WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 6, 1017-1028. Research Article ISSN 2277– 7105

ANTIALLERGIC, ANTIANAPHYLACTIC & MAST CELL STABILIZING POTENTIAL OF *VITIS VINIFERA* IN MANAGEMENT OF ASTHMA

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Article Received on
04 April 2015,

Revised on 24 April 2015,
Accepted on 20 May 2015

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ABSTRACT

The anti-allergic, anti-anaphylactic and mast cell stabilizing activity of ethanolic extract of *Vitis vinifera* (VV) was evaluated pharmacologically by using milk induced leukocytosis and eosinophilia in mice, compound 48/80 induced mast cell degranulation and egg albumin induced passive paw anaphylaxis in rats. In the milk induced eosinophilia and leukocytosis, VV showed an significant decrease in the no. of eosinophils and leukocyte count in mice; in the compound 48/80 induced mast cell degranulation VV showed significant protection of mast cells in rats, and in the egg albumin induced passive paw anaphylaxis, VV showed significant reduction in the paw edema volume. These results suggest that VV may prove to be a potential therapeutic agent in management of asthma which may be due to anti-stress, mast cell stabilizing and anti-inflammatory activity.

KEYWORDS: compound 48/80, milk, egg albumin, anaphylaxis.

1. INTRODUCTION

Asthma is a chronic pulmonary disorder which is characterized by airway inflammation and remodeling that leads to reversible air way obstruction. Asthma belongs to the category of classical allergic diseases which generally arise due to IgE mediated hypersensitivity to environmental triggers. Asthma is widely recognized as a chronic inflammatory lung diseases characterized by reversible bronchoconstriction, elevated basal airway tone, eosinophils and lymphocyte accumulation and activation, epithelial cell dysfunction and damage, smooth

muscle and submucosal gland hypertrophy, submucosal fibrosis, airway wall edema, mucus over production and episodes of non-specific airway hyper responsiveness to spasmogens.[1]

Vitis vinifera Linn. belonging to family Vitaceae, is commonly known as grapes. The leaves, due to their astringent and haemostatic properties, are used in the treatment of diarrhea, haemorrhage and varicose veins, and the juice of leaves has been used for eye washing. Traditionally, the leaves are utilized in treatment of jaundice and as tonic and being used in treatment of asthma.[2, 6] This plant has been reported for is hepatoprotective[3], antidiabetic, antioxidant[4], antimicrobial, antiviral, anticarcinogenic activity.[5]

Thus on the basis of the traditional claims and reported pharmacological activities of *Vitis vinifera*, the present study was designed to evaluate antiallergic, antianaphylactic & mast cell stabilizing activity of *Vitis vinifera* plant in treatment of asthma.

2. MATERIALS AND METHODS

2.1 Experimental Animals

The rat and mice of either sex were purchased from National Toxicology Center, Pune. They were housed in group of five under standard laboratory conditions of temperature (25 ± 2°C) and 12/12 hr light/dark cycle. Animals had free access to standard pellet diet (Amrut laboratory animal feed, Sangli-Maharashtra.) and water *ad libitum*. The distribution of animals in the groups, the sequence of trials and the treatment allotted to each group were randomized, throughout the experiment. Laboratory animal handling and experimental procedures were performed in accordance with the guidelines of CPCSEA (CPCSEA NO.198/99) with IAEC clearance proposal number DYPIPSR/IAEC/12-13/P-10.

2.2 Procurement of Plant Material

The *Vitis vinifera* leaves were collected from the local market of Nashik and the sample was authenticated from Botonical survey of India, Pune. (Voucher no- RGKVIV1 dated 5th June 2013).

2.3 Preparation of Extract

Shed dried leaves of *Vitis vinifera* (1000 g) were powdered and extracted with ethanol 90% at room temperature (5 L X 6 times). Combined ethanol extract was concentrated to dryness with rotary evaporator under pressure and controlled temperature (50-60^{0}c) to yield dark

brown semi-solid mass of ethanolic extract of *Vitis vinifera* (VV). This was further dried under vacuum oven drier to give solid residue.[4]

2.4 Preliminary Phytochemical Screening

The qualitative preliminary phytochemical screening of VV was performed to find out the presence of various phytochemicals such as steroids, flavenoids, triterpenoids, glycosides, saponins, carbohydrates and alkaloids.[7]

2.5 Acute toxicity study and Dose selection

Albino rats of either sex weighing 200-250 gm. were used in the study. Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD)-423 guideline.[8] The animals were divided in 4 groups (n=3) and were fasted overnight prior to drug administration. Following the period of fasting, the animals were weighed and the test substance was administered. The animals were administered with test extract at the dose of 5, 50, 300 and 2000 mg/kg body weight orally. The animals were observed for 5 min every 30 min till 2 h and then at 4, 8 and 24 h after treatment for any behavioral changes/mortality. They were further observed daily for 7 days for mortality. No mortality up to 7 days after treatment was observed with the ethanolic extract of *Vitis vinifera* leaves at the dose of 2000 mg/kg, p.o. and therefore they were found safe up to dose of 2000 mg/kg. Thus the animal doses were selected on the basis of acute oral toxicity study. Hence, 1/10th (200 mg /kg) of this dose was selected for this study. For the doses of mouse, the dose of rat was extrapolated by using conversion factor i.e., 140,280,560 (mg/kg, p.o.).[9]

2.6 Milk induced leukocytosis and eosinophilia in mice[10]

Mice were divided into five groups (n=5). Animals belonging to group I served as control and were administered with only boiled and cooled milk (4ml/kg, s.c.); group II served as standard and were administered Dexamethasone (50mg/kg); group III to group V served as test and were received respective doses of ethanolic extract of *Vitis vinifera* leaves (VV) and 1 hr later boiled and cooled milk (4ml/kg, s.c.) was administered to the same animals. After 24 hr, blood samples were collected from the retro orbital plexus of each animal under light ether anesthesia. Total leukocyte and eosinophils count was recorded in each group 24 hr after milk injection.

2.7 Compound 48/80 mast cell degranulation in rats[11]

Rats were divided into five groups (n=5). On the 1st day of sensitization, all the animals from each group were injected with Compound 48/80 (1mg/kg, s.c.). Animals belonging to group I was administered with distilled water (10 ml/ kg, p.o.). Animals belonging to group II served as standard and were administered Ketotifen fumarate (1mg/kg, p.o.). Animals belonging to group III to V served as test and group and were administered respective doses of ethanolic extract of *Vitis vinifera* leaves (VV) for 15 days. On day 15th, 2 hour after the assigned treatment, mast cells were collected from the peritoneal cavity. Ten ml of normal saline solution was being injected into peritoneal cavity and abdomen was gently massaged for 90 seconds. The peritoneal cavity was carefully opened and the fluid containing mast cells were aspirated and collected in siliconised test tube containing 7 to 10 ml of RPMI-1640 Medium (pH 7.2- 7.4).The mast cells were then washed thrice by centrifugation at low speed (1000-5000 rpm) and the pallets of mast cells were taken in the RPMI-1640 medium. The mast cell suspension (approximately 1 x 10^6 cells/ml) was challenged with 5 µg/ml of compound 48/80 solution and stained with 0.1 % toluidine blue and observed under high power microscope (45 X).Total 100 cells were counted from different visual areas. The numbers of intact and degranulated cells were counted and the percent protection was also calculated.

2.8 Passive paw anaphylaxis in rats[12]

The rats were divided into five groups (n=5). Antiserum to egg albumin was raised in rats by using aluminum hydroxide gel as an adjuvant. Animals were given three doses of 250 µg of egg albumin (s.c.) adsorbed on 12 mg of aluminum hydroxide gel prepared in 0.5 ml of saline on 1st, 3rd and 5th day. On 10th day of sensitization, the blood was collected from the retro orbital plexus under light ether anesthesia. The collected blood was allowed to clot and serum is separated by centrifugation at 1500 rpm. The animals were passively sensitized with 0.1 ml of the undiluted serum into the left hind paw. The right hind paw was received an equal volume of saline. Animals belonging to group I served as control and was administered with only distilled water (10 ml/kg, p.o.). Animals belonging to group II were administered Dexamethasone (0.5 mg/kg, i.p.), whereas animals belonging to Group III to Group V received ethanolic extract of *Vitis vinifera* leaves (VV) at different concentrations respectively 24 hr after sensitization. One hr. after test drug administration, animals were challenged by giving 10 µg of egg albumin in 0.1 ml of saline in the left hind paw and the paw inflammation was measured by using a Plethysmometer (UGO Basile, 7140). The

difference in the reading prior to and after antigen challenge represents the edema volume and the percent inhibition of edema was calculated by using the formula,

$$\% \text{ Inhibition} = [1-(T / C)] \times 100$$

Where, T-Mean relative change in paw volume in test group.

C- Mean relative change in paw volume in control group.

2.9 Statistical analysis

The results were expressed as Mean ± SEM and statistically analyzed by one-way analysis of variance (ANOVA) followed by Dennett's test and $p < 0.05$ was considered significant

3. RESULTS

Phytochemical Screening

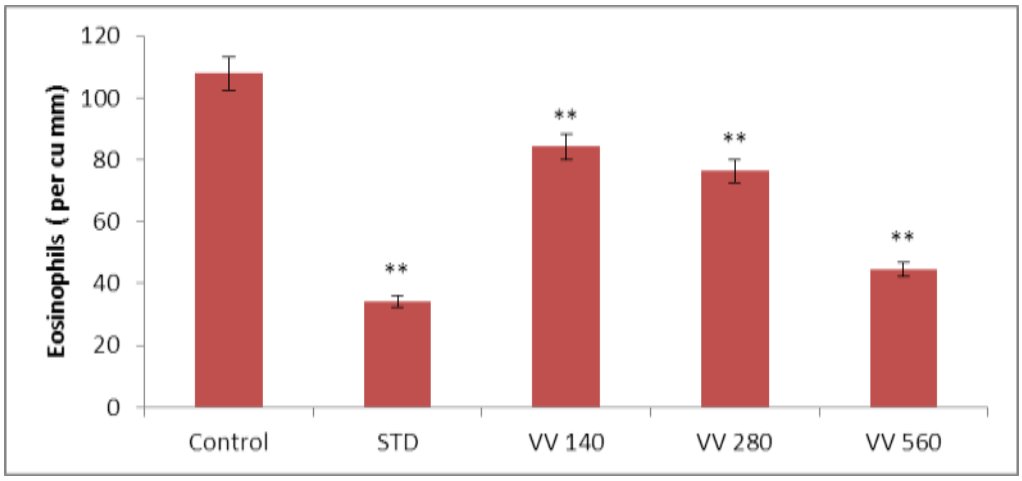
The phytochemical investigation of VV showed the presence of alkaloids, glycosides, triterpenoids, proteins, carbohydrates and flavonoids.

Acute Toxicity Study

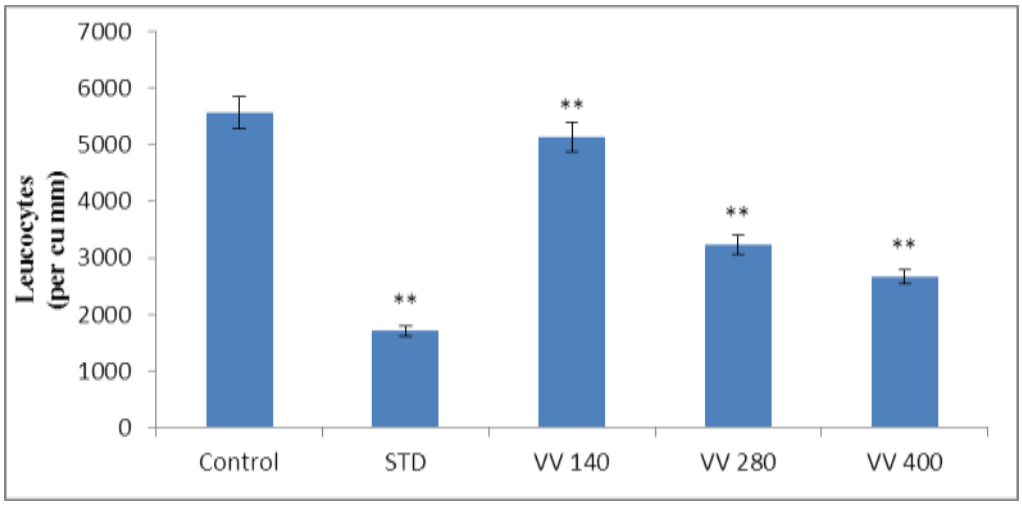
Dose was selected based on basis of acute oral toxicity study done on ethanolic extract of *Vitis vinifera*. Extract was found to be safe up to the dose level 2000 mg/kg. There was no behavioral abnormality and zero mortality was recorded till 48 h post treatment with no signs of acute toxicity. Therefore $1/10^{th}$ of the dose 2000 mg/kg of ethanolic extract of *Vitis vinifera* was selected i.e., 200 mg/kg as middle dose in rats. In rats, three doses were used, i.e., 100, 200, and 400 mg/kg (1/20th; 1/10th; and 1/5th of 2000 mg/kg, the highest dose used in acute toxicity study). Whereas, in mice, the doses were 140, 280 and 560 (i.e., dose used in rat X 1.4, as suggested by Ghosh).[9]

Effect VV on milk induced leukocytosis and eosinophilia in mice

Subcutaneous injection of milk at dose of 4 ml/kg produced a significant increase in the total leukocytes and eosinophil count after 24 hr of its administration. Animals treated with Dexamethasone at the dose of 50 mg/kg, p.o. has significantly ($p<0.01$) inhibited milk induced leukocytosis and eosinophilia as compared to control. In the groups mice, pretreated with ethanolic extract of *Vitis vinifera* at doses 140, 280 and 560 mg/kg, there was significant ($p<0.01$) inhibition of milk induced leukocytosis and eosinophilia as compared to control.



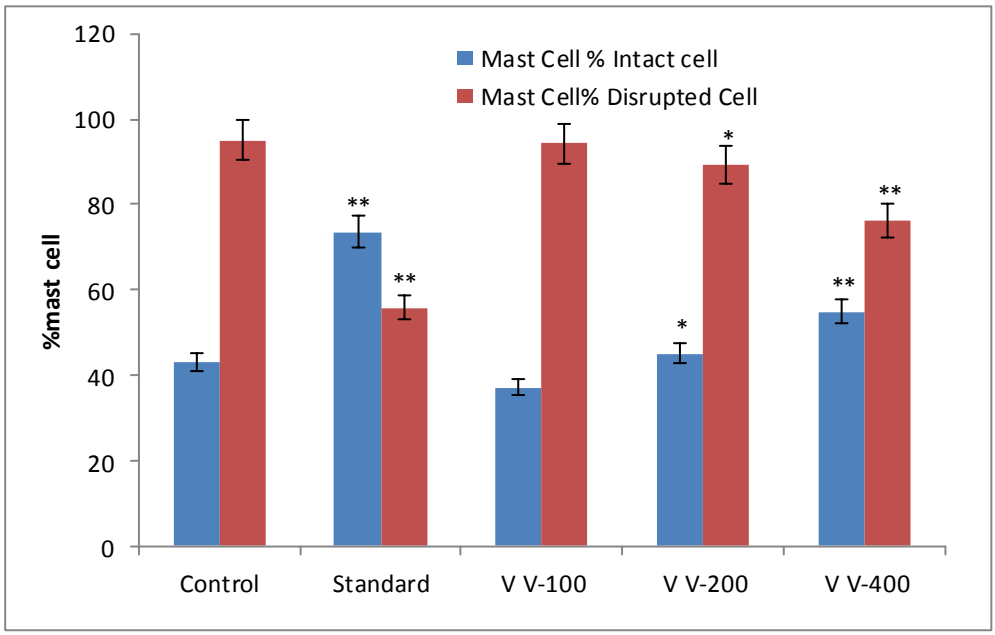
Graph 1: Effect VV on milk-induced eosinophilia in mice.



Graph 2: Effect of VV on milk-induced leukocytosis in mice

3.2 Effect of VV on Compound 48/80 induced mast cell degranulation in rats

Compound 48/80 (1 mg/kg, s.c.) induced mast cell degranulation was significantly (p<0.01) inhibited by Ketotifen fumarate (1 mg/kg, i.p.) and the percent protection was found to be 41.27%. The groups pretreated with ethanolic extract of *Vitis vinifera* at the dose of 100 mg/kg, p.o. has not shown any significant protection of mast cells and the percent protection was found to be 0.64 %. The groups pretreated with ethanolic extract of *Vitis vinifera* at the dose of 200 mg/kg, p.o., has shown significant protection (p< 0.05) of mast cells and the percent protection was found to be 6.11 %. Also the dose of 400 mg/kg, p.o. has shown significant protection (p< 0.01) of mast cells and the percent protection was found to be 19.79%



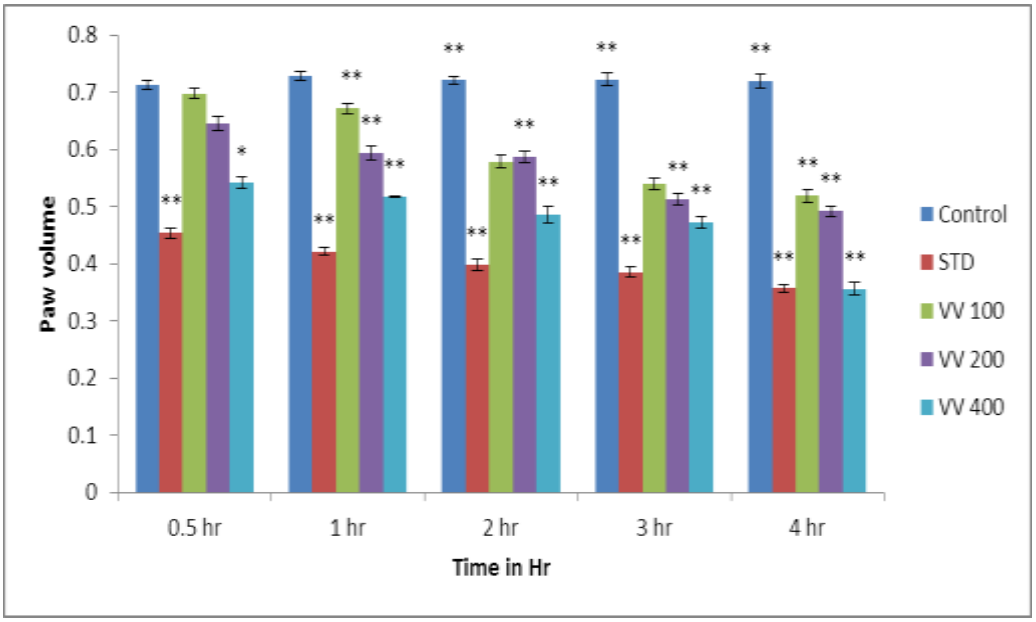
Graph 3: Effect of VV on Compound 48/80-induced mast cell degranulation in rats

3.3 Effect VV on Passive Paw Anaphylaxis in Rats

Antiserum to egg albumin was injected 24 hr before administration of the test drugs or standard. Egg albumin was injected after the administration of ethanolic extract *Vitis vinifera* leaves and Dexamethasone. In the vehicle or distilled water treated group, egg albumin increased the paw volume in the sensitized animals, which was measurable up to the time period of 4 hrs. Dexamethasone at the dose of 0.5 mg/kg, i.p. significantly ($p<0.01$) reduced the paw edema volume at 0.5, 1, 2, 3 and 4 hrs time intervals and the percentage inhibition was found to be 36.23%,42.17%, 44.79%, 46.67% and 50.34% respectively.

Pretreatment with the ethanolic extract *Vitis vinifera* leaves at the dose of 100 mg/kg, p.o., there was no significant reduction in the paw edema volume at 0.5 hr but significant ($p<0.01$) reduction in the paw edema volume at 1, 2, 3 and 4 hrs. The percentage inhibition was found to be 1.97%, 7.69 %, 19.69%, 25.34% and 27.81% for 0.5, 1 ,2 3 and 4 hr time interval respectively. Pretreatment with the ethanolic extract *Vitis vinifera* leaves at the dose of 200 mg/kg, p.o., there was no significant reduction in the paw edema volume at 0.5 hr but significant ($p<0.01$) reduction in the paw edema volume at 1, 2, 3 and 4 hrs. The percentage inhibition was found to be 9.41%, 18.4 %, 18.58%, 28.94% and 31.57% for 0.5, 1 ,2 3 and 4 hr time interval respectively. The ethanolic extract *Vitis vinifera* leaves at the dose of 400 mg/kg, p.o. significantly reduced the paw volume at 0.5 ($p<0.05$), 1 ,2 3 and 4 hr ($p<0.01$)

time interval and the percentage inhibition was found to be 24.01%, 28.84%, 32.59%, 34.48% and 50.48% respectively.



Graph 4: Effect of VV on passive paw anaphylaxis in rats

DISCUSSION

Ayurveda provides number of herbs for the treatment of asthma and herbal formulations, which include some anti-stress herbs to enable adoption to stress since excessive stress may aggravate symptoms of asthma.[13] Adaptogens are the medicinal substances that are meant to put an organism into a state of non-specific heightened resistance in order to better resist stress and adaptation to external challenges. An important feature of the adaptogen is their capacity to increase organism's resistance to various adverse effects of a physical, chemical and biological nature. An adaptogen may produce normalization that reveals itself irrespective of the direction of previous pathologic shift. After parental administration of milk, there is increase in the total number leukocyte count. This stress full condition can be normalized by administration of an adaptogenic drug.[14]

Eosinophils are one of the major effector cells in asthma, because eosinophils can influence airway function. Though eosinophils can increase in number in body fluids and tissues, emphasis is placed on the number of eosinophils in blood. Most allergic and non-allergic asthmatics, including those with mild asthma, have bronchial eosinophilia and there is a significant association between eosinophil activation and asthma severity as well as bronchial hyper responsiveness by producing effects on airway remodeling through release of cytotoxic

proteins, lipid mediators, oxygen free radicals, cytokines and transforming growth factor- β. The involvement of eosinophils into bronchial mucosa, in which allergic inflammation occurs, is a critical contributor to the late asthmatic reaction of congestion and mucus hypersecretion. Eosinophil degranulation is an important immunologic mechanism leading to allergic inflammation in cutaneously manifested cow's milk allergy. When these cells arrive, they degranulate and perpetuate underlying airway inflammation. The late asthmatic phase reaction to an allergen often coincides with an increased number of eosinophils in the airway.[15]

In the present study it has been found that, after 24 hour of parental administration of milk (4 ml/kg, s.c.) to the vehicle treated group, there was significant increase in the total eosinophil and leukocyte count; whereas the groups treated with ethanolic extract of *Vitis vinifera* restored the total eosinophil and leukocyte count. This probably indicates the adaptogenic activity of *Vitis vinifera* which may contribute in antiasthmatic activity.

A mast cell is a resident cell of several types of tissues and contains many granules rich in histamine. Although best known for their role in allergy and anaphylaxis, mast cells play an important protective role as well, being ultimately involved in wound healing, anti-inflammatory activity and defence against pathogens.[16] The pathological mechanisms involved in Type-I allergy has been explained as the degranulation of mast cells, followed by the release of mediators such as histamine, leukotrienes and prostaglandins from these cells.[14] The degranulation of mast cells occurs in response to the immunological stimuli in which the antigen–antibody reaction on the cell surface predominates. The mast cell stabilizing effect may be due to stabilization of mast cell membrane or inhibition of antigen-induced histamine release.

Compound 48/80 is a mixed polymer of phenethylamine cross-linked by formaldehyde, induces almost a 90% release of histamine from mast cell after degranulation of mast cell. Hence compound 48/80 has been used as a direct and convenient reagent to study mechanism of allergy and anaphylaxis.[17] Stimulation of mast cell with compound 48/80 is believed to initiate the activation of a signal transduction pathway, which leads degranulation of mast cell and histamine release by the process of exocytosis which requires energy and Ca^{2+} ions. Compound 48/80 initiates the generation of superoxide anion by A-kinase inactivation through decreasing the intracellular cAMP concentration in mast cells. Generated superoxide anion results in the inositol 1, 4, 5- triphosphate or GTP induced calcium release from

endoplasmic reticulum which increases intracellular calcium content, which leads to histamine release from mast cells by degranulating the mast cells.[18] Thus, compound 48/80 has been used as a direct and convenient reagent to study the mechanism of allergy and anaphylaxis. Compound 48/80 induces a rapid release of inflammatory mediators only from one type of mast cells, namely rat peritoneal mast cells.

In present study, the ethanolic extract of *Vitis vinifera* leaves has significantly reduced the compound 48/80 induced mast cell degranulation and probably the subsequent release of histamine and further array of inflammatory cytokine. Thus, *Vitis vinifera* may possess the mast cell stabilizing activity which may contribute to its antiasthmatic activity.

Allergic asthma is an aberrant lung immune response, in which an immunological response is generated by harmless inhaled antigens. Allergens are protein in nature such as ovalbumin, plant pollen, house dust and microorganism such as *Aspergillus fumigatus*. Allergic asthma is a chronic inflammatory process occurring due to exposure of allergen.[19] Egg albumin acts as an antigen. Thus, administration of egg albumin (s.c.) to rat raises the antiserum to egg albumin in the plasma and sub plantar injection of plasma containing these antibodies, then challenged with egg albumin leads to passive paw anaphylaxis in rats. This leads to activation of T-Lymphocytes, which releases various inflammatory mediators such as histamine, PAF, bradykinin etc. and edema is formed due to increased flow of neutrophils from the blood.[20]

The present study revealed that in the animals pretreated with *Vitis vinifera* extract had significantly reduced the paw volume at all the time intervals in the model of passive paw anaphylaxis in rats. The beneficial effect of *Vitis vinifera* extract could be due to either inhibition of antigen-antibody complex which may contribute to antiasthmatic activity.

During Preliminary photochemical evaluation *Vitis vinifera* leaves have shown presence of flavonoids. Flavonoids are known to possess anti-inflammatory and anti-oxidant activities.[21] Thus, the presence of these phytoconstituents in the ethanolic extract of *Vitis vinifera* leaves may further contribute in anti-allergic and anti-anaphylactic activities in the management of asthma.

CONCLUSION

Thus, it can be concluded that in the present investigation ethanolic extract of *Vitis vinifera* leaves may possess significant antiasthmatic activity. The anti-asthmatic activity of ethanolic

extract of *Vitis vinifera* leaves can be attributed due to antiallergic, anti-inflammatory, adaptogenic and mast cell stabilizing activity which can be further suggestive for its potential in prophylaxis and management of asthma.

ACKNOWLEDGMENT

Authors are very much thankful to Dr. S. S. Chitlange, Principal, Padm. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune, for providing good laboratory facilities and equipments**.**

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