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Anticonvulsant appraisal of benzylideneacetophenone analogues in Swiss mice

Azibanasamesa D.C Owaba[1*], James O. Kemelayefa[2], Oyeintonbra Miediegha[1]

[1]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

[2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University. Wilberforce Island, Bayelsa State, Nigeria.

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* *Corresponding Author:*

azibanasamesa@gmail.com
https://orcid.org/0000-0001-9989-665X
+2348064578188

ABSTRACT

Background: Diketones and α,β-unsaturated ketone are useful intermediates for the synthesis of antiepileptic moieties. Epilepsy is a neurological disorder that affects about fifty million people worldwide. The aim of the study was to assess benzylideneacetophenone analogues for their potential antiepileptic effects in mice.

Methods: The compounds used in the pharmacological assay which include; 4-Dimethylaminochalcone (A), 1(4-Nitrophenyl) – 3 ($_{\gamma}$-benzopyranoyl)-2-propen – 1-one (B), 1(4' – Nitrophenyl) – 3 (1,3-benzodioxolyl) 2 – propen – 1 – one (C), 1-Phenyl-3- (benzodioxolyl) – 2 – propen – 1-one (D), 4-Chlorochalcone (E), 4'-Nitro-4-dimethylaminochalcone (F) 4'-Nitro-4-methoxychalcone (G), were assayed using three models; Strychnine, Pentylenetetrazole (PTZ) and Maximal Electroshock (MES). The samples were given at 500, 1000 and 1500 mg/kg while standard drug diazepam and distilled water were given at 5 mg and 10 ml/kg intraperitoneal respectively. Convulsion inducing agents (Strychnine 2 mg/kg and PTZ, 100 mg/kg) were administered intraperitoneal 1hour after the administration of test samples while MES model electroconvulsive shock was applied using electroconvulsive machine.

Results: The samples (A-G) showed significant ($P<0.03$, 0.003, 0.0007 and 0.0001) effect in delaying onset of seizure compared to control in strychnine model, while in PTZ model samples (A-F) evaluated at 500,1000 and 1500 mg/kg are equipotent at all the doses by significantly ($P<0.0001$) and sample G ($P<0.0007$) reduced the onset of seizure compared to control. However in MES model samples (A-F and G) evaluated at 500, 1000 and 1500 mg/kg are equipotent at all the doses by significantly ($P<0.0001$; 0.0007), reduced the duration of seizure compared to control respectively.

Conclusion: Benzylideneacetophenone analogues showed various effects in control of seizure in all the models used.This effect is due to delayed onset and reduction of seizure duration. The samples could act by stimulating the glycine, Gamma Aminobutyric acid (GABA) receptor, inhibition of GABA Aminotransferase and prolong inactivation of sodium ion channel.

1. Introduction

Epilepsy is a neurological disorder that affects about fifty million people worldwide[2,3,4]. It is a disorder with psychosocial stigmatization and about 80% of the populations are living in the developing countries. The continuous search of novel compounds with potential biological activity is a routine exercise by researchers to discover therapeutic effect of new or already existing compounds[2,3,4]. The search of novel antiepileptic agent is a global concern because of the side effect, chronic toxicity associated with already existing drugs. Chalcone scaffold could be source of novel antiepileptic agents with fewer side effects in humans[1,5,6]. Glycine is an inhibitory neurotransmitter present mainly in the medulla, spinal cord, lower brain stem and retina. Its agonist or drugs that stimulate or mimic its effect are β-alanine, taurine and its antagonist is strychnine[7]. Stimulation of glycine receptor is also enhanced by the chloride ion channel[7]. Strychnine

induced seizure by competitively binding to the α subunit while tetanus toxins acts by preventing the release of glycine and causing excessive excitation and violent muscle spasm[7]. The aim of this research is to assess chalcones as a potential seizure control agents using the mice as the animal model. Several study models exist among which includes the Pentylenetetrazole (PTZ), maximal electroshock (MES). The MES induced seizure are abolished by medicinal agents that block voltage-gated Na^+ channels such as iminostilbenes and hydatoins and felbamates while PTZ induced seizures are prevented by drugs that block The T-type Ca^{2+} current in thalamus, like sodium valproate and diazepam drug that enhanced GABA receptor in the brain[8,9].

2. Materials and Method

2.1 Materials

Pentylenetetrazole (Sigma, USA), Strychnine (Sigma, USA), Diazepam (Roche, Switzerland).

The test samples were synthesized and obtained from Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmacy, Niger Delta University. These samples include; 4-Dimethylaminochalcone (A), 1(4'-Nitrophenyl)– 3 ($_{Y}$-benzopyranoyl)-2-propen – 1-one (B), 1(4' – Nitrophenyl) – 3 (1,3-benzodioxolyl) 2 – propen – 1 – one (C), 1-Phenyl-3- (benzodioxolyl) – 2 – propen– 1-one (D), 4-Chlorochalcone (E), 4'-Nitro-4-dimethylaminochalcones (F) 4'-Nitro-4-methoxychalcone (G).

2.2 Method

2.2.1 Experimental Animals

The mice of both sex weighing (16-30 g) were bought from the Department of Pharmacology and Toxicology, Faculty of Basic Clinical Science, University of Port Harcourt, Rivers State. The mice were kept in well ventilated plastic cage, feed with standard diet and had free access to water and were exposed to 12 hours light and dark cycle for two weeks to acclimatize. The standard laboratory protocol of the ethical committee of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, Niger Delta University was adopted[10].

2.2.2 Pharmacological Assay

The protocol used by Owaba *et al*., [11] was adopted and followed strictly for the pharmacological assay of the anticonvulsant effect in mice based on the $LD_{50,}$ the following doses 500, 1000, 1500 mg/kg were administered. The protocols of Strychnine, PTZ and Electroconvulsive model were used[11].

One hundred and thirty-five mice of either sex were divided into twenty seven groups (n=5). Group I and II were given 10 mL and 5 mg/kg intraperitoneal (i.p), for distilled water (VEH) and standard drug (Diazepam, DZP) respectively. Each samples (A, B, C, D, E, F and G), were administered at 500, 1000, and 1500 mg/kg i.p 1hour before the administration of strychnine (2 mg/kg i.p), the onset and duration of seizure were observed. The assay was repeated for pentylenetetrazole model and Maximal electroshock models were Pentylenetetrazole (100 mg/kg, i.p) andelectroconvulsive shock was established using electroconvulsive machine (Ugo Basil®) with the following parameters applied: Frequency 100 pulse/sec, Pulse width 04 unit/sec, Pulse duration 10 secs, Current 99 mA respectively[2,3,4,7].

2.2.2 Statistical Analysis

Statisticalanalysiswas done using graph pad prism 8.3 ANOVA with multiple comparison post hoc test (Tukey). The results obtained were expressed as Mean±S.E.M with a $p<0.05$ was considered significant[2,3,4].

3. Results

3.1 Result of anticonvulsant effect of benzylideneacetophenone analogues in mice using Strychnine model.

The alphabets used indicate the various test samples (A-G) for the experiment as in Figure 1.

(A) The data from 500, 1000 and 1500 mg/kg showed significant (** $P<0.003$) to the onset of seizure compared with control.

(B) Statistical measures revealed that the 3 doses of 1(4'-Nitrophenyl) – 3 ($_{Y}$-benzopyranoyl) – 2 – propen – 1 one onset of seizure are significant (**$P<0.003$) compared with the control group.

(C) Statistical measures revealed that the 3 doses of 1(4' – Nitrophenyl) – 3 (1,3-benzodioxolyl) 2 – propen – 1 – one (Sample C) delayed onset of seizure are significant ($P<0.0001$; *$P<0.03$) compared with the control group.

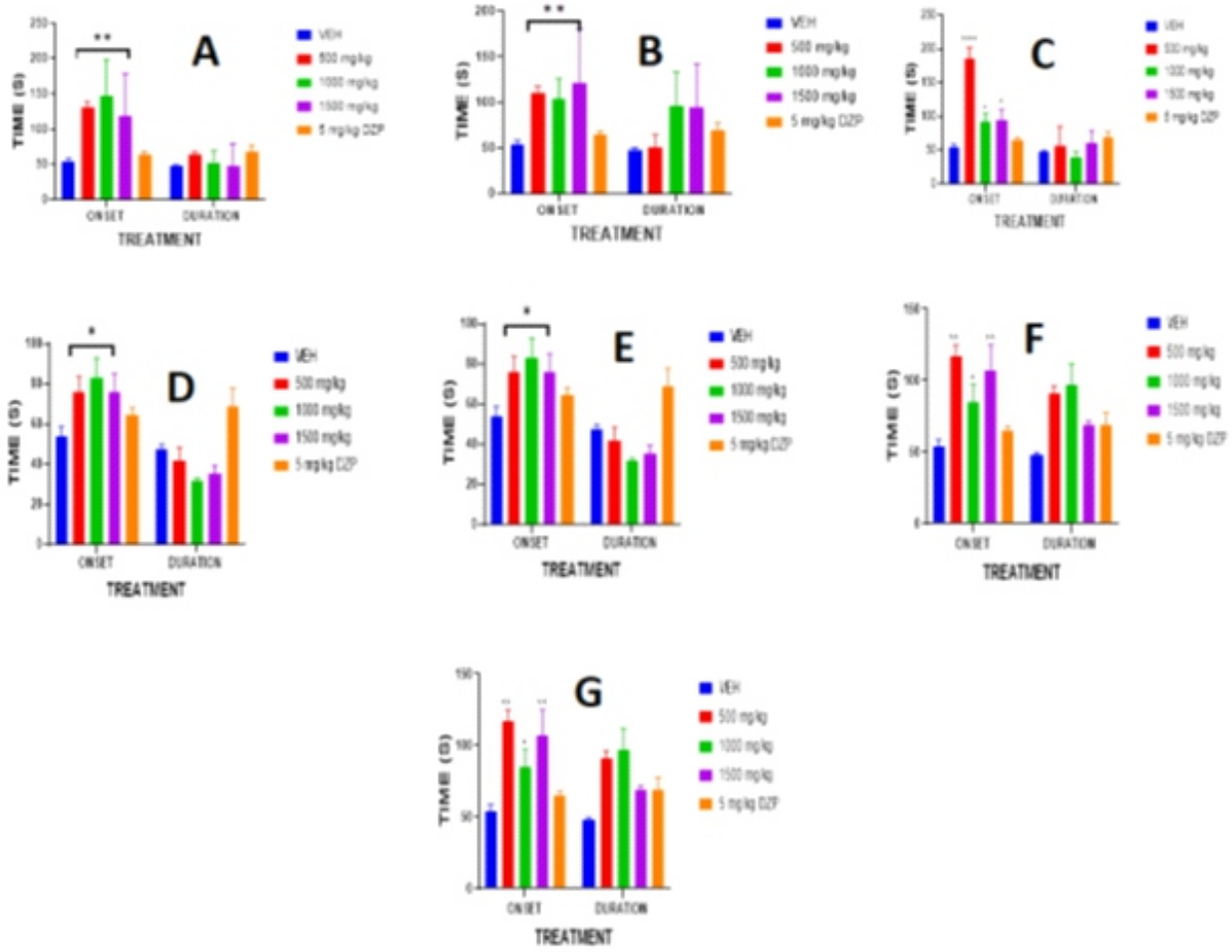
(D) Statistical measures revealed that the 3 doses of 1-Phenyl-3- (benzodioxolyl) – 2 – propen – 1-one delayed onset of seizure are significant (*$P< 0.03$) compared with the control group.

(E) Statistical measures revealed that the 3 doses of

Chlorochalcone (Sample E) onset of seizure are significant (*P< 0.03) compared with the control group.

(F) Statistical measures revealed that the 3 doses of 4'-Nitro-4-dimethylaminochalcones onset of seizure are significant (**P< 0.003; *P<0.03) compared with the control group.

(G) Statistical measures revealed that the 3 doses of 4'-Nitro-4-methoxychalcone delayed onset of seizure are significant (***P< 0.0007; *P<0.03) compared with the control group.



Note: The samples (A-G) showed significant effect in delaying onset of seizure at *P<0.03, **P<0.003, ***P<0.0007 and****P< 0.0001 compared to control.

Figure 1 Results of the anticonvulsant effect of sample (A-G) in mice using strychnine model.

3.2 Result of anticonvulsant effect of benzylideneacetophenone analogues in mice using Pentylenetetrazole model

The alphabets used indicate test samples (A-G) for the experiment in Figure 2.

(A) Statistical measures revealed that the 3 doses of 4-dimethylaminochalcone (Sample A) onset of seizure are significant (**P< 0.003) compared with the control group.

(B) Statistical measures revealed that the 3 doses of 1(4'-Nitrophenyl) – 3 ($_{\gamma}$-benzopyranoyl) – 2 – propen -1- one (Sample B) onset of seizure are significant (*P< 0.03) compared with the control group.

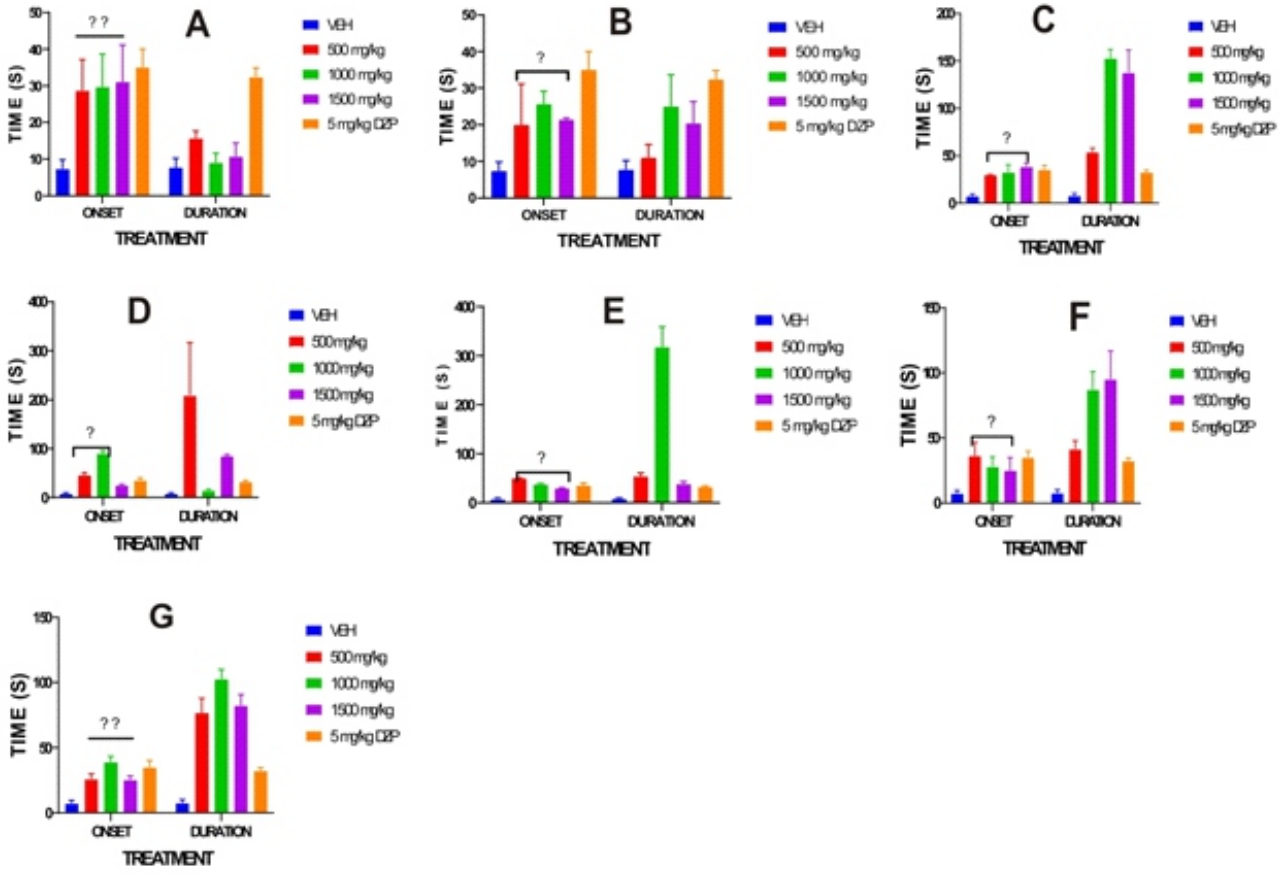
(C) Statistical measures revealed that the 3 doses of 1(4' – Nitrophenyl) – 3 (1,3-benzodioxolyl) 2 – propen – 1 – one (Sample C) onset of seizure are significant (*P< 0.03) compared with the control group.

(D) Statistical measures revealed that the 3 doses of 1-Phenyl-3- (benzodioxolyl) – 2 – propen – 1-one (Sample D) onset of seizure are significant (*P< 0.03) compared with the control group.

(E) Statistical measures revealed that the 3 doses of Chlorochalcone (Sample E) onset of seizure are significant (*P< 0.03) compared with the control group.

(F) Statistical measures revealed that the 3 doses of 4'-Nitro-4-methoxychalcone4'-Nitro-4-dimethylaminochalcones (sample F) onset of seizure are significant (P< 0.03) compared with the control group.

(G) Statistical measures revealed that the 3 doses of 4'-Nitro-4-methoxychalcone (Sample G) onset of seizure are significant (P< 0.003) compared with the control group.

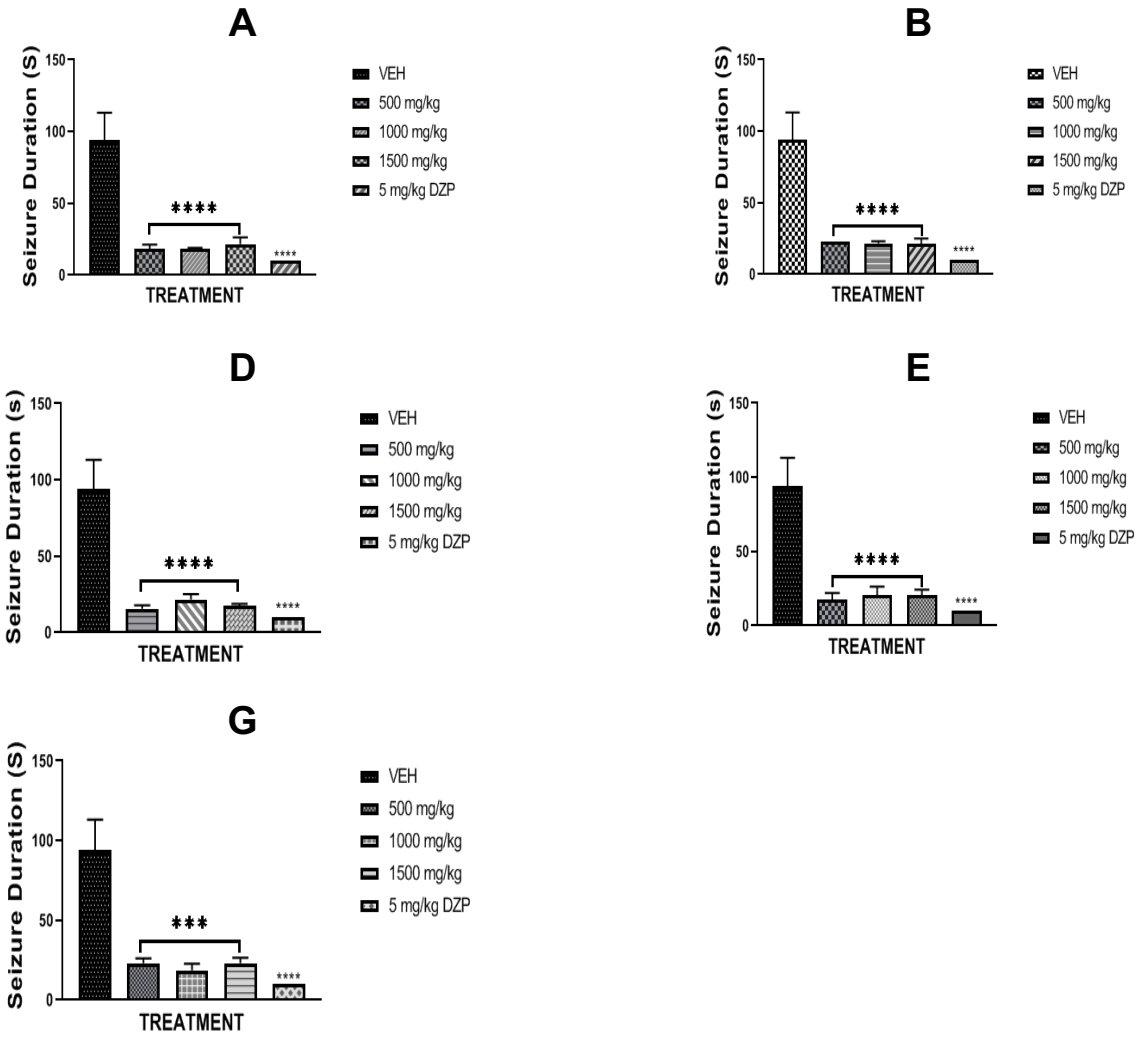


Note: Sample A and G showed significant effect at all the doses by delaying the onset of seizure at ** P<0.003 compared to control while sample (B-F) showed significant effect at *P<0.03 compared to control.

Figure 2 Results of the anticonvulsant effect of samples (A-G) in mice using PTZ model.

3.3 Result of Anticonvulsant Effect of Benzylideneacetophenone Analogues in Mice MES Model.

The samples (A-F) evaluated at 500, 1000 and 1500 mg/kg are equipotent at all the doses by significant reduction of seizure duration at **** P<0.0001 and sample (G) at ***P<0.0007 compared to control (Figure 3).



Note: The samples (A-F) **** P<0.0001 and sample (G) at ***P<0.0007 significantly reduced seizure duration compared to control.

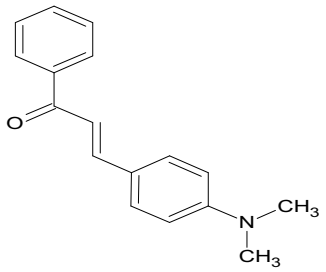
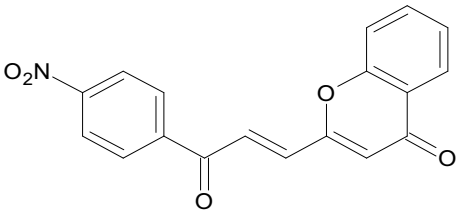
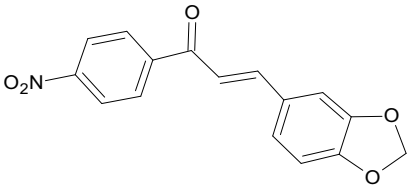
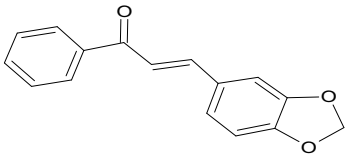
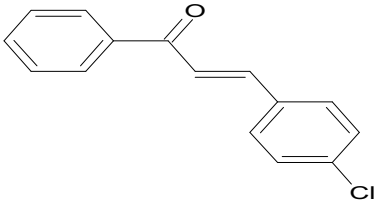
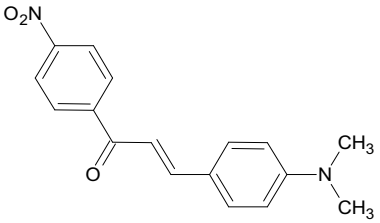
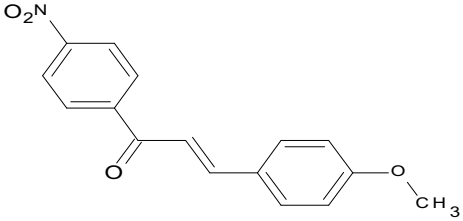
Figure 3 Results of the anticonvulsant effect of samples (A-G) in mice using MES model.

Table 1. Mortality Rate of the MES Study model

Sample/Dose(mg/kg)	Number of Animals	Mortality	% Protection
VEH 10ml	3/5	3	40
Diazepam 5mg	0/5	0	100
(A)			
500 mg	0/5	0	100
1000 mg	0/5	0	100
1500 mg	0/5	0	100
(B)			
500 mg	0/5	0	100
1000 mg	1/5	1	80
1500 mg	1/5	1	80
(C)			
500 mg	0/5	0	100
1000 mg	1/5	1	80
1500 mg	0/5	0	100
(D)			
500 mg	1/5	1	80
1000 mg	0/5	0	100
1500 mg	0/5	0	100
(E)			
500 mg	0/5	0	100
1000 mg	0/5	0	100
1500 mg	0/5	0	100
(F)			
500 mg	0/5	0	100
1000 mg	0/5	0	100
1500 mg	0/5	0	100
(G)			
500 mg	1/5	1	80
1000 mg	1/5	1	80
1500 mg	0/5	0	100

Note: Data showed mortality in sample G: 500 mg/kg and 1000 mg/kg doses respectively as well as sample D: 500 mg/kg. Sample C: 1000 mg/kg, sample B: 1000 mg/kg and 1500 mg/kg while control groups of the samples observed 60% mortality.

Table 2: Chemical structures of benzylideneacetophenone analogues

Sample	Structure	Name
(A)		4-Dimethylaminochalcone
(B)		
(C)		1(4'-Nitrophenyl)-3(1,3-benzodioxolyl)-2-Propen-1-one
(D)		1-Phenyl-3(Benzodioxolyl)-2-Propen-1-one
(E)		4-Chlorochalcone
(F)		4'-Nitro-4-Dimethylaminochalcone
(G)		4'-Nitro-4-methoxychalcone

Note: The chemical structures of benzylideneacetophenone analogues used for the assay.

4. Discussion

Strychnine model (Figure 1), act via glycine pathway. Sample D and E, significantly (P< 0.03) suppress the onset of seizure when compared to control at all the doses administered. Sample A and B exhibited similar effect by delaying the onset of seizure significantly (P< 0.003) in all the doses assessed when compared to control as illustrated in Figure 1. Sample F showed a different pattern by delaying the onset of seizure at low and high dose significantly (P< 0.003) prevent the onset of seizure compared to control while the median dose significantly (P< 0.03) suppresses the onset of seizure compared to control. Sample C and G showed similar pattern in delaying the onset of seizure significantly (P< 0.0001; 0.0007) at low dose respectively when compared to control. The median and high doses of samples assayed, significantly delayed the onset of seizure. This suggest that sample C and G are likely posing as partial agonist at the glycine receptor, also the standard drug diazepam does not reveal any significant effect compared to control in delaying the onset of seizure in strychnine model. This assertion is on the fact that diazepam ameliorates or control seizure provocation through the $GABA_A$ sub-unit receptor which is a prototype working channel for the PTZ –induce seizure contrary to the strychnine site of action[12].

PTZ Model, all samples were assessed; samples B, C, D, E F and standard drug diazepam, displayed similar effect of delaying the onset of seizure. Although, sample A and G, displayed similar effect in delaying or preventing the onset of seizure at all the doses administered (P< 0.003) when compared to the control group. Less interaction or less potentiating of the GABA receptor complex suggest the poor seizure control effect of these substances in this study model as seen in Figure 2.

In the MES model (Figure 3), all the samples assessed did not show dose dependent effect in suppressing or reducing the duration of seizure. These samples; 4-Dimethylaminochalcone (A), Benzopyranone analogue (B), Benzodioxylchalcone (D), Chlorochalcone (E), showed extremely significant (P< 0.0001) effect when compared to control. However, the samples at the doses assessed are equipotent when compared to the standard drug diazepam. 4'-Nitro-4-Methoxychalcone (G); Sample C and G are equipotent at all the doses administer with highly significant (P< 0.0007) when compared to control. These compounds contained Nitro functional groups which tend to reduce the potency of these samples compared to the analogues without 4'-Nitro derivatives such as sample A and D as shown in the mortality evaluation (Table 1). Although sample (E) without Nitro group at 4'-position contained Chloro substituent at position C-4 (Table 2.0) enhanced the effect in delaying the onset of seizure[13] However, 1-(4'-Nitrophenyl)-3(γ-benzopyranoyl)-2-propen-1-one prevents and control seizure. This could be due to benzopyranoyl moiety which has been reported to have anti-seizure properties[14]. The standard drug was more potent when compared to sample C, F and G in preventing the onset of seizure. This may be due to test sample stabilizing and decreasing the action potential of neurons or due to inhibition of voltage dependent Na^+ channels and may prevent seizure from spreading[1,2,8,15]. The samples showed various percentage of protection in the MES model when compared to the standard drug and control which had 100 and 40 percentage protection respectively as shown in Table 1.0.

5. Conclusion

The antiseizure study of the samples in strychnine model revealed that sample A, B and F delayed onset of seizure in this model while in MES model, all the samples showed antiseizure effect by significant reduction of seizure duration. This could be due to inhibition of GABA-Aminotransferase or by prolonging inactivation of sodium channels; this could be use in control of generalized and partial complex seizure. However, the samples are less active in control of seizure induced by PTZ.

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Conflict of Interest

The authors of this article declare no conflict of interest.

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