

Study of anti-asthmatic and anti-inflammatory activity of *aloe barbadensis miller* leaf

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Abstract- *Aloe barbadensis Miller* is commonly named “Korphad”. It is native to Africa and the eastern European continents and spread almost throughout the world. The present study reports important secondary metabolites present in *Aloe barbadensis Miller* belonging to the family Liliaceae. It is one of the widely distributed medicinal plants in treating many diseases like diabetes, inflammation, ulcer, tumor, cancer, etc. The powdered leaf was subjected to extraction by using ethanol. The ethanolic extract of *Aloe barbadensis Miller* was investigated for anti-asthmatic and anti-inflammatory activity in animal models with their phytochemical investigation. The anti-asthmatic activity was studied on clonidine-induced catalepsy in mice, and anti-inflammatory activity was studied using carrageenan-induced paw edema in rat models, at a 200 mg/ kg body weight dose, and 400 mg/ kg body weight.

Keywords: *Aloe barbadensis Miller*, anti-asthmatic, anti-inflammatory, catalepsy, clonidine, carrageenan, Phytochemical.

1. INTRODUCTION:

Asthma is a serious allergic disorder of the respiratory system, marked by inflammation and narrowing of airways. This has a great responsibility on medical treatment. Current therapy for asthma has many serious side effects. Therefore, searching for a new therapeutic agent with no or fewer side effects is needed while most inexpensive for the patients. Due to the availability of strong bioactive chemicals, anti-asthmatic herbal remedies have been employed in traditional medicine and have demonstrated potential therapeutic anti-asthmatic efficacy. Breathlessness, chest tightness, and wheezing during a paroxysmal attack of bronchial asthma are symptoms brought on by a paroxysmal narrowing of the bronchial airways. Asthma is characterized by inflammation (The airway lining becomes red, swollen, and narrow), obstruction (airway muscles become tightened and it may cause difficulty to air get in and out of the lungs), and hyper-responsiveness (muscles encircled and very quick response on small allergens and irritants) of the airway. Generally, patients cough more during sleep and when awake in the morning (Rida Zainab et al. 2019). Asthma affects 10% of the population. Epidemiology will help us study asthma's prevalence, morbidity, and mortality rate. Around 235 million individuals around the world have been affected. Many people die from this deadly disease in many countries due to its high prevalence rate. The number of individuals has been increasing day by day (Dr. Khadim Qureshi 2019). Millions of people have died from asthma. The highest number of patients is in New Zealand, Australia, and the United Kingdom, and the lowest patient rate is in China and Malaysia. About 7% of adults and 15% of children have asthma.

In several individuals, inflammation causes frequent episodes of wheezing, shortness of breath, coughing, and chest tightness, particularly at night or in the early morning or after exposure to allergens, cold air, and exercise (shinde Suvarna et al. 2019).

Nowadays, most of the cases of asthma were considered similar but differ only based on disease severity. Therefore, the treatment of patients with asthma requires differences in dose, route of intake, or frequency of taking the β 2-adrenoceptor agonist, and corticosteroids that are essential to manage asthma disease. However, asthma sub-phenotypes identification has challenged this view in modern medicinal systems. Over the past two decades, research has identified the fundamental source of asthma is the allergic pathways.

The National Institute of Health defines asthma as a chronic inflammatory disorder of the airways in which cellular elements play a major role particularly mast cells, T-lymphocytes, eosinophils, epithelial cells, and neutrophils. Asthma is an inflammatory disease that targets the airway's narrowing and thereby resulting in the change of eosinophils, mast cells, lymphocytes, and cytokine levels. It is characterized by Coughing, Wheezing, Breathlessness, Respiratory rate increased, Chest tightness, Chest or abdominal pain, Fatigue, feeling out of breath, Agitation, and Increased pulse rate. The patient with asthma is well known to have greater levels of IgE that bind to the receptor of mast cells and inflammatory products (Abhimanyu Kumar Jha et al.2021). The antigen-antibody interaction results in the activation of inflammatory cellular reactions ([Stephen Holgate](#) and [Riccardo Polosa](#) 2008). Thereby releasing the mediator such as histamine, a prostaglandin that ultimately led to the contraction of airway smooth muscles.

Mast cell plays a key role in the pathophysiology of asthma and is abundant in the airways of asthmatic patients. They are orchestrated by several interacting cytokines, one of which is stem cell factor released by the epithelial cell upon encounter with inhaled allergens. Now the inhaled allergens activate sensitized mast cells by binding with IgE molecules present on the surface of mast cells to release various bronchoconstrictor mediators. Dendritic cells also process the allergens after being conditioned by the thymic stromal lymphopoietin (TSLP) released by the mast and epithelial cells to release several of the chemokines that draw T helper 2 cells, these cells in turn, induce B cells to produce and secrete IgE antibodies that sensitize mast cells, induce eosinophil mediated inflammation and stimulate mast cell proliferation (P.J. Barnes 2011).

Aloe barbadensis Miller belongs to the Liliaceae family with thick, green lance-shaped, juicy, sharp, and edged leaves (D. I. Sanchez-Machado et al. 2017). Aloe vera grows in dry regions of Africa and the eastern European continents. Aloe vera is probably the most used medicinal plant commercially and the most popular plant around the world (A. Surjushe et al. 2008). Ethanolic leaf extract of the plant contains vitamins, minerals, enzymes, sugars, anthraquinones, or phenolic compounds, lignin, saponins, sterols, amino acids, and salicylic (Chauhan et al. 2007). Therapeutic Effects of Aloe Vera in wound healing, anti-inflammatory, antioxidant, etc (Sandeep Kumar Verma 2011). This dissertation investigated the anti-asthmatic, Anti-inflammatory activity of the ethanolic extract of *Aloe barbadensis Miller* leaf (EEAB) in experimental animal models namely carrageenan-induced paw oedema in rats and also asthma effect in clonidine-induced catalepsy in mice.

2. MATERIALS AND METHODS:

2.1. Chemicals and reagents:

Clonidine, chlorpheniramine maleate, ketotifen, saline water, carrageenan, diclofenac sodium, etc.

2.2. Experimental Animals:

Mice (Swiss albino Mice) of both sex weighing between 25-35 gm and Wistar rats were used. Protocol or the experimental procedure approved by the animal ethics committee of the institute. Animals under the study were maintained under CPCSEA standard procedure for the animal house before and during the experiment. Mice and rats used in this project were procured from the Animal House of Sudhakar Rao Naik Institute of Pharmacy, Pusad. The animals were housed in Poly propylene cages and maintained at 24°C 2°C under 12h light/ dark cycle and were feed ad libitum with a standard pellet diet and had free access to water.

2.3. Collection and authentication of Plant material:

The plant was collected from the kitchen gardens of the Umarkhed city of Yavatmal district in the month of December and January. The plant was authenticated by Dr. Ramkrishna Sakhre, Department of material science, Ayurvedic College, pusad, yavatmal 445204.

2.4. Preparation of the extract:

The fresh *Aloe barbadensis miller* leaves were washed thoroughly 2-3 times with distilled water and air-dried. A few of these leaves were chopped into small pieces and ground into a fine powder with the help of a grinder machine, and a mortar pestle and polyethylene bags were used to store them. These bags were kept in the oven for further analysis. The dried powder was extracted with 70% ethanol by using the Soxhlet apparatus.

2.5. Phytochemical Screening:

The obtained ethanolic extract was tested for various chemical constituents with the help of qualitative chemical tests (Nafeesa Zahid Malik et al. 2017).

2.6. Anti-asthmatic activity:

Clonidine-induced catalepsy in mice-

Albino mice Will be divided into four groups (n=3).

Group I: Control animals received distilled water 10 ml/kg p.o.

Group II: The animal received chlorpheniramine malate at the dose of 10 mg/kg i. p.

Group III: Animals received EEAB at the dose of 200 mg/ kg body wt. p.o.

Group IV: Animals received EEAB at the dose of 400 mg/ kg body wt. p.o.

All the groups will be received Clonidine (1mg/kg s.c.) 1 hr after the drug administration and the duration of catalepsy will be measured at (15,30, 60, 90, 120, 150, and 180 min) (Deepak Kumar et al. 2009; Dnyaneshwar J. Taur et al. 2012).

2.7. Anti-inflammatory activity study –

Carrageenan-induced rat paw edema:

The anti-inflammatory reaction is readily produced in rats in the form of paw edema using carrageenan. The rats were divided into four groups, each consisting of three animals.

Group I: Control animals received distilled water 10ml/kg p.o.

Group II: Animals received standard Diclofenac sodium at the dose of 5mg/kg p.o.

Group III: Animals received an EEAB at the dose of 200 mg/kg p.o.

Group IV: Animals received an EEAB at the dose of 400 mg/kg p.o.

After 30 min of extracts administration, 0.1ml of 1% w/v carrageenan was injected into the right hind paw sub-plantar region of each rat. The left paw served as a reference (non-inflammatory paw) for comparison. The paw volumes of both paws of control and extract-treated rats were measured at 30, 60, 90, and 120 min after carrageenan administration". The percentage of edema inhibition for each rat and each group was obtained by using the formula.

RESULTS:**2.8. Table no. 1) Percent yield of ethanolic extract of *Aloe barbadensis* Millar:**

Plant Name	<i>Aloe barbadensis</i> Millar
Part used	Leaves
Solvent used	Ethanol
Weight of dried leaves	150 gm
Yield	12.7 gm
Percent yield	8.7 %

2.9. Table no. 2) Phytochemical analysis of an ethanolic extract of *Aloe barbadensis* Millar:

Sr no.	Phytochemicals	Chemical test	Ethanolic extract
1.	Alkaloid	Dragendroff's test	+
2.	Cardiac Glycoside	Keller-Kilani test	+
3.	Reducing sugar	Fehling's test	+
4.	Steroids	Salkowaski test	+
5.	Terpenoids	Vanillin-sulphuric test	-
6.	Carbohydrates	Molish test	+
7.	Amino acids	Ninhydrin test	+
8.	Flavonoids	Shinoda Test	+
9.	Tannins	Ferric Chloride test	+
10.	Saponin	Lead acetate test	+
11.	Cyanogenic glycosides	-	-
12.	Volatile oil	-	-

2.10. Anti-asthmatic activity:

• Clonidine-induced catalepsy in mice:

Table no.3) Effect of EEAB on Clonidine-induced catalepsy in mice.

Treatment	Duration of catalepsy in mice (sec)						
	15 min	30 min	60 min	90 min	120 min	150 min	180 min
control	28.60 ± 0.89	73.07 ± 1.92	105.4 ± 3.29	199.7 ± 5.82	221.5 ± 11.37	206.2 ± 2.45	192.6 ± 1.70
Standard (1 mg/kg)	10.10 ± 1.92	21.97 ± 2.55	52.57 ± 6.02	57.57 ± 11.63	63.0 ± 4.17	62.20 ± 3.06	67.90 ± 0.79 ^{***}
EEAB (200 mg/kg)	25.73 ± 1.25	62.30 ± 2.35	91.43 ± 4.74	109.7 ± 17.16	133.3 ± 2.08	105.8 ± 1.61	98.10 ± 1.25 ^{nsns}
EEAB (400mg/kg)	16.80 ± 2.44	55.17 ± 3.62	60.93 ± 3.50	70.0 ± 13.34	76.73 ± 5.98	72.50 ± 1.76	70.17 ± 1.07 ^{***##}

- Values were expressed as mean ± SEM (n=3). The statistical significance between means was analysed using a one-way analysis of variance (ANOVA) followed by Tukey test.
- ^{**}Statistically significant at (p<0.001), ^{***}Statistically significant at (p<0.0001) when compared with inducer control group I.
- ^{##}Statistically Significant at P<0.001, when compared with standard group II.
- ns indicate non significate.

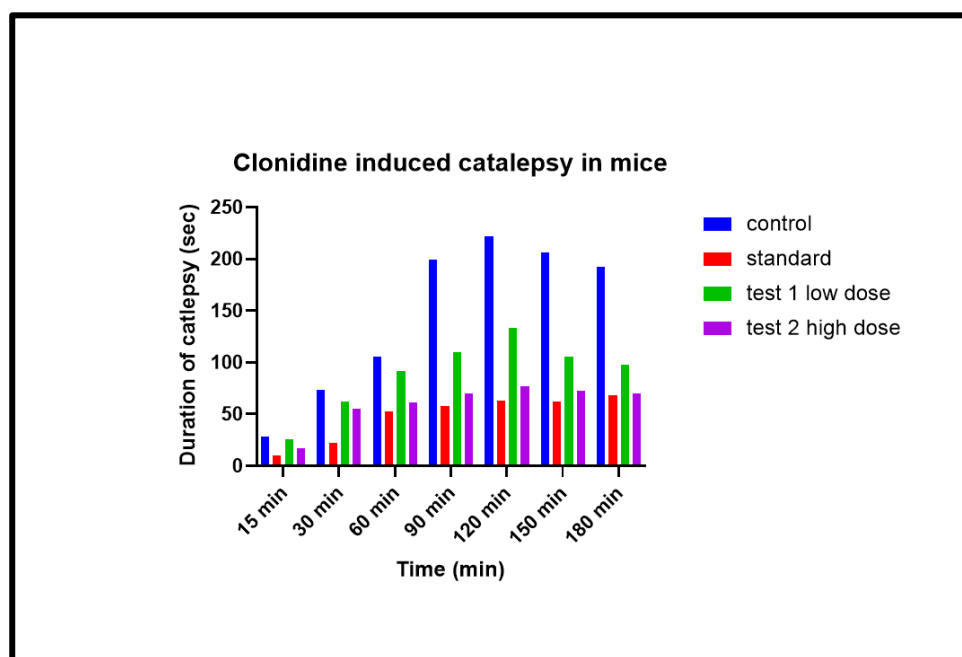


Fig no.1) Effect of ethanolic extract of *Aloe barbadensis miller* on Clonidine-induced catalepsy in mice.

2.11. Anti-inflammatory activity study –

• Carrageenan-induced rat paw edema:

Table no. 3) Anti-inflammatory activity of EEAB on Carrageenan-induced rat paw edema:

Treatments	Increase in Paw volume in ml % inhibition of paw edema				% inhibition
	1 hr	2 hrs	3 hrs	4 hrs	
Disease Control (10 ml/kg)	0.40 ± 0.02	0.42 ± 0.02	0.42 ± 0.02	0.47 ± 0.02	-
Standard (Diclofenac sodium 5mg/kg)	0.19 ± 0.01	0.17 ± 0.014 ^{***}	0.14 ± 0.013	0.12 ± 0.013 ^{***}	74.46%
EEAB (200mg/kg)	0.31 ± 0.01	0.28 ± 0.01 [#]	0.25 ± 0.017	0.24 ± 0.01 ^{*#}	48.93%
EEAB (400 mg/kg)	0.24 ± 0.014	0.21 ± 0.016 ^{##}	0.21 ± 0.014	0.17 ± 0.016 ^{***#}	63.82%

- Values were expressed as mean ± SEM (n=3). The statistical significance between means was analyzed using a one-way analysis of variance (ANOVA) followed by the Tukey test.
- *Statistically significant at (p<0.05), **Statistically significant at (p<0.001), ***Statistically significant at (p<0.0001), when compared with disease control group I.
- #Statistically Significant at P<0.05, ##Statistically Significant at P<0.001, when compared with standard group II.

- ns indicate non significant.
- #Indicates compared to the standard group (Group-II). ##Statistically Significant at $P < 0.001$.
- ns indicate non significant.

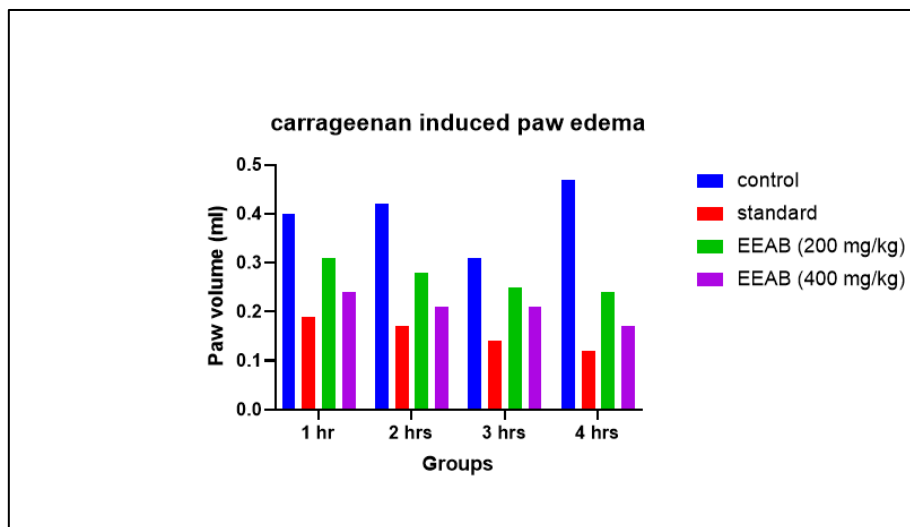


Fig no. 2) Histogram of Anti-inflammatory activity of EEAB on Carrageenan-induced rat paw oedema.

3. DISCUSSION:

Asthma is a chronic inflammatory disorder of the airways characterized by an acute exacerbation of coughing, dyspnoea, wheezing, and chest tightness particularly early in the morning and at night (Rang et al. 2003). Reversible bronchoconstriction, elevated basal airway tone, activation and accumulation of lymphocytes (Eosinophils), hypertrophy of smooth muscles and submucosal glands, submucosal fibrosis, airway wall oedema, mucus overproduction and episodes of non-specific airway hyper-responsiveness to specific spasmogens are common manifestations (Holgate, 1999; Shargel et al, 2004).

Aloe vera has been traditionally used to treat skin injuries (burns, cuts, insect bites, and eczemas) and digestive problems because of its anti-inflammatory, antimicrobial, and wound-healing properties. Research on this medicinal plant has been aimed at validating traditional uses and deepening the mechanism of action, identifying the compounds responsible for these activities. Likewise, new actions have been investigated for Aloe vera and its active compounds, especially highlighting its promising role as a cytotoxic, antitumoral, anticancer, and antidiabetic agent. Aloe-emodin and aloin have been the most studied ones. Particularly, aloe-emodin has resulted to be a promising agent as an antimicrobial, antidiabetic, cytotoxic, cardioprotective, and bone protective (in vitro studies) as well as anti-inflammatory and skin protective compound (in vivo studies). Aloin was effective in the inflammatory process and bone diseases (in vitro studies) and in cancer and cardiovascular diseases (in vivo studies).

Previous studies reported that Aloe vera is rich in secondary metabolites such as Anthraquinone (Aloe-emodin), tannins, saponins, and flavonoids. glycosides. terpenoids, alkaloids, carbohydrates, amino acids, sterols, and steroids (Gajendra Mahor and Sharique A Ali; 2016). Our findings also show similar chemical constituents such as Alkaloid, Cardiac Glycoside, Reducing sugar, Anthraquinone, Steroids, Amino acids, Flavonoids, Tannins, and Saponin in ethanolic leaf extract of *Aloe barbadensis* Miller.

The morphological assessment showed that the leaves of the Aloe vera plant are glabrous green while the color of dried leaves was dull green. The length of both fresh and dried leaves was measured where; the fresh leaf had an average length of 43.3cm while the length of the dried leaf was 38.5cm. Results indicated that dried leaves shrink in size due to

dehydration. That's why; reduced length in dried leaf was noted as compared to fresh one. Leaf width was 8.2cm in fresh form and 6.1 cm in dried form. The weight of fresh leaf noted was 5.4 g whereas 5.2 g weight was noted for dried leaf. The root color of *A. vera* was greyish brown in the fresh sample, and dark brown in the dried sample. The length of the root was 35 cm in fresh form and 34 cm in the dried sample was noted. The width of the fresh root noted was 5.4 whereas 5.2 cm was recorded in the dried root. The weight of fresh root noted was 0.5 g while 0.4 g was noted for dried root. Flowers of *A. vera* were found to be pendulous type and were yellow to whitish in color (Adil Hussain et al. 2017).

Lakdawala et al. 1980 and Jadhav et al. 1983, explains that catalepsy is a condition in which the animal maintains imposed posture for a long time before regaining normal posture. Catalepsy is a sign of extrapyramidal side effects of drugs that inhibit dopaminergic transmission or increase histamine release in the brain. Clonidine α_2 – an adrenoceptor agonist, induces dose-dependent catalepsy in mice, which is inhibited by histamine H1 receptor antagonists but not by H2 receptor antagonists. Clonidine releases histamine from mast cells which is responsible for different asthmatic conditions, Therefore, it has been suggested that the cataleptic effect of Clonidine in mice be mediated by histamine via H1 receptors (Dhanalakshmi et al. 2004).

Our findings show that the Aloe extract at a dose of 200 mg/kg p.o. gives a non-significant decrease in the duration of catalepsy in mice. whereas when the animal was treated with Aloe extract at a dose of 400 mg/kg p.o. shows a highly significant ($P < 0.0001$) decrease in the duration of catalepsy in mice when we compared it with the inducer control group, and similar ($P < 0.0001$) results were observed in the pheniramine maleate 1mg/kg i.p. (standard) treated group of animals. In this study we have observed that treatment of aloe extract significantly inhibits clonidine-induced catalepsy; hence extract may possess anti-asthmatic activity.

Earlier studies reported that intracerebroventricular injection of histamine in conscious rats induced convulsions, which were inhibited by H1 receptor antagonists but not by H2 receptor antagonists. Several drugs are known to induce convulsions in animals. The neuroleptic agents induce catalepsy by inhibiting dopamine D_2 receptors in the substantia nigra. Many studies have shown the role of acetylcholine and histamine in perphenazine-induced catalepsy and suggested that the anticholinergic activity of antidepressants might be due to an increase in dopamine content in the brain or their ability to inhibit the release of acetylcholine (M.S. Rakh et al. 2010; Chopra and Dandiya, 1975).

In our study the Aloe extract at the dose of 200 mg/kg p.o. as shown significantly ($P < 0.05$) delayed the onset of PCT when compared with the inducer control group; whereas aloe extract at the dose of 400 mg/kg p.o. produced highly significant ($P < 0.001$) delayed onset of PCT induced by histamine, and similar ($P < 0.0001$) results were observed in the ketotifen (1 mg/kg p.o.) treated group of animals. The results of the present study suggested that the ethanolic extract of *A. vera* shows a significant inhibitory effect on pre-convulsion symptoms like dyspnoea, asphyxia, convulsion, etc. The result shows that aloe extract has a significant protective effect against asthma.

Alprogen inhibit calcium influx into mast cells, thereby inhibiting the antigen-antibody-mediated release of prostaglandins, histamine, and leukotriene from mast cell (Sharrif Moghaddasi M. 2011). Earlier studies reported that Aloe vera also inhibits the cyclooxygenase pathway, reducing the production of prostaglandins, and thereby reducing inflammation (Surjushe A. et al. 2008; Reynolds T. et al. 1988). According to N. Checkchaki et al. 2018; oxidative stress significantly involves regulating inflammation as well as the pathogenesis of various chronic inflammatory diseases such as asthma. Indomethacin and Diclofenac sodium, like most non-steroidal anti-inflammatory compounds, inhibits the biosynthesis of prostaglandins and this effect might explain its anti-inflammatory activity in carrageenan-induced rat paw edema (Higgs et al., 1979). So, our observation shows that Aloe extracts at a dose of 200 mg/kg p.o. shown significant ($P < 0.05$) inhibition against inflammation (48.93%), whereas animals treated with extract at the dose of 400 mg/kg p.o. shown a highly significant ($P < 0.001$) reduction in inflammation (63.82 %). Similar effects were shown ($P < 0.0001$) when animals were treated with diclofenac sodium 5 mg/kg p.o. (74.46%).

The result obtained support for the use of *Aloe barbadensis miller* in the treatment of respiratory disease. Drugs effective in asthma are mostly steroidal in nature. The phytochemical profile of the plant may be because of these chemical moieties. However, the claims demand further research & studies to isolate & characterize the active principle responsible for the anti-asthmatic activity.

4. CONCLUSION:

Various chemical and physical tests confirmed the presence of alkaloids, flavonoids, steroids, triterpenoids, and saponins in the whole leaf of *Aloe barbadensis miller* showing anti-asthmatic activity. Also, various categories of agents are employed in the symptomatic relief of asthma. Such as antihistaminic, anti-inflammatory, anti-allergic, etc. The PCT was increased in the ethanolic extract of *Aloe barbadensis miller* as compared to the control group. Ethanolic extract *Aloe barbadensis miller* of significantly raised the PCT in histamine-induced bronchospasm. Thus, anti-histaminic activity and broncho-dilating activity were obtained.

In clonidine-induced catalepsy, in mice, Ethanolic extract *Aloe barbadensis miller* of a significant reduction in the duration of action of catalepsy at all time intervals. The standard ketotifen was found to be highly significant at all intervals. The anti-inflammatory activity of ethanolic extract of *Aloe barbadensis miller* was investigated by Carrageenan-induced paw edema. Carrageenan-induced paw edema is a suitable experimental animal model for evaluating the anti-inflammatory effect of natural products. Thus, it shows the antihistaminic activity in dose dependent manner. In the present study, I tried to cover most of the categories of agents in the screening models for the evaluation of the anti-asthmatic activity. A short effort has also been made to discuss the symptoms, causes & triggers of asthma. Thus, Ethanolic extract *Aloe barbadensis miller* shows significant antihistaminic, bronchodilators, and mast cell stabilizing activity in various anti-asthmatic models. All over I can say that ethanolic extract *Aloe barbadensis miller* shows significant antihistaminic activity.

Overall, these reports highlight the relevance of the investigation of bioactive compounds that could present anti-asthmatic potential. As the current asthma treatment involves drugs that have been extensively studied in the past decades, the experimental studies that evaluate the activity of compounds obtained from diverse natural sources might allow the development of new anti-asthmatic drugs in the near future.

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