ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF *ALBIZIA ZYGA* STEM BARK (MIMOSACEES)

Soh Fongang Rene[2], Ndebia Eugène Jamot[1*] , Sylvere Ngouela[2], Chungag-Anye Nkeh[1] and Tsamo Etienne[2]

[1] Department of Biology and Animal Physiology, Faculty of Sciences, University of Yaounde I; P.O. Box 812 Yaoundé – Cameroon.

[2]Department of Organic chemistry, Faculty of Sciences, University of Yaounde I; P.O. Box 812 Yaoundé – Cameroon.

Summary

Albizia zyga is used in Cameroonian folk medicine to treat oral wound and throat infection. The present work assesses the analgesic and anti-inflammatory activities of *albizia zyga* using in vivo models. Pain was induced in mice by injection of 1 ml/100 g of 1 % acetic acid and by the application of increasing pressure on rat's paws using an Analgesy Meter. Inflammation was induced in rat's right hind paw by the injection of 0.05ml of 1 % carragenan. Oral administration of extract at doses of 100 mg/kg and 150 mg/kg significantly ($p<0.05$) inhibited acetic-induced pain by 52 % and 61 % respectively. Analgesy Meter pressure-induced pain was reduced by 45 % and 49 % ($P<0.01$) at the second hour. Peripheral inhibitory effect was less strong than aspirin activity. Anti-inflammatory property of the extract, one hour after carrageenan injection, was significant ($P<0.05$) without dose dependent effect. The plant could then act by suppressing the initial phase of carrageenan paw oedema that is mediated by histamine and serotonin. The results of the present study indicate that *albizia zyga* has both analgesic and anti-inflammation properties.

Keywords: *albizia zyga*, analgesic, anti-inflammatory, writhing, Analgesy Meter, rat paw eodema

Corresponding author:
Ndebia Eugène Jamot
Department of Biology and Animal Physiology, Faculty of Sciences, University of Yaounde I;
P.O. Box 812 Yaoundé – Cameroon.
Tel (237) 953 49 33.
e-mail: **jamot17@yahoo.fr**

Introduction

The geographic distribution of *albizia zyga* is confined in the large aera of the west cameroon, this plant is also known as "keyemtieu" in Bandjoum language. *Albizia zyga* is used in Cameroonian folk medicine to treat headache, diarrheoa, oral wound and throat infection (Berhaut, 1975). The use of this plant by people is mainly due to its analgesic, antirheumatic and antifever properties (Surville, 1980). The phytochemical studies we conducted on the same species allowed us to isolate a number of compounds including terpenoïds, saponins, flavonoïds phenols and steroïds. Since no investigation is available on the analgesic and anti inflammatory properties of *albizia zyga ,*the present study was to provide scientific validation of the claimed ethno pharmacological properties by investigating the analgesic and anti inflammatory aspects of this plant.

Materials and methods

Plant extract

Albizia zyga stem barks were collected in Bafoussam, west Cameroon in the month of september 2005 and identified in National Herbarium, Yaounde, where a voucher specimen N° 2338(SRFK) is deposited. The stem barks of *albizia zyga* was reduce to a fine powder 8 kg and soxhlet extracted with methanol. The resulting methanol extract was concentrated in a green dark powder (250 g).

Animals

Swiss albinos mice (20 – 30 g) and wistar albinos rats (150 – 200 g) of either sex were used for this study. The animal were fed with rat pellet food and water ad libitum. All animals were acclimatized to the laboratory environment for at least one week before the experimental session.

Drugs

Aspirine (Aspegic®, Sanofi-Synthelabo France), Indomethacine (Indocid®, Laboratoire Merck Sharp et Dohme France) and Morphine (Sigma-Aldrich-Quiinina S.A. Madrid-Spain) were dissolved in distilled water. Carrageenan (Sigma Chemical Co, St Louis, USA) in physiological saline.

Nociceptive activities

The analgesic activity was measured against chemical and mechanical stimulus.

Acetic acid-induced abdominal writhing test

Mice were divided into five groups each containing five animals. The first group served as a negative control and received NaCl $9^0/_{00}$. The second and the third groups served as positive

control and received respectively Morphine (1.5 mg/kg, po) and Aspirin (100 mg/kg, po), while the last two groups received respectively oral doses of 100 and 150 mg/kg plant extract of *albizia zyga*. One hour after administration of the test drugs, each group was injected intraperitoneally with 1% acetic acid in a volume of 1 mL/100 g body weight. The number of writhing response such as contortions and stretching were recorded for 30 min. The result were evaluated by calculating the mean number of contortions per treated group compared with that of the control group. Protection rate were calculate as follows.

$$\text{Protection}(\%) = \frac{\overline{Nc} - \overline{Nt}}{\overline{Nc}} \times 100$$

$\overline{Nc}$: mean number of contortions of the control group

$\overline{Nt}$ **:** mean number of contortions per treated group

Pressure test

In these experiments, the drugs cited above were tested at the same doses in rats using an Ugo Basile Analgesy Meter (N° 7200). Force that was applied to the left hind paw of experimental animals by an Analgesy Meter plunger which exerts a constantly increasing force on the rat paw. The rat was suspended vertically while its left hind paw was placed between the plinth and the plunger. As the applied force increases, it gets to a point where the animal struggles to free its paw. This is the level at which the animal fells pain. The weight causing pain before treatment and then 1h, 1h 30, 2h and 2h 30 h after treatment of animals with the various test drugs was determined. Protection rates were calculates as follows.

$$\text{Protection.}(\%) = \frac{\overline{Ft} - \overline{Fo}}{\overline{Fo}} \times 100$$

$\overline{Fo}$: force where the animal struggles to free its paw before administration of drugs.

$\overline{Ft}$: force where the animal struggles to free its paw after administration of drugs.

Carrageenan- induced paw oedema

Oedema was induced on the right hind paw of rat by a subplantar injection of 0.05 mL of a solution of 1% sterile carrageenan in saline. Plant extract (100 and 150 mg/kg) and Indomethacine (10 mg/kg) were administered orally 30 min before carrageenan injection. Control animals received the vehicle only. The volume of the injected paw was measured before and 30, 60, 120 and 240 min after induction of inflammation using a Plethysmometer (Ugo Basile N° 7140). Inflammation was expressed as an increase in paw volume due to carrageenan injection. Oedema (ΔV) and inhibition rate (I %) were calculate as follows.

$$\Delta V = \overline{vt} - \overline{vo}$$

$$I\% = \frac{\left(\overline{vt} - \overline{vo}\right)C - \left(\overline{vt} - \overline{vo}\right)E}{\left(\overline{vt} - \overline{vo}\right)C} \times 100$$

$\overline{vt}$: right hind paw volume at time t. C: control group; E: essays group

$\overline{vo}$: right hind paw volume before subplantar injection of carrageenan

Statistical analysis

The results are expressed as mean ± SEM. Data were analyzed statistically by analysis of variance followed by Dunnett's multiple comparison tests. P values less than 0.05 were considered as indicative of significance.

Results

Antinociceptive effects

Acetic acid-induced abdominal writhing test

As we can see from table 1, the methanolic fraction of *albizia zyga* extract at dose of 100 and 150 mg/kg po significantly (P<0.05) decreased the number of writhes by 52 % and 61 % respectively. The reference drugs (Morphine and Aspirin) induced significant (P<0.01) reduction of the noted parameters by 61 % and 40 % respectively.

Table 1: Effect of *albizia zyga* on acetic acid induced writhing

Group	Dose (mg/kg po)	Writhing	% Protection
Control	-	148 ± 16	-
Aspine	100	75 ± 7**	40
Morphine	5	56 ± 9**	61
albizia zyga	100	71 ± 7**	52
albizia zyga	150	58 ± 8**	61

Significant difference versus control: * P<0.05; **P<0.01 vs control, n: 5

Pressure test

On mechanical pain induced by the plinth plunges of Analgesymeter (table 2), aspirin (100 mg/Kg) did not show significant antinociceptive effect. One hour after their administration, the analgesic effects of leaves aqueous extract of *albizia zyga* (100 and 150 mg/Kg p.o.) were significant, and the results were less to morphine (1.5 mg/Kg). The maximal inhibitory effects with the extract were observed at the 2nd hour (P<0.01) at the dose of 150 mg/Kg.

Table2: Effect of *albizia zyga* on pressure test induced by Analgesy- meter (g/f)

Group	Dose (mg/kg po)	Before administration	After administration			
			1 h	1h 30	2 h	2h 30
Control	-	84	89 ± 4 (6)	80 ± 8 (-5)	82 ± 6 (-2)	86 ± 5 (2)
Aspirine	100	89	94 ± 5 (6)	95 ± 4 (7)	102 ± 6 (15)	89 ± 3 (0)
Morphine	5	81	89 ± 7 (10)	115 ± 7** (50)	136 ± 2** (68)	108 ± 5* (33)
albizia zyga	100	73	72 ± 5 (-1)	102 ± 7** (40)	106 ± 6** (45)	83 ± 6 (14)
albizia zyga	150	79	95± 5 (20)	118 ± 5** (46)	115 ± 4** (49)	94 ± 4 (19)

Significant difference versus control * P<0.05; **P<0.01 vs control, n: 5 ( ): Protection

Carrageenan- induced paw edema

In the control group, the sub-plantar injection of carrageenin produced local aedema in the following 30 min that increased progressively to reach a maximal intensity 2h after the injection of the phlogistic agent. A pre-treatment by *albizia zyga* significantly (P<0.01) attenuated paw swelling after 1 hours following oral administration at a dose of 100 mg/kg (table 3). Afterwards, the inhibition of the oedema was not significant until 2h. Antiinflammatory effect of the extracts were not dose-dependent. Indomethacine was more powerful (P<0.01) in reducing paw oedema than *albizia zyga.*

Table 3: Antiinflammatory activity of *albizia zyga* in carrageenan-induced hind paw oedema: expressed in volume variation (ΔV: mL)

		Oedema (mL)				Inhibition (%)			
Groupe	**Dose**	**0,5h**	**1h**	**2h**	**4h**	**0,5h**	**1h**	**2h**	**4h**
Control		0,18± 0,01	0,35± 0,06	0,61± 0,07	0,50 ± 0,06	-	-	-	-
Indo	10	0,20± 0,04	0,21± 0,01*	0,11± 0,02**	0,05 ± 0,00**	-11	41	82	89
albizia zyga	100	0,24± 0,07	0,34± 0,06*	0,46± 0,09	0,43 ± 0,04	-31	40	25	15
albizia zyga	150	0,31± 0;08	0,32± 0,07	0,55±0,08	0,49 ± 0,09	-70	10	11	02

Significant difference versus control * P<0.05; **P<0.01 vs control, n: 5

Discussion

The present result shows that, the pre-treatment of rat with *albizia zyga* inhibited pain caused mechanically by a constantly increasing pressure on rat paw by the plunges and plinth of the analgesy Meter. This system provides a model for the study of non inflammatory pain. The nociceptors seem to be sensitized by sensory nerves, therefore it is more likely that opoïd-like analgesic drugs be more effective in inhibiting mechanically induced pain (Nkeh *et al*, 2002). The involvement of endogenous substances such as prostaglandins may be minimized in this model (Dongmo *et al*, 2001). The central protecting effect of *albizia zyga* were lower than morphine test results. It is therefore more likely that opoïd-like analgésic drugs be more effective in inhibiting mechanically induced pain. *albizia zyga* significantly blocked the pain sensation at both used doses (100 and 150 mg/kg). This compound is then effective against pain due to sensory nerve stimulation. Aspirin did not show analgesic effect on this model of pain, this corroborates the previous study that Aspirin and Aspirin like drugs are ineffective both against pain due to sensory nerve stimulation (Flower *et al*, 1985). Other way *albizia zyga* induced analgesic effect against the writhing syndrome indicating peripheral effect of the plant (Atta *et al*., 1997). In peripheral tissues, prostaglandins and kinines are suggested to play an important role in the pain process (Hajare *et al*., 2000) and writhing induced by chemical substances injected intrapertoneal was said to be due to sensitization of chemosensitive nociceptors by prostaglandins (Maria Elena *et al*, 1997). These results suggest that this plant would exercise their pain killing effect by the prostaglandins synthesis inhibition. This test also confirms the peripheral action of Aspirin (Rang *et al.,* 1995).

In the last part of the study, the carrageenan experimental model of inflammation was used to evaluate the anti-inflammatory effect of *albizia zyga*. This model of inflammation is highly sensitive to non steroïdal anti-inflammatory drugs (Maria *et al.,*1998). The presence of terpenoïds in this plant corroborate its anti-inflammatory activities. It is well known that terpenoïds are almost ubiquitous in plants and have been long considered the anti-inflammatory principles of several important drugs (Salvador, 1997).

According to Hwang et al (1996), Lo et al (1987), the initial phase of carrageenan paw oedema is mediated by histamine and serotonin, while the later phase is suspected to be due to arachidonate metabolites (prostaglandins, leukotrienes) producing oedema dependent on mobilisation of neutrophiles. Although the cyclooxygenase and lipoxygenase pathways are both involved in the inflammation process, Flower et al (1985) have however shown that inhibitors of cyclooxygenase are more effective in inhibiting carrageenan-induced inflammation than lipoxygenase inhibitors. In our experiment, the oedematous response was slighly significantly suppressed in rat pre-treated with *albizia zyga* 1h after treatment, that is 30 min after the carrageenan injection. This result suggest that *albizia zyga* act by suppressing the the initial phase of carrageenan paw oedema is mediated by histamine and serotonin.
In conclusion, the presented data indicate that oral administration of *albizia zyga* show analgesic and anti inflammatory activities. Theses capabilities confirm his use in folk medicine. Follow up studies will concentrate on studying the effects of *albizia zyga* on prostaglandin synthesis, cyclooxygenase and lipoxygenase activities in order to establish its possible mechanism (s) of action.

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