Original Research Article

EFFECTS OF CHRONIC CAFFEINE INGESTION ON SHORT TERM MEMORY, LOCOMOTION ACTIVITY AND EXPLORATORY BEHAVIOR IN WISTAR RATS

Comment [i1]: Unnecessarily explanatory. It is suggested as alternative *EFFECTS OF CHRONIC CAFFEINE INGESTION ON NEUROLOGICAL FUNCTIONS IN WISTAR RATS*

ABSTRACT

The study was designed to investigate the effects of caffeine on short term memory, locomotion activity and exploratory behaviour in rats. The rats underwent some memory tasks such as navigational maze test, passive avoidance test, object recognition test and motor task such as Acoustic reflex (movement) test. .Twenty-five Wistar rats were grouped into five groups which comprises of the control groups, group 2(0.2ml caffeine), group 3(0.4ml caffeine), group 4(0.6ml caffeine), group 5(epinephrine drug) with five rats per group. Group 1(control) was given saline water and feed, group 2 was given 0.2ml of caffeine, group 3 was given 0.4ml of caffeine, group 4 was given 0.6ml of caffeine, group 5 was given 0.1ml/100g epinephrine (also known as adrenaline) drug every week after which they underwent a total of nine (9) trials of some memory tasks which include: navigational maze, passive avoidance test, object recognition test and motor task which include; acoustic reflex (movement) test. Statistical analysis was performed with ANOVA, while Post Hoc multiple comparison test was used in the comparison of the effects of the control group with Epinephrine treatment groups. Results obtained were statistically analyzed and showed that caffeine could adversely interfere with physiological activities significantly. Furthermore, it also showed that the ability of chronic caffeine ingestion is largely dose-dependent and that caffeine in the facilitation of short-term memory, exploratory behaviour and locomotion is dependent on the dosage.

Keywords: chronic caffeine,short term memory, locomotion activity and exploratory behavior

1. Background to the study

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Caffeine has been consumed by humans all over the world for thousands of years. An ancient Chinese legend says the Emperor Shen Nung first discovered tea in 2437 BCE when the wind blew leaves into his boiling water. He was intrigued by the pleasant aroma and invigorated after drinking it. [1,2,3]. ~~There are~~Many reports showing that caffeine may ameliorate amnesia in human beings, particularly in cases of age-related cognitive decline, scopolamine-induced amnesia [4,5] and electroconvulsive therapy. Many of these studies with human subjects are not specifically addressed to memory issues and interpretations of the results may be difficult due to interference of the previous caffeine consumption habits and heterogeneity of the samples. The

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improving effect of caffeine on animal models of learning and memory has been reported since the 1960's [6][7]but the results of these animal studies are also contradictory.Caffeine is the most widely consumed central-nervous-system stimulant. Three main mechanisms of action of caffeine on the central nervous system have been described. Mobilization of intracellular calcium and inhibition of specific phosphodiesterase only occur at high non-physiological concentrations of caffeine**.**

Each day, billions of people rely on caffeine to wake up, or to get through that night shift or an afternoon slump. In fact, this natural stimulant is one of the most commonly used ingredients in the world.Different works has been done on caffeine and their effects on some systems. This work is to review and analyse the effect of chronic caffeine ingestion on short term memory, locomotion activity and exploratory behaviour in rats.

2. MATERIALS AND METHODS

Animal collection and authentication

A total of 30 albino rats will be obtained from animal house of faculty of Basic health science, University of Port Harcourt.A total of 25 male wistar rats weighing 100- 135g were used. The rats were purchased from the animal house of the department of human physiology and toxicology, faculty of basic health science, university of Port Harcourt, Port Harcourt city, Nigeria. The rats were kept in clean disinfected wooden cages with saw dust as beddings in the animal house. The rats were kept at normal room temperature (approximately 27□) and exposed to natural lighting conditions (12 hours' daylight and 12 hours' darkness) they were fed with standard animal feed and water at ad libitum. They were allowed to acclimatize to the new environment for the period of two weeks before the commencement of the experiment.

Experimental Design

A total of twenty-five (25) albino rats will be randomly divided into five groups of five rats per group. The remaining five (5) will be kept on reserve in case of any death.

Chart 1 : List of Experimental group and their treatment efficacy

Experimental group	Number of rats	Treatment
Group 1(control)	5	feed + water
Group 2	5	feed + water + caffeine

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Group 3	5	feed + water + caffeine
Group 4	5	feed + water + caffeine
Group 5	5	Epinephrine (0.1ml)

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Group 1 (control) was given clean feed and water and exposed to recognition test and motor task test using Passive Avoidance, Navigation maze, Acoustic reflex test and Object Recognition.

Group 2 was given clean feed and water and exposed to recognition test and motor task test using Passive Avoidance, Navigation maze, Acoustic reflex test and Object Recognition with treatment (0.2ml caffeine)

Group 3 was given clean feed and water and exposed to recognition test and motor task test using Passive Avoidance, Navigation maze, Acoustic reflex test and Object Recognition with treatment (0.4ml caffeine)

Group 4 was given clean feed and water and exposed to recognition test and motor task test using Passive Avoidance, Navigation maze, Acoustic reflex test and Object Recognition with treatment (0.6ml caffeine)

Group 5 was given clean feed and water and exposed to recognition test and motor task test using Passive Avoidance, Navigation maze, Acoustic reflex test and Object Recognition with 0.1 ml of Epinephrine

Comment [i11]: Indicate: All groups were exposed to recognition test and motor task test using Passive Avoidance, Navigation maze, Acoustic reflex test and Object Recognition

Chart 2 : Response of animal Groups in respect to time

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	Number of animals	Week 1	Week 2	Week 3	Week 4
Group 1(control)	5	-	-	-	-
Group 2 (ml of caffeine)	5	0.2~~ml~~	0.2~~ml~~	0.2~~ml~~	-

Group3 (ml of caffeine)	5	0.4~~ml~~	0.4~~ml~~	0.4~~ml~~	-
Group 4	5	0.6ml	0.6ml	0.6ml	-
Group 5	5	-	-	-	0.1ml epi

Recognition and motor tasks

These are series of tests that ascertain the behavior, memory retention and intelligence in rats. The test carried out are the; Passive avoidance test, Navigation maze task, objective recognition test and Acoustic reflex test.

Passive Avoidance Task

It is a fear motivated test classically used to access memory function on small laboratory animals (rats, mice). Its working protocol involves timing of transitions i.e. time that the animal takes to move from the white compartment to the black one after a conditioning session, in which the entry into the black compartment is punished with a mild foot shock. It comes with two independent grid floors that allow for flexible adverse stimuli. A top loading door allows an easy access inside the box. The cage contains a sound generator and a visual stimulus (light) that functions separately for each compartment (a dark compartment which is preferable to rodents and a brightly lit compartment).

Procedure

In this task the animals were placed in the light compartment and allowed to roam, there is a flash of light which causes the rats to leave the light compartment into the rather preferred dark compartment. A mild foot shock is given at the dark compartment that forces the animal to leave the dark compartment. Immediately it leaves the dark compartment, transition time was taken for when it will go back into the dark compartment. Hence measuring its learning and memory. It's time limit is 5mins.

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Navigation Maze Task

It is utilized in the assessment of exploration, path planning and navigation which rely on learning and memory capacities to form cognitive maps. It has two doors; an entrance door and an exit door. It is made of fine wood and covered with glass

Procedure

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1. The animals were given the appropriate drugs with their appropriate doses accordingly and were allowed to rest for a period of 5 minutes.

2. Each rat was then put into the navigation maze box (one at a time) and the stop watch was started immediately.

3. The rat was allowed for 5 minutes to locate the end of the maze box.

4. Immediately the rat reached the end of the maze, the result was recorded and the rat was removed from the box.

5. If the rat doesn't locate the endpoint and the 5 minute elapses, the rat is also removed from the box and the result is taken as incomplete.

Object Recognition task

The Object Recognition task is used to evaluate exploratory behavior and recognition memory, in rodent models. This test is based on the tendency of rodents to explore novel (new) objects and also recognize familiar objects.

Procedure

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1. The animals were given the appropriate drugs with their appropriate doses accordingly.

2. They were allowed to rest for a period of 5 minutes.

3. Each rat was then put into the object recognition box containing 2 objects (circle and cross) and

were given time to explore these objects

4. Then 2 novel (new) objects were introduced into the box.

5. The rat was observed to test its memory and exploratory abilities

6. The time spent with the objects was recorded.

Acoustic Reflex (Movement) Test

This test involves the use of a bell. The bell was used to create noise causing fear in the rats and their behavior were observed.

Procedure

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1. After the rats were given their appropriate dose of drugs

2. The rat (one at a time) were place on a free space, and a bell is rung.

3. The stopwatch was immediately started to record the time of movement,

4. There are 3 possible outcomes; the rat either runs away from the sound, towards the sound or doesn't move at all. The time taken for the rat to either run away or towards the sound was recorded.

Method of Data Analysis

Quantitative data on the trials gotten were obtained, recorded and tabulated on a broadsheet using the Microsoft Excel (Microsoft office 2006). The quantitative data was then analyzed statistically using Statistical Package for Social Sciences Software (SPSS version 22). Variables such as caffeine treatment and trials were represented as Mean ± SD and with the ANOVA

analysis techniques, these variables were compared. The results were presented in tables and charts. Statistical significance was set at 95% confidence level ($p < 0.05$*).

3. RESULTS AND DISCUSSION

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Table 1 Amnestic test recorded from the test and control groups using Passive Avoidance box technique

		Passive Avoidance Test								
		Week 1			Week 2			Week 3		
Groups	treatment	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
1	saline	183.0 ±71.33	214.00 ±59.13	253.20 ±46.80	214.00 ±59.13	194.00 ±6.84	243.60 ±56.40	214.00 ±52.66	234.00 ±40.42	232.00 ±45.76
2	0.2ml caffeine	186.0 ±69.84	211.00 ±55.65	300.00 ±.00	188.00 ±65.81	300.00 ±.00	300.00 ±.00	300.00 ±.00[b]	300.00 ±.00	300.00 ±.00
3	0.4ml caffeine	274.0 ±26.0[b]	232.80 ±45.05	300.00 ±.00	158.60 ±40.0	300.00 ±.00	300.00 ±.00	181.40 ±62.7	256.00 ±44.0	300.00 ±.00
4	0.6ml caffeine	267.60 ±20.92[b]	245.00 ±34.79	300.00 ±.00	198.20 ±62.99	265.00 ±35.0	300.00 ±.00	240.00 ±60.0[b]	215.00 ±58.95	240.00 ±60.0
5	epinephrine	81.20 ±55.98	180.00 ±73.49	240.00 ±60.0	181.20 ±55.98	180.00 ±73.49	240.00 ±60.0	81.20 ±55.98	180.00 ±73.96	240.00 ±60.0

Values are presented as mean ± sem. N=5. a means values are statistically significant when compared to the negative control. b means values are statistically significant when compared to the positive control.

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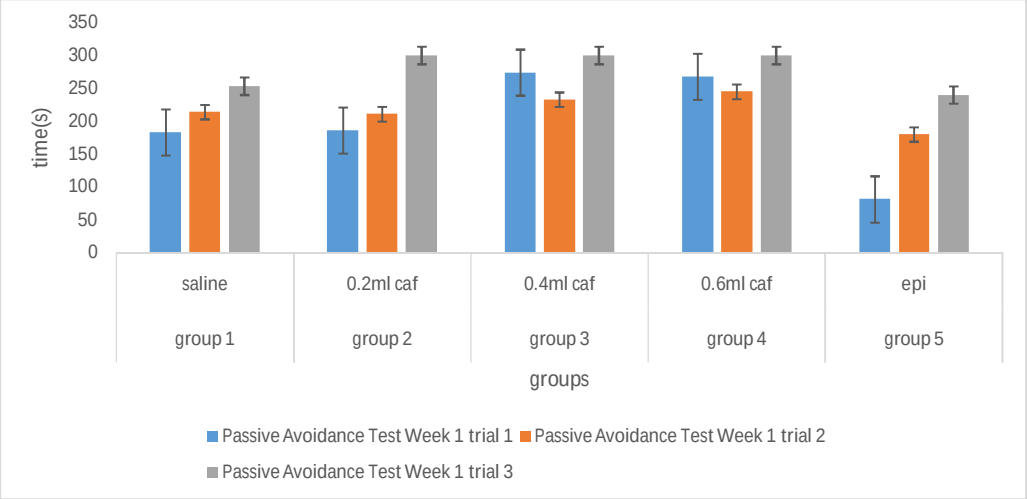

Fig .1 Pattern of Amnestic expression in the test and control groups in week 1

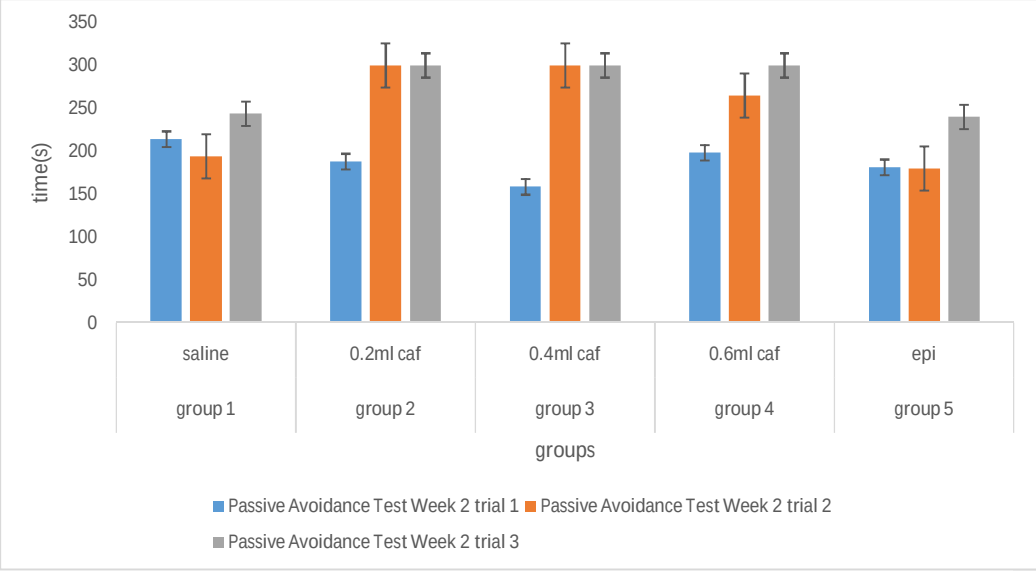

Fig 2 Pattern of Amnestic expression in the test and control groups in week 2

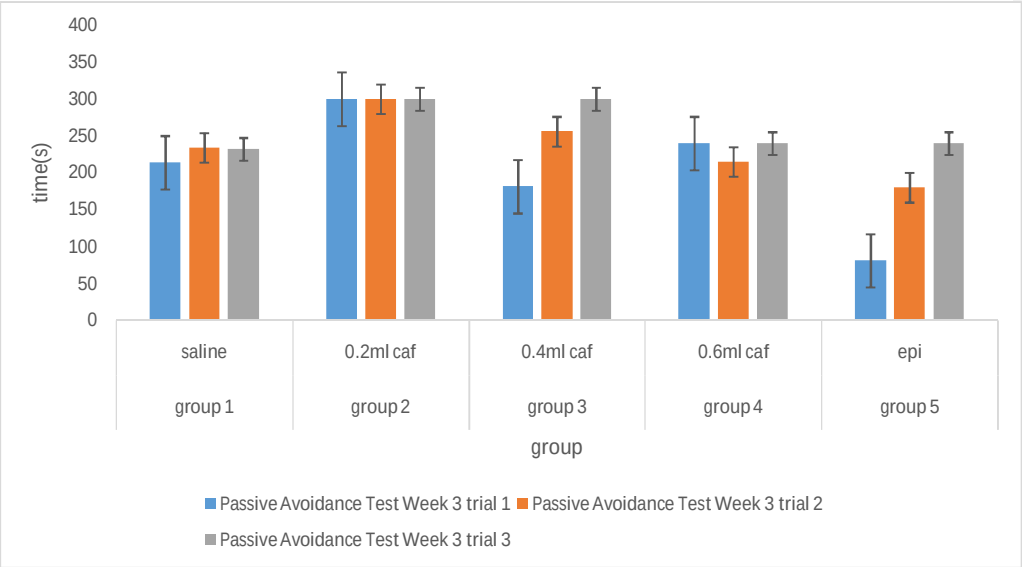

Fig 3 Pattern of Amnestic expression in the test and control groups in week 3

Table 2 Adaptive locomotion test recorded from the test and control groups using navigation maze technique

	NAVIGATION TEST									
		Week 1			Week 2			Week 3		
Groups	**treatment**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**	**Trial 1**	**Trial 2**	**Trial 3**
1	**saline**	262.20 ±37.80	273.20 ±26.80	260.20 ±39.80	300.00 ±.00	257.00 ±43.0	235.40 ±37.32	227.00 ±46.68	247.20 ±33.52	261.20 ±38.80
2	**0.2ml caffeine**	102.80 ±50.29 ab	182.40 ±56.8 b	127.60 ±49.28 ab	172.60 ±52.90 ab	194.00 ±65.13	207.20 ±56.96	302.20 ±26.28	300.00 ±.00	263.00 ±37.0
3	**0.4ml caffeine**	241.60 ±41.89	263.60 ±23.09	242.80 ±35.14	254.40 ±45.60	213.00 ±55.80	252.80 ±36.03	209.60 ±77.77	221.80 ±54.23	231.80 ±42.37
4	**0.6ml caffeine**	252.40 ±47.60	270.80 ±29.20	233.20 ±45.50	230.00 ±43.01	254.40 ±45.60	237.80 ±38.37	241.60 ±36.43	262.00 ±24.11	300.00 ±.00
5	**Epinephrine**	300.00 ±.00	300.00 ±.00	300.00 ±.00	300.00 ±.00	300.00 ±.00	300.00 ±.00	300.00 ±.00	300.00 ±.00	300.00 ±.00

Values are presented as mean ± sem. N=5. a means values are statistically significant when compared to the negative control. b means values are statistically significant when compared to the positive control.

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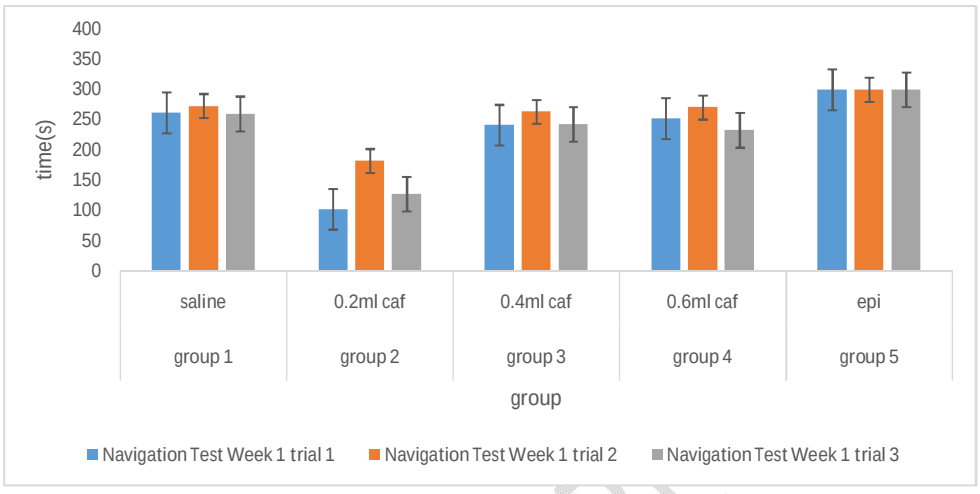

Fig 1 Patterns of adaptive locomotion in the test groups and control group in week 1

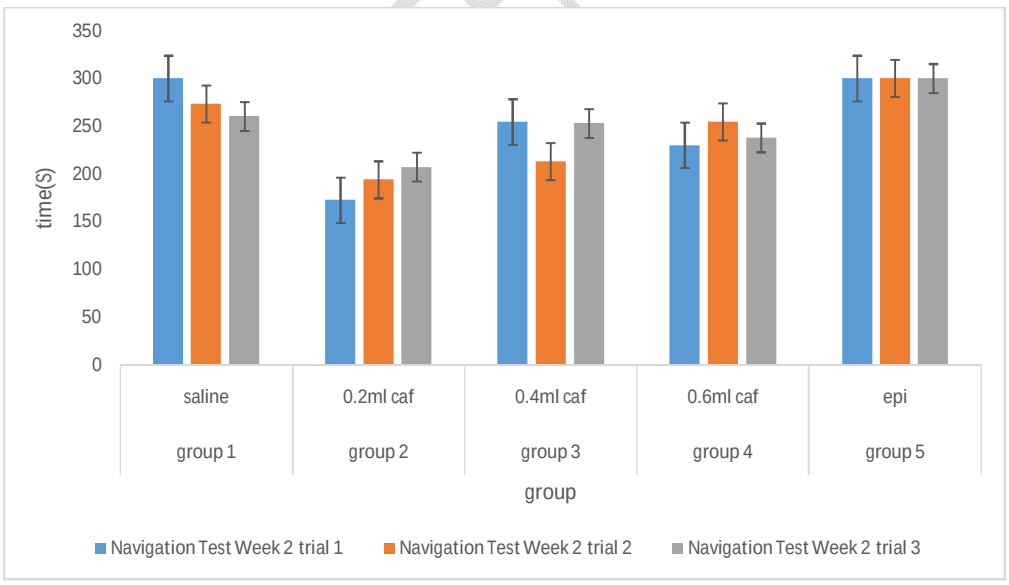

Fig 2 Patterns of adaptive locomotion in test groups and control in week 2

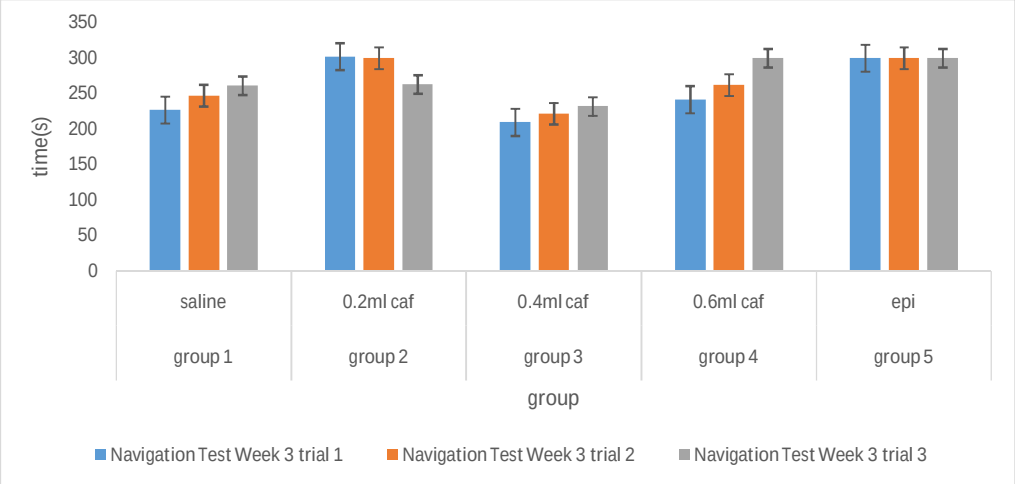

Fig 3 Patterns of adaptive locomotion in test groups and control in week 3

Table 3.1 Amnestic and exploratory behaviour test recorded from the test and control groups using Object recognition technique in week1 trial1

		Object recognition test			
Groups	**treatment**	**Week 1 Trial 1**			
		FAMILIAR OBJECTS		**NEW OBJECTS**	
		circle object	**Cross object**	**Square object**	**Star object**
1	**saline**	70.80 ±34.97	41.80 ±16.23	50.40 ±16.23	11.60 ±8.25
2	**0.2ml caffeine**	9.40 ±4.33	14.20 ±11.35	101.60 ±61.21	2.40 ±1.47
3	**0.4ml caffeine**	15.20 ±6.38	57.00 ±48.47	14.00 ±6.79	37.60 ±24.47
4	**0.6ml caffeine**	10.40 ±5.07	142.20 ±41.39	15.00 ±15.0	69.80 ±57.77
5	**epinephrine**	67.80 ±57.98	69.80 ±57.68	70.80 ±57.65	.00 ±.00

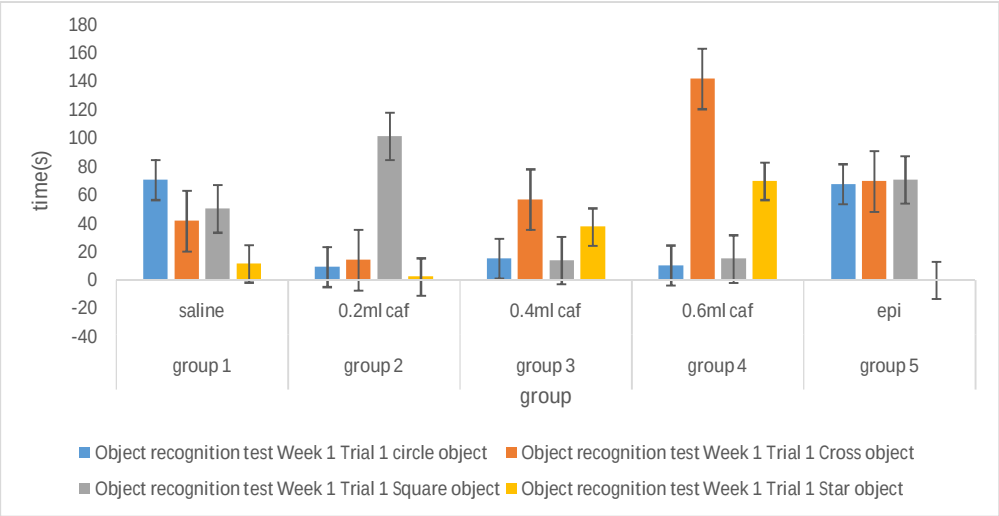

Fig1 Pattern of Amnestic expression and exploratory behaviour in the test and control groups in week 1 trial1

Table 3.2 Amnestic and exploratory behaviour test recorded from the test and control groups using Object recognition technique in week1 trial2

		Object recognition test			
groups	**treatment**	**Week 1 Trial 2**			
		FAMILIAR OBJECTS		**NEW OBJECTS**	
		circle object	**Cross object**	**Square object**	**Star object**
1	**saline**	73.60±56.96	53.40±37.69	82.40±37.89	21.20±21.20
2	**0.2ml caffeine**	35.60±25.19	11.20±5.00	114.00±58.04	38.00±38.0
3	**0.4ml caffeine**	14.20±8.7	27.40±25.93	71.20±48.73	31.40±12.03
4	**0.6ml caffeine**	38.20±12.75	20.40±16.69	11.80±7.27	153.60±51.21[a]
5	**epinephrine**	69.20±58.17	84.80±55.39	51.60±37.02	84.80±50.45

Values are presented as mean ± sem. N=5. a means values are statistically significant when compared to the negative control. b means values are statistically significant when compared to the positive control.

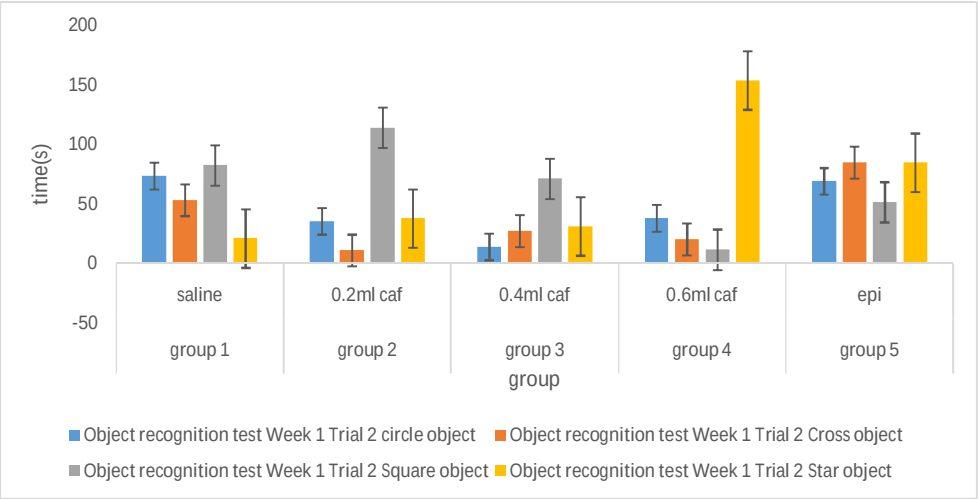

Fig 2 Pattern of Amnestic expression and exploratory behaviour in the test and control groups in week 1 trial2

Table 3.3 Amnestic and exploratory behaviour test recorded from the test and control groups using Object recognition technique in week 1 trial3

		Object recognition test			
groups	**treatment**	**Week 1 Trial 3**			
		FAMILIAR OBJECTS		**NEW OBJECTS**	
		circle object	**Cross object**	**Square object**	**Star object**
1	**saline**	29.52±16.51	109.40±36.64[b]	43.00±27.28	15.60±10.83
2	**0.2ml caffeine**	13.20±6.62	5.00±5.0[a]	77.00±56.38	22.60±12.84
3	**0.4ml caffeine**	15.40±8.17	11.20±4.66[a]	33.60±13.76	52.00±34.86
4	**0.6ml caffeine**	67.20±58.13	6.00±6.0[a]	44.00±37.14	92.40±43.94
5	**epinephrine**	131.20±68.87	24.80±15.31[a]	49.00±36.82	21.60±14.05

Values are presented as mean ± sem. N=5. a means values are statistically significant when compared to the negative control. b means values are statistically significant when compared to the positive control.

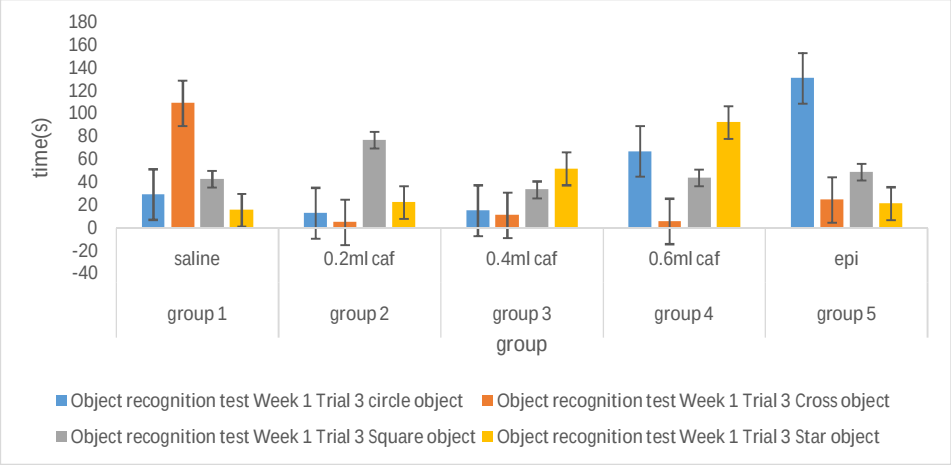

Fig3. Pattern of Amnestic expression and exploratory behaviour in the test and control groups in week 1 trial3

Table 4.1 Amnestic and exploratory behaviour test recorded from the test and control groups using Object recognition technique in week2 trial1

	Object recognition test				
groups	**treatment**	**Week 2 Trial 1**			
		FAMILIAR OBJECTS		**NEW OBJECTS**	
		circle object	**Cross object**	**Square object**	**Star object**
1	**saline**	89.80±54.92	54.00±35.3	53.80±15.22	16.40±11.25
2	**0.2ml caffeine**	114.40±60.4	79.00±54.46	47.00±44.54	11.00±5.8
3	**0.4ml caffeine**	113.40±62.54	13.00±9.7	49.80±43.65	1.20±1.20
4	**0.6ml caffeine**	96.00±48.31	75.60±31.74	30.00±25.3	4.00±2.92
5	**epinephrine**	67.80±57.98	69.80±57.68	70.80±57.65	.00±.00

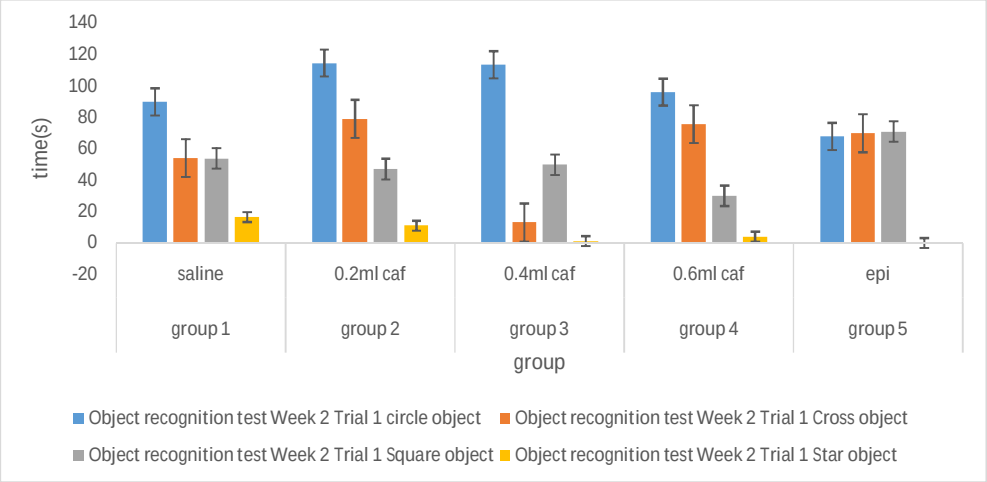

Fig 4.1 Pattern of Amnestic expression and exploratory behaviour in the test and control groups in week 2 trial1

Table 4.2 Amnestic and exploratory behaviour test recorded from the test and control groups using Object recognition technique in week2 trial2

		Object recognition test			
groups	**treatment**	**Week 2 Trial 2**			
		FAMILIAR OBJECTS		**NEW OBJECTS**	
		circle object	**Cross object**	**Square object**	**Star object**
1	**saline**	94.00±54.12	26.20±14.09	42.40±22.12	41.80±35.67
2	**0.2ml caffeine**	134.20±53.13	16.40±12.78	3.00±2.00	39.20±25.59
3	**0.4ml caffeine**	112.20±51.53	15.00±9.88	25.80±14,28	41.40±20.62
4	**0.6ml caffeine**	33.20±10.84	25.40±13.64	57.60±21.92	22.60±7.89
5	**epinephrine**	69.20±58.17	84.80±55.39	51.60±37.02	84.80±50.45

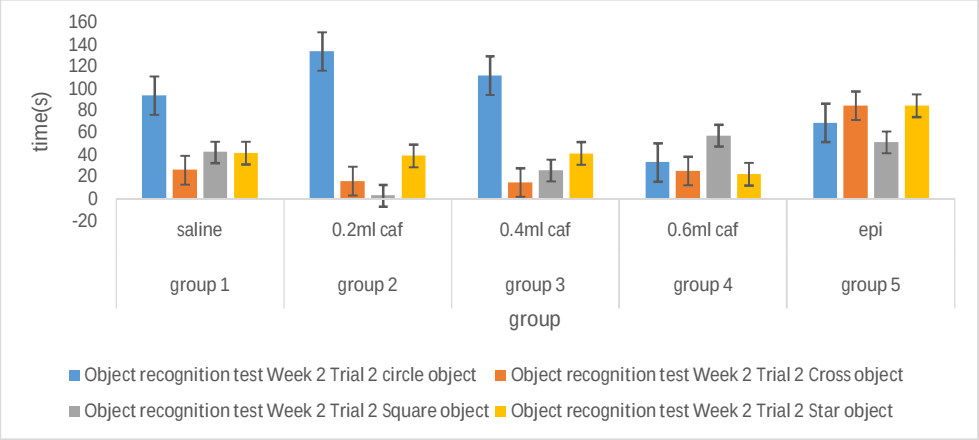

Fig 4.2 Pattern of Amnestic expression and exploratory behaviour in the test and control groups in week 2 trial2

Table 4.3 Amnestic and exploratory behaviour test recorded from the test and control groups using Object recognition technique in week 2 trial 3

	Object recognition test				
Groups	**treatment**	**Week 2 Trial 3**			
		FAMILIAR OBJECTS		**NEW OBJECTS**	
		circle object	**Cross object**	**Square object**	**Star object**
1	**saline**	125.00±54.87	33.20±15.87	15.40±10	27.40±16.79
2	**0.2ml caffeine**	140.80±57.22	75.60±42.06	21.40±10.22	9.00±9.0
3	**0.4ml caffeine**	106.00±44.49	33.40±25.07	8.40±5.88	32.20±18.54
4	**0.6ml caffeine**	90.20±32.91	80.00±27.96	3.40±3.40	47.00±31.11

5	**epinephrine**	131.20±68.87	24.80±15.31	49.00±36.82	21.60±14.05

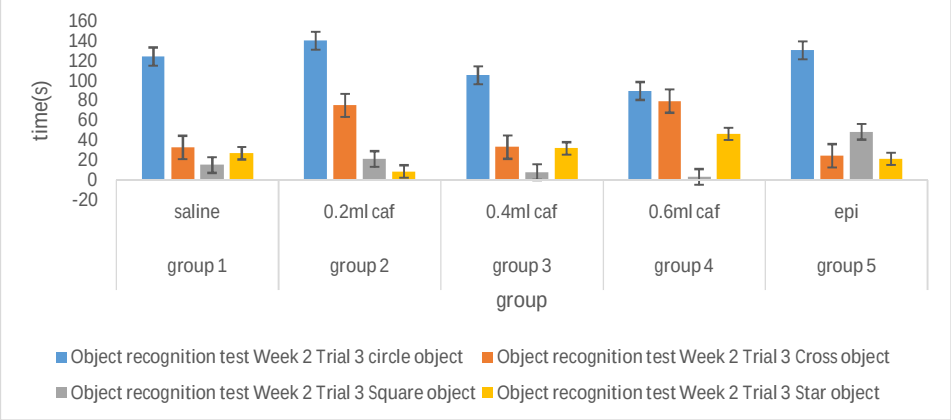

Fig.4.3 Pattern of Amnestic expression and exploratory behaviour in the test and control groups in week 2 trial3

Table 5.1 Amnestic and exploratory behaviour test recorded from the test and control groups using Object recognition technique in week 3 trial1

		Object recognition test			
groups	**treatment**	**Week 3 Trial 1**			
		FAMILIAR OBJECTS		**NEW OBJECTS**	
		circle object	**Cross object**	**Square object**	**Star object**
1	**saline**	116.20 ±56.89	14.00 ±8.57	67.40 ±26.56	21.20 ±13.12
2	**0.2ml caffeine**	62.20 ±38.03	30.80 ±14.31	35.00 ±13.02	73.40 ±56.83
3	**0.4ml caffeine**	73.80 ±56.35	64.80 ±41.34	34.20 ±14.76	29.60 ±19.66

4	**0.6ml caffeine**	52.00 ±37.07	14.60 ±7.63	82.80 ±47.27	4.80 ±2.63
5	**epinephrine**	67.80 ±57.98	69.80 ±57.68	70.80 ±57.65	.00 ±.00

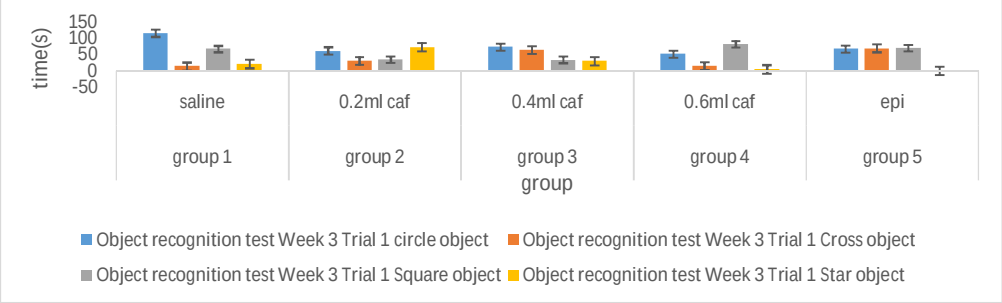

Fig 5.1 Pattern of Amnestic expression and exploratory behaviour in the test and control groups in week 3 trial1

Table 5.2 Amnestic and exploratory behaviour test recorded from the test and control groups using Object recognition technique in week 3 trial2

		Object recognition test			
groups	**treatment**	**Week 3 Trial 2**			
		FAMILIAR OBJECTS		**NEW OBJECTS**	
		circle object	**Cross object**	**Square object**	**Star object**
1	**saline**	43.60 ±39.02	110.80 ±51.85	71.20 ±30.17	97.40 ±31.55
2	**0.2ml caffeine**	82.00 ±34.99	17.20 ±8.75	128.20 ±52.27	4.80 ±3.34
3	**0.4ml caffeine**	81.60 ±55.06	93.40 ±53.85	2.80 ±2.80	5.20 ±5.20
4	**0.6ml caffeine**	12.80 ±12.80	93.40 ±55.77	67.60 ±58.34	40.00 ±50.45

5	**epinephrine**	69.20 ±58.17	84.80 ±55.39	51.60 ±37.02	84.80 ±15.35

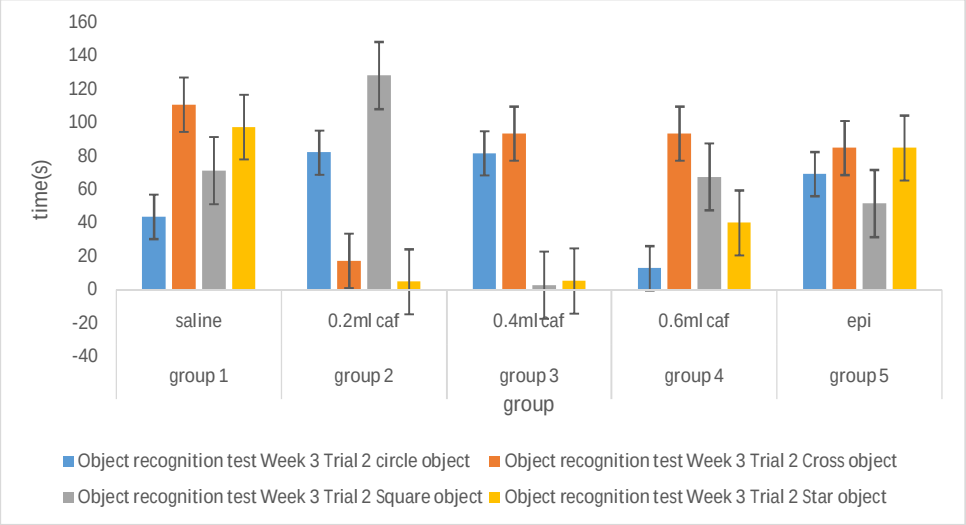

Fig5.2 Pattern of Amnestic expression and exploratory behaviour in the test and control groups in week 3 trial2

Table 5.3 Amnestic and exploratory behaviour test recorded from the test and control groups using Object recognition technique in week3 trial3

		Object recognition test			
groups	**treatment**	**Week 3 Trial 3**			
		FAMILIAR OBJECTS		**NEW OBJECTS**	
		circle object	**Cross object**	**Square object**	**Star object**
1	**saline**	40.80±27.33	133.60±68.72	98.20±55.17	11.60±11.60

2	**0.2ml caffeine**	86.40±54.36	14.20±7.71	8.00±3.45	93.60±38.28[a] [b]
3	**0.4ml caffeine**	89.80±58.21	61.80±59.33	86.20±54.93	32.20±29.76
4	**0.6ml caffeine**	131.20±39.38	12.40±10.09	17.20±9.22	20.40±13.48
5	**epinephrine**	131.20±68.87	24.80±15.31	49.00±36.82	21.60±14.05

Values are presented as mean ± sem. N=5. a means values are statistically significant when compared to the negative control. b means values are statistically significant when compared to the positive control.

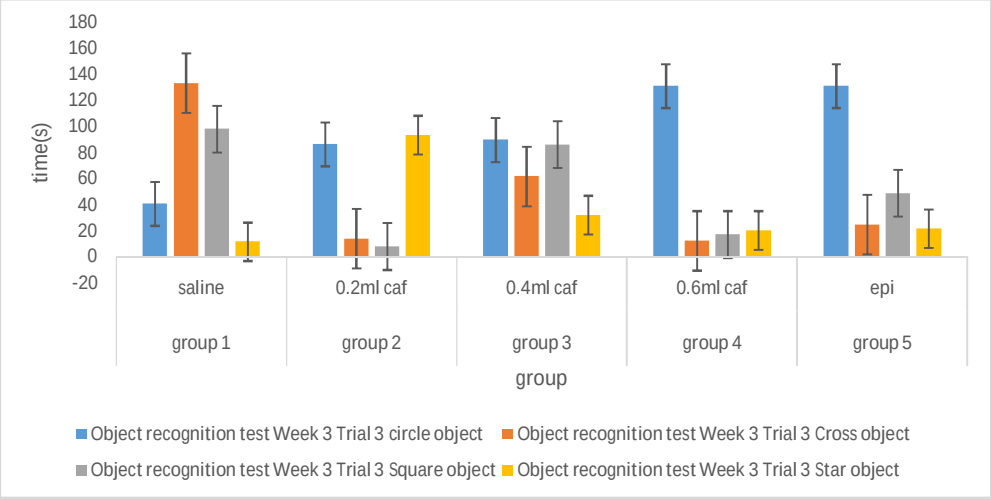

Fig 5.3 Pattern of Amnestic expression and exploratory behaviour in the test and control groups in week 3 trial3

Table 6.1 Movement test recorded from the test and control groups using Acoustic reflex technique in week1 trial1

	ACOUSTIC REFLEX (MOVEMENT) TEST			
groups	**treatment**	**Week 1 Trial 1**		
		Towards	**Away**	**No Movement**
1	**saline**	1.00±.00	2.00±.00	3.00±.00
2	**0.2ml caffeine**	1.00±.00	2.00±.00	3.00±.00
3	**0.4ml caffeine**	1.00±.00	2.00±.00	3.00±.00

4	**0.6ml caffeine**	1.00±.00	2.00±.00	3.00±.00
5	**epinephrine**	1.00±.00	2.00±.00	3.00±.00

Fig 6.1 Pattern of Movement in the test and control groups in week 1 trial 1

Table 6.2 Movement test recorded from the test and control groups using Acoustic reflex technique in week 1 trial2

	ACOUSTIC REFLEX (MOVEMENT) TEST			
Groups	**treatment**	**Week 1 Trial 2**		
		Towards	**Away**	**No Movement**
1	**saline**	1.00 ±.00	2.00 ±.00	3.00 ±.00
2	**0.2ml caffeine**	1.00	2.00	3.00

		±.00	±.00	±.00
3	**0.4ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
4	**0.6ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
5	**epinephrine**	1.00 ±.00	2.00 ±.00	3.00 ±.00

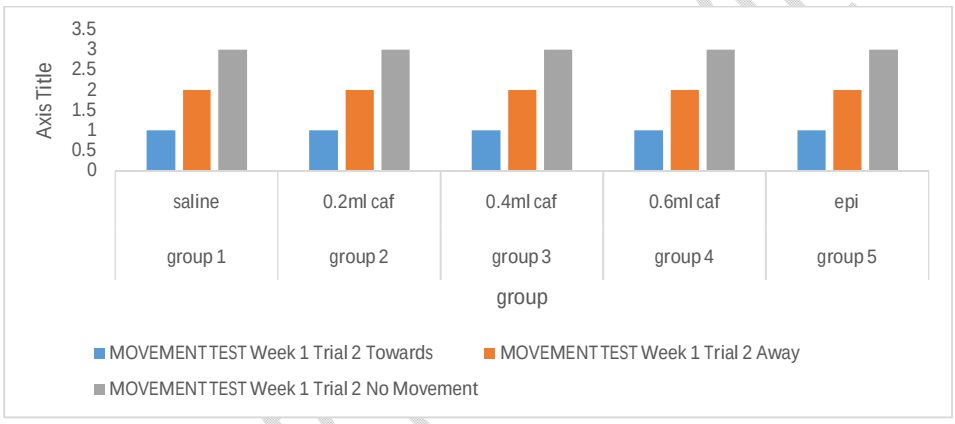

Fig 6.2 Pattern of Movement in the test and control groups in week 1 trial2

Table 6.3 Movement test recorded from the test and control groups using Acoustic reflex technique in week 1 trial3

	ACOUSTIC REFLEX (MOVEMENT) TEST			
Groups	**treatment**	**Week 1 Trial 3**		
		Towards	**Away**	**No Movement**
1	**saline**	1.00 ±.00	2.00 ±.00	3.00 ±.00

2	**0.2ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
3	**0.4ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
4	**0.6ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
5	**epinephrine**	1.00 ±.00	2.00 ±.00	3.00 ±.00

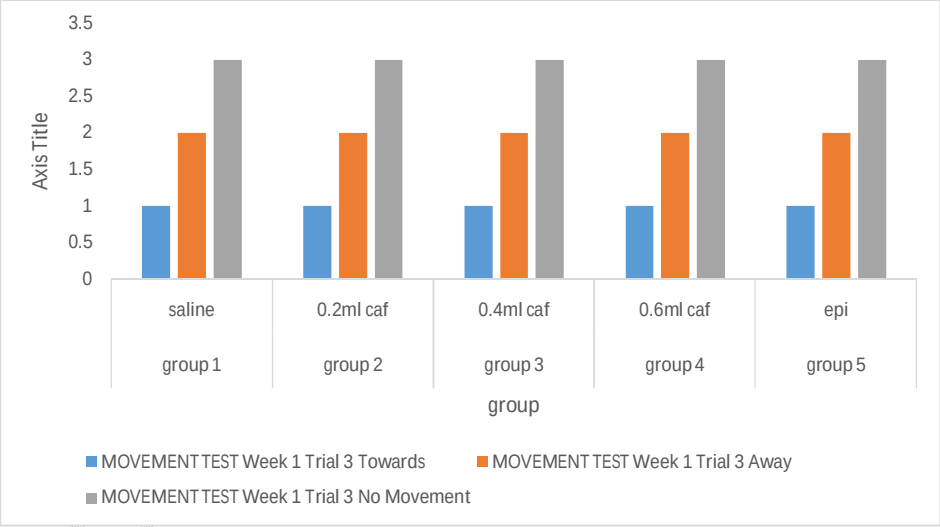

Fig 6.3 Pattern of Movement in the test and control groups in week 1 trial3

Table 7.1 Movement test recorded from the test and control groups using Acoustic reflex technique in week 2 trial1

	ACOUSTIC REFLEX (MOVEMENT) TEST			
groups	**treatment**	**Week 2 Trial 1**		
		Towards	**Away**	**No Movement**
1	**saline**	1.00 ±.00	2.00 ±.00	3.00 ±.00
2	**0.2ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
3	**0.4ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
4	**0.6ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
5	**epinephrine**	1.00 ±.00	2.00 ±.00	3.00 ±.00

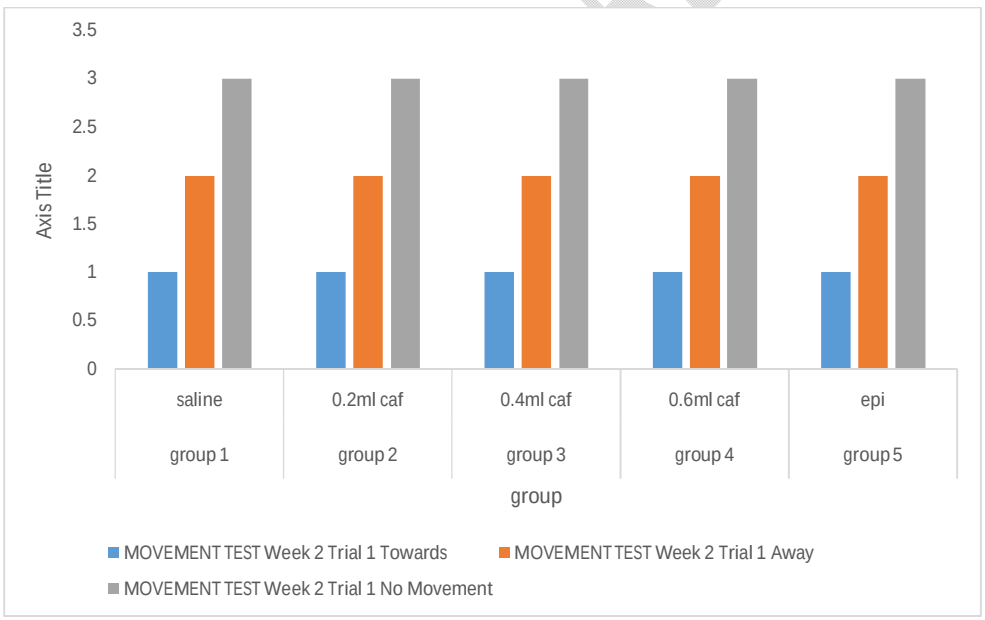

Fig 7.1 Pattern of Movement in the test and control groups in week 2 trial1

Table 7.2 Movement test recorded from the test and control groups using Acoustic reflex technique in week 2 trial2

	ACOUSTIC REFLEX (MOVEMENT) TEST			
groups	**treatment**	**Week 2 Trial 2**		
		Towards	**Away**	**No Movement**
1	**saline**	1.00 ±.00	2.00 ±.00	3.00 ±.00
2	**0.2ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
3	**0.4ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
4	**0.6ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
5	**epinephrine**	1.00 ±.00	2.00 ±.00	3.00 ±.00

Fig 7.2 Pattern of Movement in the test and control groups in week 2 trial2

Table 7.3 Movement test recorded from the test and control groups using Acoustic reflex technique in week 2 trial3

	ACOUSTIC REFLEX (MOVEMENT) TEST			
groups	**treatment**	**Week 2 Trial 3**		
		Towards	**Away**	**No Movement**
1	**saline**	1.00 ±.00	2.00 ±.00	3.00 ±.00
2	**0.2ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
3	**0.4ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
4	**0.6ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
5	**epinephrine**	1.00 ±.00	2.00 ±.00	3.00 ±.00

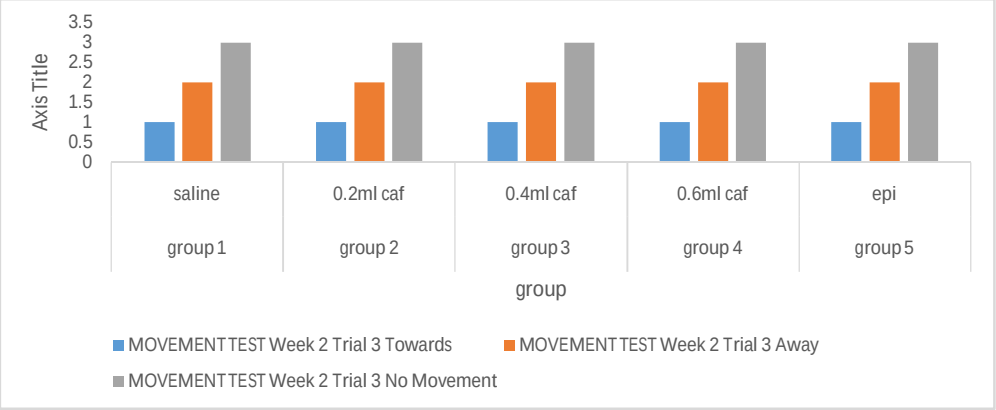

Fig 7.3 Pattern of Movement in the test and control groups in week 2 trial3

Table 8.1 Movement test recorded from the test and control groups using Acoustic reflex technique in week 3 trial 1

ACOUSTIC REFLEX (MOVEMENT) TEST				
groups	**treatment**	**Week 3 Trial 1**		
		Towards	**Away**	**No Movement**
1	**saline**	1.00 ±.00	2.00 ±.00	3.00 ±.00

2	**0.2ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
3	**0.4ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
4	**0.6ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
5	**epinephrine**	1.00 ±.00	2.00 ±.00	3.00 ±.00

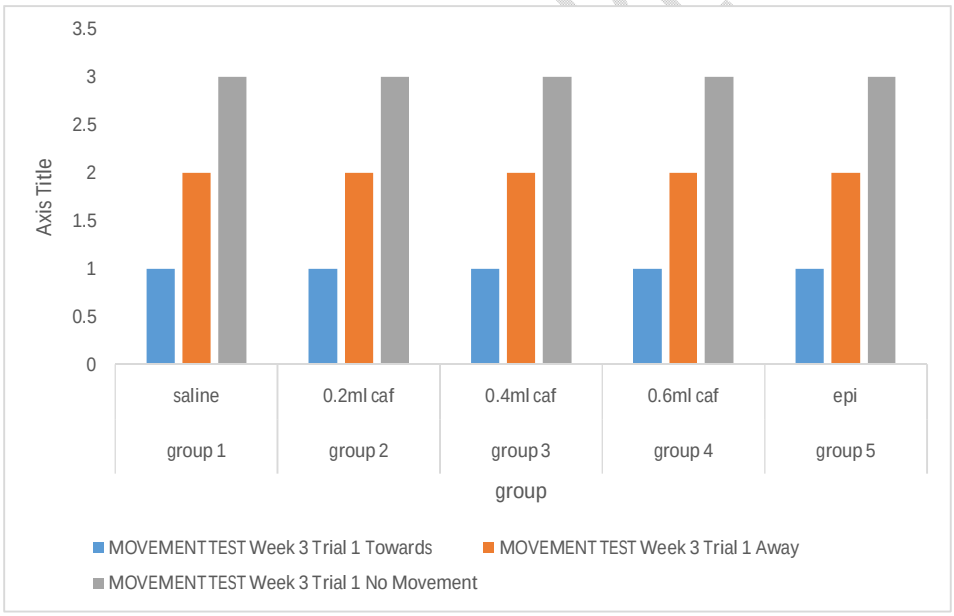

Fig 8.1 Pattern of Movement in the test and control groups in week 3 trial1

Table 8.2 Movement test recorded from the test and control groups using Acoustic reflex technique in week 3 trial2

	ACOUSTIC REFLEX (MOVEMENT) TEST			
groups	**treatment**	**Week 3 Trial 2**		
		Towards	**Away**	**No Movement**
1	**saline**	1.00 ±.00	2.00 ±.00	3.00 ±.00
2	**0.2ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
3	**0.4ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
4	**0.6ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
5	**epinephrine**	1.00 ±.00	2.00 ±.00	3.00 ±.00

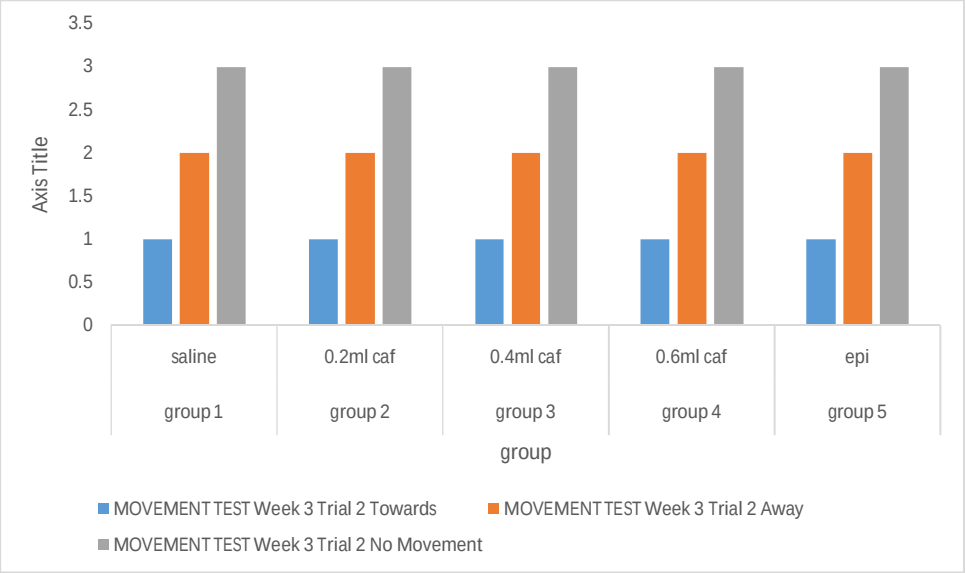

Fig 8.2 Pattern of Movement in the test and control groups in week 3 trial2

Table 8.3 Movement test recorded from the test and control groups using Acoustic reflex technique in week 3 trial3

	ACOUSTIC REFLEX (MOVEMENT) TEST			
groups	**treatment**	**Week 3 Trial 3**		
		Towards	**Away**	**No Movement**
1	**saline**	1.00 ±.00	2.00 ±.00	3.00 ±.00
2	**0.2ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
3	**0.4ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
4	**0.6ml caffeine**	1.00 ±.00	2.00 ±.00	3.00 ±.00
5	**epinephrine**	1.00 ±.00	2.00 ±.00	3.00 ±.00

Fig 8.3 **Pattern of Movement in the test and control groups in week 3 trial3**

DISCUSSION

The present study was designed to examine the effect of caffeine on short term memory, locomotion activity and exploratory behavior in albino wistar rats. The experimental procedure

was done using the following test; Passive avoidance test, Navigation maze test, object recognition test and acoustic reflex (movement) test.

Comment [i20]: Please, Take out this paragraph

Passive Avoidance Test

The passive avoidance test is useful for evaluating the effect of novel chemicals entities on learning and memory as well as studying the mechanism involved in cognition. From the current study the passive avoidance test involving three trials for the total period of three weeks;

From table 4.1 (figure 4.1); At 0.2ml caffeine in group 2 trial 1 week 3 a significant effect was observed compared to other trials in group 2. Furthermore, to passive avoidance test, in group 3 at 0.4ml caffeine, there was a significant effect on trial 1 week 1 amongst all the trial treated with this dosage. At 0.6ml caffeine, the result showed that only week 1, trial 1 was significantly affected among all the trials treated at 0.6ml caffeine. Last result obtained on passive avoidance test with epinephrine showed slight increase in the performance of the treatment. The general result of the passive avoidance test showed significant effect on some of the trials used in the study basically due to increase in the treatment rate of caffeine application as higher-levelbrain functions appeared to be improved by theadministration of caffeine. These results compare favourably with the study of [8][9] but however disagree with that of [10].

Comment [i21]: Table or figure, only one is enough.

Comment [i22]: This information is important but is insufficient. Is important explain why there is a differences or similarities with the studies indicated as references. The dose of caffeine used? The parameters used for each test?

Navigation test

Navigational maze is employed in behavioural neuroscience to study spatial stated that the test could be a very precise study of learning memory and spatial working and is also capable of accessing damages to cortical regions of the brain.

Comment [i23]: Please, take out this

Table 4.2. (Figure 4.2) reported the result obtained for navigation test from week 1 to week 3. At 0.2ml caffeine, week 1 recorded a significant effect on the albino wistar rat in trial 1,2,3 and week 2 trial 1 across the trial extending to week 3. However, slight increase in the time spent to locate the end of the maze was recorded in other trials. At 0.4ml caffeine, only trial 3 at week 3 was significantly affected across the weeks and trials. However, result obtained at 0.6ml caffeine from week 1 to week 3 showed slight increase in the time spent in locating the end of the maze.

Comment [i24]: Table or figure please, not both with the same results

The result gotten showed that animals administered with 0.2ml caffeine performed better as they spent less time in locating the end of the maze compared to other groups. At epinephrine treatment, only at week 1 trial 3 and week 2 trial 1 was a significant increase in the time spent in locating the end the maze recorded. The result gotten from caffeine treatment for the trails generally showed that caffeine at this treatment rate didn't improve memory.These result obtained aligned with [11] result but however contradict that of [12].

Comment [i25]: This information is important but is insufficient. Is important explain why there is a differences or similarities with the studies indicated as references. The dose of caffeine used? The parameters used for each test?

Object Recognition test

The result obtained at 0.2ml caffeine, there were slight performance amongst the trial, however only week1, trial 1 had a significant effect on the rat where it was deduced that the old object (cross) had a significant impact on the rat while at other trial, no significant effect was recorded. At 0.4ml caffeine, there was no significant effect on the familiar and new object in the first week. This was also observed in week 1 among the trials. However, week 1, trial 1 showed a significant effect at this treatment. At 0.6ml caffeine for week 1 and week 3, significant effect was observed. Last treatment on epinephrine showed no significant effect. Generally, from the result obtained, the animals administered with 0.2ml caffeine spent more time exploring the novel (new) object (star and square) than the familiar object (circle and cross), although in week 2 across the three trials they spent more time with the familiar objects. This pattern of behaviorof exploring the novel (new) objects was preserved in week 3, but not after another dose which further indicates a lack of preference for the initial objects after receiving the 0.6ml of caffeine. The same study showed that the high doses of caffeine significantly decrease the time spent sniffing the new object. This result compares favourably with that in [12].

Comment [i26]: Should explain why there is similarities with the studies indicated as references. The dose of caffeine used? The parameters used for each test?

Acoustic Reflex (Movement) test

Result obtained on movement test showed that no significant effect was recorded from week 1 trial 3 to week 3 trial three at all the treatment (control, 0.2ml, 0.4ml, 0.6ml and epinephrine). The general result obtained in the movement test showed no significant influence of caffeine on the alertness on the rat as there were no movement velocity in lower-body exercises. The result obtained agreed with that of [12] but contradict [13] at a higher dosage rate.

Comment [i27]: The same comments that previous

Conclusion

Overall this study revealed effects of caffeine on different parameters. The result shows that caffeine could adversely interfere with physiological activitiessignificantly.The result also shows that the ability of chronic caffeine ingestion is largely dose-dependent and that caffeine in the facilitation of short-term memory, exploratory behavior and locomotion is dependent on the dosage.

Comment [i28]: How did you come to these conclusions if you don't explain them in the discussion?

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