Original article

Antioxidant and neuroprotective effects of standardized extracts of *Mangifera indica* leaf

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Abstract:

Standardized methanol and aqueous extracts of *Mangifera indica* L. (mango) leaf were analyzed for antioxidant and neuroprotective activities. Antioxidant activities were evaluated by 2, 2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), superoxide ($O^{\bullet}_2$), hydrogen peroxide (H_2O_2) scavenging assays and ferric reducing antioxidant power (FRAP) including ion-chelating activity. The neuroprotective effect was evaluated by measuring the protection of neuroblastoma cells from H_2O_2-induced oxidative injury. The IC_{50} values for the DPPH of the methanol and aqueous extracts were 6.18 ± 0.15 and 5.57 ± 0.18 μg/ml. The IC_{50} values for the ABTS of the methanol and aqueous extracts were 1.33 ± 0.13 and 2.96 ± 0.05 μg/ml. The EC_1 of FRAP for the methanol and aqueous extracts were 202.92 ± 3.39 and 298.55 ± 8.65 μg/ml, respectively. The IC_{50} values for the ($O^{\bullet}_2$) scavenging of the methanol and aqueous extracts were 0.07 ± 0.01 and 0.06 ± 0.01 μg/ml. The IC_{50} values for the H_2O_2 scavenging and ferrous ion chelating activity of the methanol extract were 70.89 ± 6.56 and 0.89 ± 0.81 μg/ml, respectively. The methanol extracts at 30-50 μg/ml and aqueous extracts at 100 μg/ml as well as mangiferin, a biologically active component present in the leaf of *M. indica,* at 100 μM effectively protected neuroblastoma cells from H_2O_2-induced oxidative damage. The beneficial effects of these extracts appear to be promising for neuroprotection.

Keywords: Aqueous extract; Antioxidant; Free radical scavenging; *Mangifera indica;* Methanol extract; Neuroprotective

Introduction

Mangifera indica L. (mango) has various biomedical applications including antioxidative or free radical scavenging, anti-inflammation [1], anti-allergic [2], cardio-protective [3, 4], anti-cancer [5, 6], hepatoprotective [7], analgesic [8, 9] and immunomodulator activities [10]. It is a rich source of various biologically active compounds. Phytochemical studies of different parts of *M. indica* have demonstrated the presence of phenol constituents, triterpenes, flavonoids, phytosterols, and polyphenols [11]. In the field of antioxidant, a previous study showed that the extract from stem bark of *M. indica* (Vimang®) could reduce the production of reactive oxygen species by peritoneal macrophages in mice. In addition, Vimang® showed a greater ability to reduce the formation of reactive oxygen species (ROS) in these mice compared to vitamin E [12]. In another study, it was shown that incubation of erythrocytes with Vimang® significantly enhanced the ability of the erythrocytes to resist H_2O_2 [13]. Recently, standardized ethanol and aqueous extracts from the leaf of selected species of *M. indica* were reported to contain free radical scavenging activities. Both extracts were shown to protect human keratinocyte and human fibroblast cells from DPPH and ABTS radical-induced cell death [14]. In the area of neuroscience, a phenolic compound present in *M. indica,* mangiferin, as well as *M. indica* stem bark extract showed neuroprotective effects *in vitro* model of glutamate-induced neuronal death [15, 16]. However, there is still lack of information of neuroprotective effect of *M. indica* leaf extract against oxidant-induced cell death. Therefore, it is worthwhile to investigate the protective effects of standardized methanol and aqueous extracts of *M. indica* leaf on H_2O_2-induced neuronal cell death. Before that, the antioxidant activity of mango leaf extracts was studied to investigate the underlying mechanisms. The beneficial effects of these extracts appear to be promising for neuroprotection.

Materials and Methods

Chemicals

2,2-diphenyl-1-picryl-hydrazyl (DPPH), 2,2-azinobis 3-ethylbenzothiazoline 6-sulfonate (ABTS), Trolox®, ferrous sulfate, NADH, polyornithine hydrobromide, bovine serum albumin, and trypan blue were purchased from Sigma (St. Louis, MO, USA). Potassium persulfate, 2,4,6,-tripyridyl-s-triazine complex (TPTZ), N-methylphenazonium methyl sulfate (PMS), nitrotetrazolium blue chloride (NBT), Folin-Ciocalteu, gallic acid, and tannic acid were purchased from Fluka (Switzerland). Sodium carbonate, dimethyl sulfoxide (DMSO), and hydrogen peroxide (H_2O_2) were purchased from Merck (Darmstadt, Germany).

Plant material

Fresh leaves of mango (Namdokmai) from Nakornpathom, Thailand were selected in August 2008 for the study.

Cell line and culture

The NG108-15 cells were a gift from Associate Professor Tohda Michihisa, Institute of Natural Medicine, University of Toyama. Cells were grown in DMEM containing 100 μM hypoxanthine, 1 μM aminopterin, 16 μM thymidine and 10% fetal bovine serum. Cell cultures were maintained in a humidified incubator with 5% CO_2 and 95% air at 37 °C.

Extract preparation from leaf of mango

The leaf were washed with water and allowed to air dry at room temperature. They was then placed in an oven at 40 °C until completely dry, after which they were powderized using a blender. A sample of 30 g was extracted with 300 ml of distilled water, methanol or chloroform. Extraction was carried out at room temperature and left shaking for 24 h. The suspension thus obtained was filtered using 2 layers of a filter cloth and the filtrate was collected. Aqueous filtrate was concentrated using a freeze drier while methanol and chloroform filtrate were concentrated using a rotary evaporator at 40 °C.

Antioxidant assays

Free radical scavenging activity for DPPH

A 200 μM DPPH in methanol (100 μl) was added to 100 μl of sample extracts. The extracts were dissolved by their solvent and then diluted to desired concentration

by methanol. The mixture was left standing at room temperature for 30 min.; the absorbance was then measured spectrophotometrically at 550 nm (Fusion Universal Microplate Analyser Model: A153601, Perkin Elmer Life and Analytical Sciences, Inc, USA). The results of the assay are expressed as IC_{50}, which represents the concentration of the extract (μg/ml) required to inhibit 50% of the free radical scavenging activity. The free radical scavenging activity was assessed using the formula:

$$\% \text{ inhibition} = (1-A_{sample}/A_{control}) \times 100 \quad (1)$$

where A_{sample} is the absorbance in the presence of the extracts and $A_{control}$ is the absorbance of control. The IC_{50} values were calculated by linear regression of plots where the x-axis represented the various concentrations (μg/ml) of test plant extracts while the y-axis represented the % inhibition.

Scavenging activity for ABTS radicals

ABTS is oxidized to the colored nitrogen-centered radical cation $ABTS^{\bullet+}$ in a persulfate system. $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS in distilled water with 4.95 mM potassium persulfate. The mixture was kept in the dark at room temperature for 12-16 h before use. Prior to the assay, the solution was diluted in phosphate buffer pH 7 to give an absorbance of 0.7 ± 0.02 at 734 nm in a 1-cm cuvette. Then, 3.9 ml of the working solution was mixed with 0.1 ml of extracts at various concentrations or negative controls (phosphate buffer) or positive controls (Trolox®). The extracts were dissolved in distilled water. After 10 min. at room temperature, the absorbance at 734 nm was read. The inhibition percentage was calculated using the following formula:

$$\% \text{ inhibition} = (1-A_{sample}/A_{control}) \times 100 \quad (2)$$

where A_{sample} is the absorbance in the presence of the extracts and $A_{control}$ is the absorbance of control. The radical scavenging activity is expressed as an IC_{50} value.

FRAP assay

The reducing ability of extracts was determined by the ferric reducing antioxidant potential (FRAP) assay. FRAP solution was freshly prepared on the day of use by mixing acetate buffer (pH 3.6), ferric chloride solution (20 mM) and TPTZ in 40 mM HCl (10 mM). Two hundred microliters of the diluted sample extract in methanol (or for the blank 200 μl methanol and for the positive control 200 μl Trolox®) was added to FRAP reagent and sodium acetate buffer to a final volume of 4 ml. The tubes were vortexed and left for exactly 30 min., and the absorbance was measured at 593 nm. The measurement was compared to a standard curve of $FeSO_4 \cdot 7H_2O$ solutions and expressed as an EC_1 value, which means the concentration of antioxidant in the reactive system having a ferric-TPTZ reducing ability equivalent to that of 1 mM $FeSO_4 \cdot 7H_2O$. Determination of each sample was performed in triplicate.

Superoxide scavenging

The samples were added into reaction mixtures containing 150 μM PMS, 500 μM NBT, or 730 μM NADH in 20 mM phosphate buffer. After incubation for 5 min. at room temperature, the absorbance was measured at 550 nm. The inhibition percentage was calculated using the following formula:

$$\% \ O^{\bullet}_2 \text{ inhibition} = (1-A_{sample}/A_{control}) \times 100 \quad (3)$$

where A_{sample} is the absorbance in the presence of the extracts and $A_{control}$ is the absorbance of the control. The $O^{\bullet}_2$ scavenging activity is expressed as an IC_{50} value.

Hydrogen peroxide scavenging

A solution of 43 mM H_2O_2 was prepared in 0.1 M phosphate buffer (pH 7.4). Extracts in 3.4 mL phosphate buffer was added to 0.6 mL of H_2O_2 solution (43 mM) and absorbance of the reaction mixture was recorded at 230 nm. A blank solution contained the sodium phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging by extracts and standard compounds, 2,6-Di-tert-butyl-4-methylphenol (BHT), was calculated using the following equation:

$$\% \text{ inhibition} = (1-A_{sample}/A_{control}) \times 100 \quad (4)$$

where A_{sample} is the absorbance in the presence of the extracts and $A_{control}$ is the absorbance of control.

Scavenging of hydroxyl radicals

The formation of hydroxyl radicals (OH·) from Fenton reagents was quantified using 2-deoxyribose oxidative degradation. The reaction mixtures contained deoxyribose (2.8 mM); $FeCl_3$ (100 mM); KH_2PO_4-KOH buffer (20 mM, pH 7.4); EDTA (100 mM); H_2O_2 (1.0 mM); ascorbic acid (100 mM) with different concentrations of the test extracts in a final volume of 1 ml. Samples were incubated at 37°C for 1 h and then 1.6 ml of 2.8% trichloroacetic acid and 1.6 ml of 0.6% aqueous solution of TBA were added to 0.8 ml of sample. The mixture was heated in a boiling water bath for 15 min. The sample was cooled and absorbance was taken at 532 nm. The percentage of OH· scavenging by extracts and standard compounds, mannitol, was calculated using the following equation:

$$\% \text{ inhibition} = (1-A_{sample}/A_{control}) \times 100 \quad (5)$$

where A_{sample} is the absorbance in the presence of the extracts and $A_{control}$ is the absorbance of control.

Ferrous ion chelating activity

The different concentrations of test compounds (0.1 ml) or EDTA (0.1 ml), standard compound, were added to a solution of 50 mM of $FeSO_4$ (0.1 ml) and 0.7 ml of 0.15 M NaCl solution. The reaction was initiated by the addition of ferrozine at 300 mM (0.1 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm in a spectrophotometer. The ferrous ion chelating activity is expressed as % inhibition.

Determination of total phenolic content

In order to ensure that every batch of the extract has the uniformity and consistency of the biological effects exhibited, total phenolic and tannic acid content as well as yield of extraction was carried out. The amounts of phenolic compounds in the extracts were determined using the Folin-Ciocalteu method which was adapted from Singleton *et. al.* [17], and gallic acid was used as the standard phenolic compound. Fifty microliter aliquots of the extracts (1 mg/ml) were added to a mixture of 2.5 ml of 10% Folin-Ciocalteu reagent and 2 ml of 7.5% Na_2CO_3. After incubation at 45 °C for 30 min., the absorbance was measured at 765 nm. A linear dose-response regression curve was generated using absorbance reading of gallic acid (0.1-1.0 mg/ml). The content of total polyphenols in the extract is expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g) of extracts.

C = A/B where C is expressed as mg GAE/g dry weight of the extract; A is the equivalent concentration of gallic acid established from the calibration curve (mg); and B is the dry weight of the extract (g).

Determination of tannic acid content

The tannic acid content in the extracts were determined using the method which was adapted from Silber *et.al.* [18]. First, 2 mg of bovine serum albumin was mixed with 1 ml of the sample extract at 1 mg/ml and then kept at room temperature for 20 min. Then, the mixtures were centrifuged and the sediment was dissolved with 0.1% sodium dodecyl sulfate (2 ml), triethanolamine (2 ml) and 10 mM $FeCl_3$ (1 ml). The suspensions were measured at 510 nm. The calibration curve was established using standard tannic acid (1.6-2.4 mg/ml).

Measurement of cell viability

NG 108-15 cells were plated out at $2x10^3$ cells/well in a polyornithine-coated 96-well plate. The extracts were dissolved in dimethysulfoxide (DMSO) and then diluted to desired concentration by culture medium. After 48 h, sample extracts (final concentration, 1, 10, 30, 50 and 100 μg/ml) were added. The plates were then incubated for 4 h. After incubation, cells were treated with MTT solution (final concentration, 1 mg/ml) for 3 h. The dark blue formazan crystals formed in intact cells were solubilized with DMSO, and the absorbance at 550 nm was measured with a microplate reader (Fusion Universal Microplate Analyser Model: A153601, PerkinElmer Life And Analytical Sciences, Inc, USA). Percent cell viability was calculated, assuming that the absorbance of control cells was 100%.

Hydrogen peroxide-induced neurotoxicity and study of protection offered by extracts

NG108-15 cells ($2x10^3$ cells/well) were seeded into 96-well plates and incubated at 37 °C for 48 h. After plating, they were treated with various concentrations of the two extracts or Trolox for 2 h, and a stock solution of H_2O_2 solution was added to yield a final concentration of 100 μM. Neuronal survival was quantified using MTT.

Statistical analysis

Each of the measurements described above was carried out in triplicate and the results are reported as means and standard deviations. In addition, one way analysis of variance (ANOVA) was used to analyze the results with post-hoc analysis, and where appropriate, Turkey's test was employed, using a commercial statistical software package (SPSS). p values less than 0.05 were considered significant.

Results and Discussion

In the study for substances with neuroprotective activity, antioxidant is a candidate. Most of substance with antioxidant properties in plant is polyphenols. The methanol and aqueous extracts of mango leaf were expected to contain large amount of polyphenol substances. In order to prove this, we extracted mango leaf, and finally we found that the both of extracts have potential desired effects.

First, a DPPH and ABTS assay were used for screening of the free radical scavenging (FRS) activities of methanol, aqueous and chloroform extracts from *M. indica* leaf. The methanol and aqueous extracts of *M. indica* leaf exhibited much higher FRS activities compared to the chloroform extract. IC_{50} values for DPPH assay were 6.18 ± 0.15, 5.57 ± 0.18 and 72.40 ± 3.24 μg/ml respectively. IC_{50} values for ABTS assay were 1.33 ± 0.13, 2.96 ± 0.05 and 6.56 ± 0.49 μg/ml respectively (Table 1). Therefore, for subsequent experiments we used the methanol and aqueous extracts. Then, in order to standardize the extracts, *M. indica* extracts were investigated for total phenolic and tannic acid content (Table 2). Total phenolic contents were determined using Folin-Ciocalteu reagent and calculated using a standard curve for gallic acid (R^2 = 0.9984); tannic acid content was calculated using the standard curve of tannic acid (R^2 = 0.9964). The methanol extract had much higher phenolic content (420.00 ± 4.30 mg of GAE/g) compared to the aqueous extract (187.00 ± 3.20 mg of GAE/g). These results correspond with Ling's study [14]. The two types of extracts had comparable tannic acid contents (1.634 ± 0.007 and 1.611 ± 0.014 mg/ml in aqueous and methanol extracts, respectively).

For other FRS activity assays, we found that the methanol extract (EC_1 = 202.92 ± 3.39 and IC_{50} = 1.33 ± 0.13 μg/ml) exhibited statistically significant higher FRS activity than the aqueous extract (EC_1 = 298.55 ± 8.65 and IC_{50} = 2.96 ± 0.05 μg/ml) in the FRAP and ABTS assays, respectively. The aqueous extract exhibited slightly higher but not statistically significant free radical scavenging activity than the methanol extract in the DPPH scavenging assays. Taken together, the results suggest that 1) the extracts of *M. indica* leaf act as antioxidants as a result of their single electron transfer (SET) capabilities [14] and 2) the methanol extracts have high total phenolic content, which corresponds to higher FRS activity (FRAP and ABTS assays). To support the last suggestion, numerous studies have shown that the antioxidant capacities of plant extracts correlate to the content of phenolics [19, 20]. In order to prove biological radical scavenging properties, the $O^{\bullet}_2$, $OH^{\bullet}$ and H_2O_2 scavenging as well as ferrous ion chelating activities were evaluated. $O^{\bullet}_2$ damage biomacromolecules directly or indirectly by forming H_2O_2, $OH^{\bullet}$ or other free radicals during pathophysiologic event. As expected, the methanol mango leaf extracts showed strong $O^{\bullet}_2$ and H_2O_2 radical scavenging. The aqueous extract (IC_{50} = 0.06 ± 0.01 μg/ml) exhibited slightly higher $O^{\bullet}_2$ scavenging activity than the methanol extract (IC_{50} = 0.07 ± 0.01 μg/ml). (Table 1). On the other hand, the methanol extract (IC_{50} = 70.89 ± 6.56 μg/ml) exhibited much higher H_2O_2 scavenging activity than the aqueous extract (Table 3).

Neurodegenerative diseases are characterized by the loss of neuronal cells in the brain. Reactive oxygen species (ROS) may be involved in the etiologies of these diseases. Antioxidants in plants or herbs may be useful in delaying or preventing oxidation damage [21-24]. The

potent antioxidant activity of *M. indica* has been reported in various studies [7, 13, 25-31]. However, little is known about antioxidant and protective activity in the brain. Our results demonstrate that the extracts could potently reduce free radicals especially H_2O_2. Therefore, this study reveals the ability of *M. indica* extracts to protect against neuroblastoma resulting from H_2O_2-induced oxidative injury.

In the present study, we chose H_2O_2 to induce oxidative cell damage because: 1) oxidative stress is believed to be an important mediator of neuronal cell death and has been postulated to contribute to the pathogenesis of various neurodegenerative diseases; 2) H_2O_2 is a precursor of highly oxidizing, tissue-damaging radicals such as $^{\bullet}OH$ and is known to be toxic to many systems; 3) among a great variety of reactive oxygen species, H_2O_2 plays a pivotal role because it is generated from nearly all sources of oxidative stress; and 4) exogenous H_2O_2 can enter cells and induce cytotoxicity due to its high membrane permeability.

For the cytoprotective study, NG108-15 cells were treated with various concentrations of H_2O_2 (50-800 μM). NG108-15 cells are a neuroblastoma-glioma hybrid cell line. They have been widely used as a neuron model in electrophysiology and pharmacology research. Following 2 h of incubation after H_2O_2 challenge, cell viability was determined. The results showed that H_2O_2 significantly reduced cell viability in a concentration-dependent manner with an IC_{50} value of about 100 μM (Fig. 1A). Therefore, for subsequent protection experiments, we used 100 μM as the concentration of H_2O_2. When Trolox, a reference standard, was added simultaneously with H_2O_2, it caused a concentration-dependent increase in cell viability from 1 to 1000 μM (Fig. 2A). When the methanol and aqueous extracts of *M. indica* were added together with H_2O_2, they significantly increased the viability of cells in a concentration-dependent manner compared with treatment with H_2O_2 alone. The neuroprotective effects of methanol and aqueous extracts were observed in the concentration range of 30-50 and 100 μg/ml, respectively (Fig. 2B and 2C). The methanol extracts showed higher neuroprotective activities than the aqueous extract. These results suggest that the protective effects against H_2O_2-induced oxidative damage are directly

Table 1 Free radical scavenging activities of mango leaf extracts. Data are expressed as IC_{50} means for DPPH, ABTS and superoxide radical scavenging as well as EC_1 means ± S.D. (n=3) of three independent replicates. *indicates significant differences in the means compared to positive control, Trolox ($p \leq 0.05$)

Extract	Antioxidant activity			
	FRAP value (EC_1) (μg/ml)	DPPH (IC_{50}) (μg/ml)	ABTS (IC_{50}) (μg/ml)	Superoxide scavenging (IC_{50}) (μg/ml)
Methanol	202.92 ± 3.39*	6.18 ± 0.15	1.33 ± 0.13*	0.07 ± 0.01*
Water	298.55 ± 8.65	5.57 ± 0.18	2.96 ± 0.05	0.06 ± 0.01*
Chloroform	-	72.40 ± 3.24	6.56 ± 0.49	-
Trolox	269.11 ± 5.16	3.13 ± 0.86	1.82 ± 0.23	7.28 ± 0.90

Table 2 Percent yield and total phenolic and tannic acid content of mango leaf extracts. Data of are total phenolic and tannic acid content expressed as means ± S.D. (n=3) of three independent replicates

Extract	Total phenolic contents (mg of GAE/g)	Tannic acid (mg/ml)	% yield
Methanol	420.00 ± 4.30	1.611 ± 0.014	44.79%
Aqueous	187.00 ± 3.20	1.634 ± 0.007	61.27%
Chloroform	96.00 ± 2.52	-	51.07%

correlated with their antioxidant abilities and their phenolic contents. Mangiferin is a famous biologically active phenolic compound that is present in large amounts in the leaf of *M. indica.* Therefore we also evaluated cytoprotective effect of mangiferin on H_2O_2-induced oxidative damage. We here found that mangiferin could protect the cell from H_2O_2-induced cell death without effect on cell viability (Fig. 2D and 1D). These indicated that the cytoprotective effect of extracts from *M. indica* leaf may be partly due to mangiferin.

Since the methanol extracts could protect the cell from H_2O_2-induced oxidative damage. We would like to further explore underlying mechanism of its protective effect. In Fenton reaction, H_2O_2 convert into $^{\bullet}OH$ and the metal ion catalyzed Haber-Weiss reaction. $^{\bullet}OH$ is the most reactive and harmful. We perform $^{\bullet}OH$ scavenging assay by measuring their effect on the degradation of 2-deoxyribose induced by Fe (III)-EDTA. We found that the methanol extracts did not show positive results on trapping $^{\bullet}OH$ radicals (Table 4). Iron ion plays a vital role for generation $^{\bullet}OH$ radicals in this experiment. Ferrous Iron (II) is oxidized by H_2O_2 to ferric iron (III), a $^{\bullet}OH$ and a hydroxyl anion. Therefore, we perform ferrous ion chelating assay. We found that the methanol extracts had strong ferrous ion chelating activities (IC_{50} value = 0.89 ± 0.81 μg/ml), rendering them in poorly active in the Fenton reaction (Table 5). These results suggest that the extracts of mango leaf act as antioxidants. Their antioxidant activities at least partly due to their $O^{\bullet}_2$ and H_2O_2 radical scavenging as well as metal ion chelating activities.

Conclusion

This study indicates the presence of antioxidant activities and measures total phenolic and tannic acid contents in methanol and aqueous extracts from the leaf of mango. The mechanism of free radical scavenging activity may be SET. The extracts from *M. indica* leave exert significant neuroprotective effects against H_2O_2-induced cell death. These protective effects may be

Table 3 Hydrogen peroxide scavenging activities of mango leaf extracts. Data are expressed as % inhibition means for hydrogen peroxide scavenging ± S.D. (n=3) of three independent replicates

Concentration (μg/ml)	% Inhibition
2,6-Di-tert-butyl-4-methylphenol(BHT)	
10	0.48 ± 2.46
50	10.78 ± 5.59
100	27.48 ± 6.85
150	39.83 ± 5.77
200	54.89 ± 1.61
Water extract	
1	-2.20 ± 6.46
10	2.32 ± 4.23
30	6.80 ± 7.30
50	14.40 ± 7.32
100	27.95 ± 6.79
Methanol extract	
1	-6.69 ± 5.72
10	-5.58 ± 4.97
30	26.38 ± 3.95
50	32.24 ± 2.64
100	72.51 ± 10.59

partly attributed to their free radical scavenging properties. In addition, one of biologically active component from *M. indica* leaf extract, mangiferin, could protect cells from oxidative damage. Thus, the extracts as well as mangiferin may be of value in preventing neurodegenerative diseases in which free radical generation is implicated.

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Table 4 Hydroxy radical scavenging activities of mango leaf extracts. Data are expressed as % inhibition means for hydroxy radical scavenging ± S.D. (n=3) of three independent replicates

Concentration	% Inhibition
Mannitol (mM)	
1	20.15 ± 1.43
10	57.47 ± 1.02
30	76.33 ± 3.03
100	86.56 ± 3.76
Methanol extract (μg/ml)	
0.1	10.76 ± 8.37
1	10.15 ± 2.2
10	16.74 ± 2.11
100	09.48 ± 19.64

Table 5 Ferrous ion chelating activities of mango leaf extracts. Data are expressed as % inhibition means for ferrous ion chelating ± S.D. (n=3) of three independent replicates

Concentration	% Inhibition
EDTA (μM)	
0.05	2.78 ± 15.61
0.1	23.82 ± 9.33
0.5	28.66 ± 24.25
1	41.45 ± 5.43
5	93.51 ± 18.11
Methanol extract (μg/ml)	
1	57.75 ± 10.34
10	83.85 ± 15.11
30	103.24 ± 26.06
50	96.14 ± 19.69
100	122.77 ± 11.95

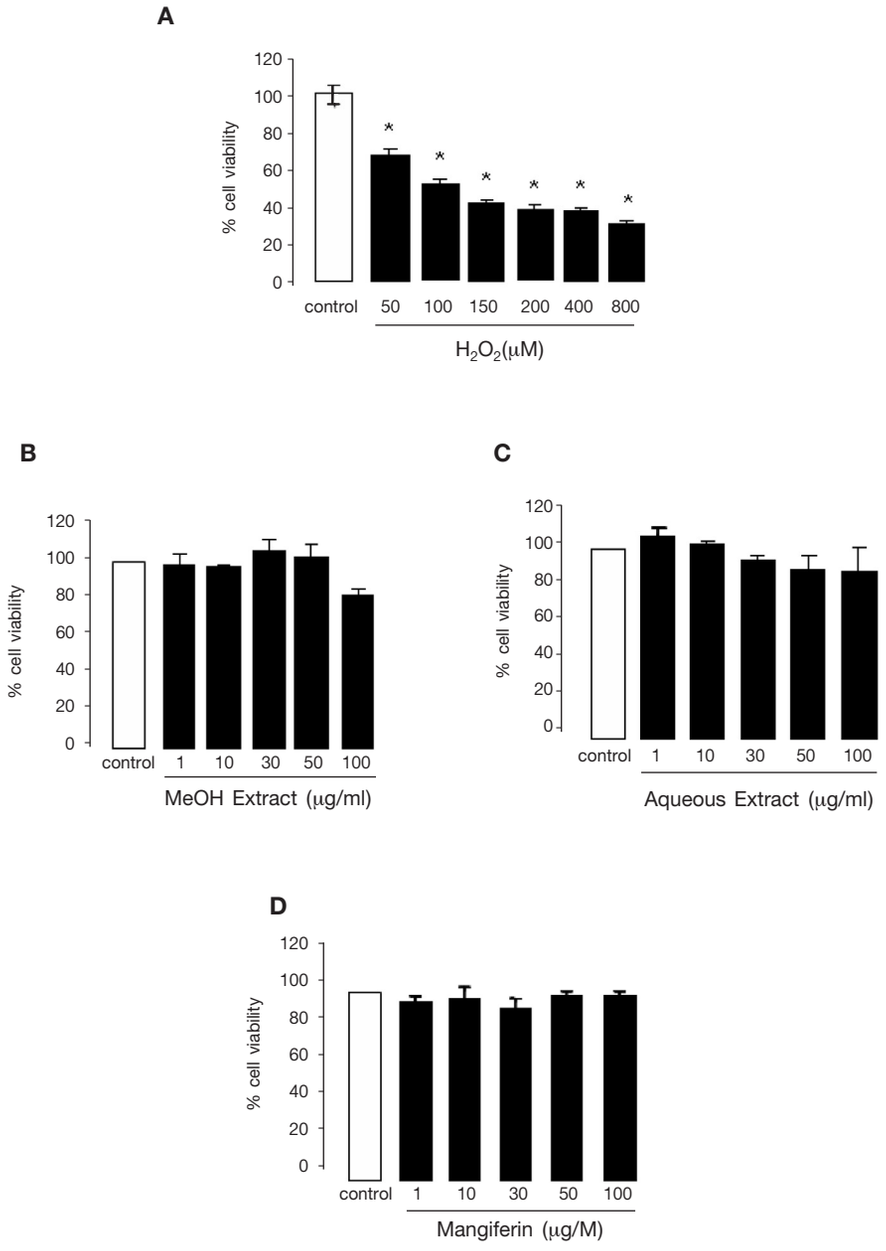

Figure 1 Effect test compounds on NG108-15 cells viability. NG108-15 cells were treated with various concentrations of hydrogen peroxide (1A), methanol (1B) and aqueous extracts from *M. indica* leaf (1C) and mangiferin (1D) for 4 h. Data are expressed as means of cell viability ± S.D. (n=5) of three independent replicates. *$p \leq 0.05$ compared to the control group

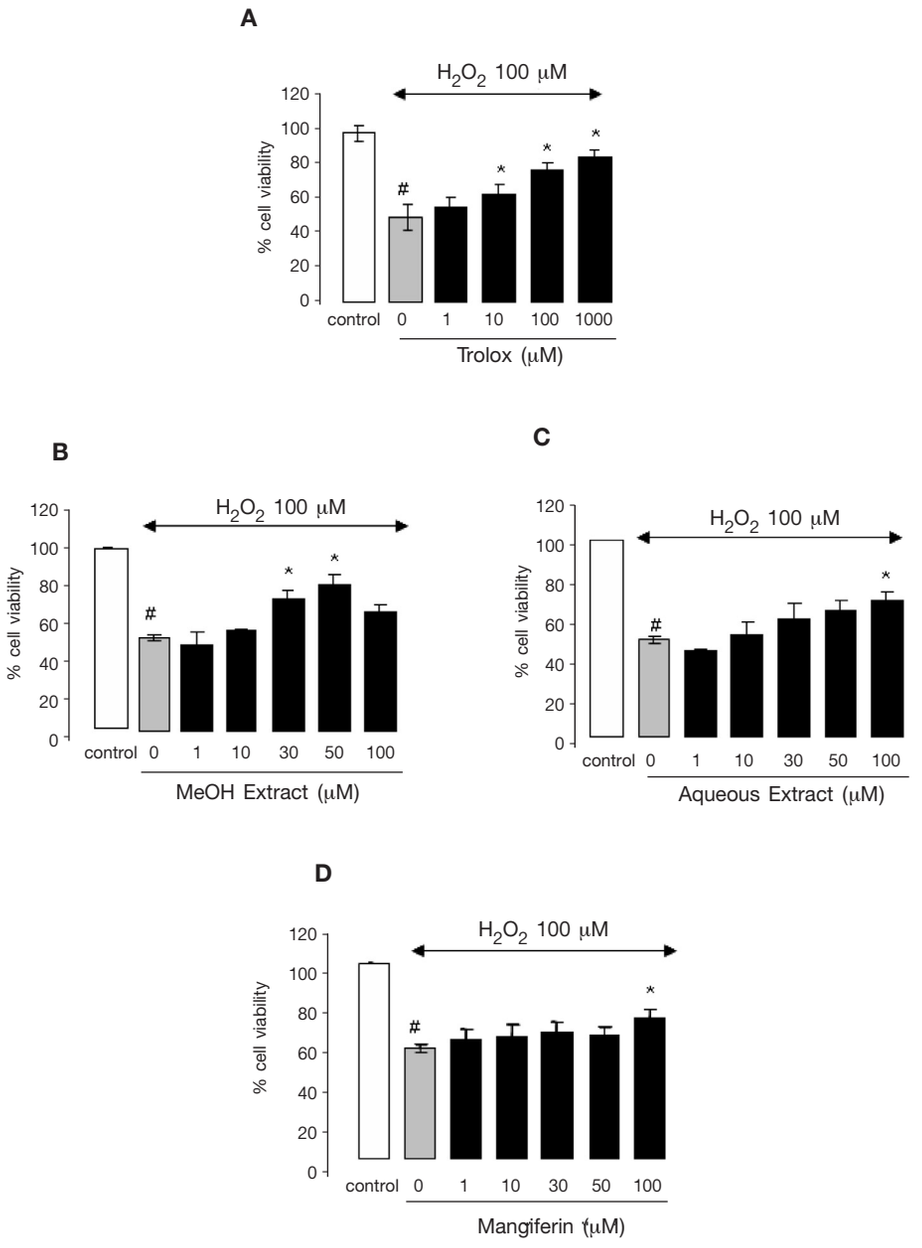

Figure 2 Effects of test compounds on H_2O_2-induced cell damage in NG108-15 cells. NG 108-15 cells were pretreated with Trolox (1- 1000 μM) (2A), methanol (2B) and aqueous extracts from *M. indica* leaf (2C) and mangiferin (2D) for 2 h, then stock solution of H_2O_2 was added. After 2 h of incubation, cell viability was measured by the MTT assay. Data are expressed as means ± S.D. (n=5) of three independent replicates. # $p \leq 0.05$ compared to the control group. *$p \leq 0.05$ compared to the H_2O_2-treated control group

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