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RESEARCH ARTICLE

PHARMACOLOGICAL ASSESSMENT OF THE ANTINOCICEPTIVE AND ANTI-INFLAMMATORY EFFECTS OF THE METHANOL LEAF EXTRACT AND FRACTIONS OF *LUPINUS ARBOREUS*

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ABSTRACT

Objective: To investigate the antinociceptive and anti-inflammatory potentials of the leaf extract and fractions of Lupinus arboreus in rodents.
Methods: The methanol extract (ME) and fractions of Lupinus arboreus leaf were studied using acetic acid-induced (writhing reflex) pain, pressure-induced (rat tail immersion) pain, thermally-induced (hot plate) pain as well as formaldehyde- and egg albumin-induced rat paw oedema for antinociceptive and anti-inflammatory studies respectively. Acute toxicity and lethality (LD50) test and phytochemical analysis were also carried out.
Results: The extract (30 and 60 mg/kg i.p) exhibited a dose-related significant ($p<0.05$) antinociceptive activity in mice. At 60mg/kg, Hexane fraction (HEF) and Ethylacetate fraction (EAF) exhibited significant ($p<0.05$) pain inhibition of 73 and 64 % respectively, while methanol extract fraction (MEF) produced non significant ($p>0.05$) 24 % pain inhibition. In both albumin (acute) and formaldehyde (chronic) induced oedema in rats, the extract (30, 60 mg/kg i.p) produced a significant ($p<0.05$) dose-related inhibition. Similarly, HEF and EAF at 60 mg/kg produced a significant ($p<0.05$) oedema inhibition. The MEF (60 mg/kg) showed no significant ($p>0.05$) effect. The intraperitoneal acute toxicity test revealed LD50 of 77.4mg/kg, while the phytochemical studies showed the presence of steroids, terpenes, flavonoids, glycosides, saponins, alkaloids, tannins, resins, protein and reducing sugar.
Conclusion: The results demonstrated that the leaves of L. arboreus possess antinociceptive and anti inflammatory effects in rodents.

INTRODUCTION

Lupinus arboreus Sims (Fabaceae), is a bushy shrub up to six feet (1.8 m) tall, with bright yellow sweet-smelling flowers blended with purple and white colours. Also known as yellow bush (Pickart *et al.,* 1998), *L. arboreus* is called "Chikadoma" in igbo, south-eastern Nigeria. It occurs as an invasive species in Northern California coastal dunes (Wear, 1998) but in Nigeria, it is planted widely as ornamental plant. The ancients employed lupine medicinally against deformities of the skin, scabies, ulcers, scald heads and other cutaneous distempers (Pliny, 2009). *L*- asparaginase from developing seeds of *L. arboreus* is known to catalyse the formation of the neuroactive amino acid L- aspartate by deamination of asparagines. The major pathophysiological significance of L-asparaginase activity is in its use for the treatment of acute lymphatic leukemia and neoplasias (Lough, 1992).

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In South-eastern Nigeria, decoction of leaves of *L. arboreus* is being used in the ethnomedical management of pain and inflammation. The concept of pain in its widest sense encompasses both perception and sensation of feeling of discomfort (Wheeler *et al.,* 2004); while inflammation depicts redness and swelling with heat and pain (Ringler, 1997).

Most drugs available for the treatment of pain and inflammation, for instance, non steroidal anti-inflammatory drugs (NSAIDs), are associated with serious adverse effects such as gastric ulceration. Hence, plants and many of their derivatives are being used as natural remedies (Akah *et al.,* 2002). In a previous work, intraperitoneal LD_{50} of 77.45 mg/kg and antimicrobial activity of the methanol leaf extract of *Lupinus arboreus* were reported (Ohadoma *et al.,* 2014). This study was aimed at establishing the antinociceptive and anti-inflammatory effects of the methanol leaf extract and fractions of *L. arboreus* in rodents.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *L. arboreus* were collected from Owerri Imo State, Nigeria. The Plant was authenticated by Osuala, F.N. of Pharmacognosy Department, Madonna University, Elele, Nigeria. A voucher specimen of the leaf was deposited at the herbarium.

Preparation of extracts and fractions

The leaves were air-dried at room temperature for 28 days and pulverized into fine powder. The powdered leaf (2 kg) was extracted with absolute methanol (Sigma Aldrich, Germany) by cold maceration for 48 hours. The mixture was filtered to obtain the methanol extract, which was evaporated using a rotary evaporator (RV 05 Basic IB, IKA, Staufen, Germany) and the concentrated methanol extract stored in a refrigerator. Using silica gel column chromatography, dried methanol extract (10 g) was partitioned to yield hexane fraction (HEF), ethylacetate fraction (EAF) and methanol fraction (MEF).

Animals

Healthy adult albino mice and Wister rats of both sexes weighing 20-32g and 200-320g respectively were used in this study. The animals were obtained from the animal house department of pharmacology and toxicology, Madonna University, Elele, Nigeria. The animals were maintained under standard laboratory conditions and had free access to water and standard pellets (Guinea Feeds Plc, Nigeria). The animals on transfer to work area were allowed two weeks of acclimatization.

Phytochemical tests

The Phytochemical tests were carried out using standard methods (Trease and Evans, 1989; Harbone, 1988).

Antinociceptive activity

Acetic acid-induced (writhing reflex) pain

Mice of either sex were randomly divided into 4 groups of six each. Distilled water was given to one group serving as the negative control. Another group received diclofenac (50 mg/kg) as positive control. The other two groups received two doses of *L. arboreus* extract 30 and 60 mg/kg i.p respectively. Thirty minutes later, 0.01% of acetic acid was injected. The writhing movement (abdominal contraction) was observed and the number recorded for 15 minutes, starting from 5 minutes after injection of acetic acid. The percentage inhibition of writhing movement relative to the control animals was then calculated (Oriowo, 1982; Otimenyin, 2004).

Pressure-induced (rat tail immersion) pain

The animals prior to the antinociceptive experiment, were screened for sensitivity test by immersing the tip of their tails into hot water maintained at 55 ^{0}c. The rats that lifted the tail within 5 seconds were selected for this study on 4 groups of 6 rats each.

The rats were then treated thus:

Group 1 - Pentazocin (0.5 mg/kg, i.p)
Group 2 - Extract (30 mg/kg, i.p)
Group 3 - Extract (60 mg/kg, ip)
Group 4 - Normal saline

The reaction time which is the time taken to lift tail was measured at 15, 30, 45, 60, 75 and 90 minutes [12].

Thermally-induced (hot plate) pain

Hot plate test (Janssen and Jageneau, 1957) was performed on 4 groups of 5 mice each. Mice were treated intraperitoneally with 2 different doses (30, 60 mg/kg) of extract. A negative control group received distilled water (0.3 ml); while the positive control group received diclofenac (50 mg/kg). The animals were placed on a hot plate maintained at 55 $\pm$ 0.5 ^{0}c. Licking paws or jumping which indicate latency or discomfort reaction, was measured for each mouse just prior to extract administration, and later at 30, 60 and 90 minutes after administration.

Anti inflammatory activity

Egg albumin induced (acute) oedema

The rat pedal oedema method (Akah and Njike, 1990) was employed in studying the anti inflammatory effect of the extract and fractions. Wistar rats weighing 200-320g of both sexes were grouped with five animals in each groups. The phylogistic agent (egg albumin 0.01 ml) was injected into the subplantar surface of the hind paw 30 minutes after treatment was administered. Groups 1 and 2 received 30 and 60 mg/kg i.p respectively of methanol extract; HEF, EAF, and MEF were given (60 mg/kg, i.p) to respective groups.

Groups 3 and 4 received piroxicam (0.5 mg/kg, i.p) or aspirin (100 mg/kg i.p) and normal saline (5 ml/kg i.p) respectively. Anti-inflammatory effect was evaluated by the effect of different treatments on albumin-induced inflammation by measuring changes in volume of water displaced by the inflamed hind paw over time. From the value obtained from measuring changes in volume of water displaced, percentage inhibitions of inflammation were calculated as follows:

$$\% \text{ inhibition} = \frac{(V_0 - V_1)}{V_0} \times 100$$

Where,

V_1 = volume of oedema at corresponding of time, and
V_0 = volume of oedema of vehicle (control) treated rats at the same time.

Formaldehyde-induced (Chronic) oedema

Adult Wister rats (n = 5, per group) of both sexes weighing 200-320 g were used in this study[15] receiving 30 or 60 mg/kg i.p. Day 1, after 1 hour of administration, inflammation was induced by subplanta injection of 0.1 ml of 2.5 % formaldehyde solution and repeated on day 3.

Inflammation was assessed by measuring the rats paw volume by water displacement before the induction of inflammation and once daily for 10 days, starting from day 1, after induction of inflammation. Drug administered was continued once daily for the first 5 days and once every other day for the next 5 days. Control animals received either i.p administration of piroxicam (0.5 mg/kg) or indomethacin (5 mg/kg) or equivalent volume of vehicle (10 % Tween 80). The percentage inhibition was calculated thus:

$$\% \text{ inhibition} = \frac{(V_0 - V_t)}{V_0} \times 100$$

Where

V_t = volume of oedema at corresponding of time, and
V_0 = volume of oedema of vehicle (control) treated rats at the same time.

Statistical analysis

The results obtained were analyzed using one way Analysis of Variance (ANOVA, SPSS Version 13) and expressed as mean ± SEM. Difference between means were regarded significant at $P<0.05$ and post -hoc tests were then performed using the Dunnel test.

RESULTS

Phytochemical Constituents

The phytochemical studies showed that methanol extract had the abundance of saponins, glycosides, steroids, terpenes and flavonoids. Resins, protein and reducing sugar occurred in moderate amounts, while alkaloids appeared but in trace amount. Hexane Fraction (HEF) contained steroids and terpenes. Ethylacetate Fraction (EAF), flavonoids and glycosides. While Methanol Fraction (MEF) contained tannins, saponins and glycosides (Table 1).

Table 1. Phytochemical constituents of leaf extract and fractions

Phytochemical constituents	Extract (12.5 %w/w)	HEF	EAF	MEF
Saponins	+++			+
Glycosides	+++	+++	+++	+
Flavonoids	+++		+++	
Steroids	+++	+++		
Terpenes	+++			
Tannins	++			+
Resins	++			
Protein	++			
Reducing sugar	++			
Alkaloids	+			

Value in parenthesis is the extractive yield
+++ = Abundantly present,
++ = moderately present,
+ = present in trace amount.

Acetic acid-induced (writhing reflex) test

The methanol extract on acetic acid-induced writhing test indicated that it possessed dose-related antinociceptive activity with 71.13 % inhibition at 60 mg/kg, while 47.80 % inhibition at 30 mg/kg.

The fractions HEF, EAF and MEF showed significant ($p<0.05$) inhibition of 73, 64 and 24 % respectively (Table 2).

Pressure-induced (rat tail immersion) test

The extract (30 and 60 mg/kg, i.p) significantly ($p<0.05$) produced analgesic effect in rats. This effect was time-dependent but non-dose dependent. The effect was observed to be more at 45 to 60 minutes (Table 3).

Thermally-induced (hot plate) test

The methanol extract (30 and 60 mg/kg, i.p) exhibited dose-dependent resistance against thermal pain and significant ($p<0.01$) inhibition of pain as it prolonged the stay of rats on the hot plate (Table 4).

Egg albumin- induced (acute) inflammatory test

The methanol extract (30 and 60 mg/kg, i.p) showed a significant ($p<0.05$) dose- dependent inhibition of egg albumin-induced oedema over a period of 4 hours. HEF and EAF at 60 mg/kg i.p, showed significant ($p<0.01$) and ($P<0.05$) inhibition of oedema at 4 hours. The MEF (60 mg/kg) showed non significant ($p>0.05$) oedema inhibition (Table 5).

Formaldehyde-induced (chronic) inflammatory test

The methanol extract (30 and 60 mg/kg) inhibited significantly ($P<0.05$) the oedematous response to formaldehyde-induced arthritis. The inhibition was dose-dependent. HEF and EAF at 60 mg/kg i.p, significantly ($P<0.05$) inhibited the oedematous response to formaldehyde-induced arthritis (Table 6).

DISCUSSION

The results generated in this study showed that methanol extract and fractions of *L. arboreus* possess antinociceptive and anti-inflammatory effects.

The albumin-induced and formaldehyde-induced oedema tests were to cater for both acute and chronic inflammatory assessment respectively. The acetic acid-induced writhing test also known as abdominal constriction test can detect antinociceptive compounds at doses that may be inactive with other processes such as tail flick method (Collier *et al.,* 1968).

Table 2. Effect of extract and fraction of *L. arboreus* on acetic acid-induced pain

Treatment (Mg/kg, i.p)	N/group	No of writhes	%inhibition
Distilled	8	49.23±5.0	-
Diclofenac (50)	6	18.12± 3.7*	63
Extract (30)	6	25.70± 3.2*	48
Extract (60)	6	14.12±3.7**	71
HEF (60)	6	13.12±3.2**	73
EAF(60)	6	17.70±3.5*	64
MEF (60)	6	37.65±3.1	24

*p<0.05, **p<0.01, N/group = number per group (given), values different from the negative control.

Table 3. Effect of extract of *L. arboreus* on pressure-induced pain

Treatment (mg/kg, i.p)	Latency time (minutes)					
	15	30	45	60	75	90
Pentazocin (0.5)	3.00±0.58	7.33±0.66	6.67±0.33*	6.33±0.66*	4.00±0.58	2.67±0.33
Extract (30)	3.67±0.67	2.33±0.33	5.33±0.67*	5.33±0.88*	4.33±0.33	2.32±0.33
Extract (60)	2.33±0.33	4.67±0.23	5.67±1.20*	5.0±1.00*	3.33±0.33	2.66±0.33
Normal saline	2.33±0.33	2.67±0.30	2.33±0.30	2.33±0.30	2.33±0.30	2.33±0.33

* p<0.05, n=6, values significantly higher than negative control and comparable to pentazocin.

Table 4. Effect of extract of *L. arboreus* on thermally-induced (hot plate) pain

Treatment (mg/kg, i.p)	Reaction time after treatment			
	0	30	60	90
Distilled water (0.3ml)	5.60±0.35	4.01±0.31	3.70±0.31	2.07±0.31
Extract (30)	5.66±0.41	8.54±0.09*	8.86±0.89*	8.11±0.14*
Extract (60)	5.95±0.28	9.03±1.09*	9.31±0.41*	9.64±0.25**
Diclofenac (50)	5.56±0.61	6.58±0.89	6.12±0.67	7.12±0.62

*p<0.05, **p<0.01, values are mean± SEM, n = 5, zero minute = reaction time prior/before treatment, values significantly higher than the negative control and diclofenac.

Positive analgesic was considered when animals fail to respond to painful stimulus for a period corresponding to the pretreatment reaction time (PTRT) plus 4 seconds (Woolfe and Macdonald, 1964). The abdominal constriction method involves the local peritoneal receptors (Bentley, 1983), hot plate test is a specific central antinociceptive test (Parkhouse and Pleuvry, 1969; Ramezani *et al.,* 2001). The technique of tail immersion test was employed to detect central analgesic activity of drugs and the participation of the spinal mechanism (Sanchez-Blazques and Garson, 1989). Relatively higher proportion of μ-opioid receptor densities exist in the brain than the elevated sigma opioid receptor concentration in the spinal cord (Mansour *et al.,* 1993). Phytochemical investigations indicated among others, the presence of steroids, flavonoids, glycosides and saponins which are substances reported to exert potent anti inflammatory and analgesic properties (Ahmadiani *et al.,* 1998; Ahmadiani *et al.,* 2000). Steroids inhibit the activity of phospholipase A_2 activity and flavonoids are potent prostaglandins inhibitors as well as inhibitors of phosphodiesterases (Manthey *et al.,* 2001). Prostaglandins are pro-inflammatory signaling molecules and phosphodiesterases are involved in cell activation, much of whose effect is on the biosynthesis of protein cytokines that mediate adhesion of circulating leucocytes to site of injury (Ohadoma, 2008). From this study, the leaf extract and fractions (save MEF) exhibited both acute and chronic anti inflammatory effect, as well as showed analgesic activity against hot plate, writhing and tail immersion tests which was indicative that the extract and fractions participate in both spinal and supraspinal mechanisms.

Table 5. Effect of extract and fractions of *L. arboreus* on egg albumin-induced oedema

Treatment (Mg/kg, i.p)	Mean oedema (ml) (mean±*SEM*) at					
	0 h	0.5 h	1 h	2 h	3 h	4 h
Extract (30)	0.82±0.02	1.30±0.01 (25.40)	1.13±0.02 (50.80)	1.05±0.09 (63.50)	0.95±0.10 (72.40*)	0.91±0.07 (81.10*)
Extract (60)	0.79±0.06	1.15±0.05 (42.90)	1.05±0.05 (58.80)	0.98±0.03 (69.90)	0.88±0.03 (80.90*)	0.83±0.05 (91.50**)
Piroxicam (0.5)	0.91±0.03	1.50±0.04 (06.40)	1.35±0.07 (30.20)	1.08±0.05 (73.10)	1.08±0.05 (73.80)	0.98±0.05 (85.10)
Normal saline (0.5 ml)	0.70±0.03	1.33±0.02	1.33±0.02	1.33±0.02	1.17±0.03	1.17±0.03
HEF (60)	_	_	0.24±0.07 (72)	0.19±0.06 (78)	0.16±0.06 (78)	0.16±0.08 (79)
EAF (60)	_	_	0.64±0.09 (27)	0.52±0.07 (42)	0.44±0.09 (40)	0.45±0.07 (40)
MEF (60)	_	_	0.78±0.08 (11)	0.74±0.09 (17)	0.65±0.08 (12)	0.64±0.13 (14)
Aspirin (100)	_	_	0.78±0.10 (11)	0.55±0.12 (38)	0.40±0.12 (45)	0.45±0.13 (40)
Normal saline (0.4 ml)	_	_	0.88±0.04	0.90±0.06	0.74±0.09	0.75±0.08

h= time in hours, *p<0.05, **p<0.01, n = 5, values significantly different from the negative control. ( ) parenthesis = % inhibition.

Table 6. Effect of extract and fractions of *L. arboreus* on formaldehyde-induced oedema

Treatment (mg/kg, i.p)	Mean oedema (ml) (mean±SEM) at					
	0 h	0.5 h	1 h	2 h	3 h	4 h
Extract (30)	0.82±0.04	0.47±0.05 (40)	0.34±0.03* (62)	0.36±0.05* (61)	0.32±0.80* (65)	0.31±0.13* (68)
Extract (60)	0.78±0.05	0.45±0.04 (43)	0.32±0.06* (64)	0.33±0.06* (65)	0.31±0.16* (80.9*)	0.30±0.03* (91.5**)
Piroxicam (0.5)	0.89±0.06	0.48±0.05 (39)	0.30±0.03* (67)	0.28±0.05 (69)	0.29±0.40 (69)	0.26±0.05 (73)
Normal saline (0.5 ml)	0.71±0.02	0.79±0.01	0.91±0.02	0.93±0.02	0.93±0.09	0.96±0.08
MEF (60)	_	_	0.14±0.06** (83.7)	0.13±0.05** (85.3)	0.13±0.05** (84.5)	0.12±0.04** (85.7)
EAF (60)	_	_	0.78±0.10 (9.3)	0.64±0.11 (28)	0.62±0.11 (26)	0.30±0.03* (64.2)
MEF (60)	_	_	0.79±0.03 (10.4)	0.74±0.04 (16.8)	0.77±0.06 (8.3)	0.76±0.05 (4.7)
Piroxicam (0.5)	_	_	0.77±0.02 (10.4)	0.6±0.06 (31.9)	0.51±0.02* (39.2)	0.20±0.02* (76.1)
Normal saline (0.4ml)	_	_	0.86±0.04	0.89±0.04	0.84±0.09	0.84±0.07

*p<0.05, **p<0.01, n = 5, values significantly different from the negative control. () parenthesis = % inhibition.

Thermal pain is mediated via the supraspinal level, the activation of the μ-opioid receptors mediate antinociceptive effects, the spinal and supraspinal mechanisms of pain are mediated by μ and sigma-opioid receptors (Campos *et al.,* 2002; Sha *et al.,* 1994) Non-narcotic analgesics which are peripherally active are distinguished from narcotic (centrally active) analgesics by the ineffectiveness of non-narcotics in the prolongation of the stay time of rodents on the hot plate (Turner, 1965). *L. arboreus* was effective in the protection of experimental animals against peripherally and centrally induced pain. It significantly ($p<0.05$) inhibited acetic acid-induced writhing in mice and prolonged the stay time of rats on the hot plate. It also significantly ($p<0.05$) inhibited both albumin-induced and formaldehyde-induced oedema, indicating that the leaf extract and fractions possess anti-inflammatory properties.

Conclusion

We have established in this study that the methanol leaf extract, hexane and ethylacetate fractions of *Lupinus arboreus* possess antinociceptive and anti-inflammatory effects. The constituents of the fractions mainly steroids, glycosides and flavonoids may not be unrelated to the observed effects. Work is going on in our laboratory to isolate and characterize the active principle(s) responsible for these effects which might serve as a lead compound for development of agents that could be used in the management of some specific pain and inflammatory health conditions.

Conflict of interest

We declare that we have no conflict of interest.

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