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Research Article

Aromatherapy of *Humulus lupulus* Extract for the Management of Nocturnal Enuresis: A Pilot Study in Indian Children

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Abstract

Background/Aim: The present study was a pioneer effort to evaluate the efficacy and safety of hops flower (Sinorina Ambientador [*Humulus lupulus*]) extract aromatherapy in the treatment of nocturnal enuresis (NE) in children.

Methods: Fifty children aged 5-10 years with NE were randomized to receive test intervention: hops flower extract aromatherapy (n = 26) or placebo (almond oil) (n = 24) every night for 60 consecutive days. The primary efficacy assessment was change in NE grading in patients at the end of the study compared to baseline and between two study groups at the end of the study along with global assessment of efficacy by both investigator and patient. The secondary objective was safety and global assessment of tolerability at the end of the study by both investigator and the patient.

Results: A total of 91.7% children were reduced to grade 0 NE (completely dry) at the end of the study (p = 0.0005) and remained dry even after 15 days of withdrawal from test intervention (p = 0.0005). As per both the investigator's and the patient's assessment of efficacy in test group, very good improvement (Grade 1) was observed in 91.7% patients. No adverse events or deaths were observed in the study and the test intervention was well tolerated by all the participating patients. For the test group, both the investigator's and the patient's assessment of tolerability was excellent (Grade 1) for all patients.

Conclusion: The results revealed aromatherapy with hops flower extract to be a safe, effective, and a viable treatment for NE in children.

Keywords: *Pediatrics; Sinorina Ambientador; Nocturnal Enuresis; Efficacy; Tolerability*

Abbreviations

GABA: Gamma-Aminobutyric Acid; GABA: Gamma-Aminobutyric Acid; GCP: Good Clinical Practice; ICMR: Indian Council of Medical Research; LUTS: Lower Urinary Tract Symptoms; NE: Nocturnal Enuresis; WHO: World Health Organisation

Introduction

Nocturnal enuresis (NE) or bed-wetting is a common condition or disorder affecting children aged ≥ 5 years [1]. According to the World Health Organization (WHO) it is classified under mental and emotional disorder. Enuresis is defined as "Involuntary voiding of urine that occurs at a frequency of at least twice a month in children aged under seven years, and at least once per month in children aged seven or above [2]". Enuresis is classified into monosymptomatic and non-monosymptomatic. Monosymptomatic enuresis or primary enuresis accounts for the majority of the NE cases; children with primary enuresis are without any history of bladder dysfunction or lower urinary tract symptoms (LUTS) [3]. Non monosymptomatic enuresis refers to enuresis accompanied by any other LUTS, such as altered voiding frequency, urgency, hesitancy, straining, daytime incontinence, weak stream, intermittency, holding maneuvers, genital or LUT pain, and feeling of incomplete emptying [3].

Evidence suggests an interplay of genetic, psychological, and physiological factors to contribute towards the development of NE [1,4]. Prevalence data for NE in children in India is limited; however, estimates from few cross-sectional studies reveal a prevalence rate ranging from 7.6% to 18.4% [5-8]. Though the incidence rate decreases with age, the condition is distressing for the child and the family and impacts the overall quality of life of children. Children with this condition are depressed and have reduced self-esteem, compared with other healthy children [9].

Nonpharmacological approaches (parental and patient education, psychological and behavioral intervention) and various pharmacological agents are used for efficient management of NE [3,10]. Children aged ≥ 7 years who do not respond to behavioral intervention are prescribed pharmacological therapy depending upon the frequency of bed-wetting. Older, motivated patients, exhibiting frequent bed-wetting are recommended enuresis alarm [10]. Though there are established pharmacological treatments (desmopressin, vasopressin analogs, and imipramine) available for the management of NE, their use is limited due to the occurrence of serious side effects and high relapse rates. Desmopressin, which is considered the drug of choice for NE, has effectiveness ranging from 60%-70% with 80% of patients relapsing after discontinuing the treatment. Moreover, the intake of desmopressin is accompanied by side effects like epistaxis, abdominal cramps, hyponatremia, and anorexia. Imipramine, another pharmacological agent, shows an effectiveness response of 40% - 60% but is accompanied by side effects such as seizures, depression, sleep disturbance, and arrythmia [10,11].

Hence, in an attempt to find a safe and effective treatment agent, we turned towards nature and explored the hops flower (Sinorina Ambientador [*Humulus lupulus*]) extract aromatherapy in controlling NE in children. Hops flower has long been a part of ancient traditional medicine. No toxic effects on human health have been detected so far.

Hops flower constituents are known to exert anti-inflammatory, antioxidant, antifungal, antibacterial, and anticancer effect [12-14]. The essential oil component of hops flower is known for its sedative and other neuropharmacological properties. Hence, we aimed to evaluate the efficacy and safety of hops flower extract in controlling urinary incontinence in children while they were asleep. To the best of our knowledge, this is the first study to evaluate the aromatherapy effects of hops flower extract in the management of nocturnal enuresis in children.

Materials and Methods

The study was a two-arm, randomized, double-blind, prospective, placebo-controlled, multicentric one, conducted in outpatient clinics in India.

Study participants

Fifty-three children aged 5 - 10 years, attending three outpatient clinics in Mumbai between October 2017 and February 2018 with a clinical diagnosis of grade 1 and grade 2 enuresis were evaluated for inclusion into the study. Children with grade 2 enuresis also having associated disorders like diabetes, urinary tract infections, urogenital malformations, inability to recognize a full bladder, chronic constipation, and diagnosed resistance to bedwetting treatments were excluded from the study. Additionally, patients deemed unable to keep returning for appointments and children suffering from attention-deficit hyperactivity disorder, conditions associated with large urine volumes, abnormal neurological control, and abnormalities of bladder and urinary tracts were also excluded from the study.

Informed consent and ethics

The study was approved by Suraksha Institutional Ethics Committee and was compliant with the principles of Declaration of Helsinki (2013 Brazil revision), Good Clinical Practice (GCP) guidelines, and Ethical Guidelines for Biomedical Research on Human Subjects by Indian Council of Medical Research (ICMR), Govt. of India. Parents or legal guardians of the children were explained about the study details and were provided ample opportunity to raise any queries/doubts. Written informed consent was obtained from all the parents or

legal guardians before enrollment. The study was registered in the clinical trials registry of India (CTRI/2017/06/008911; Registered on: 23/06/2017).

Study interventions

Patients were randomized to receive either, test intervention: aromatherapy of hop flower extract (Sinorina Ambientador [*Humulus lupulus*]) or placebo (almond oil [*Prunus dulcis*]) every night for 60 consecutive days. Thirteen drops of test intervention or placebo were put on a clean handkerchief or cotton every night by the parents/legal guardian of the participating children. The handkerchief or cotton was kept on the bedside and was placed as close as possible to the children during their sleep.

Outcome measures

The primary objective of the study was to evaluate the effectiveness of *Humulus lupulus* extract aromatherapy in the treatment of NE in children. The secondary objective of the study was to assess the safety of *Humulus lupulus* extract aromatherapy. Efficacy analyses included assessing (1) change from baseline in NE grading at the end of the study; (2) improvement of NE grade, and the number of days with nocturnal enuresis between two treatment groups at the end of the study considering:

- Baseline values: End of treatment value.
- After the removal of aromatherapy values: End of study values.

Nocturnal enuresis was graded on a 4-point scale (0: completely dry; 1: 1 - 2 times/week; 2: 3 - 4 times/week, and 3: daily or 5 - 7 times/week). The effectiveness analysis included the global assessment of efficacy by the investigator and the patient on a 4-point scale (1: very good improvement; 2: good improvement; 3: moderate improvement; and 4: negligible improvement). Safety analysis included assessment of adverse events and global assessment of tolerability at the end of the study by patients based on the 4-point scale (1: excellent; 2: good; 3: satisfactory or fair; 4: poor).

Study procedure

After obtaining written informed consent, the eligible patients were randomized to either test or placebo group. Randomization was generated by the website www.randomization.com and was not revealed either to the sponsor or the investigator. Physical examination, vital signs, routine laboratory investigations, and NE assessment were performed on baseline. Post administration of study interventions, patients were followed up every 15 (+2) days up to 90 days for efficacy and safety outcome measures.

Statistical analysis

Analysis was performed using Microsoft Excel. Demographic data was summarized using mean, standard deviation, and percentages. The efficacy variables were from baseline values and compared with treatment value for every visit between drug and placebo. Statistical analysis was carried out using analysis of variance (ANOVA) test. The level of significance was set at $p < 0.05$.

Results

Demographics and baseline characteristics

Fifty-three patients were screened for eligibility, out of which 50 were randomized to receive the test intervention or placebo. All patients in the intervention and placebo groups were followed up to the end of the study (90 days). Two patients were screen failures and one of them was considered as a dropout as the patient did not report at the scheduled follow-up visit (Figure 1). The present study had

23 male (46%) and 27 female (54.0%) patients, among which 11 (45.8%) male and 13 (54.1%) female patients were in the test group and 12 (46.1%) male and 14 (53.8%) female patients were in the placebo group. For the test group, the majority of the patients were 6, 8, and 10 years old, while in the placebo group, the majority of the patients were 6, 7, and 9 years old. Vital signs and patients' parameters were within the normal range. Demographic characteristics of the two study groups were comparable (Table 1).

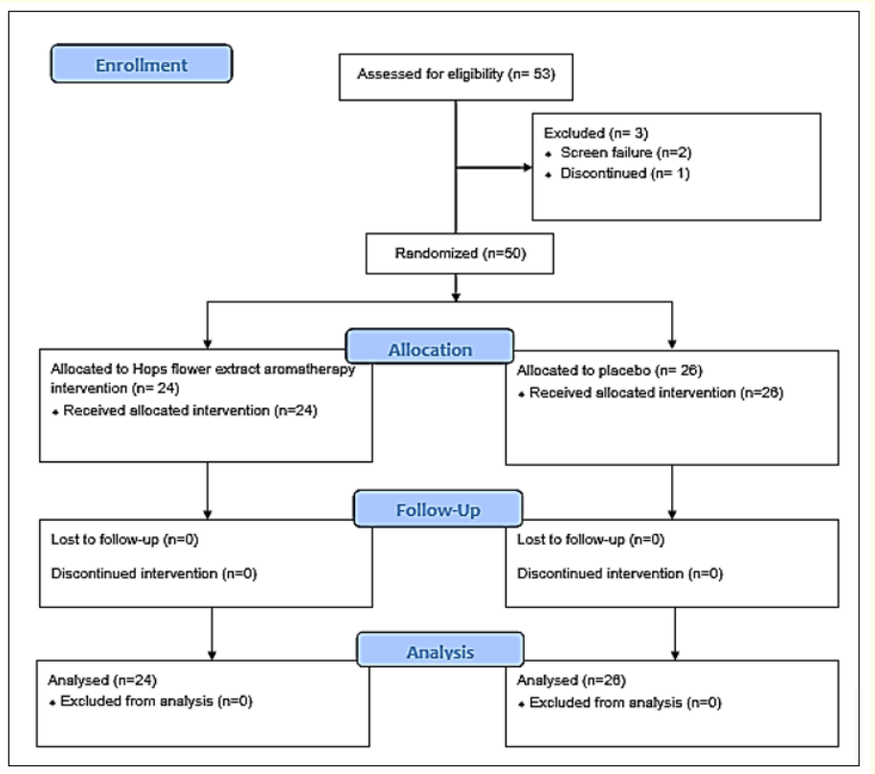

Figure 1: *Flow chart for the randomized study.*

	Test intervention (N = 24)	Placebo (N = 26)
Gender (Count [%])		
Male	11 (45.8)	12 (46.1)
Female	13 (54.2)	14 (53.9)
Age distribution (years) (Count [%])		
5	2 (8.3)	1 (3.8)
6	5 (20.8)	7 (26.9)
7	4 (16.6)	7 (26.9)
8	5 (20.8)	4 (15.3)
9	3 (12.5)	5 (19.2)
10	5 (20.8)	2 (7.6)
Patient parameters (Mean ± SD)		
Body temperature (degree Fahrenheit)	97.2 ± 0.4	97.2 ± 0.6
Pulse rate (beats/min)	75.3 ± 7.3	73.6 ± 7.0
Respiratory rate (breaths/min)	18.7 ± 1.7	21.1 ± 10.0
Systolic blood pressure (mmHg)	110.6 ± 7.3	111.9 ± 6.4
Diastolic blood pressure (mmHg)	72.5 ± 4.6	75.2 ± 4.9

Table 1: *Demography and baseline data for the patients.*
SD: Standard Deviation.

Efficacy outcome measures

In the test group, NE symptoms were reduced to 0 grade (completely dry) for 22 of the 24 patients at the end of the treatment and 15 days after the withdrawal of the treatment with hops flower extract (p = 0.0005). Again, in the placebo group, NE symptoms were reduced to 0 grade at the end of the treatment in only five of the 26 patients, and only four patients remained dry 15 days after withdrawal. Treatment with hops flower extract had significantly better NE grade improvement compared to placebo on day 30 (p = 0.0001), 75 (p = 0.0005) and 90 (p = 0.0005) of the study (Table 2).

Visit (days)	Test intervention (N = 24) (Mean ± SD)	Placebo (N = 26) (Mean ± SD)	p-value
Baseline	1.04 ± 0.20	1.19 ± 0.40	0.1056
Day 15	1 ± 0	1.19 ± 0.40	0.0234
Day 30	0.5 ± 0.59	1.11 ± 0.43	0.0001
Day 45	0 ± 0	0.76 ± 0.58	0.000
Day 60	0 ± 0	0.80 ± 0.57	0.000
Day 75	0.20 ± 0.72	0.84 ± 0.46	0.0005
Day 90	0.25 ± 0.73	0.88 ± 0.43	0.0005

Table 2: *Comparison of mean nocturnal enuresis grading of the patients in the intervention and placebo groups. SD: Standard Deviation.*

Global assessment of treatment investigator's assessment of efficacy revealed 22 patients (91.7%) had very good improvement in NE even after 15 days of withdrawal of the treatment with hops flower extract. A similar result was obtained for patients' global assessment of test group where 91.7% patients reported very good improvement in NE after treatment with hops flower extract (Table 3).

Grades*	Test intervention (N = 24) (Count [%])	Placebo (N = 26) (Count [%])
Investigator grading (Count [%])		
1	22 (91.7)	0 (0)
2	0 (0)	2 (7.7)
3	0 (0)	5 (19.3)
4	2 (8.3)	19 (73.0)
Patient grading (Count [%])		
1	22 (91.7)	0 (0)
2	0 (0)	0 (0)
3	0 (0)	3 (11.5)
4	2 (8.3)	23 (88.5)

Table 3: *Global assessment of efficacy by the investigator and patient.*
**(1: Very Good Improvement; 2: Good Improvement; 3: Moderate Improvement; 4: Negligible Improvement).*

Safety outcome measures

No adverse events or deaths were reported during the study. Both the investigator and the patient graded excellent (grade 1) tolerability for test and placebo group (Table 4).

Grading*	Test intervention (N = 24)	Placebo (N = 26)
Investigator grading (Count [%])		
1	24 (100)	26 (100)
2	0 (0)	0 (0)
3	0 (0)	0 (0)
4	0 (0)	0 (0)
Patient grading (Count [%])		
1	24 (100)	26 (100)
2	0 (0)	0 (0)
3	0 (0)	0 (0)
4	0 (0)	0 (0)

Table 4: *Global assessment of tolerability by the investigator and patients.*
**(1: Excellent; 2: Good; 3: Satisfactory or Fair; 4: Poor).*

Discussion

Nocturnal enuresis is a prevalent, but under-diagnosed and under-tackled condition in India. In line with the global guideline, the evidence-based Indian recommendation stresses on individualized management approach for NE by using behavioral therapy, alarms, desmopressin, and tricyclic antidepressants [15]. However, these pharmacological agents are not curative in nature. With high relapse rates upon discontinuation and the wide range of safety concerns for a child, it becomes highly imperative for the scientific fraternity to explore treatments that are not only effective but also safe for a growing child.

In view of this, a pharmaceutical composition based on Hops (*Humulus lupulus*) extract, comprising a therapeutically effective amount of volatile organic agents of the plant's metabolism have been studied for alleviation of childhood NE. The three most prominent organic volatile substances of the plant include tannins and proanthocyanidins (condensed tannins) with astringent properties; flavonoids, especially rutin with vasodilator and antidiuretic properties and gamma-aminobutyric acid (GABA), which act as brain neurotransmitter [16]. Tannins have been widely studied to have anti-inflammatory, microbicidal and anti-parasitic properties and have been medicinally used in in antidiarrheal, haemostatic, and anti-hemorrhoidal compounds [17]. Rutin, an anti-inflammatory and antioxidant flavonoid glycoside, has been known to inhibit platelet aggregation and reduction of vascular permeability resulting in improved circulation. Its property as a muscle relaxant has been demonstrated in animal studies [18,19]. Finally, GABA acid, amongst other properties, generates a voltage potential in the neuron and transmit muscle control impulses. Subjects with NE have been studied to have low levels of GABA which may potentially inhibit the flow of ions Ca^{2+} due to low Cl^- ions concentrations, thereby affecting the development of the CNS [20]. Aromatherapy with GABA may therefore reset the proper development of the CNS and restore muscle control impulses. Aromatherapy is hypothesized to induce activation of neurotransmitters that stimulate the limbic system, which governs emotions and the hypothalamus that controls many vital activities [16].

The efficacy as well as the safety findings, i.e. NE grade reduction even after withdrawal from the treatment, NE symptom improvement, excellent tolerability, and no adverse events from our study were in line with the evidence discussed above. Hence, through this study we present that hops flower extract aromatherapy is a promising agent in treating children with NE in the Indian scenario. Though our study was randomized and double blind in nature, it was limited by the absence of a standard treatment group; short duration of the follow-up, and exclusion of children with grade 3 NE and adolescent children. Hence, we suggest future large, randomized, blinded studies comparing *Humulus lupulus* extract aromatherapy with established and recommended interventions for NE such as desmopressin in varied age groups of children. The findings from the study revealed *Humulus lupulus* extract aromatherapy to be effective in relieving the

symptoms of NE compared to placebo for a duration of three months. Therefore, the results of the present pilot study can be generalized to a larger pediatric population aged 5 - 10 years in the Indian scenario.

Conclusion

The current pilot study presents evidence of effectiveness and safety of *Humulus lupulus* extract aromatherapy in relieving the symptoms of NE in children. Components of this therapy are able to activate and/or inhibit unwanted signals during the NE and hence may help to reinforce learning and urinary habits. Aromatherapy of *Humulus lupulus* extract aromatherapy can thus serve as a safe and promising therapy for the treatment of NE in children.

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Author's Contributions

All authors equally contributed to the study and manuscript development.

Conflict of Interest

None.

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