

Translated documentation by Laboratec S.L

Valencia, 18th January 2013

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de la Comunidad Valenciana (FISABIO)
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Distinguished Laboratec S.L.

After completion of the clinical study done in children with a rebel nocturnal enuresis diagnosed by primary care paediatrician and referred to the Department of Paediatrics, University Hospital Dr. Peset, I am sending you the report of the project entitled: "The effects of SINORINA (hops flower extract, *Humulus lupulus*) in children from 5 to 7 years old having nocturnal enuresis."

This clinical study was initiated in September 2011, following the submission of the appropriate documentation for approval by the local Clinical Research Ethics Committee (CREC or CEIC in Spain) and ended in December 2012 (15 months), after analysis of 48 patients, 25 for SINORINA's group and 23 for the placebo's group.

After evaluating your product, SINORINA, derived from plant extracts of *Humulus lupulus* (Hops) which its active properties can relieve and / or improve bedwetting conditions in children, has shown that the product has an effectiveness of 65% in children already diagnosed and treated for nocturnal enuresis.

Your Sincerely,

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Jefe del Servicio de Pediatría,
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Valencia, España

The effects of SINORINA (hops flower extract, *Humulus lupulus*) in children from 5 to 7 years old having nocturnal enuresis.

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1 INTRODUCTION

General information about hops

Hop (*Humulus lupulus*) is one of three species of plants of the genus *Humulus* from the Cannabaceae family. Hailing from the wet and cold areas of Europe, western Asia and North America. With the industrialization of beer its uses flourished with more resistant varieties, so now is very abundant in places as diverse and distant as China and the United States¹.

It can be found in the wild, weeds, forest edge or along rivers. Its dark green leaves are provided with 3-5 toothed lobes. It has male and female flowers. The former are greenish yellow and are gathered in panicles, the female, gathered in catkins, are light green. Is a perennial plant that can reach up to eight meters high. Its stems are fickle, annual and can curl in any support. The major active components of the hop plant have been isolated, that are flavonoids, chalcones (aromatic ketones) and α acid, which has a mild antibiotic effect against Gram-positive bacteria and fungi², and promotes the activity of malt yeast³. Already used by the Romans over 2000 years ago in the manufacture of beer, now, in the western development, the main additive used to counterbalance (balancing if you prefer) the sweetness of the malt is hop³. What is used from the plant is the unfertilized female flower. On the bracteoles basis there are some glands that contain lupulin, which is the ingredient that will bring the beer its own bitter flavour and aromas. For the bitterness are responsible bitter acids and elementary oils derived especially by volatile and delicate compounds produce the aromas, with an ester-based and resins with an antioxidant role⁴. Hops are the cause of the stimulation of appetite produced by beer. It has also been used as a remedy for various ailments or discomfort, both physical and mental over many years⁵.

The flowers of this perennial vine are used for its natural sedative properties. Hops are used to relieve occasional sleep problems caused by nervousness and tension nerviosa⁶. A high dose of hops reduces sleep latency and supports healthy sleep function. This specie in Europe is known as wolves' herb, hops have a legendary reputation for sedative plant at the same level of valerian and passionflower. Used for its bitter principles to flavour beer, also has other active principles, such as Humulin and lupulin present in the female inflorescences and cones, which produce a state of general calm, without affecting care or reflections and either does generate addiction. It is a plant that has no side effects or drug interactions. Although the exact mechanism of action of hops are not yet determined, its sedative qualities are not put in doubt due to the extensive historical documentation as well as some clinical research trials^{7,8}. It is believe that flavonoids, chalcones and α acids contained in hops extracts are responsible for their sedative activity⁹. Is supposed to act on the GABA-A receptors in the brain, the same receptor where benzodiazepines or sedating chemicals act. Despite this similarity of action, the effect in terms of pharmacological potency is lower in hops. Hops have gamma aminobutyric acid (GABA). Some studies suggest that GABA supplements can enhance the release of growth hormone¹⁰ or through the endocrine system, which is responsible for regulating, coordinating and integrating many physiological processes.

Possible uses of this herb, among others are:

- 1 - Antianxiety: GABA acts as an inhibitory neurotransmitter, having a calming effect on the central nervous system.
- 2 - Sleep Inducer: Its effects on insomnia may be due to the soothing properties on the nervous system and brain.
- 3 - Anticonvulsant: GABA is often deficient in certain seizure disorders and motors such as epilepsy or tardive dyskinesia.
- 4 - Antihypertensive: GABA may help regulate some cardiovascular mechanisms involved in hypertension.
- 5 - Anticancer agent: Recent studies has show that some components can neutralize the enzymes that trigger abnormal growth of cancerous tumours producing cells, especially breast¹¹ and prostate cancers¹².
- 6 - Aphrodisiac agent: Studies with hop extracts have shown to help female sexual dysfunction that accompanies menopause¹³.
- 7 - Anti-inflammatory: Rutin (quercetin-3-O-rutinoside) has anti-inflammatory properties, demonstrated in several animal models and *in vitro*¹⁴.
- 8 - Anticoagulant: Rutin can help prevent blood clots and reduce the risk of heart attacks and thrombosis¹⁵.
- 9 - Anti-bacterial: Proanthocyanidins, especially type A, have a preventive role in urinary tract infection¹⁶.

Enuresis in children

Bedwetting is a common problem. Approximately 15-20% of children under 7 years old regularly wet the bed¹⁷ and the problem can persist into adolescence and even occasionally in adulthood. Boys suffer more often than girls and may be a family factor. Three pathogenic mechanisms have sufficient scientific support to be considered, such as: nocturnal polyuria, nocturnal hyperactivity of the detrusor muscle of the bladder and a high threshold for awakening. They can depend, in turn, from a common underlying disorder at the brainstem level.

It can be distinguish two degrees of patients, grade 1 and grade 2. Grade 1 patients are those who have some form of urinary incontinence and grade 2 patients are all grade 1 patients who have had some form of treatment for enuresis in which, for some reason, has not been responding to any results or therapies (such as alarms) or with drugs / medications (such as desmopressin). These patients may be referred to as "rebels" for treatments. Also, bedwetting may be complicated further, ie without underlying factors such as diabetes, kidney disease or urogenital malformations, being enuresis single the most common.

The first line of treatment for bedwetting is the enuresis alarm, which has a very high curative potential but requires hard work and motivation. Therefore, there are many families who

cannot carry it out properly. Desmopressin in these cases, a synthetic substitute vasopressin hormone that reduces the production of urine, can be the treatment of choice. Desmopressin has risks and side effects, can cause hyponatremia, which can become severe if combined with excessive liquid intake¹⁸. For children who do not respond to desmopressin, other treatments such as anticholinergics (oxybutynin, tolterodine and propiverine), which are useful as adjuvant therapy¹⁹, are available. There are drugs that carry a significant toxicity but can cause or aggravate constipation. Likewise, imipramine, which is a tricyclic antidepressant therapy, has been used to treat enuresis. Studies give a response rate of approximately 50%. The reason for this effect is unclear, but most likely is linked to cerebral noradrenergic action²⁰. Imipramine is used only in the treatment of enuresis resistant to desmopressin, since the side effects (mostly in mood) can be annoying, are common and an overdose can be life threatening for the patient²¹.

Finally, alternative treatment modalities such as acupuncture and hypnotherapy are widespread. However, there is no scientific evidence for its use to justify their use as therapy standard²².

Aromatherapy uses essential oils extracted from plants. Its use can be made through the vaporization of these extracts in the bedroom or on the pillow and applying a few drops on a cloth or towel and leave it near the bed headboard. One of the traditional remedies was to sleep on a pillow stuffed with hops flowers. Precisely the use the hops flower extract as relaxing therapy and sleep inducer in children has revealed that these children, who had enuresis, did not show any problem while they were given aromatherapy. Based on its soothing properties, it has been proposed hop as an alternative therapy for the treatment of enuresis.

SINORINA (of Laboratec S.L.) is a new product recently marketed and available in pharmacies, in which its main active ingredients from hops flower extract are rutin, concentrated tannins (proanthocyanidins) and gamma-aminobutyric acid (GABA).

Annex, product quality

Product Quality control certificates:

ISO 9001:2000 Certified

- Hop Products Australia
- Botanix Ltd.
- John I. Haas, Inc.
- Joh. Barth & Sohn GmbH & Co.
- NATECO2 GmbH & Co.
- Hopfenveredlung St. Johann GmbH & Co.

ISO 14001:2004 Environmental Certificates (Europe)

- NATECO 2 GmbH & Co.
- Hopfenveredlung St. Johann GmbH & Co.

Kosher Certificates

- NATECO2 GmbH & Co.
- Hopfenveredlung St. Johann GmbH & Co.

- Betatec Hop Products and John. I. Haas, Inc.
- Botanix Ltd.

Organic Certificates (Europa)

- Hopfenveredlung St. Johann GmbH & Co.
 - Joh. Barth & Sohn GmbH & Co.
 - Hopfenveredlung St. Johann GmbH & Co.
- <http://www.barthhaasgroup.com/>

2 HIPOTHESIS

The hops flower extract has been proven with calming and relaxing properties. This may have a beneficial effect on nocturnal enuresis in children.

Hypotheses Laboratec S.L. proposed are:

- That one of the components of *Humulus lupulus* helps control urine retention overnight.
- That using hops flower extracts contained in a commercialised product (SINORINA) can help prevent and / or eliminate bedwetting.

3 OBJETIVES

The main objective of this pilot study is to demonstrate that with the use of SINORINA, a hops flower extract, that by its properties and natural active ingredients, in addition to the qualities to be known as relaxing and calming, can help control urine leakage (urinary incontinence) in children when the child is sleeping. These properties are found in the volatile agents that are in the aroma of the product.

4 MATERIALS Y METHODS

Patients

Children in the study group were children of 5-7 years old (age at which it is normal to control full night-time urination) suffering from enuresis grade 2, ie without associated disorders such as diabetes, urinary tract infections, urogenital malformations or nerve or constipation and diagnosed resistant to bedwetting treatments.

Sampling calculations

We assess whether the use of SINORINA is better than the natural evolution in containing urine at these ages. We know from previous data that containment spontaneous at these ages is around 70% and considered clinically relevant if SINORINA does it by 90%. Our levels of risk are fixed at 0.05 and we estimate a statistical power of 80%.

$$n = \frac{\left[Z_{\alpha} * \sqrt{2p(1-p)} + Z_{\beta} * \sqrt{p_1(1-p_1) + p_2(1-p_2)} \right]^2}{(p_1 - p_2)}$$

$$p = \frac{0.7 + 0.9}{2} = 0.8$$

$$n = \frac{\left[1.645 * \sqrt{2 * 0.8(1-0.8)} + 0.842 * \sqrt{0.7(1-0.7) + 0.9(1-0.9)} \right]^2}{(0.7 - 0.9)^2}$$

Sampling n = 48 patients.

Study distribution

The study was conducted in two groups: the study group was given the SINORINA with 25 children and the other group (placebo group) with 23 children were given the same composition but without the hops flower extract SINORINA (prepared by the same company – Laboratec S.L., in an identical container as the product of the study). Consecutive sampling made the distribution of the product by groups randomly. Neither the doctor nor the family knew if they were administered the product or the placebo. Children were included in the study as they came for assessment in outpatient General Paediatric Hospital Universitario Dr. Peset de Valencia.

Composition

Hop extracts were used with the following composition:

Sweet almond oil, tocopheryl acetate, ascorbyl palmitate, 2% of flower extracts from *Humulus lupulus*. Each vial provided by Laboratec S.L. contains 20 ml.

We selected children with urinary control problems during the night from the database of the Paediatrics ward, Hospital Universitario Dr. Peset for submission to the study of SINORINA which are detailed below:

Placebo group, with the supply of the same compounds as the SINORINA but without the principle active ingredients from hop flower extract, with no therapeutic effects.

Study group, this group was provided with the product produced by Laboratec S.L. (SINORINA).

Statistics

Statistical analysis of the data was performed by Student's t test in the case of quantitative variables (the variables included in the data report forms).

Regulations

Data collection was done in accordance with the rules on human experimentation fulfilling the Nuremberg Code, the Helsinki (1964), Tokyo (1975), Venice (1983), Hong Kong (1989), Sydney and additional Directive, Directive 91/507/CE, RD 561/1993, the Bioethics convention of the European Council (BOE 10/20/99), and ethics and current medical ethics. Each patient was asked for permission, in writing, to be liable for the study by contrasting information.

5 METODOLOGY

Usage

When the child goes to bed at night, put a total of 12 or 14 drops per day in one or two cleansing wipes, or cotton. The wipes will be placed on the nightstand or as close as possible to the child, with the bedroom door closed or almost closed (to enhance its effect).

Follow up

Data collection was carried out in a diary in which parents recorded if the child had urine leakage at night and the approximate amount (much, pretty, little), and the time they went to sleep, the amount of liquid intake (water). The trial was made in three stages:

15 days at baseline (without taking anything)
15 days with SINORINA
15 days after removing the SINORINA.

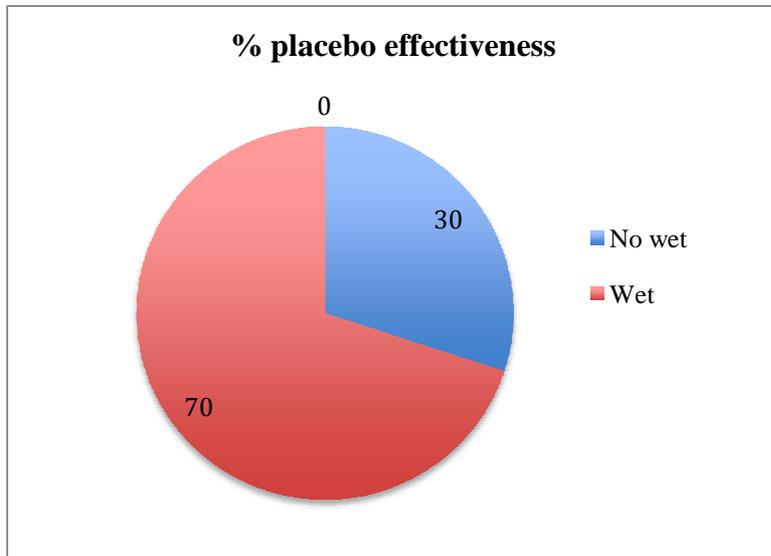
6 MEDICAL-SCIENTIFIC INTEREST

As it is a safe product with no side effects, it can be used as an alternative to treatment simple nocturnal enuresis in children. This situation (nocturnal enuresis) produces a significant unease within the family and makes the children's life relationship difficult by limiting their output in hiking, camping and other. If it is effective, the product can be the basis for research on *Humulus lupulus* active components responsible for this effect and its actions at the nervous system and urinary tract in both young patients and adults.

7 RESULTS

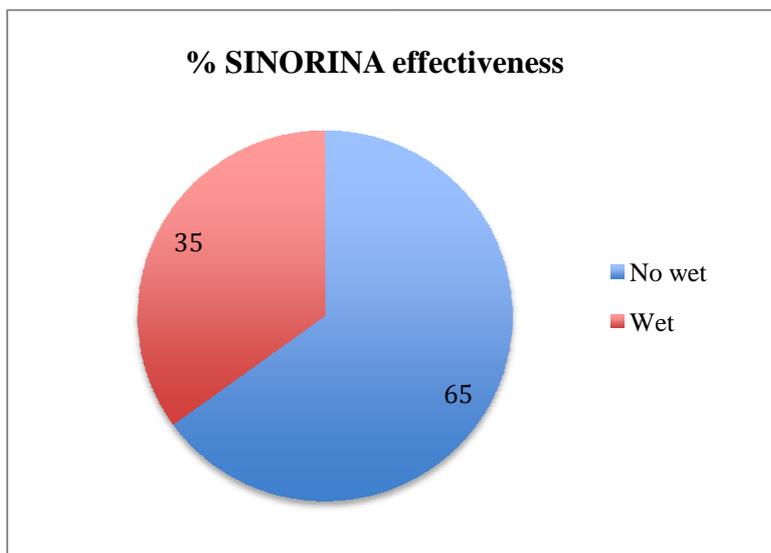
After analysing the data from the data collection notebooks and watching the kids diapers that they have soaked overnight, we have taken the percentage of SINORINA product effectiveness.

Fig. 1: Graphic representation placebo group



There is a high percentage (70%) of children who wet in the placebo group, n = 23.

Fig. 2: Graphic representation SINORINA group



The percentage of children who do not wet with the product is 65%, n = 25.

8 CONCLUSIONS

Following the completion of the clinical trial in children with nocturnal enuresis grade 2 diagnosed by primary care paediatrician and sent to the hospital for the same reason we can say that the activity of the demonstrated effectiveness of SINORINA product is 65%. In order to use with children with nocturnal enuresis grade 1, the percentage of effectiveness should be higher, data not studied in this pilot study, since patients with grade 2 is more effective than desmopressin.

As demonstrated, SINORINA's properties can control urine leakage in infants and would be of great interest to analyse in more detail the active ingredients of the extract of hops flower.

SINORINA is recommended as the first therapy treatment (for its high degree of effectiveness) to treat urinary incontinence in children and / or possibly in adults.

9 REFERENCE

Note: Laboratec S.L. provided the literature in collaboration with the Department of Paediatrics of the Hospital Universitario Dr. Peset.

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The present clinical study was initiated in September 2011 and ended in December 2012.

Valencia, 18th January 2013.

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