



Full Length Research Paper

Evaluation of anti-inflammatory activity of aqueous extract of medicinal preparation constitute of three plants in the rat wistar

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Abstract

Aqueous extract of an preparation constitute of three medicinal plants produced a significant reduction in wistar rats volume edema induced by carrageenan 2%, and an increase granuloma formation induced by implantation of cotton pellet under the under the aponevrose of the posterior leg of wistar rats. This extract of the preparation, without limit none dose dependently vascular permeability (100 mg / kg), also blocked the migration of leukocytes to the inflammation site and shows a significant antioxidant activity. Phytochemical study revealed the presence in this extract of flavonoids, tannins and saponins which could support the anti-inflammatory effect. The proposed formulation with this aqueous extract is also active in acute inflammation.

Key words: Preparation, Medicinal plants, Treatment, anti inflammatory.

INTRODUCTION

Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, and extravasation of fluid, cell migration, tissue breakdown and repair (Vane and Bolting, 1995). The search for a safe anti-inflammatory drug that is free from gastric intolerance continues unabated and a part of such research is the evaluation of medicinal plants known to be used for the treatment of inflammatory disorders (Singh et al., 1989). Because, *Lippia multiflora*, *Ageratum conyzoides* and *Cymbopogon citratus* used alone or such as a preparation by the Congolese people in particular, were widely used in folk medicine as an anti-inflammatory agent against: the hemorrhoid, ophthalmia, asthma, sore hypertension, and headaches (Adjonohoun and al. 1989).

The objective of this study was to investigate the anti-inflammatory effect of the aqueous extract preparation constitute of these three medicinal plants and realised a phytomedicament.

MATERIALS AND METHODS

Preparation of plant extract

Fresh leaves of three plants: *Ageratum conyzoides* Lin., *Cymbopogon citratus* DC.Staph. (Poaceae) and *Lippia multiflora* Mold. were collected from Inoni, 185 km north of Brazzaville in June 2006. These samples were compared and identified by Dr. (Mr.) Mousamboté Jean Marie, a Taxonomist in the laboratory of Botany and Ecological Studies, CERVE (study center of vegetable resources). A herbarium specimen voucher number 648 of 18/01/1963 De NERE (*A. conyzoides*); *L. Multiflora* number 2047 of 16/06/1953 by J. Koechler assigned to it and deposited in the laboratory of Botany and ecological studies. 300g powder of fresh leaves of the three plants (1:1:1) is mixed and boiling 1500ml distilled water for 30 min at 55 ° C , decanted and filtered with

paper Wattman N°1. The filtrate is evaporated under vacuum by a rotary evaporator R114. The dry residue obtained stored at 4°C in the refrigerator until used, constitutes the aqueous extract preparation.

Animal stock

The randomized rats Wistar (male and female) weighing between 100 and 150 g are used. These animals are bred at the Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences (Brazzaville), where they are fed a standard way, with water *ad libitum* under standard laboratory conditions (Temperature 25± 5°C, Relative humidity 50-60%, and a 12/12h light/dark cycle).

Phytochemical study

The extract was screened for identify the major chemical groups: Reducing sugars, flavonoids, saponins, tannins, anthraquinones, alkaloids, terpenes and stréroïds using the classical methods of Sofowora, (1993).

Acute toxicity

The limit test dose of 5000 mg/kg was used as stipulated in Organization for Economic Cooperation Development (OECD) guidelines (OECD, 2002). Five healthy albino rats weighing (120g) were divided into five groups of five rats per group. Wistar rats were administered orally with extract: 200, 400, 800, 1600 and 3200mg kg⁻¹; Signs of physical toxicity (mortality in each lot) were observed for 24-72h.

In vivo anti-inflammatory activity

Edema induced par carrageenan 2%

In the present study anti inflammatory activity is determined in Wistar male rats of either sex according to the method of Winter and al, (1962). Using five animals in each group, the animals were injected carrageenan (1% w/v suspension in 0.9% saline) in the right hind foot under the plantar aponeurosis. Five groups are constitutes:

- The controls were received 10 ml/kg of saline by orally way;
- The test groups of rats were given orally 25; 50, 100 and 200 mg/kg of aqueous extract of association of plants one hour before the carrageenan injection;
- Other group of rats was treated with 25 mg/kg of indometacin as the standard drug in this model, one hour before carrageenan injection.

The acute inflammation was quantitated in terms of ml i.e. replacement of water by edema using a plethysmometer immediately before carrageenan 2% injection and then 1,2 and 4 hours after carrageenan injection. The percent inhibition of edema as calculated for each group with respect to its vehicle-treated control group. The inhibition percentage of anti-inflammatory activity was calculated by using the relation used:

$$\frac{v_c - v_t}{v_c} \times 100$$

where v_c and v_t denote mean increase in paw volume of control and drug treated animals respectively.

Cotton pellet granuloma:

The effect of aqueous extract of association of three plants on chronic inflammation was also evaluated using cotton-pellet granuloma test in rat (Swingle and Shideman, 1972).

Groups of five male and female Wistar rats weighing 100–180 g were used to determine the effects of the aqueous extract on the transudative and proliferative phases of the granuloma. The difference between the final and initial weights was regarded as the granulomatous tissue produced. The first day, test groups of rats were received orally 25; 50, 100 and 200 mg/kg of aqueous extract of association of plants. Control animals received saline solution 0.9% (10 ml/kg). Thirty minutes later, two sterilized cotton-pellet 100 ± 1.0 mg were implanted under the previously depilated back of rats anaesthetized with diethyl ether. Extract of Preparation was administered once daily for the next 7 days. On day 8, rats were sacrificed by overdose of ether. The cotton-pellets were dissected out, freed of tissue attachments and dried in the oven overnight at 60°C. The dry pellets were weighed and the mean weight of granuloma tissue formed around each pellet determined. The level of inhibition of granuloma tissue development was calculated using relation:

$$\frac{T_c - T_t}{T_c} \times 100; \text{ where } T_c: \text{ weight of granuloma tissue of control group and } T_t: \text{ weight of granuloma tissue of treated group}$$

Vascular permeability activity

Effect of the extract on plasma leakage in rats

The method of Wittle, (1964) was used to evaluate the effect of the aqueous extract of association of three plants on vascular permeability responses. The microvascular permeability of the skin was assessed by an extravasation of Evan's blue. After 3.5 h of extraction period, the dye concentration was determined by a spectrophotometer at 590 nm. Groups of five male Wistar rats weighing 150–200 g were used:

- The controls were received 10 ml/kg of saline by orally way;

Table I: result of phytochemical screening of aqueous extract of the preparation

Extracts	Chemical Elements	inference	Observation	Aspects	Yield
Aqueous extracts	Saponins	+++			
	Alcaloids	+	Yellow precipitate	crystallin	13.2 %
	flavonoïds	+++	Turned blue black		
	Tannins	++			

Key: (+) = trace; (++) = positive; (+++) = strongly positive

- The test groups of rats were given orally 25, 50, 100 and 200 mg/kg of aqueous extract of association of plants one hour after oral administration; 0,1 ml of Evans Blue dye was intravenously administered through the tail vein.
- Other group of rats was treated with 25 mg/kg of indometacin as the standard drug. Thirty minutes later, rats were killed by overdose of ether and the peritoneal cavity washed with 3ml normal saline into heparinized tubes and centrifuged. The dye content in the supernatant was measured by spectrometer.

Leukocyte migration test

The effect of the aqueous extract of association of plants on cell migration in vivo was evaluated in Wistar rats of either sex using the method described by Schuster and al.,(2002)

One hour after oral administration of aqueous extract of preparation (25, 50, 100 and 200mg/kg), wistar rats received intraperitoneal injection of 1 ml of 6%, w/v dextran in normal saline. Four hours later, the animals were sacrificed by overdose of diethyl ether. Total and differential leukocyte counts in the peritoneal wash were performed. The inhibition of neutrophil and lymphocyte migration was calculated.

IP IN + oral administration Test

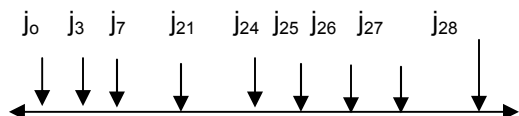


Figure 4: Diagram to sensitize rats (j = day IP: intraperitoneal injection, IN: intranasal instillation).

Antioxidant activity by the 2, 2 Diphenyl-1 picryl hazryl

Antioxidant activity of the aqueous extract of association of three plants was determined based on its ability to react with the stable DPPH free radical by the methods used by McCune, (2002). Fifty μ L of the extract (40, 80, 160 and 320 mg/mL in methanol/water (1:1, v/v)) was added to 5 mL DPPH solution (0.004%) in methanol.

After incubation at room temperature for 30 min, the absorbance of each solution was determined at 517 nm. Percentage of inhibition and the concentration of the sample required for 50% scavenging of the DPPH free radical (IC50) were determined. Quercetin was used as a positive control at concentrations of 4, 8, 16 and 32 mg / ml. All determinations were performed in triplicate. The percentages of inhibition are determined by the following formula:

$$\text{Percent inhibition} = (A_c - A_e) \times 100 / A_c$$

Where, A_c : optical density in the presence of methanol and A_e : optical density in the presence of a sample or quercetin (positive control).

Statistical analysis

The results were analyzed with the Systat 5.0 and are expressed as mean \pm standard error (m \pm SEM). We used analysis of variance (ANOVA) for comparing the groups and the difference between groups was determined using the Fischer's test. The level of significance was set at $p < 0.05$. The results were

RESULTS

The administration of the aqueous extracts of preparation up to 32000 mg/kg p.o. did not produce signs of toxicity (no mortality) at the dose during the next three days of the period of observation. Phytochemical study revealed the presence of saponins, flavonoïds and tannins in the aqueous extract of preparation. These chemical composites could contribute to the observed anti-inflammatory effect (table I).

The anti-edematogenic response obtained by the administration of the aqueous extract of preparation, indometacin and distilled water on the carrageenan-induced hind paw edema in rats is shown in figure1.

Although, this extract at doses of 25, 50, 100 and 200 mg /kg) caused a significant reduction of edema induced by injection of carrageenan 2 % in the rats. These results support the hypothesis of the greater effect of the aqueous extract of this preparation on the mediators involved in the immediate and chronic responses of

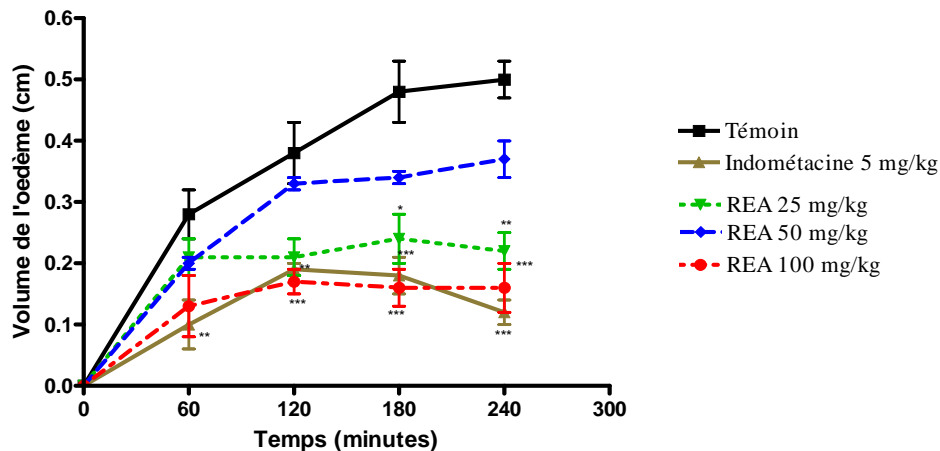


Figure 1: effect of aqueous extract of preparation (REA) on edema induced by cotton-pellet in the wistar rats . ** = $p < 0,01$ *** = $p < 0,001$ (n=5).

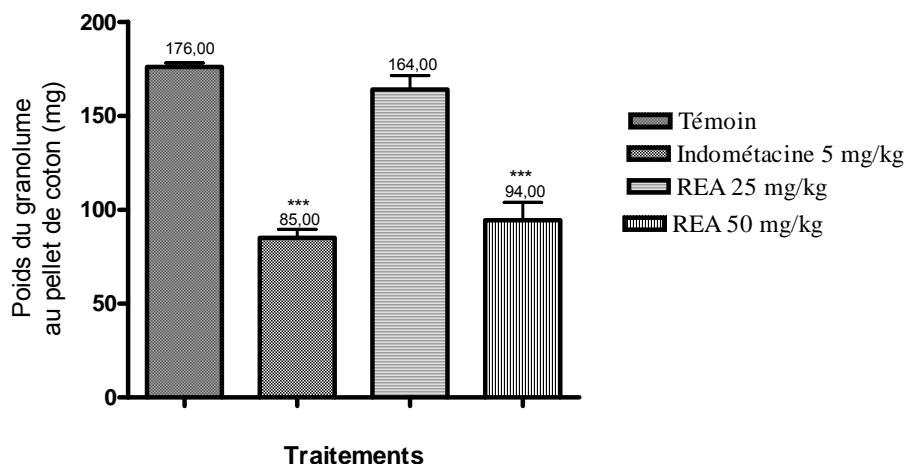


Figure 2: effect of aqueous extract of preparation (REA) on edema induced by cotton pellet in the wistar rats . ** = $p < 0,01$, *** = $p < 0,001$ (n=5).

inflammation in rats.

The effects of the aqueous extract of preparation and indometacin on the cotton pellet granuloma are summarized in figure 2. As the indometacin, the extract produced a significant inhibition of the transudative phase of the granuloma.

In the cotton pellet test, another widely used method for the assessment of anti-inflammatory agents, the aqueous extract and indometacin appeared to be equally effective in inhibiting the transudative phase of the granuloma, but only indometacin inhibited the proliferative phase (Swingle and Shideman, 1972). That anti-inflammatory effect may be elicited by a variety of chemical composite in the extract (Sertie et al., 1990). The figure 3, 4 and table II, shows the effect of the

aqueous extract and indometacin on the increase of vascular permeability induced by blue Evan's, on the leukocytes migration and on oxidative action.

The aqueous extract of preparation without limit none dose dependently vascular permeability (100 mg / kg) and blocked or decreased leukocyte migration to the peritoneal cavity in a significant way, mainly inhibiting neutrophil and lymphocyte migration, when compared with the distilled water (négatif) or indometacin (positif) controls.

These data indicate a better effect of the extract on the mediators of early or immediate response in injured tissues. This effect also did not appear to have any relation to the events involved with cellular migration to injured sites. These results confirmed these of others

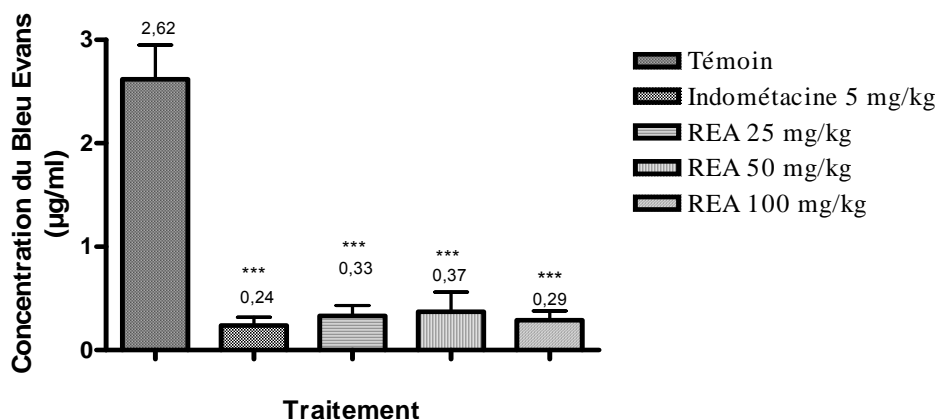


Figure 3: Effects of aqueous extract of the preparation (REA) on Concentration of Evans blue in fluid -blood in the wistar rats . * = $p < 0.001$ ($n = 5$).

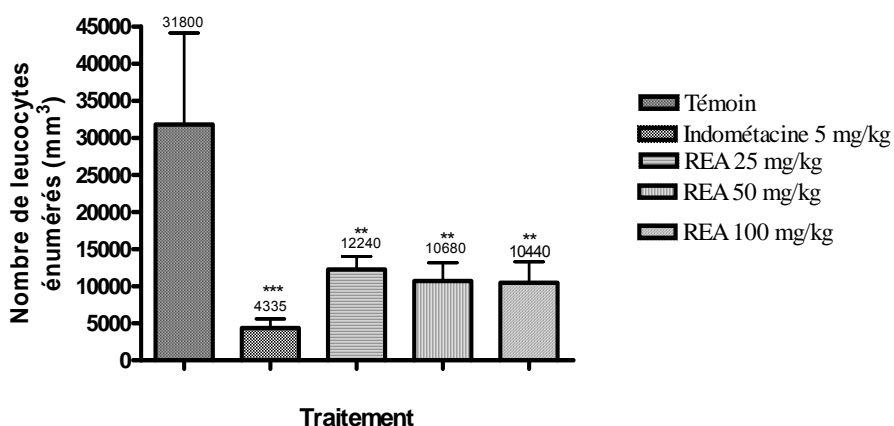


Figure 4: Effect of the aqueous extract of the preparation (REA) on number of leukocytes of bronchoalveolar fluid in the wistar rats. * = $p < 0.01$, ** = $p < 0.001$ ($n = 5$).

Table II: IC₅₀% of the oxidized state of DPPH.

Traitement	CI ₅₀ ± E.S.M.	S	r ²	p
Control	1,05 ± 0,68	0,33	0,58	0,44
REA	9,33 ± 3,50	0,22	0,92	0,01
Quercetine	14,76 ± 6,27	0,22	0,99	0,001

S : slop ; r² : correlation indices ; P : significativity ; REA : aqueous extract preparation

autors: (Okemy and al. 2006), (Abena and al. 1993, 1996, 1997, 1998 and 2003).

DISCUSSION

In the present investigation, the preparation constitutes of three medicinal plants traditionally used as anti-

inflammatory was evaluated for its activity by hind paw edema method (in vivo study). Inflammation is a local response of living vascularized tissues to endogenous and exogenous stimuli. So, carrageenan induced oedema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic. The early phase of carrageenan model is mainly mediated by histamine, Cytokine, serotonin and

increased synthesis of prostaglandins in damaged tissue surroundings (Winter, 1962). The late phase is sustained by prostaglandin release and mediated by bradykinin, prostaglandin produced by tissue macrophages and polynuclear cells (Ami and al, 2011).

The result of the present study showed that the aqueous extract of association of three plants was anti-inflammatory effect by inhibition of mean increase in paw volume induced by injection of carrageenan in the subpantar region of rat's paw. This extract at different doses (25, 50, 100 and 200 mg /kg) caused a significant reduction of edema induced by injection of carrageenan 2 % in the rats . These results support the hypothesis of the greater effect of the aqueous extract of this preparation on the mediators involved in the acute and chronic responses of inflammation in rats. It was possible that constituents inhibited the biosynthesis of prostaglandins since studies demonstrated that the injection of carrageenan into the rat paw induced the liberation of bradykinin, which later induced the biosyntheses of prostaglandin and other autacoids, which were responsible for the formation of the inflammatory exudates (Ueno and al.2000; Vinegar and al. 1973).

In addition, the cotton pellet test, another widely used method for the assessment of anti-inflammatory agents, the aqueous extract and indometacin appeared to be equally effective in inhibiting the transudative phase of the granuloma, but only indometacin inhibited the proliferative phase (Swingle and Shideman, 1972).

That anti-inflammatory effect may be elicited by a variety of chemical composite in the extract (Sertie et al., 1990). Also, the aqueous extract of preparation without limit none dose dependently vascular permeability (100 mg/kg) and blocked or decreased leukocyte migration to the peritoneal cavity in a significant way, mainly inhibiting neutrophil and lymphocyte migration, when compared with the distilled water (négatif) or indometacin (positif) controls. These data indicate a better effect of the extract on the mediators of early or immediate

response in injured tissues. This effect also did not appear to have any relation to the events involved with cellular migration to injured sites.

These results confirmed these of others: (Okémy and al.,2006), (Abena and al. 1993, 1996, 1997, 1998 and 2003).

CONCLUSION

The aqueous extract of the preparation is composed of flavonoids, tannins, and saponins. It reduces significantly ($p < 0, 001$) edema volume -induced by carrageenan 2% (25, 50, 100 and 200 mg/kg) and the granulomateous tissue formation caused by the cotton pellet (25 and 50 mg/kg) compared to controls. In addition, this extract inhibits vascular permeability, leukocyte migration, and formation of free radicals during inflammatory reaction.

Final, these results suggest the possibility of the use this preparation the treatment of acute or chronic inflammation by the Congolese population.

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