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Anti-convulsant activity of different extracts of *Centella asiatica* and *Bacopa monnieri* in animals

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Abstract

Objective: To evaluate anti-convulsant profile of different extracts of *Centella asiatica* and *Bacopa monnieri* in rats. Materials and methods: The effects of single oral administration of different preparations of *C.asiatica* and *B.monnieri* were evaluated for their anticonvulsant profile in the Maximal Electroshock Seizure (MES) in rats at 1, 3, 6, and 24 h after administration and Pentylene tetrazole (PTZ) test in mice and rats. The ED_{50} dose of Phenytoin (30 mg/kg) was used for comparison. Results: The crude drug of *C.asiatica* (500 mg/kg) showed mild to moderate anticonvulsant activity, from 1 h to 24 h. The methanolic extract of *C.asiatica* (CA-I) showed higher activity than the crude drug at 3 and 6 h, but there was no anti-convulsant activity at 1 h. The solubulised extract of *C.asiatica* (CA-II) (using Cresmer RH 40 and propylene glycol) at 500 and 1000 mg/kg, also showed a similar profile of activity which was dose-dependent. The crude drug of *B.monnieri* (500 mg/kg) showed mild to moderate activity from 1 h to 6 h but there was no activity at 24 h. The methanolic extract of *B.monnieri* (BM-I) (which was later partitioned between butanol and water) showed a lesser degree of activity only at 3 h and 6 h. The solubilised extract of *B.monnieri* (BM-II) (500 mg/kg) showed mild to moderate activity at 3 h and 6 h with minimal activity at 24 h. At 1000 mg/kg, a comparatively higher degree of activity was seen at 1 h – 6h but not at 24 h. The activity of *B.monnieri* was almost equivalent to Phenytoin at 6 h. In the Pentylenetetrazol (PTZ) chemoshock seizure test, no activity was detected for both plants (500 mg/kg). Conclusion: Overall, *B.monnieri* has a faster onset of action and time/dose responses were qualitatively similar to Phenytoin, while *C.asiatica* had quantitatively lesser activity but had a longer duration of action.

Key words: *Centella asiatica, Bacopa monnieri,* Anti-convulsant activity, Maximal Electroshock Seizure (MES), Pentylene Tetrazole. (PTZ)

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1.Introduction

The recent worldwide interest in traditional forms of healthcare has given an impetus to "bio-prospecting" of plant sources for diverse therapeutic applications and currently well planned studies for detecting their potential pharmacological activity are being actively pursued [1]. In the traditional system of Indian medicine, Ayurveda, *Centella asiatica* Linn.(CA) and *Bacopa monnieri* Linn.(BM) have been used in the treatment of insanity, epilepsy and hysteria [2].

Ashtanga Hridaya states the *B.monnieri*, Brahmi, is the best remedy for epilepsy [2,3]. *C.asiatica* commonly known in India as Mandookaparni, has considerable reputation in indigenous medicine and is frequently confused for *B.monnieri* [4]. The plants often get substituted for each other in the market as both are commonly sold under the same vernacular name 'Brahmi". But Ayurvedic texts and related literature are clear in mentioning the name Brahmi to *B.monnieri.* Comparative anatomical features can differentiate the two species [5]. Chemically both the species are rich in saponins. Madecassoside and asiaticoside are the important saponins of *C.asiatica*, whereas *B.monnieri* contains bacosides A & B.

B. monnieri has been shown to have sedative, cardiotonic, vasoconstrictor and neuromuscular blocking actions [6]. A tranquilising and hypotensive effect has also been reported [7]. In earlier acute pharmacological studies on *B. monnieri* [8], 0.6 g/kg of the aqueous extract and 7 g/kg of the alcoholic extract, administered i.p. to rats, did not alter the flexor or clonic phases of maximal electroshock (MES) induced seizures, but reduced the duration of the extensor phase, though not to a significant extent. Similar results were obtained when the same doses were administered orally to rats for 15 days. Thus, at these doses, there was no abolition of the extensor phase of MES seizures, which is the criteria that has been established to denote evidence of anticonvulsant activity in the MES test [9]. In the pentylenetetrazole (PTZ) test, acute and chronic administration of the aqueous and alcoholic extracts (at the same doses mentioned above), significantly increased the time to onset of convulsions for 4 hours post drug, while mortality was marginally reduced in comparison to controls. These studies showed that the aqueous and alcoholic extracts of *B.monnieri* at the doses employed, did not provide complete seizure protection in either the MES or PTZ tests [8].

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 [10,11]. *C.asiatica* (aqueous extract, 50 mg/kg)given orally to rats did not influence flexion, extension or clonus but significantly reduced the duration of post seizure recovery in the MES induced seizures [12]. In the PTZ test, *C.asiatica* at 50 mg/kg i.p. administered to mice, prolonged the time to onset of convulsions and caused 60% mortality, while control mice had 100% mortality [13]. In Amerindian and African traditional medicine, anti-convulsant activity of *C.asiatica* has yielded differing results. The hydroalcoholic extract has been shown to have anti-convulsant activity against PTZ [14] while the alcoholic extract has been reported to have no anti PTZ activity [15].

In view of the lack of significant consistent activity in classical anticonvulsant tests in rodents and the lack of agreement with proven use of the above two herbal remedies in epilepsy in Ayurveda, the present study was undertaken to systematically evaluate the anti-convulsant of activity in different extracts of the whole plants of *C.asiatica* and *B.monnieri.*

Classical pharmacological screening tests such as the MES test and the chemoshock or PTZ test were employed. Reference standards like Phenytoin and Sodium valproate were used for comparison [9]. The MES test has predictive value for efficacy in clinical Generalized Tonic Clonic Seizures (GTCS) and Phenytoin is the prototype drug for these seizures, while the PTZ test predicts activity in absence seizures and Sodium valproate is the standard anti-absence agent of choice [9].

2.Materials and methods

2.1 Plant material

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 Pharmacognosy 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* [16].

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 [16].

2.2 Preparation of extracts

The crude drugs thus obtained were extracted thoroughly with methanol. The dried methanolic extract of *C.asiatica* (yield 25%w/w) had 5.2% w/w asiaticoside, 7.8% madecassoside and 55%w/w of total saponins. This was referred to as CA-I.

Whole plant of *B.monnieri* was thoroughly extracted with methanol (yield 14%w/w). The methanolic extract was then partitioned between butanol and water. Extraction with butanol was carried out to separate the organic components from inorganic and high molecular weight components like carbohydrates, etc. The dried butanolic extract (yield – 7%w/w) had a content of 40% w/w bacoside-A. This was called BM-I.

The dried methanolic extract of *C.asiatica* and the dried butanolic extract of *B.monnieri* were solubilised using 4%w/w glycerol polyethylene glycol hydroxy stearate (Cresmer RH 40) and propylene glycol 6%w/w. The pH of 10% aqueous solution of Cresmer RH 40 was found to be in the range of 6 to 7. These extracts are referred to as CA-II and BM-II respectively.

Crude drug of both plants as well as CA-I and BM-I were triturated with distilled water and administered orally to rats in a volume of 0.4ml/100g. The slurries thus obtained were thick and difficulty was encountered during gavaging, limiting the maximum oral dose of aqueous suspensions of crude drug and CA-I and BM-I to 500 mg/kg. However CA-II and BM-II could be administered in oral doses of 500 and 1000 mg/kg.

The standard reference agents - Phenytoin/Dilantin suspension, 30 mg/kg (Parke Davis, India, Ltd.) in the MES test and Sodium

Valproate (Reckitt Colman India Ltd.) 200 mg/kg in the PTZ test were administered orally.

2.3 Animals

Adult male Wistar rats (220-250g) and male albino mice (20-25g), housed in standard environmental conditions of temperature of 27 ± 1°C and on a 12h dark/light cycle were used. They were fed with standard rat pellet diet (Hindustan Lever India Ltd.) and water *ad libitum*, except during experimental sessions. All animal experiments were conducted in humane conditions, in accordance with NIH guidelines (NIH publication, No.80-23; revised 1978).

2.4 Anti-convulsant activity against Maximal Electroshock Seizures (MES) in rats

Animals were randomly divided into groups of eight (n>=8). Independent groups of animals were used for each time point tested. Groups received the crude drug, CA-I and BM-I at 500 mg/kg (po), CA-II and BM-II at doses of 500 and 1000 mg/kg (po), or Phenytoin 30 mg/kg (po). Control animals received equivalent amounts of the respective vehicle.

Experiments were conducted at the same time each day and the rats were subjected to MES at 150 mA, 60 Hz for 0.2 sec [9] through pinnal electrodes at 1, 3, 6 and 24 h after vehicle/drug administration. In all electrically induced convulsions, the rats are manually restrained and released immediately after stimulation to permit observation of the seizure throughout its entire course. MES results in Hind Limb Tonic Extension (HLTE), the duration being measured in seconds.

Rats were pre-tested 24 h prior to drugging (baseline values) and those failing to give HLTE were rejected. The criterion for anti-convulsant activity and protection against MES induced seizures is abolition of HLTE, which is taken as the end point of the test. The tonic extensor component is considered abolished if the HLTE does not exceed a 90° angle with the plane of the body. The MES test [9] was done according to the criteria followed in NIH, USA and followed in the Anti-convulsant Drug Development Program [17].

Another parameter that was measured was the period of post ictal depression (PID) in sec, which follows HLTE. PID is measured from the end of HLTE till the rat regains the righting reflex and walks away.

2.5 Anti-convulsant activity against Chemoshock (PTZ) induced seizures in mice and rats

Seizures were induced in mice or rats with PTZ at 70 mg/kg ip, which is the convulsive dose in 97% of the animals [9]. PTZ was dissolved in 0,9% saline and injected ip in mice at 0.1 ml/10g and rats at 0.2ml/100g. Animals were placed in isolation cages and observed for the next 30 min for the presence or absence of clonic spasms, persisting for at least 5 sec and for % mortality observed. Absence of clonic seizures and reduction or mortality (the above dose produced 100% mortality) are indices of anti-convulsant activity.

2.6 Statistical analysis

The mean and Standard deviation was calculated for all the groups. Fischer's test was used to compare the presence/absence of hind limb tonic extension. The one-way ANOVA was used to compare the HLTE data of the various groups at baseline. The HLTE data obtained in the MES test were analyzed using appropriate models of analysis of variance.

3. Results

3.1 Anti-convulsant activity of different extracts of C.asiatica and B.monnieri against MES induced seizures in rats

Table 1.
Mean ± standard deviation hind limb tonic extension duration (sec) in rats receiving different crude and standard extracts of CA, BM (500mg/kg) and Phenytoin (30 mg/kg) at different times.

Group	Baseline	1 h	3 h	6 h	24 h
Saline	10.1 ± 3.0	10.6 ± 3.4	9.7 ± 1.9	8.4 ± 1.6	8.8 ± 1.1
Crude CA	11.5 ± 2.4	7.4 ± 4.8	11.1 ± 5.0	11.1 ± 4.8	8.0 ± 5.0
CA – I	11.3 ± 1.3	9.4 ± 1.0	8.2 ± 5.2	7.4 ± 4.7	9.9 ± 4.6
Crude BM	10.8 ± 2.7	7.7 ± 5.1	7.9 ± 6.6	8.5 ± 7.2	10.2 ± 2.1
BM – I	10.3 ± 2.9	10.7 ± 1.5	8.3 ± 3.6	6.5 ± 4.1	8.7 ± 1.9
Phenytoin	11.4 ± 1.3	2.9 ± 3.3	6.3 ± 6.8	7.4 ± 5.5	11.8 ± 1.9

There was no significant difference between the groups at baseline groups; Main effect for Time and Group: not significant; Time x Group interaction effect: $p < 0.05$; CA- *Centella asiatica.*, BM - *Bacopa monnieri*

In Figs.1and 2, comparative anti-convulsant profiles of the crude drugs and the different extracts of *C.asiatica* and *B.monnieri* are represented along with the reference agent Phenytoin at 30 mg/kg. The saline and vehicle treated rats had 0% protection at all time points and are not included in the figures. Phenytoin, at its reported oral ED_{50} dose [9] showed 50% protection against MES induced seizures at 1 and 3 h and 31% at 6h post drug, with no activity at 24 h.

Fig 1 shows that CA (crude drug), 500 mg/kg showed moderate anticonvulsant activity at 1 h, and marginal activity at 3 h, 6 h, and 24 h. CA-I at 500 mg/kg did not show protection at 1h, but had moderate activity at 3 and 6 h which declined at 24 h. CA-II at 500 and 1000 mg/kg had no activity at 1 h. CA-II at 500 mg/kg showed maximum activity at 3 h but was reduced thereafter. CA-II at 1000 mg/kg showed an almost equal level of protection at 3, 6 and 24 h.

Overall, extracts had approximately one fourth to one third the activity of Phenytoin, but interestingly, in contrast to Phenytoin, protection persisted for 24 h.

The activity of crude BM, 500 mg/kg, commenced at 1 h (Fig 2), while maximal protection occurred at 3 and 6 h, but none at 24 h. BM-I at 500 mg/kg, had a lesser degree of activity than crude BM which was present at 3 and 6 h. BM-II at 500 and 1000 mg/kg showed moderate activity.

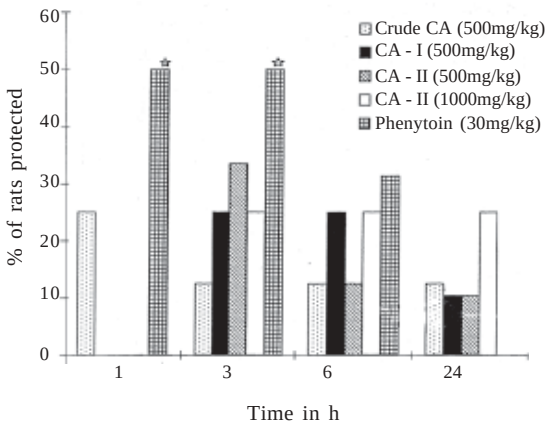
Crude BM and BM-II (500 and 1000 mg/kg) showed 38% protection at 6 h, while Phenytoin showed 31% at 6 h. However, like Phenytoin, *B.monnieri* did not show protection at 24 h, except for BM-II (500 mg/kg) which showed marginal protection.

Thus, in the MES test in rats, both plant extracts showed protection, with *B.monnieri* displaying a quantitatively greater degree of activity than *C.asiatica*, but in the latter case activity persisted up to 24 h.

3.2 Effect of plant products on duration of HLTE

The crude drug and the different extracts of *C.asiatica* and *B.monnieri*, and Phenytoin were compared to saline treated controls. Baseline HLTE duration data in secs are presented in Table 1. One-way ANOVA showed that the HLTE duration data did not differ significantly between the six groups ($F=0.6774$, $df=5,191$, $p=0.6422$), indicating that the groups were similar at baseline.

HLTE duration data measured at baseline, 1 h, 3 h, 6 h and 24 h after the administration of different herbal extracts, Phenytoin or saline are



* Significantly (P<0.05) different from controls (0% protection)

Figure 1. Anticonvulsant activity of *C.asiatica*

also presented in Table 1. The main effect for time was not significant, indicating that overall, irrespective of the group, HLTE duration did not vary across the different time points.

The main effect for Group was also not significant, indicating that there was no significant change in HLTE duration across the different groups. But the Time x Group interaction was significant (F=2.144, df=15,229, p=0.019) indicating that the pattern of change was significantly different between the various groups.

The effects of CA-II and BM-II (solubilised extracts) 500 mg/kg and 1000 mg/kg versus vehicle are shown in Tables 2 and 3 respectively. At both doses, one-way ANOVA showed that the HLTE duration did not differ significantly between the three groups, indicating that the groups were similar at baseline.

Table 2 shows that the HLTE duration data measured at baseline, 1h, 3h, 6h and 24 h after the administration of different herbal extracts or vehicle, reveal that the main effects for Time, Group and Time x Group interaction effect were all non significant. Likewise, at higher dose, the main effect for Group was non significant, indicating that overall, there was no significant difference between the groups.

However, Table 3 shows that the main effect for Time was significant (F = 3.106, df = 3,87, p = 0.031) indicating that overall, there was a change in HLTE duration across the different time points. The Time x Group interaction effect was not significant.

3.3. Effects of different herbal extracts on post ictal depression (PID)

None of the crude drug or extracts of both plants produced any significant effect on PID duration.

Table 2.
Mean ± standard deviation hind limb tonic extension duration (sec) in rats receiving solubilised extracts of CA-II, BM-II at 500 mg/kg or vehicle at different times.

Group	Baseline	1 hour	3 hours	6 hours	24 hours
Vehicle	11.3 ± 2.1	10.2 ± 1.7	9.5 ± 2.4	9.3 ± 1.4	10.2 ± 1.5
CA – II	10.0 ± 1.8	9.6 ± 2.4	6.9 ± 5.5	10.3 ± 4.7	9.0 ± 3.9
BM – II	10.1 ± 3.4	10.6 ± 2.6	9.4 ± 5.0	6.3 ± 5.5	10.4 ± 3.3

There was no significant difference between the groups at baseline; Main effect for Time, Main effect for Group and Time x Group interaction-NS; CA- *Centella asiatica.*, BM - *Bacopa monnieri*

Table 3.
Mean ± standard deviation hind limb tonic extension duration (sec) in rats receiving solubilised extracts of CA-II, BM-II at 1 g/kg or vehicle at different times.

Group	Baseline	1 hour	3 hours	6 hours	24 hours
Vehicle	10.8 ± 2.9	10.5 ± 2.6	8.8 ± 1.7	8.9 ± 2.4	8.3 ± 0.6
CA – II	11.4 ± 1.8	10.4 ± 2.0	6.1 ± 3.9	5.3 ± 3.6	7.6 ± 4.7
BM – II	10.0 ± 2.4	7.3 ± 4.7	6.0 ± 4.2	5.4 ± 4.6	9.2 ± 1.6

There was no significant difference between the groups at baseline ; Main effect for Group and Group x Time interaction effect - NS; Main effect for Time: $p < 0.05$; CA- *Centella asiatica.,* BM - *Bacopa monnieri*

3.4. Anticonvulsant activity against PTZ induced seizures in mice and rats

In the PTZ chemoshock test, the reference again Sodium Valproate 200 mg/kg p.o showed complete protection and absence of clonic seizures or mortality in both mice and rats. None of the crude drug or different extracts of *C.asiatica* or *B.monnieri* afforded any protection against PTZ induced seizures and 100 % mortality occurred in both mice and rats at all doses.

4. Discussion

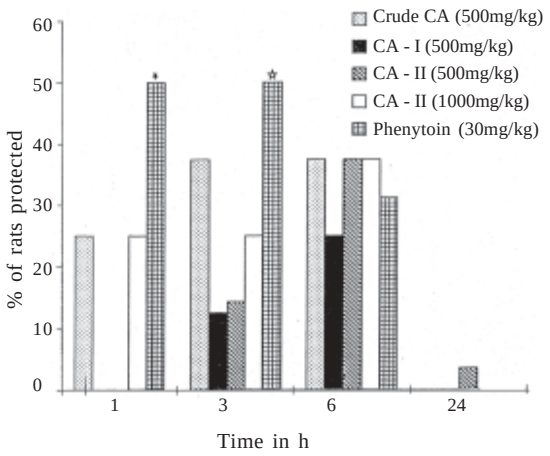
The results obtained in the MES test in rats showed that crude drug as well as different extracts of *C.asiatica* and *B.monnieri,* protected against MES induced seizures. *C.asiatica* had a slower onset of action than *B.monnieri* and had a quantitatively lesser degree of anticonvulsant activity (approximately, one third the activity of Phenytoin) but the activity lasted for 24 h. This could suggest the possibility of active metabolite/s. In comparison, *B.monnieri* had a fast onset of action which was about half to three fourths the activity of Phenytoin, but like Phenytoin lasted only for 6 h. The anticonvulsant profile of activity of the crude drug of *B.monnieri* as well as BM-II at 1000 mg/kg was similar to that of Phenytoin, but of lesser intensity.

The results obtained in these studies, demonstrate unequivocally that like Phenytoin, both *C.asiatica* and *B.monnieri,* possessed anticonvulsant activity in the MES test, predicting their potential to control clinical GTCS, and corresponding to the observation in Ayurvedic medicine.

Abolition of HLTE, the decisive laboratory parameter for establishing anticonvulsant activity [9], was not evident in previous studies for *H.monnieri* following acute or chronic administration [8]. However, our results differ from previous studies [8,14] as neither plant product protected rats against PTZ induced seizures. This could be attributed to species difference between the plants or the method of extraction.

Moreover, in the study of De Lucia *et al.* [14], only the leaves of *C.asiatica* were used, whereas in our study the whole plant has been used. The crude drug of both plants had moderate activity. In comparison the different extracts, in general, showed less activity suggesting that the glycosides, asiaticosides and bacosides, did not contribute to the anticonvulsant activity.

Solubilization improved activity of both plants, presumable due to better absorption, especially for *B.monnieri* (BM-II, 500 and 1000 mg/kg)



* Significantly (P<0.05) different from controls (0% protection)

Figure 2. Anticonvulsant activity of *B. monnieri*

which showed almost 39% protection at 6h post drug. Notably, at the higher dose of 1000 mg/kg, both plant products showed 50% reduction in HLTE duration suggesting the possibility that like Phenytoin, they could limit seizure spread.

The results conclusively demonstrate that in accepted standard pharmacological screening tests [9], single doses of both *C.asiatica* and *B.monnieri* possessed anticonvulsant activity in the MES test and it is likely that following chronic administration their efficacy may improve considerably.

In traditional medicine, if a plant is claimed to treat epilepsy in one or more countries, or potential anticonvulsant activity has been reported from different continents, as in the case of *C.asiatica* [13,14], this would support the scientifically obtained evidence that the claims are authentic and worth investigating.

Laboratory studies are currently fortifying the tenets of ancient wisdom derived from herbal remedies. Researchers are gaining new insight into traditional medicine in assisting the body to maintain its own self-healing systems while preventing debilitating effects of chronic diseases, like epilepsy.

This study addressed the question of defining the pharmacological activity of *C.asiatica* and *B.monnieri,* which are well known remedies in Ayurveda for the treatment of epilepsy, and confirmed the presence of their anticonvulsant activity in animals.

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