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Research Article

Effect of Cacao Bean Extract as a Paracetamol Adjuvant on Pain Scale and Tumor Necrosis Factor-Alpha in Neuropathic Pain: An Animal Model Study

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ABSTRACT

Introduction: One treatment for neuropathic pain is paracetamol. Meanwhile, cacao bean extract is a traditional remedy developed for pain management. **Objective:** Analyzing effect of combining cacao bean extract and paracetamol on pain scale and tumor necrosis factor-alpha (TNF-α) in neuropathic pain. **Methods:** Subjects were randomized post-test only control group design from 28 mice (Mus musculus) to 4 groups: G_0 (control), G_1 (paracetamol only), G_2 (cacao + paracetamol), and G_3 (cacao + ½ doses paracetamol). The subject assessed pain scale using von Frey test and TNF-α. The statistical analysis includes paired t-tests, Wilcoxon, one-way ANOVA, Kruskal Wallis, and Pearson correlation tests with p <0.05. **Results:** The combination of cacao bean extract and paracetamol resulted in a pain scale of 2.57 ± 1.10 gf, with significant differences observed among the four groups (p <0.001). Significant differences in pain scale scores were found in four groups (p <0.001), including G_0 (p = 0.006), G_1 (p <0.001), G_2 (p <0.001), and G_3 (p <0.001). After treatment, the average TNF-α levels was 86.96 ± 23.73 ng/mL, with significant differences observed among the four groups (p <0.001). There was a strong correlation between the pain scale and TNF-α levels (p <0.001). **Conclusion:** In an animal model of neuropathic pain, using cacao bean extract as a paracetamol adjuvant significantly reduces pain scale (as measured by the von Frey test) and TNF-α levels.

Keywords: Cacao, neuropathic pain, pain scale, paracetamol, TNF-α.

INTRODUCTION

Pain is an unpleasant sensory and emotional experience caused by the presence or prospective injury to tissue, or by a condition that explains the tissue damage [1]. Neuropathic pain is caused by injuries or disorders of the somatosensory nerve system. This pathological state has a significant impact on patients' quality of life and psychological well-being [2]. The global prevalence of neuropathic pain is estimated to be between 3 to 13%, but when concomitant illnesses such as diabetes (26%), shingles (19%), and postoperative pain (10%) are included, the prevalence might be significantly greater [3]. A study in 13 Indonesian hospitals found that out of 8,160 pain patients, 1,779 (21.8%) had neuropathic pain [4].

Neuropathic pain can arise from both central and peripheral causes. Central pain can be detected in stroke or post-spinal trauma patients, whereas peripheral mechanisms occur in nervous system tissues. Neuropathic pain can be caused by peripheral nerve lesions, particularly those in the C nerve fibers, or by central nervous system malfunction. When a typical stimulus is present, stimuli that would ordinarily not produce pain are perceived as pain (allodynia) or an excessive response (hyperalgesia) [5]. The pathophysiology of neuropathic pain is defined by a neuroinflammatory response that develops following the activation of the nonspecific immune system (innate immune system). TNF-α, a proinflammatory cytokine, is directly linked to neuropathic pain [6].

TNF-α is a proinflammatory cytokine that regulates immunity, cell proliferation, and apoptosis [7]. TNF-α may contribute to neuropathic pain at both peripheral and central levels, according to certain study. A study found a link between higher TNF-α levels and pain severity [8].

One of the treatments for neuropathic pain is paracetamol, which is the most often used analgesic medication in pain management [9]. Pharmacological therapy is beneficial for less than 50% of patients with neuropathic pain [10]. Pain therapy with traditional medicine became popular and became an option since it is not only inexpensive and easy to obtain, but it also has very few negative effects. Cacao is a plant that can be used to relieve pain. Cacao includes polyphenols, catechins, epicatechins, polymeric procyanidins, procyanidin B2, and other flavonoid compounds. These compounds can inhibit pain-inducing pro-inflammatory immune reactions, for example polyphenols can inhibit the effects of Prostaglandin E2 (PGE2), TNF-α, interleukin-6 (IL-6), and interleukin-1 beta (IL-1β), epicatechin can inhibit TNF-α and IL-6, and procyanidin B2 and catechins can reduce TNF-α [11].

Cacao is known to alleviate neuropathic pain, as an earlier study has shown that cacao has antihyperalgesic and oedema inhibitory effects on mice produced with Complete Freud's Ajduvant (CFA) interplanetary [12]. Based on the explanation above, our study aims to examine how administering cacao as a paracetamol adjuvant affects pain scale and TNF-α levels in neuropathic pain.

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METHODS AND MATERIALS

Animal Subjects

The experimental animals in this study were mice (Mus musculus). The animals were adapted for seven days in a clean cage. During this time, mice (Mus musculus) were fed and watered in sufficient quantities. Illumination was similarly controlled by a 12-hour cycle of light and darkness, with the light cycle beginning at 6:00 a.m. and ending at 18:00 p.m. Before being tested, the experimental animals were checked to verify they were in good health, with clean eyes, no standing hair, and a relatively consistent weight [13]. Healthy male mice (Mus musculus) with a body weight of 20-25 g are eligible to participate. Dead animals were excluded, as was developing neuropathic discomfort.

Study Designs and Protocols

This study is a true experiment with a randomised post-test-only control group design. The total number of study subjects was 28 mice (Mus musculus), which were randomly divided into four groups of seven mice each. The groups were coded sequentially as follows: G_0 receives 0.9% NaCl (control), G_1 receives only paracetamol, G_2 receives both paracetamol, and cacao bean extract, and G_3 receives half of the paracetamol conversion and the entire dose of cacao bean extract. The trial ran from January to June 2024.

This study's stages included developing animal models of neuropathic pain, creating analgesic suspensions, intervening, and evaluating pain levels. Analgesic suspensions were prepared by suspending carboxymethylcellulose sodium (CMC-Na) 1%, cacao bean extract, and paracetamol. 1% CMC-Na was used to dilute the cacao bean extract and paracetamol suspension. The mice (Mus musculus) were treated with an analgesic suspension, with the dose and type of analgesic varying between groups. Group G_0 only received 0.9% NaCl, group G_1 received paracetamol with a conversion dose, group G_2 received paracetamol with a conversion dose and cacao bean extract at a dose of 1 mg/g, and group G_3 received a half dose of paracetamol conversion and 1 mg/g cacao bean extract. The subjects received therapy 1×/day for 7 days. Pain monitoring used pain observe and TNF-α levels, in which pain observe was conducted twice, before and after observation, while TNF-α levels were only tested once after therapy.

Animal models of neuropathic pain

The chronic construction injury (CCI) paradigm was developed to induce neuropathic pain in mice (Mus musculus). Mice (Mus musculus) were given rat cocktail anesthesia (Ketamine/Xylazine/Acepromazine) at doses of 60 mg/kg, 7.5 mg/kg, and 1.0 mg/kg intraperitoneally, respectively. After a skin incision, the right sciatic nerve was cut in the mid-thigh and freed of adhering tissue near the trifurcation. Four right sciatic nerve ligations were performed using 5.0 monofilament safyl thread with a diameter of 1/3 to 1/2 of the right sciatic nerve [14]. After 7 days, neuropathic pain was assessed using an electronic Von Frey filament (Ugo Basile Co., Varese, Italy), with neuropathic pain defined as effectively established when the subject pulled or jerked his hind leg soon after the stimulation was ceased.

Preparation of Analgetic Suspension

In order to prepare 1% carboxymethylcellulose sodium (CMC-Na), we boiled 100 mL of distilled water before pouring in 1 g of CMC-Na powder and stirring until a homogenous suspension emerged. When the aquadest evaporated during the process, more aquadest was added until the solution was uniform and measured 100 mL.

Cacao bean extract suspension was made by extracting cacao bean material from the Coffee and Cocoa Research Centre (Puslit Koka) in Jember, Indonesia with 70% ethanol. The suspension contained 1,000 mg of cacao bean extract and 10 mL of 1% CMC-Na suspension, which was gradually combined until a homogeneous suspension formed with a dosage of 100 ng/mL [15,16].

Paracetamol suspension was produced from 500 mg paracetamol tablets (pim pharmaceutical Ltd, Pasuruhan, Indonesia), which were ground into powder and given at a dose of 100 mg. The ingredients for paracetamol suspension were 100 mg paracetamol powder and 10 mL of 1% CMC-Na suspension, which were gradually mixed until a homogenous suspension with a dosage of 10 ng/mL developed. The administration of paracetamol in mice (Mus musculus) used a conversion dose of 15×12.3 mg/kg = 185 mg/kg [17].

Pain Score

The subject's pain scale was measured using the electronic Von Frey filament (Ugo Basile Co., Varese, Italy) method, resulting in a pain scale value ranging from 0 to 5 gramforce (gf). The electronic Von Frey filament (Ugo Basile Co., Varese, Italy) works by providing a force stimulation to the subject's foot, to which the subject responds by pulling or jerking when the stimulus is discontinued. Furthermore, the subject's reaction is automatically recorded concurrently. The lower the force applied to the subject reflects a high pain scale score [18]. The pain scale was observed three times: before CCI, 7 days after CCI for confirmed neuropathic pain, and 7 days after treatment (administration of cacao bean extract and paracetamol combination therapy), with a 7-day rest between each pain scale observation.

Tumor necrosis factor-alpha (TNF-α) measurement

Blood was obtained from the experimental animals by putting a syringe directly into their hearts and gently aspirating. The serum was then obtained by centrifugation (3000 rpm for 15 minutes) at room temperature. TNF-α levels in mouse serum were measured using a 1775×Mark™ ELISA equipment (catalog number E0117Mo, Bioassay Technology Laboratory, Shanghai Korain). Results were analyzed using the 1775×Mark™ ELISA reader system at 450 nm (Bio-Rad Laboratories, Inc. Hercules, California, USA) [19]. TNF-α levels were reported as ng/mL [20].

Statistical Analysis

The data were analyzed with IBM SPSS Statistics software version 23.0 (IBM Corp., Armonk, NY, USA). Bivariate analysis employed the Anova test, while if the data distribution was not normal and homogenous, the Kruskal Wallis test might be utilized. Spearman and Pearson correlations were used to analyze the relationships between variables. Furthermore, to establish the efficacy of therapy or treatment, data analysis utilizing paired t-tests or Wilcoxon depending on data distribution. The researcher used a significance level of 5%, indicating that a value of p <0.05 is statistically significant.

RESULTS

Neuropathic pain confirmation

The first stage of pain scale assessment yielded a pain scale score of 4.27 ± 0.41 gf, with a median of 4.18 (3.91 – 4.56) gf. The lowest pain scale number was 3.78 gf, and the highest was 4.96 gf. The pain scale values in each group were G_0 = 4.20 ± 0.45 gf, G_1 = 4.36 ± 0.45 gf, G_2 = 4.22 ± 0.35 gf, and G_3 = 4.30 ± 0.46 gf (f = 0.205; p = 0.892). According to the von Frey test interpretation, the patient fell into the mild pain scale category during the first stage of pain scale evaluation. The second stage pain scale value was 1.21 ± 0.25 gf, with a median of 1.20 (0.99 – 1.45 gf). The pain scale had a minimum value of 0.81 gf and a maximum value of 1.62 gf, indicating that the animal model of neuropathic pain in mice (Mus musculus) was successfully established. The second stage pain scale yielded G_0 = 1.13 ± 0.23 gf, G_1 = 1.26 ± 0.27 gf, G_2 = 1.27 ±

0.27 gf, and G_3 = 1.19 ± 0.26 gf (f = 0.439; p = 0.727; Table 1). These findings suggest that the pain scale levels in each group were quite comparable.

The difference in pain scale values between stages 1 and 2 supports the success of the animal model of neuropathic pain, as stage 1 pain scale values were high (no pain) and stage 2 pain scale values were low (extremely painful). The average decrease in pain scale was -3.05 ± 0.37 gf, with a median of -2.98 (-3.34 – -2 .79) gf. The lowest pain scale difference was -4.04 gf, while the highest was -2.41 gf. There was a considerable decrease in pain scale values in stages 1 and 2, with the difference in pain scale values being progressively as follows. G_0 of -3.07 ± 0.58 gf (t = 14.073; 95% CI = 2.537 – 3.605; p <0.001), G_1 of -3.10 ± 0.43 gf (t = 19.041; 95% CI = 2.699 – 3.495; p <0.001), G_2 of -2.95 ± 0.22 gf (t = 35.864; 95% CI = 2.747 – 3.149; p <0.001), and G_3 of -3.11 ± 0.20 gf (t = 41.566; 95% CI = 2.931 – 3.297; p <0.001). Meanwhile, the analysis of the difference in pain scale values for stages 1 and 2 in the four groups revealed no significant difference (p = 0.711). These findings also support the idea that neuropathic pain can be successfully created in subjects using a pain scale that is substantially similar.

Effect of cacao bean extract and paracetamol combination on pain scale

In stage 3 pain scale measurements, the mean pain scale was 2.57 ± 1.10 gf, with a median of 3.00 (1.29 – 3.49 gf). The minimal value of the pain scale stage 3 was 0.78 gf, while the maximum value was 4.09. The results of stage 3 pain scale measures in each group were as follows: G_0 of 0.94 ± 0.11 gf, G_1 of 2.39 ± 0.41 gf, G_2 of 3.62 ± 0.36, and G_3 of 3.31 ± 0.30 gf (f = 100.772; p <0.001). Furthermore, the comparison between groups revealed a substantial difference in pain scale stage 3, and the results were as follows: G_0 vs G_1 (z = -3.130; p = 0.002), G_0 vs G_2 (z = -3.134; p = 0.002), G_0 vs G_3 (z = -3.130; p = 0.002), G_1 vs G_2 (z = -3.134; p = 0.002), and G_1 vs G_3 (z = -3.130; p = 0.002). Meanwhile, G_2 vs G_3 (z = -1.471; p = 0.141) was the only group comparison that did not show a significant difference. The aforementioned data show that administering cacao bean extract as a paracetamol adjuvant can reduce pain scale as demonstrated by an increase in von Frey value, which is greater than administering paracetamol alone or a placebo.

The difference in pain scale values between stages 2 and 3 is 1.35 ± 1.05 gf, with a median of 1.92 (0.11 – 2.19) gf. The minimum value was -0.32 gf, while the greatest was 2.57 gf. Meanwhile, the difference in values between each group was successively as follows: G_0 of -0.19 ± 0.12 gf (t= -4.195; 95% CI= -0.298 – -0.078; p = 0.006), G_1 of 1.12 ± 0.44 gf (t = 6.842; 95% CI = 0.723 – 1.528; p <0.001), G_2 of 2.36 ± 0.25 gf (t = 25.243; 95% CI = 2.127 – 2.584; p <0.001), and G_3 of 2.12 ± 0.12 gf (t = 45.976; 95% CI = 2.010 – 2.236; p <0.001; Table 2). Additionally, there was a significant difference in pain scale values for stages 2-3 between each group (p <0.001). Meanwhile, different results were observed in the comparison of G_2 and G_3 (z = -1.535; p = 0.125). These findings show that there is a difference in pain scale values before and after administering cacao bean extract as a paracetamol adjuvant, with group G_2 seeing the greatest decrease in pain scale (highest von Frey).

Effect of cacao bean extract and paracetamol combination on tumor necrosis factor-alpha

The subjects' mean TNF-α value was 86.96 ± 23.73 ng/mL, with a median of 87.72 (65.80 – 105.88) ng/mL. The subjects' TNF-α levels ranged from 50.38 ng/mL to 127.27 ng/mL. The average TNF-α levels in each group were: G_0 of 112.96 ± 10.54 ng/mL, G_1 of 103.68 ± 5.95 ng/mL, G_2 of 64.28 ± 8.71 ng/mL, and G_3 of 66.94 ± 11.35 ng/mL (f = 49.743; p <0.001). There were significant differences in TNF-α values in comparisons between group subjects including G_0 vs G_2 (t = 9.424; 95% CI = 37.431 – 59.943; p <0.001), G_0 vs G_3 (t = 7.863; 95% CI = 33.271

Table 1. TNF-α measurement results and pain scale stages 1-3.

Variable	Mice	Mean ± SD	Median (IQR)	F	p
Von Frey 1	G_0	4.20 ± 0.45	3.95 (3.84 – 4.52)	0.205	0.892
	G_1	4.36 ± 0.45	4.31 (3.83 – 4.89)		
	G_2	4.22 ± 0.35	4.08 (3.91 – 4.51)		
	G_3	4.30 ± 0.46	4.18 (3.93 – 4.87)		
Von Frey 2	G_0	1.13 ± 0.23	1.23 (0.92 – 1.36)	0.439	0.727
	G_1	1.26 ± 0.27	1.18 (1.01 – 1.58)		
	G_2	1.27 ± 0.27	1.36 (0.99 – 1.50)		
	G_3	1.19 ± 0.26	1.08 (0.97 – 1.08)		
Von Frey 3	G_0	0.94 ± 0.11	0.99 (0.85 – 1.04)	100.772	<0.001**
	G_1	2.39 ± 0.41	2.19 (2.06 – 2.86)		
	G_2	3.62 ± 0.36	3.52 (3.49 – 4.07)		
	G_3	3.31 ± 0.30	3.23 (3.07 – 3.58)		
TNF-α	G_0	112.96 ± 10.54	109.87 (104.67 – 126.93)	49.743	<0.001**
	G_1	103.68 ± 5.95	105.07 (98.39 – 109.57)		
	G_2	64.28 ± 8.71	61.88 (57.72 – 72.14)		
	G_3	66.94 ± 11.35	70.72 (53.69 – 77.83)		

Note: G=group; IQR=interquartile range; SD=standard deviation; TNF-α=tumor necrosis factor-alpha; *significant <0.05; **significant <0.001.

Table 2. Comparison of pain scale values at stage 1-2 and 2-3 measurements.

Pain Scale	Mice	Δ Pain	95% CI	t	p
Von Frey 1-2	G_0	-3.07 ± 0.58	2.537 – 3.605	14.073	<0.001**
	G_1	-3.10 ± 0.43	2.699 – 3.495	19.041	<0.001**
	G_2	-2.95 ± 0.22	2.747 – 3.149	35.864	<0.001**
	G_3	-3.11 ± 0.20	2.931 – 3.297	41.566	<0.001**
Von Frey 2-3	G_0	-0.19 ± 0.12	-0.298 – -0.078	-4.195	0.006*
	G_1	1.12 ± 0.44	0.723 – 1.528	6.842	<0.001**
	G_2	2.36 ± 0.25	2.127 – 2.584	25.243	<0.001**
	G_3	2.12 ± 0.12	2.010 – 2.236	45.976	<0.001**

Note: G_0 = mice (*Mus musculus*) group with number zero; G_1 = mice (*Mus musculus*) group with number one; G_2 = mice (*Mus musculus*) group with number two; G_3 = mice (*Mus musculus*) group with number three; *significant <0.05; **significant <0.01.

Table 3. Comparison between each group in the study.

Comparison	G_1		G_2		G_3	
	Coefficient	p	Coefficient	p	Coefficient	p
Pain 1						
G_0	-0.642	0.553	-0.060	0.953	-0.415	0.685
G_1			0.654	0.526	0.223	0.827
G_2					-0.402	0.695
Pain 2						
G_0	-0.962	0.355	-1.022	0.327	-0.438	0.669
G_1			-0.050	0.961	0.505	0.623
G_2					0.558	0.587
Pain 3						
G_0	-3.130	0.002*	-3.134	0.002*	-3.130	0.002*
G_1	-	-	-3.134	0.002*	-3.130	0.002*
G_2	-	-	-	-	-1.471	0.141
Δ Pain 1-2						
G_0	-0.319	0.749	-0.192	0.848	-0.384	0.701
G_1	-	-	-0.319	0.749	-0.576	0.565
G_2	-	-	-	-	-1.537	0.124
Δ Pain 2-3						
G_0	-3.130	0.002*	-3.130	0.002*	-3.130	0.002*
G_1	-	-	-3.130	0.002*	-3.130	0.002*
G_2	-	-	-	-	-1.535	0.125
TNF-α						
G_0	2.030	0.065	9.424	<0.001**	7.863	<0.001**
G_1	-	-	9.884	<0.001**	7.585	<0.001**
G_2	-	-	-	-	-0.493	0.631

Note: Coefficient included *f*, *t*, or *z* in the study; G_0 = mice (*Mus musculus*) group with number zero; G_1 = mice (*Mus musculus*) group with number one; G_2 = mice (*Mus musculus*) group with number two; G_3 = mice (*Mus musculus*) group with number three; TNF-α = tumor necrosis-alpha; *significant <0.05; **significant <0.001.

– 58.777; p <0.001), G_1 vs G_2 (t = 9.884; 95% CI = 30.716 – 48.087; p <0.001), and G_1 vs G_3 (t = 7.585; 95% CI = 26.185 – 47.292; p <0.001). Meanwhile, G_0 vs G_1 (t = 2.030; 95% CI = -0.680 – 19.251; p = 0.065) and G_2 vs G_3 (t = -0.493; 95% CI = -14.443 – 9.117; p = 0.631) yielded different findings (Table 3). Subjects who received cacao bean extract as a paracetamol adjuvant (G_2) had the most substantial decrease in TNF-α levels compared to other groups.

Association between pain scale and tumor necrosis factor-alpha

The TNF-α value was 86.96 ± 23.73 ng/mL, and the stage 3 pain scale was 2.57 ± 1.10 gf, indicating a significant correlation (r = 0.875; p <0.001; Fig. 1). However, when analyzing the correlation between pain scale and TNF-α in each group, it revealed no significant correlation, with the following values: G_0 (r = 0.029; p = 0.951), G_2 (r = -0.526; p = 0.225), and G_3 (r = -0.309; p = 0.500), but different results were obtained in the G_1 group (r = -0.888; p = 0.008). The study found a favorable correlation between lower TNF-α levels and decreased pain scale scores.

DISCUSSION

Cacao includes two essential chemicals called methylxanthines: caffeine and theobromine. Some pain relievers, particularly those containing the chemicals acetaminophen and aspirin, contain caffeine. Caffeine, at larger doses (approximately 15-45 mg/kg), improves the pain-relieving effects of acetaminophen and other nonsteroidal anti-inflammatory medications (NSAIDs), even giving pain relief on its own in preclinical models. Caffeine demonstrated immediate pain relief at even higher levels (50-100 mg/kg) in some studies. This pain-relieving action is mediated by central cholinergic and noradrenergic pathways, as well as suppression of central adenosine receptors type A2B and A2A, and microglia COX [21].

Cacao contains salsolinol in levels of up to 25 µg/g. This substance is a tetrahydroisoquinoline alkaloid that modulates dopamine activity [22]. Salsolinol, in particular, has been shown to interact with dopamine D3 receptors, which are part of the brain's reward system. Salsolinol also activates l-opioid receptors on GABAergic neurons in rats. Its capacity to pass the blood brain barrier remains limited [23].

TNF-α is a peripheral mediator of neuropathic pain [6]. A study evaluated L5 spinal nerve crush injury (distal to DRG) and L5 nerve root (proximal to DRG) in rats and showed that distal crush injury resulted in more neuronal death and elevated production of TNF-α and caspase levels, which linked with higher neuropathic pain [24].

Cacao was found to significantly reduce TNF-α levels in plasma in CKD patients [25]. In vitro studies have demonstrated that cacao extract and extracted flavonoids have anti-inflammatory effects. Cacao extract and flavonoids (epicatechin and isoquercitrin) inhibited macrophage production of TNF-α and monocyte chemoattractant protein-1. Similarly, epicatechin inhibited IL-6 and IL-8 production in stimulated whole blood cell cultures. Intestinal epithelial cells contain hexameric cacao procyanidins, which have been found to influence the activation of the transcription factor Nuclear factor kappa B (NF-kB) via TNF-α. NF-kB regulates the expression of genes that encode chemicals and enzymes involved in the inflammatory process [cytokines, i-NOS, cyclooxygenase 2 (COX-2), adhesion molecules, acute phase proteins, among others]. Cacao has been shown to reduce cytokines, chemokines, ROS, NO, and other inflammatory molecules in both in vitro and in vivo studies. Pro-inflammatory cytokines like IL-1β, IL-6, and TNF-α are known to play specific roles in the patho-logical pain process [26].

Quercetin or flavonoids have been found to have anti-inflammatory, antioxidant, and analgesic properties [27]. A wide range of animal models demonstrate that quercetin can raise the pain threshold. The analgesic effects of quercetin appear to be mediated by a variety of mechanisms involving both the central and peripheral neural systems. These mechanisms include NO generation, GABA and serotonin receptor activation, opioid-like actions, TRPV-1/NMDA receptor inhibition, cytokine production, and oxidative stress. Quercetin has

been demonstrated to lower the expression of numerous interleukins, including IL-6 and IL-2, as well as iNOS, NF-jB, p38 MAPK, and TNF-α [28].

TNF-α is a pro-inflammatory cytokine that plays a crucial part in the "immune-to-brain" communication pathway in pain and illness response models. TNF-α causes neuropathic pain by affecting the glial system. In the central nervous system, glial cells outnumber neurons 50-fold and are classified into three types: astrocytes, oligodendrocytes, and microglia. In response to nerve injury and inflammation, microglia transform into macrophage-like cells that display major histocompatibility complex antigens and release pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6 [29].

CONCLUSION

The administration of cacao bean extract as paracetamol adjuvant in subjects with neuropathic pain showed a significant reduction of TNF-α and pain scale (increased von Frey value) compared to other groups. TNF-α levels and pain scale (von Frey) have a significant correlation in the therapy of cacao bean extract as a paracetamol adjuvant. The administration of cacao bean extract as a paracetamol adjuvant with a low difference in paracetamol dose (1 and ½ paracetamol) did not have a significant difference in effect.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

ETHICS STATEMENT

This study protocol was reviewed, and the need for approval was obtained by the Animal Care and Use Committee in the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia, with an ethical number of 2.KEH.168.11.2023.

DATA AVAILABILITY

All data can be made available upon request to the corresponding authors.

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AUTHOR CONTRIBUTION

Faiz Muhammad Ammar: Conceptualization, data curation, formal analysis, funding acquisition, investigation, visualization, writing – draft original; Christrijogo Sumartono Waloejo: Conceptualization, formal analysis, investigation, resources, supervision, validation, writing – review & editing; Herdiani Sulistyo Putri: Conceptualization, investigation, methodology, project administration, software, visualization, writing – review & editing; Kohar Hari Santoso: Data curation, investigation, resources, validation; Prananda Surya Airlangga: Formal analysis, validation, supervision; Budi Utomo: Formal analysis, software, visualization.

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