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ANTICONVULSANT ACTIVITY OF AQUEOUS EXTRACT OF DATURA STRAMONIUM LEAVES IN ALBINO RATS.

A.PURKAYASTHA *[1], A.SAIKIA[2] AND R.TIGGA[2]

[1,2]Department of Pharmacology, Silchar Medical College and Hospital, Silchar, Assam,India.

ABSTRACT

Datura stramonium is a well known plant for its different pharmacological properties such as antiasthmatic, antimicrobial, anticancer, hypnotic, narcotic and analgesic. In the present study we investigated the anticonvulsant activity of the aqueous extract of *Datura stramonium* (AEDS) leaves in albino rats. The anticonvulsant activity of aqueous extract of leaves of *Datura stramonium* (200 mg/kg and 400 mg/kg) in rats was assessed using maximum electroshock seizure (MES) and pentylenetetrazole (PTZ) induced seizure models.The extract of *Datura stramonium* leaves significantly ($p<0.01$) reduced the hind limb tonic extension (HTLE) in the MES seizure model. In the PTZ model also, the extract signicantly ($p<0.01$) reduced the duration of clonic convulsions as well as delayed the onset of seizures in a dose dependent manner. Our study demonstrates anticonvulsant activity of *Datura stramonium* leaves in MES and PTZ induced seizure models.

KEYWORDS: **Anticonvulsant, Datura stramonium, Maximal electroshock, Pentylenetetrazole albino rats.**

A.PURKAYASTHA
Department of Pharmacology, Silchar Medical College and Hospital, Silchar, Assam,India.

INTRODUCTION

Epilepsy is a neuropsychological condition in which there is occurrence of seizures. It is a very frequent chronic disorder which affects human beings.[1] Seizure can be defined as a paroxysmal event which occurs due to abnormal, excessive hyper-synchronous discharges from aggregates of central neurons.[2] Epilepsy is the second most common neurological disorder in our country.[3,4] Although several antiepileptic drugs are available, seizures stay uninhibited in greater than 20% of the patients. Inauspicious responses like drug reactions or reduced reaction to the presently available drugs also specify a call for the development of new drugs or molecules as alternatives.[5] Thus, herbal based medicines are given much significance as they serve as efficient alternatives to modern medicine.Jimson weed, scientifically known as *Datura stramonium* [6] belongs to family Solanaceae. It is a well known folklore medicinal herb. It is a wild growing flowering plant distributed over tropical and warm temperate regions of the world. It is used for religious purposes in the worship of Lord Shiva apart from its uses in traditional medicine. Traditionally, extract from the leaves is used in the treatment of asthma and sinus infections, and stripped bark are applied externally to treat swellings, burns and ulcers. Some other traditional uses include use as an anti-inflammatory agent, stimulation of the central nervous system, respiratory decongestion, treatment of dental and skin infections, alopecia, toothache, parkinsonism and hemorrhoids. Datura is internally used for treating giddiness, dry mouth, hallucinations and coma. Externally, the plant is used as a cold compress in treating fistulas, abscesses wounds and severe neuralgia.[6] It has been scientifically proven that, *Datura stramonium* has antiasthmatic,[7] antimicrobial,[8] antifungal,[9] anti-inflammatory,[10] anticancer,[11] antioxidant,[12] analgesic and narcotic[13] activities. Though the antiepileptic activity of *Datura stramonium* as a single agent has not been extensively studied, combination therapy with other herbs has demonstrated protective effects as an anticonvulsant.[14] Also, there are reports that *Datura stramonium* was used internally in the treatment of epilepsy.[15] Thus the present study was undertaken to assess the anticonvulsant activity of aqueous extract of leaves of *Datura stramonium* as a single agent.

MATERIALS AND METHODS

The study was carried out after due approval from the Institutional Animal Ethics Committee(No. SMC/13/3420). *Datura stramonium* leaves were collected from a field near the undergraduate boys hostel of Silchar Medical College and Hospital, Silchar, Assam. The leaves were air dried at room temperature for 14 days and then powdered with the help of a commercial grinder. The powder was then subjected to percolation using boiling water as solvent for a period of 2 hours. The percolation process was continued by gradually adding boiling water until extraction was complete.[16] A percentage yield of 14.64 % was obtained after evaporation to dryness at room temperature. The extract obtained was then stored at 4ºC until further used as suspension in 2% gum acacia.The aqueous extract was then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents using standard methods.[17] Wistar albino rats of either sex weighing between 150-200 grams were procured from M/s Chakraborty Enterprise, Kolkata. The animals were acclimatized under laboratory conditions. The animals were housed under standard conditions of temperature (25±2°C) and relative humidity (30%–70%) with a 12:12 light-dark cycle. They were fed with standard laboratory food. Water was allowed *ad libitum* under strict hygienic conditions. For the purpose of our study, Phenytoin was obtained from Zydus Cadila Healthcare Limited, Diazepam obtained from Ranbaxy Laboratories, New Delhi and Pentylenetetrazol was obtained from Sigma Aldrich India, Bangalore. Acute oral toxicity study was done as per OECD guidelines 423. A group of three Wistar rats of either sex selected randomly and were used for acute toxicity study. The extracts were administered orally at the dose level of 5 mg/kg body weight to the animals and observed for 14 days. Since no mortality was observed, the procedure was repeated for further higher doses of 50, 300 and 2000 mg/ kg body weight. The extract showed no mortality at doses upto 2000mg/kg. Hence 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected as the dose levels for the study and the following tests were carried out to assess anticonvulsant activity.

i) Maximum electro shock (MES) induced seizure test

Twenty-four albino rats were taken and divided into four groups containing 6 animals each. Group I served as control and received 2% gum acacia solution (10 ml/kg, p.o). Rats in groups II and III received aqueous extract of *Datura stramonium* (AEDS) orally at the doses of 200 mg/kg and 400 mg/kg body weight respectively. Group IV received the standard drug Phenytoin at a dose of 25mg/kg intraperitoneally. All drugs were administered 1 hour prior to induction of seizures by MES. After 1 hour, electric current of 150 mA for 0.2 seconds was administered through ear electrodes to induce convulsions in all the experimental animals with the help of a convulsiometer. The different phases of convulsions were noted down along with the duration of each phase. Abolition or reduction in the duration of hind limb tonic extensor (HLTE) phase was taken as a measure of protection against MES induced seizures.[18]

ii) Pentylenetetrazole (PTZ) induced seizure test

Twenty-four albino rats were taken and divided into four groups containing 6 animals each. Group I served as control and received 2% gum acacia solution (10 ml/kg, p.o). Rats in groups II and III received aqueous extract of *Datura stramonium* (AEDS) orally at the doses of 200 mg/kg and 400 mg/kg body weight respectively. Group IV received the standard drug diazepam at a dose of 4 mg/kg intraperitoneally. All drugs were administered 1 hour prior to induction of seizures by PTZ. After 1 hour, all the animals received convulsive doses of pentylenetetrazole (80 mg/kg) intraperitoneally. The animals were observed for 30 minutes after the administration of PTZ. The different parameters noted were the onset and duration of clonic convulsions. The anticonvulsant property was assessed by the ability to reduce the duration of clonic convulsions and increase

the latency of seizures.[18]The statistical analysis of data was done by using one way ANOVA followed by Dunnet's t test. Values were expressed as Mean ± SD (n= 6). The tests were considered to be significant when $p< 0.05$ and highly significant when $p< 0.01$.

RESULTS

1. *Phytochemical screening*

The phytochemicals screening investigation for aqueous extract of *Datura stramonium* leaves revealed the following results(Table I).

Table I
Results of phytochemical screening

Sl no.	Constituent	Aqueous extract of *Datura stramonium*
01.	Saponins	+
02.	Tannins	+
03.	Carbohydrate	+
04.	Protein	+
05.	Steroid	+
06.	Flavonoids	+
07.	Alkaloid	+
08.	Phenols	+
09.	Glycosides	+

(+) indicates presence

2. *Effect of AEDS on MES induced seizures*

The AEDS at doses 200mg/kg and 400mg/kg did not completely abolish the hind limb tonic extensor (HLTE) phase as seen with phenytoin, however there was a significant ($p<0.01$) reduction in the duration of HLTE phase in a dose dependent manner. Phenytoin treated animals showed 100% protection against MES induced seizures whereas AEDS 200 mg/kg and 400 mg/kg showed 39% and 61% protection respectively. (Table II).

Table II
Effect of aqueous extract of Datura stramonium leaves on MES induced seizures.

GROUPS	TREATMENT	FLEXION(SECS)	EXTENSION(SECS)	CLONUS(SECS)	% PROTECTION
I	CONTROL (2%gum acacia 10 ml/kg p.o)	7.16 ± 0.46	13.23 ± 0.69	16.13 ± 0.43	0
II	AEDS (200mg/kg p.o)	5.76 ± 0.56*	8.03 ± 0.75*	11.36 ± 0.56*	39
III	AEDS (400mg/kg p.o)	4.13 ± 0.43*	5.13 ± 0.82*	10.06 ± 0.51*	61
IV	PHENYTOIN (25 mg/kg i.p)	3.63 ± 0.49*	0±0*	9.26 ± 0.41*	100

Values are expressed as Mean ± SD (n=6). Statistical analysis done by one-way ANOVA followed by Dunnett's test. * p value < 0.01 when compared to control.

3. *Effect of AEDS on PTZ induced seizures*

The AEDS at both the doses significantly ($p< 0.01$) increased the latency of seizures as well as reduced the duration of seizures in a dose dependent manner. With diazepam, a complete abolition of seizures was observed. The AEDS 200 mg/kg and 400 mg/kg exhibited 36% and 71% protection respectively against PTZ induced seizures whereas 100% protection was observed with diazepam. (Table III).

Table III
Effect of aqueous extract of Datura stramonium leaves on PTZ induced seizures.

GROUPS	TREATMENT	ONSET OF CLONUS(SECS)	DURATION OF CLONUS(SECS)	% PROTECTION
I	CONTROL (2%gum acacia 10 ml/kg p.o)	66.4 ± 4.63	264.5 ± 6.83	0
II	AEDS (200mg/kg p.o)	128.06 ± 5.82*	168.83 ± 6.88*	36
III	AEDS (400mg/kg p.o)	182.1 ± 5.39*	75.56 ± 6.21*	71
IV	DIAZEPAM (4 mg/kg i.p)	0 ± 0*	0 ± 0*	100

Values are expressed as Mean ± SD (n=6). Statistical analysis done by one-way ANOVA followed by Dunnett's test. * p value < 0.01 when compared to control.

DISCUSSION

Traditional plant medicines have the prospective to turn into the first-line therapies for ailments with unmet medical requirements.[19] However, most of the antiepileptic drugs are unreachable, more expensive and have many noxious undesirable effects. In this regard, there is a need for the development of efficient and more cost-effective anticonvulsant agents from plant and other sources. MES and PTZ seizures models are commonly used to test anticonvulsant activity of drugs. The MES test is the most universal preliminary screening model for detection of anticonvulsant activity of drugs. The MES test corresponds to the generalized tonic clonic seizures or "grand mal" epilepsy in humans.[18] Pentylenetetrazole (PTZ) is a chemoconvulsant, which induces convulsions by the inhibition of $GABA_A$ receptors and is a broadly conventional experimental model for absence seizure.[20,21]In our study, it was observed that treatment with AEDS significantly reduced the hind limb tonic extensor (HLTE) phase in MES induced seizure model. Standard antiepileptic drugs such as phenytoin, valproate and lamotrigine, which are clinically efficient in the treatment of generalized tonic clonic and partial seizures, all curb down the hind limb tonic extension in the MES model.[22,23] Protection against HLTE indicates the capacity of a test substance to reduce or eliminate the spread of seizure discharges within the brain. In our present study, the ability of the aqueous extract of *Datura stramonium* leaves to inhibit the HLTE in the MES model as compared to phenytoin (100% protection) suggests the occurrence of anticonvulsant compounds in the extract. Similarly, it was observed that treatment with AEDS significantly decreased the duration of convulsions as well as increased the onset of convulsions in the PTZ seizure model. Although the exact mechanism of PTZ induced seizure is unknown, recent studies suggest that PTZ may cause seizure by inhibiting the chloride ion channel associated with gamma amino butyric acid type A ($GABA_A$ receptors).[20,21] PTZ has been shown to interact with GABA neurotransmission and drugs such as benzodiazepines and phenobarbitone, which enhance the $GABA_A$ receptor mediated inhibitory neurotransmission, can prevent PTZ induced seizures.[18,24] Hence, the ability of aqueous extract of *Datura stramonium* leaves to antagonize the PTZ induced seizure suggests a possible interaction with GABA-ergic neurotransmission.Preliminary phytochemical analysis of *Datura stramonium* showed that the aqueous leaf extract contains saponins, tannins, carbohydrates, proteins, steroids, flavonoids, alkaloids, phenol, and glycosides. Several studies have indicated that plants containing flavonoids and saponins have significant anticonvulsant activity. Many flavonoids and phytosteroids have been found to be ligands for the $GABA_A$ receptors and hence can act like benzodiazepine like molecules.[25,26] Therefore, these phytoconstituents may be responsible for the anticonvulsant activity of *Datura stramoniun*. However, further research work is needed to establish the active constituent(s) of the extract and the exact mechanism of action.

CONCLUSION

Thus, the present investigation establishes the anticonvulsant activity of *Datura stramonium* leaves and also suggests the possibility of a GABA-ergic interaction to be responsible for the observed effect. Though *Datura stramonium* appears to be an effective alternative to modern therapy its use should be implemented with caution keeping in mind its toxic potential as well.

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CONFLICT OF INTEREST

None declared.

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