Original Research Article

Interference of Recreational Intake of 1, 3, 7-Trimethylxanthine on Sensorimotor, Cognitive Functions and Fasting Blood Glucose In Wistar Rats

Comment [1]: 1, 3, 7-Trimethylxanthine, there is a missing dash

Abstract

1, 3, 7-Trimethylxanthine (Caffeine) is a natural alkaloid found in coffee beans, tea leaves, cocoa beans, cola nut etc. It is probably the most frequently ingested pharmacologically active substance in the world at large. This study was carried out to evaluate the effectS of recreational intake of caffeine (methyl-xanthine) on sensorimotor and cognitomotor functions and fasting blood glucose in a rat model. In this study, a total of twenty (20) wistar rats were randomly divided into 4 groups. After three (3) weeks of acclimatization, caffeine was administered to the rats as follows; Group1 (control) received normal water, group 2 was treated with 0.4g/ml of caffeine, group3 – 0.8g/ml of caffeine, and group4 – 1.6g/ml of caffeine, all for a period of thirty (30) days. Opaque maze (memory), elevated maze (intelligent and anxiety), beam walking (learning) and swimming/climbing test (learning), were used to evaluate sensory, motor, and cognitive performances of the rats in both control group and treated groups. Also glucometer with fine test strips was used to determine the blood glucose level of the rats at day 1, day 5 and day 10 during the period of caffeine administration. Data was obtained and inferentially analysed using ANOVA (SPSS version 23) The result from this study showed that caffeine significantly interfered with ($p < 0.05$) cognitive performance in the group treated with 1.6g/ml of caffeine and also significantly decreased blood glucose level in low dose treated group and increase blood glucose at higher doses. In conclusion, Caffeine was found to improve sustained cognitive functions at medium doses (0.8g/ml), sustained motor steadiness at low (0.4g/ml) and moderate doses (0.8g/ml) but at higher doses produced uncoordinated movement.

1. INTRODUCTION

The most popular psychoactive chemical used globally is caffeine (1, 3, 7-trimethylxanthine). It is lawful and not subject to regulation, unlike many other psychoactive substances. Caffeine may be absorbed in a number of ways, including through the consumption of beverages like coffee, tea, soft drinks, kola nuts, energy drinks, and over-the-counter painkillers and sedatives [1,2]. In the United States, the United Kingdom, and Canada, as well as in Scandinavia, the average daily caffeine consumption for all adult consumers is about 2.4 and 4.0 mg/kg for people weighing 60

to 70 kg [1]. The most commonly used psychoactive drug, caffeine, may stimulate the central nervous system [3]. During the second part of the twentieth century, the caffeinated soft drink market expanded dramatically, with growing popularity occurring among beverages containing higher quantities of caffeine. The rising popularity prompted the introduction of energy drinks, which have since become immensely popular. Today, roughly 80% of the world's population consumes a caffeinated substance on a regular basis, with 90% of people in North America doing so [4]. Caffeine concentration differs among different beverage, with coffee generally having the highest value when compared to tea, soft drinks, and some energy drinks. Caffeine concentration can vary significantly within a beverage category, as in the case of coffee and tea. Green tea contains caffeine; however, the caffeine concentration varies greatly depending on the variety of green tea and the brewing technique. Because caffeine exists naturally in those beverages, the caffeine level will differ depending on the plant variety, environmental growing conditions, or brewing method utilized [5,6]. Caffeine is most commonly consumed in beverages including coffee (71%), soft drinks (16%), and tea (12%) [7]. Caffeine is rapidly absorbed from the gastrointestinal system into the bloodstream and processed in the liver after ingestion [8]. Caffeine is substantially processed by the liver (99%) to generate three primary metabolites: 3,7-dimethylxanthine, 1,7-dimethylxanthine, and 1,3-dimethylxanthine, demonstrating that 70 to 100 mg of caffeine has a linear pharmacokinetics [9]. Caffeine clearance is greatly lowered and its elimination half-life is prolonged at higher dosages (250 to 500 mg), demonstrating nonlinearity [10]. 90% of the caffeine in one cup of coffee is eliminated from the stomach in 20 minutes, and peak plasma concentration is attained in 1 to 1.5 hours [11, 8]. Caffeine, once absorbed, has a wide range of physiological effects on the body's organs.

NEW PARAGRAPH

Type 2 diabetes is a global health hazard that impacted 2.2% of the global population in 2000 and is expected to rise to 4.4% by 2030 [12]. Several research have looked into the relationship between caffeine, particularly caffeine from coffee drinking, and the risk of acquiring type 2 diabetes. The exact amount of caffeine necessary to produce an adverse effect varies from person to person depending on their weight and sensitivity to caffeine [13]). Due to the popularity and wide consumption of caffeinated beverages, the objective of this study was to investigate Interference of Recreational Intake of 1, 3, 7 Trimethylxanthine on Sensorimotor, Cognitive Functions and Fasting Blood Glucose In Wistar Rats.

2. MATERIALS AND METHOD

2.1 Collection of Experimental Animals

Twenty Healthy Wistar rats, weighing 160 – 180g, were used in the experimental research. The animals were acquired from the animal house of biochemistry department, University of Port Harcourt Choba, Rivers State.

They were fed with rat diets (palletized poultry feeds) and distilled water throughout the period of the study.

2.2 Acclimatization of Animals

After identification, the animals were weighed and housed in wire mesh cage under standard conditions (temperature of $25^{o}C$ – $29^{o}C$, 12 hours of light and dark cycle) for four (3) weeks to acclimatize with the environmental condition of the animal house of the pharmacology department of the University of Port Harcourt. The study was generally conducted in accordance with recommendation from the 1983 declaration of Helsinki or guiding principles in the welfare use of animals.

2.3 Collection of Caffeinated Coffee

Caffeinated coffee (Nescafe) was purchased from Tejod Pharmacy, in Port Harcourt. The caffeinated coffee was weighed using Tolendo electronic weighing balance from department of biochemistry in the University of Port Harcourt, into three (3) sets of 4g, 8g, and 16g respectively and were poured into conical flasks separately.

2.4 Preparation of Substance /Drug

After the caffeinated coffee was weighed into 4g, 8g, and 16g, they were dissolved into 10ml distilled water respectively into the following concentration.

i. 4g of caffeinated coffee + 10ml of distilled water

   C = $\frac{4}{10}$ = 0.4g/ml of caffeine

ii. 8g of caffeinated coffee + 10ml of distilled water

   C = $\frac{8}{10}$ = 0.8g/ml of caffeine

iii. 16g of caffeinated coffee + 10ml of distilled water

   C = $\frac{16}{10}$ = 1.6g/ml of caffeine

2.5 Experimental Design

The experimental animals were divided into four groups according to body weight.

GROUP 1 (CONTROL)

This was the control group and consist of consist of five rats. Receiving normal poultry chow and distilled water.

GROUP 2

This group consists of five rats, receiving 0.4g/ml of caffeine.

GROUP 3

This group consists of five rats, were treated with 0.8g/ml of caffeine.

GROUP 4

This group consists of five rats, treated with 1.6g/ml of caffeine.

2.6 Administration of Caffeine

During the administration of caffeine, group 1 received 0.4g/ml caffeine group 2 received 0.8g/ml of caffeine and group 3 received 1.6g/ml caffeine group 4 was a control group and did not receive any caffeine except rat diet and water. WHAT'S THE TOTAL AMOUNT EACH RECEIVED? OR WHERE THEY DRINKING AT WILL?

2.7 Determination of Fasting Blood Glucose

During the period of administration of caffeine, cotton wool and methylated spirit was used to clean the tail of the rats after which a slight cut was made on the tail using dissecting blade to expose the blood. A glucose fine test strip was inserted in a glucometer and the blood was placed on it and allowed to read. The result was recorded. This was done morning and evening for 30 days along with caffeine administration. The results were recorded.

2.8 Determination of Weight

Before the administration of caffeine, a simple zhengya weighing apparatus was used to measure the weight of all the rats and the result was recorded. This was done morning and evening for 30 days of caffeine administration.

2.9.0 Determination of Sensorimotor & Cognitive Functions

The following mazes were used to determine the sensorimotor/cognitive functions in the rats' opaque maze, elevator maze, swimming, climbing test and walking on a beam test.

2.9.1 Cognitive Test Using Navigation (Opaque) Maze

Opaque maze is a wooden cage constructed with many rats inside it. Despite its numerous routs, it has only one way in and another one way out. The rat is placed at one end (i.e IN) of the cage. A stop watch was start. The time taken for the rat to trace its way to the other end (i.e OUT) of the cage determines the intelligence of the rats. Most rats were able to find their way out within a short time and were recorded as intelligent. While most rats could not find their way out and remain at certain and was recorded as not intelligent. The test was carried out for each of the rats in all the groups at several times.

2.9.2 Cognitive Test Using Elevator Maze

Elevator maze is used to test anxiety. It is a wooden structure constructed with four crossed bars, looking for a way to come down determines the intelligence of the rat. Most rats were able to go round within a short time and were recorded as intelligent with time taken. While most rats remain at a point and was recorded as not intelligent. This test was carried out for each of the rats in all the four groups.

2.9.3 Cognitive Test Using Morris Water Maze Test

The Morris Water Maze Test is a cognitive task used to evaluate therapies for learning and memory using visual spatial navigation [14]). Morris water task is a test widely used and established by behavioural physiologist and pharmacologist to evaluate and compare learning and memory in rodents [15]. It is based on the rodent's (rats or mice) aversion to the water environment. The apparatus consists of a water-filled pool with a hidden escape platform beneath the surface of the water. When the animal is released into the water, it will swim around the pool to look for the platform to escape from the water. The platform offers no local cues to direct the escape behaviour of the animal. The only spatial cues are mainly the visual cues exterior of the tank.

The crux of the task is that the animal is released from four different sites, so that an egocentric response (‘straight ahead and then a gentle left turn') will not help to find the platform in more than one of the starting locations. Before the testing session starts, animals are trained for 3-5 days with 3-5 daily trials.

The test was done for each of the rat in all the four groups for several times.

2.9.4 Cognitive Test Using Beam BalanceTests

Walking on a beam test is used to test balancing in the rat. The modified method of protocol of [16] and [17] were used. A beam is sustained at an average height supported from both side. Then the rat was placed on one end of the beam and a stop watch start. The time taken for the rat to move from one end placed to another end was noted and recorded as intelligent. While most rats remain at one end and was recorded as not intelligent. This process was repeated severally for each of the rats in all the four groups.

2.10 Precautions during the Experimental Study

i. It was ensured that the rat cage was regularly CLEANED to avoid infection to the rat.
ii. It was ensured the rats were labeled properly to avoid mix-upS.
iii. It was ensured that the animal were disease-free .
iv. It was ensured that the animals were allowed to acclimatized before administration of caffeine
v. It was ensured that the cognitive test was done in a quiet zone to avoid distraction from noise
vi. It was avoided zero error when using the weighing balance and stop watch.
vii. It was ensure that the caffeine was administered gently to avoid waste drop from the mouth of the rat.
viii. It was ensured that the animals were starved for 24 hours before the random blood glucose test.
ix. It was ensured that the caffeinated coffee was properly measured to ensure accuracy in doses.
x. It was ensured that I observed the various principles guiding animals handling and the use of animal for experimental study.

Comment [2]: This manuscript needs a major grammar review by a professional.

Comment [3]: ?

2.11 Statistical Method

Results were analyzed using ANOVA method, and were presented as mean ± S.E.M at $p \leq 0.05$. Post tests were used for multiple comparison (SP.SS version 18).

3. RESULTS

TABLE 1: Result of responses to cognitive task performance using opaque maze test

	NAVIGATION (OPAQUE) MAZE TEST Time(s ± SEM)		
GROUPS	**Week 1**	**Week 2**	**Week 3**
GROUP 1 (Control)	82.2 ± 23.9	68.3 ± 28.7	67.7 ± 26.5
GROUP 2 (0.4g/ml caffeine)	61.3*± 35.8	55.9 ± 23.3	40.2* ± 34.1
GROUP 3 (0.8g/ml caffeine)	63.0*± 8.5	86.8 ± 13.0	49.2*± 12.1
GROUP 4 (1.6g/ml caffeine)	246.7* ± 25.5	170.9* ± 29.6	193.5* ± 21.4

Values represent mean ± SEM, N=5, * = mean significant differences relative to the normal control at $p<0.05$.

TABLE 2: Result of cognitomotor responses to task performance using elevated maze test

	ELEVATED MAZE TEST		
	Time(s ± SEM)		
GROUPS	**Week 1**	**Week 2**	**Week 3**
GROUP 1 Control	64.2 ± 9.9	116.5 ± 9.0	122.8 ± 13.3
GROUP 2 (0.4g/ml caffeine)	182.8* ± 10.3	133.8 ± 11.4	134.5 ± 14.6
GROUP 3 (0.8g/ml caffeine)	105.5 * ± 9.3	107.6 ± 8.3	103.4± 12.2
GROUP 4 (1.6g/ml caffeine)	102.1* ± 16.2	149.5 ± 11.5*	148.9*± 9.7

Values represent mean ± SEM, N=5, * = mean significant differences relative to the normal control at $p<0.05$.

TABLE 3: Result of responses to sensorimotor task performance using swimming/climbing test

	SWIMMING/CLIMBING TEST		
	Time(s ± SEM)		
GROUPS	**Week 1**	**Week 2**	**Week 3**
GROUP 1 (Control)	90.5 ± 22.2	112.8 ± 27.2	68.5 ± 11.7
GROUP 2 (0.4g/ml caffeine)	58.4*± 8.2	64.6* ± 10.5	51.0* ± 8.3
GROUP 3 (0.8g/ml caffeine)	108.0* ± 19.1	110.2 ± 31.9	114.0 ± 22.0
GROUP 4 (1.6g/ml caffeine)	168.8*± 21.8	173.8* ± 30.5	173.6* ± 27.4

Values represent mean ± SEM, N=5, * = mean significant differences relative to the normal control at $p<0.05$.

TABLE 4: Result of responses to motor coordination and balance task performed using beam walking test

	BEAM WALKING TEST		
	Time(s ± SEM)		
GROUPS	**Week 1**	**Week 2**	**Week 3**
GROUP 1 Control	181.2 ± 59.6	159.6 ± 7.2	139.1 ± 36.1
GROUP 2 (0.4g/ml caffeine)	53.9* ± 17.6	53.4* ± 7.8	69.5*± 10.9
GROUP 3 (0.8g/ml caffeine)	209.9± 69.4	182.6 ± 14.9	144.7± 39.8
GROUP 4 (1.6g/ml caffeine)	307.8* ± 34.5	216.4*± 26.8	252.8* ± 17.9

Values represent mean ± SEM, N=5, * = mean significant differences relative to the normal control at $p<0.05$.

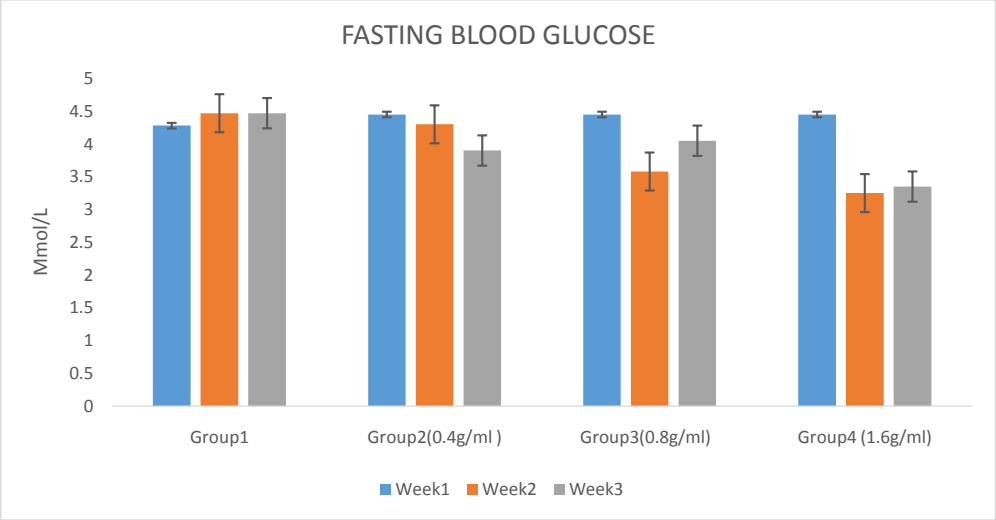

Figure 1: Variations in the pattern of fasting glucose in the control and caffeine-induced groups

4. DISCUSSION

Table 1 revealed the impact of the use of caffeine on motor activities and adaptive locomotion assessment in rats. In week 1 of study, assessing both the quality and time of completion of the task showed that caffeine in medium doses could alter sensory motor and cognitive parameters significantly when compared to the control group. This effects could be due to the ability of Caffeine modulates higher brain functions through its effects on distinct areas of the brain such as the prefrontal cortex, thereby speeding up reaction time and improving short term memory [18].

Results from table 2 and 3 demonstrated a significant improvement on the quality of sensorimotor responses in the animals treated with lower doses of caffeine when compared with the control groups. Sensorimotor activities entails all afferent, efferent transmissions and central integration and processing components required for muscle and joint articulations.

This result could be attributed to the report that Caffeine indirectly affects the release of neurotransmitters as it can act as a competitive antagonist against the depressant effects of adenosine [19]Results showed that in week 1 assessment, caffeine seemed quite promising in memory enhancement at short duration but as the test approached weeks 2 and 3, there was a significant aberration and redundancy in memory and cognitive performance.

This observation was not far from the fact that caffeine could distort cognitive activity and decline sharpness as a result of exhaustion of glucose availability in the brain and glucose store in the muscle [20].Studies showed that moderate consumption of caffeine exhibited ability to attenuate cognitive failure, suicide and symptoms of major depressive disorder while high doses of caffeine may lead to psychosis and anxiety [21]. The study is also in line with the report of [22] who reported that caffeine was able improve the alertness and performance in attention and memory-based tasks Generally, from the result, it is noted that, rats in group 4, treated with 1.6g/ml of caffeine, performed very poorly in the entire neurobehavioural tests. While rats in group 3, treated with 0.8g/ml of caffeine, performed excellently in the entire four (4) cognitive tests. It can therefore be said that, moderate dose of caffeine, significantly increases ($p \leq 0.05$) sensorimotor and cognitive performance While high dose of caffeine decline cognition. This support the work done previously by Van, 2003 to evaluate the effect of chronic ingestion of caffeine on cognitive performance [23]).Previous studies showed that short-term effects of caffeine consumption include enhanced mood and alertness [24,25,26], improved exercise performance [27], increased blood pressure [28], improved ability to remain awake and mentally alert after fatigue [29], faster information processing speed and reaction time, and heightened awareness and attention [30]. Pattern of fasting blood glucose level as shown in figure 1 revealed that the effect of caffeine across the test groups significantly decreased in medium and higher

doses. Decrease in blood glucose by caffeine action has been reported to be associated with increase in insulin sensitivity and that accounted for the slight decrease in glucose level as the study progressed. However, one study found that acute caffeine ingestion with meals can led to increased insulin and blood glucose levels and a decrease in insulin sensitivity [31]. Previous researchers reported that Coffee bioactive compounds such as caffeine, caffeic acid, and cafestol have been shown to improve glucose metabolism [32, 33]. This could be attributed to the decrease in blood glucose as seen in the present work. Short-term metabolic studies showed that caffeine intake can acutely lower insulin sensitivity and increase glucose concentrations [34], this also can explain the spark in blood glucose in first week of group 4 which receive the highest dose of caffeine.

5. CONCLUSION

From the data extrapolated from this work, it was concluded that caffeine at moderate dose reduces cognitive delay, increases mental alertness and presents better general body coordination, which result in significant increase in cognitive performance on the caffeine-treated rats. Furthermore, caffeine at high dose inhibits mental alertness, induces fatigue and slow response to cognitive activities. Also, caffeine at medium and high doses decrease fasting blood glucose level.

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