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Original article:

ANTI-SEIZURE ACTIVITY OF FLOWER EXTRACTS OF *NEPETA BRACTAETA* IN SWISS ALBINO MICE

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ABSTRACT

Epilepsy is a neurological disorder characterized by unprovoked, recurring seizures that disrupts the nervous system and can cause mental and physical dysfunction. Based on the ethno pharmacological information of the plant, the methanolic and aqueous extracts of the flowers of *Nepeta bractaeta* was evaluated for its antiepileptic activity. The methanolic and aqueous extracts of the flowers of *Nepeta bracteata* were observed for their antiepileptic activity by increased current Electroshock seizures (ICES) test and Pentylenetetrazole (PTZ) test using Swiss albino mice. Both the extracts showed significant activity in ICES and PTZ induced convulsions in comparison to control. In ICES model, NBAE at higher dose showed 16.7 % and NBME at higher dose showed 33.3 % protection against seizure and in PTZ model, NBME at higher dose showed 33.3 % protection against seizure. From the experiments performed, it can be said that *Nepeta bractaeta* does possess anticonvulsant property.

Keywords: *Nepeta bractaeta,* antiepileptic effects, ICES, PTZ

Abbreviations: *Nepeta bractaeta*– NB, Increased current electroshock seizures – ICES, Pentylenetetrazole – PTZ, Gamma amino butyric acid – GABA, AEDs – antiepileptic drugs, DW – Distilled Water, NBAE – *Nepeta bractaeta* aqueous extract, NBME – *Nepeta bractaeta* methanolic extract

INTRODUCTION

Natural medicines are gaining prominence because of their economic benefit, ease of availability and relatively less side effects when compared to allopathic medicines. They have been investigated till now and found to treat effectively many diseases like cancer, ulcers, infections, heart disorders along with neurological disorders mainly epilepsy, schizophrenia, dementia etc. Epilepsy is a neurological disorder characterized by unprovoked, recurring seizures that disrupt the nervous system and can cause mental and physical dysfunction. Approximately 50 million individuals suffer from this debilitating disease (Fisher and Boas, 2005).

There are numbers of synthetic antiepileptic drugs currently available in the management, control and/or treatment of epilepsy. The current therapeutic treatment of epilepsy with modern antiepileptic drugs

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(AEDs) is associated with side-effects, dose-related chronic toxicity, teratogenicity effects and approximately 30 % of the patients continue to have seizures with current AEDs therapy (Stefan and Wang, 2004).

Natural products from folk remedies have contributed significantly in the discovery of modern drugs and can be an alternative source for the discovery of AEDs with novel structures and better safety and efficacy profiles (Raza and Shaheen, 2001-2003). Now, various phytochemical and pharmacological studies have been carried out on these anticonvulsant plants. Herbal medicines are often considered to be a gentle and safe alternative to synthetic drugs. More than half of the medically important pharmaceutical drugs are either natural products or derivatives of natural products (Cragg and Newman, 1997; Nassiri-Asl and Shariati-Rad, 2008).

Nepeta bractaeta is an aromatic perennial herbaceous plant of Lamiaceae family; it is a brightly colored shrub or sub-shrub that ranges from 30-100 cm in height. Found in western temperate Himalayas from Garhwal to Kashmir at altitudes of 1800-2400 m. Leaves are ovate-obtuse. During summer the plant produces bunches of pink blue and more rarely white fragrant flowers (Bronlund et al., 2008; Kokkini and Vokou, 1989). A syrup prepared from the leaves, flowers and seeds is reported to be useful in cough and fever (Jamzad, 2001). It is also reported to be used in, boils and abscesses, cystitis, gastritis, fever, rheumatism, cold (Amini, 1997), cough, asthma, earache, insect bites, flatulence (Vohora, 1986; Amini, 1997). The major classes of chemical constituents present in this plant are carbohydrate, flavonols, phenolics, saponins (Kokate, 1994).

MATERIALS AND METHODS

Plant material

The flowers of *Nepeta bractaeta* were purchased from Shamsi Dawak-hana, Ballimaran, Delhi-110006, India. The authenticity and identity was confirmed on the basis of classical description in Unani literature at Department of Ilmul Advia F/O Medicine (u), Jamia Hamdard, New Delhi and modern Botanical information was established by matching with the specimens available at the National Institute of Science Communications. The wealth of India division, Dr. K. Krishnan Marg, New Delhi, 100012. Reference no. of drug sample NISCAIR/RHMD 1656/254. Voucher deposited in D/O Ilmul-Advia F/O Medicine, Jamia Hamdard, New Delhi-110062.

Preparation of extracts

The flower was dried at room temperature. After complete drying, it was powdered and passed through a 60 mesh sieve and stored in air tight container. Dried powdered drug was used to prepare the extract. 150 g of the powdered flower was taken and extracted with water and methanol in a soxhlet apparatus for 72 h. The extracts were evaporated to dryness in a rotary flash evaporator at a temperature not exceeding 60 °C. Preliminary phytochemical investigations of the extract which were conducted as per the procedures described by Kokate (1994) revealed the presence of flavonoids, saponins, carbohydrates, phenolic compounds and alkaloids (Bhat et al., 2012). The dried extracts (methanolic and aqueous) were preserved in desiccators until further use.

Animals

Swiss albino mice of male sex (24-34 g), supplied by the Central Animal House Facility of Jamia Hamdard, New Delhi (Registration no. 733/CPCSEA) were used. All animals were housed in cages in groups of 10, at 23-30 °C with a natural light-dark cycle. They had free access to standard pellet diet (Amrut Laboratory rat and mice feed, Navmaharashtra Chakanoil mills Ltd., Pune, India) and tap water. The study has been approved by the Ethics committee. Ethical norms were strictly followed during all experimental procedures.

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Assessment of anticonvulsant activity - Increasing Current Electroshock Seizures (ICES)

The ICES as proposed by Kitano and Usui (1996), was used to evaluate the anticonvulsant effect of the drugs. To start with a current of 2 mA electroshock to each mouse via ear electrodes as a single train of pulses (for 0.2 sec) was given with linearly increasing intensity of 2 mA/2 sec using an electroconvulsometer (INCO, Ambala, India). The current at which tonic Hind Limb Extension (THLE) occurred was recorded as the seizure threshold current (STC). When no tonic HLE was observed by a current of 30 mA, electroshock was terminated (Kitano and Usui, 1996; Shahid and Pillai, 2004).

Experimental design

The mice were divided into 6 groups (n=6) and treated with the respective test solutions as given below:

Group I (Control): DW (10 ml/kg, b.w, p.o)
Group II (Standard): Phenytoin (15 mg/kg, b.w, p.o)
Group III (Test-I): NBAE (190 mg/kg b.w, p.o)
Group IV (Test-II): NBAE (560 mg/kg b.w, p.o)
Group V (Test-III): NBME (70 mg/kg b.w, p.o)
Group VI (Test-IV): NBME (210 mg/kg b.w, p.o)

PTZ-induced seizures

PTZ at the dose of 60 mg/kg b.w. was injected i.p. to induce clonic tonic convulsions in mice. The test animals received methanolic and aqueous extracts orally and standard group received phenytoin (25 mg/kg b.w.) injected i.p. PTZ was injected i.p. 60 min after the administration of drugs. Occurrence of hind limb tonic extension (THLE) and duration of seizures were noted. If no HLTE occurred during the time limit, the animals were considered protected (Vohora and Pal, 2000).

Experimental design

The mice were divided into 6 groups (n=6) and treated with the respective test solutions as given here:

Group I (Control): DW (10 ml/kg b.w, p.o)
Group II (Standard): Sodium valproate (300 mg/kg, b.w, p.o)
Group III (Test-I): NBAE (190 mg/kg b.w, p.o)
Group IV (Test-II): NBAE (560 mg/kg b.w, p.o)
Group V (Test-III): NBME (70 mg/kg b.w, p.o)
Group VI (Test-IV): NBME (210 mg/kg b.w, p.o)

Statistical analysis

The data were analysed using One-way analysis of variance (ANOVA) followed by Dunnett's test. P values <0.05 were considered significant.

RESULTS

Effect of extracts on increasing current electroshock seizures (ICES)

Phenytoin (15 mg/kg, p.o) showed 66.7 % protection against ICES as evidenced by abolition of THLE. Methanolic extracts of *Nepeta bractaeta* at higher dose showed 33.3 % protection. Aqueous extract of *Nepeta bractaeta* at higher dose showed 16.7 % protection respectively (Table 1).

Nepeta bractaeta was not able to abolish tonic hind limb extension at all the doses used in this study but significantly reduced the duration of the tonic hind limb extension. Tonic hind limb extension is the universal feature of electroshock in mice, rats, rabbits, cats, monkeys and humans (Raza and Shaheen, 2001). Abolishing hind limb extension indicates the ability of testing material to inhibit or prevent seizure discharge within brainstem seizure substrate (Raza and Shaheen, 2001). All the currently available drugs that are clinically effective in the treatment of generalized tonic seizures (phenytoin, carbamazepine, phenobarbitone, valproate, lamotrigine, oxycarbamazepine, etc.) are effective in electroshock (McDonald and Althomsons, 1998).

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Table 1: Effects of extracts of *Nepeta bractaeta* in ICES induced convulsions

S.NO.	Group	Dose (mg/kg, p.o)	Seizure threshold current (mA)	% protection from seizure
1.	Control	10 ml/kg DW	13.5±0.71	0
2.	PHT	15	30.5±1.23**	66.7
3.	NBAE	190	15.5±0.42	0
4.	NBAE	560	22.16±0.65*	16.7
5.	NBME	70	17.33±0.61	0
6.	NBME	210	25.16±0.47**	33.3

Values are expressed as mean ± SE (n=6), *p<0.05, **p<0.01vs control

Table 2: Effects of extracts of *Nepeta bractaeta* in PTZ induced convulsions

S.NO.	Group	Dose (mg/kg, p.o.)	Latency of myoclonic jerks (s)	Latency of clonic generalized seizures (s)	% protection from seizure
1.	Control	10 ml/kg	65.5±3.72	228±1.08	0
2.	SVP	300	282.66±5.57**	1455.66±1.43**	50
3.	NBAE	190	70.83±6.55	247.3±18.14	0
4.	NBAE	560	83.16±3.65*	591.5±1.94*	0
5.	NBME	70	72.33±2.22	256.5±17.64	0
6.	NBME	210	186.66±6.05**	1035.5±1.47**	33.3

Values are expressed as mean ± SE (n=6), *p<0.05, **p<0.01vs control

Effect of extracts on pentylenetetrazole-induced seizures (PTZ)

The ability of an agent to prevent or delay the onset of tonic and tonic-clonic convulsion induced by PTZ in animals is an indication of anticonvulsant activity (Vellucci and Webster, 1984; Amabeoku and Green, 2007), (Table 2).

DISCUSSION

Mental, neurological and behavioral disorders are common to all countries and cause immense suffering. People with these disorders are often subjected to social isolation, poor quality of life, and increased mortality. These disorders are the cause of staggering economic and social costs. Habituation, dependence and the resulting potential for addiction are the greater disadvantages of the modern synthetic psychopharmacological agents. The abrupt discontinuation of long-term therapy with these drugs leads to serious withdrawal symptoms. Therefore, modern society is now cautiously discovering traditional herbal medicines, particularly those which have been proved to be effective in controlled studies and which in some cases demonstrated even better galenic properties than the conventional medicines. Unique opportunities for research exist in the field of CNS-active Indian medicinal plants (Weir, 1965).

Pharmacological evaluation of the anticonvulsant properties of the aqueous and methanolic extracts of *Nepeta bractaeta* against PTZ induced seizure revealed that both the methanolic and aqueous extracts exhibited statistically significant and dose dependent delay in the onset of seizure; both extracts showed significant and dose dependent reduction in the duration of THLE. However, the methanolic extract was more active against PTZ induced convulsion than the aqueous extract. On the other hand, both extracts showed significant and dose dependent reduction of the THLE induced by ICES. Tonic hind limb extension is the universal feature of electroshock in mice, rats, rabbits, cats, monkeys and

humans (Raza and Shaheen, 2001). Abolishing hind limb extension indicates the ability of testing material to inhibit or prevent seizure discharge within brainstem seizure substrate (Raza and Shaheen, 2001). All the currently available drugs that are clinically effective in the treatment of generalized tonic seizures (phenytoin, carbamazepine, phenobarbitone, valproate, lamotrigine, oxycarbamazepine, etc.) are effective in electroshock (McDonald and Althomsons, 1998). And in the PTZ model both the methanolic and aqueous extracts exhibited statistically significant and dose dependent delay in the onset of seizure. The ability of an agent to prevent or delay the onset of tonic and tonic-clonic convulsion induced by PTZ in animals is an indication of anticonvulsant activity (Vellucci and Webster, 1984; Amabeoku and Green, 2007). Anticonvulsant activity in PTZ-induced seizures identifies compounds that can raise seizure threshold in brain (Raza and Shaheen., 2001). AEDs effective in the therapy of generalized seizures of petit mal type (absence of myoclonic) i.e. phenobarbitone, sodium valproate, ethosuximide and benzodiazepines suppress PTZ-induced seizures in a dose-dependent manner (Loscher, 2002). Sodium valproate which was used in this study as a reference anticonvulsant agent showed significant activity by delaying the onset of myoclonic jerks and tonic convulsions and decreasing the frequency and duration of tonic convulsions.

According to De Sarro and Ferreri (2003) PTZ may be exerting its convulsant effect by inhibiting the activity of GABA at $GABA_A$ receptors. GABA is the major inhibitory neurotransmitter which is implicated in epilepsy. The enhancement and inhibition of the neurotransmission of GABA attenuates and enhances convulsion, respectively (Meldrum, 1982). Anticonvulsant agents such as sodium valproate, diazepam and phenobarbitone inhibit PTZ-induced seizure by enhancing the action of $GABA_A$-receptors, thus facilitating the GABA-mediated opening of chloride channels (Olsen, 1981; Malizia and Nutt, 1995; Acharya et al., 2008). Postsynaptic $GABA_A$-receptors are multi-unit complexes with binding sites for the endogenous ligand GABA, benzodiazepines, barbiturates and other ligands with a central chloride ion channel (Olsen and Leeb-Lundberg, 1981). Thus, the inhibition of PTZ-induced seizures by both the extracts in a dose dependent manner suggests that they may produce this effect by enhancing GABAergic neurotransmission although it is also possible that it could have done so by depressing glutamate-mediated excitation. Phytochemical screening of the plant showed that the plant contains alkaloids, flavonoids, sterols, glycosides and saponins, to which the anticonvulsant activity of the plant extracts may be attributed.

CONCLUSION

In conclusion, *Nepeta bractaeta* extracts may have potential anticonvulsant activity which may be due to the presence of certain active phytoconstituent. The anticonvulsant activity of *Nepeta bractaeta* may involve GABAergic transmission and glutaminergic transmission or sodium channel blockage. Further studies are, however, needed to isolate the active principle(s) of the plant and to enlighten the mechanism underlying its anticonvulsant effect. To this end we have to perform studies that will elucidate the exact mechanism of action of these active principle(s) before recommending these extracts for clinical application.

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