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Does Drug Potentiation Reduce its Dose or Duration or Both? A Case Study of *Brahmi ghrita*

Swati Gadgil*, Asmita Wele

Department of Rasashastra Evam Bhaishajyakalpana, College of Ayurved, Bharati Vidyapeeth, Maharashtra, India

***Corresponding author:** Swati Gadgil, Department of Rasashastra Evam Bhaishajyakalpana, College of Ayurved, Bharati Vidyapeeth, Maharashtra, India, E-mail: swati.gadgil@bharatividyapeeth.edu

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Abstract

Diazepam a drug from benzodiazepine class is used as an anticonvulsant and for anxiolysis, sedation, and myorelaxation and is known to produce anterograde amnesia. Amnesia becomes more worrisome among the aging population, because age-related organ decline reduces the ability to metabolize and eliminate drugs, including benzodiazepines. *Brahmi ghrita* a lipid based formulation of two potencies is a proven anti-stress agent capable of reversing the memory loss due to depression. It was tested for its antiamnesic effect in this study. Self-prepared and standardized 1x *brahmi ghrita* (5.0 g/kg) and 10x *brahmi ghrita* (5.0, 2.5 and 1.25 g/kg) were evaluated in diazepam induced amnesia. Memory assessment was done using elevated plus maze test. One way ANOVA followed by Tukey-Kramer multiple comparisons test was used. Induction of amnesia using diazepam was confirmed. Upon comparison with diazepam control group, 1x *brahmi ghrita* and 10x *brahmi ghrita* at all three dose levels, resulted in reduced ($p<0.001$) transfer latency (TL) on day eight as well as day nine, 1x *brahmi ghrita* (5 g/kg) resulted in reduced ($p<0.01$) transfer latency and 10x *brahmi ghrita* (5, 2.5 &1.25 g/kg) also resulted in reduced ($p<0.001$) TL on day 16, on day 17, all the treatment groups showed reduced transfer latency ($p<0.001$) reflecting reversal in diazepam induced impairment of learning and memory. Both the samples of *brahmi ghrita*, showed reversal of diazepam induced amnesia. This unique study reports that drug potentiation results in better efficacy in a reduced dose.

Key-words: Amnesia; Ayurved; *brahmi;* potentiation; *siddha ghrita*; *brahmi ghrita.*

Introduction

Diazepam is a drug from benzodiazepine (BZDs) group. BZDs are used for numerous indications, including anxiety, insomnia, muscle relaxation, relief from spasticity caused by central nervous system pathology, and epilepsy along with intra-operative use because of their amnesic and anxiolytic properties [1]. However, these properties become undesired side effects in nearly all other clinical instances. Although amnesia can occur in any patient, it is especially worrisome among the aging population, because age-related organ decline reduces the ability to metabolize and eliminate drugs, including BZDs.This problem can lead to toxic accumulation of BZDs and their breakdown products, the result of which may manifest in morbidity and even mortality [1]. Patients taking benzodiazepines as a treatment for anxiety disorder, convulsive disorder or insomnia often suffer from amnesia and are relatively unaware about memory impairments [2].

Sidhha ghrita (medicated ghee) is the foremost drug of choice for preventive as well as curative treatment for conditions involving cognition. Siddha ghrita exhibits poor drug compliance owing to its high dose of about 20 to 40 g per day, greasy nature, strong smell and taste. To combat these issues; avartana, which literally means repetition of the procedure can prove helpful.Thus present study was conducted to check whether drug potentiation leads to dose minimization or duration reduction or both using siddha ghrita(medicated ghee) of two potencies.

Brahmi (Bacopa monnieri Linn) is one among the most praised medhya rasayana drugs–class of herb taken to sharpen intellect and attenuate mental deficits. Bacopa monnieri is a perennial creeping plant found throughout India in wet, damp and marshy areas.It is perhaps the most extensively, in depth researched herb which is also unambiguous. Current evidence suggests Bacopa monnieri acts *via* the following mechanisms antioxidant neuro-protection (*via* redox and enzyme induction), acetylcholinesterase inhibition and/or choline acetyltransferase activation, b-amyloid reduction, increased cerebral blood flow, and neurotransmitter modulation (acetylcholine (Ach), 5-hydroxytryptamine (5-HT), dopamine (DA)[3].

Thus present work was carried out for evaluation and comparison of efficacy of standardized *brahmi ghrita* with 1x potency (1x BG) (prepared with a single processing cycle - ekapaki) and standardized *brahmi ghrita* with 10x potency (10x BG) (prepared with ten processing cycles -dashapaki) (at three dose levels) using diazepam induced amnesia model.

Materials and Methods

Formulation preparation: 1x BG and 10x BG were prepared using only two ingredients i.e. cow ghee and brahmi juice extracted from fresh brahmi plant. Drugs were prepared by complying with the guidelines in ayurvedic treaties and were standardized using Bacoside A as a marker compound [4].

Animals: Adult swiss albino mice of either sex, (18 -30 gm) were procured from CPCSEA approved Central animal house, BVDU Medical College, Pune. They were housed under standard (23-300C, 50–60 % humidity) laboratory conditions, maintained on a 12-h natural day–night cycle. Animals had free access to standard food and water. They were acclimatized to laboratory conditions before onset of the experiment. Approval was obtained from Institutional Animal Ethics Committee (Ref: BVDUMC/2228/2017/001/001)

Experimental design: Before beginning the study, mice were subjected to one time screening for their activity on the EPM. The mice having close range of Transfer Latency (TL) were selected for the study. This was done to reduce the drastic variations in TL that is the parameter of assessment [5]. Here 1X BG was used in therapeutic dose while 10X BG was used in therapeutic; half and also one fourth of the therapeutic dose with an intention to check whether or not

potentiation results in drug dose reduction or reduction in drug duration or both.

- Forty two mice (six in each group) were randomly divided in to seven groups. Group I consisted of untreated mice (normal control),
- Group II comprised of Diazepam control mice (disease control) (1mg/kg) (Calmpose 2ml) (Ranbaxy, New Delhi),
- Group III consisted of mice receiving Piracetam (500 mg/kg), (positive control) (Piracetam syrup commercially available as Nootropil (Exemed Pharmaceuticals, Gujrat) + Diazepam,
- Group IV consisted of mice receiving 1x BG (5.0 g/kg) (therapeutic dose equivalent to 40g in humans) + Diazepam,
- Group V, VI and VII consisted of animals receiving 10x BG in the dose of 5.0 g/kg therapeutic dose + Diazepam, 2.5 g/kg half of the therapeutic dose + Diazepam and 1.25 gm/kg one fourth of the therapeutic dose + Diazepam.
- Study drugs were liquefied using water bath just before dosing; and administered.

Study procedure

Elevated plus maze (EPM) for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 12 cm) extended from a central platform (5 cm × 5 cm), and the maze is elevated to a height of 25 cm from the floor.

Drug dosing was done orally continuously for sixteen days. On eighth day and 16 th day, after 90 minutes of administration of the drug dose, diazepam (i. p. in a dose of 1 mg/kg) was administrated to all the animals except normal control animals.

Transfer latency was recorded after 45 minutes of administration of diazepam on eighth day and 16 th day of drug administration and considered as acquisition/learning trial [5]. Transfer latency (TL) is defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arms with all its four legs. Each mouse was placed at the distal end of an open arm, facing away from the central platform and TL was recorded. The mouse was allowed to explore the maze for another two minutes and then returned to its home cage. Retention of this learned task (memory) was examined 24 hours after the learning trial (*i.e.* on ninth day and on 17th day). On ninth day; drug dosing was done after completion of the task on EPM. Significant reduction in TL value is indicative of reversal of impairment of memory. Experiment was conducted in a dimly lit, semi soundproof room under natural light.

Data analysis

Data was expressed as Mean ± standard deviation. Statistical analysis was carried out using Graphpad Instat software version 3 and differences were considered significant at $p<0.05$. The normal control group was compared with the diazepam control group. The drug treated animals were compared with the diazepam control and with each other also. The data was analyzed using one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test.

Results

The TL was elevated (60.50 ± 12.46) on day eight by diazepam injected before training as well as on day nine (61.83 ± 13.58) reflecting remarkable impairment in learningand memory in mice. Drug treatment with Piracetam and study drugs resulted in reduced ($p<0.001$) TL on day eight as well as day nine, when compared to diazepam control groupreflecting remarkable reversal in diazepam induced impairment of learning and memory. Treatment with 1x BG and piracetam displayed elevated TL (39.50 ± 7.00) & (39.83 ± 7.02) respectively compared to normal control on day eight. Treatment with 10x BG (5 g/kg) reflected reduced ($p<0.05$) TL on day nine compared to treatment with1x BG (5 g/kg) and Piracetam. But treatment with 10x BG at the dose (2.5 & 1.25 g/kg) did not show statistically significant difference in TL on day nine when compared with 1x BG (Figure1).

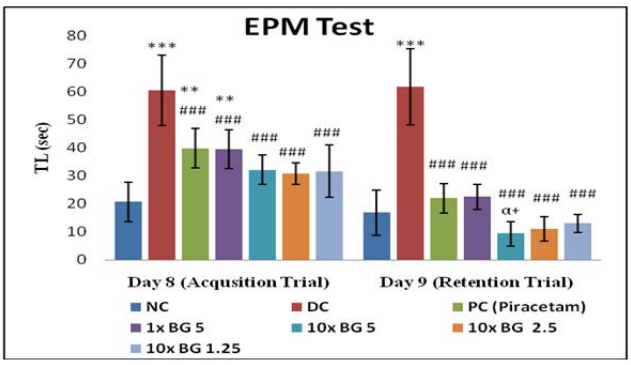

Figure 1: Effect of diazepam induction and drug treatment on EPM test (Day eight & Day nine)

The TL was elevated (60.66 ± 11.82) on day 16th by diazepam injected before training as well as on day 17th (61.50 ± 9.83) reflecting remarkable impairment inlearning and memory in mice. Drug treatment with Piracetam and 1x BG resulted in reduced ($p<0.01$) TL and treatment with 10x BG (5, 2.5 &1.25 g/kg) also resulted in reduced ($p<0.001$) TL on day 16th when compared to diazepam control group. On day 17, all the treatment groups showed reduction in TL ($p<0.001$) when compared to diazepamcontrol group reflecting remarkable reversal in diazepam induced impairment of learning and memory. Treatment with 1x BG displayed elevated TL (39.66 ± 11.29) compared to normal control on day 16th .Treatment with 10x BG (5, 2.5 &1.25 g/kg) reflected reduced ($p<0.01$) & ($P<0.001$) TL on day 17th compared to treatment with 1x BG and piracetam respectively (Figure 2).

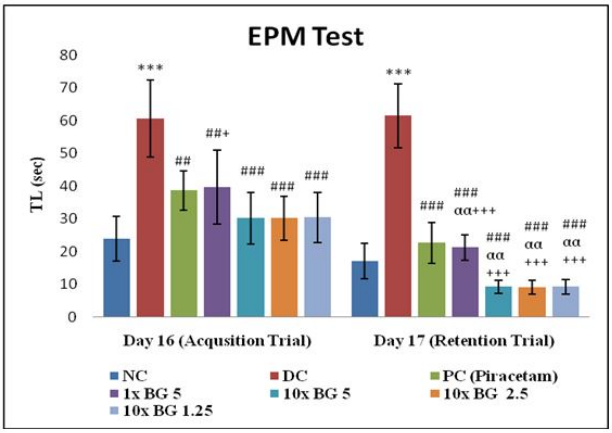

Figure 2: Effect of diazepam induction and drug treatment on EPM test (Day 16 & Day 17)

Discussion

Present study was framed to evaluate and compare efficacy of 1x BG and 10 BG (used at three dose levels) using diazepam induced amnesia model and check if the drug potentiation results in drug dose reduction or reduction in the duration of the treatment or both.Over the past decade; while working on various siddha ghrita formulations (Brahmyadi ghrita, Vachadi ghrita, Panchagavya ghrita etc.), it was noticed that healthy individuals or patients were hesitant to consume strong smelled, bitter tasted ghee in the dose of 10-20 g twice a day, for the duration up to three months. Similar experience of non compliance is highlighted in the recent research work by Kadus et al. This led us to think about the textual process of avartna i.e. repetition, which could make the ghrita palatable as well as potent in a smaller dose.

Diazepam is a drug from benzodizepine group. All the benzodiazepines increase the binding of gamma-aminobutyric acid (GABA) (a major inhibitory neurotransmitter) at GABA-receptors and thus work through GABAergic mechanism. They are known to produce anterograde amnesia (that is forgetting events occurring subsequent to administration of the drug) disrupting both short-term and long-term memory functions. The deficit in long-term memory is probably because the events are not transferred from short-term memory to long-term memory thus resulting in disruption of memory consolidation . Patients taking benzodiazepines as a treatment for anxiety disorder, convulsive disorder or insomnia often suffer from amnesia and are relatively unaware about memory impairments [2]. This information provides a rationale for conduction of the present experiment.

Elevated plus maze was originally used to test anxiety but many studies have also used it as a test of cognition [5]. Though it is used for studying anxiolytics as well as nootropics (cognitive enhancers), procedure and parameters used for the two tests are clearly different. Elevated plus maze is an ‘exteroceptive behavioral model’ (where stimulus lies outside thebody) which uses natural anxiety of rodents to light, open environment and height. The height and open arms act as an aversive stimulus to the rodents. This is a simple less time-consuming procedure that does not involve any sophisticated equipments or prior training or noxious stimuli like electric shock and also there is no need to manipulate appetite behaviours [5].

Out of three broad memory classes’ i.e. sensory, short term and long termmemory; benzodiazepines are known to affect the long term memory. There are two subcategories of long-term memory; explicit i.e. conscious memories and implicit *i.e.* unconscious memories. Episodic memory is categorized as the sub class of explicit memory. It is the memory of personally experienced events, involving recall and recognition of information and benzodizepines impair episodic memory [1]. The task on the elevated plus maze can be categorized under episodic memory which was found to be impaired in retention trials (evident from increased TL) after administration of diazepam. The results are in tune with some of the previous researches [5]. In retention trial carried out on ninth day, all the drugs showed positive results when compared with the diazepam control group by reducing the TL time. 10x BG (5 g/kg) showed better results ($p<0.05$) when compared with 1x BG and even the standard drug piracetam. This is indicative of increase in the efficacy of drug due to potentiation. After prolong treatment for continuous sixteen days; all the drugs showed positive results when compared with the diazepam control group as before. 10x BG at all three dose levels showed better results as compared to 1x BG($p<0.01$) and even against piracetam ($p<0.001$). This clearly indicate that within eight days treatment both half and one forth (of therapeutic dose) dose of 10xBG were not able to produce significant results than 1x BG in this model. But as the drug treatment prolonged up to sixteen days; the same produced statistically significant results than 1x BG [6].

Piracetam is the most widely practiced nootropic drug with multiple pharmacological

effects thus was used as a positive control drug in diazepam induced amnesia model. Benzodiazepines may interfere with the processes of learning/memory by reducing the arousal during acquisition. Conversely improved retention of the task by 1x BG and 10XBG could be attributed to increased arousal or anxiety. Several classes of drugs that enhance arousal are known to produce improvement in learning and subsequent retention. Prabhakar et al. proved that Bacopa monnieri possesses antiamnesic effect against diazepam- induced anterograde amnesia and the effect is mediated by the GABAergicsystem possibly affecting long term potentiation [2]. Further, in a research work by Saraf et al. authors conducted behavioral and molecular studies showing anti-amnesic effect of *Bacopa monniera* (120 mg /kg -1 oral). Bacopa showed anti-amnesic results in behavioural studies. The molecular studies revealed that diazepam up-regulated mitogen activated protein kinase (MAP kinase), phosphorylated CREB (pCREB) and inducible nitric oxide synthase (iNOS), while it down-regulated nitrite, nitrate, total nitrite, cAMP response element binding protein (CREB) expression, phosphodiesterase, cyclic adenosinemonophosphate (cAMP) without affecting calmodulin levels. *Bacopa monniera* suppressed the diazepam induced up-regulation of MAP kinase, pCREB and iNOS and attenuated the down-regulation of nitrite. Extension of this work published by Prabhakar et al. demonstrated that level of SOD (superoxide dismutase) was significantly reduced with diazepam induction and Bacopa when administered along with diazepam alleviated theSOD activity. Thus antioxidant activity of brahmi plays an important role in reversing the diazepam induced amnesia. In concurrence with above findings we can propose that 1x BG and 10x BG (containing brahmi as a key ingredient) must have displayed their antiamnesic effect by modulating GABAergic neurotransmission. Similarly antioxidant property of brahmi should have contributed for positive reversal of diazepam induced amnesia by 1xBG and 10x BG (all three doses).

In EPM test, post-hoc test indicated a significant difference in the results of 1x BG and 10x BG (at all three dose levels). 10x BG at all three dose levels showed better results after completion of sixteen day’s treatment when compared with 1x BG group. The major chemical entity shown responsible for neuropharmacological effects of *Bacopa monniera* is bacoside A (64.28%) and bacoside B (27.11%). 10x BG is prepared using brahmi juice repeatedly for ten times and hence contain higher concentration of phytochemical from brahmi including bacoside A in comparison with 1x BG[4].Thus this higher concentration must be responsible for better results of 10x BG (2.5 g/kg) and (1.25 g/kg) dose, which is actually the purpose of potentiating the drug [7].

It is evident that orally taken Bacopa monnieri extract (BME) was up taken into the system by showing presence of bioactive compound bacoside A in the serum of BME treated rats. The bioactive compounds in the BME could directly or indirectly interact with neurotransmitter systems to enhance learning and memory. Since the bacosides present in the BME are nonpolar glycosides, they can cross the blood-brain barrier (BBB) by simple lipid-mediated passive

diffusion. To add to this effect; both the study drugs are lipid based and have a natural affinity towards brain tissue facilitating the easy entry of the drug into the tissue.

Efficacy in diazepam induced amnesia model was checked at two time-points one after eight day's treatment and another after sixteen days treatment. This was done to check whether drug potentiation reduces the duration of the treatment. It is evident from the results that after eight days treatment 10x BG at therapeutic dose showed significant results when compared with 1x BG. But it did not prove effective in half and one fourth of the therapeutic dose. With continuation of the treatment up to sixteen days it proved effective at all three dose levels when compared with 1x BG. Thus it can be claimed that one can get better results with use of potentiated form of the drug (10x BG) used in the therapeutic dose in comparison to the comparator (1x BG). Whereas long term administration of potentiated version of the drug will prove efficacious in half and even one fourth of the therapeutic dose. This study is the first and foremost attempt to generate evidence between the relationship of drug potentiation and drug dose reduction. Further extended research is required to find out exact mechanism of action of the drug; as well its efficacy in reduced dose needs to be clinically evaluated [8].

1x BG (5 g/kg) and 10x BG (5, 2.5 &1.25 g/kg) showed reversal of memory impairment induced by diazepam after completion of sixteen days treatment. The study established that drug potentiation increases the efficacy of the drug that too in a reduced dose. The small drug dose can be dispensed in a better and palatable form like soft gel capsules.

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