

Effect of Tramadol on Neuromuscular and Cognitive Behaviours of Albino Rats (*Rattus norvegicus*)

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ABSTRACT

Concerns around the effects of chronic pharmaceutical opioid use on cognitive function arise from the known impairing effects following the acute administration of opioids in healthy people. This study was designed to evaluate the impact of tramadol on the cognitive and neuromuscular functions of Wistar rats. A total of 24 Wistar rats were grouped into 6 groups and treated with 0 mg/kg, 5mg/kg, 10mg/kg, 20mg/kg, 40mg/kg, and 80mg/kg doses of tramadol. They were then tested for cognition and muscular effects using standard behavioral tests like the handgrip test, inverted screen test, beam walking, Morris water maze, forced swimming test and Navigational tests. Results indicated that in inverted screen and hand grip tests, the treatment groups were all significantly ($P < 0.05$) stronger than the control group. The Morris water maze had the treatment group significantly retarded in cognition hence they found it difficult to locate the platform. Throughout the period, control increased in weight faster than the treatment groups. Tramadol is therefore concluded to increase muscle strength but impaired cognition and weight gain in the treated rats compared to the control rats.

Keywords: tramadol, handgrip, Morris water maze, cognitive function.

INTRODUCTION

Pharmaceutical opioids, which are among the most commonly-used medicines worldwide, are often prescribed to people who experience chronic non-cancer pain (CNCp) [1, 2, 3, 4]. However, medical professionals cautioned against the frequent use of opioid medicines suggesting the consequences of side effects of its regular administration [5]. Tramadol is an analgesic drug that acts on pain sensation pathways by reducing pain sensitivity [6, 7]. It is used as over the counter drugs for the prevention and treatment of moderate to severe pains [8,9]. It is also used for the treatment of post surgery pains [10]. Despite its wide and global acceptance [10, 11], its addictive predisposition has generated much concern with physical and psychological consequences [12]. It has been observed that it has harmful effects on various organs like the liver, kidney and brain, [13]. Tramadol is known to easily cross the placenta and the cell membrane because of its lipophilic property hence

causes withdrawal syndrome in neonates [10]. Experiment on rats demonstrates that tramadol results in weight loss over prolonged administration [11]. Its effects on memory functions have been attributed to its activation of μ -opioid receptors in rodent models [12]. In another study, it was observed that tramadol causes histological abnormalities such as increase of apoptosis in rats cerebral cortex as a result of its associated oxidative stress [12], induce seizures in patients [10].

Administration of acute and subacute doses of opioid was noticed to have a deleterious effect on cognitive function by impairing performance in tasks assessing attention and information processing abilities in healthy volunteers [4]. In another study, it was noticed that the effects of opioid varies according to dose, type, and route of administration, and these effects are felt on cognition by enhancing cognitive function in some and alter it in another [5]

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Opioid dependence is expressed in a pattern of compulsive, continuing and prolonged self-administration of opioid substances despite significant substance-related challenges such as physiological, behavioral, and cognitive dysfunctions [5]. United Nations Office on drugs and Crimes, (2016) reported that 10% of the persons with substance use have a substance use disorder. According to them, Long-term opioid use leads to physical, mental, and social impairments as well as opioid-related increased death rates. A study was conducted on the effects of opioids on bowel motility and it was found that opioid induces constipation and bowel dysfunction by inhibiting VIP (vasoactive intestinal peptide) secretion which lead to decreased pancreatico-biliary secretion and gut absorption [7]. A lot of nonspecific symptoms are recorded with association of gastric, urinary and hepato-pancreatic symptoms. The gastric symptoms include

spastic achalasia-like esophageal dysmotility, gastric hypomobility and paralytic ileus, generalized visceral congestion or cramp, nausea, vomiting, constipation, anorexia and a loss of weight [10, 13]. The most common neurological symptom of opioid is reduced level of consciousness that is commonly noticed. It can appear as drowsiness and/or disorientation, depression of central nervous system, lethargy, or calm coma leading to death. Side effects are miosis, allodynia, hyperalgesia, anesthesia, anxiety, dizziness depression, dissociation, memory loss, impairment of psychomotor performance and sleep disturbance [8]. Cerebral edema is observed in autopsies following fatal overdoses due to synthetic opioids [11]. In some case reports, specific effects were reported. Fentanyl or analogs overdoses cause immediate stiffening of the body, seizure-like activity or delayed leukoencephalopathy.

MATERIALS AND METHODS

The design used in this study was an independent group randomized design. Commercially available tramadol hydrochloride capsules were purchased from a pharmaceutical shop in Port Harcourt. Albino rats were purchased from the Animal House of the Department of Human Anatomy, Faculty of Basic Medical Sciences, University of Port Harcourt. The rats were weighed and shared into six groups, and fed with growers' mash and water. A total of 24 adult Albino rats were used. They were housed under standard laboratory conditions and had free access to

feed and water; they were acclimatized for 14 days to laboratory conditions before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of the University of Port Harcourt Ethics Committee on guiding principles on the care and use of animals. The rats were divided into 6 groups, with 4 rats per group. The groups were: Group 1 (Control), groups 2-6 which were treated with 5mg/kg, 10mg/kg, 20mg/kg 40mg/kg and 80mg/kg of tramadol respective.

Neuromuscular and Cognitive tests

Six different neuromuscular tests were conducted on the animals after three weeks of daily administration of the drug (tramadol). These tests were: inverted screen test, Morris water test, forced swimming test, navigational test, handgrip test and beam walking test. The inverted screen test was used to measure motor strength and coordination using the four limbs of the animal. An inverted screen was put in between two ends. It was carried out in respect to the body weight of the rat. The experimental rat was made to hang using four limbs and suspend in the air, then a stopwatch was started simultaneously.

Morris water test is used to study assess damage to particular cortical regions of the brain, it is also used to study spatial learning and memory. In a pool of water, a safety platform was placed at one end of the pool, the experimental animal was placed in a large circular pool and it was required to find the platform as an escape route. A signal like a cues was put to one end to enable the animal to locate it escape platform. The test was carried out after the animals were trained to locate the safety platform. The forced swim is a behavioral test used to determine the depression effect of a drug on animals. It is mostly used in

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evaluating the effect of anti-depressant drugs. A pool of water was filled so that the rats cannot stand in the water. An experimental animal was placed in the water then a stop watch was switched on simultaneously at the beginning of the placement and time taken by the animal to swim actively before it gets tired and begin to float was recorded.

Navigational test is a test used to evaluate the learning and memory ability of animals. It can also be used to determine the cognitive disposition of animals and ability to explore and navigate. An experimental rat was placed at the entrance of a navigational maze and allowed to find its way out of the maze. The ability and time taken for the rat to locate the way out of the maze was recorded. Some were removed from the maze after 300 seconds, if they could not locate the way out within the time frame. The animals were trained to master the escape route before the test. The time taken for the rat to locate the escape route was recorded. Handgrip test was used to determine the muscle strength of the

animal; A string was tied to two wooden poles, Then the Experimental animal was made to hang using it's fore limbs and suspend in the air, then a stopwatch was started and the time taken to fall off was recorded. The beam walking test was used to check the animal's motor coordination and balance. The aim of the test was for the rat to stay upright and walk across an elevated narrow beam to a safe flat platform. It was noted that the beam must not be slippery for accurate result, in that note, a masking tape was used to wrapped around the beam. A rat was placed at the middle of the beam to walk to either end of the plane and a time frame of 240 seconds was given to reach the platform. The rat was monitored to see if it would coordinated to move to the either edge within 240 seconds if not, it was removed. These experiments were conducted five consecutive times for each of the tests. The weight of each rat in each treatment was recorded every seven days during the course of the experimental work.

RESULTS

In inverted screen test, the treatment groups expressed more neuro-muscular strength as they held onto the inverted screen more than the control. Groups 3,4, 5 and 6 with treatment doses of 10mg/kg,20 mg/kg 40 mg/kg and 80mg/kg respectively had holding times of 14.25±5.67, 22.7±5.67, 20.4±3.20 and 22±3.78 seconds respectively as compared with the control which had a mean holding time of 5.45±0.65. However group 2 with treatment of the 5 mg/kg was seen with the least holding time of 2.75±0.85 (Fig 1). Morris water maze expressed a different trend as the treatment groups were observed to delay in finding the safety platform. The group 2 was seen to expressed a relatively high mental smartness to detect the safety platform faster than all of them by detecting the platform at a mean time of 4.85±1.32, seconded by the control with a mean time of 5.45±0.85. Other treatment groups were slower with respective time of 14.25±6.93, 11.8±2.14, 13.3±3.53 and 13±5.47 for groups 3, 4, 5 and 6 (Fig 2). Forced swimming test has a dramatic response. Group 3 had the longest swimming strength

as it swam for 116.75±12.16. Group 2 had the second longest time of 106.85±6.43 as compared to the control with 81.4±3.32. The higher treatment groups -groups 4, 5 and 6- were observed to have a depressed disposition as they had their swimming time of 56.75±7.98, 27.8±7.04 and 44.35±6.32 respectively (Fig 3). Navigational maze test shows a gradual increase in cognitive alertness from the control through the treatment groups, with the smartest performance expressed in group 4 where it achieved navigational success in 59.25±22.35. Groups 1, 2 and 3 had their navigational times of 178.6±49.94, 107.25±40.69, 91.05±39.53 respectively while groups 5 and 6 had navigational time of 112.1±46.54 and 252.05±20.81 respectively. Groups 1 and group 6 were seen to have the longest navigational time (Fig 4). Handgrip demonstrated the longest neuromuscular strength in group 4 with 92.1±26.80. Another long handgrip time was record in group 2 with 71±29.96 seconds , while the other groups (1,3,5 and 6) had their respective handgrip time of

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20.65±3.22, 38.9±14.31, 50.35±9.49 and 40.55±2.66 (Fig 5).

Beam walking indicate some close and similar performances. Group 4 (40mg/kg) has the fastest beam walking record as it completes the test within 23.7±14.59 seconds. This was followed by the control with 48.55±16.77 seconds. other groups performances were in this order: group 6 (65.55±13.06), group 5 (66.5±40.00), group 3 (77.25±27.37) and group 2

(100.15±48.84). However, these result were not significantly different because of a wide range of intra group performance differences expressed in a high value of standard error of means as indicated by the error bar (Fig 6). The result indicates that the weight of group 1 increased more than the treatment groups within the period of analyses. Though the differences were not statistically significant (Fig 7).

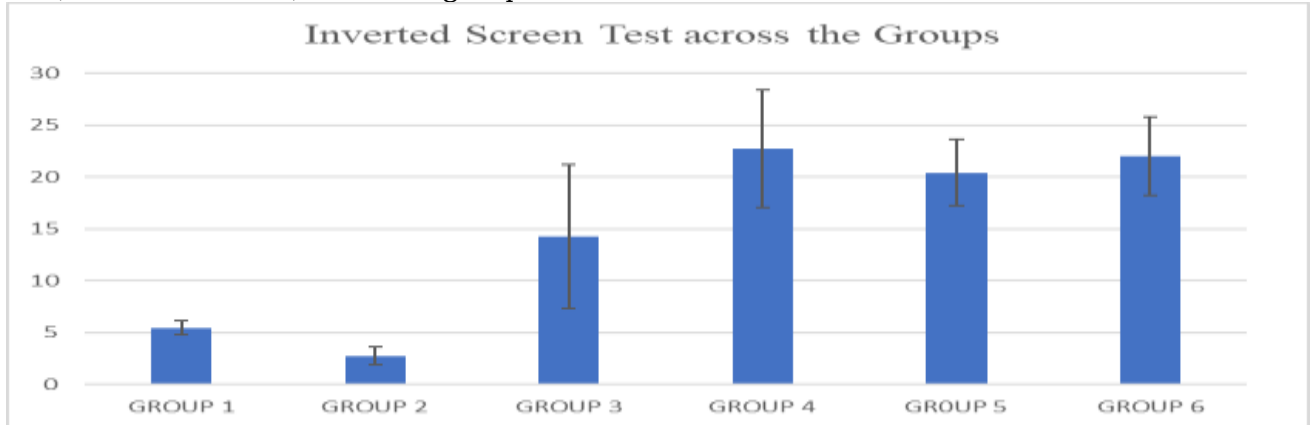


Fig 1: Results of the inverted screen test across the groups

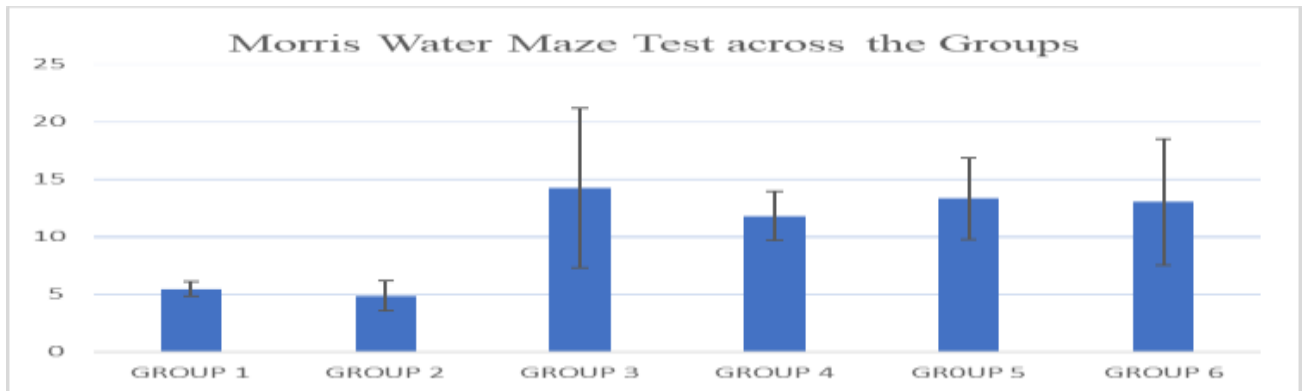


Fig 2: Performance in the Morris water maze test across the groups

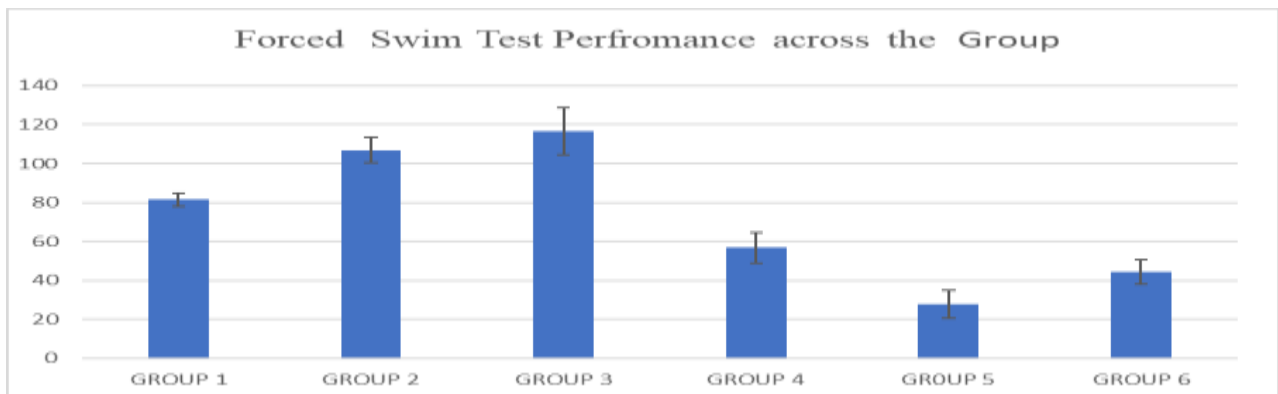


Fig 3: Performance in forced swim test across the groups

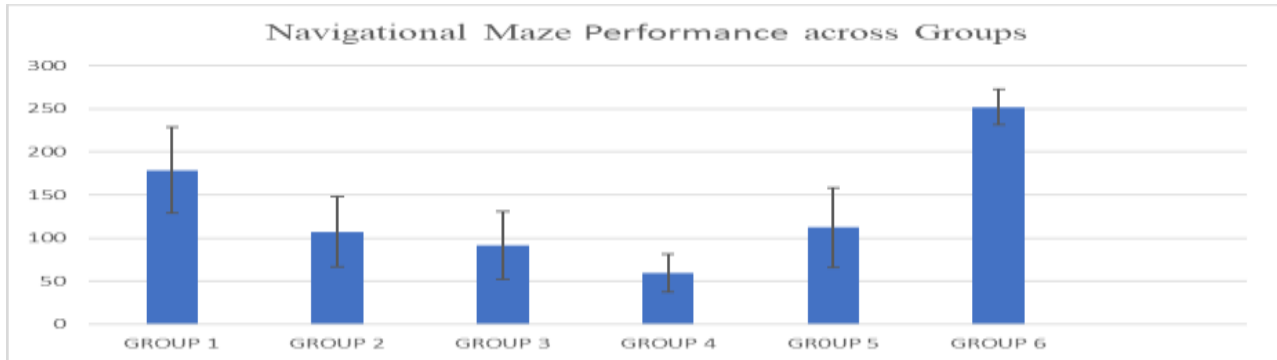


Fig 4: Performance of the rats during the navigational test

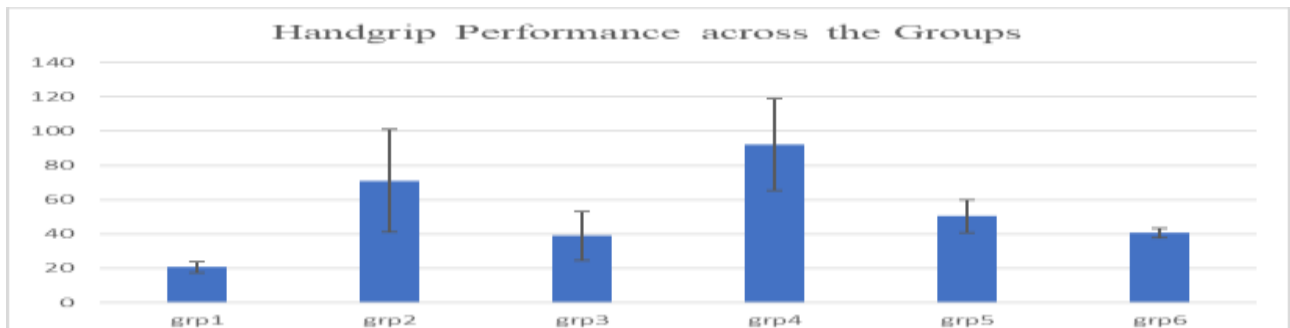


Fig 5: Results of rat's performance during the handgrip test across the groups

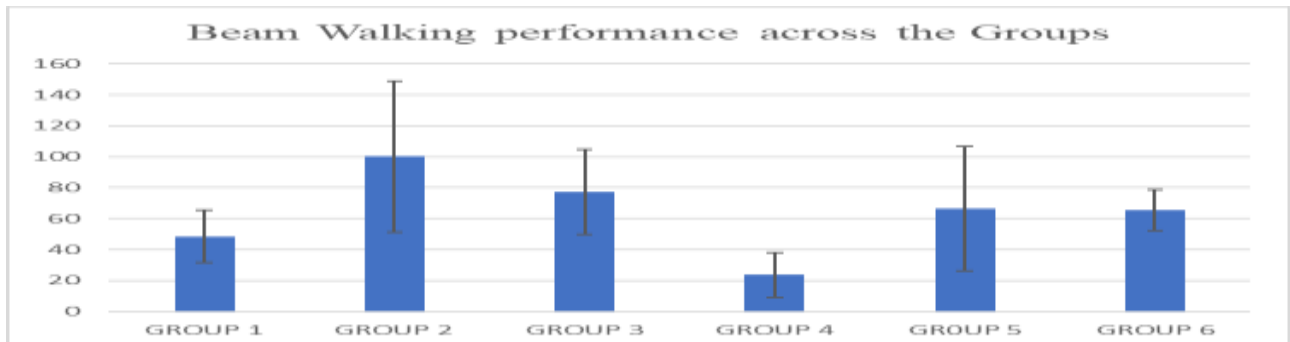


Fig 6: Performance of rats in the beam walking across the group

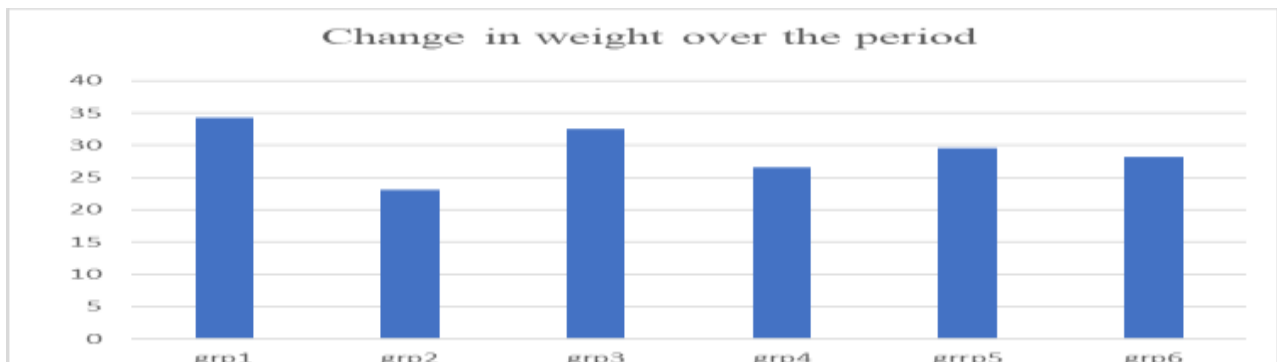


Fig 7: Weight of the rats in each group during the period of the study

DISCUSSION

Looking at the inverted screen test, 20mg/kg group (group 4) with mean value of 22.7 ± 5.67 , it shown that group 4 had the highest muscular strength performance compared to other groups, with control group (group1) having a mean value of 5.45 ± 0.65 . The Morris water maze test showed that 10 mg/kg group (group 3) had the poorest performance compared to other groups, including the control group. This results suggest that there is a negative effect of tramadol on learning and memory. The forced swim test showed that 10mg/kg group (group3) exhibited a highest level of anti-depression compared to other groups including the control group (group 1), while groups 4, 5 and 6 were caught up with depression and lethargy as the dosage of tramadol increases. The navigational maze test showed that 20 mg/kg group (group 4) had the lowest time spent in the maze due to the effect the drug (tramadol) had on their memory, they could repeatedly find their ways out of the maze, unlike other

From the results, it can be concluded that tramadol can increase or reduce muscle strength, it also affect weight in albino rats, there is also a positive effects of tramadol on learning and memory. In terms of coordination, it has both negative and positive effects on coordination depending on the dosage. It also encourages anxiety on rats as shown in forced swim test. The use

groups especially 80 mg/kg group (group 6) which expressed the lethargic effect of tramadol. From results above, the handgrip test showed that the control group exhibit the lowest muscle performance compared to other administered groups. This is an indication that the consumption of tramadol may have a positive impact on the neuro-muscular strength and endurance [7]. The beam walking test also shown the motor coordination and balance indicates that 20 mg/kg group (group 4) had better coordination compared to other groups, the group 4 could easily move to a safe surface on all trials. Though the results of the treatment groups were not significantly different from the control. The weight of control group (group1) increased across the period of four weeks compared to the treatment groups it could be said that continuous administration of tramadol could affects weight gain [11]. This is different from the effect of *Cannabis sativa* which increased weight gain [13].

CONCLUSION

of tramadol and other opioids products should be strictly regulated to curb societal drug abuse because of its negative effects especially at high doses as it was observed in the rats experiments. The federal government should also ensure that tramadol is sold at approved pharmaceutical shops and according to medical prescription only

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