Analgesic And Anti-Inflammatory Effects Of *Allium Ascalonicum*

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Abstract

The methanol and aqueous extract of *Allium ascalonicom* were investigated for analgesic and anti-inflammatory properties. Thermal and chemical models of pain assessment were used while albumin was used to induce inflammation. The extracts were administered at doses of 50, 100 and 200 mg/kg.

The methanol extract produced analgesic activity at all the doses tested by reducing significantly ($P< 0.05$) the early and the late phases of formalin induced paw licking in rats, while the aqueous extract reduced the early and late phases of paw licking at a dose of 200mg/kg and only the early phase at a dose of 100mg/kg. In the thermally induced pain, both the methanol and aqueous extract showed significant ($P< 0.05$) inhibition only at a dose of 200 mg/kg for temperatures of 45^{o}C and 50^{o}C but no significant inhibition at 55^{o}C and 60^{o}C. Both the methanol and aqueous extracts significantly ($P< 0.05$) exhibited dose dependent inhibition of albumin-induced paw oedema in rats (at 3 hours post treatment with the extracts).

In conclusion this study has shown that the aqueous and methanol extracts of *Allium ascalonicum* have mild analgesic activity and strong anti-inflammatory activities.

Keywords: *Allium ascalonicum*; anti-inflammatory activity; analgesic activity; oedema

Introduction

Allium ascalonicum is a common plant everywhere but it is widely used in tropical countries[1]. The plant is mildly aromatic and of the lily family. It is a hardy and bulbous perennial plant that is closely related to common onion and garlic. Its leaves are short, small, cylindrical and hollow. It is commonly called spring onions or shallots[2].

This plant as well as others in the family of *Allium* has been linked to the treatment of several ailments including jaundice, gonorrhea, cholera, atherosclerosis, essential hypertension, chest pain, and asthma and wound healing among others[3]. They are also used as flavours in cooked foods as well as a source of Vitamin C[1].Recently we evaluated the haematological effects of the plant and our report indicated that the alcoholic extract induced anaemia in rats[4]. There are oral reports from traditional medical practitioners that the plant is used along with the leaves of B. *coletricha* for the treatment of inflammatory and migrating pain in the South West region of Nigeria. This study, therefore, has been undertaken to investigate the analgesic and anti-inflammatory activities of this plant.

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Materials and Methods

Plant Materials

Allium ascalonicum plant was bought from a vegetable farm opposite the Lagos University Teaching Hospital in January 2001 and was identified by Prof. (Mrs.) S. Mabadeje of the Botany Department University of Lagos and brought to University of Ilorin where a voucher specimen was kept.

Preparation of Extracts

The air-dried plants were reduced to a thick paste, and 800 g of the paste was divided into two parts of 400 g each, each part was exhaustively extracted with distilled water and methanol by maceration. The solvent was removed at about 90 and 40^{o}C respectively to give a dry extract of 10g and 8.5 g for methanol and distilled water respectively. Dilutions of the extracts were further made in saline for pharmacological studies.

Animals

Albino rats of either sex weighing 150 – 200 g were used. They were bought from the animal house of the Lagos University Teaching Hospital and brought to the laboratory of the department of Physiology and Biochemistry, University of Ilorin. The animals were fed with mouse cubes bought from Bendel feeds, Yoruba road, Ilorin . The feed and water were provided to the animals ad libitum. The rats were divided into fifteen groups comprising five animals each. Five groups each were used for the anti- inflammatory, formalin and tail immersion tests. In each tests the groups were ; control, 50mg/Kg, 100 mg/Kg, 200 mg/Kg and the reference group (indomethacin ,5 mg/Kg). All the animals were fasted for 12 hours prior to drug, extract or saline administration.

Anti-inflammatory activity

Acute inflammation was induced by injection of 0.1 ml of fresh egg albumin into the subplantar aponeurosis of the right hind - paw of the rats according to the methods of Muko and Ohiri[5]. Oedema was then assessed for 3 hours at 1-hour intervals in the albumin-injected paw. The aqueous and methanolic extracts were administered orally at the doses of 50, 100 and 200mg/kg.body weight. The reference and control groups received 5 mg/kg indomethacin and 10 ml/kg of normal saline respectively. Measurement of paw size was carried out by wrapping a piece of cotton thread round the paw and measuring the circumference with a meter rule[6,7]. Inhibitory activity was calculated according to this formula[4,8].

Percentage inhibition =

$$\frac{(C_1 - C_0)Treated - (C_1 - C_0)Control}{(C_1 - C_0)control} \times 100$$

Where C_1 = paw circumference at time 3 h

C_o = paw circumference before carrageenan injection

$C_1 - C_o$ = Oedema

Inhibitory activities at 3 hours were taken as a measurement of oedema.

Tail immersion

The tail immersion model described by Sewell and Spencer [9] was used. An hour after the oral administration of the extracts, the reference drug and the normal saline, the tail of the animals were immersed in water baths of varying temperature (45°C, 50°C, 55°C and 60°C). The time spent by each of the rats before flicking or removing their tail from the water at 45°C was recorded. This was repeated when the water bath temperature was increased to 50 °C, 55 °C, and 60 °C. The average of the time spent by each group before the animals flicked or removed their tails from the water bath was determined and this was expressed as Mean Immersion Duration (MID). Extract and reference drug analgesic activities were expressed as follows[10].

% Inhibition =

$$\frac{(Latency)Treated - (Latency)Control}{(Latency)control} \times 100$$

Latency = MID

Formalin - induced pain

This was according to the method of Hunskaar and Hole [11]. 100ml of 3% formalin was injected into the dorsal surface of the left hind - paw of the animals 1 hour after the oral administration of 50, 100 and 200 mg/kg of extract. The control animals received 10 ml/kg of saline while 5 mg/kg of indomethacin was administered to the animals in the reference group. The time spent by each of the animals in licking the injected paw (licking time) was noted and the mean for each group was determined. The animals were observed for the first 5 (i.e. 0-5) minutes post formalin (early phase) or for 10 min starting from the 20th minute (i.e. 20-30) post formalin injection (late phase).

Statistical analysis

The results are expressed as mean ± SEM. Statistical significance was determined using the student t-test according to the method of Neville and Kennedy (12). Values with $P < 0.05$ were considered significant.

Results

Albumin Induced Paw oedema

Albumin-induced rat paw oedema was markedly inhibited by oral pre-treatment with the methanol, aqueous extract

Table 1: Effects of Aqueous and Methanol Extract of *Allium ascalonicum* on Fresh Egg Albumin Induced Paw Oedema in Rats[a]

Groups	Dose (mg/kg) orally	Paw Size (mm) At 3hrs	Inhibition (%)
Control (saline)	-	10.05±0.64	-
aqueous extract			
Allium ascalonicum	50	6.0 ± 0.71*	40.5
Allium ascalonicum	100	6.25 ± 0.25+	37.5
Allium ascalonicum	200	3.25 ± 0.63+	68.0
methanol extract			
Allium ascalonicum	50	5.25 ± 0.75*	48.0
Allium ascalonicum	100	3.25 ± 0.10*	68.0
Allium ascalonicum	200	1.50 ± 0.29+	85.5
Indomethacin	5	5.25 ± 0.31+	48.0

Each value is the mean S.E.M of 5 rats *P <0.05, +P<0.05 compared with control: students t-test

Table 2: Effect of Aqueous and Methanol Exact of *Allium ascalonicum* on formalin induced paw licking in rats

Groups		LICKING	
	Dose (mg/	Early Phase	Late Phase
Control	-	39 ± 1.1	23.5 ± 0.65
Aqueous Extract			
A. ascalonicum	50	36.5 ± 1.19*	22.25 ± 0.85
A. ascalonicum	100	35.25 ± 0.25*	21.25 ± 0.96
A. ascalonicum	200	27.0 ± 1.08*	12.0 ± 0.71*
Methanol Extract			
A. ascalonicum	50	27.5 ± 1.19*	13.0 ± 0.71*
A. ascalonicum	100	21.0 ± 0.91*	11.0 ± 0.91*
A. ascalonicum	200	17.25 ± 0.85*	8.25 ± 0.86*
Indomethacin	5	12.5 ± 1.04*	7.25 ± 0.85*

Each value is the mean ± S.E.M of 5 rats * P<0.05 compared with control: Student's t-test

Table 3: Effect of Methanol Extract of *Allium ascalonicum* on thermally induced pain

Group	Saline	A. ascaloni-cum	A. ascaloni-cum	A. ascaloni-cum	Indometha-cin
Dose orally (mg/kg)	-	50	100	200	5
45°C (MID)	9.5 ± 0.5	10.5 ± 0.5	13.0 ± 1.1	23.25 ± 1.2*	18.67 ± 1.3*
% Protection	-	10.53	36.84	144	74.30
50°C (MID)	3.5 ± 0.35	4.13 ± 0.32	4.25 ± 0.48	4.75 ± 0.75*	4.88 ± 0.13*
% Protection	-	3.85	7.69	30.77	74.30
55°C (MID)	3.5 ± 0.35	4.13 ± 0.32	4.25 ± 0.48	4.75 ± 0.75	4.88 ± 0.13*
% Protection	-	18	21.43	35.71	39.43
60°C (MID)	2.38 ± 0.13	2.63 ± 0.24	2.75 ± 0.25	2.63 ± 0.24	3.0 ±0.0*
% Protection	-	10.50	15.55	10.50	26.05

MID = Mean Immersion Duration (Secs). Each value is the mean ± SEM of 5 rats
*** P<0.05 compared with control student t-test**

Table 4: Effect of Aqueous Extract of *Allum ascalonicum* on ther-

Groups	Saline	A. ascalonicum	Dose orally (mg/kg) A. ascalonicum	A. ascalonicum	Indomethacin
	-	50	100	200	5
45°C (MID)	9.5 ± 0.5	9.63 ± 0.13	10.75 ± 0.63	12.5 ± 0.5*	18.67 ± 1.3*
% Protection	-	1.36	13.16	31.58	96.52
50°C (MID)	6.5 ± 0.35	6.5 ± 0.20	6.63 ± 0.13	7.63 ± 0.13*	11.33 ± 0.67*
% Protection	-	0.0	2	17.38	74.30
55°C (MID)	3.5 ± 0.35	3.5 ± 0.20	3.63 ± 0.24	3.75 ± 0.25	4.88 ± 0.13*
% Protection	-	0.0	3.71	7.14	39.43
60°C (MID)	2.38 ±	2.5 ± 0.20	2.5 ± 0.20	2.75 ± 0.14	3.0 ± 0.0*
% Protection	-	5.04	5.04	15.55	26.05

MID = Mean Immersion Duration (Secs). Each value is the mean ±SEM of 5 rats * P<0.05 compared with control students t-test

and indomethacin. The aqueous extract inhibited the paw oedema from 10.05 ± 0.64 -3.25 ± 0.63mm while the methanol extract inhibited the paw oedema from 10.05 ± 0.64 - 1.50 ± 0.29 mm. Inhibition of paw oedema by the methanol extract was dose dependent with the highest inhibition at the dose of 200 mg/kg (Table 1).

Formalin – Induced pain

The aqueous extract of Allium ascalonicum only inhibited the early phase of the formalin test except the 200mg/Kg dose that inhibited the late phase. The methanol extract on the other hand inhibited the two phases of the test. The aqueous extract inhibited the licking time from 39.0 ± 1.1-17.3 ± 0.85 while the methanol extract inhibited the licking time from39.0 ± 1.1 - 27.0 ± 1.08 (Table. 2)

Tail immersion

The results of the tail immersion model of analgesia are shown in Table 3 and 4. The result show that only the 200mg/Kg dose of the extract significantly ($P < 0.05$) inhibited the thermally induced pain at 45°C and 50°C .

While indomethacin significantly ($P < 0.05$), inhibited thermally induced pain at all the tested water bath temperature.

Discussion

In the present study, the analgesic activity of methanol and aqueous extract of *Allium ascalonicum* was established. Both the methanol and aqueous extracts were found to significantly inhibit thermally induced pain of not more than 50°C and at a dose of 200 mg/kg. Indomethacin on the other hand was found to significantly inhibit thermally induced pain at all the water-bath temperature considered [Tables 3 & 4]. The trend of the result obtained from the tail immersion model show that only the highest dose (200 mg/kg) produced significant analgesia and the analgesia produced was observed at water bath Temperatures of 45 and50. The implication of this findings is that the extract (both aqueous and methanol) has weak inhibitory activity against this model. It is not unusual to find such responses because agents/extracts that acts peripherally like Non steroidal anti- inflammatory drugs do not show strong activity with this test [13-14]. The Tail immersion model is an effective method of inducing pain thermally [9-10]. It is used together with the hot plate method in detecting strong analgesics [13]. The aqueous extract only significantly inhibited paw licking in the formalin test except for the highest dose (200mg/Kg). This indicates that the aqueous extract has strong inhibitory activity on non-inflammatory pain, since the early phase represents non – inflammatory pain while the late phase of the test represents inflammatory pain [11,15]. The fact that the 200mg/Kg dose of the extract inhibited the late phase of this test may be due to an increase in the concentration of the active ingredients in the 200mg/Kg dose compared with those of the lower doses because the observed analgesic activity in the late phase show a form of dose dependent pattern from 50mg/Kg to 200mg/Kg but the activity only became significant after the administration of the highest dose (200mg/Kg). The methanol extract on the other hand significantly inhibited both phases of the test showing that the methanol extract has stronger analgesic activities than the aqueous extract as revealed by the formalin test. The difference in the activities of the two extracts may be due in part to the differences in the number or concentration of active compounds in the two extracts. Although in the present study we did not conduct a Phytochemical analysis to determine the actual active ingredients, however information from the literature[1] show that alcoholic extract (e.g methanolic extract) of *A. ascalonicum* contain an active ingredient that was not present in water extracts. The active ingredient was simply named an 'anaemic factor'. Therefore, it is on this basis that we presume that the differences in the activity of the extracts may be due to differences in their chemical composition. We hope that further studies using bioguided fractionation will provide quantitative and qualitative information about the active ingredients of the plant.

The anti-inflammatory activities of methanol and aqueous extract of *Allium ascalonicum* was established by the inhibition of albumin-induced paw oedema. This model has been used previously to induce paw oedema[5]. A progressive increase in rat paw circumference was observed in the control rat after the injection of phlogistic agent; maximum swelling was observed after approximately 1 hour [,6,16]

Both the methanol and aqueous extracts show relatively good anti-inflammatory activities when compared with the control. However, the percentage inhibition produced by various doses of methanol extract was higher at all doses tested than those of the aqueous extract Table 4. This trend was equally found in the analgesic test and it may be due to differences or the increase in concentration of the constituents of methanol extract compared to the aqueous extract as stated earlier.

Traditional uses of *Allium ascalonicum* have been found to range from curing infections to healing wound. These uses indirectly relate to analgesic and anti-inflammatory activities. The analgesic and anti-inflammatory activities observed in this study may be due to the presence of some active constituents of *Allium ascalonicum,* prominent among which are flavonoids and vitamin C[1]. The flavonoids play a role in restoring abnormal capillary permeability to normal; they also help to minimize the effect of other mediators of inflammation[1]. In conclusion, this study has shown that extract of *Allium ascalonicum* possesses mild analgesic and strong anti-inflammatory activities; thus justifying the folkloric uses of this plant in the treatment of many ailments including pain.

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