Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

IJBMS

Anti-inflammatory effect of longan seed extract in carrageenan stimulated Sprague-Dawley rats

Ching-Hsiao Lee [1], Yuh-Shuen Chen [2], Chien-Wei Hou [3], Kee-Ching Jeng [4], Kuo-Shu Chen [5]*

[1] Department of Medical Technology, Jen-Teh Junior College of Medicine, Nursing and Management, Miaoli, Taiwan
[2] Department of Food Nutrition, Hungkuang University, Taichung, Taiwan
[3] Department of Biotechnology and Pharmaceutical Technology, Yuanpei University, Hsinchu City, Taiwan
[4] Department of Medical Research, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan
[5] Division of Allergy, Immunology and Rheumatology, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan

ARTICLE INFO

Article type:
Original article

Article history:
Received: Dec 9, 2015
Accepted: Apr 28, 2016

Keywords:
Anti-inflammatory
Longan seed extract
Lipopolysaccharide
MAPKs
λ-carrageenan

ABSTRACT

Objective(s): Longan seeds have been used as a folk medicine in China. Longan seed extract (LSE) is known for antioxidative, antiproliferative, hypoglycemic, and hypouremic effects. However, its anti-inflammatory effect has not been shown.
Materials and Methods: In this study, Sprague-Dawley (SD) rats were given LSE orally (vehicle, 10, and 30 mg/kg) for 3 days to its test anti-inflammatory effect by injecting λ-carrageenan (CARR) in the right hind paw or lipopolysaccharide (LPS), IP. For the positive control, animals were given aspirin (20 mg/kg) orally and treated likewise. Serum or tissue samples from treated rats were collected after 3 hr of stimulation. Regarding the in vitro study, BV2 microglial cells were stimulated with LPS in the presence of LSE or normal saline for 10 min or 24 hr for Western blot and ELISA assay, respectively.
Results: LSE reduced CARR-induced edema in the experimental animals. LSE also reduced LPS/CARR-induced nitric oxide (NO), interleukin-1β (IL1β), IL6, and COX2 productions. These inflammatory factors were also reduced dose dependently by LSE in LPS-stimulated BV2 cells. Furthermore, Western blot analysis revealed that LSE inhibited LPS activated c-Jun NH2-terminal protein kinase (JNK), extracellular signal-regulated kinases (ERKs), and p38 MAP kinases signaling pathways, caspase-3, inducible NO synthase, and COX2 expressions.
Conclusion: LSE pretreatment suppressed CARR- and LPS-induced inflammations and these effects might be through the inhibition of MAP kinases signaling pathways and inflammatory factors.

► *Please cite this article as:*
Lee Ch, Chen Y, Hou Ch, Jeng K, Chen K. Anti-inflammatory effect of longan seed extract in carrageenan stimulated Sprague-Dawley rats. Iran J Basic Med Sci 2016; 19:870-874.

Indroduction

Longan (*Dimocarpus longan* Lour.) from the Sapindaceae family is widely cultivated in Southern China, India, and Southeast Asia (1), and this fruit is very popular in the summer. Longan seeds have long been used as a folk medicine in China for treatment of acariasis, hernia, wound hemorrhages, eczema, and scrofula (2). It also has anticancer, hypoglycemic, and anti-uremic effects (3-6). Longan seeds have been found to be a rich source of antioxidant phenolic compounds, such as gallic acid, corilagin, and ellagic acid (7).

Gallic acid has a strong antioxidant effect, and ellagic acid has cytotoxic effect on cancer cells, but not normal human lung fibroblast cells (8, 9). We previously showed the hyporuricemic effect of longan seed extract (LSE) on animal model, and the inhibitory effect of gallic acid from a plant extract on lipopolysaccharide (LPS)-induced inflammation by suppression of c-Jun N-terminal kinases (JNK) signaling pathways (6, 10). The anti-inflammatory effect of longan seeds has not been reported, so, we investigated its effect on the λ-carrageenan (CARR)- and LPS-induced inflammation in animal models. Since mitogen activated protein kinases (MAPK) signaling pathways are involved with inflammation (11), this mechanism was further studied in LSE-treated cells.

Materials and Methods

Materials

Longan seed extract (LSE) was purchased from Joben Bio-Medical Co (Kaohsiung, Taiwan) with oxygen radical absorbance capacity (ORAC) units of 1300±40 μmol Trolox equivalents per gram. LPS and aspirin were purchased from Sigma-Aldrich (St. Louis, MO). Cytokine, COX_2, and prostaglandin E_2 (PGE_2) ELISA assay kits were obtained from (R&D, Minneapolis, MN, USA). Anti-phospho-p38, ERK, JNK, COX_2, iNOS, and β-actin antibodies were purchased from Abcam (Cambridge, UK). Fetal bovine serum (FBS) was obtained from Gibco Invitrogen (Grand

**Corresponding author:* Kuo-Shu Chen. Division of Allergy, Immunology and Rheumatology, Tungs' Taichung MetroHarbor Hospital, Taichung, Taiwan. Tel: +886-4-26581919, Fax: +886-4-26567684; email: t5439@ms.sltung.com.tw

Island, NY, USA). Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO (Grand Island, NY, USA).

HPLC analysis of LSE

Analysis of gallic acid, corilagin, and ellagic acid was carried out by the modified method (12), using the HPLC technique. The chromatographic conditions used in this study were as follows. A column (Atlantis®T3 5 μm 4.6×10 mm, Waters) with a pre-string column (Cosmosil 5C18-AR-II 4.6×10 mm, Nacalai Tesque) was equipped with a separation model (Agilent 1100). The sample was eluted with a flow rate of 1.0 ml/min and maintained at 25 °C, and the detection wavelength was UV 270 nm. The retention time of samples was as following: gallic acid, 14.4 min; corilagin, 43.3 min; and ellagic acid, 63.5 min.

Animal experiments

Male Sprague-Dawley (SD) rats, aged 6 months (400±20 g), were purchased from National Laboratory Animal Center (Taipei, Taiwan). The study protocol was approved by the Institutional Animal Care and Use Committee, Hungkuang University. Animals were kept in the housing facilities, at least three days to adapt to the environment before the experiment, and were maintained at 25±2 °C, a 12 hr light/dark cycle with food and water provided *ad libitum*. Each experiment consisted of five groups of animals. The positive control group was given aspirin (20 mg/kg) orally. The other three groups were given LSE (dissolved in distilled water) orally (vehicle, 10, and 30 mg/kg), for three days before testing its anti-inflammatory effect by IP injection of LPS or 100 µl of 1% (w/v) CARR in the paw of right hind leg. Animals were anesthetized by chloral hydrate (400 mg/kg, IP) and sacrificed after CARR/LPS stimulation for 3 hr. Serum or tissue samples were collected and stored at -70 °C until assay.

Measurement of carrageenan-induced edema

CARR-induced edema was determined by a Digital Water Plethysmometer (EB Instruments, Florida, USA). The difference of the paw volume was calculated before and 3 hr after the CARR injection (13).

Cell culture

The murine microglial cell line BV_2 was maintained in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 g/ml streptomycin at 37 °C in a humidified incubator under 5% CO_2. Confluent cultures were passed by trypsinization. For *in intro* study, BV_2 microglial cells were stimulated with LPS in the presence of LSE or normal saline for 10 min or 24 hr.

Western blot assay

For preparation of the cell extracts, cells were washed twice with ice-cold phosphate buffered saline (PBS) after removal of the test medium, scraped off with a rubber policeman, and centrifuged at 200×*g* for 10 min at 4 °C. The cell pellets were re-suspended in an appropriate volume (approx. 4×10^7 cells/ml) of lysis buffer (20 mM Tris–HCl, pH 7.5, 137 mM NaCl, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 5 μg/ml pepstain A), and sonicated. Protein concentration of samples was determined by Bradford assay (Bio-Rad, Hemel, Hempstead, UK) and samples were equilibrated to 2 μg/ml with lysis buffer. For Western blotting, protein samples were separated on 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to immobilon polyvinylidene difluoride membranes (Millipore, Bedford, USA). The membranes were incubated for 1 hr with 5% dry skim milk in TBST buffer (0.1 M Tris–HCl, pH 7.4, 0.9% NaCl, and 0.1% Tween-20) to block non-specific binding. Then, they were incubated with mouse anti-β-actin and anti-phospho MAPKs antibodies. Subsequently, the membranes were incubated with secondary antibody streptavidin-horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Jackson, West Grove, PA, USA). β-actin, phosphorylated MAPK proteins, COX_2, and iNOS were detected by a chemiluminescence detection system according to the manufacturer's instructions (ECL, Amersham, Berkshire, UK). The band intensity was quantified with a densitometric scanner (PDI, Huntington Station, NY, USA).

Elisa assay

Cytokines, interleukin-1β (IL_1 β), IL_6, COX_2, and PGE_2 were measured by ELISA kits (R&D, Minneapolis, MN, USA). Nitric oxide (NO) was determined by Griess reagent assay (11). The absorbance at 450 nm was determined using a microplate reader (spectraMAX 340, Molecular Devices, Sunnyvale, CA, USA).

Statistical analysis

All data were expressed as the mean±SE. Data were analyzed by one-way ANOVA and Scheffe's multiple range test *Post hoc*. *P*-value less than 0.05 was considered significantly different.

Results

Rat pretreated with LSE (vehicle, 10, and 30 mg/kg) or aspirin had a reduced CARR-induced paw edema as compared with the vehicle control ($P<0.05$; Figure 1).

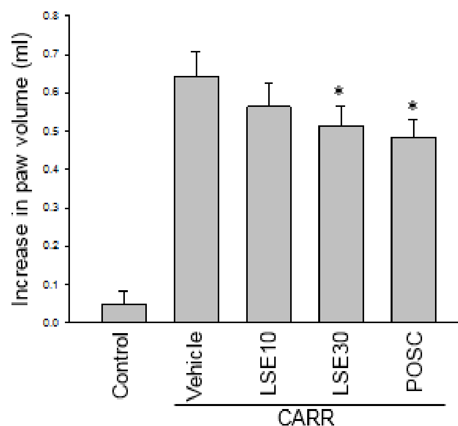


Figure 1. Effect of LSE on carrageenan-induced paw edema in SD rats. Animals were pretreated with LSE (vehicle, 10, and 30 mg/kg, orally) for 3 days, as described in the methods, or oral aspirin (20 mg/kg) 30 min prior to CARR (1%) injection, and the rats were evaluated for paw edema, 3 hr after 1% injection. Each group contained 6 rats and the aspirin group served as the positive control (POSC). The results are expressed as mean±SEM. *, *P*<0.05 versus the vehicle control group from three separate experiments

Similarly, LSE reduced CARR-induced NO, $IL_1\beta$, IL_6, COX_2, and PGE_2 production in paw tissue, dose dependently (Figures 2 and 3). However, serum $IL_1\beta$, IL_6, and PGE_2 productions from LPS treated animals were modestly reduced by LSE (Figure 4). Consistently, LSE could reduce NO, $IL_1\beta$, IL_6, and PGE_2 productions in LPS stimulated BV_2 cells significantly (Figure 5). The anti-inflammatory mechanism of LSE on LPS-induced signaling pathways and inflammatory factors was further examined by Western blot assay (Figure 6). The

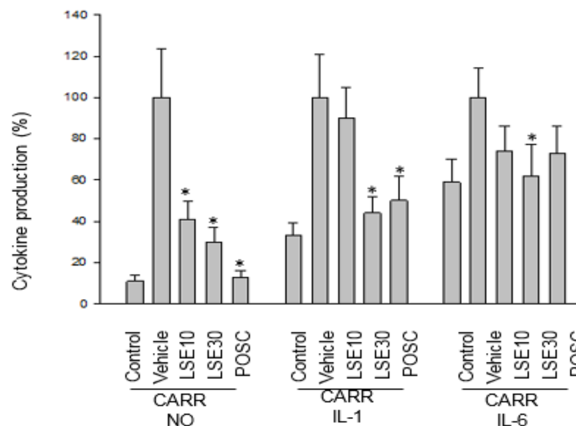


Figure 2. Effect of LSE on nitric oxide (NO), interleukin-1β (IL1β), and IL6 production of the carrageenan-injected paw tissue. Animals were pretreated with LSE or aspirin and treated with λ-carrageenan, as above. The concentration of each CARR-stimulated cytokine was 110±9, 33±1, and 740±25 pg/ml, respectively. *, *P*<0.05 as compared with the CARR group

result showed that LSE (10 μM) reduced LPS-induced protein expressions as following: JNK (80±6%), ERK (80±7%), p38 MAPKs (75±10%), COX_2 (48±7%), iNOS (99±1%), and caspase3 (69±6%), respectively to the LPS only (*P*< 0.05; Figure 6)

Discussion

In the present study, anti-inflammatory effect of LSE was tested in animal models. The results showed that LSE pretreatment reduced CARR-induced edema and inflammatory factors in the paw tissue, dose dependently. But LPS induced serum level of $IL_1\beta$, IL_6,

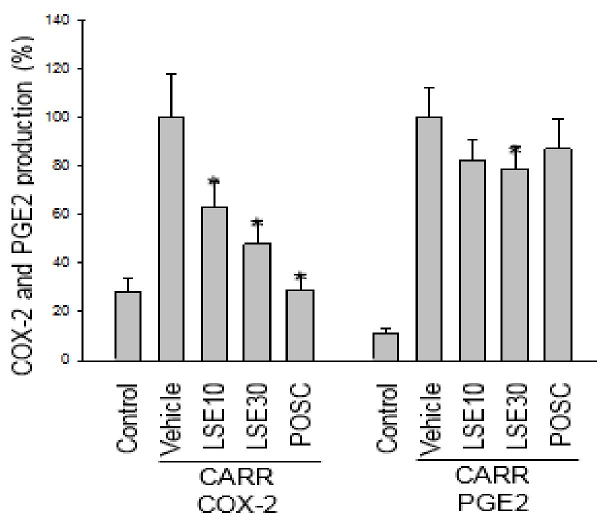


Figure 3. Effect of LSE on cyclooxygenase-2 (COX_2) and prostaglandin E_2 (PGE_2) production in the carrageenan-induced inflammatory paw tissue. The values for CARR stimulated COX_2 and PGE_2 were 460±90 pg/ml and 1.7±0.1 ng/ml, respectively. *, *P* < 0.05 as compared with the CARR group

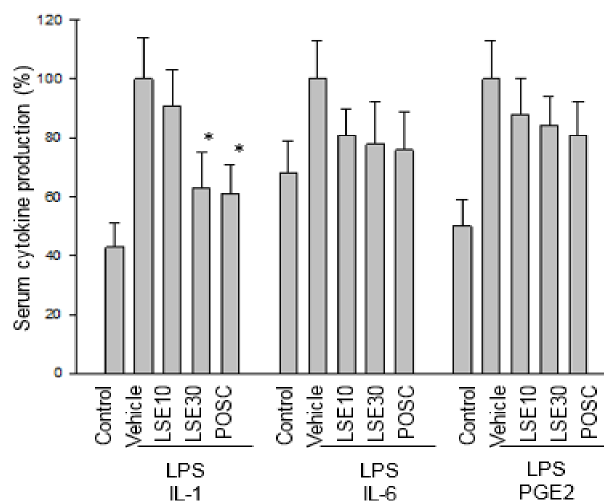


Figure 4. Effect of LSE on IL1⍰, IL6, and PGE2 serum concentrations of LPS-injected animals. The value for each factor was 11.6±2.1 ng/ml, 155±10 ng/m, and 750±20 pg/ml, respectively. *, *P*<0.05, as compared with the LPS only group from three separate experiments

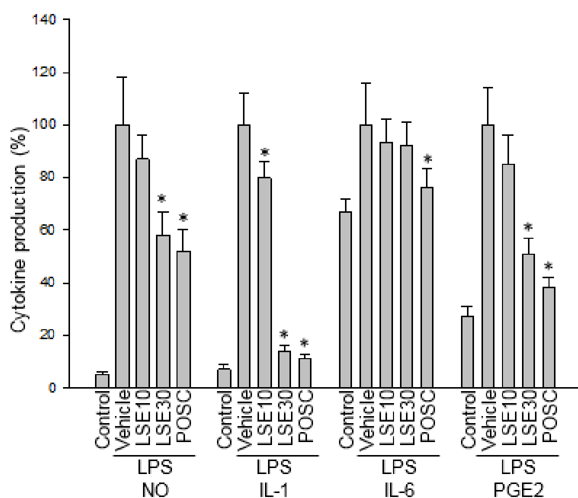
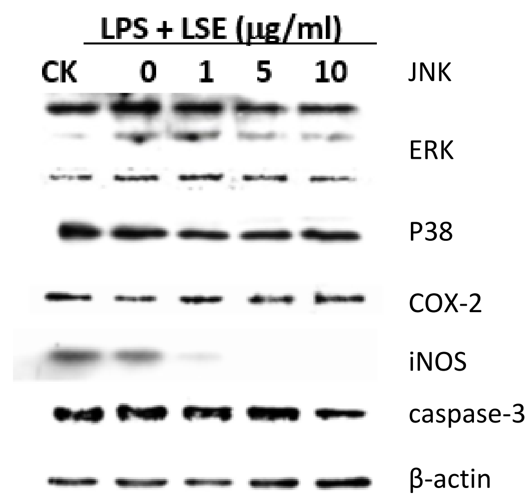


Figure 5. Effect of LSE on LPS induced NO, IL1β, IL6, and PGE2 productions from BV2 cells. The LPS induced inflammatory factors levels were 250±55 pg/ml, 700±20 pg/ml, 360±10 ng/ml, 750±20 pg/ml, and 55±8 pg/ml, respectively. * $P<0.05$ as compared with the LPS group from three separate experiments



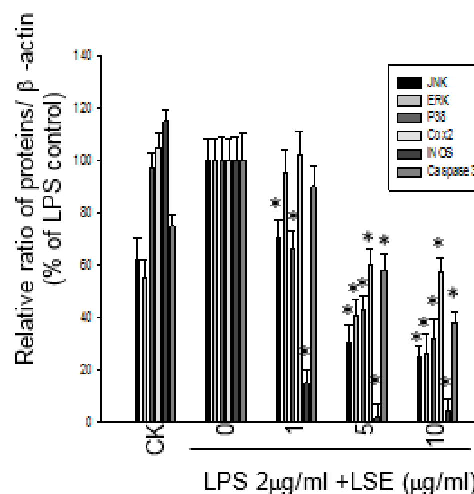


Figure 6. Western blot analysis of LSE effect on LPS induced signaling pathways and inflammatory factors. BV_2 cells were stimulated with LPS in the presence of LSE or normal saline, for 10 min. The extracted protein samples from cells were subjected to SDS-PAGE and Western blotting, as described in Methods. Protein expression of JNK, extracellular signal-regulated kinases (ERKs), p38 MAPK signaling pathways, caspase-3, COX_2, and inducible NO synthase (iNOS) was determined by a chemiluminescence method. *, $P < 0.05$ as compared with the LPS group

and PGE_2 was modestly reduced by LSE pretreated and inflammatory factors in the paw tissue, dose dependently. But LPS induced serum level of $IL_1β$, IL_6, and PGE_2 was modestly reduced by LSE pretreated animals. The anti-inflammatory mechanism of LSE might be related to the inhibition of LPS induced MAPKs, COX_2, iNOS, and caspase-3 expressions. It has been reported that LPS induced JNK, ERK, and p38 MAPK signal pathways mediate inflammatory responses in various cell types, and transcription factors positively regulate the inflammatory genes (11, 14, 15). Inhibition of MAPK signaling pathways is expected to be beneficial in inflammation, and specific inhibitors of p38 MAPK have been proven to reduce inflammation (16). The major components of LSE were gallic acid (42.4 μg/ml), corilagin (52.7 μg/ml), and ellagic acid (22.4 μg/ml), shown by HPLC assay. These compounds are known for their anti-inflammatory effects (10, 17).

LSE significantly reduced NO, $IL_1β$, IL_6, and COX_2 productions in the CARR-stimulated paw tissue and serum of LPS stimulated animals. Furthermore, LSE was able to suppress LPS induced JNK, ERK, and p38 MAPK signal pathways. p38 MAPK is thought to mediate inflammatory responses in various animal models and cell types, possibly through transcription factors to activate inflammatory genes (18). In CARR- or LPS-induced rat arthritis model, amygdalin reduces TNF-α and $IL_1β$ mRNA expressions (19) and this anti-inflammatory mechanism is mediated via the JNK_1-dependent pathway suppression (20). Another plant extract of *Wercklea insignis* also inhibits CARR- and LPS-stimulated inflammation and NO, PGE_2, IL_6, $IL_1β$, and TNFα productions in RAW 264.7 cells (21). This effect is closely associated with suppression of ERK, JNK, p38 MAPK, and NF-κB. We also found that LSE inhibited iNOS and NO, in consistent with reports on extracts from longan flower that could suppress the excitotoxicity or LPS-induced NO production (22, 23). Importantly, the present data showed that LSE inhibited the activation of p38, JNK, and ERK MAPK signaling pathways, and the mechanism of LSE anti-inflammatory effect might be through the inhibition of MAP kinases signaling pathways and other inflammatory factors.

Conclusion

The present result indicated that LSE could reduce LPS/CARR-induced inflammatory cytokine production in the experimental animals. Anti-inflammatory mechanism of LSE was related to the

inhibition of LPS-activated JNK, ERK, and p38 MAPK signaling pathways and caspase-3 and COX2 expressions. These mechanisms might be important due to their medicinal effects.

Acknowledgment

The results described in this paper were part of student thesis.

References

1. Jiang Y, Zhang Z, Joyce DC, Ketsa S. Postharvest biology and handling of longan fruit (*Dimocarpus longan* Lour.). *Postharvest* Biol Technol 2002; 26:241-252.
2. Jiangsu New College of Medicine. *Chinese Materia Medica Dictionary.* Shanghai, China: Shanghai People's Press; 1977. p. 637.
3. Lin CC, Chung YC, Hsu CP. Potential roles of longan flower and seed extracts for anti-cancer. World J Exp Med 2012; 2:78–85.
4. Wang H, Zhang X, Li Y, Chen R, Ouyang S, Sun P*, et al*. Antitumor activity of a polysaccharide from longan seed on lung cancer cell line A549 *in vitro* and *in vivo*. Tumour Biol 2014; 35:7259–7266.
5. Huang RQ, Zou XY, Liu XM. Study on the hypoglycemic effect of longan seed extract. Nat Prod Res Dev 2006; *18*:991–992.
6. Hou CW, Lee YC, Hung HF, Fu HW, Jeng KC. Longan seed extract reduces hyperuricemia via modulating urate transporters and suppressing xanthine oxidase activity. Am J Chin Med 2012; 40:979–991.
7. Rangkadilok N, Sitthimonchai S, Worsuttayangkurn L, Mahidol C, Ruchirawat M, Satayavivad J. Evaluation of free radical scavenging and antityrosinase activities of standardized longan fruit extract. Food ChemToxicol 2007; 45:328–336.
8. Jadon A, Bhadauria M, Shukla S. Protective effect of *Terminalia belerica* Roxb. and gallic acid against carbon tetrachloride induced damage in albino rats. J Ethnopharmacol 2007; 109:214–218.
9. Losso JN, Bansode RR, Trappey A 2nd, Bawadi HA, Truax R. *In vitro* anti-proliferative activities of ellagic acid. J Nutr Biochem 2004; 15:672–678.
10. Lin WH, Siao AC, Kuo HH, Ho LH, Jeng KC, Hou CW. *Gardenia jasminoides* extracts and gallic acid inhibit lipopolysaccharide-induced inflammation by suppression of JNK2/1 signaling pathways in BV-2 cells. Iran J Basic Med Sci 2015; 18:555–562.
11. Jeng KC, Hou RC, Wang JC, Ping LI. Sesamin inhibits lipopolysaccharide- induced cytokine production by suppression of p38 mitogen-activated protein kinase and nuclear factor-kappaB. Immunol Lett 2005; 97:101–106.
12. Rangkadilok N, Worasuttayangkurn L, Bennett RN, Satayavivad J. Identification and quantification of polyphenolic compounds in Longan (*Euphoria longana* Lam.) fruit. J Agric Food Chem 2005; 53:1387-1392.
13. Hajhashemi V, Minaiyan M, Banafshe HR, Mesdaghinia A, Abed A. The anti-inflammatory effects of venlafaxine in the rat model of carrageenan-induced paw edema. Iran J Basic Med Sci 2015; 18:654–658.
14. Bhat NR, Zhang P, Lee JC, Hogan EL. Extracellular signal-regulated kinase and p38 subgroups of mitogen-activated protein kinase regulate inducible nitric oxide synthase and tumor necrosis factor-gene expression in endotoxin-stimulated primary glial cultures. J Neurosci 1998; 18:1633–1641.
15. Woo CH, Lim JH, Kim JH. Lipopolysaccharide induces matrix metalloproteinase-9 expression via a mitochondrial reactive oxygen species-p38 kinase-activator protein-1 pathway in Raw 264.7 cells. J Immunol 2004; 173:6973–6980.
16. Branger J, van den Blink B, Weijer S, Madwed J, Bos CL, Gupta A, *et al*. Anti-inflammatory effects of a p38 mitogen-activated protein kinase inhibitor during human endotoxemia. J Immunol 2002; 168:4070–4077.
17. Jin F, Cheng D, Tao JY, Zhang SL, Pang R, Guo YJ*, et al*. Anti-inflammatory and anti-oxidative effects of corilagin in a rat model of acute cholestasis. BMC Gastroenterol 2013; 13:79.
18. Kyriakis JM, Avruch J. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev 2001; 81:807–869.
19. Hwang HJ, Lee HJ, Kim CJ, Shim I, Hahm DH. Inhibitory effect of amygdalin on lipopolysaccharide-inducible TNF-α and IL-1β mRNA expression and carrageenan-induced rat arthritis. J Microbiol Biotechnol 2008; 18:1641–1647.
20. Kim MJ, Rangasamy S, Shim Y, Song JM. Cell lysis-free quantum dot multicolor cellular imaging-based mechanism study for TNF-α-induced insulin resistance. J Nanobiotechnol 2015; 13:4.
21. Park JW, Kwon OK, Jang HY, Jeong H, Oh SR, Lee HK, *et al*. A leaf methanolic extract of *Wercklea insignis* attenuates the lipopolysaccharide-induced inflammatory response by blocking the NF-κB signaling pathway in RAW 264.7 macrophages. Inflammation 2012; 35:321–331.
22. Ho SC, Hwang LS, Shen YJ, Lin CC. Suppressive effect of a proanthocyanidin-rich extract from longan (*Dimocarpus longan* Lour.) flowers on nitric oxide production in LPS-stimulated macrophage cells. J Agric Food Chem 2007; 55:10664–10670.
23. Lin AM, Wu LY, Hung KC, Huang HJ, Lei YP, Lu WC, *et al*. Neuroprotective effects of longan (*Dimocarpus longan* Lour.) flower water extract on MPP+-induced neurotoxicity in rat brain. J Agric Food Chem 2012; 60:9188–9194.