



Effects of chelidonic acid, a secondary plant metabolite, on mast cell degranulation and adaptive immunity in rats

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ABSTRACT

The present study evaluated the immunomodulatory effects of chelidonic acid, a secondary plant metabolite, with therapeutic potential in allergic disorders, in experimental animals. In mast cell degranulation studies, ovalbumin immunized and challenged rats, chelidonic acid (1, 3 and 10 mg/kg, i.p.) dose relatedly prevented ovalbumin challenge induced mast cell degranulation by differing degrees when compared with vehicle treated group, and these effects were comparable with prednisolone (10 mg/kg). A reduction in post-challenge mortality was also observed in all treated groups. Further, there were reductions in the blood eosinophil counts and serum IgE levels after chelidonic acid treatment. Chelidonic acid also inhibited histamine release from rat peritoneal mast cells (RPMC) in vitro, in a dose related manner. In tests for adaptive immunity, in rats immunized with sheep RBC, chelidonic acid differentially suppressed the (a) plaque forming cell (PFC) count in rat splenic cells, (b) anti-SRBC antibody titre and serum IgG levels and (c) increases in foot pad thickness in the DTH assay – all of which were comparable with prednisolone. These experimental results are discussed in light of the possible therapeutic potential of chelidonic acid in allergic disorders.

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1. Introduction

Allergic disorders result from the dysregulation of the immune system and are associated with considerable morbidity. Such inappropriate immune reactions may result from exposure to foreign antigens or those misdirected against intrinsic body components leading to autoimmune disorders. Complex cellular and humoral mechanisms have been forwarded to explain such responses, and targeted drug therapy is often associated with untoward adverse effects. Further, mechanisms for explaining the etiopathogenesis of allergic responses are being revealed and hence the search for safer and effective therapeutic modalities is being constantly explored [1].

Herbal drugs are emerging as strong alternatives or adjuncts to conventional modern medical therapy. Their therapeutic efficacy combined with the comparative lack of adverse effects have prompted the generation of renewed interest in such plant based products. Further, application of modern medical technologies/strategies to authenticate the use of such agents has resulted in integration between traditional (from herbal sources) and modern medicines in a variety of disease states. The concept of 'reverse pharmacology' has added impetus to such interactive studies and a translational approach is being adopted to develop a

more rational interface between traditional and modern medical concepts. Chelidonic acid is a secondary metabolite found in several plants and its presence in many alkaloid containing plants like *Chelidonium majus* (Celandine) and several others of the Papaver species. Chelidonic acid has also been reported as the leaf closing signaling molecule for *Cassia minosodia* (an Indian medicinal plant) and has been isolated from *Sorghum vulgare* seedlings (a common food material grown in India), flowers of *Cassia spectabilis* and leaves of *Gloriosa superba*. There is the possibility of its ability to modulate the pharmacological activity of the alkaloids with which it co-exists in plants. Modern herbal pharmacologists have paid little attention to its potential as a therapeutic agent though it is a relevant active component of medicinal herbs. Interest in this molecule was triggered by the reports that it is a potent glutamic acid decarboxylase (GAD) inhibitor, and that the zinc site of the enzyme is its site of action. Structurally, this zinc site of the enzyme is analogous to many other immunological sites involved in histamine release and many other inflammatory phenomenon. There are also several reports on its zinc chelating properties. One preliminary pilot study had indicated that chelidonic acid was as effective in inhibiting histamine release as disodium cromoglycate, a mast cell stabilizer used in the prophylaxis of asthma and related allergic disorders [2–6]. In view of the above, it is possible that this plant product could have potential in the treatment of allergic disorders, and hence this study evaluated its immunomodulatory profile in experimental animals.

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2. Materials and methods

2.1. Animals

Wistar rats (180–220 g) were used for the study. The animals were maintained under standard laboratory conditions of light–dark cycle (12 h light–12 h dark) and temperature of $(22 \pm 2^\circ\text{C})$ and had free access to food and water. The animal care was as per guidelines laid down by the Indian National Science Academy, New Delhi, and the study protocol was approved by the Institutional Animal Ethical Committee.

2.2. Drugs and chemicals

Chelidonic acid, Prednisolone, and Ovalbumin were procured from Sigma-Aldrich (St. Louis, USA). Aluminium hydroxide was procured from Wyeth (India). Sheep red blood cells (SRBC) were generous gifts from Dept. of Microbiology, UCMS, Delhi. Anti-IgE and anti-IgG antibody was procured from Genex Bio (New Delhi). All other routine chemicals were procured from Sisco Research Labs (Delhi). Chelidonic acid, prednisolone, and SRBC was administered intraperitoneally (i.p.), after dissolving in appropriate vehicle, in a volume of 2 ml/kg.

2.3. Mast cell degranulation studies [7]

Rats were immunized with an intraperitoneal (i.p.) injection of antigen, viz. ovalbumin (10 mg) per rat adsorbed to 10 μg of aluminium hydroxide (i.e. ovalbumin + aluminium hydroxide). The five experimental groups were as under: controls (vehicle), chelidonic acid (1, 3 and 10 mg/kg) and prednisolone (10 mg/kg), and received daily treatment (i.p.) for 14 days for all drugs. Fourteen days after the sensitization and drug treatments, the animals were challenged with an i.p. injection of ovalbumin (1 mg) in 0.5 ml of isotonic saline in all groups. Anaphylactic mortality was checked at 2 h post challenge. After 10 min, the rats were anesthetized and exsanguinated. The abdomen was opened and the mesentery was dissected away from the small intestine. Fragments of mesentery were fixed and stained for 15 min in a solution containing 50% ethanol, 10% formaldehyde, 5% acetic acid and 0.2% toluidine blue. Mesentery fragments were then mounted on a glass slide, care being taken not to fold or stretch the tissue. Mast cell degranulation was assessed by counting the % of cells with extruded granules (12 microscopic fields counted). The degranulated cells in the rats could be visualized because the granules were still stained by the dye when they were discharged from the cell and became extracellular. The number of intact and degranulated mast cells in the different treatment groups was counted microscopically using standard technique.

2.4. Estimation of IgE and IgG levels

The blood samples collected were processed as per standard methodology for the assay of the immunoglobulins (IgE and IgG) using commercially available assay kit (Shibayagi Co., Ltd.). This was performed by enzyme linked immunosorbent assay (ELISA) using the sandwich method. Briefly the samples and biotin conjugated anti IgE antibody are incubated in monoclonal antibody coated wells. After 2 h of incubation HRP conjugated avidin is added and incubated for 1 h. The HRP conjugated avidin is reacted with a chromogenic substrate reagent and reaction is stopped and the absorbance of yellow product formed is measured using the software based microplate reader at 450 nm. Results are expressed in ng/ml. Similar procedure was also followed for IgG estimation.

2.5. Histamine assay from rat peritoneal mast cells (RPMC) in vitro [8]

Rats were anesthetized by anesthetic ether and injected with 50 ml of Tyrode buffer (137 mM NaCl, 5.6 mM glucose, 12 mM NaHCO_3 , 2.7 mM KCl, 0.3 mM NaH_2PO_4 , and 0.1% gelatin) into the peritoneal

cavity, and the abdomen was gently massaged for about 90 s. The peritoneal cavity was carefully opened, and the fluid containing peritoneal cells were aspirated by a Pasteur pipette. The peritoneal cells were sedimented at 150 g for 10 min at 4°C and resuspended in Tyrode buffer. Mast cells were separated from the major components of rat peritoneal cells. Mast cell preparations were assessed by toluidine blue staining. Histamine content was measured by the *o*-phthalaldehyde spectrofluorometric procedure. RPMC suspensions (54×10^5) were reacted with chelidonic acid 1, 3, and 10 mg/ml and with prednisolone 10 mg/ml for 10 min at 37°C then treated with 48/80 1 $\mu\text{g}/\text{ml}$. The reaction was stopped by cooling the tubes on ice. The cells were separated from the released histamine by centrifugation at 400g for 5 min at 4°C . Residual histamine in the cells was released by disrupting the cells with 1% Triton-X 100 and centrifuged at 400g for 5 min at 4°C . In brief, the culture supernatant (250 μl each) were mixed with 225 μl of 0.1 N HCl and 25 μl of 60% perchloric acid and centrifuged 22,000 g at 4°C for 20 min. The collected supernatant (400 μl), water (1.5 ml), and *n*-butanol (5 ml) were put into a tube containing NaCl (0.6 g) and 5 N NaOH (250 μl), and mixed for 20 min and centrifuged at 385g for 5 min at 4°C . The collected supernatant (4 ml) and heptane (5 ml) were put into a tube containing 1.5 ml of 0.1 N HCl, and mixed for 20 min and centrifuged as above. The liquid collected from bottom level (150 μl), 1 N NaOH (30 μl), and 7.5 μl of 1% ophthalaldehyde (OPT) were put into optiplate 96-well plates (Costar) and incubated for 3 min at 30°C . To stop the reaction, 3 N HCl (15 μl) was added and the light emission was measured in a spectrofluorometer. The fluorescence intensity was measured at 440 nm (excitation at 360 nm) by a spectrofluorometer.

2.6. Tests for adaptive immunity

2.6.1. Antibody response [9]

Animals were immunized with sheep red blood cells (SRBC, 0.5×10^9 cells/ml/100 g) on day 0 then they then received vehicle or chelidonic acid (1, 3 and 10 mg/kg) from the day 1 to 5. On the day 6, all rats were anesthetized with ether and blood was collected from the retro-orbital plexus using the microcapillary technique. The serum was assayed for hemagglutination titre was follows: two fold dilutions (0.025 ml) of sera were made in microtitre plate with saline. To each well 0.025 ml of 1% (v/v) SRBC was added. The plate were incubated at 37°C for 1 h and then observed for hemagglutination by using the double dilution technique. The highest dilution giving haemagglutination was taken as the antibody titre. The antibody titres were also expressed in graded manner ($-\log_2$), the minimum dilution (1/2) being ranked as 1, and the mean ranks of different treatment groups were compared for statistical significance.

2.6.2. Plaque forming cell assay [10,11]

The plaque forming cell (PFC) assay was carried to assess the ability of the spleen to form antibody forming cells. The animals were immunized with SRBC on day 0 and sacrificed on the day 4 under anesthesia. The spleen was removed and Spleen cell suspensions are prepared by gently tamping the spleen through a 60-mesh stainless steel screen, and collecting the cells in balanced salt solution (BSS). The spleen cells were washed and made up to 15 ml with BSS. SRBC was washed twice and made up to a 10% concentration. Complement (Gibco) was diluted 1/20 with BSS. All stock solutions were kept on ice water until used. All the cells were transferred to a conical test tube and the volume adjusted to 15 ml. The tube filled with the cell suspension was centrifuged. The supernatant was aspirated and discarded. The remaining pellet, constituted of intact spleen cells, was resuspended in fresh balanced salt solution. The tube was once more centrifuged; the supernatant was also discarded and the pellet is resuspended in more balanced salt solution. The cells were washed this way three times with balanced salt solution. The test consisted of mixing 0.05 ml of spleen cells, 0.070 ml of SRBC and 0.5 ml of the complement solution in a test tube at 37°C . The whole

mixture was immediately withdrawn and put into chambers prepared by gluing two 75 × 25 mm slides together with double-sided tape. Four to five slide chambers were necessary for each sample, filling up the last chamber if necessary with a blank solution of SRBC and complement in the same concentrations as for the test mixture. The slide chambers were sealed with paraffin wax, and incubated at 37 °C for 45–60 min. In earlier experiments, a 30 min incubation period was used, but this proved insufficient time for full development of all plaques. The number of PFC were counted by both macro- and microscopic examination under light microscope and expressed as plaque/million cells. The splenic PFC counts of the different treatment groups (viz. chelidonic acid and controls) were compared with controls.

2.6.3. Delayed type hypersensitivity (DTH) response [11,12]

Animals were immunized with sheep red blood cells (SRBC 1 × 10⁸ cells, sc) on the back of the tail on day 0. They were then divided in the different groups which received saline, chelidonic acid 1, 3, and 10 mg/kg (test groups) and prednisolone 10 mg/kg (positive controls). On day 5 post immunization, rats were challenged by injection SRBC into the left hind foot pad. Isotonic saline was injected in right hind paw. Foot pad thickness was measured at 24 h after antigen challenge using a Plethysmometer (Ugo-Basile) by the volume displacement method and differences between right and left paw volumes (R – L) were measured. The effects of the different treatments on this parameter were taken as a measure of their impact on cell mediated immunity.

2.7. Statistical analysis

All data were expressed as Mean ± SE. The data was analysed using the one way ANOVA followed by post hoc Tukey's test. Chi-square test was used to compare quantal data. A *p* value of at least 0.05 was considered as the level of significance in all statistical tests.

3. Results

3.1. Effects of chelidonic acid on mast cell degranulation and anaphylactic mortality

In this experiment, the total number of mast cells present in the mesentery and the percentage of intact and degranulated mast cells with extruded granules were counted following the antigen challenge. In normal (unsensitized) rats, 10% mast cells were seen as degranulated whereas 90% mast cells were intact. In the control group (i.e. sensitized + challenged) there was marked increase in mast cell degranulation which was 80% of the total mast cells while the other 20% were intact. The differences between the normal and control groups were statistically significant (*p* < 0.05). CA treatment in varying doses of 1, 3, and 10 mg/kg, in sensitized rats, decreased the mast cell degranulation in a dose related manner. Specifically, 55% of total mast cell were degranulated (45% intact) at the dose 1 mg/kg, whereas, with a dose of 3 mg/kg only 33% mast cells were degranulated (67% intact). In the CA (10 mg/kg) treated group, 17% mast cell were degranulated 83% cells being intact). In the comparator group, treatment of rats with prednisolone (10 mg/kg), also markedly inhibited mast cell degranulation and only 22% degranulated mast cells were seen (i.e. 78% mast cells were intact) (*p* < 0.02 in all cases). Further, there were also significant differences between CA 1 and 10 mg/kg groups (*p* < 0.05). However, the differences between the CA (10 mg/kg) and prednisolone groups were not statistically significant (*p* > 0.05). Further, control animals (ovalbumin immunized + challenged) showed 40% mortality, indicating serious anaphylactic reactions. However, no mortality was seen in any of the CA treated groups (1, 3 or 10 mg/kg) or in the prednisolone (10 mg/kg) treated group (*p* < 0.05 in each case, Chi-square test). These results are summarized in Table 1.

Table 1

Effect of chelidonic acid (CA) on mast cell degranulation and mortality in ovalbumin immunized rats.

Treatment group (mg/kg)	% intact mast cells	% degranulated mast cells	% mortality
Normal (unsensitized)	90.71 ± 2.14	9.28 ± 2.86	0
Controls (sensitized + challenged)	19.70 ± 3.57 ^a	80.29 ± 3.55 ^a	40
CA (1)	55.00 ± 6.92 [*]	45.00 ± 6.46 [*]	0 [*]
CA (3)	66.71 ± 7.23 [*]	33.29 ± 4.80 [*]	0 [*]
CA (10)	83.29 ± 6.67 [*]	16.71 ± 1.67 [*]	0 [*]
Prednisolone (10)	77.83 ± 3.03 [*]	22.17 ± 3.25 [*]	0 [*]

All data are Mean ± SE; *n* = 10/group; C – controls (vehicle).

^a *p* < 0.02 (vs normal).

^{*} *p* < 0.02 (vs controls).

3.2. Effects of chelidonic acid on serum IgE levels

The blood samples of ovalbumin immunized and challenged rats were processed for IgE estimation by ELISA and the results showed that there was a clearcut, dose related reductions in the levels of IgE after the drug treatments. Specifically, CA (1, 3 and 10 mg/kg) inhibited serum IgE concentrations in a dose related manner. There was near 50% reductions in the levels in blood at both 3 and 10 mg/kg of the drug (*p* < 0.05, in both cases, compared to controls). Similar reductions were also seen in the prednisolone treated groups. However, these differences between chelidonic acid and prednisolone groups were not statistically significant (*p* > 0.05). These results are summarized in Table 2.

3.3. Effects of chelidonic acid on histamine release from rat peritoneal mast cell (RPMC) preparations in vitro

Isolated rat peritoneal mast cell (RPMC) suspensions were reacted with either chelidonic acid (1, 3 or 10 mg/ml) or prednisolone (10 mg/ml) for 10 min at 37 °C, and then treated with 48/80 (1 µg/ml). The quantum of released histamine was assayed spectrofluorimetrically (Exc 360 nm/Emi 440 nm). The values were expressed in percentage release of histamine from the total content of mast cell (with drug treated plus without drug treated in per groups), where the total no. of mast cells were 54 × 10⁵ per ml. The data showed that there was a dose related inhibition in the histamine release in the CA treated preparations, with maximum effect being seen after CA 10 mg/ml (65% inhibition) (*p* < 0.05), whereas the inhibitions at the other dose levels were 30% (at CA 1 mg/ml) and 43% (at CA 3 mg/ml), respectively. These effects on histamine release from RPMC were comparable with those seen in the prednisolone treated samples (near 50% inhibition of histamine release). The data of the CA (1 mg/ml) group was also significantly different from that of the CA (10 mg/ml) group (*p* < 0.05). However, these differences between chelidonic acid and prednisolone groups were not statistically significant (*p* > 0.05). These results are summarized in Table 3.

Table 2

Effects of chelidonic acid (CA) on IgE levels in blood in ovalbumin immunized rats.

Treatments (mg/kg)	IgE levels in blood (ng/ml)
Control	64.56 ± 4.07
CA (1)	29.83 ± 3.88 [*]
CA (3)	33.31 ± 2.99 [*]
CA (10)	33.80 ± 2.98 [*]
Prednisolone (10)	27.19 ± 3.26 [*]

All data are Mean ± SE (*n* = 6/group).

^{*} *p* < 0.05 compared to controls (vehicle).

3.4. Effects of chelidonic acid on adaptive immunity

3.4.1. Antibody response

The effects of chelidonic acid on antibody responses were assessed in SRBC immunized rats by the haemagglutination technique. In this method, rats were immunized by SRBC in tail vein and animal divided in different group and treated with different doses of test drug (chelidonic acid, CA) and standard drug (prednisolone) from day 0 to day 5 (i.e. 5 days) and on 6th day, rats were bled and blood collected. The sera were prepared and anti-SRBC antibody titre was determined as two-fold dilutions in the various groups in the microtitre plates. The mean antibody titres were significantly lowered in the prednisolone group by approx. 50%. Pretreatment with CA resulted in dose related attenuations in the antibody response as compared to controls. There were 21%, 37% and 64% reductions in the anti-SRBC antibody titres after CA 1, 3 and 10 mg/kg, respectively. The maximal effects were seen after chelidonic acid 10 mg/kg, and this was significantly different from controls ($p < 0.05$). Thus it is evident that only the chelidonic acid 10 mg/kg treated group and prednisolone had effects on the humoral immune response, whereas 1 and 3 mg/kg of the test drug, chelidonic acid, did not show any significant effect ($p > 0.05$). These experiments showed that only chelidonic acid (10 mg/kg) and prednisolone induced comparable suppressions in the antibody titres. Intergroup comparisons also showed that the differences between CA (1 mg/kg) and CA (10 mg/kg) were statistically significant ($p < 0.05$). However, the differences between CA (10 mg/kg) and prednisolone groups were not statistically significant ($p > 0.05$). These results are summarized in Table 4. The IgG levels were also measured in various groups and the data showed that chelidonic acid (3 mg/kg) treatment reduced the IgG levels to $53.2 \pm 6.6 \mu\text{g/ml}$ as compared to $73.3 \pm 3.0 \mu\text{g/ml}$ in controls ($p < 0.05$). Further, chelidonic acid (10 mg/kg) also induced greater reductions in IgG levels to $30.0 \pm 5.5 \mu\text{g/ml}$, whereas, in the prednisolone treated group the IgG levels were $49.6 \pm 3.8 \mu\text{g/ml}$ ($p < 0.05$ in both cases, as compared to controls).

3.4.2. Plaque forming cells (PFC) assay

In the plaque forming cell (PFC) assay, rats were immunized with 25×10^6 SRBC. On day 5, all rats of the different treatment groups, viz. saline, chelidonic acid (1, 3 and 10 mg/kg) and prednisolone, were sacrificed under mild anesthesia (with chloroform) and after opening the abdomen, the spleen was taken out and by teasing in a petri dish containing HBSS on ice. Cell suspension was made and antibody forming cell (PFC) was counted by the method described (in the methods section) and was expressed as PFC for million spleen cells. Specifically, it was observed that there was a consistent and marked reduction in PFC counts in the various groups of sensitized rats treated with different doses treatment of drug i.e. chelidonic acid, when compared to the data of the control group. The reductions were by 63%, 76% and 69% with CA, 1, 3 and 10 mg/kg groups respectively ($p < 0.02$ in each case). The effects of chelidonic acid on the PFC counts were maximal after 3 and 10 mg/kg doses of the drug. Reductions in the PFC count (by 77%) were also seen in the prednisolone treated group. However, these differences between

Table 4

Effect of chelidonic acid (CA) on humoral and cell mediated immune (CMI) parameters in SRBC immunized rats.

Treatment (mg/kg)	PFC count (cells per million)	Antibody titre ($-\log_2$)	CMI (DTH assay) mean change in paw volume (%)
Control	85.50 ± 25.60	4.46 ± 0.76	88.60 ± 2.29
CA (1)	$32.26 \pm 9.38^*$	3.50 ± 0.33	$63.40 \pm 5.01^{**}$
CA (3)	$21.14 \pm 4.08^{**}$	2.82 ± 0.60	76.40 ± 3.84
CA (10)	$27.71 \pm 6.45^*$	$1.67 \pm 0.45^{**}$	$68.40 \pm 7.01^*$
Prednisolone (10)	$19.60 \pm 7.50^{**}$	$2.28 \pm 0.40^*$	71.60 ± 5.78

All data are Mean $\pm$ SE ($n = 6/\text{group}$).

* $p < 0.05$ (compared to controls).

** $p < 0.02$ (compared to controls).

the data of the CA and prednisolone treated groups were not statistically significant ($p > 0.05$). These results are summarized in Table 4.

3.4.3. Cell mediated immune responses (in vivo)

The delayed type of hypersensitivity (DTH) reaction is a well validated test for assessment of cell mediated immune (CMI) function and sheep red blood cells (SRBC) are highly immunogenic and stable T cell dependent antigen. Rats ($n = 6$ per group) were immunized with SRBC (1×10^8 cells i.p.) on the day 0. They were then divided in to five different groups – the control group received saline whereas the other groups received CA in different doses (1, 3 and 10 mg/kg) or prednisolone 10 mg/kg. After 5 days of treatment all groups are challenged with 1×10^8 SRBC (on the day 5, in the left hind paw, whereas saline was injected in to the right hind paw). The differences in the footpad thickness ($R_t - L_t$ paw) were measured 24 h after SRBC challenge by the fluid displacement method. Inconsistent effects were seen on the CMI response after drug treatments. Overall analysis of data showed that there were significant differences across all treatment groups ($F_{4,20} = 3.59$; $p < 0.02$, one way ANOVA). At doses of 1, 3, and 10 mg/kg, CA inhibited the CMI response by 29%, 14% and 23% respectively as compared to controls. The maximum enhancement was seen after CA (3 mg/kg) which was statistically significant ($p < 0.05$). These reductions were comparable to that seen after prednisolone (10 mg/kg) where the inhibition in this response was by 20%. However, the differences between chelidonic acid and prednisolone groups were not statistically significant ($p > 0.05$). These results are summarized in Table 4.

Chelidonic acid up to a dose of 20 mg/kg (for 14 days) did not show any overt signs of toxicity in these animals. The prednisolone treated group also did not show any apparent signs of toxicity other than a marginal increase in body weight and locomotor activity. However, it is well known that longer term administration of prednisolone induces a variety of untoward effects.

4. Discussion

Agents derived from medicinal plants have made major contributions in modern therapeutics and it is being increasingly realized that systematic pharmacological studies for efficacy and safety are required prior to their use in humans [13]. Such validation of plant based medicinal substances can help to promote meaningful interactions between traditional and modern medicine. The growing interest in such phytopharmaceuticals has also been due to the fact that they are efficacious and safer than their more expensive modern medicinal counterparts [14]. Advancing research has explored different strategies for developing pharmacological agents from herbal sources. Secondary metabolites from plants are one such area which has yielded good results with reference to drug development and the field holds considerable promise. Chelidonic acid is one such secondary metabolite which is present in several medicinal plants, which may have the ability to modify the other active ingredients of these plants and enhance their potency and safety. For example, it is known that *Gloriosa superba* leaf, which is rich in colchicine, also contains substantial amounts of

Table 3

Effects of chelidonic acid (CA) on histamine release from rat peritoneal mast cell (RPMC) in vitro.

Treated groups (mg/ml)	% release of histamine from RPMC
RPMC alone	10.80 ± 3.02
Controls	69.58 ± 8.59^a
CA (1)	50.80 ± 8.80
CA (3)	40.80 ± 7.05
CA (10)	$25.40 \pm 6.96^*$
Prednisolone (10)	$35.40 \pm 8.90^*$

All data are Mean $\pm$ SE; all groups except RPMC alone treated with compound 48/80.

^a $p < 0.05$ as compared to controls (vehicle).

* $p < 0.02$ as compared to RPMC group.

chelidonic acid. However, before proceeding to investigate such interactions, it was thought worthwhile to evaluate the effects of this molecule, per se, if any, on different experimental models of allergy. Earlier studies had shown that chelidonic acid may have some benefit in an experimental model of allergy, and further studies have shown that this plant secondary metabolite may be beneficial in conditions like allergic rhinitis and ulcerative colitis [6,15,16]. There is thus a strong possibility that chelidonic acid may have potential in the treatment of allergic disorders like bronchial asthma, which has not been studied. Thus, the purpose of the present study was to evaluate the effects of chelidonic acid in different experimental models of allergy. Allergic disorders result from a dysregulated immune system and complex cellular and humoral components interact during such pathology [1]. A plethora of modern therapeutic agents are known to be effective but safety considerations have prompted the search for safer agents from plant sources. For example, there is high prevalence of usage of complementary medicines for allergic disorders like bronchial asthma [17]. Mast cells play a key role in allergic disorders and chemical mediators like histamine, arachidonate products, proteases and several cytokines, are found in relatively high quantities in these cells [18]. These mediator molecules are potent vasoactive and bronchoconstrictor agents and they modulate local immune responses and inflammatory cell infiltration. Mast cells thus act as important targets for drugs effective in allergic disorders. Antigen challenge, in sensitized animals, results in IgE mediated degranulation of mast cells, which is an important feature of anaphylaxis [19,20]. In the present study, we evaluated the effects of chelidonic acid, a secondary plant metabolite with possible potential against allergic disorders, in experimental models of allergy in rats. Our results showed that chelidonic acid dose dependently attenuated the ovalbumin challenge induced mast cell degranulation in rat peritoneal mast cells *ex vivo*. In fact, these responses were comparable to those seen after prednisolone, which acted as the positive control agent. In addition, anaphylactic mortality, which was 40% in controls, were totally absent in the chelidonic acid treated groups — suggesting protection against such mortality. Prophylaxis in allergic disorders is achieved by preventing mast cell degranulation and resultant mediator release and both cromoglycate and corticosteroids are equieffective in this regard. We chose prednisolone as a comparator drug in our study, as in addition to stabilizing the mast cell membrane; it also inhibits mast cell formation and cytokine synthesis. It is also one of the most effective therapies in mast cell related disorders and has been used by earlier research workers to this effect [21].

In addition to immunological mechanisms for mast cell degranulation and mediator release, non-immunological factors can also facilitate mediator release from mast cells. A recent study has also identified a novel receptor (MrgprX2) on mast cells which are activated by secretagogues like inflammatory peptides and drugs thus resulting in allergic/pseudoallergic reactions [22]. In view of this and in order to further explore the mast cell stabilization property; the effects of this molecule were studied on compound 48/80 induced histamine release from rat peritoneal mast cells *in vitro*. The quantum of histamine release in the presence and absence of chelidonic acid was quantified. Our results showed that quantum of histamine released from mast cells treated with compound 48/80 treated mast cells were markedly reduced in the chelidonic acid treated group as compared to controls. Taken together, our *ex vivo* and *in vitro* experiments with mast cells suggested that this molecule inhibited mast cell degranulation and histamine release. It was clear that chelidonic acid inhibited mediator release from mast cells irrespective of the mechanisms (immunological or otherwise) involved in such release.

An allergic inflammatory response is the outcome of complex interactions between inflammatory and structural cells as well as a plethora of chemical mediators. The immunoglobulin, IgE, plays a central role in such responses and antigenic sensitization is the first step in the development of IgE-mediated inflammatory/immune response. After antigen capture, the antigen-presenting cells process them and migrate to

draining lymph nodes, where they present allergic peptides to naive T cells and promote their activation and differentiation into Th2 cells under the influence of cytokines such as IL-4. The Th2 cells then bind with B cells and induce plasma cell differentiation, thereby leading to production of IgE immunoglobulins. These allergen-specific immunoglobulins via circulation reach the site of allergen exposure and bind to FcRI receptors on resident mast cells. Subsequent allergen exposure leads to crosslinking of the IgE–FcRI complex on mast cells, thereby causing their degranulation and release of preformed mediators [1]. Our results show that there was increase in levels of IgE in the blood in ovalbumin immunized and challenged rats which was attenuated after chelidonic acid treatment in a dose related manner. Further, the test drug effects were also comparable with that of prednisolone. This suggests that the effects of chelidonic acid on ovalbumin immunized rats were dependent of IgE and mediated by the Th2 cells.

Since IgE formation takes place in the B-lymphocytes under the influence of several cytokines, it was relevant to evaluate the effects of chelidonic acid on other aspects of B-cell function namely other antibody formations. In addition to IgE, humoral immune responses are mediated by IgM and/or IgG which may play a significant role in the protection against invading pathogens and the resultant bacterial or viral infections. It is also well known that factors like infections can precipitate allergic reactions like asthma. In contrast, there are various clinical situations in which immune suppression is desirable to allow the sustenance of foreign tissue (grafts) in the body. In addition to humoral immune responses, the cell mediated immune response has also been implicated in allergic disorders. During the CMI response, sensitized T-lymphocytes, when challenged by the antigen, are converted to lymphoblast and secrete lymphokines, attracting more scavenger cells to the site of reaction. The infiltrating cells are thus immobilized to promote defensive (inflammatory) reaction. Studies have indicated that in asthma a heightened Th2 response coexists with the activation of Th1 cells. The Th1 cells may be activated through an allergen–non-specific pathway, via cytokines involved in Th2 response, or by a sensitizing allergen, a true cell-mediated immune reaction. In the lung, cell-mediated immune reactions preferentially involve the bronchioles, alveoli and interstitium, but rarely the bronchi. Mostly cells from Th2 pathway may participate as effector cells, but occasionally, effector cells from Th1 pathway can also characterize the IgE-mediated reaction [23,24]. In view of this we investigated the effects of chelidonic acid on classical tests of humoral and cell mediated immunity and our results showed that the chelidonic acid inhibited the anti-SRBC antibody response and also reduced the number of splenic plaque forming cells in SRBC immunized mice. In addition, our studies showed that specific IgG levels were also lower after chelidonic acid treatment as compared to control levels — thereby indicating that IgG may have also contributed to the humoral immune response. Further, in tests for delayed type hypersensitivity (DTH), an indicator of cell mediated immunity, chelidonic acid induced reductions in the DTH response when compared to controls — suggesting CMI suppressing effects of the drug. However, these effects were not dose related and this could have been due to the non-specific nature of the response — though a general suppression was observed at all doses of chelidonic acid. These results indicate that chelidonic acid has immunosuppressant effects on tests for adaptive immunity, which could highlight its modulatory effects in other immune mediated disorders.

In conclusion, the present results highlight the significant immunomodulatory effects of chelidonic acid, a secondary plant metabolite, in experimental models of allergy and suggest the possible therapeutic importance of this novel molecule of plant origin in allergic disorders.

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