ORIGINAL ARTICLE

Quercetin prevent proteoglycan destruction by inhibits matrix metalloproteinase-9, matrix metalloproteinase-13, a disintegrin and metalloproteinase with thrombospondin motifs-5 expressions on osteoarthritis model rats

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ABSTRACT

Prior study has shown that *Ageratum conyzoides* L. extract that containing quercetin has been proved to prevent inflammation and proteoglycan degradation by inhibiting tumor necrosis factor-alpha and matrix metalloproteinase (MMP-9) expression. Target of osteoarthritis (OA) treatment was in the synovial joint that requiring a drug delivery system. The aim of this study was to prove the efficacy of quercetin-loaded lecithin-chitosan nanoparticles on the OA model rats by observed its effect on interleukin (IL-1) β, MMP-9, MMP-13, and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS-5) expressions. In this study, 70 white male Sprague Dawley rats were divided into 14 groups, 7 groups each for destabilization of medial meniscus (DMM) and monoiodoacetate (MIA)-induced OA. After 28 days from induction, SHAM and negative group received gel base topically; positive group received sodium diclofenac gel; three-dose group received each 0.84, 1.68, 3.36 mg/g quercetin-loaded nanoparticles gel; and *A. conyzoides* L. group received *A. conyzoides* L. extract gel. Each group gets treatment until day 70, and then, blood sample was collected for serum analysis; knee joint was isolated and subjected to histology samples treatment. Quercetin-loaded nanoparticle gel dose 1 (0.84 mg/g gel), dose 2 (1.68 mg/g gel), dose 3 (3.36 mg/g), and *A. conyzoides* L. extract gel could decreased the level of IL-1 β, MMP-9, MMP-13, ADAMTS-5, and increasing color intensity significantly on histopathological observations on DMM and MIA-induced OA.

Key words: A disintegrin and metalloproteinase with thrombospondin motifs-5, matrix metalloproteinase-13, matrix metalloproteinase-9, osteoarthritis, quercetin-loaded nanoparticle

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INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease[1] and the most common form of arthritis.[2] Several studies have been reported that there were increased levels of some proinflammatory mediators in the serum of OA patients. Interleukin-1 (IL-1) β was considered as the major agents.[3]

IL-1 β level is increased by activation of nuclear factor kappa-B (NFκB) in chondrocyte cells that triggered by some risk factors such as biomechanical injury. Diclofenac sodium is one of the nonsteroidal anti-inflammatory drugs that can inhibit NFκB activation so that the level of proinflammatory cytokines also will be reduced.

Degradation of cartilage is one of the OA pathogeneses. The inadequacy of anabolic factors by association with an elevated influence of catabolic factors leading to an overexpression of proteases that degrading the matrix. Matrix metalloproteinase (MMP-13) is a primary candidate for degradation collagen type II fibril when a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS-5) is the main aggrecanases that responsible for the proteoglycan breakdown.[1] MMP-9 has been shown to degrade extracellular matrix, initiate, and promote new vessel formation.

Prior research has shown that *Ageratum conyzoides* L. extract that containing quercetin had been proved to prevent inflammation and proteoglycan degradation by inhibiting tumor necrosis factor-alpha (TNF-α) and MMP-9 expression.[4] Quercetin is a natural flavonoid that has been proved to have anti-inflammatory activities.[5]

The target of OA treatment is into synovial joint space and requiring drug delivery system that can increase drug retention time within the joint. Therefore, this research carried out drug delivery efforts with nanoparticle gel topically. One example of nanoparticle is a mixture of phospholipids and natural polymers such as chitosan. Tan *et al*.[6] have proven that this vehicle could entrap quercetin in the core of vehicle.

In this study, the curative effect of quercetin-loaded nanoparticle was analyzed on the destabilization of medial meniscus (DMM) and monoiodoacetate (MIA) models of OA by evaluating its effects on reducing proteoglycan degradation.

MATERIALS AND METHODS

Materials

Quercetin for this research was bought from Sigma-Aldrich (Singapore), phospholipon 90G from Lipoid, (GmbH, Germany), chitosan from Sigma-Aldrich (Singapore), D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) from Sigma-Aldrich (Singapore), Fine Test® ELISA kits from Wuhan (China). All other reagents were analytical grade.

Animals

Two–3-month white male Sprague Dawley rats as test animal had been approved to pass the Ethical Review from Faculty of Medicine, University Indonesia. The animals were grouped and housed under standard laboratory conditions and got free access to food and water.

Preparations of nanoparticles

Nanoparticle formula was presented in Table 1. Lecithin was dissolved in 96% ethanol, and then, quercetin was added into that solution. Chitosan was dissolved in 0.1% acetic acid, and TPGS was subsequently dissolved in that solution. 4 mL of lecithin-quercetin solution was dropped into 46 mL of chitosan-TPGS solution (2 mL/min) using a syringe (internal diameter 0.603 mm [20G]) with agitation at 1000 rpm. After 30 min, pH was adjusted to 4.5 with 0.5 M NaOH solution and then filtered.[7]

Characterization of nanoparticles

Entrapment efficiency was performed with direct method. Nanoparticle has been centrifuge at 4500 rpm for 60 min. The precipitate was collected and diluted with methanol to break down the nanoparticle. Quercetin concentration was measured using HPLC at the quercetin maximum wavelength (370 nm). Entrapment efficiency was calculated according to the following equation:

$$\text{Entrapment efficiency} = Qe/Qt \times 100\%$$

Where, Qe was measured quercetin concentration (mg/mL) and Qt was theoretical quercetin concentration (mg/mL).[8]

Particle size distribution, zeta potential, and polydispersity index were analyzed by dynamic light scattering system using Malvern Zetasizer Nano (UK).

Gel preparation

Quercetin nanoparticle has been centrifuge at 4500 rpm for 60 min. Carbopol was dispersed in distilled water and then homogenized. Triethanolamine was added into the dispersion and then homogenized. Methylparaben and butylhydroxytoluene severally dissolved in propylene glycol and added into gel base. The rest of propylene glycol and distilled water was added to gel base and then homogenized. The precipitate of quercetin nanoparticle was added to the gel base and then homogenized as well as *A. conyzoides* extract that added to gel base separately. The gel pH was adjusted with HCl 2M to 5.5 ± 0.1. Formulation of the gel was presented in Table 2.

Animal grouping and treatments

Animal grouping and treatments could be seen in Table 3. Rats were divided into 14 groups, 7 groups each for DMM

and MIA-induced OA. DMM procedure was established except for SHAM group and saline water injection for MIA normal control group. Each rat was weighed and anesthetized with ketamine 120 mg/kg intramuscularly. In DMM procedure, joint capsule incised from medial to patellar tendon then infrapatellar fat pad blunt dissection performed, and medial meniscotibial ligament (MMTL) was transected.[9] The dose of monoiodoacetate solution was 3 mg/0.05 mL in saline water. MIA injection was done intraarticularly. On the 29th day, gel base was topically applied on normal and negative control group, while sodium diclofenac gel was applied on positive control group and quercetin-loaded nanoparticle gels were applied on dose group 1, 2, and 3. *A. conyzoides* extract gel was applied to *A. conyzoides* group.

Table 1: Nanoparticles formula

Materials	Concentration (%)
Quercetin (g/mL)	0.24
Lecithin (g/mL)	0.4
Chitosan (g/mL)	0.02
TPGS (g/mL)	2
Ethanol 96% (mL/mL)	8
Acetic acid 0.1% (mL/mL)	100

TPGS, *D-α-tocopheryl polyethylene glycol 1000 succinate*

Blood sampling

After 42 days of treatments, blood sample was collected and centrifuged at 3000 rpm for 10 min. Keep in −20°C until analyzed. Expression of IL-1 β, MMP-9, MMP-13, and ADAMTS-5 was determined by ELISA.

Histology of knee joint

Rats had been sacrificed and their knee joint was isolated. Histology specimens stained by Safranin O-fast green. ImageJ software was used for color intensity evaluation.

Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by multiple comparison tests. $P < 0.05$ was considered statistically significant.

RESULTS

Serum analysis

Tables 4 and 5 show that all biomarkers in OA rats were

Table 2: Gel formulation

Materials	Concentration (% w/w)			
	Quercetin Gel Dose 1	Quercetin Gel Dose 2	Quercetin Gel Dose 3	*A. conyzoides* Gel
Quercetin nanoparticles concentrated	Equal with 0.84 mg/g quercetin	Equal with 1.68 mg/g quercetin	Equal with 3.36 mg/g quercetin	-
A. conyzoides Extract	-	-	-	160 mg/g
Carbomer	1.50	1.50	1.50	1.50
Propylene glycol	7.50	7.50	7.50	7.50
Methylparaben	0.10	0.10	0.10	0.10
Butyl hydroxy toluene	0.02	0.02	0.02	0.02
Triethanolamine	1.50	1.50	1.50	1.50
Distilled water	ad 100	ad 100	ad 100	ad 100

Tabel 3: Animal treatments

Groups	Number of rats	Treatment	
		Day 1	Day 29-70
SHAM/normal control	5	Surgery without MMTL dissection	Gel base
	5	Saline water injection intraarticular	Gel base
Negative control	5	DMM	Gel base
	5	MIA injection intraarticular	Gel base
Positive control	5	DMM	Sodium diclofenac
	5	MIA injection intraarticular	Sodium diclofenac
Dose 1	5	DMM	0.84 mg/g quercetin-loaded nanoparticles gel
	5	MIA injection intraarticular	0.84 mg/g quercetin-loaded nanoparticles gel
Dose 2	5	DMM	1.68 mg/g quercetin-loaded nanoparticles gel
	5	MIA injection intraarticular	1.68 mg/g quercetin-loaded nanoparticles gel
Dose 3	5	DMM	3.36 mg/g quercetin-loaded nanoparticles gel
	5	MIA injection intraarticular	3.36 mg/g quercetin-loaded nanoparticles gel
A. conyzoides extract gel	5	DMM	160 mg/g *A. conyzoides* extract gel
	5	MIA injection intraarticular	160 mg/g *A. conyzoides* extract gel

higher compared with normal control group, while positive control group showed a lower level of all biomarkers compared with negative control. In DMM groups, doses 2, 3, and *A. conyzoides* extract gel could significantly reduce IL-1 β level compared with negative control ($P < 0.05$). For MMP-9 and ADAMTS-5, all doses showed a lower level compared with negative control ($P < 0.05$). Furthermore, on MMP-13 result just dose 3 that could significantly reduce MMP-13 level compared with negative control ($P < 0.05$). On MIA group, doses 2 and 3 could significantly reduce IL-1 β and MMP-13 level compared with negative control ($P < 0.05$). All doses showed lower MMP-9 and ADAMTS-5 level compared with negative control ($P < 0.05$).

Histology analysis of knee joint

Analysis of knee joint histology showed that normal group on both DMM and MIA-induced OA had smooth surface and regular arrangement of chondrocytes in articular cartilage, while negative control had uneven surfaces and fewer number of chondrocytes. Safranin O-fast green staining showed that DMM and MIA procedure could induce proteoglycan degradation. It could be seen from the faded red compared with SHAM or normal control group as shown in Figures 1 and 2. Application of diclofenac gel showed the proteoglycan repairment because of its color intensity similar to the SHAM or normal control group.

In DMM group, nanoparticle gel dose 1 gave red color that not differs significantly with negative control. Dose 2 and dose 3 showed better result that shown by red color similarity to SHAM group. *A. conyzoides* gel application also showed progress that similar to gel dose 2. In MIA group, doses 1 and 2 showed better red color than negative control, but dose 3 showed the best result that equal with red color intensity on positive control. *A. conyzoides* gel group still showed pale red color.

Color intensity measurements were performed with ImageJ that could be seen in Table 6. Red color intensity in ImageJ was indicated by mean number. If red color intensity was high and then the mean number would be low. All treatments in DMM groups significantly different ($P < 0.05$) with negative control, and dose 3 did not differ significantly with SHAM group ($P > 0.05$). All treatments in MIA groups significantly different ($P < 0.05$) with negative control.

DISCUSSION

Preparation of nanoparticles

One method that commonly used in nanoparticles preparation was ionic gelation method which cross-linking occurs through electrostatic interactions. Chitosan was dissolved in acetic acid solution to obtain cations from chitosan while alcohol solutions containing lecithin were anionic solutions. Lecithin solution was dripped into chitosan solution with constant stirring. Due to a combination of different charge solutions, chitosan undergoes ionic gelation and precipitated to form spherical particles.[10]

Hydrophobic core was formed due to the bond between hydrophobic groups of TPGS and lecithin molecule. In

Table 4: Serum index (pg/mL) on DMM model rat

Groups	Serum Index (pg/mL)			
	IL-1β	MMP-9	MMP-13	ADAMTS-5
SHAM	9.22±3.52#	427.76±94.50#	571.4±94.86#	233.56±50.66#
Negative control	48.03±12.57*	1170.67±147.79*	1145±106.41*	616.78±56.36*
Positive control	23.22±3.17#	755.13±34.70*#	692.6±107.69#	300.17±34.92#
Dose 1	31.97±10.33*	891.31±56.49*#	953±55.63*	439.45±22.80*#
Dose 2	25.16±6.32#	825.84±43.1*#	927±47.04*	418.69±27.80*#
Dose 3	24.09±8.63#	762.11±61.31*#	816.6±29.85*#	358.99±20.38*#
A. conyzoides Gel	26.97±7.99*#	814.49±61.23*#	901±27.40*	447.23±36.88*#

*$P<0.05$ as compared to SHAM group; #$P<0.05$ as compared to negative control group

Table 5: Serum index (pg/mL) on MIA model rat

Groups	Serum Index (pg/mL)			
	IL-1β	MMP-9	MMP-13	ADAMTS-5
Normal control	9.99±2.47#	54.12±14.930#	184.20±32.94#	75.69±14.79#
Negative control	74.97±1.56*	824.97±55.84*	1192.6±70.45*	995.24±18.19*
Positive control	40.15±2.75#	344.83±25.34#	480.20±15.14#	388.84±9.48#
Dose 1	70.28±2.47*	630.29±57.28*#	905±37.16*	530.71±4.93*#
Dose 2	63.28±3.11*#	501.96±59.64*#	755±42.58*#	432.09±18.25*#
Dose 3	56.06±1.09*#	409.43±33.75*#	561.8±20.27*#	392.30±7.86*#
A. conyzoides Gel	74.34±3.77*	700.13±37.13*#	767.8±40.16*#	811.85±16.36*#

*$P<0.05$ as compared to normal control group; #$P<0.05$ as compared to negative control group

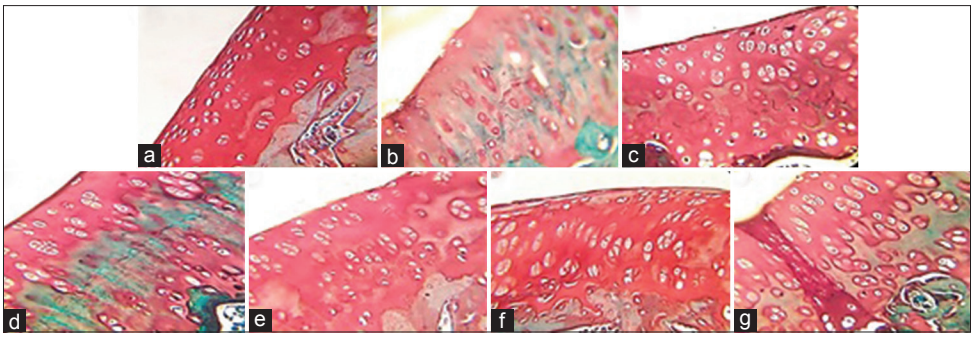

Figure 1: Knee joint histology of destabilization of medial meniscus model rat with Safranin O/fast green staining. (a) SHAM; (b) negative control; (c) positive control; (d) quercetin-loaded nanoparticle gel dose 1; (e) dose 2; (f): dose 3; (g) *Ageratum conyzoides* gel

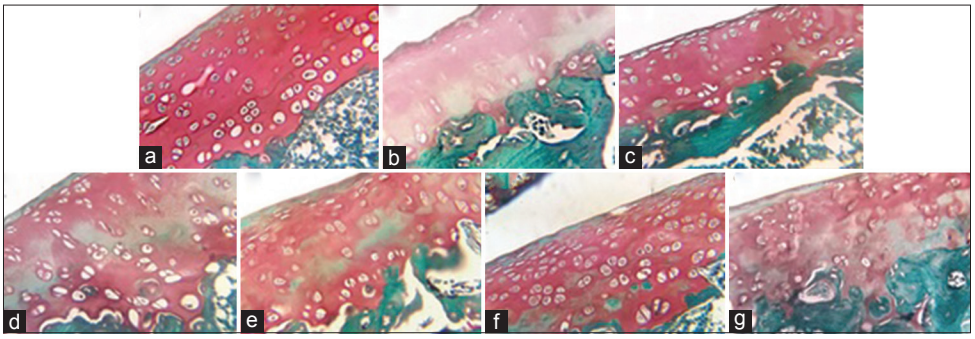

Figure 2: Knee joint histology of monoiodoacetate model rat with Safranin O/fast green staining. (a) SHAM; (b) negative control; (c) positive control; (d) quercetin-loaded nanoparticle gel dose 1; (e) dose 2; (f) dose 3; (g) *Ageratum conyzoides* gel

Table 6: Color intensity average for DMM and MIA groups

Groups	Color intensity average (±SD)	
	DMM	MIA
SHAM	137.10±9.85#	94.996±3.118#
Negative control	188.03±13.09*	164.438±1.371*
Positive control	133.13±14.92#	144.777±1.801*#
Dose 1	165.20±4.11*#	149.836±1.069*#
Dose 2	154.09±17.66*#	147.342±1.230*#
Dose 3	128.63±5.74#	141.442±1.227*#
A. conyzoides Gel	155.55±11.75*#	152.742±0.805*#

*$P<0.05$ as compared to SHAM or normal control group; #$P<0.05$ as compared to negative control group

addition, hydrated shell layer was formed from chitosan together with the hydrophilic polyethylene glycol chain of TPGS and hydrophilic parts of lecithin. Quercetin had lipophilic properties, so it would disperse in hydrophobic core of the system and would be protected by outer shell layer.[6]

Nanoparticle formula that prepared as gel dosage form had entrapment efficiency 41.41% ± 0.33%, particle size 212.2 nm with polydispersity index 0.405, and zeta potential + 26.5 mV.

Induction of osteoarthritis

There were some animal models for OA, one of them was animal models for secondary OA, especially posttraumatic OA, while DMM was invasive/surgically induced models and MIA injection was chemically induced models.

Among animal models, surgically induced methods of OA performed by a combination of joint destabilization, altered contact forces of articular surface, and intraarticular inflammation. In surgically induced models, the most commonly used was knee joint. Greater load, especially in humans and many of animal species, was distributed through medial compartment of knee.[11] DMM method was done by cutting the MMTL. Build upon on earlier studied in guinea pigs, DMM model could induce OA with great ease and reproducibility.[9]

The chemically induced model was done by injecting a toxic or inflammatory compound straight into the joint. Monoiodoacetate worked by inhibits glyceraldehyde-3-phosphate dehydrogenase of the Krebs cycle causing the death of chondrocytes and leading to osteophyte formation and degradation of articular cartilage.[12]

Serum analysis

Inflammation was involved in OA development and progression even in early stages.[13] Increased levels of IL-1 β in negative control group showed that there was an inflammatory process in rats that undergoing DMM procedures and MIA injection. Furthermore, IL-1 β stimulates chondrocytes to released some proteolytic enzymes such

as MMP, so IL-1 β level increase was accompanied by increased levels of matrix-degrading enzymes on negative control group.[3] IL-1 β reduced the synthesis of extracellular matrix major component by inhibiting anabolic activity of chondrocytes, i.e., suppressing the expression of Type II collagen and aggrecan. IL-1 β suppressive effect on proteoglycan synthesis in chondrocytes mediated through suppression of β-1,3-glucuronyltransferase I, a key enzyme in glycosaminoglycan biosynthesis that linked to the core protein of aggrecan.[3]

MMP-13 was a major candidate for collagen Type II degradation. There was also ADAMTS-5, a major aggrecanase that responsible for proteoglycans destruction.[1] Among various MMPs that had a correlation with MMP-13 were the total of MMP-9,[14] and it had been hypothesized that MMP-9 might be embroiled in pro-MMP-13 activation. Particularly, MMP-13 has been considered as a major enzyme that embroiled in cartilage erosion during OA, so MMP-9 might have a role in joint degradation.[15]

ADAMTS enzymes in human family had 19 members that can be divided into some subgroup based on their substrates, one of them was aggrecanases or proteoglycanases. These aggrecanases worked by cleave hyaluronan-binding chondroitin sulfate proteoglycan extracellular proteins, including aggrecan.[16] It has been studied, from *in vitro* studies, that catabolic cytokines such as IL-1 β in human cartilage and chondrocytes could induce ADAMTS-4 but not ADAMTS-5.[17] This finding was diverged from *in vivo* studies which show that ADAMTS-5 expression was affected by catabolic cytokines.[18] Increased MMP-9, MMP-13, and ADAMTS-5 levels in negative control group showed that DMM and MIA procedures could cause cartilage damage successfully.

Quercetin nanoparticle gel and *A. conyzoides* gel were able to decrease IL-1 β level, so matrix degradation enzyme level was also decreased. This was in accordance with histopathologic result, where all the treatment groups able to increase red color intensity that means proteoglycans number was increased. All parameters showed that *A. conyzoides* gel effect was similar with quercetin nanoparticle gel dose 2. This could be due to *A. conyzoides* extract not only contained quercetin compound but also contained other compounds that could synergize to give a good effect on OA. Ethanolic extract of *A. conyzoides* leaves besides containing quercetin also proved to contained kaempferol.[19] Kaempferol also showed activity as an anti-inflammatory[20] so it might work synergistically with quercetin in *A. conyzoides* extract.

In addition, the target of treatment through joints requires a delivery system that was able to extend drug retention time within the joint. In this study, we tried to make formula to solve this problem by nanoparticle gel and applied topically. Nanoparticles could stay in joints for several weeks.[21] To extend OA therapy, not only need drug delivery system but also need new treatment strategy. One of the treatment strategy was developing a Disease Modifying OsteoArthritis Drug (DMOAD). Mechanism action of DMOAD was directly reduced or prevented OA disease progression either by blocking the causes of disease (catabolic activity) or stimulating the repair mechanism (anabolic activity). Most widely investigated DMOAD mechanisms based on their targets were enzyme inhibitors (MMP inhibitor, aggrecanase inhibitor), cytokine inhibitor (IL-1 inhibitor, TNF-α antagonist, iNOS inhibitor), growth factor, and drug targeted subchondral bone.[22] From this study, it could be seen that quercetin nanoparticle gel was included as enzyme and cytokine inhibitors because it was able to decrease IL-1 β, MMP-9, MMP-13, and ADAMTS-5 levels in blood serum of OA animal model.

CONCLUSION

Quercetin-loaded nanoparticle gel exhibits a curative effect on OA by preventing proteoglycan degradation by inhibiting MMP-9, MMP-13, and ADAMTS-5 expressions both on DMM and MIA-induced OA.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Aigner T, Schmitz NM. Pathogenesis and pathology of osteoarthritis. Rheumatology 5th edition. Philadelphia: Mosby Elsevier; 2010. p. 173.
2. Hochberg MC. Osteoarthritis year 2012 in review: Clinical. Osteoarthritis Cartilage 2012;20:1465-9.
3. Kapoor M, Martel-Pelletier J, Lajeunesse D, Pelletier JP, Fahmi H. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. Nat Rev Rheumatol 2011;7:33-42.
4. Bahtiar A, Nurazizah M, Roselina T, Tambunan AP, Arsianti A. Ethanolic extracts of babandotan leaves (*Ageratum conyzoides* L.) prevents inflammation and proteoglycan degradation by inhibiting TNF-α and MMP-9 on osteoarthritis rats induced by monosodium iodoacetate. Asian Pac J Trop Med 2017;10:270-7.
5. Lesjak M, Beara I, Simin N, Pintać D, Majkić T, Bekvalac K, *et al.* Antioxidant and anti-inflammatory activities of quercetin and its derivatives. J Funct Foods 2018;40:68-75.
6. Tan Q, Liu W, Guo C, Zhai G. Preparation and evaluation of quercetin-loaded lecithin-chitosan nanoparticles for topical delivery. Int J Nanomedicine 2011;6:1621-30.
7. Sonvico F, Cagnani A, Rossi A, Motta S, Di Bari MT, Cavatorta F, *et al.* Formation of self-organized nanoparticles by lecithin/chitosan ionic interaction. Int J Pharm 2006;324:67-73.
8. Karliana D. Curative Effect Test of Quercetin Nanoparticle Gel on Rats Joint in The Osteoarthritis Model with Monoiodoacetate Induction. Depok: Universitas Indonesia; 2018.

9. Glasson SS, Blanchet TJ, Morris EA. The surgical destabilization of the medial meniscus (DMM) model of osteoarthritis in the 129/SvEv mouse. Osteoarthritis Cartilage 2007;15:1061-9.
10. Agnihotri SA, Mallikarjuna NN, Aminabhavi TM. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J Control Release 2004;100:5-28.
11. Teeple E, Jay GD, Elsaid KA, Fleming BC. Animal Models of Osteoarthritis: Challenges of Model Selection and Analysis. American Association of Pharmaceutical Scientists; 2013.
12. Marker CL, Pomonis JD. The monosodium iodoacetate model of osteoarthritis pain in the rat. Methods Mol Biol 2012;851:239-48.
13. Felson DT. Clinical practice. Osteoarthritis of the knee. N Engl J Med 2006;354:841-8.
14. Kim KS, Lee YA, Choi HM, Yoo MC, Yang HI. Implication of MMP-9 and urokinase plasminogen activator (uPA) in the activation of pro-matrix metalloproteinase (MMP)-13. Rheumatol Int 2012;32:3069-75.
15. Galasso O, Familiari F, De Gori M, Gasparini G. Recent findings on the role of gelatinases (matrix metalloproteinase-2 and -9) in osteoarthritis. Adv Orthop 2012;2012:834208.
16. Stanton H, Melrose J, Little CB, Fosang AJ. Proteoglycan degradation by the ADAMTS family of proteinases. Biochim Biophys Acta 2011;1812:1616-29.
17. Verma P, Dalal K. ADAMTS-4 and ADAMTS-5: Key enzymes in osteoarthritis. J Cell Biochem 2011;112:3507-14.
18. Fosang AJ, Rogerson FM, East CJ, Stanton H. ADAMTS-5: The story so far. Eur Cell Mater 2008;15:11-26.
19. Tambunan AP, Bahtiar A, Tjandrawinata RR. Influence of extraction parameters on the yield, phytochemical, TLC-densitometric quantification of quercetin, and LC-MS profile, and how to standardize different batches for long term from *Ageratum conyoides* L. leaves. Pharmacogn J 2017;9:767-74.
20. Guo P, Feng YY. Anti-inflammatory effects of kaempferol, myricetin, fisetin and ibuprofen in neonatal rats. Trop J Pharm Res 2017;16:1819-26.
21. Schubert MA, Harms M, Müller-Goymann CC. Structural investigations on lipid nanoparticles containing high amounts of lecithin. Eur J Pharm Sci 2006;27:226-36.
22. Janssen M, Mihov G, Welting T, Thies J, Emans P. Drugs and polymers for delivery systems in OA joints: Clinical needs and opportunities. Polymers 2014;6:799-819.