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Pharmacological interaction of *Centella asiatica* and *Bacopa monnieri* with antiepileptic drugs - an experimental study in rats

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Abstract

Objective: The main objective of this study was to determine experimentally the pharmacological interaction of *C.asiatica* (CA) and *B.monnieri* (BM) with standard antiepileptic drugs (AEDs) such as Phenytoin (PHT), Phenobarbitone (PB) and Carbamazepine (CBZ) in rats. Methods: Adult, male Wistar rats were given either CA or BM (500 mg/kg) alone or in combination with one of the antiepileptic drugs (AEDs) at the ED_{50} doses. The anticonvulsant activity was assessed by the classical Maximal Electro Shock (MES) test at 1, 3, 6 and 24 h after drug administration. Results: PHT and PB showed significant protection at 3 h ($p < 0.05$), whereas CBZ showed significant protection at 1, 3 and 6 h ($p < 0.05$). CA alone, showed protection from 1 - 24 h, with significant protection at 3 h ($p < 0.05$). When CA was combined with PHT, though seizure protection was seen, this effect was not statistically significant. When CA was combined with PB, the protective activity of PB declined from 50% to 0 % ($p < 0.05$) at 3 h. When CA was combined with CBZ, significant seizure protection was seen only at 1 and 3 h ($p < 0.05$). Similar to CA, the seizure protection of BM was significant only at 3h ($p < 0.05$). In combination with AEDs significant seizure protection ($p < 0.05$) was observed – with PHT at 6 h, with PB at 3 h, and with CBZ, at 1, 3 and 6 h. Conclusion: This study demonstrated that herbal plant products such as CA and BM interact pharmacologically with standard AEDs and hence caution should be exercised to avoid any possible adverse interactions.

Key words: *Centella asiatica, Bacopa monnieri,* Phenytoin, Phenobarbitone, Carbamazepine, Maximal electroshock test, anticonvulsant activity, Herb/drug interaction.

1. Introduction

The World Health Organization has estimated that perhaps 80% of the world's population relies chiefly on traditional medicines for primary health care needs and in 1998, suggested appropriate guidelines for the use of herbal medicines for various chronic disorders [1]. Epilepsy is a chronic disorder requiring continuous medication to keep patients seizure

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free. Since ancient times *Centella asiatica* L. (Apiaceae) and *Bacopa monnieri* L. (Scrophulariacea) have been used in Ayurveda for the treatment of epilepsy [2].

In Ayurvedic literature, Astanga Hridya, *B. monnieri* (Brahmi) is stated as the best remedy for epilepsy (Astanga Hridya) [3]. *C.asiatica* has been used as brain tonic, as an adjuvant in the treatment of leprosy ulcers, as a diuretic, for the treatment of skin disorders and tried in the treatment of obesity [4,5,6].

The pharmacological properties of *B.monnieri* have been well documented and its most important effects on the central nervous system are its anxiolytic, nootropic and anticonvulsant properties [5,7,8]. It also has sedative, cardiotonic, vasoconstrictor and neuromuscular blocking actions [5].

In a previous study, both *C.asiatica* and *B.monnieri* at a single oral dose of 500 mg/kg showed mild to moderate anticonvulsant activity in the Maximal electroshock test in rats [9,10]. *C.asiatica* had a slow onset of action, showed mild to moderate activity, but its activity lasted for 24 h. *B.monnieri,* had a fast onset of action, showed mild to moderate activity, and only minimal activity was seen at 24 h [9,10]. This suggested that the anticonvulsant activity of *C.asiatica* and *B.monnieri* is worth further investigation.

Established guidelines for the clinical trials of new antiepileptic agents make it obligatory for the new agent to be administered as “add-on” therapy to the existing antiepileptic medication [1]. Concurrent use of herbs may mimic, magnify, or oppose the use of drugs. A previous study has shown that *Shankapushpi,* when added to Phenytoin treatment, caused breakthrough seizures in patients, suggesting that herbal remedies when co-administered with phenytoin may result in lowering blood levels and loss of seizure control [11].

Previous reports of herb-drug interactions are sketchy and lack systematic scientific investigations. Hence the objective of this study was to determine if the concurrent administration of single, effective anti-convulsant doses of either *C.asiatica* or *B.monnieri* would alter the anticonvulsant efficacy of standard antiepileptic drugs such as phenytoin, phenobarbitone and carbamazepine in rats.

2. Materials and methods

2.1 Animals

Adult, male Wistar rats (200-250g) were housed in groups of 4 per cage at ambient temperatures of 25 ± 2°C on a 12 h light/dark cycle. They had free access to standard laboratory pellets (Hindustan Lever India Ltd.) and water ad libitum and were fasted overnight prior to experimental sessions. The Institutional Animal Ethics Committee approved the protocol for all experiments, which were conducted in humane conditions.

2.2 Plant materials

Whole plants of *Centella asiatica* Linn. (Family: Apiaceae) and *Bacopa monnieri* Linn. (Syn: *Herpestis monniera;* Family: Scrophulariaceae) were collected, in the fresh condition, from electronic city, Bangalore, India in February 1999 and taxonomically identified. The plants were sun dried for 3-4 days, herbaria were prepared and voucher specimens (CA/E/01) and (BM/E/01) were deposited at the Pharma-cognosy Department of Natural Remedies Pvt. Ltd. Voucher specimens were also sent to the National Institute of Science Communication (NISCOM), New Delhi for authentication.

The dried crude drug of *C.asiatica* was found to have asiaticosides-0.6%w/w, madecassoside - 0.9%w/w, estimated by HPLC technique using chromatographic reference standards obtained from M/s Extrasynthese, France [Lot No 99091005 and 99091006 respectively]. The

crude drug of *C.asiatica* was found to contain 10.5%w/w of total saponins, estimated according to the procedure of Marston *et. al.* (1995) [12].

The dried crude drug of *B.monnieri* was found to have bacoside A- 4.2% w/w estimated by HPLC and HPTLC techniques using chromatographic reference standard provided by Phytochemistry department of Natural Remedies Pvt. Ltd. The crude drug of *B.monnieri* was found to contain 11%w/w of total saponins [12].

2.3 Drug administration to rats

All drugs were administered orally to rats after overnight fasting. The crude drugs were triturated with distilled water and given in a volume of 0.4 ml/100 g body weight. The rats were randomized and the following groups of rats were tested:

1. Saline control
2. Phenytoin (PHT) - 30 mg/kg
3. Phenobarbitone (PB) – 13.5 mg/kg
4. Carbamazepine (CBZ) – 8.5 mg/kg
5. CA - 500 mg/kg
6. CA (500 mg/kg) + PHT (30 mg/kg)
7. CA (500 mg/kg) + PB (13.5 mg/kg)
8. CA (500 mg/kg) + CBZ (8.5 mg/kg)
9. BM - 500 mg/kg
10. BM (500 mg/kg) + PHT (30 mg/kg)
11. BM (500 mg/kg) + PB (13.5 mg/kg)
12. BM (500 mg/kg) + CBZ (8.5 mg/kg)

The doses of CA and BM were chosen based on previous studies done in our laboratory [9,10]. The crude drugs of CA or BM (500 mg/kg) were first administered to the rats followed 5 min later by the AED. The standard reference drug PHT (Knoll Pharma, India) was given at a dose of 30 mg/kg, PB (Rhone-Poulenc, India) at a dose of 13.5 mg/kg and CBZ (Novartis, India) at a dose of 8.5 mg/kg, which are the ED_{50} doses in the MES test in rats [13].

2.4 Assessment of anticonvulsant activity

All experiments were conducted in the forenoon at the same time each day and rats were subjected to maximal electroshock seizure (MES) at 150 mA, 60 Hz for 0.2 sec through ear electrodes. On Day 1, all rats were pre-tested with MES for baseline readings and those animals failing to give HLTE were rejected.

Only those animals that exhibited HLTE were chosen for the study. These animals were then divided into 12 groups. On Day 2, each group of rats was subdivided into three sub-groups, one for each time point tested, and subjected to MES at 1, 3 or 6 h, respectively after drug administration. On Day 3, all groups the animals were once again subjected to MES (24 h values). Three parameters were observed:

1. Presence or abolition of HLTE which is the criterion for anticonvulsant activity and protection against MES induced seizures [13]. The percentage protection in each group was also calculated using the following formula:

$$\frac{\text{No. of rats in which HLTE was abolished}}{\text{Total no. of rats}} \times 100$$

All control rats displayed HLTE and no seizure protection.

2. MES results in hind limb tonic extension (HLTE), the duration of which is measured in seconds. In control rats, the mean HLTE duration was 11.23 ± 1.82 s, with 100% rats displaying HLTE, indicating the absence of seizure protection

3. The righting reflex (RR), i.e. the time from end of HLTE to the time the rat spontaneously rights itself, was also calculated. The cut off time for RR was fixed at 10 min or 600 sec. The mean value for saline treated controls was 498.4 ± 166.3 s.

2.5 Statistical analysis

The mean and standard deviation was calculated for all the groups. The presence or abolition of HLTE was analyzed using the Chi-square test to compare the various categories. The HLTE and RR data were analyzed using ANOVA followed by the post-hoc Tukey's test. Significance was set at 0.05.

3. Results

3.1 Percentage protection

The percentage protection of different drugs is shown in Table 1. PHT and PB showed significant protection at 3 h ($p < 0.05$), whereas CBZ showed significant protection at 1, 3 and 6 h ($p < 0.05$).

CA alone, showed protection from 1 - 24 h, with significant protection at 3 h ($p < 0.05$). When CA was combined with PHT, though seizure protection was seen, this effect was not statistically significant. When CA was combined with PB, the protective activity of PB declined from 50% to 0 % ($p < 0.05$) at 3 h. When CA was combined with CBZ, significant seizure protection was seen only at 1 and 3 h ($p < 0.05$).

Similar to CA, the seizure protection of BM was significant only at 3h ($p < 0.05$). In combination with AEDs significant seizure protection ($p < 0.05$) was observed – with PHT at 6 h, with PB at 3 h, and with CBZ, at 1, 3 and 6 h.

3.2 HLTE and RR

The HLTE and RR of the different groups of rats at baseline, 1, 3, 6 and 24 h after drug administration are given in Tables 2 and 3. There was no difference between the various groups at baseline. However, at 1 h HLTE for the phenytoin group was significantly lower ($F (df=11,83) = 3.922$, $p < 0.01$) than the saline controls. At 6 h the groups treated with CA and BM showed higher HLTE than controls ($F (df = 11,74) = 6.037$, $p < 0.01$). At 24 h HLTE for the CA, PHT, BM + PHT, PB, CBZ

Table 1.
Percentage of rats (number of rats protected / total number of rats) protected against MES – induced seizures at various times after drug administration.

Groups	1h	3h	6h	24h
Control	0% (0/10)	0% (0/10)	0% (0/10)	0% (0/30)
PHT	40% (4/10)	50% (5/10)*	30% (3/10)	0% (0/30)
PB	33% (6/18)	50% (9/18)*	30% (3/10)	3% (1/32)
CBZ	63% (5/8)*	63% (5/8)*	50% (4/8)*	6% (1/18)
CA	10% (1/10)	50% (5/10)*	20% (2/10)	30% (3/10)
CA+PHT	30% (3/10)	30% (3/10)	40% (4/10)	10% (1/10)
CA+PB	0% (0/8)	0% (0/7)#	29% (2/7)	14% (3/22)
CA+CBZ	63% (5/8)*	63% (5/8)*	13% (1/8)	4% (1/24)
BM	30% (3/10)	50% (5/10)*	20% (2/10)	10% (1/10)
BM+PHT	30% (3/10)	40% (4/10)	60% (6/10)*	30% (3/10)
BM+PB	0% (0/8)	63% (5/8)*	38% (3/8)	29% (7/24)
BM+CBZ	63% (5/8)*	63% (5/8)*	63% (5/8)*	22% (5/23)

* Significantly different from controls, $p < 0.05$; # Significantly different from PB at 3 h, $p = 0.0267$
Note: All control rats did not show any protection against MES-induced seizures.

Table 2.
The hind limb tonic extension (HLTE) (Mean ± SD) in sec of different groups of unprotected rats (which exhibited HLTE) at baseline, 1, 3, 6 and 24 h after drug administration.

Groups	Baseline	1 h	3 h	6 h	24 h
Saline	11.23 ± 1.82	11.92 ± 1.46	10.98 ± 0.91	10.41 ± 0.36	13.67 ± 3.19
PHT	10.30 ± 1.77	7.33 ± 1.21*	12.80 ± 1.30	11.29 ± 2.69	10.87 ± 0.96*
PB	12.56 ± 1.05	11.25 ± 1.66	10.00 ± 1.22	10.43 ± 1.40	10.81 ± 1.82*
CBZ	11.85 ± 1.31	9.00 ± 1.00	8.50 ± 0.05	9.00 ± 1.41	10.83 ± 1.50*
CA	13.09 ± 2.55	12.06 ± 2.40	12.50 ± 3.81	14.52 ± 2.39*	10.00 ± 2.16*
CA + PHT	13.09 ± 2.37	10.98 ± 1.76	10.29 ± 2.43	9.50 ± 2.74	11.44 ± 1.51
CA + PB	12.36 ± 1.09	12.38 ± 3.11	11.86 ± 1.68	11.00 ± 2.92	11.79 ± 1.44
CA + CBZ	11.96 ± 1.20	9.00 ± 1.00	7.50 ± 0.50	10.57 ± 1.51	11.61 ± 1.62
BM	13.10 ± 2.92	11.34 ± 2.45	13.00 ± 1.41	14.63 ± 1.85*	12.78 ± 0.67
BM + PHT	13.20 ± 2.74	12.60 ± 2.35	10.50 ± 2.98	10.40 ± 2.41	10.29 ± 2.06*
BM + PB	12.42 ± 1.06	10.88 ± 2.47	11.00 ± 1.00	12.00 ± 0.71	11.71 ± 1.45
BM + CBZ	11.31 ± 1.74	8.00 ± 1.00	12.00 ± 1.01	9.00 ± 1.00	11.08 ± 1.68*

* Significantly different from saline control ($p < 0.01$)

and BM + CBZ groups was significantly lower than saline controls ($F (df = 11, 177) = 3.639$, $p < 0.01$). The righting reflex was not significantly different for any of the groups at 1, and 3 h after drug administration.

However, at 24 h CA, CA +PHT, BM + PHT, PB, CA + PB, BM +PB and BM + CBZ groups showed significantly higher ($F (df=11,214) = 4.010$, $p < 0.01$) RR than saline controls.

4. Discussion

Concurrent administration of antiepileptic drugs and herbal medicines may result in interactive synergistic or antagonistic clinical effects and complicate the purpose of long-term medication [14]. It has been reported that 16% of epileptic patients being treated with PHT were also using Chinese herbal medicine concomitantly [15]. Pharmacological or toxicological effects of either component may increase or decrease during combined therapy.

This study has been undertaken with the object of finding any beneficial effect or otherwise of combinations of *Centella asiatica* or *Bacopa monnieri* with standard AEDs. Effects of AEDs per se and AEDs combined with either CA or BM were examined in rats to determine pharmacodynamic effects on anticonvulsant efficacy of AEDs.

Our results showed that CA and BM have mild to moderate anticonvulsant activity. However unlike standard AEDs the activity persisted up to 24 h. CA when combined with PHT or CBZ did not influence the time of onset or degree of activity. Though a decrease in seizure protection was observed, this effect was not statistically significant.

However, when combined with PB, the activity of PB was significantly lowered at 3 h. BM, on the other hand demonstrated increased protection when combined with AEDs, suggesting no interference with individual pharmocodynamic profiles. Earlier studies showed that decreased concentrations of phenytoin, one of the most commonly used antiepileptic drugs, occurred when combined with a traditional Ayurvedic

Table 3.
The righting reflex (RR) (Mean ± SD) in sec of different groups of rats at baseline, 1, 3, 6 and 24 h after drug administration.

Groups	Baseline	1 h	3 h	6 h	24 h
Saline	498.4 ± 166.3	522.0 ± 135.8	427.0 ± 211.2	339.8 ± 158.0	383.7 ± 188.3
PHT	454.4 ± 178.9	417.1 ± 216.0	441.1 ± 182.6	480.7 ± 163.7	442.0 ± 168.4
PB	586.2 ± 46.7	527.1 ± 122.8	589.8 ± 35.5	553.8 ± 97.4	557.5 ± 80.0*
CBZ	508.6 ± 158.6	477.9 ± 130.1	428.4 ± 243.3	288.8 ± 280.8	453.1 ± 179.4
CA	522.0 ± 135.8	513.7 ± 138.0	529.0 ± 150.6	525.5 ± 158.3	547.5 ± 99.9*
CA+ PHT	534.3 ± 139.3	494.1 ± 182.5	465.4 ± 185.3	504.0 ± 160.5	558.0 ± 132.8*
CA + PB	600.0 ± 0.0	600.0 ± 0.0	600.0 ± 0.0	600.0 ± 0.0*	584.6 ± 36.5*
CA + CBZ	503.8 ± 157.8	504.6 ± 148.9	570.1 ± 69.4	471.3 ± 145.9	485.3 ± 150.2
BM	432.4 ± 206.3	368.9 ± 244.3	582.3 ± 56.0	487.1 ± 182.3	494.3 ± 175.8
BM+ PHT	425.9 ± 222.9	408.7 ± 226.4	514.6 ± 180.9	544.3 ± 130.1	538.6 ± 142.6*
BM + PB	600.0 ± 0.0	567.8 ± 91.2	597.0 ± 8.5	600.0 ± 0.0*	591.3 ± 26.6*
BM + CBZ	517.5 ± 150.1	410.1 ± 147.0	510.9 ± 165.2	555.8 ± 82.5	535.6 ± 111.2*

* Significantly different from saline control ($p < 0.01$).

preparation, *Shankapushpi*, which contains *Centella asiatica*, resulting in breakthrough seizures in epileptics [11].

Other studies showed that PHT pharmacokinetics in the rats were not affected after a single dose with the plant P*aeoniae radix*, possessing anticonvulsant activity, except for significant differences in T max and Vd [16]. When co-administered with PHT, CA and BM shifted the time for peak effect from 4 to 6 h, possibly due to delayed absorption [10].

Depending on the pharmacological properties of herbal medicines, it is feasible to co-administer them along with AEDs, without compromising anticonvulsant efficacy provided comprehensive preclinical profiling is done. The main concern is the possibility of unknown herbal drug/allopathic drug interaction that may adversely influence therapy.

In those animals that exhibited HLTE, phenytoin significantly reduced HLTE duration suggesting that it could limit seizure spread. Unlike phenytoin, both CA and BM showed significantly higher HLTE duration than controls at 6 h. The RR of many groups co-administered CA and BM was higher than controls at 24 h. These results further emphasize the need for caution when co-administering herbal products with standard drugs.

In conclusion, the mild to moderate anti-convulsant activity of *C.asiatica* or *B.monnieri* merits consideration for further investigations. Therapeutic monitoring may be necessary if patients receive both allopathic AEDs and herbal drugs containing *C.asiatica* and *B. monnieri.*

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We dedicate this work to late Dr. Joy David, who originally initiated this study.

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