



THE EFFECT OF SUB-CHRONIC USE OF TRAMADOL ON GAIT AND COGNITIVE-MOTOR FUNCTIONS IN WISTER RATS

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ABSTRACT

This research study was done to investigate the Effect of sub-chronic use of Tramadol on Gait Pattern and Cognitive- Motor functions using Navigation Test, Barnes maze test and Gait pattern test. This whole research was carried out in three weeks. Each week, the animals (Wister rats) performed the three tasks. At the end of the three weeks the results gotten were being evaluated. The research was done on 25 Albino Wister rats. They weighed between about 140-160 g. After acclimatization, the rats were divided into five groups which consisted of five rats each, according to the dosage of the drug that was administered. Group one (Control) were not administered with any drug and were made to perform the task. Group two (low dose) received 0.1ml of Tramadol, Group three (medium dose) received 0.2ml of Tramadol, group four (high dose) received 0.3ml of Tramadol, and Group five received 0.1ml of Epinephrine. The drugs were administered intraperitoneally. The groups given Tramadol were allowed to perform all experimental task 5 minutes after Administration and the group given Epinephrine was allowed to perform the tasks 30 minutes after administration. The results were presented as a mean value standard error of means (\pm sem) and statistical analysis was done using the SPSS and excel. The results further showed that cognitive motor function and gait were significantly ($p < 0.05$) affected by the medium dose and high dose of tramadol. From the results obtained at the end of the three weeks study, groups that received tramadol showed a decline in adaptive locomotion, as they spent more time to complete the task when compared to the control group for Navigation, spatial memory was significantly enhanced as the dose increased as observed in Barnes maze test while quality of gait pattern declined significantly ($p < 0.05$) as recorded in the Gait Pattern test, conclusively, continuous use of tramadol for a long time surely is hazardous as it affects locomotion and motor coordination coupled with a wobbled gait pattern with increasing dose of tramadol administration in the study.

KEYWORDS: Tramadol, motor functions, gait pattern, Barnes maze, wistar rat.

Background of study

Presently there is an increase rate in the abuse of drugs among youths in Nigeria. Tramadol which is a central acting analgesic was manufactured in Germany more than 35 years ago. Tramadol is a known opioid prescribed by doctor's use for the treatment of moderate pain. It also possesses catecholamine and serotonin reuptake property.^[1] This activity profile suggests antidepressants potency and in animal paradigm, tramadol indeed shows antidepressants activity.^[2]

Tramadol is a centrally acting synthetic opioid analgesic and SNRI (serotonin/norepinephrine reuptake-inhibitor) that is structurally related to codeine and morphine. Due

to its good tolerability profile and multimodal mechanism of action, tramadol is generally considered a lower-risk opioid option for the treatment of moderate to severe pain. It is considered a Step 2 option on the World Health Organization's pain ladder and has about 1/10th of the potency of morphine.^[3]

Animal and human experiments have shown and provided findings into the acute effect of psychoactive substances and the common effect of chronic use of tramadol on cognitive function.

It is believed that it kills brain cells, yet majority still abuse it which later affects their memory coordination

and performance of one's activity. You may neglect healthy social interactions and obligations or experience increased interpersonal conflict, damaging even your most important relationships.^[4] Global trends indicate an increased misuse of opioids with a 5% prevalence reported in Europe (United Nations Office on Drugs and Crime, 2017). An online survey of UK university students listed Tramadol as among the most commonly misused prescription drug.^[4]

Gait is the pattern of movement of the limbs in animals during locomotion. Human gait can be defined as the different ways in which a human can move, either naturally or as a result of specialized training.^[5] Regulation of Gait by the cerebral cortex is as a result of sensory input from multiple areas of the cerebral cortex, such as the visual cortex, vestibular cortex, and the primary sensory cortex, is required for skilled locomotor tasks. This information is integrated and transmitted to the supplementary motor area (SMA) and premotor area (PM) of the cerebral cortex where motor programs are created for intentional limb movement and anticipatory postural adjustments. For example, the motor cortex uses visual information to increase the precision of stepping movements. When approaching an obstacle, an individual will make adjustments to their stepping pattern based on visual input regarding the size and location of the obstacle. The continuous abuse of tramadol has a negative effect on cognitive functions mainly attention, memory, visuospatial functions executive functions, decision making and reaction time. When taken at high dose, it could cause seizure which could cause damage to the brain or eventually lead to death. According to^[6], Tramadol abuse could cause to impairment of memory as speculated hence, the effect of sub-chronic use of tramadol on gait and cognitive-motor functions in wister rats was investigated in the study.

MATERIALS AND METHOD

Experimental animals

This research work was carried out in the University of Port Harcourt, The Faculty of Basic Medical Science, Twenty-five (25) Wister rats were purchased from the Animal house. They were weighed and kept in different plastic cages according to the range of their weights and grouped afterwards. They weighed between 140-160g and were fed with feed and water every day and their beddings were changed frequently. They were kept and maintained under good laboratory condition. The procedures and protocols used in this experimental study were approved by Ethical Committee, University of Port Harcourt, Rivers State, Nigeria and conform to the guideline of the care and use of animals in research and teaching. Acclimatization was done.

Experimental Design

The animals were divided into five (5) groups, which consisted of five rats each.

Group 1 (Control)- This group consisted of five rats. No drug was administered to them and were made to carry out the tests.

Group 2 (Low Dose) – This group consisted of five rats. Each rat was administered 0.1ml of Tramadol and were made to carry out each of the test.

Group 3 (Medium Dose) – The group consisted for five rats. They were administered 0.2ml of Tramadol and were made to carry out each test.

Group 4 (High Dose) – This group consisted of five rats. They were administered 0.3ml of Tramadol and were made to carry out each test.

Group 5 (Epinephrine) – This Group consisted of five rats. Each was administered with 0.1ML of Epinephrine and were made to carry out each test.

Drugs used: The drugs used during this research were 50mg/1ml of Tramadol hydrochloride and Epinephrine. A solution containing 1.5ml/10ml of Tramadol prepared. 1.5ml of Tramadol Hydrochloride contain 75mg of the drug. Tramadol solution were administered intraperitoneally to Group two which was given 0.1ml of the diluted solution, three was given 0.2ml and four was given 0.3ml and the test was carried out after five minutes of administration. For Group Five, 0.1ml of Epinephrine was administered to each rat intraperitoneally and the tests were shortly after administration.

Experimental test: In order to study the effect of chronic use of Tramadol on Gait and Cognitive- Motor Functions, the following test were done.

Navigation test: This test is used for learning and memory. It is also used to study the effect of pharmacological agents and the impact of these substances in learning and memory. The rats perform three trials per test for each week. Time taking for each animal to complete the test was recorded in seconds. And the allocated time for the experiment was 5 minutes per trial. If after 5 minutes the animal was not able to complete the test, the rat was taken out of the box and the time was recorded as 300 seconds.

Barnes maze test: This test is done in order to measure spatial memory and learning (Barnes, 1979). The basic function is to measure the ability of a rodent (Wistar rat) to learn and remember the location of a target zone using a configuration of distal visual cues located around the testing area and it is also used to test the effect of some drugs on cognition in rodents. Each rat performed three trials for each day and the time allocated for each trial was 5 minutes. The test was done in the three weeks of the study. At first, they were placed in the tunnel, then they were placed in the center of the maze and allowed to locate the escape tunnel. If before five minutes, it located the tunnel, the time it took to locate the escape tunnel was then recorded in seconds. If after five minutes the rat was not able to locate the tunnel, it was recorded as 300 seconds.

Gait pattern test: this test is done to access the quality of movement. The rats were placed on the gait plate and observed how they moved. Observations were based on the time it took for the rats to move from Start to finish. Each rats performed three trials. They were placed. Each trial was meant to be done within five minutes, and if after 5 minutes, the rat did not move, the rat was removed from the plate. If the rat was able to move from the beginning of the pate to the end without moving away from the marked line, the time they spent was recorded. If the rat during the course of the test moved to

another lane or turned back, the time was also recorded if the rat didn't move at all in five minutes, it was recorded as 0.

Statistics Analysis

Values were expressed as mean ± standard deviation (SD). The data were analyzed by using SPSS for windows (version 10.0). The significance of differences was calculated by using One-Way ANOVA test. was considered significant.

RESULTS

Table 1: Pattern of adaptive locomotion in the test groups treated with tramadol and control rats in the three weeks study.

NAVIGATION TEST TIME (s)										
Groups	Treatment	Week 1			Week 2			Week 3		
		Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
1	Control	300.00 ±0.00	243.20 ±56.80	113.60 ±47.79	300.00 ±0.00	247.40 ±52.60	300.00 ±0.00	161.40 ±58.24	185.80 ±51.09	276.00 ±24.00
2	0.1ML Tram.	209.80 ±44.11	190.40 ±67.48	243.20 ±56.80	186.00 ±69.84	245.60 ±54.40	245.00 ±58.80	249.00 ±51.00	242.00 ±58.00	141.20 ±64.91
3	0.2ML Tram.	120.00 ±73.49	120.00 ±73.49	120.00 ±73.49	67.99 ±58.53	120.60 ±73.24	120.60 ±73.24	80.40 ±58.09	120.60 ±73.24	120.60 ±73.24
4	0.3ML Tram.	250.40 ±30.40	251.80 ±48.20	212.20 ±53.82	300.00 ±0.00	254.00 ±55.00	266.00 ±88.00	247.20 ±52.80	281.40 ±18.60	300.00 ±0.00
5	0.1ML Epine.	300.00 ±0.00	221.08 ±25.95	300.00 ±0.00	300.00 ±0.00	300.00 ±0.00	300.00 ±0.00	300.00 ±0.00	300.00 ±0.00	300.00 ±0.00

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

Table 2: Pattern of cognitive response in the test and control rats in the three weeks study.

BARNES TEST TIME(s)										
Groups	Treatment	WEEK 1			WEEK 2			WEEK 3		
		Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
1	Control	100.00 ±0.00	80.00 ±44.72	80.00 ±44.72	80.00 ±12.25	50.00 ±0.00	30.00 ±44.72	70.00 ±20.00	70.00 ±12.25	30.00 ±20.00
2	0.1ML Tram.	80.00 ±44.72	100.00 ±0.00	70.00 ±44.72	21.26 ±19.60	21.60 ±19.60	2.00 ±0.00	20.80 ±11.93	40.80 ±18.28	21.60 ±19.60
3	0.2ML Tram.	2.00 ±0.00	2.00 ±0.00	2.00 ±0.00	11.60 ±9.60	2.00 ±0.00	11.60 ±9.60	11.20 ±9.71	1.20 ±0.49	11.60 ±9.60
4	0.3ML Tram.	40.00 ±22.36	10.40 ±22.15	10.00 ±22.36	30.00 ±12.25	1.60 ±0.40	11.69 ±9.60	30.00 ±20.00	11.60 ±9.60	11.20 ±21.71
5	0.1ML Epine.	11.60 ±21.47	1.60 ±0.89	2.00 ±0.00	11.60 ±9.60	1.60 ±0.40	2.00 ±0.00	11.60 ±9.60	1.60 ±0.40	2.00 ±0.00

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

Table 3: Showing Gait pattern response in the test treated with Tramadol and control rats in the three weeks study.

GAIT PATTERN TEST PERCENTAGE (%)										
Group	Treatment	Week 1			Week 2			Week 3		
		Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
1	Control	100.00 ±0.00	80.00 ±20.00	80.00 ±20.00	80.00 ±12.25	50.00 ±0.00	50.00 ±0.00	80.00 ±12.25	70.00 ±12.25	60.00 ±10.00
2	0.1ML Tram.	90.00 ±10.00	100.00 ±0.00	80.00 ±12.25	60.00 ±10.00	60.00 ±10.00	50.00 ±0.00	50.00 ±0.00	60.00 ±10.00	60.00 ±10.00
3	0.2ML Tram.	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	10.00 ±10.00	0.00 ±0.00	10.00 ±10.00	10.00 ±10.00	0.00 ±0.00	10.00 ±10.00

4	0.3ML Tram.	40.00 ±10.00	10.00 ±10.00	10.00 ±10.00	30.00 ±12.25	0.00 ±0.00	10.00 ±10.00	30.00 ±20.00	10.00 ±0.00	10.00 ±10.00
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Values are presented in mean ± sem. N = 5. * mean values are statistically significant when compared to the control values

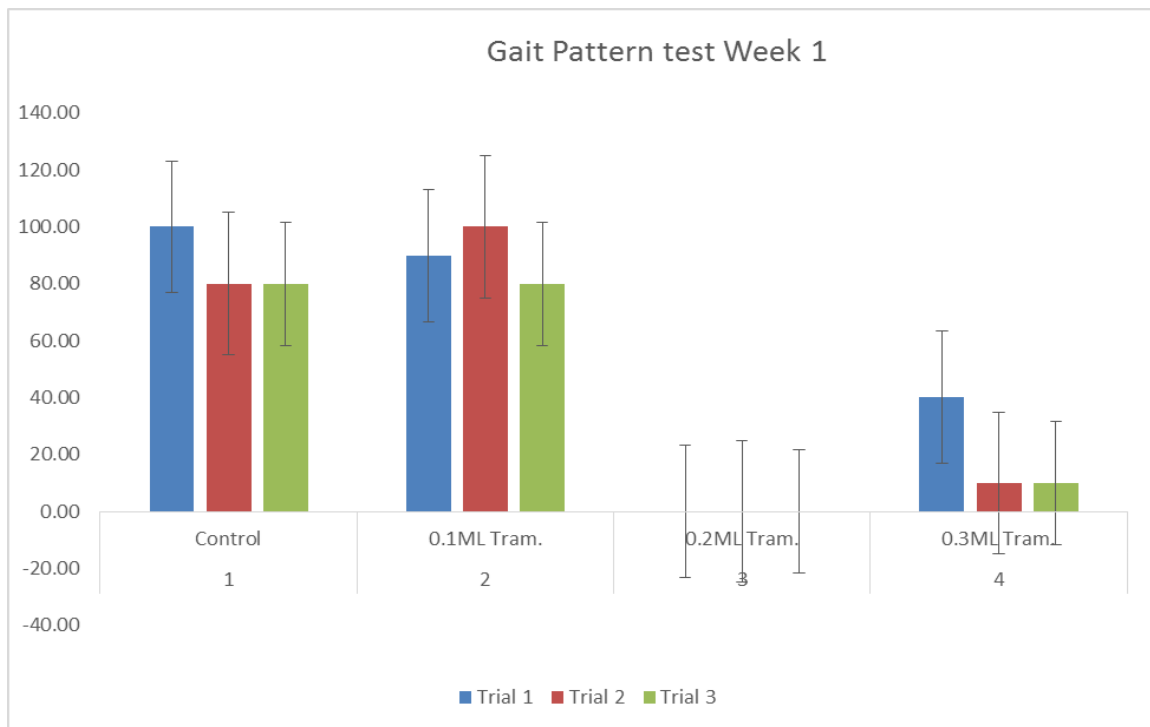


Fig. 1: Pattern of gait response in the test treated with Tramadol and control groups in week 1 study.

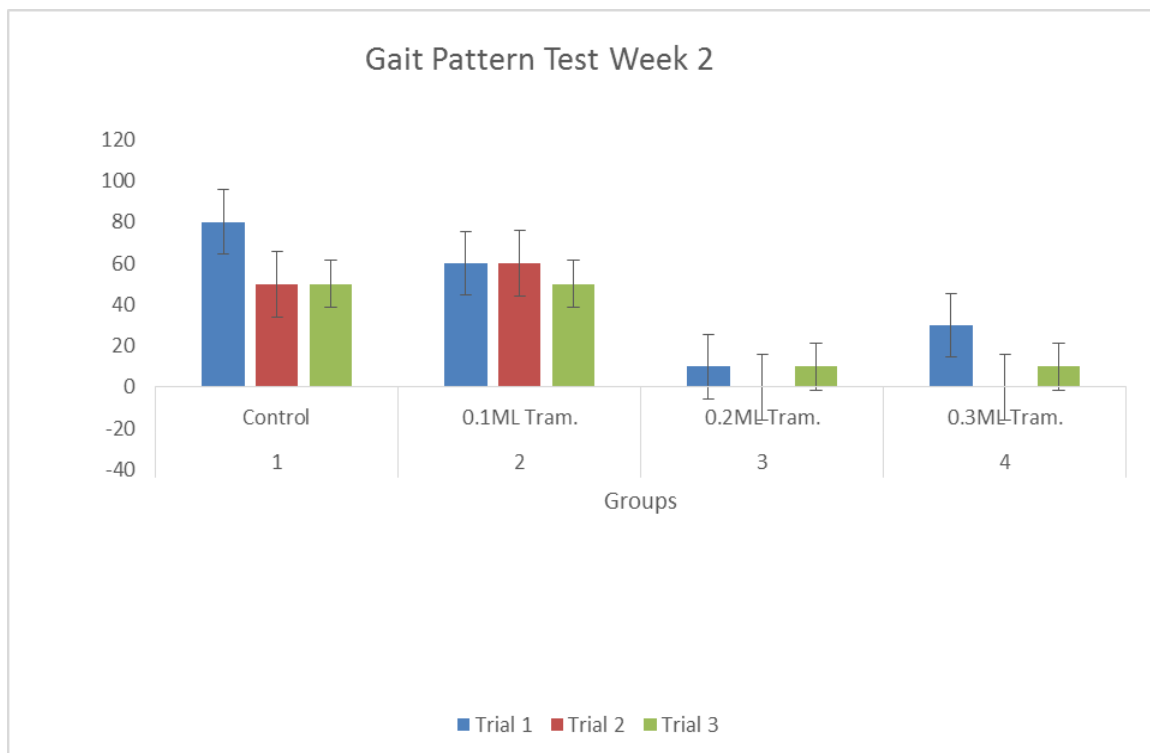


Fig. 2: Pattern of Gait response in the test treated with Tramadol and control groups in week 2 study.

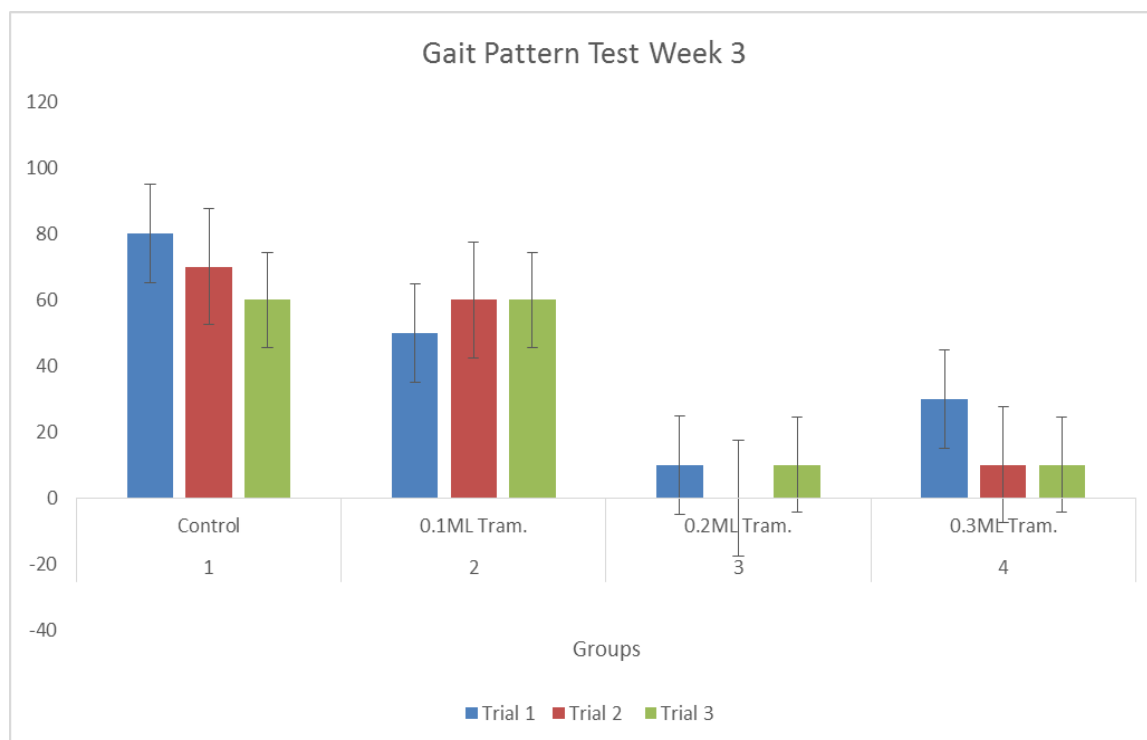


Fig. 3: Pattern of Gait response in the test treated with Tramadol and control groups in week 3 study.

DISCUSSION

Previous studies have shown that morphine and other opioidergic drugs can modulate learning, memory processes in a positive and negative way. Researches on the effect of chronic use of tramadol are very scarce. This research study analyzed on the chronic use of Tramadol which is an opioid that is known to be one of the most abused drugs, Its effect on Gait and cognitive motor function. The experimental procedures were done on Albino Wister rats and the following test were carried out; Navigation test (learning and memory), Barnes maze test (learning and memory) and Gait pattern test (motor control and walking pattern).

Navigation is the ability of organisms to learn and be able to find its way through the environment without getting lost and this is a characteristic that requires spatial learning and memory for locations and routes. This is a function of the hippocampus. The test results from Table 1 remarkably showed a change across the test and control group. The first week, at the end of the third trial, the Control group spent lesser time in navigating their way, Group 2 showed a decline as they spent more time in the third trial compared to the first trial and, Group 3 there was no significant change, group four spent less time compared to the first trial and the Epinephrine group also spent more time in the third trial. The second week, the control group later spent more time at the end of the third trial. This could occur as a result of stress and other environmental factors. Group 2 spent more time in their third trial. Same with Group 3. Group 4 spent lesser time in their third trial compared to the 1st and group 5 more time was spent during their third trial. The third week, the Control group (Group 1) spent

more time in the third trial compared to the first trial, Group 2 spent less time, Group 3 spent more time in their third trial, Group 4 also showed a decline as they spent more time in their third trial. Groups induced with tramadol showed delayed time in able to navigating their way after their first trial. This revealed a deficit in memory. This observed deficit could be as a result of hippocampal impairment of tramadol. From the test results in Table 2, it also shows that at the end of the three weeks study. The first week, at the end of the three trials, The Control group spent lesser time in completing the test, same for Group 2, group 4 and Group 5. Group 3 showed no significant changes in time. The second week, the Control group and Group 5 spent less time in their third trial, Group 2 they spent more time in the 1st trial and 3rd trial but in their 2nd trial, the time spent was less. Group 3 and Group 4 also spent less time in their 2nd trail compared to the 1st and 3rd. The third week, The Control group (Group 1) took less time at the end of the 3rd trial compared to the 1st and 2nd. Group 1 took more time in the 2nd trail and less at the 3rd trail, Group 3 spent less time in the 2nd trail compared to the 3rd. Group 4 spent less time in the 3rd trial and Group 5 also spent less time in the 3rd trial.

From the results, the pattern also revealed impairment of memory. This also could as a result of impairment of the Hippocampus is it is known to play a major role in memory consolidation, also the prefrontal cortex. Many studies have shown that the activation of Opioid receptors will consequently increase the GABA activity (8. 9). GABA is an inhibitory neurotransmitter in the central nervous system which chief role is to reduce neuronal excitability throughout the nervous system (this

neurotransmitter is been produced in areas of the brain responsible for Memory Formation). It has been shown that stimulation of GABA receptors diminishes memory.^[9,10] Memory formation are as a result on excitatory neuronal activities. The hippocampus is also made up of Muscarinic receptors and tramadol is known to inhibit the actions of this receptors and thereby affecting memory formation. Shiraishi and colleagues, showed tramadol inhibited both the Acetylcholine-mediated response of M1 receptor in vivo and the decrease of the muscarine-induced accumulation of cGMP in vitro. This study is in agreement with above mentioned reports.^[11,12]

The first week, comparing the test treated with Tramadol and control group, Group 1 was the most coordinated, followed by Group 2. Group 3 and 4 were not able to maintain coordinated gait as revealed in the result. They were not able to complete 50% of the task. The second week; almost the same results in week one was recorded. Group 3 and 4 were not able to complete half of the task while the Control group and Group 2 had a more coordinated gait pattern and were able to do more than 50% of the task. At the third week, the control group were more coordinated and were able to move from start to the end of the gate plate, followed by group 2. Group 3 and Group 4 were not able to complete the task as shows in the table that majority of them could not perform up to 50% of the task. From the results, it shows that the higher the dose of the drug, the higher the effect of the drugs on the inability of the animal to have good motor coordination and walking pattern as observed in group 3 and Group 4 which were given 0.2ml and 0.3ml of tramadol respectively, the effect of the drug on motor movement (gait) was more when compared to group 2 that was given 0.1ml of tramadol.

The cerebellum coordinates voluntary movements like balance, posture and coordination and this area of the brain are made up of a network of cholinergic receptors. Also, skeletal muscles are made up of cholinergic receptors. Opioids are known to inhibit the action of these receptors and which could lead to an impairment in motor coordination resulting to an uncoordinated walking pattern, loss of balance.^[13]

Continuous administration of tramadol leads to the increase GABA activity which inhibits memory formation and causing sensorimotor inhibition leading to dystonic movements especially where balancing and coordination of the hind limbs were necessary.^[14,15]

CONCLUSION

It is concluded that sub-chronic use of tramadol can cause many dangerous effects on the body and from this current study, it is extrapolated that long term use of Tramadol is deleterious to cognitive motor functions and gait. Thus, it is advisable that tramadol should be used with caution with appropriate dose, monitoring, to avoid its undesirable side effects that could lead to cognitive

impairment and also affect our motor coordination and control.

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