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In vitro studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum graecum*) seeds

S. Kaviarasan [a], G.H. Naik [b], R. Gangabhagirathi [c], C.V. Anuradha [a], K.I. Priyadarsini [b,*]

[a] *Department of Biochemistry, Annamalai University, Annamalai Nagar, Tamil Nadu 608 002, India*
[b] *Radiation and Photochemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India*
[c] *Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400085, India*

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Abstract

An extract of fenugreek (*Trigonella foenum graecum*) seeds was isolated and evaluated for antioxidant activity using various in vitro assay systems. The seed extract exhibited scavenging of hydroxyl radicals ($^{\cdot}OH$) and inhibition of hydrogen peroxide-induced lipid peroxidation in rat liver mitochondria. The $^{\cdot}OH$ scavenging activity of the extract was evaluated by pulse radiolysis and the deoxyribose system. The antimutagenic activity of the extract was recorded by following the inhibition of γ-radiation induced strand break formation in plasmid pBR322 DNA. The extract at high concentrations acted as a scavenger of 2,2′-diphenyl-1-picryl hydrazyl hydrate (DPPH) and 2,2′-azinobis 3-ethylbenzothiazoline-6-sulfonate ($ABTS^{\cdot-}$) radicals. The total phenolic content in the extract was determined spectrophotometrically according to the Folin–Ciocalteau procedure and expressed as mg or mM gallic acid equivalents. The results indicate that the extract of fenugreek seeds contains antioxidants and protects cellular structures from oxidative damage. These findings provide evidence for the in vivo beneficial effects of the seeds reported in the literature.

Keywords: Fenugreek seeds; Mitochondria; Free radicals; Antioxidants; Lipid peroxidation

1. Introduction

Polyphenols constitute a large group of naturally occurring substances in the plant kingdom, which include the flavonoids. The plant phenolics are commonly present in fruits, vegetables, leaves, nuts, seeds, barks, roots and in other plant parts. These substances have considerable interest in the field of food chemistry, pharmacy and medicine due to a wide range of favorable biological effects including antioxidant properties. The antioxidant property of phenolics is mainly due to their redox properties. They act as reducing agents (free radical terminators), hydrogen donors, singlet oxygen quenchers and metal chelators (Cook & Samman, 1996). Evidence suggests that high intake of antioxidant nutrients from food sources offers health advantages (Aruoma, 1998).

Fenugreek (*Trigonella foenum graecum*) is an annual herb that belongs to the family *Leguminosae*. The seeds of fenugreek are commonly used in India and in oriental countries as a spice in food preparations due to their strong flavor and aroma. The seeds are reported to have restorative and nutritive properties and to stimulate digestive processes (Khosla, Gupta, & Nagpal, 1995). Fenugreek seeds are used as a traditional remedy for the treatment of diabetes (Basch, Ulbricht, Kuo, Szapary, & Smith, 2003; Miraldi, Ferri, & Mostaghimi, 2001). Clinical and experimental studies have documented antidiabetic and antiatherosclerotic effects (Ajabnoor and Tilmisany, 49 1988; Sharma & Raghuram, 1990).

Studies show that fenugreek seeds have antioxidant properties. Supplementation of fenugreek seed powder in the diet leads to a reduction in biomarkers of oxidative damage in alloxan-diabetic rats (Ravikumar & Anuradha, 1999). Further fenugreek seed polyphenols prevented oxidative hemolysis and lipid peroxidation induced by H_2O_2

* Corresponding author. Tel.: +91 22 25595399; fax: +91 22 25505151.
E-mail address: kindira@apsara.barc.ernet.in (K.I. Priyadarsini).

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in vitro in human erythorcytes (Kaviarasan, Vijayalakshmi, & Anuradha, 2004).

The promotion of antioxidants as food additives or as therapeutic agents requires proof on their efficacy in various in vitro systems besides their pharmacological, toxicological properties and in vivo benefits. The antioxidant activity of the seeds in vivo and the traditional medicinal uses has prompted us to investigate the in vitro antioxidant activity of the seeds in detail.

We now report the antioxidant activity of fenugreek seed extract by in vitro studies. The ability of seeds to protect mitochondrial lipids and antioxidant enzyme superoxide dismutase (SOD) from H_2O_2-induced damage and plasmid DNA from γ-radiation were assayed. The total radical scavenging activity of the extract was tested by the ability to scavenge the 2,2′-diphenyl-1-picryl hydrazyl hydrate (DPPH) and 2,2′-azinobis 3-ethyl benzothiazoline-6-sulfonate ($ABTS^{\bullet-}$) radicals while, hydroxyl radical ($^{\bullet}OH$) scavenging activity was assayed by the deoxyribose system and pulse radiolysis.

2. Materials and methods

2.1. Chemicals

DPPH and $ABTS^{2-}$ were purchased from Aldrich Chemicals, USA. Thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), ascorbic acid and epinephrine were obtained from Sigma Chemicals, USA. All other reagents used were of the highest available purity. Nitrous oxide (N_2O) and oxygen (O_2) gases obtained from Indian Oxygen Ltd., Mumbai, were of IOLAR grade purity. Nanopure water, obtained from a Branstead nanopure purification system, was used for preparation of aqueous solutions. Freshly prepared solutions were used for each experiment.

2.2. Preparation of fenugreek seed extract

Fenugreek seeds (100 g) were finely powdered, mixed with 80% methanol and kept at room temperature for 5 days. After 5 days it was filtered and the solvent was evaporated to get the residue. The residue was dissolved in water and the aqueous layer was washed with petroleum ether several times until a clear upper layer of petroleum ether was obtained. The lower layer was then treated with ethyl acetate containing glacial acetic acid (10 ml/l). Extraction was then carried out for 36 h at room temperature and the combined ethyl acetate layer was concentrated (Xia, Allenbrand, & Sun, 1998). The residue was lyophilised and stored at −70 °C. This yielded about 6–8 g per 100 g of seed powder. An aqueous extract was prepared and used for in vitro studies. The polyphenolic content of the extract was assessed by method of Singleton and Rossi (1965) and expressed as mg gallic acid equivalents (GAE) per gram of dry extract, using a standard curve generated with gallic acid.

2.3. Isolation of mitochondria

Mitochondria was isolated from the liver of normal male albino Wistar strain rats (180–200 g b.w). Normal animals ($n = 6$) were killed by decapitation, livers were quickly removed and washed with isolation medium (ice-cold 0.25 M sucrose containing 10 mM Tris–HCl, pH 7.4) and a 10% liver homogenate was prepared by differential centrifugation. All operations were carried out at 0–4 °C. The protein content was estimated by the method of Lowry, Rosenbrough, Parr, and Fandall (1951). During the experiment, mitochondrial fraction was appropriately diluted with pH 7.4-phosphate buffer.

2.4. Lipid peroxidation

Lipid peroxidation was determined by quantitating the thiobarbituric acid reactive substances (TBARS) after exposure of mitochondrial samples to H_2O_2, in the absence and in the presence of the extract at various time intervals. The reaction was initiated by adding H_2O_2 (100 μM) to a reaction mixture containing 0.5 ml mitochondrial sample and extract (20 μg/ml) in a total volume of 1.5 ml. After incubation at 37 °C for various time intervals, the reaction was stopped with TCA and colour developed with thiobarbituric acid (0.375%w/v). The absorbance was measured at 532 nm ($\varepsilon\ 532 = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$).

2.5. Superoxide dismutase enzyme activity

Protection of superoxide dismutase (SOD) against peroxide-induced damage was studied in isolated rat liver mitochondria. Mitochondrial samples suspended in oxygenated phosphate buffer equivalent to 2 mg protein/ml were taken in glass vials and treated with H_2O_2 (100 μM) both in presence and absence of the extract. The basal activity was determined in mitochondrial samples in the absence of H_2O_2. SOD activity in control and test samples was assayed according to the procedure reported in the literature (Sun & Zigman, 1978). One unit of SOD is that amount of enzyme which inhibits 50% epinephrine autoxidation.

2.6. 2-Deoxy-D-ribose degradation assay

The ability of the extract to scavenge $^{\bullet}OH$ was conducted in Fe^{3+}–EDTA–H_2O_2–deoxyribose system (Halliwell, Gutteridge, & Aruoma, 1987). The extent of deoxyribose degradation by the formed $^{\bullet}OH$ was measured directly in the aqueous phase by the thiobarbituric acid test (Nath, Balla, Croatt, & Vercelloti, 1995). Inhibition of deoxyribose degradation (I) in percentage was calculated according to the equation

$$I = (A_0 - A_1/A_0) \times 100, \quad (1)$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance of the test compound.

2.7. Mitochondrial hydroxyl radical assay

Mitochondrial production of ·OH· like species was determined by following the oxidation of ·OH scavenging agent, dimethylsulfoxide (DMSO) to formaldehyde after treating the mitochondria with 100 μM H_2O_2 in the presence or absence of the extract (100 μg/ml) (Dicker & Cederbaum, 1992). The reaction system contained 100 mM potassium phosphate, pH 7.4, 1 mM sodium azide (to inhibit any catalase present), 0.05 mM ferric-EDTA (1:2), 33 mM DMSO, and 0.2–0.3 mg mitochondrial protein in a final volume of 0.5 ml. Reaction was initiated by the addition of NADH (final concentration 1.5 mM) and terminated after 15 min by the addition of TCA. The production of formaldehyde was then determined (Nash, 1953).

2.8. DNA damage in plasmid pBR322

Plasmid DNA is a good model system for studying the DNA damage and its modulation. Agarose gel (1%) was prepared in 89 mM Tris-borate/2 mM EDTA (TBE) buffer. Ethidium bromide was added in the gel preparation at a concentration of 0.5 μg/ml to enable the visualization of the DNA bands in a UV transilluminator. The gel was submerged in an electrophoresis tank filled with TBE buffer. About 2 μg of pBR322 DNA was suspended in 50 μl of 10 mM sodium phosphate buffer, pH 7.4, and exposed to γ-radiation at a dose of 6 Gy in the absence and presence of extract. Control samples were mixed with the dye (0.25% bromophenol blue and 30% glycerol) and loaded into the wells. Electrophoresis was carried out at 60 V for about 2.5–3 h till the open circular and the supercoiled form of DNA were well separated. The movement of the DNA bands was visualized on a UV transilluminator. The intensity of the bands was determined using gel documentation system (Devasagayam, Subramanian, Singh, Ramanathan, & Das, 1995).

2.9. Pulse radiolysis studies

The kinetics of hydroxyl radical scavenging capacity of the extract was carried out by the pulse radiolysis technique, the details of which are given elsewhere (Priyadarsini, Naik, Moorthy, & Mittal, 1991). Typically 50 ns pulses of 7 MeV electrons from a linear electron accelerator were used for the pulse radiolysis studies. Aerated aqueous solution of 0.01 M KSCN was used for determining the dose/pulse which was kept at 7 Gy. Radiolysis of water leads to the formation of three highly reactive species (·H, ·OH, e^-_{aq}). In N_2O-saturated solutions e^-_{aq} is quantitatively converted to ·OH radicals with G (·OH) = 0.56 μmol J^{-1}.

Hydroxyl radical scavenging activity was studied by competition kinetic methods. In this method competition between thiocyanate anion and extract towards hydroxyl radical was followed by monitoring the yield of $(SCN)^{\cdot-}_2$ which absorbs at 500 nm. For this N_2O saturated solutions of 2 mM KSCN at pH 7 (10 mM phosphate buffer) were pulse radiolysed, in the absence and in the presence of varying concentrations of extract.

2.10. DPPH and $ABTS^{\cdot-}$ assay

In separate experiments, one ml of 100 μM DPPH in methanol and 500 μM $ABTS^{\cdot-}$ were mixed with equal volume of the extract solution in phosphate buffer (pH 7.4), mixed well and kept in dark for 20 min. The absorbance at 517 nm (DPPH) and 635 nm ($ABTS^{\cdot-}$) was monitored in presence of different concentrations of extract. Blank was also carried out to determine the absorbance of DPPH and $ABTS^{\cdot-}$ before reaction with the extract. The amount of the extract in μg/ml at which the absorbance decreased to half its initial value was taken as the IC_{50} value for the extract. Percentage inhibition of the radicals was calculated according to Eq. (1). Here A_0 is the absorbance due to either DPPH or $ABTS^{\cdot-}$ in the absence of the extract and A_1 is that in the presence of the extract.

Kinetics of DPPH and $ABTS^{\cdot-}$ reaction with the extract was studied using stopped-flow kinetic spectrometer (model SX 18 MV from Applied Photo Physics, UK) in single mixing mode and by steady state kinetics. In this experiment, syringe I contained 50 μM DPPH in methanol or 50 μM $ABTS^{\cdot-}$ (prepared by mixing stoichiometric amounts of $ABTS^{2-}$ with potassium persulphate in water) (Erel, 2004) and syringe II contained varying concentrations (100–1000 μg/ml) of the extract in methanol. The dead time of the instrument was 1.3 ms and the mixing volume was 100 μl. After mixing, the reaction was followed by monitoring the absorbance in reduction due to disappearance of DPPH and $ABTS^{\cdot-}$ radicals, at 517 and 635 nm, respectively, as a function of time at 25 °C. Analysis of the kinetic traces was carried out with an exponential function using the built-in software. At least three independent runs were used to determine the rate constant at any concentration.

3. Results and discussion

3.1. Lipid peroxidation

Peroxidation is important in food deterioration and in the oxidative modification of biological molecules particularly lipids. Inhibition of lipid peroxidation by any external agent is often used to evaluate its antioxidant capacity. In the present study the inhibition of lipid peroxidation induced by H_2O_2 by the seed extract was carried out in rat liver mitochondria. Table 1 gives the level of lipid peroxidation in terms of TBARS produced in rat liver mitochondria induced by 100 μM H_2O_2 in the presence and absence of seed extract (20 μg/ml) at different time intervals (0–90 min). TBARS production was found to increase with time and maximum inhibition (15%) was observed at 90 min. Preincubation of the mitochondria with the extract showed significant inhibition of lipid peroxidation at all

Table 1
Levels of thiobarbituric acid reactive substances (TBARS, nmol/mg protein) in rat liver mitochondria exposed to H_2O_2 (100 μM) in the presence and absence of extract at different time points

Time	Without extract	With extract
0	1.68 ± 0.18	0.96 ± 0.08*
30	4.47 ± 0.41	3.26 ± 0.30*
60	5.04 ± 0.49	4.42 ± 0.43*
90	5.62 ± 0.52	4.8 ± 0.45*

Values are means ± SD of five determinations.
* Significant at $p < 0.01$ compared to that without extract (Student's *t*-test).

time points indicating that the extract protects the mitochondria from peroxide-induced lipid peroxidation.

3.2. Superoxide dismutase enzyme activity

SOD is an important cellular antioxidant enzyme, which converts superoxide radical into H_2O_2 and O_2. We also looked for the protective effect of the seed extract on antioxidant enzyme SOD in mitochondria exposed to H_2O_2. Table 2 gives the changes in the activity of SOD upon treatment with 100 μM H_2O_2, in the presence and absence of the extract. Upon exposure to H_2O_2, the enzyme activity declined by 52% (basal-6.15 ± 0.5 U/mg protein; with 100 μM H_2O_2 – 2.96 ± 0.15 U/mg protein). The extract at all concentrations protected the enzyme from the damage caused by peroxide. In the presence of extract the decline was significantly lower (27% at 80 μg/ml and 24% at 100 μg/ml). The protective effect of the extract was maximal at a concentration of 100 μg/ml and there was 76% restoration.

3.3. 2-Deoxy-D-ribose degradation assay

Degradation of deoxyribose by ·OH releases certain products, which upon heating with thiobarbituric acid under acid conditions would yield a pink chromogen with the maximum absorbance at 532 nm. Several workers (Halliwell et al., 1987; Pin-Der-Duh, Pin-Chan-Du, & Gow-Chin Yen, 1999) have employed this system to assess the biological activity of various natural plant-derived biomolecules. The extract competes with deoxyribose for hydroxyl radicals and diminishes chromogen formation. Table 2 indicates that at all the concentrations tested, the extract exerted inhibition of ·OH radical formation during incubation period and in a concentration-dependent manner. The percentage inhibition of hydroxyl radical formation in deoxyribose system was higher at 100 μg of extract and was found to be 76%. The presence of phenolic groups in fenugreek seeds could be responsible for ·OH radical scavenging activity (Bors, Heller, Michel, & Stettmaier, 1996; Rice-Evans, 1995).

Table 2
Effect of various concentrations of the extract (μg/ml) on superoxide dismutase (SOD) activity in mitochondria exposed to H_2O_2 (100 μM) and on inhibition of ·OH radical formation

Volume of extract (μg/ml)	SOD activity (Units/mg protein)	% Inhibition of ·OH radical formation
0	2.96 ± 0.15	–
20	3.34 ± 0.28*	28 ± 2.52
40	3.57 ± 0.32*	43 ± 3.56
60	4.05 ± 0.39*	56.5 ± 4.87
80	4.49 ± 0.42*	61 ± 5.78
100	4.68 ± 0.45*	76 ± 6.80

Values are means ± SD of five determinations.
* Significant at $p < 0.01$ compared to that without extract (Student's *t*-test).

3.4. Mitochondrial hydroxyl radical assay

The production of ·OH by mitochondria could be determined by assaying the oxidation of chemical scavenging agents to products known to arise from their interaction with ·OH. In the present study the oxidation of DMSO to formaldehyde with NADH as a reductant was employed to evaluate the ·OH production in mitochondria. Fe^{3+}–EDTA was used as the iron catalyst to obtain maximal rates of ·OH production since this iron complex is most reactive in stimulating ·OH production by many biological and chemical systems. The rate of oxidation of DMSO as an index of ·OH radical production was increased three folds after treatment with hydrogen peroxide (Fig. 1). The production of formaldehyde was reduced considerably indicating the ·OH radical quenching activity of the extract.

3.5. Inhibition of radiation induced strand breaks in plasmid DNA

The role of the extract in altering the radiation induced strand break in plasmid pBR322 DNA was investigated. Gamma radiation induces many strand breaks in DNA by generation of hydroxyl radicals and subsequent free radical induced reactions on the DNA backbone (Von Sonn-

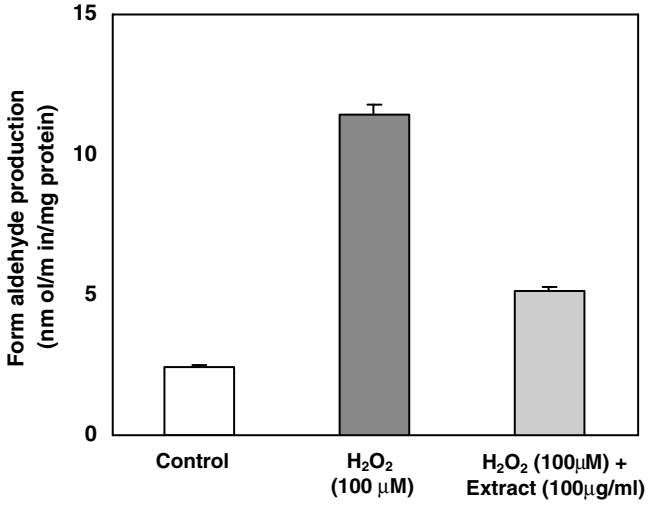

Fig. 1. Rate of formaldehyde formation from DMSO in the mitochondria after peroxide treatment. The oxidation of DMSO to formaldehyde was assayed as described in Section 2 in the presence of NADH as reductant.

tag, 1987). This leads to the formation of open circular form of DNA and these are visualized by horizontal gel electrophoresis. Detailed analysis was carried out regarding the extent of the open circular and the supercoiled form before and after irradiation. The samples of plasmid DNA were exposed to gamma radiation at a dose of 6 Gy both in the absence and in the presence of different concentrations of the extract from 50 to 500 μg/ml. The conversion of the supercoiled form to open circular form was monitored. Relative yield of open circular form of DNA decreased as the concentration of the extract was slowly increased from 50 to 500 μg/ml. Fig. 2 shows the percent inhibition of strand break formation. The antimutagenic activity is evident as the extract significantly reduced the production of radiation-induced open circular forms of DNA. The extent of protection conferred is significant only at high concentrations. This may be due to the low potential of the extract in scavenging free radicals as seen in the supporting experiments discussed below.

3.6. Pulse radiolysis studies

Nanosecond pulse radiolysis technique was carried out to study the reaction of the ·OH with the extract. Fig. 3 gives the transient spectrum obtained for the extract on reaction with ·OH. The transient signal intensities are very small and the transient spectra did not show formation of any characteristic species. The phenoxyl radicals of a number of polyphenols like gallic acid, hydroxy flavonoids show absorption in this wavelength region, this prompts us to speculate that polyphenols present in the extract, as in most of the plant extracts, may be responsible for such transient spectra. However very small absorbance values in the spectrum indicate presence of phenolics in much less concentrations. We tried to evaluate the rate constant for ·OH radicals reaction with the extract, however due to the presence of mixture of components in the extract, it is not possible to estimate by direct monitoring, we therefore

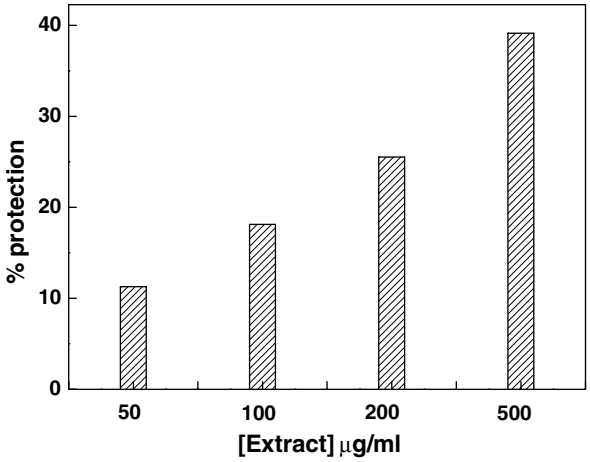

Fig. 2. Percentage protection as assessed by the inhibition of formation of open circular form is plotted against concentrations (50–500 μg/ml) of the extract, in pBR322 DNA assay after exposure to 6 Gy dose.

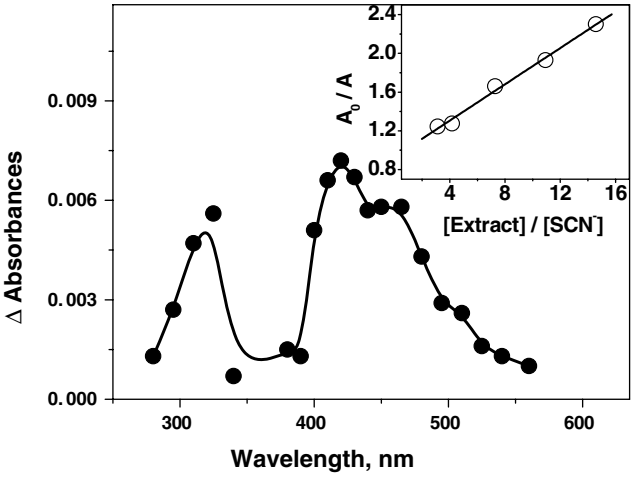

Fig. 3. Difference absorption spectrum of the transient obtained on pulse radiolysis of N_2O saturated aqueous solutions containing 124 μg/ml extract at pH 7. Inset shows linear plot for Eq. (4) for competition kinetics between thiocyanate and extract.

employed competition kinetic method. Using this technique, we have studied the relative rate constant of the extract with ·OH radicals. In this, ·OH radical was made to react with 2 mM SCN^- at pH 7, in the absence and in the presence of different concentrations of the extract. The formation of $(SCN)_2^{\cdot-}$ was observed by monitoring its absorbance at 500 nm. In the absence of the extract, the ·OH was found to react completely with SCN^- to produce $(SCN)_2^{\cdot-}$. However in the presence of the extract, a decrease in the absorbance of $(SCN)_2^{\cdot-}$ was observed, the extent of which was used to calculate the rate of scavenging of ·OH by the extract. In presence of extract the competing processes are

$$OH + SCN^- \rightarrow SCN^{\cdot} + SCN^- \rightarrow (SCN)_2^{\cdot-} \quad (2)$$

$$OH + \text{Extract} \rightarrow \text{Products} \quad (3)$$

Monitoring the absorbance of $(SCN)_2^{\cdot-}$ at 500 nm in the absence (A_0) and in presence of extract (A) a linear plot is obtained for the following relationship

$$(A_0/A) = 1 + k_2/k_1[\text{Extract}]/[\text{KSCN}]. \quad (4)$$

Here k_1 and k_2 correspond to the rate constants for the reactions given in Eqs. (2) and (3), respectively. Slope of the line for A_0/A vs. [Extract]/[KSCN] (both concentrations expressed in 307 μg/ml) gave the comparative ability of the extract towards ·OH radical with respect to KSCN. Thus from the slope (Fig. 3), the reactivity of the extract towards ·OH was found to be 10.3 times less than that of thiocyanate ($k_1 = 1.1 \times 10^{10}\ M^{-1}\ S^{-1}$) (Spinks & Woods, 1991).

3.7. DPPH and $ABTS^{\cdot-}$ radical scavenging assay

DPPH is a stable free radical having maximum absorption at 517 nm (Kumar, Priyadarsini, & Sainis, 2002; Wang, Jin, & Ho, 1999; Yokozawa et al., 1998) that accepts an electron or hydrogen atom to become a stable

diamagnetic molecule. We have studied the ability of the extract to neutralize the free radicals such as DPPH radicals. In the presence of the extract capable of donating an H atom its free radical nature is lost hence the reduction in DPPH radical was determined by the decrease in its absorbance at 517 nm. It is very convenient to follow the DPPH reactions and it has often been used to estimate the antiradical activity of the natural products. The decrease in DPPH absorption in the presence of varying concentrations of extract has been monitored. Fig. 4 gives the changes in the absorbance at 517 nm, at different concentrations of the extract. It can be noticed that the extract at high concentrations showed significant decrease in the absorbance of DPPH radical. From this figure, the IC_{50} value for the extract was found to be 350 μg/ml.

$ABTS^{\bullet-}$ radicals are more reactive than DPPH radicals and unlike the reactions with DPPH radical which involve H atom transfer, the reactions with $ABTS^{-}$ radicals involve electron transfer process. Fig. 5 depicts a decrease in the absorbance of $ABTS^{-}$ radical at 635 nm in the presence of the extract upto a concentration of 250 μg ml^{-1}. The IC_{50} value for the extract was found to be 117 μg ml^{-1}.

The kinetics of the DPPH and $ABTS^{\bullet-}$ radical reactions were studied by the stopped flow kinetics spectrophotometer. The decrease in the DPPH and $ABTS^{\bullet-}$ absorbance at 517 nm immediately after mixing with extract solution was monitored as a function of time to determine the rate constant. In the absence of the extract, no decrease in DPPH and $ABTS^{\bullet-}$ absorbance was seen with time; however, it started decreasing in presence of extract. Insets of Figs. 4 and 5 show the absorption–time plot for the decay of DPPH and $ABTS^{\bullet-}$ radicals, respectively, in presence of increasing concentration of extract (>100 μg /ml), where the absorption decayed almost completely due to reaction with extract in ~200 s. There exists

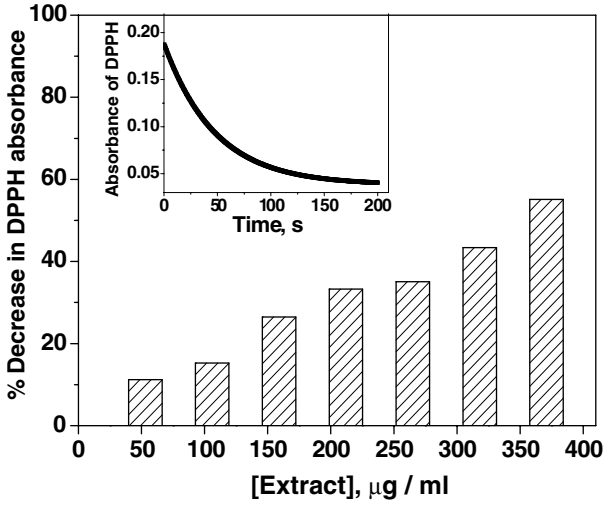

Fig. 4. Bar graph showing the percentage decrease in the absorbance at 517 nm after mixing 50 μM methanolic solution of DPPH radical with different concentrations of extract. Inset gives the absorption–time plot showing the decay trace of DPPH radicals at 517 nm in presence of 390 μg/ml of extract.

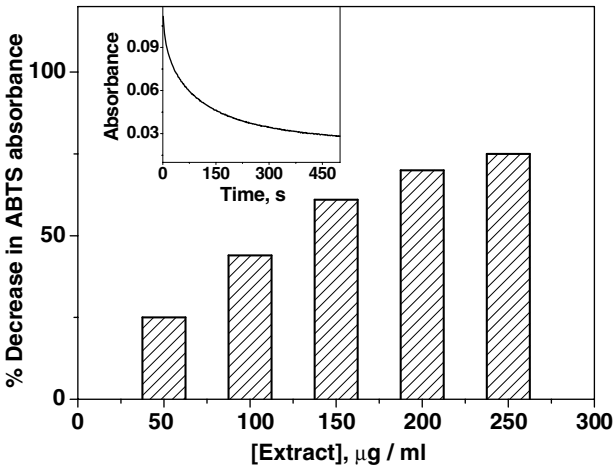

Fig. 5. Bar graph showing the percentage decrease in the absorbance at 635 nm due to $ABTS^{\bullet-}$ radical by different concentrations of the extract and inset gives the decay trace of $ABTS^{\bullet-}$ radicals at 635 nm in presence of extract (250 μg/ml).

a fast and slow component in the absorption decay trace. We compared the decay kinetics in presence of ascorbic acid and the ascorbate equivalents in the extracts were calculated to be <1%. The low potential of the extract in scavenging free radicals (ABTS) as compared to ascorbic acid might be attributed to the protective effect on mitochondria and DNA only at high concentrations.

The total phenolic content of the extract was estimated to be 78.6 ± 1.2 mg GAE/g seeds from five determinations. Earlier study by Gupta and Nair (1999) had shown that the fenugreek seeds are rich in flavonoids (>100 mg/g). Later Shang et al. (1998) identified five different flavonoids namely vitexin, tricin, naringenin, quercetin and tricin-7-*O*-β-D-glucopyranoside to be present in fenugreek seeds. In the present study the fenugreek seed extract showed significant antiradical and antioxidant properties, which were found to be concentration-dependent. The scavenging activities of the phenolic substances are attributed to the active hydrogen-donating ability of the hydroxyl substitutions (Bors et al., 1996). The presence of phenolic groups in the phytochemicals especially naringenin and quercetin in fenugreek seeds could be responsible for $^{\bullet}OH$ radical scavenging activity.

A broad spectrum of beneficial effects of fenugreek seeds in vivo is reported by a number of investigators. Besides antidiabetic effects, the seeds possess significant antiatherosclerotic (Sharma, Sarkar, Hazra, & Misra, 1996), anti-inflammatory (Ahmadiani, Javan, Semnanian, Barat, & Kamalinejad, 2001), antinociceptive (Puri, 1998) and anti-ulcerogenic activities (Suja Pandian, Anuradha, & Viswanathan, 2002). In one study fenugreek seeds displayed antineoplastic effects in Ehrlich ascites carcinoma cells (Sur et al., 2001).

4. Conclusion

An aqueous methanolic extract of fenugreek seeds was examined for its antiradical and in vitro antioxidant activity

in different model systems. The antiradical activity could be correlated with the polyphenolic components present in the extract. The results gained by these methods provide some important factors responsible for the antioxidant potential of fenugreek seeds and offer evidence for the large number of in vivo beneficial effects of the seeds reported in the literature. Further investigations in the field of toxicology and pharmacology are required.

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