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LETHAL DOSE DETERMINATION, ACUTE TOXICITY OF SOME DIBENZYLIDENE ANALOGUES AND SEX VARIATION IN EXPERIMENTAL MICE MODEL

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ABSTRACT

This is a preliminary phase of a research series aimed at appraising acute toxicity of five (5) analogues (designated A1 to A5) of dibenzylidene administered orally to male and female mice selected randomly; in lieu of proffering alternative medications with improved efficacy and minimal or nil deleterious side effects, particularly for pain management. The lethal dose (LD50) was estimated using Lorke's method, and acute toxicity dose appraised at 4415.88mg/kg and 5000mg/kg for 24 hours. Behavioral and physiological toxicity effects were then examined. Results showed that A3 caused mortality in a mouse (male) with the highest dosage (5000mg/kg). The mice weighed 23.93kg, and the LD50 was calculated as 4415.88mg/kg. The other 4 analogues did not cause mortality. Meanwhile, LD50 for all five (5) derivatives was higher than 3800mg/kg, which is considerable as safe dose. In conclusion, A1, A2, A4 and A5 administered orally were relatively non toxic in both male and female mice while A3 was toxic in a male mouse. Whether LD50 observed is associated with sex seem positive in this preliminary study.

KEYWORDS: Toxicity, Chalcones, Dibenzylidine, LD50, Analogues, Mortality.

INTRODUCTION

Pain is a complex and uniquely personal experience, involving various physiological and perceptual pathways and factors different from nociception – which is the sensory mechanism that allows animals to sense and avoid potentially tissue-damaging stimuli (Tracey, 2017). It is also regarded as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Kumar & Elavarasi, 2016).

The management of pain presents a complex and multifaceted challenge within the healthcare sector globally, as it impacts millions of individuals worldwide who grapple with acute and chronic pain conditions (Merskey & Bogduk, 2018); affecting both quality of life and productivity. Therefore, addressing pain effectively demands a holistic approach that encompasses various interventions, spanning from pharmaceutical to non-pharmaceutical methods.

In response to this germane pressing concern, researchers have delved into the exploration of various pharmacological agents aimed at effectively managing pain whilst mitigating adverse effects. However, despite notable progress in pain management strategies, attaining ideal pain relief while minimizing adverse effects continues to be a formidable task.

Dibenzylidine (a compound having 2 benzylidene) which can be identified as chalcone, is an aromatic ketone that forms the central core for a variety of important biological activity (Kemelayefa, *et al,* 2022).

Chalcone are known as benzalacetophenone and phenyl styryl ketone, functioning majorly in blood pressure regulation, osteoporosis and cancer prevention, as well as boosting immunity (Singh *et al* 2011). They have also been reported to exhibit anti-inflammatory and anti-nociceptive activities (Kemelayefa, *et al,* 2022). They are alpha, beta unsaturated ketones holding two fragrant rings (rings A and B) having different arrangement of substituents. In chalcones, two fragrant rings are connected by an aliphatic three carbon series (Selahi *et al,* 2021). Also they are recognized as benzyl acetophenone (Aluru *et al,* 2020).

In view of the preceding, the objectives of this current study preliminarily focused on synthesis of some analogues from dibenzylidene, determination of their lethal dose (LD50), in acute toxicity models, and assessing sex variation; to set the stage for a next phase of the broader research that will examine their effectiveness/efficacy in the healing/management of pains.

METHODS

The following analogues of dibenzylidine were synthesized in the laboratory of Department of

pharmaceutical and Medicinal chemistry, Niger Delta University;
2,5-bis[[4-dimethylaminophenylmethylidene] cyclopentan-1-one (A1)
2,5-bis[[4-methoxyphenyl) methylidene]cyclopentan-1-one (A2)
2,5-diethylidenecyclopentan-1-one (A3)
2,5-diphenylmethylidenepentan-1-one (A4)
2,5-dibenzodioxoylmethyledenecyclopentan-1-one (A5)

Experimental Animals
Male and female mice weighing between 17-30g were used for this study. They were obtained from the animal house in Department of Pharmacology, Niger Delta University, Bayelsa State, Nigeria. They were housed in plastic cages under normal light / room temperature condition and fed with mash hybrid foods with access to clean water and allowed to acclimatize for duration of 2 weeks. Weight of the mice from each groups were measured with electronic weighing balance, recorded, and permanent marker used to tag the mice for identification after measurement and during observations.

Lorke's method was used to determine the LD50 of A1 to A5; designed and divided into two phases. The phase one consist of 9 mice divided into 3 groups of 3 mice each, which were administered 10, 100 and 1,000 mg/kg body weight of the test substance in order to establish the dose range that will produce any toxic effect within 24 hours. The number of mortality would be recorded after 24hours, but where no mortality occurs, testing proceeds to phase 2. The phase 2, involves 3 mice that are divided into 3 groups, one for each group, and are administered different doses each that is higher than those in phase 1, and after 24 hours mortality would be recorded.

The dosage for each mouse was calculated using their diverse weight for phase 2 and the average weight of the mice in each group for phase 1, and the stock solution of 50mg/ml. A clean beaker was placed on the weighing balance and zeroed before using the experimental spoon to take the 2,5-bis[[4-dimethylaminophenylmethylidene] cyclopentan-1-one (A1), which was gradually put into the beaker to get the accurate stock measure. Distilled water was used to mix the analogue by adding gradually

to monitor the solubility; wherein 2,5-bis[(4-dimethylaminopheny lmethylidene] cyclopentan-1-one (A1), was found soluble. But in cases where the analogue was insoluble, Tween 80 and DMSO was added and stirred to increase the solubility.

After mixture, the dosages measured for each mouse was orally administered in each group using oral canola then mice were put back in its group for observation. Same procedure was taken to administer the whole analogues to all the mice. After which, tissue paper was used to wipe off splashes that might have resulted during administration.

Determination of acute toxicity signs and LD50
Acute toxicity is involved in estimation of LD_{50} (the dose which has proved to be lethal - causing death to 50% of the tested group of animals). Determination of acute oral toxicity is usually an initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. (Akhila, et al., 2007). The Lorke's method also called Lorke's 1983 was used for the determination of the oral median lethal dose (LD50). Food was taken away from all the animals 24hours prior to the experiment but water was not. Water was also taken away, 3 hours before the experiment. They were exposed to food and water again 2 hours after the commencement of the experiment. In the first phase, nine mice were weighed using the electrical weighing balance and the mice were designated into three groups of three each. The average weights of each group were obtained and used to calculate the doses for 10, 100 and 1000mg/kg for each of the dibenzylidene analogue. The mice were observed for signs of acute toxicity and mortality for 24hrs. Same procedures were repeated for the second phase using three mice which were designated into three groups of a mouse each, given 1600, 3900 and 5000mg/kg using the calculated dosage volume for each dose using their individual weights respectively. The mice were observed for signs of acute toxicity and mortality for 24hrs. The whole procedures were repeated for each of the dibenzylidene derivatives.

Finally, Fisher's Exact test was used to analyze the association between sex and the various LD50 observed.

RESULTS
Table 1a: Oral acute toxicity signs evaluation of 2,5-bis[[4 dimethylaminophenylmethylidene] cyclopentan-1-one (A1).

Dosage (mg/kg)	DIA	HYP	SED	Right	SAL	SEZ	DEP	IMB	HYP-A	HPT	Death
10	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1600	0/1	0/1	1/1	0/1	0/1	0/1	0/1	0/1	1/1	0/1	0/1
3900	0/1	0/1	1/1	0/1	0/1	0/1	1/1	0/1	1/1	0/1	0/1
5000	0/1	0/1	1/1	0/1	0/1	0/1	1/1	0/1	1/1	0/1	0/1

Numerator= number of animals affected, Denominator= number of animals in a group, DIA= diarrhea, HYP= hyperventilation, SED= sedation, Right= righting effect, SAL= salivation, SEZ= seizure, DEP= depression, IMB=

imbalance, HYP-A= hyperactivity, HPT= hypotonia,   LD50= 5000mg/kg.
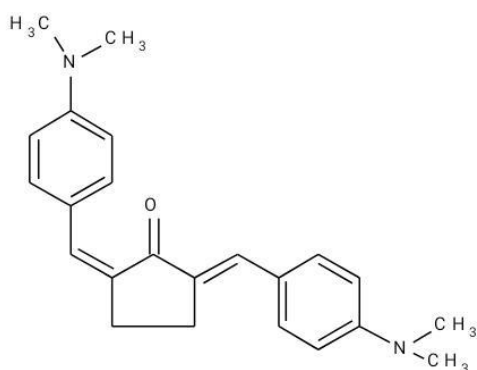
2,5-bis[(4-dimethylaminophenylmethylidene]cyclopentan-1-one (D6)

Table 1b: Parameters for the study of the determination of lethal dose of 2,5-bis[(4-dimethylaminopheny lmethylidene] cyclopentan-1-one (A1), and sex variation in mice.

Dosage	Tag	Weight(g)	Sex
1600mg/kg	T1	21.81	Male
3900mg/kg	T2	18.63	Female
5000mg/kg	T3	25.24	Male

Stock solution= 50mg/ml

$LD50 = \sqrt{D0 \times D100}$

D0 = highest dose without mortality.

D100 = lowest dose with mortality.

D0 =5000mg/kg, D100= >5000mg/kg

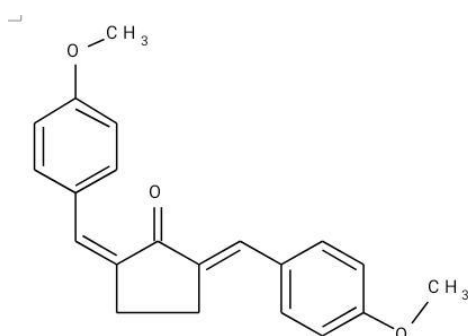$LD50 = \sqrt{5000mg/kg \times >5000mg/kg}$

LD50 = >5000mg/kg

LD50 = 5000mg/kg

Table 2a: Oral acute toxicity signs evaluation of 2,5-bis[(4 methoxyphenyl)methylidene] cyclopentan-1-one (A2).

Dosage(mg/kg)	DIA	HYP	SED	Right	SAL	SEZ	DEP	IMB	HYP-A	HPT	Death
10	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1600	0/1	0/1	1/1	0/1	0/1	0/1	1/1	0/1	1/1	0/1	0/1
3900	0/1	0/1	1/1	0/1	0/1	0/1	1/1	0/1	1/1	0/1	0/1
5000	0/1	0/1	1/1	0/1	0/1	0/1	1/1	0/1	1/1	0/1	0/1

Numerator= number of animals affected, Denominator= number of animals in a group, DIA= diarrhea, HYP= hyperventilation, SED= sedation, Right= righting effect, SAL= salivation, SEZ= seizure, DEP= depression, IMB= imbalance, HYP-A= hyperactivity, HPT= hypotonia, LD50= 5000mg/kg.

2,5-bis[(4-methoxyphenyl)methylidene]cyclopentan-1-one (D7)

Table 2b: Parameters for the study of the determination of lethal dose of 2,5-bis[(4-methoxyphenyl) methylidene] cyclopentan-1-one (A2), and sex variation in mice.

Dosage	Tag	Weight(g)	Sex
1600mg/kg	T1	23.6	Male
3900mg/kg	T2	22.0	Male
5000mg/kg	T3	26	Male

Stock solution= 50mg/ml
LD50 = $\sqrt{D0 \times D100}$
D0 = highest dose without mortality.
D100 = lowest dose with mortality.
D0 =5000mg/kg, D100= >5000mg/kg
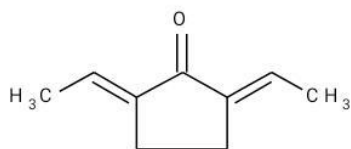LD50 = $\sqrt{5000mg/kg \times >5000mg/kg}$
LD50 = >5000mg/kg
LD50 = 5000mg/kg

Table 3a: Oral acute toxicity signs evaluation of 2,5-diethylidenecyclopentan-1-one (A3).

Dosage (mg/kg)	DIA	HYP	SED	Right	SAL	SEZ	DEP	IMB	HYP-A	HPT	Death
10	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1600	0/1	0/1	1/1	0/1	0/1	0/1	1/1	0/1	0/1	0/1	0/1
3900	0/1	0/1	1/1	0/1	0/1	0/1	1/1	1/1	0/1	0/1	0/1
5000	0/1	0/1	1/1	0/1	0/1	0/1	1/1	1/1	0/1	0/1	1/1

Numerator= number of animals affected, Denominator= number of animals in a group, DIA= diarrhea, HYP= hyperventilation, SED= sedation, Right= righting effect, SAL= salivation, SEZ= seizure, DEP= depression, IMB= imbalance, HYP-A= hyperactivity, HPT= hypotonia, LD50= 4415.88mg/kg.

2,5-diethylidenecyclopentan-1-one (D8)

Table 3b: Parameters for the study of the determination of lethal dose of 2,5-diethylidenecyclopentan-1-one (A3), and sex variation in mice.

Dosage	Tag	Weight(g)	Sex
1600mg/kg	T1	17.15	Female
3900mg/kg	T2	17.25	Female
5000mg/kg	T3	23.93	Male

Stock solution= 50mg/ml
LD50 = $\sqrt{D0 \times D100}$
D0 = highest dose without mortality.
D100 = lowest dose with mortality.
D0 =5000mg/kg, D100= 3900mg/kg
LD50 = $\sqrt{5000mg/kg \times 3900mg/kg}$
LD50 = 4415.88mg/kg
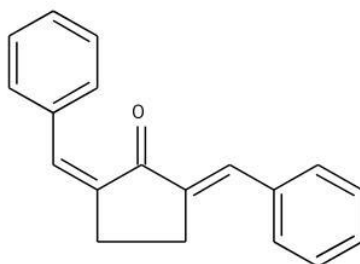
Table 4a: Oral acute toxicity signs evaluation of 2,5-diphenylmethylidenepentan-1-one (A4).

Dosage(mg/kg)	DIA	HYP	SED	Right	SAL	SEZ	DEP	IMB	HYP-A	HPT	Death
10	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1600	0/1	0/1	1/1	0/1	0/1	0/1	1/1	0/1	1/1	0/1	0/1
3900	0/1	0/1	1/1	0/1	0/1	0/1	1/1	0/1	1/1	0/1	0/1
5000	0/1	0/1	1/1	0/1	0/1	0/1	1/1	1/1	0/1	0/1	0/1

Numerator= number of animals affected, Denominator= number of animals in a group, DIA= diarrhea, HYP= hyperventilation, SED= sedation, Right= righting effect, SAL= salivation, SEZ= seizure, DEP= depression, IMB= imbalance, HYP-A= hyperactivity, HPT= hypotonia, LD50= 5000mg/kg.

2,5-diphenylmethylidenepentan-1-one (D9)

Table 4b: Parameters for the study of the determination of lethal dose of 2,5-diphenylmethylidenepentan-1-one (A4), and sex variation in mice.

Dosage	Tag	Weight(g)	Sex
1600mg/kg	T1	17.04	Male
3900mg/kg	T2	17.90	Male
5000mg/kg	T3	23.25	Female

Stock solution= 50mg/ml
$LD50 = \sqrt{D0 \times D100}$
D0 = highest dose without mortality.
D100 = lowest dose with mortality.
D0 =5000mg/kg, D100= >5000mg/kg
$LD50 = \sqrt{5000mg/kg \times >5000mg/kg}$
LD50 = >5000mg/kg
LD50 = 5000mg/kg

Table 5a: Oral acute toxicity signs evaluation of 2,5 dibenzodioxoylmethyledenecyclopentan-1-one (A5).

Dosage(mg/kg)	DIA	HYP	SED	Right	SAL	SEZ	DEP	IMB	HYP-A	HPT	Death
10	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1600	0/1	0/1	1/1	0/1	0/1	0/1	1/1	0/1	1/1	0/1	0/1
3900	0/1	0/1	1/1	0/1	0/1	0/1	1/1	0/1	0/1	0/1	0/1
5000	0/1	0/1	1/1	0/1	0/1	0/1	1/1	1/1	0/1	0/1	0/1

Numerator= number of animals affected, Denominator= number of animals in a group, DIA= diarrhea, HYP= hyperventilation, SED= sedation, Right= righting effect, SAL= salivation, SEZ= seizure, DEP= depression, IMB= imbalance, HYP-A= hyperactivity, HPT= hypotonia, LD50= 5000mg/kg.
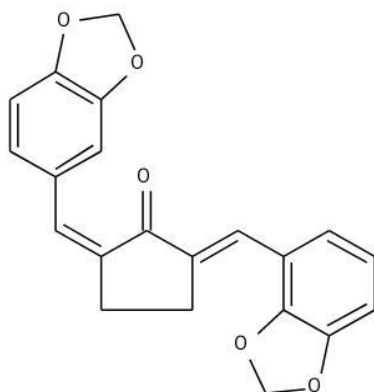
2,5-dibenzodioxoylmethyledenecyclopentan-1-one (D10)

Table 5a: Parameters for the study of the determination of lethal dose of 2,5-dibenzodioxoylmethy ledenecyclopentan-1-one (A5), and sex variation in mice.

Dosage	Tag	Weight(g)	Sex
1600mg/kg	T1	22.6	Female
3900mg/kg	T2	23.6	Male
5000mg/kg	T3	29	Male

Stock solution= 50mg/ml
$LD50 = \sqrt{D0 \times D100}$
D0 = highest dose without mortality.
D100 = lowest dose with mortality.
D0 =5000mg/kg,
D100= >5000mg/kg
$LD50 = \sqrt{5000mg/kg \times >5000mg/kg}$
LD50 =>5000mg/kg
LD50 = 5000mg/kg

Fisher's exact test

LD50 (mg/kg)	A1 (5000)	A2(5000)	A3 (4415.88)	A4 (5000)	A5 (5000)
MALE	2	3	1	2	2
FEMALE	1	0	2	1	1

Association between Sex and LD50 shows statistical significance p = p-0.5578.

DISCUSSION

In this study of determination of lethal dose of dibenzylidene analogues and sex variation in mice, the research questions which states;
1. What dosage of (A1) would cause mortality
2. What dosage of (A2) would cause mortality
3. What dosage of (A3) would cause mortality
4. What dosage of (A4) would cause mortality
5. What dosage of (A5) would cause mortality,

Previously in the introduction were careful answered in the results section, which are 5000mg/kg (A1); 5000mg/kg (A2); 4415.88mg/kg (A3); 5000mg/kg (A4); and 5000mg/kg (A5) respectively. Also in this study, the observed toxicities and LD50 did not seem to be dissociated from sex of the animals. So that sex of mice may influence /affect toxicity of the drugs on them. A3 analogue appeared more toxic amongst all analogues tested as mortality was observed before 24hours of its administration, whereas in the rest four analogues no mortality Inferring that 4000mg/kg is a safe dose for these other four analogues. Meanwhile from the lethal dose of the A3, 3500mg/kg is considered a safe dose as mortality did not occur in the mouse given a dosage of 3900mg/kg.

During the acute toxicity study, most mice were found very active while some were not as shown in the tables above. Some mice were immobile after the administration and some where able to control their reflex. Most of the mice appeared depressed as soon as the drug was administered to them. All through the study, none of the mice were found salivating, having diarrhea, experiencing seizure, hypotonic, hyperventilating and having writhing effect.

CONCLUSION

In conclusion, the study showed that the analogues of dibenzylidene used for the purpose of the study all have 3500mg/kg as a safe dosage of which the right calculation for the volume of dosage should be carefully examined during preparation in other not to overwhelm the digestive organs with loads of liquids and also to avoid high concentration of this drug. The LD50 for the A3 analogue of dibenzylidene was calculated to be 4415.88mg/kg, indicating that any living mice having a normal physiological state should experience mortality taking this dosage or greater. But whether this will be same irrespective of the animals' sex was not statistically evident.

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