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ANALYTICAL COMPARISON OF PAIN BEHAVIOUR MODELS STUDY USING STANDARD AND REFERENCE DRUG REGIMENS

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ABSTRACT

Typically, pain it is triggered by noxious stimuli and transmitted via specialized neural networks to the central nervous system (CNS), where it is interpreted as such. Various animal experiment models are developed to investigate pain phenomenon; and this study aimed to analytically consider two models of pain behaviour studies in exploring for analgesic potential of reference regimens – 2,5-bis[(4-methoxyphenyl)methylidene]cyclopentan-1-one (D7) and 2,5-diethylidenecyclopentan-1-one (D8); while using tramadol as standard control, with distilled water as normal control. Hot plate and water bath experiment models of thermal pain induction in randomly selected and grouped mice were implored to investigate effects of D7 and D8 on pain threshold. The results present both models tallying in their independent assessments, while D7 expressed significant ($P<0.0001$) benefit in analgesic potential.

KEYWORDS: Pain, Experiment models, dibenzylidene compounds, tramadol, threshold.

INTRODUCTION

Pain and inflammation remain the most important and devastating health problems affecting 80% of the world's population. Pain is an undisputed protective phenomenon, which may be a personal experience that is influenced to varying degrees by biological, psychological and social factors.

Acute pain usually occurs suddenly and has a known cause, like an injury, surgery, or infection while on the other hand Chronic pain may appear persistent without necessarily being definitive or delimitative (Berge, 2011).

Furthermore, the mechanism of pain together with the modes of action of treatment have been seen to be numerous and complex. In this regard, analgesics are designed to abolish painful sensations without causing loss of consciousness or suppressing other sensitivities (Mogil, 2009). Likewise, anti inflammatory drugs are symptomatic drugs that do not act on the cause.

Animal models of "experimental preparations have been developed such that it is used in one species for the purpose of studying phenomena occurring in another species (Mogil, 2009). For instance, animal models of pain are designed to mimic distinct clinical diseases to better evaluate underlying mechanisms and potential treatments; that may then be extrapolated to other close species (Mogil, 2009).

Animal experimental models of pain studies play a crucial role in helping researchers better understand pain mechanisms, test potential pain-relief drugs, and develop new treatment options. By inducing pain in animals in a controlled environment, scientists can observe pain responses, measure pain-related behaviours, and examine physiological changes associated with pain (Mogil, 2009). Pain behaviours tested include measuring paw withdrawal threshold, paw licking and duration, latency to response, jump, vocalization, and locomotor activity. Electrophysiological recordings can be used to assess changes in neuronal activity in response to pain stimuli and changes in gene expression, protein levels, as well as neurotransmitter release in pain pathways could be asssessed.

These models may vary depending on the type of pain being studied and can include inflammatory pain models, neuropathic pain models, and visceral pain models, among others. Common methods used to induce pain in animals include

the administration of chemical irritants, thermal stimuli, nerve injury, or surgical procedures.

It is essential for researchers to carefully consider ethical implications and animal welfare when conducting these studies. Ethical guidelines and animal welfare regulations are in place to ensure that animals used in pain research are treated with care and respect.

Analgesics are medications that relieve pain. There are three main types: non-opioid analgesics, opioid analgesics, and compound analgesics that combine the two previous forms. Opioid analgesics, used to relieve pain of moderate intensity. Drugs belonging to this group are paracetamol and non-steroidal anti-inflammatory drugs – NSAIDs (eg, aspirin, ibuprofen).

Dibenzylidene Compounds also known as Dibenzylideneacetone (dba) derivatives are a class of compounds that share structural similarities with dibenzylideneacetone. They are known for their interesting chemical properties and potential applications in various fields such as organic synthesis, materials science, and medicinal chemistry.

Dibenzylideneacetone (dba) itself is a well-known compound that is frequently used as a ligand in coordination chemistry, particularly in catalytic processes involving transition metals. Its analogues may have similar ligand properties, making them useful in catalysis and other chemical reactions.

Researchers have been exploring the synthesis and properties of dibenzylidene compounds to study their potential applications. By modifying the structure of the dibenzylidene moiety, researchers can tune the properties of these compounds for specific purposes, such as enhancing their stability, reactivity, or selectivity.

DBL, also known as Dibutylammonium chloride, is a potent pain reliever; inhibiting nociception signals centrally. It acts on specific receptors in the brain and spinal cord to reduce the perception of pain. There are several parameters that can be used when comparing the effects of analgesics on pain in experimental mice models. Some common parameters include measuring pain thresholds using techniques such as the hot plate test, tail flick test, or von Frey filaments. Additionally, pain-related behaviors such as paw licking or vocalizations in response to a painful stimulus are also assessed.

Other factors to consider include dosage of the analgesic, frequency of administration, and any potential side effects observed during the study. By carefully monitoring and evaluating these parameters, investigators may understand the efficacy, safety that different analgesic compounds proffer in treating pain in animal models.

One of the primary goals is to unravel the intricacies of pain perception, transmission, and modulation. By studying animals in controlled experimental settings, researchers can investigate the biological, neural, and behavioral aspects of pain.

Drug Development and Testing: Animal models are used to assess the efficacy and safety of potential pain-relief medications. This research aims to identify novel drug targets, evaluate therapeutic interventions, and optimize treatment strategies for various types of pain conditions.

By using animal models, researchers can discover and validate biomarkers associated with pain, which can aid in diagnosis, prognosis, and treatment monitoring in clinical settings.

Animal studies help in mapping pain pathways in the nervous system, facilitating the identification of key molecular targets that can be manipulated for pain management. While acknowledging the limitations, another aim is to predict human responses to pain stimuli and potential treatments based on observations in animal models. This can guide the design of human clinical trials and personalized pain management approaches.

Animal models allow researchers to study the processes underlying pain perception, transmission, and modulation in a controlled environment. This can lead to discoveries of new pain pathways and potential drug targets.

Drug Development: These models help in testing the efficacy and safety of new pain-relief drugs before human trials, accelerating the drug development process.

Comparative Studies: Animals can be used to compare different pain conditions and responses, giving insights into the variability of pain experiences. Translation to Humans: Findings from animal studies can provide a foundation for designing human clinical trials and understanding potential outcomes.

Ethical Concerns: There are ethical considerations regarding the use of animals in research, requiring strict adherence to regulations

and guidelines to ensure animal welfare.

Complexity of Pain: Animal models may oversimplify the complexity of human pain conditions, leading to gaps in understanding certain aspects of pain mechanisms.

Despite limitations, animal models remain vital in advancing pain research, offering valuable insights that contribute to the development of effective pain management strategies and treatment.

Animal experimental models are commonly used in pain studies to better the mechanisms of pain and the effectiveness of new compounds. Dibenzylidene derivatives are a class of compounds that have shown potential in pain management. By using animal models, researchers can assess the analgesic properties of these derivatives and determine their potential for treating pain in humans. The of the study would involve designing experiments carefully evaluate the effects of the dibenzylidene derivatives on pain behavior in animals, analyzing the data collected, and drawing conclusions about their efficacy as pain relievers.

Animal experimental models of pain studies have been crucial to further comprehension of pain conditions; with various animal designs modeling different types of pain, including inflammatory pain, neuropathic pain, and cancer pain.

One commonly used animal model of pain is the formalin test, which involves injecting formalin into the paw of a rodent and observing their pain responses. This model is often used to study inflammatory pain and has been instrumental in identifying the role of inflammatory mediators in pain processing (Tjolsen et al., 1992).

Another widely used animal model of pain is the chronic constriction injury (CCI) model, which involves ligating a nerve to induce neuropathic pain-like behaviors in rodents. This model has been valuable in studying the mechanisms underlying neuropathic pain and testing potential therapeutic interventions (Bennett & Xie, 1988).

Animal models of cancer pain, such as the bone cancer pain model, have also been developed to study the mechanisms of cancer-induced pain and evaluate novel treatments for cancer-related pain (Schwei *et al.*, 1999).

Overall, animal experimental models of pain studies have provided valuable insights into the complex mechanisms of pain processing and have been essential in the development of new pain therapies.

For instance, the study by Schwei *et al*. (1999) titled "Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain" focused on investigating the neurochemical and cellular changes that occur in the spinal cord in response to bone cancer-induced pain. In this study, the researchers utilized a murine model of bone cancer pain to mimic the pain experienced by individuals with cancer metastasis to the bone as its theoretical framework.

The study examined the alterations in neurotransmitter expression, glial cell activation, and neuronal plasticity' among others in lieu of elucidating potential targets for therapeutic interventions.

The empirical framework of the study involves the development of a rat model of neuropathic pain to mimic the pain sensations experienced by humans with nerve injuries. In this study, the researchers ligated a nerve in the rats to induce chronic constriction injury (CCI), which resulted in neuropathic pain-like behaviors in the animals. (Bennett & Xie et al, 1988)

The researchers observed pain-related behaviors in the rats following the CCI procedure, such as hypersensitivity to mechanical and thermal stimuli, spontaneous pain, and altered responses to pain medications. They also investigated the neurochemical and cellular changes in the spinal cord of the rats with CCI to understand the underlying mechanisms of neuropathic pain.

Overall, the study provided valuable insights into the development and characterization of a rat model of neuropathic pain that closely mimics the pain sensations observed in humans with nerve injuries. This model has since been widely used in pain research to study the mechanisms of neuropathic pain and test potential therapeutic interventions.

METHOD AND PROCEDURES

Animal Selection: Rodents are chosen as the animal model due to their well-established pain response mechanisms. Mice were weighed and marked, then divided into groups receiving different doses (as indicated in result) of dibenzylidene derivatives (D7, D8), then normal and standard control groups (i.e. distilled water and tramadol) respectively.

HOT PLATE TEST

This was in accordance with established protocol (Carlsson & Jurna, 1987). Animals were

administered regimens as appropriate, and put on the hot plate, with a constant temperature (55 °C), noting the jumping or paw licking as basal reaction time. The animals usually showed the response in 6-8 seconds. A cut off period of 15 sec was normally considered to avoid any damage to the paws. As the reaction time increased with drug treatment, 15 sec was taken as maximum analgesia and the animals were taken from the analgesiometer to avoid injury; then calculation of percent increase in reaction time at each time interval.

HOT Water BATH

This is a standard protocol that was adopted (Tieu et al, 2022). Mice were labeled by tail, for identification and administered regimens - D7 & D8 and the control regimens. They were dipped by small portion of tail in water bath (between forty-six and fifty-two degrees Celsius) for about thirty seconds, while observing/recording reactions (when tail is raised out of the water) in 30min, 60min and 90 min intervals (Tieu et al, 2022).

RESULTS

Table 1a: Hot Plate Test For D7.

		30mins	60mins	90mins
500mg/kg	A	14.1	24.3	8.2
	B	16.5	19.0	23.4
	C	4.6	4.2	7.0
1000mg/kg	A	4.7	17.9	8.9
	B	-	-	-
	C	17.2	11.2	19.0
1500mg/kg	A	6.9	15.6	29.7
	B	11.3	17.0	17.4
	C	12.8	14.3	7.3

Table 1b: Hot Plate Test For D8.

		30mins	60mins	90mins
500mg/kg	A		10	9.3
	B		10	3.4
	C		13.6	12.3
1000mg/kg	A		6.8	9.8
	B		9.4	9.8
	C		10.4	13.5
1500mg/kg	A		-	-
	B		11.9	13.1
	C		25	12.9

Table 2a: Water Bath Test For D7.

		30mins	60mins	90mins
500mg/kg	A	1.6	4.5	3.6
	B	5.4	3.2	2.4
	C	6.2	2.5	2.1
1000mg/kg	A	6.9	6.9	4.6
	B	2.6	2.4	2.8
	C	2.8	2.5	6.5
1500mg/kg	A	2.7	2.8	1.8
	B	2.1	3.5	2.6
	C	2.4	1.6	8.0

Table 2b: Water Bath Test For D8.

		30mins	60mins	90mins
500mg/kg	A		2.1	4.6
	B		3.1	20.7
	C		4.0	5.2
1000mg/kg	A		10	35.3
	B		20	47
	C		30	14.6
1500mg/kg	A		51	50.9
	B		4.6	28.4
	C		53	40.9

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

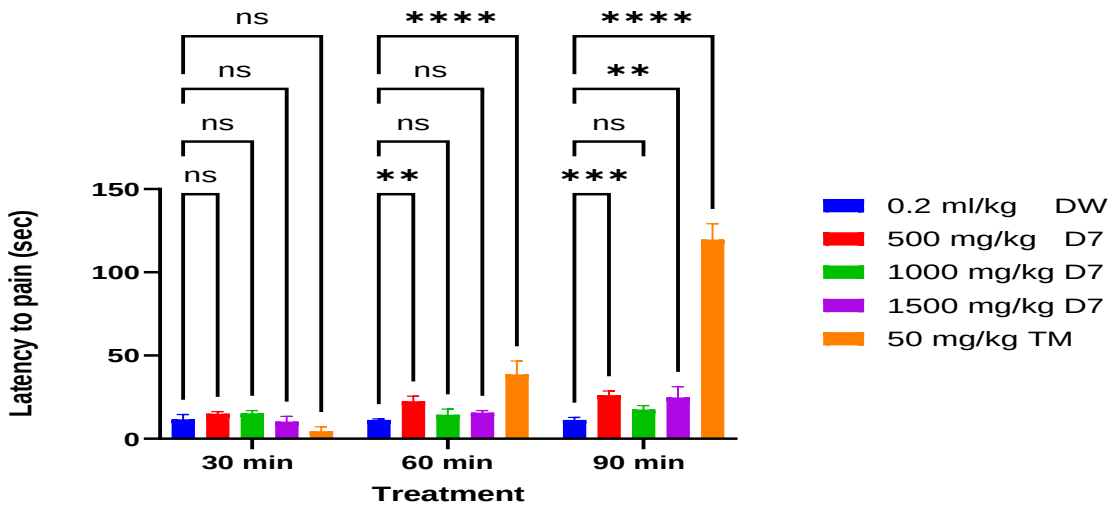

Figure 1a: Graphical Analysis for D7.

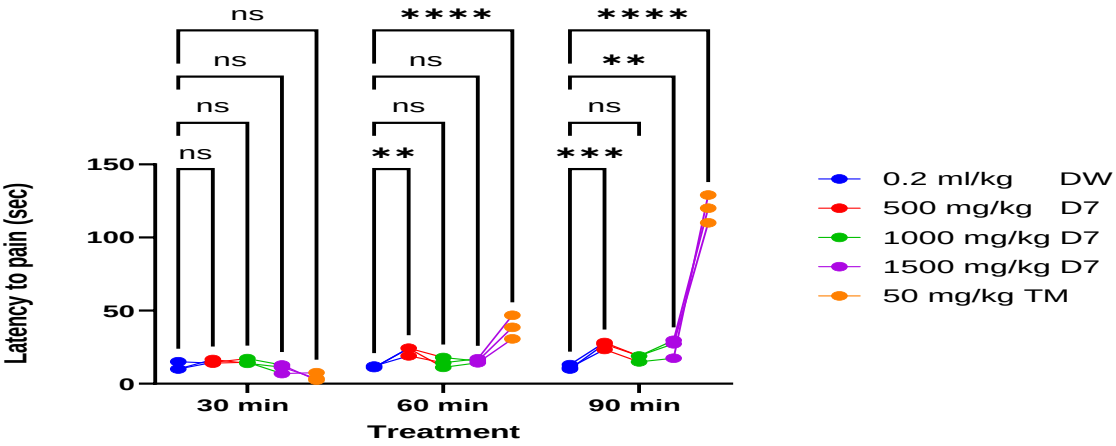

Figure 1b: Graphical Analysis for D7.

STATISTICS: Graph Pad Prism 10.2. 2Way ANOVA, Dunnett's Multiple Comparisons Test. At 60 min: 500mg/kg D7 indicated **significance when compared to the control DW 0.2 mg/kg, and At 90 min: 500mg/kg, 1500mg/kg of D7 indicated **, ***significance when compared to the control DW 0.2 mg/kg.

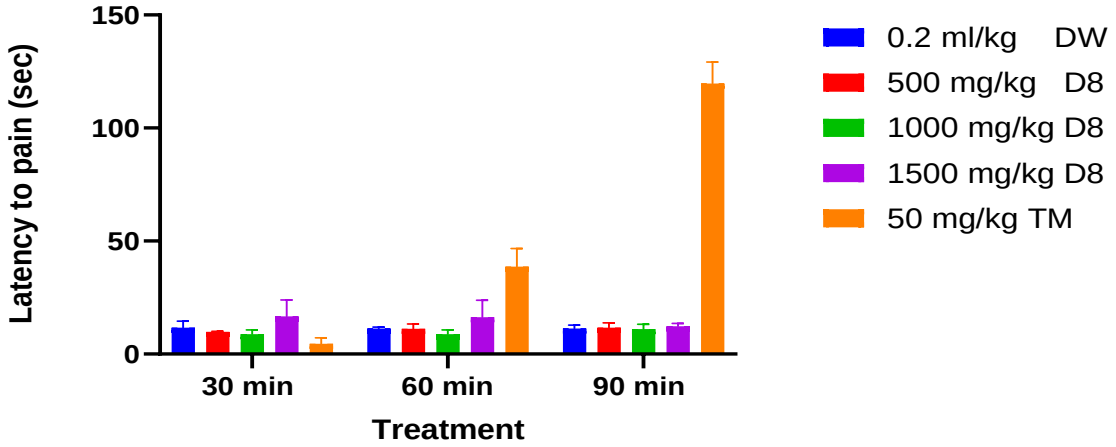

Figure 2a: Graphical Analysis for D8.

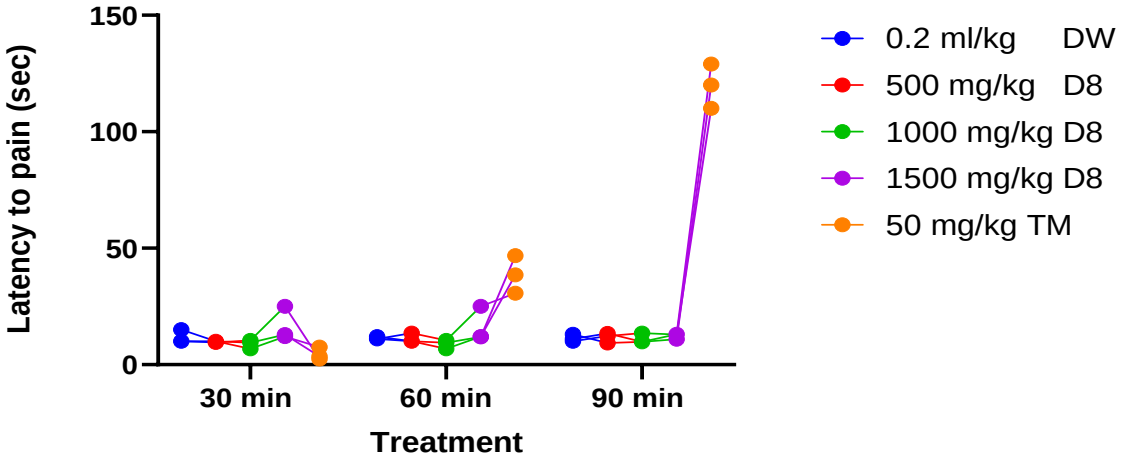

Figure 2b: Graphical Analysis for D8.

STATISTICS: Graph Pad Prism 10.2. 2Way ANOVA, Dunnett's Multiple Comparisons Test. D8 indicated no significance when compared to the control DW 0.2 mg/kg.

DISCUSSION AND CONCLUSION

This research analysis demonstrates an approach to studying the effects of dibenzylidene derivatives D7 and D8 in two specific animal experimental models of pain. Essentially, research in this area involves detailed experimental protocols, data analysis, and interpretation of results to advance our understanding of the analgesic properties of these compounds.

Some animal experimental models of pain studies include thermal models such as Hot plate and tail-flick tests involving application of heat stimulus to measure the response latency. Chemical models wherein formalin or acetic acid-induced writhing tests are used to induce pain by injecting intradermally or intraperitoneally. Mechanical models like Von Frey filaments test applying pressure to the animal's paw.

In this study, the focus was on hot plate and water bath thermal induction of pain. The results showed that one of the dibenzylidene derivatives; 2,5-bis[(4-methoxyphenyl)methylidene]cyclopentan-1-one or D7 exhibit analgesic properties in the two animal models of pain, highlighting their potential for further development as novel pain-relieving agents, whereas D8 did not show similar bebefit.

Meanwhile, comparative analysis of the behavioural parameter measuring threshold for pain in the two models, though independently experimented gave range and margin of results on the impact of those reference regimen (D7, D8) and the standard (tramadol) that may be considered as precise.

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PPENDIX 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 D6	-3.400	-12.23 to 5.434	No	ns	0.7187
0.2 ml/kg DW vs. 1000 mg/kg D6	-3.600	-12.43 to 5.234	No	ns	0.6790
0.2 ml/kg DW vs. 1500 mg/kg D6	1.333	-7.501 to 10.17	No	ns	0.9851
0.2 ml/kg DW vs. 50 mg/kg TM	7.167	-1.668 to 16.00	No	ns	0.1384
60 min					
0.2 ml/kg DW vs. 500 mg/kg D6	-11.17	-20.00 to -2.332	Yes	**	0.0099
0.2 ml/kg DW vs. 1000 mg/kg D6	-3.133	-11.97 to 5.701	No	ns	0.7700
0.2 ml/kg DW vs. 1500 mg/kg D6	-4.300	-13.13 to 4.534	No	ns	0.5387
0.2 ml/kg DW vs. 50 mg/kg TM	-27.33	-36.17 to -18.50	Yes	****	<0.0001
90 min					
0.2 ml/kg DW vs. 500 mg/kg D6	-14.87	-23.70 to -6.032	Yes	***	0.0006
0.2 ml/kg DW vs. 1000 mg/kg D6	-6.267	-15.10 to 2.568	No	ns	0.2252
0.2 ml/kg DW vs. 1500 mg/kg D6	-13.47	-22.30 to -4.632	Yes	**	0.0017
0.2 ml/kg DW vs. 50 mg/kg TM	-108.4	-117.2 to -99.53	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 D6	11.67	15.07	-3.400	3.426	3
0.2 ml/kg DW vs. 1000 mg/kg D6	11.67	15.27	-3.600	3.426	3
0.2 ml/kg DW vs. 1500 mg/kg D6	11.67	10.33	1.333	3.426	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.67	4.500	7.167	3.426	3
60 min					
0.2 ml/kg DW vs. 500 mg/kg D6	11.33	22.50	-11.17	3.426	3
0.2 ml/kg DW vs. 1000 mg/kg D6	11.33	14.47	-3.133	3.426	3
0.2 ml/kg DW vs. 1500 mg/kg D6	11.33	15.63	-4.300	3.426	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.33	38.67	-27.33	3.426	3
90 min					
0.2 ml/kg DW vs. 500 mg/kg D6	11.33	26.20	-14.87	3.426	3
0.2 ml/kg DW vs. 1000 mg/kg D6	11.33	17.60	-6.267	3.426	3
0.2 ml/kg DW vs. 1500 mg/kg D6	11.33	24.80	-13.47	3.426	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.33	119.7	-108.4	3.426	3

APPENDIX 2

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 D8	1.800	-7.679 to 11.28	No	ns	0.9663
0.2 ml/kg DW vs. 1000 mg/kg D8	2.800	-6.679 to 12.28	No	ns	0.8603
0.2 ml/kg DW vs. 1500 mg/kg D8	-5.000	-14.48 to 4.479	No	ns	0.4698
0.2 ml/kg DW vs. 50 mg/kg TM	7.167	-2.312 to 16.65	No	ns	0.1812
60 min					
0.2 ml/kg DW vs. 500 mg/kg D8	0.1333	-9.346 to 9.612	No	ns	>0.9999
0.2 ml/kg DW vs. 1000 mg/kg D8	2.467	-7.012 to 11.95	No	ns	0.9045
0.2 ml/kg DW vs. 1500 mg/kg D8	-5.000	-14.48 to 4.479	No	ns	0.4698
0.2 ml/kg DW vs. 50 mg/kg TM	-27.33	-36.81 to -17.85	Yes	****	<0.0001

90 min					
0.2 ml/kg DW vs. 500 mg/kg D8	-0.3333	-9.812 to 9.146	No	ns	>0.9999
0.2 ml/kg DW vs. 1000 mg/kg D8	0.3000	-9.179 to 9.779	No	ns	>0.9999
0.2 ml/kg DW vs. 1500 mg/kg D8	-0.9667	-10.45 to 8.512	No	ns	0.9966
0.2 ml/kg DW vs. 50 mg/kg TM	-108.4	-117.8 to -98.89	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 D8	11.67	9.867	1.800	3.676	3
0.2 ml/kg DW vs. 1000 mg/kg D8	11.67	8.867	2.800	3.676	3
0.2 ml/kg DW vs. 1500 mg/kg D8	11.67	16.67	-5.000	3.676	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.67	4.500	7.167	3.676	3
60 min					
0.2 ml/kg DW vs. 500 mg/kg D8	11.33	11.20	0.1333	3.676	3
0.2 ml/kg DW vs. 1000 mg/kg D8	11.33	8.867	2.467	3.676	3
0.2 ml/kg DW vs. 1500 mg/kg D8	11.33	16.33	-5.000	3.676	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.33	38.67	-27.33	3.676	3
90 min					
0.2 ml/kg DW vs. 500 mg/kg D8	11.33	11.67	-0.3333	3.676	3
0.2 ml/kg DW vs. 1000 mg/kg D8	11.33	11.03	0.3000	3.676	3
0.2 ml/kg DW vs. 1500 mg/kg D8	11.33	12.30	-0.9667	3.676	3
0.2 ml/kg DW vs. 50 mg/kg TM	11.33	119.7	-108.4	3.676	3