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Analgesic and Anti-Inflammatory Activities of Tengho: A Drink Made from Some Cameroon Spices

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ABSTRACT

Background: "Tengho" is a Cameroonian drink traditionally used for the treatment of inflammation, pain, hypertension, arthritis, diabetes and anxiety. Accordingly, the aim of this study is to evaluate its anti-inflammatory activity using carrageenan-, histamine and serotonin-induced paw oedema models and its analgesic activity using peripheral (acetic acid-induced writhing) and central (tail immersion in hot water) pain models.

Methodology: Tengho was daily orally administered to rodent at the dose of 200, 400 and 600 mg/kg BW, 1 hour before the induction of inflammation or pain.

Results: All tested doses significantly reduced oedema in carrageenan ($p<0.001$ after 3, 4, 5 and 6 hours), histamine ($p<0.01$ after 2 hours) and serotonin ($p<0.01$ after 1 h at the dose of 600 mg/kg) induced paw oedema models. We noticed as well a significant decrease ($p<0.001$) in the number of writhing provoked by the acetic acid and an increased ($p<0.001$) the tail flick latency in time-dependent manner.

Conclusion: Taken altogether, these results indicate the analgesic and anti-inflammatory properties of Tengho, supporting its local use as drink to relief inflammatory and painful conditions.

Keywords: Tengho; Spices; Anti-inflammatory; Analgesic

INTRODUCTION

Pain, especially chronic pain, is a very significant medical problem with a high social cost [1]. It is a major source of suffering and one of the most frequent causes for patients seek medical care [2]. This unpleasant experience is often associated with discomforts and long-term detrimental effects if not appropriately managed. Most of systems, especially the cardiovascular and respiratory systems, can be affected [3]. In contrast to chronic pain that has no beneficial biological significance, acute pain aims to avoid harmful stimuli and promote healing of wounded tissue [4]. The same goes for inflammation. Under normal conditions, this healthy and adaptive process both counters harmful stimuli and is involved in repairing damage to tissues [5]. When prolonged, severe, and/or inappropriate, it is often detrimental, leading to a host of diseases such as periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer [6]. After injury, enhanced responses to noxious stimuli (through the direct activation or sensitization of nociceptors) promote recovery by fomenting protection and rest of the afflicted body part [7]. The evoked nociceptive (acute) pain along with redness, swelling, heat and functio laesa (loss of function) constitutes the cardinal features of the inflammation [8]. Globally, it is of paramount importance to correctly manage inflammation and pain to avoid the chronic phase and minimize the detrimental associated effects.

Whether acute or chronic, pain modulation involves both peripheral and central nervous system mechanisms. Analgesia can be accomplished by analgesics (Mu-opioid receptor (MOR) agonists and non-MOR agonists) and co-analgesics depending on the specific cause and the severity of the pain [9]. Besides the severe adverse events associated with the usage of these drugs, they are very often expensive and not accessible for a huge part of the population in low-income countries like Cameroon. Cyclooxygenase (COX) inhibitors (e.g., ibuprofen, indomethacin, diclofenac and aspirin) belong to the non-MOR agonists and display analgesic, antipyretic and anti-inflammatory activities. They can cause gastrointestinal haemorrhage, myocardial infarction, analgesic asthma, hypertension, renal failure, peptic ulcer disease sodium and water retention [9-11]. MOR agonists can induce respiratory depression, hypothermia, bradycardia, constipation, miosis and urinary retention. Moreover, they are used reluctantly in many

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countries due to the perceived risk of addiction, especially when injected i.v. [9]. In developing countries, 80% of the population still resorts to traditional medicine for their primary health care. This is mainly due to the accessibility of traditional medicines, the high cost, increase in drug resistance and the undesirable side effects of synthetic drugs. In this context several plant extracts and preparations claimed to possess analgesic and anti-inflammatory properties are widely used.

Tengho is a Cameroonian drink mainly made from *Zingiber officinale*, *Allium sativum*, *Cymbopogon citratus*, *Ocimum basilicum*, *Petroselinum crispum* and *Bee propolis*. It is traditionally used against inflammation, pain, hypertension, diabetes, cancer and Alzheimer disease. A large and growing body of literature indicates the traditional uses and scientific evidences of some of these plants, including *Zingiber officinale*, *Allium sativum*, *Cymbopogon citratus*, *Ocimum basilicum* and *Bee propolis*, against in various types/models of pain and inflammation. However, investigation has not been reported till now with Tengho. Accordingly, the present study was designed to evaluate the analgesic and anti-inflammatory properties of Tengho and therefore provide a substantial scientific background to its traditional use.

MATERIALS AND METHODS

Plant collection and preparation of the extract

The ingredients used for the preparation of Tengho were collected in July 2018, at Dschang in West Region of Cameroon. The preparation process as well as the parts and amount of each plant are being protected. The mixture of these ingredients was macerated in 4:6 (v/v) ethanol/water. The filtrate was therefore lyophilized to obtain the crude extract. The therapeutic dose of 400 mg/kg was extrapolated from the traditional dose used in humans and surrounded by the doses of 200 and 600 mg/kg.

Experimental animals

Adult Wistar rats (7-8 weeks old, 115-150 grams) were used for the anti-inflammatory tests and the tail withdrawal test while, adult swiss albino mice (8-9 weeks old; 20-25 grams) were used in the acetic acid-induced writhing test. Healthy animals were bred in the animal house of the Department of Biology of Animal Organisms, University of Douala, and segregated according to gender. They were maintained under normal laboratory condition of temperature (25 ± 2°C) with a natural ~12 hours light/dark cycle. Animal had free access to diet and tap water ad libitum. Prior each experiment, animals were fasted overnight (10-12 hours).

Chemicals

Acetic acid, histamine and serotonin were obtained from Carl Roth GmbH (Karlsruhe, Germany). Carrageenan was purchased from SIGMA-Aldrich (Germany), Diclofenac (Voltaren® 50) was obtained from Novartis PHARMA SAS (France), Tramadol (TrabarTM-50) was purchased from Merckle GmbH (Blaubeuren-Weiler, Germany), Promethazine (Phénergan®) was manufactured by Sophartex Laboratory (Vernouillet, France), Cyproheptadin (NURABOL®) was manufactured by PHARMA 5 (Bouskoura, Morocco).

Antinociceptive activity tests

Acetic acid-induced writhing test: The antinociceptive effect tengho was investigated using the method described by Koster et al. [12]. Mice were randomly assigned to 5 different groups (n=6) and treated. The first group or control received distilled water (10 mL/kg), the second received aspirin (200 mg/kg, p.o.) and the three-remaining received tengho at the doses of 200, 400 and 600 mg/kg BW, p.o. groups. One hour after, animals received acetic acid (1% w/v, 10 mL/kg, i.p.) to induce the writhing movements (pain). Each animal was then immediately placed in a separate plastic observation chamber and the writhes (arching of the back, body stretching, and extension of the forelimbs) was counted for 30 min after acetic acid challenge. The percentage of analgesic activity was expressed as percentage reduction of the number of nociceptive movements in treated animals with respect to the control according to the following formula [13].

Tail withdrawal test: The test procedure was previously used by Aydin et al. [14]. Rats randomly distributed in five groups of 6 animals each and orally treated with vehicle (distilled water 10 mL/kg), tramadol (20 mg/kg) and tengho (200, 400 and 600 mg/kg), respectively. Prior treatment (initial reaction time) and 30, 45, 60, 120 and 180 min after, each rat was gently restrained in a cloth and the lower 3 cm of the tail immersed into a thermostatically-controlled warm water bath (55 ± 0.5 °C). The reaction time or tail flick latency (in seconds) was recorded using a stopwatch. To avoid tissue damage, a cut-off time of 15 hours was set for withdrawal. Difference in tail flick latencies between treated and control groups were used to determine the anti-nociceptive effects of extract and standard drug.

Anti-inflammatory activities

Carrageenan-induced rat paw oedema: The method used was previously described by Winter et al. [15]. Adult Wistar rats were subdivided into 5 groups of 5 animals each and orally treated one hour before a subcutaneous injection of 0.1 mL of carrageenan 1% (w/v in saline) into the plantar surface. The first group served as control and received distilled water (10 mL/kg, p.o), the second group received the reference drug Diclofenac (5 mg/kg i.p.); the three remained groups received tengho at the doses of 200, 400 and 600 mg/kg (p.o), respectively. The paw oedema (volume) was measured before and 0.5, 1, 2, 3, 4, 5 and 6 hours after injection of carrageenan using a traditional water displacement Ugo Basile 7510 plethysmometer. The anti-inflammatory activity was expressed as the percentage of reduction in oedema and calculated.

Histamine and serotonin-induced rat paw oedema: In these experiments, animals were handled in the same manner as described above in the carrageenan-induced rat paw oedema. The main differences were the phlogistic agents (0.1 mL of histamine or serotonin at 1% (w/v in saline)), the time-period for the oedema measurement (before and 0.5, 1, 2 hours after the injection of the phlogistic agent) and the reference drugs used (1 mg/kg promethazine p.o and 2 mg/kg cyproheptadine p.o, respectively).

Statistical analysis

All data were expressed as the mean ± standard error of the mean (S.E.M). The GraphPad Prism® program version 5.03 (Graph Pad Software, San Diego, CA, USA) allowed the analysis of data using Analysis of Variance (ANOVA) one-way followed by the Dunnett test. Values of $p<0.05$ were considered statistically significant.

RESULTS

Analgesic effect of tengho on acetic acid-induced writhing

The administration of tengho significantly ($p<0.05$) reduced writes in dose-dependent manner (Table 1). Percentages of inhibition at the tested doses of 200, 400 and 600 mg/kg were 33.83, 57.94 and 63.55%, respectively. The doses of 400 and 600 mg/kg were more active than 200 mg/kg aspirin used as the standard drug (48.41% inhibition).

Analgesic effect of tengho on the tail withdrawal test

Compared to the control group, tengho significantly ($p<0.05$) prolonged tail withdrawal reflex time (Table 2) from 45 min (200 and 400 mg/kg) and 30 min (600 mg/kg) after treatment. At the doses of 400 and 600 mg/kg, the percentages of inhibition are globally higher than that exhibited by 20 mg/kg tramadol.

Effect of tengho on carrageenan-induced paw oedema

The results in Table 3 shows that tengho significantly reduced carrageenan-induced paw oedema volume from 0.5 hours for 400 and 600 mg/kg, and 1 hour for 200 mg/kg. At 5-6 h the activity was close to that of diclofenac (5 mg/kg).

Anti-inflammatory effect of tengho on histamine-induced paw oedema

By contrast to promethazine (1 mg/kg) that significantly decreased the paw oedema volume at 0.5 hours (58.68% inhibition), 1 hour (56.63%) and 2 hours (58.44%), a significant anti-oedema effect of Tengho at all tested doses was only observed at 2 hours (41.09%-47.48%) (Table 4).

Anti-inflammatory effect of tengho on serotonin-induced paw oedema in rats

Table 5 shows that tengho decreased the volume of serotonin-induced paw oedema compared to the control group. Significant reductions of 44.04 and 39.53% were observed with the highest tested dose of 600 mg/kg 1 hour and 2 hours after treatment, respectively.

Table 1: Effect of Tenghõ on acetic acid-induced writhing.

	Dose	Number of writhes	% of protection
Control (water)	10 mL/kg	106.8 ± 8.61	-
Aspirine	200 mg/kg	55.20 ± 3.73 ***	48.41%
Tenghõ	200 mg/kg	70.80 ± 6.43 *	33.83%
Tenghõ	400 mg/kg	45.00 ± 9.26 ***	57.94%
Tenghõ	600 mg/kg	39.00 ± 4.71 ***	63.55%

Note: Values are the mean ± SEM from 5 animals in each group. Significance against control group: *p<0.05, ***p<0.001.

Table 2: Effect of Tenghõ on pain induced by tail immersion in warm water.

	Dose	Tail withdrawal latency (seconds) at various time intervals (before and after treatment)					
		Before treatment	30 min	45 min	60 min	120 min	180 min
Control (water)	10 mL/kg	1.62 ± 0.12	1.50 ± 0.18	2.25 ± 0.1	2.33 ± 0.21	1.66 ± 0.10	1.50 ± 0.18
Tramadol	20 mg/kg	2.29 ± 0.1 *	4.33 ± 0.51 ***	4.83 ± 0.42 ***	4.50 ± 0.34 ***	2.58 ± 0.39 *	2.58 ± 0.39
			(47.11 %)	(52.58 %)	(49.07 %)	(11.29 %)	(11.29 %)
Tenghõ	200 mg/kg	2.20 ± 0.26 *	2.91 ± 0.32 *	4.96 ± 0.30 ***	3.91 ± 0.45 **	2.20 ± 0.10	2.33 ± 0.24
			(24.3 %)	(55.54 %)	(43.62 %)	0%	(5.35 %)
Tenghõ	400 mg/kg	1.58 ± 0.08	2.41 ± 0.20	3.91 ± 0.08 ***	4.16 ± 0.16 ***	2.00 ± 0.00	1.83 ± 0.10
			(34.48 %)	(59.57 %)	(62 %)	-20.83%	(13.63 %)
Tenghõ	600 mg/kg	1.62 ± 0.05	3.33 ± 0.16 **	4.58 ± 0.30 ***	3.5 ± 0.18 *	2.41 ± 0.23 *	1.83 ± 0.42
			(51.25 %)	(64.54 %)	(53.57 %)	(37.5 %)	(11.36 %)

Note: Values are the mean ± SEM (n=6). The values in brackets represent the percentage of protection or inhibition following treatment. Significance against the control group: *p<0.05, **p<0.01, ***p<0.001.

Table 3: Anti-inflammatory effect of Tenghõ on carrageenan-induced paw oedema in Wistar rats.

	Dose	Paw oedema volume (mL) at various periods of time						
		0.5 hours	1 hour	2 hours	3 hours	4 hours	5 hours	6 hours
Control (water)	10 mL/kg	0.20 ± 0.01	0.27 ± 0.01	0.39 ± 0.018	0.43 ± 0.028	0.47 ± 0.004	0.45 ± 0.014	0.41 ± 0.017
Diclofenac	5 mg/kg	0.12 ± 0.004 ***	0.10 ± 0.005 ***	0.06 ± 0.009 ***	0.16 ± 0.008 ***	0.17 ± 0.007 ***	0.23 ± 0.014 ***	0.19 ± 0.022 ***
		-40.20%	-61.30%	-85.40%	-64.10%	-64.90%	-48.90%	-54.30%

Tenghō	200 mg/kg	0.16 ± 0.01	0.18 ± 0.018 ***	0.32 ± 0.02 **	0.17 ± 0.007 ***	0.31 ± 0.031 ***	0.24 ± 0.009 ***	0.1 ± 0.004 ***
		-20.50%	-34.40%	-17.60%	-20.80%	-33.30%	-45.90%	-52.30%
Tenghō	400 mg/kg	0.14 ± 0.008 *	0.2 ± 0.012 *	0.32 ± 0.016 *	0.23 ± 0.014 ***	0.32 ± 0.02 ***	0.26 ± 0.005 ***	0.22 ± 0.016 ***
		-32%	-25%	-16.70%	-22.40%	-31.90%	-43.30%	-46.90%
Tenghō	600 mg/kg	0.13 ± 0.005 **	0.26 ± 0.006 ***	0.26 ± 0.006 ***	0.19 ± 0.022 ***	0.28 ± 0.016 ***	0.23 ± 0.013 ***	0.22 ± 0.019 ***
		-34.40%	-38.10%	-33.90%	-25.90%	-40.40%	-48.90%	-45.70%

Note: Values are the mean ± SEM (n=6). The values in brackets represent the percentage of protection or inhibition following treatment. Significance against the control group: *p<0.05, **: p<0.01, ***p<0,001.

Table 4: Anti-inflammatory effect of Tenghō on histamine-induced paw oedema.

	Dose	Paw oedema volume (mL) at various periods of time		
		0.5 hours	1 hour	2 hours
Control (water)	10 mL/kg	0.426 ± 0.06	0.392 ± 0.08	0.438 ± 0.07
Promethazine	1 mg/kg	0.176 ± 0.02 ***	0.170 ± 0.02 **	0.182 ± 0.01***
		-58.68%	-56.63%	-58.44%
Tenghō	200 mg/kg	0.316 ± 0.04	1.302± 0.03	0.258 ± 0.02**
		-25.82%	#######	-41.09%
Tenghō	400 mg/kg	0.328 ± 0.01	0.314 ± 0.01	0.258 ± 0.01**
		-23.00%	-19.89%	-41.09%
Tenghō	600 mg/kg	0.300 ± 0.04	0.254 ± 0.01	0.230 ± 0.02***
		-29.57%	-35.20%	-47.48%

Note: Values are the mean ± SEM (n=5). The values in brackets represent the percentage of protection or inhibition Significance against the control group: *p<0.05, **p<0.01, ***p<0.001.

Table 5: Anti-inflammatory effect of Tenghō on serotonin-induced paw oedema.

	Dose	Paw oedema volume (mL) at various periods of time		
		0.5 hours	1 hour	2 hours
Control (water)	10 mL/kg	0.380 ± 0.05	0.504 ± 0.05	0.344 ± 0.03
Cyproheptadin	2 mg/kg	0.312 ± 0.03	0.348 ± 0.05 * (37.30 %)	0.126 ± 63.37 ***
		-16.84%		-63.37%
Tenghō	200 mg/kg	0.366 ± 0.02	0.380 ± 0.01	0.272 ± 0.01 (20.93 %)
		-3.68%	-24.60%	
Tenghō	400 mg/kg	0.360 ± 0.02 (5.26 %)	0.370 ± 0.05 (26.58 %)	0.326 ± 0.04
				-5.23%
Tenghō	600 mg/kg	0.358 ± 0.05	0.282 ± 0.05 **	0.208 ± 0.04 *
		-5.78%	-44.04%	-39.53%

Note: Values are the mean ± SEM (n=5). The values in brackets represent the percentage of protection or inhibition Significance against the control group: *p<0.05, **p<0.01, ***p<0.001.

DISCUSSION

The present study aimed at evaluating the analgesic and anti-inflammatory activities of tengho, a Cameroonian traditional drink made by a mixture of some traditionally used and scientifically evidenced plants against pain and inflammation. For this purpose, standard animal models of pain and inflammation were applied. Whether acute or chronic, pain modulation involves both peripheral and central nervous system mechanisms. Accordingly, new pain relief strategies involving multiple combinations of analgesics that point both central and peripheral nociceptive pathways provide a better opportunity for effective analgesia at reduced doses of individual agents [16,17]. In line of this, various plant mixtures such as tengho are used in Traditional Medicine over the World aiming at inducing greater effect at reduced and more tolerable doses of individual plants.

Acetic acid induced-writhing test is a well-known and widely used, reliable, simple, sensitive and particularly suitable model for evaluating even weaker analgesics [12,18]. Injected i.p., acetic acid induces peritoneal acute inflammation (tissue damage). It directly activates the visceral and somatic nociceptors that innervate the peritoneum, and promotes the local release of endogenous nociception-producing substances (inflammatory pain) such as prostaglandins, prostacyclins, thromboxanes, bradykinin, TNF-α, IL-1β and IL-8 [19-23]. Our results showed that Tengho significantly reduced writes in dose-dependent manner suggesting a peripheral analgesia by, at least partly, blocking nociceptors and/or the production and release of pro-inflammatory cytokines (COX inhibition). In addition to pain with an inflammatory component (e.g., sport injuries, arthrosis, and rheumatic diseases), COX inhibitors such as aspirin are suitable for the therapy of acute and transient pain including tension headache, lower back pain, migraine, acute gout, toothache, menstrual, and postsurgical pain [9]. Interestingly, Tengho at the doses of 400 and 600 mg/kg was more active than 200 mg/kg aspirin (48.41% inhibition) suggesting that this traditional drink could be used in these types of pain. Both central and peripheral nociceptive pathways are known to be implicated in inflammatory pain. Therefore, to further investigate the involvement of central pain pathways in the anti-nociceptive effect of Tengho, tail immersion test was used.

Tail-immersion test causes centrally mediated pain at both spinal and supra-spinal levels [24-26]. As the tail withdrawal following immersion in warm water is a reflex response to heat stimuli, it obviously involves central mechanisms. Central analgesia is mainly mediated by opioids especially MOR agonists, the most efficient available painkillers [18,27]. It can also be accomplished by co-analgesics, including neuron inhibitors with pleotropic effects, ketamine, α2AR agonists, benzodiazepines, nonselective monoamine reuptake inhibitors, selective serotonin-norepinephrine reuptake inhibitors, muscle relaxants, and anti-osteoporotic drugs [9]. In our study, oral administration of Tengho at all tested doses significantly increased the response time in tail withdrawal suggesting spinal and/or supra-spinal analgesia. At the doses of 400 and 600 mg/kg, the percentages of inhibition are higher than that of the low-potency MOR agonist tramadol (20 mg/kg). However, between the activation of opioid receptors and the different mechanisms of co-analgesics, the under mechanisms of tengho in central analgesia need to be elucidated.

Along with pain, oedema is a cardinal feature of the inflammation. In other words, analgesia on pain inflammatory assumes an anti-inflammatory activity. Carrageenan-induced paw oedema is an acute inflammation model extensively used for assessing anti-inflammatory components. It is known to be a biphasic process [28,29]. Histamine, serotonin and bradykinins were release during the first phase (0-2 h after carrageenan injection) while, the later phase (3-6 hours) is attributed to polymorphonuclear neutrophils (PMNs) leukocyte infiltration, generation of prostaglandins and various pro-inflammatory cytokines such as nitric oxide, IL-1β, IL-6, IL-10, and TNF-α [29-32]. According to our results, tengho at all tested doses significantly inhibited carrageenan-induced paw oedema throughout the two phases like diclofenac (5 mg/kg), suggesting an inhibiting action on the histamine and serotonin as well as prostaglandins release. However, the activity of tengho was more pronounced (43.3%-52.3% inhibition) during the second phase. At 5-6 hours, the inhibition value is close to that of 5 mg/kg diclofenac, a COX inhibitor. This lower activity of tengho observed during the first phase was further confirmed in histamine and serotonin-induced paw oedema tests. However, results from these two tests showed that tengho was more active on histamine-induced paw oedema. Histamine and serotonin induce inflammation by initiating vasodilation, increasing vascular permeability and attracting neutrophils at the target site [33,34]. Taken altogether, results indicate that tengho exerts its anti-inflammatory activity by inhibiting the action or release of inflammatory mediators including histamine, serotonin and prostaglandins.

CONCLUSION

In conclusion, this study showed the anti-nociceptive and anti-inflammatory properties of tengho using standard murine models. Tengho is endowed with peripheral and central analgesia, associated with anti-inflammatory effects on acute inflammatory processes. These effects could explain and support the traditionally use of this drink as a pain and inflammatory remedy in Cameroon. However, further studies are needed to elucidate the exact mechanism by which tengho inhibits inflammation and pain.

CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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