

# An Acute, Double-Blind, Placebo-Controlled Cross-over Study of 320 mg and 640 mg Doses of *Bacopa monnieri* (CDRI 08) on Multitasking Stress Reactivity and Mood

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Little research exists in humans concerning the anxiolytic, antidepressant, sedative, and adaptogenic actions the traditional Ayurvedic medicine *Bacopa monnieri* (BM) possesses in addition to its documented cognitive-enhancing effects. Preclinical work has identified a number of acute anxiolytic, nootropic, and adaptogenic effects of BM that may also co-occur in humans. The current double-blind, placebo-controlled cross-over study assessed the acute effects of a specific extract of BM (KeenMind<sup>®</sup> - CDRI 08) in normal healthy participants during completion of a multitasking framework (MTF). Seventeen healthy volunteers completed the MTF, at baseline, then 1 h and 2 h after consuming a placebo, 320 mg BM and 640 mg of BM. Treatments were separated by a 7-day washout with order determined by Latin Square. Outcome measures included cognitive outcomes from the MTF, with mood and salivary cortisol measured before and after each completion of the MTF. Change from baseline scores indicated positive cognitive effects, notably at both 1 h post and 2 h post BM consumption on the Letter Search and Stroop tasks, suggesting an earlier nootropic effect of BM than previously investigated. There were also some positive mood effects and reduction in cortisol levels, pointing to a physiological mechanism for stress reduction associated with BM consumption. It was concluded that acute BM supplementation produced some adaptogenic and nootropic effects that need to be replicated in a larger sample and in isolation from stressful cognitive tests in order to quantify the magnitude of these effects. The study was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12612000834853). Copyright © 2013 John Wiley & Sons, Ltd.

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The Ayurvedic medicine, *Bacopa monnieri* (L.) Wettst. (syn. *Bacopa monniera*) has been used for thousands of years to treat a wide variety of mental ailments (Russo and Borrelli, 2005). Recently, standardized extracts of *Bacopa Monnieri* (BM) have been developed and have been subjected to randomized placebo-controlled trials to evaluate the reported cognitive-enhancing properties of the herb (Pase *et al.*, 2012). Growing evidence suggests that standardized extracts of BM do promote cognitive enhancement in older (>54 years) cohorts (Barbhaiya *et al.*, 2008; Calabrese *et al.*, 2008; Morgan and Stevens, 2010) and healthy adults (18–60 years) over chronic (3–4 months) administration periods (Stough *et al.*, 2008; Stough *et al.*, 2001a). In addition to these cognitive-enhancing properties, BM has also been reported to possess anxiolytic, antidepressant, sedative, and adaptogenic actions (Russo and Borrelli, 2005) which may co-occur with or facilitate the reported cognitive enhancement associated with BM consumption.

Preclinical work originally established BM's potential as a cognitive enhancer and mood modulator with *in vivo* and *in vitro* studies indicating BM has adaptogenic (Rai *et al.*, 2003), antioxidant (Shinomol *et al.*, 2012), cholinergic (Uabundit *et al.*, 2010), metal-chelating (Dhanasekaran *et al.*, 2007), vasorelaxant (Dar and Channa, 1999), and anti-inflammatory properties (Channa *et al.*, 2006). Emergent evidence from animal studies suggests that BM may acutely improve cognition, particularly when participants are engaged in cognitively challenging or stressful paradigms (Andrade and Chandra, 2006b; Saraf *et al.*, 2008). BM has been observed to improve or restore learning and memory within a wide range of animal paradigms including scopolamine, electroshock, and immobilization stress-induced retrograde amnesia models (Singh and Dhawan, 1997). BM supplementation has also produced neuroprotection and restoration of cognitive performance in animal models of induced cognitive deficits by *N*<sub>ω</sub>-nitro-L-arginine (Anand *et al.*, 2010; Saraf *et al.*, 2009), diazepam (Prabhakar *et al.*, 2008; Saraf *et al.*, 2008), hypobaric hypoxia (Hota *et al.*, 2009), and electroconvulsive shock (Andrade and Chandra, 2006a). These types of animal models also increase anxiety-like behaviour and stress-related biological indices in animals. For instance the

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scopolamine and intermittent hypoxia models have been reported to produce anxious behaviour in rodents (Duarte *et al.*, 2010; Klinkenberg and Blokland, 2010), and increase production of stress hormones such as corticosterone (Zoccal *et al.*, 2007). Given BM treatment has also been reported to reduce anxiety in mice (Russo and Borrelli, 2005) and illustrated adaptogenic (Rai *et al.*, 2003) and corticosterone-reducing properties in rat models (Sheikh *et al.*, 2007), these anxiogenic and stress-moderating effects need to be considered as possible mediators of the treatment-related effects of BM in both preclinical and future human trials.

The steroidal saponins, Bacoside A, and Bacoside B (Deepak and Amit, 2004) are considered to be the primary psychoactive constituents of BM and have various mechanisms of action upon the central nervous system including antioxidant activity (Dhanasekaran *et al.*, 2007; Russo and Borrelli, 2005) and enhancement of kinase activity, restoration of synaptic activity, and ultimately nerve impulse transmission (Anbarasi *et al.*, 2005; Rai *et al.*, 2003; Saraf *et al.*, 2008). Of specific relevance to this study are the anxiolytic and adaptogenic properties of BM, given the potential relationship between these effects inferred from preclinical studies demonstrating acute improvement in cognitive performance under laboratory-induced stressful conditions (Andrade and Chandra, 2006b; Saraf *et al.*, 2008).

To date, limited research exists concerning the acute effects of BM consumption with humans. The first study to assess the potential of an acute nootropic effect of BM in humans observed no acute effect on cognitive performance 2 h post-treatment consumption (Nathan *et al.*, 2001). No assessment of mood, anxiety, or any biological measures related to the possible adaptogenic properties of BM was collected from the participants limiting any speculation concerning whether BM (at the dose administered) produced any anxiolytic effect independent of the lack of nootropic effect. Recently, a second study (Downey *et al.*, 2012) examined the acute effect (again, at 2 h post-treatment) of two doses (320 mg and 640 mg) of a specific extract of BM (KeenMind<sup>®</sup>: CDRI 08) upon performance on a series of mentally effortful cognitive tasks, cardiovascular parameters, and mood. Change from baseline scores indicated that the 320 mg dose of BM improved performance at the first, second, and fourth repetition (coinciding with 120–160 min) post-dosing of a mentally effortful cognitive battery. Although no treatment effect was observed upon cardiovascular activity or in attenuating task-induced ratings of stress and fatigue. It was concluded that assessment of an earlier pharmacological window may be appropriate given the nootropic effect was evident in the earlier cognitive assessments. This finding is consistent with extant research that has demonstrated that chronic consumption of standardized extracts of BM can improve components of human cognition (Calabrese *et al.*, 2008; Morgan and Stevens, 2010; Stough *et al.*, 2008; Stough *et al.*, 2001a; Stough *et al.*, 2012) including speed of information processing, decision-making time (Stough *et al.*, 2008; Stough *et al.*, 2001a), and aspects of attention/freedom of distractibility (Barbhaiya *et al.*, 2008; Calabrese *et al.*, 2008) after chronic supplementation.

In addition to the cognitive enhancement detected in chronic supplementation studies, reductions in trait anxiety as a result of chronic BM supplementation have

also been observed (Calabrese *et al.*, 2008; Stough *et al.*, 2001a). Whilst an anxiolytic finding has not been observed or assessed consistently in chronic supplementation studies, evidence from preclinical studies support the notion that BM possesses anxiolytic and adaptogenic properties and that these properties may be more evident in stressful or cognitively challenging paradigms (Andrade and Chandra, 2006b; Saraf *et al.*, 2008). Biological assessment of BM treatment-related and task-induced modulation of mood in acute settings has only been recently undertaken in humans, with BM treatment having no effect upon cardiovascular activity 2 h post-treatment (Downey *et al.*, 2012). Given the wide-ranging biological effects of BM observed in preclinical studies (Russo and Borrelli, 2005), the pharmacological window used, and possible insensitivity of the cardiovascular assessments used in a relatively young and healthy population, the lack of a detectable change in cardiovascular functioning specific to BM consumption is not surprising.

Studies utilizing cross-over designs have previously assessed modulation of cognitive performance, stress reactivity (assessed via saliva cortisol levels and self-rated affective state), and mood by purportedly nootropic, anxiolytic and adaptogenic substances (Hellhammer *et al.*, 2004; Kennedy *et al.*, 2006a; Kennedy *et al.*, 2006b; McMorris *et al.*, 2006; Scholey *et al.*, 2009). For example, the effects of controlled administration of phosphatidylserine complex has been shown to attenuate the cortisol response to laboratory-induced stress (using the Trier Social Stressor Test), particularly with a 400 mg dose, and also produced positive self-rated emotional response to the same treatment (Hellhammer *et al.*, 2004). Similarly, using a computerized multitasking framework (MTF) designed to evoke stress and additionally collect cognitive performance metrics, the effect of chewing gum on alleviating stress and negative mood associated with completion of the stressful cognitive task battery has recently been examined (Scholey *et al.*, 2009). Chewing gum throughout completion of the MTF was associated with significant improvements in levels of alertness and cognitive task performance and reductions in state anxiety, stress, and levels of salivary cortisol. The efficacy of the phosphatidylserine was attributed to a selective stress dampening effect on the pituitary–adrenal axis (Hellhammer *et al.*, 2004), and although the mechanism by which chewing gum was efficacious in alleviating task-induced stress and cortisol production is less clear, the ability to experimentally induce the stress-cortisol response and collect mood and cognitive data concurrently provides an ideal platform to assess the acute effects of various doses of interventions such as BM.

The above evidence suggests the effects of nootropics on responses to stress can be dissociated from their effect upon behaviour. Given the established evidence of a facilitative cognitive and anxiolytic effect due to chronic BM consumption in humans, and preclinical evidence for cognitive, adaptogenic, and anxiolytic effects within acutely stressful paradigms, the aim of the current study was to ascertain whether a standard clinical dose of 320 mg or a 640 mg dose of a specific extract of BM (KeenMind<sup>®</sup> - CDRI 08) would acutely effect cognition, mood, anxiety, and stress at an earlier time-point than previous acute BM supplementation studies. The present study therefore aimed to test the hypotheses

that compared to a placebo; BM would attenuate task-induced negative mood change, result in reduced cortisol levels, and improve cognitive task performance.

## METHOD

**Participants.** Participants included 17 healthy volunteers comprising of four males and 13 females aged between 18 and 44 years ( $M = 25.23$   $SD = 5.97$ ). Participants were restricted from taking part based on several self-report exclusion criteria which included the following: smoker; any history of psychiatric disorders or neurological diseases; suffering from endocrine, gastrointestinal, or bleeding disorders; individuals with chronic illness and infection; pregnant or lactating. Any individuals taking any over-the-counter or prescription medications or herbal extracts were also excluded from participation. On the day of testing, participants were required to consume only a light breakfast while abstaining from alcohol and caffeine. The study was approved by the Swinburne University Human Research Ethics Committee and was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12612000834853).

**Treatment and study design.** A double-blind, placebo-controlled, cross-over design was employed for this study. On each testing day, participants received a combination of four capsules corresponding to inert placebo, 320 mg of KeenMind<sup>®</sup> (CDRI 08) BM or 640 mg of KeenMind<sup>®</sup> (CDRI 08) BM. KeenMind<sup>®</sup> (CDRI 08) is standardized for no less than 55% of total bacosides. Each BM capsule contained 160 mg BM extract (25:1) equivalent to 4 g of dried herb. The extract of KeenMind<sup>®</sup> (CDRI 08) BM was prepared from stems, leaves, and roots of a cultured variety of BM collected from West Bengal and extracted with 50% ethanol. The placebo capsule was identical in shape, smell, taste, and weight and was supplied in the form of 160 mg capsules (made up of inert plant-based materials) per participant per testing day. Allocation to a treatment order was performed using a computer-generated randomization program that allocated each participant to a cell of a William's Latin Square which balanced the order of the three treatment conditions across visits and participants.

**Cognitive assessment.** The MTF (Purple Research Solutions, UK) is a platform for the presentation of performance-driven, cognitively demanding tasks (Wetherell and Sidgreaves, 2005). The test requires attention to be given to four tasks presented simultaneously, each on one of four quadrants on a computer screen, whilst monitoring the central counter displaying the score, which is dictated by the accuracy and speed of the response. Each task was set to the 'medium' difficulty level, and the test was performed over 20 min. The tasks included (clockwise from top left) Mental Arithmetic, Stroop, Letter Search and Visual Tracking (Scholey *et al.*, 2009).

**Mental Arithmetic:** This task requires participants to add a series of numbers. Using a number pad on the computer screen, participants used the computer mouse to click the numbers that gave the desired answer. Participants were awarded 10 points for a correct answer or deducted 10 points for an incorrect answer.

**Stroop:** A series of colour names (red, blue, yellow, and green) were presented in differing font colours (red, blue, yellow, and green), and the participants were required to respond to the colour of the font by using their computer mouse to click on one of the four available answers. Participants were awarded 10 points for a correct answer or deducted 10 points for an incorrect answer and for not selecting a response within the allocated time frame.

**Letter Search:** Four letters were presented during the initial four seconds of testing. The letters then disappeared, but could be reviewed at any time by clicking on a 'retrieve list' button. At 10-s intervals, a single target letter appeared and the participant indicated whether or not the letter belonged to the original four letters by clicking on a 'yes' or a 'no' button. Participants were awarded 10 points for a correct answer, deducted 10 points for an incorrect answer or no response, and deducted five points for each time they retrieved the letter list.

**Visual Tracking:** A dot progressively moved outwards from the centre of the task through five concentric circles. Participants were asked to allow the dot to travel as far from the centre as possible without letting it touch the edge of the outermost circle before clicking on a 'reset' button. Two points were awarded per circle that the dot had passed through, with a maximum of 10 points. Participants were deducted 10 points per half second that the dot touched the outer edge before resetting the task. The MTF has been shown to increase negative mood and anxiety (Scholey *et al.*, 2009) and induce a stress-related physiological response (Wetherell and Sidgreaves, 2005).

**Mood measures.** The Bond-Lader VAS (Bond and Lader, 1974) and the State Trait Anxiety Inventory (STAI; Spielberger *et al.*, 1970) were used to evaluate mood. The Bond-Lader VAS consists of 16 visual analogue scales with the end points anchored by antonyms reflecting three key mood factors: 'alert' (alert-drowsy, attentive-dreamy, lethargic-energetic, muzzy-clear-headed, coordinated-clumsy, mentally slow-quick witted, strong-feeble, interested-bored, incompetent-proficient), 'calm' (calm-excited, tense-relaxed), and 'content' (contented-discontented, troubled-tranquil, happy-sad, antagonistic-friendly, withdrawn-sociable). Scores for each item represent the number of millimeters, ranging from 0 to 100, from the negative antonym. Item scores were summed and averaged to create total scores for each respective factor. Factor scores had a potential range between 0 and 100, with 100 representing highly alert, calm, or content. A pen and paper version was used, and participants were asked to mark each line between the antonyms indicating how they felt at the present time. As with many mood visual analogue scales, high reliability and validity have been demonstrated (Ahearn, 1997).



The state anxiety subscale (STAI-S) of the STAI (Spielberger *et al.*, 1970) was utilized to assess participants' state level anxiety before and after completion of the MTF. The STAI-S consists of 20 items measured on a four-point Likert scale with 1 = 'not at all' and 4 = 'very much so'. After reversing certain items as appropriate, the scores were summed to give the overall score, higher scores reflected greater state anxiety. The trait anxiety subscale (STAI-T) of the STAI (Spielberger *et al.*, 1970) was utilized in the practice session to assess participants' trait anxiety level. The STAI-T consists of 20 items on a four-point Likert scale with 1 = 'almost never' and 4 = 'almost always', with higher scores reflecting greater levels of trait anxiety.

**Biological measure.** The participants provided salivary samples using salivettes (Sarstedt, UK) as a non-invasive measure of free, bio-available cortisol levels. Participants were required to place a cotton dental roll in their mouth for approximately 30 s; these were immediately frozen at  $-20^{\circ}\text{C}$ . Samples were defrosted and centrifuged prior to testing for cortisol levels by luminescence immunoassay according to the instructions of the manufacturers (IBL Hamburg, Flughafenstrasse 52a, D-22335 Hamburg, Germany).

**Procedure.** Each participant was required to attend a total of four sessions (one practice visit and three study visits) that were conducted one week apart to ensure sufficient wash out between each acute condition. Participants were asked to consume a light breakfast (e.g. one standard serve of cereal or two pieces of toast at home on each testing day) before arriving at the testing location. Testing took place in a suite of dedicated university laboratories at the Swinburne Centre for Human Psychopharmacology. Prior to the first study visit, participants initially underwent a practice session where the STAI, Bond Lader VAS and  $2 \times 10$  min. Multitasking sessions were completed in order to familiarize them with the testing procedure and reduce the possibility of practice effects. Each participant provided a saliva sample for cortisol testing, completed the Bond-Lader VAS and the STAI-S, respectively, before and after undergoing 20 min of the MTF. Each participant's pre-MTF scores for alert, calm, contented, state anxiety, and cortisol scores were subtracted from the corresponding post-MTF score to provide the change in mood and anxiety induced by completion of the MTF. Participants repeated the testing procedure 1 h and 2 h post-administration of the day's treatment. The same testing sequence was carried out in all three study visits.

**Data treatment and statistics.** To examine the first aim of the current study, the effects of condition and time on MTF task performance and to investigate the hypothesis that the 320 mg *Bacopa monniera* condition would not have a significant enhancing effect on cognition; the analysis was carried out using change scores. These scores were calculated by finding the change from the pre-dose baseline to the one and 2-h post-dose testing sessions for each task and the total MTF score (e.g. 1 h post-treatment Stroop score minus the baseline Stroop score). Two-way repeated-measures ANOVAs employing condition (placebo, 320 mg, and 640 mg) and time (change score for baseline to 1 h, change score for baseline to 2 h) were conducted for the total MTF score and for Maths, Stroop, Letter search and tracking

scores. Post-hoc comparisons using paired-samples *t*-tests were conducted for further analyses where appropriate. To establish the effects of condition and time on mood, state anxiety, and stress, analyses were run in the absence and in the presence of the stressor. In order to establish the effects in the absence of the stressor, the change from the baseline scores to the 1 h and 2 h post-treatment pre-stressor scores were calculated. A series of two-way repeated measures ANOVA with condition (placebo, 320 mg, 640 mg) and time (baseline to 1 h change, baseline to 2 h change) were employed. Further analyses were performed using paired-samples *t*-tests.

To determine the effects of the conditions on the stress-related change in the mood, anxiety, and cortisol measurements caused by the MTF, a change score was calculated for each of the variables at each session time by subtracting the post-MTF score from the pre-MTF score. The change from baseline scores at 1 and 2 h post-dosage were then calculated by subtracting the pre-dose change from the 1 h and the 2 h post-dosage change scores. These delta scores were analysed for each factor by employing a two-way repeated measures ANOVA incorporating factors of Time (1 h, 2 h)  $\times$  Condition (placebo, 320 mg, 640 mg).

## RESULTS

No adverse effects were reported throughout the study for any of the three treatments. Prior to examination of the cortisol, mood, and cognitive testing results, all data were examined with regard to gender and treatment order effects, with no significant pattern of results emerging. Baseline performance scores (mean and standard error) for the MTF and difference from baseline scores for each outcome measure and treatment are summarized in Table 1 below with regard to the two testing time-points post-dosing. Significant time, treatment, and time  $\times$  treatment effects are reported in the text below; other than trends ( $.1 > p > .05$ ) non-significant effects are not reported for the sake of brevity.

### MTF

Performance on the MTF overall score was not affected differentially by any of the three treatments. Analysis of the Stroop results revealed a trend for an interaction effect between time and condition [ $F(2,30) = 2.91$ ,  $p = .070$ ]. A series of paired-samples *t*-tests revealed no significant condition differences, although, the change in scores from baseline to 1 h post-treatment was considerably greater in the 640 mg condition compared to the placebo condition [ $t(15) = 1.76$ ,  $p = .099$ ]. In the 640 mg condition, the 1 h post-treatment scores were significantly higher than the baseline scores [ $t(16) = 3.92$ ,  $p = .001$ ]. The 2 h post-treatment scores were also significantly higher than the baseline scores [ $t(16) = 3.48$ ,  $p = .003$ ]. In the 320 mg condition, there was a significant increase in scores from baseline to 1 h post-treatment [ $t(16) = 2.41$ ,  $p = .028$ ]. In the placebo condition, the score significantly increased from baseline to 2 h post-treatment [ $t(16) = -4.57$ ,  $p = .000$ ]. Analysis of the letter search scores revealed a significant main effect of time [ $F(1,16) = 5.44$ ,  $p = .033$ ]. Paired-samples *t*-tests revealed that in the 320 mg condition, there was a significant increase in

**Table 1. Mean ( $\pm$ SE) baseline scores and change from baseline scores for the multitasking framework.**

		Baseline	1 h post-dose	2 h post-dose
Total MTF	Placebo	10130.76 $\pm$ 803.62	145.59	831.82
	320 mg	10664.18 $\pm$ 496.86	724.47	854.35
	640 mg	9940.71 $\pm$ 608.57	1053.12	909.31
MTF: Maths score	Placebo	385.88 $\pm$ 34.86	31.18	58.24
	320 mg	406.47 $\pm$ 29.47	46.47	98.24
	640 mg	380.00 $\pm$ 35.54	77.06	63.53
MTF: Stroop score	Placebo	4248.24 $\pm$ 294.93	268.75	514.71
	320 mg	4514.12 $\pm$ 316.86	285.29	189.41
	640 mg	4305.33 $\pm$ 225.73	502.94	546.47
MTF: Letter score	Placebo	5280.88 $\pm$ 568.64	-38.65	375.29
	320 mg	5405.29 $\pm$ 321.52	450.29	676.18
	640 mg	5312.65 $\pm$ 476.41	401.47	473.53
MTF: Tracking score	Placebo	449.88 $\pm$ 11.71	-0.29	-29.37
	320 mg	440.47 $\pm$ 12.72	-25.18	4.38
	640 mg	393.47 $\pm$ 26.00	31.40	7.12

performance from baseline to 1 h post-treatment [ $t(16) = 2.85$ ,  $p = .012$ ] and from baseline to 2 h post-treatment [ $t(16) = 3.53$ ,  $p = .003$ ]. Additionally, the baseline to 1 h post-treatment change score was significantly greater in the 320 mg condition when compared to the placebo condition [ $t(16) = 2.42$ ,  $p = .028$ ]. The baseline to 2 h post-treatment change score was considerably greater in the 640 mg condition when compared to the placebo condition [ $t(16) = 1.91$ ,  $p = .074$ ], but this difference did not reach statistical significance.

### Mood and anxiety ratings

In the absence of the MTF, ANOVA revealed a significant main effect of condition [ $F(2,32) = 4.24$ ,  $p = .023$ ] for ratings of alertness (Table 2). Post-hoc probing using paired-samples  $t$ -tests revealed the change from baseline to 2 h post-administration was significantly greater in the 320 mg condition compared to the placebo condition [ $t(16) = 3.89$ ,  $p = .001$ ]. Also, the 640 mg condition change from baseline to 2 h post-administration

was considerably greater than the placebo, although the difference was not significant [ $t(16) = 1.82$ ,  $p = .087$ ]. Taking into account the effect of the MTF, analysis of change from baseline scores for ratings of content revealed a strong trend towards a significant main effect across conditions [ $F(2,24) = 3.31$ ,  $p = .054$ ], driven by the 640 mg condition 1 h post-administration change being considerably greater than the placebo condition change [ $t(14) = 1.97$ ,  $p = .069$ ], but not to the point of statistical significance (Table 3). The analysis of the change scores for calmness ratings indicated that no significant main effect of condition were apparent (Table 4). The analysis of state anxiety scores revealed a trend for a main effect of condition [ $F(2,16) = 2.88$ ,  $p = .086$ ]. Paired-samples  $t$ -tests did not reveal any significant differences (Table 5).

### Cortisol reactivity

Analyses of the cortisol data in the absence of the MTF revealed a significant main effect of condition

**Table 2. Mean ( $\pm$ SD) change scores for alertness ratings for the three treatment conditions pre- and post-dosing and pre- and post-MTF completion**

	Placebo	320 mg Bacopa	640 mg Bacopa
Baseline	-0.94 $\pm$ 13.20	0.95 $\pm$ 7.69	4.74 $\pm$ 14.85
1 h pre-MTF	-3.26 $\pm$ 13.60	3.61 $\pm$ 15.79	1.73 $\pm$ 15.44
1 h post-MTF	-4.15 $\pm$ 15.07	0.55 $\pm$ 11.01	1.12 $\pm$ 8.85
2 h pre-MTF	-5.99 $\pm$ 12.94	8.95 $\pm$ 16.27	3.23 $\pm$ 18.40
2 h post-MTF	2.58 $\pm$ 8.39	-1.78 $\pm$ 6.50	1.47 $\pm$ 10.32

**Table 3. Mean ( $\pm$ SD) change scores for contentedness ratings for the three treatment conditions pre- and post-dosing and pre- and post-MTF completion**

	Placebo	320 mg Bacopa	640 mg Bacopa
Baseline	-0.95 $\pm$ 8.66	-2.75 $\pm$ 9.94	2.59 $\pm$ 8.96
1 h pre-MTF	-0.77 $\pm$ 5.58	1.76 $\pm$ 7.58	2.47 $\pm$ 10.84
1 h post-MTF	-1.98 $\pm$ 4.10	-0.31 $\pm$ 4.72	3.87 $\pm$ 11.03
2 h pre-MTF	-1.74 $\pm$ 7.92	1.04 $\pm$ 5.01	4.20 $\pm$ 8.78
2 h post-MTF	-0.81 $\pm$ 5.69	-2.84 $\pm$ 6.51	0.87 $\pm$ 8.71

**Table 4.** Mean ( $\pm$ SD) change scores for calmness ratings for the three treatment conditions pre- and post-dosing and pre- and post-MTF completion

	Placebo	320 mg Bacopa	640 mg Bacopa
Baseline	0.26 $\pm$ 13.11	-5.30 $\pm$ 10.48	0.07 $\pm$ 20.01
1 h pre-MTF	8.94 $\pm$ 16.75	6.47 $\pm$ 13.77	13.84 $\pm$ 17.21
1 h post-MTF	-3.50 $\pm$ 9.18	-2.06 $\pm$ 12.61	7.18 $\pm$ 36.85
2 h pre-MTF	9.56 $\pm$ 18.31	11.18 $\pm$ 16.68	10.04 $\pm$ 14.83
2 h post-MTF	-6.94 $\pm$ 12.95	-5.25 $\pm$ 6.82	-0.08 $\pm$ 13.01

[ $F(2,26) = 5.21$ ,  $p = .012$ ]. Post-hoc probing of this main effect using paired-samples  $t$ -tests revealed differences in the baseline to 1 and 2 h post-treatment change scores across the conditions. At 1 h post-treatment, the change from baseline was significantly greater in the 640 mg condition compared to the 320 mg condition [ $t(14) = 2.70$ ,  $p = .017$ ] and the placebo condition [ $t(15) = 2.66$ ,  $p = .018$ ]. Similarly, the baseline to 2 h post-treatment delta scores were significantly greater in the 640 mg condition compared to the 320 mg condition [ $t(15) = 3.73$ ,  $p = .002$ ] and the placebo condition [ $t(14) = 2.58$ ,  $p = .022$ ]. These differences are illustrated in Fig. 1 below. No significant treatment, time, or time by treatment interaction was observed on the change scores generated after completion of the MTF.

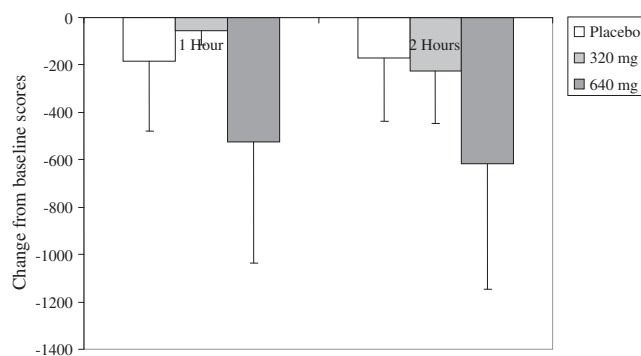
## DISCUSSION

The current study examined the effect of two doses (320 mg and 640 mg) of a specific extract of BM (KeenMind<sup>®</sup> - CDRI 08) upon repeated cognitive performance, mood, and stress-reactivity (cortisol) before and after supplement administration. Assessment of the change from baseline performance following consumption of placebo, 320 mg or a 640 mg dose of a specific extract of BM (KeenMind<sup>®</sup> - CDRI 08) revealed performance on the Stroop task aspect of the MTF was improved in comparison to placebo at both 1 and 2 h post-dosing with the 640 mg BM, and 1 h post-dosing in the 320 mg BM condition. Additionally, performance on the Letter Search task within the MTF was observed to improve 1 and 2 h post-administration of the 320 mg dose of the BM. Participants' self-rated Alertness was increased within the 320 mg BM condition 2 h post consumption prior to completion of the MTF. Increased levels of Contentedness were identified

in the 640 mg condition after completion of the MTF at the 1 h post-dosing testing time-point. The 640 mg BM condition also produced significantly lower cortisol levels (greater reduction from baseline) at the 1 and 2 h post-dosing time-points prior to completion of the multitasking cognitive battery. No significant differences between conditions were identified in cortisol reactivity following completion of the MTF, possibly indicating that at the doses assessed and the timeline of the cortisol assessments that the BM had no obvious acute effect upon attenuation of cortisol production.

Consumption of the clinically standard dose of BM (320 mg; CDRI 08) improved Letter Search and Stroop task performance (1 and 2 h post-dosing) and Alertness ratings (2h) prior to completion of the MTF. This improvement in Stroop task performance is consistent with faster information processing and decision-making time (Stough *et al.*, 2008; Stough *et al.*, 2001a), and improvement in aspects of attention/freedom of distractibility (Barbhaiya *et al.*, 2008; Calabrese *et al.*, 2008) previously noted in chronic BM supplementation studies. Improvement in the Letter Search task is more consistent with the memory-enhancing qualities of BM reported in chronic intervention studies (Barbhaiya *et al.*, 2008; Calabrese *et al.*, 2008; Morgan and Stevens, 2010; Stough *et al.*, 2008; Stough *et al.*, 2001a), that have not been previously demonstrated in the only previously published acute BM supplementation study (Nathan *et al.*, 2001).

That some degree of cognitive enhancement was evident at both the 1 and 2 h post BM administration suggests that the previously investigated neuropsychological tests were insensitive to the acute nootropic effects of BM within the pharmacological window that was employed (Nathan *et al.*, 2001). Aspects of the tests employed may have precluded detection of any nootropic effect attributable to BM given tests such as the Rey Auditory Verbal Learning Test and Digit Span are not administered under any time pressure, and faster responding does not increase performance scores. The improved performance in these cognitive tests may have been elicited by improvement in aspects of cognition that are more relevant to multitasking on time-sensitive cognitive tasks including cognitive speed and reasoning (Pase *et al.*, 2012). Changes in these abilities may also account for the memory-enhancing effects attributed to BM in previous chronic dosing studies given these aspects of cognition would be involved in completion of the range of memory-specific cognitive tasks employed to assess the nootropic effects of BM (Calabrese *et al.*, 2008; Mandal *et al.*, 2011;



**Figure 1.** Cortisol (ug/ml) change from baseline scores to 1 and 2 h post-administration across each condition in the absence of the stressor.

**Table 5.** Mean ( $\pm$ SD) change scores for anxiety ratings for the three treatment conditions pre- and post-dosing and pre- and post-MTF completion

	Placebo	320 mg Bacopa	640 mg Bacopa
Baseline	0.75 $\pm$ 3.22	-0.43 $\pm$ 2.59	2.41 $\pm$ 5.64
1 h pre-MTF	-0.71 $\pm$ 4.81	-3.00 $\pm$ 5.94	-0.82 $\pm$ 4.20
1 h post-MTF	0.76 $\pm$ 5.32	-0.33 $\pm$ 1.59	1.13 $\pm$ 5.08
2 h pre-MTF	-0.94 $\pm$ 5.57	-2.12 $\pm$ 7.25	0.00 $\pm$ 4.70
2 h post-MTF	0.53 $\pm$ 4.36	0.60 $\pm$ 2.61	0.06 $\pm$ 2.63

Stough *et al.*, 2008; Stough *et al.*, 2001a). Future acute and chronic administration studies of BM could therefore aim to dissociate aspects of cognitive performance relevant to tests of memory and attention to identify the possible mechanism for improved cognitive performance attributable to BM (Downey *et al.*, 2012).

Acute improvement in cognitive performance elicited by BM could be attributed to the purported adaptogenic and anxiolytic properties of BM that may have been evinced in the cognitively challenging paradigm utilized in the current study. In regards to the subjective outcomes, ratings of Alertness (2 h post-dosing with 320 mg BM) and Contentedness (1 h post-dosing with 640 mg BM) were observed to be improved. Whilst the ratings of mood and state anxiety were not observed to consistently improve in the absence of completion of the MTF, or attenuate any task-induced mood modulation, examination of the means reveal that the placebo condition was generally more negatively affected by completion of the multiple cognitive assessments than the two BM conditions. This suggests that at the doses administered, the subjective effect of any anxiolytic or antidepressant action may be subtle, or obscured by task completion. Limited evidence from human studies exist for any positive mood-moderating action of BM (Calabrese *et al.*, 2008; Stough *et al.*, 2001b), although this effect has not been evident in all chronic supplementation studies (Roodenrys *et al.*, 2002; Stough *et al.*, 2008), and was not assessed in the previous acute supplementation study (Nathan *et al.*, 2001). The current results suggest that at the doses assessed, BM does not fully attenuate the experience of experimentally induced mood deficits after two completions of multitasking assessments, but did have some positive effect upon mood at each testing time-point that may have facilitated the positive cognitive performance findings. Given the small sample size, imbalance of genders, and the relatively small change from baseline average scores in the subjective mood ratings, it would be important to replicate this finding of selective mood enhancement in a larger sample, and in the absence of cognitively demanding tasks to quantify the magnitude of the subjective effects.

Assessment of the effect of BM upon cortisol reactivity was undertaken pre- and post-completion of the MTF to assess any physiological adaptogenic effect of BM consumption. The treatment-related effects were strongest in the 640 mg BM condition, where at both 1 and 2 h post-treatment, the change from baseline scores for saliva cortisol was larger in comparison to both the 320 mg and placebo conditions. This is somewhat consistent with the adaptogenic properties

inferred from preclinical studies for BM where acute improvement in cognitive performance has been observed under laboratory-induced stressful conditions (Andrade and Chandra, 2006b; Saraf *et al.*, 2008). Interestingly, following completion of the MTF at each time-point, no significant differences in levels of cortisol were evident between treatments, suggesting the adaptogenic effect of BM was limited to reducing cortisol between assessments, rather than attenuating the response due to the completion of the multitasking. This pattern of results is similar to the findings of Sheikh and colleagues who, in models of acute and chronic unpredictable stress, observed changes in plasma corticosterone, noradrenaline, serotonin, and dopamine in the cortex and hippocampal regions of rat brains (Sheikh *et al.*, 2007). They noted that the adaptogenic properties of BM manifested via normalization of stress-induced changes in corticosterone and monoamine levels in the cortex and hippocampus when applied as a pre-treatment to exposure to stressful conditions (Sheikh *et al.*, 2007).

The current study examined the effect of two doses of BM for possible cognitive-enhancing, adaptogenic, and anxiolytic effects on the basis of growing preclinical and human trial evidence for BM-related nootropic efficacy within acutely stressful paradigms. We observed some positive cognitive effects, notably at both 1 h post BM consumption suggesting an earlier nootropic effect of BM than previously investigated, and 2 h post BM consumption on tasks more dependent upon aspects of reasoning and cognitive speed than the previously investigated tests of memory (Nathan *et al.*, 2001). Subjective ratings of mood and anxiety were not consistently affected by BM consumption (although Alertness and Contentedness ratings were increased), with any anxiolytic or mood-enhancing qualities of BM possibly being too subtle to detect in a small sample, or being obscured by completion of the multiple testing procedures. The general pattern of subjective ratings of mood appeared to provide some evidence of the attenuation of the negative mood effects associated with completion of the MTF (Wetherell and Sidgreaves, 2005), which could be linked with the apparent adaptogenic effect of reduced cortisol levels after consumption of BM (640 mg) prior to completion of the cognitive assessments (1 and 2 h post-treatment). Future research in healthy human populations could again utilize the earlier pharmacological window to ascertain when 'peak' acute neurocognitive and behavioural effects are observable and could benefit from employing temporally sensitive brain imaging measures to qualify what brain regions are acutely affected or are benefitting from BM consumption.



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## Conflict of Interest

The authors declare no conflict of interest.

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