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INTERFERENCE OF 1, 3, 7-TRIMETHYLXANTHINE (CAFFEINE) INGESTION ON MOTOR BALANCE AND POSTURAL REFLEX

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ABSTRACT
The research was carried out to investigate the effect of pure caffeine administration on the postural reflex using healthy wistar rats weighing 180-240g for 3 weeks. A total of 20 rats were divided into four groups. All the rats were given clean water and rat feed throughout the study. The group one animals (control group) were not given any drug, while the animals in group two, three and four were given drugs intra-peritoneally: group two were given 1ml/100g of Celebrex, group three and four were given pure caffeine at concentrations of 0.5ml/100g and 1ml/100g respectively. The effect of caffeine on postural reflex was carried out on both control and test groups, the animals first underwent a mild shock of 3.5mA using an Electroconvulsive therapy, then the spatial learning and memory was assessed with Barnes-maze task, the postural reflex was assessed using Rotarod task and Beam-walk task. The measurement of latency on all the tasks was taken at time range of 5minutes. The results were presented as mean value standard error of Means (±S.E.M). Mean drugs (Celebrex and Caffeine) effect was analyzed using the Analysis of Variance (ANOVA). The findings showed that there was significant increase ($P \leq 0.05$) in spatial learning and memory in the animals that were given Celebrex and Caffeine, and there was significant increase ($P \leq 0.05$) in the spatial learning and memory in the animals in group four compared to the animals in group one, two and three. It was observed that there was no significant difference ($P \geq 0.05$) on the postural reflex on the animals that were given caffeine. From this research it was observed that caffeine may have effect on spatial learning and memory but do not have significant effect ($P \geq 0.05$) on the postural reflex.

KEYWORDS: Celebrex and Caffeine.

INTRODUCTION
A reflex, or reflex action, is an involuntary and nearly instantaneous movement in response to a stimulus (Purves, 2004). A reflex is made possible by neural pathways called reflex arcs which can act on an impulse before that impulse reaches the brain (consciousness). The reflex is then an automatic response to a stimulus that does not receive or need conscious thought.

Caffeine is a bitter, white crystalline xanthine alkaloid that acts as a stimulant drug and an acetyl cholinesterase inhibitor (Cardoso-Lopes *et al.*, 2009). Caffeine (1, 3, 7-trimethylxanthine) ingestion promotes an improvement in human performance (Davis and Green, 2009).

In humans, caffeine acts as a central nervous system stimulant, temporarily warding off drowsiness and restoring alertness. It is the world most widely consumed psychoactive drug, but unlike many other psychoactive substances, it is both legal and unregulated in nearly all parts of the world. Beverages containing caffeine such as coffee, tea, soft drinks, and energy drinks, enjoy great popularity; and about 90% of adults consume caffeine daily (Lovett, 2005).

The effects of caffeine on performance are linked to both central and peripheral mechanisms. The effect of caffeine on the central nervous system (CNS) is linked to a blockade of adenosine receptors, which prevents a decrease in neuronal activity and subsequent an increase in muscle recruitment (Bazzucchi *et al.*, 2011).

Peripherally, caffeine inhibits phosphodiesterase activity, thereby promoting increased plasma catecholamine and glycolysis activity, increasing energy availability for active muscle during exercise (Davis and Green, 2009). As a consequence of central and peripheral effects, caffeine improves performance in tasks involving psychomotor function, such as agility and decision-making accuracy (Brice and Smith, 2001; Gillingham *et al.*, 2004; Tikuisis *et al.*, 2004; Van-Duinen *et al.*, 2005).

MATERIALS AND METHODS

Animal Ethics

All Procedure involving the use of animals in this study followed the guiding Principles for research involving animals as recommended by the declaration of research ethics committee of the University of Port Harcourt, Rivers State on the guiding Principles in the care and use of animals and the use and care of animals was in conformity with International acceptable standards.

Collection of Experimental Animals

20 Healthy experimental animals (female wistar rats) weighing (180-240g) were bought for the experimental research work from the animal house of the department of pharmacology, university of port Harcourt, choba rivers state. They were fed with rat diet (palletized poultry feeds) and tap water throughout the course of the experimental research work.

Animal Housing and Acclimatization of Animals

The rats were kept in a favourable housing environment, which was in a clean plastic cage with wooden shavings to enable daily cleaning of the cage. The cage was constructed to be well Ventilated as wire gauze was used to create the Roof of the cage. The area was devoid of Noise and foul smells from the surrounding environment. The Cage were regularly cleaned every morning, as their wooden shavings were changed, feeding and Drinking trough washed and replaced with new feed and water which were not contaminated.

Experimental Design

GROUPS	NUMBERS PER GROUP	TREATMENT
GROUP1 (Control)	5	Feed, water and shock.
GROUP2 (Celebrex 1ml/100g)	5	Feed, water, Celebrex and shock
GROUP3 (Caffeine low dose 0.5ml/100g)	5	Feed, water, low dosage of caffeine, and shock
GROUP4 (caffeine high dose 1ml/100g)	5	Feed, water, High dosage of caffeine and shock

ECT Procedure

After administration of the drugs to the rats respectively, each rats were expose to ECT (once daily throughout the experiment) (3.5mA, 50Hz) via ear clip electrodes. They were exposed to this mild current to actively induce alertness on the rats.

Barnes-maze is a tool used to measure spatial learning and memory.

The basic function of Barnes maze is to measure the ability of a rat to learn and remember the location of a target zone (Harrison *et al.*, 2009). It is useful for evaluating novel chemical entities for their effects on cognition (Reiserer *et al.*, 2007). The Barnes maze consists of a circular surface with up to 8-20 circular holes around its circumference. Visual cues, such as colored shapes or patterns, may be placed around the table in plain sight of the animal.

Principle

Under one of the holes is an “escape box" which can be reached by the rodent through the corresponding hole on the table top. The model is based on rodents' aversion of open spaces, which motivates the test subject to seek shelter in the escape box. A normal rodent will learn to find the escape box within three to five trials and will head directly toward the escape box without attempting to escape via incorrect holes. Various parameters are measured including latency to escape, path length, number of errors, and velocity. The selection of choice of behavioural tasks are significant in determining the outcome of an experiment.

These variables help to verify that innate anxiety and cognitive ability differ considerably among mouse strains (Harrison *et al.*, 2009).

Beam Walking Task

The balance beam is a test of motor coordination (Goldstein and Davis, 1990). In general the round beams are harder than the square beams and the thinner the beam the harder the test. Choice of difficulty, as always, depends on empirical determination of control behavior for a given species, age, strain, sex etc.

This test can be more sensitive than rotarod for some types of motor coordination deficits (Stanley, 2005).

The beam walk was 4cm above the ground level with a very narrow surface. The animals were exposed to pre-training to reduce neophobia. The animals were allowed to cross the beam, with gentle guiding or prodding as needed, until they cross readily.

Procedure

- The Test was assembled in an isolated room away from any extraneous interference of Noise, Scents or Movements to avoid distracting the animal.
- The Beam walk was cleaned with 70% ethanol before starting the test in order to remove any dirt or smell accumulated on the apparatus.
- The rats were placed on one end of the beam walk singly and was timed for 5minutes for it to walk over to the other end of the beam walk.
- The time it took the rat to cross over to the other end of the beam walk was recorded.

- All the Urine and Fecal boli was removed and the Maze was cleaned with 70% ethanol to remove residual smell from the first rat
- The Procedure was repeated for all the Animals.

Rotarod Test

A ROTAROD APPARATUS

To evaluate drug effects on motor coordination, balance and motor learning in rodents. A rat rotarod apparatus is a rotating rod with forced motor activity being applied. The test measures parameters such as riding time (seconds) or endurance. Some of the functions of the test include evaluating balance, grip strength and motor coordination of the subjects; especially in testing the effect of experimental drugs or after traumatic brain injury (Mouzon and Chaytow, 2012).

Principle

The principle of this test was that rats were first trained to walk on a rod rotating at a certain speed. Once the animals have learned this, the effect of a test-compound on their motor performance is evaluated.

Animals experiencing impaired motor coordination are unable to cope with the rotating rod and will drop off when the rotation speed exceeds their motor coordination capacity. When the animal drops from rod safely into its own lane, the time latency to fall was recorded.

RESULTS

Table 1: Showing the Effect of Caffeine on the Spatial Learning and Memory of Wistar Rats using Barnes-Maze Task.

GROUP	TREATMENT	Barnes Maze Task(Seconds)								
		Trial 1 (s±sem)	Trial 2 (s±sem)	Trial 3 (s±sem)	Trial 4 (s±sem)	Trial 5 (s±sem)	Trial 6 (s±sem)	Trial 7 (s±sem)	Trial 8 (s±sem)	Trial 9 (s±sem)
1	GROUP1 (Control)	38.60±17.56	72.40±44.95	32.20±7.71	74.00±43.01	49.20±18.57	113.00±41.14	18.00±3.87	61.80±30.81	77.00±41.47
2	GROUP2 (Celebrex 1ml/100g)	47.60±28.50	59.60±35.35*	49.40±18.18*	68.20±33.24	47.40±12.42	28.60±10.40*	27.40±20.47*	54.80±15.07*	42.00±20.41*
3	GROUP3 (Caffeine low dose 0.5ml/100g)	89.40±46.80	112.80±63.12	165.00±6110*	37.00±5.54	87.80±34.00	26.80±10.27*	75.60±35.22*	70.80±30.08*	73.80±42.10*
4	GROUP4 (caffeine high dose 1ml/100g)	80.80±52.57	222.40±13.92*	231.00±19.28*	116.60±62.73	155.00±61.14	225.60±51.07*	211.60±40.53*	209.80±39.17*	256.20±21.44*

All the values were expressed as Mean ± S.E.M, $P \leq 0.05$. *= statistically significant when compared to control

Table 4.2: showing Effect of Caffeine on the Motor Coordination, Balance and Motor Learning of Wistar Rats using Rotarod task.

Groups	Treatment	Rotarod Task(Seconds)								
		Trial1 (s±sem)	Trial2 (s±sem)	Trial3 (s±sem)	Trial4 (s±sem)	Trial5 (s±sem)	Trial6 (s±sem)	Trial7 (s±sem)	Trial8 (s±sem)	Trial9 (s±sem)
1	GROUP1 (Control)	30.00±6.25	21.00±3.99	33.20±7.53	40.60±11.34	24.60±3.31	19.80±3.84	30.60±15.05	26.80±8.31	38.00±8.14
2	GROUP2 (Celebrex 1ml/100g)	52.40±35.74	52.60±39.40	72.00±43.95	66.60±49.68	69.80±48.43	80.80±47.92	82.40±52.68	89.40±48.94	86.80±51.46
3	GROUP3 (Caffeine low dose 0.5ml/100g)	23.20±8.29	19.20±9.32	16.20±8.556	21.00±8.05	65.00±42.28	40.80±12.78	41.20±11.90	45.40±24.92	25.60±5.64
4	GROUP4 (caffeine high dose 1ml/100g)	17.20±8.47	15.20±5.61	12.80±2.85	15.80±4.47	13.80±7.21	13.40±4.93	13.80±5.83	11.80±5.86	31.80±20.48

DISCUSSION

The effect of the administration of low dose and high dose of pure caffeine on the postural reflex on wistar rats was investigated. Caffeine a popular stimulant used by many has been known to increase alertness on individuals and also have effect on various body organ systems. This study was done to investigate the effect of caffeine on the postural reflex of wistar rats.

The basic function of Barnes maze is to measure the ability of a rat to learn and remember the location of a target zone (Harrison *et al.,* 2009). It is useful for evaluating novel chemical entities for their effects on cognition (Reiserer *et al.,* 2007). From the result (figure 4.1) showed that rats treated with high dose of caffeine (1ml/100g) had a longer latency to discover the escape hole on the barnes-maze when compared to the rats treated with low dose of caffeine (0.5ml/100g) and celebrex (1ml/100g). The rats treated with celebrex had a shorter latency to discover the escape hole when compare to the control group, and rats treated with high dose of caffeine (1ml/100g) and low dose of caffeine (0.5ml/100g), showed there was a shorter latency on the rats treated with low dose of caffeine (0.5ml/100g) compared to rats treated with high dose of caffeine (1ml/100g).

From table 1 the result showed that during trial 1, 4, 5 there was no significant difference on the groups treated with celebrex and caffeine, but there was significant difference on trials 2, 3, 6,7,8,9, also from table 4.1 the result showed that there was significant increase ($p<0.05$) in the spatial learning and memory of the rats in all the group treated with celebrex when compared to the control group using the Barnes-maze task. Furthermore, from table 4.1 result showed that there was significant decrease ($p<0.05$) in spatial learning and memory in the group treated with high dose of caffeine when compare to the control group due to longer latency to discover the escape hole, from this study it has shown that there was a significant increase ($p<0.05$) on the spatial learning and memory of rats that were treated with low dose of caffeine compared to the rats treated with high dose of caffeine, this study is similar with the work done by Cupo (2012) which reported that in a study of the effect of caffeine on spatial learning and memory that while acute caffeine may not cause any impairment in learning, chronic caffeine impairs memory over time. Also in correlation with previous findings by Han *et al.* (2007) which showed that chronic caffeine impaired spatial learning. This study therefore revealed that there may be increase in spatial learning and memory of rats treated with low dose of caffeine but a significant decrease ($p<0.05$) on spatial learning and memory when treated with high dose of caffeine.

Performance on the balance beam is a useful measure of fine coordination and balance and has been validated by previous work. The beam test can detect motor deficits due to age, central nervous system lesions, and genetic and pharmacological manipulations in young and older rodents (Wallace *et al*, 1980; Brooks and Dunnett, 2009; Fox *et al* 1998). Rat with cortical impact lesions often exhibit contralateral slipping on the beam (Buccafusco, 2009; Southwell, 2009). Stanley (2005) showed that only a 30% GABA (A) receptor occupancy by diazepam or lorazepam was needed in order to observe motor deficits on the Beam compared to over 70% receptor occupancy for deficits on the Rotarod (Stanley, 2005). Beams of narrower widths can be used to detect even more subtle differences.

The beam-walk was used to test for motor coordination (Goldstein and Davis, 1990), from this study, it was observed that none of the rats from all the groups showed any positive results on the beam-walk task. No rat was able to walk to the other end of the round beam of about 29cm length, there was a firm grip on the beam by the some rats to prevent them from falling, while some rats slipped and fell. This was due to the overt narrowness and roundness of the beam used to critically investigate the effect of caffeine administration on the rats, the round beams are harder than the square beams and the thinner the beam the harder the test.

From this study it was shown that neither the administration of celebrex, low dose nor high dose of caffeine had an effect on the motor coordination of the rats.

The effect of caffeine on the postural reflex of the rats was investigated using a rat rotarod apparatus; a rotating rod with forced motor activity being applied. The test measured parameters such as latency (seconds) or endurance, was used to evaluate balance, grip strength and motor coordination of the subjects (Mouzon and Chaytow, 2012). From figure 4.2 it showed that the rats treated with celebrex (1ml/100g) had a longer latency to fall off from the rotating rod when compared to the control group and rats treated with high dose of caffeine (1ml/100g) and low dose of caffeine (0.5ml/100g), also from figure 4.2 it revealed that the rats treated with low dose of caffeine had a longer latency when compared to the rats treated with high dose of caffeine. However, the rats treated with high dose of caffeine had a shorter latency to fall off from the rotating rod when compared to the control.

Table 2 showed there was non-significant decrease ($P>0.05$) in the motor coordination, balance and motor learning on wistar rats when treated with high and low dose of caffeine, From this study, it was observed that caffeine a psycho stimulant drug had no significant difference ($p>0.05$) on the motor coordination or balance on the rats when compared to the control. Neither the low dose nor the high dose caffeine group had significant effect on the postural reflex. This study showed that caffeine do not significantly affect the postural stability or reflex of the body nor either does it has a significant effect on the various organs in the body that brings about

postural balance and motor coordination when tested on a rotating rod suggesting there may be an impairment on the motor function, this study is similar to the work done by Fredholm *et al.,* (1999) on the effect of caffeine containing energy drink on postural stability, affirmed that there was no significant effect, either with eyes open or closed, on movement of the body's Centre of pressure.

Furthermore, in correlation to this study Mednick *et al*., (2008) reported that there was a significant impairment in motor learning in the caffeine group in a given motor task requiring finger tapping of 4-1-3-2-4 sequence on a keyboard with a non-dominant hand.

CONCLUSION

From the analysis and results, it was clear that both the low dose and high dose administration of caffeine had no significant effect ($P>0.05$) on the postural reflex on wistar rats, but had a significant effect ($P<0.05$) on the spatial learning and memory on the wistar rats. In conclusion, it can be stated that caffeine has no effect on postural reflex.

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