Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **28** (3): 465-9 (2009)

Short Communication

Received: March 24, 2009
Accepted: April 2, 2009

Dual Effects of Low and High Dose Caffeine

Siew H. GAN *, Norzihana RAMLI & Norman MUKIAR

Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.

***SUMMARY*. Using a Digiscan Actimeter, many previous studies have shown that low dose caffeine (6.25-25 mg/kg) stimulates locomotion while caffeine in high doses (100 mg/kg) depresses locomotion. This locomotor stimulatory effects induced by caffeine in rodents have been attributed to antagonism of adenosine A1 and A2A receptors while its depressant effects are mediated by the blockade of the A1 receptors. The objective of this study is to further confirm caffeine's dual effects using Porsolt's Forced Swim Tests (FST). This study is also the first to report on mice's active behaviours such as climbing and swimming that can further elucidate caffeine's mode of antidepressive action. Male Swiss albino mice (n = 18) weighing 22-29 g were divided into 3 groups. Each group received either 100 mg/kg of caffeine (Group 1), sodium benzoate vehicle as control (Group 2) or 10 mg/kg of caffeine (Group 3) intraperitoneally. After 30 min of administration, the mice were subjected to a FST and the immobility time was measured. The mice's active behaviours were also scored by an independent observer who is blinded to the treatment group. Animals receiving high dose caffeine (Group 1) illustrated a significantly longer immobility time (reduced by 82%), while the groups receiving low dose caffeine showed shorter immobility time (increased by 19%) when compared to the control group. Group 1 also went into an immobility stage faster (p = 0.036: ANOVA). Animals in Group 3 also showed significantly higher frequencies of swimming and climbing behaviours when compared to the other two groups. Overall, these data support the hypothesis that high dose caffeine promotes a state of "prolonged helplessness". Caffeine shows a dual effect and when it is administered in low dosages, it may be a potential drug to be used and developed as an antidepressant agent. High dose caffeine gives the opposite effect.**

INTRODUCTION

Coffee is a popular beverage in Western societies and it is estimated that about 90% of the Dutch population drinks coffee [1]. It is also widely consumed in the Asian countries. Caffeine (1,3,7-trimethylxanthine) contained in coffee and other foods, beverages as well as over-the-counter medicines [2] have a mild stimulating effect on the central nervous system. This effect has so far been quantified in locomotor activity studies in rodents [3]. Subjective effects reported following the consumption of caffeine include increased energy, alertness and concentration [4]. Many findings have prompted speculation that the behavioural effects of caffeine might be associated with its ability to block adenosine receptors [3] by increasing extracellular levels of acetylcholine and serotonin [5].

Previous researchers have shown that low intraperitoneal doses (i.p.) (6.25-25 mg/kg) of caffeine stimulate locomotion while locomotion is depressed at a higher i.p. dose (100 mg/kg) [6]. The locomotor stimulatory effects induced by caffeine in rodents have been attributed to the antagonism of adenosine A1 and A2A receptors [6] while its depressant effects are mediated by the blockade of the A1 receptors [3,7]. The dual effects of caffeine on locomotion was largely confirmed by using a Digiscan actimeter that monitors the animals' horizontal movements as done by Snyder *et al.* [3]; Yacoubi *et al.* [6] & Powell *et al.* However, not many studies have used the

KEY WORDS: Caffeine, Dual effects, Forced swim test.

* Author to whom correspondence should be addressed. *E-mail:* usmpcrprob@yahoo.com

forced swim tests (FSTs) to investigate caffeine's dual effects.

The FSTs is often used to determine if pharmacological compounds exhibit antidepressant activity [8-10]. Rodents placed in a chamber of water for an extended period of time display a range of behaviours, typically becoming immobile several minutes into the test period. The time of immobility was usually recorded during the last 3 to 4 min of the 6-min testing period.

Recently, a sampling technique was developed to score active behaviours such as swimming and climbing for the FST in addition to immobility with pharmacologically diverse antidepressants showing different behaviours of FST patterns [11-13]. For example, selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, paroxetine and sertraline reduce immobility and increase swimming without affecting climbing. In contrast, selective noradrenaline (NA) reuptake inhibitors such as desipramine, reduces immobility and increase climbing without altering swimming. Finally, drugs with effects on both catecholamines and serotonin (5-HT) can increase both active behaviours simultaneously in certain instances [14]. To our knowledge, active behaviours elucidated with caffeine using the FST have so far, not been investigated to further confirm its dual effects.

This study attempts to further characterize and confirm the dual effects of caffeine using Porsolt's FST in mice. We also hope to elucidate mice's active behaviours such as climbing and swimming to further investigate caffeine's mode of antidepressive effects.

If caffeine is found to have antidepressive effects, it will be very useful because caffeinated drinks and chocolates are widely consumed by human and advise regarding the amounts to be appropriately consumed can be made.

MATERIALS AND METHODS

Animals

Male Swiss albino mice weighing 22-29 g were used after at least one week of habituation in our animal house. Mice were housed in groups of 15 ± 20 in Makrolon cages with free access to water and food and kept in a ventilated room at a temperature of 25 °C, under a 12 h light/12 h dark cycle (light on between 07.00 and 19.00). Experiments were carried out between 09.00 and 18.00.

Experimental procedures were in accordance with protocols approved by the University Sains Malaysia, Kubang Kerian Health Campus Animal Ethics Committee [PPSG/07(A)/044].

Forced swim test (FST) in mice

In this experiment, 18 mice were divided into 3 groups. Each group received either 100 mg/kg of caffeine (Group 1), sodium benzoate vehicle (control group or Group 2) or 10 mg/kg of caffeine (Group 3). The effects of caffeine at different dosages as well as the controlled group were illustrated by using a FST. The high (100 mg/kg) and low (10 mg/kg) doses were chosen based on previous studies as mentioned in the introduction.

Two swimming sessions were conducted: a pre-test followed by a test, 24 h later. The pretest facilitates the development of immobility during the test session and increases the sensitivity for detecting antidepressant behavioral effects [15]. In the pretest session, animals were forced to swim during a 6-min period and removed from the cylinder and gently dried. About 24 h later, each animal was placed in a glass cylinder and exposed to swim conditions for 6 min.

The mice were individually weighed and then administered with the different i.p. doses of caffeine and vehicle. After 30 min, the mice were individually dropped into 10L glass cylinders containing 10 cm of water maintained at room temperature. Three mice were simultaneously tested for a 6-min period which was videotaped for subsequent analysis of behavioural responses. A non-transparent screen placed between the cylinders prevented mice from seeing each other.

Climbing was defined as vigorous forepaws movements directed towards the wall of the cylinder. Swimming was defined as movement throughout the swim chamber, which included crossing into another quadrant [13]. Immobility time was defined as floating passively in an upright position, making only the necessary movements to keep its head above water. Onset of immobility time is defined as the time when the first incidence of immobility is observed. The total duration of swimming, climbing, onset of immobility time, immobility time (measured during the last 3 min of the 6 min session) and percentage change of immobility time when compared to the control group was measured using a stopwatch by an observer who was blinded to the drug treatment.

Each mouse was used only once for each swimming session after which they will be returned to the animal house for termination.

Chemicals and reagents

Caffeine (1,3,7-trimethylxanthine) was pur-

chased from Sigma (U.S.A). Caffeine at 10 and 100 mg/kg respectively, were dissolved in an aqueous solution of sodium benzoate (10 mg/ml) purchased from Biolabs UK limited, London. The solutions of drugs were prepared fresh daily and injected i.p. in a volume of 10 ml/kg.

Statistics

Results are expressed as means± standard deviation. Differences between means were analysed by ANOVA followed by Bonferoni post-hoc tests. Significance levels were set at $p<0.05$.

RESULTS

There was no significant difference in the body weights of the mice from the 3 groups.

Onset and Duration of Immobility

Animals treated with high dose caffeine (Group 1) went into an early stage of helplessness at 16.67 ± 14.15 s compared to animals in Group 2 (56.23 ± 47.23 s) and Group 3 (130.67 ± 50.20) (Fig. 1). Their duration of immobility was also significantly longer (128.7 ± 46.6 sec) when compared to animals in Group 2 (104.3 ± 24.0 sec) and Group 3 (22.3 ± 18.5 sec) respectively (Fig. 2). This means that animals in Group 3 had an 82% reduction in immobility time as compared to animals receiving high dose caffeine (an increment of immobility time by 19%).

Swimming and Climbing behaviours

It was observed that mice from Group 3 vigorously tried to escape from the chamber by actively climbing and swimming when compared

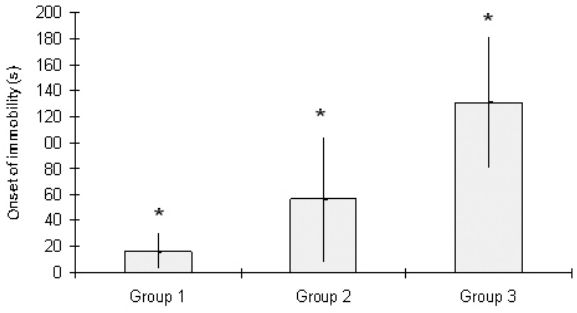

Figure 1. Onset of immobility where Group 1 = animals receiving low dose (10 mg/kg) caffeine, Group 2 = animals receiving sodium benzoate (control group) and Group 3 = animals receiving high dose caffeine (100 mg/kg). *p = 0.036 (ANOVA); n = 6 animals/Group.

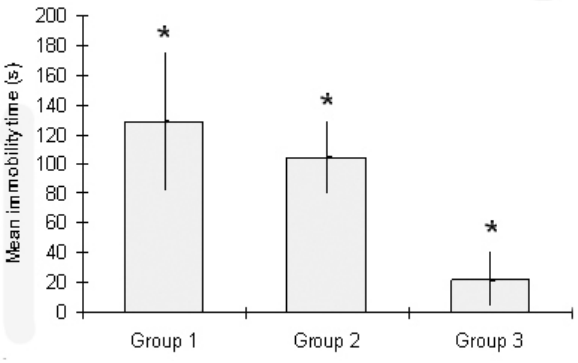

Figure 2. Duration of immobility where Group 1 = animals receiving low dose (10 mg/kg) caffeine, Group 2 =animals receiving sodium benzoate (control group) and Group 3 = animals receiving high dose caffeine (100 mg/kg). *p = 0.015 (ANOVA); n = 6 animals/Group.

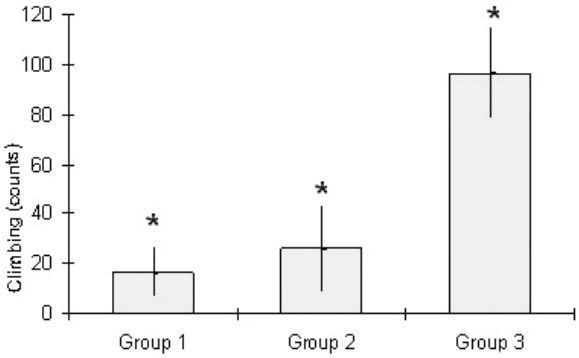

Figure 3. Frequency of climbing where Group 1 = animals receiving low dose (10 mg/kg) caffeine, Group 2 = animals receiving sodium benzoate (control group) and Group 3 = animals receiving high dose caffeine (100 mg/kg). *p = 0.001 (ANOVA); n = 6 animals/Group.

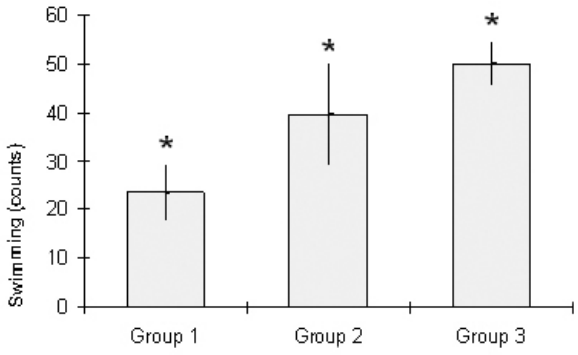

Figure 4. Frequency of swimming where Group 1 = animals receiving low dose (10 mg/kg) caffeine, Group 2 = animals receiving sodium benzoate (control group) and Group 3 = animals receiving high dose caffeine (100 mg/kg). p = 0.012 (ANOVA); n = 6 animals/Group.

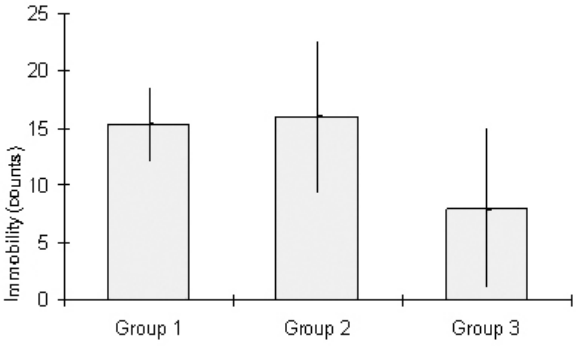

Figure 5. Frequency of immobility where Group 1 = animals receiving low dose (10 mg/kg) caffeine, Group 2 = animals receiving sodium benzoate (control group) and Group 3 = animals receiving high dose caffeine (100 mg/kg); n = 6 animals/Group.

to the other 2 groups. The mean frequency of climbing was 16.7 ± 10.1, 26.3 ± 17.0 and 96.3 ± 17.9 for animals in Group 1, Group 2 and Group 3 respectively (Fig. 3). For swimming, the mean frequency was 23.7 ± 5.7, 39.7 ± 10.3 and 50.0 ± 4.0 for animals in Group 1, Group 2 and Group 3 respectively (Fig. 4). Both climbing and swimming activities were more frequently observed in the first 3 min of the test session. The frequency of immobility however, was not significantly different when compared between the three groups (Fig. 5).

DISCUSSION

Our results confirmed that depending on its dose, caffeine produced dual effects. High dose caffeine was clearly found to promote a state of "prolonged helplessness" where animals went into an early depressive state. On the other hand, it was observed that animals receiving low dose caffeine, actively attempted to swim and climb out of the chamber. Therefore, low dose caffeine may increase alertness and stamina by increasing locomotor activity. This increased in locomotor activity however, could possibly contribute to reducing the period of immobility in animals receiving low dose caffeine.

The involvement of adenosine in regulating complex central functions, such as anxiety states [6,16] has been investigated in the past. Caffeine has important effects on alertness, and there is no doubt that caffeine is widely consumed by subjects who need to stay awake [17]. Numerous findings have also prompted speculation that the behavioural effects of caffeine might be associated with its ability to block adenosine receptors and one of them was as that reported by Synder *et al.* [3].

The active behaviours illustrated during FST helps to further elucidate caffeine's mode of dual effects. It has been shown that SSRIs such as fluoxetine, paroxetine and sertraline reduce immobility and increase swimming without affecting climbing [13]. In contrast, selective NA reuptake inhibitors such as desipramine or maprotiline, reduce immobility and increase climbing without altering swimming. Finally, drugs with effects on both catecholamines and serotonin can increase both active behaviours simultaneously in certain instances [14]. Because low dose caffeine increases both climbing and swimming behaviours, we conclude that it may affect both catecholamines and serotonin levels as shown by Rénéric & Lucki [14]. The increase in acetylcholine and serotonin levels will result in blockade of adenosine receptors. For high dose caffeine, Nikodijevic *et al.* [18] reported that the activation of different adenosine receptor subtypes can synergistically contribute to locomotor suppression.

The drawback of this experiment however is that even though a single low dose caffeine is found to be sufficient to decrease immobility time and swimming and climbing behaviours, chronic treatment of antidepressants are usually required for a clinical response. Detke *et al.* [19] reported that low doses of chronic desipramine and fluoxetine in otherwise untreated rats reduced immobility time. It would be interesting to confirm the antidepressant effects in mice treated with low dose caffeine administered chronically or compared with a standard antidepressant agent. The mechanism of action of low dose caffeine should also be confirmed by using adenosine A2A receptor knockout mice.

CONCLUSION

Low dose caffeine which increases both climbing and swimming behaviours, may affect both catecholamines and serotonin levels. Our data support the hypothesis that caffeine has a dual effect where at low dose (10 mg/kg) it is an antidepressant and at high dose (100 mg/kg), it shows a depressant effect. Caffeine should therefore be consumed by humans only in low amounts to increase alertness.

Acknowledgement. The authors are most grateful to Universiti Sains Malaysia's *Rakan Sains Programme* for providing the animals for the study..

REFERENCES

1. Hameleers, P.A.H.M., M.P.J. Van Boxtel, E. Hogervorst, W.J. Riedel, P.J. Houx, F. Buntinx & J. Jolles (2000) *Hum. Psychopharmacol.* **15**: 573–81.
2. Nehlig, A., J.L. Daval & G. Debry (1992) *Brain Res.Rev.* 17: 139–70.
3. Snyder, S.H., J.J. Katims, Z. Annau, R.F. Bruns & J.W. Daly (1981) *Proc. Natl. Acad. Sci. USA* **78**: 3260-4.
4. Griffiths, R.R. &, P.P. Woodson (1988) *J. Pharmacol. Exp. Ther.* **246**: 21-9.
5. van Boxtel, M.P.J., J.A.J. Schmitt, H. Bosma & J. Jolles (2003) *Pharm. Biochem. Behav.* **75**: 921-7.
6. E.L. Yacoubi M., C. Ledent, J.F. Meanard, M. Parmentier, J. Costentin & J.M. Vaugeios (2000) *Br. J. Pharmacol.* **129**: 1465-73.
7. Ledent, C., J.M. Vaugeois, S.N. Schiffmann, T.E.L. Pedrazzini, M. Yacoubi, J.J. Vanderhaeghen, J. Costentin, J. K. Heath, G. Vassart & M. Parmentier (1997) *Nature* **388**: 674-8.
8. Powell, K.R., P.M. luvone & S.G. Holtzman (2001) *Pharmacol. Biochem. Behav.* **69**: 59-70.
9. Porsolt, R.D., M. Le Pichon & M. Jalfre (1977) *Nature* **266**: 730-2.
10. Porsolt, R.D., G. Anton, N. Blavet & M. Jalfre (1978) *Eur. J. Pharmacol.* **47**:379-91.
11. Detke, M.J., M. Rickeis & I. Lucki (1995) *Psychopharmacol.* **121**: 66-72.
12. Lucki, I. (1997) *Behav. Pharmacol.* **8**: 523-32.
13. Page, M.E., M.J. Detke, A. Dalvi, L.G. Kirby & I. Lucki (1999) *Psychopharmacol.* **147**: 162-7.
14. Reneric, J.P. & I. Lucki 1998 *Psychopharmacol.* **136**: 190-7.
15. Borsini, F., A. Lecci, A. Sessarego, R. Frassine & A. Meli (1989) *Psychopharmacol.* **97**: 183-8.
16. Jain, N., N. Kemp, O. Adeyemo, P. Buchanan & T.W. Stone (1995) *Br. J. Pharmacol.* **116**: 2127-33.
17. Fredholm, B.B. & M.K. Lindstrom (1999) *Eur. J. Pharmacol.* **380**: 197-202.
18. Nikodijevic, O., R. Sarges, J.W. Daly & K.A. Jacobson (1999) *J. Pharmacol. Exp. Ther.* **259**: 289-94.
19. Detke, M. J., J. Johnson & I. Lucki (1997) *Exp. Clin. Psychopharmacol.* **5**: 107-12.