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RESEARCH PAPER

ANTIEPILEPTIC ACTIVITY OF AQUEOUS EXTRACT OF *DATURA INOXIA* LEAVES AGAINST SEIZURE INDUCED BY MAXIMAL ELECTROSHOCK AND CHEMICALLY IN MICE

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The existing description is a study of the antiepileptic effect of *Datura inoxia* as a recognized herb that is used in Ayurveda for asthma, rheumatism, tumors, cough, fever, antimicrobials and epilepsy. Toxicity studies were carried out for standardizing a dose of aqueous extract of *D. inoxia*, maximal electroshock (MES) and isoniazid (INH) induced seizures. The Albino mice model were used for screening the antiepileptic activity. As per OECD guideline no. 423, up to 2000 mg/kg body weight of recommended dose of extract were found toxic. Animals were treated with aqueous extract of 200, 400 and 600 mg/kg body weight. Phenytoin was used as reference anticonvulsant drugs for comparison. The investigation stated the significant interruption in the INH-induced clonic seizure and in MES model, reduction in the period of hind leg extensor phase. In MES model, *D. inoxia* exhibited a significantly decrease in the duration of hindlimb extension with 400 and 600 mg/kg dose, respectively. Comparable results were obtained in INH model by delayed onset of a clonic seizure. Aqueous extract of *D. inoxia* exhibited antiepileptic action against MES and INH-induced epilepsy in animal models.

Key words: Antiepileptic activity, *Datura inoxia*, Phenytoin, Maximal electroshock, Isoniazid.

INTRODUCTION

Naturals products have a wide history in treating of a variety of disorders [1-4], including epilepsy (goatopathy) which is a relatively common neurological disorder. Epilepsy is defined as a group of long-lasting disorders related to a nervous system which is characterized by the spontaneous occurrence of seizures mostly linked with the loss of consciousness and body activities (convulsions) [5]. Epilepsy has often explained as the incidence of at least 2 motiveless seizures separated 24 hr apart but as per latest international consensus, only a single epileptic seizure as long as there is a continuing predisposition to create epileptic seizures [6]. Several etiological influences foremost to a first epileptic seizure in the aged cause a continuing predisposition to seizures [3-7]. The annual incidence is 50/100,000 per year [7, 8]. Almost 5-10% of the inhabitants will have at least 1 seizure, with the maximum prevalence happening in premature and old age [9]. WHO estimates that eight people per 1000 worldwide have this disease [10]. More than half of the 50 billion individuals with epilepsy worldwide are

predictable to living in the region of Asian countries. Almost, eighty-five percent of epilepsy affected persons living in developing nations [11]. Precisely sixty percent of all epilepsies are idiopathic or cryptogenic [12]. It is predictable that there are 55 lakhs peoples with epilepsy in India, 20 lakhs in the USA and 3 lakhs in the UK [13, 14]. The yearly financial load of epilepsy in India is 88% [15]. Due to the seizure-activating action of estrogen and seizure-protective effect of progesterone, epilepsy is to some extent more common in males in comparison to females [16-18]. The neuronal membrane potential is regulated by an accurate balance between excitatory postsynaptic potential (EPSP) and inhibitory postsynaptic potential (IPSP). If this balance is compromised, an epileptic seizure can be generated [19]. A test commonly used to diagnose epilepsy is called an electroencephalogram (EEG). The doctor may also request a brain scan by using magnetic resonance imaging (MRI), computed tomography (CT) and positron emission tomography (PET) scan to see structures inside the brain. Bromide was introduced in 1857 for treating epilepsy and Phenobarbital in 1912. Subsequently, many other anticonvulsants were discovered [20, 21]. Carbamazepine and valproate are normally used in the treatment of epilepsy but can cause a lot of adverse effects, now most preferred drug are phenytoin and GABA analogs [19-23].

Datura is mentioned in all the ancient scriptures of Ayurveda. In ancient flora, it is used in the treatment of spasmodic asthma, fever with catarrhal symptoms, anesthetic cough and convulsion [22]. It grows in waste lands, along with the roadside, gardens and railway lines, and in scrub jungles throughout the tropical parts of India. *Datura inoxia* is a yearly growing shrubby herb which characteristically spreads an altitude of 0.5 to 1.4 meters [23]. The whole plant is showing a grayish presence because its twigs and leaves are protected with little and soft grimy hairs. This herb has a snowy, trumpet-shaped (12–19 cm long) beautiful flowers [24]. As per a traditional use, the herb has tranquilizer effect, leaves are used as a local application for rheumatic swellings of the joints, backache, aching back, neuralgia, and throbbing lumps. Seeds are used externally for piles. Scopolamine has analgesic and sedative actions and produces amnesia. It also has anti-inflammatory and anti-nociceptive property. It is acrid, anti-hyperglycemic, hypolipidemic and antimitotic [25]. The aqueous extract is reported to be used in the treatment of gastric pains and indigestion and may be useful for the treatment of organophosphate poisoning. Numerous herbal medications establish a possibly significant path important to the innovative healing agent for treatment of seizure which might not only avert but also be harmless, budget, highly tolerated and suitable for various patients. Every year a lot of plants from the traditional medicinal system have been screened for their potential antiepileptic activity but only a few of them are included in health care system after clinical research.

Fig. 1. Leaves of *D. inoxia* herb [23]

MATERIALS AND METHODS

Plant collection

Fresh leaves of *D. inoxia* used for the study were collected from the waste lands, along with the roadside and bank of Ganges River of Varanasi district, Uttar Pradesh during August 2023. The sample drug has been identified and authenticated by Dr. K. N. Dubey at Banaras Hindu University, Varanasi. The voucher sample of the herb was placed at the academy for further reference.

Preparation of extract

Leaves were separated from other parts of the plant and stored in a polyethylene bag. The leaves were air dried in shade. The size was reduced and made to a coarse powder and then further passed through the appropriate sieve number to obtain uniform particle size. Dried powder was extracted by using water in Soxhlet apparatus. Coarse powdered was packed in Soxhlet column with water at boiling point. The temperature was maintained at the level of boiling point and time took approximate 18-19 hr until the leaves were colorless. The extracts

were concentrated on a water bath and were kept in desiccators.

Preliminary phytochemical screening

The phytochemical examination of the aqueous extract of *D. inoxia* was performed by the standard methods [26].

Animals used

Albino male mice (20-30 g) were purchased from Jamia Hamdard animal house, New Delhi. The mouse was preserved in a well-ventilated area with 12:12 hr light/dark cycle in polypropylene cages. The mouse was nourished with normal pellet food and water was given ad libitum. Ethical committee permission was attained from Institutional Animal Ethics Committee (IAEC) of Committee for Control and Supervision of Experiments on Animals (CPCSEA). All test animals are allowed free access to food and water ad libitum, both being withdrawn just prior to experimentation.

Drugs and chemicals

Phenytoin was obtained as a gift sample from Akum Drugs and Pharmaceutical Company, Haridwar. Every day new solution of drug was prepared for the experiment. 2 ml/100 g volume of drugs were administered through the intraperitoneally (*i.p.*) route to the experimental animal. An equivalent volume of doses of the suitable vehicle was administered to the control group of experimental animals. The doses and pretreatment times of the *D. inoxia* leaves extract and the antiepileptic drugs used were attained from pilot studies in our research laboratory. The pretreatment dose and the times following the administration of either 300 mg/kg, *s.c.*, isoniazid, *D. inoxia* extract (200, 400 and 600 mg/kg, *i.p.*), and 25 mg/kg, *i.p.*, phenytoin.

Acute toxicity study

As per the OECD guideline no. 423 the acute toxicity of test aqueous extract was determined. Even at 2000 mg/kg dose also, test drug was found toxic. Henceforth, 1/10th (200 mg/kg), 1/5th (400 mg/kg) and another dose (600 mg/kg) of this dose were carefully chosen for further research [27].

Antiepileptic activity

Effect on maximal electroshock induced seizures

Male Swiss albino mice were divided into 5 groups of 6 mice each. Group I treated as a control group and was administered 0.9% saline as a vehicle. Group II treated as a standard group and was administered phenytoin 25 mg/kg, *p.o.* Group III, IV, and V were treated with diverse doses of aqueous extract of *D. inoxia*, received 200, 400 and 600 mg/kg p.o. for 7 days, respectively. After 7 days, trans auricular electrodes were used for electrical provocation. 50 mA for 0.2 sec of electroconvulsive shock was transported through trans auricular electrodes to induce Tonic Hind Limb Extension (THLE) phase. The electrical provocation was applied by a stimulator device to 6 mice of 5 group. The current was applied after 30 min of administration of aqueous extract, control and standard drug [28, 29].

Effect on Isoniazid (INH) induced seizures

Male Swiss albino mice were divided into 5 groups of 6 mice each. Group I treated as a control group and was administered 0.9% saline as a vehicle. Group II treated as a standard group and was administered phenytoin 25 mg/kg, *p.o.* Group III, IV, and V were treated with diverse doses of aqueous extract of *D. inoxia*, received 200, 400 and 600 mg/kg *p.o.* for 7 days, respectively. On the 7th day, 60 min 300 mg/kg *s.c.* of Isoniazid was administered after administration of control, standard and extract to respective groups. The different parameter was noted throughout test session of initial, 30 min and upto 24 hr. The mice were detected for latency (onset of epileptic seizure), animal status after 30 min and 24 hr period and the percentage [30].

Statistical analysis

The outcomes of this research were stated as mean ± standard error of the mean (mean ± SEM). One-way ANOVA and followed by Dunnet's test were used for assessing the significance of differences among the different groups. The significance is recognized when P value is less than 0.05. P values are denoted as *P<0.05 as significant, **P<0.01 as very significant and ***P<0.001 as extremely significant.

RESULTS

Phytochemical screening

The results of preliminary phytochemical screening of the aqueous extract of *D. inoxia* revealed that presence of alkaloids, glycosides, tannins, terpenoids, phenols, amino acids and absence of steroids, flavonoids and carbohydrates.

Effects of D. inoxia on MES induced epilepsy

The duration of THLE in vehicle treated mice was 11±0.73 seconds. At doses of 400 and 600 mg/kg *D. inoxia* were significantly (P<0.01 and P<0.001) reduced the duration of THLE. While the standard drug treated animals exhibit abolished THLE. Phenytoin administered mice have shown 83.33% protection against MES-induced seizures while *D. inoxia* 600 mg/kg has shown 50% protection, respectively. Mice treated with *D. inoxia* (200 mg/kg) failed to produce any significant reduction in duration of THLE as compared to control mice (**Table 1**).

Table 1. Effect of aqueous extract of *D. inoxia* on maximum electroconvulsive shock (MES) induced seizures in mice

Sr. No.	Treatment		Time (sec) in various phases of convulsion (MEAN±SEM)					
	Drug	Dose (mg/kg)	Flexon	Extension	Clonus	Stuper	No. of animals convulsed	% of animals protected
1	Normal Saline + MES	2 ml/100g	3±0.36	11±0.73	12.5±0.76	30.5±12.5	6/6	0
2	Phenytoin+ MES	25	0	0	6±0.57	0	1/6	83.33
3	Test drug + MES	200	2.5±0.42^{NS}	10.16±0.98^{NS}	10.83±0.7^{NS}	25.16±6.5^{NS}	5/6	16.66
		400	1.5±0.76**	5.83±1.16**	8.6±2.3**	12.83±16.9**	4/6	33.33
		600	0.83±0.4***	1.06.16±2***	6.8±2***	4.33±18.5***	2/6	66.66

Values are expressed as mean ± SEM by using one way ANOVA and followed by Dunnet's test.
n=6, *P<0.05, **P<0.01, ***P<0.001, NS - not significant.

Effect of D. inoxia on INH induced epilepsy

Isoniazid (300 mg/kg *s.c.*) provoked tonic-clonic seizures followed by THLE and death in mice. 400 and 600 mg/kg *D. inoxia* administered mice significantly delayed (P < 0.01 and P < 0.001) onset of seizure mice as well as significantly reduce the duration of clonic and tonic convulsion (P < 0.01 and P < 0.001) as compared to INH control mice. Mice treated with *D. inoxia* (200 mg/kg) failed to produce any significant delaying in onset of seizure and significant reduction in the clonic-tonic convulsion as compared to isoniazid (INH) control mice (**Table 2**).

Table 2. Effect of aqueous extract of *D. inoxia* on isoniazid (INH) induced seizures in mice

Sr. No.	Treatment		Time (sec) in various phases of convulsion (MEAN±SEM)				
	Drug	Dose (mg/kg)	Latency	Tonic	Clonic	No. of animals convulsed	% of animals protected
1	Normal Saline + NIH	2 ml/100g + 300	139.83±8.1	3.16±0.4	4±0.5	6/6	0
2	Phenytoin+ NIH	5 + 300	0	0	0	0/6	100
3	Test drug + NIH	200 + 300	133.8±9.9^{NS}	2.83±.60^{NS}	3.6±0.5^{NS}	4/6	33.33
		400 + 300	147.6±7.2**	1.16±0.3**	1.5±0.4**	3/6	50
		600 + 300	250.5±4.7***	0.8±0.3***	1.0±0.2***	1/6	83.33

Values are expressed as mean ± SEM by using one way ANOVA and followed by Dunnet's test.
n=6, *P<0.05, **P<0.01, ***P<0.001, NS - not significant.

DISCUSSION

Presently existing anticonvulsants treatments can capably regulate seizures in about half of the populations of total patient, another one fourth might show enhancement, while the remaining one fourth of antiepileptic medications do not help significantly. Moreover, an unwanted adverse effect of the medications used clinically often render treatment difficult so that a request for new categories of antiepileptic's exists. One of the methods to exploration for novel antiepileptic drugs is to examine the naturally existing compounds that might belong to novel structural classes [31]. In current study, for the screening of aqueous extract of *D. inoxia* for antiepileptic activity, two standard methods namely maximal electroshock (MES) and Isoniazid (INH) methods have been used. In Ayurveda, numerous plants appealed to have anticonvulsant action deprived of any scientific basis. The goal of the current study was to carry phytochemical investigation and evaluation of putative antiepileptic activity of leaves of *D. inoxia*. The parameters observed were the

duration of tonic hind limb flexion, tonic hind limb extension, clonus, stupor, and incidence of recovery and death. MES method has a high degree of predictivity for drugs useful in the management of tonic-clonic seizures in man. The second group of mice received a subcutaneous injection of isoniazid (300 mg/kg). INH is used commonly for the treatment and chemoprophylaxis therapy of tuberculosis but can have a serious side effect on the CNS triggering seizures [32]. The reason for INH-induced epileptic seizures is the decrease of GABA level below the life-threatening level in some neurons. Phenytoin preserved group exhibited 100% safety of the experimental animals. INH-induced epileptic seizures in mice significantly delayed the onset of seizures. The test group treated showed protection of the animals from convulsion. The induction of seizure with isoniazid (INH) results from the action of pyridoxine. The precipitating mechanism of seizure is not exactly known but it may relate to INH-induced pyridoxine deficiency.

CONCLUSION

The outcome of the current research discovered the anticonvulsant activity of the aqueous extract of *D. inoxia*. At a dose of 400 and 600 mg/kg body weight, *D. inoxia* revealed a statistically significant anticonvulsant outcome against both MES and INH induced convulsions. It is, consequently, conceivable that anti-convulsant effect of *D. inoxia* might be applied by the several phytochemical ingredients existing in the herb i.e. glycosides, triterpenes, tannins alkaloids, steroids, flavonoids, protein, amino acid and defend its use as a traditional widespread medication for CNS-associated disorders. Though, further research is needed to determine its clinical efficiency, its active compounds and mechanism of action of the herb extract.

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