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Research Article

# The potential of *Colocasia esculenta* tuber and *Zingiber officinale* rhizome combined extracts to ameliorate inflammation in monosodium iodoacetate-osteoarthritis rat model

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## Abstract

This study aims to evaluate effect of *Colocasia esculenta* and *Zingiber officinale* combined extract (CEZO) in osteoarthritis rats. Twenty-four Wistar rats were split into normal group, positive group, negative group, and CEZO group on 29$^{th}$ to 43$^{rd}$ day (n = 6). All rats were injected with Na-iodoacetate intraarticularly on first day, unless for the normal group, and then observing the diameter of knee edema until the 28$^{th}$ day (OA rats). On the 43$^{rd}$ day, rats were euthanasia for measurements of hematology, spleen weight, and inflammatory mediators of nitric oxide, matrix metalloproteinase 9, Interleukin-6, and tumor necrosis factor-α using ELISA kit. Our experiments showed that CEZO 90 mg/kgBW was able to decrease knee edema, leukocytes, lymphocytes, spleen enlargement, the concentration of mediators inflammatory NO, TNF-α, IL-6, and protect cartilage degradation by decreased MMP-9 significantly in OA rats. Conclusion of the research is the CEZO 90 mg/kgBW supplementation has potential to be used in osteoarthritis.

## Keywords

Nitric Oxide, TNF-α, IL-6, in-vivo, combination extract

## Introduction

Osteoarthritis (OA) is the most frequent chronic joint illness(Gallego et al. 2022). Gender, obesity, weak muscles, excessive or inadequate physical exercise, previous injuries, diminished proprioceptive function, and genetics are risk factors for OA. However, age is the strongest risk factor, even the occurance rate of OA continues to increase with age. Therefore, OA is more occurs in elderly patients and characterized by progressive destruction the joint tissues such as cartilage, synovium, and subchondral bone which ends in degenerative conditions so that the joint surfaces experience fissures, and ulcerations, and become thin which causes inflammation. This is characterized by pain and swelling which causes the joints to appear prominent (Abdulkhaleq et al. 2018). Under these conditions, chondrocytes and synovial cells produce one of the potent mediators of inflammation, namely Nitric Oxide (NO), which contributes to cartilage degradation by various mechanisms, including inducing chondrocyte apoptosis, inhibiting chondrocyte proliferation, synthesis of collagen, proteoglycans, and receptor antagonists.

IL-1 activates matrix-degrading enzymes, namely Matrix metalloproteinases (MMPs), and increases the inflammatory response in chondrocytes (Abramson 2008; Chien et al. 2016). OA therapy is targeted at improving the patients quality of life by overcoming illness, reducing inflammation, slowing down cartilage degradation, improving joint function, and reducing disability (Bahtiar et al. 2017). For OA with mild to moderate pain, NSAID (non-steroidal anti-inflammatory drug) are the first line of therapy. NSAIDs are not suggested for long-term use, particularly in senior people, because of their numerous side effects, especially those affecting the digestive system and cardiovascular risk (da Costa et al. 2017). Non-pharmacological management of OA can be done with exercises that focus on joint strength which includes the use of isokinetic weight machines, resistance exercise training using or not using elastic bands, and isometric exercises. Other sports currently being developed are neuromuscular training, weight loss, self-management programs and self-efficacy, Tai chi which is a meditation consisting of gentle movements, diaphragmatic breathing, and relaxation as well as Yoga, thermal interventions, and others. However, symptoms of severe OA require NSAIDs, so it is necessary to develop natural ingredients that can reduce pain and inflammation in patients with OA.

Jamu is an Indonesian traditional herbal remedy, that has been used empirically by Indonesians as an alternative therapy for variety of illness such as OA because it is thought to less hazardous and has fewer adverse reaction (Elfahmi et al. 2014). One of the plants used in herbal concoctions for the treatment of OA is ginger rhizome/ *Zingiber officinale* (ZO) and the tuber of *Colocasia esculenta* (CE). The CE extract contains many compounds such as flavones, anthocyanins, mucilage (Fayek et al. 2021) panthenol (Kang et al. 2021), and tarin (Pereira et al. 2021) which are known to have anti-inflammatory activity. In inflammatory conditions, CE extract can reduce the thickness of ear edema in the dermatitis rat model-induced topical tetradecanoylphorbol-acetate (TPA) and suppress production of NO in RAW 264.7 cells induced by Lipopolysaccharide (LPS) (Kang et al. 2021). CE extract also contains three essential amino acids, namely glycine, proline, and lysine (HILARY VAN WYK and OSCAR AMONSOU, 2021) which play an essential role in the synthesis of collagen for cartilage regeneration (de Paz-Lugo et al. 2018). Not only *C. esculenta (L.)* tubers, the ginger rhizome is also known for its antioxidant and anti-inflammatory activities (Herve et al. 2018; Romero et al. 2018). According to previous research, *Z. officinale* extract under inflammatory conditions can reduce NO levels in RAW 264.7 cells induced by LPS (Dugasani et al. 2010). reduce the diameter of knee edema in monosodium iodoacetate (MIA)-induced OA rat (Abdel-Rahman et al. 2020), reduced leukocyte levels in the uterine synovial cavity in the monoarthritis rat model induced by CFA (Complete Freund's Adjuvant) (Levy et al. 2006) and decreased spleen weight in the ulcerative colitis rat model induced by Dextran Sulphate Sodium (Zhang et al. 2018).

There is a lack of studies to examine the effect of *Colocasia esculenta* tuber and *Zingiber officinale* rhizome combined extract (CEZO) on OA by measuring the levels of its biomarkers, namely NO, IL-6, TNF-α, and MMP-9. So, this research aimed to scientifically prove the potency of CEZO in rats with OA induced using MIA, so that this research can provide great benefits to patients with OA.

# Materials and methods

## Materials

CE aqueous extracts, ZO aqueous extracts, monosodium-Iodoacetate (Sigma-Aldrich), Xylazine (Interchemie, Holland), Ketamin HCl Injection, USP (Hospira, Inc., USA), Sodium diclofenac tablets 25 mg (Novell, Indonesia), NO, IL-6, TNF-α, and MMP-9 Elisa Kit (RnD Systems, USA), 10,000 Molecular weight cut-off filters (Millipore), NaCl 0,9%, CMC Na, and Ethanol (Brataco Chemical, Indonesia).

## Animal study - ethical approval

This experiment was carried out from July to December 2022. These assays were approved and conducted after receiving ethical approval from the Ethics Committee of Health Research, Medicine Faculty, Universitas Indonesia (No. 1419/UN2F1/ETIK/PPM00.02/2022). Rats that were used were acclimatized for one week with controlled room condition 25 ± 2 °C, moisture 65 ± 10%, illumination 12 hrs per day (07:00 – 19:00), and air ventilation 11–13 times per day. The rats were given regular pellets and ad libitum drinking.

## Preparation of CEZO

Sodium carboxymethylcellulose (Na-CMC) suspension was prepared by weighing as much as 0.5% of the desired dosage volume, then add hot distilled water at 70 °C. Allow Na-CMC to swell for 10–15 minutes then grind until homogeneous. Each 500 mg of extract contains 350 mg of *CE* and 150 mg of *ZO*. Add the combined CE and ZO extracts and suspend with 0.5% Na-CMC suspension. Sodium diclofenac suspension was prepared by suspending crushed diclofenac tablets into 0.5% Na-CMC for a dose equivalent to 150 mg/day (Da Costa et al. 2021).

## Design of animal experiment

In this test, 24 male Wistar rats were acclimatized, then split into 4 sets, each consisting of six rats as follows: Normal group as the control without any treatment, Positive control group given sodium diclofenac suspension 13.5 mg/kgBW, Negative group given sodium CMC (0.5%), and sample group was given CEZO suspension at a dose of 90 mg/kgBW given orally using oral sonde. All the groups were injected with 0.025 mL MIA (10 mg/mL) intra-articularly on day 1 and then observing it until the 28th day, except the normal group. Induction of OA with 0.025 ml (10 mg/mL) intra-articular injection MIA was performed by anesthetizing rats with a combination of xylazine at dose 8.8 mg/kgBW and ketamine (75 mg/kgBW) intraperitoneally. Inject 0.025 ml (10 mg/mL) MIA with the needle to the area that has been marked and massage the knee gently to ensure that the injected MIA solution is distributed evenly (Janusz et al. 2001; Pitcher et al. 2016). Induction was carried out in all test groups, except for the normal group on day 1, and then observed until day 28th, day 29th-43rd received the treatment (Bahtiar et al. 2017). Knee diameter was measured using a micrometer screw on the part that had the largest size in the knee joint area on days 0, 7th, 14th, 28th, 36th, and 43rd. Then calculate the average of each group (Khotib et al. 2019). On the day 43rd, rats were euthanized for blood, spleen, and knee tissue samples.

### Blood, serum, plasma, and tissue isolation

Blood was placed in a clean tube and had been given $K_3$EDTA for leukocyte and lymphocyte count levels, then measured automatically using a Sysmax XS-1000i Hematology analyzer with the principle of Flow cytometry. The blood allowed to stand until it clots (30 min) at room condition and then centrifuged (5 min, 3500 rpm). Transfer the serum to a new tube and store it at ≤ 20 °C before measuring.

Measurement of the spleen weight was carried out by taking the spleen through the abdominal section of the rat. After the spleen organ is taken, it is weighed using a scale, then calculate the mean of each group (Bahtiar et al. 2017).

### Protein Isolation and sandwich Enzyme--Linked Immunoassay (ELISA) for measurement of NO, IL-6, TNF-α, dan MMP-9

Knee tissue is taken and isolation of proteins from knee tissue using 10xRIPA buffer pH 7.4 (150 mM sodium chloride, 50 mM Tris-HCl, 1% NP-40, 0.25% Na-deoxycholate, 1 mM NaF, and 1 mM Na3VO4). The isolate was stored at –40 °C for analysis. Measurement of proinflammatory mediators IL-6, TNF-α, NO, and MMP-9 was conducted using an ELISA kit. The manufacturer's procedure was followed for conducting the analysis to measure absorbance using a microplate reader spectrophotometer (Bio-Rad Reader 680).

### Statistical analysis

Parameter measurement data from each treatment group were statistically analyzed using SPSS 20. The Shapiro-Wilk normality test and Levene homogeneity test were carried out followed by a parametric statistical test using the ANOVA method, then proceed with the Post HOC Least Square Difference (LSD) test. The significant degree value used was with a 95% confidence interval ($p < 0.05$).

## Results and discussion

### Edema profile in MIA rats model and after treatment

In this study, the OA model with Monosodium Iodoacetate (MIA) induced was successful after 28 days from the first injection of MIA in the joint of the rat intraarticular (Fig. 1). To simulate the symptoms of OA in people, several animal experimental models for the investigation of anti-arthritic drugs have been created (Chien et al. 2016). Induction with MIA is most often used in modeling OA. MIA works by inhibiting glycolysis of glyceraldehyde-3-phosphate dehydrogenase and interfering with chondrocyte glycolysis resulting in necrosis of chondrocytes and subchondral bone, neovascularization, and inflammation. This results in cartilage damage accompanied by reduced proteoglycan matrix and functional changes, such as stiffness, as seen in OA in the human body. Injection of MIA into the target joint results in bone destruction within a short period (Morais et al. 2016; Pitcher et al. 2016). Induction with MIA is considered less invasive, fast, and easy to implement and can cause degeneration and histological changes comparable to OA in humans (Nagy et al. 2017). This modeling is very popular for use by researchers because it can induce the condition of OA progression very quickly and requires a low cost (Fang and Beier 2014).

When cartilage deteriorates to the point where the cartilage rubs against one another, edema can occur. In this study, within 28 days after intraarticular injection of MIA it appeared that increase in the diameter of knee edema in the positive and treatment group, and then continue to reduce the volume of edema until day 43rd. These results were linear with another study which showed that the rat model of osteoarthritis MIA-induced showed signs of inflammation in the form of increased levels of leukocytes, lymphocytes, and spleen weight (Bahtiar et al. 2017). This demonstrates the effectiveness of MIA induction, which increases immune system parameters and promotes inflammation. This supports the discovery that elevated T-cells are a general immunological characteristic in OA. A study also showed signs of inflammation in the form of an increase in the diameter of knee edema in a mouse model of MIA-induced osteoarthritis. Edema or swelling of the joints is a sign of OA. The same thing happened when MIA was injected into the rat's knee. As a result, there is a volume increased of synovial fluid in the joints. Therefore, the success of OA induction was assessed by measuring the diameter of the edema in the knee of MIA-injected rats (Khotib et al. 2019).

Measurements of the edema caused by inflammation were done on days 0, 7th, 14th, 28th, 36th, and 43rd. Measurements were made to assess changes in edema diameter in the treatment group. Fig. 2 demonstrates that there was a significant elevated in the edema diameter in all groups given MIA induction in rat knees from day 7th to day 28th. On days 29th-43rd, the positive control was given


**Figure 1.** The knee joint of the rat in 43rd day. **A.** Normal knee joint; **B.** OA knee joint of rat-induced MIA; **C.** Knee joint after diclofenac treatment; **D.** Knee joint after CEZO treatment.

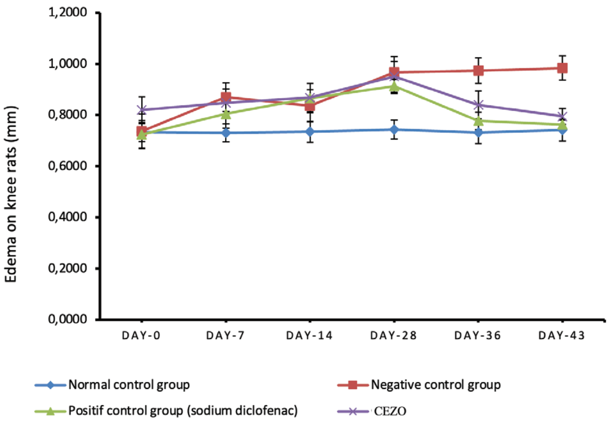


**Figure 2.** Edema profile on MIA rats' model (mm) day 0, 7, 14, 28, 36, and 43 after treatments (*p < 0.05 vs negative group).

Na-diclofenac 13.5 mg/kgBW and the sample group was given CEZO (90 mg/kgBW). On day 43rd, there was a significant difference between the negative group and the test group (13.5%). In this study, we used diclofenac 150 mg/day as a comparator drug because in many research studies reported about the effectiveness of nonsteroidal anti-inflammatory drugs (NSAIDs) to treat osteoarthritis pain and this drug is also the most effective oral NSAID for knee and hip osteoarthritis pain and physical function (Kołodziejska and Kołodziejczyk 2018; Da Costa et al. 2021; Sandhiutami et al. 2023).

## Effect on hematology evaluation

Table 1 showed the hematology evaluation parameter (day 43rd). This study showed that OA induction by monosodium iodoacetate has no effects on Hemoglobin (Hb), Red Blood cells (RBC), Hematocrit (Hct), MCH, MCV, MCHC, thrombocyte, RDW, MPV, and PDW in all groups. There was a significant difference of lymphocytes and leukocytes counting in negative group and the group that received treatment for 14 days at

**Table 1.** The effect of CEZO on the hematology.

| Hematology | Unit | Normal group | Negative control group | Positive control group | CEZO group |
|---|---|---|---|---|---|
| Leukocyte (WBC) | $10^3/\mu L$ | 15.96 ± 4.62 | 18.54 ± 5.93* | 16.08 ± 3.89# | 16.60 ± 3.31# |
| Lymphocytes | % | 68.00 ± 15.25 | 73.25 ± 9.33* | 65.50 ± 17.08# | 68.25 ± 7.74# |
| Neutrophils | % | 26.33 ± 11.09 | 40.67 ± 6.77* | 32.83 ± 20.01# | 27.67 ± 9.00# |
| Red Blood Cells (RBC) | $10^3/\mu L$ | 7.29 ± 1.25 | 8.2 ± 0.39 | 7.78 ± 0.94 | 7.85 ± 1.03 |
| Hb | g/dL | 14.50 ± 2.54 | 15.4 ± 0.48 | 13.68 ± 1.38 | 14.78 ± 1.22 |
| Hct | % | 41.25 ± 5.12 | 42.12 ± 1.26 | 38.23 ± 3.76 | 41.77 ± 3.87 |
| MCV | fL | 57.25 ± 6.87 | 51.45 ± 2.95 | 49.92 ± 1.41 | 53.47 ± 2.97 |
| MCH | pg | 19.88 ± 0.63 | 18.82 ± 0.97 | 17.87 ± 0.55 | 18.93 ± 1.12 |
| MCHC | g/dL | 35.07 ± 3.56 | 36.58 ± 0.74 | 35.80 ± 0.47 | 35.43 ± 0.54 |
| Thrombocyte | $10^3/\mu L$ | 741.33 ± 91.43 | 788.5 ± 52.94 | 786.00 ± 105.94 | 783.67 ± 114.85 |
| RDW | % | 13.27 ± 2.15 | 13.15 ± 0.90 | 13.78 ± 0.83 | 13.35 ± 1.60 |
| MPV | fL | 5.35 ± 0.16 | 5.33 ± 0.15 | 5.18 ± 0.08 | 5.28 ± 0.13 |
| PDW | % | 15.82 ± 0.60 | 15.43 ± 0.19 | 15.45 ± 0.44 | 15.77 ± 0.48 |

* Differences are significant with normal groups, # Differences are significant with negative control groups($p < 0.05$).

the end of the test. The negative control group presented a higher number of leukocytes vs with the normal group. The same results were seen in lymphocytes, there were significantly more lymphocytes in negative group vs to the normal group. These results suggest MIA can make the hematologic profile of OA model rats change. Table 1 showed the ability of CEZO to decrease in leukocyte count. The leukocyte number in the positive group and the extract was significantly less than those in the negative group. Similar results were also seen in the lymphocytes and neutrophils number 14d after the treatment of the extract.

In addition to producing enzymes that break down cartilage and harm the cartilage matrix, monosodium iodoacetate has enhanced the release of cytokines into the joint cavity, including TNF-alpha, interleukin-1, and interleukin-6 (Lee et al. 2014; Di Paola et al. 2016). By preventing leukocyte binding to the protein selectin, which can suppress the inflammatory process and inhibit neutrophil migration, the flavone content in the extract is known to reduce leukocyte migration, which includes lymphocytes, macrophages, monocytes, and granulocytes, in inflammation (Suyenaga et al. 2014). This is also due to the process of inflammation that occurs is inhibited by flavones (Made et al. 2018; Sharma et al. 2021), anthocyanins (Sharma et al. 2020), mucilage (Fayek et al. 2021), panthenol (Kang et al. 2021), and tarin (Pereira et al. 2021) in *C. esculenta* L. and shogaol, gingerol, and paradol can also reduce inflammation by inhibiting COX-2 and PGE-2 in *Z. officinale* (van Breemen et al. 2011). Another study showed that the content of shogaol in *Z. officinale* demonstrated anti-inflammatory activity as indicated by a decrease in leukocyte levels in the knee synovial cavity in the CFA-induced monoarthritis rat model (Levy et al. 2006).

## Effect of spleen weight

The spleen distributes lymphocytes and leukocyte maturation throughout the body via the lymph nodes. As indicated in Table 2, we assessed the spleen of the OA rat model. The analysis's findings revealed a sizable difference between the treatment and negative groups. Comparing

**Table 2.** The effect of CEZO on the spleen.

| Group | Weight of spleen (mg) |
|---|---|
| Normal control group | 0.47 ± 0.06 |
| Negative control group | 0.84 ± 0.03* |
| Positive control group | 0.58 ± 0.02# |
| CEZO group | 0.60 ± 0.03# |

* Differences are significant with normal groups ($p < 0.05$), #Differences are significant with negative groups ($p < 0.05$).

the two groups, the positive group has the smallest spleen weight while the negative group has the greatest spleen weight. Treatment with CEZO demonstrated that it could reduce spleen enlargement. Leukocyte growth and production take place in the spleen. Through the lymph nodes, mature leukocytes will be disseminated throughout the body. Other rheumatic conditions, including rheumatoid arthritis, are associated with spleen enlargement. In order to remove circulating immune complexes, leukocytes are produced in the spleen, which is likely a similar mechanism to Felty's syndrome. Infection or OA in rats that have splenomegaly causes the spleen to continually release white blood cells as a response of systemic immune. Therefore, in OA, splenomegaly also occurs as indicated by the spleen average weight is bigger than normal rats, indicating that sodium iodoacetate can induce splenomegaly. Severe OA involves the spleen due to its close association with lymphocyte proliferation in the case of infection (Bahtiar et al. 2017). Comparing the groups in this research, the positive group has the smallest spleen weight while the negative group has the greatest spleen weight. Combining CE with ZO extract led to a reduction in spleen size. Zhang et al. showed that the shogaol content in *Z. officinale* showed anti-inflammatory activity which was indicated by a decrease in spleen weight in DDS-induced ulcerative colitis rat models (Zhang et al. 2018).

CEZO causes a significant decrease in NO rat's serum. Fig. 3 showed that the negative control group rats had higher serum levels of NO (390.305 ± 30.34 mmol/L) compared with the normal control group (84.287 ± 15.73 mmol/L), therefore the positive control and extracts groups showed lower serum levels of NO. In this study, there was no

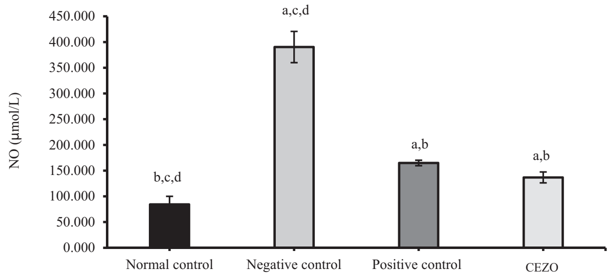


**Figure 3.** Decreased NO in rat serum after treatment with Sodium diclofenac and CEZO with p-value < 0.05 vs (a) normal group; (b) negative control groups; (c) positive control group (Sodium diclofenac) (d) CEZO.

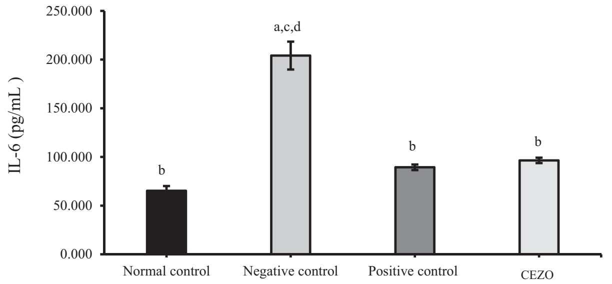


**Figure 4.** Decreased IL-6 in rat knee tissue homogenate after treatment with Sodium diclofenac and CEZO with p-value < 0.05 vs (a) Normal group; (b) negative group; (c) positive group (Sodium diclofenac) (d) CEZO.

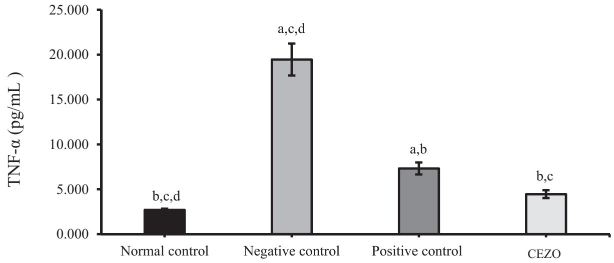


**Figure 5.** Decreased TNF- α in rat knee tissue homogenate after treatment with Sodium diclofenac and CEZO with p-value < 0.05 vs (a) Normal group; (b) negative group; (c) positive group (Sodium diclofenac) (d) CEZO.

significant difference between CEZO and diclofenac treatment (P > 0.05). These findings suggest that CEZO could recover high amounts of NO to alleviate inflammation during OA. *C. esculenta* contains Cyanidin 3-rhamnoside. This anthocyanidin can reduce COX-2, PGE-2, NO, and iNOS points by alleviating the NF-κB and IκBα expression (Kang et al. 2014). Nitric Oxide (NO) is a chemical mediator which act an essential role in inflammation process and degradation of articular cartilage. The role of NO in the inflammatory process is to promote leukocyte release of IL-1 and TNF-α, as well as vasodilation and capillary permeability. Meanwhile, the role of NO in articular cartilage degradation is by inducing apoptosis, inhibiting chondrocyte proliferation, collagen and proteoglycan synthesis, synthesis of IL-1 receptor antagonists, and stimulating MMP which will further degrade articular cartilage. MIA induction in rats has been shown to significantly increase NO levels in blood serum (Chien et al. 2016; Min et al. 2021). Other research have also found that the panthenol content in *CETE* has an anti-inflammatory activity which can be seen from decreasing the thickness of ear edema in TPA-induced dermatitis mouse models and suppressing the production of NO in LPS-induced RAW 264.7 cells. (Kang et al. 2021). Other study showed that the gingerol and shogaol content in *Z. officinale* showed anti-inflammatory activity which was characterized by a decrease in NO levels in RAW 264.7 cells induced by LPS (Dugasani et al. 2010).

## Effect on IL-6 level and TNF-α concentration

Fig. 4 revealed treatment CEZO can reduce IL-6 in the knee tissue of MIA model rats. The treatment group and the negative control group differed significantly from each other. Similarly, with serum, NO levels, IL-6 levels in the negative group had higher serum levels of NO (204.102 ± 14.27 pg/mL) compared to the control group (65.260 ± 4.87 pg/mL) (P < 0.05). This indicated monosodium iodoacetate could make an increased pro-inflammatory mediator IL-6. After treatment, IL-6 levels from CEZO (96.478 ± 2.74 pg/mL) were not a significantly different positive group (89.399 ± 2.89 pg/mL) and normal group (p < 0.05).

Fig. 5 showed that positive control and CEZO treated rats presented a lesser score of TNF-α. There are differences between the normal control (2.70 ± 0,22 pg/mL) and negative control (19.457 ± 1.78 pg/mL). These results showed that CEZO could recover high amounts of TNF-α compared to negative control and decreased inflammation during OA. CEZO also could recover TNF-α higher compared to positive group and significantly different (P < 0.05).

An influx of inflammatory cells and fluid entered the inflamed area as a result of the joint inflammation. IL-6 in OA pathology plays a role in osteoclast differentiation, and bone resorption also stimulates the production of receptor activators for NF-κB ligand, IL-1β, parathyroid hormone, and its related proteins, as well as PGE-2 (Mobasheri et al. 2021). The decreased joint edema in the test group showed that anthocyanins and flavonoids in CE and gingerol, shogaol, and paradol in ZO showed an anti-inflammatory impact by suppressing the inflammatory cells and pro-inflammation cytokines activity in this model rats. Pelargonidin 3-glucoside content in CE also inhibits phosphorylation of Mitogen-Activated Protein Kinase (MAPK), protein inhibitor of alpha (IκBα), and nuclear transcription factors in the form of nuclear factor-kappa B (NF-κB) and IL-1β-induced activator protein-1 (AP) -1). Thus, this anthocyanin stops the expression of TNF-α (van Breemen et al. 2011; Lv et al. 2016; Duarte et al. 2018; De Stefano et al. 2021). TNF-α is a proinflammatory cytokine that plays a role in inducing MMP production, expressing proinflammatory genes namely NF-κB, MAPK, and AP-1 (Molnar et al. 2021). Cyanidin 3-glucoside can decrease the IL-6, IL-1β, and TNF-α levels by inhibiting NF-κB activation and MAPK phosphorylation (Sun and Li 2018). The flavonoids isovitexin, orientin, isoorientin, vicenin-2, and luteolin 7-O-glucoside can all be found in CE. Orientin can reduce the proinflammatory mediators production such as TNF-α, IL-1β, IL-6, IL-18, COX-2, and iNOS (inducible nitric oxide synthase). These flavonoids act on the nuclear factor-kappa B (NF-κB) pathway which is a factor of pro-inflammatory transcription that will start the transcription and expression of pro-inflammatory genes, as well as on nucleotide like receptor protein 3 (NLRP3), a multiprotein complex made up of NOD-, ASC adapter protein, and caspase-1 that starts the expression of IL-1β and IL-18. Isoorientin can also reduce the production of proinflammatory mediators. These flavonoids work by upregulating the phosphorylation of glycogen synthase kinase 3β (GSK3β) at the amino acid ser9 (p-GSK3β) (Li et al. 2020). Flavonoid Isovitexin have mechanism of reducing the activation of the NF-κB pathway by suppressing the phosphorylation of MAPK and stopping the protein inhibitor of alpha (IκBα) phosphorylation (Lv et al. 2016). Luteolin 7-O-glucoside can reduce IL-1β by upregulating MAPK and stopping IL-1β-induced AP-1 and NF-κB activation (De Stefano et al. 2021). Vicenin-2 can reduce levels of TNF-α, IL-1β, and IL-18; also increased the expression of the anti-inflammatory cytokine IL-10 (Hassan et al. 2018). 6-shogaol is the most potent shogaol in *Z. officinale* compared to 6-gingerol, 8-gingerol, and 10-gingerol as an antioxidant and anti-inflammatory by inhibiting NO and prostaglandin E2 (PGE-2) mechanisms. However, 6-dehidroshogaol is more potent than 6-shogaol in terms of anti-inflammatory (Zhang et al. 2013). 8-shogaol and 10-shogaol also have the potential to inhibit COX-2, with 10-shogaol having a higher affinity for COX-2 than 8-shogaol. The shogaol and gingerol can be useful as antioxidants and anti-inflammatories, with 10-gingerol having the highest potential in inhibiting NO and prostaglandin E2 (PGE-2), as well as antioxidants. Only 10-gingerol and 12-gingerol can inhibit COX-2. 10-gingerol has a lower affinity for COX-2 than 12-gingerol (van Breemen et al. 2011).

### Effect on MMP-9 level

Fig. 6 showed that the positive control and CEZO treated rats showed a lower level of MMP-9 compared to the negative control. The negative control showed high levels of MMP-9 (362.787 ± 22.204 ng/mL), significantly different from the normal control group (97.053 ± 9.39) ($P < 0.05$). There was no significant difference between the positive control and CEZO. This indicates that treatment with CEZO is as good as diclofenac in reducing MMP-9. According to these findings, MMP-9 could be significantly inhibited by CEZO to stop cartilage breakdown caused by OA.

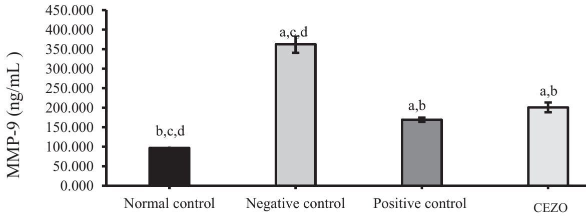


**Figure 6.** Decreased MMP-9 in rat knee tissue homogenate after treatment with Sodium diclofenac and CEZO with p-value < 0.05 vs (a) Normal group; (b) negative control group; (c) positive control group (Sodium diclofenac) (d) CEZO.

The CEZO retrieved a significant quantity of MMP-9 in this investigation to stop cartilage breakdown during OA. MMP-9 is a type of MMP that plays a role in excessive joint degradation in OA patients. Increased levels of MMP-9 caused by proinflammatory cytokines such as TNF-α and IL-6 stimulate chondrocytes to produce MMP (Messier et al. 2018). In OA, adjacent subchondral bones rub against each other so that they become brittle and stiff and experience a decrease in their ability to withstand loads. Subchondral bone will also release vasoactive peptides and MMPs, an enzyme that will degrade cartilage thereby aggravating cartilage damage. (Stefik et al. 2021).

## Conclusion

In conclusion, this study showed that the combination of *C. esculenta* tuber and *Z. officinale* rhizome extracts can reduce inflammation by decreasing pro-inflammatory mediators NO, IL-6, and TNF-α and reducing cartilage degradation by recovering MMP-9 in OA rats induced monosodium iodoacetate. Reduction of pro-inflammatory mediators by CEZO as well as diclofenac. Further investigations are underway in our research team to address the precise mechanisms involved for effective and safe usage.

## Conflicts of Interest

All authors affirm that there are no conflicts of interest.

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