

**EFFECT OF ANTI-ESTROGENS ON MES AND THEIR INTERACTION WITH  
ANTIEPILEPTICS IN WISTAR RATS.**

**Goel S<sup>1</sup>, Patil PA<sup>2\*</sup>, Vivek V<sup>2</sup>**

<sup>1</sup> Dept of Pharmacology, KLES's College of Pharmacy, Belgaum, India.

<sup>2</sup>Dept of Pharmacology and Pharmacotherapeutics, KLE University's JN Medical College, Belgaum, India.

**Summary**

The female gonadal sex steroid (FGSS) antagonist like tamoxifen, clomiphene and mifepristone are expected to modify the seizures as sex steroids like estrogen and progesterone are very well known to alter the seizure threshold. Similarly if they are coadministered with anticonvulsants as in case of comorbid condition, the interaction is poorly reported. Thus the present study was planned to elicit and confirm the effect of FGSS antagonists on convulsion and their interaction with the commonly used anticonvulsants like phenytoin, phenobarbitone and carbamazepine. Experimental models like maximum electric shock seizure and pentylenetetrazol induced seizure model were used to study the effect on convulsion. Antiestrogens tamoxifen and clomiphene potentiated the anticonvulsant activity significantly indicating decreased dose requirement of anticonvulsants when coadministered with antiestrogens.

**Keywords:** Clomiphene; Estrogen; MES; Progesterone; Seizures; Tamoxifen,

**\*Corresponding author:**

Patil P. A.,  
Professor,  
Dept of Pharmacology and Pharmacotherapeutics,  
J N Medical College,  
Belgaum-590010, Karnataka, India.  
Phone: 0831-24091828, Fax: 08312470759, e-mail: [drpapatil@yahoo.co.in](mailto:drpapatil@yahoo.co.in)

### Introduction

Epilepsies are common and frequently devastating neurological disorders affecting about 5.4 per 1000 and its age specific prevalence is highest in age group between 25-49, which is child bearing age group in women<sup>1</sup>. In pregnancy FGSS hormonal changes are well known and these changes not only could influence seizures but also influence the anti-convulsant activity of the drugs used.

The female gonadal sex steroid (FGSS) hormones *viz.* estrogen and progesterone have been shown to modulate threshold of seizures. Estrogen has been reported as epileptogenic<sup>2,3</sup>, where as progesterone reported to suppress seizures<sup>4</sup>. The reported increased incidence of seizures in older age group<sup>5</sup> supports the hormonal influence on seizures.

Logically the antagonists could be expected to antagonize epileptogenic effect of estrogen. The anti-estrogen, tamoxifen has been shown to provide protection against maximal electric shock seizures and Pentylentetrazol seizures in mice<sup>6</sup> and clomiphene has been reported to lessen seizure frequency in women<sup>7</sup>, whereas anti-progesterone, Mifepristone (MFP) has been shown to provide no protection against seizures<sup>6</sup>. In view of these reports estrogen antagonist if coadministered could reduce dose requirement of antiepileptic agents. But there are scanty reports on interaction of FGSS antagonists with commonly used anti-convulsants like phenytoin, phenobarbitone and carbamazepine. So, the present study was planned to elicit; effect of estrogen, progesterone, tamoxifen, clomiphene and mifepristone on experimentally induced convulsions, as well as the interaction of antiestrogens with phenytoin, phenobarbitone and carbamazepine.

### Materials and methods

#### Animals:

Inbred, strain of female Wistar rats aged between 4 to 5 months weighing  $170\pm 30$  gms were acclimatized with 12;12 light dark cycle in the laboratory. They were fed on standard food pellets (Amrut brand) and water *ad lib*. The study was approved by Institutional Animal Ethics Committee constituted as per CPCSEA guidelines.

#### Drugs and dosage:

All the drugs were administered parentally (*i.p.*) except estrogen, which was given orally suspending in 2% of gum acacia. Progesterone was diluted with olive oil and chlorbutol, whereas others were dissolved in distill water. Animal dose for each drug was obtained by converting maximal recommended clinical dose with the help of conversion table devised by Paget and Barnes<sup>8</sup>. Accordingly 18, 10.8, 18, 0.9, 90, 13.5, 3.6 and 50 in mg/kg were the doses of phenytoin, phenobarbitone, carbamazapine, estrogen, progesterone, clomiphene, tamoxifen and mifepristone used in the present study. The sub anticonvulsant dose (SACD) were estimated using different experiments and the maximum dose that just failed to significantly reduce HLE was considered as SACD which was determined as 13.5, 4.5 and 11.25 mg/kg of phenytoin, phenobarbitone and carbamazapine respectively.

#### Methods:

Maximal electroshock seizures (MES)<sup>9</sup>: MES were induced as described by Toman et al. with an alternating current of 150 mA delivered through the ear electrode for 0.2 sec with help of convulsimeter. Absence of HLE was taken as protection against seizures. Only the animals which showed HLE during screening procedure on the earlier day were included in the study. Treated animals were tested for MES after 30 min of drug administration. In addition to HLE, flexion extension ratio during seizures was also monitored in all the groups.

*Pentylentetrazol (PTZ) induced seizures*<sup>10</sup>: The chemical convulsant, PTZ was injected *i.p.* in the dose of 50 mg/ kg to induce convulsions. Control animals within 30 min developed a sequence of excitement, myoclonic jerks, clonic seizures. The anti-convulsant efficacy was assessed by considering number of animals protected from seizures/ number of deaths, number of seizures, duration of seizure and time for onset and recovery from seizures.

The animals were starved overnight prior to the day of experimentation.

Acute study: Different groups (n=6, in each) treated with estrogen, progesterone, tamoxifen, clomiphene and mifepristone in their therapeutic equivalent doses were subjected to MES. Progesterone, clomiphene and tamoxifen treated groups were subjected to PTZ induced seizures.

Interaction study: In all 9 groups of animals included in interaction studies, of which 3 groups each received either clomiphene/ tamoxifen/ mifepristone and after 30min of each antagonist treated group was coadministered with SACD of either phenytoin/ phenobarbitone/ carbamazepine separately. The animals were subjected to MES after 30 min of the treatment.

Sub acute study: The animals were divided into 6 treated groups and one control group. Of these three groups received either tamoxifen/ clomiphene alone for first 5 days every 24 hrs and on day 6, each of the antagonist treated groups received SACD of phenytoin/ phenobarbitone/ carbamazepine after 30 min of antagonist administration. From day 7 the antagonist (tamoxifen/ clomiphene) were withdrawn while only treatment with SACD of phenytoin/ phenobarbitone/ carbamazepine once 24hrs was continued (for 3 days) till day 9. On day 10 and 11 no drugs were administered. All the groups were subjected to MES once in 24hrs for 11 days.

## Results

As expected anticonvulsants phenytoin, phenobarbitone and carbamazepine in therapeutic equivalent doses protected all the animals from seizures.

At therapeutic equivalent dose progesterone though provided 16.67% protection, it was not statistically significant; but E/F ratio was significantly ( $p < 0.001$ ) reduced compared to that of control, where as estrogen failed to modify significantly, both HLE and E/F ratio.(Table 1)

**Table 1. Effect of various treatments on MES and PTZ induced seizures.**

Drugs (mg /kg)	MES		PTZ
	% protection	E/F ratio (mean±SEM)	Mean duration of Seizure (min)
<b>Control</b>	0	4.78±0.8	1.82±0.1
<b>Progesterone (90)</b>	33.3*	1.30±0.1***	0.0
<b>Estrogen(0.9)</b>	0	5.17±0.4	-
<b>Clomiphene (13.5)</b>	50**	1.4±0.3***	0.19±0.05***
<b>Tamoxifen (3.6)</b>	33.3*	1.05±0.5**	0.4±0.2***
<b>Mifepristone (18)</b>	0	5.5±0.4	-
<b>Phenytoin (18)</b>	100***	0	-
<b>Phenobarbitone (10.8)</b>	100***	0	-
<b>Carbamazepine (18)</b>	100***	0	-

ANOVA followed by Dunnet's test, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

Both tamoxifen as well as clomiphene significantly (p<0.01) abolished HLE and reduced E/F ratio significantly (p<0.001) as compared to that of control.(Table 1)

In contrast mifepristone neither modified HLE in any animal subjected to seizures nor significantly altered E/F ratio.

PTZ seizures: progesterone, clomiphene and tamoxifen in their therapeutic equivalent dose provided significant (p<0.001) protection against PTZ seizures. (Table 1)

In acute interaction studies none of the treatments provided significant protection in MES.

Table 2. Effect of various treatment on MES induced seizures (for 11 days).

Days	Tamoxifen						Clomiphene					
	% P	E/F	% P	E/F	% P	E/F	% P	E/F	% P	E/F	% P	E/F
1	16.7	2.03±0.4**	16.7	2.48±0.5**	16.7	2.16±0.4**	16.7	2.83±0.5**	33.3	1.86±0.5	16.7	1.61±0.3
2	16.7	1.67±0.4**	33.3	1.48±0.0*	23.3	1.3±0.4**	33.3	1.28±0.4*	33.3	1.46±0.4	33.3	1.25±0.4
3	16.7	1.96±0.4**	33.3	1.6±0.4*	33.3	1.5±0.4*	16.7	0.8±0.4**	16.7	1.68±0.4	33.3	1.0±0.4
4	16.7	1.73±0.3**	16.7	1.31±0.3**	16.7	1.96±0.4**	0	1.53±0.2***	0	2.2±0.2	16.7	1.75±0.4
5	0	1.68±0.1**	66	1.15±0.3**	0	1.56±0.2***	16.7	1.83±0.3**	16.7	1.65±0.4	16.7	1.5±0.3
		<b>+Phenytoin</b>	<b>+Phenobarbitone</b>	<b>+Carbamazepine</b>		<b>+ Phenytoin</b>	<b>+Phenobarbitone</b>	<b>+Carbamazepine</b>				
6	50*	0.68±0.3**	66	0.41±0.3**	66	0.48±0.3**	66	0.3±0.3**	66	0.18±0.2**	83	0.18±0.2**
7 <sup>€</sup>	50*	0.39±0.3**	50	0.3±0.3**	83	0.2±0.3**	100	0	66	0.33±0.3**	83	0.16±0.2**
8 <sup>€</sup>	50*	0.86±0.3**	33.3	0.83±0.4**	100	0	50	0.71±0.4**	66	0.07±0.3**	83	0.38±0.3**
9 <sup>€</sup>	0	1.95±0.2**	0	1.08±0.4**	66	0.55±0.4**	50	0.6±0.1**	50	0.41±0.3**	66	1.51±0.3*
10 <sup>¥</sup>	0	2.53±0.4**	0	2.58±0.4**	16.7	2.3±0.4**	0	2.5±0.2***	0	2.18±0.3**	0	1.51±0.3**
11 <sup>¥</sup>	0	2.23±0.4**	0	3.5±0.4**	0	4.48±0.4*	0	2.73±0.2***	0	3.11±0.3**	0	2.71±0.2**
<b>Control</b>	0	5.15±0.1	0	5.15±0.1	0	5.15±0.1	0	5.15±0.1	0	5.15±0.1	0	5.15±0.1

<sup>€</sup> Treatment with tamoxifen or clomiphene was discontinued. <sup>¥</sup> No treatment was administered.

(%P: Percentage protection.) :ANOVA followed by Dunnet's test, \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

Sub acute study:

On all the first six days all the treated groups failed to provide protection against seizures and reduce E/F ratio. In all the treated groups significant ( $p < 0.05$ ) protection as assessed by absence of HLE and decreased E/F ratio was observed on days 7 to 9. But on next two days (10 & 11) only E/F ratio reduction continued to be significant ( $p < 0.05$ ). (Table 2)

### Discussion

In the present study estrogen was found to be ineffective in controlling MES, though it increased E/F ratio significantly, while progesterone significantly decreased E/F ratio indicating its anticonvulsant activity and these findings are in agreement with earlier report<sup>11</sup>. The observations of the study clearly indicate that tamoxifen and clomiphene in their therapeutic equivalent dose possess significant anticonvulsant activity and clomiphene appears to be better as it also abolished HLE in contrast to tamoxifen, while mifepristone worsened MES by increasing E/F ratio.

The results of subacute study, clearly indicate that, both tamoxifen as well as clomiphene in their therapeutic equivalent dose potentiated the anticonvulsant activity of phenytoin, phenobarbitone and carbamazepine. There are scanty reports on such interactions.

Frequently carried out vaginal cytology in various treated groups confirmed the systemic efficacy of administered dose of hormones and their antagonists.

The present study was aimed to probe the gross effect of these hormones on seizures, rather than to elucidate the mechanisms involved. The anticonvulsant activity of progestins have been suggested to be mediated with GABA mechanism<sup>12,13</sup> and increased norepinephrine turnover rate in the CNS<sup>14</sup>. Moreover, decreased prostaglandin (PG) synthesis by progesterone<sup>15</sup> could also be responsible for anticonvulsant activity. Since, NSAIDs well known PG synthesis inhibitors have also been reported to possess anticonvulsant activity.<sup>16,17</sup> Progesterone receptor antagonism could possibly antagonize above mentioned mechanisms and that explains pro-convulsant activity of MFP.

Estrogens have been reported to inhibit GABA mediated neuronal inhibition<sup>18</sup>, decrease the turnover of norepinephrine in the brain<sup>14</sup> and enhance glutamate excitations<sup>19</sup> as well as PG synthesis<sup>20</sup>. All these actions of estrogen might contribute to its proconvulsant activity. Antiestrogens like clomiphene and tamoxifen possibly by antagonizing the above actions of estrogen<sup>21</sup> could produce anticonvulsant activity. Clomiphene and tamoxifen have been reported to possess both estrogenic and antiestrogenic activity and the type of activity is determined by their dose<sup>22</sup>, duration<sup>23</sup> of treatment and the animal species used. However, in the present study, the anticonvulsant action of the antiestrogens appears to be due to their antiestrogenic activity, as indicated by the vaginal cytology.

The nature of the interaction between antiestrogen and anticonvulsants observed in the present study cannot be precisely discerned. However, based upon the available literature, the nature of interactions appears to be of pharmacodynamic rather than pharmacokinetic as these sex steroid antagonists have been shown not to alter the free plasma levels of antiepileptics used.<sup>24</sup> The hormonal estimation in the plasma would have differentiated the nature of interaction. Potentiation of the anticonvulsant activity (phenytoin, phenobarbitone and carbamazepine) by tamoxifen and clomiphene even for three to five days after discontinuation could be explained on the basis of their prolonged plasma half life (5-7 days) due to high protein binding, storage in adipose tissue, enterohepatic circulation, etc. Kinetics of tamoxifen and clomiphene explains their failure to interact with anticonvulsants on single dose administration in acute studies.

If the findings of the present study could be extrapolated to the clinical situation, tamoxifen could be useful adjunct to antiepileptics, particularly in comorbid patients suffering from breast cancer as tamoxifen is routinely used in the treatment of breast cancer. Failure of treatment and potential hazards associated with anti-epileptics justify clinical exploration of such interactions.

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