DETAILED SCREENING OF ANTI-CONVULSANT ACTIVITY OF *ARGEMONE MEXICANA*

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ABSTRACT:

Introduction: *Argemone mexicana* belongs to family Papaveraceae. *A. mexicana* is used by traditional healers in Mali to treat malaria, externally in the treatment of cataracts and internally in the treatment of dropsy and jaundice. **Objective of the study:** The present study was undertaken for investigating and validating anti-convulsant activity of chloroform, ethanol and aqueous extracts of *Argemone Mexicana* (200mg/kg) in swiss albino mice in comparison with that of standard drug Diazepam (4mg/kg i.p). **Methods:** Extracts was evaluated for its anti-convulsant activity and compared with control and standard drug (diazepam) using MES and PTZ method. Mice of either sex were taken and divided into five groups of 6 animals each. First group was considered as control, second as standard (Diazepam), third, fourth and fifth as test group (with three different extracts of the plant) and all the drugs were given intra-peritoneally. **Results:** Preliminary phytochemical screening revealed that aerial parts of *Argemone Mexicana* extracts contained triterpenoids, flavonoids, steroids, alkaloids, carbohydrates, proteins and tannins. All the three studied extracts have shown significant anti-convulsant activity in both the models. However, the chloroform extract (200mg/kg) of *A.*

Mexicana has shown highly significant activity ($p<0.01$) by reduction in the hind limb tonic extension in the MES test in a dose dependent manner. In the PTZ model also, the extract significantly ($p<0.01$) reduced the duration of clonic convulsions as well as delay the onset of seizures in a dose dependent manner. **Conclusion**: The study demonstrates that *Argemone mexicana* has significant anticonvulsant activity possibly through a GABA-ergic interaction.

Keywords: Anti-convulsant, Pentylenetetrazole(PTZ), Maximal electroshock (MES)

INTRODUCTION

The term of medicinal plants includes a various types of plants used in herbalism and some of these plants have a medicinal activity. Medicinal plants play a key role in human health care. Medicinal plant research has succeeded in overwhelming the problems associated with synthetic drugs in maintaining low toxicity and less side effects[1]. Herbal drugs comprise the use of whole plant or parts of plant for their therapeutic effect[2,3]. WHO has distinct herbal drugs as complete, labeled medicinal products that have vigorous ingredients, aerial or secretive parts of the plant or other plant material or combination[4]. These are the oldest form of healthcare known to mankind and is a chief constituent in Ayurvedic, Homeopathic, Naturopathic and other medicine systems[5,7]. They are usually considered as safe, since they belong to natural sources, and this leads to rapid increase in their use[7,8].

One such herbal drug with various applications is *Argemone Mexicana* is an erect annual spiny herb with grayish white stem secreting yellow coloured latex. It grows to a height of 0.3 to 0.12m.. The word *Argemone'* is derived from the Greek argena, which means 'cataract of the eye', and this name was used in the first century AD by Dioscorides (AD 40-90) and Pliny (AD 23- 79) for some spiny poppies (the juice of which was supposedly a cure for *Introuduction* cataract); *mexicana* is a combination of Mexico with Latin suffix ana, suggesting the name of country of origin[9].

A. mexicana is reported to have antimicrobial activity[11], wound healing capacity in rat[10], larvicidal and chemosterilant activity[11], Nematicidal and Allelopathic potential[12]. In Mexico infusion of aerial part of the plant is used as hypoglycemic[13]. Chemical investigations of this plant have revealed the presence of alkaloids[14],[15]. amino acids[16], phenolics[17] and fatty acids[18]. The aerial part of the plant contains Isoquinoline and Benzyl isoquinoline alkaloids. Alkaloids like Berberine and Tetrahydroberberine, Protopine, Benzophenanthridines has been isolated from the plant[19].

A. mexicana is used by traditional healers in Mali to treat malaria, externally in the treatment of cataracts and internally in the treatment of dropsy and jaundice. *mexicana* has been investigated in terms of modern pharmacology for its anti-malarial activity[20,21], Molluscicidal and Nematicidal activity[22,23], anticancer activity, antimicrobial activity[24,25,26,27], Hepatoprotective activity[28], anti-HIV activity[29] and Neuropharmacological activity[30].The aerial part of the plant contains Isoquinoline and Benzyl isoquinoline alkaloids. Alkaloids like Berberine and Tetrahydroberberine, Protopine, Benzophenanthridines has been isolated from the plant[31].

However, no scientific record is available for the anticonvulsant, skeletal muscle relaxant, and diuretic activity of aerial part of the plant *Argemone mexicana*. Hence the present study was designed to evaluate these activities of chloroform, ethanol and aqueous extract of *Argemone mexicana* leaves by using experimental models in mice.

Argemone Mexicana

METHODOLOGY

Preparation of *Argemone mexicana* extracts:

Collection and Authentication of *Argemone mexicna*

The plant of *Argemone mexicana* was collected from the Navule, Shimoga district, Karnataka state. It was identified and authenticated by Prof. Dr.Rudrappa, head of the Botany, S.R.N.M National College of Applied Science, Balraj-Urs, road, Shivamogga, Karnataka.

Drying and powdering of leaves of *Argemone mexicana*

The leaves of *Argemone mexicana* was shade dried and reduced to a coarse powder in a pulvirizer (Sunbeam, Munger, India) using mesh no. 3 and passed through a sieve No. 40 to obtain about 2 kg of powder.

Extraction of the plant using different solvents.

Various extracts of the plant material were prepared by continuous soxhlet extraction method. The powdered material of *Argemone mexicana* was extracted with different solvents (Aqueous, Ethanol and Chloroform) in a soxhlet extractor for 48hrs in 3 batches. The extracts were concentrated in vacuum using rotary flash evaporator (Buchi, Flawil, Switzerland). The Aqueous extract of plant material was prepared by cold maceration method, the coarsely powdered plant material is kept in contact with the solvent in a stoppered container for a defined period with frequent agitation until soluble matter is dissolved66. The solvent was removed completely over the water bath and finally desiccator dried. The extract, so obtained was labelled, weighed and the yield was calculated in terms of grams percent of the weight of the powdered leaves of the plant. These extracts are then used for the various pharmacological activities.

Animals:

Healthy young adult male and non-pregnant female Swiss albino mice (20 – 30) were used for acute toxicity, anticonvulsant activity and skeletal muscle relaxant activity whereas rats (180 – 250 g) of wistar strain of either sex were used for the diuretic activity using different extracts of the aerial parts of *Argemone mexicana*. The animals were procured from Central animal house, National College of Pharmacy, Shivamogga, Karnataka. After randomization into various groups, animals were acclimatized for period of 10 days under standard husbandry conditions.

Room temperature 270 ± 300C.

Relative humidity 65 ± 10%

12 hours – Light/dark cycle

All the animals were fed with rodent pellet diet (Gold Mohr, Lipton India Ltd.,) and water was allowed *ad-libitum* under strict hygienic condition. Ethical clearance (NCP/IAEC/CL/01/2016-17) for performing experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC).

Statistical analysis:

All the values were expressed as mean ± S.E.M. Statistical analysis was carried out by performing one-way ANOVA followed by pair wise comparisons of Tukey's HSD (honestly significant difference) test. A probability level of $P<0.05$ was considered moderately significant, $P<0.01$ is considered as significant and $P<0.001$ is considered as highly significant.

4.3.2. Evaluation of Anticonvulsant activity:

Anticonvulsant activity was evaluated in-vivo using total leaves extract of *Argemone mexicana* Two models have been used to evaluate the anticonvulsant activity,

A. Maximal electric shock (MES) induced convulsions.

B. Chemical method, i.e. Pentylenetetrazole (PTZ) induced Convulsions.

A. Maximal Electric Shock Induced convulsion (In-vivo)[32,33]

In this model, the animals were divided into five groups with six animals in each group. The animals of group I served as solvent control, received distilled water (1 ml/100gm b.w.); group II receive Diazepam (4 mg/kg b.w) treated as positive control; III, IV and V

groups treated with Chloroform, ethanol and aqueous extract at the dose of 200mg/kg respectively. All the treated groups were administered orally1hour prior to the electric shock induced in the entire animals passing a current of 50 mA for 0.2 sec duration through electro

convlsiometer (Techno India) using ear electrodes. The duration of flexion, extensor, clonus and stupor phase were noted and Percentage of inhibition of seizures relative to controls was calculated.

B. Pentylenetetrazole – induced seizure model:[32,33]

In this type of seizure model, the animals were divided into five groups with six animals in each group. Group I served as solvent control, received distilled water (1ml/100gm b.w); Group II received Diazepam (4 mg/kg b.w) treated as positive control and III, IV and V groups treated with total Chloroform, ethanolic, and aqueous extract at the dose of 200 mg/kg b.w. respectively. All the treated groups were administered 60min prior to the administration of Pentylenetetrazole (80 mg/kg b.w) by i.p route. The animals were observed for 1 hour by placing in a separate cage. The duration of seizures (tonic, clonic convulsions) were recorded and Percentage of inhibition of seizures relative to controls was calculated.

RESULTS

In the present study phytochemical investigation was carried out for different extracts of *Argemone mexicana* plant and anticonvulsant activity was carried out.

5.1 Preparation of different extracts of *Argemone mexicana.*

Coarsely powdered aerial parts of *Argemone mexicana* was subjected to extraction with different solvents (ethanol, chloroform and aqueous) in a soxhlet extractor for 48 hours. The extracts were concentrated using rotary flash evaporator (Buchi, Flawil, Switzerland). The solvent was removed completely over the water bath and labeled, weighed and the yield was calculated in terms of grams percent of the weight of the powdered taken.

Table No.1 Table showing the Percentage yield of various extracts of *Argemone mexicana*

. Sl. No.	**Name of the extract**	**Quantity taken in Grams**	**Yield in grams**	**% yield**
1	Ethanol extract	100	14gm	14
2	Chloroform extract	100	4.8gm	4.8
3	Aqueous extract	100	4.2gm	4.2

Table No. 2: Table showing the Qualitative chemical investigations of extracts Of *Argemone mexicana.* (+: present; -: absent)

Sl.No	**Phytoconstituents**	**Chloroform extract**	**Ethanol extract**	**Aqueous extract**
1	Carbohydrates	-	-	+
2	Proteins	-	-	+
3	Saponins	-	+	+
4	Triterpenoids	+	-	-
5	Flavonoids	+	+	+
6	Steroids	+	-	-
7	Glycosides	-	-	-
8	Alkaloids	+	+	-
9	Tannins	+	+	+

The Phytochemical analysis reveals that triterpenoids, flavonoids, steroids, alkaloids and tannins were present in the chloroform extract. In ethanol extract saponins, flavonoids, alkaloids and tannins are present. in aqueous extract carbohydrates, proteins, saponins, flavonoids, Tannins were present.

Group	**Dose mg/kg**	**Flexion (in sec)**	**Extension (in sec)**	**Clonus (in sec)**	**Stupor (in sec)**	**R/D**
Control	0.5ml	3.18±0.45	11.5±0.41	15.25±0.45	93.46±3	112.23±4.4
Diazepam	4mg/kg	1.15±0.18	0.3±0.073	7.36±0.37	52.58±3.39	50.9±2.63

CEAM	200mg	1.65±0.18**	9.48±0.46**	13.01±0.37*	72.85±3.52**	93.68±2.7
EEAM	200mg	1.96±0.24*	9.26 ±0.46**	12.58±0.55**	77.41±2.87*	96.2±3.22
AEAM	200mg	1.816±0.22*	10.35±0.60 ns	12.91±0.51*	76.81±3.65*	95.43±3.7

Note: Data was analysed using one way ANOVA followed by pairwise comparision. Values are expressed as mean ± S.E.M. *n*=6, ***P < 0.001, **P< 0.01and *P < 0.05.

Figure No.4: Histogram showing the effect of chloroform ethanolic & aqueous extract of leaf of *Argemone mexicana* Convulsion of drug administration in maximal electric shock induced convulsion

Table no 7: Effect of chloroform ethanolic & aqueous extract of leaf of *Aegemone 35exicana* on Pentylenetetrazole (PTZ) induced convulsion in mice After 60 min.

Groups	**Drugs used**	**On set of time in seconds (Mean SEM)**		
		Jerks	**Clonus**	**Extensor**
I	**Control**	50.63±2.68	78.65±3.47	277.6±6.45
II	**Diazepam**	0.21±0.06	0	0
III	**Chloroform extract200mg/kg**	70.71±4.33**	60.05±3.70*	249.63±6.58**
IV	**Ethanol extract200mg/kg**	66.76±4.01*	63.81±2.15*	256.75±4.61
V	**Aqueous extract 200mg/kg**	67.76±3.87ns	65.38±4.40*	252.76±3.37

Note: Data was analysed using one way ANOVA followed by pairwise comparison. Values are expressed as mean ± S.E.M. n=6, ***P < 0.001, **P< 0.01and *P < 0.05.

Figure No.5: Histogram showing the effect of chloroform, ethanolic & aqueous extract of leaf of *Argemone mexicana* on Convulsion of drug administration in Pentylenetetrazole (PTZ) Induced convulsion.

DISCUSSION

Swiss albino mice were used for the screening of anticonvulsant activity in MES induced convulsion and PTZ method. In MES-induced convulsion model, neither Phenytoin nor EEAM (200mg/kg) protected the animal completely, but a significant reduction in the duration of tonic convulsion was observed. MES induces seizure particularly due to the spread of stimulus throughout the body and anticonvulsant drugs that block the effect of MES act by blocking the seizure spread. Thus, the present study indicates that chloroform extract of *Argemone mexicana* (200mg/kg) has significant ability to slowdown the spread of seizure34

The chloroform extract of *Argemone mexicana* increased the threshold of PTZ-induced convulsion in rats and offered protection against PTZ-induced convulsion. The fact that the extract protected animal against PTZ-induced seizures may suggest that the plant extract contains compounds that facilitate GABAergic transmission35.
Thus the results indicate that chloroform, ethanol and Aqueous extracts were effective in absence of seizure as well as tonic clonic seizure. Finally the results from both the model indicate that the plant leaves extract have been found to have broadspectrum anticonvulsant activity, however the further research is in progress to isolate the compound responsible for this activity.

Flavonoids emerged as a very valuable class of secondary metabolite having potential for the comprehensive treatment of epilepsy. As future investigations continue, this class may prove to be a rich source of new molecules for the development of new therapeutic agents for the treatment of epilepsy. During our literature review we observed the modulatory role of flavonoids in almost all the neuronal pathways involved in the pathogenesis of epilepsy. Studies carried out till date suggests that flavonoids inhibit voltage gated sodium channels, activate Ca+ activated K+ channels, stimulate GABAergic inhibition, interact with opioid receptors, inhibit NMDA receptors and exhibit antioxidant actions via modulation of nitric

oxide and xanthine oxidase pathways and by leukocytic immobilization, one or more of these mechanisms are involved in suppression of epileptic seizures.

CONCLUSION

The present study concludes that the effects of chloroform, ethanol and aqueous extracts of aerial parts of *Argemone mexicana* exhibited significant actions on anticonvulsant activity.

Anti-convulsant activity was performed by maximal electro-shock inducedconvulsions and PTZ induced convulsion. Diazepam was taken as standard reference drug. The chloroform extract have been shown a significant activity by abolishing tonic extensor phase when compared to control, ethanol and aqueous extracts. It can be concluded that active constituents responsible for anticonvulsant activity might be present in the leaves
extracts.

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