

EVALUATION OF HYPNOTIC ACTIVITY OF *BACOPA MONNIERI* (BRAHMI) LEAVES EXTRACT IN RATS

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ABSTRACT

Insomnia refers the problem with sleep duration or quality, as well as difficulties initiating or sustaining sleep, are common symptoms, as can significantly distress and impairments in daytime functioning. This research focuses on pharmacological screening of hypnotic effect of hydroalcoholic leaves extract (HLE) of *Bacopa monnieri* (Brahmi) in Wistar albino rats by using diverse types of animal models to confirm its actual pharmacological potential. Leaves of *Bacopa monnieri* (Brahmi) were obtained from the Unnao region. It was identified and authenticated by a botanist. The leaves were washed making dust-free and dried at room temperature or shade. The dried leaves are rendered into coarse powders and then finally into fine ones and extracted separately into 3 solvents i.e., distilled water, ethanol and methanol using percolator. Rats were divided into group 1 (normal saline), group 2 (Diazepam 4mg/kg), group 3a & 3b (Aq. BM leaves extract- 80mg/kg & 120mg/kg), group 4a & 4b (Eth. BM leaves extract- 80mg/kg & 120mg/kg) and group 5a & 5b (Meth. BM leaves extract- 80mg/kg & 120mg/kg). Several parameters were estimated as Thiopental sodium-induced sleeping time estimation, Motor co-ordination (Rota-rod) test, Light-Dark Arena Model and Locomotion Activity. In results, it confirmed that BM is much significant in the preclinical treatment of insomnia and related issues. Its mode of action may be suppression of neurotransmitters release, increase the destruction of catecholamines. In conclusion, leaves of BM are efficacious in the subsiding the insomnia and reset mind to induce sleep. It suggests to isolate the significant active component towards the activity and convert into suitable dosage for better absorption and tolerability.

KEYWORDS: Insomnia, Brahmi, *Bacopa monnieri*, Thiopental sodium-induced sleeping time.

INTRODUCTION

Insomnia refers the problem with sleep duration or quality, as well as difficulties initiating or sustaining sleep, are common symptoms, as can significantly distress and impairments in daytime functioning (Ohayon & Reynolds, 2009; Roth et al. 2011). Insomnia symptoms are frequent, with one in every three people experiencing them in the previous year (Johnson, 2006). The incidence of a formal diagnosis of insomnia is 615 percent, while percentages vary depending on the definition (Roth, 2007). In addition to having a personal history of insomnia, 35% of people have a family history of insomnia (Beaulieu-Bonneau et al. 2007). Insomnia is linked to a lower socioeconomic status, a lack of education, and being divorced or widowed (Bixler et al. 1989).

Bacopa monnieri (BM) is also called as brahmi that is creeping perennial native to Australia and India with small rectangular leaves and purple flowers (Barrett & Strother, 1978). Although several chemical components have been identified from Brahmi, bacoside-A and bacoside-B are found in the active parts of this medicinal plant. Alkaloids, glycosides, flavonoids, saponins, and other phytochemicals are among the constituents of (Mathur et al. 2016).

On the basis of above literature survey, I found that previous researches just indicate the hypnotic effect of leaves extract of *Bacopa monnieri* (Brahmi) not separately but in cumulative form with other herbs, using animal models.

So, this research focuses on pharmacological screening of hypnotic effect of hydroalcoholic leaves extract (HLE) of *Bacopa monnieri* (Brahmi) in Wistar albino rats by using diverse types of animal models to confirm its actual pharmacological potential.

MATERIALS AND METHODS

Materials

Bacopa monnieri (Brahmi) hydroalcoholic leaves extract (HLE), Thiopental sodium, Diazepam, distilled water, Wistar albino rats (either sex), and ethanol.

Weighing machine, Rotatory evaporator/Water bath, Rota-rod apparatus, Light dark arena.

Collection, Identification & Authentication of plant

Leaves of *Bacopa monnieri* (Brahmi) were obtained from the Unnao region. It will be identified and authenticated by a botanist. The leaves are washed making dust-free and dried at room temperature or shade. The dried leaves are rendered into coarse powders and then finally into fine ones. The powder is weighed and extracted separately into 3 solvents i.e., distilled water, ethanol and methanol using percolator (Khan et al. 2020).

Extraction of AI leaves

The powder is weighed and soaked separately into 3 solvents i.e., distilled water, ethanol and methanol for fifteen days with gradual stirrings.

Cold aqueous percolation

A percolator is a tall cylindrical jar with a cone bottom and a constructed fake bottom with a filter cloth that is used to remove plant material. For solvent removal from the marc, the percolator is attached to a condenser and a receiver.

Water/ethanol/methanol is used to macerate the powdered material, which is then poured into a tall column. The powdered substance is immersed in cold water until it is completely submerged. Water-soluble components are allowed to reach equilibrium in the water after 24 hours. The aqueous enhanced extract is condensed to a specific concentration in multiple-effect evaporators. This concentrated extract is diluted and then ready to be used as a medicine (Handa et al. 2008).

Preparation of animals

Animal House, Institute of Pharmaceutical Sciences and Research (IPSR), Unnao will provide albino rats of either sex weighing 150–200 g. The animals are kept in good health, with room temperatures of 25°C and a 12-hour light/dark cycle. The relative humidity is kept at 44–56 percent, and the rats are provided a regular rodent diet and free access to water. The animals will continue to fast but have free access to water until 1 hour before the ulcers are induced (Bhajoni et al. 2016).

Group design

All the rats are divided into 4 groups (n=6) as followings-

Group 1: Rats are administered only distilled water once a day for 15 days.

Group 2: Rats are administered Diazepam (4mg/kg) orally, for 15 days.

Group 3 & 3a: Rats are administered Aqueous leaves extract of *Bacopa monnieri* 80mg/kg & 120mg/kg orally, for 15 days (Hazra et al. 2012).

Group 4 & 4a: Rats are administered Ethanolic leaves extract of *Bacopa monnieri* 80mg/kg & 120mg/kg orally, for 15 days (Hazra et al. 2012).

Group 5 & 5a: Rats are administered Methanolic leaves extract of *Bacopa monnieri* 80mg/kg & 120mg/kg orally, for 15 days (Hazra et al. 2012).

Protocols

1. Thiopental sodium-induced sleeping time estimation

The sleeping period induced by thiopental sodium was measured using the method reported previously. Thiopental sodium (20mg/kg) was given intraperitoneally to each rat at a dose of 4 mg/kg, 30 minutes after vehicle or BM therapy and 15 minutes after diazepam treatment. For a period of time, animals were observed to lose their ability to balance. Shortly following Diazepam injection (latent response) period and sleep duration (time between loss and resumption of sleep) TS causes the recovery of reflexes (Turner, 1965).

2. Motor co-ordination (Rota-rod) test

A horizontal rotating rod (Ugo Basile, Varese, Italy) was used in this test, which rotated at a pace of 20 rotations per minute. Mice that could stay on the rod for more than 180 seconds were chosen and divided into groups. Each mouse was placed on the rod for 180 seconds half an hour after receiving the vehicle or drug, and diazepam was given 15 minutes before the experiment. The time it took each mouse to fall from the rotating pole was then recorded (Fujimori & Cobb, 1965).

3. Light-Dark Arena Model

In light-dark arena model, a 100Watt bulb is being placed 30 cm above to base of box. Rats are kept in centre of light arena (box) and have to expose for 5 minutes. No. of entries and time spent in light arena segment are recorded till 5 minutes. It is cleansed every time before keeping a new rat (Khan et al. 2020).

4. Locomotion Activity

Actophotometer is tuned on to check and make sure that all the photocells are working properly for accurate readings. Rats are placed once at a time in the activity cage for 10 min. Activity score is recorded for each rat till 10 min. Finally, motor activity is observed and compared with standard drug- Imipramine (Kulkarni, 1999).

RESULTS AND DISCUSSION

1. Thiopental sodium-induced sleeping time estimation

In this model, onset of sleep and sleep duration (min) was evaluated in rats- a most reliable model. Onset on sleep was estimated as $7.52 \pm 0.31^{**}$ in control and $4.82 \pm 0.20^{**}$ in Diazepam treatment group whereas sleep duration (min) was estimated as $78 \pm 2.57^{**}$ and $183 \pm 4.11^{***}$ in control and standard group respectively. Aq. BM leaves extract exhibited onset of sleep as $6.56 \pm 0.13^{**}$ and sleep duration (min) as $131 \pm 3.20^{***}$ at its lower dose 80mg/kg. Ethanolic BM leaves extract

significantly lowered the onset of sleep and sleep duration as well. At 120mg/kg dose it demonstrated onset of sleep as $5.20 \pm 0.12^{**}$ and sleep duration as $161 \pm 2.16^{**}$. When the effects were compared among different extracts, it represented that ethanolic extract has much potential specially at higher dose.

BM confirms for its hypnotic potential in all the doses when compared with control group. Its effect was similar and near to standard in ethanolic extract at 120mg/kg when tested.

Table 1: Thiopental sodium-induced sleeping time estimation.

Treatment	Effects on sleep	
	Onset of sleep (min)	Sleep duration (min)
Control (Normal saline)	$7.52 \pm 0.31^{**}$	$78 \pm 2.57^{**}$
Diazepam (4mg/kg)	$4.82 \pm 0.20^{**}$	$183 \pm 4.11^{***}$
Aq. BM leaves extract (80mg/kg)	$6.56 \pm 0.13^{**}$	$131 \pm 3.20^{***}$
Aq. BM leaves extract (120mg/kg)	$5.37 \pm 0.51^{**}$	$156 \pm 5.27^{***}$
Eth. BM leaves extract (80mg/kg)	$6.23 \pm 0.39^{**}$	$135 \pm 4.29^{***}$
Eth. BM leaves extract (120mg/kg)	$5.20 \pm 0.12^{**}$	$161 \pm 2.16^{**}$
Meth. BM leaves extract (80mg/kg)	$6.35 \pm 0.52^{***}$	$139 \pm 3.52^{**}$
Meth. BM leaves extract (120mg/kg)	$5.51 \pm 0.17^{***}$	$151 \pm 5.21^*$

Significance Level= *

Values were given in Mean \pm S.E.M. and found statistically significant at $P < 0.05$, compared to control (n=6)

2. Motor Co-ordination (Rota-rod) test

Rota-rod was used to test motor co-ordination behavior of animals. In control group the motor co-ordination was observed in seconds as $120 \pm 2.41^*$ and in standard group as $24 \pm 3.22^{**}$ seconds. Whereas, aqueous BM leaves extract exhibited motion as $106 \pm 2.19^{**}$ at the dose of 80mg/kg and $69 \pm 3.18^*$ at the dose of 120mg/kg. Ethanolic BM leaves extract showed motor co-ordination as $102 \pm 2.63^{**}$ and $55 \pm 4.62^{**}$ at 80mg/kg and 120mg/kg respectively. When we compared among all the treated groups, it was found that maximum inhibition was found in ethanolic extract at higher dose.

However, BM proved itself as potent hypnotic drug when compared with compared and standard drug- Diazepam.

Values were given in Mean \pm S.E.M. and found statistically significant at $P < 0.05$, compared to control (n=6)

The % Inhibition confirms for its hypnotic potential extent. Among all groups, highest % inhibition was noted in ethanolic extract at higher dose.

Table 2: Motor Co-ordination (Rota-rod) test.

Treatment	Rota-rod movement (sec)
Control (Normal saline)	$120 \pm 2.41^*$
Diazepam (4mg/kg)	$24 \pm 3.22^{**}$
Aq. BM leaves extract (80mg/kg)	$106 \pm 2.19^{**}$
Aq. BM leaves extract (120mg/kg)	$69 \pm 3.18^*$
Eth. BM leaves extract (80mg/kg)	$102 \pm 2.63^{**}$
Eth. BM leaves extract (120mg/kg)	$55 \pm 4.62^{**}$
Meth. BM leaves extract (80mg/kg)	$105 \pm 5.34^*$
Meth. BM leaves extract (120mg/kg)	$65 \pm 5.32^*$

Significance Level= *

Table 2a: % Inhibition in Rota-rod test.

Treatment	% Inhibition
Control (Normal saline)	0.00
Diazepam (4mg/kg)	73.32
Aq. BM leaves extract (80mg/kg)	22.12
Aq. BM leaves extract (120mg/kg)	58.19
Eth. BM leaves extract (80mg/kg)	27.52
Eth. BM leaves extract (120mg/kg)	67.39
Meth. BM leaves extract (80mg/kg)	26.49
Meth. BM leaves extract (120mg/kg)	62.57

Significance Level= *

Values were given in Mean \pm S.E.M. and found statistically significant at $P < 0.05$, compared to control (n=6).

3. Light-dark arena model

In light/dark arena test, no. of entries, time spent and % of time spent in light arena were recorded for 5 min. There was lowest no. of entries recorded in light arena.

In Diazepam treated rats, no. of entries in light arena was recorded as $9.00 \pm 0.45^{**}$ and time spent 155 ± 0.81 sec and thus % of time spent as $51.79 \pm 0.29^{**}$ which was

highest among all. Aq. BM leaves extract also showed increased no. of entries and time spent in light arena as $6.45 \pm 0.27^*$ and 95 ± 0.68 respectively at the dose of

80mg/kg. Whereas, ethanolic BM leaves extract exhibited $8.19 \pm 0.20^{**}$ (no. of entries) and 135 ± 0.50 (time spent) in light arena at the dose of 120mg/kg.

Table 3: Light/dark arena test.

Treatment	No. of entries (light arena)	Time spent (light arena) (s)	% of time spent (light arena) (s)
Control (Normal saline)	$5.24 \pm 0.32^{**}$	68.12 ± 0.72	$22.70 \pm 0.12^{**}$
Diazepam (4mg/kg)	$9.00 \pm 0.45^{**}$	155.39 ± 0.81	$51.79 \pm 0.29^{**}$
Aq. BM leaves extract (80mg/kg)	$6.45 \pm 0.27^*$	95.52 ± 0.68	$31.84 \pm 0.09^*$
Aq. BM leaves extract (120mg/kg)	$7.21 \pm 0.49^{**}$	115.20 ± 0.57	$38.40 \pm 0.41^{**}$
Eth. BM leaves extract (80mg/kg)	$6.71 \pm 0.51^{**}$	97.18 ± 0.71	$32.39 \pm 0.39^{**}$
Eth. BM leaves extract (120mg/kg)	$8.19 \pm 0.20^{**}$	135.52 ± 0.50	$41.70 \pm 0.24^*$
Meth. BM leaves extract (80mg/kg)	$6.24 \pm 0.11^{**}$	91.27 ± 0.45	$30.42 \pm 0.29^*$
Meth. BM leaves extract (120mg/kg)	$7.93 \pm 0.57^{***}$	119.18 ± 0.61	$39.72 \pm 0.34^{**}$

Significance Level= *

Values were given in Mean \pm S.E.M. and found statistically significant at $P < 0.05$, compared to control (n=6)

4. Locomotion activity

In screening of hypnotic effect, the rats were divided in 8 groups. Group 3 & 3a was given aqueous extract of BM leaves in dose of 80mg/kg and 120mg/kg respectively. Whereas, group 4 & 4a served ethanolic BM leaves extract and group 5 & 5a given BM leaves that extracted by methanol solvent using percolation process. Group 1 was served only normal saline and Group 2 was administered Diazepam (4mg/kg).

In locomotor activity score test, highest activity was achieved in control as $160 \pm 0.59^*$ whereas lowest activity score as found in Diazepam treated group as $91 \pm 0.63^{**}$ in 10 min. Activity score was exhibited as $124 \pm 0.67^{**}$ and $104 \pm 0.71^*$ at the dose of 80mg/kg and 120mg/kg respectively, of ethanolic BM leaves extract. It was highest inhibition when compared with other 2 extracts (methanolic and aqueous). BM at both the doses, significantly demonstrated hypnotic activity when observed.

Table 4: Locomotion activity score.

Treatment	Locomotor activity score (Sec \pm SEM)
Control (Normal saline)	$160 \pm 0.59^*$
Diazepam (4mg/kg)	$91 \pm 0.63^{**}$
Aq. BM leaves extract (80mg/kg)	$135 \pm 0.77^{**}$
Aq. BM leaves extract (120mg/kg)	$109 \pm 0.85^*$
Eth. BM leaves extract (80mg/kg)	$124 \pm 0.67^{**}$
Eth. BM leaves extract (120mg/kg)	$104 \pm 0.71^*$
Meth. BM leaves extract (80mg/kg)	$127 \pm 0.94^{**}$
Meth. BM leaves extract (120mg/kg)	$106 \pm 0.81^{**}$

Significance Level= *

Values were given in Mean \pm S.E.M. and found statistically significant at $P < 0.05$, compared to control (n=6)

In another context, it may enhance the release of inhibitory neurotransmitter GABA (Gamma Amino Butyric Acid) that facilitates the influx of Chloride ions and causes hyperpolarization that in turn increases suppression of neurotransmitters release. Therefore, leaves have been proved by this study for having hypnotic activity.

In results, it confirmed that BM is much significant in the preclinical treatment of insomnia and related issues. Its mode of action may be suppression of neurotransmitters release, increase the destruction of catecholamines. Moreover, it lowers the concentration of biogenic amines thus sleepiness is reversed.

CONCLUSION

In conclusion, leaves of BM are efficacious in the subsiding the insomnia and reset mind to induce sleep. But its mode of action is needed to unfold for better selection of drug in different aspects of mental disorders.

It suggests to isolate the significant active component towards the activity and convert into suitable dosage for better absorption and tolerability.

SOURCE OF FUNDING

Nil.

CONFLICT OF INTEREST

None.

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