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Original Article

DUMB CANE (*DIEFFENBACHIA SEGUINE* (JACQ.) SCHOTT) EXTRACT NANOEMULSION: PREPARATION, CHARACTERIZATION AND ITS ACTIVITY AS AN INFLAMMATION-INDUCING AGENT IN RATS

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ABSTRACT

Objective: This study aimed to characterize Dumb cane extract nanoemulsion for injection preparation and test its activity as an inflammation-inducing agent in rat intraplantar with IL-6 and TNF-alpha parameters in blood serum as well as the swelling response in paw.

Methods: Nanoemulsion was made from an emulsion base of olive oil, coconut oil, tween 80 propylene glycol, and water for injection, as well as varying concentrations of Dumb cane extract (1%, 2%, and 4%) using vortex mixing and sonication methods. The characterization included particle size, zeta potential, and polydispersity index using the Zetasizer tool. Induction was carried out intraplantar in male rats. The parameters observed were the volume of swelling in paw and IL-6 and TNF-alpha in blood serum.

Results: Dumb cane extract nanoemulsion concentrations of 1%, 2%, and 4% have particle size characteristics in the range of 20.3±0.17–30.1±0.68 nm, and zeta potential -31.4±1.59–33.1±1.33 mV. Dumb cane nanoemulsion can induce intraplantar inflammation with high IL-6 and TNF-alpha levels, significantly different from normal controls. Volume swelling occurred 4 h after intraplantar induction at a nanoemulsion concentration of 4%.

Conclusion: Dumb cane extract nanoemulsion can be an alternative agent for inducing intraplantar inflammation in rat models.

Keywords: *Dieffenbachia seguine*, Dumb cane, *Sri rejeki*, Nanoemulsion, Inflammation inducer agent

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INTRODUCTION

Every year, tons of inflammatory drug research and the development of new anti-inflammatory therapeutics are being made [1]. In order to do that research, inflammation inducers are needed to show the success of the therapeutic drugs. However, only a few natural or organic inflammation inducers are used in scientific research. Following this, there are many important reasons why inflammatory inducer agent research is still needed.

Dieffenbachia seguine (Jacq.) Schott (Dumb cane), previously known as *Dieffenbachia picta* (*D. picta*), is an ornamental plant called Dumb cane in the West with toxic properties often found as an indoor houseplant in tropical countries. *Dieffenbachia* is a genus of tropical flowering plants in the family of Araceae. In Indonesia, this plant is known as Sri Rejeki and referred to as Pisang Tanah in Malaysia. According to Alwan [2], consuming vast quantities of these in humans will result in an inability to talk for several days. Dermal exposure to the juice or cut stems of *D. picta* can cause edema, inflammation, and superficial ulceration [3]. This prompts the need for research to determine whether the inflammatory effect caused by the Dumb cane extract can be used as an agent to induce inflammation in animal models.

Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-alpha) are two types of pro-inflammatory cytokines produced by immune system cells in response to infection or tissue damage [4]. Measurement of IL-6 and TNF-alpha concentrations in blood serum can provide valuable information about the level of inflammation in an individual. When inflammation occurs due to infection or noninfectious inflammatory conditions such as arthritis, IL-6 and TNF-alpha are released into the blood to coordinate the inflammatory response and cause local and systemic changes.

Currently, more research is needed regarding this topic. This study measured the proper concentration of Dumb cane extract nanoemulsion to induce inflammation in wistar rats. The inflammatory parameters measured were the swelling volume in the rats' paws and the levels of IL-6 and TNF-alpha in the blood serum. This research aimed to study the potential of Dumb cane extract nanoemulsions as an inflammatory inducer in wistar rats as an animal model.

MATERIALS AND METHODS

Materials and reagents

The materials and apparatus used in this research are wistar rats from Animal laboratory Unit of Tropical Biopharmaca Research Center (Trop-BRC), Dumb cane (Trop-BRC Collection Garden, Bogor Indonesia), carrageenan (Merck), ethanol 96% (Bratachem, Indonesia), tween 80 (Bratachem, Indonesia), coconut oil (Barco®, Indonesia), olive oil (Bertolli®), propylene glycol (Bratachem, Indonesia), FineTest® Rat TNF-alpha (Tumor Necrosis Factor Alpha) ELISA Kit (China), and FineTest® Rat IL-6 (Interleukin-6) ELISA Kit (China).

Equipment

The equipment used in this research were a stirring rod, vortex, rotary evaporator, plethysmometer (Orchid, India), ultrasonic cleaner (BRANSON), water bath, autoclave, centrifuge machine and particle size analyzer (PSA) (HORIBA Scientific, SZ-100), Elisa rider (Labtron, lMPR-A20).

Plant sample and extract preparation

Dumb cane leaves were obtained from Biopharmaca Collection Gardens, IPB University. After removing the dust and debris by isolating and rinsing the leaves with distilled water, the leaves were cut into small parts with sharp knives. Afterwards, the drying process occurs in an oven at 45 °C for three days. Dried leaves were then finely ground in a blender, and Dumb cane simplicia was made. Dumb cane simplicia (150 g) was extracted in powder form and then macerated in 1500 ml of 96% ethanol. After four days of macerating, the extract was filtered. Evaporation process takes 30 min in a rotary evaporator.

Phytochemical screening

Phytochemical screening of extracts involved the examination of alkaloids, flavonoids, saponins, tannins, quinones, and steroids/ triterpenoids, employing the Harborne method [5].

Nanoemulsion preparation

There were four formulations, namely base, 1%, 2%, and 4%, with the composition shown in table 1 with modification from Hafizah *et al.* [6].

Table 1: Formulation of nanoemulsion

Ingredient (%)	Base	1%	2%	4%	Function
Dumb cane extract	-	1	2	4	Active compound
Olive oil	1.5	1.5	1.5	1.5	Oil phase
Coconut oil	1.5	1.5	1.5	1.5	Oil phase
Tween 80	11	11	11	11	Emulsifier
Propylene glycol	3	3	3	3	humectant
Water for injection	83	82	81	79	Water phase

Before all the ingredients were mixed, olive and coconut oil were combined in the same vial. The tween 80 and propylene glycol also were mixed in another vial. Both vials were vortex for one minute and then sonicated for 15 min in the ultrasonic cleaner. Afterwards, each amount needed for the concentration was homogenized using mortar and pestle (table 1). After homogenization, the mixture was poured into the glass bottle, respectively. Water for injection was added up to 100%. Lastly, each glass bottle was sonicated for 15 min and placed in a water bath at 37 ℃ for 30 min. The emulsion preparations can be stored at room temperature.

Droplet size, distribution and zeta potential

The particle size and zeta potential were assessed using Dynamic light Scattering (DLS) and particle Size Analyzer (PSA). This analysis was conducted at 25 ℃ using a disposable plain folded capillary. Before taking measurements, all samples were diluted with distilled water and subjected to 30 seconds vortexing to achieve an appropriate scattering intensity. Each measurement for samples was repeated two times at the same temperature. Particle size distributions and zeta potential were calculated using refractive indices of 1.54 for particles and 1.33 for water. These indices were applied to determine the parameters [7].

Animal studies

Twenty-four male Wistar rats weighing 190–240 g were obtained from the Animal laboratory Unit Trop-BRC**,** IPB University. The rats were maintained in a room with a controlled temperature of 26 ℃ and a 12 h light/dark cycle. They were given free access to food and water, which is 20 g of feed per day each. The animals were carried out according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations in conscious animals. All animal procedures were reviewed and approved (Protocol number: 009/KEH/SKE/I/2023) by the Animal Ethics Committee School of Veterinary Medicine and Biomedical Sciences, IPB University.

Inflammation induction in rats, grouping and dosing schedule

Twenty-four male wistar rats were divided into six groups (n=4). Group of (Water for injection/WFI), Group of base, and Group of positive control (1% of carrageenan), while the three treatment groups received 1%, 2%, and 4% concentrations of Dumb cane. Each group was induced with 0.1 ml for respective treatment groups through intraplantar injection. Paw thickness was measured just before the induction, at 0, 1st, 2nd, 3rd, 4th, and 24th h after the agent injection using a mercury plethysmometer. The rat's hind paw was immersed in the pool of mercury vessels. A slight rise in the mercury level in a pool will be transmitted to a drastic rise in the water in a pipette. An increase in paw thickness was measured and noted based on the water in pipette measure as the difference in paw thickness at "0 h" and paw thickness at respective hours. Edema volume was calculated from the difference in the volume of the rat's paw before and after injection. Then, proceed with the area under the curve (AUC) calculation for the total edema volume.

Quantification of inflammatory cytokines

Blood sample serum was used to analyze the level of cytokines using the enzyme-linked immunosorbent assay (ELISA) technique as per the test kit manufacturer's instructions (FineTest company, China) [8]. A Blood sample is collected from the heart by inserting the needle into the chest, bevel upward, and puncture the heart. The equipment needed is a 5 ml syringe with a 23G needle. Blood flow into the syringe if the needle is inserted into the heart. 1.5 ml of blood was taken from the rats in each group and placed in a microtube. The microtube was then centrifuged at 3.0 rpm for 10 min for the serum. After the fibrin clot is detached, the serum is carefully transferred into a sterile tube using a sterile pipette. The serum was isolated and kept in the freezer until further testing.

One hundred µl standard or sample is added into each well, and the plate is sealed and incubated for 90 min at 37 ℃. The plate is washed twice without immersion. Next, 100µl biotin-labeled antibody working solution is added into each well, the plate is sealed, and static incubated for 60 min at 37 ℃. The plate is washed 3 times and immersed for 1 time each time. After washing, SABC working solution is added into each well, sealed the plate, and static incubated for 30 min at 37 ℃. The plate is washed several times and immersed for 1 min in every wash. Then, 90µl TMB substrate solution is added to the plate and static incubated for 10-20 min at 37 ℃. Finally, 50µl stop solution is added, and the result is read at 450 immediately and calculated.

Statistical analysis of data

All data were analyzed using the SPSS version 27 program. Data analysis was carried out using one-way analysis of variance (ANOVA) with a completely randomized plan (RAL) at a confidence level of 95% with a P value α=0.05, followed by the Duncan test method-data visualization using the GraphPad Prism 7 application.

RESULTS AND DISCUSSION

Plant extraction and phytochemical screening

Simplicia, extract, and nanoemulsion of Dumb cane (fig. 1). The results of the phytochemical analysis showed that the ethanol extract of Dumb cane contained flavonoids, saponin, and steroids using 70% ethanol solvent. The phytochemical contained in the present results was different from previous reports. In previous research, the presence of alkaloids, cardiac glycosides, flavonoids, reducing sugars, and tannins was detected, whereas saponins and anthraquinones were not detected using 70% methanol solvent [9]. The variations in results can be attributed to the choice of extract solvent, which significantly influenced the extraction of bioactive compounds.

Droplet size analysis, pH and zeta potential

Based on the results of the sample characteristics, the pH measurements showed that the Dumb cane extract nanoemulsions and the base emulsion had a pH range of 5.8-7.01. The particle size analyzer (PSA) was used in this research to examine the particle size parameters. The test results showed the mean value of 1% extract was 21.86 nm, while 23.03 nm for 2% extract, 30.13 nm for 4% extract, and 386.5 nm for the base emulsion (table 2). A zeta potential analysis was carried out to evaluate the stability of a colloid during storage. The test results showed that the zeta potential values of 1% and 2% extract were -31.4 mV. The 4 % extract and base zeta potential were -33.06 and -49.5 mV, respectively. These values provide insights into the electrical charge and potential stability of the colloidal systems.

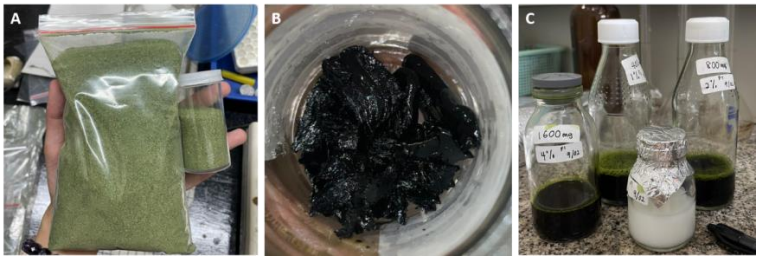

Fig. 1: Powdered leaves (a), extract (b), and formulation (c) of Dumb cane

Table 2: Characterization of *D. Sequine* extract nanoemulsions

Sample	pH	Particle size* (nm)	Zeta potential* (mV)
Base	7.01	386.5±8.85	-49.5±0.36
1% extract	5.8	20.3±0.17	-31.4±1.59
2% extract	5.8	23.0±0.55	-31.4±1.45
4% extract	5.9	30.1±0.68	-33.1±1.33

*Data are represented as mean±SD (n=3)

Injectable solutions are designed for intravenous, intramuscular, or subcutaneous injection. Parenteral dosage forms' key concerns are formulation stability, drug substance compatibility, and adequate drug concentration within a suitable pH range without implementing excipient levels, resulting in blood incompatibility and tissue irritation issues [10]. These parameters demand a thorough drug characterization. Based on table 2, the results of pH measurements showed that the Dumb cane extract and the base solution had a pH range of 5.8-7.01. The pH measurements are carried out to determine whether the solution is safe for the rat paw. The skin's epidermis provides a fundamental barrier against water and nutrient loss. The acid mantle is a layer of defense covering the epidermis's surface, the stratum corneum. Changes in pH can negatively impact the acid mantle, making the skin sensitive and easily infected with bacteria [11]. The more the pH fluctuates, the more inflamed the skin becomes, which can result in false results in this research. According to Roethlisberger *et al.* [12], pH levels should remain within the target pH range of 3.5-9.0 to minimize the risk of tissue damage. This indicates that all sample emulsions used in this research are reasonably safe since the pH range is between the reliable values.

The Particle Size Analyzer (PSA) is used in this research to examine the particle size parameters of Dumb cane and base nanoparticles. According to Zielińska *et al.* [13], this approach is done to analyze the particle size in a sample and the distribution of a representative sample. The PSA employs dynamic light scattering to quantify the size distribution of particles moving in Brownian motion. Furthermore, since light has a very rapid propagation speed, it could convey the results in a very short period [14]. As mentioned by McClements [15], nanoparticles are particles ranging from 10 to 1000 nm formed of natural or synthetic polymers that may be utilized as a drug carrier's method of dissolving, trapping, absorbing, or pasting active compounds. Mathers *et al.* [16] also mentioned that small particle size enhances particle movement, which prevents sedimentation. Based on table 2, the mean value of 1% extract is 20,3 nm, while 23 nm for 2% extract, 30,1 nm for 4% extract, and 386,5 nm for the base emulsion. This result indicates that the sample used in this research is nanoparticles.

A potential zeta analysis was carried out to evaluate the stability of a colloid during storage. The zeta potential determines the electro-kinetic potential of particles and molecules in a liquid medium. It quantifies the amount of the electrical charge that surrounds particles in a liquid medium. Vanderfleet and Cranston [17] state that a high zeta potential value indicates that the sample is very stable and resistant to aggregation. Based on table 2, the zeta potential value of 1% and 2% extract is-31.4 mV, while for 4% extract is-33,1 mV. Nanoparticles having zeta potential with a value ranging from-10 mV to+10 mV are regarded as neutral, whereas nanoparticles with zeta potentials more than-30 mV to+30 mV are termed highly cationic and anionic, respectively [18]. As for the base, the emulsion is considered high since the zeta potential value for the base is -49,5 mV. Based on Sah *et al.*'s [19] studies, a zeta potential with highly cationic and anionic properties indicates that the particle is more likely to resist one another and remain suspended in the liquid medium rather than clumping. The higher the zeta potential value, the better the particles are stable in the liquid medium.

Inflammation induction intraplantar

Based on table 3, the edema started to appear one hour after the *in vivo* induction. The edema peaked at the 2nd h, except for 1%, 2%, and 4% extract nanoemulsion groups because the volume still increased until the 4th h. As expected, water for injection (WFI) and base emulsion groups were not significantly changed because both were the control groups. The highest mean paw volume for the carrageenan group was observed in the 2nd h, which was 0.70 ml. The mean paw volume in 1%, 2%, and 4% extract groups at the 4th h were 0.66 ml, 0.70 ml, and 0.70 ml, respectively. The Duncan test was used because a significant difference in the ANOVA results on H1, H2, H3, H4, and H24 was detected. In H0, all treatment groups did not have any significant difference. The 1%, 2%, and 4% extract groups differed significantly from WFI and base groups in H1, H2, H3, and H4, except the 1% extract group was not significantly different from the WFI group in H3. However, the 1%, 2%, and 4% extract groups showed an insignificant mean paw compared to the carrageenan control group in H1, H2, H3, and H4. The results showed that 1%, 2%, and 4% extract injection induced inflammation, comparable to carrageenan for H1-H4 periods. Interestingly, the mean paw volume in the 4% Dumb cane extract nanoemulsion group showed a significant difference from the WFI and base group in H24 but an insignificant difference compared to the carrageenan control and 2% Dumb cane extract nanoemulsion groups.

The inflammatory inducer efficacy was obtained by comparing the total area under the curve (AUC) value of the treatment group with the carrageenan control group. Based on fig. 2, the lowest AUC value was the base emulsion with values of 12.17 ml, which had no significant difference between WFI and 1%. The highest value of AUC was 4% extract with values of 15.32 ml, which had no significant difference with 2% and carrageenan. The higher the AUC value, the stronger the inflammatory induction effects. Based on the results, the base emulsion did not impact the paw volume because the AUC value was nearly around the WFI value. This indicates that 2% and 4% were genuinely affected by the Dumb cane itself. As shown in fig. 2, carrageenan, 2%, and 4% Dumb cane extract nanoemulsion do not have any significant difference.

A mercury plethysmometer, which generally works based on Archimedes' law, is used during the induction test. The volume of

fluid displaced is a force equal to the volume of the body. The extract nanoemulsion groups give similar results to Kuballa *et al.* [20] and Ajuru *et al.* [21] research when the swelling of the hind paw in rats treated with Dumb cane extract nanoemulsions peaked at 4 h after injection and slowly decreased after the 24th h (table 3). On the other hand, the carrageenan group gives similar results to Tandoh *et al.* [22] and Solanki *et al.* [23] research, which stated that the carrageenan control group reached the highest edema volume in the second hour after the induction and gradually decreased after 4 h. and bradykinin (0-1 h), there is edema correlated with an increase in prostaglandins and inducement of COX2 enzyme release in the injected paw during the second stage (1-6 h).

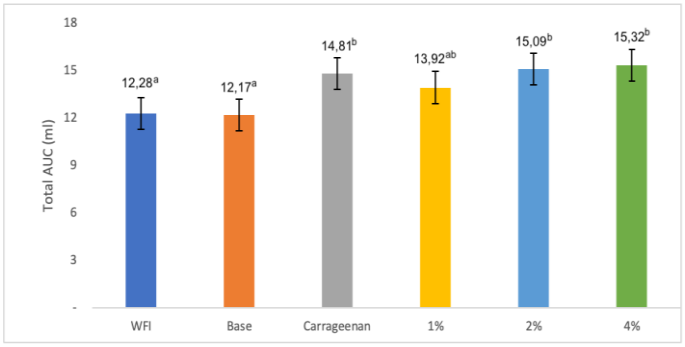

Fig. 2: The total area under the curve (AUC) on edema volume in wistar rats after 24 h, Data are represented as mean±SD (n=4). WFI: Water for Injection; Base: Induction by base of emulsion; Carrageenan: Positive control; NA1%: *D. sequine* extract nanoemulsion 1%; NA 2%: *D. sequine* extract nanoemulsion 2%; NA 4%: *D. sequine* extract nanoemulsion 4% (one-way analysis of variance (ANOVA) with a completely randomized plan (RAL) at a confidence level of 95% with P value α=0.05)

Table 3: Evaluation of edema volume through intraplantar injection in Wistar rats

Treatments group (n = 4)	Mean paw volume (ml)±SD					
	H0	H1	H2	H3	H4	H24
WFI	0.48±0.063^{a}	0.47±0.050^{a}	0.51±0.025^{a}	0.52±0.028^{ab}	0.56±0.025^{a}	0.47±0.028^{a}
Base	0.45±0.040^{a}	0.51±0.047^{a}	0.52±0.050^{a}	0.52±0.064^{a}	0.53±0.025^{a}	0.47±0.028^{a}
Carrageenan	0.50±0.000^{a}	0.60±0.070^{b}	0.70±0.070^{b}	0.67±0.064^{cd}	0.67±0.050^{b}	0.55±0.040^{ab}
1% extract	0.47±0.050^{a}	0.61±0.025^{b}	0.63±0.025^{b}	0.60±0.000^{bc}	0.66±0.025^{b}	0.48±0.075^{a}
2% extract	0.51±0.047^{a}	0.66±0.075^{b}	0.66±0.062^{b}	0.66±0.062^{cd}	0.70±0.070^{b}	0.55±0.057^{ab}
4% extract	0.48±0.075^{a}	0.62±0.064^{b}	0.66±0.062^{b}	0.68±0.075^{d}	0.70±0.091^{b}	0.57±0.050^{b}

Description: H0 = Hour 0, H1 = Hour 1, H2 = Hour 2, H3 = Hour 3, H4 = Hour 4, H24 = Hour 24. Data are presented as mean with a standard deviation (mean±SD) (n=4). The same superscript letter in the same column indicates no significant difference (P<0.05) between groups.

Inflammatory cytokines

The graphs showed changes in Wistar rat blood serum IL-6 and Tnf-alpha in response to Group of (Water for injection/WFI), Group of the base, and Group of positive control (1% of carrageenan), 1%, 2%, and 4% concentrations of Dumb cane (fig. 3). These parameters are observed at the 4th h of induction due to its highest edema volume in the intraplantar inflammatory response.

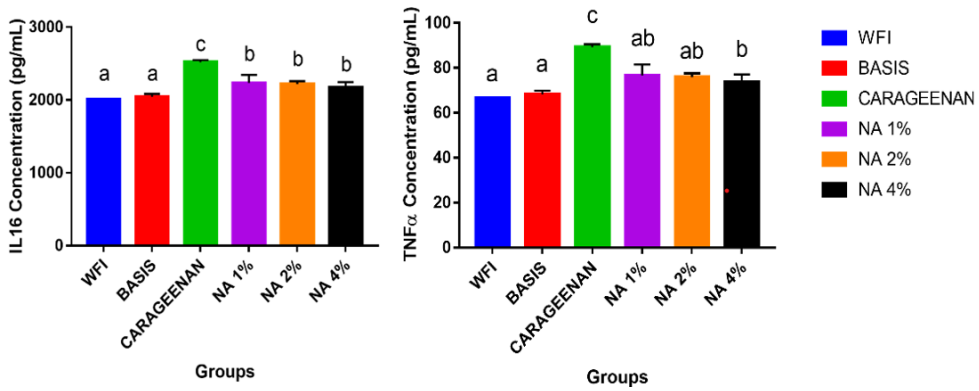

Fig. 3: level of IL-6 and TNF-Alpha in wistar rats' serum, Data are represented as mean±SD (n=4). WFI: Water for Injection; Basis: Induction by base of emulsion; Carrageenan: Positive control; NA1%: *D. sequine* extract nanoemulsion 1%; NA 2%: *D. sequine* extract nanoemulsion 2%; NA 4%: *D. sequine* extract nanoemulsion 4% (one-way analysis of variance (ANOVA) with a completely randomized plan (RAL) at a confidence level of 95% with a P value α=0.05)

IL-6 of 1%, 2%, and 4% Dumb cane extract nanoemulsion were not statistically significantly different between groups, but all groups were significantly different from the WFI and base groups. IL-6 levels in the treatment group were higher than in the control group (WFI and Basis). These results indicate that induction of Dumb cane nanoemulsion extract can increase cytokine levels in blood plasma,

which indirectly confirms that an inflammatory process has occurred. Likewise, TNF-alpha levels also showed an increase compared to the WFI and baseline control groups, but the Dumb cane extract nanoemulsion 4% group was significantly different. In contrast to the AUC value of the treatment group, which was not significantly different from the carrageenan group, the cytokine levels of IL-6 and TNF-alpha in the carrageenan group were significantly different from the treatment group.

Carrageenan acts as an irritating chemical and causes cell damage by releasing mediators that trigger the inflammatory process. The active component that acts as an inflammatory inducer is poligeenan. As soon as this happens, inflammatory mediators are released, and edema peaks for several h. According to Mota *et al.* [24], carrageenan acts in two phases to cause edema, which first releases inflammatory mediators such as histamine and serotonin.

The critical concern of parenteral dosage forms is formulation stability and adequate drug concentration within a suitable pH range and without implementing tissue irritation issues [10]. According to Roethlisberger *et al.* [12], pH levels should remain within the target pH range of 3.5-9.0 to minimise the risk of tissue damage. This indicates that all sample emulsions used in this research are reasonably safe since the pH range is between the reliable values. Mathers *et al.* [16] also stated that small particle size enhances particle movement, which prevents sedimentation. The particle size result indicates that the sample used in this research is nanoparticles. As well as the zeta potential value, the sample used is stable. Vanderfleet and Cranston [17] state that a high zeta potential value indicates that the sample is very stable and resistant to aggregation. Nanoparticles with zeta potentials more than-30 mV to+30 mV are termed highly cationic and anionic, respectively [18]. Base emulsion controls Dumb cane treatments to ensure the inflammation comes from the extract itself and not from olive oil, coconut oil, tween 80, and propylene glycol.

Dieffenbachia is the most toxic genus in the araceae family. As stated by ummuhan [25], the inflammatory response caused by Dumb cane is raphides, oxalic acid, and protease enzymes. Dumb cane contains high amounts of calcium oxalate crystals, which have a poisoning effect of calcium oxalate crystals that can cause swelling, a temporary burning sensation, excessive salivation, and inability to speak [26]. Even though the mechanism of raphides causing inflammation is still not completely understood. However, raphides are very sharp crystals that can penetrate cells and tissues, causing the macrophages to come to the infected area and digest the active compounds. In response to the infection, macrophages produce cytokines interacting with the capillary endothelial cells. The damaged tissue cells in the region of inflammation then produce bradykinin, which aids in loosening the capillary cells. According to Iyer *et al.* [27], during this process, bradykinin attaches to mast cells and then releases histamine to increase the permeability of blood vessel walls. Histamine reaction leads to symptoms like itching, redness, swelling, and increased mucus production. When bradykinin binds to a capillary cell, it stimulates the production of prostaglandins, which excite nerve endings, producing pain. This might be why the wistar rats in the treatment groups showed signs of fast breathing, restlessness, and scratching within four hours of administration, and Ajuru *et al.* [21] also got similar results.

Suppose we combine the swelling volume and cytokine parameters of the three concentrations of nanoemulsion extract. In that case, the most optimal for inducing intraplantar inflammation in the paw of Wistar rats is Dumb cane extract nanoemulsion 4% because the TNF-alpha is significantly different from the WFI and base groups, whereas at 1% and 2% is not significantly different. However, for swelling volume and IL-6 parameters, Dumb cane extract nanoemulsion 2% was not significantly different from 4%. Although the cytokine parameters of the nanoemulsion group are still smaller than those of 1% carrageenan, Dumb cane extract nanoemulsion can be an alternative material for intraplantar inflammation induction in the paw of rats with gout or arthritis models.

CONCLUSION

Intraplantar induction of Dumb Cane-extract Nanoemulsion significantly amplifies paw swelling volume and elevates IL-6 and TNF-alpha levels in the blood serum, particularly 4 h post-induction. This nanoemulsion derived from Dumb Cane extract emerges as a promising alternative agent for inducing intraplantar inflammation in animal models.

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Nil

AUTHORS CONTRIBUTIONS

Siti Sadiah: Conzeptualitation, Design methods, Supervised the findings of this work; Nida Nazeera Japri: Preparation and extraction, Nanoemulsion, Inflammation test *in vivo*; leong Ke Yong: blood serum test; Deni Widaya lukman and Ni luh Putu Ika Mayasari: Verified the analytical methods; Hamzah Alfarisi: Statistic analysis; leliana Nugrahaning Widi: Attending veterinary induction inflammation (*in vivo*); Dewa Ayu Regina Amel: attending veterinary blood sampling. All authors discussed the results and contributed to the final manuscript.

CONFLICT OF INTERESTS

The authors have no conflict of interest associated with the material presented in this paper.

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