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COMPARATIVE STUDY OF THE EFFECT OF CODEINE AND TRAMADOL CO-INGESTION ON PAIN TOLERANCE, ALERTNESS AND LOCOMOTIVE ACTIVITY IN RATS.

[1]Ibeachu C. P. and *[2]Olorunfemi O. J.

[1]Department of Human Anatomy, College of Health Sciences University of Port Harcourt, Port Harcourt, Choba, Rivers State, Nigeria.
[2]Department of Human Physiology, College of Health Sciences University of Port Harcourt, Port Harcourt, Choba, Rivers State, Nigeria.

***Corresponding Author: Olorunfemi O. J.**
Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Choba Nigeria.

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ABSTRACT
This study was done to investigate the effect of codeine and tramadol co-ingestion on pain tolerance, alertness and locomotive activity in rats. A total number of twenty-five[25] wistar rats of both sex (male and female) weighing between 70-140g were collected and divided into 5 experimental groups of control group, codeine group, tramadol group, combined codeine and tramadol (group 1) and combined codeine and tramadol (group 2) and with 5 rats in each group. The rats in the group 1 (control) were given saline water, while the rats in the group 2, 3, 4 and 5 were given 0.1ml of codeine, 0.2ml of tramadol, 0.1ml + 0.1ml of codeine and tramadol (group 1) and 0.2 + 0.2ml of codeine and tramadol (group 2) respectively for 10 days. The rats were administered with the drugs every other day and observed after each treatment day for the effect of the drugs on alertness using passive avoidance, pain tolerance using analgesy meter and locomotive activity using navigation box and inverted screen. The measurement of latency on all the task was taken for 5 minutes maximum. The results of the study were analysed using statistical package for social science (SPSS) and presented as mean value standard error of mean (±S.E.M). The variation and the statistical significance of the groups were determined by Analysis of Variance (ANOVA) and turkey post Hoc test. The finding showed that ingestion of codeine increased pain tolerance and locomotive activity but had no significant effect on alertness; ingestion of tramadol had a positive increasing effect ($p<0.05$) on pain tolerance, alertness and locomotive activity; co-ingestion of codeine and tramadol (0.1ml + 0.1ml) significantly increased ($p<0.05$) pain tolerance, decreased ($p>0.05$) alertness and also decreased or increased locomotive activity; co-ingestion of codeine and tramadol (0.2ml + 0.2ml) significantly increased ($p<0.05$) pain tolerance, decreased ($p>0.05$) alertness and either decreased or increased locomotive activity. It is therefore concluded that co-ingestion of codeine and tramadol has the ability to alter the pain tolerance, alertness and locomotive activity in rats.

KEYWORDS: Tramadol, Codeine, Pain tolerance, Alertness, Locomotive activity.

1 INTRODUCTION
Tramadol is a synthetically produced opioid with a specific chemical formula; C16- H25- NO2.[1][2] It is an analgesic drug with a mechanism of action influencing pain sensation pathways.[3][2] Nowadays, this opioid drug has become one of the most widely prescribed drugs in the world.[4] it is an analgesic of choice during and the postoperative period, with a dose adjusted to the severity of the patient's pain and sensitivity.[5][6] Tramadol is a centrally acting synthetic analgesic drug with antinociceptive effects. It binds to the u-opioid receptors with weak affinity while inhibiting the neuronal uptake of norepinephrine and serotonin. Tramadol is well known drug for the treatment of intermediate or severe pain, with lower incidence of adverse effects such as respiratory depression, nausea.[7][8] Studies have shown that tramadol causes several effect in the central nervous system such as dizziness, euphoria, hallucination, dysphoria and seizures.

Codeine or 3-methylmorphine is the most commonly consumed opiate worldwide, widely used for its analgesic, antitussive and anti-diarrhoeal properties.[9][10]

It is an opiate mostly derived from morphine, isolated from opium and poppy straw.[11] The misuse and abuse of codeine containing products is a foremost emerging health challenges in various nations around the globe this might be because of such products are available in the range over-the-counter (OTC) medications which are

constantly bought without the need of a doctor's prescription.[12] This study is to determine the effect of Tramadol and Codeine co-ingestion on pain tolerance, alertness and locomotive activity.

2 MATERIALS AND METHODS

A total number of 25 rats were bought from the University of Port Harcourt Animal house in the Faculty of basic medical science. The rats were weighed and with various weight of about 70-150g and were randomly grouped into 5 groups in a wooden cage for 1week so as to acclimatize with members of the groups as well as the environmental condition with 12hours light/dark cycle and 50-60% humidity at a normal room temperature of about 27^{o}C where they were kept. During this period of acclimatization, the rats were fed with rat diet (poultry feeds) and provided with clean tap water consistently throughout the period of the study. All animals received care in accordance with the Nigerian law on experimentation with laboratory animals which is based on the US National Institutes of Health guidelines.

Experimental design

A total of 25 rats of both sex (male and female) were used in the study. The rats were grouped into 5 groups. The rats were put in a cage and each cage was clearly labelled with the drug category and their dosage. Rats were also marked for identification and the rats in each cage according to their groups were labelled 1 to 5. The rats were taken from the cage to the Neurophysiology Laboratory, institute of sports in the University of Port Harcourt where the test for pain, alertness and locomotive activity was performed.

Table 3.1: showing all the groups, division, test and duration.

GROUPS	DIVISION	TESTS	PROCEDURE/DURATION
Group 1	Control (5 rats)	Analgesymeter, Passive avoidance, Navigation, Inverted screen	The rats was made to perform all the pain, alertness and locomotion test without any drug treatment for 10 days
Group 2	Codeine (0.2ml) group (5 rats)	Analgesymeter, Passive avoidance, Navigation, Inverted screen	The rats was treated with 0.2ml 0f codeine and then made to perform all the pain, alertness and locomotion test for 10 days
Group 3	Tramadol (0.1ml) group (5 rats)	Analgesymeter, Passive avoidance, Navigation, Inverted screen	The rats was treated with 0.1ml 0f tramadol and then made to perform all the pain, alertness and locomotion test for 10 days
Group 4	Codeine+tramadol (0.1ml + 0.1ml) group (5 rats)	Analgesymeter, Passive avoidance, Navigation, Inverted screen	The rats was treated with 0.1ml + 0.1ml 0f codeine+tramadol and then made to perform all the pain, alertness and locomotion test for 10 days
Group 5	Codeine+tramadol (0.2ml + 0.2ml) group (5 rats)	Analgesymeter, Passive avoidance, Navigation, Inverted screen	The rats was treated with 0.2ml + 0.2ml 0f codeine+tramadol and then made to perform all the pain, alertness and locomotion test for 10 days

Site of Study

The experiment took place at the animal house and Neurophysiology Laboratory, Institute of sports; University of Port Harcourt, Rivers State, Nigeria.

Drugs/Preparation of drugs

The drugs used for this study were Tramadol HCL and Codeine phosphate. Mode of administration was done orally for both drugs with the use of an oral cannula.

The drugs were prepared as follows

Tramadol chlorhydrate (Tramal®, Pfizer, Guarulhos, Brazil)

The recommended dose of injectable tramadol chlorhydrate for rats, according to the manufacturer, is 20mg/kg; thus,

Tramadol (0.1ml): 0.1ml of tramadol was formed by the mixture of 0.05ml of TRAM + 0.05ml of saline solution, for a final solution volume of 0.1ml.

Tramadol (0.2ml): 0.2ml of tramadol was formed by the mixture of 0.10ml of TRAM + 0.10ml of saline solution, for a final solution volume of 0.2ml.

Codeine phosphate (Codein®, Cristália, São Paulo, Brazil).

The recommended dose of injectable COD for rats, according to the manufacturer, is 5mg/kg; thus,

Codeine (0.1ml): 0.1ml of codeine was formed by the mixture of 0.05ml of codeine phosphate + 0.05ml of saline solution for a final solution volume of 0.1ml.

Codeine (0.2ml):0.2ml of codeine was formed by the mixture of 0.1ml of codeine phosphate + 0.1ml of saline solution for a final solution volume of 0.2ml.

Neurocognitive test

The study was carried out for a period of 2 weeks, the experiment started with exposing the rats on the Instrumental process using the Anagelsy-Meter, Passive Avoidance box, Navigation box and Inverted screen for first day without any drug administration before exposing them to drug treatment for aperiod of 10 days.

Analgesy meter

Analgesy-meter is the classic device to perform Paw Pressure experiments according to the Randall-Selitto

method. Typically, the Randall-Selitto method is used as a rapid and sensitive screening of analgesic and anti-inflammatory drugs.

Passive Avoidance box

The passive avoidance (PA) apparatus consisted of a light and a dark compartment with the same size (20 × 20 × 30 cm) separated by a small gate. The floor of the two light and dark compartments were made of stainless-steel bars (0.5 cm diameter) separated by a distance of 1 cm.

The passive avoidance test is fear motivated test classically used to assess short term or long term memory on small laboratory animals (rats, mice). Passive avoidance paradigm requires the rat to behave contrary to their innate tendencies for the preference of dark areas and avoidance of bright ones.

Navigation box

The principle is based on the ability of the rats navigate. The navigation box is made up various chambers that are interconnected. It consists of an entering or starting point and an exit point. The rats were put inside the navigation box to explore the box and locate the exit point.

Inverted screen

This test is used to test for coordination. It is based on the fact that rats on their normal neurological condition have the ability to grip firmly or climb wire mesh in an inverted position.

Method of data analysis

The variation and the statistical significance of the differences between the groups were determined by Analysis of Variance (ANOVA) and Turkey post Hoc test. All the data obtained from the study was analyzed using Statistical Package for Social Science (SPSS).

Results are expressed in mean±S.E.M with * representing values that are statistically significant upon comparison with the control.

RESULTS

Table 2: Pain tolerance tests conducted on the test and control groups.

		treatment procedure					
		Analgesylmeter recordings week 1 Time(s)			Analgesylmeter recordings week 2 Time(s)		
GROUPS	Treatment	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
Group 1	Normal saline	15.22±3.43	12.74±3.76	12.26± 3.52	11.82±3.79	8.0±2.38	8.12±3.27
Group 2	Codeine (0.2ml)	18.70±2.56	21.30±3.34	15.88±4.07	23.24±1.76*	18.20 ±4.61	14.68±2.93
Group 3	Tramadol (0.1ml)	12.90± 5.60*	15.0±6.12	12.26±5.60	21.10±3.90*	21.82 ±2.62*	20.98±2.52*
Group 4	Codeine + tramadol (0.1ml + 0.1ml)	25.0±.0*	25.0±.0*	23.30± 1.70	16.60±3.76	14.94 ±4.23	11.66±3.78
Group 5	Codeine + tramadol (0.2ml + 0.2ml)	17.98±3.93	13.14±3.43 *	15.88±4.20	10.0±3.84 *	15.96 ±3.74	15.46±4.14

Results are presented in mean±sem. N = 5. * means values are statistically significant when compared to the control values.

Table 3: Showing the alertness conducted in the test and control groups.

		treatment procedure					
		Passive avoidance recordings week 1 Time(s)			Passive avoidance recordings week 2 Time(s)		
GROUPS	**Treatment**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**
Group 1	Normal saline	167.40±52.22	240.0±60.0	278.40±21.60*	180.0±73.48	300.0±.0	240.0±60.0
Group 2	Codeine (0.2ml)	240.0±60.0*	240.0±60.0	240.0± 60.0*	237.0±59.32	240.0±60.0	240.0±60.0
Group 3	Tramadol (0.1ml)	60.60±59 85*	67.60±58.56	60.80±58. 80	151.0±61.10*	277.40±22.60	211.0±55.46
Group 4	Codeine + tramadol (0.1ml + 0.1ml)	38.20±23.97*	187.60±69.10	240.0±60.0*	300.0±.0*	300.0±.0	300.0±.0
Group 5	Codeine + tramadol (0.2ml + 0.2ml)	300.0±.0*	109.20±59.58	300.0±.0*	300.0±.0*	300.0±.0	300.0±.0

Results are presented in mean±sem. N = 5. * means values are statistically significant when compared to the control values.

Table 4: Showing the locomotive activity conducted in the test and control groups.

		treatment procedure					
		Inverted screen recordings week 1 Time (s)			Inverted screen recordings week 2 Time (s)		
GROUPS	**Treatment**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**
Group 1	Normal saline	9.0±5.24	7.60±1.96	7.40±1.17	5.80±1.0	5.20±1.0	6.20±1.0
Group 2	Codeine (0.2ml)	7.0±1.70*	8.20±1.80*	9.20±1.56*	9.20±3.35	44.20±38.20	16.20±11.0
Group 3	Tramadol (0.1ml)	3.80±1.83*	1.20±.0*	1.0±.0*	8.0±3.54	5.0±1.58	5.0±0.3*
Group 4	Codeine + tramadol (0.1ml + 0.1ml)	19.0±5.34	10.40±4.68*	4.60±1.12*	10.60±3.59	8.40±2.66	5.60±2.77*
Group 5	Codeine + tramadol (0.2ml + 0.2ml)	33.40±12.14*	33.80±13.34*	43.0±15.92*	16.40±8.10	18.0±5.81	31.80±12.59*

Results are presented in mean±sem. N = 5. * means values are statistically significant when compared to the control values.

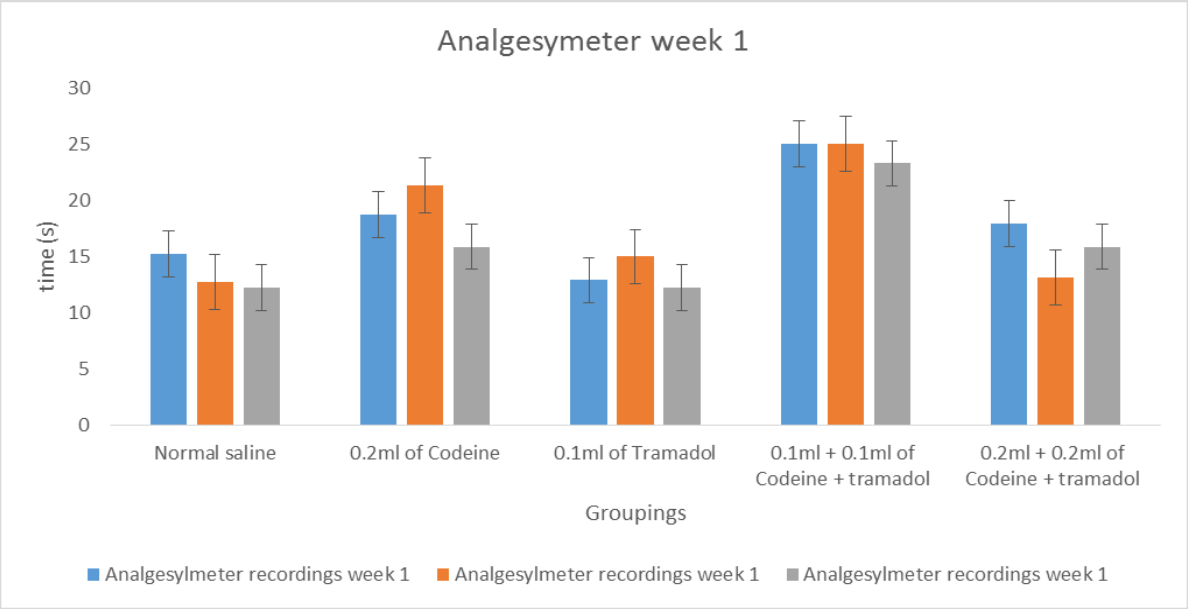

Figure 1: Chart showing patterns of pain tolerance response in the test and control groups in week 1 during treatment phase.

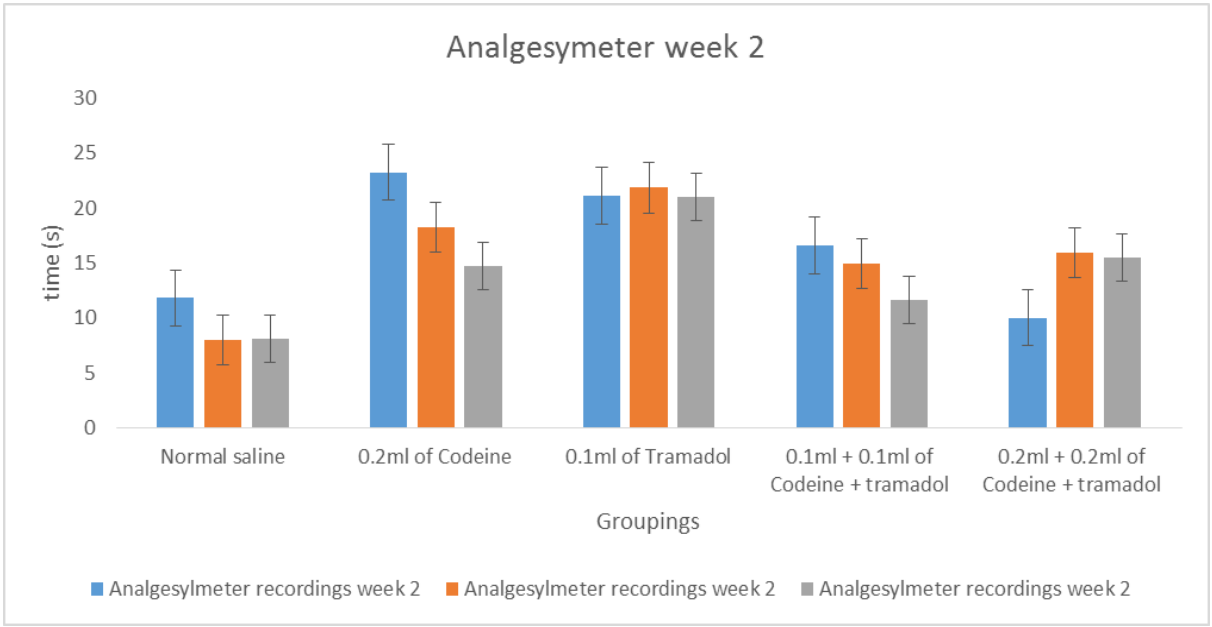

Figure 2: Chart showing patterns of pain tolerance response in the test and control groups in week 2 during treatment phase.

DISCUSSION

The effect of codeine and tramadol on pain tolerance was investigated using the analgesy meter. The test measured the pain tolerance threshold upon application of pressure on the paw of the rat.

From the results in table 2 showed that during trial 1, 2, and 3 in the first and second week of drug administration, the rats treated with 0.1ml/100g of codeine had increased pain tolerance when compared with to the rats treated with saline water (control group) this increased pain tolerance was significant during trial 1 in the second week of drug administration. The table also revealed the that pain tolerance of rats treated with 0.2ml/100g of tramadol decreased significantly in trail 1 in the first week but no significant difference in trail 2 and 3, however, in the second week of drug administration there was a significant increase ($p<0.05$) in the pain tolerance of rats treated with tramadol.

The result table 2 also showed that during co-ingestion of codeine and tramadol the pain tolerance of rats treated with codeine and tramadol (0.1ml/100g + 0.1ml/100g) increased more when compared to the pain tolerance of rats treated with 0.2ml + 0.2ml of codeine and tramadol. However, the pain tolerance of rats treated high dose of codeine and tramadol showed more increase than the rats in the control group. This is similar to the finding in Souza et al.,[13]; American Society of Health-system Pharmacists[14], which reported that these opiod (codeine and tramadol) drugs have an anesthetic property and they are used in the treatment of pain.

The passive avoidance test was used to evaluate the degree of alertness in rats after the ingestion of codeine and tramadol separately and in combined dose. From the result table 3 it was revealed that the rats treated with codeine during trial 2 and 3 in the 1st and 2nd week respectively showed no significant difference when compared with the control group, however the 3rd trail had a significant decrease in the 1st week and a non-significant decrease in the trial 2 of the 2nd week. This table also revealed the latency was shorter with the group administered with tramadol when compared with the control group that was treated with saline water. The result showed that codeine has no effect on alertness, whereas tramadol was shown to exhibit a positive influence on alertness this is in contrast to the work done by Ornstein et al.,[16] which showed a cognitive decline associated with opiate abusers.

Observations also revealed that the rats treated with low dose of codeine and tramadol (0.1ml/100g + 0.1ml/100g) showed a shorter latency than the rats treated with high dose of codeine and tramadol (0.2ml/100g + 0.2ml/100g) in the first week. However, in the second week, of treatment the latency of the rats treated with low dose of codeine and tramadol (0.1ml/100g + 0.1ml/100g) increased and the rats treated with high dose of codeine and tramadol (0.2ml/100g + 0.2ml/100g) had longer latency when compared with the rats in the group one (control group) treated saline water. This suggests a significant decrease in alertness and it is consistent with the findings by Abel-Ghany[17] that activation of opoid receptor impairs memory.

It was observed during the first week of drug treatment that the rat groups treated with codeine, tramadol, low dose of codeine and tramadol and high dose of codeine and tramadol had a longer latency when compared to the rats treated with saline water in the control group, this suggest that the locomotive activity decreased ($p>0.05$) in rats treated with these drugs.

This is consistent with the finding in Szkutnik-fiedler et al.[18] that higher doses of tramadol and administration for a longer period had greater impact on the decrease of locomotive activity in rats.

From the result table 4.4, the result showed that the rats treated with tramadol, had a significant decrease in locomotive activity as the latency was shorter (the time it took for the rats to fall off the inverted screen) when compared with the control group during the first week of drug administration and a slight decrease in the second week, this reveals a decrease in locomotive activity and it is similar to the finding in Szukutnik-Fiedler[18] which showed that tramadol had great impact on the decrease of locomotor activity.

This study therefore concludes that ingestion of codeine and tramadol separately, increases pain tolerance therefore codeine and tramadol have a positive increasing effect on pain tolerance. Co-ingestion of codeine and tramadol (0.1ml + 0.1ml) significantly increases pain tolerance and therefore has a positive significant effect on pain tolerance. Co-ingestion of codeine and tramadol (0.2ml + 0.2ml) increases pain tolerance and therefore has a positive significant effect on pain tolerance.

After a careful analysis on the result, this study concludes that ingestion of codeine has a no significant effect on alertness while the ingestion of tramadol has a positive increasing effect on alertness. Co-ingestion of codeine and tramadol at 0.1ml + 0.1ml significantly decreases alertness and therefore has a negative significant effect on alertness. Co-ingestion of codeine and tramadol at 0.2ml + 0.2ml also decreases alertness and therefore has a negative significant effect on alertness.

This study therefore concludes that ingestion of codeine and tramadol separately, increases locomotive activity and therefore codeine and tramadol have a positive increasing effect on locomotive activity. Co-ingestion of codeine and tramadol at 0.1ml + 0.1ml either increases or decreases locomotive activity or therefore it has both positive and negative effect on locomotive activity. This study also concludes that co-ingestion of codeine and

tramadol at 0.2ml + 0.2ml either increases or decreases locomotive activity and therefore it has both positive and negative effect on locomotive activity. This is consistent with the finding according to Zhang and Kong[19] that administration of morphine to rat increases locomotor activity as well as Szukutnik-Fiedler (2012) which showed that tramadol had great impact on the decrease of locomotor activity.

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