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Full Length Research Paper

Antioxidative and neuroprotective effects of *Loranthus parasiticus* (L.) Merr (Loranthaceae) against oxidative stress in NG108-15 cells

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The antioxidative and neuroprotective effects of *Loranthus parasiticus* (L.) Merr (Loranthaceae) were investigated for the first time. *L. parasiticus* ethanol extract (LPEE), *L. parasiticus* ethyl acetate fraction (LPEAF), and *L. parasiticus* aqueous fraction (LPAF) were evaluated and exhibited antioxidative activity in a dose-dependent manner. LPAF exhibited the lowest IC_{50} values of 16.82 ± 0.27 µg/ml and 1.05 ± 0.04 mg/ml in 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and lipid peroxidation activities, respectively. Moreover, LPAF possessed the strongest reducing power activity and the highest total phenolic content. The neuroprotective activity was investigated in NG108-15 hybridoma cells against hydrogen peroxide (H_2O_2)-induced oxidative stress by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay. Both LPEE and LPAF exhibited neuroprotective activity on NG108-15 cells in a dose-dependent manner with the highest cell viability (78.00 ± 1.85%) observed with LPAF at the highest treatment concentration of 100 µg/ml. A close correlation was evident between antioxidative and neuroprotective activities with the total phenolic content in LPAF. These results suggested that LPAF may be used as a potent antioxidant and neuroprotectant in treating or preventing neurodegenerative diseases where oxidative stress is implicated.

Key words: *Loranthus parasiticus*, 2,2-diphenyl-1-picrylhydrazyl (DPPH), lipid peroxidation, reducing power, total phenolic content, antioxidant, neuroprotectant.

INTRODUCTION

Reactive oxygen species (ROS) are chemical species of atoms or molecules that possess an unpaired electron on their outermost orbit (Desai et al., 2008) and are produced under normal metabolic conditions when oxygen is reduced to water (Scandalios, 1992). Elevated level of ROS causes oxidative stress, which leads to various biochemical and physiological lesions that result in metabolic impairment and cell death. All aerobic organisms are susceptible to oxidative stress because semi-reduced oxygen species, such as superoxide and hydrogen peroxide, are produced by mitochondria during respiration (Chance et al., 1979).

Free radical generation leads to oxidative damage of lipids, nucleic acids and proteins, abnormal aggregation of cytoskeletal proteins, mitochondrial dysfunction, antioxidant enzymes upregulation, reactive nitrogen species formation, advanced glycation end products formation, and inactivation of key enzymes (Aksenov et al., 2001; Butterfield and Stadtman, 1997; Butterfield et al., 2001; Markesbery, 1997; Rao and Balachandran, 2002). Accumulation of oxidative damage in neurons accounts for the increased incidence of neurodegeneration in Alzheimer's disease, Amyotrophic

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Abbreviations: LPEE, *Loranthus parasiticus* ethanol extract; **LPEAF**, *Loranthus parasiticus* ethyl acetate fraction; **LPAF**, *Loranthus parasiticus* aqueous fraction; **NG108-15**, mouse neuroblastoma x rat glioma hybrid cells; **H_2O_2,** hydrogen peroxide; **MTT,** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

lateral sclerosis (ALS), Parkinson's disease, and also in HIV-1 infection where neuronal cell loss and acquired immune deficiency syndrome (AIDS) associated dementia has been observed (Perl and Banki, 2000). In addition, oxidative stress has also been implicated in the pathogenesis of a number of acute and chronic neurodegenerative disorders in the brain (Halliwell, 1992; Behl and Moosmann, 2002). Model systems such as experimental animals and cell cultures are often used to understand how oxidative stress can produce neurotoxic effects, which later lead to neuronal dysfunction, degeneration, and cell death.

To cope with oxidative stress, aerobic organisms evolved protective mechanisms against the ROS. These include enzymatic systems and chemical scavengers such as dietary antioxidants (α-tocopherol, β-carotene, ascorbic acid, glutathione, uric acid), some hormones (estrogen, angiotensin), and endogenous enzymes (superoxide dismutase, glutathione peroxidase, catalase), which are capable of removing ROS formed in cells and thus protecting against oxidative damage (Tawaha et al., 2007).

Recently, there is an upsurge of interest in herbal medicinal plants for their medicinal properties as the phytochemicals in these plants may be potential new antioxidants (Tawaha et al., 2007; Bouayed et al., 2006). The health promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting reactive oxygen species (Wong et al., 2006). Several reports indicate that the antioxidant potential of medicinal plants may be related to the concentration of their phenolic compounds which include phenolic acids, flavonoids, anthocyanins and tannins (Wong et al., 2006; Djeridane et al., 2006).

Loranthus species in semiparasitic plants are known to produce a variety of bioactive compounds, including sesquiterpene lactones from *Loranthus parasiticus* for the treatment of schizophrenia (Okuda et al., 1987) and (+)-catechin, 3,4-dimethoxycinnamyl alcohol and 3,4,5-trimethoxycinnamylalcohol from *L. globosus* for the antimicrobial and antifungal properties (Sadik et al., 2003). Many other chemical components such as triterpenoids from *Loranthus grewinkii* (Rahman et al., 1973) and *Loranthus falcatus* (Anjaneyulu et al., 1977), flavonoids from the leaves of *Loranthus kaoi* (Lin and Lin, 1999) and from *Loranthus europaeus* (Harvala et al., 1984), a cytotoxin from *L. parasiticus* (Zhou et al., 1993), and phenolics from *L. longiflorus* (Indrani et al., 1980) have been reported so far. *L. parasiticus* (L.) Merr (Loranthaceae), a parasite plant which is generally known as "benalu teh" (in Malay), "Sang Ji Sheng" (in Chinese), and "baso-kisei" (in Japan) distributed in south and southwest part of China and has been used as a folk medicine for the treatment of schizophrenia in southwest China. Additionally, *L. parasiticus* has various uses in folk and traditional medicines for bone, brain, kidney, liver, expels wind-damp, and prevents miscarriage. However, there is no scientific report to clarify the medicinal effects on *L. parasiticus* except sesquiterpene lactones isolated from *L. parasiticus* used for the treatment of schizophrenia (Okuda et al., 1987). It was thus necessary to evaluate the remedial properties of *L. parasiticus* in the present paper. We are here for the first time to discuss and report the antioxidative and neuroprotective activities exerted by *L. parasiticus* against H_2O_2-induced oxidative damage in NG108-15 hybridoma cells.

MATERIALS AND METHODS

L. parasiticus leaves were acquired from a Chinese medicine retail outlet in the Selangor state of Malaysia. Voucher specimen with KLU number 47366 was deposited at the herbarium of University of Malaya. Ethanol, ethyl acetate, dimethyl sulfoxide (DMSO) and trichloroacetic acid (TCA) were purchased from Merck. NG108-15 hybridoma cell line was obtained from American type culture collection (ATCC). Dulbecco's modified eagle's medium (DMEM), Hypoxanthine-Aminopterin-Thymidine (HAT), phosphate buffered saline (PBS), MTT, DPPH, and Folin-Ciocalteu's phenol reagents were provided by Sigma-Aldrich. Fetal bovine serum (FBS), penicillin/streptomycin (100×) and amphotericin B (250 μg/ml) were supplied by PAA Laboratories. Accutase was bought from Innovative Cell Technologies, Inc. H_2O_2, disodium hydrogen phosphate (Na_2HPO_4), sodium dihydrogen phosphate (NaH_2PO_4), sodium carbonate (Na_2CO_3), and thiobarbituric acid (TBA) were purchased from Systerm®. All chemicals used were of analytical grade.

Extraction and fractionation

The leaves of *L. parasiticus* were plucked from the aerial portion, dried, and ground into fine particles. It was then soaked with ethanol for 3 days. Activated charcoal was added subsequently and filtered as *Loranthus parasiticus* ethanol extract (LPEE). LPEE was further partitioned into *Loranthus parasiticus* ethyl acetate fraction (LPEAF) and *Loranthus parasiticus* aqueous fraction (LPAF). LPEE, LPEAF, and LPAF of different polarity were subjected to antioxidative and neuroprotective activities evaluation. Filtration was done by Whatman No.1 filter paper and evaporation was accessed under reduced pressure at 40°C using a rotary evaporator (Buchi). All the extract and fractions were weighted and stored frozen.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

DPPH radical scavenging assay was performed according to the method described by Blois (1958). Briefly, 1.0 ml samples with various concentrations (15, 30, 60, 120, 240 and 480 μg/ml in ethanol) were added with 1.0 ml 0.2 mM DPPH solution and incubated in the dark for 30 min at room temperature. Butylated hydroxytoluene (BHT) was used as a positive control. Absorbance at 517 nm was measured by spectrophotometer (Amersham Biosciences Ultrospec 2100 pro). The percentage of antioxidant activity was calculated as below:

Percentage (%) of antioxidant activity $= [1- A_S/A_C] \times 100\%$

Where A_S is the absorbance of sample or BHT and Ac is the

absorbance of control (DPPH solution + ethanol).

Reducing power assay

Reducing power assay was carried out according to the method reported by Karawita et al. (2005) with some modifications. 1.0 ml of each sample (15, 30, 60, 120, 240, 480 and 960 µg/ml in ethanol) was added to 1.0 ml sodium phosphate buffer (0.2 M, pH 6.6) and 1.0 ml potassium ferricyanide (10 mg/ml in distilled water). The mixture was incubated at 50°C for 20 min. After incubation, 1.0 ml TCA (100 mg/ml in distilled water) was added to each sample and centrifuged at 1500 rpm for 8 min. 1.0 ml of supernatant was transferred into 1.0 ml distilled water, followed by 0.1 ml of ferric chloride (1.0 mg/ml in distilled water). BHT was used as positive control. The absorbance at 700 nm was measured after 10 min. The higher absorbance represents stronger reducing power activity.

Lipid peroxidation assay

Lipid peroxidation assay was done according to the method modified from Kuppusamy et al. (2002). Egg yolk homogenates which mainly comprised of phospholipids, triacyglycerols and proteins was used as an alternative to rodent liver microsomes and linoleic acid. The reaction mixture for the induction of lipid peroxidation contained 1.0 ml egg yolk emulsified with PBS (0.1 M, pH 7.4), to give a concentration of 12.50 g/L. 0.1 ml of serial concentrations of the extract and fractions ranging from 2.79 to 357.14 µg/ml was then added to the buffered egg yolk. 0.2 ml of 3.0 mM $FeSO_4$ was added to the mixture and incubated at 37°C for 1 h. After incubation, the mixture was treated with 0.5 ml of 15% TCA and 1.0 ml of 1% TBA. The reaction tubes were kept in boiling water bath for 10 min. Upon cooling, the tubes were centrifuged at 3,500 g for 10 min to remove precipitated protein and absorbance was measured at 532 nm. Curcumin was used as positive control. The percentage of inhibition was calculated from the following equation:

Percentage (%) inhibition = $[(A_C - A_S)/A_C] \times 100\%$

Where A_S is the absorbance of sample or curcumin and Ac is the absorbance of control (buffered egg + $FeSO_4$).

Total phenolic content

Total phenolic content was determined by the method of Lee et al. (2004) with some modifications. 200 µl of 100 µg/ml samples and various concentration of gallic acid (3.75, 7.50. 15.00, 30.00, 60.00, 120.00 and 240.00 µg/ml in ethanol) were prepared. 1.5 ml of Folin-Ciocalteu's phenol reagent (10× dilutions in distilled water) was added into each sample and incubated for 5 min at ambient temperature. After incubation, 1.5 ml sodium carbonate (60 mg/ml in distilled water) was added and the final mixture was incubated in darkness for an additional 2 h at ambient temperature. Absorbance at 725 nm was measured and total phenolic content was determined from the gallic acid calibration curve. Results were expressed as milligram of gallic acid equivalent per gram of dry weight of plant (mg GAE/g_{DW}).

Culture of NG108-15 hybridoma cells

NG108-15 hybridoma cells were cultured in Dulbecco's modified eagle's medium (DMEM) supplemented with 10% heat inactivated FBS, 2% penicillin/streptomycin, 1% amphotericin B, and HAT as a complete medium. The cells were cultured and maintained at 37°C in 5% CO_2 atmosphere with 95% humidity (CO_2 incubator chamber, RSBiotech). NG108-15 cells were subculture every 3 to 4 days.

Induction of oxidative damage

Oxidative damage was induced with H_2O_2 in NG108-15 cells. Cells were treated with serial concentrations of H_2O_2 prepared in complete medium for up to 10 h.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay

The cell viability in neuroprotection was analyzed with MTT assay. This test is based on a reduction of tetrazolium salts into purple formazan derivatives in intact mitochondria of viable cells. NG108-15 cells were raised to confluence, harvested by accutase, rinsed with PBS and plated at a density of 5×10^3 cells/well in a 96-well plate. The cells were incubated at 37°C with 5% CO_2 for 48 h. After the incubation period, cells were pretreated with varying concentrations of the samples and incubated at 37°C with 5% CO_2 for an additional 2 h. After 10 h exposure to H_2O_2, tetrazolium salt solution was added into each well and incubated for subsequent 4 h. Plates were analyzed in microplate reader (ASYS UVM340) at 570 nm (with a reference wavelength of 650 nm). The percentage cell viability was calculated according to the equation below:

Percentage (%) of cell viability = $[A_s/A_C] \times 100\%$

Where A_s is the absorbance of sample-treated cells and Ac is the absorbance of control cells.

Statistical analysis

The experimental results were expressed as mean ± standard error of mean (S.E.M.) of two independent experiments, (n=6). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test. *P<0.01 were considered statistically significant.

RESULTS

DPPH radical scavenging activity

LPEE, LPEAF, and LPAF exhibited strong DPPH radical scavenging activity with IC_{50} values of 20.51 ± 0.18, 55.66 ± 0.55, and 16.82 ± 0.27 µg/ml, respectively (Table 1). LPAF showed the strongest scavenging ability as compared with LPEE and LPEAF. Moreover, all the *L. parasiticus* extract and fractions resulted in higher antioxidant scavenging activity than the standard positive control, BHT with the IC_{50} value of 57.20 ± 4.92 µg/ml.

Reducing power activity

The ability to reduce Fe (III) may be attributed to hydrogen donation from phenolic compounds (Shimada et al., 1992) w hich is also related to the presence of

Table 1. Antioxidative properties of *L. parasiticus* extract and fractions (LPEE, LPEAF, and LPAF) for DPPH radical scavenging activity, lipid peroxidation inhibitory activity, and content of total phenolic quantified by Folin-Ciocalteu reaction.

Group	DPPH IC_{50} (µg/ml)	Lipid peroxidation IC_{50} (mg/ml)	Total phenolic content (GAE/g_{DW})
LPEE	20.51 ± 0.18	3.39 ± 0.24	52.08 ± 1.76
LPEAF	55.66 ± 0.55	7.68 ± 0.37	18.82 ± 0.53
LPAF	16.82 ± 0.27	1.05 ± 0.04	56.51 ± 1.43
BHT	57.20 ± 4.92	-	-
Curcumin	-	0.47 ± 0.02	-

Experiments were conducted as reported in Materials and Methods. Data are representative of mean ± S.E.M. (n=6).

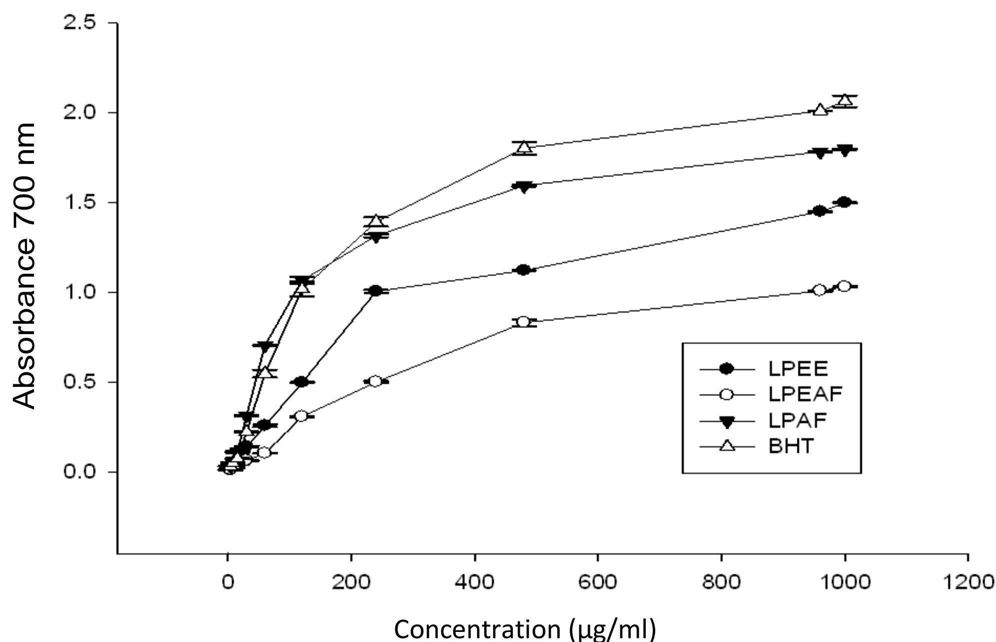

Figure 1. Dose-response curve of LPEE, LPEAF, and LPAF in reducing power assay as compared to BHT as a reference positive control. The ability in reducing power assay was performed as reported in Materials and Methods. Results represent mean ± S.E.M., (n=6).

reductant agents (Duh, 1998). Figure 1 shows the reducing power activity of the LPEE, LPEAF, LPAF, and BHT. LPAF possessed the strongest reducing power followed by the standard positive control, BHT, LPEE, and LPEAF in a descending order. All the *L. parasiticus* extract and fractions (LPEE, LPEAF, and LPAF) showed a dose dependent manner in reducing power activity as shown in Figure 1.

Lipid peroxidation inhibitory activity

From the results shown in Table 1, the positive control, curcumin, possessed the lowest IC_{50} value of 0.47 ± 0.02 mg/ml which indicated its ability to inhibit the oxidative degradation of lipids from egg yolk. The highest inhibition was shown by LPAF with IC_{50} value of 1.05 ± 0.04 mg/ml followed by LPEE and LPEAF with IC_{50} values of 3.39 ± 0.24 and 7.68 ± 0.37 mg/ml, respectively.

Total phenolic content

The usage of Folin-Ciocalteu reagent was measured based on the color measurement which was non-specific on phenol. However, the measurement of color changes after 2 h of incubation in the dark could be used to determine the existence of phenol in samples. At the concentration of 100 µg/ml, LPAF yielded the highest total phenolic content with 56.51 ± 1.43 GAE/g of dry weight, followed by LPEE and LPEAF with 52.08 ± 1.76 GAE/g_{DW} and 18.82 ± 0.53 GAE/g_{DW}, respectively as

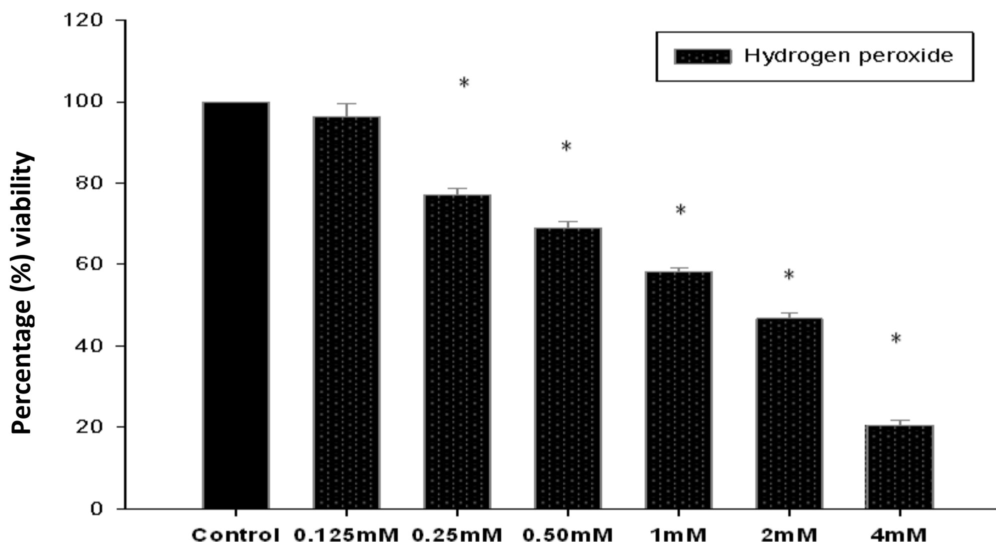

Figure 2. Percentage viability of NG108-15 cells against H_2O_2-induced oxidative damage by MTT cell viability assay. Concentration of H_2O_2 ranging from 0.125 mM to 4.0 mM caused a significantly (P<0.01) decreased in NG108-15 cell viability. Results represent mean ± S.E.M., (n=6). *P<0.01.

shown in Table 1.

Effects of H_2O_2-induced damage in NG108-15 cells

In order to determine the optimal H_2O_2 concentration to induce oxidative stress in NG108-15 cells, the cells were treated with different concentrations of H_2O_2. A significant (P<0.01) cell death in a dose-dependent manner was observed (Figure 2) when NG108-15 cells were treated with varying concentrations of H_2O_2 (0.125 to 4.0 mM) for 10 h. Figure 2 showed that the highest percentage of death (79.45 ± 1.03%) of NG108-15 cells occurred at 4.0 mM concentration of H_2O_2. After determination of H_2O_2 concentrations, 2.0 mM of H_2O_2 which killed approximately 50% of cells was selected and found to be suitable in the present neuroprotection model. The untreated cells were used as the control group which was considered as 100% cell viability.

Neuroprotective activity

As determined by MTT assay, oxidative stress induced by 2.0 mM H_2O_2 decreased cell viability significantly (P <0.01) by 46.64 ± 1 .29% as compared with the untreated group (Figure 2). LPAF and LPEE showed the ability to inhibit cell death following H_2O_2 insult in a dose-dependent manner (Figure 3). 2 h pretreatment with LPAF exhibited stronger protective activity than LPEE with 78.00 ± 1.85% and 67.23 ± 1.85% cell viability, respectively. However, LPEAF failed to protect NG108-15 cells against H_2O_2-induced oxidative damage even at the highest treatment concentration of 100 µg/ml (Figure 3). The standard positive control, resveratrol, showed a dose-dependent increase in cell viability with the percentage cell viability of 78.49 ± 1.09 at its highest treatment concentration of 100 µg/ml.

DISCUSSION

NG108-15 cells are mouse neuroblastoma-rat glioma hybridoma cells which have been utilized extensively as a neuronal model in electrophysiology and pharmacology research by possessing both neuronal and glial properties. Among a great variety of ROS, H_2O_2 is known to be generated from nearly all sources of oxidative stress in biological condition. Due to its high membrane permeability, exogenous H_2O_2 causes oxidative stress by entering cellular membrane and leads to the generation of highly oxidizing, tissue-damaging radicals such as hydroxyl radicals, which is able to induce toxicity to the cellular system. Therefore, H_2O_2 was selected to induce oxidative damage on NG108-15 cells in this study.

L. parasiticus, a traditional medicinal herb which is known as a supplement for brain diseases was investigated for its antioxidative and neuroprotective ability. DPPH radical scavenging assay was performed initially. DPPH is a free radical with an unpaired electron used for the evaluation of antioxidant activity. Due to the unpaired electron, DPPH in ethanol gives a deep color as it absorbs strongly at 517 nm. After the unpaired electron paired off, decolorization occurred as the absorption

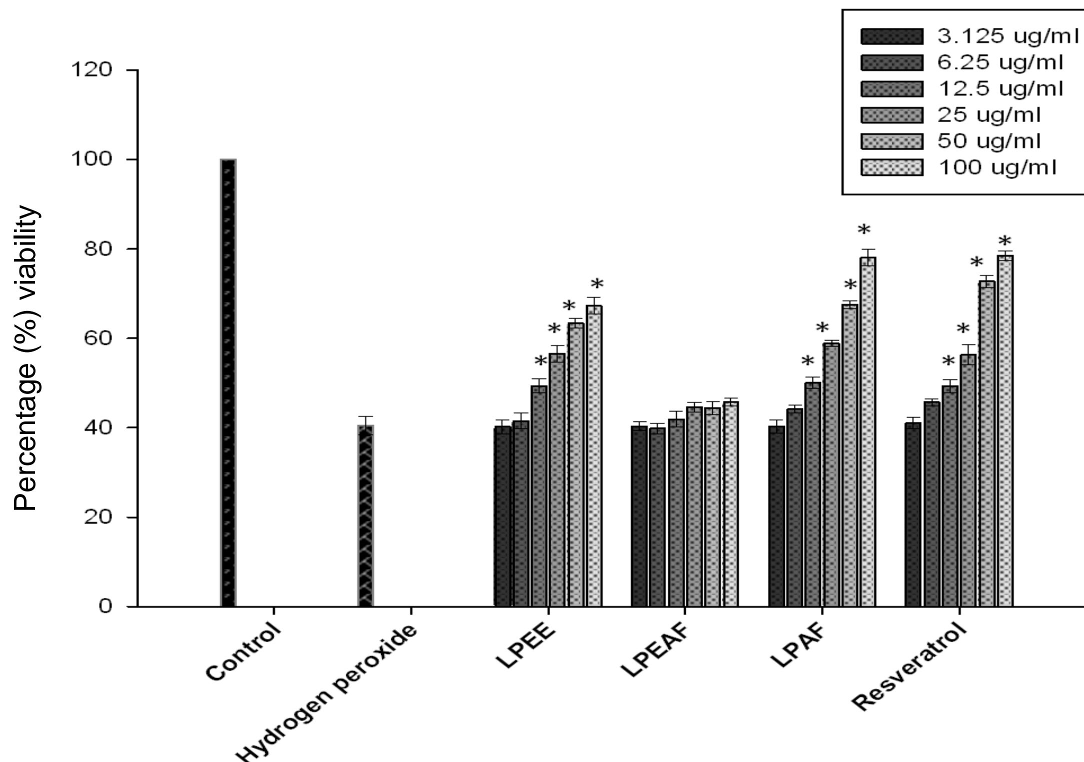

Figure 3. Neuroprotective effects of LPEE, LPEAF, and LPAF by MTT cell viability assay. NG108-15 cells viability was decreased significantly (P<0.01) over 10 h by 2.0 mM H_2O_2 insult. NG108-15 cells were incubated for 48 h followed by pretreatment with LPEE, LPEAF, and LPAF for 2 h and subsequently 2.0 mM H_2O_2 for 10 h, as described in Materials and Methods. Results represent mean ± S.E.M., (n=6). *P<0.01.

vanishes, and this is proportional to the number of electrons taken up (Blois, 1958). When DPPH is being scavenged by an antioxidant, it accepts an electron from the antioxidant and becomes a stable yellow substance known as 2,2-diphenyl-1,2-picryhydrazyl. The extent of this reaction depends on the electron or hydrogen donating ability of the antioxidant. In the present study, LPAF showed the highest DPPH radical scavenging activity as shown in Table 1. Even at a low concentration of 16.82 ± 0.27 *µ*g/ml, LPAF exhibited 50% antioxidant activity. LPEE possessed the second highest DPPH radical scavenging activity, followed by LPEAF with the IC_{50} value of 20.51 ± 0.18 and 55.66 ± 0.55 *µ*g/ml, respectively.

In reducing power assay, the presence of antioxidant reduces ferricyanide ions to ferrocyanide ions, which further react with ferric chloride to form ferric ferrocyanide. This Fe^{3+} to Fe^{2+} transformation gives rise to the Prussian blue complex that absorbs light at 700 nm. The intensity of the colored complex increased with the electron or hydrogen donating ability from the antioxidant. Figure 1 reveals the absorbance of *L. parasiticus* extract and fractions at various concentrations. LPAF exhibited the highest DPPH radical scavenging activity and reducing power activity as compared with LPEE and LPEAF. Hence, LPAF might have the ability to reduce both free radicals and ferric ions, which may due to the main constituents consisting of phenolic acids, polyphenols which are primarily flavonoids and flavonoid glycosides. It has been reported that flavonoids and phenolic acids are the sources of antioxidants in plants (Cook and Samman, 1996). However, LPEE which showed a much lower antioxidant activities may due to the composition of chlorophylls, chlorophyll derivatives, and luteins. LPAF exhibited the strongest reducing power compared to the other fractions but its reducing power was lower than BHT, the standard positive control. Nevertheless, these data indicate that LPAF had a higher potential to act as a reductant or antioxidant.

Lipid peroxidation assay was carried out subsequently to determine the amount of malondialdehyde formation. Malondialdehyde, a breakdown product of lipid peroxides, was measured as previously described (Jentzsch et al., 1996). In the presence of heat and acid, malondialdehyde reacts with TBA to form a red pigment which has a peak

wavelength at 530 nm (Janero, 1990). Since the reaction is not specific to malonaldehyde and therefore results are reported as TBA reactive substances (TBARS). The present TBARS assay utilized egg yolk as lipid substrate. It is known that linoleic acid and linolenic acid are two of the polyunsaturated fatty acids present in this substrate. Upon reaction with oxygen, these fatty acids produce malonaldehyde which reacts with TBA producing a pink color. Results indicated that LPAF was again possessed the highest inhibitory effects towards lipid peroxidation by exhibiting the lowest IC_{50} value of 1.05 ± 0.04 mg/ml. On the other hand, LPEE also inhibited lipid peroxidation as indicated by low color formation with TBA. However, LPEAF yielded the highest IC_{50} value, showing the least inhibition towards the oxidative degradation of lipids among LPAF and LPEE.

Phenolic compounds are chemical compounds that consist of hydroxyl group (-OH) attached to an aromatic hydrocarbon group. Among the natural antioxidants isolated from various plants, phenolic compounds are in the forefront due to their wide distribution in the plant kingdom (Duan et al., 2006). There are many types of phenolic compounds found in plants, such as simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, and flavonoids. All the phenolic compounds possess the structural requirements of free radical scavengers and have potential as food antioxidant (Duan et al., 2006). Folin-Ciocalteu's phenol reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants (Singleton et al., 1999). It reacts with phenolic compounds to form chromogens that can be detected by using spectrophotometer. The color development is due to the transfer of electrons at basic pH to reduce the phosphomolybdate and phosphotungstate complexes to form chromogens in which the metals have lower valence. In this study, a comparison between *L. parasiticus* extract and fractions for their total phenolic content was assayed at 100 µg/ml as shown in Table 1. LPAF possessed the highest total phenolic content by giving 56.51 ± 1.43 GAE/g of dry weight, followed by LPEE and LPEAF with 52.08 ± 1.76 and 18.82 ± 0.53 GAE/g_{DW}, respectively. Hence, the highest antioxidant ability exhibited by LPAF may due to the phenolic compounds which were found to be present the most in LPAF.

It is well acknowledged that neurodegeneration such as in ischemic stroke, Alzheimer's and Parkinson's diseases, is closely associated with neuronal damage which is mediated by free radicals-induced oxidative stress. The remarkable antioxidative effect of *L. parasiticus* has led to the investigation of its relevancy in neuroprotection. Neuroprotective effect of *L. parasiticus* extract and fractions were assessed by using MTT cell viability assay. MTT is a yellow color tetrazolium salt which can be converted into insoluble purple formazan by mitochondrial enzyme present only in living cells, succinate dehydrogenase (Lee et al., 2004). Thus, the amount of formazan produced is directly proportional to the number of viable cells. The decrease in cell viability is due to the conversion of H_2O_2 into •OH radicals through Fenton reaction in the cells. •OH radicals, which in turn, damage the proteins, lipids and DNA of the cells, resulting in the cell damage followed by cell death. As determined by MTT assay, 10 h exposure of NG108-15 hybridoma cells to 2.0 mM H_2O_2 significantly ($P<0.01$) revealed oxidative damage (Figure 2). LPAF and LPEE showed significantly ($P<0.01$) ability to protect cell death induced by H_2O_2 with 78.00 ± 1.85 and 67.23 ± 1.85% cell viability at the highest treatment concentration of 100 µg/ml, respectively. LPAF exhibited stronger neuroprotective effect compared with LPEE which may due to the stronger antioxidant capability possessed by LPAF shown previously and the presence of higher total phenolic content compared with LPEE. A dose-dependency was observed in cells pretreated with both LPAF and LPEE (Figure 3) but the neuroprotective effect of LPEAF was not observed with the percentage cell viability of 45.78 ± 0.91 at 100 µg/ml treatment concentration which may due to the weak antioxidative activities and low phenolic content presented in LPEAF.

Collectively, LPAF exhibited the highest neuroprotective activity and presented obvious radical scavenging activity against DPPH with the IC_{50} value of 16.82 ± 0.27 µg/ml, indicating that LPAF may be a potent neuroprotectant owing to its free radical scavenging activity. LPAF also exhibited strongest reducing power ability and lipid peroxidation inhibitory activity. Moreover, total phenolic content screening showed that LPAF yielded the highest phenolic content of 56.51 ± 1.43 GAE/g_{DW} which may explain the highest presence of phenolic compounds in LPAF that possessed the strongest antioxidative and neuroprotective activities due to their reactivity of hydrogen- or electron-donating agents, metal ion chelating properties (Rice-Evans et al., 1996), and may be the occurrence of synergistic effects from phenolic compounds contained in LPAF that contributes to the potent antioxidant and neuroprotection ability. The presence of the highest phenolic content of LPAF demonstrated the strongest antioxidant capacity compared with LPEE and LPEAF, showed a consistency results with many researchers who reported such positive correlation between total phenolic content and antioxidant activity (Cai et al., 2004; Tawaha et al., 2007; Zheng and Wang, 2001). Additionally, total phenolic concentration of medicinal plants is generally ranging from 0.23 to 2.85 GAE/g_{DW} (Zheng and Wang, 2001) while the phenolic content of LPAF found in our present study established substantial amount of phenolic concentration which contributed to the effective antioxidant activity. Hence, elaborate studies to isolate and identify the most active

compound in LPAF and the possible mechanisms underlying neuroprotection are necessary in the future.

In summary, the present study demonstrated that LPAF exhibited the strongest antioxidative and neuroprotective effects in an *in vitro* model which implies the LPAF has a potential in neuroprotection under oxidative stress-induced cell damage. Hence, these findings suggest that LPAF warrants for further investigation as a potential neuroprotectant agent from natural sources.

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