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COMPARATIVE PAIN THRESHOLD INCREMENT IN EXPERIMENTAL MICE MODEL BY 2, 6-DIBENZODIOXYLMETHYLIDENECYCLOHEXAN-1-ONE (A5) AND TRAMADOL

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ABSTRACT
The study aimed to investigate the analgesic effects of two compounds designated as A5 and A6, (synthesized from dibenzylidene) in mice model of Pain induction; as a series in research into effective and less adversely portentous treatment options for pain management. In this controlled experimental study, mice groups were subjected to two models of pain-inducing stimuli (hot plate, water bath) and then administered either of A5 or A6, as well as Tramadol, a widely used analgesic drug - serving as a reference standard control; while distilled water was administered as normal control. The study evaluated the timed relieving potential by compounds, deducing from the latency to pain exhibited by mice in the experiment models. Results indicated that for doses of A5; at 30 minutes point (1000 mg/kg), at 60 mins point (1000 mg/kg, 1500 mg/kg), and at 90 mins point (low, mid, high) all increased pain threshold significantly ($p<0.0001$, $p<0.002$) than normal control. Tramadol also significantly ($p<0.0001$) attenuated pain responses in the mice models than control. However, there were notable differences in their analgesic profiles. A5 demonstrated a rapid onset of action and prolonged duration of pain relief, while Tramadol exhibited a slower onset but prolonged duration of effect. But A6 did not show any analgesic potential. In conclusion, A5 may be beneficial as potent analgesic, although further investigation to fully elucidate the mechanisms of action and safety profiles is recommended.

KEYWORDS: Threshold, analgesic, pain, dibenzylidene, comparative.

INTRODUCTION

Until the 1960s, pain was considered an inevitable sensory response to tissue damage; with little room for the affective dimension of this ubiquitous experience, and none whatsoever for the effects of genetic differences, past experience, anxiety, or expectation. Pain has been explained to be displeasing perception or reality presence of injured tissue, according to International Association for Study of Pain, 2005. However, pain therapies are sustained even though debate is ongoing whether to modify this definition – opioids and the none categories (Lehmann *et al*, 1990); with opioids administered across grades of pain but recording undesired side effects (Wilder-Smith *et al*, 2001).

In understanding pain, the roles of factors outside the patient's body have also been clarified. Pain is probably the most common symptomatic reason to seek medical consultation (James, 2022). People have headaches, burns, cuts, and other pains at some time during childhood and adult life. Individuals who undergo surgery are almost certain to have postoperative pain (Lehmann *et al*, 1990). Ageing is also associated with an increased likelihood of chronic pain. Health-care expenditures for chronic pain are enormous, rivalled only by the costs of wage replacement and welfare programmes for those who do not work because of pain. Despite improved knowledge of underlying mechanisms and better treatments, many people who have chronic pain receive inadequate care (Lehmann *et al*, 1990; Wilder-Smith *et al*, 2001).

Pain may be sharp or dull, intermittent or constant, or throbbing or steady. Sometimes pain is very difficult to describe. Pain may be felt at a single site or over a large area. The intensity of pain can vary from mild to intolerable and people differ remarkably in their ability to tolerate pain; in accordance with mood, personality, and circumstance (Hennies *et al*, 1988). For instance, in a moment of excitement during an athletic match, an athlete may not notice a severe bruise but is likely to be very aware of the pain after the match, particularly if the team lost.

There has been rise in the consumption of certain drugs in some climes for managing pain, including tramadol, which besides its numerous adverse side effects is also prone to addictive and abusive tendencies (Lehmann *et al*, 1990). Since the advent of this drug, it has over the years gain acceptance in some societies particularly among teaming youths in Africa and Nigeria. Tramadol can also interfere or synergize with other medication pathways (Hennies *et al*, 1988; Sagata *et al*, 2002)).

In view of these, research has been directed in recent years at designing compounds devoid of the typical side effects. Therefore, the discovery of new, potent and safer NSAIDs represents a challenging goal for such a research area. Because resistance to NSAIDs is widespread, there is an increasing need for identification of novel structure leads that may be of use in designing new, potent, and less toxic NSAIDs (Lai *et al*, 1996)

Dibenzylidene sorbitol (DBS) as low-molecular-weight organic molecule and Dibenzylidene-cyclohexanone as a cyclohexanone based bischalcone possesses two reactive ketovinyl moieties (−CO−CHCH−) and hence categorized as bichromophoric molecules (Kemelayefa *et al*, 2022). Thus far, dibenzylidene moieties have gradually been gaining usage with promising benefit for relief of pain **(**Al-Karawi**,** 2018).

This investigation considers how analogues synthesized from dibenzylidene would affect pain in mice.

METHODOLOGY

Mature albino mice (150-200 g) numbering 72; obtained from the Laboratory Animal Units of the Faculty of Pharmacology, Niger Delta University, were used for the experiment. The study adopted an experimental approach where animals were randomly assigned to five groups of six mice each for the two different experimental models of the same analogue.

The animals were housed under standard laboratory conditions at room temperature with relative humidity of 70–80%. They were fed with standard commercial diet and water ad libitum. Prior to the experiment, the animals were fasted for 12 h with water given ad libitum and weighed.

The first groups were administered 0.2 mg/kg distilled water (normal control). Second, third and fourth groups were administered 500mg/kg (low), 1000mg/kg (mid) and 1500 mg/kg (high) respectively. The fifth groups were administered 50mg/kg tramadol (standard control). This procedure was carried out for A5 and A6 along with the two controls and they were all tested for latency to pain at 30 minutes interval (Yam *et al*, 2020; Raffa *et al*, 1992).

RESULTS ANALYSIS

LATENCY TO PAIN EXPERIMENT RESULTS

Table 1a: HOT PLATE TEST FOR A5.

		30mins	60mins	90mins
500mg/kg	A	28.0	17.9	44.6
	B	20.7	27.3	35.9
	C	15.2	21.3	96.9
1000mg/kg	A	52.7	33.5	69.5
	B	12.2	39.9	11.5
	C	37.5	70.9	90.4
1500mg/kg	A	13.3	37.3	60
	B	21.2	29.1	26.9
	C	11.9	32.0	51.8

Table 1b: HOT PLATE TEST FOR A6.

		30mins	60mins	90mins
500mg/kg	A	5s	10s	5s
	B	10s	5s	-
	C	10s	10s	10s
1000mg/kg	A	12s	12	8.8s
	B	8.2s	8.17s	8.4s
	C	13s	7.4s	5.9s
1500mg/kg	A	5.7	6.3	5
	B	11	12.8	10
	C	6.2	8.6	8

Table 2a: WATER BATH TEST FOR A5.

		30mins	60mins	90mins
500mg/kg	A	4.0	2.1	2.6
	B	2.6	2.5	1.2
	C	3.7	2.1	3.5
1000mg/kg	A	2.6	2.0	3.3
	B	1.2	1.9	3.2
	C	2.7	1.7	6.0
1500mg/kg	A	2.0	1.2	9.4
	B	2.4	1.1	8.5
	C	1.7	3.9	3.4

Table 2b: WATER BATH TEST FOR A6.

		30mins	60mins	90mins
500mg/kg	A	6	10	4
	B	5	3	4
	C	3	5	3
1000mg/kg	A	3	3	3
	B	2.3	2.9	3
	C	2	2.5	3
1500mg/kg	A	3.3	3.1	3
	B	1	3.7	3.5
	C	2	14.9	9.0

Table 3: HOT PLATE TEST FOR CONTROL.

	30mins	60mins	90mins
A	15	12	13
B	10	10	11
C	10	11	10

Table 4: WATER BATH TEST FOR CONTROL.

	30mins	60mins	90mins
A	10	11	9
B	15	12	13
C	10	11	10

Table 5: HOT PLATE TEST FOR (Standard Drug) TRAMADOL.

	30mins	60mins	90mins
A	7.6	38.5	120
B	3.5	46.8	129
C	2.4	-	-

Table 5: WATER BATH TEST FOR (Standard Drug) TRAMADOL.

	30mins	60mins	90mins
A	8.5	5.3	16
B	5.8	10.2	19.7
C	5.5	9.2	18.9

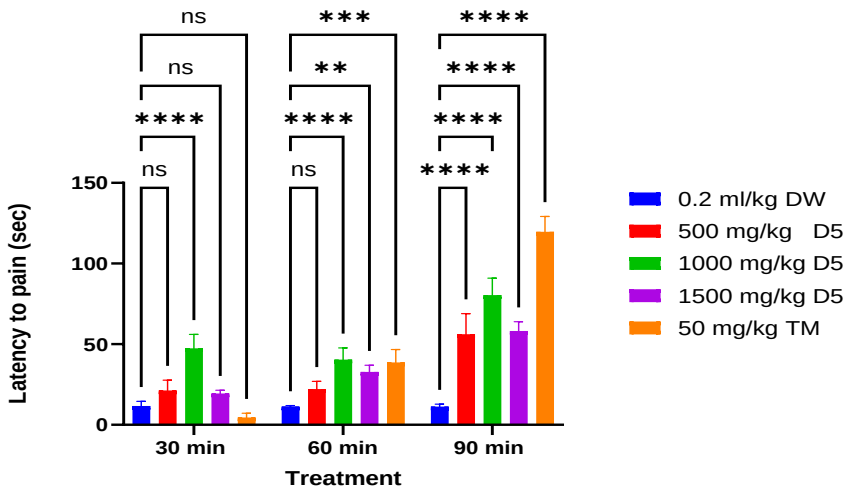

Figure 1a: Graphical Analysis for A5.

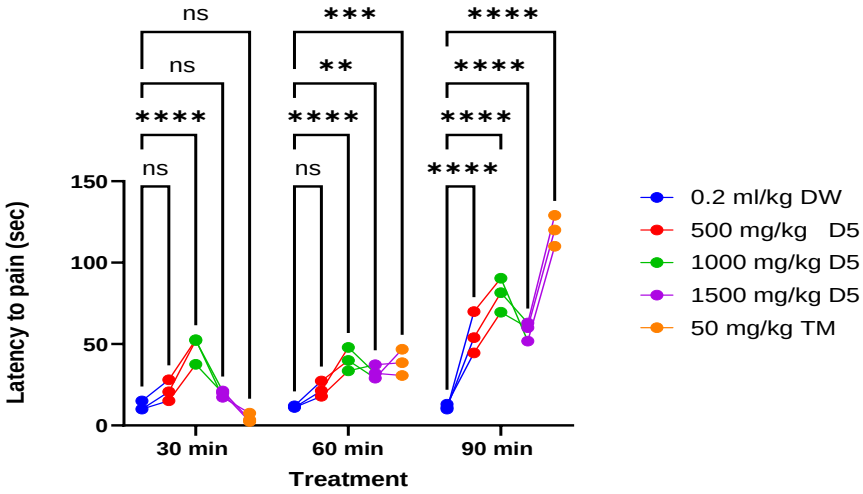

Figure 1b: Graphical Analysis for A5.

STATISTICS: Graph Pad Prism 10.2. 2Way ANOVA, Dunnett's Multiple Comparisons Test. 30 min: 1000 mg/kg of A5 indicated *** Significance when compared to the control DW 0.2 mg/kg with Adjusted P<0.0001; 60 min: 1000,1500 mg/kg of A5 indicated ***, ** Significance when compared to the control DW 0.2 mg/kg with Adjusted P<0.0001, 0.002;90 min: 500,1000,1500 mg/kg of A5 indicated *** Significance when compared to the control DW 0.2 mg/kg with Adjusted P<0.0001.

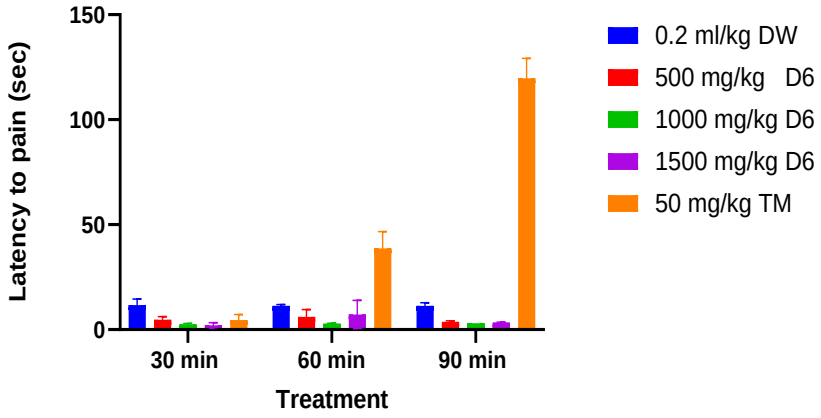

Figure 2a: Graphical Analysis for A6.

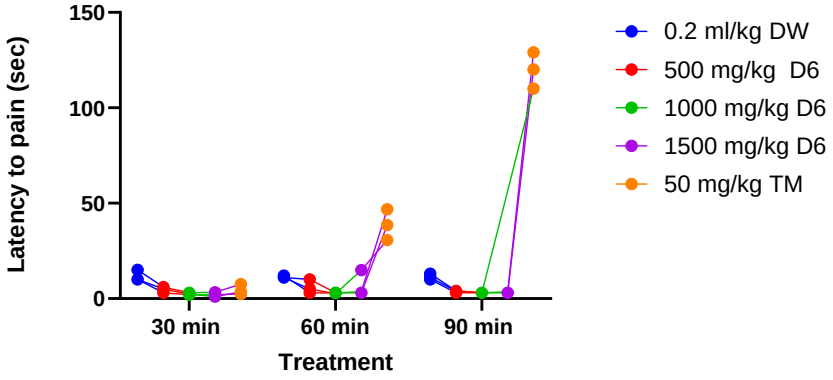

Figure 2b: Graphical Analysis for A6.

STATISTICS: Graph Pad Prism 10.2. 2Way ANOVA, Dunnett's Multiple Comparisons Test. A6 indicated no significance when compared to the control DW 0.2 mg/kg.

DISCUSSION

As part of the series in research for alternative antinociceptive therapy, analgesic potentials of A5 and A6 were investigated by 2 pain study models; the hot plate and water bath test, where it was observed apparently in the result that while A6 showed no analgesic activity, there was significant difference between the control group (0.2 ml/kg DW) and the group treated with all dosages of A5 at the 30-minute, 60-minute and 90 minute time points.

At 60 minutes: The group treated with 500 mg/kg of A5 significant difference compared to the control group treated with DW 0.2 mg/kg, with an adjusted p-value of 0.009. This suggests that the treatment with 500 mg/kg of A5 has a notable effect at this time point. At 90 mins: Mice administered low, mid and high A5 show significant differences in increment of pain threshold than normal control. The adjusted p-values for these comparisons are 0.0006 and 0.002, respectively. This indicates that the treatments with 500 mg/kg, 1000 mg/kg, and 1500 mg/kg of A5 have significant effects at these time point. Overall, these findings suggest that the treatment with A5 at different doses (low, mid, high) has significant effects at both 60 minutes and 90 minutes, respectively, relative to distilled water - grp 0.2 mg/kg.

Furthermore, the result shows a slow onset of analgesic action which takes over 60-minute for biological response to be noticed in the standard drug whereas, the onset was rapid for A5 as shown in the result. Whether this observed difference in onset is associated with quantity of dose was not within this scope.

Noteworthy is an inference that the quality of A5 indicating biological responses at the 30 mins, 60 mins and 90 mins, where standard drug only responded after 60 mins point may portend it as preferential emergency treatment option over the available standard. Also, all three dosage of A5 showing satisfactory significant increase in latency to pain at 90 mins point is indicative of its potential application in chronic pain management at dose 500mg/kg and above.

CONCLUSION

In conclusion, one of the reference drugs (A5) comparatively exhibited rapid onset of analgesic potential than tramadol, although both had prolonged duration of action; suggesting it could be a progressively potential alternate analgesic.

RECOMMENDEDATION

This study offers valuable insights into the comparative effects of A5 dibenzylidene compound and tramadol on pain. The findings contribute to the existing knowledge base and could serve as a foundation for future studies aiming to develop novel and effective analgesic compounds.

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APPENDIX 1

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
30 min					
0.2 ml/kg DW vs. 500 mg/kg D3	-9.633	-23.94 to 4.669	No	ns	0.2641
0.2 ml/kg DW vs. 1000 mg/kg D3	-35.80	-50.10 to -21.50	Yes	****	<0.0001
0.2 ml/kg DW vs. 1500 mg/kg D3	-7.800	-22.10 to 6.502	No	ns	0.4408
0.2 ml/kg DW vs. 50 mg/kg TM	7.167	-7.136 to 21.47	No	ns	0.5141
60 min					
0.2 ml/kg DW vs. 500 mg/kg D3	-10.83	-25.14 to 3.469	No	ns	0.1800
0.2 ml/kg DW vs. 1000 mg/kg D3	-29.10	-43.40 to -14.80	Yes	****	<0.0001
0.2 ml/kg DW vs. 1500 mg/kg D3	-21.47	-35.77 to -7.164	Yes	**	0.0020
0.2 ml/kg DW vs. 50 mg/kg TM	-27.33	-41.64 to -13.03	Yes	***	0.0001
90 min					
0.2 ml/kg DW vs. 500 mg/kg D3	-44.80	-59.10 to -30.50	Yes	****	<0.0001
0.2 ml/kg DW vs. 1000 mg/kg D3	-69.13	-83.44 to -54.83	Yes	****	<0.0001
0.2 ml/kg DW vs. 1500 mg/kg D3	-46.90	-61.20 to -32.60	Yes	****	<0.0001
0.2 ml/kg DW vs. 50 mg/kg TM	-108.4	-122.7 to -94.06	Yes	****	<0.0001
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N1
30 min					
0.2 ml/kg DW vs. 500 mg/kg D3	11.67	21.30	-9.633	5.547	3
0.2 ml/kg DW vs. 1000 mg/kg D3	11.67	47.47	-35.80	5.547	3
0.2 ml/kg DW vs. 1500 mg/kg D3	11.67	19.47	-7.800	5.547	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.67	4.500	7.167	5.547	3
60 min					
0.2 ml/kg DW vs. 500 mg/kg D3	11.33	22.17	-10.83	5.547	3
0.2 ml/kg DW vs. 1000 mg/kg D3	11.33	40.43	-29.10	5.547	3
0.2 ml/kg DW vs. 1500 mg/kg D3	11.33	32.80	-21.47	5.547	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.33	38.67	-27.33	5.547	3
90 min					
0.2 ml/kg DW vs. 500 mg/kg D3	11.33	56.13	-44.80	5.547	3
0.2 ml/kg DW vs. 1000 mg/kg D3	11.33	80.47	-69.13	5.547	3
0.2 ml/kg DW vs. 1500 mg/kg D3	11.33	58.23	-46.90	5.547	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.33	119.7	-108.4	5.547	3

APPENDIX 2

Dunnett's multiple comparisons test	Predicted (LS) mean diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
30 min					
0.2 ml/kg DW vs. 500 mg/kg D6	7.000	-1.496 to 15.50	No	ns	0.1295
0.2 ml/kg DW vs. 1000 mg/kg D6	9.233	0.7373 to 17.73	Yes	*	0.0300
0.2 ml/kg DW vs. 1500 mg/kg D6	9.567	1.071 to 18.06	Yes	*	0.0237
0.2 ml/kg DW vs. 50 mg/kg TM	7.167	-1.329 to 15.66	No	ns	0.1172
60 min					
0.2 ml/kg DW vs. 500 mg/kg D6	5.333	-3.163 to 13.83	No	ns	0.3196
0.2 ml/kg DW vs. 1000 mg/kg D6	8.533	0.03731 to 17.03	Yes	*	0.0487

0.2 ml/kg DW vs. 1500 mg/kg D6	4.100	-4.396 to 12.60	No	ns	0.5447
0.2 ml/kg DW vs. 50 mg/kg TM	-27.33	-35.83 to -18.84	Yes	****	<0.0001
90 min					
0.2 ml/kg DW vs. 500 mg/kg D6	7.667	-0.8518 to 16.19	No	ns	0.0874
0.2 ml/kg DW vs. 1000 mg/kg D6	8.333	-0.1851 to 16.85	No	ns	0.0566
0.2 ml/kg DW vs. 1500 mg/kg D6	8.083	-1.441 to 17.61	No	ns	0.1146
0.2 ml/kg DW vs. 50 mg/kg TM	-108.4	-116.9 to -99.85	Yes	****	<0.0001
Test details	Predicted (LS) mean 1	Predicted (LS) mean 2	Predicted (LS) mean diff.	SE of diff.	N1
30 min					
0.2 ml/kg DW vs. 500 mg/kg D6	11.67	4.667	7.000	3.289	3
0.2 ml/kg DW vs. 1000 mg/kg D6	11.67	2.433	9.233	3.289	3
0.2 ml/kg DW vs. 1500 mg/kg D6	11.67	2.100	9.567	3.289	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.67	4.500	7.167	3.289	3
60 min					
0.2 ml/kg DW vs. 500 mg/kg D6	11.33	6.000	5.333	3.289	3
0.2 ml/kg DW vs. 1000 mg/kg D6	11.33	2.800	8.533	3.289	3
0.2 ml/kg DW vs. 1500 mg/kg D6	11.33	7.233	4.100	3.289	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.33	38.67	-27.33	3.289	3
90 min					
0.2 ml/kg DW vs. 500 mg/kg D6	11.33	3.667	7.667	3.289	3
0.2 ml/kg DW vs. 1000 mg/kg D6	11.33	3.000	8.333	3.289	3
0.2 ml/kg DW vs. 1500 mg/kg D6	11.33	3.250	8.083	3.677	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.33	119.7	-108.4	3.289	3