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Research Article

Maximal electroshock (MES) induced convulsions model for evaluating anti epileptic activity of new isatin derivative - N'- (7- Chloro- 2- Oxo -2, 3-Dihydro- 1H - Indol- 3-yl) Benzohydrazide

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ABSTRACT

Anticonvulsant activity was determined after per oral administration of the isatin derivative in albino wistar mice by maximal electroshock (MES) induced seizure method in rats. The acute anticonvulsant effect of the derivative is compared with the standard drug Valproic acid. Control, standard and isatin derivative was injected throughout the experimental period for seven days. On the 7th day, animals were subjected to MES induced convulsions by electroconvulsometer by the application of electrical current to the brain via corneal electrodes and observed their behavior for 30 minutes. Abolition of the hind limb tonic extensor spasm was recorded as a measurement of anticonvulsant activity. The result showed that the isatin derivative at the dose of 50mg/kg depicted significant anticonvulsant activity as compared to control, while the dose of 100mg/kg elicited significant activity comparable to standard drug in reducing the duration of tonic hindleg extension and in decreasing the percent mortality.

Keywords: Anticonvulsant activity; isatin derivative; MES induced seizures; Valproic acid

INTRODUCTION

Epilepsy is a major neurological disorder and affect all ages (Hauser WA*et al.,* 1991, 1993) up to 5% of the world population develops epilepsy in their lifetime. The current therapy of epilepsy is associated with side effects, dose-related and chronic toxicity, as well as teratogenic effects (Raza *et al.,* 2001) and approximately 30% of the patients continue to have seizures with current antiepileptic drugs therapy **(**Devinsky O, 1995; Holmes GL, 1993; Mattson RH, 1995; Smith MC *et al.,* 1991). The prevalence is higher in less developed countries because of higher incidence of antecedent factors such as brain infections, cranial and perinatal traumas and parasitic infections.

The incidence rate of epilepsy in the developed and developing countries approximately ranges from 25-50 and 30-115 per one lakh people per annum respectively (Kotsopoulos, 2002). In India, studies have reported the prevalence rate of epilepsy varying from 1710 to 9780 cases per million populations (Walters *et al.,* 2010). It is estimated that there are about 2.73 million women with epilepsy and 52% of them are in the reproductive (15-49 years) age group. Women experience more psychosocial problems and burden related to epilepsy than men (Delgado *et al.,* 1983). The mortality rate of epilepsy patients is high in South Africa. Epilepsy is a collective term used for a group of chronic seizure disorders having in common, sudden and transient episodes (seizures) of loss or disturbance of consciousness, usually but not always with characteristic body movements (convulsions) and sometimes with autonomic hyperactivity. A seizure is due to abnormal discharge of some neurons within the brain.

Epilepsy can be classified into two major groups.

1. Tonic clonic seizures/Grandmal/Major epilepsy: This is characterized by sudden loss of consciousness, followed by generalized tonic, followed by clonic convulsive movements. This is followed by a period of headache, drowsiness, confusion and sleep. The attack may be accompanied by tongue biting, frothing from the mouth and in continence (French JA, 2008).

2. Petit-mal/Absence seizure: It consists of sudden cessation of on-going conscious activity without convulsive movement and without loss of postural control. Antiepileptic drugs may have a stabilizing in--- fluence on neuronal membrane; prevent detonation of normal brain cells by the focal discharge, these drugs act only on those neurons which are firing repeatedly. Some drugs reduce a low threshold Ca2+ current and abolish absence seizures whereas some drugs increase GABA activity at the synapse causing neuronal inhibi-

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tion hence ant seizure effect. Thus, there is still a great demand for new agents, which can treat all types of seizures with greater efficacy, negligible or reduced side effects and devoid of unfavourable drug interac---tions.

Isatin has an indole moiety which exhibits a variety of biological activities like anticonvulsant (Kaur H *et al.,* 2010), anxiolytic (Geronikaki A *et al.,* 2005), anticancer (Gudipati R *et al.,* 2011), anti-inflammatory, analgesic, antipyretic (Venkateshwaralu E*et al.,* 2012), antifungal (Rodríguez-Argüelles M.C *et al.,* 2007), antiviral (Jiang T *et al.,* 2006), anti-angiogenic (Maskell L *et al.,* 2007) and anti-parkinsonian effects (IgoshevaN *et al.,* 2004). Literature survey showed that isatin molecule has posi--tive anticonvulsant effects during its initial screening in the Maximal Electroshock (MES) Induced seizures model in wistar rats. (VenkateshwarluEggadi *et al.,* 2013; Pandeya SN *et al.,* 2002; Popp FD., 1984; Hewa---wasam P *et al.,* 2002; Jursic BS *et al.,* 2002).

MES stimulation can be applied through Trans corneal or Tran's auricular (ear-clip) electrodes from an elec--troshock apparatus at an intensity sufficient to elicit tonic hind limb extension (HLE) in 100% of the control animals. A seizure is generally considered to be maxi--mal if increments in current intensity do not alter the pattern or the duration of its various components (Tedeschi, D.H *et al.,* 1956).

The conventional MES test has standardized parame---ters such as a 50-mA (mice) or 150-mA (rats) fixed cur--rent, a 50-60–Hz pulse frequency, a 0.6-ms pulse width and a 0.2-s stimulus duration (Löscher, W *et al.,* 1988; Mareš, P *et al.,* 2006; Löscher, W *et al.,* 1991; Brown---ing, R.A *et al.,* 1985; Woodbury, L.A *et al.,* 1952; Mody, I *et al.,* 1997). Corneal electrodes are mainly used brief--ly, following stimulus application an immediate severe tonic seizure with maximal extension of the anterior and posterior legs occurs and the body becomes stiff--ened; at the end of this tonic phase, which usually lasts for 10-15 s, clonic seizures start, characterized by pad--dling movements of the hind limbs and shaking of the body; 20-30 s later, the animal is usually able to come back to an upright position and start moving around, apparently recovering its normal behavior (André, V *et al.,* 2002).

The test will be considered positive if the animal exhib---its tonic extensor seizure with rearward HLE more than 90° from the body and sustained for more than 3 s fol--lowing 10 s after stimulation (Dalby, N.O *et al.,* 1997; Welty, D.F *et al.,* 1993). The tonic HLE finishes at the time of onset of generalized clonus (Tedeschi, D.H1956). Therefore, the present study was carried out to evaluate the anticonvulsant profile of isatin deriva---tive in the models of electroconvulsions (MES) and to co-relate with the available literature and as the deriv--ative is found to be non-genotoxic (Nirmala *et al.,* 2012).

MATERIALS AND METHODS

Materials

The test compound isatin derivative in a dose of 50 and 100 mg/kg in 10% DMSO, standard drug Valproic acid (SD fine chemicals) 200 mg/kg were given by per oral route. Maintenance of Animals: (experiments were conducted at MRIPS institution with 1662/PO/a/08/CPCSEA). Albino Wistar mice were pur---chased from Mahaveer enterprises, Hyderabad. The animals were acclimatized to the conditions by main---taining them at the experimental conditions for about 7 days prior to dosing. Cage number and individuals marking on the tail to identify the animals. The animals were housed six per cage of same sex in polypropylene cages with bedding of paddy. Pellet chews to feed standard diet under good management conditions, and water *ad libitum* was provided to the animals. The temperature 20-25°c and 12 hours each at dark and light cycle was maintained.

Acute Toxicity Studies

The procedure was followed by using OECD guidelines (Organization of Economic Cooperation and Develop---ment) 423 (Acute Toxic Class Method). The acute toxic class method is a step-wise procedure with three ani--mals of single sex per step. Depending on the mortality and/or morbidity status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance. This procedure results in the use of a minimum number of animals while allowing for an acceptable data-based scientific conclusion. The method use (acute oral toxicity -420 fixed dose procedure) fixed doses (5, 50, 300, 2000 mg/kg b.wt) and the results allow a substance to be ranked and classified according to the Globally Harmo---nised System (GHS) for the classification of the chemi--cal which causes acute toxicity.

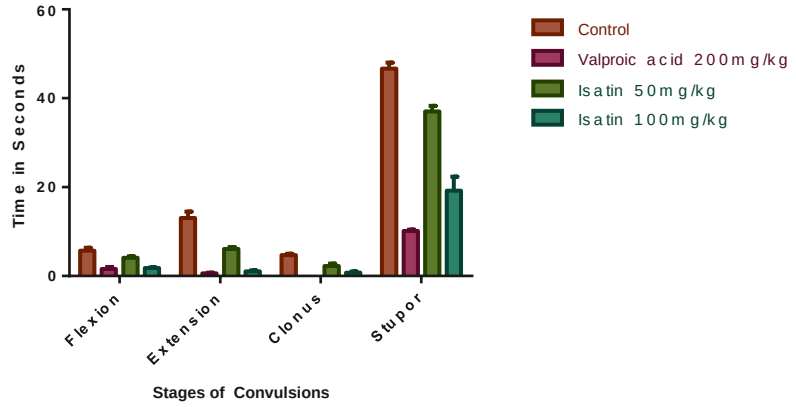
Six mice weighing between 18-22 gms were used for toxicity. The starting dose level of Isatin derivative of 50 mg/kg b.wt orally, as most of the crude extracts possesses LD50 value more than 4000 mg/kg b.wt per oral dose, was administered to the mice, which were fasted overnight with water *ad libitum*, food was with---held for a further 3-4 hours after administration of drugs and observed for another 14 days.

Body weight of the mice before and after treatments were noted and any changes in skin and fur, eyes and mucous membranes and also autonomic, central nerv---ous systems, somatomotor activity and behavior pat---tern were observed and also signs of tremors, convul--sions, salivation, diarrhea, lethargy, sleep and coma were noted. The onset of toxicity and signs of toxicity was also to be noted (OECD 423). The extract of isatin derivative was devoid of mortality of animals at the dose of 200mg/kg and hence >200mg/kg was taken as LD 50 cut off value.

Table 1: Effect of Isatin derivatives on Maximal electroconvulsive shock(MES) – induced seizures in mice

Group	Design of Treatment	Flexion (Seconds)	Extension (Seconds)	Clonus (Seconds)	Stupor (Seconds)	Recovery (Seconds)	% Protection
I	Control(DMSO) 50mg/kg, *p.o*	5.66± 0.66	13±1.46	4.66± 0.33	46.66± 1.35	159.08± 4.03	40
II	Valproic Acid 200mg/kg,.*p.o*	1.56± 0.44**	0.56± 0.14**	0	10.11± 0.29**	89.76± 2.59	100
III	Isatin 50mg/kg, *p.o*	4.08± 0.32^{ns}	6.08± 0.37**	2.23± 0.55**	37± 1.291**	156.33± 7.57	81.47
IV	Isatin 100mg/kg, *p.o*	1.76± 0.17**	1.05± 0.19**	0.71± 0.27**	19.16± 3.17**	97.5± 2.69	100

Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns – non significant (One-way ANOVA way ANOVA followed by Dunnet's test). Experimental groups values are compared with control group and stand---ard. P.o.: per oral route of administration.



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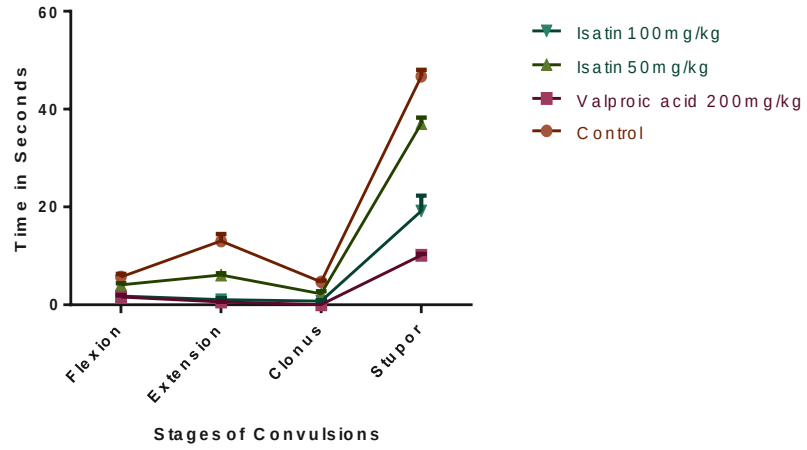

Figure 2: Effect of Isatin derivatives on Maximal electroconvulsive shock(MES) – induced seizures in mice

Values are expressed as Mean ± S.E.M; n=6, *p<0.05. **p<0.01, ns – non significant (One-way ANOVA way ANOVA followed by Dunnet's test). Experimental groups values are compared with control group and stand---ard group. P.o.: per oral route of administration.

Methods for Antiepileptic activity

Experimental design (Bhat *et al.,* 2012)

Albino wistar mice of either sex (18-22g) were used in the present study.

Animals were provided with standard diet and water ad libitum.

The mice were divided in to four groups containing 6 each.

Group I- Control, administered vehicle orally 10% DMSO).

Group II- Administered standard drug at a dose of 200mg/kg b.wtorally.

Group III- Administered test drug (Isatin) at a dose of 50mg/kg b.wt per orally.

Group IV- Administered test drug (Isatin) at a dose of 100mg/kg b.wt per orally**.**

Preparation of test drug

Test drug was suspended in 10%DMSO and each mice received a daily 1ml as suspension at a dose of 50mg/kg and 100mg/kg body weight, per orally throughout the experimental period**.**

Maximal electroshock (MES) induced seizures

Albino wistar mice of either sex 18 to 22gm were divided in to four groups of six animals each. The treatment was received for the four groups perorally for seven days. On the 7th day, seizures are induced to all the groups by using an Electro convulsiometer. Maximal electroshock seizures were elicited by a 60 Hz alternating current of 50 mA intensity for 0.2 sec. A drop of electrolyte solution (0.9% NaCl) with lignocaine was applied to the corneal electrodes prior to application to the mice. This increases the contact and reduces the incidence of fatalities.

The duration of various phases of epilepsy such as Phase of tonic limb flexion, Phase of tonic limb extension, Phase of clonic convulsions, Stupor and Recovery or death were observed. The characteristics of electroshock seizures are a tonic limb flexion of 1 to 2 seconds, followed by a tonic limb extension of roughly 10 to 12 second, and finally generalized clonic movements for 12 seconds. The total duration of the seizure is approximately 25 seconds.

A substance is known to possess anticonvulsant property if it reduces or abolishes the extensor phase of MES induced convulsion spread discharge through neural tissue**.** The percentage protection of the derivative from seizures was estimated by observing the number of animals showing abolition of hind leg tonic extension does not exceed a 90°angle with the plane of the body (Balkrishnan S *et al.,* 1998) relative to control.

RESULTS

During the experiment period of seven days, the duration of tonic hindleg extension in mice treated with vehicle was 13±1.46 seconds. The Isatin doses of 50mg/kg and 100mg/kg protected animals from seizures and significantly ($p<0.01$) reduced the duration of tonic hindleg extension. The standard drug valproic acid treated animals shown 100% protection against MES induced seizures and whereas isatin 50mg/kg and 100mg/kg have shown 81.47 and 100% protection respectively.

DISCUSSION AND CONCLUSION

The results of the present study demonstrate that Isatin derivative has shown significant anticonvulsant activity and elicited an effective protection against MES seizures in wistar rats. This effect was very much significant with isatin derivative at the dose of 50mg/kg as compared to control, while the dose of 100mg/kg has depicted significant anticonvulsant activity comparable with reference drug in reducing the duration of tonic hindleg extension and in decreasing the percent mortality.

The Maximal Electroshock-induced Seizure test (MES) is probably the test-validated preclinical test that predicts drugs effective against generalized seizures of the tonic–clonic (grand mal) type. This model is based on observation of the stimulation by repeated electrical pulses induced in different neuronal structures one characteristic standard of epileptic activity. It has often been stated that antiepileptic drugs that block MES-induced tonic extension phase act by blocking seizure spread (Castel-Branco, M.M *et al.,* 2009). All the currently available drugs which are clinically effective in the treatment of generalized tonic-clonic convulsions (e.g. phenytoin, carbamazepine, valproicacid, feblamate and lamotrigine) act by inhibiting volt--- age---dependent Na+ channels, or by drugs that block glutaminergic excitation mediated by the N- methyl---D---Aspartate (NMDA) receptor such as feblamateare effective in the MES test (McDonald RL *et al.,* 1993). Since, Isatin derivative significantly inhibited generalized tonic-clonic seizures in MES test, it suggests the presence of anticonvulsant property and might be either inhibiting voltage-dependent Na+ channels or act as a NMDA antagonist. In view of the promising results, the isatin derivative may be chosen for further modification aimed at improving the anticonvulsant activity.

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