Effect of Herbals on Sleep and their Interactions with Hypnotic Drugs

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The aim of the present study was to investigate the sleep promoting effect of herbals and their interaction with GABAergic drugs. Pentobarbitone-induced hypnosis test was used in order to prove the above hypothesis. Seep latency and total sleep time were used as parameters for the evaluation. *Ashwagandha* (*Withania somnifera*) or BR-16A (Mentat®) potentiated the effect of triazolam (0.1 mg/kg, i.p.). However, ashwagandha did not produce any significant effect with alprazolam (0.25 mg/kg, i.p.). Also melatonin (5.0 mg/kg, i.p.) and buspirone (1.0 mg/kg, i.p.) did not produce any significant effect on sleep parameters along with ashwagandha or BR 16A (100mg/kg, p.o., P<0.05). Sleep promoting effect of BR-16A might be due to presence of *ashwagandha*. Its combination with GABAergic drugs (triazolam and alprazolam,) suggested that these drugs have common mechanism in sleep promoting effect of pentobarbitone and could be used along with other GABAergic hypnotics for the treatment of insomnia. This may reduce the dose of the latter drug(s). BR-16A or *ashwagandha* can be used for the treatment of sleep and related disorders.

Sleep is an essential part of the everyday life both for animals and human beings. Any disturbance in the fulfillment of sleep requirement leads to sleep disorders. Benzodiazepines are widely used therapeutic agents for sleep disorders[1]. However despite intense research, the use of these agents is limited by the development of tolerance to their effect and risk of developing dependence. A number of other hypnotics are used for the treatment of insomnia with varying success rates[2]. In recent years there is an increased awareness of use of herbals preparations for treating various ailments including sleep and related disorders[3-4]. This may be due to the fact that herbal prepartions are safe.

BR-16A (Mentat®), a herbal psychotropic preparation contains the following indigenous ingredients: *Brahmi (Hydrocotyle asiatica), Shatavari (Asparagus racemosus), Buchh (Acorus calamus), ashwagandha (Withania somnifera), giloi (Tinospora cordifolia), amla (Embelica officinalis), Shankhpusphi (Evolvulus alsinoides), Kuth (Saussurea lappa) and Triphala.* LD_{50} value of BR-16A has been reported to be 2400 mg/kg by oral route of administration (personal communication). Similarly *ashwagandha* is an important herb from the Ayurvedic system of medicine and used for the treatment of stress, immunomodulation, inflammation and epilepsy[5-8]. Acute LD_{50} within 24 hours was reported as 1260 mg/kg and subacute toxicity studies with repeated intraperitoneal injection of *Withania* extract at a dose of 100 mg/kg for 30 days in rat did not result in any mortality[9]. In the present study attempt has been made to explore the action of BR-16A or ashwagandha in sleep pattern i.e. pentobarbitone-induced hypnosis in animals and its interactions with GABAergic drugs used in the treatment of sleep disorder in human beings.

Laca mice of either sex (weighing 20-25 g), bred in Central Animal House facility of Panjab University, were used. The Animals were housed under standard light/dark cycle with food and water provided *ad libtum*. Animals were acclimatized to laboratory condition before test. Each animal was used once in the experiment. The experiments were performed between 0900 and 1700 hrs. The experimental protocols were approved by the Institutional Animal Ethics Committee and were conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals. Following drugs and their dosages were used in the study: BR-16A (100 mg/kg, p.o., Himalayan Drug Co., Bangalore), ashwagandha root extract (100 mg/kg, p.o., Himalayan Drug Co., Bangalore), melatonin (2.5, 5.0 mg/kg, i.p., Dabur India Ltd, Sahiabad UP), triazolam (0.1 mg/

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kg, i.p., Upjohn, Kalamazoo, USA), alprazolam (0.25 mg/kg, i.p., Upjohn Co. Kalamazoo, MI), buspirone (1.0 mg/kg, i.p.), and pentobarbitone sod (45 mg/kg, i.p., Sigma, MO, USA). Melatonin was dissolved in a few drops of dimethylsulfoxide (DMSO) and the volume made up with distilled water. Triazolam was dissolved in one drop of dilute hydrochloric acid and the volume made up with distilled water (pH 7.5). Other drugs were dissolved in distilled water. Drugs were administered 30 min before pentobarbitone administration to overnight-fasted animals. Doses were selected on the basis of previous studies conducted in our laboratory and reported in the literature. The data were expressed as Mean±SEM and analyzed by one-way analysis of variance (ANOVA) followed by Dunnett test. In all the tests, the criterion for statistical significance was $P<0.05$

Effect of BR-16A or ashwagandha on pentobarbitone induced hypnosis: BR-16A (100 mg/kg, p.o) or ashwagandha (100 mg/kg, p.o.) dose dependently shortened the sleep latency (onset) and increased the total sleep time (duration of sleep) due to pentobarbitone. The effect of BR-16A or ashwagandha on total sleep time was found to be significant as compared to control group at $P<0.05$ (Table 1)

Effect of melatonin, triazolam, alprazolam, and buspirone on pentobarbitone-induced sleep: melatonin (2.5 mg/kg, i.p.), triazolam (0.1 mg/kg, i.p.), alprazolam (0.25 mg/kg, i.p), and buspirone (1.0 mg/kg, i.p.), also decreased the sleep latency (onset of sleep) and increased total sleep time (duration of sleep) significantly. $P<0.05$ (Table.1)

When BR-16A (100 mg/kg, p.o) was administered in combination with triazolam (0.1 mg/kg, i.p.) or alprazolam (0.25 mg/kg, i.p.), a potentiation of effect was observed i.e. significant prolongation of total sleep time was observed as compared to effect *per se* ($P< 0.05$, Table 2). However, sleep latency was not decreased significantly as compared to effect *per se* ($P<0.05$). Similarly *ashwagandha* (100 mg/kg, p.o.) when given with triazolam (0.1 mg/kg, i.p.) or alprazolam (0.25 mg/kg, i.p.), a potentiation of effect i.e. significant reduction in sleep latency and prolongation of duration of sleep was observed only with triazolam (fig.1). However, when BR-16A or ashwagandha (100 mg/kg, p.o) was administered in combination with melatonin (5.0 mg/kg, i.p.), or buspirone (1.0 mg/kg, i.p.), no significant potentiation was observed as compared to effect *per se* ($P<0.05$)

Pentobarbitone-induced hypnosis has been widely used in sleep studies and to study the CNS depressant effects[10]. Role of GABAergic system is known to play a role in sleep and its related problems[11]. In the present study, BR-16A or

TABLE1: EFFECT OF HERBALS AND HYPNOTIC DRUGS ON PENTOBARBITONE-INDUCED SLEEP IN MICE

Treatment Drug (mg/kg)	Onset of action (Min) Mean±SEM	Sleep time (Min) Mean±SEM
Control	3.5±0.3	44.4±2.19
BR 16A (100)	2.2±0.3[a]	169.0±4.1[a]
Ash (100)	2.7±0.3	107.4±9.20[a]
Tria (0.1)	1.9±0.4[a]	113.3±0.4[a]
Alpra (0.25)	2.0±0.2[a]	95.8±6.4[a]
Melatonin (2.5)	2.3±0.2[a]	183.7±8.7[a]
Buspirone (1.0)	2.4±0.2[a]	105.4±4.0[a]

N=5 animals in each group. Superscript a denotes statistical significance in comparison to control at $p<0.05$. (ANOVA followed by Dunnett's test)

TABLE 2: EFFECT OF BR 16A AND OTHER HYPNOTICS ON PENTOBARBITONE- INDUCED SLEEP.

Treatment Drug (mg/kg)	Onset of action (Min) Mean±SEM	Sleep time (Min) Mean±SEM
Control	3.5±0.3	44.4±2.2
BR 16A (100)	2.2±0.3[a]	169.0±4.1[a]
Tria (0.1)	1.9±0.4[a]	113.3±0.4[a]
BR (100) + Tri (0.1)	2.1±0.2[a]	190.0±2.5[a,b,c]
Alpra (0.25)	2.0±0.2[a]	95.8±6.4[a]
BR (100) + Alpr (0.25)	2.3±0.1[a]	183.0±3.3[a,b,d]

N=5 animals in each group. Superscript a, b, c and d denotes statistical significance in comparison to control, BR16A (100 mg/kg, p.o.), tri (0.1 mg/kg, i.p.), and alpra (0.25 mg/kg, i.p.) respectively at $p<0.05$ (ANOVA followed by Dunnett's test)

ashwagandha shortened the sleep latency and prolonged the total sleep time as compared to control group. This suggested for a CNS depressant effect of BR-16A or *ashwagandha*. Melatonin, triazolam, alprazolam and buspirone are well known agents recommended for the management of insomnia. Literature reports clearly indicated that these agent act through GABAergic mechanism or

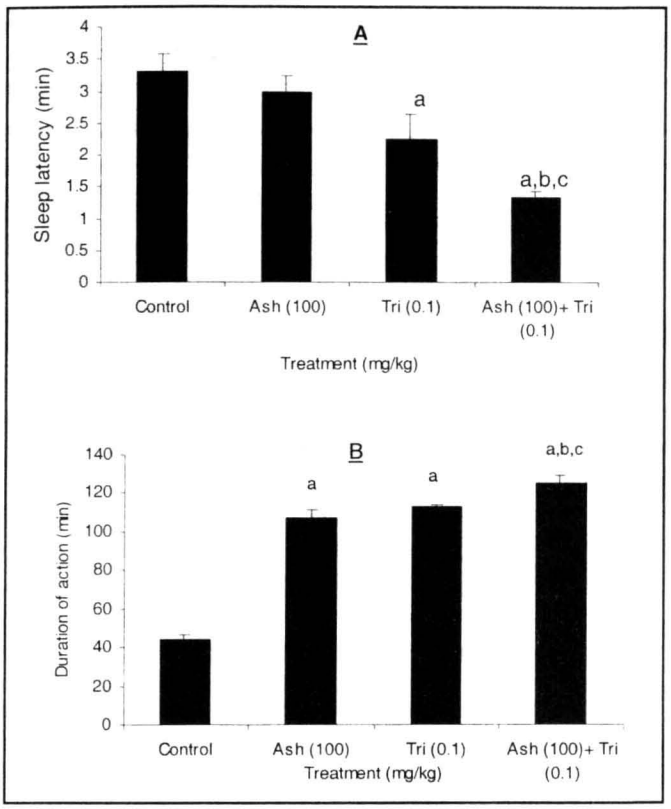

Fig.1: Effect of *ashwagandha* alone or its combination with triazolam on sleep latency (A) and duration of sleep (B) of pentobarbitone-induced sleep

Superscript a, b, and c denotes statistical significance in comparison to control, Ash (100 mg/kg, p.o.), and tri (0.1 mg/kg, i.p.) respectively at $p<0.05$ (ANOVA followed by Dunnett's test). Data is expressed as mean ± SEM (n=5).

modulation[12-14]

In combination studies of BR-16A with triazolam (0.1 mg/kg i.p.) or alprazolam (0.25 mg/kg i.p.), a significant prolongation of total sleep time was observed. However, sleep latency was not influenced significantly. Similarly, when ashwagandha was administered in combination with triazolam (0.1mg/kg, i.p.) and alprazolam (0.25mg/kg, i.p.), a significant potentiation of sleep latency (onset) and total sleep time (duration) was observed only with triazolam. However, alprazolam could not potentiate further, the sleep promoting action of ashwagandha. This may suggest a GABAergic modulatory action of BR16A or ashwagandha on the action of some of the hypnotics. Besides, sleep-promoting effect of BR16A preparation might be due to the presence of ashwagandh, which is one of the components of BR-16A, although, effects of other ingredients of the BR 16A preparation on pentobarbitone-induced sleep remains to be assessed. Further, BR-16A did not potentate the effects of melatonin (5 mg/kg, i.p.) or buspirone (1.0 mg/kg, i.p.). These agents have other mechanisms besides GABAergic modulation. These observations suggest that BR-16A may be used as sleep promoting herbal preparation, and if used along with other GABAergic hypnotics, it may reduce the dose of these agents for the treatment of insomnia.

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REFERENCES

1. Porkka-Heiskanen, T., Alanko, L., Kalinchuk, A. and Stenberg, D., **Sleep Medicine Review,** 2002, 0, 1.
2. Quera-Salva, M.A., Orlue, A., and Goldberg, F., **Sleep,** 1991, 14, 386.
3. Beaubrun, G. and Gray, G.E., **Psychiatr. Serv.,** 2000, 51, 1130.
4. Gold, J.L., Laxer, D.A. and Dergal, J.M., **Curr. Opin. Clin. Nutr. Metab. Care.,** 2001, 4, 29.
5. Kulkarni, S.K and George, B., **Indian drugs,** 1995, 32, 37
6. Kulkarni, S.K and George, B., **Phytotherap. Res,** 1996,10, 447.
7. Mishra, L.C., **Alternative Med. Rev.,** 2000, 5, 334.
8. Ziauddin, M., Phansalkar, Patki. P., Diwanay, S. and Patwardhan, B., **J Ethanopharmacol.,** 1996, 50, 69.
9. Devi, P.U., Soloman, F.E., and Sharada, A.C., **Indian. J. Exp. Bio.,** 1993, 31, 607.
10. Vogel, H.G., and Vogel, W.H., In: Drug Discovery and Evaluation, Springer Verlag Berlin, 1997, 26.
11. Lancel, M., **Sleep,** 1999, 22, 25.
12. Sack, R.L., Hughes, R.J., Edgar, D.M. and Lewy, A.J., **Sleep,** 1997, 201, 908.
13. Feinberg, I., Maloney, T. and Campbell, I.G., **J. Pschiatr. Res.,** 2000, 34, 423.
14. Suher, M.A., Abdullah, S.E., Saleem, S.A., Samir, F.S. and Amina, S.A., **Acta Neurobiol. Exp.,** 2000, 60, 447.