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AN EXPERIMENTAL EVALUATION AND COMPARISON OF ANTIEPILEPTIC ACTIVITY OF GANAXOLONE AND ITS COMBINATION WITH SODIUM VALPROATE IN RATS

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ABSTRACT

Rationale: The positive allosteric modulation of the $GABA_A$ receptor complex by neurosteroids may be an important new approach for antiepileptic interventions. Ganaxolone (GNX) is the first synthetic analogue of Allopregnanolone, an endogenous neurosteroid.

Objective: To evaluate and compare the antiepileptic effect of GNX with sodium valproate (SV) and the combined antiepileptic effect of GNX in combination with SV on two seizure models in male albino wistar rats

Methods: A total of 60 rats were divided into 5 groups, each containing 6 rats for each seizure model. Group 1 was treated with 40% Hydroxypropyl-β-Cyclodextrin i.p, group 2,3 received GNX 5mg/kg and 10mg/kg i.p respectively, group 4 received SV 200 mg/kg i.p. and group 5 was given GNX 5 mg/kg and SV 100 mg/kg i.p. The ability to abolish the Tonic Hind Limb Extension component was selected as the anticonvulsant activity criteria in the Maximal Electroshock (MES) induced model. The latency period and duration of seizures were studied in the pentylenetetrazol (PTZ) induced model.

Results: Our study revealed that GNX (10 mg/kg) and combination of GNX 5 mg/kg with SV 100 mg/kg has promising antiepileptic effect in both the models. The combination of SV and GNX is additive, but not synergistic or supra-additive.

Conclusions: The results of our study calls for future experiments to assess the therapeutic effect of GNX and its combination with available antiepileptic drugs and compare it for mono or polytherapy, based on the balance of adverse effects and efficacy.

Keywords: Ganaxolone, Neurosteroids, Sodium valproate, PTZ, MES

INTRODUCTION

Appreciable advantages of neurosteroids in terms of their favorable pharmacokinetics and pharmacodynamic properties are the plausible explanation to consider them as an ideal new antiepileptic drug (AED). Endogenous neurosteroids as well as synthetic neurosteroids like Ganaxolone (GNX) activate both synaptic and extrasynaptic $GABA_A$ receptors, mediating two types of GABAergic inhibition, phasic and tonic inhibition. Neurosteroids, to a greater degree than normal generates tonic inhibition even in presence of saturating GABA concentrations **[1]**. Till we bring to fruition the substantial need to develop new AEDs we should consider polytherapy with existing drugs (treatment with two or more AEDs) which can be additive, supra-additive (synergistic) or infra-additive manner in efficacy **[2]**. Therefore, two drugs, GNX and sodium valproate (SV) have been combined in our study. We have used submaximal doses of GNX and SV at 5 mg/kg and 100 mg/kg respectively in two antiepileptic models, to test their combined efficacy.

MATERIALS AND METHODS: Male Albino Wistar rats weighing 120-180 grams each, bred in the central animal house of the Krishna Institute of Medical Sciences, Karad were used in these experiments. Males were chosen in order to reduce variability introduced by hormonal cycles, which is greater in the female. Total of 60 animals were obtained. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Drugs and Vehicle: GNX (obtained from Pro Lab Marketing Pvt. Ltd, India), SV (Okasa Pharmaceuticals, India), PTZ (Sigma–Aldrich Chemicals Pvt. Ltd, India), diazepam and thiopentone sodium (KIMSDU, India). Vehicle used were 2-Hydroxypropyl-β-cyclodextrin (Pro Lab Marketing Pvt. Ltd, India) and distilled water (D.W).

Maximal Electroshock Seizures (MES) Methods in rats: All the test animals were screened for standard convulsive responses and subjected to further experiments of this study after a gap of 7 days to avoid any possible "kindling" effect. Color coding on the base of tail was done to identify the same animal before subjecting MES. The maximal seizure pattern was induced in rats by using electroconvulsiometer (Techno, India) to give an alternating current of 150 mA for 0.2 sec. via ear electrodes **[3]**. Duration of tonic hind limb extension (THLE) was measured using stop watch in seconds. The ability of test drug to abolish or reduce the THLE component was noted and selected as the criteria to establish anticonvulsant activity of the drugs.

Pentylenetetrazol (PTZ) induced seizure in rats: The rats were administered PTZ (50mg/kg, i.p) for induction of seizures. The animals were observed for 60 min for the development of PTZ induced seizures. Two parameters measured in minutes using stop watch were Latency period and duration of seizures. Latency period is the time taken for onset of seizures (jerky movements of whole body and clonic seizures with episodes of rearing and falling down) after administration of PTZ in rats or time duration observed maximum till 60 minutes after administration of PTZ. Duration of seizures is the actual time from onset of PTZ induced seizures till its disappearance **[4, 5]**. Increase in latency period (or seizure free state for period 1 hour) and decrease in duration of PTZ induced seizures was selected as the criteria to establish anticonvulsant activity of the drugs **[5]**.

If the drug prevented the appearance of hind limb tonic extensor component of the seizure in the MES model and caused seizure free state for a period of 1 hour in PTZ model, then the percentage protection was calculated as follows **[6]**:

$$\text{Percentage protection (\%)} = \frac{\text{No of animals with absent seizures}}{\text{Total number of animals}} \times 100$$

Study Design for MES and PTZ models : Each group of 6 male rats **(Table 1)** received test drugs, control & standard drug intraperitoneally, 30 minutes before being subjected to electric shock in the MES model and half hour before subjecting rats to PTZ induced Seizures (50mg/kg PTZ, i.p.) in the PTZ model.

Table 1: Grouping of Rats For MES and PTZ Model

GROUP S	DRUGS	DOSE
I. CONTROL	Hydroxypropyl-β-Cyclodextrin	40 % w/v, 4ml/kg
II.	Ganaxolone in 40% 2-Hydroxypropyl-β cyclodextrin in D.W	5mg/kg
III.	Ganaxolone in 40% 2-Hydroxypropyl-β cyclodextrin in D.W	5mg/kg
IV. STANDARD	Sodium Valproate dissolved in D.W	200mg/kg
V. COMBINATION	Ganaxolone + Sodium Valproate	GNX 5mg/kg+ S.V 100mg/kg

RESULTS

A decrease in the mean duration of THLE was seen in 5, 10 mg/kg GNX group, 200 mg/kg SV group and the combination group of GNX 5 mg/kg and SV 100 mg/kg, when compared to its before treatment values using paired t test. Though experimentally the mean duration of THLE in 10 mg/kg GNX alone group (THLE=2.17s) and combination group of 100 mg/kg SV and 5 mg/kg GNX (THLE=2.17s) was high as compared to 200 mg/kg SV group (THLE=0.00s), according to post hoc Dunnet's test it showed statistically similar effects for protecting against THLE phase in MES model **(see Table 2)**. Graphical representation for duration of THLE after treatment with the study drugs in MES model is as shown in **Graph no: 1**.

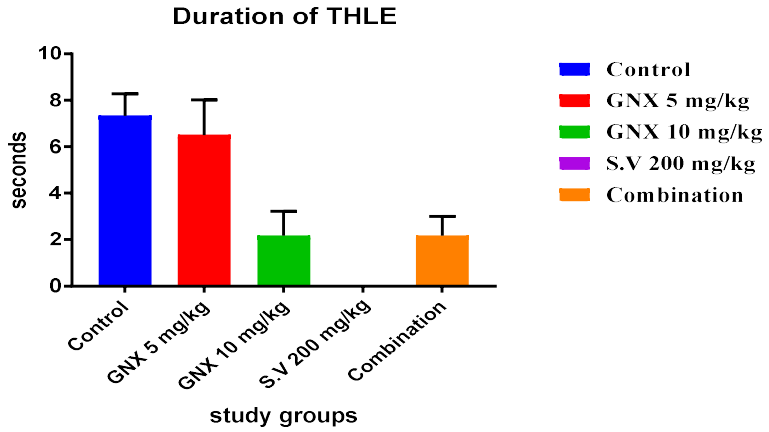
Latency period of PTZ induced seizures Post hoc Dunnet's multiple comparison test revealed that the mean latency period was very significant in 5 mg/kg GNX group and combination group ($p<0.01$) when compared to 200 mg/kg SV. In the PTZ model of our study, though experimentally the mean latency period of GNX 10 mg/kg (latency period=18.67 min) was less as compared to SV 200 mg/kg (latency period=26.50 min), it showed statistically similar effects for increasing latency period in PTZ induced seizures **(see Table 3)**.

Duration of PTZ seizures: The mean duration of PTZ induced seizures was very significantly high in 5 mg/kg GNX group ($p<0.01$) as compared to 200 mg/kg SV according to post hoc Dunnet's test. Though the mean duration of PTZ induced seizures of GNX 10 mg/kg (Duration of seizure = 21.83 min) and combination group (Duration of seizure=33.17 min) was more as compared to SV 200 mg/kg (Duration of seizure=15 min), it showed statistically similar effects **(see Table 3)** for decreasing the mean duration of PTZ induced seizures ($p>0.05$). Graphical representation for Latency period and Duration of PTZ induced seizures (in minutes) with the study drugs in PTZ model is as shown in **Graph no: 2 and 3** respectively.

Table 2: Comparison Effect Between Cyclodextrin, Sodium Valproate and Ganaxolone on Duration of THLE in MES Method

Groups	Duration of THLE (in seconds) Mean ± SEM		% Protection
	Before treatment	After treatment	
CONTROL (Cyclodextrin)	8.33 ± 0.99	7.33 ± 0.95	0
S.V 200 mg/kg	8.17 ± 0.98	0.00 ± 0.00	100
GNX 5 mg/kg	10.83 ± 0.75	6.50 ± 1.52 ^{NS}	16.67%
GNX 10 mg/kg	9.67 ± 1.05	2.17 ± 1.05*	50%
GNX 5 mg/kg +S.V 100 mg/kg	10.17 ± 0.70	2.17 ± 0.83*	33.33%

p<0.05*(significant), p<0.01(very significant), p<0.001***(extremely significant), NS- not significant p>0.05 when compared with control using Post hoc Tukey Kramer test**

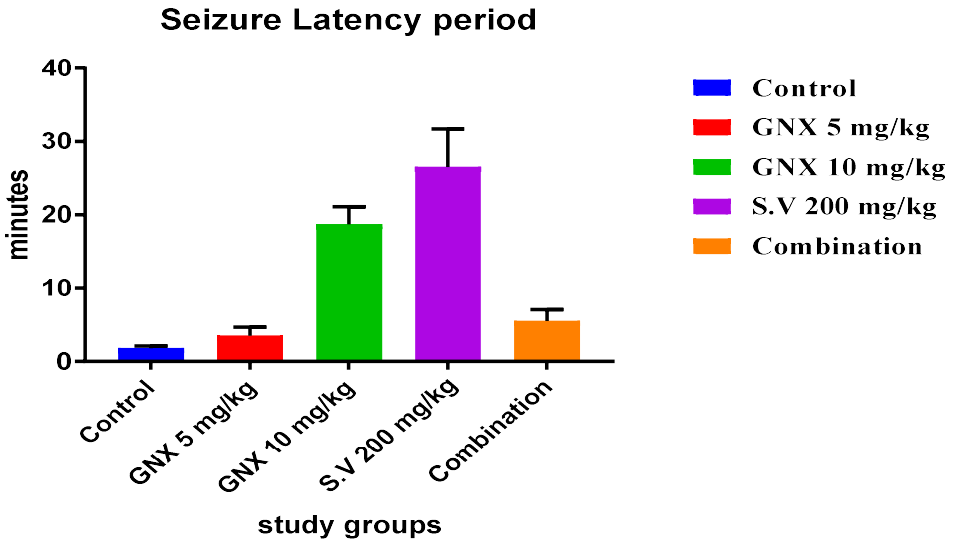


Graph 1: Duration of THLE (IN SECONDS) With Cyclodextrin, Ganaxolone (5 mg/kg, 10 mg/kg), Sodium Valproate (200 mg/kg) and Combination (Ganaxolone 5 mg/kg + Sodium Valproate 100 mg/kg)

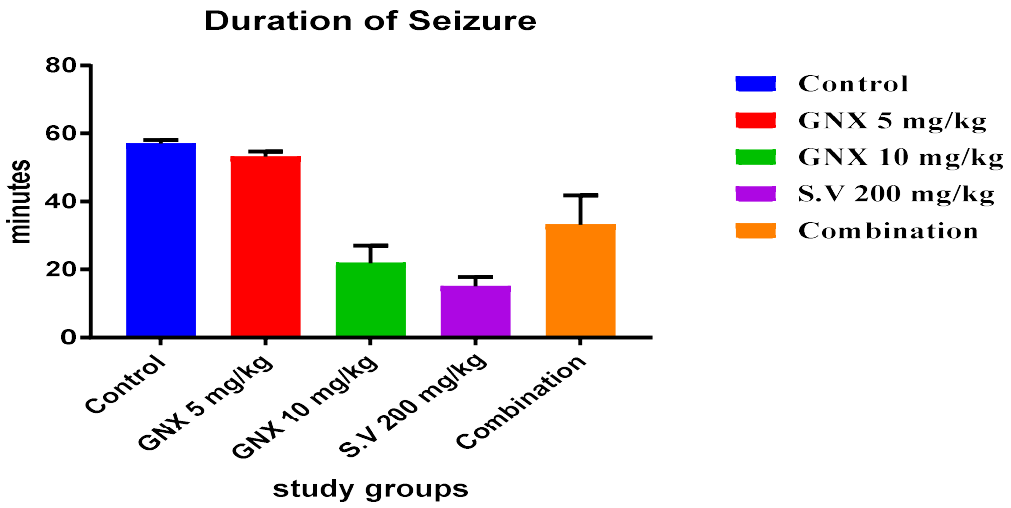
Table 3: Comparison Effect Between Cyclodextrin, Sodium Valproate and Ganaxolone and on Latency Period And Duration of Seizures in PTZ Method

Groups	PTZ induced seizures (in minutes) Mean ± SEM		% Protection
	Latency period (min)	Duration (min)	
CONTROL (Cyclodextrin)	1.83 ± 0.31	57 ± 1.13	0
S.V 200 mg/kg	26.50±5.19	15±2.83	33.33%
GNX 5 mg/kg	3.50 ± 1.18 ^{NS}	53.17 ± 1.56 ^{NS}	0
GNX 10 mg/kg	18.67 ± 2.40 **	21.83 ± 5.22 **	33.33%
GNX 5 mg/kg + S.V 100 mg/kg	5.50 ± 1.61 ^{NS}	33.17 ± 8.67 *	16.66 %

p<0.05*(significant), p<0.01(very significant), p<0.001***(extremely significant), NS- not significant p>0.05 when compared with control using Post hoc Tukey Kramer test**



Graph 2: Latency Period of PTZ Induced Seizures (in minutes) With Cyclodextrin, Ganaxolone (5 mg/kg, 10 mg/kg), Sodium Valproate (200 mg/kg) and Combination (Ganaxolone 5 mg/kg + Sodium Valproate 100 mg/kg)



Graph 3: Duration of PTZ Induced Seizures (in minutes) With Cyclodextrin, Ganaxolone (5 mg/kg, 10 mg/kg), Sodium Valproate (200 mg/kg) and Combination (Ganaxolone 5 mg/kg + Sodium Valproate 100 mg/kg)

DISCUSSION

Though the anticonvulsant properties of steroid hormones, steroid anesthetics and endogenous neurosteroids have been spun out for long, only in the recent past has the potential clinical use of neuroactive steroids as AED been considered **[7]**. The most studied and best accepted mechanism of action of neurosteroids as seen in diverse animal seizure models is enhanced neural inhibition of extrasynaptic (δ subunit-containing) $GABA_A$ receptors. By the observations of lesser risk of side effects with ganoxolone due to its non conversion to the hormonally active 3-keto form by 3α-hydroxysteroid oxidoreductase isoenzymes, we can juxtapose the synthetic neurosteroids like GNX and endogenous 3α-hydroxy-pregnane neurosteroids such as allopregnanolone **[8]**.

It is assumed that, interactions (efficacies) of drugs can be additive, supra additive or infra additive. If the combined efficacy C of drugs A and B administered together is equal to the expected efficacies of A and B individually, then interaction is considered to be additive. If the combined efficacy is greater than the expected efficacies of drug A and drug B individually, then the interaction is assumed to be supra additive or synergistic **[9]**. With the availability of a wide range of modern AEDs known to cause lesser drug interactions than their older counterparts, it is expedient that we consider assimilating combination therapy as an acceptable treatment strategy **[10]**.

Statistically a similar effect on the mean duration of THLE was seen on comparison of 10 mg/kg GNX alone group and combination group against the 200 mg/kg SV but experimentally the efficacy seems to be lower with a higher mean duration of THLE and a percentage protection of 50% with GNX 10 mg/kg groups and 33.33 % with combination group as compared to SV group with a 100 % protection against THLE. Similar results were observed by **[7]** who demonstrated GNX when given alone was less potent against MES (ED50 of 29.7 mg/kg i.p) as opposed to SV (ED50 of 259.2 mg/kg i.p.) in mice.

Our results for MES model were in accordance with **[11]** who demonstrated significant anti-epileptic activity with 10 mg/kg of GNX as compared to control group (Hydoxypropyl-β-Cyclodextrin). Though the percentage protection for THLE was 16.66 % with GNX 5 mg/kg, no significant antiepileptic activity was observed with 5 mg/kg GNX when compared to control in our study, which was in contrast with **[11]** who mentions significant antiepileptic activity for the same group. In the present study, GNX 10 mg/kg has less activity when compared to that of SV on duration of THLE but they were statistically significant.

[11] also stated that the combination of GNX + phenobarbitone at submaximal doses has a similar MES induced convulsions blocking activity when compared to that of phenobarbitone alone in rats. Our study, based on the decrease in mean duration of THLE demonstrated that combination of GNX (5 mg/kg) and SV (100 mg/kg) at sub maximal doses has less activity when compared to that of sodium valproate on duration of THLE but they were statistically significant. Therefore we can conclude that the combination of GNX and SV used in the MES model of our study is additive, but not synergistic or supra-additive.

In our study the percentage protection against PTZ seizures was 33.33% for both GNX 10 mg/kg group and SV 200 mg/kg group. **[11]** reports that 5 mg/kg,10 mg/kg ganaxolone and the combination group increased the latency to seizure as compared to control group (Hydroxypropyl-β-Cyclodextrin). However, our study reveals that GNX 10 mg/kg alone shows promising effect for increasing the seizure latency period of PTZ induced seizures when compared to the control and the standard drug. This study finding is in contrast with **[11]** who reports that PTZ induced convulsions blocking activity of GNX was slightly less than that of SV. The probable explanation for

this could be the dose of PTZ (70 mg/kg, i.p.) used in their study. Statistically similar effects for decreasing the mean duration of PTZ induced seizures was seen on comparison between the combination group of SV 100 mg/kg and GNX 5mg/kg and the standard SV 200 mg/kg. However, the efficacy seems to be lower with a percentage protection of 16.66% against PTZ seizures. The seizure latency period is not increased in the combination group as compared to SV.

Based on our study results for the combination group, we can say that the combination of SV and GNX is additive, but not synergistic/ supra- additive. In contrast, **[11]** stated that the combination of GNX and SV has a superior PTZ induced convulsions blocking activity compared to that of SV alone. Genesis for this variation in the response when compared to our study could be the dose of SV given in the combination group of the **[11]** study which was 200 mg/kg, whereas in our study, we have used submaximal dose of 100mg/kg SV.

Our study findings are in accordance with those reported by Richard B. Carter et al, who reports that the profile of anticonvulsant activity obtained for GNX in their study using the PTZ model compares favorably with that of SV **[7]**. **[13]** demonstrated that in naive female control rats, GNX (0.625–15 mg/kg s.c.) protected against PTZ induced seizures in a dose dependent fashion. Thus, GNX could be of use in the treatment of perimenstrual catamenial epilepsy, a condition that is often resistant to other anticonvulsant therapies. The results of experiments by **[12]** revealed antiepileptogenic and anticonvulsant effects of GNX against PTZ induced kindled seizures. All these findings add to accumulating evidence suggesting an improved profile of antiepileptic properties through positive allosteric modulatory effect on $GABA_A$ neurotransmission. The other mechanism of actions of AED like blocking voltage-dependent Na+ channels or NMDA receptors has not been fully characterized for GNX. With a lack of experimental evidence to identify the other sites at which neurosteroids can exert its antiepileptic action, the positive effect of GNX in blocking the MES induced extension could be attributed to its allosteric modulaton of $GABA_A$ receptors **[11, 12]**.

Variations in response in our study probably may be because of various factors like sex, age, diet, species, climate, circadian rhythms, temperature . The equipment used for seizure induction are known to affect the response of animal to MES seizures while the dose of

PTZ and route of administration are known to affect the response of the animal to PTZ induced seizures.

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