

ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC EFFECTS OF *LEPIDAGATHIS ANOBRYA* NEES (ACANTHACEAE)

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Abstract

This study investigated the general acute, anti-inflammatory, analgesic and antipyretic effects of methanol extract of *Lepidagathis anobrya* Nees (Acanthaceae). Carrageenan-induced rat paw edema and croton oil-induced ear edema in rats were used for the evaluation of general acute anti-inflammatory effects. Acetic acid-induced writhing response and yeast-induced hyperpyrexia in mice were used to evaluate the analgesic and antipyretic activities respectively. The extract at doses of 10, 25, 50 and 100 mg/kg⁻¹ for carrageenan test and doses of 0.5 mg/ear for croton oil test induced a significant reduction ($p < 0.001$) of paw and ear edemas in rats. In the analgesic and antipyretic tests, the extract has shown a significant inhibition of writhes and hyperpyrexia with all the doses used when compared to the untreated control group. These results clearly show the anti-inflammatory, analgesic and antipyretic effects of the methanol extract of *Lepidagathis anobrya* and give the scientific basis for its traditional use. Further studies are needed to clarify the mechanism of action and the components responsible for these pharmacological effects.

Key words: *Lepidagathis anobrya*, anti-inflammatory, analgesic, antipyretic, acute toxicity.

Introduction

Inflammation processes are implicated in many degenerative diseases such as rheumatoid arthritis, shoulder tendonitis, gouty arthritis, polymyalgia rheumatica, heart disease, asthma and cancer (Polya 2003; Iwalewa et al. 2007). Non-steroidal anti-inflammatory drugs (NSAIDs) are prescribed worldwide for the treatment of inflammation but their use can be associated with a high occurrence of intestinal side effects and mucosal erosions that can progress into ulcers. Also, life-threatening complications such as perforation, hemorrhage and an increase in blood pressure are seen with NSAIDs (Burke et al. 2006).

Because of the toxicity of synthetic drugs, many researchers have focused on medicinal plants for finding natural anti-inflammatory drugs. *Lepidagathis anobrya* is a yearly herbaceous plant used in traditional medicine within Burkina Faso for the treatment of inflammation, malaria, migraine and hyperpyretic convulsions (Nacoulma 1996; Sawadogo et al. 2006). Our previous study has shown that this plant possesses one of the highest total phenolic contents ($23.67 \pm 0.85\%$) and antioxidant activity ($IC_{50} = 16.33 \pm 1.04 \mu\text{g/mL}^{-1}$) in plants belonging to the Acanthaceae family within Burkina Faso (Sawadogo et al. 2006). There are few reports of scientific studies on *Lepidagathis anobrya* and there is no data on the anti-inflammatory, analgesic and antipyretic effects of the extract.

The present study was aimed at evaluating general acute, anti-inflammatory, analgesic and antipyretic activities of methanol extract of *Lepidagathis anobrya*. Phytochemical analysis and acute toxicity were also carried out.

Material and methods

Chemicals and reagents

Carrageenan, croton oil, brewer's yeast, acetic acid (Sigma, St Louis, USA) and methanol (BDH) were used in the study. The standard drugs used are aspirin (Panpharma S.A. France), paracetamol (Perfalgan, Bristol-Myers Squibb, France) and solumedrol (Bristol-Myers Squibb, France).

Plant material and extraction

Lepidagathis anobrya was collected in the east of Ouagadougou (Burkina Faso) and identified by Pr Millogo J., Botanist of University of Ouagadougou. A voucher specimen was deposited in the herbarium of the Research Institute in Health Sciences of Ouagadougou (Burkina Faso) with the number RO3. The dried plant (stems with leaves and inflorescence) was pulverized into a fine powder using a grinder. 25g of the powder was extracted by maceration in methanol (250mL) at room temperature for 24h. The filtrate of this methanol extract was concentrated under reduced pressure until all the methanol had evaporated. The concentrate was then re-dissolved in distilled water and lyophilized.

Animals

Male Swiss albino mice (35 ± 5 g) and male Wistar albino rats (165 ± 15 g) were purchased from the Centre International de Recherche et Développement sur l'Élevage en Zone Sub-humide, CIRDES, Bobo-Dioulasso, Burkina Faso. They were kept in a room maintained under environmentally controlled conditions of 25 ± 2 °C with a 12h light–12h dark cycle. All animals had free access to water and a standard diet. They were acclimatized at least 2 weeks before the experiments were started. The animals were fasted for 12h prior to the experiments and the test substances were given intraperitoneally. They had free access to water during the experiments. All procedures described were reviewed and approved by the Research Institute in Health Sciences of Ouagadougou (Burkina Faso) and met international standards of animal study (guidelines of the International Association for the Study of Pain) (Zimmermann 1983).

Phytochemical screening

Standard phytochemical tests for steroids and triterpenoids, flavonoids, anthraquinones, coumarins, tannins, saponins and alkaloids were performed according to Ciulei (Ciulei 1982). Borntrager's reaction was used to characterize anthraquinones; FeCl_3 was used to test for tannins; Dragendorff reaction for alkaloids; observation under UV light of alkalized extracts for coumarins; frothing test for saponins and the Liebermann-Buchard reaction for triterpenoids and steroids.

Acute toxicity

The toxicity study was carried out to determine the LD_{50} using the graphical method of Litchfield and Wilcoxon (Litchfield and Wilcoxon 1949) in mice. Seven groups of six mice each received plant extract in the quantities 500, 600, 650, 700, 750, 850 or 950 mg kg^{-1} b.w., i.p.. The control group received normal saline (5 mL kg^{-1} , i.p.). Signs of toxicity and mortality within 24–72h were recorded. Confirmatory tests were carried out and the LD_{50} was calculated from the graph of percentage (%) mortality (converted to probit) against log-dose of the extract; probit 5 being 50%.

Anti-inflammatory activity

Carrageenan-induced rat paw edema

The methanolic extract of *Lepidagathis anobrya* was tested for anti-inflammatory activity on carrageenan-induced rat paw edema, according to Winter *et al.* (Winter *et al.* 1962). The animals were divided into seven groups of six rats. The negative control group received vehicle control (NaCl 0.9%, i.p.), the positive control group received the NSAID aspirin (100 mg kg^{-1} , i.p.) and the test groups received the extract at the doses of 5, 10, 25, 50, 100 and 200 mg kg^{-1} i.p. Acute inflammation was produced by the sub-plantar administration of 0.1 mL of 1% carrageenan in normal saline in the right paw of the rats 1h after test sample administration. The paw volume was measured at 0h and 3h after carrageenan injection using a plethysmometer (Model Ugo Basil, N°7141, Italy). The average volume of the right hindpaw of each rat was calculated from three readings which did not deviate more than 4% (Arul *et al.* 2005). The anti-inflammatory effect of the extract was calculated using the following equation:

Anti-inflammatory activity (%) = $(1 - D/C) \times 100$, where D represents the percentage difference in paw volume after extract was administered to the rats and C represents the percentage difference of volume in the control groups (Gupta *et al.* 2005).

Croton oil –induced rat ear edema

Topical anti-inflammatory activity was evaluated as inhibition of the croton oil-induced ear edema in rats (Tubaro *et al.* 1986; Lompo *et al.* 1998). Male Wistar rats were anaesthetised with ketamine hydrochloride (150 mg kg^{-1} , intraperitoneally) before the induction of the phlogosis. A total of 5 μL of croton oil was given on the anterior surface of the right ear lobe. The methanol extract, solumedrol (positive control) and methanol (negative control) were administered with croton oil at the dose of 0.5 mg per ear. Six hours later, the animals were killed by cervical dislocation, and the right and left ears of each animal were removed. The left ear was considered as a control. Circular sections were taken with a cork borer (diameter of 7 mm) and weighed. The oedematous response was measured as the weight difference between the two plugs. The anti-inflammatory activity was expressed as a percentage of the edema reduction in treated mice compared to the control group.

Analgesic activity

An acetic acid-induced writhing test was used to evaluate the analgesic effect of the plant extract (Arul *et al.* 2005). Different groups of six mice each received either normal saline solution (5 mL kg^{-1} i.p.) (control group), paracetamol (100 mg kg^{-1} , i.p.), or plant extract at 25, 50, 100, 200 or 300 mg kg^{-1} , i.p.. Sixty minutes later, a 0.6% acetic acid (10 mL kg^{-1}) solution was injected intraperitoneally to all the animals in the different groups. The number of writhes occurring between 5 and 20 min after acetic acid injection was counted. A significant reduction of writhes in tested animals compared to those in the control group was considered as an analgesic effect.

Antipyretic activity

To induce hyperpyrexia, 10 mg kg^{-1} b.w. of an aqueous suspension of brewer's yeast (20% in distilled water) was injected subcutaneously in the back below the nape of the mice (Sakandé *et al.* 2004). The animals were fasted during the experiment, but they were given water ad libitum. Control temperatures were recorded with a thermometer (Model BAT-12, Physitemps,

Sensortek inc. USA) before the yeast injection and 16 hours later to evaluate the pyretic response. The temperature measurements in febrile animals prior to drug administration were used as the pre-drug control. The extract (100, 200 and 300 mgkg⁻¹ b.w.) and paracetamol (150 mgkg⁻¹ b.w.), which served as the reference drug, were given intraperitoneally 16 hours after the yeast injection. The temperatures were recorded at 1, 2, 3 and 4 hours after the drugs administration. A significant decrease in hyperthermia in tested animals compared to those in the control group was considered as an antipyretic effect.

Statistical analysis

Values were expressed as mean±SEM (n = 6). Statistical significance was determined using the Sigma Stat 2.0 Jandel Scientific software (one way ANOVA followed by turkey test). Values of p<0.05 were considered significant.

Results

Phytochemical screening

Phytochemical analysis of the methanol extract revealed the presence of flavonoids, triterpenoids, steroids, coumarins, tannins, saponins and alkaloids.

Acute toxicity

The LD₅₀ value of the methanol extract was estimated to be 750 ± 24.55 mgkg⁻¹ body weight i.p. in mice.

Anti-inflammatory activity

The acute anti-inflammatory activity of the extract was measured at the doses of 5, 10, 25, 50, 100 and 200 mgkg⁻¹ i.p. b.w. against acute paw edema induced by carrageenan. A strong inhibition of the paw edema was observed with the different doses of the extract and with aspirin (100 mgkg⁻¹ b.w.). All the doses tested produced a significant (p<0.001) anti-inflammatory activity (table 1). At the same dose (100 mgkg⁻¹ b.w.) the effect of *L. anobrya* (94.20±5.65% inhibition) was found to be more active than aspirin (59.42±10.99) which was used as the positive control. The results of croton-oil test (Table 2) showed a significant inhibition of the ear edema. The effect of the plant extract was found to be more effective than solumedrol, a steroidal anti-inflammatory drug which was used as the positive control.

Analgesic activity

The methanol extract of *L. anobrya* (25, 50, 100, 200 and 300 mg kg⁻¹, i.p.) and paracetamol (100 mgkg⁻¹ b.w.) both induced a significant (p<0.001) decrease in the number of writhes when compared to the untreated group (Table 3). In this test, the plant extracts at the doses of 50 and 100 mgkg⁻¹ exhibited a higher antinociceptive power (66.75 and 75.19% inhibition respectively) than paracetamol (54.61%) which was used as a positive control at the dose of 100 mgkg⁻¹.

Antipyretic activity

All the doses tested have produced significant (p<0.001) antipyretic effect 2hours after drug administration (Table 4). The methanol extract of *L. anobrya* was found to be more active than paracetamol which was used as the reference drug.

Table 1: Anti-inflammatory activity of methanol extract of *Lepidagathis anobrya* stems with leaves and Aspirin (100 mgkg⁻¹) on carrageenan-induced edema in the right hind-limb paw of rats

Group (n=6)	Dose (mgkg ⁻¹)	Volume of edema (mL)	% Inhibition
Control (saline)	5	0.69 ± 0.08	-
Aspirin	100	0.28 ± 0.08	59.42 ± 10.99*
<i>L. anobrya</i>	5	0.40 ± 0.05	42.02 ± 6.58*
<i>L. anobrya</i>	10	0.31 ± 0.08	55.07 ± 10.99*
<i>L. anobrya</i>	25	0.22 ± 0.08	69.56 ± 10.99*
<i>L. anobrya</i>	50	0.14 ± 0.05	79.71 ± 7.15*
<i>L. anobrya</i>	100	0.04 ± 0.01	94.20 ± 1.95*
<i>L. anobrya</i>	200	0.02 ± 0.01	97.10 ± 1.95*

(*p<0.001)

Table 2: Anti-inflammatory activity of *L. anobrya* and Solumedrol (0.5 mg/ear) on croton oil induced ear edema in rat.

Group (n=6)	Dose (mg/ear)	Weight of plugs (mg)		Weight of ear edema (mg)	%Inhibition
		Right (Untreated)	Left (Treated)		
Control (Methanol)	-	24.66 ± 1.46	34.06 ± 1.96	8.42 ± 0.46	-
Solu-Médrol	0.5	25.28 ± 1.81	27.42 ± 1.46	2.22 ± 0.57	81.41 ± 4.76*
Methanol extract	0.5	26.90 ± 2.28	27.04 ± 2.27	0.14 ± 0.08	98.32 ± 0.95*

(**p*<0.001)**Table 3:** Analgesic effect of the methanol extract of *L. anobrya*

Lots (n=6)	Dose (mgkg ⁻¹)	Number of writhing	Inhibition (%)
Control (saline)	5 mL/kg	63.17±2.64	-
Paracetamol	100	28.67±1.21	54.61±4.89
<i>L. anobrya</i>	25	38.00±1.41	39.84±2.56
<i>L. anobrya</i>	50	21.00±2.37	66.75±3.75
<i>L. anobrya</i>	100	15.67±1.03	75.19±5.16
<i>L. anobrya</i>	200	10.83±1.94	82.85±4.16

Table 4: Antipyretic activity of *L. anobrya* and paracetamol (150mg/kg) on brewer's yeast-induced pyrexia in mice.

Group (n=6)	Dose	Rectal temperature (°C) before drug administration		Decrease of rectal temperature after drug administration			
		-16h	0h	1h	2h	3h	4h
Control (saline)	5ml/kg	37.70±0.23	39.78±0.34	0.15±0.04	0.17±0.08	0.28±0.03	0.38±0.09
Paracetamol	150mg/kg	37.88±0.31	39.03±0.34	0.13±0.09	0.38±0.03	0.81±0.04	1.61±0.02
<i>L. anobrya</i>	100mg/kg	37.71±0.22	39.46±0.10	0.15±0.05	0.40±0.05	0.60±0.03	0.80±0.06
<i>L. anobrya</i>	200mg/kg	37.60±0.35	39.02±0.21	0.19±0.02	0.72±0.03	1.22±0.19	1.65±0.11
<i>L. anobrya</i>	300mg/kg	37.80±0.11	39.64±0.15	0.42±0.08	1.34±0.10	1.74±0.10	2.22±0.09

Discussion

The results of this study showed the value of *Lepidagathis anobrya* for the treatment of inflammation diseases. The methanol extract of this plant was found to be more active than aspirin and solumedrol when used at the same dose. Carrageenan edema consists of two distinct phases, an initial release of histamine, 5HT and kinins and finally a second phase where the mediator is suspected to be prostaglandins (Vane and Booteing 1987). Nitric oxide (NO) is also another crucial mediator contributing to the generation of inflammatory response in carrageenan-induced inflammation (Toriyabe et al. 2004; Sakaguchi et al. 2006). Our results are an indication that *L. anobrya* can be effective in the treatment of acute inflammatory disorders.

The work of Sung *et al.* (Sung et al. 2000) and those of Thiéfin and Beaugier (Thiéfin and Beaugier 2005) showed that the gastrointestinal toxicity of anti-inflammatory drugs was not confined only to the stomach and duodenum, but also reached the small intestine, colon and rectum. Thus, the search for anti-inflammatory drugs with a possible external use may be an interesting alternative to avoid this toxicity. The results of croton oil induced ear edema in rats have demonstrated the anti-inflammatory effect of methanol extract of *L. anobrya*, allowing an external application in order to avoid the toxicity associated with oral administration of drugs.

The extracts of *L. anobrya* also showed very strong analgesic and antipyretic activities. Acetic acid-induced abdominal writhing was used to evaluate the peripheral analgesic effect. The injection of acetic acid leads to the release of endogenous mediators like bradykinin, serotonin, histamine, substance P and prostaglandins (mainly PGE_{2α}, PGF_{2α}) and some cytokines such as TNF-α, IL-1β, IL-8 (Bentley et al. 1983; Negus et al. 2006). Therefore, this test is a nonspecific (e.g., anti-cholinergic, antihistaminic and other agents also show activity in the test) and widely used for analgesic screening. Our results indicate that the analgesic effect of *L. anobrya* might be mediated by its peripheral effect.

The phytochemical screening revealed the presence of triterpenoids, steroids, saponins, tannins and flavonoids in the extract. These compounds are well known to possess anti-inflammatory, analgesic and antipyretic effects due to their inhibitory effect on enzymes involved in the productions of the chemical mediators of inflammation and their antioxidant activity (Oweyele et al. 2005). This hypothesis is strongly supported by our previous study which has shown that *L. anobrya*

possesses one of the highest total phenolic content ($23.67 \pm 0.85\%$) and antioxidant activity ($IC_{50} = 16.33 \pm 1.04 \mu\text{g mL}^{-1}$) in plants belonging to the Acanthaceae family in Burkina Faso (Sawadogo et al. 2006).

Our study has clearly demonstrated the strong anti-inflammatory, analgesic and antipyretic activities of *L. anobrya* in dose dependant manner and also its low toxicity. These results provide data supporting the traditional use of this plant for the treatment of inflammation and hyperpyretic convulsions. Future studies will focus on the isolation and chemical characterization of the active molecules of this plant and to clarify their mechanism of action.

Acknowledgments

We are grateful to the “Département Medecine, Pharmacopée Traditionnelle et Pharmacie / IRSS, Burkina Faso” for providing financial support.

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