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# Synthesis of benzylideneacetophenone and anti-seizure determination in experimental rodents

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**Abstract**
Benzylideneacetophenone was synthesized using benzaldehyde and acetophenone. The melting point recorded 52-54oC, percentage yield 72.8% and Rf value of 0.68 in solvent system; ethylacetate: petroleum spirit (2:1) were determined. The sample was subjected to anti-seizure evaluations using mice as the phase one screening: Pentylenetetrazole (PTZ), Strychnine (STC) and Maximal Electroshock (MES) models at a dose of 500, 1000 and 1500 mg/kg. The phase two (II) assessment: the same doses of 500, 1000 and 1500 mg/kg/day treatment for 15 days using rats before induction of seizure using PTZ (100 mg/kg) and Strychnine (2 mg/kg) as convulsion inducing agents. The result of the anti-seizure effect showed that the sample delayed the onset of seizure in chemical model while in the MES decreased the duration of seizure. The result of the phase II models also confirmed that sample delayed the onset of seizure in strychnine compared to PTZ model appreciably at P<0.03-0.003 compared to control. These showed that the samples elicit anti-seizure effect in both phases of the experiment conducted this is line that flavonoid tend to have anti-seizure properties. The chemical structure of sample A was characterized using Infrared (IR), 1H, and 13C-NMR spectroscopic studies.

**Keywords:** benzylideneacetophenone, anti-seizure effect, PTZ, MES, strychnine model

## Introduction
Benzylideneacetophenone is a naturally occurring compounds found in medicinal plants. It belongs to the chemical family of flavonoids and are found to play important role in the biosynthesis of cyclic compounds such as flavonoids, isoflavonoids and aurones which have been reported to have anti-inflammatory, antimicrobial and anticancer properties [1, 2, 3, 4] Numerous compounds have been reported to have anti-seizure properties such as phenytoin, benzodiazepines, barbiturates, iminostilbenes, succinimides, valproic acids and oxazolidinediones [5, 6]. The aim of these studies is to assess benzylideneacetophenone as potential antiseizure in mice and rats using pentylenetetrazole, strychinine and maximal electroshock models because epilepsy affect about 40-50 million people worldwide [7, 8, 9, 10].

## Materials and Methods Materials
All reagents and drugs were purchased from reputable chemical and pharmaceutical companies.

## Methods
About 0.198 M of benzaldehyde and 0.198 M of acetophenone were reacted in 250 ml beaker containing magnetic bead, 50 ml of ethanol was measured and added into the content of the beaker it was immersed in an ice-bath and stirred using magnetic stirrer until 0oC temperature was ascertained by the thermometer and 50 ml of cold 10% of potassium hydroxide was added in a drop-wise manner using burette with continuous stirring, at the end of the addition, it was allowed to stir for 30 minutes and kept in a refrigerator for 7 days. The mixture was neutralized with 10% acetic acid at 0oC and the precipitate filtered under suction. The crystals washed using cold water, this was air dried, weighed, melting point and Rf value determined in a solvent system; Ethyl acetate Petroleum spirit (2:1) determined to give sample A. The sample was subjected IR, Proton and 13C NMR for structural elucidation [11, 12].

## Pharmacological Assay Acute Toxicity Test
Acute toxicity test was determined using the Lorke's method for LD50 and dose determination.

## Anti-seizure Assay Phase I
### PTZ Model
The Mice were divided into five groups of six mice per group. The samples were administered using the outline below.
Group I and II; were given distilled water (10 ml/kg) and standard drug diazepam (5 mg/kg) Group III, IV and V: were administered at a dose of 500, 1000, 1500 mg/kg of the test sample.
The convulsion was induced in the animals by administering Pentylenetetrazole 100 mg/kg interpertioneal 1 hour after the administration of the extracts [9].

### Strychnine Model
The same procedure was adopted as stated in PTZ model and Strychnine was administered at a dose of 2 mg/kg to induced seizure.

**Electroshock**

The Same protocol was adopted as stated in PTZ model, maximal electroshock was used to induce seizure at 100 mA in mice.

**Phase II**

The same protocol was used for PTZ and strychnine model as stated in phase I above, the samples were administered daily for 15 days before the induction of seizure in rats weighing (130-220 g) using PTZ (100 mg/kg) and strychnine (2 mg/kg).

**Result**

**Anti-Seizure Evaluation**

Figure 1; result showed very significant ($P$<0.0001) increased latency to seizure at 1000 & 1500 mg/kg of the benzylidineacetophenone, sample A compared to the control group Vehicle in the Phase 1: acute study, Pentylenetetrazole model.

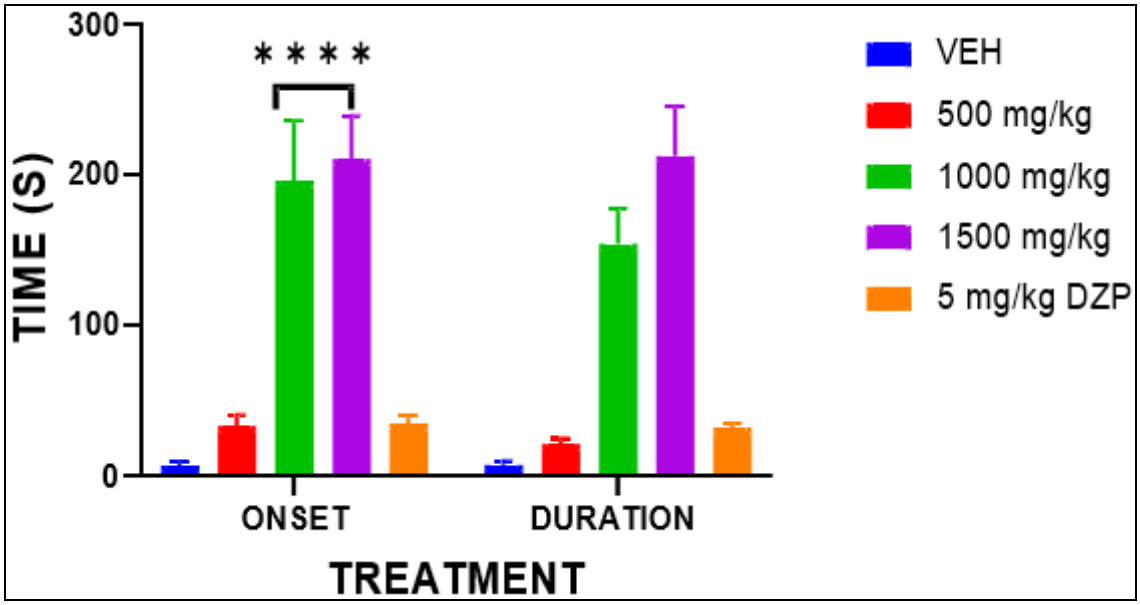


**Fig 1:** PTZ Model of Sample A, acute study analyzed using graph pad prism 8.3: ANOVA followed by multiple comparism post hoc test. Significant ****=$P$<0.0001. VEH= Vehicle, DZP= Diazepam

**Anti-Seizure Evaluation**

Figure 2; result showed significant ($P$<0.02) increased latency to seizure at 500, 1000 & 1500 mg/kg of the benzylidineacetophenone, sample A compared to the control group Vehicle in the Phase 1: acute study, strychnine model.

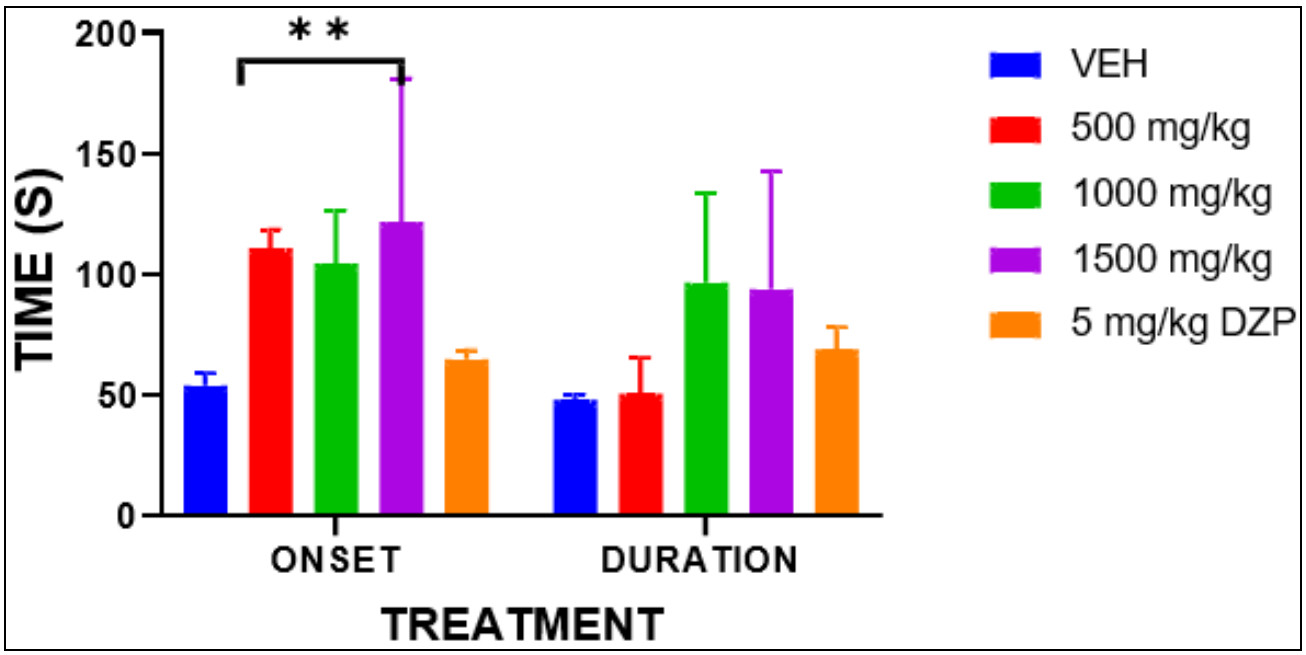


**Fig 2:** Antiseizure effect of sample A using strychnine model. Acute study analyzed using graph pad prism 8.3: ANOVA followed by multiple comparison post hoc test. Significant **=$P$<0.02. VEH=Vehicle, DZP=Diazepam

**Anti-Seizure Evaluation.** Figure 3; result showed very significant ($P$<0.001, 0.0001) decreased duration of seizure at 500, 1000 & 1500 mg/kg of the benzylidineacetophenone, sample A compared to the control group (Vehicle) in the Phase 1: acute study, maximal-electroshock model.

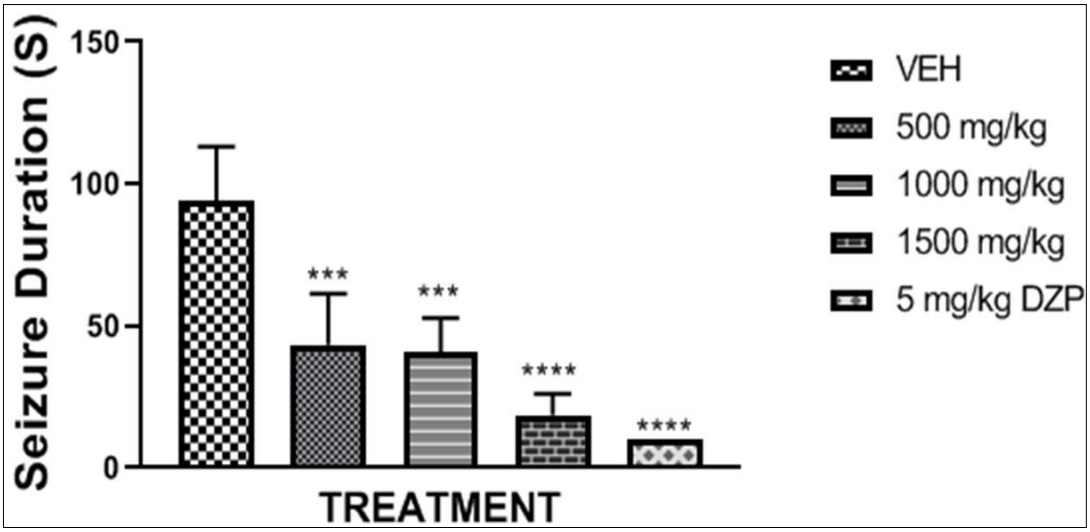


**Fig 3:** Sample A. Showed seizure duration of benzylideneacetophenone (Chalcone) using MES, indicating significant (***= P <0.001, ****= P <0.0001) reduction compared with VEH, control; DZP, Diazepam.

**Mortality Rate.** The test substance at all doses applied in this study indicated protection from maximum electroshock induced death at 100%

**Table 1:** Mortality rate in Electroshock Model

| Sample | Mortality Rate | % Protection |
|---|---|---|
| VEH | 3/5 | 40 |
| Dzp5mg/kg | 0/5 | 100 |
| A | | |
| 500 mg/kg | 0/5 | 100 |
| 1000mg | 0/5 | 100 |
| 1500 mg | 0/5 | 100 |

Data showed no mortality with 100 % protection against electro convulsion in the test group. VEH= Vehicle or Control.

**Phase II Study Anti-Seizure**

Figure 4 result showed no statistical significant increased latency to seizure of the benzylidineacetophenone, sample A compared to the control group (Vehicle) in the Phase 2: sub-acute study, pentylenetetrazole model.

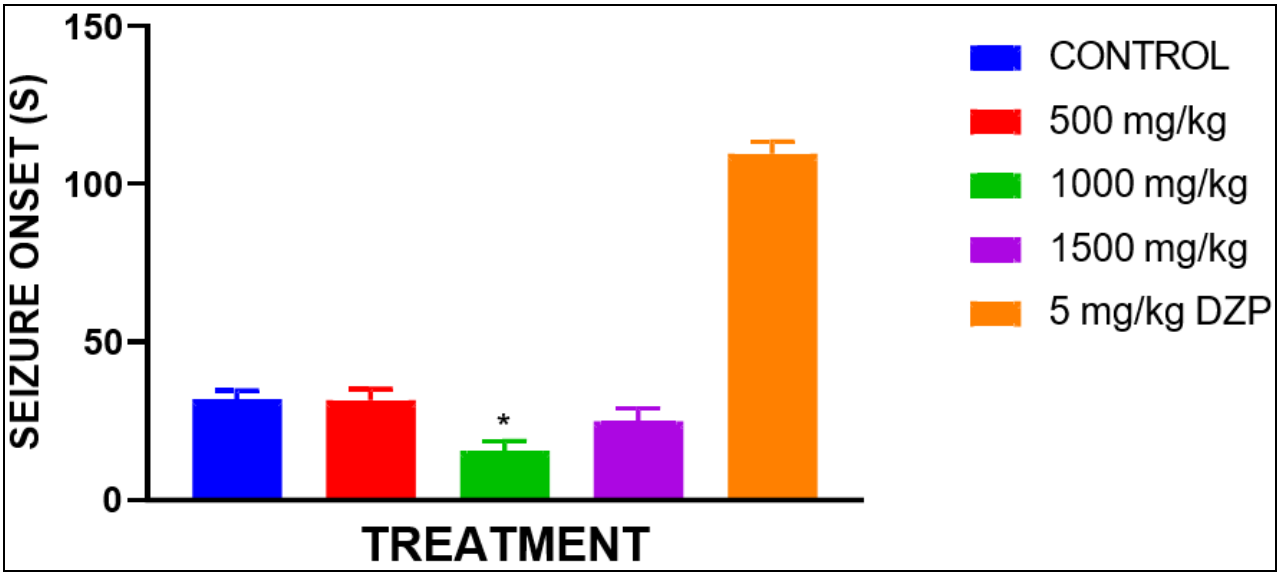


**Fig 4:** PTZ Model of Sample A, sub-acute study analyzed using graph pad prism 8.3: ANOVA followed by multiple comparison post hoc test. Significant VEH= Vehicle, DZP= Diazepam

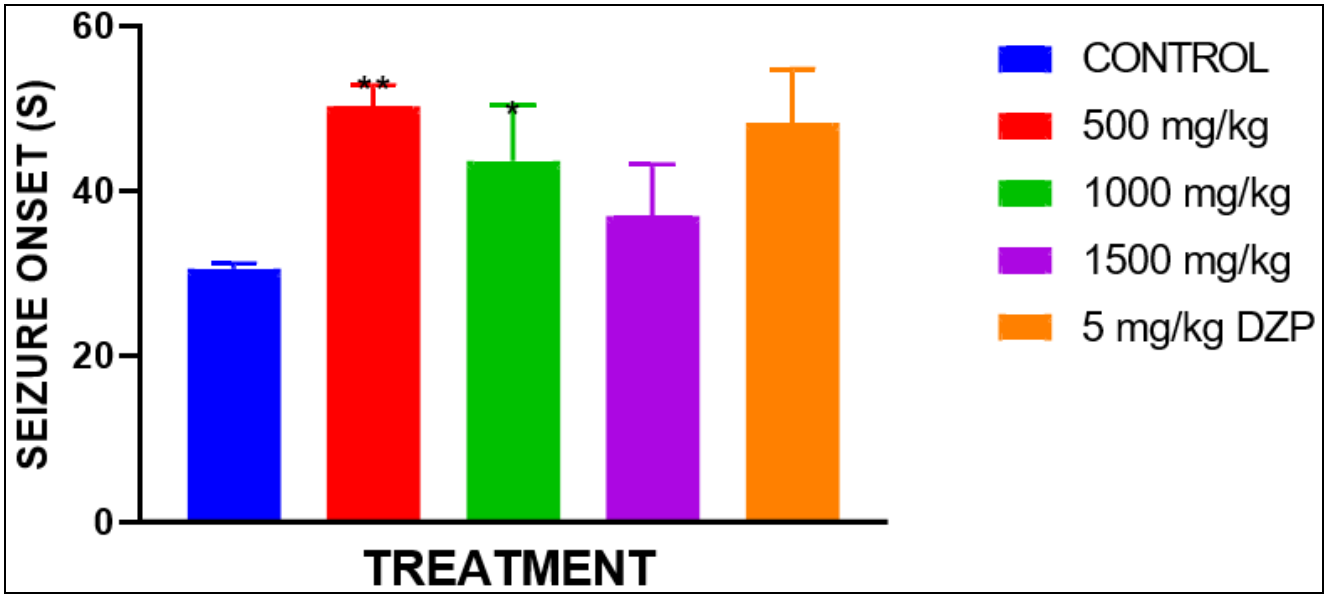


**Fig 5:** Antiseizure effect of sample A using strychnine model. Sub-acute study analyzed using graph pad prism 8.3: ANOVA followed by multiple comparison post hoc test. Significant **=*P*<0.02; *=*P*<0.03. VEH=Vehicle, DZP=Diazepam

**Table 2:** IR Analysis

| S/N | IR(cm-1) | Remark |
|---|---|---|
| 1. | 3057-2616 | Due to C-H stretch of Aromatic ring |
| 2. | 1658.7 | Due to Carbonyl functional group of Ketone. |
| 3. | 1497-1446 | Due to C=C stretch |

**1H- NMR and 13C-NMR**

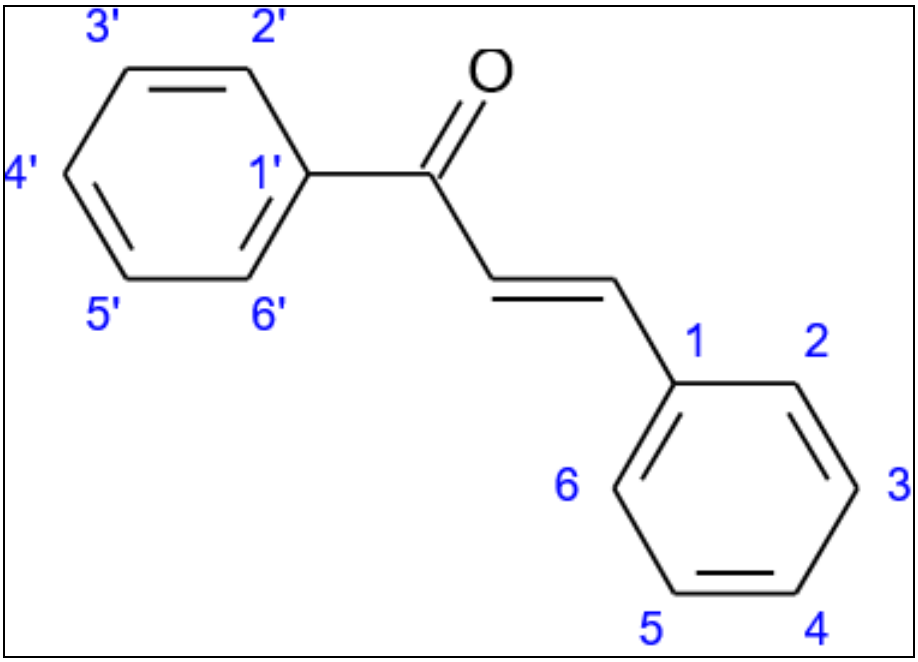


**Fig 6:** Chemical structure of benzylideneacetophenone

**Table 3:** 1H- NMR

| S/N | Chemical shift(ppm) | Interpretation |
|---|---|---|
| 1. | 8.01 | Due to aromatic proton at 2, 6 and 2', 6' proton |
| 2. | 7.79 | Due to α-carbon and proximity to carbonyl group. |
| 3. | 7.52 | Due to β-carbon methine proton |
| 4. | 7.37 | Multiplet due to aromatic protons |

**Table 4:** 13C-NMR

| SN | Chemical shift(δ) | Remark |
|---|---|---|
| 1. | 190.74 | Due to Carbonyl carbon of Ketone |
| 2. | 145.09 | Due to Methine carbon (α)due to proximity to carbonyl carbon. |
| 3. | 133.96 | Due to β-carbon, Methine carbon. |
| 4. | 138.42 | Due to aromatic quaternary carbon C-1' position. |
| 5. | 135.09 | Due to quaternary carbon at C-1 position of the aromatic ring |
| 6. | 130.84 | Due to carbon at 2 and 6 position |
| 7. | 129.23 | Due to aromatic carbon at position 2' and 6 because are equivalent carbon |
| 8. | 128.90 | Due to 3 and 5 position of the aromatic position |
| 9. | 128.74 | Due to aromatic carbon at 4' |
| 10. | 128.78 | Due to aromatic carbon at position 3' and 5' |
| 11. | 122.26 | Due to carbon at position 4 |

## Discussion

Benzylidene acetophenone showed pharmacological significant effect in delaying the onset of seizure induced using PTZ at median and high dose compared to control and standard drug at $P<0.0001$, while in the strychnine model, the sample screened at all the doses of 500, 1000 and 1500 mg/kg showed significant delay on the onset of seizure when compared to control at $P<0.003$ as shown in figure 1-3. It also showed the anti-seizure effect in the electroshock model by reducing the duration seizure compared to negative control and standard drug, figure 5. In phase II Model, after administration of the sample for 15 days and induction of seizure using strychnine, benzylidene acetophenone at low and median dose of the sample delayed the onset of seizure in strychnine model when compared to control at $P<0.02$-$0.03$ respectively. The doses showed more effect when compared the standard dose and control because benzylidene acetophenone tends to prevent seizure via glycine pathway, figure 1-4 [13]. However, in the PTZ model the median dose of the sample showed significant effect at $P<0.02$ compared to control as shown in Figure 5 [8, 14, 15]. The synthesis yielded 72.8%, 52-54oC melting point recorded using Gallenkamp apparatus and Rf value of 0.68 in solvent system; ethylacetate: petroleum spirit (2:1) were determined. The result of IR analysis showed that a prominent peak at 26116 cm-1 which due to C-H stretch and residual ethanol used for the synthesis. The peak at 1658 cm-1 due to carbonyl carbon of ketone while 1497-1446 cm-1 due C=C stretch (Table 2.0). The Proton NMR revealed four signals at δ 8.00 ppm due to aromatic proton at C- 2, 6 and 2', 6' protons. The peak at δ7.79 ppm due to methinne proton at α carbon position, and could be due to proximity to the carbonyl functional group. The peak at 7.52 ppm is due to β-carbon methine proton and the peak at δ7.37 ppm is multiplet due to aromatic protons [15]. The carbon -13 NMR revealed eleven (11) peaks. The two dimension NMR (APT) which differentiates the nature of carbon presence as shown in table 4.0. The sample showed 3 quaternary carbon with a peak signal at δ 190.72 ppm, due to carbonyl group, 138.42 and 135.09 ppm due to C-1' and C-1 aromatic carbon respectively as shown in Figure 6.0, the 9 peaks is made of two non- aromatic carbon (ketoethylenic group) i.e the α,β-carbon at δ 145.09 and 133.96 ppm respectively. The 7 peaks shows various methine carbon of the aromatic ring some are chemically equivalent as illustrated in Table 4.0 [11]. The data were compared as reported in literature [16].

## Conclusion

The evaluation of the test substance, benzylidine acetophenone suggests anti-seizure potential in 500- 1500 mg/kg doses in strychnine model as reflected in both acute and sub-acute duration of study with acceptable safety level in oral treatment as seen in MES. Further characterization study may be useful for clinical incorporation.

## Acknowledgement

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## Conflict of Interest

Authors declare no conflict of interest in this study

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