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Research Article

TO EVALUATE THE EFFECT OF *MORUS ALBA* LEAVES EXTRACT ON SLEEP AND ANXIETY IN RAT MODELS

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ABSTRACT

Objectives: The objectives of the study were to study the effect of *Morus alba* leaves extract (MAE) on sleep by phenobarbitone-induced sleeping time and the antianxiety effect by elevated plus maze apparatus model in rats.

Methods: In this study, the effect of MAE on sleep was evaluated by the phenobarbitone-induced sleeping time of rats. The onset and the duration of sleep were recorded in minutes. The antianxiety effect was evaluated by the elevated plus maze apparatus model in rats. During 5 min test period, the number of entries into the open arm and closed arm and time spent in the open arm and closed arm were recorded in seconds.

Results: MAE at the dose 200 and 400 mg/kg, highly significantly ($p<0.001$) decreased the onset of phenobarbitone-induced sleeping time. The duration of sleeping time was increased significantly ($p<0.01$) for 200 mg/kg and highly significantly ($p<0.001$) for 400 mg/kg as compared to the control group. *M. alba* has significant antianxiety activity in comparison with control in a dose-dependent manner. *M. alba* in a dose of 200 mg showed significant ($p<0.01$) and 400 mg/kg treated groups showed highly significant ($p<0.001$) anxiolytic activity by increasing the mean time spent in open arms as compared to control but less significant with standard (diazepam).

Conclusion: Results indicate that the MAE has a significant dose-dependent effect on phenobarbitone- induced sleeping time and antianxiety effect in the elevated plus maze test.

Keywords: *Morus alba* leaves extract, Sleep, Anxiety, Elevated plus maze.

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INTRODUCTION

India is one of the nations endowed with the inheritance of the traditional medicinal system. Customarily, herbal formulations are appraised as moderate in effectiveness and less toxic than most pharmaceutical agents [1]. Insomnia is difficulty in commencing or maintaining sleep. Insomnia statistics show a 1-year prevalence rate of 30–45% in adults [2]. Long-term utilization of commonly prescribed medications can lead to habituation and troublesome withdrawal symptoms. Hence, herbal and other natural sleep aids are obtaining attention [3].

Anxiety disorders are frequent and worrisome. Women are at greater risk for anxiety disorders, and developmental, communal, and conceptive factors are considered to contribute to the paramount of this susceptibility [4]. Anxiety can be stress related and an unpleasant incident may lead to phobic anxiety. Anxiety patients were frequently suffering from depression [5].

The white mulberry (*Morus alba*) is cultivated throughout the world, as nourishment to silkworms. Various parts of the mulberry plant were used by tribal for the treatment of asthma, cough, bronchitis, edema, insomnia, wound healing, diabetes, influenza, and eye infections [6]. The white mulberry leaf contains triterpenes (lupeol) sterols (β-sitosterol), bioflavonoids (rutin, moracetin, quercetin-3-triglucoside, and isoquercitrin), coumarins, volatile oil, alkaloids, amino acids, and organic acids [7]. Hence, the present study aimed to evaluate the effect of *M. alba* leaves extract (MAE) on sleep by phenobarbitone-induced sleeping time and the antianxiety effect by elevated plus maze apparatus model in rats.

METHODS

Animals

This study was conducted in the Pharmacology Department of Narayana Medical College. The Central Animal House of the institution was the source for the procurement of animals for this experiment. For this study, albino rats weighing150–250 g of either sex were included in the study. The animals were excluded from this study, If the weight of rats were below 150 g and if they are suffering from any evident disease. The animals were conserved as per the Committee for the Purpose of Control and Supervision of Experiments on Animals norms. The current study proposal was accepted by the Institutional Animal Ethics Committee on December 20, 2013, with protocol number – 27/2013/NMC.

Plant material

The leaves of the mulberry plant were possessed from Udayagiri, Nellore district, Andhra Pradesh, and authenticated by a botanist. The leaves were washed with water and put dry in the shade for 5 days, and with the help of an electric mixer, leaves were granulated to a fine dry powder. Using 90% ethanol, the powdered plant material was extracted twice at room temperature. Extracts were filtered with Whatman filter paper No.1. To obtain the final extract, using a Soxhlet evaporator, the filtrate was evaporated until dry. The extract was dissolved in distilled water for administration intraperitoneally (i.p) before the experiment [8].

The effect of MAE on sleep was evaluated by phenobarbitone-induced sleeping time model in rats.

Procedure

The rats were divided into five groups of six rats each (n=6). The extract (100, 200, and 400 mg/kg) was administered to various groups of rats,

diazepam (2 mg/kg) was given to the standard group and the control group was given saline (0.5 ml/kg). After 30 min, the groups were treated with phenobarbitone sodium (40 mg/kg, i.p). The onset and the duration of sleep were noted and recorded in minutes [9].

The antianxiety effect of MAE was evaluated by the elevated plus maze apparatus model in rats.

Five groups of rats (each group containing six rats) were taken. A standard 5 min test was employed for each rat. Group I received normal saline 5 ml/kg I.P, Group II received diazepam (1 mg/kg i.p), and Groups III, IV, and V received *M. alba* in doses of 100, 200, and 400 mg/kg I.P, respectively. Rats were placed on the maze after the administration of the test and standard drugs. The animals were placed at the center of the maze, facing one of the open arms. During 5 min test period, the number of entries into the open arm and closed arm and time spent in the open arm and closed arm in seconds were measured [10].

Arm entries were counted when the animal placed all of its four paws on it. The procedure was conducted in a sound attenuated room. Increase in no. of entries and time spent in the open arm were taken as the index of anti-anxiety activity.

Statistical analysis

The data were enlisted into an Excel spreadsheet 2007. Sigma GraphPad Prism version-6 USA was used for statistical analysis. Data were reported as Mean±Standard deviation. For data analysis, one-way analysis of variance followed by *post hoc* Tukey's multiple comparison tests was used. All the results of the test drug (*M. alba*) were correlated with control and standard drugs.

RESULTS

The onset of mean phenobarbitone-induced sleeping time in the control group was 9.19±0.63, MAE at the dose of 100 mg/kg was 8.08±0.61, 200 mg/kg was 7.49±0.70, and 400 mg/kg was 6.53±0.49, respectively, and 4.94±0.33 with diazepam-treated group. When compared with the control, the diazepam group showed highly significant ($p<0.001$) results. MAE group at 200 mg/kg and 400 mg/kg showed highly significant ($p<0.001$) results in comparison to the control group, but less significant when compared with the standard group.

The mean sleeping time in the control group was 78.05±6.89, MAE at the dose of 100 mg/kg was 82.65±4.03, 200 mg/kg was 93.27±4.30, and 400 mg/kg was 108.08±6.04, respectively, and 162.25±10.42 with diazepam. When compared with control, diazepam showed highly significant ($p<0.001$) results and MAE in the dose of 200 mg/kg showed significant ($p<0.01$) and at 400 mg/kg showed highly significant ($p<0.001$) results, but when compared to standard MAE 200 mg/kg, 400 mg/kg showed less significant results on mean sleeping time (Table 1).

Table 1: Phenobarbitone-induced sleeping time

S. No.	Group and dose (I.P)	Onset of sleep in Mean±S.D (min)	Duration of sleep in Mean±S.D (min)
1	Group-I: Control Saline: 0.5 ml/kg	9.19±0.63	78.05±6.89
2	Group-II: Standard Diazepam: 2 mg/kg	4.94±0.33 ***	162.25±10.42***
3	Group III MAE: 100 mg/kg	8.08±0.61	82.65±4.03
4	Group-IV MAE: 200 mg/kg	7.49±0.70 ***	93.27±4.30**
5	Group V MAE: 400 mg/kg	6.53±0.49 ***	108.08±6.04***

*p<0.05, **p<0.01, and ***p<0.001 compared to control group. Analysis of variance followed by Tukey's multiple comparison tests was used for the analysis of data between the groups, MAE: Morus alba leaves extract, I.P: Intraperitoneally

The mean number of entries in the open arm and closed arm is 5.33±1.50 and 10.5±1.64 in control, the number of entries in the open arm and closed arm MAE at the dose of 100 mg/kg is 6.5±1.22 and 10±1.09, 200 mg/kg is 8.66±1.50 and 7.66±1.63, and 400 mg/kg is 9.66±1.21 and 6.16±1.94, respectively, and the number of entries in open arm and closed arm in diazepam group is 11±1.78 and 5.33±1.21.

The mean time spent in the open arm and closed arm is 41.83±6.01 and 258.16±6.01 in control, and time spent in the open arm and closed arm in the MAE group at the dose of 100 mg/kg is 48.33±7.20 and 251.66±7.20, 200 mg/kg is 62.83±8.25 and 237.16±8.25, and 400 mg/kg is 99±7.15 and 201±7.15, respectively. Time spent in the open arm and closed arm in diazepam 1 mg/kg group is 115.83±11.25 and 184.16±11.25.

Standard drug diazepam showed highly significant results ($p<0.001$) by increasing the number of entries and time spent in the open arm. MAE at the dose of 200 mg/kg showed significantly ($p<0.01$) and MAE at 400 mg/kg showed highly significant ($p<0.001$) antianxiety activity when compared with control but less significant when compared with standard (Table 2).

DISCUSSION

The study was conducted to evaluate the effect of MAE on sleep by phenobarbitone-induced sleeping time, as described by Okokon *et al*. [9]. In this study, the interval between loss and recovery of righting reflex was used as the index of hypnotic effect.

In this study, MAE at the dose 200 and 400 mg/kg, highly significantly decreased the onset of phenobarbitone-induced sleeping time. The duration of sleeping time was increased significantly for 200 mg/kg and highly significant for 400 mg/kg as compared to the control group. Thus, MAE exhibited significant hypnotic activity when compared to the control group in a dose-dependent manner. MAE may act through gamma-aminobutyric acid (GABA) receptor complex or by decreasing dopaminergic transmission.

This study was supported by Adhikrao and Vandana [11]. They stated that MAE shortened the sleep latency and prolonged the total sleep time as compared to control. Pre-treatment with MAE was found to prolong phenobarbitone-induced sleeping time, suggesting that MAE, by decreasing dopaminergic transmission, increases the sensitivity of the central nervous system to the depressant action of phenobarbitone, which prolongs sleeping time. The extract of *M. alba* L. leaves contains constituents that may inhibit dopaminergic neurotransmission and possibly block the dopamine D2 receptor.

In this study, the evaluation of the antianxiety activity of MAE was by elevated plus maze apparatus in rats. In this model, increased time spent in the open arm after giving the drug indicates that the drug is having antianxiety activity. The present study showed that *M. alba* has significant antianxiety activity in comparison with control in a dose-dependent manner. *M. alba* in a dose of 200 mg showed significant ($p<0.01$) and 400 mg/kg treated groups showed highly significant ($p<0.001$) anxiolytic activity by increasing the mean time spent in open arms as compared to control but less significant with standard (diazepam). Thus, *M. alba* L. has potential clinical application in the management of anxiety. MAE may act through the GABA receptor complex for its effect on anxiety. Further investigation of the mechanism of action of the plant extract, as well as the active substance responsible for its biological actions, is necessary.

This study was supported by Yadav *et al*. [12]. They showed that *M. alba* leaves demonstrated significant anxiolytic activity in rats tested on the validated behavior paradigms, namely, elevated plus maze test, open field test, light/dark exploration test, and locomotor activity. MAE may act through the GABA receptor complex for its effect on anxiety.

However, Gupta *et al*. [13] showed that *M. alba* stem bark contains a compound called moralbosteroid and the bark extracts tested for

Table 2: Elevated plus maze method

S. No.	Group and dose (I.P)	Number of entries		Time spent Mean±S.D	
		Mean±S.D		Open arm (s)	Closed arm (s)
		Open arm	Closed arm		
1	Group-I: Control Saline: 0.5 ml/kg	5.33±1.50	10.5±1.64	41.83±6.01	258.16±6.01
2	Group-II: Standard Diazepam: 1 mg/kg	11±1.78***	5.33±1.21***	115.83±11.25***	184.16±11.25***
3	Group-III MAE: 100 mg/kg	6.5±1.22	10±1.09	48.33±7.20	251.66±7.20
4	Group-IV MAE: 200 mg/kg	8.66±1.50*	7.66±1.63	62.83±8.25**	237.16±8.25
5	Group-V MAE: 400 mg/kg	9.66±1.21***	6.16±1.94	99±7.15***	201±7.15

*p<0.05, **p<0.01, and ***p<0.001 compared to control group. Analysis of variance followed by Tukey's multiple comparison tests was used for the analysis of data between the groups, MAE: Morus alba leaves extract, I.P: Intraperitoneally

anxiolytic effects using elevated plus maze test, open field test, and light/dark exploration tests. The results obtained in this study suggest that the moralbosteroid isolated from *M. alba* stem bark possesses anxiolytic activity which is possibly mediated through the GABA benzodiazepine mechanism.

CONCLUSION

Results indicate that the MAE has a significant dose-dependent effect on phenobarbitone-induced sleeping time and antianxiety effect in the elevated plus maze test. Hence, further exploration on the mode of action of the plant extract, as well as the functional solidity accountable for its biological activity, is essential.

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I will always remain sympathetic towards all the innocent animals that had to suffer for the completion of my research and also for the betterment of human society.

AUTHORS' CONTRIBUTIONS

Sriharsha Rayam worked as principal investigator including conduction of experiment, drafting the proposal acquiring approval. B.L Kudagi played a crucial role in reviewing the proposal and reviewing before and after completion of the project and for publication approval. Ramya Jonnalagadda contribution was toward data procurement and analysis. Raveendra Kumar Nallabothula contributed to the study during drafting the article, reviewing content, and final endorsement of the version to be published.

DECLARATIONS

Conflicts of interest

None.

Authors' funding

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Ethical approval

The study was approved by the Institutional Animal Ethics Committee with protocol number – 27/2013/NMC, dated December 20, 2013.

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