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Research Article

Evaluation of Anti-asthmatic Activity of *Salvadora oleoids* Leaves

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ABSTRACT

Salvadora oleoids (Family: Salvadoraceae) is being used in the traditional medicine for the treatment of severe bronchitis and asthma. So, the study aims to evaluate anti-asthmatic activity of *S. oleoids* leaves. Leaves are reported to contain flavonoids, saponins, steroids, alkaloids, and tannins, known to possess similar effects. Flavonoids have been shown to smooth muscle relaxant, bronchodilator, antioxidant, and anti-inflammatory activity. The flavonoids are also reported to inhibit mast cell degranulation. Saponins are reported to mast cell stabilizing, anti-allergic, anti-inflammatory and antihistaminic activity. Tannins are reported to inhibit cyclooxygenase (COX) enzyme, decrease vascular permeability and antioxidant activity. Steroids are reported to an extraordinarily strong anti-inflammatory activity by inhibiting cytokine release IL-1, 2 and 6. The present investigation was undertaken to evaluate the anti-asthmatic activity of ethanolic extract of S. oleoids leaves in experimental animals. Asthma is a chronic inflammatory disorder of the airways. Limitations of currently available therapies and associated side effects have created an urge to search new and better treatment options. Thus, present study was designed to evaluate anti-asthmatic activity of *S. oleoids* leaves. Ethanolic extract of *S. oleoides* was prepared and used in the dose of 200 mg/kg (p.o.). Various *in-vivo* models like histamine induced bronchoconstriction in guinea pigs and milk induced leukocytosis in mice were used for evaluating anti-asthmatic activity of the drugs. Ethanolic extract of *S. oleoides* showed a significant broncho-dilatory, anti-histaminic and anti-inflammatory activity. Thus, ethanolic extract of S. oleoides have significant anti-asthmatic activity. Thus, ethanolic extract of *S. oleoides* leaves have proved to be an effective drug in prevention of asthma.

Introduction

Asthma comes from a Greek word meaning 'panting' or 'breathless.[1] One of the common disorders experienced in clinical medicine in the both adults and children is asthma and it is characterized by inflammation of the airways which causes airway dysfunction.[2] Asthma is a chronic inflammatory disease of the respiratory tract that is characterized by increased airway hyper-responsiveness and mucus production that leads to episodes of wheezing, coughing and shortness of breath.[3]

Asthma is characterized by airway inflammatory cells, including eosinophils, macrophages, mast cells, epithelial cells and activated lymphocytes that release various cytokines, adhesion molecules and other mediators. Inflammation result in an acute, sub-acute or chronic process that alters airway tone, modulates vascular permeability, activates neurons, increases secretion of mucus, and alters airway structure reversibly or permanently.[1]

The epidemiology of asthma differs according to geographical area in period of occurrence and incidence, age, and sex distribution. Such differences have been defined in terms of race, diet, and climate factors. The risks for developing asthma depend on a complex interaction of hereditary and environmental elements. Risk factors are genetic predisposition (family history of asthma), perinatal elements (low birth weight, prematurity), exposure to allergens, infections (respiratory infection, especially those caused by respiratory syncytial virus), environmental air pollution, tobacco smoke, diet, and obesity.[4] Asthma is a major public health problem worldwide.[5] There has been a sharp increase in the global prevalence, morbidity, mortality and financial burden associated with asthma over the last 40 years,

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Approximately 300 million people worldwide currently have asthma, and its prevalence increases by 50% each decade.[6]

Considerable number of drugs are used for in the treatment of asthma. The currently used drugs for the treatment of asthma in modern medicine are far from satisfactory as they provide only symptomatic relief, produce several adverse effects, and may lose effectiveness on continued use. The search for new drug is still the need of the day. Ayurveda suggests that the herbal plants have comparatively fewer toxic values and are more efficacious. They also have fewer chances of side effects and complications to patients as compared to available synthetic drug treatments. Muscle tremor and hypokalemia are major adverse effects of β2. Theophyline has narrow therapeutic index and requires monitoring of drug. Adverse effect of corticosteroids includes fluid retention, increased cell mass, increased appetite, weight gain, osteoporosis, capillary fragility, hypertension, peptic ulceration, diabetes, cataract and psychosis.[1]

Thus, the better anti-asthmatic drug needs to be explored. The plant *S. oleoids* under this study belongs to Salvadoraceae family.[7] The *S. oleoids* leaves are used in dry cough, asthma, digestive disorders, enlarged spleen, rheumatism, low fever, snake bites and conjunctivitis. They also possess anti-inflammatory, analgesic, and anti-ulcer activities.[7] It had effects on histamine induced bronchospasm and inflammation, mast cell degranulation and inflammatory cells like leukocytes. These parameters was helpful in evaluating anti-asthmatic activity of *S. oleoides* using various experimental animals like guinea pig mice.[1] The ethanolic extract of *S. oleoides* showed anti-asthmatic activity due to the presence of flavonoids, saponins, steroids, alkaloids, glycoside and tannins. Hence, considering the traditional claim, chemical constituents and reported activities of *S. oleoides*, the present study was planned to screen leaves extract of *S. oleoides* for anti-asthmatic activity.[8-12]

Materials and Methods

Plant Collection and Authentication

The collection of the leaves of *S. oleoides* was done in the month of January from jodhpur region, Rajasthan. Since the plants will be enriched with phytoconstituents during that time. The identification and authentication of the plant was carried out by Dr. S. L. Meena Scientist D & Botanical survey of India, Jodhpur (Raj) (No.:BSI/AZRC/I.12012/Tech./2019-20/PI.Id/235), India.

Preparation of Plant Extract

The leaves of *S. oleoides* were washed and dried under shade for 10 days. Cleaned and grind with help of grinder. After proper grinding, the weight of the powder was measured. The powders were used for the soxhlet extraction. About 130 gm of dried powder was extracted with petroleum ether in soxhlet apparatus for 18 to 20 hours at 60 to 80°C to the powder and then mark was extracted with benzene (for 15–16 hours at 78–80°C), chloroform (for 15–16 hours at 60–62°C) and ethanol (for 16–18 hours at 75–79°C). The extract at the bottom was collected and the solvents were removed using reflex condenser and dried on water bath. Each time, before the extraction with other solvents, the powdered substance is air dried. The percentage (%) yield was found for petroleum ether extract was 7.92%, benzene extract was 4.32%, chloroform extract was 4.75% and for ethanolic extract was 10.16%. The obtained extracts were subjected to phytochemical investigation.

Experimental Animals

Dunkin-hartley guinea pig (350–400 gm) and albino mice (20–30 gm) of both sexes were housed together in-group of four in clean polypropylene cages (males separated from females). The bedding material of cages was changed periodically. The animals were maintained under standard environment conditions (12 hours light: 12 hours dark cycle, at a temperature 22 ± 3ºC and 30–70% humidity. One-week time was provided to the animals for acclimatization with our laboratory environment. The animals were fasted 3 to 4 hours prior to dosage but allowed free access to drinking water and standard pelleted diet ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) Reg. no.16/BNCP/IAEC/2021.

Acute Toxicity Study

Dose was selected by using acute toxicity study (OECD, 423). The acute toxicity study for ethanolic extract of *S. oleoids* was performed using mice. The animals were fasted overnight prior to the experiment and maintained under standard conditions. To find the LD_{50} of ethanolic extract of *S. oleoids*, three groups of mice, containing three in each group, were given *S. oleoids* in the dose of 2000 mg/kg orally. The animals were observed for 5 minutes, and every 30 min till 2 hours and then at 4, 8 and 24 hours after treatment for any behavioral changes/mortality. They were further observed daily for 14 days for mortality. No mortality up to 14 days after treatment was observed with the ethanolic extract of *S. oleoids* and therefore was found safe up to dose of 2000 mg/kg.[9,10]

In-vivo Anti-asthmatic Activity

Histamine Induced Bronchoconstriction in Guinea Pig:[13,14]

Overnight fasted guinea pigs were divided into three groups (n=6). Prior to drug treatment, each animal was placed in the histamine chamber and exposed to 0.2% w/v histamine aerosol. The pre convulsive time (PCT) was defined as the time of exposure to onset of dyspnea leading to the appearance of pre convulsive dyspnea (PCD). As soon as the PCD was noted, the animal was removed from the chamber and placed in air. After 24 hours, animals belonging to group I served as control and were

administered with phosphate buffer (1 mL/kg, p.o.); Animals belonging to group II were administered with chlorpheniramine maleate (2 mg/kg, i.p.) while group III was received respective dose of ethanolic extract of *S. oleoides*. These animals were again subjected to histamine aerosol later at interval of 1st, 4th and 24th hours of drug administration and PCT was determined again. The protection offered by treatment was calculated by using the following formula:

$$\%protection = 1 - \frac{T1}{T2} \times 100$$

T_1 = the mean of PCT before administration of test drugs. T_2 = the mean of PCT after administration of test drugs at 1, 4 and 24 hours.

Milk Induced Leukocytosis in Mice:[15,16]

Mice were divided into four groups, six animals in each group. Animal belonging to group-I received distilled water 10 mL/kg (p.o). Animal belonging to group II, III and IV received boiled and cooled milk injection in dose of 4 mL/kg (s.c). Animal belonging to group III served as standard and received dexamethasone in dose of 50 mg/kg (i.p). Animal belonging to group IV served as test group and received respective dose of ethanolic extract of *S. oleoides* and 1-hour later boiled and cooled milk (4 mL/kg, s.c.) were administered to the same animals. After 24 hours, blood samples were collected from all animals from their tail vein. Total leukocytes count was done in each group 24 hours after milk injection.

Statistical Analysis

The results of numerous studies were exposed as mean ± SEM and analyzed statistically using one way ANOVA followed by Dunnett's test. *p < 0.01, **p < 0.05 were considered significant.

Results

Phytochemical Investigation (Table 1)

Phytochemical investigation of the ethanolic extracts of *S. oleoides* showed the presence of carbohydrate, alkaloids, flavonoids, glycosides, tannin, polyphenols, and steroids whereas phytochemical investigation of the chloroform and benzene extracts of plant *S. oleoides* showed the presence of carbohydrate and protein. Petroleum ether extract showed the presence of fixed oils and fats.

Histamine Induced Bronchoconstriction in Guinea Pigs:

The guinea pigs when exposed to 0.2%w/v histamine aerosol showed signs of progressive dyspnea leading to convulsions. Guinea pigs treated with chlorpheniramine maleate (2 mg/kg, i.p.) prolonged the preconvulsive dyspnea at 1st, 4th and 24th hour compared to control. In the groups of guinea pigs pretreated with ethanolic extract of *S. oleoides* (200 mg/kg, p.o.) prolonged the preconvulsive dyspnea at 1st, 4th and 24th hour compared to control. Ethanolic extract of *S. oleoides* (200 mg/kg, p.o.) shows the increase in percent protection but percent protection was found to be less than chlorpheniramine maleate (2 mg/kg, i.p.). The percent protection observed for Chlorpheniramine maleate at the dose of 2 mg/kg was 70.83, 76.65 and 36.32 in 1st, 4th, and 24th hour, respectively. The percent protection observed for *S. oleoides* at the dose of 200 mg/kg was 62.49, 68.62 & 25.19 in 1st, 4th and 24th hour, respectively (Table 2).

Data are expressed as Mean ± SEM. Where, n = 6

Statistical analysis done by ANOVA followed by Dunnett's test, where *p < 0.01, **p < 0.05 when group II & III were compared with group I.

Data are expressed as Mean ± SEM Where, n = 6

Statistical analysis done by ANOVA followed by Dunnett's test, where *p < 0.01, **p < 0.05 when group II, III and IV were compared with group I.

- *GROUP-I* (Normal Control): Distilled water 10 mL/kg, p.o.
- *GROUP-II* (Positive Control): Boiled & cooled milk (4 mL/kg, s.c.)
- *GROUP-III* (Std): Dexamethasone (50 mg/kg, i.p.) + Boiled & cooled milk (4 mL/kg, s.c.)
- *GROUP-IV* (Test-2): Boiled & cooled milk (4 mL/kg, s.c.) + Ethanolic extract of Salvadora oleoides (200 mg/kg, p.o.)

The guinea pigs when exposed to 0.2%w/v histamine aerosol showed signs of progressive Dyspnea leading to convulsions. Chlorpheniramine maleate (2 mg/kg, i.p) significantly prolonged the preconvulsive dyspnea in 1st, 4th, and 24th hours as compared to control**.** The ethanolic extract of *S. oleoids* at dose of 200 mg/kg significantly prolonged the preconvulsive dyspnea at 1st, 4th, and 24th hours as compared to control but preconvualsion time was found to be less than chlorpheniramine maleate (2 mg/kg, i.p.). Thus showed more protection against preconvulsive dyspnea as compared to control (Fig. 1)**.**

Milk Induced Leucocytosis in Mice:

Subcutaneous injection of milk at dose of 4 mL/kg produced an increase in the leucocytes count after 24 hours of its administration. Mice treated with dexamethasone (50 mg/kg, i.p.), has shown inhibition of milk induced leukocytosis as compared to positive control. In the groups of mice pretreated with ethanolic extract of *S. oleoides* (200 mg/kg, p.o.), there was inhibition of milk induced leukocytosis, but inhibition of leukocytosis was found to be less than dexamethasone (50 mg/kg, i.p.) (Table 3).

Data are expressed as Mean ± SEM Where, n = 6

Statistical analysis done by ANOVA followed by Dunnett's test, where *p < 0.01, **p < 0.05 when group II, III and IV were compared with group I.

- *GROUP-I (Normal Control):* Distilled water 10 mL/kg, p.o.

Table 1: Qualitative chemical analysis of petroleum ether, chloroform, benzene and ethanolic extract of *S. oleoides*

	Test for plant constituents	*S. oleoides leaves*			
S. No.		*Petroleum ether extract*	*Benzene extract*	*Chloroform extract*	*Ethanolic extract*
1.	Test for Carbohydrate				
	A. Molish test	_	+	+	+
	B. Fehling's test	_	+	+	+
	C. Benedict's test	_	+	+	+
	D. Barfoed's test	_	+	+	+
2.	Test for Protein				
	A. Biuret test	_	_	_	+
	B. Million's test	_	_	_	+
	C. Ninhydrin test	_	_	_	+
3.	Test for Alkaloids				
	A. Mayer's test	_	_	_	+
	B. Dragenoff's test	_	_	_	+
	C. Wangner's test	_	_	_	+
4.	Test for Fats and Oils				
	Spot test	+	_	_	_
	Saponification test	+	_	_	_
5.	Test for glycoside				
	Legal test	_	_	_	+
	Baljet's test	_	_	_	+
	Borntrager's test	_	_	_	+
	Foam test	_	_	_	+
6.	Test for Flavonoids				
	Ferric chloride test	_	_	_	+
	Shinoda's test	_	_	_	+
7.	Test for Tannins and phenolic compounds				
	Ferric chloride test	_	_	_	+
	Reaction with lead acetate	_	_	_	+

Table 2: Percentage protection against histamine induced bronchoconstriction in guinea pigs at different time interval.

	Preconvulsion dyspnea (in sec) (Mean ± SEM)				*% Protection*		
		After Treatment					
Group N=6	*Before Treatment*	*1 h*	*4 h*	*24 h*	*1 h*	*4 h*	*24 h*
GROUP-I	16.43 ± 0.20	-	-	-	-	-	-
GROUP-II	16.24 ± 0.28	55.66^{a} ± 0.40	69.55^{a} ± 0.21	25.5^{a} ± 0.14	70.83	76.65	36.32
GROUP-III	15.36 ± 0.30	40.94^{ab} ± 0.50	48.94^{ab} ± 0.08	20.53^{ab} ± 0.22	62.49	68.62	25.19

- *GROUP-II (Positive Control):* Boiled & cooled milk (4 mL/kg, s.c.)
- *GROUP-III (Std):* Dexamethasone (50 mg/kg, i.p.) + Boiled & cooled milk (4 mL/kg, s.c.)
- *GROUP-IV (Test-2):* Boiled & cooled milk (4 mL/kg, s.c.) + Ethanolic extract of *S. oleoides* (200 mg/kg, p.o.)

Subcutaneous administration of boiled and cooled milk (4 mL/kg) into the mice as antigen and produced allergic

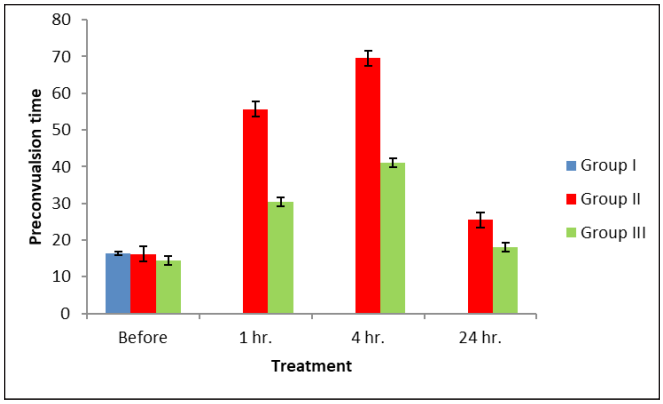

Fig. 1: Effect of ethanolic extracts of *S. oleoids* on histamine induced bronchoconstriction in guinea pigs.

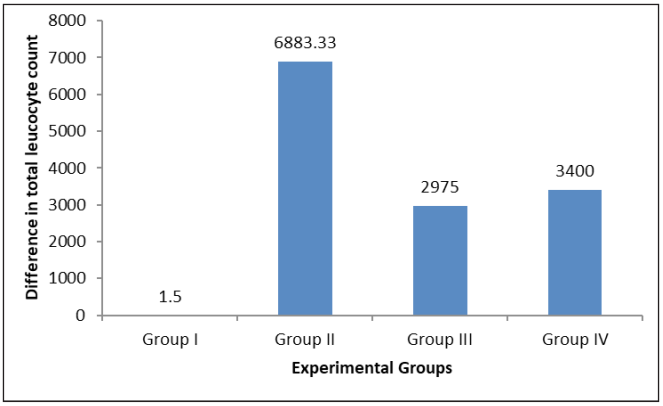

Fig. 2: Effect of ethanolic extracts of *S. oleoids* on milk induced leukocytosis in mice

Table 3: Effect of ethanolic extracts of *S. oleoids* on milk induced leukocytosis in mice

Group n=6	*Difference in number of leucocytes (per cumm) (Mean ± SEM)*
GROUP-I	1.50 ± 21.39
GROUP-II	6883.34 ± 47.85
GROUP-III	2975 ± 53.87
GROUP-IV	3400 ± 41.42

response in mice. The total leucocytes count was increased 24 hours after milk injection. The total leucocytes count in positive control group was significantly higher as compared to ethanolic extract of *S. oleoides* and dexamethasone (50 mg/kg, i.p.). In the groups of mice pretreated with ethanolic extract of S. oleoides (200 mg/kg, p.o.), there was inhibition of milk induced leukocytosis, but inhibition of leukocytosis was found to be less than dexamethasone (50 mg/kg, i.p.) (Fig. 2).

Discussion

Asthma is a chronic inflammatory disorder of airways initiated by several factors ranging from allergen, dust-mites, mold or pollen that result in IgE antibodies. Mediators include histamine, prostaglandins and leukotrienes. The mediators promote vascular permeability, smooth muscle contraction and mucus production. Which cause symptoms of asthma including airway constriction, coughing, shortness of breathing and wheezing.

In the present study, an attempt was made to investigate anti-asthmatic activity of *S. oleoides.* The chemical tests confirmed the presence of alkaloids, flavanoids, steroid, glycosides, tannins and saponins in the ethanolic extract of *S. oleoides*. They have been the action of smooth muscle relaxant, bronchodilator, antioxidant and anti-inflammatory, mast cell stabilizing, anti-allergic, anti-inflammatory and antihistaminic activity. Ethanolic extract of *S. oleoides* shows the anti-asthmatic activity against histamine-induced bronchoconstriction. In the present study, antihistaminic drugs chlorpheniramine maleate and ethanolic extract of *S. oleoides* significantly protected the guinea pigs against histamine induced bronchospasm. Ethanolic extract of *S. oleoides* has significantly prolonged the latent period of convulsions as compared to control. This indicates the utility of the ethanolic extract of *S. oleoides* in the treatment of asthma by virtue of its H_1 – receptor blocking or bronchodilating activity.

The result of this study shows that ethanolic extract of *S. oleoides* suppresses the milk induced leukocytosis by stabilizing the oxidative stress in the surrounding tissue. The leukocytes are responsible to release the several inflammatory mediators like histamine, cytokines etc. which enhance inflammatory process. Infiltration of leukocytes in surrounding tissues in asthmatic inflammation causes increased oxidative stress which is characterized as the main pathogenic feature of asthma. In this study observed that the inhibition of leukocytosis was significant in mice treated with ethanolic extract of *S. oleoides* as compared to control group.

Ethanolic extract of *S. oleoides* may possess anti-asthmatic activity which may be due to antihistaminic activity, bronchodilating activity, mast cell stabilizing activity, anti-inflammatory activity, anti-allergic, anti-spasmodic, and antioxidant activity. All over we can say that ethanolic extract of *S. oleoides* has significant anti-asthmatic activity.

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