Evaluation antiasthmatic potential of spray dried powder of fresh juice of leaves of *Brassica oleracea* variety *capitata*


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ABSTRACT

Asthma is a chronic inflammatory disorder of the airways. Current pharmacotherapy does not target various stages of asthma resulting in incomplete cure and is also associated with limited use in clinical practice, because of their adverse effect profile. As a result, there is continuous search especially from natural sources. *Brassica oleracea* (*B. oleracea*) is known traditionally to possess anti-asthmatic activity. Hence, the present investigation was undertaken to evaluate the effect of spray dried powder of fresh juice of leaves of *Brassica oleracea* (SBOCJ). Experimental models studied were histamine and acetyl choline induced bronchospasm in guinea pigs, bronchoalveolar lavage fluid (BALF) in egg albumin sensitized guinea pigs, passive paw anaphylaxis. Spray dried powder of fresh juice of leaves of *B. oleracea* (500 mg/kg, p.o., for 7 days) showed significant protection against histamine aerosol induced bronchospasm and antiedematogenic effect on allergen induced paw swelling. Significant decrease in the total and differential leukocyte counts in BALF was observed on chronic administration of spray dried powder of fresh juice of leaves of *B. oleracea* (500 mg/kg, p.o., for 15 days). These results suggest that spray dried powder of fresh juice of leaves of *B. oleracea* possesses not only bronchodilator activity but also decreases bronchial hyperresponsiveness by decreasing the infiltration of inflammatory mediators like eosinophils, neutrophils in BALF.

Keywords: asthma, inflammation, bronchoalveolar lavage, inflammatory cells, *Brassica oleracea*
INTRODUCTION

Asthma is a triad of intermittent airway obstruction, bronchial smooth muscle cell hyperreactivity to bronchoconstrictors and chronic bronchial inflammation. From an etiological standpoint, asthma is a heterogeneous disease, but often appears as a form of immediate hypersensitivity [1]. The available treatment options have major limitations owing to low efficacy, associated adverse events and compliance issues. As a result, there is continuous search especially from natural sources. In the recent years, many herbs, herb compounds, plants, foods ingredients etc. have been shown to have anti-allergic activity, some of which are used as antiallergic supplements. Some nutrients in food, drinks, or supplements have antiallergic activities and moreover, flavonoids present in vegetables, fruit and teas, have been used to treat a variety of human diseases since ancient times [2,3].

Fresh juice of *Brassica oleracea var. capitata* Linn. (Family Brassicaceae; Syn. - Cabbage) is one of the hallowed folk remedy. Cabbage is rich in vitamins, potassium, sulphur and copper plus a variety of antioxidants, flavonoids etc [4]. A systematic, scientific pharmacological evaluation to confirm the claims of activity of cabbage has not been reported to our knowledge. The present study was hence undertaken to carry out pharmacological evaluation of spray dried powder of fresh juice of leaves of *B. oleracea* with special reference to antiasthmatic activity using various experimental models.

MATERIALS AND METHODS

Collection and identification of Plant Material

Fresh leaves of *Brassica oleracea*, variety: *capitata* (Syn: Cabbage) were obtained from the local market and identified and authenticated by Dr. K. B. Kathiria, Research Scientist, Main Vegetable Research Station, Anand Agricultural University, Anand, Gujarat.

Experimental Animals

All animals (Albino Wistar rats weighing 250-300g; Hartley strain guinea pigs (350-500 gm) were housed at ambient temperature (21±1°C) and relative humidity (55±5%) with fixed 12 h light/dark cycles. Animals were fed with a standard pellet diet (Pranav Agro Ltd., Baroda) and were provided with water *ad libitum*. Guinea pigs were fed with green vegetables. The experimental protocol (Project no.7007) was approved by Institutional Animal Ethical Committee of animal house of Anand Pharmacy College as per the guidance of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. The distribution of animal in the groups, the sequence of trials and the treatment allotted in each group were randomized.
EXPERIMENTAL

Preparation of spray dried powder (SBOCJ) of fresh juice from leaves of B. oleracea (BOCJ)

Fresh leaves of B. oleracea variety capitata were homogenized in an EIE high speed Teflon coated micro tissue homogenizer at low speed. The pulp was strained through muslin cloth to obtain the fresh juice (75 % w/v). Juice obtained through this process is referred as BOCJ. Spray dried powder from BOCJ was prepared by Pharmanza Herbals Pvt. Ltd., using Lab plant spray drier model SD05. Spray dried powder obtained through this process is referred as SBOCJ.

Histamine induced bronchospasm in guinea pigs [5]

Guinea pigs were randomly allocated to two groups containing six animals each. On day 0, the animals of group I and group II were placed in an aerosol chamber and exposed to 0.05% histamine dihydrochloride aerosol under constant pressure (1 kg/cm$^2$). The time for preconvulsive dyspnoea (PCD) was noted. The end point for PCD was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions (Sheth et al., 1972). As soon as PCD commenced, the animals were removed from chamber and placed in fresh air to recover. This time for PCD was considered as day 0 value. After 15 days of wash out period, Group I (standard) received ketotifen (0.5 mg/kg). The animals of group II were administered with solution of SBOCJ (500 mg/kg, p.o.). Two hours later, all the experimental animals were exposed to 0.05% histamine dihydrochloride aerosol and time for PCD was noted. The % increase in time of PCD was calculated using following formula [6],

$$\text{Percentage increase in time of PCD} = \left(1 - \frac{T_1}{T_2}\right) \times 100$$

where, $T_1$ = time for PCD onset on day 0, $T_2$ = time for PCD onset on day 15

Bronchoalveolar lavage

Hartley strain guinea pigs were randomly allocated to four groups of six animals each i.e. group-I (control), group-II (sensitized) and group-III (sensitized + SBOCJ). The animals of group II and III were sensitized with egg albumin (1 ml, 10% w/v, i.p.) in saline on the first day. The animals of group I received 0.5% CMC for fifteen days. The animals of group III were dosed with solution of SBOCJ (500 mg/kg, p.o., for 15 days, once daily). Fifteen days later, animals of Group II and III were challenged with egg albumin (0.5 ml, 2% w/v) through the saphenous vein. Animals which did not respond were excluded from the study. After 3 h of the challenge of egg albumin or just prior to death of animals, which ever was earlier, the animals were anaesthetized with diazepam (8 mg/kg, i.p.) and ketamine (5 mg/kg, i.p.).
According to the method of Thomas et al., (1995) [7], the trachea was immediately cannulated after anaesthetization and the airways lavaged with saline at 25°C (two aliquots of 1 ml/100 g body weight). To standardize the lavage technique, 60% of the instilled medium was withdrawn from each animal. Bronchoalveolar cells were collected in two successive lavages using saline and recovered through a tracheal cannula. The lavage fluid was stored on ice and total WBC cell counts were performed using an automated cell counter (Cell Dyn 3200SL). Dilutions of lavage fluid (1 in 10) were made in saline, and differential WBC cell counts were made by light microscopy stained with Leishman’s stain. At least 200 cells were counted on each slide. Cells were differentiated using standard morphological criteria. All differential cell counts were performed blind and in randomized order at the end of the study.

**Passive paw anaphylaxis**

**Preparation of antiserum**

Wistar rats of either sex selected randomly (n=6) were injected intraperitoneally with 0.2 ml 10 % w/v egg albumin and 0.2 ml of *Bordetella pertussis* vaccine on day 1, 3 and 5. Twenty one days after the first immunization, rats were anesthetized with diazepam (8 mg/kg, i.p.) and ketamine (5 mg/kg, i.p.), and blood was withdrawn from the carotid artery. The collected blood was allowed to clot and serum was separated by centrifugation at 1500 rpm. The separated serum was stored at -20°C until it was used for further experiment.

**Sensitization and hypersensitivity**

Wistar albino rats were randomly allocated to six groups each containing six animals. Group I (model control) and Group II (standard) received 0.5% sodium carboxy methyl cellulose orally and ketotifen (1 mg/kg) respectively. Group III (test) received spray dried powder of *B. oleracea* (500 mg/kg, p.o.) once daily for seven days respectively. Two hours after the last dose of administration on seventh day, rats were passively sensitized into the left hind paw with 0.1 ml of undiluted serum. Twenty four hours after sensitization, the rats were challenged in the left hind paw with 10 µg of egg albumin in 0.1 ml saline by intraplantar injection. The hind paw volume was measured after 30 min by volume displacement method using mercury column plethysmometer [8]. The difference in the reading prior to and after challenge represents the edema volume, and the anti-anaphylactic effect was expressed as the percentage inhibition.

**Statistical analysis**

Data are expressed as mean ± standard error of mean (SEM). Data were analyzed statistically using one way analysis of variance (ANOVA) with Dunnett post hoc test to find out the level of significance. In experiments with histamine induced bronchospasm, paired t-test was used to make a statistical comparison between the mean values. Data were considered statistically significant at P < 0.05.
RESULTS

Effect on histamine induced bronchospasm in guinea pigs

Table 1: Effect of SBOCJ on histamine induced bronchospasm

<table>
<thead>
<tr>
<th>GROUP</th>
<th>PRE CONVULSION TIME (minutes)</th>
<th>PERCENT PROTECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEFORE TREATMENT</td>
<td>2.11±0.4915</td>
<td>-</td>
</tr>
<tr>
<td>AFTER TREATMENT</td>
<td>3.46±0.8644*</td>
<td>39.02%</td>
</tr>
</tbody>
</table>

Statistical Analysis by Paired t test
Values are mean ± SEM; n=6 in each group
Significant difference between before and after treatment * P< 0.05

Exposure to 0.05% histamine aerosol resulted in preconvulsive dyspnoea (PCD) similar to bronchospasm. Pre-treatment with SBOCJ (500 mg/kg, p.o., for 7 days) significantly prolonged the latent period of PCD (39.02 %; p< 0.05) as compared to the control, following exposure to histamine aerosol (Table 1).

Effect on Bronchoalveolar Cell Counts

Table 2: Effect of SBOCJ on total and differential count in BALF

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>Sensitized</th>
<th>Sensitized+SBOCJ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Leucocyte Count /cmm</td>
<td>5500±330.7</td>
<td>14500±483*</td>
<td>5816.7± 425.4*</td>
</tr>
<tr>
<td>Eosinophil Count /cmm</td>
<td>148.2±16.29</td>
<td>375±32.15*</td>
<td>246.3± 42.53</td>
</tr>
<tr>
<td>Monocyte Count /cmm</td>
<td>95.8±17.14</td>
<td>161±22.58*</td>
<td>109.6± 14.65</td>
</tr>
<tr>
<td>Neutrophil Count /cmm</td>
<td>3416.7±366.8</td>
<td>7073±464.7*</td>
<td>3595.3± 346.6*</td>
</tr>
<tr>
<td>Lymphocyte Count /cmm</td>
<td>1746±39.9</td>
<td>2030.8±56.3*</td>
<td>1975± 188.7</td>
</tr>
</tbody>
</table>

Statistical Analysis by ANOVA and Dunnett post hoc test
Values are mean ± SEM, n=6 in each group
Significantly different from control group *P<0.05
Significantly different from sensitized group @ P<0.001

All guinea pigs used in this study exhibited a marked reaction when challenged with antigen characterized by acute dyspnoea. There was a highly significant increase in the total and differential leucocyte count in the sensitized group as compared to control group. Pretreatment with SBOCJ (500 mg/kg, p.o., for 7 days, P<0.05) significantly inhibited the rise in total leucocyte count, neutrophil count but produced a nonsignificant decrease in eosinophil, monocyte and lymphocyte count as compared to sensitized group (Table 2).

Effect on passive paw anaphylaxis:

A marked inflammatory response in the form of paw edema was developed after 30 minutes on intraplantar administration of egg albumin into the hind paw of rats passively sensitized with serum containing IgE and these edema volumes measured plethysmometrically.
were significantly higher in model control animals. A significant inhibition (68.40 %, 61.51 %, P<0.001) of egg albumin induced paw edema was observed by pretreatment with ketotifen (1 mg/kg, p.o.) and spray dried powder of fresh juice of leaves of *B. oleracea* (500 mg/kg, p.o., for 7 days) respectively as compared to control group (Table 3).

Table 3: Effect of SBOCJ on passive paw anaphylaxis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% edema observed after 60 minutes</th>
<th>% inhibition of edema at 60 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>67.66 ± 3.86</td>
<td>-----</td>
</tr>
<tr>
<td>Ketotifen (1 mg/kg, p.o.)</td>
<td>21.38 ± 1.42*</td>
<td>68.40</td>
</tr>
<tr>
<td>Brassica oleracea (mg/kg, p.o.)</td>
<td>26.04 ± 4.12*</td>
<td>61.51</td>
</tr>
</tbody>
</table>

Statistical Analysis by ANOVA and Dunnett post hoc test
Values are mean ± SEM, n=6 in each group
Significantly different from control group *P<0.05

**DISCUSSION**

Bronchial asthma is marked mainly by two phases, namely; an immediate phase (EAR) consisting mainly of bronchoconstriction and a late phase (LAR) consisting of a special type of inflammation, comprising vasodilation, airway wall edema, mucus secretion and airway hyper-responsiveness [9, 10].

The first major feature, acute bronchospasm (early-phase asthmatic response), an initial event in asthma results from the release of inflammatory mediators like histamine, leukotrienes, prostaglandins on exposure to various allergens, irritants, cold air or exercise [11]. Hence in the present study, histamine (0.05%) and acetyl choline (0.4%) aerosol was used to induce immediate bronchoconstriction [12] that was measured in terms of time for preconvulsion dyspnoea (PCD). In the present study, spray dried powder of fresh juice of leaves of *B. oleracea* prolonged the time of PCD in guinea pigs following histamine aerosol induced bronchospasm. In context to this, the protective effect of SBOCJ may be due to their bronchodilator potential.

The second major feature, inflammation associated with asthma, has received increased attention over the past decades [13]. Bronchial asthma is now no longer simply viewed as reversible airway obstruction or irritable airways. Rather it is now viewed primarily as an inflammatory illness that as a result has bronchial hyperreactivity and bronchospasm. This airway inflammation is now also considered to be an immunologically initiated, mediator driven event, resulting in a cascade of immunologic events that includes antibodies, inflammatory cells and mediators [12].

The antibody involved in allergic asthma is the immunoglobulin, IgE. It is fundamental to the allergic immune response. These IgE antibodies are generally directed against substances that are not harmful to the body, including pollens; fur from cats; certain mold spores; certain
foods; certain drugs; and droppings from microscopic dust mites. After initial exposure of the patient to an allergen, the primary immune response is to generate unique IgE antibodies that become bound to the surface of mast cells [12]. These mast cells live within various tissues of the body, including the bronchi.

If the human is later re-exposed to an allergen by inhalation, the allergen binds to the surface bound IgE on mast cells in the bronchi. Binding of at least two IgE molecules, bridged by a single allergen molecule, is termed cross-linking. This cross-linking of IgE by allergen on the surface of the mast cell is the initial biologic event of an allergic reaction referred as an immediate hypersensitivity reaction (IHR), thereby inducing release of biologically active mediators; the preformed mediators stored in the cytoplasmic granules (histamine and β-hexosaminidase) and the newly synthesized mediators (leukotrienes and cytokines-TNF-α, IL-6) within 30 min [14, 15, 16]. These mediators ultimately cause various symptoms of asthma. In line with this notion, the reaction was replicated in experimental passive paw anaphylaxis model whereby passive immunization with serum containing IgE antibodies and then exposure to second antigen i.e. egg albumin caused an immediate hypersensitivity reaction in the form of paw edema [17]. SBOCJ produced prominent inhibitory effect suggesting its action against inflammation driven immunologically. These results were found comparable to ketotifen, a known antihistaminic and anti-inflammatory agent (68.40% inhibition).

Inflammatory cells are activated as a result of immunologic cascade. The concomitant presence of infiltrating cells, eosinophils, neutrophils and T lymphocytes in the bronchi of asthmatics plays a major role in the development of airway inflammation and the accompanying bronchial hyperreactivity [18, 19, 20]. BAL model involving sensitization using egg albumin followed by exposure to same antigen resulted in anaphylactic shock resembling the acute asthmatic attack causing the release of various inflammatory mediators and cellular infiltration. These inflammatory cells were measured as total and differential cell count in bronchoalveolar lavage fluid in guinea pigs.

Among the inflammatory cells measured, eosinophil is the inflammatory cell most closely associated with asthma. The triggering and regulation of eosinophil accumulation in allergic inflammation depends on the release of cytokines and chemokines such as IL-4, IL-5 and CCL11/eotaxin in response to an antigen challenge [21, 22, 23]. First of all, to participate in the allergic inflammatory response, eosinophil must migrate from the circulation to the airway [24]. Circulating eosinophils migrate to the airways by phenomenon of cell rolling, through interaction with P-selectin, and eventually adhere to the endothelium through the binding of integrins to adhesion proteins, VCAM 1 and ICAM 1. As eosinophils enter the matrix of the membrane, their survival is prolonged by IL-5. Upon reaching the airways, eosinophils can release a variety of cationic proteins contained in its cytoplasmic granules, such as eosinophil peroxidase (EPO) and major basic protein (MBP) that are responsible for injury and shedding of airway epithelium [25,26]. Disruption of the epithelium leads to the exposure of the underlying mucosal structures and sensory nerve endings to allergen and irritants, contributing to the developing of nonspecific bronchial hyperreactivity [27].
Moreover, it is also well-established that there are increased eosinophil numbers in the airways of patients with ongoing asthma, even those with mild disease [28], whilst autopsy and biopsy studies have revealed a characteristic eosinophil infiltration of the airway mucosa [29, 27]. Similar replication was observed experimentally in the present study, where antigen challenge resulted in an increase in the number of eosinophils in pleural fluid and BALF. Similarly, the present findings in BALF showed that treatment with SBOCJ significantly inhibited antigen-induced bronchial hyperreactivity by preventing the increase in eosinophil counts.

Airway hyperresponsiveness after antigen challenge is also supported by the inflammatory pathology involving other mediators in pathogenesis of asthma. As in human asthma, neutrophils are also implicated in the induction of antigen-induced airway hyperresponsiveness in the guinea-pig. Neutrophil numbers have also been reported to increase in BALF [30] in asthmatics, but neutrophilia is generally of shorter duration than eosinophilia [31]. This finding fits well with the current observation that pretreatment of the sensitized animals with SBOCJ resulted in significant reduction of neutrophil counts in BALF compared to sensitized group.

In addition to neutrophils, the predominant cells in BALF recovered from unchallenged guinea-pigs were those of the monocyte. The numbers of these cells were increased after challenge [32]. In contrast, other workers have not found an increased monocyte influx [33]. Thus, unlike eosinophils and neutrophils, monocyte recruitment is not a consistent feature of the airway inflammatory response following antigen challenge in this species. In this context, it is worth noting that, treatment of the sensitized animals with SBOCJ produced a nonsignificant decrease in monocyte count as compared to sensitized group in BAL fluid respectively.

Although an influx of lymphocytes does not appear to be a consistent feature of airway inflammation in BAL model, it remains possible that antigen specific T-lymphocytes resident in the airway wall become activated as a result of antigen challenge. Thus, T-cells may, by secreting IL-4, IL-5 and other proinflammatory cytokines, contribute to the orchestration of the ensuing allergic reaction [27]. In line with this notion, the present findings show that treatment of the sensitized animals with SBOCJ produced a nonsignificant decrease in lymphocyte count as compared to sensitized animals in BALF. This indicates the protective effect of SBOCJ by preventing release of several preformed mediators, thereby preventing the direct damage of airway epithelium.

In conclusion, *B. oleracea* has been found to attenuate brochoconstriction, inflammation by virtue of its bronchodilator, anti-inflammatory and antianaphylactic activity. The results of the present study hence prominently seem to validate the traditional use of *B. oleracea*. 
REFERENCES


