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Evaluation of Anti-Inflammatory Activity of *Argemone mexicana* Linn. Root Extract

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ABSTRACT

The aim of present study was to explore the anti-inflammatory activity of ethanolic extract of *Argemone mexicana* Linn. roots on laboratory animals, Wister rat (180-220g) of either sex. The anti-inflammatory study was done by carrageenan-induced paw edema method. The extract was administered orally in dose of 150 mg/kg and 250 mg/kg of body weight. Phytochemical study reveals that the extract contains alkaloids, phytosterols, flavonoids, phenolic compounds and tannins. It was found that the ethanolic extract can be effective in acute inflammatory disorders and shows significant anti-inflammatory dose-dependent effect at the dose level of 150 mg/kg and 250 mg/kg. Acute toxicity studies of ethanolic extract of *A. Mexicana* showed no mortality till doses of 2500 mg/kg body weight even after 72 hrs.

INTRODUCTION

The plant *Argemone mexicana Linn*. Known as Ghamoya (Family papaveraceae) is an indigenous herb. It is a perennial herb growing wild to 0.6m by 0.45m. It is a prickly, glabrous, branching herb with yellow juice and showy yellow flowers. Leaves glaucous, oblong-oblanceolate, pinnately lobed, 1/2-3/4 to midrib, both surfaces sparsely covered with prickles along veins, margins somewhat sinuate-dentate, the teeth tipped with a prickle, sessile, upper ones usually somewhat clasping the stem. The plant contains alkaloids as berberine, protopine, sanguinarine, optifine, chelerythrine *etc*. It is traditionally used as analgesic, anti-inflammatory, antispasmodic, antitussive, demulcent, emetic, expectorant, hallucinogenic, purgative, sedative, skin, warts [1-2].

It is known nowadays, that a persistent and uncontrolled inflammation can be an etiologic factor for many chronic illnesses [3]. Although inflammation is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases [4]. Moreover, many authors have revealed the link between pain and inflammation in the occurrence of several diseases [5-8]. In the domain of struggle against pain and inflammation, numerous plants possessing medical potentialities constitute a good alternative because showing anti-inflammatory properties. In the present study, the possible effect of different doses of ethanolic extract of roots of *Argemone mexicana* for anti-inflammatory activity was determined by carrageenan paw edema method.

EXPERIMENTAL

MATERIALS AND METHODS:

Plant material-

Argemone mexicana Linn leaves were collected locally from Pusad outskirts; a town located in Maharashtra, India. Authentication of plant was done in Pharmacognosy Department and the registration number of the specimen was HBN20121301. The roots were cleaned from adhering soil and dried in shade. After drying powdered coarsely and stored in airtight container till further use.

Biological material-

All the experiments were carried out in Wister rat (180-220g) of either sex. The animals had free access to food and water, and they were housed in a natural light–dark cycle. The animals were acclimatized to the laboratory conditions for at least two week before experiments. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and the laboratory animals were taken care according to the guidelines of CPCSEA, Ministry of Forests and Environment, Government of India. The rats were housed in polypropylene cages (43 x 27 x 15cm) placed on shelves.

Extract Preparation:

The powdered roots (500g) were successively extracted with Ethanol for 48hrs using soxhlet apparatus. The extract obtained was stored in desiccators and was used for further studies.

Preliminary Phytochemical Screening:

Standard methods were used for preliminary phytochemical screening of the extract to know the nature of phytoconstituents present.

Acute Toxicity:

The acute toxic class method/OECD423 set out in this guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The acute toxic class method is based on biometric evaluations with fixed doses, adequately separated to enable a substance to be ranked for classification purposes and hazard assessment. The method as adopted in 1996 was extensively validated *in vivo* against LD_{50} data obtained from the literature, both nationally and internationally. The test extract was administered in a single dose by gavages using a stomach tube. Animals were fasted prior to dosing, following fasting period, the animals were weighed and test substance was administered. After the dose was administered, food was withheld for a further 3-4hrs in mice. Animals were observed initially after dosing at least once during the first 30 minutes, 4hrs, 24hrs, and periodically during the first 24 hours. Additional observations like changes in skin and fur, eyes and mucous membranes, and also behavioral pattern, tremors and coma.

Anti-inflammatory activity:

Anti-inflammatory activity was performed by Carrageenan induced Paw edema method. The animals were weighed, numbered and marked on both hind paws, beyond the tarsal junction. So that, every time the paw was dipped in mercury column up to the fixed mark to ensure constant paw volume. The initial paw volume of each rat was noted by mercury displacement method. The animals were divided into five groups.

Group I was of Normal control.

GroupII was of Negative control.i.e. Carrageenan treated.

Group III was of Ethanolic extract (150mg/kg) + Carrageenan treated.

Group IV was of Ethanolic extract (250mg/kg) + Carrageenan treated.

Group V was of Standard i.e. Indomethacin + Carrageenan treated

In III to IV group oral dose of extract was given to the test group and in V group oral dose of Indomethacin was given. After 1hr 0.1 ml of 1%, w/w carrageenan was given in subplantar volume of control and test group was noted at 0, 1, 2, 3, 4, 5hrs. The paw volume was measured by plethysmometer. Then percent inhibition of ethanolic extract was calculated.

RESULTS

Phytochemical screening:

The Phytochemical constituents of *Argemone mexicana* root extract was identified by chemical tests and these tests showed the presence of various phytochemical constituents and are shown in table no.1

Table 1: Preliminary phytochemical studies of ethanolic extract

Sr. NO.	Phytochemical	Test	Result
1	**Phytosterols**	Salkowski test	+
		Liebermann Burchard test	+
		Sulphur test	+
2	**Alkaloids**	Mayer's reagent test	+
		Dragendroff's reagent test	+
3	**Flavonoids**	Conc.H_2SO_4 acid	+
		Shinoda test	+
4	**Triterpenoids**	Noller's test	_
5	**Phenolic compounds and Tannins**	Ferric chloride solution	+
6	**Saponins**	Foam test	_
7	**Proteins**	Ninhydrin test	-
		Biuret test	-
8	**Carbohydrate**	Molisch's Test	+
		Fehling's Test	+
9	**Glycoside**	Borntragers test	-
10	**Cardiac glycoside**	Legal test	-
		Keller–Kiliani test	-

Acute toxicity test:

The acute toxicity study has shown no toxicity of the drug extract at the dose of 2500 mg/kg. No mortality was recorded in any group of mice after 72hrs of administering the extract to the animals. However, the animals presented muscular weakness with slow movements, which disappeared around the end of observation period (72h). LD_{50} of root extract was found to be more than 2500mg/kg. The results of toxicity study are given in table no. 2 and 3.

Table 2: Acute toxicity study (LD_{50}) of root extract

Sr. No.	Plant material	Medium	LD_{50}	Therapeutic Dose
1	*Argemone Mexicana*	Root extract	More than 2500mg/kg	250 mg/kg

Table 3: Behavioral observation in mice for *Argemone mexicana*

Sr. No.	Observation	30 min	4 hrs	24 hrs	48 hrs
1	Skin and fur	Normal	Normal	Normal	Normal
2	Eyes	Normal	Normal	Normal	Normal
3	Mucous membrane	Normal	Normal	Normal	Normal
4	Behavioral pattern	Normal	Normal	Normal	Normal
5	Tremors	Nil	Nil	Nil	Nil
6	Salivation	Normal	Normal	Normal	Normal
7	Diarrhea	Nil	Nil	Nil	Nil
8	Lethargy	Nil	Nil	Nil	Nil
9	Sleep	Normal	Normal	Normal	Normal
10	Coma	Nil	Nil	Nil	Nil

Anti-inflammatory activity:

The results of anti-inflammatory activity of ethanol extract of *Argemone mexicana* roots on carrageenan induced paw edema is shown in Table no. 4 and Figure no. 1. The lower dose of 150mg/kg showed inhibition at both early and late phase; though maximum inhibition was at late phase (47.37%, $P < 0.05$). The higher dose i.e. 250 mg/kg also showed maximum anti-inflammatory activity at late phase (49.81%). The standard Indomethacin showed maximum activity at late phase (52.03%, $P < 0.05$). In this model, the standard dose showed more inhibition of edema formation than lower and higher dose of ethanolic extract.

Table 4: Anti-inflammatory activity of ethanol extract of *Argemone mexicana* roots in carrageenan induced paw edema percentage inhibition in paw edema

Group Treatment	Inference	Normal Control	Negative Control	EEAM 150	EEAM 250	Standard (Indomethacin
After 1 hr	Vol. increase	0.9667± 0.04842	1.237± 0.01333	1.067± 0.0721*	1.080± 0.07211	1.017± 0.01856
	Percent change	--	--	35.19%	36.82%	40.88%
After 2 hr	Vol. increase	0.8300± 0.1620	1.287± 0.1510	1.113± 0.0578*	1.180± 0.02000	1.093± 0.0033*
	Percent change	--	--	35.81%	41.28%	42.58%
After 3 hr	Vol. increase	0.9900± 0.01000	1.353± 0.07688	1.150± 0.0702*	1.183± 0.03712	1.123± 0.0088*
	Percent change	--	--	47.37%	49.81%	52.03%
After 4 hr	Vol. increase	0.9367± 0.03712	1.223± 0.02906	1.223± 0.02906	1.160± 0.03512	1.110± 0.0577*
	Percent change	--	--	26.91%	34.29%	31.01%
After 5 hr	Vol. increase	0.9600± 0.02517	1.190± 0.04583	1.120± 0.0519*	1.147± 0.03180	1.110± 0.0115*
	Percent change	--	--	23.2%	24.88%	25.72%

Values are expressed as Mean ± SEM (n=3) *P<0.05, **P< 0.01 decreased

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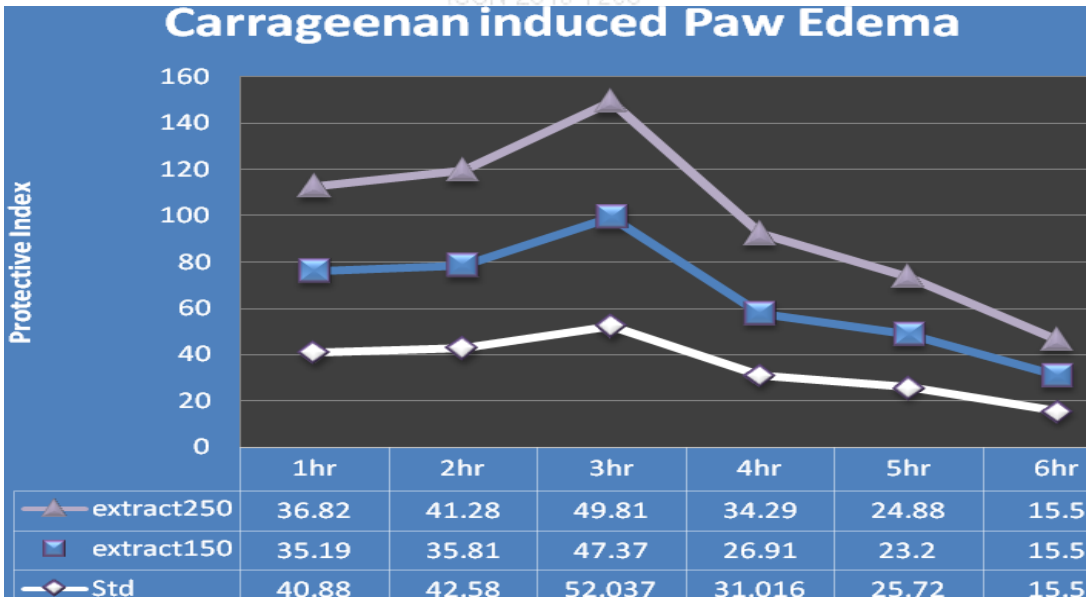

Figure 1: Effect of Ethanol Extract of *Argemone mexicana* root on percent inhibition in Carrageenan induced paw edema in rats.

DISCUSSION

Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release fluid extravasations, cell migration, tissue breakdown and repair [9]. It is also known that anti-inflammatory effects can be elicited by a variety of chemical agents and that there is little correlation between their pharmacological activity and chemical structure [10]. This associated with the complexity of the inflammatory process makes the use of different experimental models essential when conducting pharmacological trials. The present study establishes the anti-inflammatory and analgesic activity of the ethanolic extract of *Argemone Mexicana* root in a number of experimental models. Carrageenan induced rat paw edema is a suitable experimental animal model for evaluating the anti-edematous effect of natural products [11] and this is believed to be triphasic, the first phase (1hr after carrageenan challenge) involves the release of serotonin and histamine from mast cells, the second phase (2hr) is provided by kinins and the third phase (3hr) is mediated by prostaglandins, the cyclooxygenase products and lipoxygenase products [12]. The metabolites of arachidonic acid formed via the cyclooxygenase and lipoxygenase pathways represent two important classes of inflammatory mediators, prostaglandins (products of the cyclooxygenase pathway) especially prostaglandin E_2 is known to cause or enhance the cardinal signs of inflammation, similarly, leukotriene B_4 (product of lipoxygenase pathway) is a mediator of leukocyte activation in the inflammatory cascade [13]. From the results, the ethanolic extract of *Argemone mexicana* leaves inhibited Carrageenan induced rat paw edema at 150mg/kg and 250mg/kg, although not significantly different ($P > 0.05$) from the control (table 4). The aqueous fraction of the ethanolic extract significantly inhibited ($p < 0.05$) carrageenan induced rat paw edema, at the third hour the activity of the aqueous fraction is higher than that elicited by Indomethacin in a dose-dependent manner. Indomethacin is a cyclooxygenase inhibitor, the ethanolic extract has activity which is comparable to Indomethacin and can be said to inhibit the cyclooxygenase enzyme but lipoxygenase inhibitors also possess significant anti-inflammatory activity against carrageenan induced paw edema [14], so inhibition of carrageenan induced paw edema by the ethanolic extract could also be due to its inhibitory activity on the lipoxygenase enzyme. The ethanolic extract showed a dose dependent activity but was less than that produced by Indomethacin (table 4), the methanolic extract with a maximal effect at 250 mg/kg which was significant than the effect produced by Indomethacin. Preliminary phytochemical screening shows that the extract contains alkaloids, phytosterols, flavonoids, phenolic compounds and tannins [15-17].

CONCLUSION

It can be concluded that the ethanolic extract of *Argemone mexicana* has anti-inflammatory activity against carrageenan induced paw edema in rats. These activities may be due to their content of alkaloids, phytosterols, flavonoids, phenolic compounds and tannins. This study demonstrates the efficacy of *Argemone mexicana* as an anti-inflammatory agent and also scientifically justifies the use of this plant as an anti-inflammatory agent in folk medicine [18-19], however, further studies are required to determine the constituents responsible for its anti-inflammatory activity and further authenticate its mechanism of action.

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