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Anti-Allergic Effects of Herbal Product from *Allium cepa* (Bulb)

P. Kaiser, M.S. Youssouf, S.A. Tasduq, S. Singh, S.C. Sharma, G.D. Singh, V.K. Gupta, B.D. Gupta, and R.K. Johri

Division of Pharmacology and Natural Products Chemistry, Indian Institute of Integrative Medicine, Jammu-Tawi, India

ABSTRACT *Allium cepa* (Family Liliaceae) is a reputed Indian medicinal herb that is prescribed as an effective remedy for several ailments in the Ayurvedic system of medicine. The aim of this study was to evaluate its efficacy against various events responsible for Type I allergic reactions. A herbal fraction (ALC-02) from *A. cepa* (bulb) inhibited histamine release and attenuated intracellular calcium levels in Compound 48/80-induced rat peritoneal mast cells. It also prevented Compound 48/80-mediated systemic anaphylaxis while lowering histamine levels in plasma. ALC-02 suppressed carrageenan-induced rat paw edema. It inhibited eosinophil peroxidase activity and protein content in bronchoalveolar lavage fluid (BALF) of ovalbumin-challenged mice. In this experiment ALC-02 also caused a substantial reduction in lipid peroxidation in BALF/lung tissue and augmented superoxide dismutase activity in lung tissue. ALC-02 suppressed erythrocytic lysis caused by Triton X-100. A significant quenching of 1,1-diphenyl-2-picrylhydrazyl radical by ALC-02 was observed. The results have shown a promising anti-allergic profile of ALC-02 that could be attributed to its potential antihistaminic, anti-inflammatory, and antioxidant activities.

KEY WORDS: • Allium cepa • *anti-inflammatory* • *antioxidant* • *Compound 48/80* • *histamine* • *mast cell* • *paw edema* • *Type I allergy*

INTRODUCTION

In Ayurveda, an Indian traditional system of medicine, *Allium cepa* (onion) is considered a reputed herbal drug that is used in many forms, including decoction, infusion, fresh juice, raw, cooked, or roasted bulb, for several indications such as fever, dropsy, catarrh, and chronic bronchitis.[1] The Chinese pharmacopoeia mentions the use of onions for treatment of angina pectoris, dyspnea, dysentery, cough, and bronchial conditions.[2] The consumption of onion has been linked to a reduced risk of stomach carcinoma.[3] Some of the pharmacological activities reported for crude extracts of onion bulb are antifungal, antimicrobial, anti-platelet aggregatory, hypoglycemic, anticholesterolemic, antidepressant, antispasmodic, and antihypertensive.[4–14] The oil of onion has been found to possess antitumor activity: it inhibited proliferation of leukemia HL60 cells.[15]

As a part of our ongoing program of identifying medicinal/culinary herbs that can be used as a drug or a source of drug for chronic ailments, a detailed bioactivity profile of *A. cepa* (bulb) was carried out. This paper deals with the attenuation of Type I allergic reactions by an herbal product (ALC-02) derived from onion bulb, as manifested by its antihistaminic, anti-inflammatory, and antioxidant activities.

MATERIALS AND METHODS

Materials

Compound 48/80, ovalbumin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Fura-2 acetoxymethyl ester, nitro blue tetrazolium, 1,1,3,3-tetraethoxypropane (MDA), 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide (MTT), and carrageenan were obtained from Sigma Chemical Co. (St. Louis, MO). Collagenase was purchased from Roche Diagnostics GmbH (Mannheim, Germany). Ketotifen, ibuprofen, and fexofenadine were generic drugs supplied by Micro Labs India (Bangalore, India) and Zydus Cadilla, India (Ahmedabad, India). All other chemicals and biochemicals were high-purity reagent grade.

Animals

Wistar rats (weighing 160–180 g) and BALB/c mice (weighing 22–25 g) were obtained from the animal house of the Institute and kept under regulated environmental conditions (12-hour light/dark cycle; temperature of 25 ± 1°C; humidity 50–60%). Animals were fed with rodent pelleted

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Address reprint requests to: R.K. Johri, Division of Pharmacology and Natural Products Chemistry, Indian Institute of Integrative Medicine (CSIR), Canal Road, Jammu- Tawi 180 001, India, E-mail: johri.rk@gmail.com

diet (Ashirwad Industries, Chandigarh, India) and water *ad libitum*. All experiments were approved by the Institutional Animal Ethics Committee.

Preparation of test materials from A. cepa *(bulb)*

The bulbs of *A. cepa* procured from the southern Indian peninsula (Eastern Ghats) were dried, finely ground, and percolated in 50% ethyl alcohol for 16 hours at room temperature. This procedure was repeated four times, and the pooled extract was dried under reduced pressure (50 ± 1°C) in a rotary evaporator. The extract, designated as ALC (yield 55%), showed a promising antihistaminic activity in a concentration-dependent manner against Compound 48/80-stimulated rat peritoneal mast cells (RPMCs) *in vitro* (Table 1). ALC was further partitioned into chloroform (ALC-01; yield 1.0%), butanol (ALC-02; yield 5.0%) and aqueous (ALC-03; yield 93%) fractions. All these fractions were evaluated *in vitro* for antihistaminic activity, which was found to be located mainly in ALC-02 (Table 1).

High-performance liquid chromatography (HPLC) determinations

HPLC analysis was done on a Waters (Milford, MA) chromatograph system (model 2996) equipped with a diode array detector, solvent delivery module, online degasser, and an autosampler, using a reversed-phase HPLC column (RP-18, 25 × 4.0 μm, 5 μm particle size). Data analysis was carried out using Empower software (Waters).

Hepatocyte culture

Hepatocytes from rats were isolated by the two-step collagenase perfusion method, and the cell culture was established using cell preparations with a viability of about 85 ± 5% (determined by trypan blue exclusion). These were used to evaluate the effect of test materials on cell viability by MTT assay as described earlier in detail.[16]

Preparation of RPMCs

RPMCs were obtained as described previously.[17] Approximately 95% pure cells with a viability of >97% as determined by toluidine blue staining and trypan blue exclusion, respectively, were used.

Determination of histamine release

RPMCs (2×10^6 cells) were resuspended in a buffer containing 10 m*M* HEPES, 130 m*M* NaCl, 5 m*M* KCl, 1.0 m*M* $MgCl_2$, 1.4 m*M* $CaCl_2$, and 5.6 m*M* glucose and preincu-

Table 1. Effect of ALC and Fractions on Histamine Release in RPMCs

Treatment	*Concentration (μg/assay)*	*Histamine release (μg/2 × 10⁶ cells)*[a]	*Inhibition (%)*[b]
None	—	106 ± 4	—
Compound 48/80	10	215 ± 5	—
+ALC	50	187 ± 7*	25
	100	172 ± 7*	39
	200	162 ± 4**	48
	400	148 ± 8**	61
+ALC-01	0.5	212 ± 6[NS]	3
	1.0	208 ± 5[NS]	6
	2.0	207 ± 7[NS]	7
	4.0	199 ± 8[NS]	14
+ALC-02	2.5	67 ± 3*	44
	5.0	159 ± 2**	51
	10	149 ± 7**	60
	20	138 ± 7**	70
+ALC-03	45	211 ± 3[NS]	3
	90	200 ± 4[NS]	13
	185	194 ± 2[NS]	19
	370	192 ± 3[NS]	20
Ketotifen	4	133 ± 7**	75

RPMCs (2×10^6) were incubated without (*A*) or with Compound 48/80 (10 μg/mL) (*B*). In other incubations (*C*), test materials were also used along with Compound 48/80. ALC and ALC-03 were solubilized in water; ALC-01 and ALC-02 were solubilized in methanol : water (5 : 95 vol/vol).

[a]Data are mean ± SE values from three independent experiments.

[b]Percentage inhibition of liberated histamine = $100 - [(C - A/B - A) \times 100]$.

*$P < .05$, **$P < .001$, [NS]not significant versus control by Student's *t* test.

bated with or without ALC or its fractions for 10 minutes at 37°C. Compound 48/80 (10 μg/mL) was added, and cells were incubated for another 20 minutes. The reaction was stopped by cooling the tubes in ice. Cells were centrifuged (400 *g*, 5 minutes), and supernatant was collected for estimation of histamine content.[17,18]

Antihemolytic activity

Blood was collected from rats and centrifuged (986 *g*) with an equal volume of sterilized Alsver solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride in water) to obtain the erythrocytes. The cells were washed with isosaline (0.85%, pH 7.4) and diluted with phosphate buffer (0.15 *M*, pH 7.4). Erythrocytes (10^8 cells/mL) were incubated in the absence and presence of ALC-02 at 37°C for 20 minutes before adding Triton X-100 (5 mg/mL) and further incubation for 1 hour. The optical density (OD) of the supernatant was determined at 540 nm.[19]

Compound 48/80-induced systemic anaphylaxis

ALC-02 was orally administered to the mice 1 hour before Compound 48/80 (8 mg/kg i.p.).[17] Mortality was recorded after the induction of anaphylactic shock. Blood was collected by cardiac puncture for plasma histamine measurement.[18]

Carrageenan-induced paw edema

Rats were given ALC-02 orally 45 minutes before injection of freshly prepared carrageenan (0.1 mL of 10% [wt/vol] in normal saline) in the subplantar region of the left hind paw to produce edema.[20] Increase in paw volume was measured 1, 2, and 3 hours later with the help of a volume differential meter (model 7101, Ugo Basile, Comerio, Italy).

Biochemical assays in mice

BALB/c mice were sensitized with two subcutaneous (0.4-mL) injections of 100 μg of ovalbumin (OVA) mixed with 4 mg/mL $Al(OH)_3$ in 0.9% NaCl, given 7 days apart.[21] One week after the second injection, mice were challenged in both nostrils with 10 μg of OVA in 50 μL of 0.9% NaCl. The challenged animals received ALC-02 orally for 15 days once daily. On day 16, mice were anesthetized, the trachea was cannulated with a 22-gauge needle, and a final volume of 1.0 mL of bronchoalveolar fluid (BALF) was collected. BALF was centrifuged (400 *g* for 10 minutes), and supernatant was obtained. The lungs of the animals were excised, snap-frozen, and stored at −70°C. Before use, tissues were thawed in ice-cold phosphate buffer (50 m*M*, pH 7.4), blotted, weighed, and homogenized in 10 volumes of the buffer.

Lung homogenates were centrifuged (1,753 *g*, 15 minutes), and supernatant was obtained. In BALF eosinophil peroxidase (EPO) activity, lipid peroxidation (LPO), and protein content were determined; in lung supernatant, LPO and superoxide dismutase (SOD) activity were determined. EPO activity was determined by measuring peroxidative decomposition of H_2O_2 and expressed as change in OD/milligram of protein/minute.[20] LPO was measured as amount of thiobarbituric acid-reactive substances represented by MDA (extinction coefficient, $1.56 \times 10^5\ M^{-1}\ cm^{-1}$). The SOD activity was determined by monitoring the rate of nitro blue tetrazolium reduction as described earlier,[22] and results were expressed as specific activity (change in OD/milligram of protein). Protein content was estimated by the method of Lowry *et al*.[23]

DPPH assay

ALC-02 was incubated with DPPH (50 μM) for 30 minutes. The OD of the samples was recorded at 517 nm to assess the extent of decoloration of a methanolic solution of DPPH, which was taken as an index of antiradical activity.[24]

Measurement of intracellular calcium concentration ($[Ca^{2+}]_i$)

RPMCs were loaded with 3 μM Fura-2 acetoxymethyl ester in phosphate-buffered saline for 30 minutes at 37°C. Cells were washed twice with phosphate-buffered saline to remove the external dye, and approximately 10^7 cells/mL was incubated for another 10 minutes at 37°C in calcium-free phosphate-buffered saline without or with ALC-02. Cell suspensions were further incubated for 1 minute in the presence of Compound 48/80 (0.1 μg/mL). Fluorescent measurements were carried out at alternate excitation wavelengths of 340 nm and 380 nm, with an emission wavelength of 510 nm. The cells were stirred continuously with a magnetic stirrer throughout the experiment. $[Ca^{2+}]_i$ was calculated by the ratio method using the following equation: $[Ca^{2+}]_i = K_D(R - R_{min})/(R - R_{max}) \times Sf_2/Sb_2$, where K_D is the dissociation constant of Fura-2 for Ca^{2+} (224 n*M* at

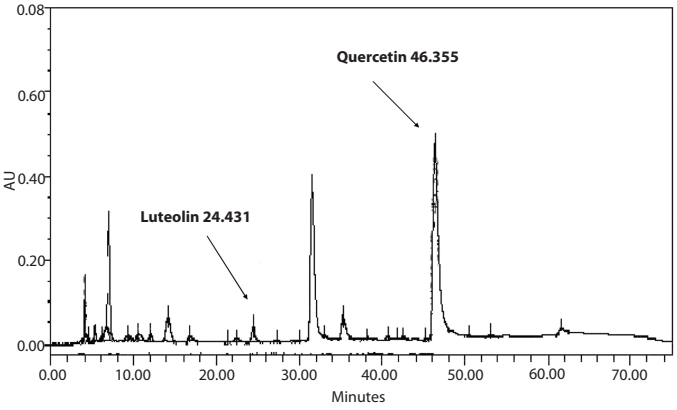

FIG. 1. HPLC chromatogram of ALC-02. The mobile phase consisted of acetonitrile:1.5% acetic acid in water (17:83 vol/vol). It was filtered under vacuum through a membrane filter (pore size 0.45 μm) before use. The flow rate was adjusted to 1 mL/minute; the detection wavelength was 254 nm, and column oven temperature was 30°C. AU, arbitrary units.

TABLE 2. QUANTITATIVE ANALYSIS OF ALC-02

	% of luteolin					*% of quercetin*				
	Batch 1	*Batch 2*	*Batch 3*	*Mean*	*SD*	*Batch 1*	*Batch 2*	*Batch 3*	*Mean*	*SD*
ALC	0.013	0.015	0.012	0.013	0.001	0.07	0.081	0.064	0.071	0.008
ALC-02	0.62	0.79	0.56	0.65	0.119	6.9	6.0	8.01	6.97	1.00

37°C), R represents the ratio of fluorescence of the intracellular indicator at the two excitation wavelengths, R_{max} represents the maximum fluorescence ratio obtained after addition of Compound 48/80, R_{min} represents the minimum fluorescence ratio obtained after chelating all the intracellular Ca^{2+} with EGTA, and Sf_2/Sb_2 represent the proportionality constant for the fluorescence of Ca^{2+}-free and Ca^{2+}-bound forms of the indicator at 380 nm, respectively.[25]

Statistical analysis

Statistical analysis was performed using Student's *t* test.

RESULTS

Marker identification

An HPLC-derived fingerprint profile of ALC-02 is shown in Figure 1. Chemical standardization of this particular active fraction (ALC-02) was done on the basis of two constituents: quercetin (6.97%) and luteolin (0.65%) (Table 2 and Fig. 1).

Cell viability

Cytoxicities of ALC, in the range of 50–400 μg, and ALC-02, in the range of 2.5–20 μg (based upon the extractive yield from the parent extract), were evaluated. No cytotoxicity was observed as determined by the MTT assay in a primary monolayer culture of rat hepatocytes (data not shown).

Antihistaminic effect of ALC-02

The effect of test materials on Compound 48/80-induced histamine release from RPMCs is shown in Table 1. The spontaneous release of histamine was 106 ± 4.1 μg/10^6 cells, which increased by twofold in the presence of Compound 48/80. ALC (50–400 μg) prevented histamine release in the range of 25–61%. Fractions were tested at a concentration that corresponded to their extractive yield from the parent extract (ALC). Fraction ALC-02 (2.5–20 μg) produced a concentration-dependent inhibitory effect that ranged from 44% to 70%. On the other hand, fractions ALC-01 and ALC-03 exhibited no significant effects. Ketotifen (4 μg) produced a 75% inhibitory effect.

Effect of ALC-02 on Triton X-100-induced hemolysis of erythrocytes

Results are depicted in Figure 2. ALC-02 prevented the hemolysis of erythrocytes induced by Triton X-100 (5 μg). A concentration range of 2.5–80 μg of ALC-02 dose-dependently inhibited hemolysis by 10–59%.

Effect of ALC-02 on Compound 48/80-induced systemic anaphylaxis

Results are depicted in Figure 3. Treatment with ALC-02 dose-dependently decreased mortality induced by Compound 48/80 (8 mg/kg). At a dose of 450 mg/kg, a 100% protective effect was observed. The spontaneous release of histamine in plasma of untreated animals was 22 μg/mg of protein, which increased by 2.8-fold after treatment with Compound 48/80. ALC-02 caused a significant reduction in plasma histamine levels in a dose-dependent manner: maximum inhibition (80%) was obtained at a dose of 450 mg/kg.

Effect of ALC-02 on carrageenan-induced edema in rats

Results are summarized in Table 3. A dose of 200 mg/kg of ALC-02 produced a 34% reduction in paw volume at 1 hour, which gradually declined during the next 2–3 hours. In contrast, ibuprofen (100 mg/kg) showed low (13%) inhi-

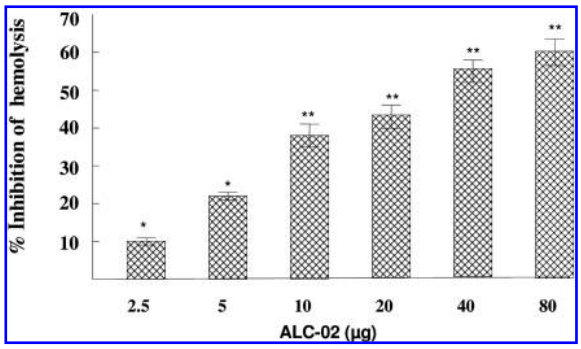

FIG. 2. Antihemolytic activity of ALC-02 in erythrocytes. Erythrocytes (10^8) were incubated (*A*) without or (*B*) with Triton X-100 (5 mg/mL). In other incubations (*C*), varying concentrations of ALC-02 (solubilized in methanol:water, 5:95 vol/vol) were used along with Triton X-100. Data are mean ± SE values from three independent experiments and were calculated as follows: $100 - [(C - A/B - A) \times 100]$. $^*P < .05$, $^{**}P < .001$ versus control by Student's *t* test.

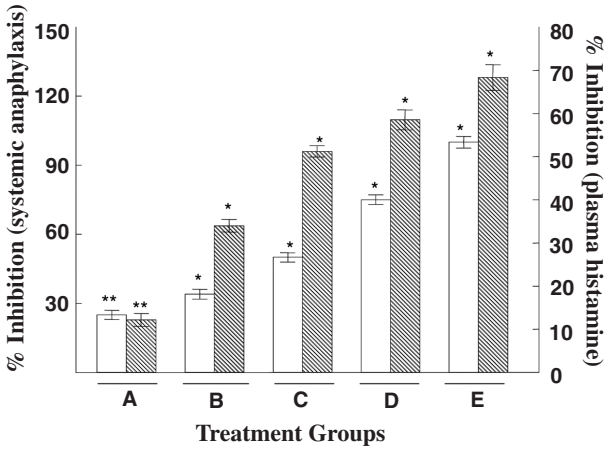

FIG. 3. Effect of ALC-02 on Compound 48/80-induced systemic anaphylaxis in mice. Forty-eight mice were divided into six groups ($n = 8$ per group). All animals received Compound 48/80 (8 mg/kg, i.p.). Group A served as the control. Mice in Groups B–E also received 50, 150, 300, and 450 mg/kg ALC-02, respectively. Test materials were solubilized in a vehicle of methanol:water (5:95 vol/vol). Data for systemic anaphylaxis are mean ± SE values ($n = 8$; open column) show percentage inhibition of mortality compared with control (Group A). Data for plasma histamine are mean ± SE values ($n = 8$; hatched column) show percentage inhibition of histamine release compared with control (Group A). $^*P < .001$, $^{**}P < .05$ versus control (Group A) by Student's t test.

bition of paw volume at 1 hour, which increased by 38–41% during the next 2–3 hours.

Effect of ALC-02 on biochemical parameters in BALF and lung supernatant in mice

Results are summarized in Table 4. In BALF of sensitized mice the specific activity of EPO was 0.99 ± 0.2 ΔOD/mg of protein/minute, which was increased by 2.46-fold after OVA challenge. ALC-02 showed a dose-dependent inhibitory effect: at doses of 50, 100, and 200 mg/kg, the activity was reduced by 41%, 99%, and 120%, respectively. Fexofenadine (9 mg/kg) produced 145% suppression.

In BALF of sensitized mice, protein content was 3.73 ± 0.66 mg/mL, which was increased by 1.99-fold after OVA challenge. ALC-02 showed a dose-dependent inhibitory effect: at doses of 50, 100, and 200 mg/kg, the protein levels were reduced by 40%, 56%, and 68%, respectively. Fexofenadine (9 mg/kg) produced 70% suppression.

In BALF of sensitized mice, the LPO level was 3.07 ± 0.4 nmol of MDA/mg of protein, which was increased by 3.32-fold after OVA challenge. ALC-02 showed a dose-dependent inhibitory effect: at doses of 50, 100, and 200 mg/kg, the MDA levels were reduced by 41%, 74%, and 78%, respectively. Fexofenadine (9 mg/kg) produced 99% suppression.

In lung supernatant of sensitized mice the LPO level was 5.00 ± 0.3 nmol of MDA/mg of protein, which was increased by 1.92-fold after OVA challenge. ALC-02 showed a dose-dependent inhibitory effect: at doses of 50, 100, and 200 mg/kg, the MDA levels were reduced by 15%, 36%, and 53%, respectively. Fexofenadine (9 mg/kg) produced 77% suppression.

In lung supernatant of sensitized mice SOD activity was 5.67 ± 0.3 ΔOD/mg of protein/minute, which was decreased by 1.54-fold after OVA challenge. ALC-02 showed a dose-dependent stimulation: at doses of 50, 100, and 200 mg/kg, SOD activity was increased by 44%, 86%, and 240%, respectively. Fexofenadine (9 mg/kg) produced a 46% increase.

DPPH radical scavenging activity

Results are depicted in Figure 4. ALC-02 (2.5–60 μg) exhibited 19–84% antiradical activity. Ascorbic acid (100 μM) showed a 95% scavenging effect.

Effect of ALC-02 on $[Ca^{2+}]_i$

Changes in intracellular calcium levels are depicted in Figure 5. $[Ca^{2+}]_i$ in control RPMCs was 41.6 nM, which was increased to 70.7 nM in the presence of Compound 48/80. ALC-02 (2.5–60 μg) caused a concentration-dependent inhibition of $[Ca^{2+}]_i$ in the range of 10–56%.

TABLE 3. EFFECT OF ALC-02 ON CARAGEENAN-INDUCED PAW EDEMA IN RATS

Treatment group	*Dose (mg/kg)*	*Paw volume (mL)*		
		1st hour	*2nd hour*	*3rd hour*
A (carrageenan)	10	0.46 ± 0.02	0.90 ± 0.05	1.10 ± 0.04
B (+ALC-02)	50	0.45 ± 0.02 (1%)	0.85 ± 0.02 (6%)	0.93 ± 0.02 (16%)
C (+ALC-02)	100	0.38 ± 0.08 (16%)	0.70 ± 0.04 (22%)*	0.93 ± 0.04 (16%)
D (+ALC-02)	200	0.30 ± 0.02 (35%)**	0.68 ± 0.07 (25%)*	0.88 ± 0.08 (20%)*
E (+ibuprofen)	100	0.40 ± 0.02 (13%)	0.65 ± 0.04 (27%)**	0.65 ± 0.04 (41%)**

A total of 30 rats were used (six per group). Animals in Groups A–E received carrageenan (10 mg/kg). In addition, animals in Groups B–D also received, respectively, 50, 100, and 200 mg/kg ALC-02 (dissolved in methanol : water, 5 : 95 vol/vol). Group E animals received ibuprofen (100 mg/kg). Data are mean ± SE values ($n = 5$). Numbers in parentheses are percentage inhibition versus Group A.

$^*P < .05$, $^{**}P < .01$ versus Group A.

TABLE 4. EFFECT OF ALC-02 ON BIOCHEMICAL PARAMETERS IN BALF AND LUNG SUPERNATANT FROM MICE

Treatment group	Dose (mg/kg)	BALF			Lung supernatant	
		EPOC[a]	Protein[b]	LPO[c]	LPO[c]	SOD[a]
A (OVA-sensitized)	—	0.99 ± 0.2	3.73 ±0.6	3.07 ±0.4	5.04 ±0.3	5.67 ±0.3
B (OVA-challenged)	—	2.44 ± 0.3*	7.45 ± 0.5**	10.21 ± 0.5*	9.61 ± 06*	3.66 ± 0.2*
C (+ALC-02)	50	1.83 ±0.3 (41%)†	5.96 ±0.5 (40%)†	7.31 ±0.4 (41%)†	8.92 ±0.6 (15%)[NS]	4.56 ±0.2 (44%)‡
D (+ALC-02)	100	1.06 ±0.2 (99%)‡	5.37 ±0.4 (56%)‡	4.91 ±0.3 (74%)§	7.95 ±0.5 (36%)†	5.40 ±0.5 (86%)§
E (+ALC-02)	200	0.70 ±0.1 (120%)§	4.92 ±0.5 (68%)§	4.62 ±0.3 (78%)§	7.19 ±0.6 (53%)§	8.49 ±0.4 (240%)§
F (+fexofenadine)	9	0.34 ±0.1 (145%)§	4.87 ±0.2 (70%)§	3.11 ±0.3 (99%)§	6.12 ±0.6 (77%)§	4.65 ±0.3 (46%)†

OVA-sensitized mice were divided into six groups ($n = 6$ mice per group). Group A consisted of sensitized mice. Groups B–F represent OVA-challenged mice. Groups A and B received vehicle only. Groups C–E received 50, 100, and 200 mg/kg ALC-02, respectively. Group F mice received fexofenadine (9 mg/kg). Test materials were solubilized in methanol:water (5:95 vol/vol) and administered perorally for 16 days once daily. At the end of the experiment, BALF and lung supernatant were collected for biochemical assays. Data are mean ± SE values ($n = 6$ per group). Numbers in parentheses are percentage restoration calculated as follows: 100 − [Group C, D, E, or F value − Group A value/Group B value − Group A value] × 100].

[a]Specific activity (ΔOD/mg of protein/minute).

[b]In mg/mL.

[c]In nmol of MDA/mg of protein.

*$P < .001$, **$P < .01$ versus Group a; †$P < .05$, ‡$P < .01$, §$P < .001$, [NS]not significant versus Group B by Student's t test.

DISCUSSION

Mast cells are the critical participants in various allergic and inflammatory reactions. These are the secretory cells that, upon activation, cause the process of degranulation, which results in the release of histamine and other mediators.[26] Compound 48/80, used in the present study, is widely used as an experimental mast cell degranulator. It destabilizes the cell membrane via modification of membrane permeability of the lipid bilayer in a nonlytic manner.[27] Our results revealed a potential mast cell-stabilizing property of ALC-02. It was found to inhibit the Compound 48/80-induced histamine burst from RPMCs, and its action was comparable to that of ketotifen. Ketotifen is a known mast cell stabilizer that inhibits histamine release in a nonimmunologic manner.[28] In another experiment ALC-02 was found to prevent Triton X-100-induced hemolytic damage of erythrocytes. Triton X-100 is a nonionic surfactant that permeabilizes the cell membrane resulting in lysis of erythrocytes.[29] From these results it may be inferred that ALC-02 might interact directly with the lipid bilayer membrane to prevent degranulation of the mast cells by producing a stabilizing effect on the membrane integrity.

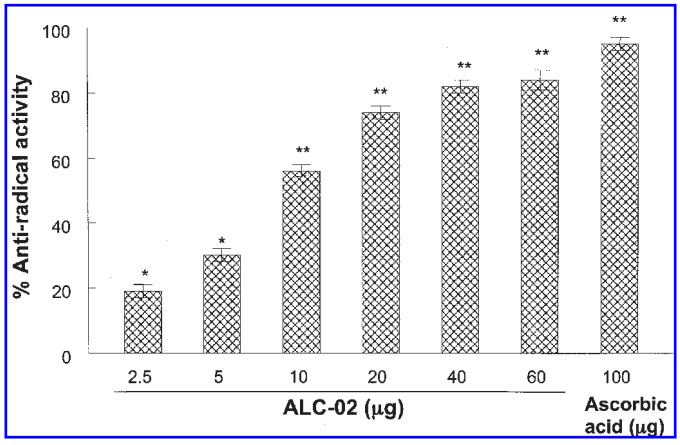

FIG. 4. Antiradical activity of ALC-02 (DPPH assay). A methanolic solution of DPPH (50 μM) was incubated (A) without or (B) with various concentrations of test materials (solubilized in methanol), and the OD was determined. Data are mean ± SE values from three independent experiments and were calculated as follows: $(A - B/A) \times 100$. *$P < .05$, **$P < .001$ versus control by Student's t test.

Mast cells play a central role in the pharmacological episode of anaphylaxis via histamine action.[30] Protection against systemic anaphylaxis by ALC-02, therefore, could be due to its inhibitory effect on the synthesis or release of histamine. This was further reinforced by the observation in the same experiment that histamine levels were found to remain significantly low in plasma of mice that had received Compound 48/80 treatment together with ALC-02.

Histamine is causally related with the inflammatory response.[27] To explore whether ALC-02 possessed anti-inflammatory action and whether this could be related to its antihistaminic action, the effect of ALC-02 was evaluated against carrageenan-induced paw edema, which is generally employed for the screening of putative anti-inflammatory agents. In this phlogistic bioassay the initial phase of the inflammatory response is caused by histamine and serotonin-like mediators, whereas the late phase is dominated by prostaglandins and related substances.[31] In this model ALC-02 produced a significant anti-edematogenic effect in the early phase. In comparison, ibuprofen's action was more pronounced during the late phase. Ibuprofen, a nonsteroidal anti-inflammatory drug, exerts its anti-inflammatory effect by inhibiting synthesis and release of prostaglandins.[32] These finding indicate that an anti-histaminic property could be responsible for the anti-inflammatory effect of ALC-02.

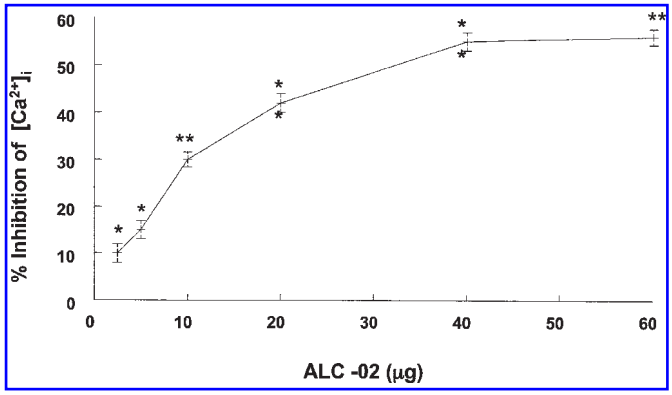

FIG. 5. Effect of ALC-02 on intracellular calcium levels in RPMCs. RPMCs (2×10^6) were incubated (*A*) without or (*B*) with Compound 48/80 (10 μg/mL). In other incubations (*C*) varying concentrations of ALC-02 (solubilized in methanol:water, 5:95 vol/vol) were used together with Compound 48/80. At the end of the experiment $[Ca^{2+}]_i$ was measured. Data are mean ± SE values from three independent experiments and were calculated as follows: $100 - [(C - A/B - A) \times 100]$. $^*P < .05$, $^{**}P < .001$ versus control by Student's *t* test.

The evidence supporting antihistaminic and anti-inflammatory activities of ALC-02 led us to examine the possible role this herbal moiety that might play in attenuating the allergic reaction. Allergy as manifested in Type I hypersensitivity is associated with a pulmonary inflammatory response.[33] This was evaluated in OVA-sensitized mice that, upon further challenge intranasally with the antigen, are reported to develop pulmonary eosinophilic inflammation.[34] Eosinophils have a pivotal role in causing airway allergic inflammation, and its granular constituent EPO has been suggested to play an important role via oxidative stress.[35] The results showed that in OVA-challenged animals that had received ALC-02 treatment, EPO activity remained inhibited. In addition, the same treatment was also able to lower the levels of lipid peroxides (in BALF and lung tissue) with a concomitant increase in SOD activity (lung tissue). Lipid peroxidation is a major consequence of oxidative stress by excessive generation of reactive oxygen species and is known to participate in the etiology of many organ and tissue pathologies. SOD is an enzyme involved in the disposal of toxic reactive oxygen species.[36] In this context, considerable evidence supports a critical role of oxidative stress in pathogenesis of airway inflammation,[37] and its reversal by agents, such as ALC-02, could therefore be via suppression of excess reactive oxygen species generation. These findings provide evidence for a potential antiperoxidative activity of ALC-02, possibly due to its antiradical activity as shown in the DPPH assay. DPPH and peroxyl radicals have similar electron arrangements (the unpaired electron is delocalized over both N atoms of the hydrazyl, and both O atoms are peroxyl). Relative reactivities of antioxidants with DPPH generally show the same order as with lipid peroxyl radicals.[38]

An increased oxidative stress resulting in elevated activities of lipid peroxides is known to enhance vascular permeability.[39] The present results show that protein exudation in BALF of OVA-challenged mice remained elevated, possibly as a result of increased vascular permeability, which was inhibited by ALC-02. Since increased vascular permeability has also been positively correlated with enhanced recruitment of eosinophils,[39] the results further confirm the anti-eosinophilic action of ALC-02. These findings were comparable to the effect shown by fexofenadine, which is known to inhibit eosinophilic inflammation.[40] Taken together, these observations highlight one important contribution of the antiperoxidative property of ALC-02 to its anti-inflammatory effects.

In order to gain further insight into the mode of action of ALC-02, the intracellular level of calcium was investigated. Calcium has a crucial role in mast cell degranulation: a rise in intracellular calcium levels results in mast cell activation and histamine release.[17] ALC-02 attenuated calcium levels, possibly by preventing peroxidative injury to the plasma membrane, further highlighting the potential membrane-stabilizing activity of this herbal moiety. A restorative effect on the calcium distribution could contribute significantly to the observed antihistaminic property of ALC-02. Earlier studies have shown that calcium antagonists are inhibitors of histamine-induced airway smooth muscle contraction and release of mediators from lung tissue.[41] ALC-02 has not shown any cytotoxicity as investigated by the MTT assay in primary monolayer cultures of rat hepatocytes. No adverse clinical symptoms or pathologies were recorded in biochemical and histological determinations during or after a subacute (28-day) toxicity study of ALC-02 at doses of 250, 500, and 1000 mg/kg of body weight in rats and mice (data not shown).

In conclusion, ALC-02 has been found to modify various cellular events that are considered responsible for Type I allergic reactions. Currently, there are several categories of drugs that are used to alleviate these symptoms. However, single molecule drug therapies for indications like allergic asthma and rhinitis are increasingly falling short of expectations. They are not only relatively ineffective in alleviation of chronic disorders, but also they produce detrimental side effects with prolonged use. Furthermore, the inaccessibility of such drugs for the general population, especially in developing countries, is an additional driving force behind the search for alternative drug forms.

Traditional preparations derived from medicinal/ culinary herbs are widely used in India and many other regions as effective remedies for a variety of chronic ailments. These have a long traditions of human use and have been found safe. *A. cepa* (bulb) is one such herb, and the present investigation has shown that a chemically standardized herbal fraction (ALC-02) derived from it has a promising therapeutic potential against acute allergic disorders.

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AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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