POTENTIATION OF MORPHINE ANALGESIA WITH ISATIN (INDOLIN—2,3 DIONE)

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Isatin (Indolin 2-3-Dione) is known to have anti-convulsant effect in supra-maximal electro-shock seizures (MES) test and this activity is found to depend on an intact activated 3 *Keto* group (10). While screening this drug for its pharmacological actions in this department, our work was to find out whether Isatin had any analgesic properties and further was there any potentiation with morphine in sub-analgesic doses.

An analgesic drug would be expected to diminish the perception of painful stimuli when applied to skin. These painful stimuli can be applied either as mechanical, thermal or electrical; each type having its own advantages and disadvantages. Thus there have been techniques (5) where pain stimulus is applied in the form of heat. But the response obtained such as skin twitch or flicking of tail involves spinal cord (1, 6, 7), where as in man the pain is mediated through thalamus and cortex. Further conditioned reflexes and local temperature of tail also play some role in modifying the effect of thermal stimulus (8).

Haffner's technique (4) where a clip is attached to tail of rat and reaction of the animal such as attempt to remove the clip is taken as response, but here again the conditioned reflexes come in the way and the reflex mechanism on which it is based is mediated through higher centres. Other ways are by the administration of an electric stimulus with squeak as response (3) or by applying pressure on tail which is measured by mercury manometer and obtaining squeak as response (2).

MATERIALS AND METHODS

Our technique consisted of testing the drug by heat-analgesiometer (Techno electronics, Lucknow) in albino rats of same sex with a weight in the range of 94 to 210 grammes. The animals were maintained on same diet *adibidum* and water except during testing. For observing the analgesic effects the rat was kept in a rat holder and its tail kept on an enclosure over an electrically heated tantalum wire which transmitted radiant heat to the tail. The enclosure was continuously kept cool by passing water through it in order that it does not hamper with the correct readings.

The current in the analgesiometer was maintained at a uniform rate throughout such that the withdrawal of tail which was taken as end point, occured within 12 to 15 seconds. This long interval was given in order to protect the tail from burning, but the range was reduced to 5 seconds in case of morphine where maximum reading was taken at 10 seconds. The tail of the rat was not blackened.

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The drug (Isatin) being insoluble in water, acids, alkalies, alcohol but soluble in acetone in lower concentrations and in propylene glycol in higher concentrations (after the solution was heated)., was given intraperitonealy as,

0.1%	= 4 *mg/kg*	Body	weight.
0.2%	= 8 *mg/kg*	,,	,,
0.4%	= 16 *mg/kg*	,,	,,
0.8%	= 32 *mg/kg*	,,	,,
and 1.0%	= 46 *mg/kg*	,,	,,

Acetone being quite a toxic substance, sufficient dilutions were made so that the effect of acetone as such was negligible.

An initial reading for withdrawal of tail was taken each day before the drug was given and readings taken after 15, 30 and 45 minutes after the administration of the drug. A rest of 24 hours was given before taking each concentration of the drug for test.

To test the potentiation, Isatin was given with the sub-analgesic dose of morphine (the dose which just fell short of producing analgesia), and the dose was found out to be 3.5 *mg/kg* body weight of morphine sulphate (Zandu Pharmaceutical, India). In order to find potentiation of drug, morphine was given in the above dosage followed by Isatin after 15 minutes and readings taken after 15, 30 and 45 minutes. A rest period of at least three days was given before testing morphine with other concentrations of Isatin. The effect of drug was compared with a control where instead of drug, distilled water in the same quantity was given intraperitonealy and the readings taken after 15, 30 and 45 minutes.

RESULTS

Table I, gives the average timings of withdrawal of tail at an interval of 15 minutes each for different doses of Isatin and control. The doses of Isatin as mentioned before were: .1%, .2%, .4%, and .8%, (0.4 cc/100 *gms* of rat).

TABLE I

Average timings of withdrawal af tail.

Timings	15 mts.	30 mts.	45 mts.	Overall average
Control	7.62	8.82	8.66	8.36
.1% Isatin	8.85	9.58	8.39	8.94
.2% Isatin	11.48	11.9	11.4	11.59
.4% Isatin	10.25	9.75	8.43	9.47
.8% Isatin	7.46	7.73	7.61	7.6

The variation among the means was tested by analysis of variance technique. Table II. gives the respective sums of squares and the mean squares as also the probability of occurrence of each.

TABLE II

Analysis of variance for the data in Table 1

Source	d.f.	Sum of sqs.	mean sq.	F.	P.
Between successive readings.	2	0.62	0.31	0.57	>.05
Between treatments: (a) Check v/s treatments	1	2.02	2.02	3.7	>.05
(b) Between different doses of Isatin	3	24.53	8.17	15.1	<.01
Error	8	4.32	0.54		
Total	14	31.49			

It follows from Table II, that Isatin is no better than control in delaying the withdrawal of tail. But the significance of the difference between the four doses of Isatin shows that certain doses are better than others. To find out this difference Tukey's test (11) was applied to the data. Table III. gives the respective means due to various concentrations of Isatin arranged in a descending order of magnitude.

TABLE III

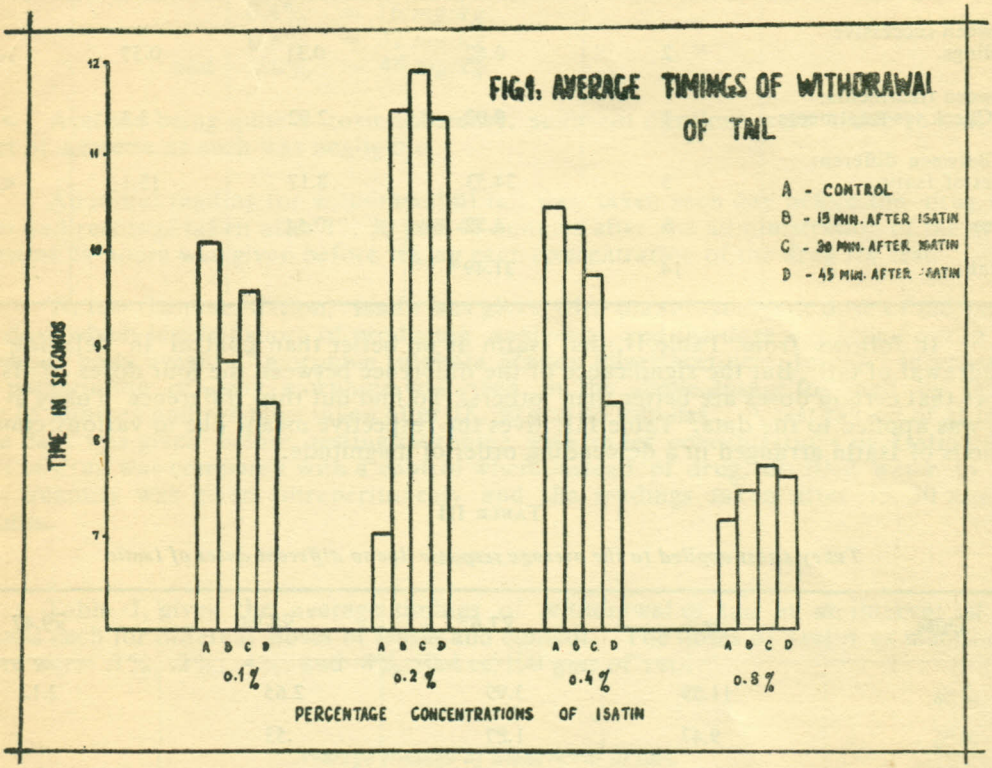
Tukey's test applied to the average response due to different doses of Isatin

Dose	x	$\bar{x}$7.6	$\bar{x}$8.94	$\bar{x}$9.47
.2 %	11.59	3.99	2.65	2.12
.4 %	9.47	1.87	.53	
.1 %	8.94	1.34		
.8 %	7.6			

All the differences in the first row of the Table are greater than D=Q. $S_{\bar{x}}=1.9$ (since $S_{\bar{x}}=(.54)^2/3=.42$ and Q (8,4)=4.53), which shows that .2% concentration of Isatin is responsible for the significance of the different treatment effects. Since neither the higher nor the lower doses of Isatin have made any analgesic effect it can be said that a difference of 4 seconds though statistically significant cannot be clinically termed as effective analgesia. Fig. 1. gives the initial and after Isatin timings of the withdrawal of tail.

For the testing of potentiation of morphine analgesia with Isatin, a 4+1 point cross-over test was arranged. One batch was given morphine 3.5 *mg/kg* body weight while the other batch was given morphine in the same dose followed by Isatin at specified concentrations. The two treatments were designated as S and T respectively. The initial

selection of animals to one or other batch was on random basis by tossing a coin. Next time the drugs were inter-changed on the two batches and the procedure continued like that till three doses of T were given. The dose of S however, was the same each time viz. 3.5 *mg/kg* body weight.



For the quantal response which followed in the form of analgesia or no analgesia, the difference in the two responses due to S and different levels of T was tested statistically. Assuming a binomial distribution to hold good, the probability of occurrence of the analgesia due to T was calculated on the basis of true probability due to S. Respective probabilities as also the degree of anaglesia due to S and T are given in Table IV.

Since all the probabilities of occurrence for the analgesia due to T in column 4 are less than .05, it follows that the difference between the two proportions of analgesia are significantly different. Hence it is justified to say that Isatin given with Morphine has a potentiation effect for the latter.

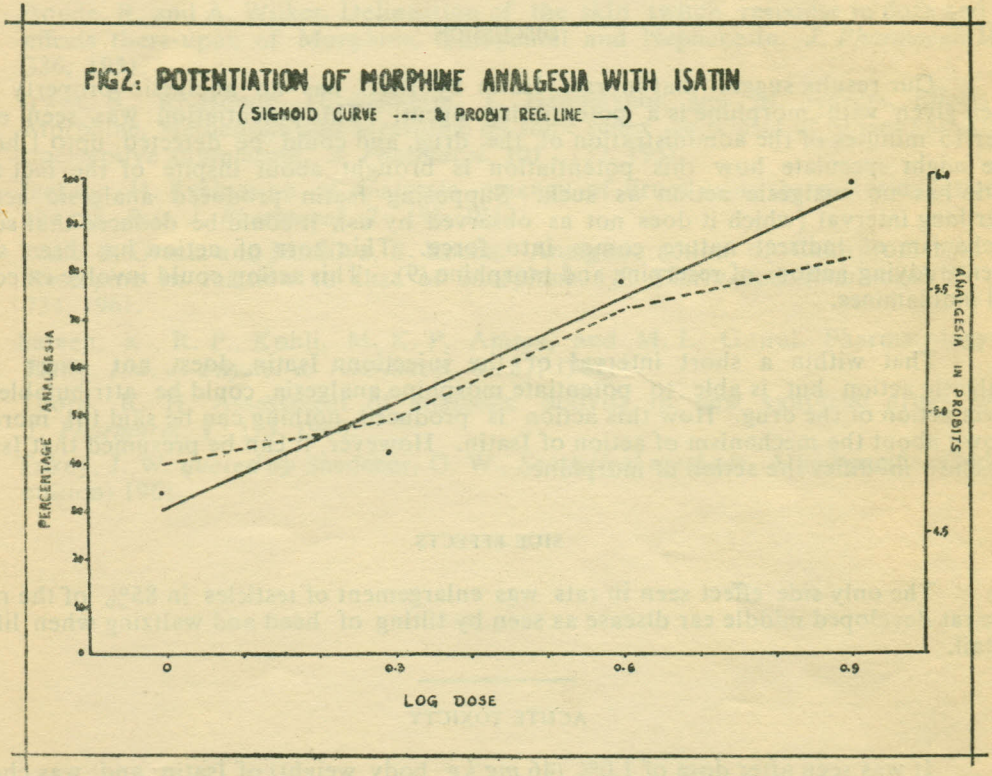
The above data has been plotted in Fig. 2. The same was, however, converted into probits for the calculation of ED_{50} for the drug. T. The equation of the probit regression line was found to be $Y=1.42X+4.67$, which gives the ED_{50} as. 17% of Isatin to

TABLE IV

Degree of analgesia and the prabability of occurrence due to S and T

Doses of T.	Degree of analgesia (proportion). T.	corresponding S.	Probability of occurrence for analgesia due to T.
0.1%	3/5	1/6	0.031
0.2%	4/6	1/5	0.0169
0.4%	5/6	1/6	0.0005
0.8%	5/6	1/5	0.0016

be given with 3.5 *mg/kg* body weight of morphine. It may however be added that for the calculation of the mediun effective dose as also for the plotting of the curve in Fig. 2,



different response was used in the vertical scale. The response used here was the average analgesia observed after three readings of 15, 30 and 45 mintutes (Table V). Contrary to

this, the response or the degree of analgesia in Table IV is the maximum analgesia recorded during the three readings.

TABLE V

Average analgesia for different levels of T.

Level of T	Av. Analgesia (%) observed over three readings.
0.1%	40.0
0.2%	49.6
0.4%	72.0
0.8%	83.1

DISCUSSION

Our results suggest that in rats Isatin by itself has no analgesic property but when given with morphine is a potentiating agent. This potentiation was seen even after 15 minutes of the administration of the drug, and could be detected upto 1 hour. One might speculate how this potentiation is brought about inspite of the fact that Isatin has no analgesic action as such. Supposing Isatin produced analgesic action after long interval (which it does not as observed by us), it could be deduced that some mechanism of indirect nature comes into force. This sort of action has been seen when studying actions of reserpine and morphine (9). This action could involve catechol and indolamines.

That within a short interval of the injection, Isatin does not exert any analgesic action but is able to potentiate morphine analgesia, could be attributable to direct action of the drug. How this action is produced, nothing can be said till more is known about the mechanism of action of Isatin. However, it can be presumed that Isatin somehow modifies the action of morphine.

SIDE EFFECTS

The only side effect seen in rats was enlargement of testicles in 85% of the rats. One rat developed middle ear disease as seen by tilting of head and waltzing when lifted by tail.

ACUTE TOXICTY

It was seen after dose of 1.0% (46 *mg/kg* body weight) of Isatin and was characterised by drowziness, difficulty in movements, partial flacid paralysis of the limbs and stertuous breathing. However the animals did not die of it and recovered after 10 to 12 hours.

SUMMARY

Isatin (Indolin 2, 3-Dione) an anti-convulsant has no analgesic action as such but potentiates the analgesia produced by morphine in albino rats.

ACKNOWLEDGEMENTS

Our thanks are due to Dr. J. D. Sharma, M. D. for his guidance and to Dr. M. L. Gujral MRCP for making the drug available.

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