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Acute Toxicity, Analgesic, and Anti-inflammatory Activities of Folk Thai Herbal Medicine: Yafon Formula

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Abstract

Yafon (YF), a formula composed of 14 herbal constituents, has been used in folk Thai herbal medicine to treat various indications, including pain relief, antipyretics, and anti-inflammatory properties. Nevertheless, this formula lacks adequate toxicological and efficacy data. Hence, this study aims to assess the safety and efficacy of YF in animal models. The study on acute oral toxicity was conducted by the Organization for Economic Co-operation and Development (OECD) number 420 guidelines. The results demonstrated that the administration of YF at a high dose of 2000 mg/kg did not result in fatality or exhibit any toxicity. The analgesic effect of YF in mice was investigated using the acetic acid-induced writhing test and the formalin test. The anti-inflammatory activity of YF was assessed in rats using the carrageenan-induced paw edema test. This investigation employed YF doses of 200, 400, and 800 mg/kg/day. The study found that all YF doses reduced writhing in the acetic acid, and YF doses of 400 and 800 mg/kg/day reduced the licking time in the late phase of the formalin test. In addition, all YF doses effectively suppressed paw edema in the carrageenan-induced paw edema test. This study demonstrated that the YF formula showed no acute toxicity and possessed acute anti-inflammatory and analgesic effects by blocking pain signals originating from the peripheral nervous system. These findings provided empirical evidence supporting the use of YF formula as an analgesic and anti-inflammatory agent.

Keywords**:** *Yafon; analgesic; anti-inflammatory; acute toxicity; herbal formula*

1. Introduction

Inflammation and pain are essential components of the body's immune response to detrimental factors like pathogens, damage, or irritants. These responses manifest through symptoms like discomfort, redness, swelling, heat, and impaired function in the affected region (Medzhitov, 2008). Acute inflammation is short-term and resolves on its own, but when the inflammatory reaction continues over time, it can evolve into chronic inflammation. This prolonged state of inflammation can cause tissue harm and plays a role in triggering several diseases, such as atherosclerosis and rheumatoid arthritis (Chen et al., 2018). Nonsteroidal anti-inflammatory drugs (NSAIDs) are the primary treatment for pain and inflammation; nevertheless, they have several side effects, including cardiovascular, renal, and gastrointestinal issues, when taken for a prolonged period (Bindu et al., 2020). Consequently, exploring new methods that offer reduced side effects for pain and inflammation management is imperative. In Thailand, the use of herbal formulas is widespread as a primary healthcare strategy for the treatment of pain and inflammation (Aiamsa-Ard, & Phetmanee, 2021; Sittiprom et al., 2023; Thongphasuk,

& Limsitthichaikoon, 2023; Charoenying et al., 2024). Yafon (YF) is a folk Thai herbal formula originated from Mr. Arj Ramadthong, Buached District, Surin Province, Thailand. This formula consists of equal mass ratios of 14 medicinal herbs as shown in Table 1. Traditionally, it is used to treat various conditions, including pain relief, fever reduction, anti-inflammatory effects, itch relief, chickenpox treatment, and fungal skin infection treatment. However, this local formula has insufficient data on toxicology and efficacy. Regarding toxicity, acute oral toxicity is the basic requirement of acute systematic toxicity data for identifying the safety of any substance. The median lethal dose (LD_{50}) is the important universal prediction value that resulted in the deaths of fifty percent of the experimental animals during exposure. Moreover, the LD_{50} value is frequently used when classifying chemicals based on their toxicity (Hamm et al., 2021).

Table 1 The component and pharmacological activities of ingredients of YF formula

NO.	Thai common name	Scientific name	Family	Part used	Analgesic	Anti-inflammation
1.	Fab Nam	*Hymenocardia punctata* Wall. ex Lindl.	Phyllanthaceae	Root	N	N
2.	Lot Thanong Daeng	*Trigonostemon reidioides* (Kurz) Craib	Euphorbiaceae	Root	N	Y (Utaipan et al., 2018)
3.	Hora Thao Sunak	*Balanophora abbreviata* Blume	Balanophoraceae	Whole plant	N	Y (Hosokawa et al., 2004)
4.	Chan Daeng	*Dracaena cochinchinensis* (Lour.) S.C.Chen	Asparagaceae	Heartwood	Y (Likhitwitayawuid et al., 2002)	N
5.	Pla Lai Phueak	*Eurycoma longifolia* Jack	Simaroubaceae	Root	N	Y (Zhang et al., 2020)
6.	Phaya Fai	*Diospyros lanceifolia* Roxb.	Ebenaceae	Root	N	N
7.	Phrachao Ha Phra Ong	*Dracontomelon dao* (Blanco) Merr. & Rolfe	Anacardiaceae	Root	N	Y (Wen et al., 2022)
8.	Ma Kluea	*Diospyros mollis* Griff.	Ebenaceae	Heartwood	N	N
9.	Ma Duea	*Ficus hirta* Vahl	Moraceae	Root	N	Y (Cheng, et al., 2017)
10	Ya Nang Daeng	*Lysiphyllum strychnifolium* (Craib) A.Schmitz	Fabaceae	Root	N	N
11.	Rotsukhon	*Tetracera loureiri* (Finet & Gagnep.) Pierre ex W. G. Craib	Dilleniaceae	Root	N	Y (Lee et al., 2022)
12.	Rang Chuet	*Thunbergia laurifolia* Lindl.	Acanthaceae	Root	Y (Boonyarikpunchai et al., 2014)	Y (Bhandare et al., 2010)
13.	Now Duean Ha	*Myxopyrum smilacifolium* (Wall.) Blume subsp. *smilacifolium*	Oleaceae	Root	N	N
14.	Mak Song	*Areca catechu* L.	Palmae	Seed	Y (Zhao et al., 2017)	Y (Nanna et al., 2017)

Regarding formula efficiency, YF is traditionally employed in folk medicine for its pain-relieving and anti-inflammatory effects. However, there are still no scientific studies confirming the efficacy of this formula. Therefore, this study focuses on assessing the safety, pain-relieving, and anti-inflammatory properties of YF through experiments with animal models. The assessment of acute oral toxicity followed the guidelines of the Organization for Economic Co-operation and Development (OECD) under number 420. To evaluate analgesic effectiveness, acetic acid-induced writhing and formalin tests were employed, while the anti-inflammatory properties were investigated using the carrageenan-induced paw edema test.

2. Objectives

The objective of this research is to assess the safety and examine the analgesic and anti-inflammatory properties of YF using animal models.

3. Materials and methods

3.1 Chemicals and reagents

Acetic acid, carrageenan, formalin, and acetylsalicylic acid (ASA) were purchased from Sigma Aldrich (USA).

3.2 Preparation of YF formula

YF was produced by combining 14 herbal constituents in a balanced ratio, as stated in Table 1. The YF formula was received from Pra Ajan Dhanes Jattaphayo at Wat Pacha Ban Kraisorn in Buached District, Surin Province, Thailand. Mr. Nirun Vipunngeun, a botanist at the Department of Pharmacognosy, College of Pharmacy, Rangsit University, authenticated each plant samples. The formula specimens were deposited at the College of Pharmacy, Rangsit University, Thailand. YF powder was prepared in the same manner as it is used in humans. The herbal ingredients were individually pulverized using a grinder and then filtered through a 40-mesh sieve.

3.3 Dose selection for *in vivo* experiment

For the rat and mouse experiments in this study, the dose of the YF formula was fixed by extrapolating the human dose to laboratory animals on the body surface area ratio (Nair, & Jacob, 2016; Shin et al., 2010). The adult human dose of YF is one teaspoonful twice daily; each teaspoon weighing 1000 mg (2000 mg/day), was converted to the animal dose. The animal dose after calculation was 411.11 mg/kg for mice and 205.55 mg/kg for rats. Therefore, three distinct YF dosages 200, 400, and 800 mg/kg/day were chosen for the experiments.

3.4 Animals

For the acute toxicity and anti-inflammatory experiments, Wistar rats weighing around 220 ± 20 g were chosen. To evaluate analgesic effects, Swiss albino mice weighing between 20–25 g were utilized. Both the rats and mice were obtained from the National Laboratory Animal Center at Salaya, Mahidol University, located in Nakorn Pathom, Thailand. They were acclimatized for 7 days before an advanced test. They were maintained under a standard temperature of 23–25 °C, a daily standard light/dark cycle (12 hrs. for light and 12 hrs. for darkness), free–feeding to standard pelleted, and water followed the standard of animal welfare. This protocol received approval from the ethics committee for animal research at Rangsit University (RSU-AEC 004-2022).

3.5 Acute toxicity testing

This study aimed to investigate the acute toxicity of YF according to the standard of OECD420 guideline regarding acute oral toxicity: fixed-dose procedure (OECD, 2002). All 20 rats were grouped and administered Tween® 80 and YF orally according to the following details: five male and five female rats were assigned to the control groups, receiving a single dose of Tween® 80. A single dose of 2,000 mg/kg body weight of YF was given to the remaining 5 male and 5 female rats for test groups. All rats were weighed before starting to administrate and then were observed. Within the first 30 minutes, rats were observed closely in detail of death, abnormal signs, or symptoms, and changing of behaviors for a period during the first 24 hours (day1). After that, the observations continued every day for 14 days. Data were presented as mean ± standard deviation (SD).

3.6 Acetic acid-induced writhing effect

The acetic acid-induced writhing effect test was conducted to evaluate visceral pain using the method previously described (Taher et al., 2015). Each group consisted of eight mice, orally administered YF at doses of 200, 400, and 800 mg/kg and ASA at 300 mg/kg as a standard drug for seven consecutive days. On the day of the test, one hour after administering the test sample, all animals received intraperitoneal injections of 0.75% acetic

acid (0.1 mL/10 g BW) to induce writhing. Each mouse was placed separately in a transparent cage, and the frequency of writhing was documented over 60 minutes. The recorded data represented the total number of observed writhing. The recorded data represented the cumulative count of detected writhing.

3.7 Formalin test

The formalin test was conducted following the method described by Oliveira et al., incorporating some minor modifications (de Oliveira Júnior et al., 2017; do Nascimento et al., 2024). In this experiment, a total of forty mice were allocated into five separate groups, each comprising eight mice. The orally administered treatments included YF at doses of 200, 400, and 800 mg/kg and ASA at 300 mg/kg as the standard drug. YF was given daily for a period of seven days. On the test day, YF was administered 30 minutes before the formalin test, followed by an injection of 20 microliters of 2.5% formalin into the dorsal hind paw of each mouse. The mice were housed individually in glass boxes for observation. The pain response was assessed by measuring the paw licking and biting duration during two distinct time intervals: the initial phase (0-5 minutes) and the later phase (15-30 minutes) after the formalin injection.

3.8 Carrageenan-induced paw edema

The evaluation of YF's anti-inflammatory effects in rats was conducted using the carrageenan-induced paw edema model described by Winter et al. (1962) Click or tap here to enter text.. For the study, the rats were divided into five groups, with each group containing eight animals. YF at doses of 200, 400, 800 mg/kg, ASA 300 mg/kg, and sterile water (as a negative control) were fed daily for seven days. On the day of the test, test samples were administered orally 1 hour before injecting 0.05 mL of 1% carrageenan into hind paw to induce edema. Edema volume was measured at 60, 120, 180, and 240 minutes after the carrageenan injection. The volume measurements were conducted using a plethysmometer from Panlab, s.l.u., Spain. Subsequently, the percentage reduction in inflammation was calculated using a specific formula:

$$\text{Inhibition of inflammation (\%)} = \left(\frac{\text{Mean paw edema}_{\text{control}} - \text{Mean paw edema}_{\text{test}}}{\text{Mean paw edema}_{\text{control}}}\right) \times 100$$

3.9 Statistical analysis

The data from the experiment were reported as the mean ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was used to ascertain statistical significance, followed by the least significant difference (LSD) test. *P-values* < 0.05 were considered significantly different. In the case of acute toxicity studies, the results were expressed as mean ± standard deviation (SD). Here, statistical significance was assessed using Student's t-test, with *P-values* < 0.05 considered significantly different.

4. Results

4.1 Acute toxicity test

The results of toxicity testing are shown in Table 2. After administering Tween® 80 in the control group and 2,000 mg/kg of body weight of YF in the test group, respectively. There were no deaths, no developed abnormality signs, or symptoms, and no observed of rats with unusual behavior in the first 24 hours and continuously until the end of 14 days in both the control and test group. In addition to observing the mortality, anomalies of rats after being given Tween® 80 and YF were also noted. The observation criteria and results are shown in Table 3. Most of the rats did not show any significant anomalies change symptoms. The weight changes of all rats are shown in Table 4. The percentage of weight change on days 1-7 and days 7-14 in both male and female test did not differ from that of the control groups.

Table 2 Mortality number of rats in acute toxicity testing within 24 hours and within 14 days of control group and test group

Group	24 hours			14 days		
	Mortality number of rats			Mortality number of rats		
	Female	Male	Total	Female	Male	Total
Control group (Tween® 80)	0/5	0/5	0/10	0/5	0/5	0/10
Test group (2,000 mg/kg body weight of Yafon)	0/5	0/5	0/10	0/5	0/5	0/10

Table 3 Anomalies observation within 24 hours and until the end of 14 days

Observation	24 hours				14 days			
	Female		Male		Female		Male	
	Control gr. (5 rats)	Test gr. (5 rats)	Control gr. (5 rats)	Test gr. (5 rats)	Control gr. (5 rats)	Test gr. (5 rats)	Control gr. (5 rats)	Test gr. (5 rats)
Skin and fur	0	0	0	0	0	0	0	0
Eyes and mucous membranes	0	0	0	0	0	0	0	0
Respiration	0	0	0	0	0	0	0	0
Salivation	0	0	0	0	0	0	0	0
Tremors	0	0	0	0	0	0	0	0
Diarrhea	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0
Lethargy	0	0	0	0	0	0	0	0
Ferocious	0	0	0	0	0	0	0	0
Sleep	0	0	0	0	0	0	0	0
Coma	0	0	0	0	0	0	0	0
Somatomotor activity	0	0	0	0	0	0	0	0

Note: 0 = no symptoms / normal, 1 = mild symptoms, 2 = moderate symptoms, 3 = severe symptoms

Table 4 Weight and percentage change of rats in the control group and experimental groups

		Average (gram) ± SD			Percentage change ± SD	
		Day 1	Day 7	Day 14	Day 1 to Day 7	Day 7 to Day 14
Control gr.	**Female**	185.46 ± 4.86	190.00 ± 4.96	213.62 ± 5.45	2.39 ± 0.32	9.89 ± 4.60
	Male	231.75 ± 5.76	257.48 ± 7.21	310.80 ± 14.28	11.04 ± 2.14	16.96 ±5.70
Test gr.	**Female**	181.46 ± 5.12	184.63 ± 5.46	207.26 ± 4.48	1.71 ± 0.37	7.25 ± 3.61
	Male	238.62 ± 5.10	257.48 ± 8.39	312.06 ± 11.52	10.93 ± 1.25	17.45 ± 2.51

4.2 Acetic acid-induced writhing test

The pain-relieving effectiveness of YF on acetic acid-induced abdominal constrictions and writhing is depicted in Figure 1. The findings indicate that YF, when given at dosages of 200, 400, and 800 mg/kg, notably decreased the occurrence of writhing compared to the control group. Remarkably, the analgesic effect at 400 mg/kg was almost equivalent to that observed at 800 mg/kg. The standard drug, ASA, at 300 mg/kg, exhibited a pronounced analgesic effect by markedly decreasing the writhing numbers.

4.3 Formalin test

In the formalin test, YF at doses of 200, 400, and 800 mg/kg did not exhibit significant activity during the early phase, as illustrated in Figure 2A. However, in the late phase, YF demonstrated notable anti-nociceptive pain activity at doses of 400 and 800 mg/kg by reducing the duration of paw licking. Conversely, YF at a dose of 200 mg/kg exhibited a minimal and statistically insignificant reduction in licking time compared to the control, as shown in Figure 2B. When comparing the analgesic effect (measured as the duration of licking) of YF with that of ASA at a dose of 300 mg/kg, YF's effect was found to be less potent than the inhibitory effect of ASA.

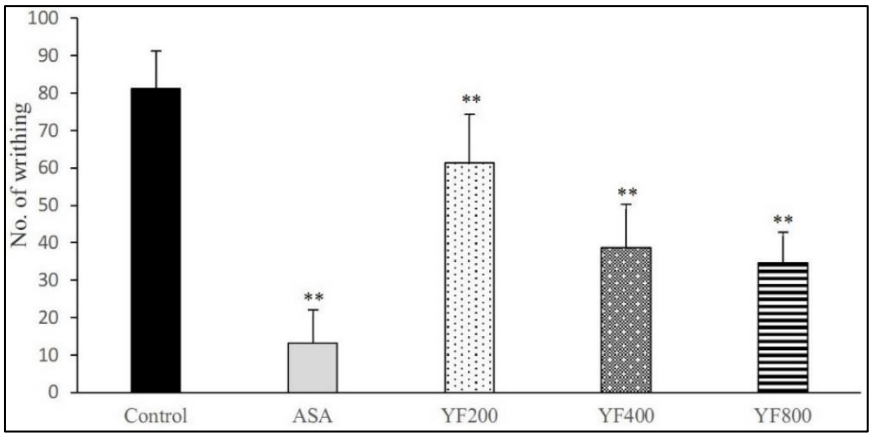

Figure 1 The effect of YF on acetic acid-induced writhing test. Data were presented as the number of the writhing effect registered for 60 minutes after the acetic acid injection. Each experimental group consisted of eight animals (n = 8/group). Statistical data analysis was carried out using ANOVA, with **P < 0.01 indicating a significant difference compared to the control group.

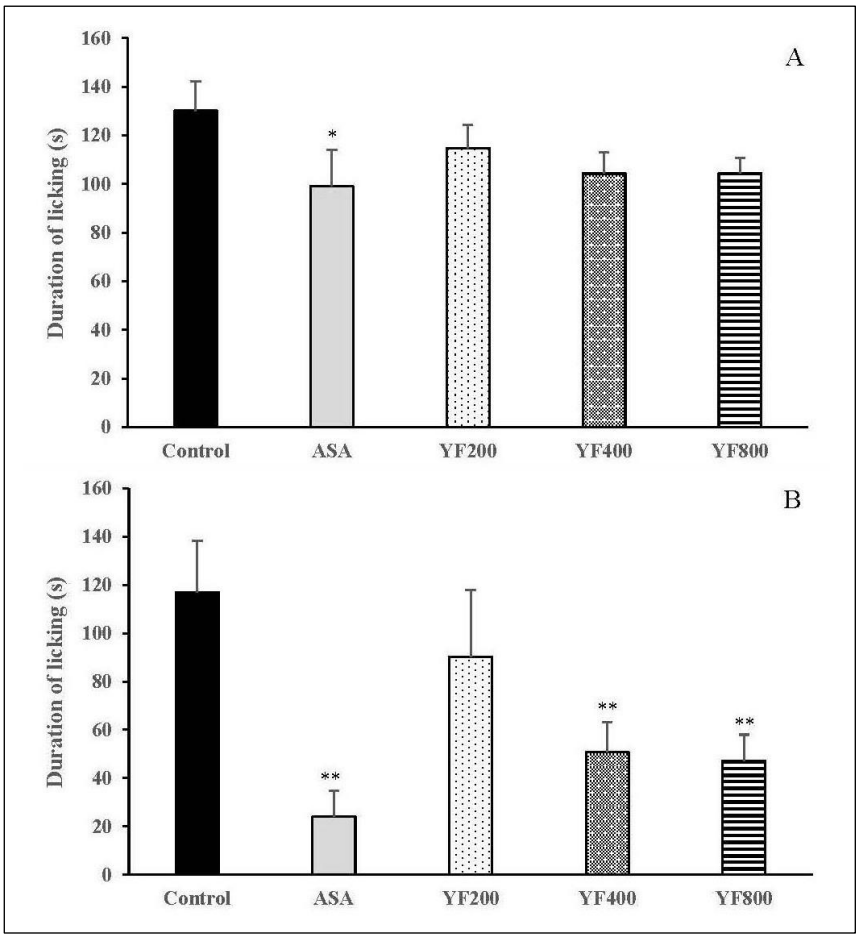

Figure 2 The effect of YF in formalin test. A: Total paw licking and biting events (sec) as mean ± SEM in 1st phase B: Total Paw licking and biting events (sec) as mean ± SEM in 2nd phase. Each experimental group consisted of eight animals (n = 8/group). All recorded values underwent statistical analysis using ANOVA, with **P < 0.01 and *P < 0.05 indicating significant differences when compared to the control group.

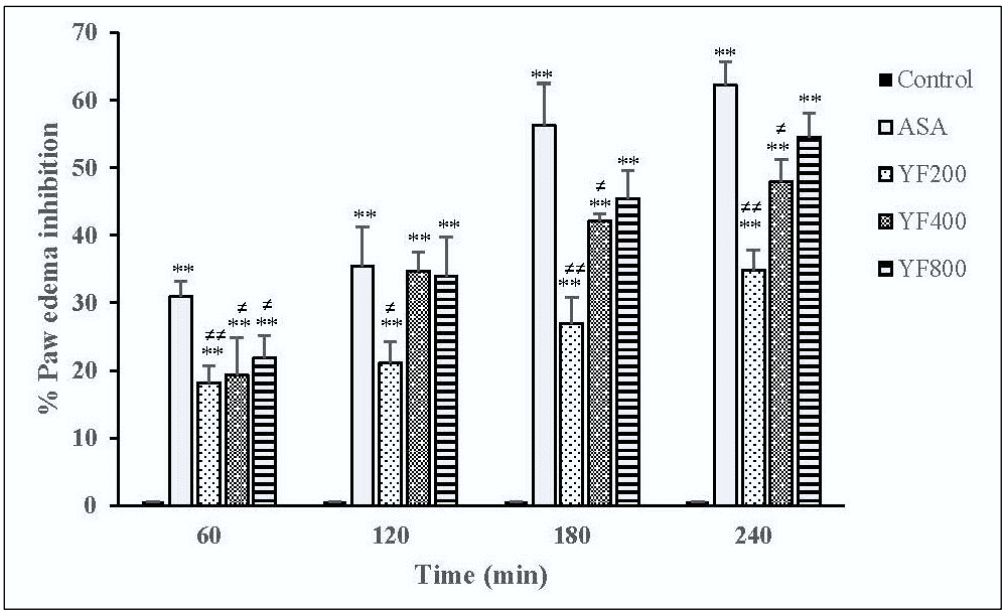

Figure 3 The effect of YF in carrageenan-induced paw edema test. Data show the mean ± SEM of the percentage inhibition in paw edema. The study involved six rats per group (n = 6/group). Statistical analysis using ANOVA revealed significance with **P < 0.01 and *P < 0.05 compared to the control group, while ≠P < 0.01 and #P < 0.05 indicate significance compared to the ASA group.

4.4 Carrageenan-induced paw edema

YF demonstrated significant anti-inflammatory effects in carrageenan-induced paw edema models in rats. Administered at doses of 200, 400, and 800 mg/kg, YF significantly reduced paw edema compared to the control group. Notably, the doses of 400 and 800 mg/kg of YF showed similar levels of effectiveness in reducing carrageenan-induced inflammation at all the observed intervals (1-4 hours). ASA at a dose of 300 mg/kg also effectively reduced carrageenan-induced paw edema, with its dose- and time-dependent effect. Importantly, YF at the dosage of 800 mg/kg showed a significant inhibition of carrageenan-induced inflammation, which was comparable to that of ASA at 300 mg/kg, particularly at 2, 3, and 4 hours post-carrageenan injection, as depicted in Figure 3.

5. Discussion

In recent years, there has been a surge in studies highlighting the pharmacological benefits of traditional polyherbal mixtures on human health. Combining multiple herbs in traditional formulas aims to enhance therapeutic efficacy while reducing potential side effects (Karole et al., 2019). In conventional Thai and folk medicine, the YF compound is frequently used to relieve pain and reduce inflammation. This study delved into the toxicological, analgesic, and anti-inflammatory effects of YF using animal models.

Acute toxicity is an important process for drug research and development. The main purpose of acute toxicity testing is to determine the safety of substances or drug-like compounds (Parasuraman, 2011). The toxicity of a substance can be found and compared by the LD_{50} value; LD_{50} is the amount of substance that can produce death of fifty percent of the animal group (Strickland et al., 2018) If the value of LD_{50} is low, the incidence of toxicity is high, which means that a small amount of substance can produce toxicity. On the other hand, if LD_{50} values are high, there is a low incidence of toxicity, which means that enormous amounts of substances are required to cause death. Currently, LD_{50} values are rated range or hazardous categorization of substance according to the standard of toxicity ratings such as the Environmental Protection Agency (EPA) and Globally Harmonized System of Classification and Labelling of Chemicals (GHS) (UNECE, 2023; Karmaus et al., 2022). Likewise, for this study, the authors needed to find out about the toxicity of YF which is a folk medicine. YF has been used for a long time to relieve fever and pain, but no studies have been conducted on it before. Therefore, this study aimed to find out the safety of YF by testing the acute toxicity according to the

OECD420 guideline - fixed dose procedure. We conducted for 24 hours only at the dose of 2000 mg/kg/day corresponds to the maximum dose in a stepwise of OECD420 guideline procedures which is recommended for studies doses that are expected to be moderately toxic and avoid using high doses (5000 mg/kg) that can cause immediate death in animals. The observation of mortality and delayed death for all rats on day 14 showed that no dead animals were found (Table 2). In addition, the rats did not display noteworthy abnormal changes in symptoms (Table 3). No difference in weight and percentage change of rats in the control group and experimental groups (Table 4) indicated that YF is not affected by the feeding behavior of rats (Ali, & Kravitz, 2018; Dietze et al., 2016; Modlinska et al., 2015). These findings suggested that YF can be administered safely in acute treatments up to a dose of 2000 mg/kg/day. Additionally, there were multiple scholars reported that *Dracaena loureiroi* (Reanmongkol et al., 2003), *Eurycoma longifolia* (Low et al., 2014), *Lysiphyllum strychnifolium* (Sudsai, & Plaingam, 2019), *Thunbergia laurifolia* (Posridee et al., 2022), and *Areca catechu* (Reanmongkol et al., 2003) exhibited no acute toxicity *in vivo* studies which consisted with our findings.

The analgesic assessment utilized the acetic acid-induced writhing reaction in mice as a measure of peripheral pain relief, and the formalin test was employed to assess both central and peripheral responses to pain. YF at doses of 200, 400, and 800 mg/kg showed significant effectiveness in reducing the acetic acid-induced writhing in mice. This writhing effect, induced by acetic acid, serves as a method to evaluate pain through a localized inflammatory response. Injecting acetic acid into the peritoneal cavity triggers the liberation of arachidonic acid from membrane phospholipids, increasing levels of pain mediators such as prostaglandins (PGE_2 and $PGF_{2\alpha}$), substance P, and bradykinins in the peritoneal fluid (Su et al., 2011). These mediators activate peritoneal nociceptors that are involved in inflammatory pain. Prostaglandins, synthesized by cyclooxygenase (COX) enzymes, function as lipid mediators and play a crucial role in inflammatory pain, acting as strong hyperalgesic components within the nociceptive pathway (Kidd, & Urban, 2001; Ricciotti, & Fitzgerald, 2011). NSAIDs inhibit COX, reducing inflammatory pain by lowering prostaglandin production (Pinheiro et al., 2011). The decrease in writhing behavior observed with the administration of ASA in this study supports the pain-relieving properties of NSAIDs. The efficacy of YF in reducing pain, possibly by affecting the production of mediators or the signal transduction in primary afferent nociceptors, was found to be comparable to that of ASA, as evidenced by the similar percentage of writhing inhibition following the administration of ASA and various doses of YF (Figure 1). However, YF's analgesic efficacy was somewhat less than that of ASA at a dosage of 300 mg/kg.

The formalin test is widely used to analyze the central and peripheral nervous system activity. A biphasic response characterizes pain from formalin; the first phase (neurogenic phase) includes the direct stimulation of type C sensory fibers, that release bradykinin, glutamate, and substance P. The second phase is linked to inflammatory pain, which is modulated by various substances such as prostaglandins, serotonin, histamine, and a range of cytokines including IL-1β, IL-6, TNF-α, along with eicosanoids and nitric oxide (NO) (Dantas et al., 2020; Yin et al., 2016). The study indicated that YF at doses of 400 and 800 mg/kg effectively reduced pain in the second phase by decreasing licking duration, while it had no impact on the first phase. This suggests that YF, particularly at higher doses, is mainly effective against inflammatory pain in the peripheral nervous system. ASA used as a standard drug, showed significant analgesic effects in both phases, affirming its role in modulating pain transmission within the central and peripheral nervous systems. The late-phase efficacy of YF was found to be less than the positive control ASA at 300 mg/kg (Figure 2). The analgesic properties of YF might be linked to the documented analgesic activities of its constituents such as *Dracaena loureiri*, *Thunbergia laurifolia*, and *Areca catechu*. Studies have shown that compounds in *Dracaena loureiri* inhibit COX-1 and COX-2 enzymes (Likhitwitayawuid et al., 2002). Furthermore, *Thunbergia laurifolia* and *Areca catechu* demonstrated analgesic effects in various tests, including capsaicin-induced nociception, tail-flick, hot plate, and formalin-induced pain tests (Boonyarikpunchai et al., 2014; Zhao et al., 2017).

The carrageenan-induced inflammation model, used to assess YF's anti-inflammatory activity, involves a two-phase process. The early phase (1-2 hours) sees the release of bradykinin, histamine, and serotonin, while the later phase (post

2 hours) is marked by increased COX-2 activity and prostaglandin release, which are sensitive to NSAIDs (Bhukya et al., 2009; Brooks, & Day, 1991). The study's results indicate that YF effectively counters acute inflammation (Figure 3). All YF dosages significantly diminished carrageenan-induced paw edema compared to the control group. The anti-inflammatory efficacy of YF at 400 mg/kg was found to be similar to that at 800 mg/kg, suggesting that YF plays a role in reducing inflammation mediators during both phases of acute inflammation. It is possible that the reduction in inflammation is linked to the inhibition of prostaglandin synthesis, potentially arising from the synergistic effects of YF's multiple components. The anti-inflammatory action may be associated with earlier research that has reported the anti-inflammatory activity of *Trigonostemon reidioides* (Utaipan et al., 2018), *Balanophora abbreviate* (Hosokawa et al., 2004), *Eurycoma longifolia* (Zhang et al., 2020), *Dracontomelon dao* (Wen et al., 2022), *Ficus hirta* (Cheng et al., 2017), *Tetracera loureirin* (Lee et al., 2022), which are components of YF. Furthermore, these findings align with prior research indicating that extracts from *Thunbergia laurifolia* and *Areca catechu* possess anti-inflammatory properties in animal models (Bhandare et al., 2010; Nanna et al., 2017). Nevertheless, when comparing YF's anti-inflammatory activity to that of ASA, it is evident that the YF has a lesser level of action. According to these findings, this study offers scientific validation for the conventional uses of YF in treating disorders associated with inflammation.

YF had a significant analgesic effect in the acetic acid and formalin tests. According to these findings, YF may exert its analgesic effects by inhibiting nociception that is mediated by the peripheral nervous system. Furthermore, YF was discovered to possess acute anti-inflammatory properties. Additional research is necessary to clarify the mechanism of YF, especially its role in inhibiting prostaglandins to provide pain relief and reduce inflammation.

6. Conclusion

The findings of this investigation into acute toxicity indicate that the YF formula at a single fixed dose of 2000 mg/kg/day is non-toxic. It demonstrates both peripheral analgesic and acute anti-inflammatory properties in animal models. This study provides evidence that the conventional application of YF formula effectively alleviates pain and reduces inflammation.

7. Acknowledgement

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