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EFFECT OF CAFFEINE SALICYLATE ON CNS STIMULANT ACTIVITY IN RATS

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ABSTRACT

The pharmacological studies on Caffeine salicylate are scanty. The main objective of the experimental work was to evaluate comparatively caffeine salicylate on experimental animal models for its potential CNS stimulant properties in comparison with caffeine as a CNS stimulant. Caffeine salicylate may have good analgesic activity along with that of CNS stimulant activity as data reported analgesia and increased spontaneous activity in modified Irwin test. The catalepsy test demonstrated that the adenosine antagonistic action of caffeine and caffeine salicylate were each able to reverse catalepsy. In forced swim caffeine and caffeine salicylate decreased immobility of rats. The results showed that the shortening of immobility time recorded in the FST was mild with caffeine (36%) and moderate with caffeine salicylate (51%) could be interpreted as a true antidepressant-like effect. In open field test caffeine salicylate and caffeine produced significant increase in the rearing and locomotion (crossings) while significant decrease in the grooming. Blockade of adenosine receptors by caffeine seemed to be the most likely mechanism of CNS stimulation.

INTRODUCTION

The pharmacological studies on Caffeine salicylate are scanty. It was therefore planed to study pharmacological properties using experimental animal models. The main objective of the experimental work was to evaluate comparatively caffeine salicylate on experimental animal models for its potential CNS stimulant properties in comparison with caffeine as a CNS stimulant. Caffeine is the most widely used psychoactive substance in the world. Caffeine When consumed acts as a stimulant influencing the central nervous system. Caffeine is water soluble and passes easily through cell membranes, including the blood brain barrier, and the placenta, allowing it to affect the CNS. The CNS effects of caffeine are mediated mainly by its antagonistic actions on adenosine receptors[1]. Caffeine elevates stress hormones cortisol, epinephrine and norephinepherine. These hormones are responsible for increased heart rate, increased blood pressure and activate the body's fight or flight response. Caffeine also stimulates the hyper secretion of gastric acids. It interferes with the binding of GABA to $GABA_A$ receptors, thus preventing it from performing its calming effects[2].

Salicylic acid in the form of esters was found in several plants, notably in wintergreen leaves and the bark of sweet birch[3]. Salicylic acid is well known anti-inflammatory agent. Salicylic acid alleviates pain, lowers an elevated body temperature and inflammation by inhibiting the synthesis of prostaglandins that occur in inflamed tissues[4]. Salicylic acid is a gastric irritant and because of the serious damage it may cause to the stomach lining, it has not been used orally[5]. Topical use of salicylic acid may induce allergic contact dermatitis, urticaria, angioneurotic oedema, rhinitis, severe and even fatal paroxysmal bronchospasm and occasionally dyspnea[6]. Caffeine salicylate is ester derivative of caffeine containing methylxanthine and salicylate moiety in the basic structure. The introduction of salicylate moiety in the structure of caffeine may enhance the pharmacological activities of caffeine or add other toxic effects. As very scanty reports are available on the pharmacological activities of caffeine salicylate; it is aimed to evaluate additional effectiveness in the parent drug for CNS stimulant properties, using standard experimental animal models of CNS stimulant activities.

METHODS

Experimental animals All the studies reported were conducted at the pharmacology department of Satara College of Pharmacy, Satara following the current standard operating procedures and according to the test guidelines on Safety Studies for Human Pharmaceuticals.

Animals were maintained in animal house of pharmacology department, Satara College of Pharmacy, Satara under standard housing conditions. Animals were selected randomly for the respective experiment, housed in groups of 6 in Makrolon cages (type IV) covered with stainless steel lid, on paddy husk bed, with provisions for water and feed, and examined every working day for their health status, beginning on the day of arrival. Animals were maintained at a room temperature of 20±3 °C, relative humidity of 30–70% with adequate ventilation; room lights were on for approximately 12 h per day. Animals were kept in the cages for at least 7 days prior to dosing for acclimatization to the laboratory conditions prior each experiment. Rats were deprived of food but not water for 12 hours and mice for 3-4 hours prior to the test.

Preparation of doses

All the solutions were prepared in distilled water. 0.1% w/v CMC (Carboxy Methyl Cellulose) was prepared by dissolving 0.1 gm of CMC in 100ml of distilled water. 0.9 gm of NaCl was dissolved in 0.1% w/v CMC solution. 20mg caffeine was dissolved in 5ml of 0.1% w/v CMC. 34mg of caffeine salicylate was dissolved in 5ml of 0.1% w/v CMC. 1ml/200gm dose of all solutions were administered in a single dose by gavage using feeding needle.

Modified Irwin test

Behavioural assessment was performed according to method described by Irwin[7, 8]. Albino Wistar male rats between weight ranges 150-200gm were divided in three groups of six animals each. Group-I normal control was administered saline solution, group-II standard control was administered caffeine (20 mg/kg, p.o.) and group-III test control was administered caffeine salicylate (34mg/kg, p.o.). The animals were placed singly into empty cages without cover. Changes in general behavior were scored for presence/absence about 4 min. Specific attention was given to changes in behavior upon touch. All symptoms observed were documented according to treatment and observation time point.

Catalepsy test

Catalepsy test was performed according to method described by vogel[9, 10]. Albino Wistar male rats were divided in four groups of six animals each. Group-I normal control received saline solution, group-II received haloperidol (4mg/kg, s.c.), group-III received Caffeine (20mg/kg p.o.) and group-IV test control received Caffeine Salicylate (34mg/kg, p.o.). Catalepsy was determined through the use of a standard bar test. The apparatus consisted of a metal bar (0.4 cm

in diameter×25.0 cm long) standing 10.0 cm. high on a platform. Thirty minutes after administration of drug animals were placed on catalepsy bar with fore paws on the bar. The time required to fall the animal from bar was noted. The catalepsy was measured at 30-min intervals for the duration of the drug effect. Catalepsy was characterized in seconds.

Forced swim test

Forced swim test (FST) was performed according to the method described vogel[9, 11, 12]. Albino Wistar male rats were divided in three groups of six animals each. Group-I normal control received saline solution, group-II standard control received caffeine (20mg/kg, p.o.) and group-III test control received caffeine salicylate (34mg/kg, p.o.). Training session was performed for 15 minutes. After training session animals were dried and returned to cage. After 24 hours drug solutions were administered. After 30 minutes of administration of doses rats were dropped individually into water tank (height: 30 cm, diameter: 22.5 cm, depth: 15cm) filled with water (depth: 15 cm). The temperature of water was maintained at 23–25°C. The duration of immobility was recorded during the last 4 min of a 5 minutes observation period. A mouse was judged to be immobile when it remained floating in an upright position and exhibited only small movements to keep its head above the water level or made other passive movements.

Locomotor activity

Locomotor activity was performed according to method described by Vogel[8, 9, 13]. Swiss albino male mice weighing 18-22gm were divided in three groups of six animals each. Group I received saline solution (0.2ml/20gm p.o.). Group II received caffeine (20 mg/kg, p.o.). Group III received caffeine salicylate (34 mg/kg, p.o.). After 30 minutes animals were placed individually in Actophotometer. The traveled distance, resting time and rearing were recorded automatically for 10 min. Counts due to interruption of beam (Horizontal activity) were recorded for 10 minutes. Readings were compared with counts before administration of drug.

Open field test

Open field test was performed according to method described by Vogel[8, 9, 14]. Swiss albino male mice weighing 18-22gm were divided in three groups of six animals each. Group I received saline solution (0.2ml/20gm p.o.). Group II received caffeine (20 mg/kg, p.o.). Group III received caffeine salicylate (34 mg/kg, p.o.). After 30 minutes animals were placed individually in open-field box (68x68x45 cm with 16 equal squares at the bottom). The No. of line crossings, grooming, rearing and defecations were recorded manually for 5 min.

STATISTICAL ANALYSIS

Data obtained from modified Irwin test was analyzed manually. Data recorded from catalepsy test, forced swim test and locomotor activity was subjected to paired t-test and compared with the values observed before administration of drug. The percent change in the activity was subjected to one way analysis of variance (ANOVA) and compared with control followed by post hoc Dunnett's test, $P<0.05$ was considered to be statistically significant. Data was analyzed using Graph Pad software (version 5).

Whereas data obtained from open field test was subjected to one way ANOVA followed by post hoc Dunnett's test and compared with the control group. $P<0.05$ was considered to be statistically significant.

RESULTS

Modified Irwin test

Animals dosed with caffeine exhibited number of signs (Table 1), with stereotypes (increased head movements and sniffing behavior), slight to moderate increased spontaneous activity, increased fear/startle response and increased respiratory rate being the predominant ones. These excitation-related effects were generally dose dependent and they were noted only during the 15-min continuous observation period (immediately after dosing) and at the 15, 30 minutes and 1, 2, 4 and 24 hours post-administration time points. For the sign of increased spontaneous activity, all two dosing groups were observed as having this effect.

In animals treated with caffeine (10mg/kg, p.o.) slight increase in spontaneous activity in two animals at 15 min while at 30 and 60 minutes all the animals showed slight increase in spontaneous activity and at 90 and 120 min after treatment three animals showed slight increase in activity. In animals treated with caffeine (20mg/kg, p.o.) moderate increase in activity was observed in all the six animals at 15 min while at 30, 60 and 90 minutes all the animals showed slight increase in spontaneous activity and at 120 min after treatment three animals showed slight increase in activity. In both groups (10, 20 mg/kg, p.o.) slight increase in fear/startle was observed at 15 minutes.

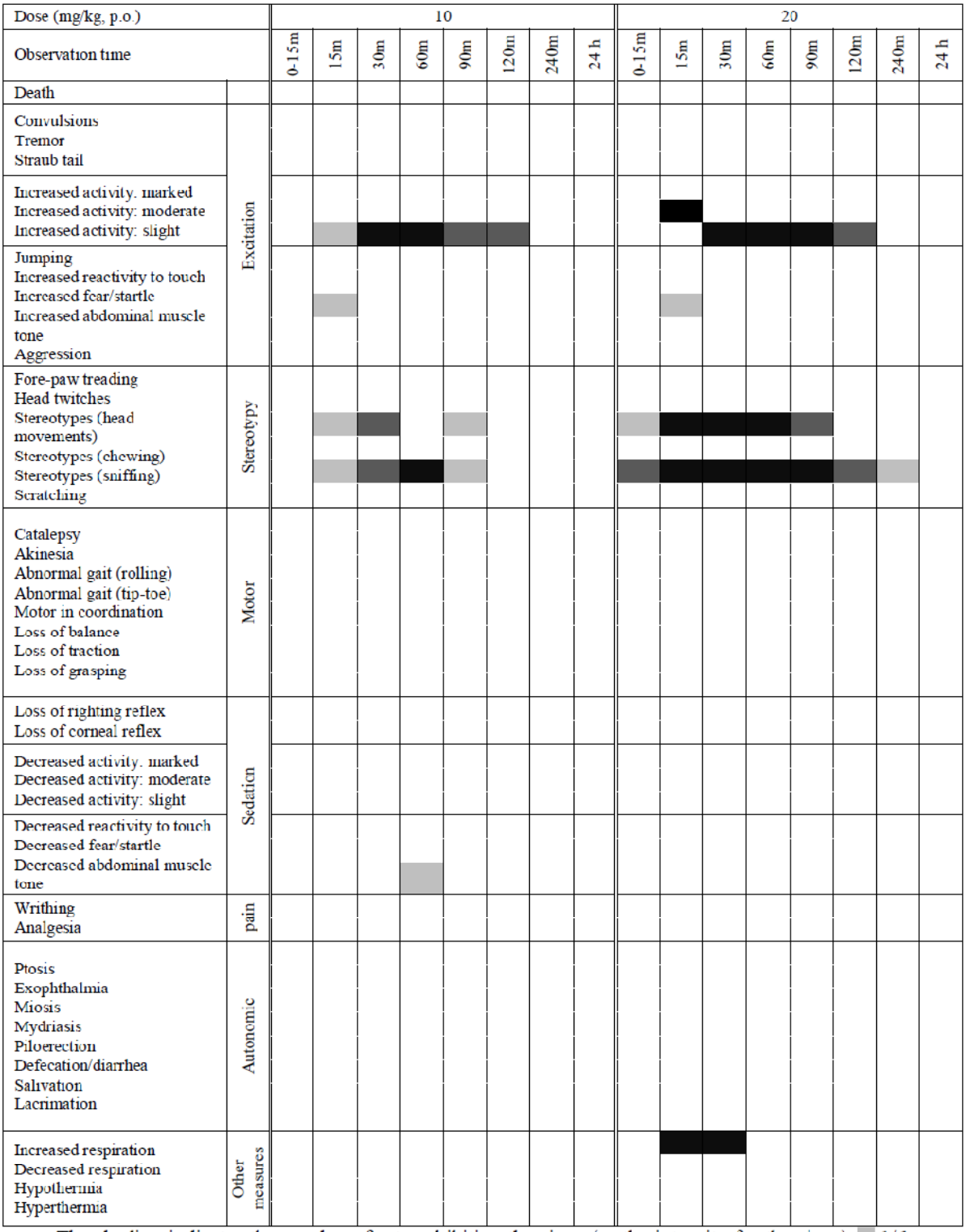
Slight stereotypes head movements were observed at 15 and 90 minutes and moderate at 30 minutes in group treated with 10 mg/kg, p.o. caffeine, while stereotypes head movements were slight at 0-15 min, marked at 30, 60 and 90 minutes and moderate at 120 minutes in the group

treated with 20 mg/kg, p.o. caffeine. Sniffing were observed slight at 15 and 90 min, moderate at 30 min and marked at 60 min in group treated with 10 mg/kg, p.o. caffeine, while in group treated with 20 mg/kg, p.o. caffeine that was moderate at 0-15 minutes and at 120 minutes, marked at 15, 30, 60 and 90 minutes and slight at 240 minutes. Slight decrease in the abdominal muscle tone was observed at 60 minutes in the group treated with 10 mg/kg, p.o. caffeine, while that was not observed in the group treated with 20 mg/kg, p.o. caffeine. Marked increase in respiration was observed at 15 and 30 min in group treated with 20 mg/kg, p.o. caffeine.

Animals dosed with caffeine salicylate also exhibited a number of signs (Table 2), with stereotype increased head movements and sniffing behavior, slight to moderate increased spontaneous activity, increased fear/startle response, increased respiratory rate, and analgesia being the predominant ones. All the responses were dose dependant and predominant as compared to the caffeine. As compare to caffeine the caffeine salicylate showed additional analgesic responses and decreased sedation effect (No change in abdominal muscle tone). Stereotype increased head movements and sniffing behavior, slight to moderate increase in spontaneous activity, increased fear/startle response and increased respiratory rate responses were predominant in caffeine salicylate as compared to caffeine.

In animals treated with caffeine salicylate (17mg/kg, p.o.) slight increase in spontaneous activity in two animals at 15 min while at 30 and 60 minutes all the six animals showed moderate increase in the activity, at 90 min after treatment all the six animals showed slight increase in spontaneous activity and at 120 min three animals showed slight increase in the activity. In animals treated with caffeine salicylate (34 mg/kg, p.o.) moderate increase in the activity was observed in all the six animals at 15 and 30min; while at 60, 90 and 120 minutes all the animals showed slight increase in the activity. In group 17 mg/kg, p. o. caffeine salicylate moderate increase in fear/startle was observed at 15 minutes; while in group treated with 34 mg/kg, p. o. it was observed at 15 and 30 minutes.

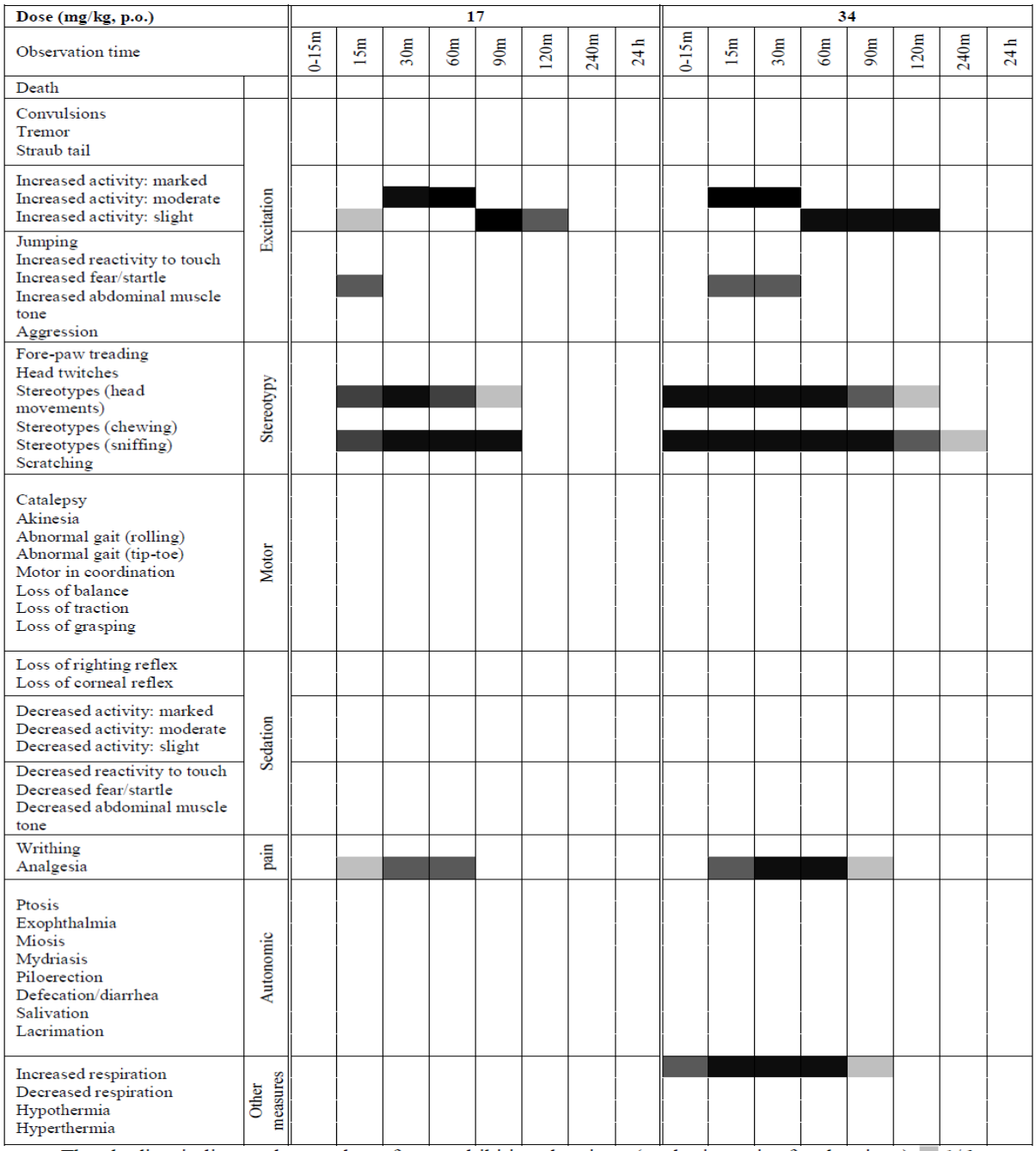
Table 1. Effect of caffeine in modified Irwin test



The shading indicates the number of rats exhibiting the signs (or the intensity for the signs): 1/6 or 2/6 (or slight), 3/6 or 4/6 (or moderate) and 5/6 or 6/6 (or marked).

Moderate stereotype head movements were observed at 15 and 60 minutes, marked at 30 minutes and slight at 90 min in group treated with 17 mg/kg, p.o. caffeine salicylate; while stereotype head movements were marked at 0-15, 15, 30 and 60 min, moderate at 90 minutes and slight at 120 minutes in the group treated with 34mg/kg, p.o. caffeine salicylate.

Table 2. Effect of caffeine salicylate in modified Irwin test



The shading indicates the number of rats exhibiting the signs (or the intensity for the signs): 1/6 or 2/6 (or slight), 3/6 or 4/6 (or moderate) and 5/6 or 6/6 (or marked).

Sniffing were observed moderate at 15 min, marked at 30, 60 and 90 min in group treated with 17 mg/kg, p.o. caffeine salicylate, while in group 34 mg/kg, p.o. caffeine salicylate that was marked at 0-15, 15, 30, 60 and 90 minutes, moderate at 120 minutes and slight at 240 minutes. Moderate increase in the respiration was observed at 0-15 min, marked at 15, 30 and 60 minutes in the group treated with 34 mg/kg, p.o. caffeine salicylate, while that was not observed in the group treated with 17 mg/kg, p.o. caffeine salicylate.

Catalepsy test

Treatment with caffeine produced a significant change (P<0.001) in the duration of haloperidol induced catalepsy in rats. Paired t-test comparisons showed that there is no significant difference in the animals pretreated with haloperidol (2mg/kg, i.p.) and normal saline group (151.83±9.58) stayed on the bar longer than the caffeine group (Table 3) Comparison of the caffeine with the control group revealed a significant reduction (P<0.001) in duration of catalepsy.

Treatment with caffeine salicylate also showed a significant overall reduction in catalepsy (P<0.001). ANOVA comparisons of % catalepsy inhibition revealed that caffeine and caffeine salicylate significantly reduce (P<0.001) catalepsy time.

Table 3. Effect of caffeine and caffeine salicylate on catalepsy in rats

Treatment	Dose	Catalepsy time (Sec)		% Catalepsy inhibition[a]
		Before treatment	After treatment[b]	
Normal saline	10 ml/kg, p.o.	150±9.92	151.83±9.58	1.267±1.328
Caffeine	20 mg/kg, p.o.	163.17±8.74	130.67±5.83***	19.73±0.840***
Caffeine salicylate	34 mg/kg, p.o.	135.67±8.45	92.5±5.97***	31.76±1.779***

Values were expressed as Mean±S.E.M. (n=6).

a. Catalepsy inhibition ***P < 0.001 were compared using one way ANOVA followed by Dunnett's multiple comparison test.

b. Catalepsy time ***P < 0.001 compared to duration of immobility before treatment using paired t-test.

Forced swim test

Caffeine 20 mg/kg, p.o. exhibited significant decrease in the duration of immobility compared to vehicle treatment. One-way ANOVA showed very highly significant difference in the duration of immobility (P<0.001) among the caffeine and caffeine salicylate treatments.

Caffeine salicylate 34 mg/kg, p.o. also showed highly significant decrease in duration of immobility (51.493±2.686, P<0.001) as compared to basal reading (155±9.22) using paired t-test. Caffeine 20mg/kg, p.o. produced very highly significant decrease in duration of immobility (111.66±7.49, P<0.001) when compared with the basal readings (159.17±5.97).

Caffeine Showed (30.224%) decrease in duration of immobility; while that caffeine salicylate showed (51.50%) decrease in duration of immobility as compared to control (1.04%). Both caffeine and caffeine salicylate showed very highly significant results P<0.001 as compared with control by one way ANOVA followed by Dunnett's multiple comparison test.

Table 4 Effect of caffeine and caffeine salicylate on duration of immobility (sec) in rats

Treatment	Dose	Duration of Immobility (Seconds)		% change in Duration of Immobility [a]
		Before treatment	After treatment[b]	
Normal saline	10 ml/kg, p.o.	129.17±10.6	128.33±12.69	1.043±3.202
Caffeine	20 mg/kg, p.o.	159.17±5.97	111.66±7.49***	30.224±2.239***
Caffeine salicylate	34 mg/kg, p.o.	155±9.22	75±5.916***	51.493±2.686***

Values were expressed as Mean±S.E.M, (n=6).

a. Percent changes in duration of immobility were ***$P < 0.001$ compared using one way ANOVA followed by Dunnett's multiple comparison test.
b. Duration of immobility ***$P < 0.001$ compared to duration of immobility before treatment using paired t-test.

Locomotor activity

Treatment with caffeine showed significant increase in the locomotor activity in mice. Caffeine (20mg/kg, p.o) showed very highly significant (P<0.001) increase in locomotor activity when compared with locomotor activity measured prior administration of drug. Percent change in locomotor activity of caffeine was very significantly (25.95%, P<0.01) increased as compared to control group.

Treatment with caffeine salicylate also showed significant increase in the locomotor activity in mice. caffeine salicylate (34mg/kg, p.o.) showed very highly significant (P<0.001) increase in locomotor activity when compared with locomotor activity measured prior administration of drug. The percent change in locomotor activity of caffeine salicylate (34 mg/kg, p.o.) produced very highly significant (36.75%, P<0.001) results when compared with control group. In addition Dunnett's multiple comparison revealed that caffeine salicylate shows significant difference (P<0.05) in the locomotor activity when compared with caffeine.

Table 5. Effect of caffeine and caffeine salicylate on locomotor activity in mice

Treatment	Dose	Locomotor activity in 10 minutes (Counts)		% change in locomotor activity [a]
		Before treatment	After treatment [b]	
Normal saline	10 ml/kg, p.o.	458.00±32.76	458.17±32.53	0.067±0.83
Caffeine	20 mg/kg, p.o.	380.50±20.56	513.83±26.18**	25.95±1.86**
Caffeine salicylate	34 mg/kg, p.o.	315.17±25.86	500±38.99***	36.75±3.20***

Values were expressed as Mean±S.E.M, (n=6).

a. Percent change in locomotor activity $^{**}P < 0.01$; $^{***}P < 0.001$ compared to control using one way ANOVA followed by Dunnett's multiple comparison test.

b. Locomotor activity counts (N) were measured 30 minutes after treatment $^{**}P < 0.01$; $^{***}P < 0.001$ compared to counts before treatment using paired t-test.

Open field test

Caffeine (20 mg/kg, p.o.), produced very significantly (P<0.01) increased exploratory locomotor activity at different time intervals, at 30 and 120 min; while at 60 and 90 min it also produced very highly significant (P<0.001) increase in the locomotor activity. Caffeine also produced significant increase (P<0.05) in percent change in locomotor activity at 30 min, very highly significant increase (P<0.001) in percent change in locomotor activity at 60 and 90 min and very significant (P<0.01) results were observed at 120 min. Caffeine salicylate (34 mg/kg, p.o.) significantly increased exploratory activity at different time intervals, at 30, 60 90 and 120 min. caffeine and caffeine salicylate produced very highly significant (P<0.001) increase in the locomotor activity at 30, 60, 90 and 120 min.

Table 6. Effect of caffeine and caffeine salicylate on rearing in rats

Treatment	Dose	Rearing (N) Mean ± SEM				
		0 Min	30 Min	60 Min	90 Min	120 Min
Normal saline	10 ml/kg, p.o.	152.33±4.8	151.67±8.34	150.50±8.65	150.67±9.08	149.50±4.30
Caffeine	20 mg/kg, p.o.	149.67±3.1	175.50±3.68*	193±4.08***	197.50±5.38***	183.50±2.64**
Caffeine salicylate	34 mg/kg, p.o.	149.5±2.6	191.17±3.07***	211.83±4.20***	226.33±6.61***	193.67±2.20***

Values were expressed as Mean±S.E.M, (n=6). ***P*<0.05; ****P*<0.01; *****P*<0.001 compared to control using one way ANOVA followed by Dunnett's multiple comparison test

Table 7 Effect of caffeine and caffeine salicylate on % rearing

Treatment	Dose	% Increase in rearing Mean ± SEM			
		30 Min	60 Min	90 Min	120 Min
Normal saline	10 ml/kg, p.o.	3.09±3.98	1.19±6.06	1.19±6.16	1.47±3.94
Caffeine	20 mg/kg, p.o.	14.69±1.06*	22.19±2.81***	24.14±0.77***	18.42±1.52**
Caffeine salicylate	34 mg/kg, p.o.	21.78±0.91***	29.30±1.74***	33.75±1.70***	22.76±1.64***

Values were expressed as Mean±S.E.M, (n=6). ***P*<0.05; ****P*<0.01; *****P*<0.001 compared to control using one way ANOVA followed by Dunnett's multiple comparison test

Caffeine (20 mg/kg, p.o.) produced very significantly (P<0.01) decreased grooming time at different time intervals at 30 and 120 min; while at 60 and 90 min it produced very highly significant (P<0.001) decrease in the grooming time. Caffeine also produced highly significant decrease (P<0.01) in percent change in grooming time at 30 min, and very highly significant increase (P<0.001) in percent change in grooming time at 60 and 90 min; highly significant (P<0.01) results were observed at 120 min.

Caffeine salicylate (34 mg/kg, p.o.) produced very highly significantly (P<0.001) decreased grooming time at different time intervals at 30, 60, 90 and 120 min. Caffeine salicylate also produced very highly significant decrease (P<0.001) in percent change in grooming time at 30, 60, 90 and 120 min.

Table 8. Effect of caffeine and caffeine salicylate on grooming time (Sec) in rats

Treatment	Dose	Grooming time (Sec) Mean ± SEM				
		0 Min	30 Min	60 Min	90 Min	120 Min
Normal saline	10 ml/kg, p.o.	147.67±4.8	148.33±8.34	149.5±8.65	149.33±9.08	150.5±4.30
Caffeine	20 mg/kg, p.o.	150.33±3.2	124.5±3.68*	107±4.082***	102.5±5.38***	116.5±2.64**
Caffeine salicylate	34 mg/kg, p.o.	150.5±2.69	108.83±3.07***	88.17±4.21***	73.67±6.61***	106.33±2.20***

Resting time was measured after treatment at different time intervals and values were expressed as mean ± S.E.M. (n=6). ***P*<0.05; ****P*<0.01; *****P*<0.001 compared to control using one way ANOVA followed by Dunnett's multiple comparison test

Table 9. Effect of caffeine and caffeine salicylate on percent grooming time in rats

Treatment	Dose	% Decrease in grooming time Mean ± SEM			
		30 Min	60 Min	90 Min	120 Min
Normal saline	10 ml/kg, p.o.	4.21±4.16	2.68±5.27	2.29±6.01	0.75±4.06
Caffeine	20 mg/kg, p.o.	17.21±1.43**	28.52±3.67***	32.02±2.18***	22.43±1.72***
Caffeine salicylate	34 mg/kg, p.o.	27.71±1.31***	41.36±2.78***	51.18±4.06***	29.22±2.00***

Percent resting time was measured after treatment at different time intervals and values were expressed as mean ± S.E.M. (n=6). ***P*<0.05; ****P*<0.01; *****P*<0.001 compared to control using one way ANOVA followed by Dunnett's multiple comparison test

Caffeine (20 mg/kg, p.o.), produced very significantly (P<0.01) increased number of line crossings at different time intervals at 30 and 120 min; while at 60 and 90 min it produced very highly significant (P<0.001) increase in number of crossings. Caffeine also produced very highly significant increase (P<0.001) in percent change in number of crossings at 30, 60, 90 and 120 min. Caffeine salicylate (34 mg/kg, p.o.), significantly increased number of crossings at different time intervals at 30, 60, 90 and 120 min. Caffeine produced very highly significant (P<0.001) increase in number of crossings. Caffeine salicylate also produced very highly significant increase (P<0.001) in percent change in number of crossings at 30, 60, 90 and 120 min.

Table 10. Effect of caffeine and caffeine salicylate on line crossings (N) in rats

Treatment	Dose	Crossings (N) Mean ± SEM				
		0 Min	30 Min	60 Min	90 Min	120 Min
Normal saline	10 ml/kg, p.o.	50.83±3.8	51.33±2.42	51±3.23	50.167±1.56	50.33±3.04
Caffeine	20 mg/kg, p.o.	50±2.24	71.17±3.83**	83.67±3.04***	92.83±4.8***	71±4.09**
Caffeine salicylate	34 mg/kg, p.o.	49.5±2.72	76.67±4.42***	94.33±3.87***	109.5±7.3***	77.67±3.93***

Number of crossings were measured after treatment at different time intervals and values were expressed as mean ± S.E.M (n=6). ****P*<0.01; *****P*<0.001 compared to control using one way ANOVA followed by Dunnett's multiple comparison test

Table 11. Effect of caffeine and caffeine salicylate on percent line crossings in rats

Treatment	Dose	% Increase in Crossings (N) Mean ± SEM			
		30 Min	60 Min	90 Min	120 Min
Normal saline	10 ml/kg, p.o.	3.84±4.03	8.15±9.32	1.92±5.96	2.64±5.87
Caffeine	20 mg/kg, p.o.	29.09±3.54***	40.07±2.52***	45.92±1.72***	29.07±2.53***
Caffeine salicylate	34 mg/kg, p.o.	35.29±1.49***	47.6±1.55***	54.23±2.76***	36.19±1.7***

Percent crossings were measured after treatment at different time intervals and values were expressed as mean ± S.E.M. (n=6). ***P<0.001 compared to control using one way ANOVA followed by Dunnett's multiple comparison test

DISCUSSION

The results of the behavioral assessment indicated that Caffeine at 10 mg/kg and 20 mg/kg and caffeine salicylate at 17 mg/kg and 34 mg/kg per oral route produced dose dependant stereotype increase in head movements and sniffing behavior, slight to moderate increase in spontaneous activity, increased fear/startle response and increased respiratory rate, additionally caffeine salicylate produced analgesia. Therefore, it may be suggested that caffeine and caffeine salicylate both produced psycho-stimulant action in rats. Caffeine salicylate produced more action than that of caffeine. Caffeine salicylate may have good analgesic activity along with that of CNS stimulant activity as data reported analgesia and increased spontaneous activity in modified Irwin test. The catalepsy test demonstrated that the adenosine antagonistic action of caffeine and caffeine salicylate were each able to reverse catalepsy. Data showed significant reversal of catalepsy by both caffeine and caffeine salicylate. Caffeine salicylate was the most effective at reversing the cataleptic state and restoring behavior to baseline measures. Caffeine at dose 20 mg/kg and caffeine salicylate at the dose 30 mg/kg was able to decrease the time spent on the bar to times similar to baseline measures. The action of caffeine salicylate was higher than that of caffeine. These findings are consistent with many other studies which are finding of A_{2A} antagonism to be an effective method by which it reduces catalepsy[15]. It is believed that this increase in effectiveness is due to action on A_{2A} and D_2 receptor localized on striatopallidal neurons. In addition, it has been found that within the brains of Parkinsonian patients[16]. Therefore, it may be suggested that caffeine and caffeine salicylate both produce reversal of haloperidol induced catalepsy by strengthening dopaminergic neurotransmission in rats. Caffeine

salicylate was more effective than caffeine in reversing catalepsy, which could stand as a hope for the medicinal use of this drug in medicine as Parkinsonian agent. In forced swim caffeine and caffeine salicylate decreased immobility of rats. Caffeine (20 mg/kg) and caffeine salicylate (34 mg/kg) both produced very highly significant anti depressant like action in rats, which may be due to antagonism of adenosine A_{2A} receptors and enhancement due to additional anti-oxidant property of salicylate in caffeine salicylate.

Caffeine and caffeine salicylate are competitive antagonists of adenosine, a nucleoside derived from sequential dephosphorylation of adenine nucleotides (ATP→ADP→AMP→adenosine), which occurs particularly during periods of intense cellular energy expenditure. Thus, when the rate of ATP breakdown surpasses its speed of synthesis, there is an increased production of adenosine, which then diffuses to the extracellular space and stimulates the adenosine receptors in a paracrine or autocrine fashion[17]. The stress experienced by rats during swimming session produces a significant decrease of ATP content in their brains, which presumably increases the extracellular levels of adenosine[17]. The results showed that the shortening of immobility time recorded in the FST was mild with caffeine (36%) and moderate with caffeine salicylate (51%) could be interpreted as a true antidepressant-like effect. Therefore, it is possible that the anti-immobility effect of caffeine and caffeine salicylate in the FST was mediated by enhancement of noradrenergic transmission in the target nuclei. Enhancement in alertness, cortical arousal and dopaminergic neurotransmission may be responsible for strengthening of immobility time in the FST[18]. The effect of caffeine and caffeine salicylate on locomotor activity was determined using actophotometer in mice. The caffeine salicylate and caffeine both showed increased locomotor activity. Caffeine salicylate was having higher activity than that of caffeine. In open field test caffeine salicylate and caffeine produced significant increase in the rearing and locomotion (crossings) while significant decrease in the grooming. Blockade of adenosine receptors by caffeine seemed to be the most likely mechanism of CNS stimulation. Adenosine is an endogenous inhibitory modulator for neuronal excitability and synapse transmission. Adenosine also inhibits the release of most brain excitatory neurotransmitters, particularly DA, and may reduce DA synthesis. Decrease in DA has been linked to central fatigue during exercise. In addition, adenosine have been shown to reduce arousal, induce sleep, and suppress spontaneous activity, which are all behaviors associated with increases in 5-HT[19].

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