SHORT COMMUNICATION

Anxiolytic, sedative, and hypnotic activities of aqueous extract of *Morinda citrifolia* fruit

Sridharan Kannan, Shanti Manickam[1], Meher Ali RajaMohammed[2]

Departments of Pharmacology, Subharti Medical College, Mumbai, [1]Madurai Medical College, Madurai and [2]Manakula Vinayagar Medical College, Puducherry, India

ABSTRACT

Morinda citrifolia (Indian mulberry or noni) fruit has been long used as a folk medicine for a wide range of health purposes as it is claimed to have analgesic, antiinflammatory, antioxidant, detoxifier, and cell-rejuvenator properties. A recent study has revealed central nervous system suppressant nature of its extract. Hence, the present study has evaluated the anxiolytic, sedative, and hypnotic effects of the aqueous extracts of *Morinda citrifolia* in rodents in comparison to diazepam. Anxiety was assessed by 'Isolation-induced aggression' model, sedation by 'Spontaneous locomotor activity using actophotometer' and hypnotic activity by 'Prolongation of ketamine-induced sleeping time'. Six male mice were used for each of the groups and postdose, all the six that received diazepam had shown an inhibition of aggression, whereas in the test group, five of six mice and none in the control group had shown an inhibition of aggression ($P = 0.0007$). Similarly, for the sedative activity, the total number of spontaneous locomotor activity at 30 min following drug administration was found to be 364.67 ± 10.74, 123.16 ± 8.33, and 196.67 ± 3.7, while at 60 min it was found to be 209 ± 12.98, 49 ± 5.78, and 92 ± 2.5 (mean ± SD) for the control, standard, and test groups of mice respectively ($P < 0.001$). Hypnotic activity was measured by prolongation of ketamine-induced sleeping time wherein the onset and duration of loss of righting reflex were compared among each group of mice. The time in minutes for the onset in control, standard, and test groups was 4.01 ± 0.22, 1.23 ± 0.05, and 2.23 ± 0.07, respectively. The duration of loss of righting reflex was 44.23 ± 0.59, 56.03 ± 1.34, and 50.57 ± 0.36, respectively. Both these were statistically significant ($P < 0.001$). However, more clinical studies are needed to assess the long-term effects of the extract in humans.

Key words: Herb, noni, sleep

INTRODUCTION

Conventional anxiolytics carry risk of various adverse effects, abuse liability, and dependence potential. There is an increasing trend toward using herbal remedies globally as shown by an estimate from World Health Organization (WHO) that around 70–80% population had used some form of alternative or complementary medicine including ayurvedic, homeopathic, naturopathic, traditional oriental, and native American Indian medicine.[1] Various herbal agents have been enlisted to have anxiolytic effects and *Morinda citrifolia* is one of them.[2] *Morinda citrifolia* fruit has been long used as a folk medicine for a wide range of health purposes as it is claimed to have analgesic, antiinflammatory, antioxidant, detoxifier, and cell-rejuvenator property. It also lacks any major adverse effects. Various alkaloids, polysaccharides, scopoletin, were identified in the fruit. Recently, Deng *et al.*[3] have shown in an *in vitro* study that the *Morinda citrifolia* fruit has hydrophilic constituents, which have $GABA_A$ receptor agonistic effect to an extent of around 80% as that of muscimol (a selective $GABA_A$-agonist), which could probably accounts for its action. Despite this, there is no *in vivo* animal data for the same. Hence, we evaluated the anxiolytic, sedative, and hypnotic effects of *Morinda citrifolia* fruit extract in *in vivo* animal models after obtaining the institutional animal ethics committee approval.

Address for correspondence:
Dr. Kannan Sridharan, Department of Pharmacology, Subharti Medical College, Subhartipuram, Meerut - 250005, Uttar Pradesh, India.
E-mail: skannandr@gmail.com

Received: 13-Nov-2013
Revised: 21-Dec-2013
Accepted: 30-Jan-2014

Access this article online	
Quick Response Code:	Website: www.jaim.in
	DOI: 10.4103/0975-9476.131738

MATERIALS AND METHODS

The study was carried out in 54 albino male mice each weighing 18-25 g after obtaining approval from the institutional animal ethics committee of Madurai Medical College, Madurai. They were divided into three groups of six animals in each group and administered standard diet (Pellet feed from Hindustan lever limited, Mumbai) and water *ad libitum*. The yellowish-white *Morinda citrifolia* fruit was obtained from an authentic source (quality control department of Noni India). It was then boiled in water and the skin and pulp were crushed. The resulting slurry was then dried at room temperature for a period of 7 days and ultimately powdered. The aqueous solution made with distilled water was injected to the animals intraperitoneally (ip) at a dose of 500 mg/kg (dose that had shown an appropriate sedative effect in the pilot study). Diazepam (1 mg/kg ip) was used as a standard comparator and distilled water as a control agent. 'Isolation-induced aggression' was used as the anxiety model[4] wherein male mice were isolated in small cages made of polypropylene of dimensions 290 × 220 × 140 mm for a period of 6 weeks. A male mouse being accustomed to live together with other animals was placed each into the cage of these isolated mice for 5 min. The aggressive behavior of the mouse characterized by hitting of the tail on the bottom of the cage, screaming and biting of the intruder was noted and the time taken for any of these was calculated as a reaction time. Following this, the experimental animals were administered any of the standard, control or fruit extract. After 30 min, the experiment was repeated and the reaction time was compared. 'Spontaneous locomotor activity using actophotometer' was used for assessing the sedative activity whereby the total number of counts made by the animal over a period of 10 min was evaluated before, 30 and 60 min following the drug administration.[5] 'Prolongation of ketamine-induced sleeping time' was used for assessing the hypnotic activity. The time at which the righting reflex was lost was taken as onset of anesthesia and the duration between the time at which the righting reflex was lost and was regained was taken as duration of anesthesia.[6] The onset and duration of anesthesia were compared between test, control, and standard groups. Statistical analysis was performed using McNemar test for the evaluation of anxiolytic effect (number of animals that had persistent aggression following introduction of other male mice before and after the drug administration) and, one-way analysis of variance (ANOVA) for assessing the sedative activity (number of spontaneous locomotor activity at 30 and 60 min following the drug administration) and hypnotic effect (time taken for the onset and duration of loss of righting reflex). A pilot study was conducted with four different doses (in mg/kg) (100, 250, 500, and 1000) in comparison to distilled water in three male mice for each of the doses. The sedative activity was assessed using the actophotometer method as described earlier. There was a proportionate reduction in the total number of counts as the dose was increased from 100 to 500 mg/kg. There was no significant decrease in the counts at 1000 mg/kg as compared with 500 mg/kg. Hence, 500 mg/kg dose was selected for the main study. A $P < 0.05$ was considered significant and the statistical analysis was done using SPSS version 17.0 (SPSS Inc. Released 2008. SPSS Statistics for Windows, version 17.0. Chicago: SPSS Inc).

RESULTS

In the anxiety mice model, total number of isolated mice that had developed aggression following the intruder was compared between the groups ($n = 6$ in each). Postdose, all the six mice that received diazepam had shown an inhibition of aggression, whereas in the test group, five of six mice and none in the control group had shown an inhibition of aggression. This was statistically significant ($P = 0.0007$). Similarly, for the sedative activity, the number of counts made by the mice in the spectrophotometer was compared. The total number of spontaneous locomotor activity (mean + SD) at 30 min following drug administration was found to be 364.67 + 10.74, 123.16 + 8.33, and 196.67 + 3.7 while at 60 min it was found to be 209 + 12.98, 49 + 5.78, and 92 + 2.5 for the control, standard, and test groups of mice, respectively ($P < 0.001$). Hypnotic activity was measured by prolongation of ketamine-induced sleeping time wherein the onset and duration of loss of righting reflex were compared among each group of animals. The time in minutes for the onset in control, standard, and test groups was 4.01 + 0.22, 1.23 + 0.05, and 2.23 + 0.07, respectively. The duration of loss of righting reflex was 44.23 + 0.59, 56.03 + 1.34, and 50.57 + 0.36 respectively. Both these were statistically significant ($P < 0.001$).

DISCUSSION

The present study had evaluated the anxiolytic, sedative, and hypnotic effects of aqueous extract of *Morinda citrifolia* fruit in albino mice in comparison with diazepam and control. We found that the aqueous extract at a concentration of 500 mg/kg intraperitoneally exhibited a significant anxiolytic, sedative and hypnotic effects as compared with control animals. GABA has been identified as an important neurohormonal system mediating various anxiety disorders from clinical experience and well evaluated experiments.,[7] Additionally, norepinephrine and serotonin neurotransmitters have been shown to be altered in these conditions.[8] Hence, those drugs that enhance either GABAergic or serotonergic system or that attenuate the adrenergic activity are currently

used for treating anxiety disorders. A recent study in rats has revealed a substantial reduction of norepinephrine in amygdale and hippocampus and that the animals have spent significantly more time in opened arms compared with the control group indicating anxiolytic effect.[9] Another *in vitro* study on Noni fruit has revealed a high binding affinity to the $GABA_A$ receptors.[3] Hence, these studies including the present, demonstrate that *Morinda citrifolia* fruit has a central nervous system depressant activity. However, the study is limited by the fact that the number of animals used for each of the activity is less and only one of the validated models was used for assessing each activity in the present study. We did not attempt in performing a molecular analysis of the neurotransmitter levels in the rodent brain. Hence, further studies are warranted to explore the anxiolytic activity of the extract in other animal models. Moreover current drug treatment methods for anxiety disorders have limitations including delayed effect, nonresponsiveness, side effects, and drug interactions.[10]

CONCLUSION

Morinda citrifolia fruit extract can be considered as a food supplement in individuals with anxiety disorders. More clinical studies are warranted to assess the long-term effects of the extract in humans.

ACKNOWLEDGMENT

The authors are grateful to Noni-India group for providing samples of *Morinda citrifolia* fruit and Dr. Mangayarkarasi, Associate Professor, Department of Pharmacology, Sri Manakula Vinayagar medical college for her assistance in the manuscript preparation.

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How to cite this article: Kannan S, Manickam S, RajaMohammed MA. Anxiolytic, sedative, and hypnotic activities of aqueous extract of *Morinda citrifolia* fruit. J Ayurveda Integr Med 2014;5:73-5.

Source of Support: Nil, **Conflict of Interest:** None declared.