APPENDICES

Appendix A

Chemicals, reagents, instruments, plastics and glasswares

The chemicals, materials and instruments employed in the present studies were summarized in Table 3-2 and 3-3.

Table A-1 List of chemicals and reagents used in the studies

Name	Source
Absolute ethanol	Merck, Germany
Citric acid monohydrate	Merck, Germany
Dimethyl sulfoxide (DMSO)	Fluka, Germany
Distilled water (Milli-Q, ≥ 18 Mega Ohm)	Milford, USA
Ethanol 95% (commercial grade)	Sasol, South Africa
Hydrochloric acid	Merck, Germany
Ketotifen fumarate	Sigma, USA
Lipopolysaccharide from *E. coli* O55:B5 (LPS)	Sigma, USA
Magnesium chloride $6H_2O$	Merck, Germany
Minimum Essential Medium (MEM)	Gibco, USA
N-(1-Naphthyl)ethylenediamine dihydrochloride	Sigma, USA
Penicillin-Streptomycin (P/S)	Gibco, USA
Phosphate buffer saline (PBS)	Amresco, USA
Phosphoric acid solution	Sigma, USA
Piperine	Merck, Germany
Prostaglandin E_2 EIA Kit	Cayman, USA
Quantikine Mouse TNF-α Kit	R&D Systems, USA
RPMI medium 1640	Gibco, USA
Silica Gel 60 (0.040-0.063 mm)	Merck, Germany
Sodium bicarbonate	BHD, England
Sodium carbonate	Merck, Germany

Table A-1 (Continued)

Name	Source
Sodium chloride	Univar, Australia
Sodium hydrogen carbonate	Merck, Germany
Sodium hydroxide (analytical grade)	Univar, Australia
Sulfanylamide	Sigma, USA
Thiazolyl blue tetrazolium bromide (MTT)	Sigma, USA
Trypan blue	Gibco, USA
Trypsin-EDTA	Gibco, USA
24-well plate flat, bottom	Costar Corning, USA
96-well plate flat, bottom with lid	Costar Corning, USA
96-well plate flat, bottom without lid	Corning, USA
Autoclave	Hirayama, Japan
Cell culture flask, canted neck 25, 75 cm^3	Corning, USA
Centrifuge tube15, 50 ml	Corning, USA
Centrifuge machine	Boeco, Germany
CO_2 humidified incubator	Forma, USA
Cryogenic tube 2 ml	Corning, USA
Disposable pipette 2, 5, 10, 25 ml	Corning, USA
Eppendrof	Costar Corning, USA
Glass bottles	Schott Duran, Germany
Glasswares	Schott Duran, Germany
	Pyrex, USA
Hematocytometer	Boeco, Germany
Hot air oven	Memmert, Germany
Hot plate	Thermolyne, USA
Inverted microscope	Nikon, Japan
Laminar air flow	Faster, Italy
Lyophilizer	Telster, Spain
Micropipettes	Eppendorf, Germany

Table A-1 (Continued)

Name	Source
Microplate reader	Bio Tek, USA
Multi-channels pipette	Costar Corning, USA
pH meter	WTW inolab, Germany
Pipette boy	Brand, USA
Quantikine mouse TNF-α ELISA test kit	R&D Systems, USA
Refrigerator (-20°C)	Sanyo, Japan
Rotary evaporater	Buchi, Japan
Sonicator	Elma, Germany
Sulforhodamine B dye	Sigma, USA
Trisma base	Sigma, USA
Vortex	Scientific industries, USA
Water bath	Lauda, Germany

Appendix B

Chemical Reagent

1. Reagents for determination Nitric Oxide inhibitory effect

1.1 Griess reagent

Sulfanilamide	1.0 g
N-(1-Naphthyl) ethylenediamine dihydrochloride	0.1 g
Phosphoric acid	2.5 g

Adjust volume with deionize water to 100 ml

(Store at 4 ^{o}C)

1.2 MTT solution (5 mg/ml)

3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium

Bromide or Thiazolyl blue tetrazolium bromide	200 mg
PBS	40 ml

(Wrapped foil and stored at 4 ^{o}C)

1.3 0.04 M HCl in isopropanol

Hydrochloric acid	0.83 ml

Adjust volume with isopropanol to 250 ml

(Stored at room temperature)

2. Reagents for determination TNF-α inhibitory effect

2.1 Wash buffer solution

Wash buffer	25 ml
Distilled water	600 ml

(Store at 4 ^{o}C)

2.2 Substrate solution

Color reagent A and B should be mixed together in equal volumes

(Freshly prepared, wrapped foil)

3. Reagents for determination COX-2 inhibitory effect

3.1 EIA buffer solution

Diluted the content of EIA buffer concentrate (10x) with 90 ml of ultra pure water (Store at 4 ^{o}C)

3.2 Wash buffer solution

Wash buffer concentrate (400x)	5 ml
Ultra pure water	2 L
Tween 20	1 ml

(Store at 4 ^{o}C)

3.3 Prostaglandin E_2 AChE Tracer solution

Reconstituted PGE_2 AChE Tracer 100dtn with 6 ml EIA buffer (Store at 4 ^{o}C)

3.4 Prostaglandin E_2 monoclonal antibody solution

Reconstituted PGE_2 monoclonal antibody 100 dtn with 6 ml EIA buffer (Store at 4 ^{o}C)

3.5 Ellman's Reagent

Reconstituted Ellman's Reagent 100 dtn with 20 ml of ultra pure water (prepared immediately before use)

4. Reagent for determination cytotoxic activity

4.1 sulforhodamine B solution (0.4%)

Sulforhodamine B dye	0.4 g
1% Acetic acid	100 ml

4.2 Trichloroacetic acid (40%)

Trichloroacetic acid	40 g
Distilled water	100 ml

4.3 Acetic acid (1%)

Acetic acid	10 ml
Water	1000 ml

4.4 Trisma base (10 mM)

Trisma base	0.1211 g

Adjust volume with Distilled water to 100 ml

4. Reagent for cell culture

4.1 RPMI 1640 (incomplete media)

RPMI 1640 1X with L-glutamine	1 pack
$NaHCO_3$	2.0 g

Adjust volume with sterile water to 1,000 ml
Adjust pH 7.00-7.20 by 1N NaOH or 1 N HCl
Filtered sterile at a pore size of 0.2 μM
(Store at 4 ^{o}C)

4.2 RPMI 1640 (complete media)

RPMI 1640 (incomplete media)	1,000 ml
FBS	100 ml
Penicillin-Streptomycin	10 ml

4.3 DMEM (incomplete media)

Dulbecco's Modified Eagle Medium	1 pack
$NaHCO_3$	3.7 g

Adjust volume with sterile water to 1,000 ml
Adjust pH 7.00-7.20 by 1N NaOH or 1 N HCl
Filtered sterile at a pore size of 0.2 μM
(Store at 4 ^{o}C)

4.4 DMEM (complete media)

DMEM (incomplete media)	1,000 ml
FBS	100 ml
Penicillin-Streptomycin	10 ml

4.5 PBS

PBS	1 tablet
Ultra pure water	100 ml

Autoclave 121 ^{o}C, 15 mins
(Store at 4 ^{o}C)

4.6 Penicillin-Streptomycin

Slowly thaw the frozen P/S, 37 ^{o}C, 60 mins till complete thaw
(Aliquot, Store at -20 ^{o}C)

4.7 FBS

Slowly thaw the frozen FBS (inactivated), heat 56 ^{o}C, 60 mins)

(Aliquot, Store at -20 ^{o}C)

4.8 Trypsin-EDTA

Slowly thaw the frozen 0.5%trypsin-EDTA, 37 ^{o}C, 60 mins till complete thaw

(Aliquot, Store at -20 ^{o}C)

Appendix C

Standard Curves

To determine the best condition for inducing NO production by murine macrophages cell line (RAW 264.7), we treated them with RPMI 1640 complete media with various concentrations of lipopolysaccharide (LPS). Graph of Nitrite production versus LPS concentration was shown in Figure C-1. From the result, Lipopolysaccharide (LPS) stimulated the highest NO production by RAW 264.7 cells at concentration of 5 μg/ml. Therefore, the 5 μg/ml LPS was used to induce NO production by RAW 264.7 cells in this study.

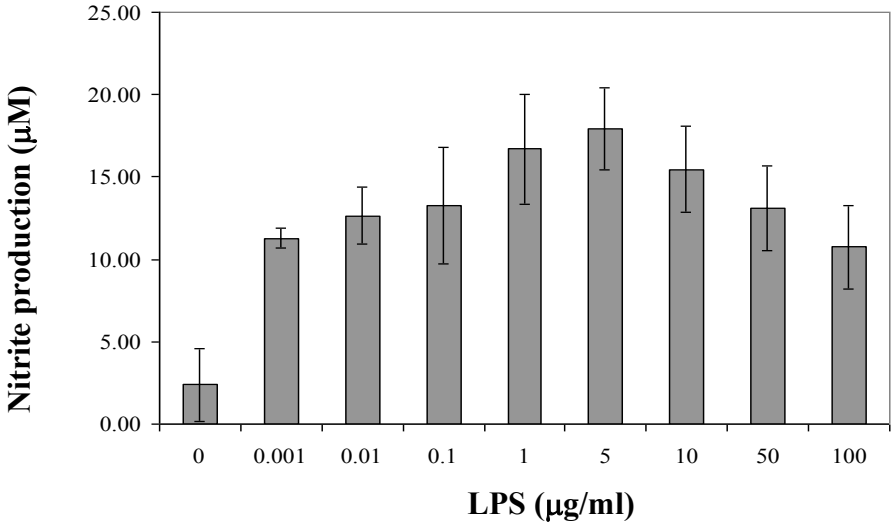

Figure C-1 Concentration of NO production by RAW 264.7 cells stimulated with LPS (0-100 μg/ml) for 48 h, after which the NO released was measured as nitrite using the Griess reagent (n = 2)

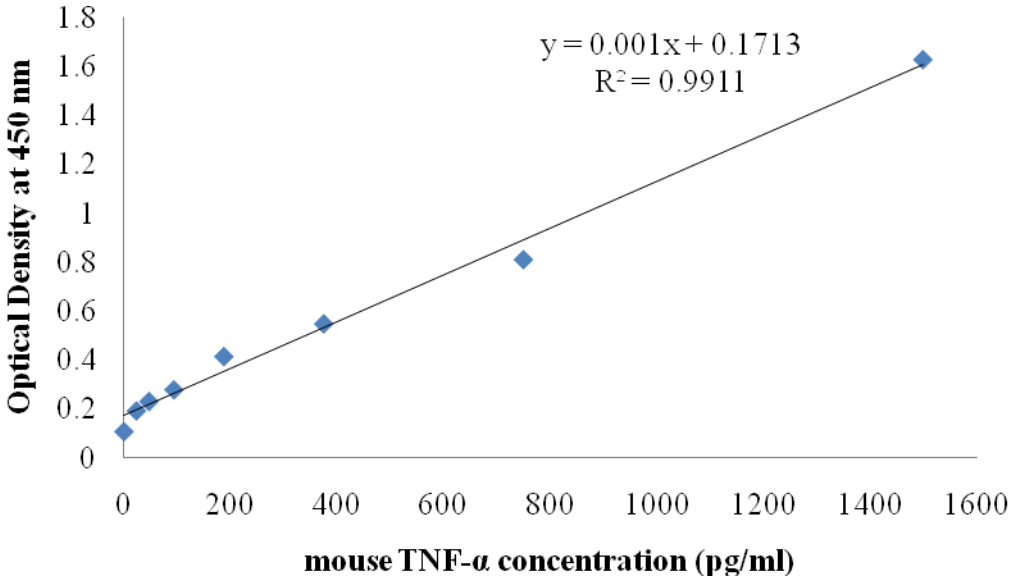

Figure C-2 Standard curve of mouse TNF-α concentrations (pg/ml)

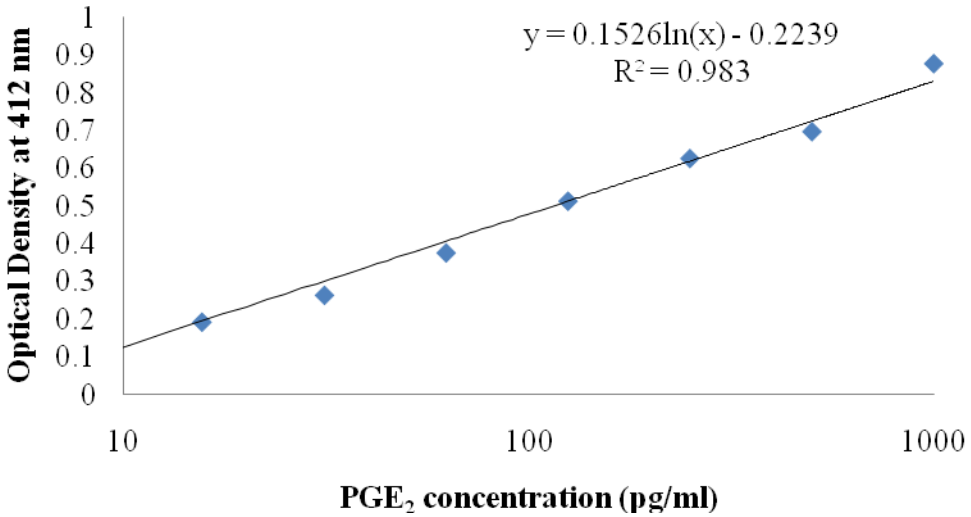

Figure C-3 Standard curve of PGE_2 concentrations (pg/ml)

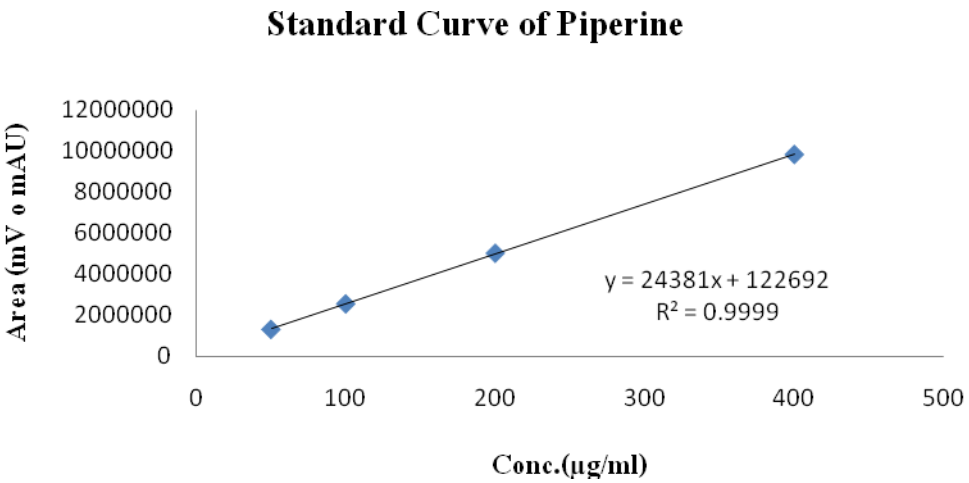

Figure C-4 Standard curve of piperine with various concentrations.