

Anti-inflammatory Activity of the Aqueous Extract of the Mixture of *Gossypium hirsutum* L. (Malvaceae) and *Terminalia catappa* L. (Combretaceae)

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Abstract

Introduction: Inflammation is a natural defense response of higher organisms to various external aggressors, such as physicochemical factors or microbial infections. The objective of this work is to enhance a medicinal formula derived from a mixture of *Gossypium hirsutum* L. and *Terminalia catappa* L. by evaluating and improving its anti-inflammatory activity.

Methods: The two plant species selected for this study are *Gossypium hirsutum* L. and *Terminalia catappa* L. The aqueous extract of the medicinal formulation was obtained by mixing 42 g of *Gossypium hirsutum* L. powder with 24 g of *Terminalia catappa* L. pericarp powder, then 4.5 L of distilled water was added to this mixture and boiled for 30 min. The anti-inflammatory activity in rats was evaluated using the 1% carrageenan edema induction method, which triggers an inflammatory response. Calipers were used to assess the size of the edema in rats. The C-reactive protein (CRP) assay was performed using a COBAS 311 (Roche Hitachi) analyzer.

Results: The percentage of inhibition of AEGhTc 300 mg/kg of pw was $42.50 \pm 05.70\%$ after 3 hours compared to $26.73 \pm 10.00\%$ for diclofenac at 10 mg/kg ($P < 0.05$). It is important to note that the 300 mg/kg dose of AEGhTc (0.03 ± 0.02 mg/l) induced the lowest CRP concentration compared to the highest doses of AEGhTc 1000 and AEGhTc 2000 (0.28 ± 0.15 mg/l and 0.07 ± 0.04 mg/l).

Conclusion: The aqueous extract of the mixture of *Gossypium hirsutum* L. and *Terminalia catappa* L. exhibited significant anti-inflammatory activity at a dose of 300 mg/kg of AEGhTc.

Keywords: *Gossypium hirsutum* L., *Terminalia catappa* L., Aqueous extract, Inflammation

Introduction

Inflammation is a natural defense response of higher organisms to various external aggressors, including physicochemical factors, such as radiation, burns, or mechanical trauma, as well as microbial infections (bacterial, viral, or parasitic). It can also be triggered by endogenous elements, including products of the immune response, such as immune complexes, cytotoxic antibodies, or cytokines. The primary objective of inflammation is to eliminate pathogens

and promote the repair of damaged tissues [1,2]. Inflammation manifests through symptoms of varying severity, including redness, heat, pain, swelling, and discomfort or functional impairment [3]. These manifestations can lead to more generalized consequences such as asthenia, anorexia, weight loss, fever, sleep disturbances, and cachexia accompanied by muscle wasting [4].

There are two types of inflammation: acute inflammation and chronic inflammation. Acute inflammation is a rapid

and immediate response to an injurious agent, typically lasting from a few days to a few weeks [5]. It is characterized by the exudation of fluids and plasma proteins (edema) and the migration of neutrophils to the injured site. Chronic inflammation, on the other hand, results from the failure of acute inflammation to resolve, and may last for months or even years [6]. It can occur as a result of incomplete clearance of the injurious agent, prolonged exposure to irritants, autoimmune disease, or a defect in inflammatory cells [7].

Medicinal plants constitute a valuable resource in the search for new therapeutic solutions, particularly in regions where access to conventional medicines is limited. In Côte d'Ivoire, traditional pharmacopoeia relies on a rich diversity of plants, some of which, such as *Gossypium hirsutum* L. (Malvaceae) and *Terminalia catappa* L. (Combretaceae), are empirically used to treat various diseases [8]. *Gossypium hirsutum* L., commonly known as cotton, is widely used in traditional medicine to treat various diseases and microbial infections in communities around the world. It is known for its antimicrobial and antiviral activities [9]. The fruit of *Terminalia catappa* L., known as "Badame" or "petit cocoman," is believed to improve blood circulation, prevent heart diseases, lowers blood pressure, reduce cholesterol levels, and strengthens immunity. The study of the mixture of *Gossypium hirsutum* L. and *Terminalia catappa* L. reveals the presence of bioactive secondary metabolites such as alkaloids, polyphenols, sterols, polyterpenes, gallic tannins and quinone compounds, suggesting promising pharmacological properties such as anti-inflammatory, antioxidant, and antihyperglycemic effects) [10].

In light of these beneficial properties, this study aims to explore the potential therapeutic effects of the mixture of *Gossypium hirsutum* L. and *Terminalia catappa* L., in the form of a decoction. The primary objective is to promote the medicinal formulation derived from these two plants, which are part of the Ivorian pharmacopoeia, by evaluating their anti-inflammatory activities.

Materials and Methods

Ethics

The study was approved by the Ethics Committee of the Biosciences Training and Research Unit on April 10, 2024.

Plant materials

The two plant species selected for this study are *Gossypium hirsutum* L. and *Terminalia catappa* L. was collected from the courtyard of the National Institute of Public Health of Côte d'Ivoire, while *Gossypium hirsutum* L. was obtained from a traditional practitioner in the commune of Yopougon, Côte d'Ivoire.

The fruits of both plants were thoroughly washed and then dried separately in the open air, under the shade. Once dried,

the *Terminalia catappa* L. fruits were deseeded, leaving only the pericarp (epicarp and mesocarp). One hundred grams (100 g) of *Gossypium hirsutum* L. fruits and one hundred grams (100 g) of *Terminalia catappa* L. pericarp were ground separately using a blender to obtain a homogeneous powder for each species. These powders were stored at 25°C in tightly sealed jars, and kept away from light and heat, until use.

Animal models

The animals used in the *in vivo* study of anti-inflammatory activity were Wistar rats of both sexes, weighing between 100 and 225 g and aged 2 to 5 months. These animals were obtained from the animal facility of the National Higher Education School. The rats were housed in groups of three in plastic cages with stainless steel lids, each measuring 36 cm x 25 cm. They had free access to both water and food. Six groups of three rats each were formed for the study. Prior to the start of the experiments, the rats underwent a two-week adaptation period, during which they were kept at an ambient temperature of 20 to 24°C, under a 12-hour light/dark cycle to respect their biological clock. The bedding, consisting of wood shavings, was renewed three times a week to ensure optimal hygiene conditions. Individual rat identification was achieved by numbering their tails using a permanent marker.

Reagents and devices

Distilled water was used as the base solvent in various preparations. Carrageenan, at a concentration of 1%, was used to induce inflammation, particularly edema, in rats. This product was provided by the Biochemistry Laboratory of the Felix Houphouët-Boigny University in Côte d'Ivoire. Diclofenac, at a dosage of 100 mg (manufactured by DENK PHARMA, Germany), was used as the standard anti-inflammatory drug and was obtained from a local pharmacy. Ether was used to anesthetize the rats prior to experimentation.

To assess the extent of edema in the rats, calipers were used. C-reactive protein (CRP) was measured using a COBAS 311 automated analyzer (Roche Hitachi).

Preparation of the aqueous extract of the mixture of *Gossypium hirsutum* L. and *Terminalia catappa* L.

The aqueous extraction was carried out according to the method described in [11]. The aqueous extract of the medicinal formulation was obtained by mixing 42 g (12 tablespoons) of *Gossypium hirsutum* L. powder with 24 g (3 tablespoons) of *Terminalia catappa* L. pericarp powder in a saucepan. 4.5 L of distilled water was added to this mixture, then it was brought to a boil for 30 minutes, with occasional stirring to prevent the powders from burning. The resulting decoction was filtered successively through a sieve, followed by a white cloth, and twice through absorbent cotton to obtain a clear filtrate. This filtrate was then oven-dried at 55°C for 48 hours, and the

resulting powder is the aqueous extract of the mixture of *G. hirsutum* and *T. catappa*, denoted AEGhTc. This powder was stored in sterile jars at room temperature (27°C) for use in various experiments.

Anti-inflammatory activity study

Carrageenan test: The anti-inflammatory activity in rats was evaluated using the 1% carrageenan-induced paw edema method, which triggers an inflammatory response. This method, as described by [12], involves injecting 0.05 ml of a 1% carrageenan suspension into the plantar fascia of the animal's left hind paw. The animals are then placed in cages and observed for 30 minutes until significant edema appears.

Carrageenan, a polysaccharide derived from red algae, induces acute inflammation when injected into soft tissues, such as the rat paw. It triggers the release of inflammatory mediators (CRP, prostaglandins, histamines, cytokines), resulting in a rapid increase in tissue volume at the injection site.

First, the paw diameter was measured using a caliper before any treatment was administered; this served as the baseline diameter (D0 to t0). Subsequent measurements of paw edema were taken hourly over a period of three hours.

For this study, a total of 18 rats were randomly assigned into six (6) groups, with three (3) rats in each group.

- Group 1 (Negative control): Rats received only 1 ml of distilled water per 100 g of body weight (BW).
- Group 2 (Positive control): Rats received only 1% carrageenan.
- Group 3: Rats received diclofenac at a dose of 10 mg/kg BW, followed 30 minutes later by 1% carrageenan.
- Group 4: Rats received 300 mg/kg BW of AEGhTc, followed 30 minutes later by 1% carrageenan.
- Group 5: Rats received 1,000 mg/kg BW of AEGhTc, followed 30 minutes later by 1% carrageenan.
- Group 6: Rats received 2,000 mg/kg BW of AEGhTc, followed 30 minutes later by 1% carrageenan.

Evaluation of anti-inflammatory activity: Three hours after the induction of edema, the development of inflammation was measured with a caliper at hourly intervals over a period of three hours. The paw diameter was measured before and after carrageenan administration. These measurements were used to calculate the percentage inhibition of edema.

The percentage of inhibition is calculated using the following formula:

$$\% \text{ INH} = \frac{(\% \text{ Control INC} - \% \text{ Treated INC})}{(\% \text{ Control INC})} \times 100$$

Where INH stands for percentage of inhibition and INC for increase in paw diameter

The extent of edema was assessed by determining the average percentage increase (% INC) in paw volume using the following formula:

$$\% \text{ INC} = \frac{(dt - d0)}{(d0)} \times 100$$

Where Dt is Paw diameter at time t after carrageenan injection and D0 is Initial paw diameter at t0 (before carrageenan administration).

CRP assay: Three hours (3h) after carrageenan administration, blood samples were collected from rats in each group into dry tubes. The serum obtained after centrifugation (3,000 rpm for 15 min) was used for CRP assay using the enzyme-linked immunosorbent assay (ELISA) method on a COBAS C 311 multiparametric biochemistry analyzer (Roche Hitachi).

Statistical analysis of data

Data analysis was performed using GraphPad Prism software (version 8, San Diego, CA, USA). Results are expressed as mean $\pm$ standard error of the mean (M $\pm$ SEM). Statistical analysis was performed using the Student's t-test. Additionally, Dunnett's multiple comparison test was used to compare the variance of control groups with the treatment groups. Differences were considered significant according to the following criteria: Significant: $p < 0.05$, Very significant: $p < 0.01$, Highly significant: $p < 0.001$, Extremely significant: $p < 0.0001$.

Results

Yield of the aqueous extract of AEGhTc

A mixture of 42 g of *Gossypium hirsutum* fruit powder and 24 g of *Terminalia catappa* pericarp powder, totaling 66 g, was used for aqueous extraction, which yielded 7 g of extract—an extraction yield of 10.60%.

Anti-inflammatory activity of AEGhTc on carrageenan-induced edema

A significant ($P < 0.05$) increase in edema was observed following carrageenan administration via the plantar route, reaching a peak of 7.46 ± 0.45 mm 2 hours after injection. In comparison, diclofenac treatment showed a percentage increase of $55.85 \pm 2.50\%$. The increase in peak with different doses of AEGhTc was observed after 1 hour: AEGhTc 300 mg/kg BW ($49.00 \pm 2.50\%$), AEGhTc 1,000 mg/kg BW ($54.52 \pm 3.00\%$) and AEGhTc 2,000 mg/kg BW ($55.85 \pm 2.50\%$), compared to the negative control, which presented an edema of 4.53 ± 0.20 mm (Table 1).

Table 1. Effect of AEGhTc and diclofenac on paw edema.					
Groups	Treatment	Paw size	1 hour	2 hours	3 hours
1	Negative Control (Distilled water)	Paw size (mm)	4.53 ± 0.20	4.53 ± 0.20	4.53 ± 0.20
2	Positive Control (Carrageenan1%)	Paw size (mm)	6.90 ± 0.26	7.46 ± 0.45	7.26 ± 0.45
		Increased edema (%)	(52.31 ± 1.21 %) *	(64.58 ± 6.30%) *	(60.26 ± 6.31%)
3	10 mg/kg of Diclofenac + carrageenan 1%	Paw size (mm)	7.06 ± 0.15	6.8 ± 0.20	6.53 ± 0.2
		Increased edema (%)	(55.85 ± 2.50 %) *	(50.11 ± 00.00 %) *	(44.15 ± 00.00 %)
		Inhibition of inflammation (%)	(-06.76 ± 10.00 %)	(22.40 ± 10.00 %)	(26.73± 10.00 %)
4	AEGhTc 300 mg/kg + carrageenan 1%	Paw size (mm)	6.73 ± 0.25	6.4±0.17	6.1 ± 0.26
		Increased edema (%)	(49.00 ± 02.50 %) *	(41.28 ± 01.50 %)	(34.65 ± 03.00 %)
		Inhibition of inflammation (%)	(06.32 ± 01.66 %) *	(36.08 ± 02.44%) *	(42.50 ± 05.70 %) *
5	AEGhTc 1,000 mg /kg + carrageenan 1%	Paw size (mm)	7 ± 0.26	6.83 ± 0.23	6.6 ± 0.17
		Increased edema (%)	(54.52 ± 03.00 %) *	(50.99 ± 01.50 %)	(45.69 ± 01.50 %)
		Inhibition of inflammation (%)	(-04.22 ± 10.00 %)	(22.55 ± 08.80 %)	(24.18 ± 08.80 %)
6	AEGhTc 2,000 mg /kg + carrageenan 1%	Paw size (mm)	7.06 ± 0.15	6.9 ± 0.17	6.73 ± 0.20
		Increased edema (%)	(55.85 ± 02.50 %) *	(52.32 ± 01.50 %)	(48.56 ± 0000 %)
		Inhibition of inflammation (%)	(-06.77 ± 0.18 %)	(18.98 ± 08.80 %)	(19.41 ± 10.00 %)
Significant (*): p<0.05					

The percentages of edema increase induced by both AEGhTc doses and diclofenac decreased over time. However, there were no significant differences (P>0.05) in the percentages of inflammation inhibition between diclofenac (10 mg/kg) and AEGhTc at 1,000 and 2,000 mg/kg BW at any of the

measurement time points (**Figure 1**). In contrast, AEGhTc 300 mg/kg BW induced a significantly higher percentage of inhibition (P<0.05) compared to diclofenac 10 mg, with a peak inhibition of 42.50 ± 5.70% observed after 3 hours (**Table 1**).

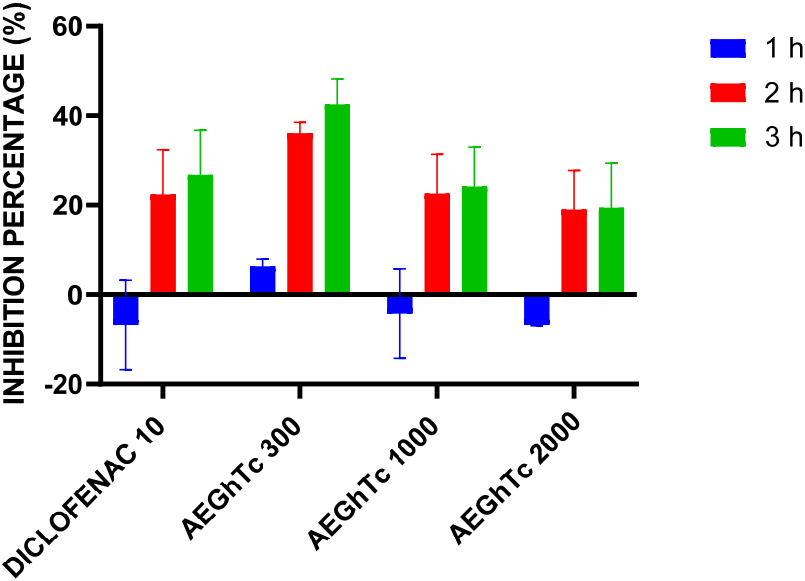


Figure 1. Effect of AEGhTc on the inhibition of carrageenan-induced inflammation. At one hour, the inhibition percentages are negative for Diclofenac, AEGhTc 1,000, and AEGhTc 2,000.

Anti-inflammatory activity of AEGhTc characterized by CRP measurement

A significant increase in mean CRP concentration ($P<0.05$) was observed in rats administered 1% carrageenan alone, with a value of 0.24 ± 0.03 mg/L, compared to 0.11 ± 0.04 mg/L in the group receiving distilled water. After treatment, CRP concentration decreased in rats treated with diclofenac 10 mg/kg BW (0.01 ± 0.00 mg/l), AEGhTc 300 mg/kg BW (0.03 ± 0.02 mg/l), and AEGhTc 2,000 mg/kg BW (0.07 ± 0.04 mg/l), compared with that of the distilled water control group (0.11 ± 0.04 mg/l). Importantly, the 300 mg/kg BW dose of AEGhTc induced the lowest CRP concentration among all AEGhTc doses tested (Table 2).

Table 2. Effect of AEGhTc and diclofenac on CRP of rats, measured after 3 hours.		
Groups	Treatment	CRP (mg/l)
1	Negative Control (Distilled water)	0.11 ± 0.04
2	Positive Control (Carrageenan 1%)	$0.24 \pm 0.03^*$
3	10 mg/kg of Diclofenac + carrageenan 1%	$0.01 \pm 0.00^{**}$
4	AEGhTc 300 mg/kg + carrageenan 1%	$0.03 \pm 0.02^{**}$
5	AEGhTc 1,000 mg/kg + carrageenan 1%	0.28 ± 0.15
6	AEGhTc 2,000 mg/kg + carrageenan 1%	$0.07 \pm 0.04^{**}$
CRP: C-reactive Protein; Significant (*): $p<0.05$; Very significant (**): $p<0.01$		

Discussion

The objective of this study was to evaluate the anti-inflammatory activity of the AEGhTc medicinal product. The AEGhTc extract was evaluated using a carrageenan-induced paw edema model in rats. The results showed a significant reduction in edema ($P<0.05$) three hours after carrageenan injection, with inhibition percentages of $42.50 \pm 0.57\%$ at 300 mg/kg BW, $24.18 \pm 0.80\%$ at 1,000 mg/kg BW, and $19.41 \pm 10.00\%$ at 2,000 mg/kg BW. Although these inhibition percentages are comparable to those observed with diclofenac ($26.73 \pm 10.00\%$), they all indicate an inhibition of acute inflammation with a more pronounced reduction with AEGhTc300, mainly during the second phase of edema, caused by the release of prostaglandins [13]. In contrast, the first phase of carrageenan-induced inflammation is partly attributed to injection-induced trauma and the release of acute phase mediators, particularly serotonin and histamine [14].

The level of CRP, a classic marker of inflammation, was significantly elevated ($P<0.05$) in the control group treated with carrageenan compared to negative control treated with distilled water. The group treated with diclofenac (a nonsteroidal anti-inflammatory drug) showed a significant reduction ($P<0.05$) compared to the group treated with

carrageenan alone (0.01 ± 0.00 and 0.24 ± 0.03 mg/l). Diclofenac is an anti-inflammatory drug whose mechanism of action is through the inhibition of the cyclooxygenase (COX) enzyme, thus reducing the production of pro-inflammatory prostaglandins [15]. AEGhTc extract, at doses of 300 mg/kg BW (0.03 ± 0.02 mg/l) and 2,000 mg/kg BW (0.07 ± 0.04 mg/l), also produced a significant reduction ($P<0.05$) in CRP levels, compared to the group treated with diclofenac (0.01 ± 0.00 mg/l), which is the reference drug, suggesting an encouraging anti-inflammatory effect. Phytochemical analysis of AEGhTc, as reported by [10], revealed the presence of flavonoids and polyphenols. These results indicate that the bioactive compounds in AEGhTc, such as flavonoids and polyphenols, play a key role in effectively reducing inflammation. In addition, AEGhTc is thought to act through a mechanism similar to that of diclofenac [16]. Other studies on plant extracts such as turmeric [17] and *Aloe vera* [18] at a dose of 500 mg/kg BW, have shown similar anti-inflammatory effects. These plants are rich in polyphenols and flavonoids, which are known for their anti-inflammatory properties [19]. According to [18], the anti-inflammatory effect of the aqueous extract of *Aloe vera* (AEAV) leaves (500 mg/kg BW) showed a 48.21% inhibition after the 4 hours, compared to 71.43% inhibition observed with indomethacin, the reference drug. The authors concluded that AEA at a dose of 500 mg/kg BW demonstrated an appreciable anti-inflammatory effect.

Conclusions

Our study showed that the aqueous extract of the mixture of *Gossypium hirsutum* L. and *Terminalia catappa* L. exhibits a statistically significant anti-inflammatory activity at a dose of 300 mg/kg BW of AEGhTc. These results are promising for the development of a drug traditionally improved with a dose of 300 mg/kg BW of AEGhTc.

Conflicts of Interest

There is no conflict of interest in this study.

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Author Contributions Statement

TREISSOU participated in the design of the study and wrote the article. TREISSOU, OKOGNI, and YAPO carried out the study. YAPO supervised the study.

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