ASSESSMENT OF ANALGESIC POTENTIAL OF DIBENZYLIDENE DERIVATIVES OF CYCLOPENTANONE

ABSTRACT

Background. *In the search for novel analgesic agents as current pain management options, Dibenzylidene-acetophenone analogues have emerged as potential candidates, and this is due to their diverse pharmacological activities, laying the foundation for innovative exploration into the analgesic potentials of cyclopentanone derivatives.* ***Aim of the study****. This study is aimed at the determination of the analgesic potentials of cyclopentanone derivatives which include; 2,5-bis[(4-dimethylaminophenylmethylidene]cyclopentan-1-one, 2,5-bis[(4-methoxyphenyl)methylidene]cyclopentan-1-one, 2,5-diethylidenecyclopentan-1-one, 2,5-diphenylmethylidenepentan-1-one, and 2,5-dibenzodioxoylmethyledenecyclopentan-1-one (D_6-D_{10} respectively).* ***Methodology.*** *The study measured analgesia potential using the hot plate and tail flick model. The mice were divided into five groups, (GPs): GP I and V were control group (0.2 ml/kg distilled water) and standard group (50 mg/kg Tramadol Hydrochloride) respectively, GP II to IV were administered the different doses (500, 1000, and 1500 mg/kg) of the test compounds respectively and evaluated 30 min afterwards. The latency to pain was observed at 30, 60 and 90 minutes in all GPs respectively.* ***Results.*** *Promising analgesic potential was discovered in: D6 showed significant increase in analgesic potential ($p<0.0487$) at 60 mins with a dose of 1500 mg/kg; D7 showed significant increase ($p<0.0099$) at 60 min with 500mg/kg and D10 also showed significant increase ($p<0.0232$) at 30 min with 1000mg/kg for the hot plate study model. On the tail-flick model, D6 showing significant increase ($p<0.0451$) at 90min with 1500 mg/kg while in contrast D8 showed significant increase($p<0.0001$) at 30 min with 1500 mg/kg and D10 showed significant increase ($p<0.0001$) at 30 min with 1000 mg/kg for the.* ***Conclusion.*** *The study showed remarkable analgesic potential in D6, D7, and D10 cyclopentanone derivatives on the hot plate model while D6, D8 and D10 cyclopentanone derivatives showed remarkable analgesic potential on the tail-flick model.*

Keywords: *Analgesia; cyclopentanone derivatives; hot plate; pain inhibition; tramadol hydrochloride.*

1. INTRODUCTION

One thing that has remained a functional unit of life is pain and this could be physiological, physical and chemically associated. The species of life that are responsive or reactive to pain and painful sensations are animals including humans. Pains may vary depending on the tissue involved and the cause; in all, pain is not a pleasant sensation and as such is not easily endured or enjoyed by many. There are consistent attempts to alleviate pain from sufferers especially through researches to achieve new and more convenient medicinal product that will be helpful in certain pain with less side effects [1]. In medicine, pain management is a basic necessity. The effectiveness and side effects of current analgesics are limited, and the possibility of abuse has become a significant factor, hence the need for

more study and research into new molecular entities with lesser toxicity, side effects and a better safety and efficacy profile.

Dibenzylidene derivatives belong to a class of compounds characterized by their unique chemical structure and potential pharmacological properties. These compounds are known for their distinctive arrangement of benzylidene groups, which confer specific characteristics that influence their biological activity and therapeutic potential.

The chemical structure of dibenzylidene derivatives typically consists of two benzene rings linked by a central carbon-carbon double bond (-CH=CH-) bearing benzylidene groups. This arrangement imparts rigidity and planarity to the molecule, allowing for specific interactions with target receptors or enzymes involved in pain perception and modulation [2]. The presence of aromatic rings in the structure contributes to the compound's lipophilicity and overall pharmacokinetic profile, affecting factors such as solubility, distribution, and metabolism in biological systems [3].

There are several analogues of dibenzylidene, however this study focuses on the cyclopentanone analogues.

The properties of dibenzylidene derivatives, including D_6 to D_{10}, are influenced by their chemical structure and molecular composition. These compounds may exhibit varying degrees of potency, selectivity, and efficacy in modulating pain pathways based on their structural features and interactions with biological targets [4]. The presence of specific functional groups or substituents on the benzylidene moieties can further impact the compound's activity and affinity for its molecular targets, potentially enhancing their analgesic effects and therapeutic utility in pain management [5].

The planar structure of dibenzylidene derivatives allows for favorable interactions with target proteins or receptors involved in pain transmission and regulation. By binding to specific sites within the target molecules, these compounds may exert modulatory effects on signal transduction pathways, neurotransmitter release, or ion channel activity, ultimately influencing pain perception

Comment [R1]: Since no synthesis includes in this paper, then what is the need of chemistry of drug?

Comment [R2]: Do you quote this sentence for the following reference?
Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *TheScientificWorldJournal*, *2013*, 162750. https://doi.org/10.1155/2013/162750

and response [6]. Additionally, the conjugated system of the carbon-carbon double bond in dibenzylidene derivatives can contribute to their stability and reactivity, affecting their pharmacological profile and potential for further chemical modifications to optimize their biological activity [7].

2. METHODS

2.1 Ethical consideration

The study protocol was ethically approved by the Department of Pharmacology Ethical Committee, with registration identity as NDU/PHARM/AEC/043b.

2.2 Drugs and Chemicals

The standard drug used in the study is Tramadol Hydrochloride BP 50 mg with brand name, WIZTRAM-100 capsules with NAFDAC REG NO- C4-1529, with Batch No. CE1023 purchased from a community pharmacy (Keto-Divine Pharmacy, Amassoma. Bayelsa state)

The Chemicals used in this study are Cyclopentanone derivatives of Dibenzylidene analogs which includes:

1. 2,5-bis[(4-dimethylaminophenylmethylidene] cyclopentan-1-one (D6)]
2. 2,5-bis[(4-methoxyphenyl) methylidene] cyclopentan-1-one (D7)]
3. 2,5-diethylidenecyclopentan-1-one (D8)
4. 2,5-diphenylmethylidenepentan-1-one (D9)
5. 2,5-dibenzodioxoylmethyledenecyclopentan-1-one (D10)

2.3 Animal

The animals used in this study were mainly male mice sourced from the animal house unit of Pharmacology and Toxicology, Niger Delta University. The animals were kept under healthy conditions of light and dark cycle 12:12 hours, with relative humidity of 55-65% and temperature of 24.0±0°C. The mice were taken to the laboratory on daily basis for acclimatization. The animals were exposed to the hot plate without switching on the power as orientation for three days before the practicals commenced, so was the animals for the tail flick model. These were done in accordance with the animal handling rules [8].

2.4 Study Design

Male mice were weighed and divided at random into group of five (5) with six (6) in each group, this was done for both hot plate and tail flick model respectively. Group I was used as control group and was orally administered 0.2 ml/kg of water. Group II, III, and IV were orally administered 500 mg/kg, 1000 mg/kg and 1500 mg/kg of the respective test compounds, while Group V was administered 50 mg/kg of the standard drug (Tramadol Hydrochloride). This process was repeated for all the five test compounds which include, D_6 (2,5-bis[(4-dimethylaminophenylmethylidene]cyclopentan-1-one), D_7 (2,5-bis[(4-methoxyphenyl) methylidene] cyclopentan-1-one), D_8 (2,5- diethylidenecyclopentan-1-one), D_9 (2,5-diphenylmethylidenepentan-1-one),and D_{10} (2,5- dibenzodioxoylmethyledenecyclopentan-1-one).

2.4.1 Hot-plate Model

The rats were put on a hot plate that was kept at 55 degrees Celsius. The time it took for the rats to jump or lick their paws in response to the thermal pain was measured as the latency period or reaction time (in seconds). At 30, 60, and 90 minutes following the prescribed treatments and response time was observed and noted. To avoid damaging the paw tissues, the response timeline was set at 45 seconds [9].

$$\%\ Inhibition = \frac{Latency\ test - Latency\ control}{Latency\ test} * 100$$

2.4.2 Tail-flick Model

Pain was evaluated using the method of tail flick to establish the analgesic potential of the compounds used in this study as previously demonstrated [10]. The terminal end of the tail of each mouse was deep into water bath with 50^{o}C temperature and the pain response timeline was 30 seconds for 30 min, 60 min and 90 min after the treatments and withdrawal time was noted for each mice in every group.

$$\%\ Inhibition = \frac{Latency\ test - Latency\ control}{cut\ of f\ tail\ latency - Latency\ control} * 100$$

2.5 Statistical Analysis

The laboratory data derived from the study were analyzed using Graph Pad Prism 10.2, two-way ANOVA, post-hoc Dunett multiple comparison test was applied. All results were presented as Mean ±SEM in table form or graph and significant levels were observed as $p < 0.05$.

3. RESULTS

3.1. Hot Plate Model

3.1.1 Latency to pain in hot plate model

The result indicated analgesic potential in D_6, D_7, and D_{10} using the hot plate model as shown in figure. However, Figure 1 did not show statistical significance, but have little biological indication for increase latency to pain.

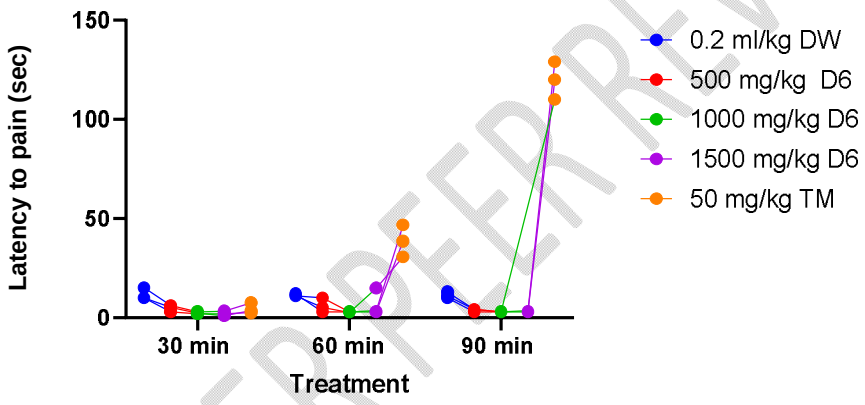

Figure 1 Showed D_6 at 30 min; 1000, 1500 mg/kg indicated *, *Significant decrease ($p<0.0300$, 0.0237) when compared to the control 0.2 mg/kg. 60 min: 1500 mg/kg of D_6 indicated *Significance increase ($p<0.0487$) when compared to the control 0.2 mg/kg. D6= 2,5-bis[(4-dimethylaminophenylmethylidene] cyclopentan-1-one (D6)] TM= Tramadol Hydrochloride

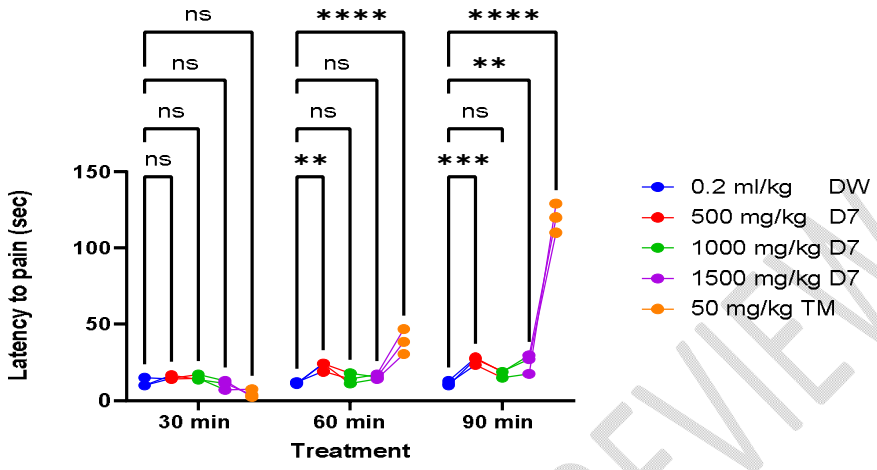

Figure 2 Showed D_7 at 60 min; 500mg/kg indicated **Significant increase ($p<0.0099$) when compared to the control 0.2 mg/kg. 90 min: 500, 1500 mg/kg of D_7 indicated ***, **Significance ($p<0.0006$, 0.0017) when compared to the control 0.2 mg/kg. 2,5-bis[(4-methoxyphenyl) methylidene] cyclopentan-1-one (D7) TM= Tramadol Hydrochloride.

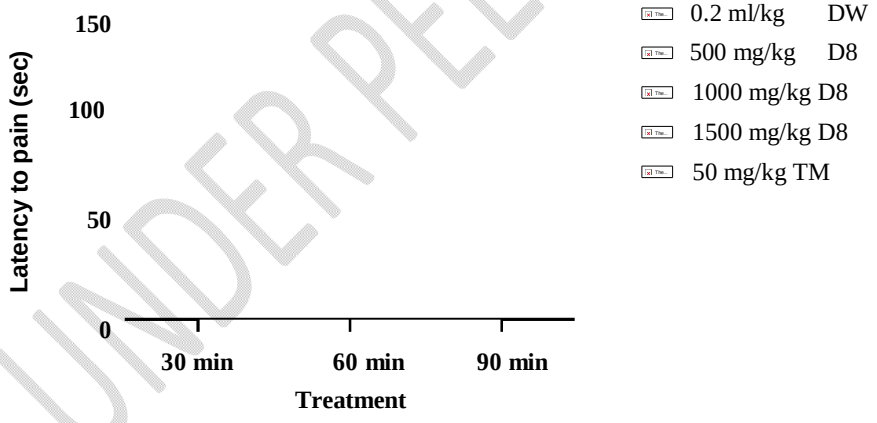

Figure 3 Showed D_8 indicated no statistically significant increase when compared to the control 0.2 mg/kg. 2,5-diethylidenecyclopentan-1-one (D_8) TM= Tramadol Hydrochloride.

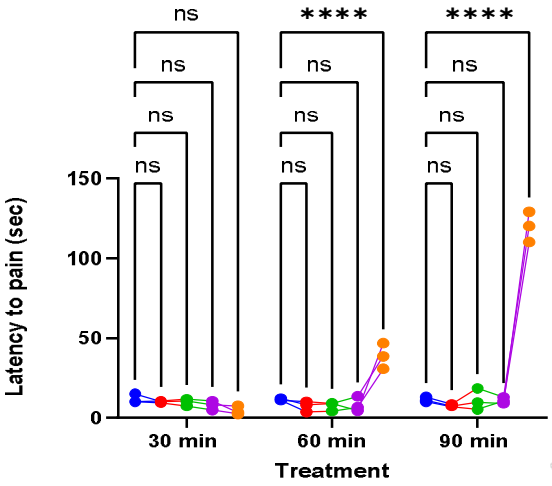

Figure 4 showed D_9 indicated no statistically significant increase when compared to the control 0.2 mg/kg. D9= 2,5-diphenylmethylidenepentan-1-one (D_9) Tm= Tramadol hydrochloride

Comment [R3]: TM??

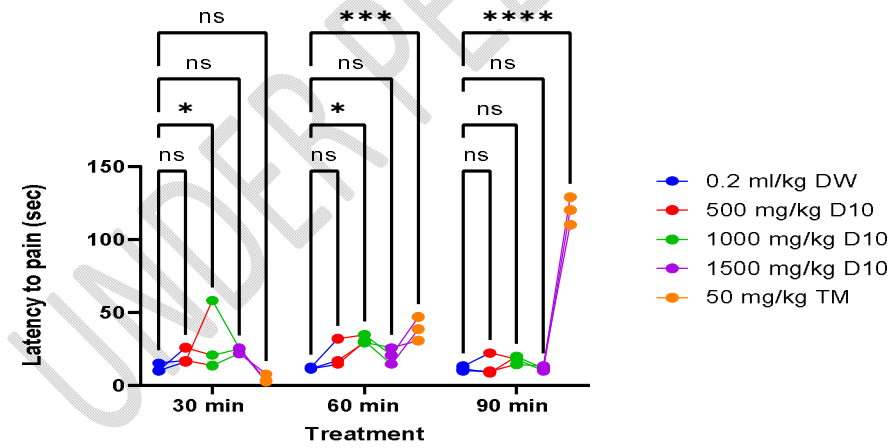

Figure 5 Showed D_{10} at 30 min; 1000mg/kg indicated *Significant increase ($p<0.0232$) when compared to the control 0.2 mg/kg. 60 min: 1000 mg/kg of D_{10} indicated *Significance ($p<0.0160$) when compared to the control 0.2 mg/kg2,5-dibenzodioxoylmethyledenecyclopentan-1-one (D10) TM= Tramadol Hydrochloride.

3.1.2 Percentage Pain Inhibition in Hot plate model

The percentage inhibition showed significant increased value in D_6, D_7, and D_{10} as seen in Table 1, 2, and 5 respectively. This is in conformity with the figures 1, 2 and 5 of the results of the hot plate model test.

Table 1 Percentage pain inhibition of 2,5-bis[(4-dimethylaminophenylmethylidene] cyclopentan-1-one (D_6).

Treatment (mg/kg)	30 minutes (%)	60 minutes (%)	90 minutes (%)
Control	0.000	0.000	0.000
Tramadol 50	-159.333	-70.700	90.535****
500	-150.052	-88.833	-208.154
1000	-379.700	-304.643	-277.667
1500	-455.700	56.643**	-247.692

Table showed results with statistical significance* = p <0.04 ** = p <0.003, *** = p <0.0001, **** = p <0.001, respectively.

Table 2 Percentage Pain inhibition of 2,5-bis[(4-methoxyphenyl) methylidene] cyclopentan-1-one (D_7).

Treatment (mg/kg)	30 minutes (%)	60 minutes (%)	90 minutes (%)
Control	0.000	0.000	0.000
Tramadol 50	-159.333	70.701***	90.535****
500	22.561*	49.644*	56.756**
1000	23.576*	21.700*	35.625*
1500	12.972	27.511*	54.315**

Table showed results with statistical significance* = p <0.04 ** = p <0.003, *** = p <0.0001, **** = p <0.001, respectively.

Table 3 Percentage Pain inhibition of 2,5-diethylidenecyclopentan-1-one (D_8).

Treatment (mg/kg)	30 minutes (%)	60 minutes (%)	90 minutes (%)
Control	0.000	0.000	0.000
Tramadol 50	-159.333	70.701***	90.560****
500	-18.273	-1.161	2.913
1000	-31.612	-27.777	-2.720
1500	29.994*	30.618*	7.886

Table showed results with statistical significance* = p <0.04 ** = p <0.003, *** = p <0.0001, **** = p <0.001. respectively.

Table 4 Percentage Pain inhibition of 2,5-diphenylmethylidenepentan-1-one (D_9).

Treatment (mg/kg)	30 minutes (%)	60 minutes (%)	90 minutes (%)
Control	0.000	0.000	0.000
Tramadol (50)	-159.333	70.701***	90.535****
500	-16.721	-55.205	-49.079
1000	-18.273	-51.067	-1.432
1500	-48.341	-38.171	-4.232

Table showed results with statistical significance* = p <0.04 ** = p <0.003, *** = p <0.0001, **** = p <0.001. respectively.

Table 5 Percentage Pain inhibition of 2,5-dibenzodioxoylmethyledenecyclopentan-1-one (D_{10}).

Treatment (mg/kg)	30 minutes (%)	60 minutes (%)	90 minutes (%)
Control	0.000	0.000	000
Tramadol 50	-159.333	70.701***	90.535****
500	40.368*	46.125*	15.258
1000	62.073**	63.952**	34.395*
1500	51.172**	43.994*	2.580

Table showed results with statistical significance* = p <0.04 ** = p <0.003, *** = p <0.0001, **** = p <0.001, respectively.

3.2 Tail-Flick Model

3.2.1 Latency to pain in tail-flick model

The result indicated analgesic potential in D6, D8, and D10 with Figure 6, 7 and 8 showing statistical increase using the tail-flick model. However, Figure 9 and 10 indicated significant statistical decrease in latency to pain compared to the control, and have little or no indication for latency to pain.

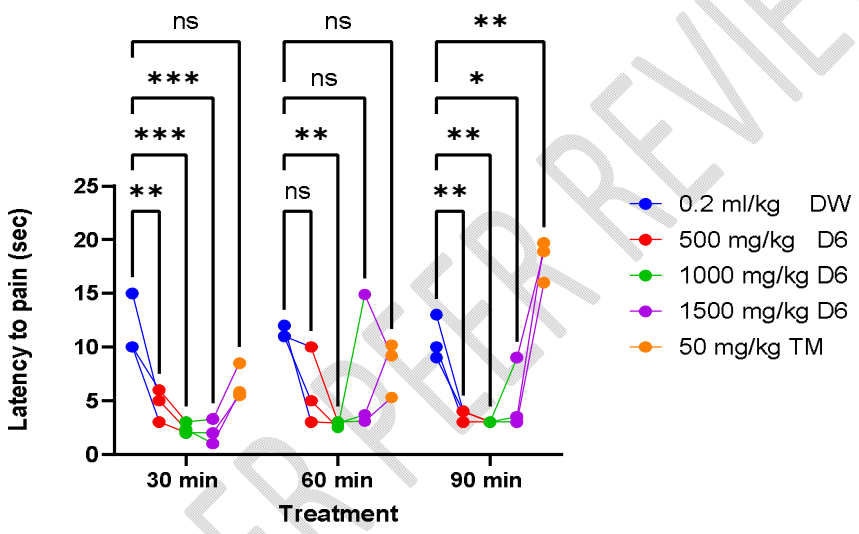

Figure 6 Showed D_6 at 30 min; 500, 1000, 1500 mg/kg indicated **, ***, ***Significant decrease ($p<0.0080$, 0.0005, 0.0003) when compared to the control 0.2 mg/kg. 60 min: 1000 mg/kg of D_6 indicated **Significance decrease ($p<0.0012$) when compared to the control 0.2 mg/kg. 90min: 1500 mg/kg of D_6 indicated *Significance increase ($p<0.0451$) D_6= 2,5-bis[(4-dimethylaminophenylmethylidene] cyclopentan-1-one (D_6)] TM= Tramadol Hydrochloride

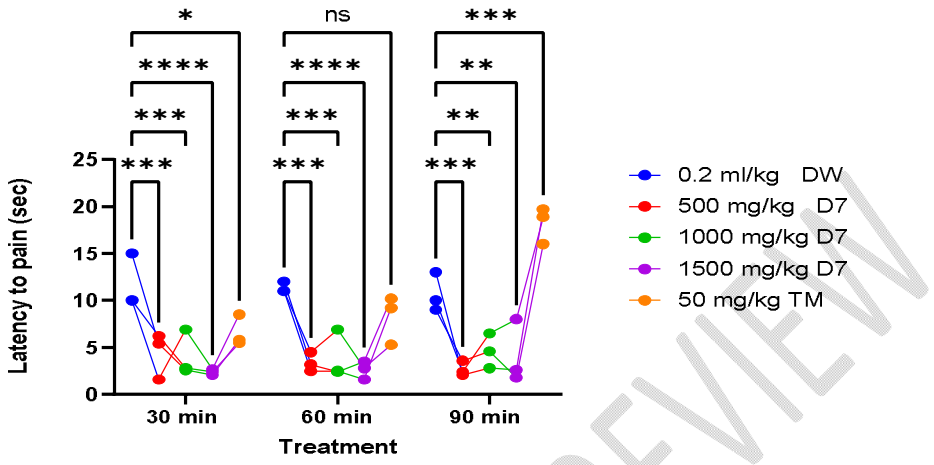

Figure 7 Showed D_7 at 30 min; 500, 1000, 1500 mg/kg indicated ***, ***, ****Significant decrease ($p<0.0005$, 0.0003, 0.0001) when compared to the control 0.2 mg/kg. 60 min: 500mg, 1000, 1500 mg/kg of D_7 indicated ***, ***, **** Significant decrease ($p<0.0002$, 0.0004, 0.0001) when compared to the control 0.2 mg/kg. 90min: 500, 1000, 1500 mg/kg of D_7 indicated ***, **, **, Significant decrease ($p<0.0002$, 0.0038, 0.0017) D_7= 2,5-bis[(4-methoxyphenyl) methylidene] cyclopentan-1-one (D_7)] TM= Tramadol Hydrochloride.

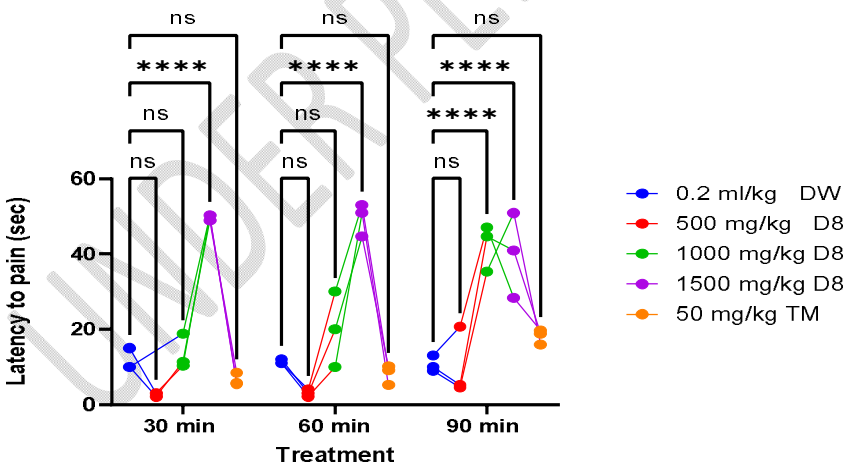

Figure 8 Showed D8 at 30 min; 1500 mg/kg indicated ****Significant increase ($p<0.0001$) when compared to the control 0.2 mg/kg. At 60 min: 1500 mg/kg of D8 indicated ****Significant increase ($p<0.0001$) when compared to the control 0.2 mg/kg. At 90 min: 1000, and 1500 mg/kg indicated ****, ****Significantly increased ($p<0.0001$, <0.0001) when compared to the control 0.2 mg/kg, D8= 2,5- diethylidenecyclopentan-1-one (D8) TM= Tramadol Hydrochloride.

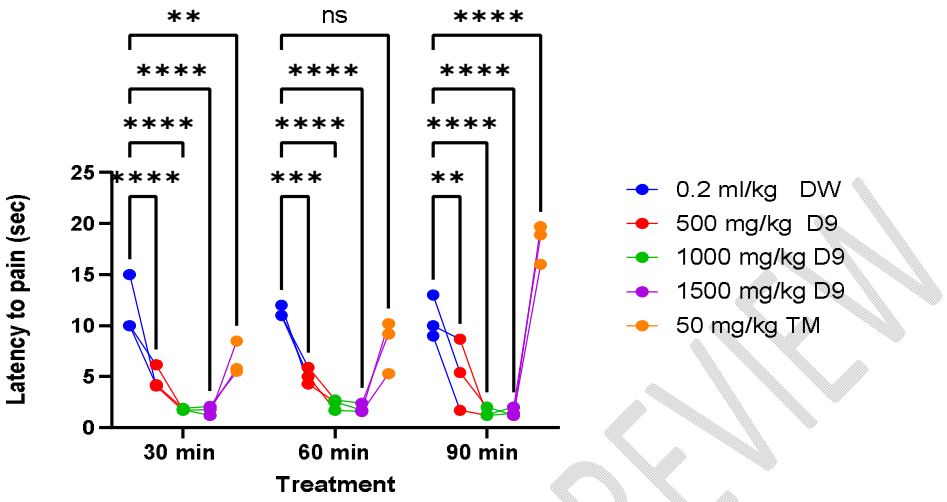

Figure 9 Showed D9 at 30 min; 500 mg/kg, 1000 mg/kg, 1500 mg/kg of D9 indicated****Significant decreased when compared to the control 0.2 mg/kg with adjusted p<0.0001, <0.0001, <0.0001. At 60 min: 500 mg/kg, 1000 mg/kg, 1500 mg/kg of D9 indicated ***, ****, ****Significantly decreased when compared to the control 0.2 mg/kg, with adjusted p<0.0003, <0.0001, <0.0001. At 90 min: 500 mg/kg, 1000 mg/kg, and 1500 mg/kg indicated **, ****, ****Significant decrease when compared to the control 0.2 mg/kg, with adjusted p<0.0015, <0.0001, <0.0001. D9= 2,5- diphenylmethylidenepentan-1-one (D9) TM= Tramadol Hydrochloride

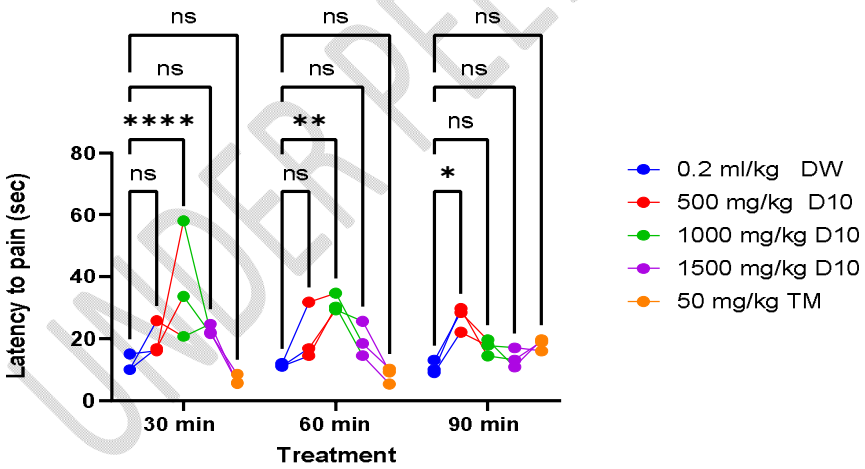

Figure 10 Showed D_{10} at 30 min; 1000mg/kg indicated ****Significant increase (p<0.0001) when compared to the control 0.2 mg/kg. 60 min: 1000 mg/kg of D10 indicated **Significant increase (p<0.0016) when compared to the control 0.2 mg/kg. At 90 min: 500 mg/kg indicated *Significant increase(p<0.0126) when compared to the control 0.2 mg/kg, D10= 2,5-dibenzodioxoylmethyledenecyclopentan- 1-one (D10) TM= Tramadol Hydrochloride

3.2.2 Percentage pain inhibition in tail-flick model

The percentage pain inhibitions are presented in Tables 6 – 10 below, and corresponds with results in Figure 6 – 10, showing significant increase in analgesic potential of D_6, D_8, and D_{10} in the tail flick model test.

Table 6 Percentage pain inhibition of 2,5-bis [(4-dimethylaminophenylmethylidene] cyclopentan-1-one (D_6).

Treatment (mg/kg)	30 minutes (%)	60 minutes (%)	90 minutes (%)
Control	0.000	0.000	0.000
Tramadol 50	52.430**	86.851****	33.194*
500	32.420*	57.128**	22.733*
1000	40.520*	91.426*****	16.466
1500	48.966*	43.912*	20.472*

Table showed results with statistical significance* = p <0.04 ** = p <0.003, *** = p <0.0001, **** = p <0.001, respectively

Table 7 Percentage Pain inhibition of 2,5-bis[(4-methoxyphenyl) methylidene] cyclopentan-1- one (D_7).

Treatment (mg/kg)	30 minutes (%)	60 minutes (%)	90 minutes (%)
Control	0.000	0.000	0.000
Tramadol 50	52.430**	86.851****	33.194*
500	22.181*	38.995*	13.926
1000	29.283*	26.282*	6.631
1500	26.863*	32.215*	15.398

Table showed results with statistical significance* = p <0.04 ** = p <0.003, *** = p <0.0001, **** = p <0.001, respectively.

Table 8 Percentage Pain inhibition of 2,5-diethylidenecyclopentan-1-one (D_8).

Treatment (mg/kg)	30 minutes (%)	60 minutes (%)	90 minutes (%)
Control	0.000	0.000	0.000
Tramadol 50	52.430**	86.851****	33.194*
500	42.795*	21.564*	30.767*
1000	-18.925	-92.926	-367.821
1500	-393.588	-409.432	-339.100

Table showed results with statistical significance* = p <0.04 ** = p <0.003, *** = p <0.0001, **** = p <0.001, respectively.

Table 9 Percentage Pain inhibition of 2,5-diphenylmethylidenepentan-1-one (D_9).

Treatment (mg/kg)	30 minutes (%)	60 minutes (%)	90 minutes (%)
Control	0.000	0.000	0.000
Tramadol 50	52.430**	86.851****	33.194*
500	20.703*	11.128	6.318
1000	12.068	9.785	9.767
1500	15.102	13.072	5.386

Table showed results with statistical significance* = p <0.04 ** = p <0.003, *** = p <0.0001, **** = p <0.001, respectively.

Table 10 Percentage Pain inhibition of 2,5-dibenzodioxoylmethyledenecyclopentan-1-one (D_{10}).

Treatment (mg/kg)	30 minutes (%)	60 minutes (%)	90 minutes (%)
Control	0.000	0.000	0.000
Tramadol 50	52.430**	86.851****	33.194*
500	-81.696	30.125*	22.037*
1000	39.822*	63.952***	30.217*
1500	48.883**	41.897**	21.717*

Table showed results with statistical significance* = p <0.04 ** = p <0.003, *** = p <0.0001, **** = p <0.001, respectively.

4. DISCUSSION

Analgesics are drugs that interact with the nervous system at different levels to alleviate pain either centrally or peripherally without significantly altering consciousness [11]. However, drugs have mixed-action such as Tramadol which interacts with opioid receptors in the CNS, while also inhibiting reuptake of norepinephrine and serotonin, neurotransmitters involved in pain modulation [12].

The tail-flick and hot plate models are two examples of the animal models used in this study to screen for analgesic activity. They use thermal stimuli to stimulate pain states. These two models are very useful in the illustration of pain reducing potential of chemical agents intended for use as medication that is claimed to be above the spinal cord level [13]. The both study models seems to act different central levels as spinal reflex is mediated by the tail flick and the supra-spinal level is mediated by the hot plate model.

In the hot plate model of this study, 2,5-bis[(4-dimethylaminophenylmethylidene] cyclopentan-1-one (D_6)], 2,5-bis[(4-methoxyphenyl)methylidene]cyclopentan-1-one (D_7)], 2,5-dibenzodioxoylmethyledenecyclopentan-1-one (D_{10}) all showed significant analgesic potential when compared to the control DW (Distilled water) with a dose 0.2 mg/kg as shown in Figure 1, 2, and 5. D_6 in contrast with the control group at 60 minutes showed significant increase ($p < 0.0237$), and a percentage latency of 56.6% as shown in Table 1, when a dose of 1500 mg/kg was administered. However, as shown in Figure 2 D_7 showed significant increase ($p < 0.0099$) at 60 min when administered the test substance at a dose as low as 500 mg/kg. At a dose of 1000 mg/kg D_{10} showed significant increase ($p < 0.0232$) in analgesic potential at 30 mins compared to the control DW (Distilled water) 0.2 mg/kg as shown in Figure 2. This result increased correspondingly as shown in Table 3, when the dose was increased with a higher percentage latency of 64% showing a proportional dose dependent relationship.

However, in the Tail-flick method, it was observed that, 2,5-bis[(4-dimethylaminophenylmethylidene] cyclopentan-1-one (D_6)], as shown in Figure 7, 2,5-diethylidenecyclopentan-1-one (D_8), as shown in Figure 8, and 2,5-dibenzodioxoylmethyledenecyclopentan-1-one (D_{10}) as shown in Figure 10, showed significant analgesic potential at various doses and time with varying latencies. The corresponding latency for 2,5-bis[(4-dimethylaminophenylmethylidene] cyclopentan-1-one (D_6)] is shown in Table 6; The corresponding latency for 2,5-diethylidenecyclopentan-1-one (D_8) is shown in Table 8 while that of 2,5-dibenzodioxoylmethyledenecyclopentan-1-one (D_{10}) is shown in Table 4. Although both approaches used thermal stimuli, the tail-flick response suggests a spinally mediated reflex, whereas the paw-licking hot plate response is the result of supraspinally integrated behaviour. This could be the cause of the differences in results between the two models. These results corroborated with Sandow's findings[14]. This manner of variation in result and experimental outcome is consistent with the findings of Kilimozhi *et al*., [15], who investigated the antinociceptive, antipyretic and anti-inflammatory effects of *Clerodendrum phlomidis* in mice and rats.

Furthermore, intra-animal variance such as the thickness of the paw individual reaction to stimuli may potentially be a factor in the hot plate method's lack of effectiveness [16].

5. CONCLUSION

The study results showed D_6 (2,5-bis[(4-dimethylaminophenylmethylidene] cyclopentan-1-one), D_7 (2,5-bis[(4-methoxyphenyl) methylidene] cyclopentan-1-one), D_8 (2,5-diethylidenecyclopentan-1-one), and D_{10} (2,5-dibenzodioxoylmethyledenecyclopentan-1-one) remarkable analgesic potential.

6. DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The lead and the co-authors declare that no part of this article was generated by AI technologies

including ChatGPT, COPILOT and text-to-image generators.

7. REFERENCES

[1] Raja, S. N., Carr, D. B., Cohen, M., Finnerup, N. B., Flor, H., Gibson, S., Keefe, F. J., Mogil, J.S., Ringkamp, M., Sluka, K. A., Song, X.-J., Stevens, B., Sullivan, M. D., Tutelman, P. R., Ushida, T., & Vader, K. (2020). The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. *Pain*, *161*(9), 1976–1982. https://doi.org/10.1097/j.pain.0000000000001939

[2] Fomina, M. V, Vatsadze, S. Z., Freidzon, A. Y., Kuz'mina, L. G., Moiseeva, A. A., Starostin, R. O., Nuriev, V. N., & Gromov, S. P. (2022). Structure-Property Relationships of Dibenzylidenecyclohexanones. *ACS Omega*, *7*(12), 10087–10099. https://doi.org/10.1021/acsomega.1c06129

[3] Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: an overview. *TheScientificWorldJournal*, *2013*, 162750. https://doi.org/10.1155/2013/162750

[4] Okesola, B. O., Vieira, V. M. P., Cornwell, D. J., Whitelaw, N. K., & Smith, D. K. (2015). 1,3:2,4-Dibenzylidene- <scp>d</scp> -sorbitol (DBS) and its derivatives – efficient, versatile and industrially-relevant low-molecular-weight gelators with over 100 years of history and a bright future. *Soft Matter*, *11*(24), 4768–4787. https://doi.org/10.1039/C5SM00845J

[5] Zhuang, C., Zhang, W., Sheng, C., Zhang, W., Xing, C., & Miao, Z. (2017). Chalcone: A Privileged Structure in Medicinal Chemistry. *Chemical Reviews*, *117*(12), 7762–7810. https://doi.org/10.1021/acs.chemrev.7b00020

[6] Ahmed, T., Khan, A.-U., Abbass, M., Filho, E. R., Ud Din, Z., & Khan, A. (2018). Synthesis, characterization, molecular docking, analgesic, antiplatelet and anticoagulant effects of dibenzylidene ketone derivatives. *Chemistry Central Journal*, *12*(1), 134. https://doi.org/10.1186/s13065-018-0507-1

[7] Higgins, C., Smith, B. H., & Matthews, K. (2019). Evidence of opioid-induced hyperalgesia in clinical populations after chronic opioid exposure: a systematic review and meta-analysis.*British Journal of Anaesthesia*, *122*(6), e114–e126. https://doi.org/10.1016/j.bja.2018.09.019

[8] Grandhin T. Recommended animal handling guidelines and audit guide: A Systematic Approach to Animal welfare. (2021).1-129

[9] Eddy, N. B., & Leimbach, D. (1953). Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. *The Journal of Pharmacology and Experimental Therapeutics*, *107*(3), 385–393.

[10] Sewell, R. (1976). Antinociceptive activity of narcotic agonist and partial agonist analgesics and other agents in the tail-immersion test in mice and rats. *Neuropharmacology*, *15*(11), 683– 688. https://doi.org/10.1016/0028-3908(76)90037-X

[11] Alorfi, N. M. (2023). Pharmacological Methods of Pain Management: Narrative Review of Medication Used. *International Journal of General Medicine*, *Volume 16*, 3247–3256. https://doi.org/10.2147/IJGM.S419239

[12] Grond, S., & Sablotzki, A. (2004). Clinical Pharmacology of Tramadol. *Clinical Pharmacokinetics*, *43*(13), 879–923. https://doi.org/10.2165/00003088-200443130-00004

[13] Vongtau, H. O., Abbah, J., Mosugu, O., Chindo, B. A., Ngazal, I. E., Salawu, A. O., Kwanashie, H. O., & Gamaniel, K. S. (2004). Antinociceptive profile of the methanolic extract of Neorautanenia mitis root in rats and mice. *Journal of Ethnopharmacology*, *92*(2–3), 317–324. https://doi.org/10.1016/j.jep.2004.03.014

[14] Sandow, J. (2013). Endocrine Pharmacology. In *Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays* (pp. 421–520). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-25240-2_16

[15] Kilimozhi, D., Parthasarathy, V., Jayant, M., & Manavalan, R. (2009). Antinociceptive, antipyretic and anti-inflammatory effects of Clerodendrum phlomidis in mice and rats. *International Journal of Biological and Chemical Sciences*, *3*(3).3-12

[16] Gårdmark, M., Höglund, A. U., & Hammarlund-Udenaes, M. (1998). Aspects on tail-flick, hot- plate and electrical stimulation tests for morphine antinociception. *Pharmacology & Toxicology*, *83*(6), 252–258. https://doi.org/10.1111/j.1600-0773.1998.tb01478.x

Comment [R4]: Different style from other references.

Comment [R5]: Different style from other references.

Comment [R6]: Different style from other references.