, 2′-diphenyl-1-picrylhydrazyl (DPPH), 3 (4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl-tetrazolium bromide (MTT), Trypan blue and calf thymus DNA were purchased from Sigma Aldrich Co. (St. Louis, USA). Lymphoprep from Stemcell Technologies (USA), pBR322 plasmid DNA was obtained from Genei, Bangalore, India. RPMI-1640 containing L-glutamine, and streptomycin/penicillin were purchased from GIBCO BRL Life Technologies (Grand Island, NY, USA). Hydrogen peroxide, ascorbic acid, ethanol, and other analytical reagents were obtained from the Sisco research laboratory, India. Nanopure water was obtained from a Millipore Elix/A-10 water purification system. Freshly prepared solutions were used for each experiment.

2.3 . Phytochemical analysis

The ethanolic leaf extracts of *Abutilon indicum* (EEAI) was subjected to phytochemical analysis according to Kumar et al. (2013). The total phenolic compound in ethanolic leaf extract of *A. indicum* was estimated by Singleton and Rossi (1965) using a calibration curve of gallic acid. The results were expressed as milligram gallic acid equivalents (GAE)/g of dry plant extract. The total flavonoid content was determined by Dewanto et al. (2002) using a standard curve with quercetin and the results are expressed as milligram of quercetin equivalents (QE/g of dry plant extract). The total alkaloid content was estimated (Shamsa et al., 2008) using a standard curve with caffeine. The results were expressed as milligram caffeine equivalents (CE)/g of dry plant extract.

2.4. In vitro free radical scavenging activity

Different concentrations increasing from 50 to 300 μg/mL of EEAI were assessed in various *in vitro* model systems for their antioxidants activity. The DPPH assay was done for EEAI with different concentration according to Naik et al. (2004). The kinetics of DPPH reaction with the EEAI was analyzed by a stopped-flow kinetic spectrometer (model SX 18 MV UK) according to Naik et al. (2004) in single mixing mode. The examination of the kinetic runs was carried out with an exponential function by in-built software. Ascorbic acid was used as a positive control reference compound for all the *in vitro* antioxidant system. DPPH was used as a blank and the reaction mixture without the plant extract was the negative control. Three independent runs were used to analyze the constant rate and concentration.

Superoxide radical was investigated based on the capacity of EEAI to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin–light–NBT system according to Winterbourn et al. (1975). The ability of EEAI to scavenge hydrogen peroxide was determined using the method of Ruch et al. (1989). The ability of the EEAI to quench nitric oxide radicals was determined as described by Parul et al. (2012). The capacity of the EEAI to reduce Fe^{3+} to Fe^{2+} was determined according to the method of Oyaizu (1986). Using a nonlinear regression algorithm, the concentration of EEAI need to quenching free radicals was estimated by IC_{50}. Ascorbic acid was used as a positive control reference compound for all the *in vitro* antioxidant system. All the *in vitro* antioxidant assays were performed in triplicate in three different experiments.

2.5. Cytoprotective activity

2.5.1. Isolation and culture of peripheral blood lymphocytes (PBLs)

Peripheral blood lymphocytes (PBLs) were isolated from normal healthy volunteers with questionnaire based proper informed consent about the research and use. The study was approved by the Institutional Ethics Committee under the reference agreement no. 2014-440/DS/BIO.

Peripheral blood lymphocytes (PBLs) were isolated under sterile conditions by using a lymphoprep density gradient method according to manufacturer's instructions. Briefly, whole blood was collected, layered over lymphoprep gradient and centrifuged

for 30 min at 800g, after which the supernatant's top two-thirds was collected and washed with RPMI-1640 and viability assessed by the trypan blue exclusion test. PBLs were cultured in RPMI 1640 supplemented with 1 mM gentamycin and 10% of fetal bovine serum (FBS). PBLs cells were maintained at 37 °C in a humidified 5% CO_2 atmosphere. Cell viability of about 95% was accepted for further experiments.

2.5.2. Cell treatment and MTT assay

To assess the effect of EEAI to protect peripheral blood lymphocytes from cytoprotective (oxidative damage) effects, PBLs cells were seeded 2×10^5 cells/well in 96 well plates for 24 h then, the cells were treated with vehicle (culture medium RPMI 1640) or with different concentrations of EEAI (50–300 μg/mL) in culture medium RPMI 1640 for 3 h at 37 °C in 5% CO_2 incubator before exposure to hydrogen peroxide (induce oxidative stress). After incubation the cells were washed with PBS, pH 7.4, and then exposed an effective dose of hydrogen peroxide (25 μM, 60 min, 37 °C) according to Boligon et al. (2012). A PBLs cell cultured alone in medium was used as negative control group, while treatment with ascorbic acid (standard control) was compared with EEAI treated cells. The medium culture added 25 μM hydrogen peroxide alone was used as positive control group. Ascorbic acid was used as the standard and the experiment was performed in triplicate. After treatment, the cells were removed and washed with PBS used for MTT assay was performed as described previously for evaluating the cytotoxicity and the cytoprotective effects of the extract (Mosmann, 1983).

2.6. Antimutagenic property in DNA

2.6.1. Protective effects of genotoxicity

Assay for protective effects of pBR322 DNA cleavage was performed according to Russo et al. (2003) with slight modification. Briefly, in a total volume of 20 μl reaction mixture, 33 μM of pBR322 DNA in 5 mM phosphate saline buffer (PBS) (pH 7.4) was treated with 80 μg of EEAI in a polyethylene microcentrifuge tube and incubated for 10 min at room temperature. Then, H_2O_2 was added to a final concentration of 2.5 mM and the reaction tube was placed directly on the surface of a transilluminator (8000 μW $cm^{-}1$) at 300 nm for 5 min at room temperature. pBR322 DNA alone was used as a control. pBR322 DNA and 80 μg of EEAI without H_2O_2 treatment was used as a test control. After treatment, the samples were analyzed by electrophoresis and photographs were taken under UV (312 nm) transillumination to visualize DNA mobility.

2.6.2. Fenton-induced DNA protection

A Fenton-induced DNA protection study of EEAI was carried out according to Shih and Hu (1996) with slight modification. Briefly, in a total volume of 20 μl reaction mixture, 600 ng calf thymus DNA, 20 mM PBS was treated with 80 μg of EEAI in a polyethylene microcentrifuge tubes and incubated for 10 min at room temperature. Then the Fenton-reaction mixture (15 mM EDTA, 15 mM H_2O_2, and 8 mM $FeSO_4$) was added and incubated for 30 min. Calf thymus DNA alone was used as a control. Calf thymus DNA with 80 μg of EEAI was used as a test control. After treatment, the samples were analyzed by electrophoresis and photographs were taken under UV (312 nm) transillumination to visualize DNA mobility.

2.7. Statistical analysis

The experiments were conducted in triplicate in three independent experiments and data presented as mean values with standard deviation. Statistical analysis was performed using SPSS version 2.0. The IC50 values of the extract were compared with one-way ANOVA, followed by Dunnet's –test ($p < 0.05$).

3. Results

3.1. Phytochemical analysis

The EEAI extract was subjected for phytochemical analysis and identified the presence of flavonoids, glycosides, tannins, phenolics, triterpenoids, saponins and glycosides containing components (Table 1a). The EEAI extracts also investigate which shows, a great quantity of total phenolics content (9.351 mg GAE/g extract), total flavonoids content (6.234 mg QE/g extract), and total alkaloids content (4.624 mg CE/g extract) as summarized in Table 1b.

3.2. In vitro antioxidant activity

The antioxidant property of the EEAI was examined for its ability to quench free radicals *in vitro* as represented in Table 2 and Figs. 1 and 2 as mentioned below. The reference compound ascorbic acid showed the highest antioxidant activity. These findings revealed that the EEAI is an effective scavenger of free radicals.

3.2.1. DPPH radical scavenging activity

The scavenging percentage of DPPH radical by EEAI at different concentrations is shown in Fig. 1a. The quenching of DPPH by EEAI was in a dose-dependent manner. EEAI was found to have strong DPPH scavenging capacity, with IC_{50} values of 89.49 ± 1.23 μg/m L. The IC_{50} of the extract was found to be similar to the standard, ascorbic acid (IC_{50} 99.36 ± 1.89 μg/mL). The absorption of DPPH radical decayed in the presence of EEAI shown in Fig. 1b. The time plot of absorption was fitted with a single exponential function to obtain a constant rate, which was noticed to increase linearly from 50 to 300 μg/ml with increased EEAI concentration.

3.2.2. Superoxide radical (O_2^-) scavenging assay

The scavenging of superoxide radicals by EEAI is shown in Table 2. EEAI was observed to have powerful dose-dependent effect on the scavenging of superoxide radicals, with IC_{50} values

Table 1a
Qualitative estimation of the phytochemicals present in the ethanolic leaf extract of *Abutilon indicum*.

Test	EEAI
Flavonoids	+
Alkaloids	+
Phenolics compounds	+
Tannins	+
Terpenoids	+
Carbohydrates	–
Steroids	+
Saponins	+
Glycosides	+
Amino acids	+
Fatty acids	+

Table 1b
Quantitative estimation of the phytochemicals present in the ethanolic leaf extract of *Abutilon indicum*.

Test (mg/g dry plant extract)	EEAI
Total flavonoid content	6.234
Total alkaloids content	4.624
Total phenolics content	9.351

Table 2
Antioxidant activity of ethanolic leaf extract of *Abutilon indicum* (EEAI) and standard compound (Ascorbic Acid).

Concentration	Superoxide Radical Scavenging Activity (%)		NO Radical Scavenging Activity (%)		H_2O_2 Radical Scavenging Activity (%)	
(μg/mL)	EEAI	Ascorbic Acid	EEAI	Ascorbic Acid	EEAI	Ascorbic Acid
5	18.25 ± 1.12	11.35 ± 0.32*	18.56 ± 1.62	20.35 ± 2.12	19.56 ± 1.62	27.35 ± 2.12*
10	20.22 ± 1.92	17.82 ± 1.3*	23.85 ± 1.49	28.5 ± 0.82	23.51 ± 0.98	38.54 ± 2.82*
25	37.43 ± 2.43	28.21 ± 2.22*	34.5 ± 0.61	39.65 ± 2.18	26.75 ± 1.28	43.66 ± 1.98*
50	46.75 ± 2.53	36.35 ± 2.62*	45.76 ± 3.54	47.75 ± 2.33	31.87 ± 2.21	46.41 ± 3.10*
75	51.42 ± 3.61	48.45 ± 3.12	53.63 ± 2.32	56.13 ± 2.65	38.51 ± 0.78	48.34 ± 0.97*
100	60.16 ± 3.21	56.25 ± 4.23	60.32 ± 3.9	65.49 ± 2.54*	51.85 ± 1.87	56.57 ± 2.18
150	68.77 ± 1.42	61.54 ± 2.62*	64.71 ± 4.23	70.83 ± 1.87*	60.25 ± 2.32	66.35 ± 1.07*
200	75.73 ± 2.78	69.85 ± 3.92*	69.85 ± 1.67	80.43 ± 1.82*	65.87 ± 2.42	69.67 ± 2.62
250	84.56 ± 3.28	78.55 ± 2.72	71.57 ± 2.65	85.51 ± 2.32*	72.76 ± 3.23	77.91 ± 3.82*
300	93.39 ± 2.18	88.65 ± 5.42	78.52 ± 2.72	89.64 ± 1.23*	79.43 ± 2.08	85.18 ± 2.14*
IC_{50} (μg/mL)	72.96 ± 2.58	77.39 ± 2.18*	69.92 ± 2.59	66.80 ± 2.54	96.43 ± 2.56	88.38 ± 2.45*

Values are mean ± SEM, statistical significant test for comparison was done by ANOVA, followed by Dunnet's *t*-test (n = 3 independent experiments). IC_{50} value: inhibitor concentration giving 50% reduction of radicals.

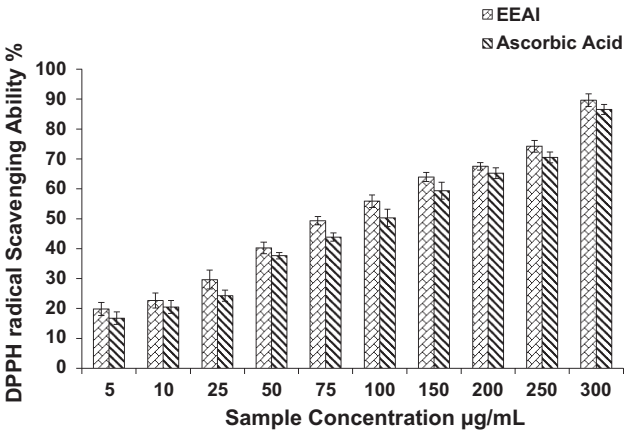

Fig. 1a. DPPH radical scavenging assay. DPPH radical scavenging activity of ethanolic leaf extract of *Abutilon indicum* and standard compound (Ascorbic acid). Effect of DPPH radical scavenging activity in percentage was plotted against the concentration of sample. Values are mean ± SEM, statistical significant test for comparison was done by ANOVA, followed by Dunnet's *t*-test for three sets of independent experiments. The IC_{50} value of the EEAI and standard were 89.49 ± 1.23 μg/mL, and 89.49 ± 1.23 μg/mL respectively.

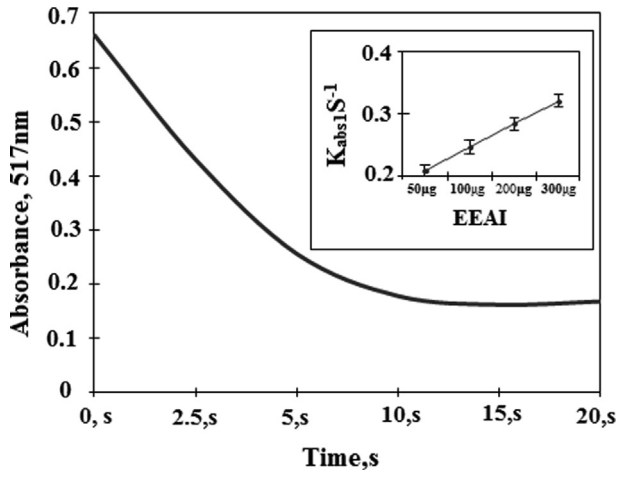

Fig. 1b. Kinetics of DPPH scavenging activity by ethanolic extract of *Abutilon indicum* (EEAI) on Stopped-flow spectrophotometer.

of for 72.96 ± 2.58 μg/mL. The IC_{50} of the extract was found to be similar to the standard, ascorbic acid (IC_{50} 77.39 ± 2.1 μg/ml). The quenching of superoxide radicals was observed by inhibiting the formation of blue formazan and the percentage of inhibition is directly proportional to the EEAI concentration.

3.2.3. Nitric oxide scavenging assay

The scavenging of nitric oxide by EEAI increased in a dose-dependent manner as illustrated in Table 2. It was found that the EEAI exhibited high nitric oxide radicals scavenging ability when compared with standard compound ascorbic acid, with IC_{50} values of 69.92 ± 2.59 μg/mL and 66.80 ± 2.54 μg/mL respectively.

3.2.4. Scavenging of hydrogen peroxide

The percentage of hydrogen peroxide scavenging by EEAI was observed in a concentration-dependent manner with, IC_{50} values of 96.43 ± 2.56 μg/mL (Table 2). The IC_{50} of the extract was found to be similar to that of ascorbic acid (IC_{50} 88.38 ± 2.45 μg/ml). The results reveal that EEAI has similar scavenging potency as ascorbic acid.

3.2.5. Reducing power

Transformation of Fe^{3+} into Fe^{2+} in the presence of EEAI was a dose-dependent activity as shown in Fig. 2. The IC_{50} value of the EEAI and standard were 72.08 ± 2.18 μg/mL, and 74. ± 2.14 μg/m

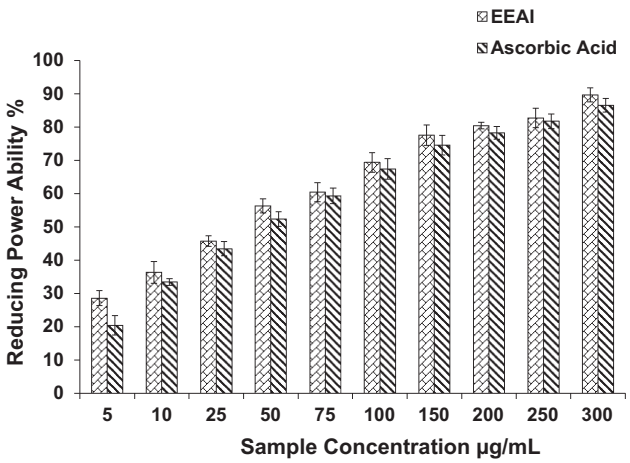

Fig. 2. Reducing power assay. The reductive abilities of ethanolic extract of *Abutilon indicum* (EEAI) and standard compound (Ascorbic acid). The data represent reducing power ability in percentage was plotted against the concentration of sample. Values are mean ± SEM, statistical significant test for comparison was done by ANOVA, followed by Dunnet's *t*-test for three sets of independent experiments. The IC_{50} - value of the EEAI and standard were 72.08 ± 2.18 μg/mL and 74. ± 2.14 μg/mL respectively.

L respectively. EEAI could reduce the most Fe^{3+} ions but had a lesser reductive activity than the standard ascorbic acid.

3.3. Cytoprotective activity

EEAI did not have a major impact on the integrity of peripheral blood lymphocytes which revealed non-toxicity to normal lymphocytes (Fig. 3). Cytotoxicity was observed in cells treated with 300 μg/mL of H_2O_2 was shown in Fig. 4 which depicts a strong cytotoxicity. However, with increasing concentrations of the EEAI, the cytotoxicity of hydrogen peroxide gradually reduced in a dose-dependent manner similar results were observed with the standard, ascorbic acid. Interestingly, the extract and ascorbic acid exhibited similar cytoprotective action ($p < 0.05$). Moreover, the extract at 150 μg/mL reduced more than 50% of the cytotoxicity of hydrogen peroxide.

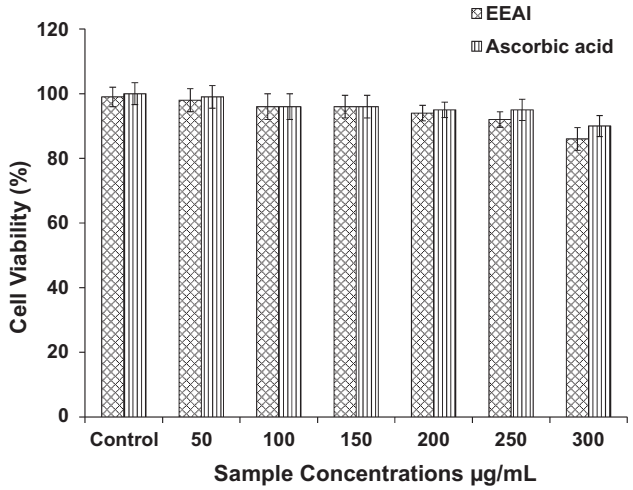

Fig. 3. MTT assay. Cytotoxic effects of ethanolic leaf extract of *Abutilon indicum* (EEAI) and Ascorbic acid on peripheral blood lymphocytes. Values are expressed as mean values ± standards for three sets of independent experiments.

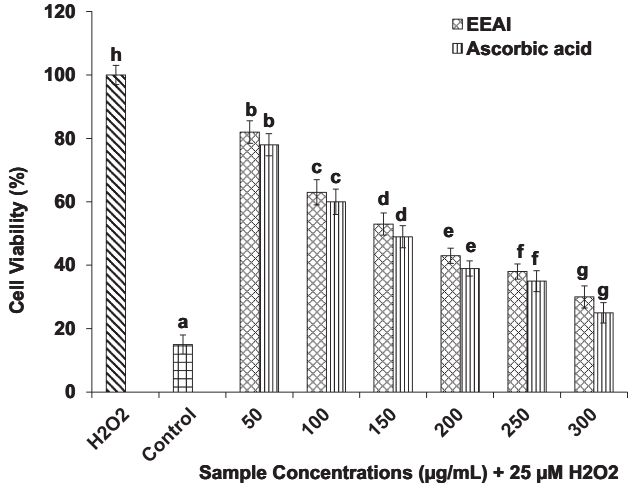

Fig. 4. Cytoprotective effects of ethanolic leaf extract of *Abutilon indicum* (EEAI) and Ascorbic acid against hydrogen peroxide-induced peripheral blood lymphocyte oxidative damage. Values are expressed as mean values ± standards deviation for three sets of independent experiments. [a–g] Denotes control cells compared with 50–300 μg/ml of extract and ascorbic acid concentration. [h] Denotes control cells compared with H_2O_2 treated peripheral lymphocytes. Statistical differences are expressed as $p < 0.05$.

3.4. Antimutagenic property of DNA

3.4.1. Protection from genotoxicity

Fig. 5 shows the protection imparted by EEAI to exposure to UV irradiation in the presence of H_2O_2. Control pBR322 plasmid shows two bands (Lane 1). The faster-moving band corresponded to the native form of supercoiled circular DNA (scDNA) and the slower moving band was the open circular form (ocDNA). Exposure to UV irradiation in the presence of H_2O_2 results in the cleavage of scDNA to ocDNA and linear form DNA (linDNA) in Lane 2, indicating that ·OH radical generated from UV photolysis of H_2O_2 produced DNA strand scission. Treatment with EEAI suppressed the formation of linear DNA and shows similar morphology as the pBR322 control plasmid when irradiated with UV in the presence of H_2O_2 (Lane 3). Treatment with EEAI without UV exposure did not change the migration pattern of plasmid DNA indicating that EEAI does not react with DNA molecule (Lane 4).

3.4.2. Fenton-induced DNA protection

Fig. 6 shows the protection imparted by EEAI to Fenton (hydroxyl radical) induced damage to calf thymus DNA. Control calf thymus DNA (Lane 1) and also evident is that the DNA integrity is not compromised when treated only with EEAI in Lane 2. Fenton (hydroxyl radical) induced damage to calf thymus DNA (Lane 3) and also protection imparted by EEAI to Fenton induced calf thymus DNA damage correlates well with the hydroxyl radical scavenging properties of EEAI (Lane 4).

4. Discussion

Dietary natural plant products have sparked interest in research due to the presence of medically significant bioactive constituents. The presence of these phyto-bioactive compounds emphasizes the importance of using natural plant products as nutrients for health care in the human diet (Mousavi et al., 2018). The use of natural plant products as nutritional supplements could be a better strategy for preventing chronic inflammation associated with oxidative cellular damage (Dhanasekaran, 2019; Dhanasekaran and

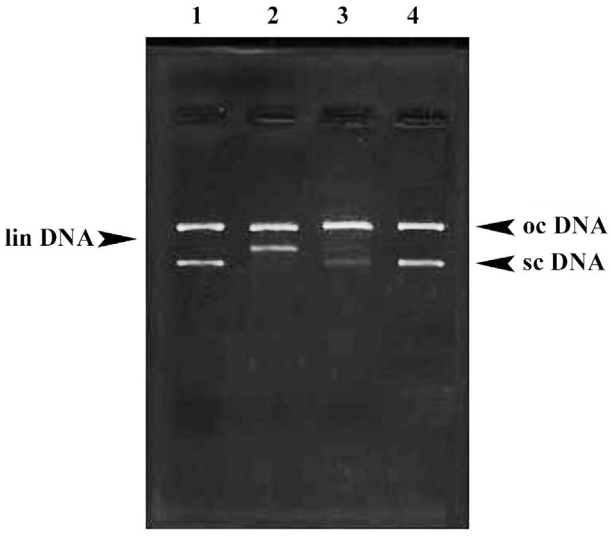

Fig. 5. Agarose electrophoretic pattern of pBR322 plasmid DNA with and without the treatment of ethanolic leaf extract of *Abutilon indicum* (EEAI). Reaction mixture containing EEAI and H_2O_2 exposed to UV (8000 μW cm^{-1}) at 300 nm for 5 min at RT. Lane 1: pPR322 plasmid DNA; Lane 2: pPR322 plasmid DNA exposed to UV; Lane 3: pPR322 plasmid DNA exposed to UV in the presence of EEAI and Lane 4: pPR322 plasmid DNA + EEAI without UV exposure. The position of open circular DNA (oc DNA), linear (lin DNA) and supercoiled (sc DNA) are indicated.

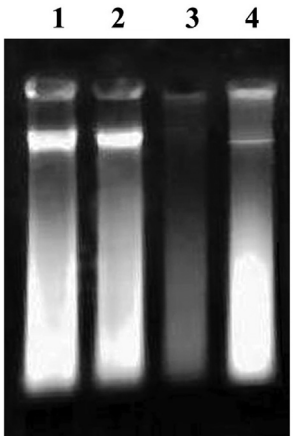

Fig. 6. Agarose electrophoretic pattern of calf thymus DNA pattern with and without the treatment of ethanolic leaf extract of *Abutilon indicum* (EEAI). Reaction mixture containing EEAI and 15 mM H_2O_2 and 8 mM $FeSO_4$ was incubated for 30 min. Lane 1: Calf thymus DNA; Lane 2: Calf thymus DNA + EEAI without reaction mixture; Lane 3: Calf thymus DNA + 15 mM H_2O_2 and 8 mM $FeSO_4$ and Lane 4: Calf thymus DNA + 15 mM H_2O_2 and 8 mM $FeSO_4$ + EEAI.

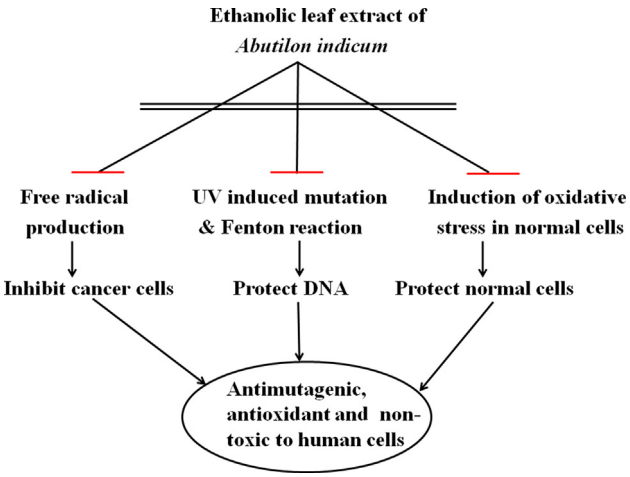

Fig. 7.

Jaganathan, 2018). Natural extracts with strong antioxidant properties generally contain compounds with a phenolic moiety, includes flavonoids, cathechins tocopherols, ascorbic acid and coumarins. Taninns, carotenoids, protein hydrolysates, and organic acids have a synergistic effect with phenolic compounds and act as antioxidants (Kaurinovic and Vastag, 2019). Present study revealed on analyses that the phytochemicals such as flavonoids, alkaloids, phenolic compounds, tannins, saponins, steroids, glycosides, and terpenoids were present in EEAI extracts, which possesses strong antioxidant activity. The extract also had a great quantity of total phenolics (9.351 mg GAE/g extract) and flavonoids (6.234 mg QE/g extract), as summarized in Tables 1a and 1b. These research findings illustrate that the ethanol extract of *Abutilon indicum* leaf is an effective free radical quencher, a powerful DNA protector and a potent cytoprotective agent. The extracts of *Abutilon indicum* is an effective scavenger of *in vitro* free radicals (DPPH, nitric oxide, superoxide, and hydrogen peroxide) as mentioned in Table 2. Several mechanisms could be associated with the cytoprotection of EEAL, such as the thorough quenching of free radicals (DPPH, nitric oxide, superoxide, and hydrogen peroxide), protection from the oxidative DNA strand breaks induced by Fenton reaction (Fig. 6), protection from the hydrogen peroxide generated oxidative stress by enhancing intracellular levels of glutathione and antioxidant enzymes which reduces the hydrogen to water molecules. Hydroxyl radicals produced by the Fenton reaction and UV exposure induces oxidative DNA strand breaks, resulting in relaxed forms or open circular DNA (Fig. 5). Therefore, these radicals react with DNA bases generating sugar and base radicals causing the breakdown of sugar-phosphate backbone resulting in strand breaks (Collin, 2019). The ethanolic leaf extract of *Abutilon indicum* protects the pBR322 plasmid DNA by combating against impairment caused by hydroxyl (•OH) radicals.

Natural bio-resources enriched with antioxidants have the reliability to prevent DNA from harmful radiation effects and Fenton reagent (Aryal et al., 2019). Bioactive phytoconstituents and their derivatives are involved in slowing the impact of carcinogenic agents. The free radical quenching of *Abutilon indicum* validated in this study was consistent with earlier studies (Kushwaha et al., 2017; Gomaa et al., 2018). The cytoprotective an effect of flavonoids in hydrogen peroxide treated peripheral blood lymphocytes has been previously established (Boligon et al., 2012). The present investigation validates that *Abutilon indicum* extract has been recorded to have a high flavonoid content that could make a significant contribution towards its cytoprotective effects (Thakor et al., 2016; Oibiokpa et al., 2014). *Abutilon indicum* extract enhances the cell survival by eliminating ROS which helps the cells to maintain resistance and combat against oxidative stress and increases cell survival (Mata et al., 2016). Recent studies have revealed the cytoprotective efficacy of bioactive compounds from traditional medicinal plant extracts (Song et al., 2019; Dhanasekaran et al., 2012). The phenolic compounds found in *Abutilon indicum* are known as powerful chain-breaking antioxidants (Das et al., 2019). Polyphenols are the major phytochemical constituents reported in *Abutilon indicum* that scavenge free radicals due to its hydroxyl group and may directly contribute to its antioxidative functions (Tlili and Sarikurkcu, 2020; Abcha et al., 2019; Bendary et al., 2013). Furthermore, it suggests that polyphenols possess inhibitory effects on carcinogenesis and mutagenesis in humans (Rybková et al., 2016; Vitaglione and Fogliano, 2004). Phytochemical screening of *Abutilon indicum* may facilitate the development of future traditional medicine as dietary supplements for the betterment of consumer health (Ezzat et al., 2019).

Thus, *Abutilon indicum* extracts can be used as an alternative and perhaps as a more cost and risk-effective source of natural antioxidants. The stronger antioxidant ability of ethanolic extracts from this plant seen in the present study suggests that much of the bioactive molecules of EEAI are polar, phenolic and flavonoid constituents. Further research is underway on the isolation and identification of these bioactive molecules. Taken together, these molecular findings lead to the suggestion that bioactive components of ethanolic leaf extracts of *Abutilon indicum* have strong anti-radical, anti-oxidant and anti-mutagenic components (Fig. 7) that might be helpful and informative in controlling health problems during degenerative diseases.

5. Conclusions

Present investigation revealed the strong antioxidant, non-toxic and antimutagenic properties of ethanolic leaf extract of *Abutilon indicum*. On analysis, EEAI contains large amounts of phenolic and flavonoid compounds that exhibited the highest antioxidant and free radical quenching, cytoprotection, strong inhibition of Fenton reaction and UV induced DNA damage. There is also a significant correlation between free radical quenching results and complementary assays. This study demonstrates that flavonoids and phenolic compounds present in ethanolic leaf extract of *Abutilon indicum* are responsible for the cytoprotective and

antimutagenic activity (Fig. 7). However, the active components responsible for these antioxidative activities are currently unclear. Further pharmacological studies are underway to isolate and identify the bioactive constituents from the ethanolic leaf extracts *Abutilon indicum* that needs to be complemented by in *vivo* studies for a better understanding of their mechanism of action may pave the way to develop a new therapeutic natural drug.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Financial Disclosures

None.

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