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Anti-asthmatic activities of an ethanol extract of *Sarcostemma brevistigma* in an Ovalbumin-induced model

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Abstract
Objectives: *Sarcostemma brevistigma* is used in old-stylemedicines to treat cough, asthma and insect bites; however, its therapeuticmechanism is not completely understood.
Methods: To explain the anti-asthmatic effect of *S.brevistigma*, we investigated the therapeutic mechanism antiasthmatic properties in the ethanolic extract of *S.brevistigma* in an ovalbumin (OVA)-induced model.
Results: In thisstudy, we showed that *S.brevistigma* significant decrease of body weight in mice sensitized to OVA and significant weight gain in the mice treated with ethanolic extract of test plant were noticed. PCV, HB and RBC were increased in all the test groups when compared with that of the OVA treated animals. These increases were statistically significant ($p<0.05$) in groups treated with 200 mg/kg and 400 mg/kg of plant extracts. A significant ($p<0.001$) decrease in WBC count and MCH was observed in same groups (200 mg/kg and 400mg/kg). There was no significant difference in the percentage of neutrophils (polymorphs), lymphocytes and monocytes count in all the test groups when compared with the control and treated groups and the histopathological variations in the lungs, whichreached normal levels in the OVA-challenged mice treated with *S.brevistigma* extract.
Conclusion: These findings suggest that *S.brevistigma* could be a promising natural agent for treating bronchial asthma in humans.

Keywords: histopathology; OVA-induced asthma; *Sarcostemma brevistigma;* significant and therapeutic

Introduction

Asthma is a prolonged inflammatory disease that affects people worldwide. The World Health Organization evaluated that 262 million persons worldwide suffered from asthma in 2019 and confirmed asthma was the third most common cause of hospitalization for children under 15 years of age [1]. The symptoms of asthma are problematic to control, and its causes are diverse, including hereditary factors and external factors, such as pet dander, dust mites, cockroaches, viral infections, pollen, mold, fungi and tobacco smoke [3]. The typical manifestations of asthma vary from a cough to obstructive apnoea, which may arise due to the excessive production of mucus, goblet cell hyperplasia, epithelial cell shedding, basement membrane thickening, as well as eosinophil and lymphocyte infiltration [4, 5].

It is a life threatening respiratory condition which interferes with the exchange of oxygen and carbon dioxide in the lungs. Although well controlled scientific studies have been performed for many of the Asian herbal therapies and some basic studies have been performed for various herbal components (active ingredients), more needs to be done to assess the composite effects of many of these remedies. Aremarkable number of modern drugs have been isolated from natural sources, particularly plants based on their use in traditional medicine [6].

A vast number of medicinal plants have been used traditionally in Ayurvedic system of medicine for the management of asthma and have been scientifically proved that could serve as 'lead' for the development of novel anti-asthmatic agents. Ayurvedic system has reported the usefulness of whole plant to cure asthma and bronchitis [7]. It has depressant action on the heart and relaxes the bronchioles [8].

The members of the family Asclepiadaceae is well known to Indian system of medicine since ancient times, and reported to contain several phytochemicals like alkaloids, sterols, tannins, terpenoids, flavonoids, wax and resins [9, 10]. In many earlier investigation made in southern parts of India it has been reported that Asclepiadaceous is one among the dominant families that includes plants with potential curative values for many health problems [11, 12, 13, 14].

The traditional healers of rural Districts of Tamil Nadu mainly using the herbal preparations of different genus of Asclepiadaceae to cure several human diseases *viz*., Ulcer, diabetes diarrhea, asthma, bronchitis, arthritis, jaundice, eczema, leprosy, elephantiasis, urinary disorders, eye diseases, piles, migraine, fever, joint and bone complications, improve fertility and as antidote for snake bite. The extracts serve as astringent, antiperiodic and anthelmintic,

Sarcostemma is a genus of flowering plants in Asclepiadaceae first described as a genus in 1810. The name is derived from the Greek words (*sarkos*), meaning "flesh," and (*stemma*), meaning "garland" [15]. Members of the genus are known generally as climbing milkweeds or caustic bushes. *Sarcostemma brevistigma* Wight. & Arn. has been

selected for further studies, whose aerial parts are appreciated as medicinal and a strong antioxidant agent in many parts of the world especially in India [16] and it cannot be overlooked.With all efforts therefore the present study was geared towards understanding the anti –asthmatic mechanism and to find suitable herbal remedies from this potential medicinal plant.

Materials and Methods

Collection and identification of plant materials

The plant *Sarcostemma brevistigma* was collected from Pillur Beat (Pillur slope RF and Nellithurai RF), Karamadai Range, Western Ghats, Tamil Nadu, and India. Theauthenticity of the plant was confirmed in BotanicalSurvey of India, Southern Circle, and Coimbatore byreferring the deposited specimen. The voucher number ofthe specimen is BSI/SRC/5/23/2015/Tech./2334. Theaerial parts of this species was washed under running tapwater, shade dried at room temperature and powdered.

In-vivo Studies

Experimental animals

All the animal experiments in the present work were carried out as suggested by the Institutional Ethical Committee (Reg.No.685/PO/Re/S/2002/CPCSEA) norms. Approval from the Institutional Ethical Committee was obtained prior to the beginning of the study.

Animals were fasted over night before the experimental schedule, but have free access for water *ad-libitum*. The animals used for anti-asthmatic study were housed under standard conditions of temperature and humidity (12hr/12hr light - dark cycles) and fed with leafy vegetables/ carrots and tap water *ad - libitum*.

In vivo Toxicity Studies

Behavioural and toxicological effects

Toxicity studies of the extracts were carried out as per the guidelines set by Organization for Economic Co-operation and Development (OECD). Acute oral toxicity study was done according to OECD guidelines 423. In this experiment, animals were divided in to five groups of six animals each. The first group served as control and was treated with normal water. Group 2, 3, 4 and 5 were treated with single graded dose (5, 50, 300, 1000, 2000 mg/Kg body weight respectively) of ethanol extract of *S. brevistigma* orally. Monitoring of the parameters commenced immediately after the administration of the sample. Animals were observed at 1.0, 3.0 and 4.0 hours post dose on day of dosing and once daily thereafter for 14 days. Observation includes mortality and morbidity, which includes changes in skin, fur, eyes and mucous membranes, occurrence of secretions and excretions. Autonomic activity like lacrimation, piloerection, pupil size and unusual respiratory pattern, changes in gait, posture, response to handling, presence of clonic or tonic movements, stereotypes like excessive grooming and repetitive circling or bizarre behavior like self-mutilation, walking backwards etc., were observed. The effect of plant extract on passivity, grip strength, pain response, stereotypy, vocalization, bodyweight and intake were also observed.

Antiasthmatic Activity

Ovalbumin induced asthmatic activity [17]

Animals (Mice) were divided into five groups (n=6). All the animals except in the non sensitized group (NS), were sensitized by an intraperitoneal injection of 1ml alum precipitate antigen containing 20µg of ovalbumin and 8mg of alum suspended in 0.9% sodium chloride solution. A booster injection of this alum-ovalbumin mixture was given 7 days later. Non sensitized animals were injected with alum only. Seven days after (15 day) second injection, animals were exposed to aerosolized ovalbumin (1%) for 30 min. Animals belonging to groups I received orally on distilled water and Group IV and V, received orally on ethanol extract,200mg/kg and 400mg/kg. Animals of group III, as positive control group received dexamethasone (0.27mg/kg p.o.) 5 hr before antigen challenge. At the end of the experiment, blood samples were collected from overnight fasted rats by retro-orbital. The blood was collected in tubes containing Heparin/EDTA as an anticoagulant. The mice were sacrificed at the end of study (24hr after sensitization) Total leukocyte Eosinophils and Neutrophils were counted under microscope and histopathologic evaluation of lung tissue was carried out.

Histopathological Techniques

Histopathology is the microscopical study of tissues for pathological alterations. This involves collection of morbid tissues from biopsy or necropsy, fixation, preparation of sections, staining and microscopical examination.

Collection of Materials

Thin sections of 3 to 5 mm, thickness of tissues were collected showing gross morbid changes along with healthy tissue.

Fixation

Kept the tissue in fixative for 24-48 hours at room temperature

The fixation was useful in the following ways:

a. Serves to harden the tissues by coagulating the cell protein,
b. Prevents autolysis,
c. Preserves the structure of the tissue, and
d. Prevents shrinkage

Common Fixatives: 10% Formalin

Haematoxylin and Eosin Method of Staining

Deparaffinise the section by xylol 5 to 10 minutes and remove xylol by absolute alcohol. Then washed the section in tap water and stained with haematoxylin for 3-4 minutes and again washed under tap water. Allow the sections in tap water for few minutes and counterstained with 0.5% eosin until the section appears light pink (15 to 30seconds), and then washed in tap water. Blotted and dehydrated in alcohol and cleared with xylol (15 to 30 seconds). Mounted on a Canada balsam or DPX Moutant and kept the slide dry.

Results

Acute toxicity

The behaviour of animals (Female Wistar rat) was assessed immediately after the administration of the drug (plant extract) at hourly basis (1h, 3 hrs and 4 hrs). The indication of toxicity was measured as any change or abnormalities in

the animals. The animals showed no changes in behaviour after the administration of an oral dose of ethanolic extract of test plant at all dose levels.

Antiasthmatic Studies - Haemotologicalanalysis

The data in Table – 1 showed haematological modifications associated with orally administered test drugs of *S. brevistigma* in experimental mice. In the present experiment, no significant change in body weight was recorded (Fig. - 1). However, when compared to control a significant decrease of body weight in mice sensitized to OVA and significant weight gain for the mice treated with *S. brevistigma* was noticed. PCV was increased in all the test groups when compared with OVA treated group. These increases were statistically significant (p<0.05) in groups IV & V (Plate 1). Furthermore, a statistically significant (p<0.05) increase was observed in HB count and RBC of the groups administered with 200 mg/kg and 400 mg/kg of the ethanolic extract of *S. brevistigma* when compared with the sensitized group. A significant (p<0.001) decrease in WBC count and MCH was observed in groups IV & V. There was no significant difference in the percentage of neutrophils, lymphocytes and monocytes count in all the test groups when compared with the control and treated groups. Slight increase in eosinophils was observed in OVA treated group and the significant reduction (p<0.01) was noticed in groups IV & V (Fig - 2).

Dexamethasone the standard (0.27mg/kg p.o.) showed a significant (p<0.01) suppressive effect on the total WBC, MCH, neutrophil (polymorphs), eosinophils, lymphocytes and monocytes count in the BAL fluid as compared to the sensitized group. The test plant extract significantly inhibited (p<0.01) the total eosinophils, neutrophil (polymorphs), monocytes, WBC and lymphocytes at 200 and 400 mg/kg treatments, which was more or less equal to the standard.

Histopathological Studies

Histopathological studies were carried out to confirm the protective effect of the test drug against ovalbumin induced injury. The histopathological investigations on the lung tissues of the experimental animals revealed the protective nature of the ethanolic extract of *S.brevistigma*. The various architectural changes in the tissues were depicted in Plate 2. Histological section of Group I (control) and Group II (standard) revealed no obvious abnormality. The blood vessels appear with normal alveoli and bronchioles. On the other hand, Group III (OVA treated) exhibited airway inflammation, infiltration of eosinophils, lymphocytes, and sub mucosal oedema of the lungs, bronchoconstriction shown as lumen plugging by mucus and cells. Treatment with *S. brevistigma* extract i.e. group IV and V prevented the tissue oedema, epithelial cell hypertrophy, infiltration of inflammatory cell and airway lumen plugging thereby, decreasing inflammation and bronchoconstriction, which leads to normal bronchiole size. Among all, group V shown the best results, which was all most comparable with that of normal group.

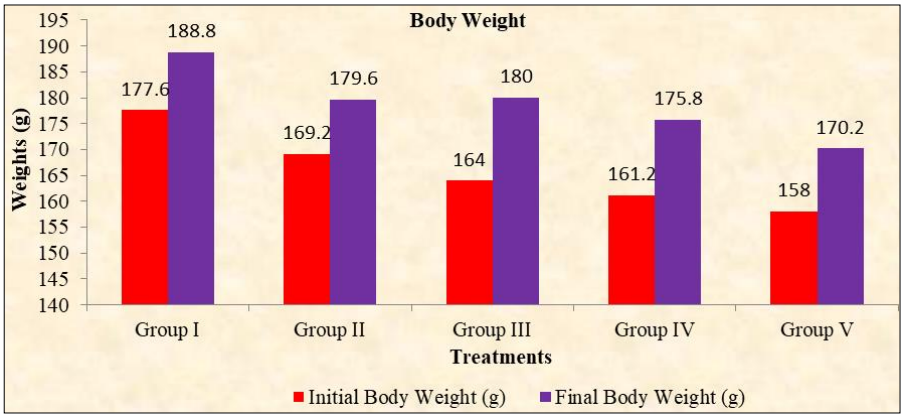


Fig 1: Effect of ethanolic extract of *S. brevistigma* on body weights against ovalbumin induced mice

Group –I: Control
Group II: Only Ovalbumin
Group III: Ovalbumin + Std (Dexamethasone)
Group IV: Ovalbumin + ESB. L.D
Group V: Ovalbumin + ESB. H.D
H.D: High Dose
L.D: Low Dose
ESB: Ethanolic extract of *Sarcostemma brevistigma*

Table 1: Anti-asthmatic activity of ethanolic extract of *S. brevistigma* using ovalbumin induced haematological study

S. No.	Characters	Group I	Group II	Group III	Group IV	Group V
1.	Initial Body Weight (g)	177.6±3.12	169.2±1.62^{ns}	164±2.82**	161.2±2.43***	158±1.41***
2.	Final Body Weight (g)	188.8±3.07	179.6±1.96^{ns}	180±3.63^{ns}	175.8±2.33**	170.2±1.24***
3.	PCV (%)	44.93± 0.95	29.8± 1.24***	42.23± 1.06^{ns}	31.3± 1.65***	42.73± 0.75^{ns}
4.	HB (mg/dl)	14.67± 0.31	09.6± 0.41	10.4± 0.34	10.1± 0.55	12.7± 0.42
5.	RBC (x10$^{\wedge 6}$)	05.89± 0.11	04.37± 0.18***	05.24± 0.13**	04.23± 0.13***	04.47± 0.11***
6.	WBC (x10$^{\wedge 3}$)	09.43± 0.24	14.57±0.24***	10.9± 0.43*	13.9± 0.56***	12.7± 0.20***
7.	MCH (Pg)	24.06± 0.31	25.96± 0.084	24.03± 0.20***	25.2± 0.19^{ns}	24.23± 0.20***
8.	Neutrophil (Polymorphs) (%)	08.33± 0.42	12.33± 2.01	08.66± 1.83	10.33± 2.20	09.33± 4.02
9.	Lymphocytes (%)	80.66± 2.56	86.66± 0.76	79.33± 2.48	84.00± 2.92	83.67± 4.005
10.	Monocytes (%)	03.03± 0.42	04.33± 0.21*	02.33± 0.21*	03.33± 0.21	02.67± 0.21
11.	Eosinophils (%)	02.66± 0.21	04.66± 0.55**	03.00± 0.36**	03.66± 0.55^{ns}	03.33± 0.21*

Group –I: Control; Group II: Only Ovalbumin; Group III: Ovalbumin + Std (Dexamethasone); Group IV: Ovalbumin+ ESB. L.D;
Group V: Ovalbumin + ESB. H.D; H.D: High Dose; L.D: Low Dose; ESB: Ethanolic extract of *Sarcostemmabrevistigma* PCV: Packed cell volume; MCH: Mean corpuscular haemoglobin; HB: Haemoglobin; WBC: White blood cells; RBC: Red blood cells
Values are expressed as mean ± SEM Statistical significance (p) calculated by one way ANOVA followed by dunnett's (n=6); ns-non significant, *p>0.05, **p<0.01, ***p<0.001, calculated by comparing treated groups with control group.

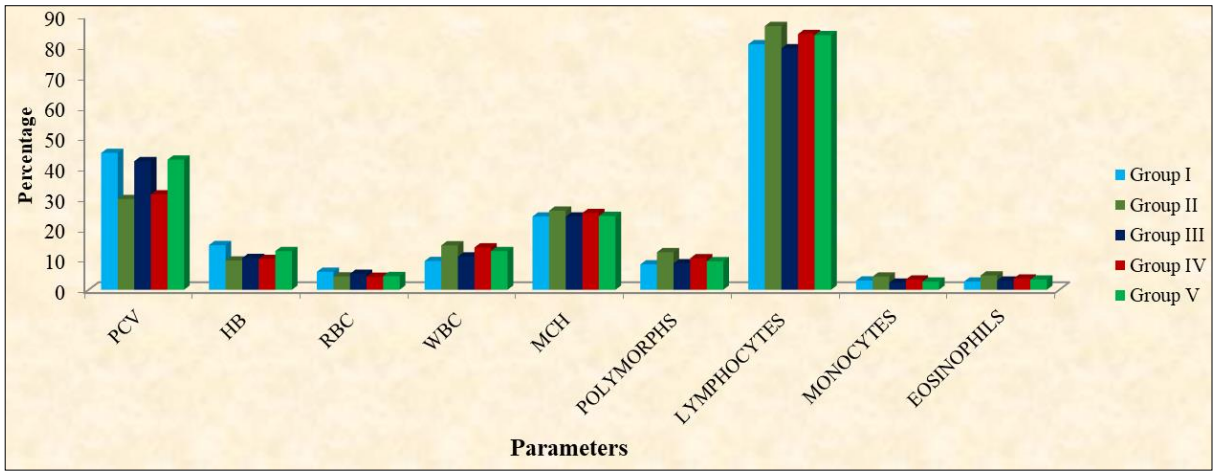
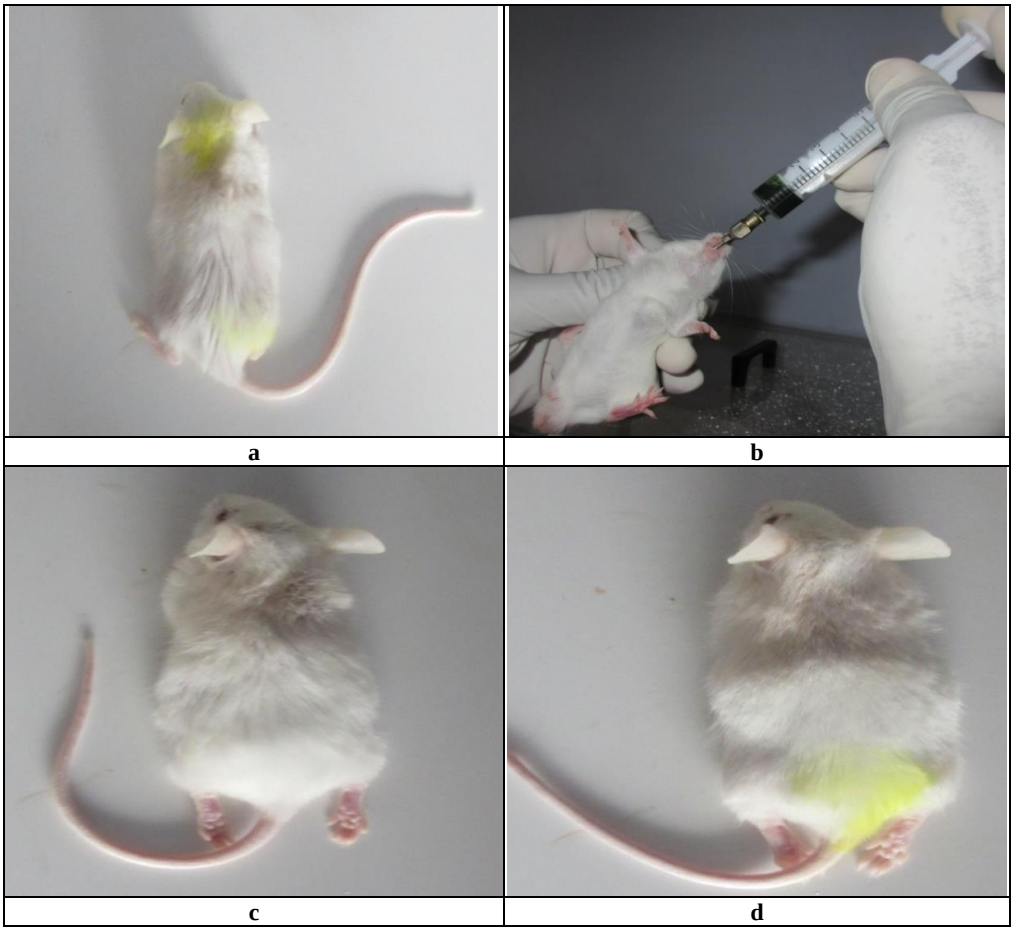
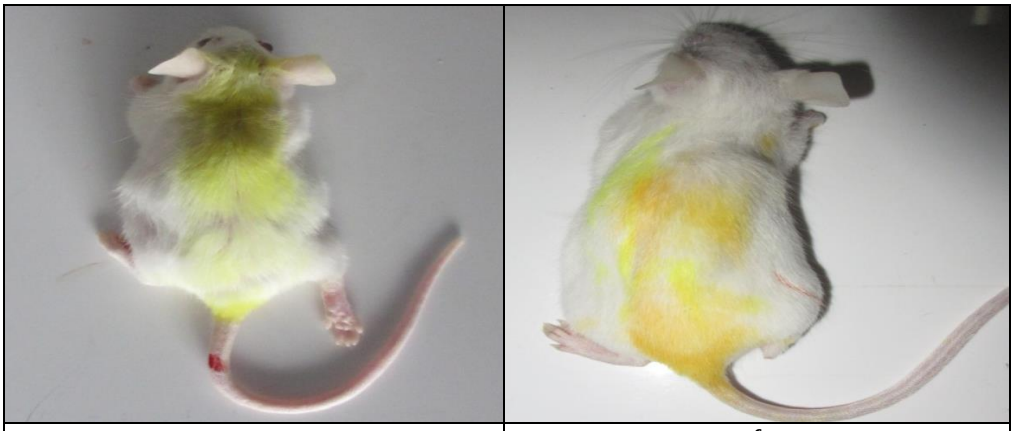


Fig 2: Effect of ethanolic extract of *S. brevistigma* on haematological parameters of ovalbumin induced mice

Group –I - Control
Group II - Only Ovalbumin
Group III- Ovalbumin + Std (Dexamethasone)
Group IV- Ovalbumin + ESB. L.D (200mg/kg p.o.)
Group V- Ovalbumin + ESB. H.D (400mg/kg p.o.)
H.D - High Dose
L.D - Low Dose

ESB - Ethanolic extract of *Sarcostemma brevistigma*
PCV - Packed Cell Volume
HB - Haemoglobin
MCH: Mean corpuscular haemoglobin
WBC: White blood cells
RBC: Red blood cells

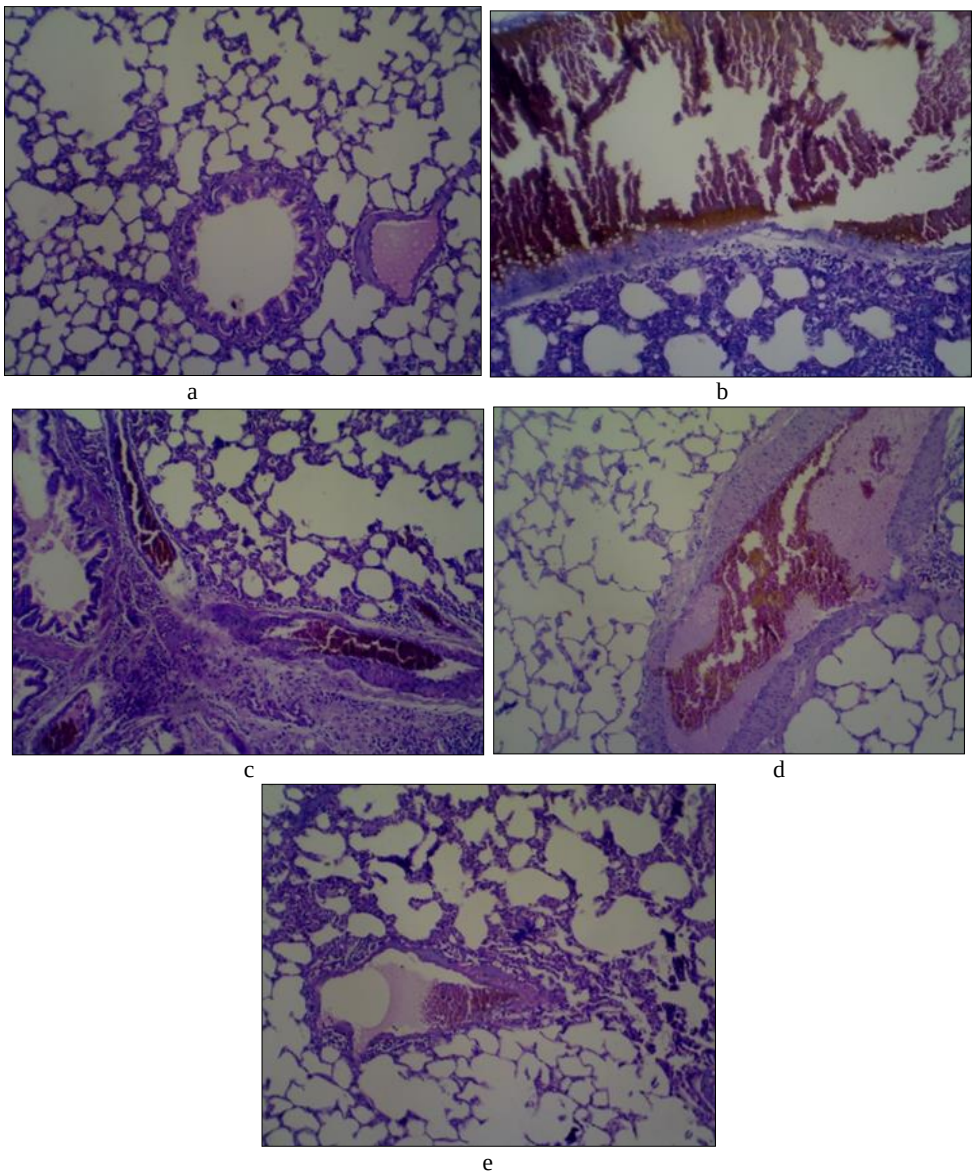




e **f**

a. Control mice
b. Oral administration of ovalbumin (8mg/kg)
c. Ovalbumin treated mice
d. Ovalbumin (8mg/kg) + standard drug dexamethasone treated mice
e. Ovalbumin (8mg/kg) + 200mg extract of treated mice
f. Ovalbumin (8mg/kg) + 400mg extract of treated mice

Plate 1: Antiasthmatic activity of ethanolic extract of *S. brevistigma* inovalbumin induced mice



a b

c d

e

Plate 2: Representative Photomicrographs of histopathological changes showing the effect of *S. brevistigma* on Ovalbumin induced anti-asthmatic activity in mice

a. Lung of control group: lung shows normal alveoli and bronchioles
b. Lung of rats treated with ovalbumin and standard drug dexamethasone (0.27mg/kg.p.o.)
c. Lung showing normal alveoli, bronchioles, epithelial cells, normal air way lumen and normal bronchiole size.
d. Lung of rats treated with ovalbumin. Lung showing destruction of alveoli, with peribronchiolitis. Blood vessels shows congestion, dense infiltration of chronic inflammatory infiltrates composed mainly of lumphocytes, esinophilis and sub mucosal oedema of the lungs
e. Lung of rats treated with ovalbumin and test drug at the rate of 200mg/kg (po) (low dose)
f. Lung showing normal alveoli, bronchioles epithelial cells, normal air way lumen and normal bronchiole size.
g. Lung of rats treated with ovalbumin and test drug at the rate of 400mg/kg (po) (high dose)
h. Lung showing normal alveoli, bronchioles epithelial cells, normal air way lumen and normal bronchiole size.

Discussion

Asthma is a chronic inflammatory lung disease, characterized by an influx and activation of inflammatory cells. The inflammatory response in the asthmatic lung is characterized by infiltration of the airway wall by mast cells, lymphocytes and eosinophils, and is associated with the increased expression of several inflammatory proteins, including cytokines, enzymes and adhesion molecules, within the airways [18, 19]. Oxidative stress is one of the reason for asthma, induced by a large variety of oxygen free radicals, including reactive oxygen species (ROS), which aggravates bronchial obstruction in asthma. Thus, the imbalance between defence and free-radical production systems causes lesions at the level of the body cells. Accumulating clinical and experimental evidence indicates that ROS plays an essential role in the pathogenesis of airway inflammation [20, 21]. Eosinophils are known to act as the primary effector cells in the pathogenesis of asthma through the release of ROS as well as specific granule proteins [22]. Therefore, several treatments to reduce the phenomenon of oxidative stress have been considered, including plant extracts used in diseases of the respiratory system [23, 24, 25, 26, 27].

In the present experiment a significant decrease of body weight in guinea pigs sensitized to OVA compared to the control group and significant weight gain for the guinea pigs treated with *S. brevistigma*. This result is in agreement with that obtained by Juma [28], who noticed a weight gain of 1.27 g during treatment with an aqueous extract of nettle [27] and in *Urtica dioica*. Contrary to our result [29] observed that *Enantia chlorantha* extract caused a slight but insignificant decrease in the body weight of the test animals; this may be due to a decrease in appetite, which may be secondary to a feeling of fullness after administration of the extract. It may also be due to the effect of the plant on the body fat metabolism. The result observed by Mahajan [30] was also contrary to the present findings.

The significant increase in the levels of PCV, haemoglobin concentration and redblood cell counts with increasing doses showed that theethanolic extract of *S. brevistigma* treated groups. The mechanism leading to the increase in PCV, HB and RBC parameters is probably mediated by the anti-oxidant property of *S. brevistigma* extract which has been variously demonstrated by other researchers [31]. In this study, *S. brevistigma* was attributed to contain saponins, which are known to have deleterious haemolysing effect on circulating erythrocytes [32]. This is in consonance with the findings of [33].The observed significant decrease ($p<0.001$) in values of WBC at 200 mg/kg and 400 mg/kg may be attributed to stimulation of the immune system that might have been caused by chemical and secondary infections. Our findings are agree with Adebayo [34, 35].

The infiltration of eosinophils is one of the principal characteristics of allergic inflammation associated with bronchial hyperresponsiveness and this is by the release of various proteins and ROS. Moreover, it has been proven that it was the activated eosinophils that induce epithelial damage characteristics in asthmatic subjects [36]. Thus, in the OVA-sensitized rats, a significant ($p<0.01$) increase in the rate of eosinophils compared to the control and treated groups was clearly seen in this study, confirming the presence of a recruitment of eosinophil and their extravasation towards lungs. These results shown in Fig. – 26 are in agreement with [24, 27, 37]. Collectively, our result therefore indicates that ethanolic extract of *S. brevistigma*is safe and effective agent for suppressing the early stages of allergic asthma. A similar concept was found in the literature demonstrating the role of *Sanguisorba officinalis* [23] and *Citrullus colocynthis* and *Cucumis trigonus* [36] in suppressing the early stages of asthma.

Lymphocytes are important regulators of the immune response and their role in asthma is related to chronic inflammation. In addition, several studies on rats and guinea pigs, in which experimental asthma was caused by exposure to ovalbumin, have shown an increase in lymphocyte ratio [27, 38 & 39]. This corresponds well with our results, confirmed with a significant increase ($p<0.01$) in the lymphocyte levels in the OVA lot compared to the control and significant decrease ($p<0.05$) in lymphocytes level was observed in BALF (Bronchoalveolar Lavage Fluid) in *S. brevistigma* lot. This decrease was due to a decrease in the recruitment of lymphocytes, once again claiming the reduction of lung inflammation (Fig.-26). In the present study, *S. brevistigma* significantly inhibited the characteristics of airway inflammation, including infiltration of inflammatory cells such as lymphocytes, eosinophils and neutrophils. A similar trend was reported by Bulani [40] in *Calotropis gigantea* and Suralkar [41] in *Abrus precatorious.*

Our results showed that the increased levels of eosinophils and other inflammatory cells in BALF induced by OVA challenge returned to normal levels by *S. brevistigma* treatment, indicating that *S. brevistigma* is an efficacious eosinophil-depleting agent. These results are supported by our histopathological findings, which include the demonstration that *S. brevistigma* significantly reduced OVA-induced leukocyte infiltration and eosinophil. Airway inflammation in asthma is a multicellular process involving mainly eosinophils, neutrophils, CD4+ T lymphocytes and mast cells, with eosinophilic infiltration being the most striking feature [24, 43].

In the present study, the ethanolic extract of the *S.brevistigma* was tested for anti asthmatic property at doses of 200mg/kg and 400mg/kg. The results from our clinical study suggested that, there was appreciable decrease in severity of symptoms of asthma and there also exists a simultaneous improvement in lung function parameters and

also defends the syndrome of bronchial asthma by releasing chemical mediators such as histamine [42].

The test plant at200 mg/kg and 400mg/kg doses significantly inhibited the Ovalbumin induced contraction of guinea pigs lung preparation indicating its H1 receptor antagonistic activity and supports the anti-asthmatic property of the plant. Asthma involves an imbalance between Th1 and Th2 cell-related factors [19], which results in airway hyper responsiveness in asthma patients as well as morphological changes in lung tissues such as mucus hypersecretion in the bronchioles, goblet cell and epithelial cell hyperplasia and eosinophil infiltration near to the bronchioles and vessels [4, 35].

Conclusion

The present study suggests that *S.brevistigma* is a promising candidate for the treatment of asthma, although additional studies are warranted in order to improve our understanding of the potential effects of *S.brevistigma*, as well as the mechanisms responsible for these effects.

Conflict of Interest Statement

We declare that we have no conflict of interest

Sources of Support

Nil

Authors Contribution

Conception or design of the work, Data collection, Data analysis and interpretation, Drafting the article, Critical revision of the article, Final approval of the version to be published.

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Reference

1. United States Environmental Protection Agency (EPA). Asthma Facts EPA-402-F-04-019., 2013.
2. World Health Organization. Asthma Fact Sheet No307, 2013.
3. Ghushchyan VH, Sucher B, Globe DR, Lin SL, Globe G, Sullivan PW, Asthma control in the United States, indicators of poor asthma control. J Allergy ClinImmunol,2008-2010:133:1579-1587.
4. Kay AB: Allergy and allergic diseases. First of two parts. N Engl J Med,2001:344:30-37.
5. National Asthma Education and Prevention Program. Expert Panel Report: Guidelines for the Diagnosis and Management of Asthma Update on Selected Topics - J Allergy ClinImmunol 110 (Suppl 5) S141-S219, 2002.
6. Cragg GM, Kingston DGI, Newman DJ. Editors. Anticancer agents from natural products. CRC/Taylor & Francis; Boca Raton, FL, 2005.
7. Kirtikar KR, Basu BD.“Indian Medicinal Plants”, Lalit Mohan Basu., Allahabad, 1956, 723.
8. TheWealth of India, “Raw Materials”, Council of Scientific and Industrial Research (CSIR), New Delhi, 1969, 225.
9. Govindhari TR, Vishwanathan N, Radhakrishnan J. “Tylophora alkaloids”. J. Ind.chem. Soc,1975:3:1-9.
10. Nikajoo LT. Analgesic activity of aqueous and alcohol root extracts of Pergulariadaemia (forsk.) Chiov. Int. J. Pharmacy Pharm. Sci.,2009:1(l):33-37.
11. Ganesan S, Venkateshan G, Banumathy N. Medicinal plants used by ethnic group Thottianaickans of Semmalai hills (reserved forest), Tiruchirappalli district, Tamil Nadu, India. J.Traditional Knowledge,2006:5:242-252.
12. Jeeva S, Femila V. Ethnobotanical investigation of Nadars in Atoor village, Kanyakumari district, Tamil Nadu, India. Asian Pac.J.Tro.Biomed,2012:2:593-600.
13. Lingaraju DP, Sudarshana MS, Rajashekar N. Ethnopharmacological survey of traditional medicinal plants in tribal areas of Kodagu district, Karnataka, India. J. Pharmacy Res,2013:6:284-297.
14. Reddy CS, Reddy KN, Murthy EN, Raju VS. Traditional medicinal plants in Seshachalam hills, Andhra Pradesh, India. J. Med. Plants Res,2009:3:408-412.
15. Umberto Q. CRC World Dictionary of Plant Names. *4 R-Z. Taylor & Francis US. p. 2388, 2000.*
16. Irvine F.Woody Plants of Ghana. London: Crown agents for overseas Administration, 1961, 23-24.
17. Chapman RW, Howard AH, Richard J, Celly C. Effect of inhaled roflumilast on the prevention and resolution of allergen induced late phase airflow obstruction in Brown Norway rats. European J. Pharmacol,2007:571:215-221.
18. Barnes PJ. Cytokine-directed therapies for the treatment of chronic airway diseases. Cytokine Growth Factor Rev,2003:14:511-522.
19. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. Nat. Rev. Immunol,2008:8:183-192.
20. Kwak YG, Song CH, Yi HK, Hwang PH, Kim JS, Lee KS, *et al*. Involvement of phosphatase and tensin homologue deleted on chromosome ten (PTEN) in airway hyperresponsiveness and inflammation in bronchial asthma. J. Clin. Invest.,2003:111:1083-1092.
21. Lee YC, Kwak YG, Song CH. Contribution of vascular endothelial growth factor to airway hyper responsiveness and inflammation in a murine model of toluene diisocyanate- induced asthma. J. Immunol,2002:168:3595-3600.
22. Rahman I, Morrison D, Donaldson K, Mac Nee W. Systemic oxidative stress in asthma, chronic obstructive pulmonary disease (COPD), and smokers. Am. J. Respir. Crit. Care. Med,1996:154:1055-1060.
23. Lee NH, Lee MY, Lee JA, Jung DY, Seo CS, Kim JH, *et al*. Anti-asthmatic effect of *Sanguisorbaofficinalis*L.and potential role of heme oxygenase-1 in anovalbumin-induced murine asthma model. Int. J. Mole. Med,2010b:26:201-208.
24. Lee MY, Lee NH, Seo CS, Lee JA, Jung D, Kim JH, *et al*. *Alpiniakatsumadai*seed extract attenuate oxidative stress and asthmatic activity in a mouse model of allergic asthma. Food Chem. Toxicol,2010a:48:1746-1752.
25. Tiwari P, Mishra BN, Sangwan NS. Phytochemical and pharmacological properties of *Gymnemasylvestre*: An important medicinal plant. Biomed. Res. Int, 2014, 1-18.
26. You H, Chen S, Mao L, Li B, Yuan Y, Li R, *et al*. The adjuvant effect induced by di-(2-ethylhexyl) phthalate

(DEHP) is mediated through oxidative stress in a mouse model of asthma. Food Chem. Toxicol,2014:71:272-281.
27. Zemmouri H, Sekiou O, Ammar S, El Feki A, Bouaziz M., Messarah M, *et al*. *Urticadioica* attenuates ovalbumin-induced inflammation and lipid peroxidation of lung tissues in rat asthma model. Pharma. Biol,2017:55(1):1561-1568.
28. Juma KK, Maina SG, Muriithi JN, Mwangi BM, Mworia JK, Mwonjoria MJ, *et al*. Protective effects of *Urticadioica* and cimetidine on liver function following acetaminophen induced hepatotoxicity in mice. J. Develop. Drugs,2015:4:130.
29. Adebiyi OE, Abatan MO. Phytochemical and acute toxicity of ethanolic extract of *Enantiachlorantha* (oliv) stem bark in albimo rats. Toxico,2013:6(3):145-151.
30. Mahajan SG, Mehta AA. Suppression of ovalbumin-induced Th2-driven airway inflammation by β-sitosterol in a guinea pig model of asthma. European Journal of Pharmacology,2011:650:458-464
31. Ekpenyong CE, Akpan UP, Ben EE, Nwama EO, Ibu JO. Hematological effect of chronic administration of ethanolic extract of *Garciniaconruana*seed on rat. J.Natural Prod,2011:4:173-176
32. Sofowora A. Screening plants for Bioactive Agents. In: medicinal plants and traditional medicinal in Africa. 2nd Ed. Spectrum Books Ltd., Sunshine house, Ibadan, Nigeria, 1993, 134-256.
33. Igwe AC, Onabanjo AO. Chemotherapeutic effects of *Annonasenegalensis*. Trop. Med. Paras,1989:83:521-534.
34. Adebayo AH, Abolaji AO, Opata TK, Adegbenro IK. Effects of ethanolic leaf extract of ChrysophyllumalbidumG. on biochemical and haematological parameters of albino Wistar rats. Afr. J. Biotechnol,2010:9:2145-2150.
35. Seo JH, Bang MA, Kim G, Cho SS, Park DH. Erythroniumjaponicumattenuates histopathological lung abnormalities in a mouse model of ovalbumin-induced asthma. Int. J. Mole. Med,2016:37:1221-1228.
36. Raju S, Kumar MNS, Gupta S, Srinivas T, Naga ST, Shankar JK, *et al*. 5-Aminosalicylic acid attenuates allergen-induced airway inflammation and oxidative stress in asthma. Pulm. Pharmacol. Therap,2014:29:209-216.
37. Yuk JE, Woo JS, Yun CY, Lee JS, Kim JH, Song GY, *et al*. Effects of lactose-beta-sitosterol and beta-sitosterol on ovalbumin-induced lung inflammation in actively sensitized mice. Int. Immunopharmacol,2007:7:1517-1527.
38. Xiang-hua L, Xian-yu T, Zhang D, Xu J-F, Wang W-Y, *et al*. Effects of *Wuweidilong* decoction on inflammatory cells and cytokines in asthma model guinea pigs. J. Tradit. Chin. Med,2009:29:220-223.
39. Wadibhasme PG, Ghaisas MM, Thakurdesai PA. Antiasthmatic potential of chrysin on ovalbumin induced bronchoalveolarhyperresponsiveness in rats. Pharm. Biol,2011:49:508-515.
40. Bulani V, Biyani K, Kale R, Joshi U, Charhate K, Kumar D, *et al*. Inhibitory effect of *Calotropisgigantea*extract on ovalbumin induced airway inflammation and arachidonic acid induced inflammation in a murine model of asthma. Int. J. Cur. Bio. Med. Sci,2011:1(2):19-25.
41. Suralkar AA, Kasture SB, Inhibitory effect of *Abrusprecatorious*extract on bronchial hyperreactivity induced by ovalbumin in experimental animals. Int. J. Pharm. Pharm Sci,2013:5(1):403-409.
42. Chaitanya B, Raviteja Sagi SM, Shashikanth P, Karunakar K. Evaluation of anti-asthmatic activity of ethanolic extract of *Ephedra Gerardiana*Wall in mice by ovalbumin induced method. Asian J. Pharma. Clinical Res,2014:7(1):166-169.
43. Kay AB. The role of eosinophils in the pathogenesis of asthma. Trends Mol. Med,2005:11:148-152.