# ANTICONVULSANT ACTION OF INDOLIN-2,3-DIONE (ISATIN)

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A detailed study of the anticonvulsant spectrum of indolin-2,3-dione revealed that it possessed anticonvulsant activity in Maximum Electro Shock Seizure (MES) and Hyponatremic Electro Shock Seizure (HET) tests in rats with $ED_{50}$ of 83.7 and 183.3 mg/kg. It was found to be ineffective in Minimal Electro Shock Seizure Threshhold (MET), psychomotor and chemoshock tests. Its considerably higher Protective Index with almost no side effects warrants its clinical trials.

In a previous communication (Sareen *et al.*, 1962) it was reported that indolin-2,3-dione (isatin) possessed a potent anticonvulsant effect in supramaximal electro-shock seizures (MES) test and that this activity was found to depend upon an intact activated 3-keto group. In view of this encouraging observation it was decided to pursue this problem further and to study the spectrum of its anticonvulsant action in detail using a battery of tests.

## METHODS

Adult albino rats of either sex weighing 75 to 100 g were used in all assay procedures except in psychomotor seizure test in which mice of 20 to 30 g weight were employed. They were maintained on adequate diet and allowed free access to food and water except during the time of testing. Drugs, suspended in 5 per cent gum acacia, were administered orally. All the doses were given in a volume of 1 ml/100 g body weight and the test was performed at the time of the peak activity of the drugs which was found to be two hours in the case of the test drug and half an hour in that of diphenylhydantoin sodium (reference standard), as determined by the method of Swinyard *et al.*, (1952). All $ED_{50}$ and $TD_{50}$[2] determinations were based on assays of the drugs at the established time. Groups of 10 animals were tested with various doses of the drug until atleast four points were established between the limits of complete protection and no protection. The results obtained were plotted and regression lines fitted to the plotted points by the method of maximum likelihood. The goodness of fit was tested by $\chi^2$ test of homogeneity (Finney, 1952) at 95 per cent level. The $ED_{50}$ and their ranges were calculated by

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2 Effective dose and toxic dose in 50 per cent of the animals.

the method of Litchfield and Wilcoxon (1949). The details of the tests employed are given below.

*Maximal electro-shock seizures (MES) Test.*—The test was performed as already described in a previous communication (Sareen *et al.*, 1962).

*Minimal electro shock seizure threshold (MET) test.*—The rats were given a single shock of 18 to 24 mA depending on their weight. This was repeated every 48 hr until the threshold had been established and did not vary by more than 0.5 mA in successive determinations. At least 7 sec of facial, lower jaw or forelimb clonus without loss of upright posture was taken as the threshold seizure. After the stabilisation of the threshold, the animals were divided into 3 groups of 10 rats each and were given 50, 100 and 300 mg/kg of the test drug. After two hours they were stimulated with a current 20 per cent above the predetermined individual threshold. Complete protection from a minimal seizure was the end point of the test.

*Hyponatremic electro-shock seizure threshold (HET) test.*—The minimal electro-shock seizure threshold was determined (vide supra) and 10 ml of a freshly prepared isomolar (5.5 per cent) glucose solution per 100 g body weight was then injected intraperitoneally. In rats the hyponatremic state reached a peak after 4 hrs (Swinyard and Goodman, 1946). The drug was administered at such a time that the peak effect coincided with the peak hyponatremia. A single test seizure was then given having a current intensity of 66 per cent of the normal electro-shock seizure threshold for each animal. This represented a 50 per cent increase above the experimentally lowered hydration threshold and absence of even a minimal seizure was taken as the end point.

*Psychomotor seizure threshold test.*—The method of Everett and Richard (1952) was employed. The test was carried out in 3 groups of 10 mice each using 100, 200 and 400 mg/kg doses of the drug respectively. The seizures were induced with 6/sec shocks for 3 sec at 125 volts pulse width 1 m/sec (Grass stimulator). They were characterised by stunning and automatisms lasting for 15 to 20 secs. The end point was the complete prevention of the psychomotor seizures.

*Chemoshock seizures.*—The seizures were produced in rats by the subcutaneous injection of pentamethylene-tetrazol (80 mg/kg), strychnine sulphate (1.5 mg/kg) or picrotoxin (7.5 mg/kg). These doses produced twitching, extensor tonic spasm and death within 30 mins in the control animals. The tests were performed in groups of 10 rats each and for each chemoshock seizure test, two doses of the drug (200 and 400 mg/kg) were administered orally,

two hours prior to the injection of the particular convulsive agent. Prevention of the extensor tonic spasm and death were considered as the protective end point.

*Neurotoxicity studies.*—The method of Swinyard *et al.*, (1952) was adopted for the determination of the end point of acute neural toxicity using different doses of the drug and of diphenylhydantoin sodium. Each dose was given to a group of 10 rats and the neural toxic dose in 50 per cent of the animals ($TD_{50}$) was calculated by the same method as used for $ED_{50}$.

*Studies on blood pressure and respiration in anaesthetised animals.*—The effect was studied on 3 dogs (8 to 10 kg) and 2 cats (2 0 to 2.2 kg) anaesthetised with sodium pentobarbital (25 mg/kg intravenously in dogs and 30 mg/kg intraperitonealy in cats). Blood pressure was recorded from the left carotid artery by a mercury manometer on a smoked drum. The trachea was cannulated and connected to a Marey's tambour for recording the respiratory excursions.

## RESULTS AND DISCUSSION

*Anticonvulsant activity.*—$ED_{50}$, $TD_{50}$ and P.I. (Protective Index) of indolin-2,3-dione as compared to that of diphenylhydantoin sodium, both in MES and HET tests, are recorded in Tables I and II. It is evident that the $ED_{50}$ of indolin-2,3-dione in both the MES and the HET tests (83.7 and 427.8 mg/kg) is higher than that of diphenylhydantoin sodium (25.2 and 183.3 mg/kg). The Protective Index of the drug (15.5 in MES and 3.04 in HET tests), however, was higher than that of diphenylhydantoin sodium (12.1 in MES and 1.77 in HET tests). Although the test drug was somewhat less potent than diphenylhydantoin sodium, it had a higher protective index than the latter[1].

The drug was found to be ineffective against minimal electro-shock seizure (MET) test in doses of 100 mg/kg and 300 mg/kg which were well above the calculated $ED_{50}$ for the MES test. Likewise the test drug did not protect against the psychomotor seizures even with the maximum dose of 400 mg/kg which was nearly five times the $ED_{50}$ for the MES test. In doses of 200 mg/kg and 400 mg/kg it also did not protect against the convulsions induced by pentamethylene-tetrazol, strychnine and picrotoxin.

*Acute toxicity.*—Intravenous administration of 800 mg/kg of the drug to a group of 10 rats did not produce any mortality. Oral doses of 5 and 10 g/kg given to groups of 5 rats each produced a considerable reduction of motor activity and muscular flaccidity and two animals died from each group within 24 hrs.

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## TABLE I

*Anticonvulsant activity of indolin-2, 3-dione and diphanylhydentoin sodium in MES and HET tests*

| Test | Drug | No. of animals | Dose (mg/kg) | Response % protection | $Ed_{50}$ (mg/kg) Graph | Calculated | Fiducial limits[1] | P.I. ($TD_{50}/ED_{50}$) |
|---|---|---|---|---|---|---|---|---|
| MES | Indolin-2, 3 dione | 10 | 25 | 20 | 85.1 | 83.7 | 46.76 to 146.39 | 15.5 |
| | | ,, | 50 | 30 | | | | |
| | | ,, | 100 | 50 | | | | |
| | | ,, | 150 | 70 | | | | |
| | | ,, | 200 | 80 | | | | |
| | Diphenylhydantoin Sodium | 10 | 10 | 20 | 25.7 | 25.24 | 14.79 to 43.39 | 12.1 |
| | | ,, | 20 | 30 | | | | |
| | | ,, | 30 | 60 | | | | |
| | | ,, | 50 | 80 | | | | |
| HET | Indolin-2,3-dione | 10 | 200 | 20 | 426.6 | 427.8 | 306.6 to 630.9 | 3.04 |
| | | ,, | 300 | 30 | | | | |
| | | ,, | 500 | 50 | | | | |
| | | ,, | 700 | 80 | | | | |
| | Diphenylhydantoin Sodium | 10 | 100 | 30 | 177.8 | 183.3 | 123.5 to 243.1 | 1.77 |
| | | ,, | 150 | 40 | | | | |
| | | ,, | 200 | 50 | | | | |
| | | ,, | 300 | 80 | | | | |

1 At 95 per cent level

## TABLE II

*Neurotoxicity of indolin-2, 3-dione and diphenylhydantoin sodium*

| Drug | No. of animals | Dose (mg/kg) | % showing neuro-toxicity | $TD_{50}$ (mg/kg) | Fiducial limits[1] |
|---|---|---|---|---|---|
| Indolin-2, 3-dione | 10 | 200 | 0 | 1303.1 | 898.67 to 2451.32 |
| | ,, | 400 | 10 | | |
| | ,, | 1000 | 30 | | |
| | ,, | 1250 | 40 | | |
| | ,, | 2000 | 80 | | |
| Diphenylhydantoin | 10 | 50 | 0 | 324.5 | 326.82 to 377.84 |
| | ,, | 80 | 10 | | |
| | ,, | 200 | 20 | | |
| | ,, | 300 | 40 | | |
| | ,, | 400 | 60 | | |
| | ,, | 500 | 80 | | |

1 At 95 per cent level

The neurotoxicity studies reported in Table II showed that indolin-2,3-dione had a greater safety margin as compared to that of diphenylhydantoin sodium. $TD_{50}$ of the test drug was 1.3 g/kg whereas that of diphenylhydantoin sodium was only 324.5 mg/kg, i.e., the test drug was definitely safer than the reference standard.

*Blood pressure and respiration.*—Intravenous administration of the drug in doses of 10 mg/kg and 20 mg/kg did not produce any effect on the blood pressure and the respiration both in the dogs and the cats. With intravenous doses of 50 to 100 mg/kg however, there was a slight and transient fall of blood pressure in these animals. The respiration was not affected in these species of animals even with the maximum intravenous dose of 10 mg/kg.

The above findings indicate that indolin-2,3-dione is a potent and non-toxic anticonvulsant against both the MES and the HET seizure tests in rats. Although it was somewhat less potent than diphenylhydantoin sodium its higher protective index coupled with that fact that it produced practically no side effects, warrants its clinical trials.

## REFERENCES

Everett, G.M. and Richard, R.K. (1952). *J. Pharmacol. Exp. Therap.*, **106**, 303.

Finney, D.J. (1952). *Probit Analysis*, p. 48. University Press, Cambridge.

Litchfield, J.T. (Jr.) and Wilcoxon, F. (1949). *J. Pharmacol. Exp. Therap.*, **96**, 99.

Sareen, K., Kohli, R.P., Amma, M.K.P. and Gujral, M.L. (1962). *Ind. J. Physiol. and Pharmacol.*, **6**, 87.

Swinyard, E.A. and Goodman, L.S. (1946). *Federation Proceed.*, **5**, 205.

Swinyard, E.A., Brown, W.C. and Goodman, L.S. (1952). *J. Pharmacol. Exp. Therap.*, **106**, 319.