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Research Paper

Acute and chronic anti-inflammatory effects of the aqueous extract of *Acacia nilotica* (L.) Del. (Fabaceae) pods

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ABSTRACT

Acacia nilotica (L.) Del. (Fabaceae) is traditionally used in Northern Cameroon to treat various inflammatory affections, such as asthma, cough, gastric ulcers, hemorrhoids, fever, etc. The aim of this study was to evaluate the acute and chronic anti-inflammatory effects of the aqueous extract of *A. nilotica* pods. The anti-inflammatory effects of the aqueous extract of *A. nilotica* pods, administered orally at doses of 50 and 100 mg/kg, were evaluated *in vivo* using various models of both acute and chronic inflammations. Xylene-induced ear oedema in mice and carrageenan-induceda paw oedema were used to evaluate the acute effect of the plant extract. Chronic inflammation was evaluated using cotton pellet-induced granuloma in rats. The aqueous extract of *A. nilotica* pods decoction produced a significant inhibition (44.16%) of xylene-induced ear swilling in mice as compared with untreated mice. On the other hand, the plant extracts also inhibited rat paw oedema induced by carrageenan and the granuloma formation induced by the cotton pellets in rats in a dose dependant manner. The highest dose of *A. nilotica* extract (100 mg/kg) produced a maximum inhibition of 64.41 and 25.62% respectively for the carrageenan-induced paw oedema and the cotton pellet-induced induced granuloma in rats. Preliminary phytochemical analysis showed the presence of flavonoids, anthraquinones, saponins, tannins, polyphenols and alkaloids. Based on these results, the aqueous extract of *A. nilotica* pods may contain orally effective anti-inflammatory principles, justifying its use in folklore medicine.

Key words: *Acacia nilotica,* pods, anti-inflammatory, carrageenan, xylene, cotton pellets, granuloma.

INTRODUCTION

Inflammation is a defensive reaction of the body against infections and injuries. Edema formation, leukocyte infiltration and granuloma formation represent typical features of inflammation (Gorzalczany et al., 2011). Non-steroidal anti-inflammatory drugs (NSAIDs), steroidal drugs, and immuno-suppressant drugs, which have been usually used in the relief of inflammatory diseases worldwide for a long time, are often associated with severe adverse side effects, such as gastrointestinal bleeding and peptic ulcer (Valiollah et al., 2009). Recently, many natural medicines derived from plants, marine organisms, etc. were considered effective and safer for the treatment of various diseases including inflammation and pain (Su et al., 2011).

Acacia nilotica (L.) Del. (Fabaceae) is a multipurpose tree of Fabaceae family widely distributed in tropical and subtropical countries (Singh et al., 2009). It is used by traditional healers in Cameroon for the treatment of asthma, cough, flu, dental carries, fever, diarrhea, rheumatism, hemorrhoid and ophthalmia. Different parts of *A. nilotica* have been reported to possess antioxidant (Sundaram and Mitra, 2007; Singh et al., 2009; Singh et al., 2010; Kalaivani and Mathew, 2010), antiplasmodial (El-Tahir et al., 1999),

antibacterial (Solomon-Wisdom and Shittu, 2010), anthelmintic (Bachaya et al., 2009), antihypertensive and antispasmodic (Gilani et al., 1999) activities. On the other hand, Eldeen et al. (2010) reported the anti-inflammatory activity of niloticane isolated from the bark of *A. nilotica*. Moreover, the inhibitory effect of a single dose (500 mg/kg) of *A. nilotica* extract on carrageenan-induced paw oedema has also been mentioned (Dafallah and Al-Mustafa, 1996).

In the present study, inhibitory effects of the aqueous extract of *A. nilotica* pods were investigated in more detail using acute and chronic inflammation models namely xylene-induced ear oedema, carrageenan-induced paw oedema and cotton pellet-induced granuloma.

MATERIALS AND METHODS

Plant material

Dried pods of *A. nilotica* were collected (December 2009) in Maroua, Far North Region, Cameroon. Identification was made by a plant taxonomist (Pr Mapongmetsem Pierre-Marie, Department of Biological Sciences, University of Ngaoundere) and a voucher specimen N° 8582HNC has been deposited at the National Herbarium, Yaounde, Cameroon.

Preliminary phytochemical analysis

The aqueous extract of *A. nilotica* was screened for the presence of various phytochemical constituents such as alkaloids, flavonoids, saponins, and tannins according to previously described method (Harborne, 1984).

Preparation of the plant extract

50 g of the dried pods of *A. nilotica* were boiled in 250 mL of distilled water for one hour, left for one more hour for cooling. Thereafter, the extract was filtered with Whatman No.1 filter paper, evaporated to dryness at 55°C to give 16.78 g of solid, which was 33.58% (w/w) yield of the extract. The extract was subjected to phytochemical screening and pharmacological tests.

Animals

Experiments were conducted using adult male Wistar albino rats (100 – 150 g) and Swiss mice (20 – 30 g). Animals were housed in standard polypropylene cages, kept under ambient temperature (20-25°C) and illuminated environment of 12:12 h dark/light cycle. They were provided with standard food pellets purchased from LANAVET, Garoua, Cameroon and tap water *ad libitum*.

The experiment were carried out in accordance with the international ethical committee guidelines (EEC Directive of 1986; 86/609/EEC; USNRC, 1996) for the care and used of laboratory animals.

Carrageenan-induced rat paw oedema

The method of Winter et al. (1962) was employed in this experiment. The rats were randomly divided into four groups of five animals each. Carrgeenan solution was freshly prepared (1% in normal saline) and injected (0.1 ml) in the right hind paw of the rats under the subplantar region. Oedema formation was measured using a digital gauge calliper (± 0.001 mm, Mitutoyo, Japan), immediately prior to the injection of carrageenan and thereafter at 30, 60, 120, 180 and 300 min. Plant aqueous extract was given orally 30 min before carrageenan injection in doses of 50 and 100 mg/kg. Positive and negative controls also received orally indomethacin (10 mg/kg) and distilled water (10 ml/kg) respectively. Oedema inhibitory activity (Pi) of the extract was calculated according to the following ratio:

$$Pi = \frac{[(\mathrm{Dt} - \mathrm{D0})\mathrm{control} - (\mathrm{Dt} - \mathrm{D0})\mathrm{treated}]}{(\mathrm{Dt} - \mathrm{D0})\mathrm{control}} \times 100$$

Where Dt is the average diameter for each group at different time points after carrageenan injection and D0 the average diameter for each group before carrageenan injection

Xylene-induced ear oedema in mice

The experiment was conducted based on a previously described method (Junping et al., 2005). Four groups of five mice each were used. *A. nilotica* aqueous extract (50 mg/kg and 100 mg/kg) was administered one hour before xylene application. Dexamethasone (2.5 mg/kg) was given as a reference anti-inflammatory drug and distilled water (10 ml/kg) given to control animals. Ear oedema was induced by applying carefully a drop of xylene (0.03 mL) to the anterior and posterior surfaces of the right ear. The left ear remained untreated and considered as control. One hour after xylene application, the animals were killed under ether anesthesia and 6 mm punches were made in the right and left ears of each mouse using a borer. Each ear punch was weighed and differences between the weight of the right and left ear punches of mice were recorded.

Cotton pellet-induced granuloma in rats

The effect of *A. nilotica* extract on cotton pellet-induced granuloma in rats was conducted as previously described by Ismail et al. (1997). Twenty rats were divided into four groups of five animals each. Granulomatous lesions were

Table 1. Effect of the aqueous extract of *A. nilotica* on carrageenan-induced paw oedema in rats.

		Paw oedema (mm)				
Group	**Doses (mg/kg)**	**0.5 h**	**1 h**	**2 h**	**3 h**	**5 h**
Distilled water	0	1.70 ± 0.16	2.69 ± 0.08	2.34 ± 0.15	2.22 ± 0.17	2.15 ± 0.08
A. nilotica	50	1.40 ± 0.18 (17.65)	2.22 ± 0.16 (17.47)	1.88 ± 0.10 (19.66)	1.59 ± 0.09* (28.38)	0.78 ± 0.18*** (59.93)
A. nilotica	100	1.10 ± 0.19 (35.29)	1.34 ± 0.31* (50.18)	1.20 ± 0.29** (48.72)	1.01 ± 0.26*** (54.50)	0.63 ± 0.18*** (64.41)
Indomethacin	10	1.18 ± 0.33 (30.59)	1.98 ± 0.26 (26.39)	1.10 ± 0.22** (52.99)	0.84 ± 0.21*** (62.16)	0.75 ± 0.19* (65.11)

Each value is the mean ± S.E.M. (n = 5). In parentheses values represents the percentage of inhibition.
* Significant ($P < 0.05$) when compared to the corresponding control
** Moderately significant ($P < 0.01$) when compared with the corresponding value of the control
*** Highly significant ($P < 0.001$) when compared with the corresponding value of the control.

induced by surgically inserting sterile cotton pellets (15 ± 1 mg) subcutaneously in both axilla regions of each rat following a single incision which was thereafter closed by interrupted sutures. After implantation of pellets, the plant extract (50 and 100 mg/kg) was orally administered once a daily for 7 consecutive days. Dexamethasone (2.5 mg/kg, p.o.) was also given daily to standard group while the control group received the same volume of distilled water (10 ml/kg). On day 8, the cotton pellets were dissected out under ether anesthesia, cleaned of extraneous tissue, weighed and dried at 50°C to a constant weight. The mean weights for different groups were determined. The increase in dry weight of the pellets was taken as the measure of the granuloma formation.

Statistical analysis

Results are expressed as mean ± S.E.M. The data obtained was statistically analyzed using one-way ANOVA, followed by Student's Newman-keuls test. The differences were considered significant at $P< 0.05$.

RESULTS

Preliminary phytochemical analysis

Phytochemical analysis of the aqueous extract of *A. nilotica* revealed the presence of flavonoids, anthraquinones, saponins, tannins, polyphenols and alkaloids.

Effect of the aqueous extract of *A. nilotica* on carrageenan-induced paw oedema in rats

The inhibitory effect of the aqueous extract of A. nilotica on carrageenan-induced paw oedema is shown in Table 1. For each of the two doses of extract tested (50 and 100 mg/kg), the aqueous extract exerted considerable inhibitory effect on paw increase 1 hour after carrageenan administration, with about a 50% inhibition for the dose 100 mg/kg. The maximum inhibition (64.41%; $p<0.001$) elicited by the aqueous extract of A. nilotica was recorded 5 hours after carrageenan injection. Indomethacin which is a reference drug showed a similar inhibitory effect 5 hours after carrageenan administration (65.11%).

Effect of the aqueous extract of *A. nilotica* on xylene-induced ear oedema in mice

The effect of the aqueous extract of *A. nilotica* on xylene-induced ear oedema in mice is recapitulated in Table 2. Oral administration of the plant extract (50 and 100 mg/kg), 1 h after xylene application, significantly ($P < 0.01$) inhibited the development of ear oedema in mice in a dose dependent manner. The inhibition produced by 100 mg/kg of the extract
(44.16%) was similar to that produced by dexamethasone (44.44%).

Effect of the aqueous extract of *A. nilotica* on cotton pellet-induced granuloma in rats

The aqueous extract of *A. nilotica* significantly inhibited ($P < 0.001$) the granuloma induced by cotton pellets in rats in a dose-dependent manner (Table 3). The extract at the doses of 50 and 100 mg/kg produced a maximum reduction on the granulomatous tissue formation on implanted cotton pellets with inhibition of 21.60 and 25.62% respectively as compared with dexamethasone (2.5 mg/kg) which produced the highest inhibition (37.64%).

DISCUSSION

Carrageenan-induced rat paw oedema is a suitable experimental

Table 2. Effect of the aqueous extract of *A. nilotica* on xylene-induced ear oedema in mice.

Group	Doses (mg/kg)	Oedema (mg)	Inhibition (%)
Distilled water	0	3.60 ± 0.39	-
A. nilotica	50	2.60 ± 0.24**	27.77
A. nilotica	100	2.01 ± 0.31**	44.16
Dexamethasone	2.5	2.00 ± 0.29**	44.44

Each value is the mean ± S.E.M. (n = 5). **P < 0.01 compared with control.

Table 3. Effect of the aqueous extract of *A. nilotica* on cotton pellet-induced granuloma in rats.

Group	Doses (mg/kg)	Granuloma weight (mg)	Inhibition (%)
Distilled water	0	32.40 ± 0.27	-
A. nilotica	50	25.40 ± 1.02***	21.60
A. nilotica	100	24.10 ± 0.41***	25.62
Dexamethasone	2.5	20.20 ± 0.47***	37.64

Each value is the mean ± S.E.M. (n = 5). ***P < 0.001 compared with control.

animal model commonly used for the study of acute inflammation and is believed to be biphasic. In general, the first phase (1-2 h) involves inflammation mediated by the release of serotonin and histamine and increased synthesis of prostaglandins in the surroundings of the damaged tissues. The second phase (3-5 h) is the result of the release of kinins mainly prostaglandins (Crunkhorn et al., 1971). In this study, the aqueous extract of *A. nilotica* exerted considerable inhibitory effect on carrageenan-induced paw oedema in rats starting from the first hour after administration. This effect was dose dependent and the maximum inhibition induced by the extract was recorded after 5 h with the highest dose (100 mg/kg) of the plant extract. Similar inhibitory effects were observed after carrageenan injection with indomethacin, a potent non-steroidal anti-inflammatory drug which acts by inhibiting cyclooxygenage. Therefore, our results suggest that the inhibitory effect of the aqueous extract of *A. nilotica* on carrageenan-induced paw oedema may be due to the suppression of the release of mediators including histamine, serotonin, bradykinin and prostaglandins responsible for the first and the second phase of acute inflammation induced by carrageenan. There are also evidences that compounds inhibiting the carrageenan-induced oedema are effective in inhibiting the enzyme cyclooxygenases (Selvam and Jachak, 2004). Based on these reports, the inhibitory effect of *A. nilotica* extract on carrageenan-induced inflammation could be mediated via this mechanism. Similar anti-inflammatory effects have been reported with other species of the *Acacia* genus (Dongmo et al., 2005; Bukhari et al., 2010).

Phytochemical analysis of *A. nilotica* revealed the presence of flavonoids, anthraquinones, saponins and polyphenols. Chemical studies on other *Acacia* species have led to the isolation of triterpenoidal saponin from *Acacia auriculiformis* (Uniyal et al. 1992) and phenolic compounds from the flowers and leaves of *A. nilotica* (Saleem et al., 2001; Venkataswamy et al., 2010). Saponins and flavonoids have previously been reported to have anti-inflammatory activities (Mohammad et al., 2004; Aquila et al., 2009). Such compounds may be responsible in part for the described anti-inflammatory activity of *A. nilotica* extract.

On the other hand it has been reported that free radicals are involved in the inflammatory process (Vajdovich, 2008). Different parts of *A. nilotica* have been reported to possess antioxidant properties (Singh et al., 2009; Singh et al., 2010; Kalaivani and Mathew, 2010). Therefore the antioxidant property of *A. nilotica* may have a beneficial role in its anti-inflammatory activity.

The xylene-induced ear oedema model is useful for the evaluation of anti-inflammatory topical steroids or for non-steroidal antiphlogistic agents, especially those inhibiting phospholipase A_2 (Kwang-Ho et al., 2008). Xylene-induced ear oedema is an acute inflammation model which may involve inflammatory mediators such as histamine, serotonin and bradykinin. These mediators induce ear oedema by promoting vasodilation and increasing vascular permeability (Carlson, 1985). The effectiveness of *A. nilotica* aqueous pods extract in this model probably provides the active principles present in the extract which interfere with the action of these mediators to produce its anti-inflammatory effect.

The cotton pellet-induced granuloma is an established and the most suitable method for studying the efficacy of drugs against the proliferative phase of inflammation (Parvataneni et al. 2005). In this model, the aqueous extract of *A. nilotica* at doses of 50 and 100 mg/kg, effectively inhibited the development of granulomatous tissues as compared to control group. The extract may act by inhibiting cellular migrations involved in this inflammation model. Similar anti-

inflammatory effects have been shown with the aqueous and organic extracts of *Acacia visco* (Pedernera et al., 2010).

According to the present study, it can be concluded that the aqueous extract of *A. nilotica* dried pods possesses anti-inflammatory effect against both exudative and proliferative phases of inflammation, justifying its wide use in folklore medicine for the treatment of inflammation conditions. Further investigations are required to isolate the active principles present in the extract and to determine their exact mechanism of action.

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