Pharmacogn J. 2024; 16(5): 1134-1137
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Research Article

Effective Dose of Cocoa as a Preemptive Analgesic and Anti-Inflammatory Agent Assessed through Pain Scale and Tumor Necrosis Factor Alpha (TNF-α) in an Acute Pain Animal Model

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History

- Submission Date: 13-08-2024;
- Review completed: 19-09-2024;
- Accepted Date: 23-09-2024.

DOI : 10.5530/pj.2024.16.185

Article Available online

http://www.phcogj.com/v16/i5

ABSTRACT

Background: Pain is a significant issue for 40-50% of hospital patients, with 10-50% of acute pain cases potentially progressing to chronic pain. Pain-associated inflammation often involves the release of mediators, including Tumor Necrosis Factor Alpha (TNF-α). Cocoa beans contain polyphenols, catechins, anthocyanidins, and proanthocyanidins, compounds believed to possess analgesic properties. This study aims to assess cocoa's potential as an oral preemptive analgesic agent in an acute pain mouse model, with an emphasis on its impact on inflammation through TNF-α levels. **Methods:** This true experimental study involved 24 male white mice split into four groups: a control group (K0) receiving a placebo, a treatment group receiving 15 mg/kg BW oral paracetamol (Kpct), a treatment group receiving 0.5 mg/g BW cocoa (K1), and a treatment group receiving 1 mg/g BW cocoa (K2). Pain response was measured using TNF-α levels and the von Frey test. The Kruskal-Wallis test and One-Way ANOVA were employed for statistical analysis. **Results:** Cocoa at doses of 0.5 mg/g BW and 1 mg/g BW substantially reduced TNF-α levels (75.82 ± 7.77 and 70.79 ± 11.50, respectively) compared to the control and paracetamol groups (98.22 ± 14.74 and 92.81 ± 2.64). On the first day, compared to the control group's 1.82 ± 0.78 von Frey values, the cocoa-treated groups' values (6.20 ± 2.72 and 7.63 ± 4.11) were notably higher. There were no notable variations in von Frey values across the groups on the second day. However, a correlation was found between von Frey values on the first and second days. **Conclusion:** Cocoa can potentially serve as an effective preemptive analgesic agent, reducing pain and inflammation primarily by reducing TNF-α levels. These results provide validity to the use of cocoa as an alternative therapy in acute pain management.

Keywords: Cocoa, Pain Degree Analgesia, Pain, TNF-α.

INTRODUCTION

Pain is a common experience that affects individuals globally and significantly impacts public health.[1] Statistics indicate that pain, particularly acute pain, affects various age groups with a high prevalence, with approximately 40-50% of hospital patients experiencing pain.[2, 3] Furthermore, around 10-50% of those with acute pain are at risk of developing chronic pain.[4]

Pain-associated inflammation often involves the release of mediators, including Tumor Necrosis Factor-α (TNF-α), which is involved in the body's response to tissue injury. Molecular research into the pathophysiology of pain, aimed at discovering new analgesic modalities, frequently employs animal models.[5, 6] One significant finding is that inhibition of TNF-α with the monoclonal antibody infliximab can reduce pain responses in animal models, highlighting TNF-α's involvement in the development and persistence of pain conditions.

In recent years, alternative treatments, including the use of cocoa as an analgesic agent, have garnered attention from researchers. Since the 17th century, cocoa has been known for its medicinal properties and used for various purposes, including pain management.[7] Cocoa contains several active compounds, such as methylxanthine, flavan-3-ol, and other polyphenols, which are believed to have analgesic effects.[8, 9] However, research on the mechanisms and effectiveness of cocoa as an analgesic agent remains limited.

A study by Bowden et al. demonstrated that mice fed a chocolate-rich diet exhibited reduced orofacial neurogenic inflammatory pain. This research also revealed that chocolate could inhibit trigeminal neuron activation and the expression of proteins associated with pain perception.[10] Nonetheless, further research is required to understand the mechanisms and effectiveness of cocoa as an analgesic agent, particularly as a preemptive treatment. Thus, this study aims to assess cocoa's potential as an oral preemptive analgesic agent in a mouse model of acute pain and its relationship with inflammation by examining TNF-α levels.

METHODS

Research sample

This true experimental study was conducted in the laboratory of the Institute of Tropical Disease at Universitas Airlangga, employing a randomized post-test-only control group design. This study utilized 6 to 8-week-old male *Mus musculus* (white mice) with body weights ranging from 20-25 grams. A total of 24 mice were split into four groups: a

Cite this article: Fathani HC, Putri HS, Airlangga PS, Waloejo CS, Yudaniayanti IS, Lestari P. Effective Dose of Cocoa as a Preemptive Analgesic and Anti-Inflammatory Agent Assessed through Pain Scale and Tumor Necrosis Factor Alpha (TNF-α) in an Acute Pain Animal Model. Pharmacogn J. 2024;16(5): 1134-1137.

control group (K0) receiving a placebo, a treatment group receiving 15 mg/kg BW (conversion dose = 4.62 mg per 25g mice) oral paracetamol (Kpct), a treatment group receiving 0.5 mg/g BW cocoa (K1), and a treatment group receiving 1 mg/g BW cocoa (K2).

Assessment of behavioral response to pain

The mice were first acclimated for seven days, followed by an initial von Frey test to establish a baseline. The mice were then distributed to the four groups at random and treated accordingly. Pain assessment (bone defect model) using the von Frey test was conducted 24 hours post-treatment. The mice's left hind paws were temporarily treated with a von Frey filament on their plantar surfaces with sufficient force to bend the filament and maintain contact for 1-2 seconds. The data were recorded via a computerized system and visualized by an ELISA microplate reader.

Measurement of TNF-α levels

Blood samples were collected directly from the mice's hearts 24 hours after acute pain was induced (Yokozawa et al., 2002). The blood serums were subsequently centrifuged for 15 minutes at 4 °C at 6,000 g. TNF-α levels in the serums were analyzed using an ELISA kit (catalog number E0117Mo, Bioassay Technology Laboratory, Shanghai Korain) following the manufacturer's instructions. At 450 nm in wavelength, the results were visualized and analyzed using an ELISA microplate reader.

Statistical analysis

The data were processed using SPSS software. Bivariate analysis was performed using ANOVA, and the Kruskal-Wallis test was employed if the data exhibited normal distribution. Correlations between variables were analyzed using Spearman and Pearson correlation tests. *p-value < 0.05, **p-value < 0.005, and ***p-value < 0.001

RESULTS

TNF-α protein expression analysis

Following the bone defect modeling, TNF-α levels were measured in each group using the von Frey method (Figure 1). The results indicated that the TNF-α protein levels were 98.22 ± 14.74 in group K0, 92.81 ± 2.64 in group Kpct, 75.82 ± 7.77 in group K1, and 70.79 ± 11.50 in group K2. Compared to both the K0 and Kpct groups, the K1 and K2 groups exhibited substantially lower TNF-α levels. Nonetheless, there was no notable variation in TNF-α levels between groups K1 and K2. These findings suggest that cocoa at doses of 0.5 and 1 mg/g BW can reduce TNF-α levels more effectively than placebo or paracetamol.

Von Frey test analysis

On the first day (D1), the von Frey values were 1.82 ± 0.78 for group K0, 4.88 ± 2.59 for Kpct, 6.20 ± 2.72 for K1, and 7.63 ± 4.11 for K2 (Figure 2). Compared to group K0, groups K1 and K2 exhibited substantially higher von Frey values. Nevertheless, there was no notable variation between groups K0 and Kpct's von Frey values.

On the second day (D2), 24 hours after treatment, the von Frey values were 1.78 ± 1.38 for group K0, 3.63 ± 3.02 for Kpct, 3.34 ± 3.42 for K1, and 3.46 ± 2.94 for K2 (Figure 2). On the second day, there were no notable variations in von Frey values among the four groups.

Analysis of the correlation between TNF-α levels and von Frey values

As shown in Table 1, the analysis of the correlation between TNF-α levels and von Frey values on days one and two yielded p-values of 0.001 and 0.046, respectively, indicating a significant correlation between

Table 1. Correlation between TNF-α levels and von Frey values on days one and two.

	N	r	p-value	Description
TNF-α levels and von Frey values on day one	24	-0.619	0.001	Correlated
TNF-α levels and von Frey values on day two	24	-0.411	0.046	Correlated

*Correlation is considered significant if p-value < 0.05.

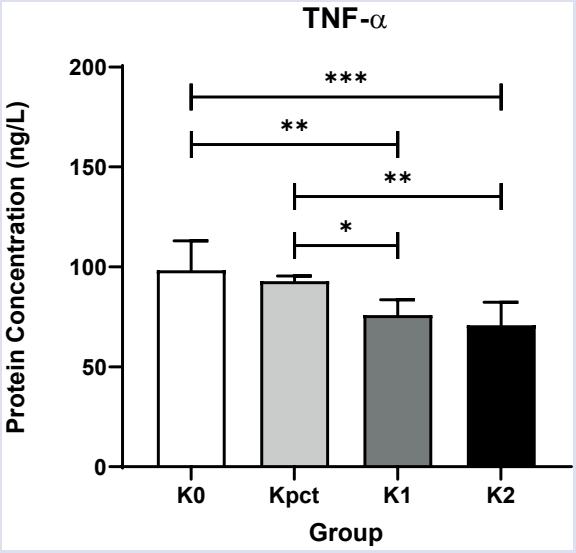

Figure 1. Comparison of TNF-α values among groups. *p-value < 0.05, **p-value < 0.005, and ***p-value < 0.001.

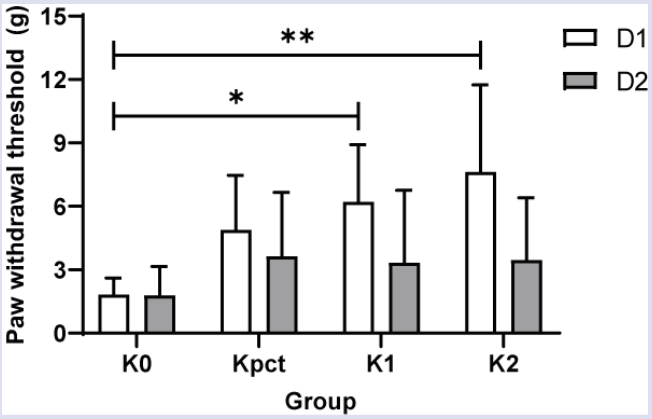

Figure 2. Comparison of von Frey values among groups before and after treatment. *p-value < 0.05 and **p-value < 0.005.

TNF-α levels and von Frey test results on both days. The correlation coefficients (r) were -0.619 and -0.416, respectively, signifying a negative correlation. This means that as TNF-α levels increased, von Frey values decreased, and vice versa. This correlation was categorized as strong on day one (0.619 or 61.9%) and moderate on day two (0.416 or 41.6%).

DISCUSSION

The effect of cocoa administration on TNF-α Levels

This study aims to assess cocoa's effectiveness as a preemptive analgesic and anti-inflammatory agent by measuring pain scales and TNF-α levels in an acute pain mice model. The findings revealed a significant

difference in TNF-α levels between the mice treated with cocoa and those in the control and paracetamol groups. These results indicate that cocoa effectively reduces TNF-α levels, whereas paracetamol did not show a similar effect. Moreover, no significant difference in effectiveness was observed between the cocoa doses of 0.5 mg/g BW and 1 mg/g BW, suggesting that the 0.5 mg/g BW dose is more efficient.

The biphasic dose-response phenomenon described by Shibata et al. (1989) supports these findings, wherein increasing the dose beyond the maximum effect may trigger the production of inflammatory mediators, thereby reducing the pharmacological effectiveness.[11] These results align with previous studies demonstrating that cocoa husk extract, rich in polyphenols, can significantly reduce pain scales in mice by inhibiting pro-inflammatory mediators like TNF-α.[7] Other studies have also found that consuming chocolate with 70% cocoa content can lower TNF-α levels in patients with chronic kidney failure undergoing hemodialysis.[12]

Furthermore, research by Praharani et al. (2023) showed that cocoa seed extract could reduce TNF-α expression and increase TGF-β expression in mice osteoclast cells, potentially accelerating orthodontic treatment processes.[13] This suggests that cocoa can potentially regulate inflammatory responses and pain relevant to various clinical conditions, including chronic pain and other inflammatory processes. Overall, cocoa has significant potential as an effective anti-inflammatory and analgesic agent in reducing pain and inflammation.

Other evidence also supports the role of TNF-α as a key mediator in pain development, with TNF-α activity on receptors (Tumor Necrosis Factor Receptor 1) TNFR1 and TNFR2 in primary afferent fiber neurons influencing acute inflammatory pain sensation.[14] Further studies have shown that cocoa flavonoids, such as epicatechin and quercetin, can suppress the production of pro-inflammatory cytokines and reduce oxidative stress, which may contribute to cocoa's analgesic effects.[15-17] Therefore, cocoa holds broad potential as a therapeutic agent in managing pain and inflammation.

The effect of cocoa administration on von Frey values

Recent studies have highlighted the potential of cocoa as an effective analgesic agent. In pain tests conducted on mice, cocoa administered at doses of 0.5 mg/g BW and 1 mg/g BW significantly reduced pain responses. The effectiveness of both doses in alleviating pain was consistent with the observed reduction in TNF-α levels, suggesting that cocoa can serve as a preemptive analgesic agent. The 0.5 mg/g BW dose was found to be as effective as the 1 mg/g BW dose, indicating that a lower dose is sufficient to achieve optimal preemptive analgesic effects.

Supporting studies by Sari (2018) demonstrated that cocoa husk extract at doses ranging from 0.25 mg/g BW to 1 mg/g BW significantly reduced pain scales, while higher doses did not show any substantial reduction in pain.[18] These findings align with the biphasic response pattern observed in other studies on epicatechin, a compound also present in cocoa. The maximum pharmacological effect appears to occur at intermediate doses, while higher doses may actually decrease analgesic effectiveness.[19]

The analgesic potential of cocoa is likely attributed to its flavonoid content, which is commonly derived from medicinal plants.[20] Flavonoids found in cocoa, such as proanthocyanidins, catechins, and anthocyanidins, are considered crucial in the anti-inflammatory and analgesic activities of cocoa.[21] Previous research has shown that these flavonoids can decrease the expression of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, and decrease the production of Reactive Oxygen Species (ROS) and Nitric Oxide (NO), all of which are involved in inflammatory responses.[16] Thus, these flavonoids contribute to the pain-relieving effects observed with cocoa use.

In addition to flavonoids, cocoa also contains methylxanthines, such as caffeine and theobromine, which are known to have analgesic effects. At high doses, caffeine has been shown to enhance the analgesic effects of medications, such as acetaminophen, through mechanisms involving the inhibition of adenosine receptors in the brain. This suggests that the methylxanthines in cocoa may contribute to analgesic effects via mechanisms involving central cholinergic and noradrenergic pathways.[22]

In addition to flavonoids and methylxanthines, chocolate contains other compounds, such as N-acylethanolamines and serotonin, which play a role in pain modulation through cannabinoid and opioid pathways. These compounds may enhance the stability and availability of anandamide, the body's natural cannabinoid involved in pain and mood regulation.[23] Chocolate also contains salsolinol, which interacts with dopamine D3 receptors and l-opioid receptors, all contributing to pain reduction.[24, 25]

Other studies further support these findings by demonstrating that the consumption of chocolate—whether white chocolate, milk chocolate, or dark chocolate—significantly reduces induced pain intensity. Interestingly, there were no notable variations between the types of chocolate, indicating that it is not just the cocoa concentration that determines the analgesic effect but also other factors, such as sugar content and additional ingredients.[26] Higher sugar content, particularly in white and milk chocolate, may contribute to enhancing the analgesic effect through mechanisms involving opioid release.[27]

CONCLUSION

Cocoa demonstrates potential as an effective preemptive analgesic agent for reducing pain and inflammation, particularly by reducing TNF-α levels. These results provide validity to the use of cocoa as an alternative therapeutic option for managing acute pain as a preemptive analgesic agent.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to the staff of the Department of Anesthesiology and Intensive Care, Faculty of Medicine, Universitas Airlangga; Department of Clinical Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Airlangga; and Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga for their support during this study.

CONFLICTS OF INTEREST

The authors have stated that they do not have any conflict of interest regarding this study.

FUNDING SOURCE

There was no specific funding from governmental, commercial, or non-profit entities for this study.

ETHICAL CLEARANCE

Ethical approval was obtained from the Ethics Commission for Basic and Clinical Science Research at the Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, with ethical compliance number 2.KEH.168.11.2023.

AUTHORS' CONTRIBUTION

All authors contributed to article preparation and paper revision and have collectively assumed responsibility for all aspects of this study.

DATA AVAILABILITY

The article contains all the necessary data to support the results; no supplementary source data is needed.

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Cite this article: Fathani HC, Putri HS, Airlangga PS, Waloejo CS, Yudaniayanti IS, Lestari P. Effective Dose of Cocoa as a Preemptive Analgesic and Anti-Inflammatory Agent Assessed through Pain Scale and Tumor Necrosis Factor Alpha (TNF-α) in an Acute Pain Animal Model. Pharmacogn J. 2024;16(5): 1134-1137.