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Neuroprotective and antioxidant effect of *Curcuma longa* (Rhizome) methanolic extract on SH-SY5Y cells and Javanese medaka

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Abstract: Diseases caused by oxidative stress can be prevented by antioxidant. Current treatments for those neurodegenerative diseases are not effective and cause many side effects. Thus, the search for alternative medicines is in high demand. Therefore, the main purposed of this study is to evaluate the neuroprotective effects of *Curcuma longa* (rhizome) 80% methanol extract. Antioxidant using dichlorofuoresence diacetate (DCF-DA) assay on SH-SY5Y cells revealed high activities of *Curcuma longa* (rhizome) extract with IC_{50} of 105.9±0.8 µg/mL. Sub-acute and chronic toxicity tests of the plant extract on adult Javanese medaka *(Oryzias javanicus)* showed high toxicity effect with LC_{50} of 24.15±0.8 mg/L and 13.69±0.7 mg/L respectively. Neuroprotective tests using cholinesterase assay disclose significant differences at $P<0.05$ between the group that are exposed to arsenic and treated with the crude extract and the group that are exposed to only arsenic. Identification of vitexin and isovitexin justified the high antioxidant potential of this plant leaf and it highest benefit to be used as medicinal supplement.

Keywords: Antioxidants, cancer, epidemiologically, neuroprotective, oxidative stress.

INTRODUCTION

Neurodegeneration result from progressive damage neural cells and loss of neurons leading to abnormal changes in both cognitive and motor functions. Examples of diseases include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis (ALS), and spinocerebellar ataxia (SCA) (Haque *et al.,* 2017). Aging is one of the predisposing factors especially in advance countries where increase numbers of individuals, especially elderly, present clinical sign of neurodegenerative disorders. It has been reported that excess production reactive oxygen species (ROS) to be the major etiology of this ailment (Lakshmi Prasanna and Vijayaraghavan, 2015). There is no adequate literature in the pathogenesis of free radicals but antioxidants compound shows high potential in managing and protecting this diseases.

Traditionally, herbal medicine has been used to treat several symptoms such as pain, inflammation, and inflammatory-mediated pain (Hamidpour *et al.,* 2015). *Curcuma longa* native of Malaysia has been used for the treatment and prevention of several illness including neurodegenerative diseases (Kumar *et al.,* 2018). One of the most important bio-active compounds available in all part of this plant but in high concentration at the rhizome is Curcumin (Ullah *et al.,* 2017). Previous report shows the potential of curcumin in curing and protection of diseases such as inflammation, microbial infection, thrombosis, atherosclerosis, cancer, neuronal diseases, cardiovascular diseases and early aging (Levy Nogueira *et al.,* 2015). Some studies reported the use of curcumin in curing metabolic diseases such as type 2 diabetes, hypercholesterolemia and heart diseases (Ghosh *et al.,* 2014).

Human neuroblastoma (SH-SY5Y cells) has been used widely as *in vitro* model in neurotoxicity research as well as studies that involved herbal medicine and their effects (Pasban-Aliabadi *et al.,* 2017). These cells was obtained from human sources and has been significantly used in studies of human related diseases for examples especially *in vitro* studies of neurological diseases and harmful substances (Sabra and Mehana, 2015). Differentiation of the cells can be achieve by treating them with retinoic acid for 7 days during and it can form 90% confluence in culture before differentiation (Gomez *et al.,* 2015).

Javanese medaka *(Oryzias javanicus)* commonly found in Malaysia, Singapore, Indonesia, Thailand and western Borneo in brackish water (Yusof *et la.,* 2012). This genus is subdivided into *O. latipa* group, *O. celesensis* group and *O. javanecus* group according to phylogenetic and Karyotype classification (Gallon *et al.,* 2018). *O. javanecus* and *O. dencena* the most related species and are used good in vitro model for scientific studies because of their high resistant to broad ranges of salinity.

Therefore, the main purpose of this work is to evaluate the toxicity, antioxidant and neuroprotective activities of *Curcuma longa* rhizome extract *in vitro* and *in vivo*.

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MATERIALS AND METHODS

Plant collection and identification

Curcuma longa (rhizome) was purchased at Pasar Borong, Seri Kembangan, Selangor, Malaysia. The plant was authenticated by botanists at Institute of Bioscience (IBS), UPM and voucher number SK2890/15 was allocated.

Plant extraction

The plant *rhizome* was cleaned, separated, cut into small pieces with the aid of anvil prunner, (UK). Drying of the sample as well as blending to semi powder form (40-60 mesh) with blender (Panasonic Malaysia) was carried out in lab at room temperature (26±1°C) for two weeks. Seventy gram of the grounded sample was soaked in 80% methanol (1000mL) in flat bottom flasks (Sigma Aldrich, USA). The semi powdered rhizome solution was shaken daily for three days at 26°C to obtain high crude extract. The extract obtained was then filtered with Whatman filter paper (1.5 Sigma Aldrich, USA) and then concentrated to semi-solid form at 42°C with a rotary evaporator (IKA® RV 10, USA), weighed, transferred to sample bottles and stored at 4°C.

Preparation of dose from the sample

A total of 100mg/mL was prepared by dissolving 100mg of crude extract in 1mL of pure dimethyl sulfoxide (DMSO) as stock solution. The crude extract was first dissolved in DMSO to make it more soluble in distilled water. Sub-stocks were formulated in µg/mL to the concentration of interest by using distilled and two-fold serial dilution. Eight concentrations (7.81-1000µg/mL) of the crude extract were formulated in 96-well microplate (Sigma Aldrich, USA) for this study. A total of 0.1% DMSO was used at 0.1% in all crude extract concentrations.

Cells viability assay

Cell viability test was carried out tetrazolium reduction assay using (MTT) reagent (Sigma-Aldrich, USA). Initially, SH-SY5Y cells were seeded into micro-plates at a density of $1X10^5$ cells/well complete Minimum Essential Medium (MEM) and stored in incubator, 5% CO_2, 37°C for 1 day. The cells were then treated with different concentrations of prepared crude extract dissolved in incomplete MEM and monitored for 3 days. Solution of was prepared by dissolving 5mg MTT in phosphate buffered saline (PBS). The solution was further diluting by with PBS at the ratio of 1:10. Ten µL of the constituted solution was 96 well plate containing cells that were previously exposed to various concentration of crude extract and stored at humidified, 5% CO_2, 37°C incubator. Media was then changed with DMSO after 4 hours of incubation and re-incubated in laminar flow hood for another 30 minutes. Absorbance's was taken with microplate reader at 570 nm (Sunnyvale, CA, USA). Similar protocol was repeated to evaluate toxicity effects of H_2O_2 on the cells.

Evaluation of acute effect of arsenic on Oryzias javanicus

Acute toxicity effect of arsenic on adult *Oryzias javanicus* was evaluated for 2 days. Initially, fishes (1.9-2.7cm length, 0.1-0.3g weighed and 6 months) were selected and treated with different concentrations of the arsenic (0.5-4mM). Each group has three replicates alongside with control group. de-chlolorinized water was used in this study as described by OECD guideline for testing of chemicals (OECD, 2013) and accepted by Universiti Putra Malaysia (Institutional animal care use committee), with AUP No. R005/2016: Reference No. UPM/IACUC/AUP/R005/2016. A total of 105 randomly selected fishes of both sexes, 5 fishes per concentration were used.

Evaluation of chronic effect of arsenic on Oryzias javanicus

Chronic effect of the arsenic was observed on adult *Oryzias javanicus* for 7 days. Initially, fishes (1.9-2.7cm length, 0.1-0.3g weighed and 6 months) were selected and treated with different concentrations of the arsenic (00.5 - 3.75mM). Each group has three replicates alongside with control group. de-chlolorinized water was used in this study as described by OECD guideline for testing of chemicals (OECD, 2013) and accepted by Universiti Putra Malaysia (Institutional animal care use committee) A total of 120 randomly selected fishes of both sexes, 5 fishes per concentration were used.

Evaluation of acute effect of crude extract on Oryzias javanicus

Acute toxicity effect of crude extract on adult *Oryzias javanicus* was evaluated for 2 days. Initially, fishes (1.9-2.7cm length, 0.1-0.3g weighed and 6 months) were selected and treated with different concentrations of the extract (62.5-1000mg/L). Each group has three replicates alongside with control group. de-chlolorinized water was used in this study as described by OECD guideline for testing of chemicals (OECD, 2013) and accepted by Universiti Putra Malaysia (Institutional animal care use committee), with AUP No. R005/2016: Reference No. UPM/IACUC/AUP/R005/2016. A total of 90 randomly selected fishes of both sexes, 5 fishes per concentration were used.

Evaluation of chronic effect of crude extracts on Oryzias javanicus

Chronic effect of the crude extract was observed on adult *Oryzias javanicus* for 2 weeks. Initially, fishes (1.9-2.7cm length, 0.1-0.3g weighed and 6 months) were selected and treated with different concentrations of the extract (35-95 mg/L). Each group has three replicates alongside with control group. de-chlolorinized water was used in this study as described by OECD guideline for testing of

chemicals (OECD, 2013) and accepted by Universiti Putra Malaysia (Institutional animal care use committee) A total of 135 randomly selected fishes of both sexes, 5 fishes per concentration were used.

2′7′-dichlorofluorescein diacetate assay

To carry out this procedure, SH-SY5Y cells were treated with retinoic acid (R.A) at 10 µM for one week. Cells were then seeded at a density of $1x10^5$ cells per well and incubated in 5% CO_2, 37°C incubators. Treatment of the cell with different dose of the extract (7.81-1000g/mL) was carried out after 2 days followed by addition of 35% H_2O_2 at 150mM and observed for another 2 days. The cells were then washed with phosphate buffered saline (PBS) in dark inside laminar floor. Addition of 2′7′-dichlorofluorescein diacetate (DCF-DA) 100µL (30µM in 0.1% DMSO PBS) was carried out follow by 30 minutes incubation. Determination of free radical was determined by taken measurement at fluorescent excitation 485 nm and 535 nm emissions with Tecan multimode microplate reader (United Kingdom). Effect of free radical on cell was then calculated with this formula:

Percentage increase in Fluorescence % = $\frac{Ft_{30}-Ft_0}{Ft_0} X100$

Where Ft_0 is the initial reading and Ft_{30} is the reading taken after 30 minutes of incubation

Total protein estimation

The protein content from the homogenate fishes brains were determined using Bradford method (Riber *et al.,* 2009) in which bovine serum albumin (BSA) (7.81-1000 µg/mL) were used as standards. Tris-HCl buffer prepared with 1% Triton X and 0.1 PMSF pH 7.4 were used as buffered and detergent. Reading was taken at 590 or 595 nm using microplate reader (Berthold Technologies GmbH) and standard curve was plotted using BSA.

Cholinesterase inhibition assay crude extract

Chronic effect of the crude extract was observed on adult *Oryzias javanicus* for 2 weeks. Initially, fishes (1.9-2.7cm length, 0.1-0.3g weighed and 6 months) were selected and treated with different concentrations of the extract (35-95 mg/L). Each group has three replicates alongside with control group. de-chlolorinized water was used in this study as described by OECD guideline for testing of chemicals (OECD, 2013) and accepted by Universiti Putra Malaysia (Institutional animal care use committee) A total of 135 randomly selected fishes of both sexes, 5 fishes per concentration were used.

Adult *Oryzias javanicus* (1.9-2.7cm length and 0.1-0.3g weighed and 6 months old) were used for the cholinesterase assay of crude extract. The fishes were initially treated with the safe dose of the extract 20mg/L for 3 days followed by exposure to 0.15mM of arsenic for 10 days in de-chlorinated water. A total of 375 randomly selected fishes of both sexes, 25 fishes per group (up to 4 groups) were used.

At the end of the experiment, the fishes were then cryo-anesthetized by exposing them for 20 second in ice followed by gentle separation of the head from the body. Cranio-medial dissection of the head and the removal of the brain were carried out followed by safely placement of the brain in sample bottle. The brain was then washed with buffer Tris-HCl 50mM, the weight was taken and blended using sample bottle with the aid of (Polytron PT-6100, tissue homogenizer USA). The sample was then centrifuged at 12,000xg for 20 minutes with (GRACE High Speed Refrigerated Centrifuge India). Separate tube was used to collect the supernatant for cholinesterase assay.

Anti-cholinesterase assay was carried out using Ellman's method (Benabent *et al., 2*014). Initially DTNB, ATC, BTC, and PTC were dissolved in 50mM Tris-HCl and transferred into 96 well plates. AChE (54mg/mL) from the fish brain was then added and incubated for 15 min at 28°C. Hydrolysis of the substrate acetylthiocholine by AChE yield thiocholine which combined with Ellman's reagent (DTNB) and yield 2-nitrobenzoate-5-mercaptothiocholine and reading was taken at 405 nm using a micro plate reader (Tecan Multimode Microplate, United Kingdom) at fluorescent excitation of 485 nm and 535 nm emission.

ChE activities were calculated using this formula;

Percentage decrease in fluorescence = $\frac{Ft_{30}-Ft_0}{} X 100$

Enzyme activity (U) = $\frac{\Delta \text{ Absorbance/10 min}}{\mu L \text{ of well}} X (TV/TS)$

Where;

Δ Absorbance = different in O.D at 405 nm after 10mn of incubation (final - initial) K

Molar extinction coefficient = $0.0136 \mu M^{-1} cm^{-1}$

TV = Total volume = 250µL

TS = Total sample = 10µL

Phytochemical study

Study of the bioactive constituent of the crude extract was carried with HPLC merchant (Waters, USA). The system is made up of 600 pumps, an auto-injector, 2998 photodiode array detector 200 to 500nm. Determination of separation was observed with a 250 x 4.6mm ODS 3,3 mm column (Inertsil, Japan) thermostated at 40°C (Marimuthu *et al.,* 2016). De-ionized water and methanol HPLC standard were used for this study. The mobile phase was set at 0 using 10% methanol in deionized water and increased to 90% methanol in deionized water for up to 45min.The 90% methanol in deionized water was maintained for further 15min. The peaks were integrated at the wavelength of 337nm.

STATISTICAL ANALYSIS

All experiments were repeated three times independently. Data were collected, processed and interpreted as mean ± standard error of mean (mean ± S.EM). LC_{50} Both

toxicity (Cells viability and survival rate of *Oryzias javanicus*) and antioxidant assays (DPPH and FRAP assays) results were analyzed using (Graph Pad Software, USA). Statistical analysis using one-way ANOVA followed by Dunnett's post hoc test, where $P<0.05$ was considered statistically significant as treated group (cells and fishes) were compared with untreated groups (cells and fishes control) using GraphPad Prism version 5.0 software (Graph Pad Software, USA).

RESULTS

Percentage yield of the crude extract

The percentage yield obtained following extraction of 70 g *Curcuma longa* (rhizome) was 20.15g.

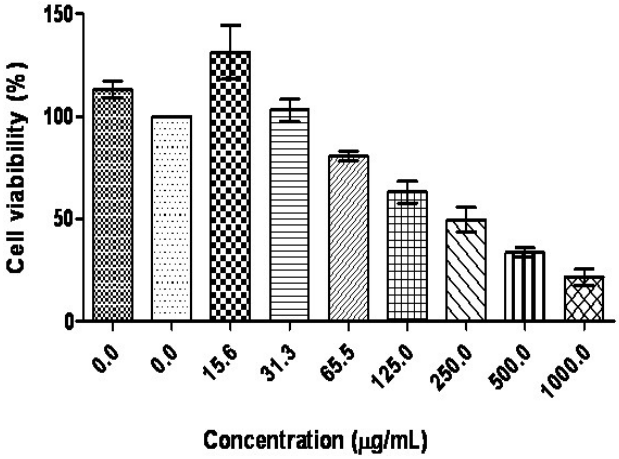

Fig. 1: Effect of *Curcuma longa* (rhizome) extract 15.62-1000 µg/mL on the viability rate of SH-SY5Y cells. Percentage of cells viability (mean ± SD), (n=3).

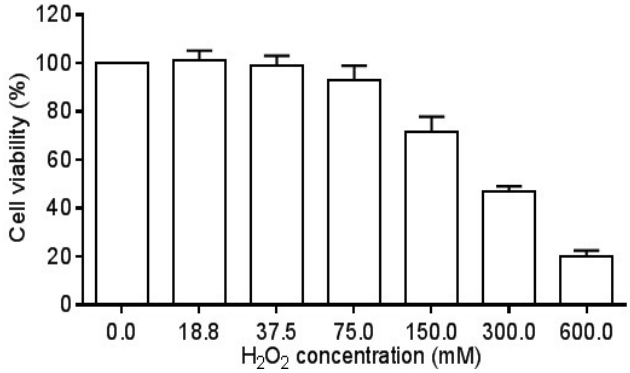

Fig. 2: Effect of Hydrogen peroxide (H_2O_2) at six concentrations (18.8-600mM) on percent cell viability of SH-SY5Y cells. Percentage of cells viability (mean ± SD), (n=3).

Toxicity test

Effects of Curcuma longa (rhizome) extract on SH-SY5Y cells

The result of *in vitro* toxicity test of the crude extract using MTT assay shows the LC_{50} of 199.7±0.46 µg/mL (fig. 1).

Effect of hydrogen peroxide in SH-SY5Y cells

The result of toxicity effect of H_2O_2 on SH-SY5Y cells using MTT assay shows the LC_{50} of 71.4% at the concentration of 150 mM (fig. 2).

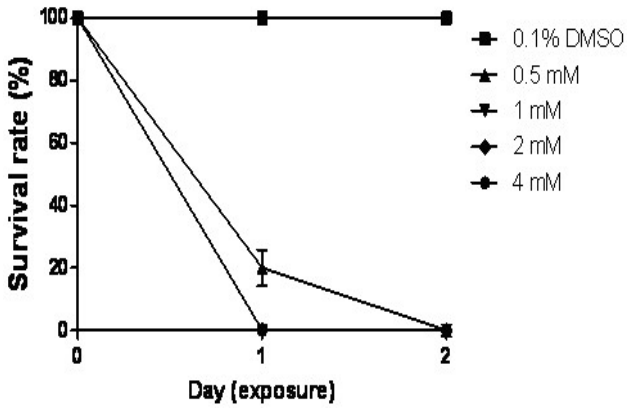

Fig. 3: Sub-acute toxicity effect of arsenic on the survival rate of adult Javanese medaka (*Oryzias javanicus*) treated with different concentration (0.5-4 mM). The percentage of survival rate (n=3) is shown versus concentration of the tested sample. The values represent mean ± SD from three independent experiments.

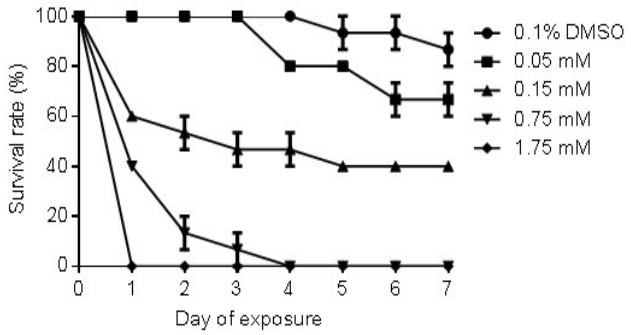

Fig. 4: Sub-chronic toxicity effect of arsenic on the survival rate of adult Javanese medaka (*Oryzias javanicus*) treated with different concentration (0.05-3.75 mM). The percentage of survival rate (n=3) is shown versus concentration of the tested sample. The values represent mean ± SD from three independent experiments with LC_{50} of 0.14 ± 0.8mM.

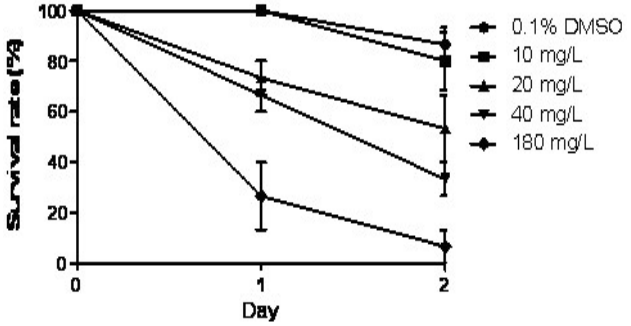

Fig. 5: Sub-acute toxicity effect of *Curcuma longa* (rhizome) extract on the survival rate of adult Javanese medaka (*Oryzias javanicus*) treated with four different concentrations (10-180mg/mL). The values represent

mean ± SD from three independent experiments with LC_{50} of 24.15±0.8 mg/L.

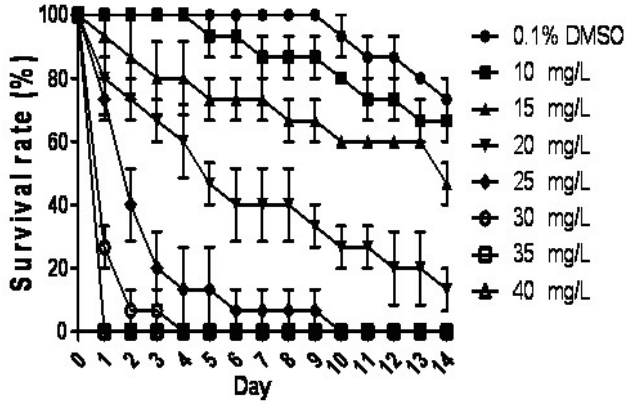

Fig. 6: Chronic toxicity effect of *Curcuma longa* (rhizome) extract on the survival rate of adult Javanese medaka (*Oryzias javanicus*) treated at seven different concentrations (10-40 mg/mL). The values represent mean ± SD from three independent experiments with LC_{50} of 13.69±0.7 mg/L.

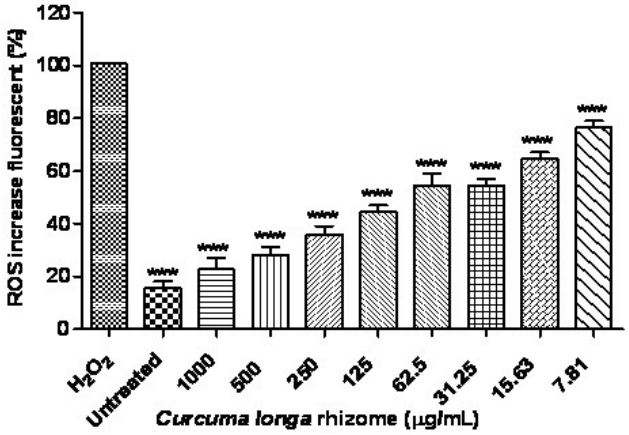

Fig. 7: Intracellular ROS levels were measured by fluorescent probe DCF-DA in SH-SY5Y cells Pretreated with extract for 24 hrs and challenge with H_2O_2 (150mM) to induce ROS production. The values represent mean ± SD from three independent experiments. ***P<0.001 represented significantly different values from H_2O_2 treated group.

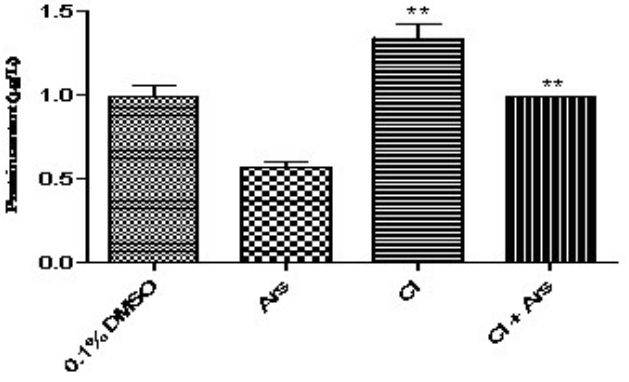

Fig. 8: Effects of *Curcuma longa* (rhizome) extract (10 mg/mL) and arsenic (0.15mM) on total protein content in adult brain of Javanese medaka (*Oryzias javanicus*). **P<0.01 represented significantly different values from an arsenic-treated group.

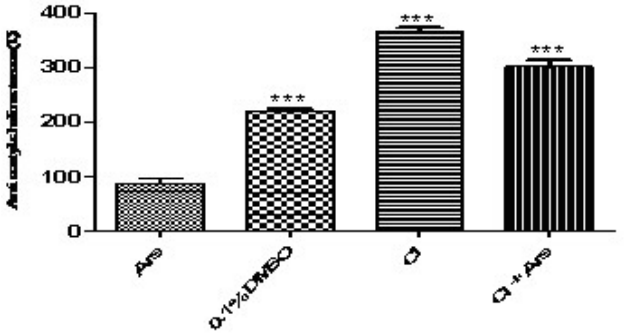

Fig. 9a: Anti-acetylcholinesterase protective effects of crude extract (10mg/mL) on arsenic (0.15mM)-induced neuron damage in adult brain of Javanese medaka (*Oryzias javanicus*) measured using DTNB read at 405 nm. Acetylcholine was used as a substrate. ***P<0.001 represented significantly different values from an arsenic-treated group. The values represent mean ± SD from three independent experiments.

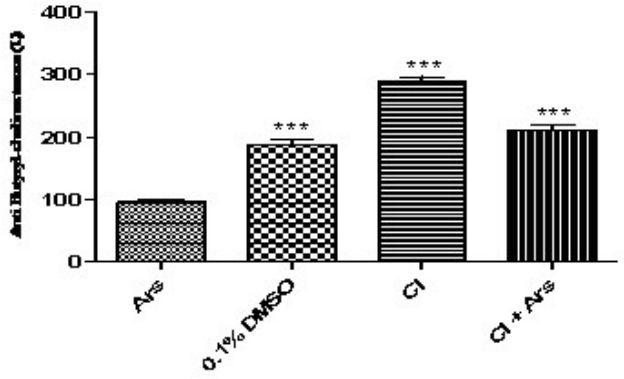

Fig. 9b: Anti-butyrylcholinesterase protective effects of crude extract (10mg/mL) on arsenic (0.15mM)-induced neuron damage in adult brain of Javanese medaka (*Oryzias javanicus*) measured using DTNB read at 405 nm. Butyrylcholine iodide was used as a substrate. ***P<0.001 represented significantly different values from an arsenic-treated group. The values represent mean ± SD from three independent experiments.

Toxicity test on adult Javanese medaka (Oryzias javanicus)

Sub-acute effects of arsenic on Oryzias javanicus

The result of sub-acute toxicity test of arsenic on adult Javanese medaka shows safe dose of 0.5mM (fig. 3).

Sub-chronic effects of arsenic on Oryzias javanicus

Result of sub-chronic toxicity test of arsenic shows the safe dose 0.05mM (fig. 4).

Sub-acute effects of crude extract on Oryzias javanicus

Result of sub-acute toxicity test of crude extract shows the safe dose 20mg/L (fig. 5).

Chronic effects of crude extract on Oryzias javanicus

The result of chronic toxicity test of crude extract shows the safe dose of 20mg/L (fig. 6).

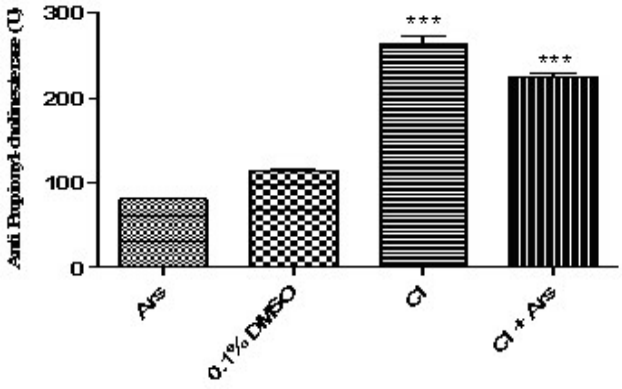

Fig. 9c: Anti-propionylcholinesterase protective effects of crude extract (10mg/mL) on arsenic (0.15mM)-induced neuron damage in adult brain of Javanese medaka (*Oryzias javanicus*) measured using DTNB read at 405 nm. Propionyl choline iodide was used as a substrate. ***P<0.001 represented significantly different values from an arsenic-treated group. The values represent mean ± SD from three independent experiments.

Protective oxidative stress effects of crude extract

Protective reactive oxygen species effects of crude extract

Results of protective reactive oxygen species inhibition of the crude extract show the IC_{50} of 146.66±0.1µg/mL (fig. 7).

Cholinesterase protective effects of the crude extract

The results of anticholinesterase effect of the crude extract were shown below. There was significant increase in acetylcholinesterase, butyrylcholinesterase and propionyl cholinesterase inhibition in groups that were exposed to crude extract only. Cholinesterase inhibition was very low in groups that were exposed to arsenic only compared to the group that were treatment with the crude extract and then exposed to arsenic as well as group that were maintained in distilled water with 0.1% DMSO. (fig. 9 a-c).

High performance liquid chromatography

Identification of bioactive compounds` in crude extract using HPLC method with vitexin and isovitexin as standard (fig. 10 a-c) show trace of concentration vitexin and isovitexin at retention time of 21.834 and 23.002) (fig. 10 a-b).

DISCUSSION

Antioxidants compounds are used manage several forms of ailment resulting from free radical accumulation for examples are neurodegenerative diseases and neoplasm (Lobo *et al.,* 2010). Current treatments for those neurodegenerative diseases are not effective and followed with many side effects (Lang and Espay, 2018). Thus, the search for cheap alternative medicines is in high demand.

High percentage yield of 20.15g was obtained following extraction of the *Curcuma longa* (rhizome) is sufficient for this experiment. Increased yield following extraction of Plant materials have been attributed to the polarities of the solvents as well as the ability of the solvent to dissolve and interact with the bioactive constituent of the plant which can be highly achieved with methanol (Kuzmina *et al.,* 2016). Therefore, since methanol has the ability to extract sufficient amount plant bioactive constituents, 80% methanol was constituted and used as extraction solving.

The viability of cells exposed to H_2O_2 was assessed using the MTT assays. This assay is sensitive, quantitative and reliable colorimetric assays for the determination of cells viability (Guo *et al*., 2015). Toxicity effect of H_2O_2 with a safe concentration of 150mM was similar with the finding of Park *et al.* (2015) which reported 250mM to be safe concentration for the experimental inducement of free radical dose on SH-SY5Y cells. The viability of SH-SY5Y cells incubated with 200µM H_2O_2 for 24 hrs decreased by 35% compared to the control (Han *et al*., 2014). In this finding, concentrations above 150mM showed high toxicity characterized by reduced cells viabilities after MTT assay. ROS accumulation might be responsible for decreased cells proliferation, differentiation and survival (Ma *et al.,* 2014; Pinho *et al.,* 2014).

Crude extract shows moderate toxicity effect with LC_{50} of 199.7±0.46µg/mL on SH-SY5Y cells. Effect of crude extract was observed to be dose dependent whereby increase in dose of crude extracts accompanied by increase in toxicity effects. There was no sufficient literature on the toxicity effect of *Curcuma longa* (rhizome) on SH-SY5Y cells so far. Exposure of the cells to different concentration of *Inula helenium* crude extract for 72 hrs resulted in a concentration-dependent decrease in cell survival. The result of sub-acute toxicity test of arsenic shows the safe dose to be below 0.5mM with more 70% of the fish survives at the concentration. Sub-chronic toxicity test also shows the safe dose to be 0.05mM because more 70% of the fish survived at the concentration.

Globally, the effects of arsenic have been reported to be detrimental to both human and animals especially if it involves freshwater contaminations. Environmental effect of this heavy metal can result from multiple interacting sources that create more challenged on quantified it spread and damage made (Barral-Fraga *et al*., 2016).

Toxicity effect both sub-acute and chronic shows the safety dose of 20mg/L on J. medaka. There was no documented fact found on the toxicity effect of *Curcuma longa* on J. medaka. Exposure of J. medaka to different concentration of *Planktothrix agardhii* methanol crude extract resulted in a dose-dependent mortality of embryos

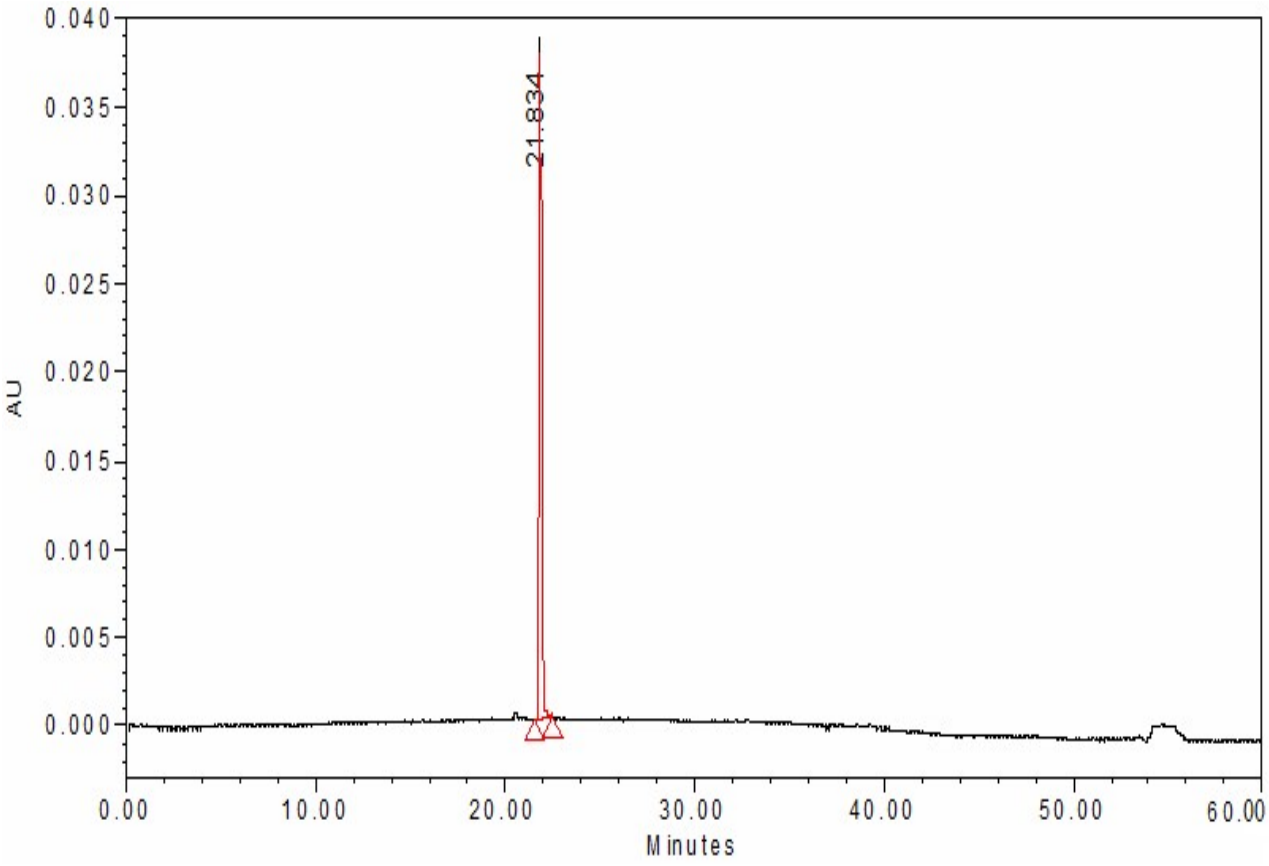

Fig. 10a: Normal phase HPLC profile of vitexin (standard) identified at retention time of 21.834 min.

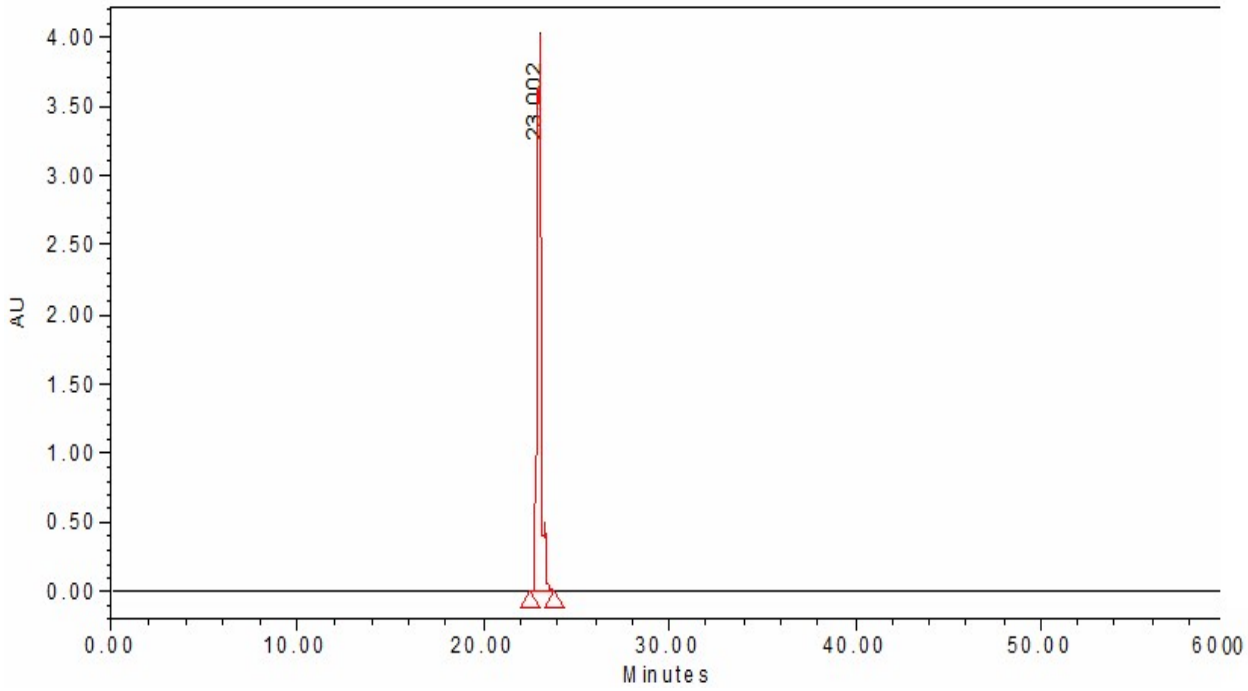

Fig. 10b: Normal phase HPLC profile of isovitexin (standard) identified at retention time of 23.002 min.

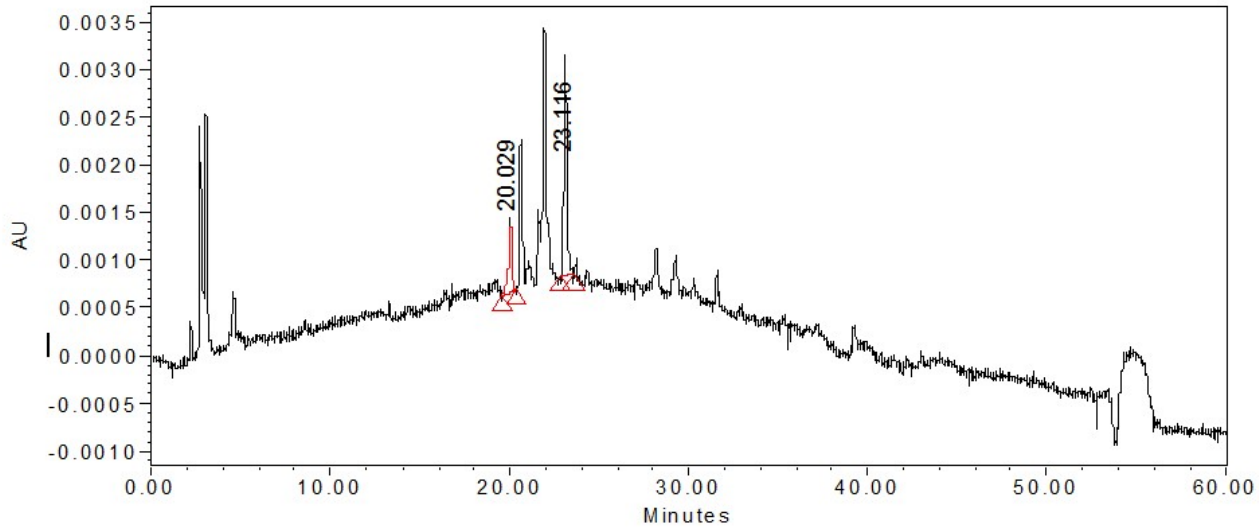

Fig. 10c: Normal phase HPLC profile of vitexin and isovitexin identified in *Curcuma longa* (rhizome) at retention time of 21.834 and 23.002 min.

and larvae (Marie *et al*., 2012). *Derris elliptica* aqueous leaf extract was reported to be lethal and caused teratogenic defect at dose concentration manner on zebrafish embryos and larvae. There is increased in mortality and decreased hatchability of both embryos and larvae with increase concentration of leaf extract (Seiler *et al*., 2014). It has been reported that the toxic effect of most medicinal plant result from the active compound present, examples alkaloids, flavonoids, terpenoids and saponins that are present in different part of the plants (Kennedy and Wightman, 2011).

Reactive oxygen species inhibition of crude at different concentration (7.81-1000µg/mL) show high activity with IC_{50} of 146.66±0.1µg/mL. Neuroprotective potential of the crude extract using cholinesterase assay also shows high activities with wide significant difference between tested and control groups. Significant increases in acetylcholinesterase, butyrylcholinesterase and propionyl cholinesterase inhibition following treatment with the crude extract and exposed to arsenic. Reduction in optical density of fluorescent is due to the ability of crude extract to inhibit ROS accumulation or neutralized H_2O_2 to form water which content less bonded radical. The result is in line with experimental study reported by Torri *et al.,* (2017) which shows crude extract with high flavonoid content can be use in the management and prevention of neurodegenerative conditions. Effects of *Curcuma longa* (rhizome) extract and arsenic on total protein content in adult brain of Javanese medaka (*Oryzias javanicus*) shows positive results, as there is increase in total protein in group that are treated with only crude extract and those that are treated with crude extract before exposing them to arsenic. This may be due to the protective effect crude extract on cell, tissues as well as protein from detrimental effect of arsenic.

Phytochemical constituents such as Curcumin (Cur), demethoxy curcumin (DCM), bis demethoxy curcumin (BDCM) also known as curcuminoids and flavonoid have been reported in *Curcuma longa* rhizome (Miquel *et al.*, 2002). Although, Curcuminoids has wide spectrum of activities and have been used as feed additives such as coloring, flavoring agents and food preservative (Kang and Chen, 2009). Presently, most research findings focus on the medicinal and antioxidant effect important of this plants, anti-inflammatory effect (Kant *et al*., 2015), anti-cancer effect (Riela *et al*., 2011), anti-ageing effect (Lee et al., 2012), anti-depressant effect (Somasundaram *et al*., 2002) and other uses (Viuda-Martos, Fernandez-Lopez, and Perez-Alvarez, 2010).

CONCLUSION

The result of this research shows *Curcuma longa* rhizome has high antioxidant and neuroprotective effect. *Curcuma longa* may contain high concentration of unidentified phenolic compound other than vitexin and isovitexin that may have high antioxidant and neuroprotective activities. High benefit of *Curcuma longa* rhizome extract as chemotherapeutic agent may be attributed to i high antioxidant activities and low toxicity effect as revealed in this study. Anticholinesterase activities of the *Curcuma longa* rhizome on mammal such as mice and rat to reaffirm their neuroprotective potentials are recommended. In vivo neuroprotective activities of other medicinal plant in J. medaka and SH-SY5Y cells model are also recommended.

Declaration of conflicting interests

Institutional animal care and use committee, Universiti Putra Malaysia.

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