Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43B, No.2S,
July-December 2024: P. 1494-1505

Print version ISSN 0970-0765
Online version ISSN 2320-3188

Original Research Article
Content Available online at: www.bpasjournals.com

STUDY THE EFFICACY OF *CUCURBITA MOSCHATA LINN.* SEEDS EXTRACT (CMSE) AGAINST OVALBUMIN INDUCED ALLERGIC ASTHMA IN GUINEA PIGS

Sandhya Suresh*[1], Dr. S. S Sisodia[2]
[1]Research scholar, B.N college of Pharmacy, Bhupal Noble's University, Private University in Udaipur, Rajasthan
[2]Professor, B.N college of Pharmacy, Bhupal Noble's University, Private University in Udaipur, Rajasthan
*sandhyasuresh110@gmail.com

ABSTRACT

Aim of the study: The present study was carried out to evaluate the efficacy of *Cucurbita moschata Linn.* Seeds extract (CMSE) against ovalbumin induced allergic asthma in guinea pigs.

Method: Extraction of seeds was carried out in methanol by maceration process. The extract was subjected to preliminary phytochemicals screening for the qualitative assessment of different phytoconstituents present in it. Further, the quantitative estimation of total flavonoid content in the extract was carried out by spectrophotometry. Pharmacological evaluation of the extract against the ovalbumin (OVA) induced asthma was carried out at two dose levels i.e. 500mg/kg and 1000mg/kg. Dexamethasone was used as the standard drug (2.5mg/kg). Test and standard drugs (CMSE and dexamethasone) were administered before challenging the animals with aerosolized 0.5% OVA. Bronchoconstriction and lung functions in animals subjected to different treatments were measured. Leucocyte count in blood samples (taken from each animal) and Bronchoalveolar lavage (BAL) fluid (collected from lung) was done using an automated hematology analyser. Estimation of histamine in lungs was done by fluorometry.

Results and conclusion: Percent yield of the seed extract was 5.73%. Preliminary phytochemical analysis of the extract confirmed the presence of steroids, terpenoids, flavonoids, glycosides, saponins, fats and fixed oils. The total flavonoid content in the extract was found to be 0.102 gram equivalent of Catechin. Increased respiratory rate, leucocyte count and histamine was observed in disease controlled animals. Treatment with CMSE (at both dose levels) and dexamethasone showed increase in tidal volume and normalized the respiratory rate.Also, CMSE at low dose (500mg/kg) was found sufficient to control the severity of the asthmatic response. Leucocyte count and histamine in treated animals were decreased. Results indicate that CMSE possess beneficial effects against asthma.

Keywords: Asthma, *Cucurbita moschata,* CMSE, Bronchoconstriction, Histamine, WBC.

1. INTRODUCTION

Asthma is a non-communicable disorder of respiratory tract characterized by chronic inflammation leading to the episodes of airway hyper responsiveness, breathlessness, wheezing and coughing that may be of varying time duration and intensity [5]. Asthma commences with the inhalation of a potential allergen that is capable of inducing an inflammatory response in the airways.

Subsequently, the inhaled allergen stimulates the proliferation of T helper (type 2) cells followed by the activation and release of certain cytokines and interleukins (IL-4, 5 and 13). The release of these inflammatory mediators ultimately elicits a chronic inflammatory response that may last for a considerable duration depending upon the intensity of sensitization [8]. According to a recent report, asthma has victimized around 300 million individuals globally. Moreover, if the existing conditions remain unattended, there will around 100 million new cases adding to the global burden of asthma by the year 2025 [4].

The conventional treatment strategies for overcoming asthma falls under two broad categories that include the controllers and the relievers. While the controllers are widely prescribed to be taken on a daily basis as anti-inflammatory drugs to control the occurrence of asthmatic episodes, the relievers are mainly inclined to provide a quick relief from bronchoconstriction and generally taken as and when required based on the intensity of asthmatic attack [6]. The commonly employed controller medications in asthma are the inhaled corticosteroids (ICs), leukotriene receptor antagonists (LTRAs), long acting muscarinic receptor antagonists (LAMAs), biological agents such as anti-IL-5 and anti-IGE- therapy. On the other hand, the relievers chiefly include the rapid-acting inhaled beta2-agonists and inhaled anti-cholinergic [5]. However, a widely acknowledged drawback of utilizing these conventional approaches is their associated side effects. Moreover, combinational therapy prescribed in asthma tends to aggravate the side effects that seem to be peculiar for individual class of drugs[7]. Considering these complications, it issues a clarion call to the researchers to look for alternative approaches that are more efficient, affordable and most importantly safe, when prescribed[4].

Plant and plant derived constituents have been a boon to the humanity since the time immemorial. Harboring a wide range of different phytoconstituents, plants have proved their versatility in the treatment of a variety of disorders. Different classes of phytoconstituents including (but not limited to) phenolics, glycosides, flavonoids, saponins, tannins, catotenoids, and alkaloids are effective in controlling the emergence of a diverse range of diseases and disorders including the inflammatory conditions[3]. In that view, screening of plants carrying a plethora of different active compounds is a promising approach in identifying novel agents that are capable of controlling inflammatory disorders without causing any significant side effects.

Cucurbita moschata Duchesne is a globally utilized plant mainly used for its fruit and seeds. It belongs to the family Cucurbitaceae[1]. The seeds of the plant are a rich source of carotenoids, flavonoids, tocopherols and amino acids[9, 18]. Moreover, the seeds have proven anti-oxidant and anti-inflammatory effects[13, 14]. Following that, the present study was designed to evaluate the efficiency of the methanolic extract of seeds of *Cucurbita moschata* in controlling the airway inflammation and subsequent asthmatic response in ova-albumin sensitized experimental guinea pigs. To the best of our knowledge, this is the first study that attempts to evaluate the effects of *Cucurbita moschata* seeds as a source of controller medication for asthma.

2. MATERIALS AND METHODS

2.1 Reagents

Methanol, Dragondroff's reagent, chloroform, Sodium hydroxide, sodium sulphate, Ferric chloride, copper sulpahate, catechin, sodium carbonate, Sodium nitroxide, Aluminiumtrichloride, perchloric acid, hydrochloric acid were obtained from the department of pharmacognosy and phytochemistry, B.V. Patel PERD center. Dexamethasone, aluminium hydroxide, histamine and acetyl choline were purchased from MP biomedicals. n-Butanol and n-heptane were purchased from Merck specialities Pvt. Ltd (HIMEDIA), Agar, Formaldehyde, Sodium chloride was purchased from fisher scientific. Isoflurane and ova-albumin were purchased from Raman and well Pvt. Ltd and Himedia respectively. All chemicals were of analytical grade.

2.2 Procurement and Identification of the Plant Material

Seeds of *Cucurbita moschata* were procured from Gandhi bazar, Ahmedabad, Gujarat (India) in the month of October, 2018. Plant material was identified and authenticated by taxonomist in the department of Pharmacognosy and Phytochemistry, B. V. Patel PERD Centre. A Voucher specimen was submitted in the department for future reference. Voucher specimen number: BVPPERD/PP/1118/08.

2.3 Extraction of plant material

Seeds were shade dried for 1 week at 37°C. Dried seeds were powdered and weighed. For extraction, 750 g of powdered plant material was taken in 1litre of methanol in a conical flask and kept undisturbed (with occasional shaking) for 3 days. Resulting Solution in the flask was filtered and kept on a rotary evaporator at 50°C to evaporate the solvent. Remaining marc was subjected to the similar process to achieve the complete extraction of seeds. The filtered and evaporated extracts were combined and the percent yield of the *cucurbita moschata* seeds extract (CMSE) was calculated[17].

2.4 Preliminary phytochemical Screening of extract

Preliminary phytochemical screening of CMSE was performed for the detection of different classes of phytoconstituents present in it according to their standard method[15, 16].

2.5 Estimation of Total flavonoids contents

Total flavonoid content in CMSE was calculated by spectrophotometric technique. For this, extract solution (125µL) was dispersed in methanol. Further, 75µL of 5% $NaNO_2$ solution was added in the extract solution. The mixture was kept undisturbed for about 6 min. 150µL of aluminum tri-chloride (10%) was added and the mixture was incubated for another 5 min. The final volume of the solution was made up to 2.5 ml with distilled water after the addition of 750µL of NaOH (1M). The mixture was kept undisturbed till it turned pink. Absorbance of the colored mixture was measured at 510nm using UV-Vis spectrophotometer (Shimadzu). As a standard, Catechin was taken and final flavonoid content in the extract was expressed as gram equivalent of catechin[17].

2.6 Animal husbandry and feeds

Guinea pigs (Dunkin-Hartley) weighing between 350-450g were kept in the animal house of B.V. Patel PERD Centre at the room temperature (25˚C). A relative humidity of 65% and 14 hr. light dark cycle were maintained in the animal house. Food (green grass) and filtered tap water was provided to the animals ad libitum. Animals were of both the sexes. Animals were

allowed to acclimatize for one week before the commencement of study. The protocol for the study was presented for evaluation to the institutional animal ethic committee (IAEC). The study was approved by IAEC and a Protocol no. (PERD/IAEC/2018/015) was issued. Animals were continuously monitored for food and water intake or any other signs of toxicity during the entire course of study.

2.7 Sensitization and Treatment of Animals

Experimental animals (guinea pigs) were divided into five different groups (n=6/group) namely: Group I (Normal control, NC) received agar solution (0.2%), Group II (Disease control, DC) sensitized with Ovalbumin, Group III (Positive control, PC) received dexamethasone (2.5mg/kg) after sensitization with Ovalbumin, Group IV (Test group, T-1) received CMSE (500mg/kg), Group V (Test group,T-2) received CMSE (1000mg/kg). Animals from all the groups (except group I) were sensitized with 100µg of ovalbumin (absorbed into aluminum hydroxide saline solution) subcutaneously on day 0 as the first sensitization. Further boosting was done on day 14. Treatment with standard and test drugs was initiated from day 15 and continued till day 21[10].

2.8 Ovalbumin Exposure

From days 18 to 21, animals in each group received their respective treatment followed by their exposure to aerosolized 0.5% OVA for 2 min., 2.5 h after the treatment. Exposure to the ovalbumin was carried out by keeping the conscious animals in a plastic circular chamber (diameter = 70 cm, height = 40 cm) connected to a nebulizer (CX-4 OMRON Healthcare Company Ltd. Kyoto, Japan). Animals belonging to group I (NC) were exposed to aerosolized saline[17].

2.9 Hematology

Blood from the animals belonging from different groups was collected before on day 17 and day 22 (before and after the OVA exposure). The blood samples were subjected to total leucocyte count using an automated hematology analyzer (VetScan HM-5; Abaxis Inc., Union City, CA, USA)[17].

2.10 Measurement of Respiratory rate

On day 21, lung function in each animal were measured using a using a data acquisition system (MP-35, Biopac Systems, Santa Barbara, Calif). Respiratory rate was measured 2 hr. after the aerosolized ovalbumin exposure (10 mins.) with the help of a nebulizer (OMRON, NE C-25 model) and recordings were taken in the biopac system. Animals in group I (normal control) were exposed to aerosolized saline [11]

2.11 Measurement of Bronchoconstriction

Bronchoconstriction in animals belonging to different groups was measured. Ovalbumin sensitized animals in their conscious state were exposed to acetylcholine (Ach, 0.25%) for a duration of 30s using a nebulizer (OMRON, NE C-25 model) connected to the animal holder. Animals in group I were exposed to normal saline. Respiratory rate in each animal was measured before and after the exposure to Ach [11]

2.12 Bronchoalveolar lavage (BAL)

BAL fluid from each animal was collected at the end of study. For the collection of BAL fluid, animals were anesthetized using isoflurane anesthesia. 10 ml of 0.9% (W/V) normal saline was introduced into the lungs of animals using a 10 ml syringe connected to a polypropylene cannula inserted into the trachea of each animal. The saline was recovered after 5 mins. and

centrifuged (5000 X g, 10 mins., 4°C). Supernatant from the centrifuged liquid was discarded and the settled pellet containing cells was washed in 0.5 ml saline. Total cell count was performed in an Automated hematology analyzer (VetScan HM-5; Abaxis Inc., Union City, CA, USA)[17].

2.13 Histamine assay

Lobes of lung tissue was collected from each animal. Lung tissue (average weight 200g) was homogenated in solution containing normal saline (2.5 ml) and 0.8N perchloric acid (2.5 ml). Total histamine in the lung tissue was estimated according to the standard method[11]

2.14 Histopathology analysis

Histopathology analyses of lung tissues obtained from each guinea pig were carried out to study the effect of extract on the immune mediated chronic inflammation brought about by sensitization with OVA. Dissected lungs and trachea were washed with normal saline and then placed in 10% formaldehyde solution for 1week. After the tissues were fixed, specimens of lungs were embedded in paraffin wax, and 5pm sections were cut and stained with haematoxylin and eosin dye for morphology. Images of selected sections were captured at 40X magnifications using a zoom digital camera optical microscopy (IX 51; Olympus, Tokyo, Japan) equipped with a digital camera (TL4) [17].

2.15 Statistical analysis

Statistical analysis was performed using one way analysis of variance (ANOVA) and t-test. Graph Pad prism 8 software was used for the analysis. Results were reported as mean ± SEM. The data was considered statistical significant at $p<0.05$.

3. RESULTS

3.1 Extraction and preliminary phytochemical Study

Extraction of *Cucurbita moschata* seeds (CMSE) was carried out using methanol. The percent yield of CMSE was found to be 5.73%. Further, CMSE was subjected to preliminary phytochemical screening to determine the different classes of phytoconstituents present in it. Alkaloids, steroids, terpenoids, flavonoids, glycosides, saponins, fats and fixed oils were identified during the preliminary phytochemical screening of CMSE.

3.2 Spectrophotometric estimation of total flavonoids

Following results from preliminary phytochemical screening of CMSE, quantitative estimation of the presence of total flavonoids in the extract was done. For this, total flavonoid content in the extract was estimated by spectrophotometry. Standard curve of catechin was obtained and the flavonoid content in CMSE was calculated from it. Concentration of total flavonoids in CMSE was found to be 0.102µg/ml. Results from spectrophotometric estimation were suggestive that the extract is rich in flavonoids.

3.3 Effect of CMSE on Body Weight

During the course of study, sensitized animals administered with different drugs (standard and test) were monitored for changes in their body weight to ensure no severe effects of interventions on normal growth of experimental animals. No significant fluctuations in body weights were observed in animals belonging to all groups. The results were suggestive that the standard (Dexamethasone) or test extract (CMSE) did not interfere with the normal growth of animals (fig.1). Furthermore, no effects on water consumption or food intake were observed in

animals belonging to different groups. Also, the external appearances of animals (eye, skin or movement) were normal.

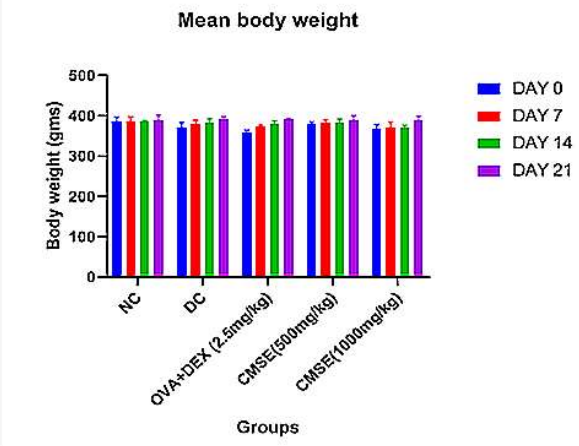


Fig.1: Histogram representing the effects of standard drug (OVA+DXM) and test drug (OVA+CMSE) on body weights of guinea pigs. Data is expressed as mean ± SEM. n=6/groups.

3.4 Effect of CMSE on Lung function (respiratory rate)

We next evaluated the changes in respiratory rate following ovalbumin exposure as a sign of lung function in animals receiving different interventions. Following the exposure to ovalbumin, difference in respiratory rates among animals belonging to different groups was observed on day 21. OVA sensitized animals receiving no treatment (Diseased group) showed increased respiratory rates after exposed with ovalbumin. However, following the ovalbumin exposure, animals present in standard and test groups (CMSE at both dose levels) had improved respiratory rates (fig.4). Also, CMSE at 500 mg/kg was found to be most effective. (*$p<0.05$) (fig.3). Results were suggestive that CMSE controlled the severity of asthmatic response (increase in respiratory rate).

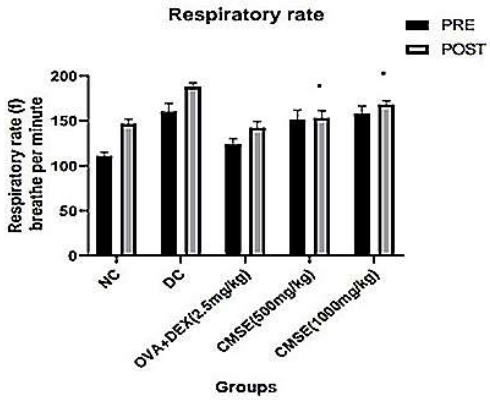


Fig.2: Histogram representing the effects of standard drug (OVA+DXM) and test drugs (OVA+CMSE) on respiratory rates of guinea pigs (Ovalbumin exposure). Data is

expressed as mean ± SEM (*n*=6/groups). *represents significant difference in respiratory rates of diseased and treatment groups(*p <0.05).

3.5 Effect of CMSE on Acetylcholine induced bronchoconstriction

Following the effects of CMSE in improving lung functions (respiratory rate), Ovalbumin sensitized animals were exposed to 0.25 % acetylcholine (Ach) to test the effects of standard and test drugs in controlling bronchoconstriction. Following the exposure to acetylcholine, respiratory rates were increased in OVA sensitized diseased animals on day 21. However, improvement in respiratory rates was observed following the standard (Dexamethasone) and test (CMSE at both dose levels) treatments. Dexamethasone was found to be most effective. Also, CMSE at both dose levels (500mg/kg and 1000mg/kg) produced comparable results (Fig: 3). Results suggest that CMSE is able to control bronchoconstriction following exposure to a potential allergen (Ach)(*p<0.05).

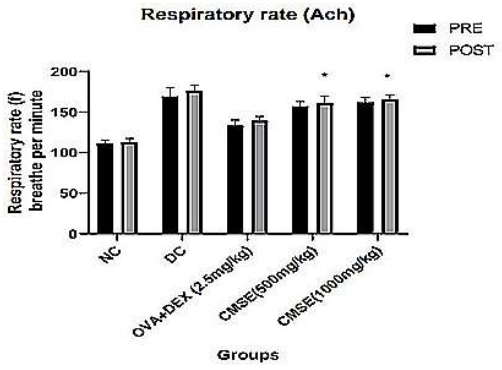


Fig.3: Histogram representing the effects of standard drug (OVA+DXM) and test drugs (OVA+CMSE)on respiratory rates(Acetylcholine exposure/Ach) of guinea pigs. Data is expressed as mean ± SEM (*n* = 6/groups). * represents significant difference in respiratory rate of animals belonging to treatment groups to that of diseased group *(*p<0.05)*.

3.6 Effect of CMSE on leucocyte count in whole blood

To evaluate the effects of CMSE in controlling ovalbumin induced allergic asthma, concentration of leucocytes (WBC) in whole blood of animals receiving different interventions was measured. WBC count was significantly different among different groups. Diseased group showed a significant increase in leucocyte count following ovalbumin exposure. The administration of standard and test drugs at both dose levels (dexamethasone and CMSE) resulted in reduction in WBC count on day 22. CMSE, at 500mg/kg dose level, was found to be most effective in reducing the leucocyte count. The results were suggestive that the extract was efficient in controlling the inflammatory response by causing a decrease in leucocyte levels in the blood. (*p<0.05) (fig. 4).

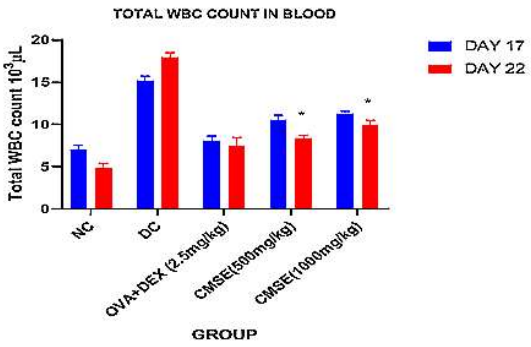


Fig.4: Histogram representing the effects of standard drug (OVA+DXM) and test drugs (OVA+CMSE) on total leucocyte counts in blood taken from guinea pigs. Data represented as mean ± SEM. n=6/group. * Represents significant difference in leucocyte count between diseased and treatment groups *(*p<0.05).*

3.7 Effect of CMSE on leucocyte count in BAL fluid

BAL fluid collected from the lung tissue was estimated for leucocyte count following the asthmatic response. WBC count was increased in diseased animals. However, significant reduction in WBC count was observed in animals receiving standard (Dexamethasone) and test extract (CMSE) on day 22 (fig.5) ($p<0.05$).

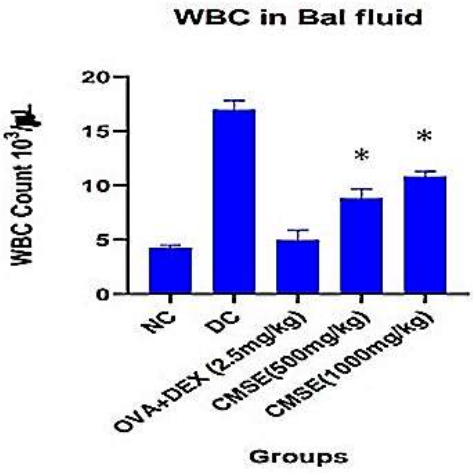


Fig.5: Histogram representing the effects of standard drug (OVA+DXM) and test drugs (OVA+CMSE) on leucocyte counts in BAL fluid. Data is represented as mean ± SEM. n=6/group. * Represents significant difference in WBC count between diseased and treatment groups (*p <0.05).

3.8 Effects on histamine levels

Histamine levels were significantly lowered in animals receiving standard (Dexamethasone) and test extract (CMSE) treatments in comparison to diseased group (Fig 7). Results were suggestive of anti-histaminic effect of extract in controlling asthma (fig.7) (*p<0.05).

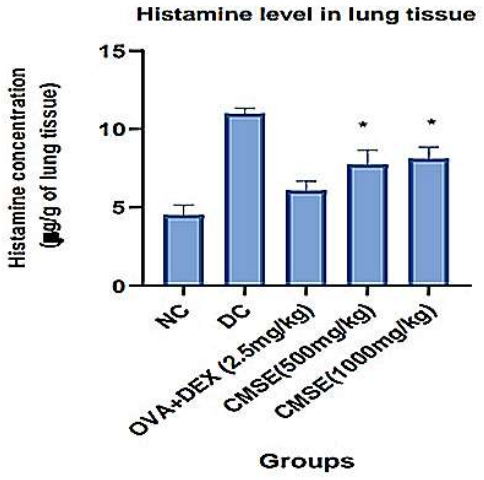
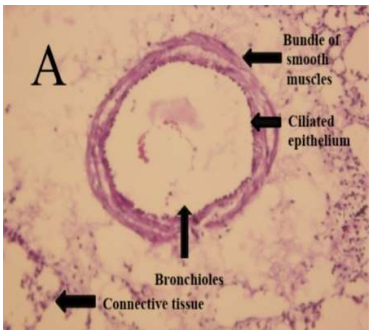
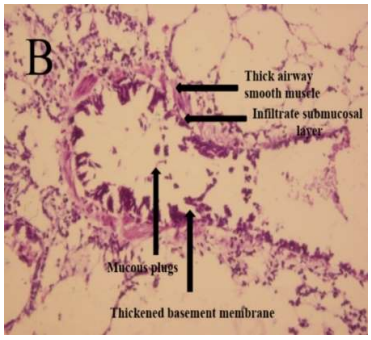


Fig.6: Histogram representing the effects of standard drug (OVA+DXM) and test drugs (OVA+CMSE) on histamine level (µg/g). Data represented as mean ± SEM. n=6/group. * Represents significant difference in histamine levels between diseased and treatment groups *(p <0.05)*.

3.9 Histopathology of CMSE

In histopathology study the lungs were taken from all groups and the study is suggest that CMSE given protective effect against asthma. The lung tissue from diseased group showed thickening basement membrane, thick airway smooth muscles and shown infiltrate sub-mucosal layer. In positive and treatment control were shown thin layer of basement membrane and airway smooth muscles. Also shown low leucocyte count in the sub mucosal layer was evident from the microscopic images of the stained tissue (fig.10).





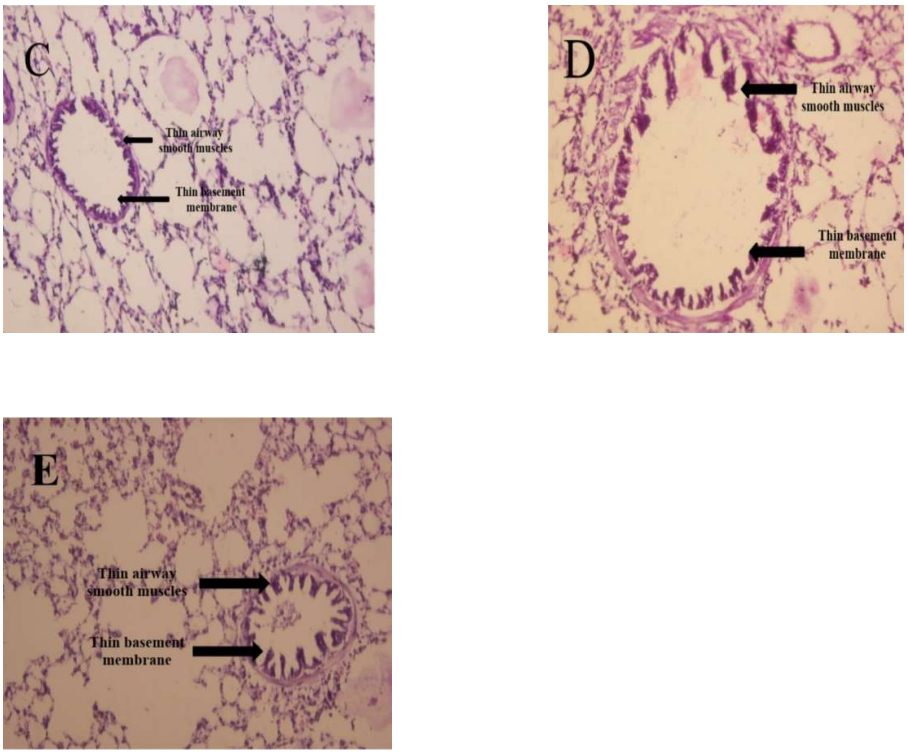


Figure.7: Histopathology of lung tissue of animals treated with CMSE (Magnification 40X). Lung tissue of treated and untreated (OVA-sensitized) animals stained with hematoxylin-eosin. (A): Normal lung tissue, (B): typical damaged lung tissue from OVA-control (disease group) arrow shows thickened airway smooth muscle, THICK basement membrane and high leucocyte count infiltrate in sub-mucosal layer, (C): Section from OVA +DEX (2.5mg/kg) positive control and the arrow shows less leucocyte infilteration, (D): Section from OVA+CMSE (500mg/kg) treatment group, (E): Section from OVA+CMSE (1000mg/kg) treatment group. In figure D and E arrows shows significant protection against leucocyte infiltration, thin airway smooth muscle and thin basement membrane**.**

4. DISCUSSION AND CONCLUSION

To substantiate our understanding on the suitability of *Cucurbita moschata* seeds extract for the treatment of inflammatory disorders, we evaluated the safety and efficacy of CMSE in ovalbumin-sensitized guinea pigs for its anti-asthmatic effect. To study the phytochemical composition of the extract, we carried out phytochemical screening of the CMSE extract that showed the presence of alkaloids, steroids, terpenoids, flavonoids, glycosides, saponins and fats and fixed oils. A similar study conducted by *Muya.S et.al* (2018) [12] also showed that the plant is rich in these phytoconstituents. Photometric estimation of plant extract showed the presence of an appreciable quantity of flavonoids in it. Needless to mention, the witnessed effects of the extract in controlling the airway inflammation is attributable to the presence of these broad classes of phytoconstituents in the extract.

The present work was carried out to examine the effect of CMSE in ova-albumin induced airway inflammation in guinea pigs and to unravel its potential against the ovalbumin allergen induced allergic asthma. An increased number of WBC count in whole blood as well as in BAL fluid of OVA control was evident in diseased animals after sensitization; as compared to normal control. The reduction in WBC count was observed in standard and test groups (CMSE 500mg/kg and 1000mg/kg) in comparison to diseased group (fig.2 and 5). Appreciable results were observed in animals administered with both the doses of extract. No fluctuations in body weights were observed during the course of the study, suggesting that the extract caused no severe toxicity to animals (fig.1).

Following this, we evaluated the differences in lung functions of animals receiving different interventions. Supportively, the improved lung functions were evident in standard group receiving dexamethasone and test groups receiving CMSE (at both the dose levels). While the respiratory rate of OVA sensitized animals receiving no interventions remained remarkably higher, the groups receiving treatment had improved respiratory rates (fig 3). Our data from the study is suggestive that the extract was capable in controlling the manifestations of inflammatory responses in asthma. Following Ach exposure, respiratory rate in diseased animals increased and decreased. Protective effects of CMSE were also observed in controlling the bronchoconstriction in experimental animals (Fig 4). The potential mode of action employed by CMSE can be attributed to its bronchodilatory effect and its further ability to block the release of inflammatory mediators into the lung tissues. Moreover, the respiratory rate in disease control animals was significantly higher which was indicative of exertional breathing, yet another symptom of asthma. Further, the extract and standard drug (dexamethasone) showed prominent bronchodilatory effect following the Ach exposure. Moreover, CMSE-treatment showed protection against bronchoconstriction and airway inflammation which was confirmed by histopathological observations.

Data from our present investigation is conclusive that the methanolic extract of *Cucurbita moschata* seed extract (CMSE) holds a potential anti-asthmatic activity and can be further explored to understand the molecular mechanism behind its anti-asthmatic activity. Further study on active phytoconstituents in the extract can be helpful in the anti- asthmatic research.

In conclusion, our data suggest that *Cucurbita moschata* seed produced beneficial effects against inflammation, bronchospasm, mast cell degranulation, immune reactions and anaphylactic reactions. The extract was found to inhibit the inflammatory mediator, histamine. Collectively, the evidences justify the use of *Cucurbitamoschata* seeds in the treatment of asthma. Further exploratory studies on the seeds can be beneficial in identification and isolation of potential anti-asthmatic compounds from the extract.

5. REFRENCES

1. Bahramsoltani, R., et al., *Evaluation of phytochemicals, antioxidant and burn wound healing activities of Cucurbita moschata Duchesne fruit peel.* 2017. **20**(7): p. 798.
2. Barnes, P.J.J.R.r., *Th2 cytokines and asthma: an introduction.* 2001. **2**: p. 1-2.
3. Bellik, Y., et al., *Molecular mechanism underlying anti-inflammatory and anti-allergic activities of phytochemicals: an update.* 2012. **18**(1): p. 322-353.

4. Dharmage, S.C., J.L. Perret, and A.J.F.i.p. Custovic, *Epidemiology of asthma in children and adults.* 2019. **7**: p. 246.
5. Hamid, Q. and M.J.A.r.o.p. Tulic, *Immunobiology of asthma.* 2009. **71**: p. 489-507.
6. Horak, F., et al., *Diagnosis and management of asthma–Statement on the 2015 GINA Guidelines.* 2016. **128**: p. 541-554.
7. Kaur, S. and V.J.T.I.J.o.P. Singh, *Asthma and medicines–long-term side-effects, monitoring and dose titration.* 2018. **85**: p. 748-756.
8. Kudo, M. and Y.J.P.o.a.F.M. Ishigatsubo, *Aoki 1.* 2013. **4**: p. 263.
9. Kulczyński, B. and A.J.M. Gramza-Michałowska, *The profile of secondary metabolites and other bioactive compounds in Cucurbita pepo L. and Cucurbita moschata pumpkin cultivars.* 2019. **24**(16): p. 2945.
10. Mahajan, S.G., et al., *Inhibitory effect of n-butanol fraction of Moringa oleifera Lam. seeds on ovalbumin-induced airway inflammation in a guinea pig model of asthma.* 2009. **28**(6): p. 519-527.
11. Mahajan, S.G. and A.A.J.I.t. Mehta, *Effect of Moringa oleifera Lam. seed extract on ovalbumin-induced airway inflammation in guinea pigs.* 2008. **20**(10): p. 897-909.
12. Muchirah, P.N., et al., *Characterization and anti-oxidant activity of Cucurbita maxima Duchesne pulp and seed extracts.* 2018. **7**(2): p. 134-140.
13. Nadjiba, C.-M., et al., *ANTI-INFLAMMATORY AND ANTI-MICROBIAL ACTIVITY OF FATTY OILS OF PUMPKIN SEEDS (Cucurbita moschata) HARVESTED IN BOUIRA (ALGERIA).* 2018. **18**(3.2): p. 725-732.
14. Sakka, D. and H.C.J.I.J.o.F.S. Karantonis, *In vitro health beneficial activities of Pumpkin seeds from Cucurbita moschata cultivated in Lemnos.* 2015. **4**(2).
15. Sharma, A., et al., *Preliminary phytochemical evaluation of seed extracts of Cucurbita maxima duchense.* 2013. **2**(3): p. 62-66.
16. Sood, A., P. Kaur, and R.J.I.J.A.B.P.T. Gupta, 3, 401, *Phytochemical screening and antimicrobial assay of various seeds extract of Cucurbitaceae family.* 2012. **409**.
17. Suresh, S., et al., *Phytochemical analysis and pharmacological evaluation of methanolic leaf extract of Moringa oleifera Lam. in ovalbumin induced allergic asthma.* 2020. **130**: p. 484-493.
18. Veronezi, C.M. and N.J.J.o.f.s. Jorge, *Bioactive compounds in lipid fractions of pumpkin (Cucurbita sp) seeds for use in food.* 2012. **77**(6): p. C653-C657.