OPEN ACCESS

Saudi Journal of Medical and Pharmaceutical Sciences

Abbreviated Key Title: Saudi J Med Pharm Sci
ISSN 2413-4929 (Print) |ISSN 2413-4910 (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

Original Research Article

Evaluation of Anti-Asthmatic Activity of Ethanolic Extract of *Argemone mexicana* Stems

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DOI: 10.36348/sjmps.2021.v07i01.007 | **Received:** 29.12.2020 | **Accepted:** 14.01.2021 | **Published:** 15.01.2021

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Abstract

Objective: To determine the anti-asthmatic activity of *Argemone mexicana* ethanolic extract and its chloroform and ethyl acetate fractions. ***Methods:*** Present study was designed to evaluate the anti-asthmatic activity of *Argemone mexicana* stems using various experimental *in vivo and in vitro* animal models. Ethanolic extract of *Argemone mexicana* and its chloroform and ethyl acetate fractions were evaluated for its anti-asthmatic activity by *in vitro* models (isolated guinea pig ileum preparation and isolated guinea pig tracheal chain preparation) *in vivo* models (Histamine and acetylcholine induced bronchospasm in guinea pigs and milk induced eosinophilia in mice models). ***Results:*** Ethanolic extract of *Argemone mexicana* significantly antagonized the effect of histamine induced contraction on isolated guinea pig ileum preparation. Ethanolic extract of *Argemone mexicana*, and its ethyl acetate fraction at the concentration of 10mg significantly inhibited the contraction of isolated guinea pig ileum and trachea chain preparation produced by histamine. Ethanolic extracts of *Argemone mexicana* at dose of 150, 250, and 350mg/kg body weight significantly extended the latent period of convulsion as compared to standard (Ketotifen fumarate) upon histamine and acetylcholine exposure in a dose dependent manner. ***Conclusion:*** Ethanolic extract of *Argemone mexicana* stems have shown significant anti-asthmatic activity.

Keywords: Asthma, Histamine, *in vivo, in vitro*, Acetylcholine, *Argemone mexicana*.

INTRODUCTION

Asthma word derived from a Greek word meaning 'Breathless'. Asthma is a chronic inflammatory disease of the airways. This chronic inflammation is associated with an exaggerated airway-narrowing response to specific triggers such as viruses, allergens and exercise that leads to recurrent episodes of wheezing, breathlessness, chest tightness and/or coughing that can vary over time and in intensity [1]. Asthma has been a major cause of morbidity and mortality in various countries. Bronchial hyper responsiveness is a characteristic feature in most asthmatic patients [2]. 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 results 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 [3]. The currently available treatment for asthma most medications work by relaxing bronchospasm (bronchodilators) or reducing inflammation (corticosteroids). Though the available treatment are not efficient for treating asthma finally as they have many toxic side effects [4]. Argemone mexicana (Papaveraceae) also known as Ghamoya belongs to family. It is an exotic weed indigenous to South America but now it has been widely distributed in many tropical and sub-tropical countries [5].

Argemone mexicana

It is commonly found everywhere by roadsides and fields in India. It is an erect prickly annual herb of about 1 m high; leaves are usually 5 to 11 cm long, and more or less blotched with green and white, glaucous broad at the base, half-clasping the stem prominently sinuate-lobed, and spiny [6]. The flowers become 4 to 5 cm in diameter, and are terminal, yellow, and scentless. The capsule is spiny, obovate or elliptic-oblong, and about 3 cm in length. The seeds are spherical, shining, and black and pitted [7].The plant contains alkaloids, flavonoids, tannins, sterols and terpenes [8]. Traditionally this plant is used for the treatment of asthma. Therefore aim of this study was to determine the anti-asthmatic activity of ethanolic extract and its chloroform and ethyl acetate fractions.

MATERIALS AND METHODS

Plant material

Argemone mexicana stems were collected in the month of April from local region of Sehore district Madhya Pradesh (India). Herbarium specimens were prepared, identification and authentication were done from Department of botany; A Voucher specimen of the plant has been deposited for future reference. The plant material was shade dried and coarsely powdered by using mechanical grinder. The powder was passed through sieve no. 40 and stored in airtight container for the extraction.

Preparation of Extract

Dried and coarse powdered drug of Argemone mexicanawas weighed (1Kg) and placed in Soxhlet apparatus. The powdered drug was then subjected to extraction by using first petroleum ether (60-80ºC) and the remaining marc was dried in oven below 50ºC and extracted with ethanol (95% v/v). Obtained ethanolic extract (26.20%w/w) was concentrated by using rotary evaporator [9, 10]. A qualitative preliminary phytochemical test was performed to find out the presence of various phytochemicals in the extract [11]. For anti-asthmatic evaluation, extract was dissolved in distilled water prior to its use.

Chemicals

Histamine dihydrochloride, acetylcholine chloride, ketotifen were purchased from Sigma-Aldrich Chemical Co., USA. Egg albumin and other chemicals were purchased from Himedia Laboratories Pvt. Ltd., India. All the other chemicals were of analytical grade.

Phytochemical screening

Qualitative phytochemical tests were performed on ethanolic extract of Argemone Mexicana for the presence of different chemical constituents [12].

Experimental Animals

The experiments were performed on Swiss albino mice weighing about 20-25g, albino rats weighing 110-150g and healthy guinea pigs (Dunkin hartley) weighing about 350-500g. Eosinophil measurement study was performed on mice while histamine induced bronchospasm study was conducted on guinea pigs, isolated ileum preparation and trachea chain preparation. The animals were housed to animal house prior to experimentation at temperature of 25±2ºC and 50±5% relative humidity in polypropylene cages with a 12 hours light/dark cycle and allowed free access to food and water. The experiments were performed by following rules and regulations of CPCSEA (Committee for the Purpose of Control and Supervision on Experimental Animals) approved by the IAEC (Institutional Animal Ethical Committee), RKDF University, Bhopal.

Acute Toxicity Testing

The acute toxicity study of Argemone mexicana ethanolic extract was carried out on female albino rats. The rats were administered orally dose of 300, 600, 1000, 1500 and 2000mg/Kg body weight of ethanolic extract and the control group provided free access to food and water as per OECD guidelines.

Experimental studies

Ethanolic extracts and its chloroform and ethyl acetate fractions of Argemone mexicana, were evaluated for anti-asthmatic potentials using the following in vitro and in vivo models

In vitro study

Contractions produced by histamine on isolated guinea pig ileum preparation and effects of ethanolic extract and different fractions of Argemone mexicana on it

Overnight fasted guinea pigs of both sex, 350-500g in weight were sacrificed by cervical dislocation. Ileum was taken and mounted in a student organ bath (Dolphin) and maintained at 37±0.5ºC containing 20 mL of Tyrode's solution under 500mg of basal tension. The composition of solution in mM was NaCl, 0.137; $CaCl_2$, 1.8; KCl, 2.70; Glucose, 5.55; $NaHCO_3$, 11.9; $MgCl_2$, 1.0. The solution was continuously aeriated. The equilibration time of tissue was 30 minutes, in between of 10 minutes bathing solution was changed. The contractile responses of ileum against histamine were recorded in presence and absence of ethanolic extracts of Argemone Mexicanaand their chloroform and ethyl acetate fractions. Contact time for 30 seconds and 5 minutes time cycle was followed for recording dose response curve (DRC) of histamine at the dose of 100, 200, 400, 800 and 1600µg. after recording DRC of histamine; ethanolic extracts of Argemone mexicana, and their chloroform and ethyl acetate fractions (10mg/mL) were put into bathing solution and same doses of histamine were repeated again. Graph of maximum response of contraction on ordinate and log concentration of histamine was plotted to record DRC of histamine. The results obtained were presented in table 1 [13].

Contractions produced by histamine on isolated guinea pig tracheal chain preparation and effects of ethanolic extract and different fractions of Argemone mexicana on it

Guinea pigs of both sexes (250–500g), starved overnight but allowed water, were used. The animals were sacrificed by a blow on the head and exsanguinated. The trachea was dissected out and cut along its length on the dorsal surface. The tracheal preparation was mounted at 1.0g of tension in Krebs-Henseleit solution (in mM, NACl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.6; $NaHCO_3$, 24.9; KH_2PO_4, 1.2 and Glucose, 11.0; $_pH$ 7.4) by using cell pins. The buffer was maintained at 37ºC and continuously saturated with bubbled air. The tissue was left to equilibrate for 60 minutes, in between of 10 minutes bathing solution was changed. At the end of equilibrium period, histamine induced contractions as well as the effect of the ethanolic extracts and its various fractions (10mg/mL) of Argemone mexicana, on the contractions produced by histamine were recorded at the dose of 100, 200, 400, 800 and 1000µg. The tissues were bathed in the test substances for 5 minutes before the addition of histamine. A drug tissue contact time of 90 seconds was observed. The results obtained were tabulated in table 2[14].

In vivo study

Histamine and acetylcholine induced bronchospasm in guinea pigs

The guinea pigs fasted for 24 h were exposed to an aerosol of 1.0% histamine dihydrochloride (dissolved in normal saline) using nebulizer at a pressure (1kg/cm2) in the histamine chamber. Guinea pigs exposed to histamine aerosol showed progressive signs of difficulty in breathing leading to convulsions, asphyxia and death. The preconvulsion time (PCD) was noted for the time of exposure to the onset of dyspnea in minutes in each animal as described by Sheth et al., 1972; Gokhale and saraf, 1996. As soon as the convulsions started, the animals were taken off from the aerosol chamber and placed in fresh air. Animals of Group I were treated with 2% v/v Tween 80 (Control) and animals of Group II were treated with Ketotifen fumarate (1mg/kg, standard). Animals of Group III to XI were treated with ethanolic extracts of Argemone mexicana and its chloroform and ethyl acetate fractions at doses of 150, 250and 350mg/kg bw, p.o., daily for 7 days. On day 7, 2 h after the administration of last dose of test extracts, the onset of convulsions recorded as for day 0. After next 15 days intervals of washout period, the same animals were given the above treatment and preconvulsion time (PCD) was noted for 0.5% acetylcholine bromide aerosol spray. The percentage protections offered by ethanolic extracts and their different fractions of Argemone Mexicana and standard drug treated animals were calculated by the formula given below [15].

Percent protection= $\{T_2\text{-}T_1/T_2\}$ X100

Where T_1= Time for onset of symptoms before treatment

T_2= Time for onset of symptoms after treatment

The results were presented in table 3 and table 4 respectively.

Milk induced eosinophilia in mice

Adult albino mice (20-25g) were divided into eleven groups, six animals in each. Animals of Group I received distilled water (10mL/kg, p.o.) and Group II only injection of boiled and cooled milk in doses of 4mL/kg, s.c. Animals of Group III to XI were received injections of boiled and cooled milk in doses of 4mL/kg, s.c. and ethanolic extract of Argemone mexicana and their chloroform and ethyl acetate fractions at the doses of 150, 250 and 350mg/kg, p.o. respectively, 1 hour before milk injection. By WBC pipette the blood was sucked up to mark and further diluted with eosin solution so as to destruct all corpuscles excluding eosinolphil and it was shaken. Neuber's chamber was charged with above fluid and eosinophil count was done [16]. The eosinophil difference before and after drug administration was calculated. The results obtained were presented in table 5

RESULTS AND DISCUSSION

The air dried powdered stems (1000g) of Argemone mexicana were defatted with petroleum ether (60-80ºC) and remaining marc was extracted with ethanol (70%v/v) and concentrated in rotary evaporator under reduced pressure to get ethanolic extract (220.0g). The ethanolic extract was dissolved in ethanol and water (1:2 v/v) and partitioned with chloroform and ethyl acetate in 50mL portion for several times till complete extraction takes place. The resulted in chloroform fraction (102.0g) and ethyl acetate fraction (106.0g) upon concentration under reduced pressure.Preliminary phytochemical screening of the ethanolic extract of Argemone mexicana revealed the presence of various secondary metabolites such as alkaloids, sterols, phenolic compounds, tannins and flavonoids. Toxicological studies of extracts of Argemone mexicana, was performed and it was found safe. The effect of ethanolic extract and its chloroform and ethyl acetate fractions of Argemone mexicana on histamine induced contractions on isolated guinea pig ileum preparation were evaluated at the concentration of 10mg. The ethanolic extract of Argemone Mexicana (23.10±2.34, 34.28±2.95, 46.25±2.57, 44.24±3.10, 58.24±2.90) significantly antagonized the effect of histamine induced contraction on isolated guinea pig ileum preparation at the concentration of 10mg when compared with the control group (vehicle) (40.62±2.12, 53.26±1.22, 64.52±2.14, 88.12±2.03, 93.88±2.14), respectively at the log concentration of histamine at 4.00, 3.699, 3.3979, 3.0969 and 2.7960. The activity was found to be dose dependent as shown in Table 1.

The chloroform and ethyl acetate fraction of Argemone mexicana did not show any significant activity. The above results revealed that ethanolic extract of Argemone mexicana significantly antagonized the effect of histamine induced contraction on isolated guinea pig ileum preparation at the concentration of 10mg at the log concentration of histamine at 4.00, 3.699, 3.3979, 3.0969 and 2.7960. The effect of ethanolic extract and its various fractions of Argemone mexicana on isolated guinea tracheal chain preparation were evaluated at the concentration of 10mg. The ethanolic extracts of Argemone mexicana significantly antagonized the effect of histamine induced contraction on isolated guinea pig tracheal chain. The ethanolic extracts of Argemone mexicana (29.24±3.2, 32.48±2.00, 24.28±2.14, 24±2.18, 32.12±1.24) antagonized the effect of histamine induced contraction, when compared with vehicle (22.10±2.58, 28.14±2.87, 32.48±2.48, 38.50±3.85, 58.27±2.59) at the log concentration of histamine (4.00, 3.699, 3.3979, 3.0969, 2.7960) as shown in Table 2.Chloroform and Ethyl acetate fraction of Argemone mexicana did not show any activity.

In the presence of ethanolic extracts of A. mexicanaby guinea pig ileum and trachea chain method exhibited right side shift of dose response curve of histamine, indicated anti-asthmatic activity. The present study revealed that ethanolic extract of Argemone mexicana, and its ethyl acetate fraction at the concentration of 10mg significantly inhibited the contraction of isolated guinea pig ileum and trachea chain preparation produced by histamine, showed anti-asthmatic properties of these plants because of H1 receptor antagonist effect. Histamine is inflammatory mediators involved in asthma which causes inflammation and hyper-responsiveness of bronchial airway. The anti-asthmatic study of ethanolic extracts of Argemone mexicana on histamine induced bronchospasm was carried out at three dose levels 150, 250, and 350mg/kg body weight in guinea pig. Results showed that ethanolic extracts of Argemone mexicana significantly extended the latent period of convulsion as compared to standard (Ketotifen fumarate) upon histamine exposure. The percentage protection offered by the ethanolic extracts of Argemone mexicana were found to be 65.20, 71.07 and 60.89 respectively at the dose of 150, 250, and 350mg/kg body weight respectively in guinea pig as shown in Table 3.The ethanolic extract of Argemone mexicana on acetylcholine induced bronchospasm were evaluated at three different dose levels 150, 250 and 350mg/kg body weight in guinea pigs. Results showed that ethanolic extracts of Argemone mexicana significantly extended the latent period of convulsion as compared to standard (Ketotifen fumarate). The percentage protection offered by the ethanolic extracts of Argemone mexicana, were found to be 46.80, 70.74 and 91.40 respectively at dose level of 3500mg/kg body weight as shown in Table 4.The percentage protection of eosinophil was measured in milk induced mice with the treatment of ethanolic extracts of Argemone mexicana, and their chloroform and ethyl acetate fractions at the doses of 150, 250 and 350mg/kg p.o. the percentage protection of eosinophils measured in milk induced mice was found to be 38.83, 39.18,52.31, 52.48, 55.11, 58.55, and 56.10, 37.00, 34.29 respectively when treated with ethanolic extracts of Argemone mexicana at the dose level of 600mg/kg body weight. The protecting effect of Ethyl acetate fraction of Argemone mexicana is (77.09%) as shown in Table 5. In India Argemone mexicana, are being used traditionally in the treatment of asthma. This study was carried out to evaluate these herbs scientifically to justify their traditional claim as anti-asthmatic drugs. Results obtained from all the parameters tested revealed that Argemone mexicana, have significant anti-asthmatic activity. The ethanolic extract of Argemone mexicana, was found to be the most effective in all the models. This anti-asthmatic activity of ethanolic extract of Argemone mexicanamay be due to the presence of alkaloids, glycosides, tannins, sterols, flavonoids, phenolic compounds.

CONCLUSION

On the basis of all above results, it is concluded that the traditional anti-asthmatic use of Argemone mexicana, is justifying. The results obtained from all the parameters revealed that the ethanolic extract of Argemone mexicana, was found to be the most potent for their anti-asthmatic activity.

Table-1: Showing contractions produced by histamine on isolated guinea pig ileum preparation and effects of ethanolic extract and different fractions of Argemone mexicanaon it

Concentration of Histamine (g)	**-Log concentration of histamine**	**Maximum percentage of contraction (Mean±S.D.) pretreatment with**			
		Vehicle	Ethanolic extract of Argemone mexicana	Chloroform fraction of Argemone mexicana	Ethyl acetate fraction of Argemone mexicana
$100X10^{-6}$	4.00	40.62±2.12	23.10±2.34	34.14±1.98	33.20±2.13
$200X10^{-6}$	3.699	53.26±1.22	34.28±2.95	51.28±2.34	45.82±2.18
$400X10^{-6}$	3.3979	64.52±2.14	46.25±2.57	54.18±3.22	70.20±2.88
$800X10^{-6}$	3.0969	88.12±2.03	44.24±3.10	61.24±2.10	69.21±4.44
$1600X10^{-6}$	2.7960	93.88±2.14	58.24±2.90	69.18±5.41	79.12±5.15

*p<0.01 when compared with vehicle

Table-2: Showing contractions produced by histamine on isolated guinea pig tracheal chain preparation and effects of ethanolic extract and different fractions of Argemone mexicanaon it

Concentration of Histamine (g)	-Log conc. of histamine	Maximum percentage of contraction (Mean±S.D.) pretreatment with			
		Vehicle	Ethanolic extract of Argemone mexicana	Chloroform fraction of Argemone mexicana	Ethyl acetate fraction of Argemone mexicana
$100X10^{-6}$	4.00	22.10±2.58	29.24±3.2	32.82±4.10	28.57±2.82
$200X10^{-6}$	3.699	28.14±2.87	32.48±2.00	49.79±3.14	34.82±3.28
$400X10^{-6}$	3.3979	32.48±2.48	24.28±2.14	31.92±3.24	52.24±4.14
$800X10^{-6}$	3.0969	38.50±3.85	24±2.18	74.82±3.74	64.61±4.17
$1600X10^{-6}$	2.7960	58.27±2.59	32.12±1.24	72.54±3.21	73.71±4.52

*p<0.01 when compared with vehicle

Table-3: Showing bronchospasm produced by histamine on guinea pig and effects of ethanolic extract and different fractions of Argemone mexicanaon it

Treatment	Group of Animals	Dose (mg/kg, p.o.)	PCT (T_1)	PCT (T_2)	Mean exposition time (T_2- T_1)	% protection against bronchospasm
Control (2% v/v Tween 80)	I	10mL	1.88±0.04	1.94±0.04	0.06±0.02	3.09
Ketotifen fumarate	II	01	3.28±0.08	19.10±0.07	15.82±0.02	82.87
Ethanolic extract of Argemone mexicana	III	150	1.26±0.002	3.248±0.03	1.988±0.03	61.20
	IV	250	1.28±1.004	4.425±0.04	3.145±0.01	71.07
	V	350	1.23±0.004	3.145±0.05	1.915±0.22	60.89
Chloroform fraction of Argemone mexicana	VI	150	1.42±0.042	1.875±0.02	0.455±0.05	24.26
	VII	250	1.38±0.003	2.42±0.048	1.04±0.07	42.97
	VIII	350	1.28±0.005	2.32±0.05	1.04±0.03	42.97
Ethyl acetate fraction of Argemone mexicana	IX	150	1.68±0.002	1.79±0.06	0.11±0.70	6.14
	X	250	1.58±0.002	1.87±0.04	0.29±0.05	15.50
	XI	350	1.35±0.003	1.58±0.04	0.23±0.05	14.55

*p<0.0003 when compared with vehicle

Table-4: Showing bronchospasm produced by acetylcholine on guinea pig and effects of ethanolic extract and different fractions of Argemone mexicanaon it

Treatment	Group of Animals	Dose (mg/kg, p.o.)	PCT (T_1)	PCT (T_2)	Mean exposition time (T_2- T_1)	% protection against bronchospasm
Control (2% v/v Tween 80)	I	10mL	1.88±0.04	1.94±0.04	0.06±0.02	3.09
Ketotifen fumarate	II	01	3.28±0.08	19.10±0.07	15.82±0.02	82.82
Ethanolic extract of Argemone mexicana	III	150	1.51±0.004	2.82±0.124	1.32±0.10	46.80
	IV	250	1.28±0.003	2.94±0.04	2.08±0.03	70.74
	V	350	1.48±0.004	3.84±0.014	3.51±0.01	91.40
Chloroform fraction of Argemone mexicana	VI	150	1.28±0.003	1.82±0.04	0.39±0.08	21.42
	VII	250	1.39±0.004	2.52±0.04	0.71±0.03	28.17
	VIII	350	1.42±0.004	2.82±0.21	1.10±0.10	39.00
Ethyl acetate fraction of Argemone mexicana	IX	150	1.68±0.003	1.72±0.057	0.09±0.07	5.23
	X	250	1.38±0.004	1.57±0.04	0.18±0.06	11.46
	XI	350	1.48±0.003	1.62±0.06	0.17±0.07	10.49

*p<0.0003 when compared with vehicle

Table-5: Showing effects on eosinophil count produced by ethanolic extract and different fractions of Argemone mexicanain mice

Treatment	Group of Animals	Dose (mg/kg, p.o.)	Eosinophil (Beore treatment)	Eosinophil (After treatment)	Protection (%)
Vehicle treated	I	10mL	111.33±3.21	114.33±2.08	-
Control group (Treated with milk)	II	4 mL	115.34±3.05	329.67±1.52	-
Ethanolic extract of Argemone mexicana	III	150	301.29±3.01	138.27±3.18	45.89
	IV	250	289.87±3.82	157.27±3.88	54.25
	V	350	338.97±3.27	187.98±2.87	55.45
Chloroform fraction of Argemone mexicana	VI	150	349.82±2.19	217.86±4.48	62.27
	VII	250	335.78±3.18	217.87±3.49	64.88
	VIII	350	358.68±4.45	246.46±3.85	68.71
Ethyl acetate fraction of Argemone mexicana	IX	150	316.94±2.49	129.38±3.48	40.82
	X	250	338.57±3.58	246.46±3.49	72.79
	XI	350	327.49±3.43	237.49±3.76	72.51

*p<0.0003 when compared with vehicle

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