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Membrane stabilizing activity : A possible mechanism of action for the anti-inflammatory property of *Gongronema latifolium* leaves

Olugbenga Morebise*[1], Michael A. Fafunso[2], Janet M. Makinde[3]

[1]Department of Biochemistry, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

[2]Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria

[3]Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria

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ABSTRACT: The methanolic extract of the dried leaves of Gongronema latifolium (100-400mg/kg, i.p.) produced a significant ($p<0.05$) inhibition of carrageenan and nystatin-induced rat paw oedema. It also significantly ($p<0.05$) stabilized erythrocyte membrane subjected to heat- and hypotonic solution-induced lyses. The anti-inflammatory activity of the extract may be due to its membrane-stabilizing action.

Keywords: Gongronema latifolium; Anti-inflammatory activity; Carrageenan; Nystatin; Membrane stabilizing action

Introduction

Gongronema latifolium Benth. (family Asclepiadaceae) is a climber found in the forest areas of Nigeria and other West African countries (1,2). The leaves are used as vegetables in the preperation of soups to which they add a bitter-sweet flavour (3,4), and in trado-medical treatment of malaria, nausea, and anorexia, especially among the Ikale people in the South-west of Nigeria (3-6).

Saponins and flavonoids have been detected in the leaves of G.latifolium and antimicrobial and phytotoxic activities of the crude saponins reported by Morebise and Fafunso (5). In our previous anti-inflammatory study on the leaves of G.latifolium, we reported that the aqeous extract exhibited strong anti-inflammatory activity by inhibiting the carrageenan-induced rat paw oedema, the acetic acid-induced vascular permeability in mice, and the carrageenan-induced leukocyte migration in rats (6).

The present study was undertaken in an attempt to investigate further the possible mechanisms of the anti-inflammatory activity of G.latifolium leaves.

*To whom correspondence should be addressed.
E-mail address: gbengamorebise@yahoo.co.uk

Materials and Methods

Plant Materials

Fresh plant material (leaves and stems) was collected at Ibadan, Nigeria. It was identified in the herbarium, Department of Botany and Microbiology, University of Ibadan, where voucher specimens were deposited.

Preparation of Extract

The air-dried powdered leaves (315g) was subjected to Soxhlet extraction for 24 hours, using methanol as the extracting solvent. The methanol was evaporated under reduced pressure to give a yield of 18.5%. The extract was dissolved in 2.5% Tween-80 in normal saline and used for the study.

Animals

Male Wistar rats (140-180g), kept at room temperature in a well-ventilated holding case and fed on normal laboratory diet (Ladokun Feeds Ltd., Ibadan) and water ad libitum were used for this study.

Effect of Extract on carrageenan-induced rat paw oedema

The method of Winter et.al (7) was used. The extract (100-400mg/kg) or indomethacin (5mg/kg) were administered intraperitoneally to rats. Animals in the control groups received 2ml of of 2.5% Tween-80 in saline. Thirty minutes later, oedema was induced in the right hind paws by injection of 0.1ml of 1% carrageenan into the subplantar region of the paw. Measurement of paw size was done by wrapping a piece of cotton thread round the paw of each rat and measuring the circumference on a metre rule (8).Measurement was done immediately before (0h) and at intervals of one hour for five hours, after carrageenan injection. Inhibitory activity was expressed as the percentage inhibition of oedema at 3h in the drug-treated groups compared with the control.

Effect of Extract on Nystatin-induced rat paw oedema

The method of Shinde et al. (9) was followed. The extract (100-400mg/kg) or dexamethasone (0.09mg/kg) were administered to rats intraperitoneally. 2mls of 2.5% Tween-80 in normal saline were administered to control animals. Thirty minutes later, rats were injected with 0.1ml of nystatin (100,000U) into the subcutaneous tissue as an 8.5% suspension in normal saline. The hind paw diameters were measured at 0h (before injection of nystatin) and at 1, 2, 3 and 24h after injection of nystatin. The inhibitory activity of the extract at the different hours of measurement was expressed as the percentage inhibition of oedema in the drug-treated groups compared with the control.

Effect of Extract on heat- and hyposaline-induced haemolysis

The method of Oyedapo et.al. (10) was followed. A 10% human erythrocyte suspension was prepared in normal saline. The reaction medium consisted of 2ml hyposaline (0.25% saline), 1ml of 0.1M sodium phosphate buffer, pH 7.4, and graded volumes of the extract (0.3ml, 0.6ml and 0.9ml of 10mg/ml of extract in Tween-80 in saline). Indomethacin (0.5ml of 10mg/ml of drug, dissolved in Tween-80 in saline) was used as the reference standard. 0.5ml of the 10% erythrocyte suspension was added to each medium. For the control medium, 1ml of 2.5%Tween-80 in saline was used instead of extract (this is control 1) while the drug-control test lacked red blood cells (control2). The mixtures were incubated at 56oC for 30 minutes. The tubes were then cooled under running tap for 20 minutes and centrifuged. The absorbance of the supernatants were read at 560nm. The percentage membrane-stabilizing activity was determined using the equation of Sadique et.al. (11), viz:

$$\% \text{ stabilizing activity} = 100 - \frac{\text{(Drug test value – control 2)}}{\text{control 1}}$$

Statistical analysis

Values are expressed as mean ± S.E.M. Statistical significance was determined using the Student's t-test. Values with $P < 0.05$ were considered significant.

Results and Discussion

The methanolic extract of Gongronema latifolium leaves at 100-400mg/kg, i.p., significantly inhibited the carrageenan-induced rat paw oedema (Table 1). Three hours post-carrageenan, the highest dose of the extract (400mg/kg) inhibited the oedema by 66.7% while indomethacin (5mg/kg, i.p.) gave an inhibition of 77.8%.

Table 1: Effect of *Gongronema latifolium* leaf methanol extract on carrageenan-induced rat paw oedema.

Group	Dose (mg/kg i.p.)	Paw circumference at 0h (cm ± S.E.)*	Paw circumference at 3h (cm ± S.E.)*	Inhibition (%)
Control	2.5% Tween-80 in saline	2.0 ± 0.09	2.9 ± 0.04	–
G. latifolium	100	2.0 ± 0.03	2.6 ± 0.04a	33.3
	200	2.1 ± 0.07	2.5 ± 0.07a	55.6
	400	2.1 ± 0.06	2.4 ± 0.01a	66.7
Indomethacin	5	2.1 ± 0.01	2.3 ± 0.02a	77.8

*Values are mean ± SEM (n = 5)

[a]$P < 0.05$ compared with control; Student's t-test.

The carrageenan-induced rat paw oedema model is the basic model for screening agents with anti-inflammatory activity (7,8). The development of oedema in the paw of the rat after the injection of carrageenan has been attributed to the release of mediators of inflammation, such as the prostaglandins (12,13). It is possible that the extract inhibited prostaglandin synthesis.

The extract (100-400mg/kg,i.p.) significantly inhibited the nystatin-induced rat paw oedema at 1, 2, 3 and 24h post-nystatin (Table 2). The extract at 400mg/kg gave a superior inhibitory activity of 87% at 24h post-nystatin when compared with the standard drug, dexamethasone (0.09mg/kg, i.p.), which gave an inhibitory value of 78.3% (Table 2).

Table 2: Effect of *Gongronema latifolium* leaf methanol extract on nystatin-induced rat paw oedema.

Group	Dose (mg/kg i.p.)	Percentage inhibition			
		1h	2h	3h	24h
Gongronema latifolium	100	41.1a	43.4a	55.6a	56.5a
	200	46.4a	56.6a	63.9a	78.0a
	400	62.3a	64.3a	74.5a	87.0a
Dexamethasone	0.09	43.4a	58.3a	73.2a	78.3a

[a]$P < 0.05$ compared with control; Student's t-test (n = 5).

Nystatin, a polyene antibiotic, induces oedema by its membrane-labilizing action, thereby releasing hydrolytic enzymes which play an important role in promoting inflammation.(9). Inhibition of the nystatin-induced oedema therefore suggests a possible stabilizing action of anti-inflammatory agents, including G.latifolium leaves, on the lysosomal membranes (9).

As a complement to the lysosomal membrane-stabilizing effect of the extract, its effect on the erythrocyte membrane was investigated. The extract (3, 6 and 9mg) significantly stabilized the erythrocyte membrane subjected to heat-and hyposaline-induced haemolysis (Table 3). The effects were very close to that of the standard drug, indomethacin (5mg) (Table 3).

Table 3: Effect of *Gongronema latifolium* leaf methanol extract on heat- and hypotonic solution-induced rat paw oedema.

Group	Absorbance (Mean ± S.E.)*	% Stabilization
Control 1	1.160 ± 0.066	–
Control 2	0.117 ± 0.009	–
G. latifolium (0.3 ml; 3 mg)	0.509 ± 0.060a	66.2
G. latifolium (0.6 ml; 6 mg)	0.398 ± 0.046a	75.8
G. latifolium (0.9 ml; 9 mg)	0.380 ± 0.034a	77.3
Indomethacin (0.5 ml; 5 mg)	0.330 ± 0.027a	81.6

*Values are mean ± SEM (n = 5)

[a]$P < 0.05$ compared with control; Student's t-test.

A possible explanation for the stabilizing effect could be an increase in the surface area / volume ratio of the cells which could be brought about by expansion of membrane or shrinkage of the cell, and an interaction with membrane proteins (14).. Moreover, it has also been reported that the deformability and cell volume of erythrocytes are closely related to the intracellular content of calcium (9). Hence, it could be speculated that the cytoprotective effects of the extract on the erythrocyte membrane might be due to the ability of the extract to alter the influx of calcium into the erythrocytes (9).

Earlier investigations have revealed that various herbal preparations are capable of stabilizing the the red blood cell membrane and exert anti-inflammatory activity (11). Since the membrane of red blood cells is similar structurally to lysosomal membranes, the effects of drugs or extracts on the human red blood cell membrane could be extrapolated to the stabilization of lysosomal membranes. Therefore, the anti-inflammatory activity of the drug or extract may stem from a stabilization of lysosomal membranes (9, 15)..

The present investigation suggests that the lysosomal membrane stabilizing activity of G.latifolium leaves may be playing a significant role in its anti-inflammatory activity.

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