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ANTICONVULSANT EFFECT OF PHENYTOIN IN ALBINO MICE

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ABSTRACT

Introduction: The electric convulsiometer can also produce generalized tonic-clonic seizure in humans and animals in easy manner. Many drugs have been tested which have protective effect against G.T.C.S. induced by electric convulsiometer. With the above background this study was conducted to study the profile of anticonvulsant action of phenytoin.

Material & Methods: The present study has been carried out in the Department of Pharmacology, Darbhanga Medical College, Laheriasarai. Induction of seizure with the help of electro-convulsiometer in albino mice was done followed by the observation of the anti-convulsant activity of phenytoin sodium was made.

Results: 0.8 to 1.1 mg/100 gm of body weight of dilantin sodium provided 50% protection. The maxi mum anticonvulsant action of phenytoin sodium was between 2 – 3 hours. The observed T.D. 50 doses of dilantin sodium was 2.5 ± 0.05 mg/100 gm of body weight.

Conclusion: The drug selected for the experiment was effective against M.E.S. test (Experimently induced grand mal seizure). Further elaborated screening of the drug by various experiments is needed to come to a precise conclusion.

Key words: Anticonvulsant Effect, Phenytoin, Albino Mice

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INTRODUCTION

Epilepsy is the most common serious neurological disorder affecting an estimated 50 million people worldwide. [1] There could be so many causes for the development of the epilepsy. Hypertension can lead to seizures through vascular brain damage that might or might not involve manifest stroke. [2] This relationship suggests that hypertension, particularly severe and uncontrolled, might increase the risk of epilepsy in the absence of prior clinically detected stroke[3]. Hypertension raises the risk of new-onset seizures in the absence of an immediate stroke.[2] The striking synergism between hypertension and stroke is more epileptogenic than other risk factors.[4] The contribution of noradrenergic transmission to the seizure susceptibility and epileptogensis is gaining more attention lately. The involvement of noradrenergic system in modulation of seizure activity is well documented. The noradrenergic system was demonstrated to participate in the occurrence of seizure in epileptic EL mice and to increase epileptiform discharge in rat limbic system via β-adrenergic receptor stimulation.[5] In experimental epileptology, to detect a substance possessing anticonvulsant activity, a huge number of compounds undergo examination in the first anticonvulsant screening test in rodents. [6,7] The first rapid screening of potential anticonvulsant compounds is routinely

performed in the mouse maximal electroshock-induced seizure model. [6,7] Phenytoin prolongs the inactive state of the voltage-dependent Na^+ channels.Generally we come across generalized tonic-clonic or grandmal seizure which is characterized by sudden loss of consciousness, tonic contracture of muscle, loss of postural control followed by a series of rhythemic contraction in all the four limbs (clonic phase). In this type of seizure, clonic phase is followed by tonic phase.

Seizure can be of different types viz. absence seizure, myoclonus, a tonic seizure, infantile spasm. In the absence seizure, the conscious activity is suddenly lost for a brief period, but there may be longer duration of absence. In case of status epilepticus, which can be dangerous and may turn into fatal outcome, there are seizures in succession without refractory period.

The mechanism by which seizure can develop in experimental animal include – the one in which the agents antagonize the GABA. So, those agents, which have GABA mimetic action or agents, which potentiate the action of GABA are supposed to have the protective effect or anti-eplileptic action.

The electric convulsiometer can also produce generalized tonic-clonic seizure in humans and animals in easy manner. Many drugs have been tested which have protective effect against G.T.C.S. induced by electric convulsiometer.

With the above background this study was conducted to study the profile of anticonvulsant action of phenytoin.

MATERIALS AND METHODS

The present one year study has been carried out in the Department of Pharmacology, Darbhanga Medical College, Laheriasarai after taking permission from institutional ethical committee. Induction of seizure with the help of electro-convulsiometer in albino mice was done followed by the observation of the anti-convulsant effect of phenytoin sodium. Appropriate lab conditions and proper animal housing was used.

Methods of work : The swinyard and co-workers method (1952 & 1965) was followed. The experimental animal (albino mice) was obtained from the animal house of Darbhanga Medical College.

Maximal electroshock method: The electro-shock convulsion was produced in the albino rats by the alternating current flow from electrical convulsiometer with the help of electrodes applied over the cornea. Every rat was screened for this test.

Production of maximal electroshock :

This was produced by convulsiometer. The instrument provides alternating current of 50 Hz frequency. The time period was also electrically operated at 0.1 second internal from 0.1 to 1 second and the stimulus current of 0.2 mA to 500 mA. The stimulus current of 150 mA to 220 mA of 50 Hz frequency was applied for 0.4 second with the help of electrodes applied over the right cornea. Before applying the electrodes to cornea the eye was irrigated with normal saline. Further the mice were kept manually on the table and released at the time of giving convulsion stimulus. After applying the stimulus for convulsion the mice passed tonic-clonic stage of convulsion. So, only those showing tonic convulsion one selected for the application to drugs. Now the control experiment was performed. In the experiment, keeping the time period constant at about 0.4 second, the current intensity of 150 mA onwards are applied. The intensity of current was increased from 150 mA in a serial increasing order. Now the point was noted at which tonic convulsion was observed in all the animals in a single batch. So in this way, 210 mA current for 0.4 second was found to be suitable. At this point all control animals showed the tonic convulsion. The animals if given the drug to be tested showed absence of tonic convulsion were considered that the drug has protective effect against the electro convulsion. So the positive or negative effects were recorded. The effect of different doses of the drug at different levels was thus calculated.

Statistical analysis: The data obtained was used for the calculation and analysed using SPSS software.

The anti-convulsant effective doses and neurotoxic doses in 50% of the animals are determined. The methods employed for its determination is Wilcoxon and Litchfield method (1949). So, to determine ED 50 and TD 50 (effective dose and toxic doses in 50% of animals respectively) 6 doses of the drug in increasing order was given and analysed. Each batch of the animal consists of six animals. Now the protection offered by the drug at different doses of a particular drug observed and noted. The dose at which fifty percent protection was calculated. Now the doses providing 40 to 60 percent protection were further subdivided (either side of 50% protection ingraduated order. Now the animal is given the different doses in that order and the required doses at which fifty percent protection is seen is considered the ED 50 dose. The experimental analysis of the effective dose of the drug is done through ED 50 in mg/100 gm body weight. Similarly the TD 50 is determined. For this also the drug to be tested is given in various increasing order and the animal is examined for their neurological status. The acute neuro-toxicity was exhibited by the following tests (Position sense test, Righting test, Gait and stance test, Muscle tone test and Equilibrium test) of neurological status and employed in our laboratory. So, in this way the abnormal neurological status was identified. Abnormality in any of the above mentioned fine test was considered as the end point, and no further doses were given thereafter. The present response of the animals at the various dose levels of the particular drug was calculated and the dose which showed 50% toxic manifestation was determined. The range of the dose of the drug showing toxic manifestation between 40 to 60% was further subdivided into varying graduated order. Batches of animals were then administered such doses in calculated order and subjected to the requisite convulsive doses. The range of the dose of the particular drug showing 50% neurological status in each of the two tests was thus determined. The observed value of TD 50 dose was thus calculated in each series of test. The calculation of TD 50 dose in mg/100gm of body weight of the experimental animal formed the basis of all experimental analysis for measuring the toxicity of an anticonvulsant drug was determined in terms of this dose level. A comparative analysis, as regards the relative toxicity of a drug in the various tests as compared to standard anticonvulsant drug (dilantin sodium,) taking the toxicity of the later as unity, was determined. The toxicity of the standard anticonvulsant drug and the other drug were noted. The results analysed in terms of histogram. The variation in the TD 50 dose levels of the standard anticonvulsant drug was thus calculated.

Dilantin sodium was administered orally in 5% gum acacia solution in distilled water through stomach canula fitted with a tuberculin syringe.The peak period of the drug action was determined previously by giving the requisite dose of the anticonvulsant drug. For the determination of the peak period MES test was employed due to its simplicity, ease and speed with which it could be performed.

In each series of experiments the animals were observed for a period of one hour after the application of convulsive dose. The percentage of protection offered by the drug, the time interval after the application of the convulsive dose and the elicited positive tonic extensor response and lastly and mortality were noted. The tested animals were kept under observation for a further time of 24 hours in the laboratory and any further mortality were noted. The results thus obtained were tabulated in various tables. Histograms of the results are also depicted.

Drug Preparation of Dilantin Sodium :

One capsule contains 100 mg of phenytoin sodium. Drug was prepared for oral feeding in the strength of 100 mg in 50 ml of distilled water with gum acacia.

The drug purchased from local medical store brand cap. Dilantin 100mg(Pfizer).

RESULTS

The drug was introduced orally with the tuberculin syringe fitted with stomach canula. Two hours after the administration of the drug electro-shock of 210 mA for 0.4 second was applied and the positive response were noted. The positive response was shown by the abolition of the tonic extensor

component of the hind leg during the tonic phase of convulsion. The mice were observed for one hour.

Table 1: Range of ED 50 of Dilantin Sodium in the MES Test

Batch No. of animal	Dose in mg/100gm of body weight	Effect on animals		% of Protection	Death
		No. of protected animals	No. of unprotected animals		
1	0.6	2	4	33.3	0
2	0.8	3	3	50	0
3	1.0	3	3	50	0
4	1.1	3	3	50	0
5	1.2	4	2	66.6	0
6	1.4	5	1	83.3	0

ED 50 is 1 ± 0.1 mg / 100 gm of body weight
SD ±0.1 SEM ±0.05.
The above table indicates that 0.8 to 1.1 mg/100 gm of body weight of dilantin sodium provided 50% protection.

The drug (Dilantin sodium) was administered orally through stomach canula. The doses given were kept 1.5 mg/100 gm of body weight. Further at definite time interval the electro shock of 210 mA for 0.4 sec. was applied. The protection offered by the drug was noted. The positive response is taken by the abolition of the tonic extensor component of the hind leg during the tonic phase of contraction.

Table 2: Peak period determination of Dilantin sodium by MES Test

Batch No. of animal	Time period in hours	Effect on animals		% of animal showing +ve response	Death
		No. of protected animals	No. of unprotected animals		
1	½	2	4	33.3	0
2	1	3	3	50	0
3	2	5	1	83.3	0
4	3	5	1	83.3	0
5	4	4	2	66.6	0
6	5	1	5	33.3	0

Table 2 shows the peak period is about two to three hours. After the administration of the drug (1.5 mg/100 gm body weight) Dilantin sodium, the maximum protection period were between two to three hours, though the action was seen from ½ hour to 5 hours. So, the maxi mum anticonvulsant action of phenytoin sodium was between 2 – 3 hours.
Each animal of the batch were given different doses of the drug Dilantin sodium with the help of tuberculin syringe fitted with stomach canula. Two hours later the electroshock of 210 mA for 0.4 second was applied. The animals protected were recorded. The positive response was indicated by the abolition of the hind leg extensor component of the tonic phase of convulsion. All the animals were observed for one hour after the electro-shock.

Table 3: Effect of different doses of Dilantin Sodium

Batch No. of animal	Dose in mg/100 gm of body weight	Effect on animals of different batches		% of protection	Death
		No. of protected animals	No. of unprotected animals		
1	0.40	0	6	0	0
2	0.60	2	4	33.3	0
3	0.80	3	3	50	0
4	1.00	3	3	50	0
5	1.20	4	2	66.6	0
6	1.50	5	1	83.3	1

Chi-square value – 28.10, P Value 0.001.

The doses given were ranging from 0.4 mg/100 ml of body weight of 1.5 mg/100 mg of body weight. Two hours after that, the animals showed the protective effect of the drug against electro-convulsion. The percentage of protection increased as the dose was increased. No animal was protected at 0.4 mg/100 gm weight but 83.3% of the animals were protected at 1.5 mg/100 gm of body weight. No mortality was observed upto 1.2 mg/100 gm of body weight but at the dose of 1.5 mg/100 gm, one animal was killed.
Test value: Chi square test 28.10P Value 0.001
The drug (Dilatin sodium) was given to each batch of the albino mice. The dose was ranging from 1.0 mg to 3.5 mg per 100 gm of body weight. Two hours after the administration of the drug, the experimental animals were examined or the acute neuro-toxicity test like Position and sense test, Gait and stance test, Righting reflex test and muscle tone test. The minimum dose of the drug showing 50% toxic effect was 2.5 mg per 100 gm of body weight.
Mean value – 2.5 mg/100 gm of body weight S.D. - ± 0.05S.E.M. – 0.16
The observed T.D. 50 doses of dilantin sodium was 2.5 ± 0.05 mg/100 gm of body weight.

DISCUSSION

Epilepsy is not a single disease entity. This is a collection of disorders of brain functions in which the most common symptom is partial or generalized fit or convulsion. These fits come in various forms hence the use of the term "epilepsies". Epilepsies have been generally managed by Diphenyl hydantoins, Barbiturates, Oxazolidions, succinamides. Recently carbamazepine, sodium valporate Benzodiazepines, lamotrigine and leviracetam have been added to the limited arementarium of antiepileptic drugs. The pharmacokinetic data as shown below relevant to their clinical use are as follows (Richens, 1976) [8]

Drug	Peak serum levels (Hrs)	Half life (Hrs)	% of protein binding	Therapeutic range (mcg/ml)
Phenytoin	1-12	9-10	89-92	10-20
Carbamazapine	6-12	24-60	70-80	4-10
Phenobarbitone	1-6	53-140	46-48	15-40
Ethosuccimide	1-2	60-100	Negligible	40-100
Sodium Vaklporate	5-4	8-15	90	50-100
Diazepam	1-2	20-42	94-98	40-60
Lamotrigine	2-18	15 – 30	55	3-5

Phenytoin is a different drug to be used correctly in practices. The plasma protein binding is too fluctuating in different individuals. It has narrow therapeutic ratio that makes individualization of doses very important. The bioavailability problem and drug interactions are very common and it exhibits saturation kinetics within the therapeutic range of serum concentration.

A large number of drugs have been mentioned in modern therapies that control the convulsive disorders. Previous workers suggested that mostly the drugs responsible to control grandmal were also responsible to control electroshock seizures or vice versa and the drugs for petitmal control chemoshock convulsion. The extensor component of the hind leg in the tonic phase of the convulsion was obtained and peak hour of the drugs related to their anticonvulsant property was determined after the oral administration of drugs.

Phenytoin under the present study was administered as suspension in 5% gum acacia solution through the stomach canula.

The mean current intensity of 210 mA was given for a time period of 0.4 sec produced 100% hind leg tonic extensor component of maximal electroshock seizure without any mortality. The current strength and the duration has been taken as standard all the experiments in this series.

For the determination of ED 50, two hours after each dose of the drug under study a current of the standard intensity (210 mA) was applied and the protection offered was determined. The abolition of the tonic extensor component of the hind leg during the tonic phase of convulsion was taken as the positive response. ED 50 for phenytoin was shown to be 1 mg ± 0.1mg for 100 gm of the body weight of albino.

For the determination of peak period of the drug effect, phenytoin sodium was administered in dose of 1.5 mg/100 gm of the body weight to the mice and the current of 210 mA was passed through the cornea of the mice for 0.4 seconds. The extent of protection offered was maximum during second and third hours. However, the action started after half hour and lasted till fifth hour. Thus the peak period for anticonvulsant action of phenytoin sodium was 2-3 hours. To find out the effective total dose orally the drugs were given in varying doses in different groups of mice. The standard current intensity was applied after two hours in each subgroup and the percentage of protection was noted. Phenytoin sodium was given in the dose of 0.6 to 1.5 mg per 100 gm of the body weight. In 1 mg group the protection offered was 50%, 83.3% protection was observed with the dose of 1.5 mg per 100 gm of body weight with one mortality in this group.

The acute toxicity of these drugs was noted after oral administration of varying doses of the drug. The position sense test righting reflex test, gait and stance test, muscle tone test and equilibrium test were the main parameters. In the phenytoin sodium group 50% of positive response was observed with 2.5 mg per 100 gm of body weight doses and 3.5 mg per 100 gm of dose produced 83.3% positive response with one mortality each in 3 and 3.5

mg/100 gm of B.W. group was seen. The TD 50 of phenytoin sodium was 2.5 ± 0.05 mg per 100 gm of the body weight (SEM ± 0.16).

Phenytoin showed maximal effect after one hour of the administration of drug and the peak period lasted for further two hours and then the effect declined. 50% effect was observed till 4th hour. The protection produced by varying doses of phenytoin against electroshock was highly significant (P Value <0.001) which was identical to the previous observations.[9,10] Acute neurotoxicity produced by phenytoin was nil at the dose levels of 1 mg./100 gm. of body weight and 50% at 2.5 mg./100 gm. of body weight, 83.3% at the dose levels of 3.5 mg./100 gm. of body weight. TD 50 range was 2.5 to 3.0 mg./100 gm. of body weight mean being 3.0 ± 0.1 mg./100 gm. of body weight. Dose response was highly significant (P Value <0.001) and protective index of phenytoin has been found to be 6.1 which was also comparable to previous records by Ramzan I. [9,10]

Toxic dose in comparison with phenytoin in 50% of the animals (TD 50) has been found high i.e. TD 50 of hydantoin as observed was 2.5 ± 0.05 mg./100 gm. of body weight.

CONCLUSION

The present study indicates that the drug selected for the experiment were effective against M.E.S. test (Experimently induced grand mal seizure). Further elaborated screening of the drug by various experiments is needed to come to a precise conclusion.

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