ejpmr, 2022, 9(9), 477-483

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ACUTE TOXICITY AND LETHAL DOSE INVESTIGATION OF DIBENZYLIDENE DERIVATIVES PROFILED ALONG AGE VARIATIONS IN MICE

Erigbali P.P*[1]., Kemelayefa OJ[2]., Joffa PPK[1]., Kiridi E.G[1]. and Pughikumo D.T[1]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, Niger Delta University.
[2]Department of pharmacology & Toxicology, Faculty of Pharmacy, Niger Delta University.

***Corresponding Author: Erigbali P. P.**

Department of Human Physiology, Faculty of Basic Medical Sciences, Niger Delta University.

Article Received on 21/06/2022 **Article Revised on 11/07/2022** **Article Accepted on 01/08/2022**

ABSTRACT

In line with search for viable alternative medications with minimized adverse side effects, five derivatives of dibenzylidene synthesized in the pharmaceutical and Medicinal chemistry laboratory of Niger Delta University and labeled as D1 to D5 were investigated in a preliminary study. Acute toxicity and lethal dose studies in accordance with the method of Lorke were done amongst groups of mice with known age range. Following administration of the derivatives, a twenty-four hour observation period ensued for examining behavioural and physiology related toxicities. Results present some derivatives of dibenzylidene to have inflicted severe toxicity signs and mortality where others had mild toxicity signs and without mortality. The death recorded was within age group one (1) week which statistically was (?)

KEYWORDS: Toxicity, Dibenzylidine, LD50, Age, Derivatives, Mortality.

INTRODUCTION

There is no gainsaying the fact that search for alternative medications with accessibility, affordability and more importantly highest possible level of safety measure with respect to side effects has continued to garner momentum amongst global research community. Neurobehaviour scientists have also been looking out natural herbal and dietary influencers of Neurobehaviour e.g. anxiety, learning, memory, pain and others (Erigbali *et al*, 2018; Osim et al, 2017).

In view of this, an interesting report from a previous study had shown that plantain diet consumed in long – term increased pain threshold in mice model of experiment, implicating serotonin pathway (Erigbali *et al*, 2018; Erigbali *et al*, 2021). Meanwhile, herbal remedies as alternative therapy though widely patronized among some locals, still has need for acceptability in scientific community and some climes owing to protocols involved in certifying safety range of medications.

Usually, an initial step in the assessment and evaluation of the toxic characteristics of any substance in lieu of being considered for medicinal consumption is the screening / determination of lethal dose – i.e amount of substance capable of causing death to 50% of the tested group of specimens in specified period (USEPA, 2012). LD50 figures are frequently used as a general indicator of a substance's acute toxicity; thus, a lower LD50 is indicative of higher toxicity (USEPA, 2012).

As part of the commitment to seek alternative medications in addressing pain and its possible related health challenge, the initial procedure of investigating some derivatives of dibenzylidene for toxicity as the main aim of this research became essential.

Dibenzylidene derivatives are compounds containing a dibenzylidene group, characterized by two benzylidene (C6H5CH=) groups linked to a central carbon atom (Johnson *et al.*, 2018; Smith *et al*, 2018). These compounds have garnered significant interest across diverse fields due to their versatile chemical properties and potential applications; as some derivatives are reported to exhibit promising biological activities, which prompts research into their potential as drug candidates or scaffolds for drug design (Smith *et al*, 2015).

METHOD

Different derivatives of dibenzylidene were synthesized and prepared ready in the laboratory of pharmaceutical and medicinal chemistry, Niger Delta University. Derivatives nomenclature and designation are;

2,6-bis[(4-dimethylaminophenyl)methylidene]cyclohexan-1-one (D1)

2,6-bis[(4-methoxyphenyl)methylidene]cyclohexan-1-one (D2)
2,6-diethylidenecyclohexan-1-one (D3)
2,6-dibenzylidenecyclohexan-1-one (D4)
2,6-dibenzodioxylmethylidenecyclohexan-1-one (D5)

The determination of LD50 was carried out in line with the protocol of Lorke's method (1983), which proceeds in two stages I and II in that order.

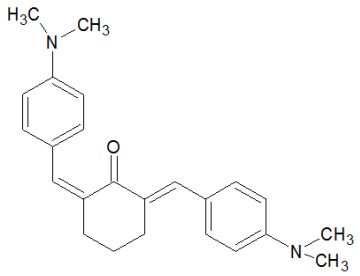
Stage I
This stage utilizes 9 of the rodents, which are further divided into 3 groups each containing 3 rodents. The various rodents group are treated with varying doses (10,100 and 1000mg/kg) of substance under test. All animals are then kept for surveillance within 24 hours during which monitoring of behavior and possible occurrence of mortality is done.

Stage II
Stage II involves the use of three animals, distributed into three groups of one animal each. The animals are administered higher doses (1600, 2900 and 5000mg/kg) of the test substance and observed within 24 hours for behavioural changes and /or mortality.

Then the LD50 is calculated using the formula;
LD50=√D0×D100
D0= highest dose that gave no mortality,
D100= lowest dose that gave mortality.

Mice within age ranges of 1 week to 8 weeks were used for this study. They were obtained from the animal house, in Department of pharmacology and toxicology, faculty of pharmacy, Niger Delta University, Wilberforce Island, Nigeria; and housed in standard plastic cages under normal light and temperature condition. They were fed with normal mash feeds with adequate accessibility to clean drinking water. All mice used in this investigation were handled in accordance with international, national and institutional guidelines for care and use of laboratory animals.

RESULTS



2,6-bis[(4-dimethylaminophenyl)methylidene]cyclohexan-1-one(D1)

Table 1: Oral acute toxicity evaluation of dibenzilydene derivatives (D1) 2,6-bis[(4-dimethylaminophenyl) methylidene] cyclohexan-1-one.

Dose (Mg/kg)	DIA	HYP	SED	SAL	SEZ	HPT	IMB	DEP	HAT	WRTR	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	1/1	1/1	1/1	0/1
3900	0/1	0/1	1/1	0/1	0/1	0/1	0/1	1/1	0/1	1/1	0/1
5000	0/1	0/1	1/1	0/1	0/1	0/1	0/1	1/1	1/1	1/1	0/1

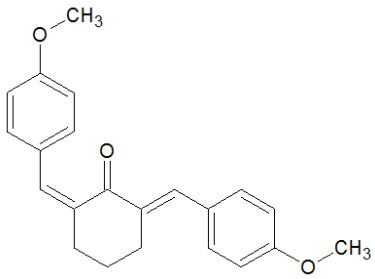
Numerator: number of animals affected
Denominator: number of animals in a group
DIA=Diarrhea, HYP=Hyperventilation, SED=Sedation, SAL=Salivation, SEZ=Seizure, HPT=Hypotonia, IMB=Immobility, DEP=Depression, HAT=Hyperactivity, WRTR=Writhing reflex.
LD 50=>5000mg/kg

Table 2: Parameters for the study of the determination of lethal dose of d1 derivative (2,6-bis[(4-dimethylaminophenyl) methylidene]cyclohexan-1-one) and age variation in mice.

Dosage	Tag	Age
1600mg/kg	T1	4 weeks
3900mg/kg	T2	1 week
5000mg/kg	T3	8 weeks

Stocksolutions=50mg/ml
LD 50= √D0 × D100

D0= highest dose without mortality
D100= lowest dose with mortality
D0=5000mg/kg, D100=>5000mg/kg
LD50 = √5000mg/kg × >5000mg/kg
= >5000mg/kg
LD50 = >5000mg/kg



2,6-bis[(4-methoxyphenyl)methylidene]cyclohexan-1-one (D2)

Table 3: Oral acute toxicity evaluation of dibenzilydene derivatives (D2) 2,6-bis[(4-methoxyphenyl) methylidene] cyclohexan-1-one.

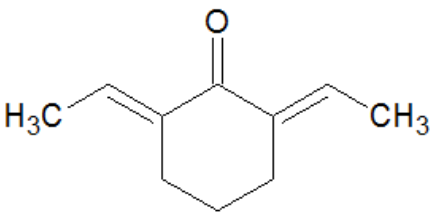
Dose (Mg/kg)	DIA	HYP	SED	SAL	SEZ	HPT	IMB	DEP	HAT	WRTR	DEATH
10	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1600	0/1	0/1	1/1	0/1	0/1	0/1	0/1	1/1	1/1	1/1	0/1
3900	0/1	0/1	1/1	0/1	0/1	0/1	0/1	1/1	0/1	1/1	0/1
5000	0/1	0/1	1/1	0/1	0/1	0/1	0/1	1/1	1/1	1/1	0/1

Numerator: number of animals affected
Denominator: number of animals in a group
DIA=Diarrhea, HYP=Hyperventilation, SED=Sedation, SAL=Salivation, SEZ=Seizure, HPT=Hypotonia, IMB=Immobility, DEP=Depression, HAT=Hyperactivity, WRTR=Writhing reflex.
LD 50=>5000mg/kg

Table 4: Parameters for the study of the determination of lethal dose of d2 derivative (2,6-bis[(4-methoxyphenyl) methylidene]cyclohexan-1-one) and age variation in mice.

Dosage	Tag	Age
1600mg/kg	T1	1week
3900mg/kg	T2	4 weeks
5000mg/kg	T3	8 weeks

Stock solutions=50mg/ml
LD50=√D0×D100
D0=highest dose without mortality
D100=lowest dose with mortality
D0=5000mg/kg, D100=>5000mg/kg
LD50=√5000mg/kg×>5000mg/kg
=>5000mg/kg
LD50=>5000mg/kg



2,6-diethylidenecyclohexan-1-one (D3)

Table 5: Oral acute toxicity evaluation of dibenzilydene derivatives (D3) 2,6-diethylidenecyclohexan-1-one.

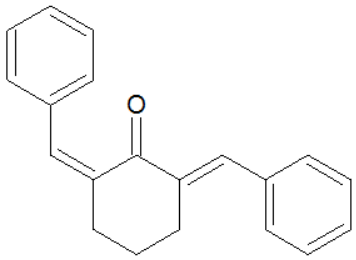
Dose (Mg/kg)	DIA	HYP	SED	SAL	SEZ	HPT	IMB	DEP	HAT	WRTR	DEATH
10	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1600	0/1	0/1	1/1	0/1	0/1	0/1	0/1	1/1	1/1	1/1	0/1
3900	0/1	0/1	1/1	0/1	0/1	0/1	0/1	1/1	0/1	1/1	0/1
5000	0/1	0/1	1/1	0/1	0/1	0/1	1/1	1/1	1/1	1/1	1/1

Numerator: number of animals affected
Denominator: number of animals in a group
DIA=Diarrhea, HYP=Hyperventilation, SED=Sedation, SAL=Salivation, SEZ=Seizure, HPT=Hypotonia, IMB=Immobility, DEP=Depression, HAT=Hyperactivity, WRTR=Writhing reflex.
LD 50=4415.8mg/kg

Table 6: Parameters for the study of the determination of lethal dose of d3 derivative (2, 6-diethylidenecyclohexan-1-one) and age variation in mice.

Dosage	Tag	Age
1600mg/kg	T1	8 weeks
3900mg/kg	T2	4 weeks
5000mg/kg	T3	1 week

Stock solutions=50mg/ml
LD50=√D0×D100
D0=highest dose without mortality
D100=lowest dose with mortality
D0=5000mg/kg, D100=3900mg/kg
LD50=√5000mg/kg× 3900mg/kg
= √19500000
= 4415.8mg/kg
LD50= 4415.8mg/kg



2,6-dibenzylidenecyclohexan-1-one (D4)

Table 7: Oral acute toxicity evaluation of dibenzilydene derivatives (D4) 2,6-dibenzylidenecyclohexan-1-one.

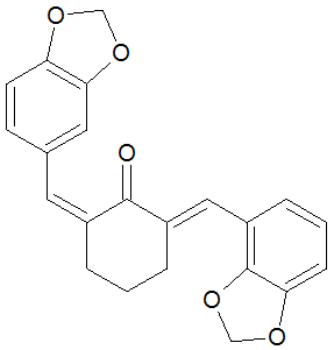
Dose (Mg/kg)	DIA	HYP	SED	SAL	SEZ	HPT	IMB	DEP	HAT	WRTR	DEATH
10	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
100	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1000	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
1600	0/1	0/1	1/1	0/1	0/1	0/1	0/1	1/1	1/1	1/1	0/1
3900	0/1	0/1	1/1	0/1	0/1	0/1	0/1	1/1	1/1	1/1	0/1
5000	0/1	0/1	1/1	0/1	0/1	0/1	0/1	1/1	1/1	1/1	0/1

Numerator: number of animals affected
Denominator: number of animals in a group
DIA=Diarrhea, HYP=Hyperventilation, SED=Sedation, SAL=Salivation, SEZ=Seizure, HPT=Hypotonia, IMB=Immobility, DEP=Depression, HAT=Hyperactivity, WRTR=Writhing reflex.
LD 50=>5000mg/kg

Table 8: Parameters for the study of the determination of lethal dose of d4 derivative (2,6-dibenzylidenecyclohexan-1-one) and age variation in mice.

Dosage	Tag	Age
1600mg/kg	T1	1 week
3900mg/kg	T2	8 weeks
5000mg/kg	T3	4 weeks

Stock solutions=50mg/ml
LD50=√D0×D100
D0=highest dose without mortality
D100=lowest dose with mortality
D0=5000mg/kg, D100=>5000mg/kg
LD50=√5000mg/kg×>5000mg/kg
=>5000mg/kg
LD50=>5000mg/kg



2,6-dibenzodioxylmethylidenecyclohexan-1-one (D5)

Table 9: Oral acute toxicity evaluation of dibenzilydene derivatives (D5) 2,6-dibenzodioxylmethylidenecyclohexan-1-one.

Dose (Mg/kg)	DIA	HYP	SED	SAL	SEZ	HPT	IMB	DEP	HAT	WRTR	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	1/1	1/1	1/1	0/1
3900	0/1	0/1	1/1	0/1	0/1	0/1	0/1	1/1	0/1	1/1	0/1
5000	0/1	0/1	1/1	0/1	0/1	0/1	0/1	1/1	1/1	1/1	0/1

Numerator: number of animals affected
Denominator: number of animals in a group
DIA=Diarrhea, HYP=Hyperventilation, SED=Sedation, SAL=Salivation, SEZ=Seizure, HPT=Hypotonia, IMB=Immobility, DEP=Depression, HAT=Hyperactivity, WRTR=Writhing reflex.
LD 50=>5000mg/kg

Table 10: Parameters for the study of the determination of lethal dose of d5 (2,6-dibenzodioxylmethylidenecyclohexan-1-one) derivative and age variation in mice.

Dosage	Tag	Age
1600mg/kg	T1	4 weeks
3900mg/kg	T2	8 weeks
5000mg/kg	T3	1 weeks

Stock solutions=50mg/ml
LD50=√D0×D100
D0=highest dose without mortality
D100=lowest dose with mortality
D0=5000mg/kg,D100=>5000mg/kg
LD50=√5000mg/kg×>5000mg/kg
=>5000mg/kg
LD50=>5000mg/kg

Table 11: Fisher's exact test

LD50 (mg/kg)	D1 (5000)	D2(5000)	D3 (4415.88)	D4 (5000)	D5 (5000)
1 WEEK	1	1	1	1	1
4 WEEKS	1	1	1	1	1
8 WEEKS	1	1	1	1	1

Association between Age and LD50 presents statistical significance p = p – 0.9043

DISCUSSION

Lethal dose determination involves establishing the amount of a substance, in this case, a dibenzylidene derivative, required to cause death of half a specific population of specimen in a given duration. In experimental mice models, researchers typically conduct dose-response studies to identify the lethal dose. This is done by exposing groups of mice to varying concentrations of the substance and observing the mortality rates. From the analysis of this investigation, the LD50 for the derivatives are; D1 ≥ 5000mg/kg; D2 ≥ 5000mg/kg; D3 ≥ 4415.8mg/kg; D4 ≥ 5000mg/kg; D5 ≥ 5000mg/kg.

Meanwhile, age profiling in experimental mice models involves studying how different age groups of mice respond to the substance. It helps assess potential variations in susceptibility or tolerance based on the age of the subjects. Factors such as metabolic rate, organ development, and overall health can influence how mice of different ages react to the dibenzylidene derivative (Brown *et al*, 2017; Raz *et al*, 2006). And by selecting mice of varying ages for experimental studies, researchers can gain insights into how biological processes change over the lifespan and how these changes impact health and disease (Garcia *et al*, 2019; Raz *et al*, 2006).

In this preliminary phase of investigation, attempt to understand whether some marginal extent of age disparity may have positive correlation with lethal dose appears to be significant. Although authors admit that whereas age profiling is a crucial required aspect, this study was limited to a specific range of age groups for mice, such that the current findings may not cover the entire lifespan of mice.

Generally, in course of this short – term toxicity investigation, many of the rodents appeared quite active, though a few were not (tables 1, 3, 5, 7 and 9). Some rodents were immobile after treatment, and some could still manage their reflexes. Seizure, diarrhea, hyperventilation, and salivation were not noticed throughout: following treatment with the test substance.

However, Derivative D3 exhibited higher toxicity propensity of all in the series recording one mortality in the 24 hours surveillance period it was served. But the others in the series were not lethal within the scope of study doses, therefore 4000mg/kg could be recommended as safe. Furthermore, derivative three which recorded single mortality at 5000 mg/kg could also be recommended as safe at dosage ≤ 3500mg/kg.

In conclusion, among the five derivatives of dibenzylidene in the current scope of study, only one was lethal with LD50 as 4415.88mg/kg, besides which others are recommended to be safe, not necessarily across age limit within this preliminary scope.

Limitation

While age profiling is a crucial aspect, this study may have a specific range of age groups for mice, which is limited so that findings may not cover the entire lifespan of mice.

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