

Topical Anti-inflammatory effect of aqueous extract ointment of *Ageratum conyzoides* L. in wistar rat

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Abstract

Leaves of *Ageratum Conyzoides* commonly known as medicinal and is reported as anti-inflammatory in traditional literature. So, the present research work has been entepis with the ointment of aqueous extract of this plant for phytochemical screening and to evaluate the anti-inflammatory effect. The carrageenan induced Hind paw edema method has been used by plethysmometer. Indometacin used as reference drug and control group receive by local application valiselin. In the Carrageenan test, the ointment inhibited the first phase (200 mg/kg) and the second phase (100 mg/kg and 200 mg/kg). Also, the result of cotton pellet test shown that ointment of aqueous extract of *Ageratum Conyzoides* present a significantly ($P<0.05$) potent anti-inflammatory activity by inhibitory effect on mean increase on granuloma. Phytochemical screenings of leaves extract revealed the presence of: tannins, flavonoids, steroids, saponins and acids amines. In acute oral toxicity study, it was observed no mortality at doses up to 5000mg/kg. The antiedematogenic effect, reduction on the exudate volume and leukocyte mobilization was observed at the doses of 100 and 200 mg/kg. The results indicated that *A. Conyzoides* possessed the anti-inflammatory activity; it's confirmed this using by population.

Keywords: *Ageratum conyzoides* Linn., anti-inflammatory, ointment, granuloma, carrageenan. phytochemical.

1. Introduction

Inflammation is a pathophysiological response of leaving tissue injury caused by many irritant agents: trauma, chemical, microbial, allergic reaction and it's characterised by oxidative damage[1]. Presently, drugs used to treat inflammatory disease are either narcotics or corticosteroids (NSAIDs). In long term the therapy by these drugs (NSAIDs) present well known side and toxic effects associated with gastroduodenal bleeding and ulceration or produced erosions of the stomach lining[2]. For centuries, the therapeutic properties of various medicinal plants have been used to treat human diseases. It has been estimated that between 60-90% of the populations of developing countries use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare[3]. Several anti-inflammatory medicinal plants have also demonstrated the ability to inhibit the synthesis of prostaglandins[4].

Ageratum conyzoides (Asteraceae) is a medicinal plant widely used in folk medicine in Psychosis, erythema, jaundice, hepatitis, dysentery, insomnia, dyspepsia, neurasthenia, osteoarthritis inflammation[5,6]. In Congo, leaf extract of *A. conyzoides* are used for treated chronic pain by the population. This plant, as well as *A. conyzoides*, is indicated in folk medicine as anti-inflammatory, analgesic and antimicrobial[7].

A rich scientific literature already exists on the chemistry and pharmacology of this plant[8-10]. Therefore, Essential oil was found to have a significant anti-inflammatory (cotton granuloma method) when administered orally[9]. In this present study we investigated the topical effect of aqueous extract of *A. conyzoides* on carrageenan induced hind paw edema and cotton pellet granuloma in rat.

2. Materials and Methods

2.1 Plant materials

Fresh leaves of *A. conyzoides* Linn. Collected at October 2012 period, in local area of Brazzaville -Congo. The plant was identified and authenticated from the Laboratory of Botany Center of Research on Vegetal Resources (CERVE). The Account no. of authentication certificate was 648. The fresh leaves were cleaned, dried and pulverized.

2.2. Preparation of extract and ointment

200g of the powdered dried leaves of plant were mixed with 1500ml of distilled water and a decoction was prepared for 30 minutes at 55°C and then filtered with Wattman No. 1 paper. The filtrate was evaporated under vacuum. The dried concentrated obtain is subjected to phytochemical analysis using the classical method. The formulation was prepared by incorporated the aqueous extract in soft Vaseline and her anti-inflammatory activity was evaluated.

2.3. Animals

Males and females Wistar rats weighing 150-170g were used. Animals received standard food and were kept under control conditions for one week. Prior each experiment, animals were fasted overnight with free access to water.

2.4. Acute toxicity and lethality (LD50) test

Acute toxicity study was carried out according to OECD guidelines (Organization for economic co-operation and development)[11]. The concentrations tested were comprised between 50 and 5000 mg/kg. Leaves extract of *Ageratum conyzoides* Linn. Shown signs of toxicity like tremor, convulsion and deep breathing at 5000 mg/kg and the therapeutic dose was obtained with 50 mg/kg.

2.5. Qualitative phytochemical screening

The aqueous extract of leaves of *Ageratum conyzoides* was subjected to identify different phytoconstituents such as flavonoids, saponins, steroids, tannins and alkaloids.

2.6. In vivo Anti inflammatory activity

2.6.1. Carrageenan induced paw edema

Anti-inflammatory activity was assessed in rat using the method described by Winter et al[12]. Increase in paw volumes was used to assess inflammation. Five groups of rats (n = 5) were deprived of food but not water for 18 h and then received topical application of the extract (2, 4, 8 g/kg). The control groups received respectively 0.5 g /kg of vaseline, and 1g/kg of indomethacin. Thirty minutes later, each animal received subplantar injection of carrageenan (0.1 ml of 2% suspension) in its right hind paw. The plant extracts were administered orally 1 h before carrageenin injection. Paw volume was measured with a water plethysmometer (Bade, 7150), prior to irritant injection and 1 h and 3 h later. The paw diameter was measured at 0, 1, 2, 3, 4 and 5h after injection of carrageenan, using a vernier calliper and the percentage of oedema inhibition in treated animals versus control was calculated.

Percentage of inhibition of oedema = $\frac{(V_c - V_t)}{V_c} \times 100$; Where, V_c and V_t are the volume of oedema in control and drug treated rats.

2.6.2. Cotton pellet granuloma

Cotton pellet (50mg) were sterilized and implanted subcutaneously, under ether anesthesia in the back of the rats housed individually[13]. Products (incorporated in vaseline) are topically administered on the back of animals: vehicle (vaseline), indomethacin (2g/kg), extract of *A. conyzoides* 2, 4, 8 g/kg for 7 days. At the 8th day, animals were sacrificed and granuloma weight determined. Percentage of inhibition of granuloma was calculated.

3. Statistical Analysis

Results are expressed as mean \pm S.E.M. Student's t-test was used to verify the statistical significance at $p < 0.05$ between the treated and control groups.

4. Results and Discussion

4.1. Phytochemical study

Chemical study (table 1) revealed the presence of many constituents: saponins flavonoïds, alkaloids, tannins, polyphenols in the aqueous extract of this medicinal plant. Theses chemical groups could contribute to the observed anti inflammatory effect. These research work rejoin this: Mondal et al[14]; Aalbersberg et al[15] and Menut et al[16].

4.2. Acute toxicity

No mortality was been observed at any of the doses (up to 5000mg/kg) at the end of 72 hours of observation.

4.3. Effect on carrageenan induced paw edema

Topical preparation of *A. conyzoides* used at the doses of 2, 4, 8 g/kg, shows a significant anti-inflammatory effect with percentages of inhibition of 60, 79, and 82% respectively on carrageenan induced edema.

4.4. Effect on granuloma of cotton pellet

The ointment significantly ($P < 0.05$) inhibited the granuloma formation induced by cotton pellet in the rats. With the percentage of inhibition of granuloma: 34, 40, 55%. The highest effect was observed with the dose of 8g/kg which was comparable to that of a, indomethacin 2g/kg (standard product).

Then, like oral administration[9,10], topical preparation of *A. conyzoides* shows significant anti-inflammatory effect. These results suggest the possibility of the use of *Ageratum conyzoides* at these doses corresponding as traditional improved extract in the treatment of inflammatory pathology.

Table 1: results of phytochemical screening of aqueous extract of *Ageratum conyzoides* L.

Chemical constituents	Saponins	alkaloïds	Terpénoïds/sterols	Flavonoïds	Tannins	Quinones	Anthocyanans	PoLy phenols	Amino acids	glucids
Aqueous extract <i>A. conyzoides</i> L.	+	+	±	++	+	+	+	++	±	-

++: hight constituents; +: presences; ±: traces; -: none (absences).

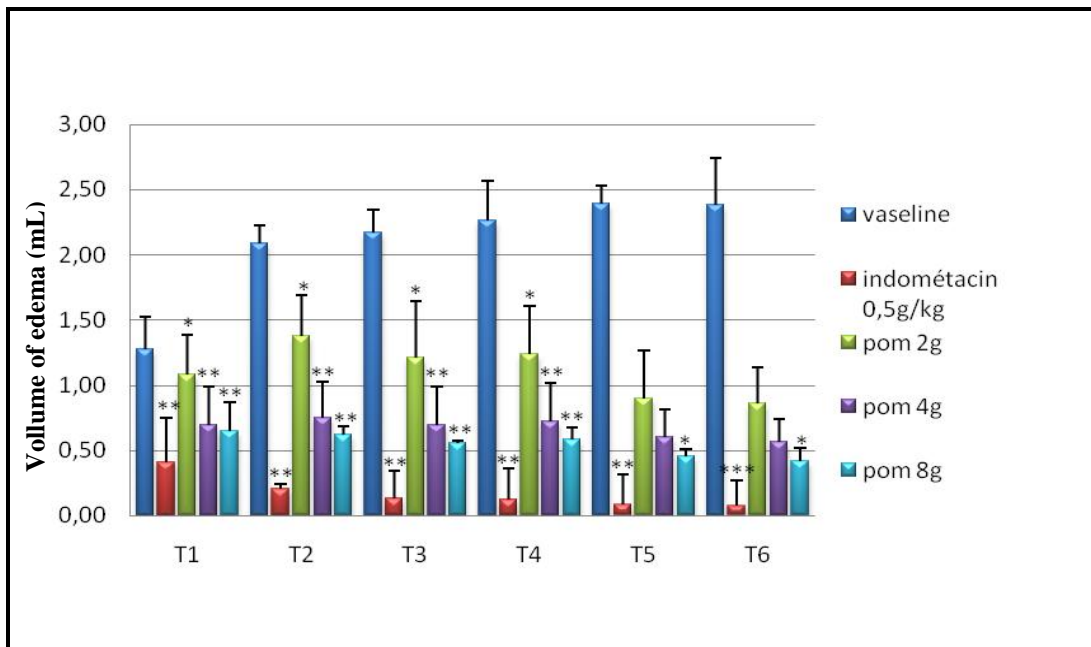


Figure 1: Effect of topical formulation of aqueous extract of *Ageratum conyzoides* of edema caused injection of 2% carrageenan in the Wistar rats (n = 6) with * $p < 0.05$; ** $p < 0.01$, * $P < 0.001$; Pom: ointment**

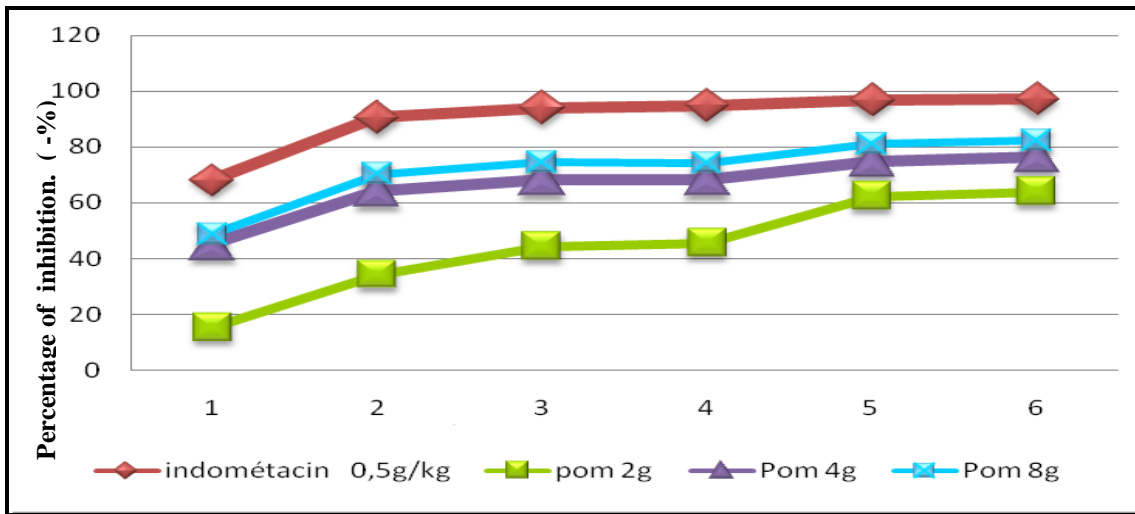


Figure 2: caused by injection of carrageenan wistar rats after application of the ointment *Ageratum conyzoides* and indomethacin. (n = 6) with * p < 0.05; ** p < 0.01, *** P 0.001; Pom: ointment

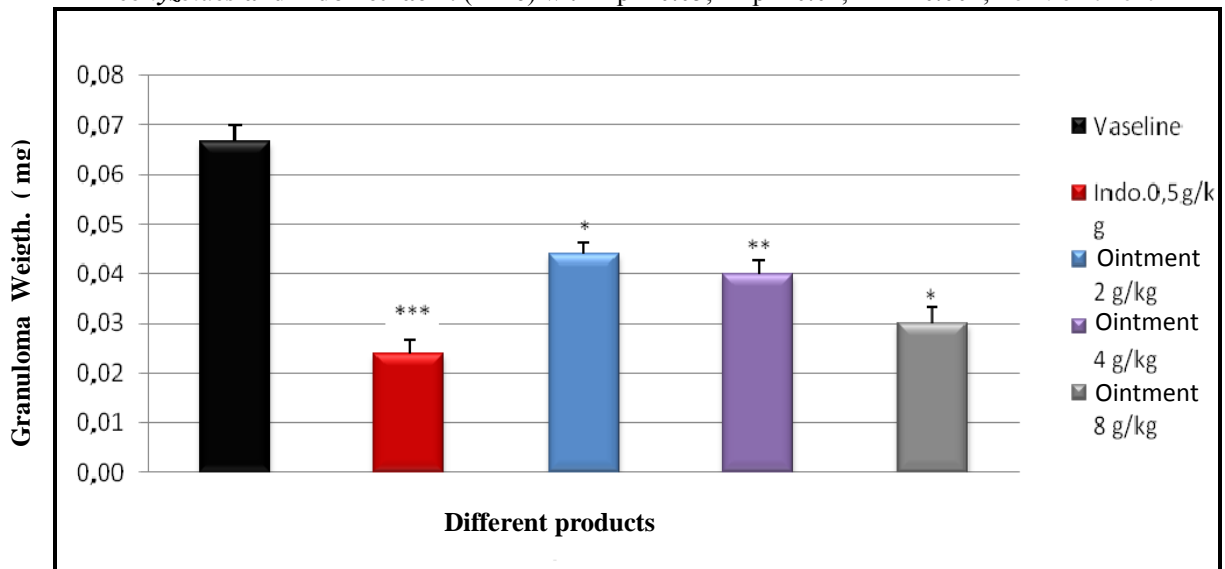


Figure 3: Effect of topical ointment of aqueous extract of *Ageratum conyzoides* on granuloma Cotton pellet in Wistar rats. (n = 5) with * P < 0.05; ** P < 0.01; *** P < 0.001

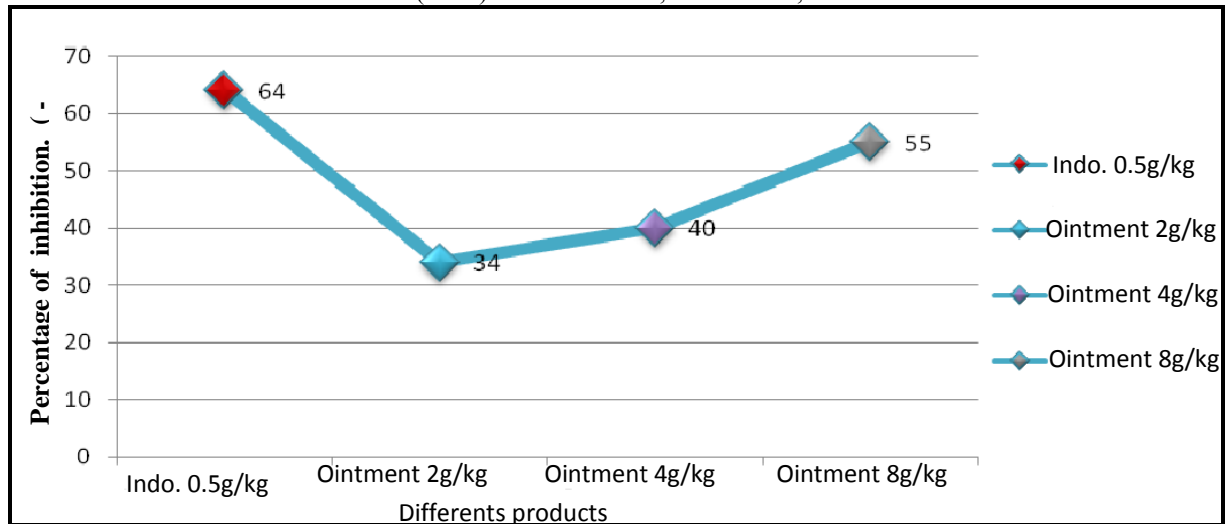


Figure 4: Percentage inhibition of granuloma cotton pellet in Wistar rats.

Acknowledgments

We are thankful to Faculty of Sciences of Health, and the Professor Ange Antoine Abena, have provided us with the necessary material.

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