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Potency chitosan-based microneedle Parem patch with Z*ingiber zerumbet* l. Extract in inhibiting pro-inflammatory(TNF-α and IL-1β) in arthritis

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Abstract

Arthritis is a condition that affects the joints, and it is caused by the production of proinflammatory cytokines and leading to discomfort in patients. According to the latest data from the Centers for Disease Control, in 2021 global incidence of arthritis over the past five years reached over 53.2 million. On the other hand, several studies have found that long-term use of arthritis medications can have side effects. Thisstudy aimed to investigate the effect of using microneedle *parem* patch in inhibiting proinflammatory factors, particularly for TNF-α and IL-1β. The experimental study is divided by three group, normal control,negative control, positive control, and microneedle patch. Patch was tested by in vivo in the form of rats injected with MIA (monosodium iodoacetate) and it was found that the microneedle *parem* patch could inhibit proinflammatory cytokine production (TNF-α and IL-1β) with an inhibition percentage of 30.01% for TNF-α and 70.99% for IL-1β. Therefore, it can be concluded that the microneedle *parem* patch can inhibit proinflammatory cytokine production (TNF-α and IL-1β) in arthritis.

Keywords: Microneedle; Arthritis; Chitosan; Zerumbone; Antiinflammation

1. Introduction

Arthritis is known as joint inflammation disease, one of the inflammation is caused by cytokine proinflammatory products therefore unpleasant therapy. Based on the Center for DiseaseControl 2021, the incidence of arthritis reached 53.2 million people, some of which are producedby pro-inflammatory cytokine products, causing discomfort in patients. Based on the latest data from the Centers for Disease Control in 2021, the incidence of arthritis in the world for five yearsreached more than 53.2 million people. In addition, as many as 44% of patients diagnosed with arthritis have limitations in daily activities (Centers for Disease Control, 2021). The target of arthritis treatment that has proven its benefits and efficacy to date is by inhibiting the pro- inflammatory cytokines TNF-α and IL-1β such as Nonsteroidal Anti-Inflammatory Drugs (NSAIDs). Corticosteroids and Disease Modifying Antirheumatic Drugs (DMARD) (Indonesian Rheumatology Association, 2014). However, several studies state that this treatment has quite serious side effects in the future, such as impaired heart function, alopecia, retinopathy, and neurological disorders (Srikartika, 2021). Apart from that, if we look at the pharmacokinetic effects, the use of oral drugs will reduce the bioavailability of the drug because it bypasses first- pass metabolism. Intravenous and intramuscular drug delivery also has an uncomfortable effect onpatients, thereby reducing the level of patient compliance in the treatment process (Splawski and Minger., 2016). Thus, there needs to be an alternative treatment that can be used as an option in treating arthritis.

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The herbal plant *Zingiber zerumbet* L., which is also known by the local name "LempuyangGajah" or shampoo ginger is used as an ingredient in phytomedicines or marketed herbal medicinesincluding *parem* (Silalahi et al., 2021). *Parem* shake is one of the empirical medicines that can beused to treat joint pain and swelling or inflammation which can be used externally or topically. (Kartika and Ariesti, 2018). Zerumbone is a natural monocyclic sesquiterpenoid with anti-inflammatory properties. The zerumbone content in *Zingiber zerumbet* has anti-inflammatory activity by inhibiting pro-inflammatory factors (TNF-α and IL-1β), so that several studies have suggested the optimum dose for anti-inflammatory is 350 mg/200 g BW (Sulaiman et al., 2010).

As time goes by, there have been advances in administering transdermal drugs with the development of needle micro-sized called microneedles. Drug delivery systems with microneedles are able to improve transdermal drug delivery by creating physical channels in the stratum corneumof the epidermis (Leo et al., 2014). The needles from microneedle preparations only penetrate the epidermis so they do not cause pain (Ariesti and Kartikasari, 2018). In general, microneedles madefrom water-soluble polymers are difficult to insert perfectly into the skin because of their wide needle shape and low mechanical strength. However, metal microneedles will become biohazardous sharp waste after use (Andrianov et al., 2011). Several researchers have developed microneedles made from chitosan polymer. Chitosan was chosen as the basic material formicroneedles because it is non-cytotoxic and biodegradable (Chen et al., 2013).

Based on the above background, research is proposed on chitosan-based *parem* patch microneedle biomaterial and shampoo ginger extract as an arthritis therapy with a mechanism forinhibiting pro-inflammatory factors (TNF-α and IL-1β) through a series of tests. The latest in ourresearch is combining shampoo ginger extract with chitosan solution in the form of modern *parem*or microneedle *parem* patch which is effective in inhibiting pro-inflammatory cytokines in arthritis cases. This creative idea is an effort to realize the third point of the Sustainable Development Goals (SDGs) regarding healthy living and community welfare.

2. Material and method

2.1. Extraction

Making Zingiber zerumbet L. extract using the maceration method. A total of 800 g of Zingiber zerumbet L. simplicia powder was soaked in 3.2 liters (1:4) of 96% alcohol, then left for24 hours and filtered. This process was carried out three times in repetition cycles. Next, the extractin filtrate form was concentrated using a vacuum rotary evaporator at a temperature of 50oC witha speed of 50 rpm and dried in an oven for 24 hours to produce a percentage yield of thick extract.

2.2. Phytochemical Screening

Phytochemical screening is a qualitative test to determine the content of secondarymetabolite compounds in extracts. Zingiber zerumbet L. contains sesquiterpenoid compounds (zerumbone) which have the effect of inhibiting inflammation (Sulaiman et al., 2010).

2.2.1. Making Chitosan Based Parem Patch Microneedle

Making microneedle patches begins by making the mold using 3D printing tools and resinmaterials. After the mold of the microneedle parem patch was successfully printed, the results werewashed using 90% isopropyl alcohol and dried. Next, the mold is irradiated with UV light for 10 minutes to ensure the mold is formed perfectly. After the mold is finished, the patch is made by mixing chitosan and acetic acid solution with a concentration of 10% with a ratio of 1 : 10 (m/v)

(Castilla-Casadiego et al., 2021). To speed up the chitosan dissolution process, the chitosan-aceticacid was dissolved on a hot plate at 70°C. The resulting chitosan solution with 10% acetic acid isthick. After that, the chitosan solution, 10% CMC (carboxymethyl cellulose) solution, andZingiber zerumbet L. extract (350 mg/200gr) were mixed with a mass ratio of 2:1:1 respectively (Trixie et al., 2023). After mixing thoroughly, the material was put into a mold and centrifuged ata speed of 4000 rpm for a duration of 30 minutes. The next step is to heat it on a hotplate at 50°Cfor 45 minutes.

2.3. Ethical Clearance

The in vivo test uses rats that have previously gone through an ethical test from the Ethics Commission, Airlangga University with an ethical number 0488/HRECCFODM/V/2024.

2.4. *In Vivo* Effectiveness Test with Biochemical Markers (ELISA)

The rats used were male Wistar rats aged 8-16 weeks, weighing 200 g. The rats were acclimatizedfor 1 week. The in vivo test treatment on the test animals was conducted by modeling arthritis through intra-articular injection of 0.6 mg MIA (Sigma-Aldrich) dissolved in 10 µL saline into theleft knee once within 14 days (Yamanashi et al., 2021). After the arthritis injection, the microneedle parem patch was applied to the arthritis area once a day for 14 days.

3. Result and discussion

3.1. ELISA Biomarker Test

The rat used were male Wistar rat aged 8 - 16 weeks, weighing 200 gr. Rat were acclimatized for 1 week. Test treatment in vivo in experimental animals was carried out by modeling arthritis via intra-injection articular 0.1 mL on the left knee once every 14 days (Yamanashi *et al*., 2021). After the arthritis is injected, a microneedle parem patch is attached to the arthritis area once a day for 14 days.

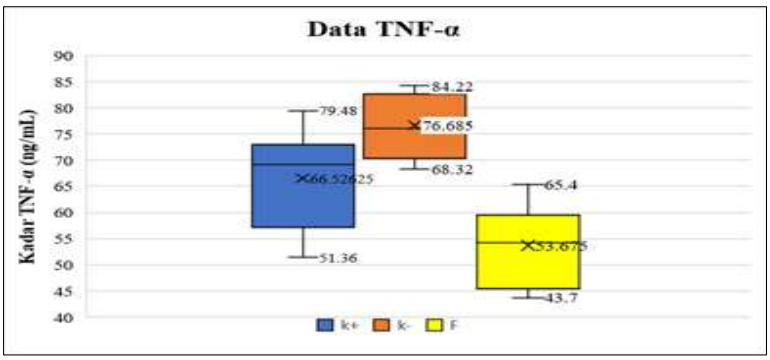

Figure 1 Level of TNF-α and IL-1β. **p < 0.05 significantly from control group

Table 1 Percentage inhibition of each group and average level TNF-α and IL-1β of the allreplication sample

	Drug Therapy	**Average Level**		**%Inhibition**	
		TNF-α	IL-1β	TNF-α	IL-1β
K-	**-**	76.685	3.809	-	-
K+	DiclofenacSodium	66.526	3.111	13.25%	18%
F	*Microneedleparem patch*	53.675	1.105	30.01%	70.99%
K- = negative control; K+= positive control; F= microneedle parem patch treatment					

Quantitatively, inhibition inflammation (TNF–α, and IL-1β) was measured by enzyme- related biochemical marker immunosorbent assay (ELISA). Normally, the levels of TNF-α and IL-1β in rat are 0.15 ng/ml and 0.23 ng/ml (Orhan *et al*., 2022). The levels of TNF-α and IL-1β in arthritis model mice were 15-20 ng/ml and 1,385 ng/ml (Li et al., 2018; Widyowati et al., 2023). Q0 TNF–α and IL-1β data in the K- treatment respectively 68.32 ng/mL and 2,628 ng/mL so that overall Quantitatively, it wasproven that there was an increase in the levels of TNF-α and IL-1β in the mouse model arthritis.

The results of the TNF-α and IL-1β data show a significance value of p>0.05 meaning thedata is normally distributed. The results of the one-way ANOVA test are available with significantdifferences with a significance value of p<0.05. Next, post hoc test significant differences betweenK+, K-, and F treatments were obtained from the data TNF-α and IL-1β. This test indicates that the levels of TNF-α and IL-1β experienced significant inhibition if given parem microneedle therapy patches made from chitosan and lempuyang extract.

In addition, inhibition of proinflammatory cytokines with percentage inhibition TNF-α andIL-1β from treatment given MIA and given therapy. Parem patch microneedle has a number that is very different from the preparation topical Diclofenac Sodium (Table 7). Therefore, parem microneedle preparation patches have higher effectiveness based on the percentage of inhibition levels of TNF–α and IL-1β. The zerumbone compound can suppress TNF-α and IL-1β in reducing arthritis progression (Doloking *et al*., 2023).

3.2. Histopathology Test

The progression of arthritis is also supported by histopathological test images (Table 2) with the criteria of cartilage density. F treatment had higher values than K- and K+, indicating thatbone degradation due to pro-inflammatory cytokines (TNF-α and IL-1β) was inhibited (Figure 2).

Table 2 Chondrocyte cell count results from each field of view

Sample	VF1	VF2	VF3	VF4	VF5	Mean
K-	27	36	26	33	25	29.4
K+	32	33	35	34	37	34.1
F	43	45	45	44	40	43.4
VF= view field; K- = negative control, without any treatment; K+= positive control, topikal sodium diclofenak, F= microneedle parem patch treatment						

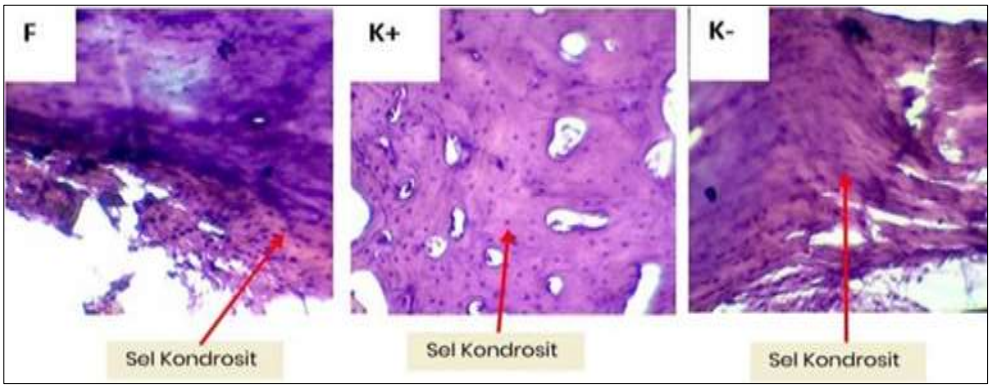

Figure 2 Field of view control group and rats with microneedle parem patch treatment from the calculation of the percentage of inhibition in Table 2,

zerumbone derived from zingiber zerumbet is a sesquiterpenoid compound that has the potential to inhibit TNF-α and IL- 1β in arthritis with various arthritis risks caused by an increase in TNF-α and IL-1β, which play arole in inducing MMP (metalloproteinase matrix) production, causing cartilage degradation(Figure 3). This is evidenced by the results of cartilage density observed quantitatively from chondrocyte cell counts. *Zerumbone* compounds derived from zingiber zerumbet also have a rolein inhibiting TNF-α and IL-1β in the cell repair process. The remodeling process due to cell damage is also inhibited by TNF-α and IL-1β by contributing to the role of osteocytes to signal osteoblasts to degenerate abnormal cells into osteophytes. Therefore, *zerumbone* compounds derived from zingiber zerumbet L. have the potential to inhibit the increase of TNF-α and IL-1β which also correlates in the inhibition of cartilage degradation.

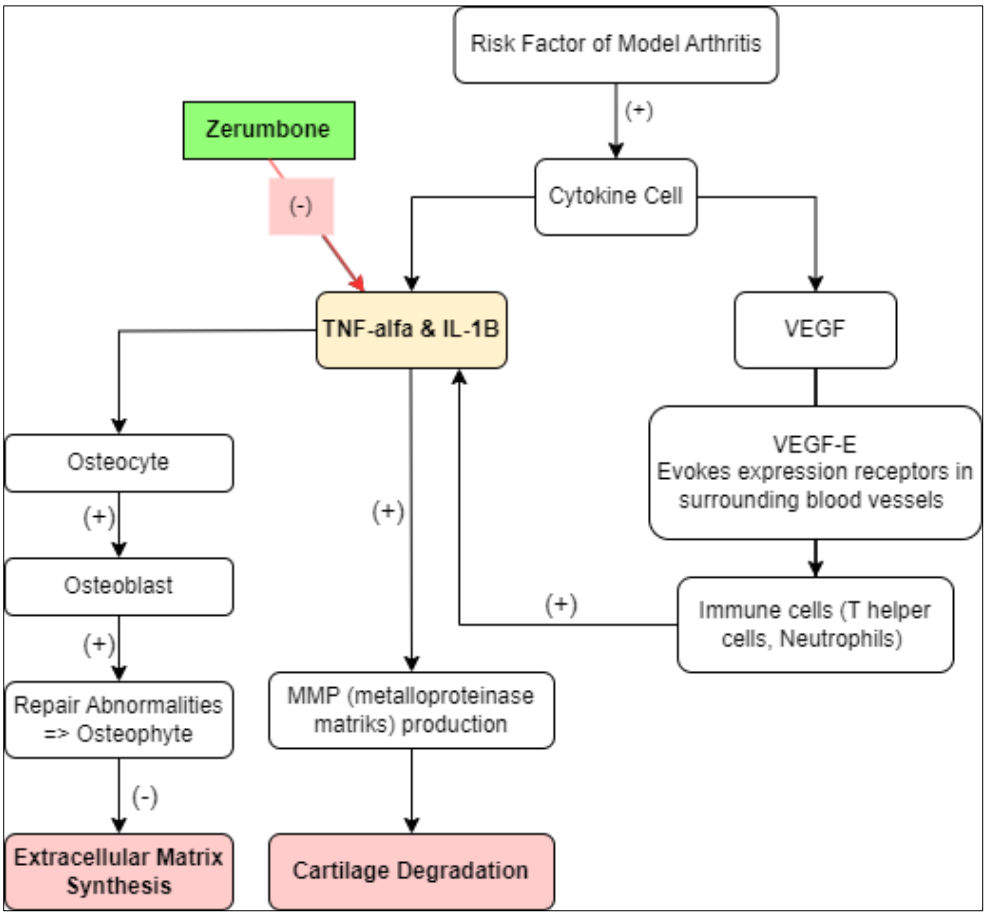

Figure 3 Cytokine cells stimulated by model arthritis risk factors. The role of TNF-α and IL-1β by degrading cartilage and making abnormalities in repair cells. This can be inhibited by the activecompound of zingiber zerumbet L., *zerumbone*.

Compliance with ethical standards

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Disclosure of conflict of interest

We have no conflicts of interest to disclose. All authors declare that they have no conflicts ofinterest.

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