International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 7 (5): 589-598, 2011
ISSN 1811-7775 / DOI: 10.3923/ijp.2011.589.598

Study on Herb-herb Interaction Potential of *Glycyrrhiza glabra* with *Solanum xanthocarpum* and *Adhatoda vasica* on Mast Cell Stabilizing Activity

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Abstract: Synergistic interactions between the components of mixtures of herb are a vital part of their therapeutic efficacy. There is no real basis for better efficacy of a mixture of herb extract than an equivalent dose of an individual herbal extract. Speculation as to explain this, whether it involves synergy, enhanced bioavailability, cumulative effects or simply the additive properties of the constituents requires further research. Liquorice, Kantkari and Vasaka are present in number of herbal formulation available in market for asthma and respiratory disorders so, present study was undertaken to explore the interaction potential of *Glycyrrhiza glabra* with *Solanum xanthocarpum* and *Adhatoda vasica* on mast cell stabilization compound 48/80 and egg albumin induced degranulation. Isobole method and Combination Index values were used to derive possible magnitude of interaction at various combinations. Compound 48/80 and egg albumin significantly disrupted mast cells ($p<0.001$). Pretreatment of mast cell with Liquorice Extract (LE), *Solanum xanthocarpum* Extract (SXE) and *Adhatoda vasica* Extract (AVE) showed significant protection ($p<0.05$) against Compound 48/80 and egg albumin induced degranulation. This activity of all the extracts was found to be dose dependant. Mast cell treated with various combinations of *Adhatoda vasica* extract and Liquorice extract (AVE+LE) as well as *Solanum xanthocarpum* extract and Liquorice extract (SXE+LE), demonstrated significant ($p<0.001$) stabilization against compound 48/80 and egg albumin induced mast cell degranulation. The isobologram and combination index analysis suggested that combination of LE with SXE and AVE has synergistic effects (and may be nearly additive at some effect level) on mast cell stabilization against compound 48/80 as well as egg albumin degranulation. Thus, it appeared to have substantial activity in this *in vitro* study, a combination of Liquorice extract with *Solanum xanthocarpum* extract and *Adhatoda vasica* extract deserves further clinical investigation in properly designed clinical trials in patients with allergic conditions.

Key words: Herb-Herb interaction, *Glycyrrhiza glabra, Solanum xanthocarpum, Adhatoda vasica,* mast cell stabilization, isobole method

INTRODUCTION

Mast cells are important producers of mediators of inflammatory responses such as allergy and immediate type allergic reaction. Immediate-type allergic reaction is mediated by histamine released in response to the antigen cross-linking of Immunoglobulin E (IgE) bound to, high affinity IgE receptor, Fcε Receptor I (FcεRI) on the mast cells. After activation via the FcåRI, the mast cells start the process of degranulation which results in the releasing of mediators, such as products of arachidonic acid metabolism and an array of inflammatory cytokines (Galli *et al*., 2005; Taechowisan *et al*., 2008). Among the inflammatory substances released from mast cells, histamine is one of the best-characterized and most potent vasoactive mediators implicated in the acute phase of immediate hypersensitivity (Mirbahar *et al*., 2000; Cook *et al*., 2002). Mast cell degranulation also can be elicited by non-immunologic stimulators such as neuropeptides, basic compounds, complement components and certain drugs compound 48/80 and polymers of basic amino acids, such as substance P, are some of the most potent stimulators of mast cells (Oskeritzian *et al*., 2005).

Glycyrrhiza glabra, *Solanum xanthocarpum* and *Adhatoda vasica* are present in number of herbal

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formulation available in market for asthma and respiratory disorders. Ethanolic extract of *Solanum xanthocarpum* has been reported to exert mast cell stabilizing activity against compound 48/80 induced degranulation (Parmar *et al*., 2008; Ranjith *et al*., 2010). In the indigenous system of medicine *Adhatoda vasica* has been widely used as an expectorant and mild bronchial antispasmodic. This biological activity is attributed to the presence of alkaloids vasicine and vasicinone (Talib *et al*., 2002). Vasicinone was found to possess potent antiallergic activity in mice, rats and guinea pigs. Its antiallergic activity has been studied by antagonism of passive cutaneous anaphylactic reaction in mice and rats. Inhibition of histamine release, induced by passive peritoneal anaphylaxis, was tested in rats and guinea pigs (Paliwa *et al*., 2000). Licorice is frequently used in traditional medicine to treat inflammatory and allergic diseases. Liquiritigenin and 18β-glycyrrhetinic acid most potently inhibited the degranulation of rat peritoneal mast cells induced by compound 48/80. Liquiritigenin and 18 β-glycyrrhetinic acid potently inhibited the passive cutaneous anaphylactic reaction as well as the scratching behavior in mice induced by compound 48/80. These components inhibited the production of IgE in ovalbumin-induced asthma mice but liquiritigenin had little effect. This suggests that the antiallergic effects of licorice are mainly due to glycyrrhizin, 18 β-glycyrrhetinic acid and liquiritigenin which can relieve IgE-induced allergic diseases such as asthma (Shin *et al*., 2007).

Liquorice provides a number of examples of interaction between its own constituents, as well as with other herbs. It has useful role in detoxification and potentiation of other herbs and further investigation regarding interactions of liquorice would be rewarding (Wagner and Ulrich-Merzenich, 2009; Williamson, 2001). So, present study was designed to study the interaction of liquorice with *Solanum xanthocarpum* and *Adhatoda vasica*, on mast cell stabilization. An effort was made to derive possible magnitude of interaction at various combinations. Mechanism of observed interaction in present study was derived as "synergistic multi target effects" theory which states that the single constituents of a mono-extract or a multi-extract combination affect not only one single target but several targets and therefore, results in an agonistic or synergistic response (Wagner and Ulrich-Merzenich, 2009).

MATERIALS AND METHODS

Plant materials: The fresh whole herbs of *Solanum xanthocarpum* were collected in the month of November 2008 from Jasapar, Jasdan and District: Rajkot, India. The leaves of *Adhatoda vasica* were collected from the campus of Smt. R. B. Patel Mahila Pharmacy College, Atkot, District: Rajkot and Liquorice roots and rhizomes were purchased from Sanjivani ausadhalay, Bhavnagar, Gujarat. All the plants were authenticated by Dr. H. B. Singh, Scientist and Head of Raw Materials Herbarium and Museum Department of National Institute of Science and Communication and Information Resources, New Delhi (NISCAIR). The voucher specimens (No. rbpmpc/museum/herbarium/02 for Liquorice, rbpmpc/museum/herbarium/25 for *Adhatoda vasica* and rbpmpc/museum/herbarium/30 for *Solanum xanthocarpum*) were deposited in the museum of Smt. R.B. Patel Mahila Pharmacy College, Atkot, District: Rajkot. All the materials were dried in the drying room with active ventilation at room temperature until they achieved constant weight.

Reagents and drugs: Ethanol, sodium cromoglycate, ketotifen, compound 48/80, toluidine blue, egg albumin (analytical grade) as well as acetonitrile, glacial acetic acid, methanol and water (HPLC grade) were purchased from Qualigen Pvt. Ltd., Mumbai, India. 18 β-glycyrrhetinic acid (>95%), vasicine (>95%) and solasodine (>95%) standards for HPLC analysis were purchased from Natural Remedies Pvt. Ltd., Bangalore, India.

Preparation of plant extracts: All plants were shed dried and reduced to coarse powder. The coarsely powdered herbs (2000 g) were subjected for extraction with ethanol (80%) using sohxlet assembly. Extracts were filtered, concentrated by evaporation under vacuum and subjected to preliminary phytochemical screening.

HPLC analysis of plant extracts: HPLC chromatograms were obtained using Shimadzu HPLC system with a 20 µL sample loop. The HPLC analysis was done using a C-18 reversed phase column (VP-ODS, (250×4.6 mm, 5 mm)) and LC-10AD pump. The column effluent was monitored with a variable wavelength photodiode-array detector (SPD-10A) which has the ability to scan from 200-800 nm. The data were analyzed by computer based class VP software.

A high performance liquid chromatography analysis was performed for determination of 18 β-glycyrrhetinic acid in *Glycyrrhiza glabra* L. (licorice) extract. The operating condition were C-18 column (VP-ODS, (250×4.6 mm, 5 mm)) at room temperature, acetonitril/phosphoric acid (3:1) as mobile phase, at flow rate of 0.6 mL min^{-1} (0-8 min), 0.4 mL min^{-1} (8-20 min) and UV detection at 230 nm (Esmaeili *et al*., 2006).

A HPLC analysis was performed to determine vasicine in *Adhatoda vasica* extract with following operating condition: acetonitrile-0.1M phosphate buffer-glacial acetic acid (15:85:1), pH 3.9; flow rate 0.7 mL min^{-1}; column temperature, 26°C; detector wavelength, 300 nm (Srivastava *et al*., 2001; Raja *et al*., 2008).

For determination of Solasodine in *Solanum xanthocarpum* extract by HPLC method, operating condition applied are as follow: The methanol:water mobile phases were buffered by either 20 mM phosphate (pH 3.5). The flow rate used was 1.0 mL min^{-1} and solasodine was detected by UV absorption at 205 nm (Kittipongpatana *et al*., 1999).

Animals: Male wistar rats weighing 180-200 g were used in this study. Animals were housed in groups of six rats on 12 h light and 12 h dark cycle; and were maintained in an air-conditioned animal quarter at a temperature of 22±2°C and a relative humidity of 60±10%. They were offered water and food *ad libitum*. The animals were acclimatized to the facilities for 5 days. Experiments reported in this study were carried out in accordance with current guidelines for the care of laboratory animals and the ethical guidelines for investigation of experimental pain in conscious animals. The Institutional Animal Ethical committee (IAEC) has approved the protocol of the study (No. IAEC/RBPMPC/09-10/06). All efforts were made to minimize the number of animals used and their suffering.

Studies on compound 48/80 induced rat peritoneal mast cell degranulation: A modification of the method described by Kanemoto *et al*. (1993) was employed for the study. Wistar rats were sacrificed by stunning and cutting the neck blood vessels. The peritoneal cavity was lavaged with 10 mL of Tyrode solution. The lavaged fluid was collected and centrifuged at 2000 rpm for 5 min. The pellet was separated, washed with Tyrode solution and finally resuspended in 1 ml Tyrode solution. 0.1 ml of this lavage fluid was transferred to 30 test tubes. The lavage fluid was then subjected to following treatment schedule:

- Test Tube No. 1: Negative Control
- Test Tube No. 2: Positive Control
- Test Tube No. 3a: 0.1 mL of Standard (10 μg mL^{-1} Sodium Cromoglycate)
- Test Tube No. 3b: 0.1 mL of Standard (10 μg mL^{-1} Ketotifen)
- Test Tube No. 4a: 0.1 mL of 10 μg $mL^{-1}$of Glycyrrhiza glabra extract (LE)
- Test Tube No. 4b: 0.1 mL of 20 μg mL^{-1} of Glycyrrhiza glabra extract (LE)
- Test Tube No. 4c: 0.1 mL of 40 μg mL^{-1} of Glycyrrhiza glabra extract (LE)
- Test Tube No. 4d: 0.1 mL of 60 μg mL^{-1} of Glycyrrhiza glabra extract (LE)
- Test Tube No. 4e: 0.1 mL of 80 μg mL^{-1} of Glycyrrhiza glabra extract (LE)
- Test Tube No. 4f: 0.1 mL of 100 μg mL^{-1} of Glycyrrhiza glabra extract (LE)
- Test Tube No. 5a: 0.1 mL of 10 μg mL^{-1} of Solanum xanthocarpum extract (SXE)
- Test Tube No. 5b: 0.1 mL of 20 μg mL^{-1} of Solanum xanthocarpum extract (SXE)
- Test Tube No. 5c: 0.1 mL of 40 μg mL^{-1} of Solanum xanthocarpum extract (SXE)
- Test Tube No. 5d: 0.1 mL of 60 μg mL^{-1} of Solanum xanthocarpum extract (SXE)
- Test Tube No. 5e: 0.1 mL of 80 μg mL^{-1} of Solanum xanthocarpum extract (SXE)
- Test Tube No. 5f: 0.1 mL of 100 μg mL^{-1} of Solanum xanthocarpum extract (SXE)
- Test Tube No. 6a: 0.1 mL of 10 μg mL^{-1} of Adhatoda vasica extract (AVE)
- Test Tube No. 6b: 0.1 mL of 20 μg mL^{-1} of Adhatoda vasica extract (AVE)
- Test Tube No. 6c: 0.1 mL of 40 μg mL^{-1} of Adhatoda vasica extract (AVE)
- Test Tube No. 6d: 0.1 mL of 60 μg mL^{-1} of Adhatoda vasica extract (AVE)
- Test Tube No. 6e: 0.1 mL of 80 μg mL^{-1} of Adhatoda vasica extract (AVE)
- Test Tube No. 6f: 0.1 mL of 100 μg mL^{-1} of Adhatoda vasica extract (AVE)
- Test Tube No. 7: 0.1 mL mixture of 10 μg $mL^{-1}$of LE+10 μg mL^{-1} of SXE (SXE10+LE10)
- Test Tube No. 8: 0.1 mL mixture of 100 μg mL^{-1} of LE+10 μg mL^{-1} of SXE (SXE10+LE100)
- Test Tube No. 9: 0.1 mL mixture of 10 μg mL^{-1} of LE+100 μg mL^{-1} of SXE (SXE100+LE10)
- Test Tube No. 10: 0.1 mL mixture of 100 μg mL^{-1} of LE+100 μg mL^{-1} of SXE (SXE100+LE100)
- Test Tube No. 11: 0.1 mL mixture of 10 μg mL^{-1} of LE+10 μg mL^{-1} of AVE (AVE10+LE10)
- Test Tube No. 12: 0.1 mL mixture of 100 μg mL^{-1} of LE+10 μg mL^{-1} of AVE (AVE10+LE100)
- Test Tube No. 13: 0.1 mL mixture of 10 μg mL^{-1} of LE+100 μg mL^{-1} of AVE (AVE100+LE10)
- Test Tube No. 14: 0.1 mL mixture of 100 μg mL^{-1} of LE+100 μg mL^{-1} of AVE (AVE100+LE100)

Each test tubes were incubated for 10 min at 37°C and then compound 48/80 (0.1 mL, 10 μg mL^{-1}) was added to each test tubes except test tube no. 1. After further

incubation for 10 min at 37°C, 0.1 mL of 10% toluidine blue was added and examined under light microscope with 450X magnification. A minimum of 100 cells was counted for intact and disrupted mast cells and from it % protection from degranulation was calculated.

Studies on egg albumin induced rat peritoneal mast cell degranulation: Male wistar rats were first sensitized with egg albumin by giving 4 subcutaneous injections (on 1st, 3rd 5th and 12th day) egg albumin (1 mL, 10%w/v). On 10th day peritoneal mast cells were collected by the method described above. Mast cells were degranulated by 0.1 mL of egg albumin (10 μg mL^{-1}) and the effect of the test agent on degranulation was studied in same way as described in Compound 48/80 induced degranulation.

The percent protection of the mast cells in the control group and the treated groups was calculated by counting the number of degranulated cells from total of atleast 100 mast cells counted. Control groups consisted of positive control group in which egg albumin was added without addition of test agent and a negative control group in which neither egg albumin nor the test agent was added to correct for spontaneous degranulation of mast cells without any degranulating agent (Shah and Parmar, 2003).

Evaluation of drug interaction by Isobole method: In order to calculate a function describing the predicted effect of a combination of the compounds, additivity was assumed. Dose-response curves were generated for each agent. The extent of the effect of the combination treatment was analyzed by the isobole method for a combination of drugs (Berenbaum, 1981; Chou and Talalay, 1984; Wagner and Ulrich-Merzenich, 2009).

Statistical analysis: Statistical significance was tested between more than two groups using one-way ANOVA followed by the Bonferroni multiple comparisons test using a computer-based fitting program (Prism, Graphpad 5).

RESULTS

Phytochemical Screening of plant extracts: Phytochemical analysis of LE (yield-25 %w/w) showed presence of terpenoids saponin glycosides, flavonoids, polysaccharides, carbohydrates, starch and protein. SXE (yield-15.42% w/w) showed presence of alkaloids, terpenoids, flavonoids, phenols and carbohydrate. AVE (yield-4.2% w/w) showed presence of alkaloids

HPLC-fingerprint of the extracts: The HPLC-fingerprints of the extracts are obtained. Figure 1 shows the

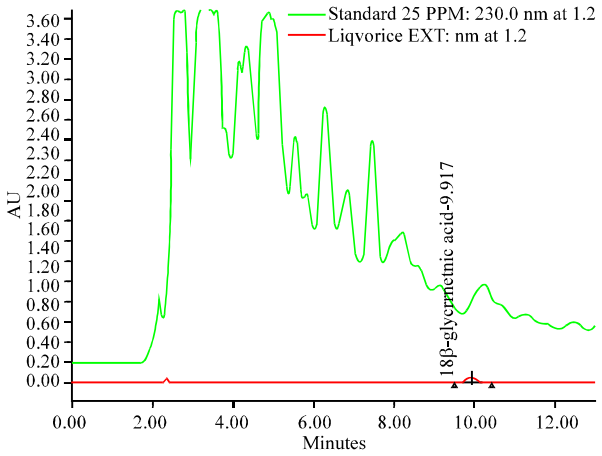

Fig. 1: HPLC chromatogram of LE with 18β-glycyrrhetinic acid (Standard). Chromatogram showed separation of 28 constituents in LE of which component at retention time 9.917 was identified as 18 β-glycyrrhetinic acid

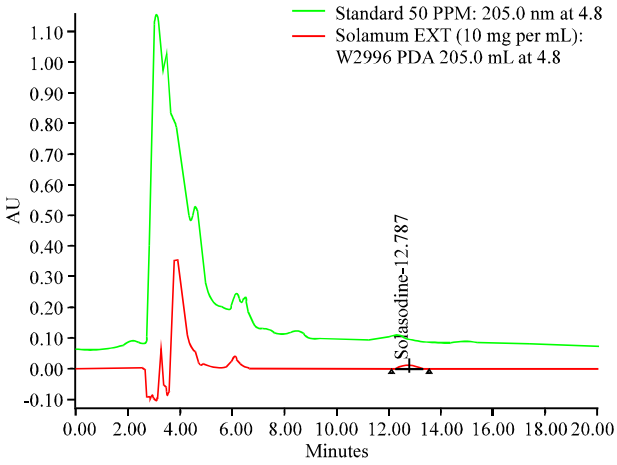

Fig. 2: HPLC chromatogram of SXE with Solasodine (Standard). Chromatogram showed separation of 9 constituents in SXE of which component at retention time 12.787 was identified as Solasodine

fingerprint of the LE. 28 components were separated and 18β-glycyrrhetinic acid was determined by comparing the retention time (9.917 min) and absorption spectra with the standard substance of 18β-glycyrrhetinic acid. Figure 2 shows separation of 9 constituents in fingerprint of the SXE, solasodine was determined by comparing the retention time (12.787 min) and absorption spectra with the standard substance. The fingerprint of the AVE showed separation of 7 constituents (Fig. 3). Vasicine was determined by comparing the retention time (7.450 min) and absorption spectra.

Effect of LE, AVE and SXE alone on compound 48/80 induced rat peritoneal mast cell degranulation: Compound 48/80 produced significant ($p<0.001$) disruption of mast cells which was significantly ($p<0.001$) inhibited by pretreatment with the LE, AVE and SXE alone, protection against degranulation was found to be dose dependent and maximum protection was observed at 100 μg mL^{-1} in all extracts (Table 1). LE exhibited maximum 55.20±0.86 percentage inhibition of mast cell degranulation at 100 μg mL^{-1}, SXE showed highest 60.40±0.927% inhibition of mast cell degranulation at 100 μg mL^{-1} while AVE demonstrated highest 62.40±1.030% inhibition of mast cell degranulation at 100 μg mL^{-1} concentration.

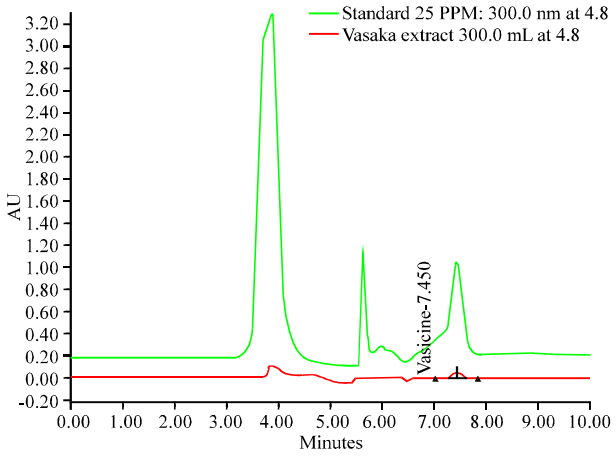

Fig. 3: HPLC chromatogram of AVE with Vasicine (Standard). Chromatogram showed separation of 7 constituents in AVE of which component at retention time 7.450 was identified as Vasicine

Effect of LE, AVE and SXE alone on egg albumin induced rat peritoneal mast cell degranulation: Egg albumin significantly ($p<0.001$) disrupted mast cells which was significantly ($p<0.001$) inhibited by pretreatment with the LE, AVE and SXE alone. Protection against degranulation was found to be dose dependent (Table 2). LE, SXE and AVE at 100 μg mL^{-1} concentration exhibited 57.50±0.764, 59.67±0.882 and 63.50±1.258 percentage inhibition of mast cell degranulation, respectively.

Effect of LE and AVE combination on compound 48/80 as well as egg albumin induced rat peritoneal mast cell degranulation: Egg albumin treatment of mast cell showed significant ($p<0.001$) degranulation compared to non treated mast cell. Various combinations of AVE and LE treated mast cell demonstrated significant ($p<0.001$) protection of mast cell against egg albumin induced mast cell degranulation. Maximum protection against egg albumin induced mast cell degranulation was observed with AVE100+LE100 pretreatment (Fig. 4).

The results were evaluated by isobole method (Fig. 5). The line of additivity in Fig. 5 indicated the alignment of theoretical values of an additive interaction between two substances. Values above this line indicate antagonistic interaction and values below indicate synergistic interaction. Combination index of AVE10+LE10, AVE100+LE10, AVE10+LE100 and AVE100+LE100 was found to be 0.249, 0.716, 0.722 and 1.05, respectively. Thus, strong synergism was observed

Table 1: Effect of LE, AVE and SXE alone on compound 48/80 induced rat peritoneal mast cell degranulation

	%Protection compound 48/80 induced mast cell degranulation					
Treatment	10 μg mL^{-1}	20 μg mL^{-1}	40 μg mL^{-1}	60 μg mL^{-1}	80 μg mL^{-1}	100 μg mL^{-1}
Negative control	90.20±1.562					
Positive control	15.80±1.281[a]					
LE	32.20±0.86[b]	36.20±1.158[b]	41.80±0.663[b]	47.20±0.583[b]	51.20±0.86[b]	55.20±0.86[b]
SXE	40.00±1.049[b]	44.20±0.374[b]	49.60±1.030[b]	54.80±0.583[b]	58.00±0.837[b]	60.40±0.927[b]
AVE	33.60±1.364[b]	40.40±0.927[b]	44.80±0.860[b]	50.20±0.663[b]	56.20±1.068[b]	62.40±1.030[b]
Sodium cromoglycate	79.33±1.745[b]					
Ketotifen	84.33±1.82[b]					

Values are expressed as mean±SEM (n = 6), [a]$p<0.001$ as compared to negative control, [b]$p<0.001$ as compared to positive control

Table 2: Effect of LE, AVE and SXE alone on egg albumin induced rat peritoneal mast cell degranulation

	%Protection egg albumin induced mast cell degranulation					
Treatment	10 μg mL^{-1}	20 μg mL^{-1}	40 μg mL^{-1}	60 μg mL^{-1}	80 μg mL^{-1}	100 μg mL^{-1}
Negative control	84.67±0.8819					
Positive control	30.17±1.400[a]					
LE	35.50±0.619[c]	40.00±0.683[b]	44.17±1.014[b]	47.50±0.764[b]	52.17±0.703[b]	57.50±0.764[b]
SXE	40.33±0.760[b]	46.00±0.931[b]	50.17±0.872[b]	51.33±0.615[b]	56.00±1.07[b]	59.67±0.882[b]
AVE	40.00±0.577[b]	45.83±0.703[b]	49.00±0.730[b]	51.50±0.995[b]	59.83±1.195[b]	63.50±1.258[b]
Sodium cromoglycate	75.33±1.43[b]					
Ketotifen	80.33±1.52[b]					

Values are expressed as mean±SEM (n = 6), [a]$p<0.001$ as compared to negative control, [b]$p<0.001$ and [c]$p<0.05$ as compared to positive control

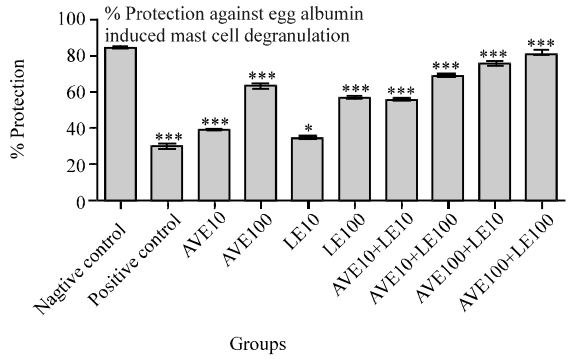

Fig. 4: Effect of various combination of AVE and LE on Egg albumin induced rat peritoneal mast cell degranulation. Each value represents mean±S.E.M., n = 6. Differences between groups were statistically analysed one-way ANOVA followed by Bonferroni multiple comparisons test. *** p<0.001 compared to negative control group. *p<0.05 compared to negative control group, +++p<0.001 compared to Positive control group

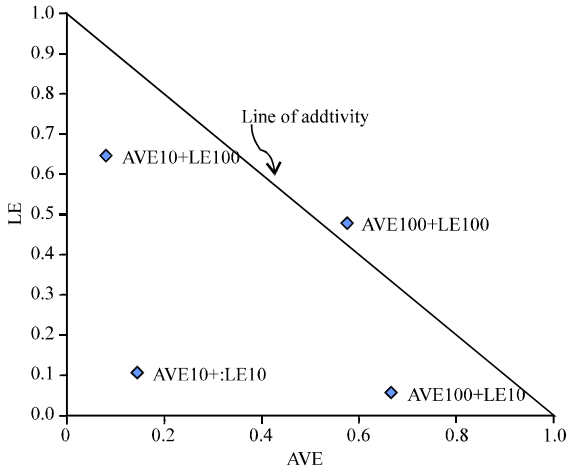

Fig. 5: Isobole for mast cell stabilizing activity of the AVE and LE combinations Egg albumin induced rat peritoneal mast cell degranulation. Combination index (CI) = (LE in combination/LE alone)+(AVE in combination/AVE alone), CI of AVE10+LE10 = 0.249, CI of AVE100+LE10 = 0.716, CI of AVE10+LE100 = 0.722 and CI of AVE100+LE100 = 1.05

with AVE10+LE10, synergism was observed with AVE100+LE10 and AVE10+LE100 combination while AVE100+LE100 combination has nearly additive activity.

Protective activities of various combinations of AVE and LE alone were tested on compound 48/80 induced mast cell degranulation. Compound 48/80 significantly

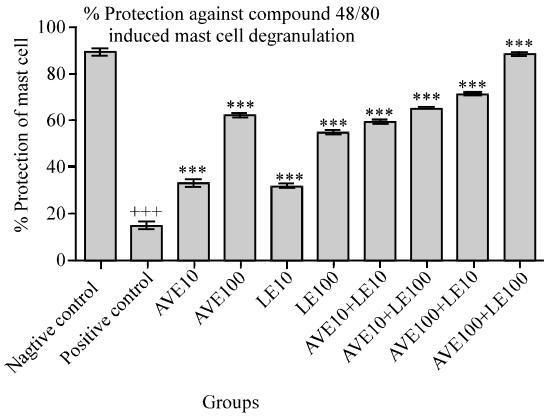

Fig. 6: Effect of various combination of AVE and LE on Compound 48/80 induced rat peritoneal mast cell degranulation. Each value represents mean±S.E.M., n = 6. Differences between groups were statistically analysed one-way ANOVA followed by Bonferroni multiple comparisons test. *** p<0.001 compared to negative control group, +++p<0.001 compared to Positive control group

induced mast cell degranulation as compared to negative control (p<0.001) while AVE and LE in various combination significantly (p<0.001) protected mast cells against compound 48/80 induced degranulation. Maximum activity was observed with AVE100+LE100 combination (Fig. 6).

Mast cell treatment with combination of AVE and LE has showed synergistic to additive Protective activity against compound 48/80 induced degranulation as revealed by isobologram (Fig. 7) and combination indexes. Combination index of AVE10+LE10, AVE100+LE10, AVE10+LE100 and AVE100+LE100 was found to be 0.195, 0.825, 0.812 and 0.962, respectively. Strong synergistic activity was commenced from the AVE10+LE10, moderate synergistic activity was commenced from AVE100+LE10 as well as AVE10+LE100 while nearly additive activity was commenced from AVE100+LE100.

Effect of LE and SXE combination on compound 48/80 as well as egg albumin induced rat peritoneal mast cell degranulation: Protective activity of SXE and LE in combinations was tested on compound 48/80 induced mast cell degranulation. Compound 48/80 significantly induced mast cell degranulation as compared to negative control (p<0.001) while SXE, LE alone and in various combination significantly (p<0.001) protected mast cells against compound 48/80 induced degranulation. Maximum activity was observed with SXE100+LE100 combination (Fig. 8).

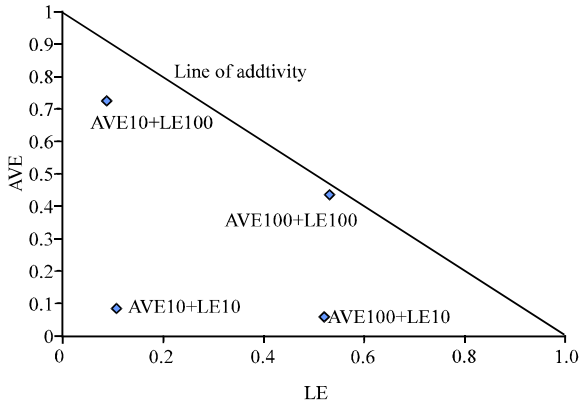

Fig. 7: Isobole for mast cell stabilizing activity of the AVE and LE combinations Compound 48/80 induced rat peritoneal mast cell degranulation. Combination index (CI) = (LE in combination/LE alone)+(AVE in combination/AVE alone),CI of AVE10+LE10 = 0.195, CI of AVE100+LE10 = 0.825, CI of AVE10+ LE100 = 0.812 and CI of AVE100+LE100 = 0.962

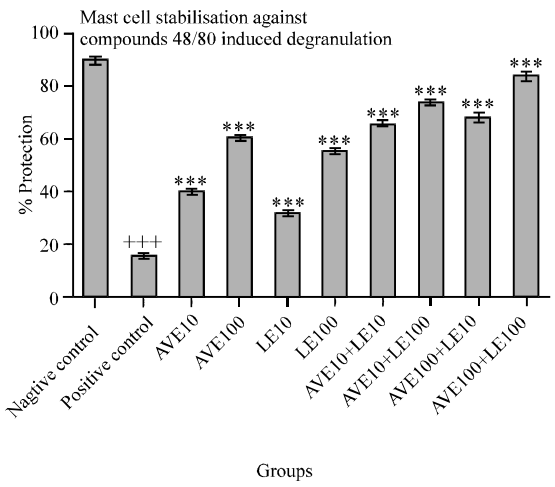

Fig. 8: Effect of various combination of SXE and LE on Compound 48/80 induced rat peritoneal mast cell degranulation. Each value represents mean±S.E.M., n = 6. Differences between groups were statistically analysed one-way ANOVA followed by Bonferroni multiple comparisons test. ***p<0.001 compared to negative control group, +++p<0.001 compared to Positive control group

Mast cell treatment with combination of SXE and LE has showed synergistic to additive protective activity against compound 48/80 induced degranulation as revealed by isobologram (Fig. 9) and combination indexes (CI). Strong synergistic activity was commenced from the

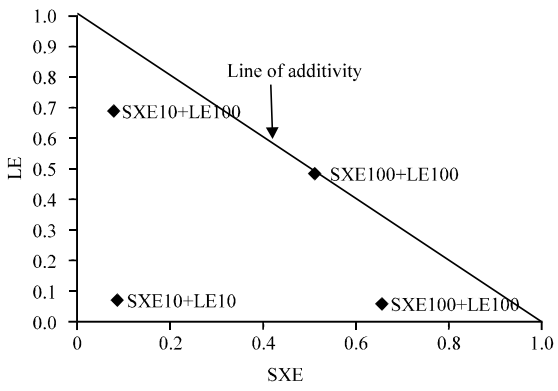

Fig. 9: Isobole for mast cell stabilizing activity of the SXE and LE combinations Compound 48/80 induced rat peritoneal mast cell degranulation. Combination index (CI) = (LE in combination/LE alone)+(SXE in combination/SXE alone), CI of SXE10+LE10 = 0.158, CI of SXE100+LE10 = 0.715, CI of SXE10+ LE100 = 0.763 and CI of SXE100+LE100 = 0.988

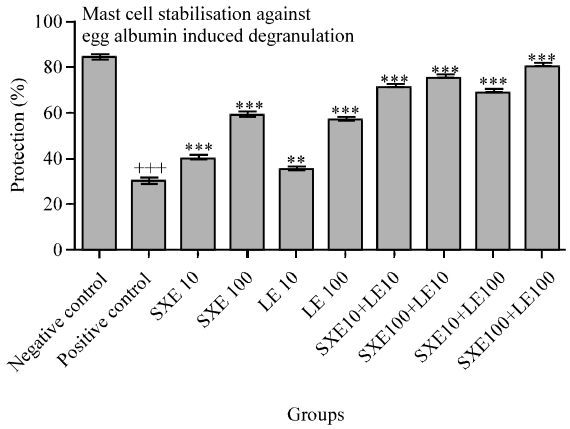

Fig. 10: Effect of various combination of SXE and LE on Egg albumin induced rat peritoneal mast cell degranulation. Each value represents mean±S.E.M., n = 6. Differences between groups were statistically analysed one-way ANOVA followed by Bonferroni multiple comparisons test. ***p<0.001 compared to negative control group, +++p<0.001 compared to Positive control group

SXE10+LE10 (CI: 0.158), moderate synergistic activity was commenced from SXE100+LE10 (CI: 0.715) as well as SXE10+LE100 (CI: 0.763) while nearly additive activity was commenced from SXE100+LE100 (CI: 0.988).

Egg albumin treatment of mast cell showed significant (p<0.001) degranulation compared to non

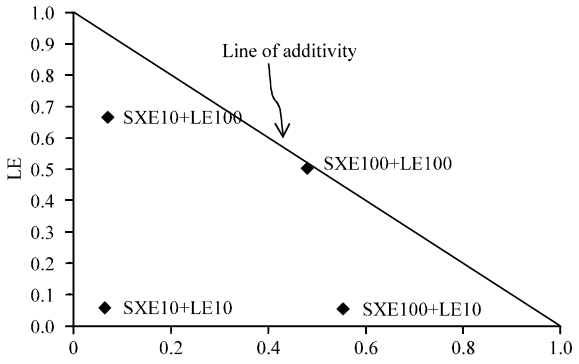

Fig. 11: Isobole for mast cell stabilizing activity of the SXE and LE combinations Egg albumin induced rat peritoneal mast cell degranulation. Combination index (CI) = (LE in combination/LE alone)+(SXE in combination/SXE alone), CI of SXE10+LE10 = 0.123, CI of SXE100+LE10 = 0.603, CI of SXE10+LE100 = 0.732 and CI of SXE100+ LE100 = 0.977

treated mast cell. Various combinations of SXE and LE treated mast cell demonstrated significant ($p<0.001$) protection of mast cell against egg albumin induced mast cell degranulation. Maximum protection against egg albumin induced mast cell degranulation was observed with SXE100+LE100 pretreatment (Fig. 10).

The results were evaluated by isobole method. (Fig. 11) Combination Index (CI) of SXE10+LE10, SXE100+LE10, SXE10+LE100 and SXE100+LE100 was found to be 0.123, 0.603, 0.732 and 0.977, respectively. Thus, strong synergism was observed with SXE10+LE10 (CI: 0.123), synergism was observed with SXE100+LE10 (CI: 0.603) while SXE10+LE100 (CI: 0.732) commenced moderate synergistic activity and SXE100+LE100 (CI: 0.977) has nearly additive activity.

DISCUSSION

Present study demonstrates interaction of LE with AVE and SXE, on their mast cell stabilization activity against compound 48/80 and egg albumin induced degranulation. There are number of reports suggesting that stimulation of mast cells with compound 48/80 or egg albumin initiates the activation of signal transduction pathway which leads to histamine release. Several studies have shown that compound 48/80 and other polybasic compounds are able, apparently directly, to activate G-proteins (Mousli *et al*., 1990). Compound 48/80 increases the permeability of the lipid bilayer membrane by causing a perturbation in the membrane. Thus, increase in membrane permeability may be an essential trigger for the release of the mediator from mast cells (Lee *et al*., 2006). In this sense, anti-allergic agents having a membrane stabilizing action may be desirable. Results of our study suggest LE, AVE and SXE might stabilize the lipid bilayer membrane, thus preventing the perturbation being induced by compound 48/80.

Phytochemical analysis of LE and SXE showed presence of flavonoids. Recent studies demonstrate flavonoids can inhibit degranulation of mast cells, reduce histamine, tryptase, IL-6 and IL-8 release from cultured mast cells, as well as from macrophages (Shichijo *et al*., 2003). In addition flavonoids are inhibitors of histamine, IL-6 and IL-3 production by activated human basophils (Hirano *et al*., 2004). Several flavonoids were found to inhibit the release of histamine, leukotrienes, prostaglandin D2 and granulocyte macrophage colony stimulating factor (GM-CSF) from mast cells in concentration dependant manner (Chang *et al*., 2000; Kimata *et al*., 2000; Gupta *et al*., 2010). Thus, Mast stabilizing activity exerted by LE and SXE might be attributed to its flavonoids content.

Results of present study confirmed the previous report of mast cell stabilizing activity of SXE and suggest that this activity might be due to presence of solasodine, other glycoalkaloids and flavonoids (Parmar *et al*., 2008). Moreover LE contains 18β-glycyrrhetinic acid which most potently inhibited the degranulation of RBL-2H3 cells induced by IgE with the antigen (DNP-HSA) and rat peritoneal mast cells induced by compound 48/80. Liquiritigenin and 18β-glycyrrhetinic acid potently inhibited the passive cutaneous anaphylactic reaction as well as the scratching behavior in mice induced by compound 48/80. These components inhibited the production of IgE in ovalbumin-induced asthma mice but liquiritigenin had little effect. This suggests that the antiallergic effects of licorice are mainly due to 18β-glycyrrhetinic acid which can relieve IgE-induced allergic diseases such as asthma (Shin *et al*., 2007).

Results suggested that AVE contains vasicinone and vasicine which might be responsible for its mast cell stabilization activity. Vasicine was found to possess potent antiallergic activity in mice, rats and guinea pigs. Its antiallergic activity has been studied by antagonism of passive cutaneous anaphylactic reaction in mice and rats. Inhibition of histamine release, induced by passive peritoneal anaphylaxis, was tested in rats and guinea pigs (Paliwa *et al*., 2000).

Synergy between herbs in multi-herbal regimen could be of potential clinical utility. We used the isobole method

and CI values to analyze and compare the different combination effects between the herbs tested. From the isobologram and combination index analysis, combination of LE with SXE and AVE has synergistic effects (and may be nearly additive at some effect level) on mast cell stabilization against compound 48/80 as well as egg albumin degranulation. As HPLC chromatogram and phytochemical screening of LE showed presence of glycosides majorly saponin and flavonoids which might have synergistic action. Thus, observed synergistic interaction might be due to their multi target effects (Wagner and Ulrich-Merzenich, 2009).

CONCLUSION

Because it appeared to have substantial activity in this *in vitro* study, combination of LE with SXE and AVE deserves further clinical investigation in properly designed clinical trials in patients with allergic conditions.

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