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Antiinfammatory Effects of *Pyrenacanthia Staudtii Engl.* (Icacinaceae) Aqueous Leaf Extract in Rodents

[Kemirgenlerde Pyrenacanthia Staudtii Engl. (Icacinaceae) Bitkisinin Sıvı Yaprak Ekstresinin Antiinflamatuar Etkisi]

SUMMARY
AIM: The continued search for potential and safe anti-inflammatory agents is important, in view of the roles played by proinflammatory factors in diseases such as asthma, rheumatoid arthritis, cancer and neurodegerative disorders. The Nigerian flora is rich in medicinal plants which are employed in traditional folk medicine for various inflammatory disorders. We therefore evaluate the antiinflammatory activity of the aqueous extract of the leaves of.
METHOD: The antiinflammatory activity was evaluated using egg albumin, agar-induced paw edema models for acute inflammation, complete Freud Adjuvant (CFA)-induced arthritis and cotton pellets granuloma models for chronic inflammation in rats.
RESULTS: PSE, produced significant (P<0.05-0.01) and dose-dependent (100-400 mg/kg) reduction of paw edema volume in rats. In chronic inflammation (CFA-induced arthritis), the extract significantly (P< 0.05) inhibited edematous response in a dose-related manner, provoking an inhibitory effect (61%, maximum inhibition) at 400 mg/kg on day 21 same as non-steroidal anti-inflammatory drug, indomethacin (5 mg/kg). PSE, also significantly (P<0.05) and dose-dependently reduce the granuloma weight in chronic model of granuloma pouch in rats.
CONCLUSION: The findings of the present study indicate that PSE possesses anti-inflammatory activity and thus lend pharmacological credence to the suggested use of the plant as a natural remedy in the treatment, management and/or control of inflammatory conditions in some rural communities of Nigeria.

ÖZET
GİRİŞ: Astım, romatoid artrit, kanser ve nörodejeneratif bozukluklar gibi hastalıklarda, proinflamatuar faktörlerin rolleri açısından, potansiyel ve güvenli anti-inflamatuar ajanlar için yapılan sürekli aramalar önemlidir. Nijerya'nın florası tıbbi bitkiler bakımından zengin olup, bu bitkiler geleneksel halk tıbbında çeşitli enflamatuar hastalıklar için kullanılmaktadır. Bu kapsamda Pyrenacanthia staudtii Engl. (Icacinaceae) (PSE) yapraklarının sulu ekstresinin antienflamatuar aktivitesi incelenmiştir.
METOD: Antiinflamatuvar aktivite; yumurta albumin, agar-akut inflamasyon için modeli, komple Freud Adjuvanı (CFA)-indüklenmiş artrit ve sıçanlarda kronik inflamasyon için pamuk pellet granülom modelleri kullanılarak değerlendirilmiştir.
BULGULAR: PSE, pençe ödemi miktarını doz bağımlı olarak azaltmıştır (100-400 mg/kg) ve bu azalış istatistiksel olarak anlamlıdır (P<0.05-0.01). Kronik inflamasyonda (CFA-indüklenmiş artrit) ekstre; ödematöz cevabı doz bağımlı olarak anlamlı şekilde inhibe etmiş (p<0.05), bu inhibisyonun (61%, maksimum inhibisyon); 400 mg/kg dozda ve 21 günlük kullanımda, non-sterodi anti-inflamatuar indometazinin (5 mg/kg) yaptığına eşit olduğu saptanmıştır. Aynı zamanda PSE, ratlarda kronik granülom kese modelinde granülom ağırlığını doza bağımlı ve anlamlı olarak azaltmıştır (p <0.05).
SONUÇ: Bu çalışmanın bulguları, anti-enflamatuar aktiviteye sahip ve farmakolojik olarak güvenli olduğu gösterilen PSE'nin, Nijerya'nın bazı kırsal topluluklarda iltihabi hastalık tedavisi, kontrolü ve/veya yönetiminde doğal bir ilaç olarak önerilebileceğini göstermiştir.

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Key Words:
Pyrenacanthia Staudtii, Aqueous Extract, Anti-İnflammatory Activity.

Anahtar Kelimeler:
Pyrenacanthia Staudtii, Sulu Ekstrakt, Anti-İnflamatuar Etkinlik.

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INTRODUCTION

Pyrenacanthia staudtii (Engl) Engl belongs to the family Icacinaceae. It is called Ahara inYoruba, nhia in Igbo and Ohogbo among Edos of Nigeria. The plant occurs as an arborescent, 6m long and 5-10cm in diameter. It is widely distributed in the tropical Africa and found in secondary jungles of southern Nigeria, Western Cameroon and across central Africa to Uganda and Angola (1). Traditionally, the plant is used for the treatment of blennorrhea, intestinal pain and hernia (1,2,3). The plant has been reported to have smooth muscle relaxant activity in isolated smooth muscle (4), control of schistomosis (5); anticonvulsant and analgesic activities (6). Phytochemically the plant's extract was reported to

contain saponins and alkaloids (5). In addition our own phytochemical analysis in this study revealed the presence of tannins. The aim of this study was therefore to investigate the anti-inflammatory effect of the aqueous leaf extract of Pyrenacanthia staudtii.

MATERIALS AND METHODS

Animals

The experimental protocol and procedures used in this study conformed to the guidelines of care and use of animals in research and teaching (NIH publications number 85-93 revised in 1985). Thirty Wistar albino rats (220-250 g) of both sexes, divided into five groups of six animals in each group were used for each experiment. Fifty six Swiss mice (18-22 g) of both sexes, divided into seven groups of eight animals in each group were used for toxicity testing. All animals were maintained on standard pellet diet (Ladokun Feeds, Nigeria limited) and water ad libitum.

Plant materials

The plant sample was collected in the month of October 2007 from a village near Ilesa in the southwestern part of Nigeria. The plant was identified in the herbarium of the Federal Institute of Forest Research Ibadan, where voucher specimen was deposited with voucher number106886. The leaves were air-dried and powdered using electric blender.

Preparation of plant extract

500 g of the powdered leaf sample of the plant was soaked in1000 ml of distilled water for 48 h. The resulting extract was filtered and the filtrate concentrated under reduced pressure at 60±1o C in rotary evaporator. Freeze-drying and solvent elimination of the resulting aqueous extract finally gave 35.2 g (7.2 % yields). Aliquot portions of the plant's extract (PSE) were weighed and dissolved in distilled water (at room temperature) for use on each day of our experiments.

Acute Toxicity Testing

The Median Lethal dose (LD50) of Pyrenacanthia staudtii leaf aqueous extract was determined in the mice using modified method (7). Mice fasted for 16 hours were randomly divided into groups of 8 mice per group. Graded doses of PSE (50, 100, 200, 400, 800, 1600 and 3200 mg/kg) were separately administered orally using oral (p.o.) cannular to the mice in each of the 'test' groups. Each of the mice in the 'control' group was treated orally (p.o.) with distilled water (10 mL/kg) only. The mice in both the 'test' and 'control' groups were then allowed free access to food and water, and observed over a period of 48 hours for signs of acute toxicity. The number of deaths (caused by the extract) within this period of time was noted and recorded. Log dose-response plots were constructed for the plant's extract, from which the median lethal dose (LD50) of the leaf aqueous extract was determined.

Egg albumin-induced paw edema in rats

The rat paw edema method (8) was used. Acute inflammation was measured in terms of change in volume of the rat hind paw (9) induced by subplantar injection of egg albumin (10,11). A 0.1 mL of fresh undiluted egg albumin was injected into the subplantar region of the right hind paw of rat 1 h after 100, 200, 400 mg/kg of the aqueous leaf extract was orally administered. Control animals received 10 mL/kg distilled water or 5 mg/kg indomethacin orally using oral cannular. Inflammation was assessed as the difference between the zero times volume of the treated paw (Vo) and the volume at the various times after administration of the phlogistic agent (egg albumin).Percentage inhibition of edema (12,13) was calculated using the relation

$$\text{Inhibition of edema} = \frac{100x\ (1\text{-}a\text{-}x)}{b\text{-}y}$$

where a=mean paw volume of treated rats at various time after egg albumin injection; x=mean paw volume of treated rats before egg albumin injection; b=mean paw volume of control rats at various time after egg albumin injection; y=mean paw volume of control before egg albumin injection.

Agar-induced paw edema in rats

The rat paw edema method (8) was used as described above. Animals in groups of six received 100, 200, or 400 mg/kg of the aqueous leaf extract administered orally One hour later, edema was induced with 0.1 mL of freshly prepared colloidal suspension of agar injected into the subplantar region of the right paw of the rats. The volume of distilled water displaced by the treated paw was measured before and 1, 2, 3 and 4 h after induction of edema. Control groups received orally either 10 mL/kg distilled water (vehicle) or 5 mg/kg indomethacin. Inflammation was assessed as described for the egg albumin induced edema method.

Adjuvant-induced arthritis in rats

The effect of the extract (PSE) on chronic inflammation was evaluated using Complete Freud Adjuvant (CFA)-induced arthritis test in rats (14). Animals were randomly divided into five groups of six animals each and treated daily for 21days. Animals in the treated groups received 100, 200 or 400 mg/kg of aqueous leaf extract, orally. Control groups received either 10 mL/kg distilled water (Vehicle) or 5 mg/kg indomethacin (standard drug). All animals were injected with 0.1 mL of CFA in the subplantar region of the left hind paw. The volume of distilled water displaced was measured on day 1 before and day 5, 12 and 21, after induction of edema. The edema rate (ER) and inhibition rate (IR) of each group were calculated as follows (15).

$$ER\% = \frac{Vt\text{-}Vo \times 100}{Vo}$$

where Vo is the volume before CFA injection

$$IR\% = \frac{Ec\text{-}Et \times 100}{Ec}$$

Where Ec is the edema rate of control group and Et is the edema rate of treated group.

Cotton-pellet granuloma test in rats

The effect of PSE on chronic inflammation was evaluated using cotton-pellet granuloma test in rats (13). On day 1, rats received 100, 200 or 400 mg/kg p.o. PSE. Control animals received orally either prednisolone (5 mg/kg) or equivalent of vehicle (2% v/v Tween 80). Thirty minutes later autoclaved cotton pellets 50±1 were implanted on the back of the rats under diethyl ether anesthesia. Drugs were administered daily for 7 days. On day 8, animals were killed by overdose of ether. The pellets were dissected out, dried in an oven at 60o C and weighed to determine the level inhibition of granuloma. The level of inhibition of granuloma tissue development was calculated using the relation:

$$\frac{(TC - Tt) \times 100}{Tc}$$

Data and statistical Analysis

Data obtained are presented as means (± SEM) for the number of animals in each group (n=6). Data obtained from distilled water or normal saline-treated 'control' rats were used as baseline. In all cases, the results obtained from extract- and reference drug treated-'test' animal groups were compared with those obtained from distilled water or normal saline (vehicle)-treated 'control' animal groups. The differences between the data obtained from 'test' animal groups and the data obtained from vehicle-treated 'control' animal groups were subjected to one-way analysis of variance (ANOVA, 95% confidence interval), followed by Dunnett'post-hoc test (16). In all cases, statistical significance was established at values of $P \leq 0.05$.

RESULTS

Oral administration of graded dosed (100-3200 mg/kg) of Pyrenacanthia staudtii leaf aqueous extract in mice gave LD50 value of 2,150±80 mg/kg. Studies of inflammatory effect showed that the extract caused a significant ($P < 0.05$) reduction or inhibition of the development of paw edema induced by egg albumin in a dose-dependent manner with activity of the extract (400 mg/kg) higher than that of indomethacin (5 mg/kg) (Table1). However, in the agar-induced paw edema, the extract exhibited significant ($P < 0.05$) dose-related inhibition of which the activity at 400 mg/kg is lower than that of indomethacin (5 mg/kg) (Table2).

Table 1. Effect of the extract of *Pyrenacanthia staudtii* on egg albumin-induced acute inflammation.

		Edema (ml)			
Treatment	**Dose (mg/kg)**	**1h**	**2h**	**3h**	**4h**
Control (normal saline)		0.31±0.04	0.28±0.04	0.24±0.06	0.21±094
Extract	100	0.23±0.04	0.20±0.03	0.18±0.03 (25)	0.16±0.03 (24)
	200	0.20±0.03	0.18±0.03	0.14±0.02 (42)	0.08±0.09*(62)
	400	0.18±0.03	0.16±0.02	0.12±0.02 (50)	0.06±0.09*(66)
Indomethacin	5	0.30±0.03	0.21±0.04	0.16±0.03 (45)	0.06±0.09*(66)

P<0.05 vs. control (ANOVA; Dunnet post hoc test). Values are mean±SEM (*n*=6) while those in parenthesis represent percent inhibition of edema

Table 2. Effect of the extract of *Pyrenacanthia staundti* on agar-induced acute inflammation

Treatment	Dose (mg/kg)	Edema (ml) 1h	2h	3h	4h
Control (normalsaline)	-	0.23±0.03	0.38±0.08	0.38±0.08	0.50±10
Extract	100	0.43±0.13	0.43±0.13	0.35±0.13	0.28±0.9*(24)
	200	0.43±0.13	0.43±0.13	0.34±0.13	0. 23±0.03*(54)
	400	0.23±0.03	0.30±0.07	0.20±0.07	0.17±0.02*(61)
Indomethacin	5	0.23±0.03	0.23±0.03	0.13± 0.02	0.10±0.008**(80)

*P< 0.05, **P<0.01 vs. control (ANOVA; Dunnet post hoc test). Values are mean ±SEM (*n*= *6*) while those in parenthesis represent percent inhibition of edema.

Table 3. Effect of the extract of Pyrenacanthia staundti on Complete Feud adjuvant-induced arthritis in rats

Treatment	Dose (mg/kg)	Edema (ml) Days 5	12	21
Control(normal Saline)		0.31±0.02	0.26±0.02	0.23±0.03
Extract	100	0.31±0.02 (NI)	0.24±0.02 (8)	0.20±0.03 (13)
	200	0.31±0.02 (NI)	0.16±0.01 (22)	0.11±0.01*(52)
	400	0.30±0.02 (NI)	0.12±0.01 (46)	0.09±0.01*(61)
Indomethacin	5	0.31±0.02	0.16 ±0.01 (22)	0.09±0.01*(61)

*P<0.05 vs control (ANOVA; Dunnet post hoc test). Values are mean ±SEM (*n*= *6*) while those in parenthesis represent percent inhibition of edema; NI= no inhibition.

Table 4. Effect of extract of *Pyrenacanthia staudtii* on cotton pellet granuloma

Treatment	Dose (mg/kg)	Cotton pellet granuloma Weight of Granuloma (mg)	Inhibition (%)
Control (2%Tween80)	10	186.67±1.21	-
Extract	100	171.34±1.24	8.0
	200	145.52±2.02	22*
	400	128.15±2.24*	31*
Prednisolone	5	92.3±1.65*	50.6*

*P< 0.05 vs. Control. Values of granuloma weight shown are mean±S.E.M (n =6)

In chronic inflammation (CFA-induced arthritis), the extract significantly (P<0.05) inhibited edematous response in a dose-related manner, provoking an inhibitory effect (61%, maximum inhibition), 400 mg/kg on day 21 same as non-steroidal anti-inflammatory drug, indomethacin (5 mg/kg) (Table 3).

However, in the cotton pellet granuloma test, PSE was not much effective as standard anti-inflammatory steroid drug (prednisolone) in inhibiting the growth of granuloma tissue (Table 4).

DISCUSSION

The LD50 value of 2,150± 80 mg/kg p.o., obtained for PSE in the mice used for acute toxicity study probably suggests that the plant's is relatively safe in, or non-toxic to mice. However results of the present laboratory animal study indicate that Pyrenacanthia staudtii leaf aqueous extract (PSE) possesses anti-inflammatory activity in rodents.

The egg albumin-induced edema peaked at 1 h and progressively decreased with time, while on the other

hand; agar-induced edema produced a progressive increase in paw edema with time. The inhibition by the extract (PSE) of the early acute phase of edema produced by egg albumin and the agar-induced progressive increase in edema suggests that the extract may suppress both the early and later phases of the acute inflammatory response. In acute inflammation there is liberation or release of various chemical mediators known as proinflammatory mediators, such as histamine, 5-HT, kinins and prostanoids mediating acute inflammation-induced phlogistic agents (17,18,19,20,21), likely also involved in both egg albumin and agar-induced edema. The results of the present laboratory animal study showed that the aqueous leaf extract of Pyrenacanthia staudtii inhibited the edema produced by egg albumin and agar-induced acute inflammation. It is therefore not unlikely that the extract may exhibit its action through inhibition of these proinflammatory mediators.

In arthritis there are modified macrophages arranged in small clusters or nodular collections or surrounded by a cuff of lymphocytes (22). This event is a consequence of cell mediated immunity (23). However, these activated macrophages at the inflammatory or injury site are known to secrete peptide growth factors, which partly mediate the process of healing and repair (22). The present laboratory animal study showed that the aqueous leaf extract of Pyrenacanthia staudtii Engl. (Icacinaceae) caused reduction in edema produced by CFA-induced arthritis. This implies that the extract (PSE) may promote tissue repair by the prohealing actions of secretions of these cells especially as tissue necrosis is known to aggravate the latter stage of arthritis (13).

This necrotic tissue produces a factor known as tumor necrotic factor (TNF), which on its own can perpetuate inflammation through several mechanisms, such as release of mediators from dead tissues or dying passenger's leukocytes (24).

Thus in addition to the modulating arthritis event through mediators' release inhibition, the extract may prevent CFA-induced arthritis and its consequences of its tissue damage seen in arthritis. Although the exact chemical component/ or components responsible for the anti-inflammatory activity of PSE could not be established in this study, a number of investigators have shown that tannins, saponins and alkaloids possess anti-inflammatory property in various experimental animal models (25,26,27).

Pyrenacanthia staudtii Engl. (Icacinaceae) has been reported to contain saponins alkaloids (5), and in addition to the presence of tannins revealed in our phytochemical analysis in this study. It is not therefore unreasonable to speculate that these chemical compounds could have contributed to the observed anti-inflammatory effect of the plant's leaf aqueous extract. However, further studies are required to clarify this speculation.

In conclusion, the experimental evidence obtained in the present laboratory animal study indicates that Pyrenacanthia staudtii Engl. (Icacinaceae) leaf aqueous extract (PSE) possesses anti-inflammatory activity. These findings lend pharmacological support to the ethnomedical uses of Pyrenacanthia staudtii as natural remedy in the treatment, management and/or control of various inflammatory conditions in some rural communities of Nigeria.

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