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Report

Effect of Lycoriside, an Acylglucosyloxy Alkaloid, on Mast Cells[1]

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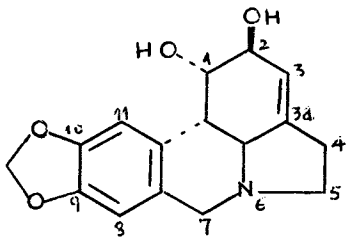
The effect of lycoriside, an acylglucosyloxy alkaloid from *Crinum asiaticum* Linn. (family Amaryllidaceae), with or without sitosterol-3-*O*-β-D-glucoside, was studied on the rate of degranulation of peritoneal mast cells of albino rats. Lycoriside, at lower concentrations (1–20 μg/ml), *in vitro*, produced statistically significant protection against Tween 80-induced degranulation, as also to sensitized mast cells challenged with an antigen (horse serum). It also provided protection against compound 48/80-induced degranulation of mast cells when administered *in vivo* (1–5 mg/kg, po). At higher concentrations (100 μg/ml and above), *in vitro*, however, it had a mast-cell degranulation effect per se. The addition of sitosterol-3-*O*-β-D-glucoside to lycoriside did not modify the effect of the latter compound. The mechanism of the dual response elicited by lycoriside is appraised in view of a concentration-dependent anti- or prorelease effect on mast-cell mediators.

KEY WORDS: *Crinum asiaticum;* amaryllidaceae; lycoriside; acylglycosyloxy alkaloids; mast cell degranulation.

INTRODUCTION

Extracts of flowering bulbs of a number of *Crinum* species, e.g., *C. asiaticum* Linn., *C. augustum* Roxb., and *C. latifolium* Linn. (family Amaryllidaceae), are used in Ayurvedic medicine in the treatment of allergy in its diverse manifestations (1). However, pharmacological screening of these plant extractives has not been conducted before to test the validity of their claimed antiallergic property. Since mast cells are the major source of mediators of allergy and anaphylaxis, it was considered appropriate to study the effect of chemical constituents of *C. asiaticum* on mast-cell activity. Recently, three alkaloidal conjugates, *viz.*, lycoriside [=lycorine-1-*O*-(6′-*O*-palmitoyl)-β-D-glucopyranoside], palmilycorine [=1-*O*-palmitoyllycorine], and 1-*O*-phosphatidyllycorine, together with sitosterol-3-*O*-β-D-glucoside, have been reported as major entities of MeOH extracts of flowering bulbs of *C. asiaticum* (2). Lycoriside has been found to form stable complexes, in solution, with a number of steryl glycosides including sitosterol-3-*O*-β-D-glucoside (3). In the present paper, we report the effect of lycoriside, with or without sitosterol-3-*O*-β-D-glucoside, on rat mesenteric mast cells.



Scheme I. Lycorine.

MATERIALS AND METHODS

Test Compounds. Lycoriside and sitosterol-3-*O*-β-D-glucoside were isolated from *C. asiaticum* Linn. as described before (2). The two compounds were separately dissolved in distilled water. For lycoriside–sitosterol-3-*O*-β-D-glucoside complexes (1:1), the doses were calculated in terms of their combined concentrations (mg or μg/ml).

Animals. Albino rats (100–150 g), of either sex, were used. The rats were sacrificed and the intestinal mesentery was taken. Pieces of the mesentery were incubated with different concentrations of the test compounds, for 15 min at 37°C. The mast cells were stained and examined microscopically according to published procedures (4,5). For *in vitro* studies in sensitized rats, the mesenteric pieces were challenged with horse serum (5%), for 10 min, prior to staining.

Mast-Cell Degranulation. Pieces of mesentery were stained supravitally with toluidine blue (4), rinsed in acetone, and then placed on a microscopic slide, stretched, and examined with a high-powered microscope. One hundred to

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Table I. Effect of Graded Concentrations of Lycoriside on Mast Cells[a]

Group, treatment	Concentration (μg/ml)	N	Percentage degranulation[b]	P
1. Control		10	11.80 ± 1.35	
2. Lycoriside				
(i)	1	8	7.21 ± 1.73	ns
(ii)	5	12	10.71 ± 2.06	ns
(iii)	10	15	12.59 ± 1.81	ns
(iv)	25	9	23.04 ± 2.12	0.2[c]
(v)	50	8	51.55 ± 2.42	<0.001[c]
(vi)	100	10	81.43 ± 3.79	<0.001[c]
3. DCG	50	6	18.23 ± 4.71	
4. DCG + lycoriside	50 + 100	8	36.95 ± 2.55	<0.05[d]

[a] Mesenteric mastocytes of rats *in vitro*.
[b] Values are means ± SE for the (*N*) number of experiments.
[c] Significance in relation to group 1.
[d] Significance in relation to group 2 (vi); ns, not significant.

150 mast cells, using at least 10 such randomly selected fields from each tissue, were counted and the mean percentage of cell degranulation was calculated (5).

Effect of Test Compounds. The effects of graded concentrations of lycoriside, with or without sitosterol-3-*O*-β-D-glucoside, on mesenteric mast cells of rats *in vitro* were investigated. For evaluating the protection provided by the test compounds against Tween 80 (10 μg/ml)-induced degranulation, the tissue was first incubated with lycoriside or the lycoriside–steroid complex, for 10 min; Tween 80 was then added and the tissue was stained after another 10 min. To study the *in vivo* effect, lycoriside or the lycoriside–steroid complex was administered orally (po) 1 hr prior to sacrifice of the animals. The tissue was then processed as before (5). In another set of experiment, the tissue was challenged with compound 48/80 (Sigma Chemical Co.) (2.5 μg/ml), for 10 min, before staining.

Effect in Sensitized Rats. The rats were sensitized by injecting horse serum (0.5 ml, sc) and triple antigen (containing 20,000 million *B. pertussis* organisms) as described before (5). After 2 weeks of sensitization, the rats were sacrificed and the intestinal mesentery was taken for workup.

Effect on the Adrenal Gland and Reticuloendothelial System. The effects of lycoriside (5 mg/kg, ip, day^{-1} for 3 days) and lycoriside–sitosterol-3-*O*-β-D-glucoside (1:1) (5 mg/kg, ip, day^{-1} for 3 days) were studied on the following parameters: (a) weight of adrenal glands, liver, spleen, and thymus; and (b) total and differential leukocyte counts. Untreated control rats were used for comparison.

Toxicity. An acute toxicity (LD_{50}) study was carried out on mice by administering graded doses of lycoriside orally and intraperitoneally.

RESULTS AND DISCUSSION

Lycoriside, at lower concentrations (1–20 μg/ml), *in vitro,* did not produce any detectable excess of degranulation per se. On increasing the concentration (25 μg/ml and above), it produced appreciable degranulation. The degranulation effect of lycoriside was not modified by combining with sitosterol-3-*O*-β-D-glucoside (Table I). Lycoriside (5–20 μg/ml) provided significant protection against Tween 80-induced degranulation of mast cells (Table II). In the *in vivo* experiment, lycoriside (1–5 mg/kg, po) provided significant protection against compound 48/80-induced degranulation (Table III). At higher concentrations (25 mg/kg, po), it caused appreciable degranulation (45.7 ± 3.4) per se. These effects also were not modified by treatment with the lycoriside–steroid (1:1) complex.

In sensitized mast cells, lycoriside (1–10 μg/ml), provided significant protection against the antigen-induced degranulation (Table IV).

Lycoriside and the lycoriside–steroid complex markedly reduced the weights of thymus and adrenal glands. The only perceptible effect of the lycoriside–steroid combination was observed in the marked reduction of the weights of liver and spleen, not shown by lycoriside alone (Table V). Neither lycoriside nor the lycoriside–steroid complex pro-

Table II. Effect of Lycoriside Against Tween 80-Induced Degranulation of Mast Cells

Group, treatment	Concentration (μg/ml)	N	Percentage degranulation ± SE	P
1. Control		6	9.75 ± 1.52	
2. Tween 80	10	6	51.53 ± 2.42	
3. Lycoriside + Tween 80	5 + 10	8	6.47 ± 0.94	<0.001[a]
4. Lycoriside + Tween 80	10 + 10	8	10.10 ± 1.79	<0.001[a]
5. Lycoriside + Tween 80	20 + 10	6	11.44 ± 1.34	<0.001[a]

[a] Significance in relation to group 2.

Table III. Effect of Lycoriside *in Vivo* Against Compound 48/80[a]-Induced Degranulation

Group, treatment	Concentration	*N*	Percentage degranulation ± SE	*P*
1. Compound 48/80	2.5 μg/ml	10	93.8 ± 2.42	
2. Lycoriside + compound 48/80	1 mg/kg + 2.5 μg/ml	10	14.72 ± 0.74	<0.001[b]
3. Lycoriside + compound 48/80	2.5 mg/kg + 2.5 μg/ml	10	11.40 ± 1.32	<0.001[b]
4. Lycoriside + compound 48/80	5 mg/kg + 2.5 μg/ml	10	8.20 ± 0.55	<0.001[b]

[a] Condensation product of *N*-methyl-*p*-methoxyphenethylamine and formaldehyde (histamine releaser; Sigma Chemical Co.).
[b] Significance in relation to group 1.

Table IV. Effect of Lycoriside Against Antigen-Induced Degranulation of Mast Cells

Group, treatment	Concentration (μg/ml)	*N*	% degranulation ± SE	*P*
1. Control		5	12.20 ± 2.45	
2. Antigen		5	57.12 ± 3.53	
3. Lycoriside + antigen	1	6	14.45 ± 3.84	<0.001[a]
	5	6	11.68 ± 2.18	<0.001[a]
	10	6	19.44 ± 3.44	<0.01[a]
4. DCG + antigen	50	6	32.40 ± 3.30	<0.01[a]

[a] Significance in relation to group 2.

Table V. Effect of Lycoriside with or Without Sitosterol-3-*O*-β-D-Glucoside on the Weights of Liver, Spleen, Thymus, and Adrenal Glands of Albino Rats

Tissue	Control (*N* = 7)	Lycoriside (*N* = 15)	Lycoriside + sitosterol-3-*O*-glucoside (*N* = 14)
Liver[a]	6.249 ± 0.345	6.156 ± 0.334	4.997 ± 0.234[c]
Spleen[a]	0.814 ± 0.240	0.980 ± 0.171	0.605 ± 0.072[d]
Thymus[a]	0.565 ± 0.113	0.305 ± 0.02[e]	0.292 ± 0.032[e]
Adrenal glands[b]	41.45 ± 10.5	22.05 ± 4.5[e]	17.26 ± 3.8[e]

[a] g/100 g body weight (bw).
[b] mg/100 g bw.
[c] $P < 0.02$ (in relation to control and lycoriside-treated group.
[d] $P < 0.05$ (in relation to control and lycoriside group).
[e] $P < 0.001$ (in relation to control).

Table VI. Effect of Lycoriside, Acetylcholine, and Atropine on Mast Cells

Group, treatment	Concentration (μg/ml)	*N*	Percentage degranulation ± SE	*P*
1. Control		10	8.20 ± 1.12	
2. Acetylcholine (Ach)	1	10	68.37 ± 2.73	<0.001[a]
3. Atropine	2	10	6.98 ± 1.02	
4. Atropine + Ach	2 + 1	10	23.65 ± 1.78	<0.001[b]
5. Lycoriside	100	10	80.08 ± 3.79	<0.001[a]
6. Lycoriside + atropine	100 + 2	10	21.81 ± 1.99	<0.001[c]

[a] Significance (*P*) in relation to group 1.
[b] Significance (*P*) in relation to group 2.
[c] Significance (*P*) in relation to group 5.

duced any noticeable change in the red-cell and total or differential leukocyte counts or in the histopathology.

No perceptible toxic effect was induced by the different doses of lycoriside (100–500 mg/kg) used. The LD_{50} in mice for this compound was greater than 500 mg/kg upon either ip or po administration in both sexes.

The results indicate that, at lower concentrations (1–10 μg/ml), *in vitro,* lycoriside provides significant mast-cell stabilization. At higher concentrations (25 μg/ml and above), it induces per se degranulation. Similar results were obtained from *in vivo* experiments. The action of lycoriside, however, did not appear to be dose related. Lycoriside, in the lower concentration range, appears to have disodium cromoglycate (DCG)-like action on mast cells, being about 100 times more potent than the latter (Table V). It is also interesting to note that peritoneal strips incubated with DCG (50 μg/ml), followed by treatment with high concentrations of lycoriside, partially resisted the degranulation of lycoriside (Table I). It may be mentioned, however, that the method employed does not distinguish between noncytotoxic degranulations and the toxic disruption of mast cells.

Several independent lines of evidence indicate that increased levels of Ca^{2+} in mast cells can trigger the release of inflammatory mediators (6). Lycoriside, like DCG, forms stable complexes with a number of bivalent metal ions (e.g., Cu^{2+}, Zn^{2+}, Ca^{2+}, Fe^{2+}) (2). It thus seems to inhibit, in the lower concentration range, free extracellular Ca^{2+} uptake by stimulated mast cells. At the higher concentrations, however, lycoriside appears to compete for the intracellular Ca^{2+} (and congener metal ions) and thereby cause cell puncture. Although the mechanism of the dose-dependent dual action of lycoriside could not be entirely elucidated, at this stage, the following additional observations may be of relevance. (i) Acetylcholine (Ach; 1 μg/ml) caused marked degranulation of mast cells *in vitro;* pretreatment of the peritoneal strips with atropine (2 μg/ml) provided significant protection against Ach-induced degranulation (Table VI). (ii) Atropine also provided significant protection against the lycoriside (100 μg/ml)-induced degranulation of mast cells (Table VI). Thus, the degranulation of mast cells by higher concentrations of lycoriside seems to be mediated through muscarinic receptors. The dose-dependent dual effects of lycoriside are similar to those of bioflavonoids, which, at lower concentrations, inhibit the release of histamine in a manner similar to that of DCG, while at higher concentrations, they produce a marked prorelease effect on inflammatory mediators (7,8). Further studies to evaluate the potential of this new class of alkaloidal conjugates as effective antiallergic agents are currently in progress.

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