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Original Article

Functional observational battery (FOB) tests using caffeine and chlorpromazine hydrochloride in sprague-dawley rats

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Abstract

Caffeine is believed to exert its therapeutic effects by acting as a nonselective, competitive antagonist of adenosine receptors. Chlorpromazine, a phenothiazine, is a classic psychotropic mediator extensively used in the clinical administration of psychotic disorders. This study aimed to validate the procedures used for performing Functional Observational Battery (FOB) tests, to demonstrate the proficiency and interobserver reliability during the FOB tests and also to assess effect on neurobehavioral parameters using positive controls in rats. The rats were administered with Caffeine in Milli-Q water as oral gavage at the dose of 20 mg/kg and Chlorpromazine HCl in 0.9% Saline as intraperitoneal route at the dose of 20 mg/kg. No inter-personnel variability was observed in home cage, handling, open field and sensory reactivity observations recorded in Proficiency test. In conclusion, the known effects of positive controls; caffeine and chlorpromazine HCl on neurobehavioral/ Functional Observational Battery parameters including autonomic, neuromuscular and sensory reactivity tests were detected in the current study. FOB test procedures for neurobehavioral, grip strength and motor activity are adequate for the detection of neurotoxic effects of positive controls. No major inter-personnel variability was observed between study personnel in neurobehavioural observations.

Keywords: Caffeine, Chlorpromazine HCl, Rat, FOB, Neurobehavior, Neurotoxicity, CRO

1. Introduction

Caffeine is believed to exert its therapeutic effects by acting as a nonselective, competitive antagonist of adenosine receptors in both central (A1 and A2A receptors) and peripheral (A2A and A2B receptors) respiratory centers [1, 2]. The impacts of caffeine on escape and averting behavior have involved a main element of caffeine investigation in the animal literature. In this regard, caffeine has been noticed to both increase in intensity of the anxiogenic effect of a stimulus and quickly reaches the central nervous system which inturn reworks cell processes, intellectual activity, reduces sleep, or diminishes fatigue [3-5]. These processces can contribute to neurobehavioral modifications by creating increased muscular activity and modifying cognition and anxiety [6-8].

Chlorpromazine is a classic psychotropic mediator drug used in to treat psychotic disorders [9]. It has an uncertain activity at pre-synaptic DA-receptors as it declines to inverse apomorphine provoked sedation and hypoactivity [10]. Besides, it causes sedation and hypothermia in rodents [11] and eases activity in animals [12].

Neurobehavioral observation battery as per the technique intended for Safety Pharmacology or Neurotoxicity testing, it is critical to recall and focus on some basic models involved. Behaviour can be hypothesized to form the ultimate integration of a variety of sensory, motor and integrative processes occurring in the nervous system at the level of the intact organism [13, 14].

A change in spontaneous locomotor activity is an excellent preclinical indicator of central nervous system/ neurobehavioral effects of test compounds [15]. A traditional method for assessing changes in locomotor activity is via commercially available test systems that automatically quantify interruptions of infrared beams by a rodent within a testing enclosure. Such technology has been employed for well over a quarter century [16]. Locomotor activity refers to the movement from one place to another. It can be assessed in conditions of involuntary motor activity with muscle co-conferment and social activity. Innovation of behavioural measurements of locomotive movement and assessment is a part appropriate in diverse rodent models as a pilot screen for pharmacologic effects predictive of therapeutic efficiency of a drug in humans [17].

The pharmaceutical industries, contract research organization (CRO) and preclinical experts needs to assess the potential neurotoxicity of drugs during development process. In such case, a validated method may be necessary to perform neurobehavioral assessment to fulfil the global

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regulatory requirements. Since caffeine and chlorpromazine are USFDA approved drugs and globally used, which have extensive properties of psychoactive and antipsychotic activity, respectively. This activity illustrates the use of caffeine and chlorpromazine as a positive control for the neurobehavioral assessment conducted in the preclinical studies or validation study. Further, the insights from the evaluation can be translated from preclinical animal study to the human (and vice versa). This study aimed to explain the purpose of caffeine and chlorpromazine in neurobehavioral assessment and to validate the procedures used for performing Functional Observational Battery (FOB) tests in rats for assessing the neurobehavioral parameters such as home cage, handling, open field, sensory reactivity, motor activity, grip performance and physiological observations. In addition, this study was also intended to train the study personnel in the FOB procedures and to demonstrate proficiency and interobserver reliability in conducting the FOB tests using positive controls. This experiment was conducted in compliance with OECD Principles of Good Laboratory Practice [C (97)186/Final] [18] and USFDA, 21 CFR part 58, Good Laboratory Practice for Nonclinical Laboratory Studies [19].

2. Materials and Methods

2.1. Ethical Statement

The study was conducted in an AAALAC-accredited facility. All the procedures comply with the guidelines provided by the Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and the experiment was approved by the Institutional Animal Ethics Committee (Proposal No.: 004/May 2022, dated: 27 May 2022).

2.2. Animals and Methodology

The experiment was performed using Sprague-Dawley rats procured from HyLasco Biotechnology (India) Private Limited, 4B, MN Park, Turkapally Village, Shameerpet Mandal, Medchal District, Telangana 500078, India. Experimentally naive healthy animals were housed individually (considering interference in blind test for the observer) in standard polysulfone cages in a double corridor barrier facility with standard laboratory conditions of 12 – 15 filtered fresh air changes, temperature range of 19 to 25.0 °C and relative humidity of 30 to 70 % with 12 hours fluorescent light and 12 hours dark cycle. The rats were provided rodent pelleted feed Altromin Rat/Mice Maintenance diets manufactured by Altromin Spezialfutter GmbH & Co. KG, Im Seelenkamp 20, 32791 Lage, Germany *ad libitum* throughout the experimental period. Deep bore-well water passed through activated charcoal filter and exposed to ultraviolet rays in Aquaguard on-line water filter-cum-purifier manufactured by Eureka Forbes Ltd., Mumbai 400 001, India was provided *ad libitum* to rats in polycarbonate bottles with stainless steel sipper tubes.

The positive control test substances such as Caffeine (99.8% purity by HPLC) and Chlorpromazine HCl (99.38% purity by HPLC) were procured from Clearsynth Labs Limited, India. These positive controls have been selected as these were used by many of the researchers with the availability of literature.

2.3. Experimental Design

A total number of 80 rats (40 males and 40 females) of 9 to 10 weeks of age with body weight ranging from 285 to 346 g for males and 222 to 269 g for females were used. The rats were randomly assigned to four different groups by the body weight stratification method using Provantis™ Software (Version 10.1.0.1, Instem LSS, Staffordshire ST15OSD, United Kingdom). Each group consisted of 10 males and 10 females. The dose formulations were prepared freshly and administered to rats of the specific groups.
Group 1: Milli-Q water, 10 mL/kg dose volume, oral gavage (dose: 0 mg/kg)
Group 2: 0.9% Saline, 5 mL/kg dose volume, intraperitoneal (dose: 0 mg/kg)
Group 3: Caffeine in Milli-Q water, 10 mL/kg dose volume, oral gavage (dose: 20 mg/kg)
Group 4: Chlorpromazine HCl in 0.9% Saline, 5 mL/kg dose volume, intraperitoneal (dose: 20 mg/kg)

The first 6 rats were used for Opto varimex 5 (Motor activity) and last 4 rats were used for Opto varimex 4 (Motor activity). The purpose of using vehicle control groups was to handle the animals to elicit normal behaviours. In addition, these animals were used for training and retraining and to check inter-observer variability after validation study and recovery (wash-out) period of one week.

2.4. Observations and Body Weight

Each rat was observed twice daily for mortality and morbidity. Rats were observed for clinical signs Individual body weights (g) were measured prior to dosing on Days 1 and 9.

2.5. Functional Observational Battery (FOB)

FOB tests were performed in a designated experimental room where the disturbances were minimal. In the FOB room, the temperature and humidity were maintained between 19 to 25 °C and 30 to 70% respectively. The motor activity assessment was conducted in a place where light intensity was low (30 to 35 lux) and open field observations were performed under bright fluorescent lighting (319 to 410 lux). Animals were allowed to acclimatize to the FOB room conditions for 30 minutes prior to commencement of observations and FOB tests were performed (Table 1).

2.5.1. Motor Activity

The motor activity for each rat was measured individually using an automated animal activity measuring system (Opto Varimex 4 and Opto Varimex 5; Make: Columbus Instruments, USA) equipped with Auto-Track position monitoring software. Each activity cage comprised a pair of infrared emitters and detector with 16 infrared beams with 1.0-inch beam spacing. Each rat was monitored for 30 minutes. During this motor activity measurement session, parameters such as the stereotypic time (ST), ambulatory time (AT), distance travelled (DT), and resting time (RT) were monitored. The data were then analyzed in 10-minute intervals and reported. The motor activity of first 6 rats/sex/group was measured using Opto Varimex 5 software at pre-dose, Days 1 and 9. The motor activity of remaining 4 rats/sex/group was measured using Opto Varimex 4 software at pre-dose and Day 1.

Table 1. FOB tests were conducted as per the schedule provided below.

Group No.	Test Substances	First 6 rats/sex/group (All FOB tests during validation and proficiency testing)	Last 4 rats/sex/group (Only for motor activity measurement)
G1	Milli Q water	Pre-dose, Days 1 and 9: 1.5-2 hours post-dose	Pre-dose Day 1: 1.5-2 hours post-dose
G2	0.9% Saline	Pre-dose, Days 1 and 9: 20-30 minutes post-dose	Pre-dose Day 1: 20-30 minutes post dose
G3	Caffeine	Pre-dose, Days 1 and 9: 1.5-2 hours post-dose	Pre-dose Day 1: 1.5-2 hours post-dose
G4	Chlorpromazine HCl	Pre-dose, Days 1 and 9: 20-30 minutes post-dose	Pre-dose Day 1: 20-30 minutes post-dose

The animals were subjected to home cage observations, handling observations, open field observations, sensory reactivity measurements, functional tests and physiological Observations (Table 2).

Table 2. Summary of Functional Observational Battery (FOB) evaluations.

Home cage observations	Handling observations	Open field observations	Sensory reactivity measurements	Functional tests	Physiological Observations
1.Posture	1.Ease of removing from the cage	1.Gait	1.Approach response	1.Motor activity	1.Body temperature
2.Abnormal	2.Handling reactivity	2.Posture	2.Touch response	2.Hindlimbs footsplay	2.Body weight
3.Vocalizations	3.Palpebral closure	3.Mobility Score	3.Click response	3.Grip performance	
4.Tremors	4.Eye examination	4.Arousal level	4.Tail-Pinch response		
5.Convulsions	5.Piloerection	5.Clonic or Tonic movements	5.Pupil response		
	6.Lacrimation	6.Stereotypic behavior	6.Aerial righting reflex		
	7.Salivation	7.Bizarre behavior			
	8.Skin/fur examination	8.Urination and Defecation			
	9.Perineum wetness	9.Abnormal vocalization			
	10.Respiration	10.Rearing			
	11.Muscle tone				
	12.Extensor thrust response				

2.5.2. Landing Hindlimbs Footsplay

The tarsal joint of hind foot of each rat was marked with coloured ink and the animal was held in a supine position and then dropped from a short height of approximately 30 cm on to a recording white paper. The distance (mm) between two footprints of hindlimbs was measured. This procedure was repeated three times for each animal and finally, three readings were averaged.

2.5.3. Grip Performance

Hindlimbs and forelimbs grip performance was tested using dual grip strength meter (Make: Columbus Instruments). The grip strength for each animal was measured for 3 consecutive trials, then averaged for both forelimb and hindlimb separately.

2.5.4. Physiological Evaluations

At the end of FOB tests, body weight and rectal temperature of individual rats was recorded at pre-dose, Day 1 and Day 9 of administration. Rectal temperature was recorded using Dr. Morepen Digital MT 222 Thermometer (calibrated).

2.5.5. Blind Observations

Binding procedure is a methodological strategy, to reduce the risk that the observer may consciously or subconsciously influence the outcome of the experiment. The “blind” observer in this experiment was unaware of a subject’s treatment group assignment. During blinding procedure, the animal accession numbers were pooled, and random numbers were generated using validated MS Excel. Animals were placed in separate cages in the same

order as per the random number with cage cards without having any information regarding group, animal accession number, dose, and treatment details. The observer for the FOB was not the same person who placed the animals and cage cards on the rack, to minimize the potential that the observer could remember the group number of the animal.

2.5.6 Training for FOB

During FOB validation phase, study personnel/s were explained and trained/retrained for observations of FOB, consisting of non-invasive procedures designed to assess the functional status of rats.

2.5.7. Proficiency Testing

The proficiency test was conducted for study personnel and was limited to home cage, handling, open field, and sensory observations. After initial training, the animals used for validation phase were used for treatment with recovery period of one week (7 days). Animals were dosed, and the groups were counterbalanced such that each observer evaluated half the rats (3 rats/sex/group) in each dose group. The observations were recorded and compared with validation study results.

2.6. Gross Pathology

Animals were fasted for 16-18 hours (with access *ad libitum* water) prior to terminal sacrifice. Last 4 rats/sex/group on Day 2 (no tissues were collected for histopathology) were subjected to detailed necropsy and findings were recorded. The first 6 rats/sex/group on Day 10 were subjected to detailed necropsy and findings were recorded. Terminal fasting body weights were recorded for first 6 rats/sex/group animals immediately prior to terminal sacrifice and used in calculation of relative organ weights. All terminally sacrificed rats were anesthetized under isoflurane anaesthesia. The detailed necropsy findings (examination of external surfaces of the body, all orifices, cranial, thoracic and abdominal cavities and their contents) were recorded for all rats and tissues were collected. The first 6 rats/sex/group were used for neuro-histopathological examination. Out of 6, first 3 rats/sex/group were subjected for perfusion technique under isoflurane anaesthesia for better preservation of nervous tissues. The tissues from the remaining 3 rats/sex/group were collected in 10% neutral buffered formalin without perfusion for routine histopathological examination. Organ weights were performed for the rats (Day 10) which were not subjected to perfusion technique. The ratio to terminal fasting body weight was determined.

2.7. Histopathology

Histopathological evaluation was carried out for the first 6 rats/sex/group. The tissues were processed for routine paraffin embedding and approximately 4-5 micron sections were stained with Haematoxylin Eosin stain. The brains were examined microscopically as per guidance provided [20].

2.8. Statistical Analyses

The statistical analysis was performed using Provantis™ Laboratory Information Management System (LIMS) built-in statistical tests. The data was evaluated using the Levene Test for homogeneity of variances and the Shapiro-Wilks Test for normality of distributions. The data was analysed by analysis of variance (ANOVA) when the data was homogeneous. When the data was found nonhomogeneous or of nonnormal distribution, the data was subjected to log transformation and ANOVA was done on the transformed data. When ANOVA was significant, pairwise comparisons of treated groups to the vehicle control group were made using a parametric test, Dunnett and Student's test, to identify statistical differences. Descriptive statistics (Mean, SD & Numbers) were presented by group and Day. The data of treated groups (G3 and G4) were compared with concurrent vehicle control groups (G1 and G2). A significance level of $P < 0.05$ was used for the entire statistical analysis.

3. Results

There were no clinical signs (other than during FOB or neurological observations) or morbidity/mortalities observed in the rats during the experimental period. The body weights and body weight gains were unaffected throughout the experimental period in all the treated groups except

Table 3. List of Tissues/Organ collected and examined.

Sl. No.	Tissues/organ	Organ Weight	Collection and Preservation	Microscopic Examination
	Brain (including representative Sections of cerebrum, cerebellum, medulla oblongata and pons)[1]	X	X	X
	Dorsal and ventral nerve root fibres and Ganglion, Dorsal Root (Spinal Ganglion)[2]	-	X	X
	Eye[3]	-	X	X
	Muscle, Skeletal (gastrocnemius)	-	X	X
	Optic nerve	-	X	X
	Sciatic nerve	-	X	X
	Tibial (with branches) nerve	-	X	X
	Spinal cord	-	X	X

[1]: The brain was trimmed as per the trimming guidance provided [20].
[2]: Representative samples from cervical, thoracic and lumbosacral regions were examined.
[3]: Collected with optic nerve and preserved in Davidson's fluid.
X: Activity carried out.

Table 4. Functional Observational Battery (FOB) Tests – Validation vs Proficiency Test (Caffeine).

Parameters	Sex→	Males (first 3 rats/sex/group)				Females (first 3 rats/sex/group)				Males (last 3 rats/sex/group)				Females (last 3 rats/sex/group)			
	Score	Validation	1^	2^	3^	Validation	1^	2^	3^	Validation	4^	5^	6^	Validation	4^	5^	6^
		Handling observations															
Ease of removal Handling reactivity	Very easy	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Easy	2	2	2	2	3	1	1	1	2	2	2	1	1	2	2	2
	Moderately difficult	-	1	1	1	-	2	2	2	1	1	1	2	2	1	1	1
	Moderately low	3	2	2	2	3	1	1	1	3	2	2	3	1	2	2	2
	Moderately high	-	1	1	1	-	2	2	2	-	1	1	-	2	1	1	1
		Open field observations															
Arousal level	Alert	3	3	3	3	2	3	3	3	2	2	2	2	1	2	2	2
	High	-	-	-	-	1	-	-	-	1	1	1	1	2	1	1	1
		Sensory reactivity															
Touch response	Normal response	2	2	2	2	3	3	3	3	3	2	3	2	1	3	3	3
	Slight response	1	1	1	1	-	-	-	-	-	1	-	1	2	-	-	-
Click response	Normal response	2	2	2	2	3	3	3	3	3	2	3	3	1	3	3	3
	Slight response	1	1	1	1	-	-	-	-	-	1	-	-	2	-	-	-
Tail-Pinch response	Normal response	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-
	Slight response	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-

^: Study personnel (proficiency test); -: Not applicable.

Table 5. Functional Observational Battery (FOB) Tests – Validation vs Proficiency Test (Chlorpromazine HCl).

Parameters	Sex→	Males (first 3 rats/sex/group)				Females (first 3 rats/sex/group)				Males (last 3 rats/sex/group)				Females (last 3 rats/sex/group)			
	Score	Validation	1^	2^	3^	Validation	1^	2^	3^	Validation	4^	5^	6^	Validation	4^	5^	6^
Handling observations																	
Ease of removal	Very easy	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Handling reactivity	Low	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Palpebral closure	Eyelids slightly drooping	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Eye examination	Normal	1	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
	Discharge (clear)	2	3	3	3	3	3	3	3	1	3	3	3	3	3	3	3
Lacrimation	Absent	1	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-
	Present	2	3	3	3	3	3	3	3	1	3	3	3	3	3	3	3
Muscle tone	Minimal	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Extensor thrust response	No extensor thrust response	2	3	3	2	3	1	1	1	2	2	1	1	2	1	1	2
	Slight	1	-	-	1	-	2	2	2	1	1	2	2	1	2	2	1
Open field observations																	
Gait	Severely abnormal gait (Hindlimbs show exaggerated, overcompensated, and / or splayed movements)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Posture	Severe abnormality (Completely flattened, pelvis flat on surface)	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Mobility score	Totally impaired	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Arousal level	Low	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Sensory reactivity																	
Approach response	No response	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Touch response	No response	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Click response	Slight response	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
Tail-Pinch response	Slight response	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3

^: Study personnel (proficiency test); -: Not applicable.

significant decrease in body weights in chlorpromazine HCl (G4 – 20 mg/kg) males on Day 9 when compared to respective vehicle control group.

3.1. Functional Observational Battery (FOB) Tests – Pre-dose

All the functional observational battery parameters evaluated during pre-dose were comparable between caffeine/chlorpromazine HCl and vehicle control group rats. The few random changes observed at pre-dose were considered as expected random biological variations.

3.2. Functional Observational Battery (FOB) Tests – Validation with Caffeine

FOB tests performed on rats administered with caffeine at 20 mg/kg at 1.5-2 hours post-dose exhibited a spectrum of neurological effects as explained below (Tables 4, 6-9 and Figures 1, 3 and 4). No abnormal vocalizations, tremors and convulsions were observed in any of the animals. The observation of resistance to handling was indicative of excited state of animals following treatment with caffeine. All other parameters examined while handling was normal. Increased arousal level, rearing counts and slight responses observed in different sensory reactivity measurement parameters are the expected treatment-related CNS-stimulatory effects in caffeine treated when compared to vehicle control group in both sexes. No statistically significant variations were observed in body weight, body temperature, grip performance (forelimbs and hindlimbs grip strength) and hindlimbs foot splay of caffeine-treated rats as compared to vehicle control group. Increased ambulatory time, distance travelled with decreased stereotypic time and resting were considered as treatment-related effects of caffeine.

3.3. Functional Observational Battery (FOB) Tests – Validation with Chlorpromazine HCl

FOB tests were performed on rats administered with chlorpromazine HCl at 20 mg/kg at 20 – 30 minutes post-dose and exhibited a spectrum of neurological effects as explained below (Tables 5 – 9 and Figures 1 - 4). In home cage, all the vehicle control group animals revealed normal postures whereas, animals treated with chlorpromazine HCl revealed abnormal postures such as flattened, limbs spread out with abdomen pressed to floor and no abnormal vocalizations, tremors and convulsions were observed in either sex. Very easy while removing from cage, low handling reactivity, slightly drooped palpebral closure, eye examination revealed clear discharge (lacrimation), soft muscle tone and no extensor thrust / slight extensor thrust. Severe abnormal gait (hindlimbs showed exaggerated, over-compensated and/or splayed movements), severe abnormal posture (completely flattened, pelvis flat on surface), mobility was totally impaired and arousal level was low and rearing count was nil in rats of chlorpromazine HCl treated rats of either sex. None of the rats in the chlorpromazine HCl-treated group revealed tremors, tonic/clonic movements, stereotypic/bizarre behaviour, and vocalizations. No response to approach and touch stimulus and slight response for click and tail pinch was observed in rats treated with chlorpromazine HCl. Defecation and urination were normal in either sex. Pupil response and aerial righting reflex were normal in all the chlorpromazine HCl-treated rats. The body temperature, forelimbs and hindlimbs grip strengths was significantly decreased. Hindlimb footsplay were increased in both sexes. The observed reduction in distance travelled, stereotypic time, ambulatory time, and increased resting time were the significant findings of chlorpromazine HCl-treated rats when compared to vehicle control-treated rats. No significant variations were observed in body weight of female rats. However, statistically significant decrease was observed in male rats.

3.4. Proficiency Testing

The proficiency test was limited to home cage, handling, open field, and sensory observations conducted on Day 9 (Tables 4 and 5). Observations were made by observers each time, who were blinded to the rat's group assignment. Rats were dosed, and the groups were counterbalanced such that each observer evaluated half the rats (3 rats/sex/group) in each dose group. Observations were made at the same time of day for each data set. The observations were recorded and compared with validation study results. No inter-personnel variability was observed in home cage, handling, open field and sensory reactivity observations recorded in Proficiency test.

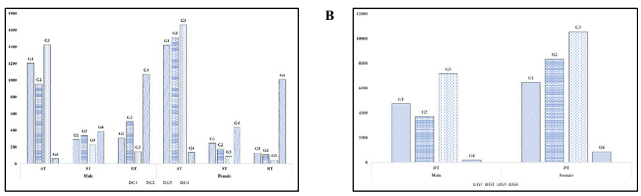

Fig. 1. Day 1 motor activity (Opto varimex 5). A: AT - Ambulatory Time, ST - Stereotypic Time, RT – Resting Time, B: DT – Distance.

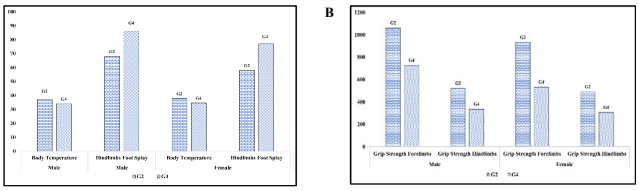

Fig. 2. Day 1 A: Body temperature, hindlimbs foot splay, B: Forelimbs grip strength and hindlimbs grip strength.

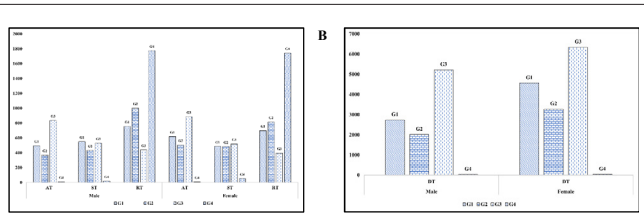

Fig. 3. Day 1 motor activity (Opto varimex 4). A: AT - Ambulatory Time, ST - Stereotypic Time, RT – Resting Time, B: DT – Distance.

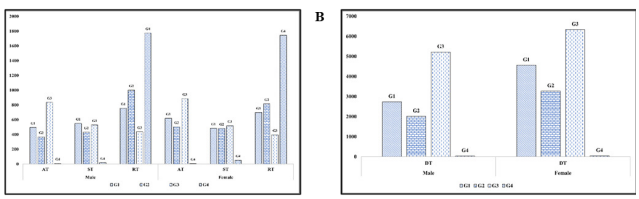

Fig. 4. Day 9 motor activity (Opto varimex 5). A: AT - Ambulatory Time, ST - Stereotypic Time, RT – Resting Time, B: DT – Distance.

Table 6. Functional Observational Battery (FOB) Tests - Validation with Caffeine and Chlorpromazine HCl (Males).

Parameters	Days	Group (dose) - Males			
		G1 (0 mg/kg) N = 6	G2 (0 mg/kg) N = 6	G3 (20[a] mg/kg) N = 6	G4 (20[b] mg/kg) N = 6
Body weight (g)	Pre-dose	299.49 ± 9.56	307.03 ± 13.41	295.95 ± 16.36	295.08 ± 18.32
	1	310.19 ± 13.61	319.07 ± 13.23	302.55 ± 14.96	300.61* ± 15.07
	9	348.56 ± 21.37	365.57 ± 12.22	347.97 ± 21.99	331.55* ± 20.65
Body temperature (ºC)	Pre-dose	37.2 ± 0.3	37.2 ± 0.3	37.2 ± 0.4	37.1 ± 0.3
	1	37.0 ± 0.4	37.0 ± 0.3	37.2 ± 0.4	34.1* ± 0.3
Grip Strength Forelimbs (gf)	Pre-dose	1069 ± 39	1075 ± 31	1065 ± 31	1074 ± 31
	1	1073 ± 29	1061 ± 46	1055 ± 29	727* ± 10
Grip Strength Hindlimbs (gf)	Pre-dose	540 ± 21	523 ± 12	536 ± 24	541* ± 13
	1	548 ± 18	521 ± 16	524 ± 29	336* ± 5
Hindlimb Foot Splay (mm)	Pre-dose	59 ± 17	68 ± 18	61 ± 10	71 ± 26
	1	70 ± 21	68 ± 17	64 ± 13	86 ± 10
Ambulatory Time Interval - 1 (seconds)	Pre-dose	543.3 ± 27.5	577.3 ± 18.6	563.8 ± 11.4	565.7 ± 14.4
	1	516.8 ± 82.7	474.5 ± 89.5	553.7 ± 47.1	16.1* ± 26.4
	9	543.8 ± 38.2	537.6 ± 70.5	589.1* ± 9.4	0.0* ± 0.0
Ambulatory Time Interval - 2 (seconds)	Pre-dose	423.5 ± 133	489.7 ± 109.2	474.0 ± 63.8	466.1 ± 72.6
	1	414.9 ± 179.7	175.8 ± 132.7	455.7 ± 75.6	13.7* ± 31.2
	9	434.3 ± 75.4	467.9 ± 85.1	558.8* ± 44.1	0.0* ± 0.0
Ambulatory Time Interval - 3 (seconds)	Pre-dose	327.3 ± 152.5	434.2 ± 125.9	377.9 ± 177.5	361.5 ± 116.4
	1	273.0 ± 210.1	303.3 ± 194.3	415.0 ± 145.9	31.5* ± 47.0
	9	384.5 ± 146.7	226.4 ± 207.7	522.6* ± 36.2	17.2* ± 42.2
Ambulatory Time Total (seconds)	Pre-dose	1294.1 ± 293.5	1501.2 ± 200.7	1415.7 ± 244.4	1393.4 ± 178.3
	1	1204.8 ± 449.3	953.7 ± 372.9	1424.4 ± 228.2	61.3* ± 103.3
	9	1362.7 ± 163.9	1231.8 ± 296	1670.5* ± 83.2	17.2* ± 42.2
Stereotypic Time Interval - 1 (seconds)	Pre-dose	37.9 ± 26.0	16.6 ± 17.7	24.0 ± 9.9	19.8 ± 11.1
	1	48.0 ± 43.0	72.4 ± 46.0	25.3 ± 25.0	163.1 ± 136.4
	9	35.6 ± 26.3	30.7 ± 28.4	6.1* ± 6.8	192.2* ± 84.5
Stereotypic Time Interval - 2 (seconds)	Pre-dose	96.3 ± 70.5	75.2 ± 86.2	90.1 ± 49.4	96.6 ± 67.2
	1	95.7 ± 52.3	145.6 ± 64.2	99.8 ± 56.6	114.5 ± 71.4
	9	101.3 ± 40.0	87.6 ± 79.0	15.8* ± 13.6	139.4 ± 92.2
Stereotypic Time Interval - 3 (seconds)	Pre-dose	124.1 ± 50.5	106.5 ± 95.9	117.1 ± 71.7	150.9 ± 73.2
	1	144.3 ± 59.6	121.6 ± 77.4	104.5 ± 81.4	110.0 ± 50.6
	9	116.5 ± 75.5	166.8 ± 75.3	49.5 ± 28.5	155.9 ± 100.8
Stereotypic Time Total (seconds)	Pre-dose	258.3 ± 114	198.4 ± 156.3	231.2 ± 121.8	267.3 ± 123.0
	1	288.0 ± 122.3	339.7 ± 164.7	229.6 ± 130.5	387.6 ± 206.5
	9	253.4 ± 86.3	285.1 ± 163.2	71.4* ± 38.6	487.5* ± 146.7
Resting Interval - 1 (seconds)	Pre-dose	18.8 ± 6.9	6.0 ± 5.0	12.3* ± 1.9	14.5* ± 6.0
	1	35.2 ± 40.5	53.0 ± 48.7	21.0 ± 23.9	331.4* ± 182.6
	9	20.6 ± 13.4	31.8 ± 46.0	4.8* ± 3.2	407.8* ± 84.5
Resting Interval - 2 (seconds)	Pre-dose	80.2 ± 74.4	35.1 ± 25.6	35.9 ± 15.8	37.3 ± 12.5
	1	89.4 ± 138.3	276.6 ± 121.1	44.5 ± 22.5	372.6 ± 184.6
	9	64.4 ± 37.9	44.5 ± 11.4	25.4 ± 35.1	460.6* ± 92.2
Resting Interval - 3 (seconds)	Pre-dose	148.6 ± 155.3	59.3 ± 34.3	105.0 ± 109.9	87.6 ± 59.4
	1	182.7 ± 187.5	174.9 ± 186.2	80.5 ± 70.2	368.2 ± 164.7
	9	99.0 ± 108.5	206.8 ± 147.7	27.9* ± 10.2	426.8* ± 115.1
Resting Total (seconds)	Pre-dose	247.6 ± 220.1	100.4 ± 50.7	153.1 ± 126.0	139.3 ± 66.5
	1	307.3 ± 353.5	504.5 ± 325.8	146.1 ± 108.1	1072.1* ± 492.4
	9	183.9 ± 112.2	283.1 ± 155.8	58.1* ± 45.6	1295.3* ± 128.1
Distance Interval - 1 (Inches)	Pre-dose	3014.62 ± 337.13	3574.14 ± 388.40	3279.40 ± 355.11	3917.96 ± 573.45
	1	2590.31 ± 647.69	2222.27 ± 676.83	3638.26* ± 411.47	30.92* ± 52.57
	9	2866.43 ± 384.47	3071.02 ± 482.13	4232.88* ± 531.26	0.0* ± 0.0
Distance Interval - 2 (Inches)	Pre-dose	1537.92 ± 773.90	2112.63 ± 712.48	1774.65 ± 491.06	2071.26 ± 667.75
	1	1377.31 ± 828.03	376.92 ± 324.26	1870.63 ± 733.60	52.67* ± 127.15
	9	1375.35 ± 382.87	1552.10 ± 440.82	2999.14* ± 564.45	0.0* ± 0.0
Distance Interval - 3 (Inches)	Pre-dose	1174.13 ± 617.06	1503.24 ± 520.43	1329.50 ± 727.12	1420.64 ± 376.15
	1	766.95 ± 637.01	1098.17 ± 837.93	1655.84 ± 780.00	109.29* ± 159.37
	9	1094.18 ± 603.72	592.23 ± 653.84	2426.39* ± 503.44	57.15* ± 139.99
Distance Total (Inches)	Pre-dose	5726.67 ± 1624.42	7190.00 ± 1026.60	6383.55 ± 1319.23	7409.86 ± 1344.10
	1	4734.57 ± 1878.95	3697.36 ± 1685.51	7164.73* ± 1614.17	192.88* ± 326.84
	9	5335.95 ± 840.60	5215.35 ± 1295.18	9658.41* ± 1468.35	57.15* ±139.99

[a]: Caffeine; [b]: Chlorpromazine HCl; N: Number; *: Significantly different from vehicle control group (G1 vs G3) and (G2 vs G4) $p < 0.05$.

Table 7. Functional Observational Battery (FOB) Tests - Validation with Caffeine and Chlorpromazine HCl (Females).

Parameters	Days	Group (dose) - Females			
		G1 (0 mg/kg) N = 6	G2 (0 mg/kg) N = 6	G3 (20[a] mg/kg) N = 6	G4 (20[b] mg/kg) N = 6
Body weight (g)	Pre-dose	223.77 ± 11.90	228.16 ± 8.41	227.99 ± 12.14	226.26 ± 8.19
	1	230.08 ± 9.46	238.06 ± 8.11	235.71 ± 7.74	237.43 ± 10.52
	9	252.83 ± 9.00	263.63 ± 8.36	257.85 ± 4.59	257.58 ± 8.44
Body temperature (ºC)	Pre-dose	38.0 ± 0.2	38.1 ± 0.2	38.2 ± 0.2	38.3 ± 0.3
	1	38.1 ± 0.3	37.8 ± 0.4	38.1 ± 0.3	34.6* ± 0.3
Grip Strength Forelimbs (gf)	Pre-dose	935 ± 8	931 ± 9	969* ± 45	942 ± 23
	1	942 ± 10	933 ± 11	934 ± 19	533* ± 4
Grip Strength Hindlimbs (gf)	Pre-dose	487 ± 22	485 ± 11	489 ± 21	491 ± 16
	1	517 ± 52	490 ± 14	497 ± 20	306 ± 6
Hindlimb Foot Splay (mm)	Pre-dose	62 ± 10	62 ± 9	65 ± 12	59 ± 15
	1	58 ± 20	58 ± 13	74 ± 10	77* ± 6
Ambulatory Time Interval - 1 (seconds)	Pre-dose	573.5 ± 14.5	590.2 ± 6.6	584.5 ± 12.5	572.9* ± 17.3
	1	568.6 ± 20.8	588.3 ± 17.2	585.6 ± 7.4	49.0* ± 43.7
	9	559.0 ± 28.5	564.0 ± 15.0	585.3 ± 16.6	18.9* ± 25.3
Ambulatory Time Interval - 2 (seconds)	Pre-dose	455.5 ± 96.3	515.5 ± 86.2	531.9 ± 43.1	537.4 ± 30.0
	1	460.8 ± 50.8	472.1 ± 145.0	549.9* ± 36.9	40.2* ± 55.6
	9	421.7 ± 119.9	462.5 ± 67.7	554.1* ± 66.3	2.2* ± 5.4
Ambulatory Time Interval - 3 (seconds)	Pre-dose	425.9 ± 60.9	405.4 ± 210.4	451.0 ± 99.3	250.3 ± 142.7
	1	391.2 ± 110.3	453.2 ± 154.3	530.1* ± 51.6	46.2* ± 75.9
	9	276.5 ± 119.2	344.6 ± 162.7	519.2* ± 64.6	1.8* ± 4.4
Ambulatory Time Total (seconds)	Pre-dose	1454.8 ± 146.6	1511.0 ± 293.6	1567.4 ± 112	1360.5 ± 135.3
	1	1420.5 ± 140.9	1513.5 ± 272.7	1665.6* ± 68.9	135.4* ± 172.2
	9	1257.2 ± 247.3	1371.1 ± 205.5	1658.6* ± 116.7	22.9* ± 32.6
Stereotypic Time Interval - 1 (seconds)	Pre-dose	16.7 ± 13.3	6.0 ± 6.3	9.0 ± 8.7	15.5 ± 14.0
	1	21.4 ± 16.9	9.4 ± 14.6	7.9 ± 6.6	150.0* ± 121.5
	9	28.3 ± 24.1	23.1 ± 8.2	9.3 ± 12.3	72.8* ± 66.1
Stereotypic Time Interval - 2 (seconds)	Pre-dose	97.2 ± 74.6	51.5 ± 48.8	50.0 ± 38.2	38.7 ± 33.2
	1	98.5 ± 46.1	78.4 ± 79.3	34.2* ± 23.2	137.6 ± 123.4
	9	112.1 ± 98.1	81.1 ± 40.6	21.4 ± 31.3	102.0 ± 140.9
Stereotypic Time Interval - 3 (seconds)	Pre-dose	111.3 ± 52.7	111.7 ± 88.1	92.2 ± 56.7	139.9 ± 97.7
	1	128.2 ± 79.3	88.3 ± 90.2	47.4 ± 43.4	148.8 ± 113.7
	9	143.1 ± 71.2	123.8 ± 62.6	53.6* ± 48.7	100.1 ± 102.8
Stereotypic Time Total (seconds)	Pre-dose	225.2 ± 120.9	169.2 ± 134.3	151.2 ± 76.4	194.0 ± 112.2
	1	248.0 ± 124.4	176.1 ± 161.6	89.5* ± 58.6	436.3 ± 295.5
	9	283.5 ± 138.5	227.9 ± 88.4	84.3* ± 76.7	274.9 ± 258.4
Resting Interval - 1 (seconds)	Pre-dose	9.9 ± 5.4	3.8 ± 3.0	6.6 ± 6.1	11.7* ± 6.7
	1	10.0 ± 6.4	2.3 ± 2.6	6.5 ± 6.5	303.6* ± 166.4
	9	12.8 ± 11.8	12.9 ± 7.9	5.4 ± 6.4	294.1* ± 214.5
Resting Interval - 2 (seconds)	Pre-dose	47.3 ± 23.7	33.1 ± 37.7	16.8* ± 5.3	23.9 ± 9.9
	1	40.8 ± 18.7	49.6 ± 67.9	15.9* ± 15.1	335.9* ± 182.9
	9	60.6 ± 28.6	56.3 ± 43.1	24.6 ± 35.1	260.2 ± 287.2
Resting Interval - 3 (seconds)	Pre-dose	53.8 ± 15.6	81.4 ± 134.9	56.8 ± 47.1	209.9* ± 135.2
	1	80.7 ± 39.7	58.5 ± 65.0	22.5* ± 16.1	369.0* ± 118.8
	9	180.4 ± 93.6	131.6 ± 111.3	27.2* ± 23.6	231.5 ± 249.4
Resting Total (seconds)	Pre-dose	111.0 ± 21.9	118.3 ± 169.7	80.2 ± 49.7	245.5* ± 130.5
	1	131.5 ± 30.4	110.3 ± 112.2	45.0* ± 19.3	1008.6* ± 347.1
	9	253.8 ± 112.4	200.8 ± 134.9	57.2* ± 46.0	785.8 ± 738.7
Distance Interval - 1 (Inches)	Pre-dose	3455.10 ± 319.68	4341.33 ± 792.38	4122.77 ± 785.04	4013.99 ± 227.56
	1	3294.83 ± 411.49	4240.48 ± 775.56	4850.01* ± 1241.48	286.97* ± 471.32
	9	3378.36 ± 583.47	3448.36 ± 268.62	4804.56* ± 853.00	64.37* ± 98.05
Distance Interval - 2 (Inches)	Pre-dose	1969.39 ± 485.41	2572.69 ± 682.85	2419.76 ± 563.61	2548.33 ± 499.62
	1	1836.52 ± 314.90	2199.24 ± 1056.92	2852.28* ± 595.95	268.17* ± 563.70
	9	1727.56 ± 722.37	1695.38 ± 359.71	3550.24* ± 1183.67	9.87* ± 24.17
Distance Interval - 3 (Inches)	Pre-dose	1864.03 ± 435.07	1486.08 ± 942.01	2012.49 ± 709.38	881.83 ± 783.90
	1	1326.45 ± 611.96	1913.96 ± 979.18	2851.57* ± 1165.13	299.38* ± 704.55
	9	718.90 ± 374.11	1148.08 ± 581.64	2975.27* ± 687.88	1.06* ± 2.59
Distance Total (Inches)	Pre-dose	7288.51 ± 937.59	8400.10 ± 1246.56	8555.03 ± 1985.71	7444.14 ± 1258.47
	1	6457.81 ± 1243.31	8353.69 ± 2627.04	10553.85* ± 2701.76	854.51* ± 1736.26
	9	5824.82 ± 1546.95	6291.82 ± 883.65	11330.07* ± 2445.40	75.29* ± 121.34

[a]: Caffeine; [b]: Chlorpromazine HCl; N: Number; *: Significantly different from vehicle control group (G1 vs G3) and (G2 vs G4) $p < 0.05$.

Table 8. Opto varimex 4 - Validation with Caffeine and Chlorpromazine HCl (Males).

Parameters	Days	Group (dose) - Males			
		G1 (0 mg/kg) N = 4	G2 (0 mg/kg) N = 4	G3 (20[a] mg/kg) N = 4	G4 (20[b] mg/kg) N = 4
Body weight (g)	Pre-dose	283.61 ± 17.54	284.89 ± 14.28	298.71 ± 15.49	286.90 ± 13.14
	1	301.55 ± 22.31	311.20 ± 17.59	310.21 ± 10.62	308.53 ± 7.96
Ambulatory Time Interval - 1 (seconds)	Pre-dose	327.8 ± 19.9	257.3 ± 35.8	300.8 ± 35.0	311.8 ± 60.8
	1	272.3 ± 51.0	204.5 ± 53.2	373.5* ± 26.0	1.8* ± 3.5
Ambulatory Time Interval - 2 (seconds)	Pre-dose	89.3 ± 39.8	91.3 ± 62.5	171.0 ± 68.3	182.3 ± 89.1
	1	122.3 ± 76.5	87.0 ± 59.8	242.0* ± 55.3	2.5* ± 5.0
Ambulatory Time Interval - 3 (seconds)	Pre-dose	3.0 ± 2.3	16.0 ± 12.5	14.0 ± 16.5	116.5 ± 86.4
	1	100.0 ± 49.7	75.8 ± 78.7	219.3* ± 17.9	3.5 ± 4.4
Ambulatory Time Total (seconds)	Pre-dose	420.0 ± 38.4	364.5 ± 108.1	485.8 ± 114.5	610.5 ± 201.1
	1	494.5 ± 131.6	367.3 ± 166.0	834.8* ± 87.6	7.8* ± 12.4
Stereotypic Time Interval - 1 (seconds)	Pre-dose	154.8 ± 6.0	208.3 ± 27.8	184.0 ± 25.2	158.8* ± 24.0
	1	178.5 ± 21.7	200.8 ± 63.7	145.0* ± 13.7	5.3* ± 10.5
Stereotypic Time Interval - 2 (seconds)	Pre-dose	169.8 ± 41.9	173.8 ± 85.0	190.5 ± 23.4	170.3 ± 14.9
	1	166.3 ± 48.1	130.3 ± 83.5	193.3 ± 17.9	6.5* ± 13.0
Stereotypic Time Interval - 3 (seconds)	Pre-dose	57.0 ± 35.1	108.5 ± 51.3	56.5 ± 19.1	171.3 ± 109.8
	1	204.5 ± 23.6	100.8 ± 55.9	190.8 ± 27.0	5.5* ± 8.0
Stereotypic Time Total (seconds)	Pre-dose	381.5 ± 12.7	490.5 ± 97.0	431.0 ± 45.4	500.3 ± 126.0
	1	549.3 ± 57.2	431.8 ± 200.2	529.0 ± 51.6	17.3* ± 31.3
Resting Interval - 1 (seconds)	Pre-dose	117.5 ± 21.3	134.5 ± 9.0	115.3 ± 22.0	129.5 ± 40.3
	1	149.3 ± 61.3	194.8 ± 111.3	81.5 ± 18.4	593.0* ± 14.0
Resting Interval - 2 (seconds)	Pre-dose	341.0 ± 65.6	335.0 ± 144.9	238.5 ± 66.9	247.5 ± 79.5
	1	311.5 ± 120.1	382.8 ± 138.8	164.8 ± 52.0	591.0* ± 18.0
Resting Interval - 3 (seconds)	Pre-dose	540.0 ± 36.3	475.5 ± 58.2	529.5 ± 26.5	312.3 ± 177.0
	1	295.5 ± 48.1	423.5 ± 113.8	190.0* ± 15.9	591.0* ± 12.3
Resting Total (seconds)	Pre-dose	998.5 ± 46.8	945.0 ± 195.7	883.3 ± 107.9	689.3 ± 239.1
	1	756.3 ± 148.5	1001.0 ± 356.6	436.3* ± 64.3	1775.0* ± 43.6
Distance Interval - 1 (cm)	Pre-dose	2295 ± 407	1843 ± 692	2177 ± 282	2081 ± 598
	1	1710 ± 511	1153 ± 394	2657* ± 314	5* ± 11
Distance Interval - 2 (cm)	Pre-dose	434 ± 159	600 ± 528	1085 ± 638	1120 ± 762
	1	545 ± 377	437 ± 296	1382* ± 354	11* ± 22
Distance Interval – 3 (cm)	Pre-dose	9 ± 7	68 ± 80	87 ± 129	657 ± 588
	1	480 ± 290	425 ± 501	1175* ± 219	20 ± 24
Distance Total (cm)	Pre-dose	2736.75 ± 312.91	2511.75 ± 1272.92	3348.25 ± 864.09	3857.25 ± 1770.32
	1	2734.75 ± 868.51	2014.75 ± 1073.64	5213.50* ± 701.87	35.75* ± 52.39

[a]: Caffeine; [b]: Chlorpromazine HCl; N: Number; *: Significantly different from vehicle control group (G1 vs G3) and (G2 vs G4) $p < 0.05$.

3.5. Anatomic Pathology

The terminal fasting body weights and organ weights/ratios in both sexes were not affected by Caffeine administration. Minimal decrease in terminal fasting body weight (9%) was observed at 20 mg/kg Chlorpromazine HCl group males. The terminal fasting body weights and organ weights/ratios in females were not affected by Chlorpromazine HCl administration. There were no gross pathological findings at necropsy in all groups. Single incidence of skeletal muscle degeneration was observed in both sexes at 20 mg/kg Caffeine and Chlorpromazine HCl groups and considered a treatment-related change. Single incidence of squamous cysts in spinal cord was observed in vehicle control group female and considered a spontaneous change.

4. Discussion

Caffeine and Chlorpromazine HCl-positive controls were used for this study. These chemicals have been selected because many of the researchers have used these chemicals as positive controls and a lot of literature was available for these chemicals. The main objective of selecting these positive controls was to study a variety of neurobehavioral abnormalities for assessment using the procedures described.

Caffeine-treated rats showed high reactivity while handling, high and altered response to external stimuli (i.e., slight touch, tail pinch and click response). Motor activity was significantly higher compared to respective vehicle control groups. These findings are in line with the available literature [21].

In Chlorpromazine HCl treated group, all rats did not respond to approach and touch stimulus. Observed finding viz., no response to approach and touch was also reported by [22] for chlorpromazine HCl. The body temperature, forelimbs and hindlimbs grip strengths were significantly decreased and hindlimbs footsplay was increased in chlorpromazine HCl treated rats as compared to vehicle control group of either sex. Chlorpromazine induces hypothermia. Reduced hindlimbs grip strength and increased hindlimbs footsplay indicate the myorelaxant effect of chlorpromazine as reported [22].

Observed reduction in distance travelled, stereotypic time, ambulatory time, and increased resting time were considered to be treatment-related effects of chlorpromazine HCl and also, reported neurological effects in this

Table 9. Opto varimex 4 - Validation with Caffeine and Chlorpromazine HCl (Females).

Parameters	Days	Group (dose) – Females			
		G1 (0 mg/kg) N = 4	G2 (0 mg/kg) N = 4	G3 (20[a] mg/kg) N = 4	G4 (20[b] mg/kg) N = 4
Body weight (g)	Pre-dose	231.52 ± 13.42	230.51 ± 10.49	227.51 ± 2.82	233.94 ± 11.58
	1	242.06 ± 13.39	239.36 ± 9.44	236.66 ± 6.29	249.62 ± 12.18
Ambulatory Time Interval - 1 (seconds)	Pre-dose	364.0 ± 34.9	332.0 ± 46.0	327.8 ± 40.3	336.3 ± 17.2
	1	313.8 ± 43.0	261.3 ± 71.5	364.0 ± 24.7	2.0* ± 4.0
Ambulatory Time Interval - 2 (seconds)	Pre-dose	107.0 ± 78.5	234.0 ± 93.6	192.5 ± 49.8	223.3 ± 60.9
	1	145.3 ± 94.8	158.0 ± 98.7	294.8* ± 29.2	3.0* ± 5.4
Ambulatory Time Interval - 3 (seconds)	Pre-dose	10.0 ± 3.4	65.0 ± 60.5	80.0 ± 82.7	118.5 ± 99.2
	1	160.0 ± 27.7	84.8 ± 105.1	226.5 ± 51.9	0.8 ± 1.0
Ambulatory Time Total (seconds)	Pre-dose	481.0 ± 65.4	631.0 ± 182.7	600.3 ± 156.1	678.0 ± 165.4
	1	619.0 ± 161.3	504.0 ± 267.6	885.3* ± 73.3	5.8* ± 9.6
Stereotypic Time Interval - 1 (seconds)	Pre-dose	126.3 ± 26.4	158.5 ± 17.7	161.5 ± 23.7	152.0 ± 15.6
	1	149.0 ± 25.3	178.8 ± 37.6	146.3 ± 13.7	1.3* ± 2.5
Stereotypic Time Interval - 2 (seconds)	Pre-dose	154.0 ± 71.6	194.3 ± 41.2	214.3 ± 23.0	187.8 ± 59.8
	1	147.0 ± 31.8	152.0 ± 42.2	168.5 ± 8.5	4.8* ± 9.5
Stereotypic Time Interval - 3 (seconds)	Pre-dose	58.5 ± 33.4	140.3 ± 79.6	184.0* ± 72.5	148.8 ± 49.9
	1	187.3 ± 39.6	150.5 ± 61.5	203.8 ± 36.2	44.5 ± 61.3
Stereotypic Time Total (seconds)	Pre-dose	338.8 ± 104.6	493.0 ± 30.9	559.8* ± 61.2	488.5 ± 21.2
	1	483.3 ± 58.9	481.3 ± 122.1	518.5 ± 42.4	50.5* ± 72.6
Resting Interval - 1 (seconds)	Pre-dose	109.8 ± 34.6	109.5 ± 28.3	110.8 ± 25.2	111.8 ± 19.0
	1	137.3 ± 40.2	160.0 ± 59.0	89.8 ± 11.5	596.8* ± 6.5
Resting Interval - 2 (seconds)	Pre-dose	339.0 ± 138.8	171.8 ± 65.3	193.3 ± 27.7	189.0 ± 7.2
	1	307.8 ± 115.2	290.0 ± 129.2	136.8* ± 29.3	592.3* ± 14.8
Resting Interval - 3 (seconds)	Pre-dose	531.5 ± 32.6	394.8 ± 126.4	336.0* ± 149.8	332.8 ± 145.8
	1	252.8 ± 32.4	364.8 ± 161.9	169.8* ± 39.5	554.8* ± 61.8
Resting Total (seconds)	Pre-dose	980.3 ± 150.6	676.0 ± 205.8	640.0* ± 175.7	633.5 ± 153.6
	1	697.8 ± 154.0	814.8 ± 344.2	396.3* ± 50.0	1743.8* ± 82.1
Distance Interval - 1 (cm)	Pre-dose	3105 ± 668	3065 ± 674	2732 ± 608	2713 ± 440
	1	2531 ± 516	1794 ± 765	3006 ± 529	18* ± 35
Distance Interval - 2 (cm)	Pre-dose	562 ± 514	1480 ± 780	1483* ± 537	1566 ± 597
	1	1038 ± 846	979 ± 739	1946 ± 355	19* ± 23
Distance Interval – 3 (cm)	Pre-dose	33 ± 11	443 ± 507	443 ± 476	579 ± 559
	1	992 ± 289	494 ± 652	1381 ± 387	8 ± 13
Distance Total (cm)	Pre-dose	3700.00 ± 934.41	4988.25 ± 1897.02	4657.75 ± 1534.37	4857.50 ± 1460.34
	1	4560.00 ± 1583.70	3267.25 ± 2126.35	6333.00 ± 1104.52	44.75* ± 58.47

[a]: Caffeine; [b]: Chlorpromazine HCl; N: Number; *: Significantly different from vehicle control group (G1 vs G3) and (G2 vs G4) $p < 0.05$.

study in chlorpromazine-treated rats viz., abnormal posture, absence of tremors and piloerection, decreased resistance to removal from cage and low handling reactivity, soft muscle tone and extensor thrust response, abnormal gait, low arousal level (stupor), reduced body temperature (hypothermia), reduced grip strength and increased landing foot splay are in line with the established results [23]. Overall, chlorpromazine induced its distinctive depressor, neurovegetative and hypothermic effects [24].

5. Conclusion

In conclusion, the known effects of positive controls; caffeine and chlorpromazine HCl on neurobehavioral/ Functional Observational Battery parameters including autonomic, neuromuscular and sensory reactivity tests were detected in the current study. Thus caffeine and chlorpromazine HCl can be used as positive control during neurobehavioral assessment in preclinical studies and also for validation study. The results of this positive control study demonstrate that the Test Facility's Functional Observational Battery test procedures for neurobehavioral, grip strength and motor activity are adequate for the detection of neurotoxic effects of positive controls under the tested conditions. No major inter-personnel variability was observed between study personnel in neurobehavioral observations.

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Conflict of interest

The authors do not have any conflict of interest to disclose

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