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Research Article

# ANTIANAPHYLACTIC AND MAST CELL STABILIZATION ACTIVITY OF *CYNODON DACTYLON*

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**ABSTRACT**

The objective of the present study was to isolate antianaphylactic and mast cell stabilizing compound from *Cynodon dactylon* through bioassay-guided fractionation. The antianaphylactic activity was evaluated by using compound 48/80 induced anaphylaxis and mast cell stabilization was studied by using peritoneal mast cells of rats. The possible antianaphylactic and mast cell stabilization mechanism was evaluated by using compound 48/80 induced mast cell activation and level of nitric oxide in serum, rat peritoneal mast cells. The present study indicates that the isolated *Cynodon dactylon* compound (CDC) was potent and has significant ($p<0.01$ and $p<0.001$) inhibitory effect on compound 48/80 induced anaphylactic reaction and mast cell activation. This CDC also inhibited significantly, compound 48/80 induced increased level of nitric oxide in rat serum and rat peritoneal mast cells. We conclude from this study that the isolated CDC is potent then disodium chromoglycate in producing antianaphylactic activity through mast cell stabilization and inhibition of nitric oxide synthesis.

**Key words:** *Cynodon dactylon*; Disodium chromoglycate; Antianaphylactic; Nitric oxide; Mast cells, Bio-assay guided fractionation, CDC.

## INTRODUCTION

Depending on the amount of allergen entered into the body, it will induce different kind of changes including running nose, sneezing, cutaneous wheel and flare reaction and wheezing occurs within few minutes[1]. This type of reaction is called as immediate type of hypersensitivity reactions[2]. Recently it has been reported that in immediate type of allergic reaction, the allergens triggers B-cells to produce IgE and IgG antibodies which reacts with these allergens and bind to high affinity receptors for IgE (FcεRI) along with circulating basophils and tissue mast cells[3]. In late phase reaction the allergens express multiple epitopes recognized by specific IgEs and IgGs, this membrane bound IgEs induces receptor aggregation, triggering a signaling cascade leading to the production and release of allergic & inflammatory mediators such as histamine, leukotrines, chemokines and cytokines responsible for the symptoms of allergic disease[2]. Mast cells are well known as critically important component in various biologic processes of allergic diseases. These are found relatively large numbers in the mucosa of respiratory, gastrointestinal, urinary tract, skin and near blood or lymphatic vessels, these cells are supposed to express surface membrane receptors with high affinity and specificity for IgE[4]. Activation of these cells is known to release proinflammatory cytokines, proteases, histamine, prostaglandin's and leukotrienes which are known to be involved in chemotaxis and phagocytosis of macrophages[4]. It has also been reported that activation of mast cells and subsequent release of cytokines are involved in stimulation of inducible nitric oxide synthase (iNOS) leading to generation of nitric oxide at relatively and sustained level. It is well established that many cell types involved directly or indirectly in immunity and inflammation synthesize nitric oxide[5]. Compound 48/80 is a condensation product of N-methoxy-phenylamine with formaldehyde which is a well known histamine releaser, discovered in the search for hypotensive agent [6]. The compound 48/80 activate the mast cells and degranulates by aggregation of high affinity receptors for Fc regions of IgE (FcεRI) cross linking of IgE by polyvalent antigens, leading to the activation of Src-like cytosolic protein tyrosine kinases (PTKs) and producing rapid tyrosine phosphorylation of the FcεRI β and γ subunits. This will enable the recruitment and activation of additional cytosolic PTKs and tyrosine phosphorylation of a number of protein substitute, including PLCγ. Even PLC is activated in mast cells phosphotidyl-4; 5-phosphate is hydrolyzed to produce $IP_3$ & DAG. $IP_3$ binds to release its receptors and the intracellular calcium storage from smooth endoplasmic reticulum. Then to released $Ca^{++}$ activate DAG and PKC which in turn increase in intracellular $Ca^{++}$ level and activation of PKC along with PI-3-kinase (PI3K) contributes to the release of histamine. Therefore compound 48/80 is employed as classic mast cell activator and induces about 90% release of histamine from mast cells and perpetuation of membrane by increased permeability[7].

*Cynodon dactylon* (Poaceae) is one of the most commonly occurring perennial grass throughout India, commonly known as Dhub. Traditionally, the paste of the plant is used as first aid for minor injuries to stop bleeding, eye disorders and weak vision. In Ayurveda it has been mentioned as pungent, bitter, fragrant, heating, appetizer, vulnerary, anthelmentic antipyretic and alexiteric. It has also been mentioned for tumors, asthma, bronchitis, leucoderma, piles and enlargement of the spleen[8, 9].

## MATERIALS AND METHODS

Compound 48/80 (C2313) was purchased from Sigma Aldrich (St. Louis USA), other chemicals including Span 80 and organic solvents used were of AR grade. Research microscope (Metzer), LTE Scientific Ltd. U.K. Mini Lyotrap (0L3 7EN), refrigerated centrifuge [Remi, C-24], tubes (15ml), Shimadzu UV Spectrophotometer (1601) and Borosil Soxhalet extractor were used in this study. The whole part of *Cynodon dactylon* was collected in and around the Bagalkot (India).

### Plant Preparation

The whole plant was cleaned and washed with 2% $KMnO_4$ in distilled water and then dried under shade, until it was free from moisture. The grass was subjected to get coarse powder and then passed through sieve no.44. The sieved powder was stored in airtight, high-density polyethylene containers before extraction. The powder was successively extracted (soxhlet) with petroleum ether (40°C), chloroform (50°C) & methanol (60°C). The extracts were completely dried at -40° C using lyophilizer[10]. The obtained extracts were then subjected to pharmacological screening for its antipruritic activity. Chloroform extract was used for bioassay-guided fractionation and evaluated for antianaphylactic and mast cell stabilization activity.

### Animals

*Balb/C* mice of either sex weighing to 18-25 gm were used antianaphylactic activity and *Spargue-Dawley* rats were used for mast cell stabilization. These animals were maintained under standard conditions in animal house of H. S. K. College of Pharmacy, B. V. V. S. campus Bagalkot (India). The animals were provided with standard diet *ad libitum* tap water. All the experiments using

animals were carried out as per guideline of institutional animal ethics committee (821/01/a/CPCSEA) (HSK/IAEC.Clear/2004-2005).

**Methods**

**Bio-assay guided fractionation and isolation**

Fractionation of chlroform extract was carried out by using large scale column chromatography. The sample (5gm) of chloroform extract was applied to top of the column (70 X 2.5 cm i.d.) of activated neutral aluminium oxide. The extract fractions were eluted with benzene: ethyl acetate: butanol (4: 2: 0.5). Total five fractions were collected from elutes and their purity was reconfirmed by TLC using the same solvent system but with varying proportion. The first fraction of chloroform extract from *Cynodon dactylon* will be as called CDC[10].

**Antianaphylactic activity**

Various extracts of *Cynodon dactylon* (100mg/kg) were administered orally 1 hr prior to administration of compound 48/80. Mice were administered an i.p. injection of 8mg/kg of the mast cell degranulator compound 48/80 for anaphylactic reaction (n=10/group). Mortality was monitored for 1 hr after induction. Based on percent protection, the extract was selected further for fractionation and evaluation[11, 12].

**Mast cell stabilizing activity**

The active fraction (CDC) in a doses of 10, 25, 50 100µg/kg were given orally to rats for 5 days, prior to collection of mast cells, along with disodium chromoglycate (10mg/kg i.p.). After anesthetizing animals with diethyl ether, 10 ml normal saline was injected into the peritoneal cavity of rats. By gentle messaging, the peritoneal fluid were collected and transferred into siliconised centrifuge tubes. Mast cells (peritonial fluid) were washed with buffer solution (pH 7) thrice by centrifugation at a low speed (400-500rpm.) discarding the supernatant and collected the pellet of mast cells into the medium. Pallets from control groups and treated groups were incubated with compound 48/80 (1µg/ml) at $37^0$C for 10 min. After incubation, mast cells were stained with toluidine blue (0.1%) and percentage protection against derganulation was counted under high-power microscope (450X) [3].

**Measurement of serum nitric oxide level**

The active fraction (CDC) in a doses of 10, 25, 50 100µg/kg were given orally to rats for 5 days, prior to collection of blood, along with disodium chromoglycate (10mg/kg i.p.). The rats were then anesthetized by ether and then the blood was collected from retro-orbital. The blood was then centrifuged at 500 rpm for 5 min, equal volume of serum, acidic griess reagent (pH 2) and 40µl of glycine buffer (100µM of glycine, 100µM of NaCl and 40µM of HCl) was added and then incubated for 15 min at $37^o$C. The normal group sample was incubated with saline whereas controlled and treated groups samples were incubated with compound 48/80 (1µg/ml). The absorbance was measured at 546nm[5, 13].

**Measurement of rat peritoneal fluid nitric oxide level**

The peritoneal fluid was collected by the same method as stated above in mast cell stabilizing activity. The equal volume of peritoneal fluid, acidic griess reagent (pH 2) and 40 µl of glycine buffer were mixed and incubated for 15 min at $37^o$ C. Incubated sample was centrifuged and the supernant was collected and used for measurement of absorbance at 546nm. The normal group sample was incubated with normal saline whereas controlled & treated groups samples were incubated with compound 48/80 (1µg/ml) [5, 13].

**Generation of the standard curve using Sodium nitrate as the standard**

The molecular weight of $NaNO_2$ is 69, therefore to prepare 1000 µM stock solution dissolved 6.9 mg in 10 ml of distilled water, from this stock solution, 100 µl added to 9.9 ml of distilled water to obtain 100 µM solution of $NaNO_2$.From the above 100 µM solution by dissolving sufficeint amount in distilled water to obtain solutions of the 25 µM, 12.5 µM, 6.25 µM, 3.125 µM, 1.5625 µM concentration. 1.5 ml solution from each concentration was added to 1.5 ml Griess reagent, placed in dark room for 10 min. Lastly, absorbance were measured in photometer at 546 nm. A standard curve was obtained by plotting the concentration of $NaNO_2$ on the X-axis and absorbance at 546 nm on Y-axis[13].

**Statistical Analysis**

The experimental results are represented as Mean ± SEM. Student un-paired 't'-test was applied. $p<0.05$ was considered statistically significant.

**RESULTS**

**Bioactivity guided extraction and fractionation**

The activity of each extract was evaluated by using compound 48/80 induced scratching which is discussed as under. Treatment of *Cynodon dactylon* extracts at 100 mg/kg 1 hr prior to compound 48/80 treatment was shown significant inhibitory effects on scratching behavior induced by the compound 48/80. More significant effect has occurred by prophylactic treatment with chloroform extract and percent of inhibition was 85% when compared to control (table 1). Therefore, the chloroform extract was considered for further fractionation using column chromatography total five fractions were obtained by developing the columns using Aluminium oxide (neutral) as stationary phase and Benzene: Ethyl acetate: Butanol as a mobile phase in a ratio of 4: 2: 0.5 selected on the basis of results of thin layer chromatography, these fractions were found to be pure. All the fractions again have undergone evaluation at 100 µg/kg in compound 48/80 induced pruritis. The first fraction has shown 69% inhibition, second fraction 61% and third fraction 48% when compared to control considered to be 100 % (Table 2). Hence forth, the first fraction of chloroform extract from *Cynodon dactylon* has been called *Cynodon dactylon* compound. The term *Cynodon dactylon* compound refers to the first fraction of *Cynodon dactylon* chloroform extract. 1 hr after compound 48/80 administration for anaphylaxis and mortality if any, was recorded. The results were analyzed by chi-square test by comparing control with normal and treated groups with control for coming to conclusion. $P<0.05$ was considered as significant. *$P<0.05$.

**Antianaphylactic activity**

Disodium-chromoglycate considered as standard drug at the dose of 10 mg/kg i.p. as in previous studies, as effectively inhibited 60% mortality when given 1 hr earlier to the compound 48/80. The first fractions of chloroform extract shown 90% protection which was again considered for further evaluation in a dose dependent manner. The minimum dose 10 µg/kg considered in this study protected one animal out of 10 animals. The dose dependent reversal on compound 48/80 induced mortality was noted with 25, 50 and 100 µg/kg leading to 60, 80 and 90% protection respectively by pretreatment with compound 48/80 (table 3). 100% mortality was observed within 1hr of compound 48/80 treatment alone in control group.

**Table 1: Effect of *Cynodon dactylon* extract on anaphylactic reaction in Balb/C mice**

| Treatment / dose | Extracts | Death (%) | Protection (%) |
|---|---|---|---|
| 100 mg/kg p.o. | Control | 100 | --- |
| | Petroleum ether | 40* | 60 |
| | Chloroform | 30* | 70 |
| | Methanolic | 70 | 30 |

100 mg/kg dose of petroleum ether, chloroform and methanolic extract of *Cynodon dactylon* was administered orally 1 hr prior to the administration of compound 48/80.

**Table 2: Effect of *Cynodon dactylon* chloroform fraction on anaphylactic reaction in Balb/C mice**

| Extract/dose | Treatment | Death (%) | Protection (%) |
|---|---|---|---|
| Chloroform fraction (100µg/kg p.o.) | First | 10** | 90 |
| | Second | 20* | 80 |
| | Third | 50 | 50 |
| | Fourth | 70 | 30 |
| | Fifth | 70 | 30 |

100 mg/kg dose of *Cynodon dactylon* chloroform fractions were administered orally 1 hr prior to the administration of compound 48/80. Animals were observed for 1 hr after compound 48/80 administration for anaphylaxis and mortality if any was recorded. The results were analyzed by chi-square test by comparing control with normal and treated groups with control for coming to conclusion. P<0.05 was considered as significant. *P<0.05, **P<0.01

**Table 3: Antianaphylactic activity of *Cynodon dactylon* compound**

| Treatment | Dose | Death (%) | Protection (%) |
|---|---|---|---|
| Chloroform first fraction | 10 µg/kg (p.o) | 90 | 10 |
| | 25 µg/kg (p.o) | 40 | 60* |
| | 50 µg/kg (p.o) | 20 | 80** |
| | 100 µg/kg (p.o) | 10 | 90** |
| Control | 8 mg/kg (i. p.) | 100 | --- |
| Standard | 10 mg/kg (i. p.) | 40 | 60* |

10, 25, 50 and 100 µg/kg dose of *Cynodon dactylon* compound was administered orally 1 hr prior to the administration of compound 48/80. Animals were observed for 1 hr after compound 48/80 administration for anaphylaxis and mortality if any was recorded. The results were analyzed by chi-square test by comparing control with normal, standard and treated groups with control for coming to conclusion. P<0.05 was considered as significant. *P<0.05, **P<0.01

**Table 4: Mast cell stabilizing activity**

| Treatment | Activated mast cells (%) |
|---|---|
| Normal | 17.16±1.9 |
| Control | 92.66±1.2*** |
| 10 mg/kg (i.p.) DSCG | 37.66±1.6*** |
| 10 µg/kg (p.o) *Cynodon dactylon* compound | 65.83±2.6*** |
| 25 µg/kg (p.o) *Cynodon dactylon* compound | 52.33±4.2*** |
| 50 µg/kg (p.o) *Cynodon dactylon* compound | 47.33±2.4*** |
| 100 µg/kg (p.o) *Cynodon dactylon* compound | 42.16±2.4*** |

Effect of *Cynodon dactylon* compound on compound 48/80 induced mast cell activation. Rats were treated regularly for five days with 10, 20, 50 and 100 µg/kg (p.o). Rat peritoneal mast cells were collected, by centrifugation were incubated with compound 48/80 at 37° C for 10 mins. Total hundred cells were counted and percent of activation calculated .The data collected are given as mean ± SEM, and analyzed by student't' test, by comparing control with normal and treated with control for coming to conclusion. P value less than 0.05 was considered as significant. ***p<0.001

**Table 5: Effect of *Cynodon dactylon* compound on Nitrate level in *Sprague Dawley* rats.**

| Treatment | Serum NO | RPMC NO |
|---|---|---|
| Normal | 15±0.99 | 4.7±0.39 |
| Control | 39±1.4*** | 13±0.97*** |
| 10 mg/kg (i.p.) DSCG | 21±1.9*** | 22±2.3** |
| 10 µg/kg (p.o) *Cynodon dactylon* compound | 17±1.1*** | 20±2.9* |
| 25 µg/kg (p.o) *Cynodon dactylon* compound | 17±0.49*** | 13±0.88 |
| 50 µg/kg (p.o) *Cynodon dactylon* compound | 17±1.3*** | 5.4±0.19*** |
| 100 µg/kg (p.o) *Cynodon dactylon* compound | 23±0.67*** | 6.5±0.95** |

Effect of *Cynodon dactylon* compound on nitrate level in serum and RPMC. Serum nitric oxide level was measured indirectly by measurement of nitrate levels by using Griess reagent test. Serum and RPMC collected from treated groups incubated with compound 48/80 for 15 min and estimated for level of nitrate. The data are presented as mean ± SEM, and analyzed by student't' test by comparing control with normal and treated groups with control for coming to conclusion. P value less than 0.05 was considered as significant. *p<0.05, **p<0.01 and ***p<0.001

**Mast cell stabilizing activity**

In an effort to evaluate possible mechanism of action this study was carried out. In this study, compound 48/80, produced similar results to earlier study and activation of rat peritoneal mast cells (RPMCs) were 92.66 ± 1.2 Vs 17.16 ± 1.9 of normal. Significant (p<0.001) effect of compound on compound 48/80 induced mast cell activation was noted. Dose dependently the *Cynodon Dactylon* compound 10, 25, 50 and 100 µg/kg significantly (p<0.001) reduced the number of activated mast cells to 65.83 ± 2.6, 92.66 ± 4.2, 47.33 ± 2.4 and 42.16 ± 2.4 respectively from 92.62 ± 1.2. However, more significant (p<0.001) mast cell stabilizing activity was noted with much higher dose disodium-chromoglycate i.e. number of activated mast cells were 37.66 ± 1.6 (Table 4).

**Serum and rat peritoneal mast cells nitric oxide**

As depicted in table 5 compound 48/80 treatment has induced a significant (p<0.001) increase in level of serum and rat peritoneal mast cell fluid nitrate levels from 15 ± 0.99 and 4.99 ± 0.39 to 39 ± 1.4 and 13 ± 0.97 respectively. Treatment with disodium-chromoglycate significantly reduced compound 48/80 augmented serum nitrate to 21 ± 1.9, contrarily significant (p<0.001) rat peritoneal mast cell fluid nitrate level increased to 32 ± 2.9 from 13 ± 0.97. Similar results were obtained with compound 10 µg/kg serum nitrate and rat peritoneal mast cell fluid was 17 ± 2.9 and 20 ± 0.9 respectively, 25, 50 and 10 µg/kg dose of compound significantly (p<0.001) reversed compound 48/80 induced serum nitrate level to 17 ± 0.49, 17 ± 1.3, 23 ± 0.67 respectively. Significant inhibitory effect of *Cynodon dactylon* compound was also observed with these doses on compound 48/80 induced increased rat peritoneal mast cell nitrate levels.

**DISCUSSION**

Anaphylaxis is a severe and systemic allergic reaction caused by systemic release of histamine and other pharmacological mediators[2]. The common cause of anaphylaxis was IgE mediated hypersensitivity reaction[2]. One of the newer method of anaphylaxis treatment involves use of immunotherapeutic agent by decreasing production of IgE[2]; induce the production of protective secretory IgA and IgG[2]; blocking the IgE; interrupting Th2 dependent allergic cascade[2]; increased elimination of IgE and markedly decrease the expression of FcεRI[2]. In the present study, the compound has shown, dose dependent protection against compound 48/80 induced anaphylaxis up to 90% better than the DSCG results. This protective effect of the fraction may involve any one or all mechanisms mentioned above along with mast cell stabilizing activity and nitric oxide synthesis.

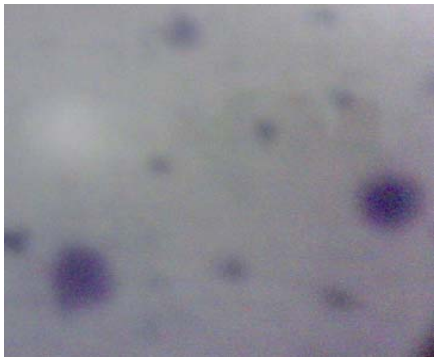

**Fig. 1: Effect of saline on rat peritoneal mast cells.**

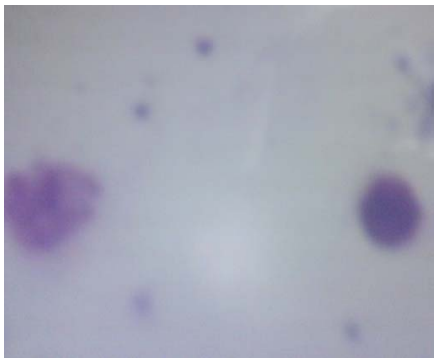

**Fig. 2: Effect of compound 48/80 (1 µg/ml) on rat peritoneal mast cells**

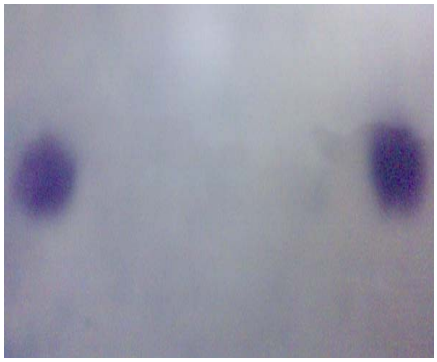

**Fig. 3: Effect of Disodium chromoglycate on compound 48/80 activated rat peritoneal mast cells**

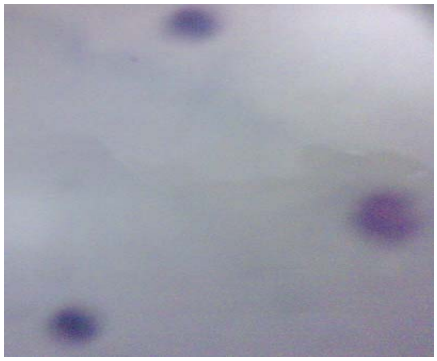

**Fig. 4: Effect of *Cynodon dactylon* compound (10 µg/kg p.o) on compound 48/80 rat peritoneal mast cells**

**Fig. 5: Effect of *Cynodon dactylon* compound (25 µg/kg p.o) on compound 48/80 activated rat peritoneal mast cells**

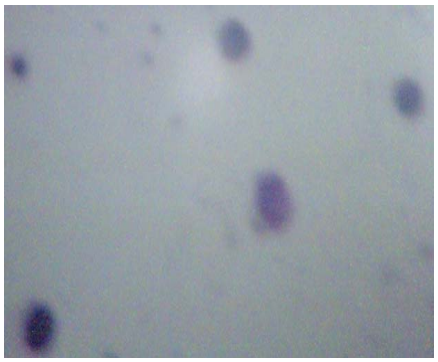

**Fig. 6: Effect of *Cynodon dactylon* compound (50 µg/kg p.o) on compound 48/80 activated rat peritoneal mast cells**

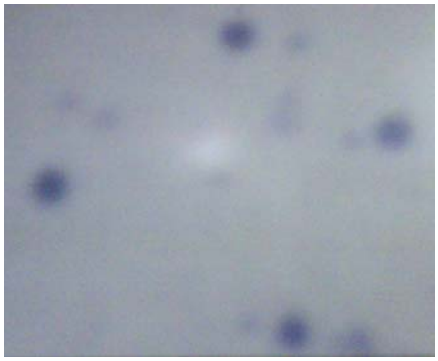

**Fig. 7: Effect of *Cynodon dactylon* compound (100 µg/kg p.o) on compound 48/80 activated rat peritoneal mast cells**

When an allergen interacts with membrane bound IgE produces formation of allergen IgE and FcεRI complex on the surface of mast cells triggering a non-cytotoxic energy dependent release of granular histamine, tryptase and membrane derived lipid mediators including leukotrienes, prostaglandins, platelet activating factor (PAF) and nitric oxide[1]. These mast cell mediators have critical role in anaphylaxis[1]. The compound isolated was significantly inhibited compound 48/80 induced anaphylaxis. It has been proven that, compound 48/80 administration stimulates mast cells and initiates activation of signal transduction pathway leading to release of histamine[6]. The compound 48/80 increases the permeability of lipid bilayer membrane perturbation of the cell membrane there by increasing membrane permeability leading to release of mediators from mast cells[6]. Prophylactic treatment of *Sprague-Dawley* rats for 5 days earlier to exposure of mast cells to compound 48/80 significantly inhibited the effect. The mast cell stabilizing activity of the compound was significant, potent and better than the results obtained with disodium-chromoglycate (DSCG). Thus, the mast cell stabilizing activity was similar to the activity of DSCG[3]. Recently, it has been reported that, naturally occurring isoforms of allergens from plants and trees reduces the capacity of allergens to be bound by IgE as a result of the substitution or deletion of amino acids[2]. This could be the reason for mast cell stabilizing activity of the fraction.

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**REFERENCES**

1. Kay AB. Allergy and allergic diseases. N Engl J Med 2001; 344:109-113.
2. Kay AB. Allergy and allergic diseases. N Engl J Med 2001; 344:30-37.
3. Singh R, Nath A, Gupta PP, Shulka M, Khare SK and Kundu B. et al: Antiallergic/antiasthmatic activity of oligopetide related to IgE. Pharmaco Res1998; 37:350-356.

4. Munoz S, Pando RH, Abraham SN, Enciso JA: Mast cell activation by mycobacterium tuberculosis mediator release and role of CD48. J Immunol 2003; 170: 5590-5596.
5. McCauley SD, Gilchrist M, Befus AD. Nitric oxide a major determinant of mast cell phenotype and function. Rio de Janeiro 2005; 100:11-14.
6. Moon PD, Choi IY, Na HJ, Jeong HJ, Kim CH and Kim HR, et al: Rubus croceacanthus leveile inhibits mast cell mediated anaphylactic like reaction and tumor necrosis factor-α secretion. Biol Pharm Bull 2004; 27:1359-1363.
7. Shefler I, Eisenberg RS: $G_i$ – mediated activation of the Syk kinase by the receptor mimetic basic secretagogues of mast cells: role in mediating arachidonic acid/metabolites release. J immunol 2001; 167: 475-481.
8. Nadkarni AK. KM. Nadkarni's Indian Materia Medica. Vol. I. Bombay: Popular Prakashan, 1976; 425- 426.
9. Qudhia, P: Traditional medicinal knowledge about useful herb doobi in Chhattisgarh. India 1998. (http:/botanical.com/site/column_poudhia/111-doobi.html).
10. Unrestricted: Chapter3. 2007. (http://adt.curtin.edu.au/theses.pdf)
11. Kim SH, Choi CH, Kim SY, Eun JS and Shin TY: Anti-allergic effects of Artemisia iwayomogi on mast cell-mediated allergy model. Experimental Biology and Medicine 2005; 230:82-88.
12. Yi JM, Hong SH, Lee HJ, Woon JH, Kim JM and Jeong DM. et al. Ixeris dentate green Sap inhibits both compound 48/80 induced anaphylaxis–like response an IgE-mediated anaphylactic response in murine model. Biol Pharm Bull 2001; 25: 5-9.
13. Ridnour LA, Sim JE, Hayward MA, Wink DA, Martin SM and Garry R. et al: A Spectrophotometric method for the direct detection and quantitation of nitric oxide, nitrite and nitrate in cell culture media. Anal Biochem 2000; 281:223-229.