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ANALGESIC POTENTIAL OF 2,6-BIS[(4-DIMETHYLAMINOPHENYL)METHYLIDENE]CYCLOHEXAN-1-ONE (A1) IN EXPERIMENTAL MICE MODEL OF THERMAL PAIN - INDUCTION

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ABSTRACT

The analgesic potential of 2,6-bis[(4-dimethylaminophenyl)methylidene]cyclohexan-1-one (A1) was investigated in mice model of thermal pain – induction; as part of research effort to ameliorate adverse and addictive proclivity of current pain regimens, while proffering effective relief. Randomly selected mice were placed in experimentally designed groups; and administered distilled water, A1 (in graded dose), and tramadol (as standard). Thereafter, all groups were subjected to thermally induced pain by hot plate and water bath methods, to understudy their Neurobehaviour associated with pain reaction and response time as a means of deciphering analgesic activity of those regimens. Observed data was analysed by one – tailed ANOVA and Dunnett's test for statistical relevance. The results from the two models show that threshold for pain was significantly ($p<0.0001$) increased in the A1 and tramadol administered animals than control group, which implies A1 may potentially proffer pain relief, as standard regimen – tramadol does.

KEYWORDS: Threshold, Regimen, Analgesic, Pain, Dibenzylidene, Thermal.

INTRODUCTION

In the face of apparent challenges confronting the synergy between pharmaceutics industry and medical profession in respect of orthodox medication for pain management and inherent proclivity of adverse and / or addictive tendencies; research for alternative therapy has garnered acceptance. Pain entails transmission of noxious signals from receptor nerves to brain for recognition as such (Smith et al, 2019); mediated through several processes utilizing chemicals and neurons (Nagamani *et al.* 2024). The eventual perception of the interpreted signal by the brain may become subjective and variant in severity and tolerance from person to person (Patel *et al*, 2021; Keyhanfar *et al*, 2013).

Amidst usage of currently available analgesics, there are yet sizable portion of patients having to cope with enduring pain which are not adequately controlled. Therefore, it is pertinent both for wellness of individuals, as well as sustainably healthy and productive society that management of pain is addressed efficiently.

There has also been the approach of exploring natural dietary means to see how dietary consumables may affect the Neurobehaviour of pain; through exposure of rodents to chronic consumption of dietary composition of musa paradisiaca or plantain (Peter *et al*, 2018). Although, this categories of investigation reports increased pain threshold in animals exposed to musa paradisiaca diet in long term, the recommendation was apparently for dietary supplementations alongside available orthodox pain relieve medication (Peter *et al*, 2018).

In the same vein, amidst plethora of alternative substances being investigated for possible benefits as analgesia is a group of compound, similar in structure composition to curcumin, having bioactive proclivity; and are synthesized from dibenzylidene or referred as its derivatives (Garcia *et al*, 2018; Patel *et al*, 2021).

The objective of current research therefore include, identifying two compounds from dibenzylidene (A1 and A2), studying their effect on animals exposed to two models of thermal pain induction, and comparing the response to a known analgesic regimen – tramadol.

Methods

Two structurally different analogues A1 = 2,6-bis[(4-dimethylaminophenyl)methylidene]cyclohexan-1-one(A1), and A2 =2,6-bis[(4-methoxyphenyl)methylidene]cyclohexan-1-one (A2) which were both synthesized from Dibenzylidene in the laboratory of Pharmacology and Neurochemistry, Niger Delta University were administered in graded dosage of 500, 1000, 1500 mg /kg to experimentally grouped mice in accordance with established methods (Alves & Duarte, 2002; Keyhaifar *et al*, 2013**)**. Distilled water (0.2ml/kg) and tramadol (50 mg/kg) were also administered to appropriate groups. Then animals were made to undergo pain induction, during which pain response behaviour were observed (Alves & Duarte, 2002; Keyhaifar *et al*, 2013**)**.

Statistical Analysis

Appropriate statistical analysis was implored for studying the observed data. ANOVA and Dunnett's test were carried out in comparing test and standard group vs control. And as predetermined, at $p < 0.05$ the analyzed results were considered and interpreted to be significant.

RESULTS ANALYSIS

Table 1a: Hot Plate Test For A1.

		30mins	60mins	90mins
500mg/kg	A	16.8	5.2	5.7
	B	21.4	3.2	
	C	7.3	8.0	9.1
1000mg/kg	A	14.8	-	-
	B	9.4	-	-
	C	20.7	34.5	10.7
1500mg/kg	A	5.7	-	-
	B	16.6	25.2	41.9
	C	-	-	-

Table 1b: Hot Plate Test For A2.

		30mins	60mins	90mins
500mg/kg	A	10.9	18.2	16.5
	B	5.8	12.1	30.8
	C	10.3	14.9	6.4
1000mg/kg	A	7.8	4.2	26.6
	B	6.4	17.5	25.1
	C	6.1	10.4	18.2
1500mg/kg	A	3.8	15.7	24.6
	B	7.8	11.3	26.6
	C	9.8	52.2	17.2

Table 2a: Water Bath Test for A1.

		30mins	60mins	90mins
500mg/kg	A	6.2	1.6	
	B	16.13	1.9	0.8
	C	5.6	0.7	1.2
1000mg/kg	A	5.5	-	-
	B	4.6	2.4	9.9
	C	2.7	-	-
1500mg/kg	A	-	-	-
	B	-	-	-
	C	-	-	-

Table 2b: Water Bath Test For A2.

		30mins	60mins	90mins
500mg/kg	A	1.9	3.4	5.1
	B	1.4	3.2	2.5
	C	1.4	2.6	1.9
1000mg/kg	A	4.7	2.3	4.2
	B	1.4	5.8	4.9
	C	2.4	2.7	4.5
1500mg/kg	A	2.3	3.7	3.4
	B	9.0	7.9	7.4
	C	2.7	2.8	4.4

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

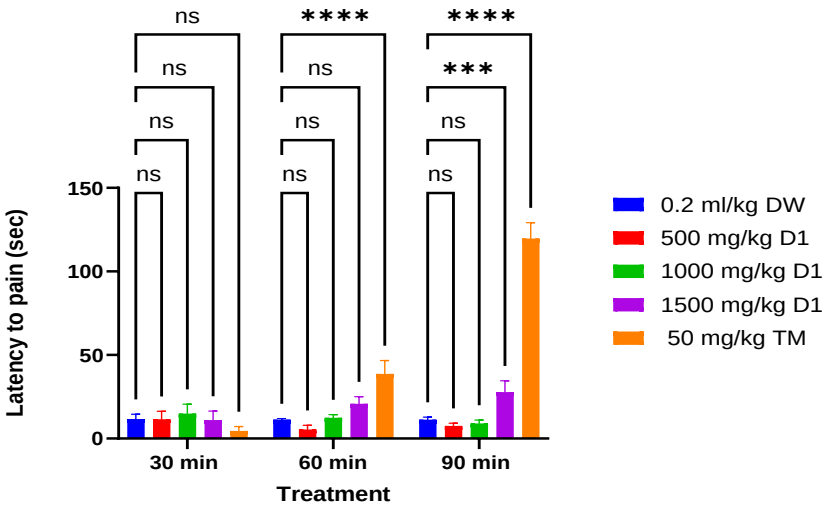


Figure 1a: Graphical Analysis for A1.

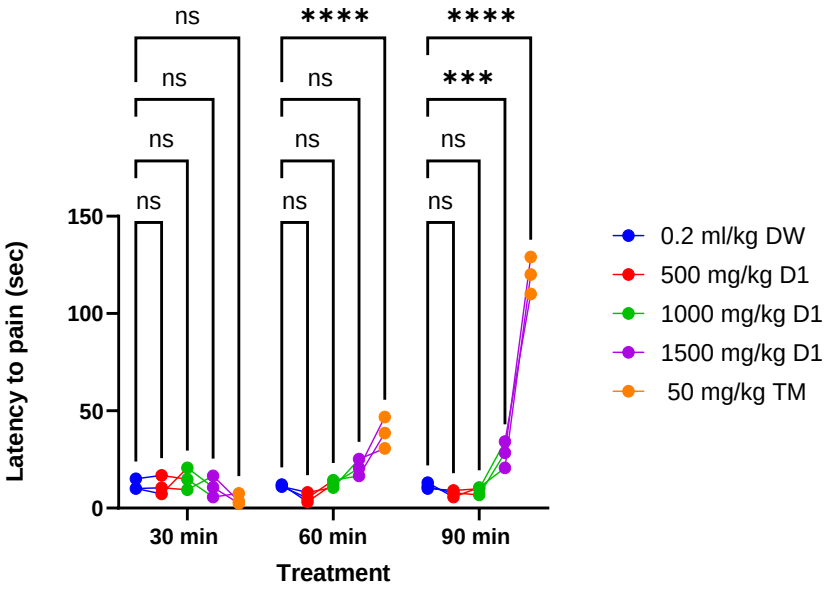
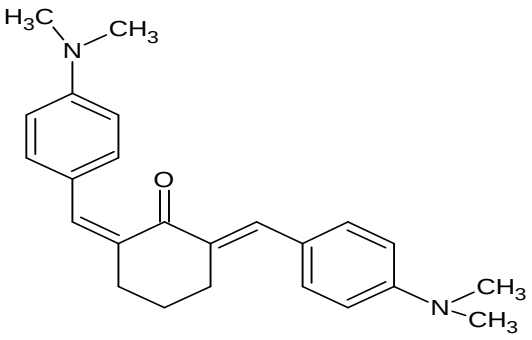


Figure 1b: Graphical Analysis for A1.

STATISTICS: Graph Pad Prism 10.2. 2Way ANOVA, Dunnett's Multiple Comparisons Test. 30 min: 1000 mg/kg of A1 indicated *** Significance when compared to the control DW 0.2 mg/kg with Adjusted P<0.0001; 60 min: 1000,1500 mg/kg of A1 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 A1 indicated *** Significance when compared to the control DW 0.2 mg/kg with Adjusted P<0.0001.



2,6-bis[(4-dimethylaminophenyl)methylidene]cyclohexan-1-one(A1)

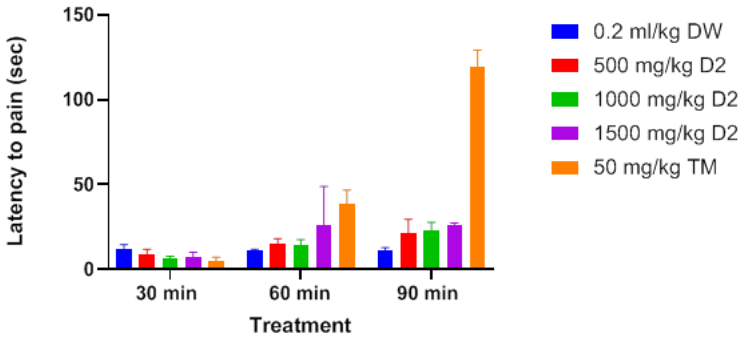


Figure 2a: Graphical Analysis for A2

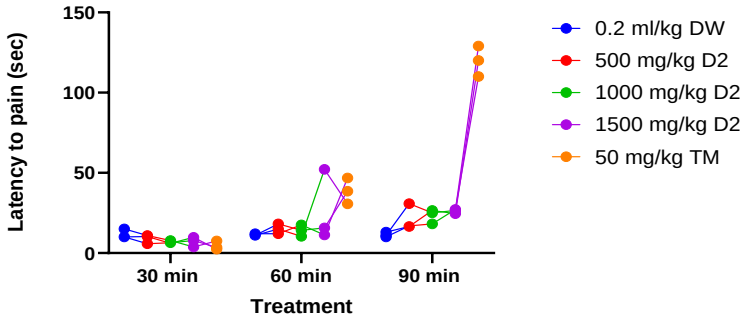
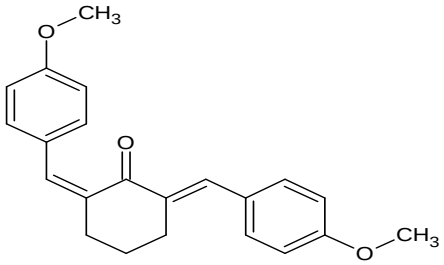


Figure 2b: Graphical Analysis for A2

STATISTICS: Graph Pad Prism 10.2. 2Way ANOVA, Dunnett's Multiple Comparisons Test. A2 indicated no significance when compared to the control DW 0.2 mg/kg as well as standard drug.



2,6-bis[(4-methoxyphenyl)methylidene]cyclohexan-1-one (A2)

DISCUSSION

Research effort to ameliorate adverse and addictive proclivity of current pain regimens, while proffering effective relief has been an area of interest. In the face of apparent challenges confronting the synergy between pharmaceutics industry and medical profession in respect of orthodox medication for pain management and inherent proclivity of adverse and / or addictive tendencies; research for alternative therapy has garnered acceptance.

In this study, the aim was to see how two analogues (A1 and A2) synthesized from dibenzylidene would impact on pain Neurobehaviour parameters in an experimental mice model of thermal pain induction. The results from both hot plate and hot water bath tests showed that the second analogue (A2); 2,6-bis[(4-methoxyphenyl)methylidene]cyclohexan-1-one did not impact on the pain behaviour of mice any differently, compared to control substance (distilled water).

However, when mice treated with (A1); 2,6-bis[(4-dimethylaminophenyl)methylidene]cyclohexan-1-one were subjected to hot plate and hot water bath tests of pain induction, it was observed that their pain response Neurobehaviour was impacted. The pain threshold for their responses was increased with statistical significance compared to the animals given distilled water; which implies that A1 possesses beneficial analgesic activity. Although, in comparison to tramadol, only the 1500 mg/kg of A1 was effective and it was only at the 90 minutes point; whereas 50 mg/kg of tramadol was effective at the 60 and 90 minutes point. This appears to suggest that A1 may be more precisely effective as an intervention analgesic in chronic pain condition.

Note worthily, in this research, animal model was implored to mimic possible occurrence of similar phenomenon in humans; if this could be extrapolated. Thus it is important to state that this study did not use human subject and as such, for purpose of extrapolation, furtherance into toxicity studies for level of safety and clinical trials may be necessary.

Moreover, exact mechanisms involved in the pain relieving effect of this reference substance is still not fully comprehended. Also, likely interactions it may exhibit alongside other drugs, impact from chronic

exposure and responses of patients might as well bring new insight into the present finding.

In concluding, it can be inferred that A1, provides elevation of pain threshold (i.e. it exhibits analgesic activity); and this was displayed in such procedure which might allow it to be suitable intervention remedy for managing acute pain.

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APPENDIX 1: HOT PLATE ANALYSIS

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 D1	0.1667	-9.847 to 10.18	No	ns	>0.9999
0.2 ml/kg DW vs. 1000 mg/kg D1	-3.300	-13.31 to 6.714	No	ns	0.8103
0.2 ml/kg DW vs. 1500 mg/kg D1	0.6667	-9.347 to 10.68	No	ns	0.9994
0.2 ml/kg DW vs. 50 mg/kg TM	7.167	-2.847 to 17.18	No	ns	0.2188
60 min					
0.2 ml/kg DW vs. 500 mg/kg D1	5.867	-4.147 to 15.88	No	ns	0.3788
0.2 ml/kg DW vs. 1000 mg/kg D1	-1.100	-11.11 to 8.914	No	ns	0.9955
0.2 ml/kg DW vs. 1500 mg/kg D1	-9.467	-19.48 to 0.5470	No	ns	0.0681
0.2 ml/kg DW vs. 50 mg/kg TM	-27.33	-37.35 to -17.32	Yes	****	<0.0001
90 min					
0.2 ml/kg DW vs. 500 mg/kg D1	3.800	-6.214 to 13.81	No	ns	0.7281
0.2 ml/kg DW vs. 1000 mg/kg D1	2.267	-7.747 to 12.28	No	ns	0.9388
0.2 ml/kg DW vs. 1500 mg/kg D1	-16.43	-26.45 to -6.420	Yes	***	0.0008
0.2 ml/kg DW vs. 50 mg/kg TM	-108.4	-118.4 to -98.35	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 D1	11.67	11.50	0.1667	3.884	3
0.2 ml/kg DW vs. 1000 mg/kg D1	11.67	14.97	-3.300	3.884	3
0.2 ml/kg DW vs. 1500 mg/kg D1	11.67	11.00	0.6667	3.884	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.67	4.500	7.167	3.884	3
60 min					
0.2 ml/kg DW vs. 500 mg/kg D1	11.33	5.467	5.867	3.884	3
0.2 ml/kg DW vs. 1000 mg/kg D1	11.33	12.43	-1.100	3.884	3
0.2 ml/kg DW vs. 1500 mg/kg D1	11.33	20.80	-9.467	3.884	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.33	38.67	-27.33	3.884	3
90 min					
0.2 ml/kg DW vs. 500 mg/kg D1	11.33	7.533	3.800	3.884	3
0.2 ml/kg DW vs. 1000 mg/kg D1	11.33	9.067	2.267	3.884	3
0.2 ml/kg DW vs. 1500 mg/kg D1	11.33	27.77	-16.43	3.884	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.33	119.7	-108.4	3.884	3

APPENDIX 2: WATER BATH ANALYSIS

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 D2	2.667	-12.76 to 18.10	No	ns	0.9758
0.2 ml/kg DW vs. 1000 mg/kg D2	4.900	-10.53 to 20.33	No	ns	0.8285
0.2 ml/kg DW vs. 1500 mg/kg D2	4.533	-10.90 to 19.96	No	ns	0.8625
0.2 ml/kg DW vs. 50 mg/kg TM	7.167	-8.264 to 22.60	No	ns	0.5778
60 min					
0.2 ml/kg DW vs. 500 mg/kg D2	-3.733	-19.16 to 11.70	No	ns	0.9241
0.2 ml/kg DW vs. 1000 mg/kg D2	-2.700	-18.13 to 12.73	No	ns	0.9747
0.2 ml/kg DW vs. 1500 mg/kg D2	-15.07	-30.50 to 0.3638	No	ns	0.0572
0.2 ml/kg DW vs. 50 mg/kg TM	-27.33	-42.76 to -11.90	Yes	***	0.0003

90 min					
0.2 ml/kg DW vs. 500 mg/kg D2	-10.07	-25.50 to 5.364	No	ns	0.2892
0.2 ml/kg DW vs. 1000 mg/kg D2	-11.97	-27.40 to 3.464	No	ns	0.1650
0.2 ml/kg DW vs. 1500 mg/kg D2	-14.80	-30.23 to 0.6304	No	ns	0.0630
0.2 ml/kg DW vs. 50 mg/kg TM	-108.4	-123.8 to -92.94	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 D2	11.67	9.000	2.667	5.985	3
0.2 ml/kg DW vs. 1000 mg/kg D2	11.67	6.767	4.900	5.985	3
0.2 ml/kg DW vs. 1500 mg/kg D2	11.67	7.133	4.533	5.985	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.67	4.500	7.167	5.985	3
60 min					
0.2 ml/kg DW vs. 500 mg/kg D2	11.33	15.07	-3.733	5.985	3
0.2 ml/kg DW vs. 1000 mg/kg D2	11.33	14.03	-2.700	5.985	3
0.2 ml/kg DW vs. 1500 mg/kg D2	11.33	26.40	-15.07	5.985	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.33	38.67	-27.33	5.985	3
90 min					
0.2 ml/kg DW vs. 500 mg/kg D3	11.33	10.67	0.6667	3.072	3
0.2 ml/kg DW vs. 1000 mg/kg D3	11.33	10.33	1.000	3.072	3
0.2 ml/kg DW vs. 1500 mg/kg D3	11.33	11.33	0.000	3.072	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.33	119.7	-108.4	3.072	3