

Original Research Article

Evaluation of anti-inflammatory activities of aqueous and ethanolic extracts of *Gomphrena celosioides* Linn.

ABSTRACT

Aims: This study aims to evaluate potential anti-inflammatory activity of Aqueous and Ethanolic extracts of *Gomphrena celosioides* (C. Mart) in animal models

Study Design: *Gomphrena celosioides* plants was collected from Bingerville, District of Abidjan (Cote d'Ivoire). The plant was identified and authenticated by botanist of "Centre National de Floristique", University Felix Houphouet Boigny, Cocody.

Place and Duration of study: analysis of the plant samples were done at "Laboratoire de Pharmacodynamie-Biochimique, UFR Biosciences, Université de Cocody-Abidjan (Côte d'Ivoire), and Institut Pasteur de Côte d'Ivoire, Département de Biochimie médicale & fondamentale" between September 2013 and June 2014.

Methodology: Aqueous and Ethanolic extracts of *Gomphrena celosioides* were investigated for in carrageenan induced paw edema in wistar rats, as compared to standard drug, Diclofenac. These extracts were administrated at doses of 100 and 200 mg/kg body weight. The effects were analyzed by following the evaluation of edema and various levels of CRP were measured by ELISA method.

Result: The present study showed that both extracts showed significant anti-inflammatory activity at 200 mg/kg. b.w. used in study and produced no significant ($p < 0.05$) inhibition of edema as comparable the inhibition produced by 10 mg/kg Diclofenac, the reference drug. Aqueous and Ethanolic extracts and Diclofenac produced maximum inhibition at 30.05%, 31.91% and 32.56% respectively. Statistical analysis showed a significant difference ($p < 0.05$) between CRP values of extracts and diclofenac as compared carrageenan. Indeed, the value of CRP of carrageenan is higher than extracts and the reference molecule CRP values. But there is no significant difference between the values of the CRP of extracts and diclofenac.

Conclusion: The study showed that extracts of *Gomphrena celosioides* have potential anti-inflammatory properties and can be utilized for therapeutic purposes.

Keywords: *Gomphrena celosioides*, anti-inflammatory, carrageenan, Diclofenac, CRP

1. INTRODUCTION

Inflammation is a local response of living mammalian tissues to the injury. It is a body defense reaction in order to eliminate or limit the spread of injurious agents as well as to remove the consequent necrosed cells and tissue [1]. Inflammation standard drugs like Indomethacin, Diclofenac, Voveran, ect, are not entirely free of side effects and have their limitation. So, it is a need to continue to research newer anti-inflammatory drugs. Medecinal plants are believed to be an important source of new chemical substances. Many herbal preparations are being prescribed widely for the treatment of inflammatory conditions [2]. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs [3].

Gomphrena celosioides Mart (Amaranthaceae) is an annual herb with popular usage in traditional medecine. An early pharmaco-chemical study of *Gomphrena celosioides* revealed the presence of saponins, steroids, aminoacids and non-reducing sugars in all the plant parts, phenols and

flavonoids in leaves, inflorescences and stem. Betacyanins occurred only in the stem; reducing sugar in the inflorescence, while ketoses were reported found in the root and stem [4, 5]. Similar metabolites were found by de Moura *et al* [6] in the plant

Phytochemical study of the Aqueous extract showed that this plant contains Flavonoids, Saponins, sterols and tri-terpens, tanins, coumarins, reducers compounds, sugars and holosises [7]. The ethyl acetate and methanol extracts of *Gomphrena celosioides* was phytochemically examined by Dosumu *et al.* [8, 9] and revealed the presence of secondary metabolites. These metabolites include alkaloids, tannins, saponins, steroids, glycosides and reducing sugars

These different types of chemical interground Blended highlighted in extracts of this plant have therapeutic effects. It can be used for the treatment of human and animal diseases [10]. In South America, it is used as abortive [11], Vieira [12] have demonstrated the analgesic, tonic, carminative and diuretic properties of this plant. Recently, Dosumu *et al.*, [13] reported its antimicrobial and anti-helminthic properties. Crude and ethanol extract of the leaf of the plant were found to have antioxidant/antidegradative activity [14]. But, little information was recorded on the anti-inflammatory activity of *Gomphrena celosioides*.

The present investigation was carried out to evaluation the anti-inflammatory potential of Aqueous and Ethanolic extracts of *Gomphrena celosioides*.

2. MATERIALS AND METHODS

2.1 Samples Collection and extraction

Gomphrena celosioides plants was collected in September 2013 from Bingerville, District of Abidjan (Cote d'Ivoire). The plant was identified and authenticated by botanist of "Centre National de Floristique", University Felix Houphouet Boigny, Cocody The authentically identified plant material (roots, leaves, flowers and stems) was washed and shade air- dried for 2-3 days in the laboratory at room temperature. It was powdered and subjected to different extraction procedures.

2.1.1 Aqueous extract

The aqueous extract was prepared by decoction method [15]. The powdered material was suspended in distilled water (100g/1000 mL) agitated with an agitator for 24h at 80°C. The extract was filtered through absorbent cotton then with Whatman N^o1 filter paper to get the filtrate. The filtrate was dried under reduced pressure using a rotary flash evaporator and stored at a temperature of -4°C until use.

2.1.2 Ethanolic extract

The powder plant material (100 g) was soaked in 1L of 70% ethanol, agitated with an agitator for 24h at 80°C. The extract was filtered and concentrated to dryness using a rotary flash evaporator and stored at a temperature of -4°C until use.

2.2 Experimental animals

Wistar albinos rats weighing 150- 250 g of each sex kept for two weeks at the laboratory Animal home of the Faculty of Pharmaceutical (Biochemistry), University of Felix Houphouet Boigny, Cote d'Ivoire were used. The animals were maintained under standard housing conditions: temperature (27° ± 1C), humidity (55-60%), light/dark cycle (12:12h) and had free access to standard diet and water.

2.3 Tests for Anti-inflammatory Activity

Anti-inflammatory activity at the extracts was measured using carrageenan induced rat paw edema essay [16, 17]. Extracts of *Gomphrena* were dissolved in normal saline (0.9%) and administrated intra-peritoneally.

2.3.1 Carrageenan-induced paw edema model

Thirty six rats of either sex were divided into six groups (n=6). Different groups of animals were pretreated with 100 or 200 mg/kg.b.w. of each extract or 10 mg/kg.b.w. Diclofenac as reference drug [18, 19]. The control group received normal saline (0.9%) intra-peritoneally. After 1h, inflammation was induced by injection of 0.2 ml[20] carrageenan (1% suspension of carrageenan in normal saline) into the plantar surface of the right hind paw. The paw diameter was measured at hourly intervals for 6 h and at 24h hours using digital paw edema meter. Percentage inhibition was calculated as follows:

$$\text{Inhibition (\%)} = \frac{(V_c - V_t) \times 100}{V_c}$$

Where V_c and V_t are average edema diameter of control and treated group respectively.

2.3.2 Quantitative measurement of Rat C Reactive Protein (CRP) in serum

Thirty rats were divided into 6 groups, each containing six animals. Group I received normal saline 0.9% (control). Group II received 0.2 ml carrageenan, a phlogistic agent. Groups III, IV and V received (200 mg/kg.b.w) aqueous and ethanolic extracts and 10 mg/kg.b.w. Diclofenac respectively.+ 0.2 ml carrageenan after 1h. After 5 hr of carrageenan administration, all the animals were sacrificed, blood samples were collected and serum was separated. The levels of C-reactive protein (CRP) was measured by ELISA method. The concentrations of CRP in samples are determined with a standard curve (Essay procedure), drawn using standard concentrations of CRP.

Essay procedure [21]

1. Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.
2. It is recommended to assay all standards, controls and samples in duplicate.
- 2.1 Prepare all reagents, working standards and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).
- 2.2 Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- 2.3 Add 50 µL of C Reactive Protein Standard or sample per well. Cover wells with a sealing tape and incubate for two hours. Start the timer after the last sample addition.
- 2.4 Wash five times with 200 µL of 1X Wash Buffer manually. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of 1X Wash Buffer and then invert the plate, decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.
- 2.5 Add 50 µL of 1X Biotinylated C Reactive Protein Antibody to each well and incubate for one hour.
- 2.6 Wash microplate as described above.
- 2.7 Add 50 µL of 1X SP Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- 2.8 Wash microplate as described above.
- 2.9 Add 50 µL of Chromogen Substrate per well and incubate for about 20 minutes or till the optimal blue colour density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- 2.10 Add 50 µL of Stop Solution to each well. The color will change from blue to yellow.
- 2.11 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

2.4 Statistical Analysis

Experimental data were expressed as the mean $\pm$ standard deviation error of the mean (S.E.M). Differences between the treatment groups were ascertained using one way ANOVA followed by Dunnett's test, performed using GraphPad Prism 5.00.288, *P* values less than 0.5 were considered as indicative of significance.

3. RESULTS AND DISCUSSION

3.1 Carrageenan-induced paw edema model

Inhibition of carrageenan induced paw edema in rats by extracts of *Gomphrena celosioides* and Diclofenac started at 1st hour and which maintained up to 24h. The results showed significant reduction in the paw diameter observed with aqueous and ethanolic extracts (200 mg/kg.b.w) and Diclofenac treated group, from 1sth to 24thh as compared reduction of extracts 100 mg/kg.b.w. The statistical analysis shows no significant difference ($p < 0.05-0.01$), between the diameter of the paw at different times with the two extracts (200mg/kg.bw) compared to results obtained with the reference molecule, Aqueous extracts (100mg/kg.b.w) drive a transient decrease paw diameter from the 1st to the 3rd time after carrageenan injection (Table 1). We obtained no effect of the ethanol extract at dose 100 mg / kg.bw before 6h after the injection of carrageenan. The results show increases of average percentage of edema inhibition by different doses of diclofenac and extracts a function of time that reach their maximum inhibition of 26.06% at the 4th hour (aqueous extract 100mg/kg bw), 30.05% at the 5th hour (aqueous extract 200mg/kg bw), 14.49% at 4th hours (ethanolic extract 100mg/kg bw), 31.91% at the 5th hour (ethanolic extract 200mg/kg bw), 32.56% at the 6th hour to diclofenac (Table 2). On average, the percentage inhibition of edema by Diclofenac is higher than other substances (Figure 1).

Table 1: Effect of Aqueous and Ethanolic extracts of *Gomphrena celosioides* and Diclofenac on carrageenan induced paw edema in rats

Treatment groups	Dose (mg/kg)	Edema diameter (mm)							
		0hr	1hr	2hr	3hr	4hr	5hr	6hr	24hr
Normal saline (control)	-	4.79 $\pm$ 0.11	6.38 $\pm$ 0.41	6.94 $\pm$ 0.55*	7.21 $\pm$ 0.47**	7.52 $\pm$ 0.38**	7.52 $\pm$ 0.46**	7.31 $\pm$ 0.51**	5.42 $\pm$ 0.16
Aqueous Extract	100	4.61 $\pm$ 0.09	5.57 $\pm$ 0.24	5.50 $\pm$ 0.23	5.43 $\pm$ 0.24	5.56 $\pm$ 0.35	6.20 $\pm$ 0.48	6.86 $\pm$ 0.45*	5.08 $\pm$ 0.26
Aqueous Extract	200	4.78 $\pm$ 0.15	5.75 $\pm$ 0.13	5.44 $\pm$ 0.08	5.38 $\pm$ 0.06	5.33 $\pm$ 0.06	5.26 $\pm$ 0.05	5.23 $\pm$ 0.06	5.18 $\pm$ 0.04
Ethanolic Extract	100	4.88 $\pm$ 0.05	5.88 $\pm$ 0.28	6.08 $\pm$ 0.19	6.21 $\pm$ 0.13	6.43 $\pm$ 0.25	6.78 $\pm$ 0.25*	6.54 $\pm$ 0.16 *	5.05 $\pm$ 0.18
Ethanolic Extract	200	4.59 $\pm$ 0.10	5.56 $\pm$ 0.07	5.44 $\pm$ 0.09	5.35 $\pm$ 0.10	5.23 $\pm$ 0.11	5.12 $\pm$ 0.07	5.01 $\pm$ 0.08	4.67 $\pm$ 0.05
Diclofenac (Standard)	10	4.41 $\pm$ 0.02	5.70 $\pm$ 0.03	5.54 $\pm$ 0.01	5.43 $\pm$ 0.01	5.29 $\pm$ 0.02	5.10 $\pm$ 0.02	4.93 $\pm$ 0.06	4.69 $\pm$ 0.06

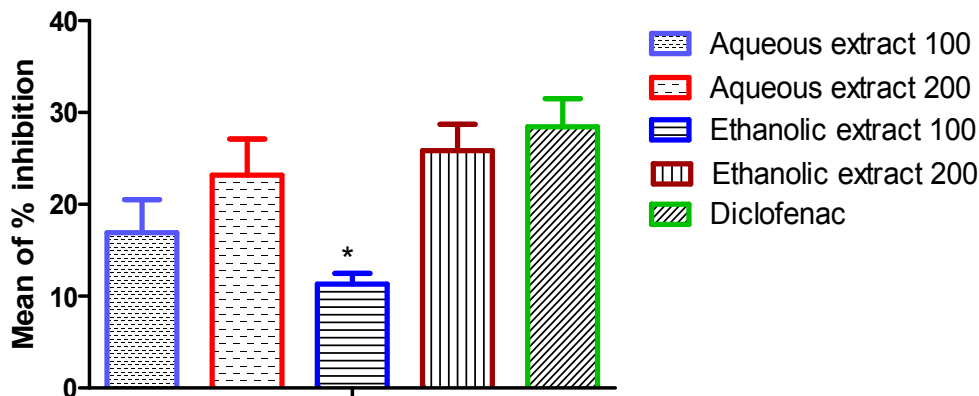
Each value is mean $\pm$ SEM, N= 6 rats

The data was analyzed by using One Way ANOVA followed by Dunnett's test, ** $p < 0.01$, * $p < 0.05$, where Extracts and control were compared with Diclofenac

Table 2: Percentage inhibition of paw edema exhibited by aqueous and ethanolic extracts of *Gomphrena celosioides* and Diclofenac

Treatment	Dose mg/kg	Percentage inhibition (%) at various times intervals						
		1hr	2hr	3hr	4hr	5hr	6hr	24hr
Normal saline (control)	-	-	-	-	-	-	-	-
Aqueous Extract	100	12.69	20.74	24.68	26.06	17.55	6.15	6.27
Aqueous Extract	200	9.87	21.61	25.38	29.12	30.05	28.45	4.42
Ethanolic Extract	100	7.83	12.39	13.86	14.49	9.84	10.53	6.82
Ethanolic Extract	200	12.85	21.61	25.79	30.45	31.91	31.46	13.83
Diclofenac	10	10.65	20.17	24.68	29.65	32.18	32.56	13.46

Figure 1: Mean of % inhibition of paw edema exhibited by aqueous and ethanolic extracts of *Gomphrena celosioides* and Diclofenac



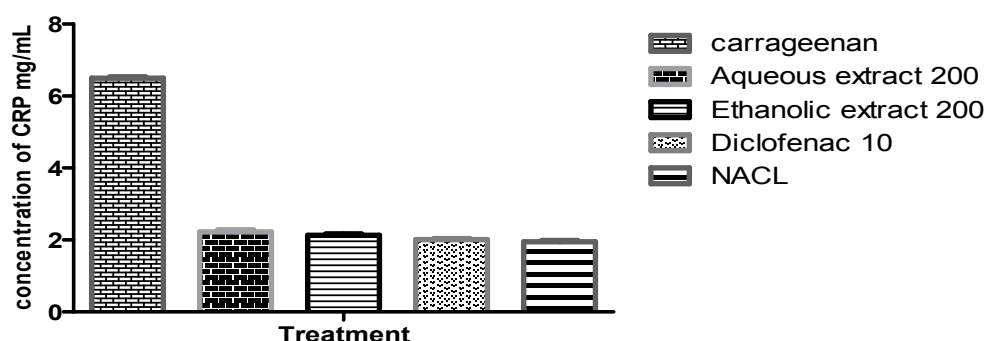
Each value is mean $\pm$ SEM, N= 6 rats

The data was analyzed by using One Way ANOVA followed by Dunnett's test, ** $p < 0.01$, * $p < 0.05$, where Extracts were compared with Diclofenac

3.2 Quantitative measurement of CRP

Statistical analysis showed a significant difference ($P < 0.05$) between values of the CRP with extracts and diclofenac as compared carrageenan. Indeed, the value of CRP of carrageenan is higher than extracts and the reference molecule CRP values. But there is no significant difference between the values of the CRP of extracts and diclofenac.

Figure 2: Changes in the level of serum CRP of rats



The anti-inflammatory activity of aqueous and ethanolic extracts of *Gomphrena celosioides* was evaluated by carrageenan induced rat paw edema method and determination serum level of C-reactive protein using commercial kit, according to manufacturer instructions.

Carrageenan induced paw edema is widely used for determining the acute phase of inflammation [22]. Carrageenan as a phlogistic agent is non antigenic and is devoid of apparent systemic effect [23]. This model is based on the principle of release of various inflammatory mediators by carrageenan [24]. Edema formation due to carrageenan in the rat paw is a biphasic [25]. The first phase is mainly due the release of histamine and serotonin in the first hour. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polynuclear (neutrophils and monocytes) and prostaglandins produced by tissue macrophages [26, 1]. Anti-inflammatory drugs inhibit different stages of inflammation [27].

In our previous studies, *In* anti-inflammatory activities of *Gomphrena celosioides* we obtained that the anti-inflammatory effects of several agents of *Gomphrena celosioides* result in the partial inhibition of inflammatory-mediator release. In the light of the results on the table 1 and 2, *Gomphrena celosioides* inhibits edema similarly to Diclofenac. So, anti-inflammatory activity of *Gomphrena celosioides* seems to be effective in the two phases of acute inflammation. Therefore, the anti-inflammatory activity of *Gomphrena celosioides* may be due to inhibition of mediators of the inflammation such as histamine, serotonin released during the first phase of inflammation and prostaglandins, bradykinin, leukotrienes which released during the second phase of inflammation,

C-reactive protein (CRP) is the classic acute-phase reactant and a sensitive marker for systemic inflammation. The body produces C-reactive protein or CRP during the general process of inflammation. CRP is a "marker" for inflammation, meaning its presence indicates an increased state of inflammation in the body. Measurement of CRP may provide a useful method of assessing the prognosis of disease with systemic inflammation [28]. The CRP synthesized by the liver cells, plays an important role in innate immunity by its properties opsonization, activation of complement and receptor binding immunoglobulins [29]. The site secretion main, but not exclusive hepatocytes is responsible for basal levels of plasma CRP. During an inflammatory response, its output increases. Secretion (extrahepatic) exists in neurons where production is increased in dementia of the Alzheimer type [30] in some cells [31], and finally even within atherosclerotic plaques [32]. Our study identifies and characterizes the direct interaction between inflammation and CRP. Treatment with aqueous and ethanolic of *Gomphrena celosioides* and diclofenac after 1 hr of carrageenan administration increased CRP level as compared reflecting the presence of

inflammation. Statistical analysis shows no significant difference in CRP concentration between the extracts and as compared the reference drug.

4. CONCLUSION

The results obtained from the present study have clearly demonstrated that the extracts of *Gomphrena celosioides* possessed anti-inflammatory activities which support the traditional utilization in Africa, particularly in Côte d'Ivoire. This activity is comparable to those obtained with reference molecules such as diclofenac.

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