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APPENDICES

APPENDIX A

PREPARATION OF TISSUE SECTIONS

PREPARATION OF TISSUE SECTIONS

Procedures:

1. The brains of the animals were perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.3
2. Following the perfusion, the brains were removed and postfixed with 4% paraformaldehyde in 0.1 M phosphate buffer overnight at 4 °C.
3. Tissues were rinsed with phosphate buffer and infiltrated with 30% sucrose solution in order to provide cryoprotection.
4. The specimens were frozen rapidly with deep freeze at -25 °C in cryostat (model JUNG FRIGOCUT 2800E).
5. After freezing, 25 μm thick of specimens are cut on cryostat.
6. Sections there were stored in phosphate buffer and they were picked up on slides coated with 0.01% aqueous solution of a high molecular weight poly-L-lysine.

Reference

Krill JJ, Halliday GM, Svoboda MD, Cartwright H. The cerebral cortex is damaged in chronic alcoholic. ***Neuroscience*** 1997; 79(7): 983-98.

APPENDIX B
PREPARATION OF TISSUE HOMOGENATES

PREPARATION OF TISSUE HOMOGENATES

After the last injection of substances, all animals were anesthetized with intraperitoneal injection of pentobarbital sodium (Nembutal®) at dose 50 mg/kg BW. Brain were isolated and kept cool in ice buckets. Then these tissues were homogenized in 4 volume of 1.15% KCl with a glass Potter-Elvehjem homogenizer.

Reference

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APPENDIX C

CRESYL VIOLET STAINING FOR NISSL SUBSTANCE

CRESYL VIOLET STAINING FOR NISSL SUBSTANCE

Cresyl violet can be used to demonstrate Nissl substance. The rationale of the technique is a simple acid-base reaction, where the cationic dyes bond with the anionic RNA of the Nissl substance, plus the DNA and RNA of cell nuclei.

Staining solution:

- 0.5% aqueous solution of cresyl violet 100 ml
- 10 % Acetic acid 7 ml

Add 10% acetic acid 7 ml in 0.5% aqueous solution of cresyl violet 100 ml and adjust pH to 3.5-3.8. Stand the solution at room temperature for 24-48 hours. The solution should be heated gently and filtrated before used.

Procedures:

1. Immerse slides into xylene solution for 2 times, approximate 2-3 minutes each.
2. Hydrate the sections in serial concentration of alcohol; absolute, 95% and 70% alcohol approximate 3 minutes per each process.
3. Wash the sections in distilled water.
4. Stain the sections in cresyl violet solution for 3-5 minutes. Nissl body should be violet.
5. Immerse the sections in serial concentration of alcohol; 70%, 95% and absolute alcohol for 1 minute or longer per each process until the background is relatively clear.
6. Clear the sections in xylene solution for 2-3 minutes.
7. Mount the slides and coverslipped with DPX permount.

Results: Nissl body: Violet

Reference

Paxinos G, Chorles W. Cresyl Violet. In: Paxinos G, Chorles W, editors. ***The rat brain in stereotaxic coordinates*** London: Academic Press; 1981. p. 9-17.

APPENDIX D

PREPARATION OF PHOSPHATE BUFFER SALINE SOLUTION

PREPARATION OF PHOSPHATE BUFFER SALINE SOLUTION

0.01 M phosphate buffer saline E may, vao day coi co con nho nay ngon lam http://nhatquanglan.xlphp.net/

Reagents

1. KCl 0.8 g
2. KH_2PO_4 0.8 g
3. NaCl 32 g
4. Na_2HPO_4 4.6 g

Procedures:

1. Add KCl, KH_2PO_4, NaCl, and $Na_2H\ PO_4$ in 3,800 ml of distilled water.
2. Dissolve these chemicals on magnetic stirrer.
3. Adjust to pH 7.4 with NaOH.
4. Make up the final volume to 4,000 ml

Reference

Merrante F, Raha S, Reod JK, Proteau G. Harwood AJ. ***Methods in Molecular Biology: Basic DNA and RNA Protocols.*** London: Humma Press; 1996.p.3-9.

APPENDIX E
DETERMINATION OF PROTEIN

DETERMINATION OF PROTEIN

Reagents:

1. Solution A: Alkaline titrate reagent
   - $Na_2C_4H_4O_6.2H_2O$) 0.1 g
   - Na_2CO_3 10 g
   - NaOH 1.2 g

Dissolve these chemicals in distilled water to make 500 ml solution.

2. Solution B: 0.5% copper sulfate

Dissolve 0.5 g of copper sulfate ($CuSO_4.5H_2O$) in distilled water 100 ml.

3. Solution C:

Freshly mixed 50 ml of solution A and 1 ml of solution B.

4. Solution D: 1 N Folin phenol reagent

Dilute commercial 2 N Folin phenol reagent with distilled water 1:1 and use immediately.

5. Standard protein solution

Dissolved bovine serum albumin (BSA) 60 mg to 100 mg with distilled water.

Procedures

1. Pipette solution into each tube as described following:

	Volume		
	Blank	Standard	Unknown
Distilled water	0.2	0.1	0.1
Standard BSA	-	0.1	-
Sample*	-	-	0.1
Solution C	5.0	5.0	5.0
Solution D	0.5	0.5	0.5

* Sample dilution 1:50 for brain

2. Add 5 ml of solution C and mix well.
3. Let stand at room temperature for 10 minutes.

4. Mix thoroughly 0.5 ml of solution D and let stand at room temperature for 1 hour.

5. Read optical density (OD) at 650 nm by UV/vis spectrophotometer (model 7,800 Japan spectonic 20) against the reagent blank.

Calculation

Protein concentration (g %)

$$= \frac{\text{O.D. unknown x concentration of standard}}{\text{O.D. standard}}$$

Reference

Lowry OH, Roseburgh NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. ***J Biol Chem*** 1951; 193: 263.

APPENDIX F
DETERMINATION OF LIPID PEROXIDE CONTENTS

DETERMINATION OF LIPID PEROXIDE CONTENTS

Reagents

1. 8.1% SDS (Sodium dodecyl sulfate).
2. 20% acetic acid solution adjusts to pH 3.5 with NaOH.
3. 0.8% TBA (thiobarbituric acid).
4. TMP (1, 1, 3, 3-tetramethoxy propane) or malondialdehyde bis (dimethyl acetal) solution was used as an external standard, and the level of lipid peroxide was expressed as nmol of MDA (malondialdehyde).

Procedures:

1. Add the following substances in the table into the series of glass tubes with screw capped.

	Blank (ml)	Standard (ml)	Unknown (ml)
Sample * (1:50)	-	-	0.2
8.1% SDS	0.2	0.2	0.2
20% acetic acid (pH 3.5)	1.5	1.5	1.5
0.8% TBA	1.5	1.5	1.5
TMP stock standard	-	0.2	-
Distilled water	0.8	0.6	0.6

* Sample = tissue homogenate prepared according in the appendix B

2. Heated the tubes in the water-bath at 95 °C for 60 minutes.
3. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1, v/v) are added and shaken vigorously.
4. After centrifugation at 4,000 rpm for 10 minutes, the organic layer is taken and its absorbance at 412 nm is measured.
5. The content of lipid peroxide is expressed in term of nmol MDA/100 mg protein.

Calibration Curve

1. Prepare a series of tubes containing TMP stock standard in water in the following concentrations: 2.0 nmol/0.2 ml, 4.0 nmol/0.2 ml, 6.0 nmol/0.2 ml, 8.0 nmol/0.2 ml, 10.0 nmol/0.2 ml.
2. Perform the procedure as in step 2.
3. Determine the absorbance at 532 nm. The O.D. was plotted against concentration of MDA, which expressed as nmol MDA/100 mg protein.

Reference

Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. ***Analytical biochemistry***. 1979; 95: 351-8.

APPENDIX G
IMMUNOHISTOCHEMISTRY OF CHOLINE ACETYL TRANSFERASE (ChAT) ENZYME

IMMUNOHISTOCHEMICAL STUDY OF CHOLINE ACETYL TRANSFERASE (ChAT) ENZYME

Reagents

1. KPBS-BT (Kreb phosphate buffer saline containing bovine serum albumin and triton x-100).

Add bovine serum albumin 2.5 g and triton x-100 1 ml in 1,000 ml of 0.1 M phosphate buffer.

2. 0.05 M Tris-HCl buffer pH 7.6.
3. 0.5 % H_2O_2 in methanol.
4. Primary antibody against Choline acetyl transferase (ChAT) dilution 1:100.
5. DAKO Strept ABC Complex /HRP duet kit. This kit consists of reagent A: Streptavidin, Reagent B: biotinylated horseradish. Reagent C: biotinylated goat antibody to mouse immunoglobulin.

- Working solution of biotinylated goat antibody to mouse

Add reagent C 10 µl in 1 ml of KPBS-BT.

- Working solution Strept AB Complex /HRP

Add 10 µl of reagent A and B into 1 ml of KPBS-BT.

6. 0.4 % H_2O_2 and diaminobenzidine in 0.05 M Tris-HCl.
7. 5 % normal horse serum in KPBS-BT.

Procedures:

1. Inhibit endogenous peroxidase activity by incubating in 0.5 % H_2O_2 in methanol for 30 minutes.
2. Wash slides in running tap water for 1 minute then wash slides again in distilled water for 1 minute.
3. Wash slides in KPBS and KPBS-BT for 5 minutes per each process.
4. Remove excess buffer, then apply the 5 % normal horse serum in KPBS-BT to the sections and incubate in moist chamber for 30 minutes in order to minimize background staining.
5. Drain off excess normal horse serum.

6. Incubate sections in mouse primary antibody against ChAT dilute 1:100 in KPBS-BT at room temperature for 2 hours and then incubate at 4°C for 48 hours (This step is omitted in control slide).

7. Wash off excess antiserum and wash slides in KPBS-BT for two 7 minutes changes.

8. Drain off excess buffer and incubate slides with 100 µl of working solution of biotinylated goat antibody to mouse for 4 hours at room temperature.

9. Wash slides in KPBS-BT for two 7 minutes changes.

10. Drain off excess buffer and incubate slides with 100 µl of working solution of Strept AB Complex /HRP for 4 hours at room temperature.

11. Wash slides in KPBS-BT for 1 minute, then wash slides again in KPBS for 10 minutes two times.

12. React for peroxidase activity in KPBS-BT containing 0.025 % DAB and 0.01 % H_2O_2 for 24 hours at room temperature.

13. Wash in running tap water, let dry and mount sections in DPX permount.

Reference

Wood GS, Warnke R. Suppression of endogenous avidin-binding activity in tissues and its relevance to biotin-avidin detection systems. ***J Histochem Cytochem*** 1981; 29: 1196-1204.

APPENDIX H
DETERMINATION OF ACETYLCHOLINESTERASE

DETERMINATION OF ACETYLCHOLINESTERASE

Reagents

1. 0.1 M phosphate buffer (PB), pH 8.0
2. 0.075 M acetylthiocholine iodide (ATChI)
3. 0.01 M dithiobisnitrobenzoate (DTNB)

Preparing a brain homogenate and dilute it to approximately 5 mg/ml. The solutions, 0.1 M phosphate buffer (PB), pH 8.0, 0.01cM dithiobisnitrobenzoate (DTNB) and a stock of acetylthiocholine iodide (ATChI) at 0.1 M will be provided. In addition, preparing ATChI at the following concentrations: 0.1 M; 0.075 M; 0.05 M: 0.025 M; 0.01 M and 0.005 M for calculation.

Prepare brain homogenates

A. Weigh a sample (30 mg) and Add 1 ml of PB/30 mg tissue (30 mg/ml). Homogenize this solution until the brain is uniformly dispersed in the buffer. Place the tube on ice.

B. Assay

1. Turn on the spectrophotometer and set at 412 nm. Let it warm up for at least 15 minutes prior to reading.
2. Label the assay tubes - four tubes (3 for the assay and one for a control)
3. Pipette 3 ml PB into each assay tube
4. Using a pipette add 200 µL of sample homogenate to each of the four labeled assay tubes. Vortex each tube and return it to the ice.
5. Zero the spec. 20 without a tube.
6. Add 100 µL of DTNB to the first cortex tube, vortex, and place it in a test tube rack for 5 minutes. This allows the solution to reach room temperature.
7. Vortex and quickly wipe the outside of the tube with a kimwipe. Place the tube in the spec. 20 and zero the spectrophotometer to 0 absorbance. This will be your baseline reading before measuring product formation.
8. Take the tube out of the spectrophotometer; quickly add 20 µL ATChI and vortex.

9. Immediately return the tube to the spec. 20. Note the time and take a zero reading of absorbance. Take readings at 30 sec, 60 sec, 2 min., and 3 min. and record the data in a table.

10. Repeat this procedure (steps 6-10) for the other sample homogenates. Run the control through the same procedure except do not add substrate (ATChI) but add 20 μL PB instead.

C. Calculate of the rate of the reaction:

Calculate the rate of the reaction according to the following equation:

R = $\Delta A/(1.36*10^4)$ x 1/(200/3320)Co= $1.22(10^{-3})$ A/Co

R = rate, in moles substrate hydrolyzed/min.g tissue

ΔA = change in absorbance/min.

Co = original concentration of tissue (mg/ml) 200/3320 are volume corrections 1.36 (10^4) is the extinction coefficient of the yellow product

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Thompson, R. F. ***The Brain: A Neuroscience Primer***, 2nd ed. W. H. Freeman and Co., New York, 1993; 13

Robertson, R.T., C. F. Holunann, J.L. Bruce and J.T. C:oyle. Neonatal enucleation reduces specific activity of acetylcholinesterase in developing rat visual cortex. ***Devel Brain Res.***, 1988; 39: 298302.

APPENDIX I

DETERMINATION OF SUPEROXIDE DISMUTASE

DETERMINATION OF SUPEROXIDE DISMUTASE

Reagents:

A. 216 mM Potassium Phosphate Buffer, pH 7.8 at 25 °C

Prepare in deionized water using Potassium Phosphate, Monobasic, Anhydrous.

B. 10.7 mM Ethylenediaminetetraacetic Acid Solution (EDTA)

Prepare in deionized water using Potassium Ethylenediaminetetraacetic Acid, Disodium Salt, Dihydrate.

C. 1.1 mM Cytochrome c Solution (Cyt C)

Prepare in deionized water using Potassium Cytochrome c.

D. 0.540 mM Xantine Solution (Xantine), pH 7.4

Prepare in deionized water using Xantine.

E. Xantine Oxidase Enzyme Solution (XOD)

Immediately before use, prepare a solution containing 0.5 units/ml of Xantine Oxidase in cold deionized water.

F. Standard Superoxide Dismutase Enzyme Solution (Std SOD)

Immediately before use, prepare a solution containing 1, 5, 10, 50, 100, 500 and 1000 units/ml of Superoxide Dismutase in cold buffer.

Prepare brain homogenates:

Weight a brain sample and diluted in the cold buffer (5 mg brain tissue/ml). Homogenize this solution until the brain is uniformly dispersed in the buffer.

Procedures:

1. Prepare a reaction cocktail by pipetting the following reagents into a container:

A. Buffer	25 ml
B. EDTA	1 ml
C. Cyt c	1 ml
D. Xantine	50 ml

2. Mix and adjust to pH 7.8, 25 °C. Monitor the absorbance, $A_{550\ nm}$, of the reaction cocktail until constant using a spectrophotometer.

3. Pipette (in micrometers) the following reagents into cuvettes:

	Cocktail	DI water	XOD	Std SOD	Sample
Blank	900	100	-	-	-
Test 1	900	50	50	-	-
Test 2 Std SOD	900	-	50	50	-
Test 2 Sample	900	-	50	-	50

4. Immediately mix by inversion and record the increase in $A_{550\ nm}$ for 3 min. obtain the rate of change ($\Delta A_{550\ nm}$ /min) for both test 1 (Uninhibited) and test 2 (Inhibited).

5. Calculate % inhibition as following:

$$\% \text{ inhibition} = \frac{(\Delta A_{550\ nm}/\text{min Test 1}) - ((\Delta A_{550\ nm}/\text{min Test 2})}{(\Delta A_{550\ nm}/\text{min Test 1})} \times 100$$

6. Plot the SOD standard curve by plotting % inhibition against Std SOD (units/ml) and determine the linear equation of the curve.

7. Calculate the activity of SOD in samples using % inhibition and the linear equation of the standard curve.

8. Report the enzyme activity and it is expressed in units/mg protein or units/mg solid.

Reference:

McCord JM, Fridovich I. Superoxide dismutase. An enzymic function erythrocuprein (hemocuprein). ***J. Biol. Chem.***, 1969; 244: 6049-6055.

APPENDIX J
DETERMINATION OF CATALASE

DETERMINATION OF CATALASE

Reagents:

A. 50 mM Potassium Phosphate Buffer, pH 7 at 25 °C

Prepare in deionized water using Potassium Phosphate, Monobasic, Anhydrous.

B. 0.005 N Potassium Permanganate Solution ($KMnO_4$)

Prepare in deionized water using Permanganate.

C. 5 N Sulfuric Acid Solution (H_2SO_4)

Prepare in deionized water using Sulfuric Acid.

D. 0.01 N Hydrogen Peroxide (H_2O_2)

Prepare in the buffer using Hydrogen Peroxide, 30 % (w/w) Solution.

E. Standard Catalase Enzyme Solution (Std CAT)

Immediately before use, prepare a solution containing 20, 40, 60, 80 and 100 units/ml of Catalase in cold buffer.

Prepare brain homogenates:

Weight a brain sample and diluted in the cold buffer (25 mg brain tissue/ml). Homogenize this solution until the brain is uniformly dispersed in the buffer.

Procedures:

1. Prepare a spectrophotometer t monitor the absorbance, $A_{515\ nm}$, by using 1 ml of the buffer as the blank.
2. Pipette 300 µl of H_2O_2 (Reagent D) and 50 µl of a Std CAT (Reagent E) into a cuvette. Immediately mix by inversion and leave the reaction goes on for exact 60 sec.
3. Then, immediately add 150 µl of H_2SO_4 (Reagent C) and mix.
4. Add 600 µl of $KMnO_4$ (Reagent B) and mix.
5. Read the absorbance, $A_{515\ nm}$
6. Repeat step 2-5 for the different concentrations of Std CAT.
7. Pipette 300 µl of H_2O_2 (Reagent D) and 50 µl of sample homogenate into cuvette. Immediately mix by inversion and leave the reaction goes on for exact 60 sec.

8. Repeat step 3-5 for the different samples.

9. Plot the CAT standard curve by plotting $A_{515\ nm}$ against Std CAT (units/ml) and determine the linear equation of the curve.

10. Calculate the activity of CAT in samples using $A_{515\ nm}$ and the linear equation of the standard curve.

11. Report the enzyme activity and it is expressed in units/mg protein or units/mg solid.

Reference:

Goldblith SA, Proctor BE. Photometric determination of catalase activity. ***J Biol Chem.*** 1950; 187(2): 705-709.

APPENDIX K
DETERMINATION OF GLUTATHIONE PEROXIDASE

DETERMINATION OF GLUTATHIONE PEROXIDASE

Reagents:

A. 50 mM Sodium Phosphate Buffer with 0.40 mM EDTA, pH 7 at 25 °C

Prepare in deionized water using Sodium Phosphate, Monobasic, Anhydrous and Ethylenediaminetetraacetic Acid, Tetrasodium Salt.

B. 1 mM Sodium Azide Solution (Buffer w/Azide)

Prepare Sodium Azide in reagent A.

C. β-Nicotinamide Adenine Dinucleotide Phosphate, Reduced Form (β-NADPH). Use 5 mg vial of β-NADPH (Reduced Form).

D. Glutathione Reductase Enzyme Solution (GR)

Immediately before use, prepare a solution containing 100 units/ml of Glutathione Reductase in cold deionized water.

E. 200 mM Glutathione, Reduced Form (GSH)

Prepare in deionized water using Glutathione, Reduced Form.

F. 10 mM Sodium Phosphate Buffer with 1 mM Dithiothreitol, pH 7 (Buffer w/ DTT)

Prepare in deionized water using Sodium Phosphate Buffer, Monobasic, Anhydrous and DL-Dithiothreitol.

G. Standard Glutathione Peroxidase Enzyme Solution (Std GPx)

Immediately before use, prepare a solution containing 1.5, 2, 2.5, 3 and 5 units/ml of Glutathione Peroxidase in cold buffer w/ DTT (Reagent F).

H. 0.042% (w/w) Hydrogen Peroxide (H_2O_2)

Prepare in deionized water using Hydrogen Peroxide, 30% (w/w) Solution.

Prepare brain homogenates:

Weight a brain sample and diluted in the cold buffer w/DTT (Reagent F, 25 mg brain tissue/ml). Homogenize this solution until the brain is uniformly dispersed in the buffer.

Procedures:

1. Prepare a reaction cocktail by pipetting the following reagents into 5 mg vial of Reagent C (β-NADPH):

   B. Buffer w/Azide 46 ml

   D. GR 0.5 ml

   E. GSH 0.25 ml

2. Mix and adjust to Ph 7, 25°C.
3. Pipette (in micrometers) the following reagents into cuvettes:

	Cocktail	Buffer w/DTT	DI water	Std GPx	Sample
Blank	-	-	1000	-	-
Test 1	1000	17	-	-	-
Test 2 Std GPx	1000	-	-	17	-
Test 2 Sample	1000	-	-	-	17

4. Mix by inversion and monitor the absorbance, $A_{340\ nm}$, of the reaction cocktail until constant using a spectrophotometer.
5. Add (in micrometers) H_2O_2 (Reagent H):

	H_2O_2
Test 1	17
Test 2 Std GPx	17
Test 2 Sample	17

Then, immediately mix by inversion and record the decrease in $A_{340\ nm}$ for 3 min. obtain the rate of change ($\Delta A_{340\ nm}$/min) for both test1 (No reaction) and test 2 (Reaction).

6. Calculate Diff $\Delta A_{340\ nm}$/min as following:

   Diff $\Delta\ A_{340\ nm}$/min = ($\Delta A_{340\ nm}$/min Test 2)-($\Delta A_{340\ nm}$/min Test 1)

7. Plot the GPx standard curve by plotting Diff Δ $A_{340\ nm}$/min against Std GPx (units/ml) and determine the linear equation of the curve.

8. Calculate the activity of GPx in samples using Diff $\Delta A_{340\ nm}$ and the linear equation of the standard curve.

9. Report the enzyme activity and it is expressed in units/mg protein or units/mg solid.

Reference:

Wendel A. ***Enzymatic Basic of Detoxication.*** New York: Academic Press; 1980; Volume 2; pp.333-53.

APPENDIX L
PREPARATION OF QUERCETIN LIPOSOMES

PREPARATION OF QUERCETIN LIPOSOMES

Quercetin liposomes were prepared and modified by using thin film hydration with ultrasonication and extrusion to obtained narrow-range of size distribution. Mixtures of lipids, egg phosphatidyl choline (EPC), cholesterol (Chol) and quercetin were dissolved with a fixed volume of chloroform in a round bottom flask which was then incubated at 45°C using a rotary evaporator. Chloroform, gradually evaporated, and the liquid mixture were rotated for about 30 min to obtain a thin lipid film attached to the wall of the round bottom flask. Warm 50% polyethylene glycol (PEG) (about 40°C, an equal volume to the chloroform used) was added to hydrate the lipid film, resulting in a cloudy liquid which contained heterogeneous lipid-bilayer particles dispersed in 50% PEG. The dispersion was then sonicated using an ultrasonic bath at 45°C for 2 h and extruded through 2 series of PC membrane with pore sizes of 0.2 and 0.1 μm, resulting in dispersion of dispersed in 50% PEG.

Reference

Betageri GV, Jenkins SA, Parsons DL. Preparation of liposomes. ***Liposome drug delivery system*** Pennsylvania: Technomic Publishing Company, Inc; 1993.

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Publications:

1. Wattanathorn Jintanaporn, **Phachonpai Wathita**, Priprem Aroonsri and Suthiparinyanont Sangrawee. Intranasal Administration of Quercetin Liposome Decreases Anxiety-like Behavior and Increases Spatial Memory. ***Am Jf Agri Biol Sci*** 2007; 2(1): 31-35.

2. Priprem Aroonsri, Wattanathorn Jintanaporn, Sutthiparinyanont Sangrawee, **Phachonpai Wathita,** Muchimapura Supaporn. Anxiety and cognitive effects of quercetin liposomes in rats. ***Nanomed: Nanotec Biol Med*** 2008; 4: 70–78.

Awards and Honour:

1. The granted of Graduated School, Khon Kaen University to proceed on research study at the University of North Dakota School of Medicine and Health Science, North Dakota, USA, 2005.

2. The granted of Integrated Nanotechnology Research Center (INRC) Khon Kaen University to proceed on poster presentation at Austria, 2007.

3. Young investigator award (oral presentation) from ICPH -3rd International Conference on Polyphenols and Health, Kyoto, Japan, 2007.