ผลของสารสกัดแอลกอฮอล์ของมะขามหวานและกล้วยไข่ต่อการเกิดพิษจากสารไฮโดรเจนเปอร์ออกไซด์ในเซลล์ประสาทมนุษย์ชนิด SH-SY5Y

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บทคัดย่อ

ความเสียหายของเซลล์จากภาวะเครียดออกซิเดชั่นสัมพันธ์กับการเกิดโรคระบบประสาทเสื่อม การวิจัยสารต้านอนุมูลอิสระจากอาหารจึงได้รับความสนใจมากขึ้น จากการศึกษาเกี่ยวกับปริมาณและฤทธิ์ของสารต้านอนุมูลอิสระในผลไม้สด 21 ชนิดที่เป็นที่นิยมรับประทานในประเทศไทยพบว่ามะขามหวานมีสารโพลีฟีนอลรวม (total polyphenol) และค่าฤทธิ์ต้านอนุมูลอิสระวัดโดยวิธี ORAC assay สูงสุด ส่วนกล้วยไข่มีสารเฟอรูลิก แอซิด (ferulic acid) และ สารแคโรทีนอยด์ (carotenoids) ในปริมาณสูง การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อทดสอบผลของสารสกัดจากมะขามหวาน กล้วยไข่ และสารสำคัญในมะขามหวานและกล้วยไข่คือ fisetin และ ferulic acid ต่อการเกิดพิษจากสารไฮโดรเจนเปอร์ออกไซด์ (H_2O_2)ในเซลล์ประสาทมนุษย์ SH-SY5Y ผลการศึกษาพบว่า H_2O_2 ที่ความเข้มข้นต่ำไม่มีผลต่อการสร้างอนุมูลอิสระของออกซิเจน (ROS) ในเซลล์และเปอร์เซ็นต์เซลล์มีชีวิต การให้สารสกัดมะขามหวานและกล้วยไข่ร่วมด้วยเพิ่มระดับ ROS ในเซลล์สูงขึ้น ในขณะที่สาร fisetin และ ferulic acid มีผลลดระดับ ROS ในเซลล์อย่างชัดเจน อย่างไรก็ตามทั้งสารสกัดจากผลไม้และสารสำคัญไม่มีผลต่อเปอร์เซ็นต์เซลล์ที่รอดชีวิตเมื่อเปรียบเทียบกับเซลล์ที่ได้รับ H_2O_2 เพียงอย่างเดียว H_2O_2 ที่ความเข้มข้นสูงทำให้ระดับ ROS ในเซลล์สูงขึ้นและเปอร์เซ็นต์เซลล์รอดชีวิตต่ำลงต่างจากเซลล์ควบคุมอย่างมีนัยสำคัญ สารสกัดมะขามหวานและกล้วยไข่ลดปริมาณ ROS ภายในเซลล์ด้วยประสิทธิภาพที่ต่ำกว่าสารสำคัญทั้ง fisetin และ ferulic acid อย่างไรก็ตามสารสกัดมะขามหวานและกล้วยไข่เพิ่มเปอร์เซ็นต์เซลล์รอดชีวิตในเซลล์ที่ได้ H_2O_2 ที่ความเข้มข้นสูงได้ใกล้เคียงกับสารสำคัญ fisetin และ ferulic acid ผลการวิจัยชี้ว่าการลดจำนวนการตายของเซลล์โดยสารพฤกษเคมีในสารสกัดจากผลไม้ส่วนหนึ่งมาจากฤทธิ์ต้านอนุมูลอิสระ อย่างไรก็ตามฤทธิ์ทางชีวภาพอื่นนอกเหนือจากการต้านอนุมูลอิสระอาจเป็นกลไกร่วมช่วยให้เซลล์รอดชีวิต

คำสำคัญ: ภาวะเครียดออกซิเดชั่น โรคระบบประสาทเสื่อม มะขามหวาน กล้วยไข่ ไฟเซติน เฟอรูลิก แอซิด

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Effects of Alcoholic Extracts of Sweet-Fruited Tamarind (*Tamarindus indica L.*) and Banana (*Musa sapientum L.*) on H_2O_2-Induced Cytotoxicity in Human Neuroblastoma SH-SY5Y Cells

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Abstract

Oxidative stress-induced cell damage has been linked to the pathogenesis of neurodegenerative diseases. Dietary antioxidants are now gaining increased attention in research for the neurodegenerative management. A previous study on antioxidants content and antioxidant activity in twenty-one varieties of fresh fruits commonly consumed in Thailand showed that sweet tamarind had the highest levels of total polyphenol and antioxidant activity (ORAC assay). Banana (Kluai Khai) showed the high content of ferulic acid, and carotenoids. The present study aimed to investigate the effects of sweet tamarind and Kluai Khai extracts, and their commercially bioactive compound fisetin and ferulic acid, on hydrogen peroxide (H_2O_2)-induced cytotoxicity in human neuroblastoma SH-SY5Y cells. Low level of H_2O_2 did not affect intracellular ROS production and cell viability. Sweet tamarind and Kluai Khai extracts enhanced ROS generation in cells whereas fisetin and ferulic acid markedly reduced ROS levels. However, both the fruit extracts and bioactive compounds did not significantly affect cell viability related to H_2O_2 treatment alone. High concentration of H_2O_2 significantly increased intracellular ROS levels and induced cell death. Sweet tamarind and Kluai Khai extracts showed lower effectiveness in reduction of ROS levels but similar capacity in increasing cell survival compared with those of related bioactive compounds. These results suggest that phytochemicals in fruit extracts play a role in suppression of cell death by possessing antioxidative activities. However, non-antioxidant functions of phytochemicals cannot be excluded from the possible mechanisms of cell survival.

Keyword: Oxidative stress, Neurodegenerative disease**,** Sweet tamarind, Kluai Khai, Fisetin, Ferulic acid

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Introduction

Neurodegenerative diseases result from a condition in which nerve cells from brain and spinal cord are lost. Oxidative stress and free radical generation have been shown to play pivotal role in contributing reactive oxygen/ nitrogen species (ROS/ RNS) the main offenders in neurodegeneration[1]. Overproduction of ROS/ RNS or reduction in antioxidant capacity, or a combination of these two factors can mediate damage to cell structures, including lipids and membranes, proteins, and DNA[2,3]. Because of the high oxygen utilization, the high content of oxidisable polyunsaturated fatty acids, brain is vulnerable to oxidative damage. Free radicals attack both the structure and function of neural cells, and contribute to a wide range of neurodegenerative diseases, including Alzheimer's disease (AD)[4]. Oxidative stress has been proposed to be the promoting factor in the pathogenesis of AD by increasing expression or activities of presenilin 1 subunit of γ-secretase, and β-secretase (BACE) 1/2 that involve in amyloid precursor protein (APP) processing[5-7]. Hydrogen peroxide (H_2O_2) is the main source of the highly reactive hydroxyl radical ($OH^{\bullet}$) in the brain by accepting an electron in a reaction catalyzed by transition metal ions. H_2O_2 is used to generate ROS to investigate the oxidative stress mechanisms within neuronal cells. H_2O_2 induced apoptotic cell death through both extrinsic and intrinsic pathways[8,9].

The antioxidant defenses of brain are not fulfilled due to the very low level of catalase activity and moderate amounts of superoxide dismutase and glutathione peroxidase. The brain also has high levels of iron which is the key catalysts for lipid peroxidation. Therefore, nutritional antioxidants are needed for against the accumulation of oxidative stress[10].

Phytochemicals in fruits and vegetables exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, and anti-tumor effects[11]. A study of nutritional composition of twenty-one varieties of fresh fruits commonly consumed in Thailand for their antioxidants content and activity (ORAC assay), revealed that sweet tamarind contained the highest levels of total polyphenol and antioxidant activity (ORAC assay). Fisetin was founded as the most flavonoids in sweet tamarind pulps. All ripe banana showed the greatest amount of ferulic acid, while Kluai Khai also contained the second highest in carotenoids content[12, 13].

Fisetin exhibits the potential antioxidant, anticancer, anti-angiogenic, and neuroprotective effects[14, 15]. Ferulic acid is the potent antioxidant that shows neuroprotective and anti-amyloidogenic activities[16, 17].

The Kame cohort study reported that fruit and vegetable juices might play an important role in delaying the onset of AD, particularly among those who were at high risk for the disease[18]. The critical review of literature and the study results on cognitive performance and risk of dementia by Boeing et al. (2012) demonstrated the possible evidence exists for a reduced risk of dementia with increasing consumption of vegetables and fruits[19].

Therefore, the present study was performed to investigate whether sweet tamarind and Kluai Khai extracts have neuroprotective effects against H_2O_2-induced neuronal toxicity in SH-SY5Y cells. The human neuroblastoma cell line (SH-SY5Y) was used as the model for studying oxidative stress-induced neurotoxicity that involve in pathology of neurodegenerative diseases including Alzheimer's disease (AD)[20]. Several experimental and clinical researches suggested that oxidative damage plays a central role in production of β-amyloid, a toxic peptide present in AD patients' brain that take part in neurodegeneration observed during AD onset and progression [21-23].

Materials and Methods

Materials

Cell culture supplements

Minimum essential medium (MEM), Ham's F-12 medium, fetal bovine serum (FBS), glutamax, sodium pyruvate, non-essential amino acid, penicillin/streptomycin, trypsin-EDTA (0.05%) were purchased from Thermo Fisher Scientific (Waltham, MA USA). Hydrogen peroxide (30%), DMSO, methanol were obtained from Merck (Darmstadt, Germany). 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), 2',7'-dichlorofluorescein diacetate (DCF-DA), fisetin and ferulic acid were provided from Sigma (St. Louis, MO, USA).

Sweet tamarind and Kluai Khai extracts

Edible portions of sweet tamarind and Kluai Khai were homogenized with cold 80% methanol; using Ace homogenizer (NISSEI Ltd., Tokyo, Japan). Process of extraction was performed in a dark room. Homogenized fruit samples were sonicated on ice for 30 min and centrifuged (HIMAC Centrifuge CR5B2, Hitachi, Japan) at 1800g 4 ºC for 10 min. The supernatant of each

fruit extract was separated and aliquoted into amber color glass vial, and stored at -20°C. Before using, the fruit extracts were blow with nitrogen gas to remove methanol. Then the extracts were dissolved in serum free media to make the concentration of 100 mg/ml stock solution and storage at -20 ºC until use. Fisetin and ferulic acid was dissolve in DMSO to 4 mg/ml stock solution and storage at -20 ºC. Hydrogen peroxide was freshly prepared before each experiment as a 10 mM stock solution in serum free media.

Cell Cultures

Human neuroblastoma SH-SY5Y cells were obtained from Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University. Cell were grown in Eagle's minimum essential medium (MEM) and Ham's F-12 Nutrient Mixture (1:1) which was supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamax, 1 mM Sodium pyruvate, 1% non-essential amino acid and 100 U/ml penicillin/streptomycin antibiotics. Cells were maintained at 37 °C under 5% CO_2/ 95% air.

Methods

Determination of phytochemicals and antioxidant activity of sweet tamarind and Kluai Khai extracts

The total phenolic contents were measured using a modified colorimetric Folin Ciocalteu method[24]. Briefly, fruit extracts were diluted with de-ionized water to appropriate volume with water. The 25 µl of diluted samples were added to 96-well plate, followed by 125 µl of 10% Folin–Ciocalteau (Merck, Darmstadt, Germany) and 100 µl 0.5 M sodium hydroxide to each well and mixed. Absorbance was measured at 750 nm using automated microplate reader (Sunrise™-Tecan, Victory, Australia) after standing for 15 min at 25 ºC. The measurement was compared to a standard curve of gallic acid (Sigma–Aldrich, St. Louis, MO, USA) and expressed as milligrams of gallic acid equivalents (GAE) per 100g of fresh weight.

Cyanidin content in fruit extracts was analysed using an HPLC system equipped with a Waters 515 pump (Water corporation, Milford, MA, USA) and Jasco UV 975 detector (Jasco International, Co., Ltd, Tokyo, Japan). Cyanidin was separated using a C18 column (Waters NovaPac C18, 100 x 4.6 mm; Water corporation, Milford, MA, USA). The mobile phase consisted of 0.4% trifluoroacetic acid (TFA) in water and 0.4% TFA in acetonitrile at ratio of

18:82 and eluate was monitored at 530 nm. The results were expressed as milligrams per 100 g of fresh weight (mg/100 g).

The oxygen radical absorbance capacity (ORAC) was carried out according to the method of Huang et al. that measured an antioxidant's ability to scavenge peroxyl radicals formed from a reaction with AAPH (2,2-azobis [2-amidinopropane] dihydro-chloride; (Wako Pure Chemical, Osaka, Japan)[25]. In brief, a 500 µl sample diluted in potassium phosphate buffer at pH 7.2 , a 3.0 ml fluorescein solution (8.16 x 10^{-2} µM), and 500 µl of AAPH (153 mM) were mixed in each tube. The assay was performed by a spectrofluorometer (Perkin-Elmer LS 55 luminescence spectro-fluorometer,Waltham, MA, USA) with an excitation wavelength at 493 nm and an emission wavelength at 515 nm. The ORAC value was determined against the area under the curve (AUC) of the standard Trolox concentrations. Data were expressed as micromole Trolox (TE) equivalents per 100 g fresh weight (µmol TE/100 g fresh weight).

Intracellular reactive oxygen species (ROS)

Cell were seeded in 96 well black plate at density of 1x10^4 cell/ml for 48 h before treatment. Cells were washed once with PBS and incubated in 200 µl of 20 µM 2',7'-dichlorofluorescin diacetate (DCF-DA) in Hank's Balanced Salt Solution (HBSS) at 37°C in a humidified incubator for 30 min. After incubation cell were treated with serum free media containing various concentrations of hydrogen peroxide with or without sweet tamarind extract, Kluai Khai extract, fisetin or ferulic acid for 24 h. The fluorescence was recorded on a microplate reader (Tecan, Infinite M200) in optimum gain with an excitation wavelength of 480 nm and emission wavelength 540 nm. Relative ROS production (percentage of the control) was expressed as the ratio of the fluorescence of the treated sample over the response in the appropriate control [(fluorescence$_{treatment}$/ fluorescence$_{control}$) x 100].

Cell viability

Cells were seeded into 96 well plate at density of 1x10^4 cell/ml for 48 h before treatment. Cells were then washed once with phosphate-buffered saline (PBS) and treated with serum free media containing various concentrations of H_2O_2 with or without sweet tamarind extract, Kluai Khai extract, fisetin or ferulic acid before incubation at 37 °C in a humidified incubator for 24 h. Then treatment media were removed, added with 10 µl of 5 mg/ml MTT in PBS and 100 µl of serum free media and allowed to incubate at 37°C for

2 h. After incubation, MTT solution was removed, and 100 µl of DMSO was added before incubation at room temperature for 15 min. The absorbance was recorded on a microplate reader (Tecan, Infinite M200) at the measure wavelength of 570 nm and reference wavelength 630 nm. Cell viability was expressed as a percentage of appropriate control [(absorbance $_{treatment}$/ absorbance $_{control}$) x 100].

Statistical analysis

Results are expressed as means ± S.E.M. Data were analyzed by using the GraphPad Prism version 5 with One-way ANOVA test follow by Tukey's Multiple Comparison Test. Significant difference was taken as $p < 0.05$.

Results and Discussion

Phytochemicals and the oxygen radical absorbance capacity (ORAC) of sweet tamarind and Kluai Khai extracts

Table 1 shows sweet tamarind extract contained total polyphenols content and antioxidant activity measured by ORAC approximate 3-4 folds higher than those of Kluai Khai extract. These results are consistent with the highest total polyphenol content and ORAC value of sweet tamarind that were reported in previous study. Fisetin and cyanidin were two major flavonoid polyphenols found in analysis of sweet tamarind fruit pulp [13]. However, only cyanidin was detected in sweet tamarind extract. Similarly, ferulic acid and carotenoids, the major bioactive compounds in banana fruit pulp were not detected in Kluai Khai extract. These may be due to the preparation of fruit extracts from fresh fruit pulp using methanol resulted in low concentration of phytochemicals especially lipophilic carotenoids.

Effect of Sweet tamarind extract, Kluai Khai extract, fisetin, and ferulic acid on H_2O_2-induced ROS production in human neuroblastoma SH-SY5Y cells

Because of its low reactivity and non-radical nature, H_2O_2 can pass through cell membranes and generate the most reactive form of oxygen, the hydroxyl radical, via Fenton's reaction[26]. Exposure of SH-SY5Y cells to H_2O_2 caused a dose-dependent increase in ROS production, except H_2O_2 at 50 µM that showed no significant difference from control (Fig 1).

The low concentrations of sweet tamarind and Kluai Khai extracts using in this experiment were selected on basis of the range of concentrations not produce pro-oxidant activities in normal cells (data not shown). In cells treated with low

concentration of H_2O_2 (50 μM), addition of fruit extracts at the low dose ranges tend to increased ROS levels up to higher than control and/ or H_2O_2 treatment alone. At similar doses, fisetin and ferulic acid could reduced H_2O_2-induced intracellular ROS generation in dose-dependent manner. Fisetin and ferulic acid showed the higher antioxidant efficacy compared with fruit extracts. However, at the same concentration the effectiveness in ROS inhibition of fisetin is greater than ferulic acid (Fig 2). However, in cells treated with high concentration of H_2O_2 (800 and 1,000 μM), sweet tamarind and Kluai Khai extracts at the lower dose ranges showed ability to reduce intracellular ROS to the level lower than H_2O_2 treatment alone. Sweet tamarind produced the higher cellular antioxidant activity compared with Kluai Khai extract. (Fig 3). Cellular antioxidant activities of fisetin and ferulic acid have been demonstrated in *in vitro* and cellular systems. Fisetin has high antioxidant capacity from having hydroxyl bond (OH) dissociation energy and dipole moment[27]. Fisetin was partitioned well into the membrane to protects against H_2O_2-induced cell damage by scavenging ROS ,enhancing GSH antioxidant capacity and preventing the H_2O_2-induced lipid peroxidation[28]. Ferulic acid possesses antioxidant potential from its radical scavenging ability by abstracting hydrogen atom from the phenolic hydroxyl group to form a resonance stabilized phenoxy radical[29,30]. Ferulic acid protected HEK293 cells from H_2O_2 induced injury through regulation of intracellular antioxidant enzyme activities, prevention of reduction in mitochondrial membrane potential and apoptosis induction[31]. Under certain conditions, in addition to antioxidant activities, polyphenols can also exert pro-oxidant activities that demonstrated by their abilities to generate ROS both in cell free systems or in *in vitro* studies with cells. Interactions with transition metal ions and oxidation of polyphenols can produce $O_2^{\bullet-}$, H_2O_2 and a complex mixture of semiquinones and quinones[32,33]. The effect on ROS level were contributed from the net results of antioxidant and pro-oxidant effects of all phytochemicals in sweet tamarind and Kluai Khai extracts.

Table 1 Total phenolics content and the oxygen radical absorbance capacity (ORAC) of sweet tamarind and Kluai Khai extracts

Name	Latin name	Total Polyphenols (mg eq GA/100g)	Anthocyanidin Cyanidin (mg/100g)	ORAC umoles TE/100 g
Sweet tamarind	Tamarindus indica L	100	0.11	2,730.26
Kluai Khai	Musa sapientum L	28	nd	924.67

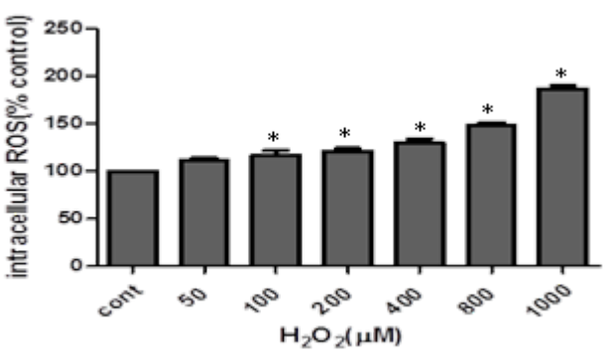
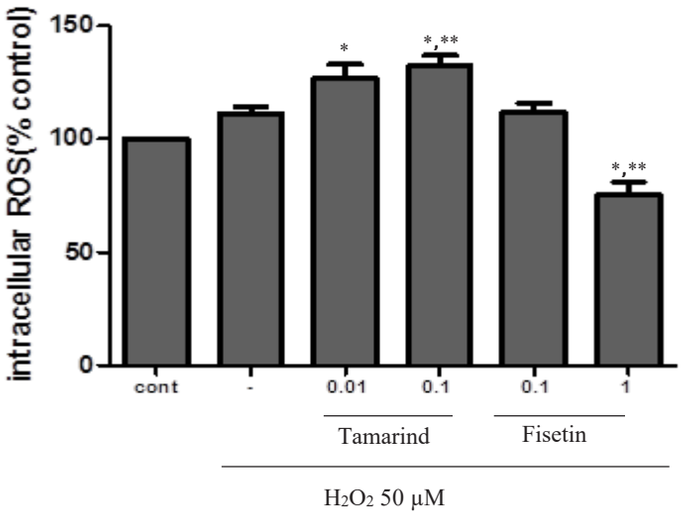


Fig 1 ROS generation in SH-SY5Y cells treated with various concentration of H_2O_2 for 24 h. Values are mean ± SEM for n ≥ 6. * Significantly difference from control group, $P < 0.05$.

(A)



(B)

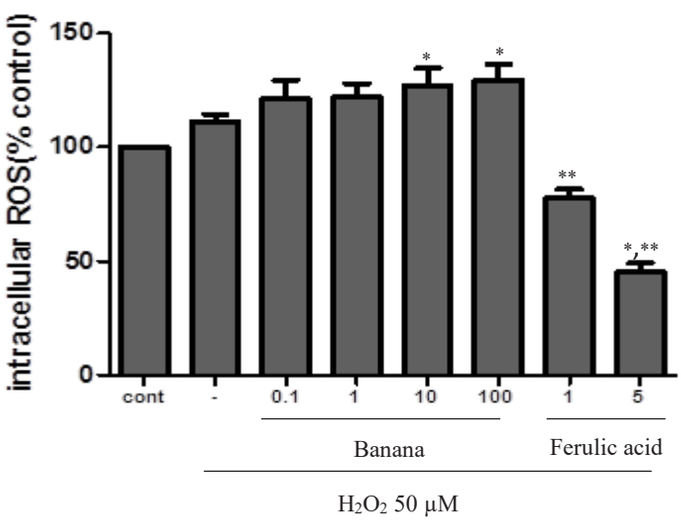
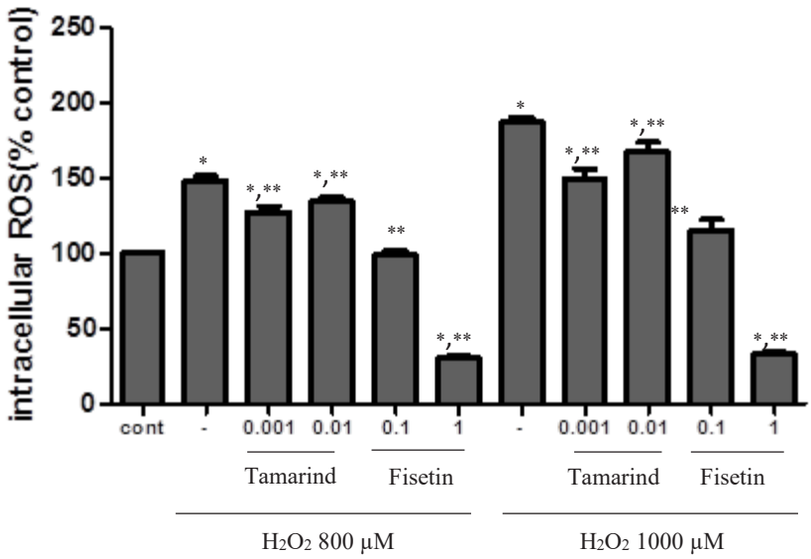


Fig 2. ROS generation in SH-SY5Y cells co-treated with low level of H_2O_2 (50 μM) and fruit extracts or bioactive compounds for 24h. (A) Intracellular ROS in cells co-treated with H_2O_2 and sweet tamarind extract or fisetin for 24 h. (B) Intracellular ROS in cells co-treated with H_2O_2 and Kluai Khai extract or ferulic acid for 24 h. Values are mean ± SEM for n≥ 6.

* Significantly difference from control. ** Significantly difference from H_2O_2 treatment alone, at $P < 0.05$.

(A)



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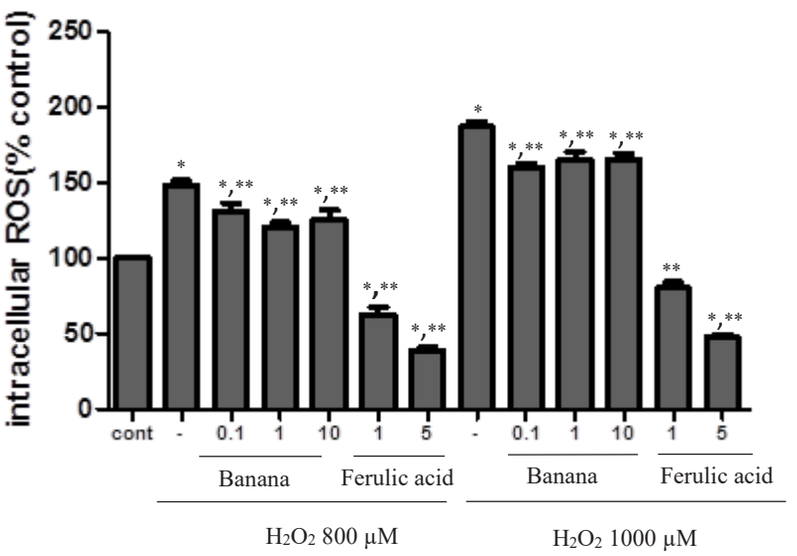

Fig 3. ROS generation in SH-SY5Y cells co-treated with high level (800/1000 µM) of H_2O_2 and fruit extracts or bioactive compounds for 24h. (A) Intracellular ROS in cells co-treated with H_2O_2 and sweet tamarind extract or fisetin for 24 h. (B) Intracellular ROS in cells co-treated with H_2O_2 and Kluai Khai extract or ferulic acid for 24 h. Values are mean ± SEM for n≥ 6. * Significantly difference from control. **: Significantly difference from H_2O_2 treatment alone, at $P < 0.05$.

Effect of sweet tamarind extract,banana extract, fisetin, and ferulic acid on H_2O_2-induced cytotoxicity in human neuroblastoma SH-SY5Y cells

Treatment of the cells with H_2O_2 at concentrations of 50, 100, 200 and 400 µM did not alter cell viability compared with control whereas 800 and 1000 µM H_2O_2 significantly caused the decline of viability to 64% and 40%, relative to control (Fig 4). To evaluate the effect of fruit extracts and bioactive compounds on oxidative induced cytotoxicity, SH-SY5Y cells were co-treated with 50, 800 and 100 µM H_2O_2 and the extract of sweet tamarind, Kluai Khai, fisetin or ferulic acid at the concentration had no effect on the survival of cells. Co-treatment with fruit extracts, fisetin and ferulic acid had no effect on the viability of SH-SY5Y cells treated with 50 µM H_2O_2 which were not different from control (data not shown). Sweet tamarind extract and fisetin showed increasing trend of percent cell viability of 13, 17% and 21, 18% higher than those of cells treated with 800 and 1,000 µM H_2O_2, respectively (Fig 5 A). Kluai Khai extract and ferulic acid exhibited lower effect on cells treated with 800 µM H_2O_2 by increasing cell survival only 9, 13% higher than H_2O_2 treatment alone. In cells treated with 1,000 µM H_2O_2, Kluai Khai extract had no effect, whereas ferulic acid treatment presented 6% increase in cell viability compared with cells treated with H_2O_2 (Fig 5 B).

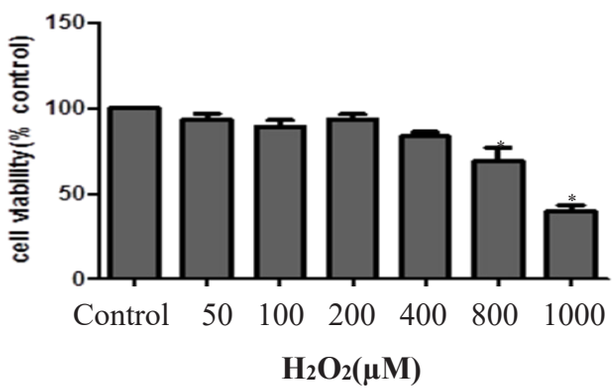

Fig 4. Percent cell viability of SH-SY5Y cells treated with various concentration of H_2O_2 for 24h. Values are mean ± SEM for n≥ 6. *: Significantly difference from control group, $P < 0.05$.

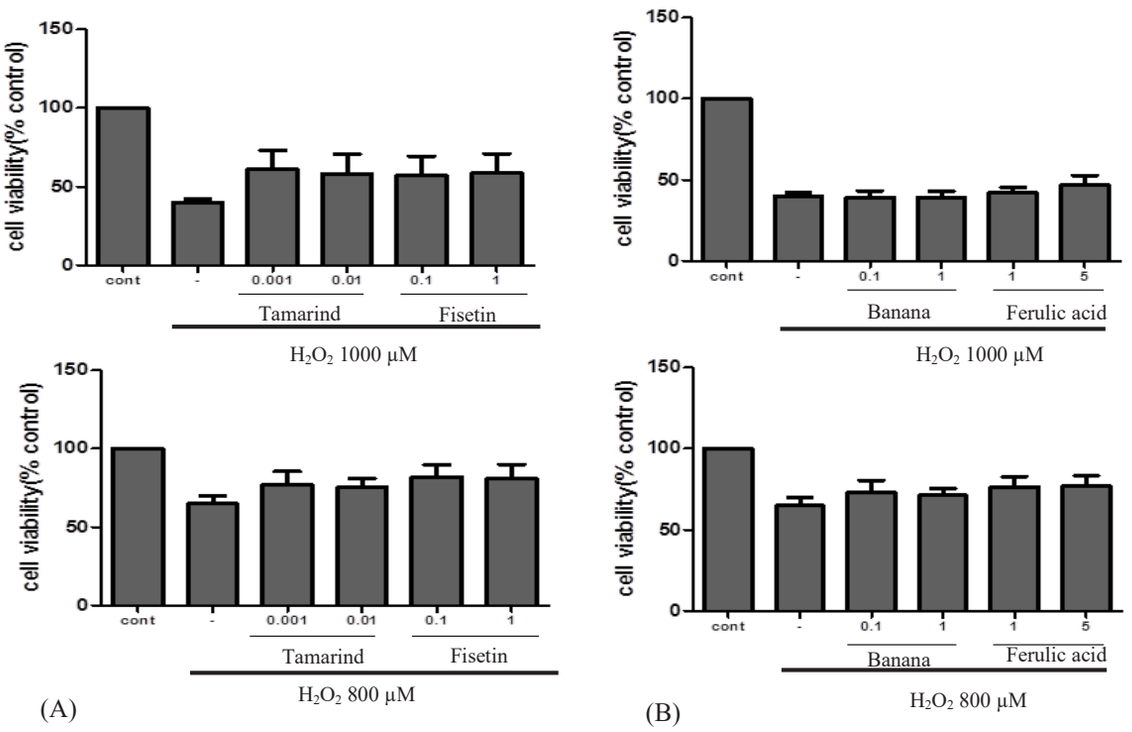

Fig 5. Percent cell viability of SH-SY5Y cells co-treated with high level (800/1000 µM) of H_2O_2 and fruit extracts or bioactive compounds for 24 h. (A) % viability of cells co-treated with H_2O_2 and sweet tamarind extract or fisetin for 24 h. (B) % viability of cells co-treated with H_2O_2 and Kluai Khai extract or ferulic acid for 24 h. Values are mean ± SEM for n≥ 6. No significant difference between H_2O_2 treatment with and without fruit extracts or bioactive compounds ($P < 0.05$).

Low to moderate levels of ROS are critical in cellular signaling and activating pro-survival pathways, such as mitogen activated protein kinase (MAPK) pathways. ROS can activate transcription factors that promote antioxidant defense processes. Nevertheless, prooxidative/ antioxidative cellular imbalance between ROS production and ability of defense mechanisms to eliminate the cellular disturbances leads to oxidative stress reciprocally aggravates ROS production. The oxidative stress can induce apoptosis cell death that is involved in pathomechanism of many general neurodegenerative pathologies[34-36]. Natural antioxidants, including polyphenols, flavonoids, anthocyanins and carotenoids exhibit prooxidant activities in vitro, under conditions of high pH, high phenolic concentrations, high number of hydroxyls in aromatic rings and the presence of redox-

active transition metals[37]. In cells treated with low concentration H_2O_2 (50 μM), sweet tamarind and Kluai Khai extracts enhanced ROS generation in cells, in contrast fisetin and ferulic acid markedly reduced ROS levels. However, at the same concentration used in ROS experiment, the fruit extracts and bioactive compounds did not alter percent cell viability related to control. The antioxidant defense must minimize the levels of ROS while maintaining slight prooxidative balance that is necessary for optimal cell signaling processes[38]. Therefore, ROS levels in cells treated with low concentration H_2O_2 with or without fruit extracts or bioactive compounds may be in the levels of function in intracellular signalling and regulation.

Treatment with high concentration of H_2O_2 (800, 1000 μM) increased intracellular ROS levels and induced cell death. Excess intracellular ROS levels cause damage to proteins, nucleic acids, lipids, membranes and organelles, which can lead to cell death as well as upregulate cellular repair processes and the protective systems[39]. The excessive ROS formation would be corrected to prevent the accumulation of oxidative damage. Decreased ROS production may take part in reduction of cell death from the main pathways of apoptosis mediated by mitochondria, death receptors and endoplasmic reticulum[40]. However, even having the lower cellular antioxidant activity, fruit extracts showed the similar capacity to those of bioactive compounds to attenuate H_2O_2-induced decrease in cell survival. These results may indicate that in addition to antioxidant activity, phytochemicals in fruit extracts can promote cell survival through the mechanisms beyond antioxidant properties[41].

In conclusion, in the present study, sweet tamarind and Kluai Khai extracts exhibited both prooxidant and antioxidant activities in human neuroblastoma SH-SY5Y cells under certain conditions. In contrast to a bioactive compound fisetin (sweet tamarind) and ferulic acid (Kluai Khai) that showed the greater intracellular ROS reduction, fruit extracts and bioactive compounds presented similar trend in decrease oxidative stress-induced cytotoxicity. This effect may be mediated by inhibition of ROS-induced oxidative stress as well as the functions beyond antioxidants of synergistic whole phytochemicals contained in sweet tamarind and Klui Khai extracts.

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