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### RESEARCH ARTICLE

#### Effect of flupirtine on seizure activity and its interactions with antiepileptic drugs in rats.

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Antiepileptic drugs, electroshock, flupirtine.

#### Abstract

**Aims:-**To evaluate the effect of flupirtine in maximal electroshock seizures (MES) induced seizures in rats and study the interactions of flupirtine with some antiepileptic drugs, by using subtherapeutic doses.

**Methods and Material:-**The effects were assessed by methods of MES.

**Results:-**Flupirtine alone showed protection against electroshock seizures. Combined treatment of flupirtine and antiepileptic drugs exerted a much stronger protective effect against electroshock seizures than either drug alone or addition of their effect. This was highly significant for a combination of flupirtine with diazepam, sodium valproate, phenobarbitone, and phenytoin.

**Conclusions:-**Flupirtine has anticonvulsant activity and has synergistic activity with antiepileptic drugs in MES model. Extrapolation of these combinations in clinical practice may suggest its utility in **grandmal epilepsy as add-on drug**.

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#### Introduction:-

Epilepsy is one of the most common neurological disorder in world and India. Epilepsy is chronic brain disease characterized by recurrent seizures. Seizures are brief episodes of involuntary shaking which may involve a part of the body (partial) or the entire body (generalized) and sometimes accompanied by loss of consciousness and control of bowel or bladder function.<sup>[1]</sup> It has important medical, social and psychological consequences. According to recent report prevalence of epilepsy is 3.6-8 per 1000 i.e. nearly seven million in India.<sup>[2,3]</sup>

There is no definite cure for epilepsy. Even in mild cases the disease can have a severe impact on quality of life.<sup>[4]</sup> Although methods such as surgery, vagal nerve stimulation and dietary changes have been employed to treat epilepsy, antiepileptic drugs (AEDs) remain the most widely utilized treatment strategy. Although there are many drugs introduced in 1990 significant numbers of patients still have uncontrolled seizures. In selecting an AED several factors including efficacy and side effect profile as well as cost and dosing convenience are kept in mind. The number of epileptologists and neurologists are looking for ideal antiepileptic agent with properties like broad spectrum activity, rapid onset of action, least side effects, and good oral bioavailability and low cost.

Flupirtine is newer drug of class, the Selective Neuronal Potassium Channel Opener (SNEPCO). It is a non-opioid, non-steroidal, non-NSAID analgesic with muscle relaxing potential. According to previous research it has some

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important action in various systems such as cytoprotection and neuroprotection, anticonvulsion, myorelaxant and antiparkinsonian etc<sup>[5]</sup>

So this study has been undertaken to evaluate the effect of flupirtine in maximal electroshock seizure (MES) and to study its interactions with currently used antiepileptic drug and also provide further information on neuropharmacological characterization of flupirtine in rats.

### Material and Methods:-

**Drugs:-** Flupirtine {2-amino-6-[(4-fluorobenzyl) amino] pyridin-3-yl} carbamate is a included in newer class of drugs, the selective neuronal potassium channel opener (SNEPCO). DMSO (dimethyl sulphoxide) is used as solvent for flupirtine as well as act as control. <sup>[6]</sup>Flupirtine was dissolved in DMSO freshly and given intraperitoneally. Similarly conventional antiepileptic inj. diazepam, sodium valproate, phenobarbitone sodium, phenytoin sodium are included in this study. Solutions of these drugs were prepared freshly in desired strength in water except for diazepam in which 1.5% v/v of 95% alcohol was added. All drugs used are from standard companies of highest purity.

**Animals:-** Male albino rats weighing between 150-200 gms were used for study. Rats were housed in colony cages with free access to food and water except 4 hours prior and during experiment and were maintained on natural light and dark cycle. The rats were randomly divided into multiple groups of 10 each (n=10). Once used rats were repeated for another bunch of study after a gap of 7 days. Female rats were excluded to avoid variation in results because of hormonal effect and slow elimination of antiepileptic drugs than male rats. <sup>[7]</sup> Approval for study has been taken by Institutional Animal Ethics Committee.

Drugs were injected intraperitoneally (i.p.) in a volume of 0.2ml/100 gms of rats in four groups of 10 rats each:

Group A: DMSO (control group)

Group B: Flupirtine alone (dissolved in DMSO)

Group C: Antiepileptic drug alone (D/V/B/P)

Group D: Flupirtine + Antiepileptic drugs (D/V/B/P)

(D= Diazepam, V= Sodium Valproate, B= Phenobarbitone sodium, P= Phenytoin sodium)

For making groups for MES, we keep control and flupirtine group in common, make Diazepam, Sodium Valproate, Phenobarbitone sodium, Phenytoin sodium groups and make flupirtine+ diazepam, flupirtine+ Sodium Valproate, flupirtine+ Phenobarbitone sodium, flupirtine+ Phenytoin sodium, so there are in total 10 groups for MES.

All drugs were given in subtherapeutic doses. Subtherapeutic dose of flupirtine by MES method was 15mg/ kg. Convulsive tests were carried out 30 minutes after drug administrations.

### Maximal electroshock seizure (MES):-

Electroshock Seizure test is extremely valuable because drugs that are effective against tonic extensor phase induced by electro shock generally have been proven to be effective against tonic-clonic seizures (grand mal epilepsy) in human. Rats were tested for tonic extensor phase (TEP) of electroshock seizure by Convulsimeter (Techno) using a current strength of 150 mA for 0.2 sec through ear electrodes.<sup>[8]</sup> In prior screening rats not showing typical TEP were discarded. Abolition of TEP indicates protective (antiepileptic) effect of a drug.

### Statistical analysis used:-

Data was presented in percentages. Comparison of percentage protection in flupirtine, antiepileptic drugs and flupirtine + antiepileptic drugs with control and addition of flupirtine + antiepileptic drug was done by proportion test. <sup>[9,10]</sup> p<0.05 was considered as statistical significance. Data was analyzed on STATA Statistical Software. (Version 13.0)

### Results:-

Table 1 shows comparison between anticonvulsant action of drugs alone and in combination by MES method in rats. Flupirtine and diazepam alone shows 30% protection in rats with MES model. However, when both drugs given together the protection was 90% (highly significant) compared with control. Protection by combination of flupirtine+ diazepam was not significant when compared with addition of flupirtine alone and diazepam alone. Rats

show 20% protection with sodium valproate alone in MES. However, the protection was highly significant i.e. 70% when flupirtine & valproate were given together in subtherapeutic doses when compared with control (p=0.001\*\*). Phenobarbitone alone shows 30% protection in MES. However, the protection was highly significant i.e. 90% when both drugs (flupirtine & phenobarbitone) were given together when compared with control.

Rats show 10% protection with phenytoin alone. However, the protection was highly significant i.e. 100% when both drugs (flupirtine & phenytoin) were given together in subtherapeutic doses when compared with control (p=0.0001\*\*). Similarly protection was highly significant when both flupirtine & Phenytoin (given together) was compared with addition of flupirtine and phenytoin alone, p=0.0016\*\* [Table 1].

**Original table:-**

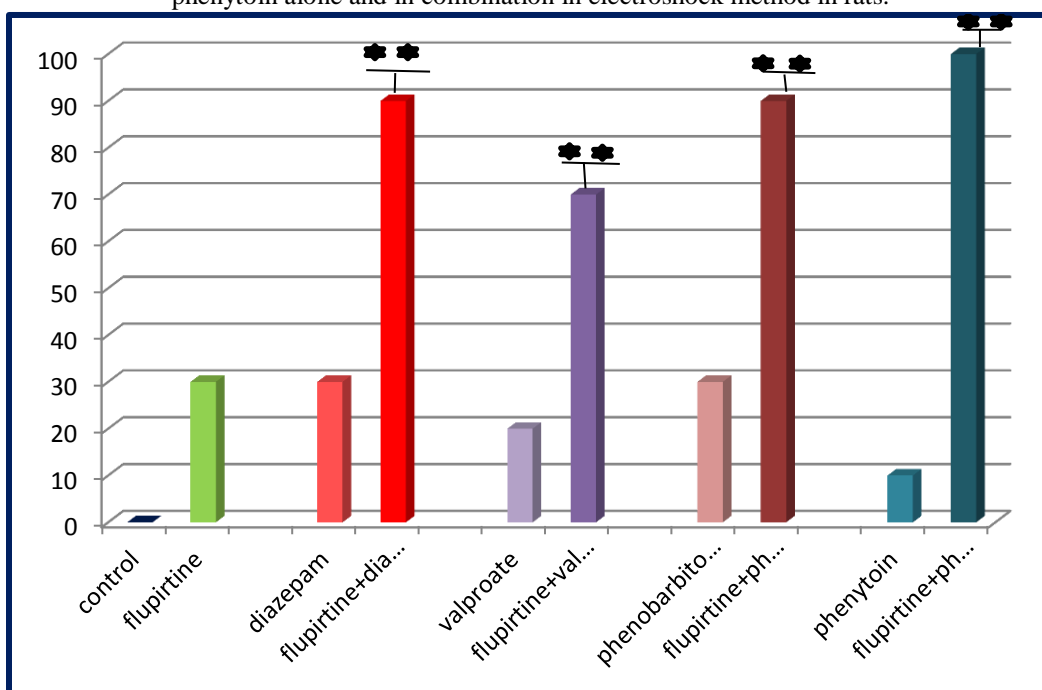
**Table 1:** Effect of flupirtine and antiepileptic drugs (AED) alone and in combination against maximal electro shock (MES) in rats.

Sr. No	Drugs	Dose mg/kg	Number of Animals in Group	Percentage of animals protected showing abolition of extensor phase.				p value			
				Group A	Group B	Group C	Group D	Group A Vs. Group B	Group A Vs. Group C	Group A Vs. Group D	Group D Vs. Group B + C
1.	Flupirtine	15	10	0	30	-	-	0.060	-	-	-
2.	Diazepam	2	10	0	30	30	90	0.060	0.060	0.000**	0.091
3.	Valproate	75	10	0	30	20	70	0.060	0.136	0.001**	0.297
4.	Phenobarbitone	8	10	0	30	30	90	0.060	0.060	0.000**	0.091
5.	Phenytoin	5	10	0	30	10	100	0.060	0.304	0.000**	0.001**

\*p value < 0.05 is significant; \*\*p value < 0.01 is highly significant as compared to control group.

**Original figure:-**

**Figure 1:-** Bar diagram showing effects of flupirtine and diazepam, sodium valproate, phenobarbitone, phenytoin alone and in combination in electroshock method in rats.



(\*\* sign over bar indicates significant protection as compared to control.)

**Discussion:-**

In present study we carried out to study anticonvulsant effect of flupirtine by using animal model of parameter-supramaximal electroshock seizure (MES) in subtherapeutic dose of 15mg/kg. Results of present study are in accordance with previous studies that demonstrate anticonvulsants activity of flupirtine.<sup>[11-15]</sup>

Anticonvulsant effect of flupirtine alone and in combination with other established anticonvulsant drugs in subtherapeutic doses was investigated here. Objectives of using combination of flupirtine with other anticonvulsants are to achieve synergism, to reduce the duration and severity of suffering of patients from seizure, to broaden the spectrum of anticonvulsant activity and to reduce the incidence of adverse effects by using low doses of drugs.

The exact mechanism of action of flupirtine has not been clear up to now. At a therapeutically relevant concentration, flupirtine activates neuronal inwardly rectifying G-protein-regulated K<sup>+</sup> channels and thus stabilizes the resting membrane potential. Indirect antagonism of excitatory NMDA receptor has recently discussed as possible mechanism of action.<sup>[16-20]</sup>

Flupirtine and some other anticonvulsant drugs shows anticonvulsant action by GABA mediated inhibition. This is a possible explanation for potentiation of anticonvulsant action of benzodiazepines by flupirtine.<sup>[14]</sup>

Naveen Kumar M, et al. (2011) also shows similar results. He studied the anticonvulsant action of flupirtine, in comparison with phenytoin and DMSO as a control in albino mice by using MES method. Flupirtine 79 mg/kg/po gives 33% protection in MES induces convulsions.<sup>(11)</sup>

Flupirtine alone showed anticonvulsant action in MES method. Flupirtine along with diazepam, sodium valproate, phenobarbitone, and phenytoin in subtherapeutic doses exerted significant protection against seizure induced by MES method. So this combination is likely to have clinical significance in grand mal seizures.

But limitations of this study are, the study has been carried out only in one species of animals viz rats and needs to be extended to other animals as well, study has been done on MES model only, has to be evaluated in other models like PTZA, kindling, picrotoxine, kainic acid or flurothyl etc. No attempt was made to establish exact mechanism of anticonvulsant activity.

**Conclusion:-**

Flupirtine thus seems to be capable of synergistic anticonvulsant against convulsion produced by MES in rats. Extrapolation of these combinations in clinical practice may suggest its utility in grandmal epilepsy as add-on drug. Conducting clinical trials in above different subsets will reveal more details.

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