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RESEARCH ARTICLE

Anti-inflammatory Activity of Nanoemulgel formulated from *Ageratum conyzoides* (L.) L. and *Oldenlandia corymbosa* L. Extracts in Rats

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Abstract

The use of traditional medicine in the treatment of disease has increased globally due to its safety and efficacy. *Ageratum conyzoides* (L.) L. and *Oldenlandia corymbosa* L. have been used traditionally as a topical preparation for joint disease in Indonesia. Hence, the study was planned to scientifically study the existing empirical data. A nanoemulgel of combined *Ageratum conyzoides* (L.) L. (ACE) and *Oldenlandia corymbosa* L. Extract (OCE) as a new drug focused on plant-based natural products with a good physical characteristic that inhibits inflammatory process in managing osteoarthritis (OA) was formulated. Thirty animals were randomly designated to the 6 groups (n=5) as follows: (1) The normal control group (Normal), (2) negative control groups of Monosodium Iodoacetate (MIA), (3) combination ACE-OCE, (4) single ACE, (5) single OCE, (6) positive control group (Diclofenac). Rats received intraarticular MIA injection dose 3mg/0.05 mL on day zero excluding normal control group. All groups were administered topical preparations allotted to each dose group on day 29. Knee edema profile (every 7 days) and serum cytokine level (on day 57) was evaluated with Enzyme-Linked Immunoabsorbent Assay (ELISA). Till day 42, knee edema profile of all group treatment have been similar with normal control group ($p>0.05$). Serum cytokines level for some biomarkers, such as S100A8 Protein, Interleukin-1β, Inducible Nitric Oxide Synthase (iNOS), matrix metalloproteinase-13 (MMP-13), a disintegrin and metalloproteinase thrombospondin-like motifs-5 (ADAMTS-5), Collagen Type II and Aggrecan Core Protein were decreased. A significant difference compared with MIA group showed for all group treatment on measurement of S100A8 Protein, IL-1β, and iNOS as a biomarker of inflammatory process (#$P<0.05$). The developed nanoemulgel ACE-OCE either in single or in combination has good physical characteristic and promising effect of anti-inflammatory activity to enhance MIA induced cartilage damage as potential therapeutic agent for OA and encouraging to conduct further studies.

Keywords: Aggrecan Core Protein, Collagen Type II, Monosodium Iodoacetate, Osteoarthritis

Abbreviations

Acan, Aggrecan Core Protein; ACE, *Ageratum conyzoides* (L.) L. Extract; ADAMTS-5, a disintegrin and metalloproteinase thrombospondin-like motifs-5; Col2, type II collagen; IL-1ß, Interleukin-1 Beta; MIA, Monosodium Iodoacetate; MMP-13, matrix metalloproteinase 13; NO, nitric oxide; iNOS, inducible nitric oxide synthase; OA, Osteoarthritis; OCE, *Oldenlandia corymbosa* L. Extract.

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1. Introduction

Osteoarthritis (OA), recognized as the most common joint disorder, is a chronic degenerative joint disease which causes significant pain and disability. It manifests as degradation of synovial joints, including progressive degradation of articular cartilage, synovial inflammation, destruction of ligament and meniscus, loss and sclerosis of subchondral bone, and also osteophyte formation[1,2]. The prevalence of this disease is increasing, particularly among people over 55 years[1]. In Indonesia, the national prevalence of joint diseases in general based on Basic Health Research (RISKESDAS) in 2013 was equal to 24.7%. The number of product with the claim for joint diseases is the sixth highest among all registered traditional medicine in National Agency of Drug and Food Control.

The great concern regarding gastrointestinal and cardiovascular adverse effects of Nonsteroidal Anti-Inflammatory Drugs (NSAIDs) in the management of OA inflammation and pain, leads to the development of OA drugs with lower side effects, in particular through the development of topical preparation for the elderly to delay or stop the progression of OA, which is chondroprotective and induce cartilage regeneration[3,4]. Recently, considerable experimentations has been focused on plant-based natural products with multifunctional anti-inflammatory effect which may reduce the side effects and suppress inflammation[3].

In Indonesia, *Ageratum conyzoides* (L.) L. (Asteraceae) and *Oldenlandia corymbosa* L. (Rubiaceae) have been used empirically as topical preparation (the leaves are pounded then smeared) for traditional medicine in the treatment of joint disease, wound, ulcer, and rheumatism[5,6]. *Ageratum conyzoides* (L.) L. has anti-inflammatory activity for both oral[7–9] and topical preparation[10]. Meanwhile, *Oldenladia corymbosa* L. has scientific evidence for its oral anti-inflammatory activity[11,12].

Previous studies have shown that topical *Ageratum conyzoides* (L.) L. extract prevent proteoglycan destruction on osteoarthritis model rats[10]. However, the effect is not yet similar to normal control group. The topical combination of *Ageratum conyzoides* (L.) L. extract and *Oldenladia corymbosa* L. extract has not yet been investigated and expected to have a greater response if developed with vehicle penetration enhancer as nanoemulsion.

The objectives of the present work was to design and characterize the *Ageratum conyzoides* (L.) L. Extract (ACE) and *Oldenlandia corymbosa L.* Extract (OCE), individually and in combination, to formulate a nanoemulgel-forming gel and to evaluate osteoarthritis bases, and its *in vivo* pharmacodynamic effect on rat model of Monosodium Iodoacetate-induced osteoarthritis by evaluation of knee edema profile and serum cytokines level.

2. Materials and Methods

2.1 Plant Material and Chemical

The plant material (*Ageratum conyzoides* (L.) L. leaves and *Oldenlandia corymbosa* L. herbs) were collected from Research Insitute for Spices and Medicinal Plants (BALITRO, Bogor) and authenticated by Indonesia Institute of Sciences (LIPI, Bogor) with a certificate of determination No. B-2307/IPH.3/KS/VII/2018.

2.2 Preparation of Herbal Extract

The dried powder was extracted using 70% ethanol (1:5) by maceration method and concentrated by rotary evaporator at Phytochemindo Reksa (Bogor). The yield was calculated in percentage w/w. The characterization of ACE and OCE was carried following Indonesian Herbal Pharmacopoeia.

The measurement of quercetin (as marker compound) content from ACE and OCE was analyzed using an HPLC system (Shimadzu LC-10A system, Japan) with an analytical column (Phenomenex® 4.6mmx250mm), mobile phase of a solution of Water: Acetonitrile: Isopropanol: Citric Acid (100:47:5:0.4%) at a rate of 1 mL/min, 20 µL at 360 nm wavelength. Samples were appropriately diluted in 5 mL of Acetone, 0.1 mL of Hexamine and 0.2 mL of HCl. The solution was hydrolyzed for 30 minutes at 60 ^{0}C. Ethyl acetate fraction diluted in methanol 0.5 mL and filtered with 0.45 µm non-sterile nylon membrane filter (Microlab Scientific, Hong Kong)

2.3 Preparation of Nanoemulsion and Nanoemulgel

Dried powder extract was disintegrated in 96% ethanol and propylene glycol followed by sonication. Nanoemulsion was prepared with spontaneous emulsification procedure[15–17]. The method comprises of injecting an oil phase containing Virgin Coconut Oil (VCO) and sample solution into the water phase (containing surfactants and co-surfactant (Tween 80 and PEG 400)) gradually under steady blending with magnetic stirring at 1250 rpm. Demineralized water was added (1mL/min through slow titration). Table 1 represents the final composition (percent w/w) of nanoemulsion. The dosage calculation for active ingredients in the nanoemulsion formula was done two times due to subsequent mixing with a gel base (1: 1). The gel base was produced by scattering Carbomer 940 1.5% as a gelling agent with demineralized water at the time of homogenizing by Homogenizer (IKA, Germany). As a penetration enhancer, propylene glycol 5% was utilized, and oleum menthae as a fragrant. Triethanolamine 1% was added to frame gel consistency and to control the pH of the formulation.

Table 1. Nanoemulsion Formula (%)

Material	Concentration (%)		
	F1	F2	F3
ACE	3.2	3.2	-
OCE	2.2	-	2.2
Tween 80	9	9	9
PEG 400	9	9	9
VCO	2	2	2
Ethanol 96%	5	5	5
Propylen glycol	10	10	10
Propyl paraben	0.05	0.05	0.05
Methyl paraben	0.05	0.05	0.05
Butylated Hydroxytoluene	0.1	0.1	0.1
Demineralized Water	Add100	Add100	Add100

2.4 Evaluation of Nanoemulsion and Nanoemulgel

The droplet size, Polydispersity Index (PDI) and zeta potential of nanoemulsion were determined at 25 °C and at 173 °C scattering angle by dynamic light scattering (DLS) and electrophoretic mobility, respectively (Zetasizer Nano ZS, Malvern Instruments, UK). The samples were adequately diluted (1:100) in purified water. A pH-meter (Eutech Instrument, Singapore) was used to record the bulk pH directly[18,19].

Nanoemulgel's viscosity and rheological characteristics were determined using a Brookfield Viscometer (Brookfield, USA) at 25 °C with HA No. 05 spindle. The samples were stored in different temperature like, room temperature (25 ± 2 °C), low temperature (2 - 8 °C), and high temperature (40 ± 2 °C) for physical stability testing. The samples were studied in detail at time intervals for their appearance, pH, and viscosity. Cycling test was also conducted for six cycles.

2.5 Experimental Animal

Procedure of animal handling have been carried out in accordance with the guidelines for the care and use of laboratory animals approved by an ethical committee from Faculty of Medicine, University of Indonesia with a certificate number 0569/UN2.F1/ETIK/2018. Skeletally matured white male Sprague Dawley rats at 3 months of age, 200-300 gram in weight was obtained from Faculty of Animal Husbandry, Bogor Agricultural Institute. Under standard laboratory conditions (25 ± 2 °C) and 12 hour light/dark cycle, the animals were grouped with food and water *ad libitum*. Acclimatization was performed one week before the experiment. Body weights was used as a randomization parameter.

In sterile saline (0,9% NaCl), monosodium iodoacetate (MIA) (Sigma-Aldrich, USA) was freshly dissolved. Under intraperitoneal anesthesia of Ketamine 120 mg/kgBW (Hameln Pharmaceuticals GmbH, Germany, which kindly donated by Combiphar), a single intra-articular injection of MIA at 3.0 mg/50µl dose was administered at the right knee joints[(10)].

Thirty animals were randomly designated to the 6 groups (n = 5) as follows: (1) The normal control group (Normal), (2) negative control group of monosodium iodoacetate (MIA), (3) combination ACE-OCE (1.6%-1.1%), (4) single ACE (1.6%), (5) single OCE (1.1%), (6) positive control group (Diclofenac) (1%). On day zero, all rats received intraarticular MIA injection, other than the normal control group. Induction lasted 28 days. On day 29, all groups were administered topical preparations according to each dose group as much as 1 gram while normal and negative control groups received a gel base. Rats were sacrificed at 57th day after MIA injection.

2.6 *In-vivo* Pharmacodynamic Effect on Rat Model Osteoarthritis Induced by Monoiodoacetate

2.6.1 Evaluation of Knee Edema Volume

On day 0, 7, 14, 21, 28, 35, 42, 49 and 56 after MIA injection, the knee volume was determined using a mercury plethysmometer and the mean values were recorded. The volume of the knee measured just before MIA injection was used as the volume of control (baseline day 0) to determine the volume of edema.

2.7 Evaluation of Serum Cytokine Levels

On day 57, blood was collected through the orbital eye sinus using a hematocrit pipette as much as 3 mL under anesthesia. Each blood sample was stored in a cup sample and centrifuged at a speed of 3000 rpm for 15 minutes. The serum obtained cytokine measurements were prepared separately to avoid the freeze-thaw cycle.

Commercial Rat enzyme-linked immunoabsorbent assay (ELISA) Kit (Finetest®, Wuhan-China) were used for quantification of the serum cytokine levels according to the manufacturer's instructions. Value of optical density (OD) was calculated at a wavelength of 450 nm on the ELISA reader (Biochrom, UK) and the serum cytokine levels were calculated from the calibration curve of standard.

2.8 Statistical Analysis

In order to determine statistical significance, one-way analysis of variance (ANOVA) followed by post hoc multiple comparisons tests was performed using Statistics Software (SPSS 24.0, IBM Ltd). If normally distributed data were not reached, non-parametric test (Kruskal Wallis) was performed. Results have been shown as mean±SD. Differences were considered statistically significant compared to the normal control group values of *p<0.05 and #p < 0.05 compared to the negative control group.

3. Result

3.1 Evaluation of ACE and OCE

The yield of ACE was 8.75% w/w and for OCE it was 8.55%. The quercetin content for ACE and OCE was 0.6% and 2.2% respectively. The standard quercetin, ACE and OCE HPLC chromatogram are shown in Figure 1. Table 2 shows the characterization of each extract.

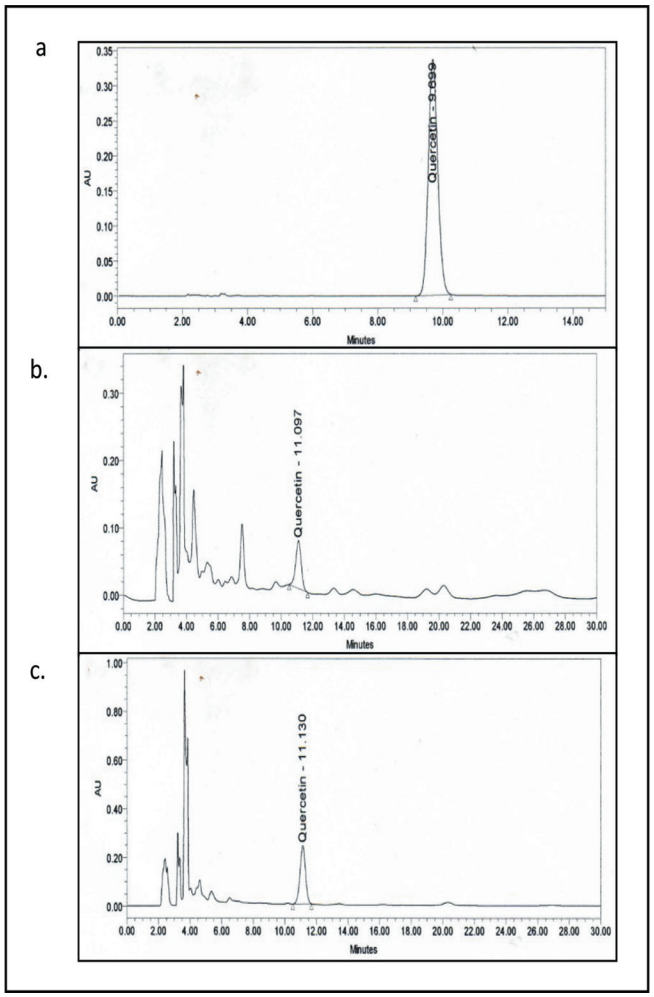

Figure 1. HPLC chromatogram of standard quercetin with RT = 9.699 **(a)**, ACE with RT = 11.097 **(b)**. and OCE with RT = 11.130 **(c)**. Phenomenex® column 4.6x250mm in mobile phase Water: Acetonitrile: Isopropanol: Citric Acid (100:47:5:0.4%) at a rate of 1 mL/min, 20µL at 360 nm wavelength.

3.2 Evaluation of Nanoemulsion and Nanoemulgel

Table 3 shows the nanoemulsion characterization. The mean droplet size as z-wave and PDI of nanoemulsion F1-F3 was 262,69 nm and 0.479. Zeta potential value was about -29,85 mV.

pH value for nanoemulgel formula F1-F3 was 5.90; 5.08; and 5.35, respectively. For stability testing, there was a decrease in pH starting at week

Table 2. Characterization of ACE and OCE

Characteristic	ACE	OCE
Form	Dry powder	Dry powder
Color	Green gray to yellowish-brown	Green to greenish brown
Odor	Aromatic	Aromatic
Taste	Typical	Typical
Water Content	1.19%	1.33%
Ash Content	1.51%	8.20%
Solvent Residue	≤ 0.5%	≤ 0.5%
Heavy Metal Content		
Pb	≤ 10 mg/kg	≤ 10 mg/kg
Cd	≤ 0,3 mg/kg	≤ 0.3 mg/kg
As	≤ 5 mg/kg	≤ 5 mg/kg
Hg	≤ 0.5 mg/kg	≤ 0.5 mg/kg
Microbial Contamination		
Total aerobic bacteria	< 10 cfu/g	< 10 cfu/g
Mold	< 10 cfu/g	10 cfu/g
Escherichia coli	Negative	Negative
Staphylococcus aureus	Negative	Negative
Salmonella sp	Negative	Negative
Pseudomonas aeruginosa	Negative	Negative

4 to 12. However, the decrease in pH can still be tolerated at the pH limit that matches to the skin. At a temperature 40 °C and week 10, a crystal was formed for Formula F2. There was no change about viscosity and rheological properties demonstrated a pseudoplastic thixotropic rheology and for the cycling test.

Table 3. Characterization of Nanoemulsion and Nanoemulgel

Formula	Nanoemulsion			
	z-ave (nm)	PDI	Zeta (mV)	pH
F1	265.5±8.3	0.521±0.062	-23.1±3.6	5.58
F2	261.5±2.6	0.274±0.014	-29.3±1.1	5.50
F3	261.1±6,2	0.644±0.033	-37.2±0.3	5.66

3.3 MIA-induced Inflammation

MIA injection into the right knee results in a time-dependent increase of inflammation as shown by the change in knee edema as described in Figure 2. In comparison with normal groups (*P < 0.05), knee edema for all group was significantly increased on day 7, 14, 21, and 28 after MIA injection. Since day 35 (7 days of administration nanoemulgel), all group treatment had a decrease in the edema profile. Starting on day 42 (14 days of administration nanoemulgel), all group treatment has been similar to the normal control group and only ACE-OCE group was not statistically significant with MIA group (#P < 0.05). On day 49 and 56, all groups were not statistically significant compared with normal group (P > 0.05) but all groups were statistically significant with MIA group (#P < 0.05).

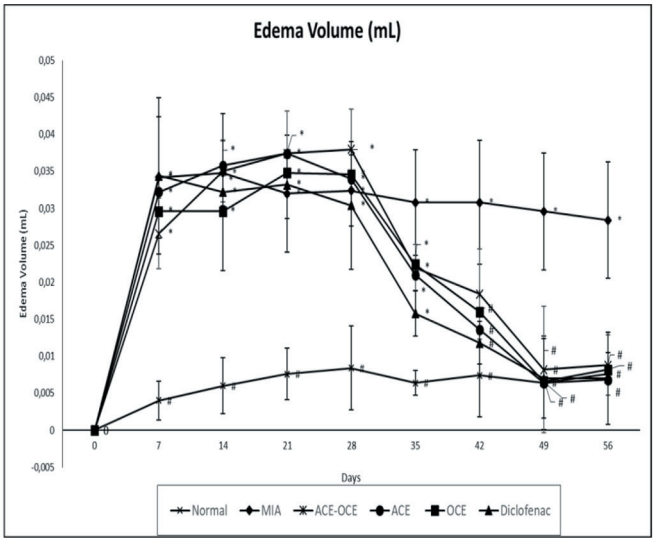

Figure 2. Edema Volume (mL) by days after MIA injection (n = 5). *P < 0.05 as compared to normal group; #P < 0.05 as compared to MIA group.

3.4 Serum Cytokines Analysis

For some biomarkers, serum analysis was done after four weeks of treatment (on day 57), which has been represented in Table 4 and Figure 3. Compared to the normal control group (*P < 0.05), all biomarkers on MIA group were higher and statistically significant compared with the normal control group (*P < 0.05). In general, all group treatment showed a decrease in all biomarkers.

A significant difference in the measurement of Protein S100A8, IL-1β, MMP-13 and iNOS (#P < 0.05) as a biomarker of inflammatory process was observed for all group treatment and compared with MIA group. There was also a significant difference in the ACE-OCE group and the Diclofenac group for ADAMTS-5 level (#P < 0.05) compared to MIA group. A significant difference with MIA group (#P < 0.05) occured only in the OCE group and Diclofenac group for the measurement of Col2 level. However, with the normal control group, the ACE-OCE group showed a statistical significance of *P < 0.05. There was significant difference in the Acan (Aggrecan Core Protein) level compared to the normal group in the Diclofenac group (*P < 0.05) and there was a significant difference in the ACE-OCE group and OCE group compared to the MIA group (#P < 0.05).

4. Discussion

Scientific evidence from traditional medicine that has been used empirically is needed in order to develop new drugs in the treatment of disease like OA. The challenge of a study that uses dosage form for transdermal delivery is to improve the penetration through the skin to act locally or systemically. For transdermal delivery, nanoemulsion[18], nanoparticle[10], ethosome[20], nanostructured lipid carriers (NLC)[21], solid lipid nanoparticle (SLN)[22] loaded quercetin (an active compound of ACE-OCE) have been developed to improve skin penetration.

Hydrophilic gels are known to rapidly increase the release of drugs. The using of a gel as a vehicle of nanoemulsions could give an added effect to the penetration of active substances. The use of propylene glycol and oleum menthae as penetration enhancer is due to its polarity and greater permeation into the skin. The high water content of the gel can hydrate the stratum corneum and leak the compact structure to facilitate the penetration of the active substances through the skin[23].

Nanoemulsion consists of fine oil/water or water/oil dispersion that is stabilized by an interfacial film of the surfactant molecule. Its formulation such as nanoemulgel has ameliorated physical stability, helps solubility and transport of lipophilic drug. Small-sized droplets have greater surface area to facilitate penetration of actives substances into the skin. [(19)] Nanoemulsion may reduce Transepidermal Water Loss (TEWL), indicating that the barrier function of the skin is strengthened[24]. The negative value of zeta potential came from the existence of anionic groups of fatty acids in the VCO, Tween 80 and –OH group of flavonoid contain (ex: quercetin). The higher the zeta potential will contribute for higher stability[18].

The use of ethanol in nanoemulsion, besides being able to increase the solubility of active substances also can increase the penetration of the skin along with propylene glycol by entrapment of active substance's molecule[25]. Ethanol can collaborate with the lipid molecules polar head group that causes the transition temperature of lipids in the stratum corneum to decline. Therefore, the fluidity of nanoemulsion and the reduction of lipid bilayer density will be increased; causing the release of the drugs along the penetration pathway into the deeper layers of the skin[26]. Tween 80 and PEG 400 are surfactant and co-surfactant very well used in formulations containing quercetin[27].

Animal model by intraarticular injection of toxic chemical inducer like monoiodoacetate can stimulate intra-articular inflammation, damage of cartilage and chondrocyte death by inhibiting glyceraldehyde-3-phosphate dehydrogenase and glycolysis. Since there is no gold standard in animal model for OA, the limitation of this model is the rapidity of joint disruption by the death of chondrocyte which is not representative of either spontaneous or posttraumatic OA, but have great reproducibility, convenient and very useful model to study matrix degeneration, drug for pain and inflammation[14,28–30]. This model causes inflammation followed by joint destruction or autoimmune response consistent with the clinical OA that results in decreased weight bearing, pain, hyperalgesia, and allodynia[28] Therefore it is necessary to determine marker level of cytokines that play a role in the inflammatory process and joint destruction. A skeletally mature animal is very important in an experimental animal study of OA to avoid bias due to tissue remodeling and matrix turnover that occurs during normal growth. Therefore 3 months old white male Sprague Dawley rat was used[30].

Pain and inflammation due to monoiodoacetate injection occurs in both acute (early up to 1 week after injection) and chronic phases (late between 2-4 weeks after injection)[31]. Injection dose of 3 mg can induce

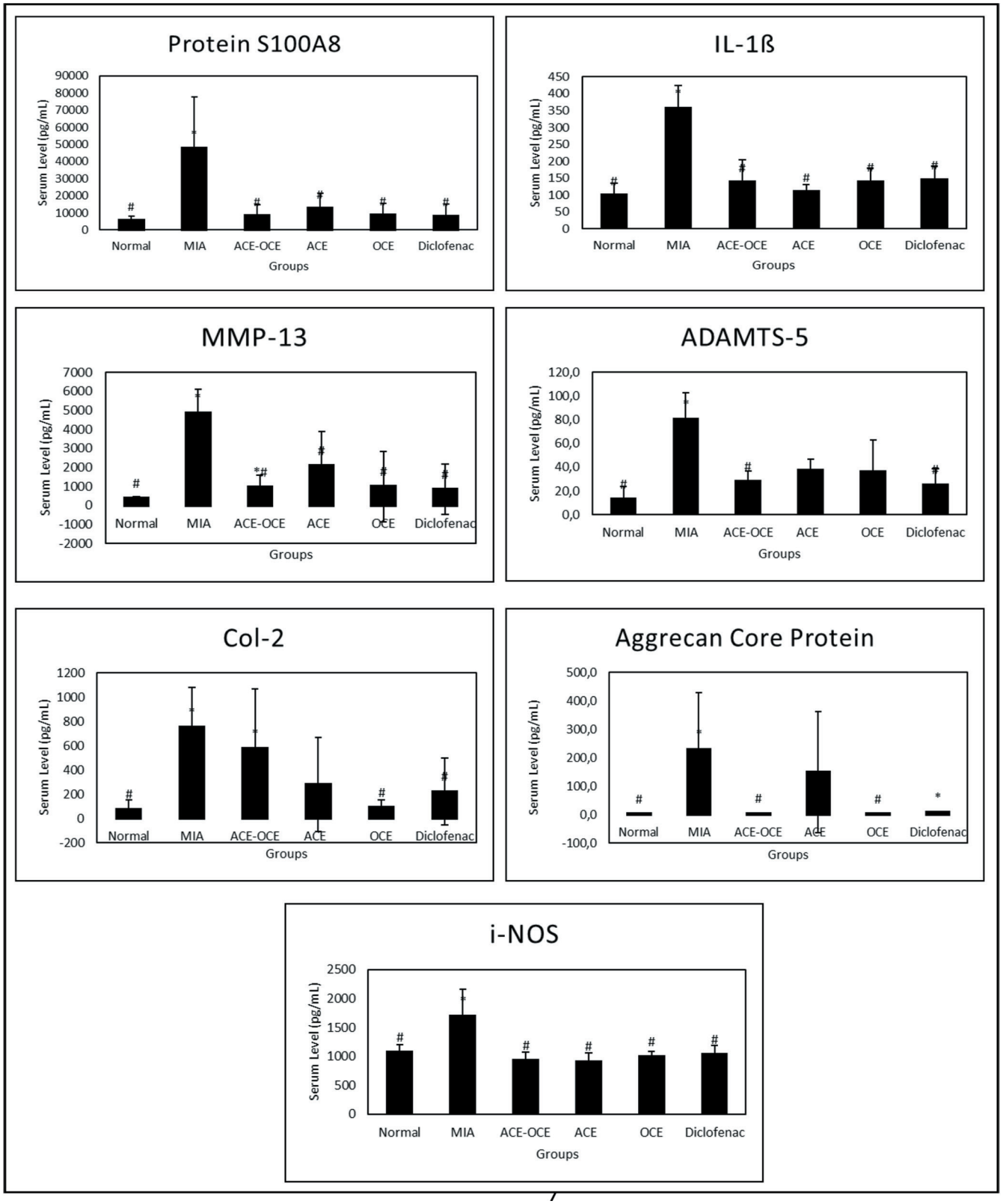

Figure 3. Bar chart for serum Level (pg/mL) (n=5) P<0.05 as compared to normal group; #P<0.05 as compared to MIA group.

Table 4. Serum Level (pg/mL) (n = 5)

Groups	Serum Level (pg/mL)						
	S100A8 Protein	IL-1β	MMP13	ADAMTS5	Col2	Aggrecan Core Protein	iNOS
Normal	5544 ± 2340#	100 ± 34.7#	367 ± 78#	13.2 ± 10.7#	75 ± 76#	3 ± 1.6#	1072±130#
MIA	47811 ± 29716*	358 ± 66.7*	4855 ± 1269*	80.2 ± 22.5*	754 ± 328*	230.2 ± 198.6*	1694±470*
ACE-OCE	8522 ± 6104#	139 ± 65.7#	967 ± 623*#	28 ± 9#	577 ± 492*	4.5 ± 2.6#	928±142#
ACE	12744 ± 8667#	111 ± 19.9#	2085 ± 1801#	37.2 ± 9.4	277 ± 388	149.5 ± 212.0	908±148#
OCE	8877 ± 6595#	140 ± 39.1#	993 ± 1850#	36.4 ± 26.4	91 ± 60#	4 ± 2.4#	997±83#
Diclofenac	8167 ± 6900#	146 ± 40.7#	841 ± 1327#	25.2 ± 13.2#	221 ± 274#	8 ± 2.2*	1036±154#

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

chondral and subchondral alterations and prolonged inhibition of proteoglycan synthesis in the central part of the patellae[32]. In this study, nanoemulgel dosage form was administered for 4 weeks after injection, where stabilized OA conditions have occurred and all groups were statistically significant (*P<0.05) compared to normal groups, although on 7th day there was a significant difference with the normal group. Other study has reported rapid inflammation and pain lasting seven days, followed by chronic musculoskeletal pain starting at the 10th day of post-injection[14].

In this study, cytokines (Interleukin-1ß), inflammatory mediators (nitric oxide as inducible nitric oxide synthase, matrix degradation (MMP-13 and ADAMTS-5), cell-derived and/or matrix-derived products (S100A8 protein, collagen fragment as type II collagen, and proteoglycan fragments as aggrecan core protein) were estimated[2].

Injection of monoiodoacetate induced an increase of activity in the family of zinc-dependent endopeptidases called Matrix Metalloproteinases (MMPs) which leads to degradation of chondrocyte and causes metabolism obstruction and chondrocyte death. Furthermore, the accumulation of inflammatory cells causes release of inflammatory cytokines[32]. The release of other inflammatory cytokines such as IL-1ß is mediated by MMP so that IL-1ß initiating a vicious cycle inflammation of catabolic and degradative events in cartilage. The expression of MMP-13 (collagenase-3) and ADAMTS5 (aggrecanase-2), leads to collagen type II and proteoglycan core proteins degradation. The expression of these proteases to break down of tissues within the joint remained significantly elevated above basal levels during the 10 weeks study[33]. In this study, an increase of levels of MMP-13 in MIA control groups was statistically different from other groups.

S100A8 Protein is Danger-Associated Molecular Pattern molecules (DAMPs) which act via the Receptor for Advanced Glycation End products (RAGE) and Toll-like receptors-4 (TLR4) which play an important role in the pathogenesis of inflammatory disorders. S100A8 in neutrophils and monocytes also can be activated in keratinocytes at the time of inflammation[34].

Since type II collagen and proteoglycans (especially aggrecan) are the main component of articular cartilage, the presence of chondrocyte death due to monoiodoacetate injection leads to an increase in Aggrecan Core Protein and Type II Collagen as a response to the damage. The chondrocyte maintains minimal collagen turnover and no mitotic activity in normal condition, but there is an increase in the breakdown of Glycosaminoglycans (GAG) constituents in aggrecan so those core proteins (three disulfide-bonded globular region G1, G2, and G3) increase in high turnover conditions due to chondrocyte death[2, 14, 35].

Nitric oxide (NO) plays a role as stimulator of MMP synthesis by chondrocyte. The synthesis of NO is stimulated by IL-1, tumor necrosis factor and shear stress which is also possible by the chemical insult of monoiodoacetate injection during inflammation. Upregulation of NO production plays an important factor in OA disease. Once expressed, inducible NO synthase (iNOS) generates a high concentration of NO.

Inhibition of iNOS decreases the severity of articular cartilage degradation[1].

Different finding for serum cytokine analysis was due to different and complex inflammation response within groups (n = 5) and the different multi-active compound from its extract.

The administration of diclofenac sodium gel 1% that was applied topically to one knee has 17-fold less the AUC_{0-24} value if compared with oral diclofenac 160 mg. The constant plasma levels following the topical treatment of diclofenac sodium gel 1% treatment suggest that it accumulates in the skin and/or underlying periarticular and articular tissues, from which it is slowly released into the systemic circulation[4]. Consequently, topical administration of diclofenac sodium had a low platelet aggregation and lower COX-1 inhibition. It may be associated with improved safety and for local use only[4]. Diclofenac sodium acts as an analgesic and anti-inflammatory agents that modulate the prostaglandin production locally and systemically[4,36]. This finding might be correlated with this study in which topical use for OA has improved safety and effectiveness by acting locally. Study of drug that can inhibit inflammatory cytokines and inflammation process is needed to obtain new drugs in OA. An active compound derived from traditional medicine is commonly safer to use for the long term due to the empirical history of its use and it also has some mechanism of actions. Scientific evidence based on preclinical studies is needed to strengthen existing empirical data. ACE and OCE has been shown to reduce proinflammatory cytokines, inflammatory mediators, matrix degradation and cell/matrix-derived that involved in the pathogenesis of OA.

The serum measurements represent total body levels of the marker and may be affected by external processes outside the joint[30]. The use of rat model in this study has a disadvantage of the possible occurrence of spontaneous intrinsic healing of cartilage lesion, but rat model is also helpful for screening of potential therapeutics prior to definitive evaluation in a large animal model and clinical trial[29].

Potential DMOAD treatments which are currently being developed for OA are glycosaminoglycans, anti-inflammatory drugs, bone modifiers, MMP inhibitors, growth promoting peptides, interleukin-1 converting enzyme inhibitors, NO synthase inhibitors, etc.[3]. Based on the results of this study on edema profile and serum cytokine levels, either ACE-OCE either in single composition or combination composition decrease edema profile and serum cytokine level therefore it would be proposed as DMOAD for OA. Further study is also needed to determine the molecular target, and also to develop more specific and precise ratio of combinations of ACE and OCE.

5. Conclusion

In conclusion, the developed nanoemulgel ACE-OCE either in combination or single composition, exhibited a good physical characteristics and has a promising effect of anti-inflammatory activity on ameliorating MIA-induced cartilage damage by inhibiting the levels of inflammatory mediators and degradation of proteoglycan; suggesting that ACE-OCE may be a potential therapeutic agent for OA. However, further studies are needed to understand the molecular mechanisms of action and clinical trials to estimate the effects of ACE-OCE on OA.

6. Acknowledgement

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7. Conflict of Interest Statement

We declare that there are no conflicts of interest that correlated with this publication.

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