A lupin protein hydrolysate reduces the severity of a preclinical mouse model of multiple sclerosis

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MULTIPLE SCLEROSIS (MS) is a disease of the central nervous system characterized by the loss of myelin.

- Inflammation
- Oxidative stress

Relapsing-remitting MS (RR-MS):

the most common form of MS, which it involves periods of disease activity followed by periods of recovery.

Experimental autoimmune encephalomyelitis (EAE): the common animal model used to study MS.

EAE mimics the clinical course of MS

EAE leads demyelination and paralysis, by immune cells activation in response to myelin antigens.

Currently, there is no cure for MS, and available treatments mainly focus on modulating the immune response to control disease progression and reduce relapse rates.

FUNCTIONAL FOODS: foods containing peptides have been found to have various health benefits, such as:

- anti-inflammatory
- antioxidant

Certain peptides have shown potential in improving memory, learning, antioxidant levels, reducing inflammation, and providing neuroprotection in animal models of CNS disorders.

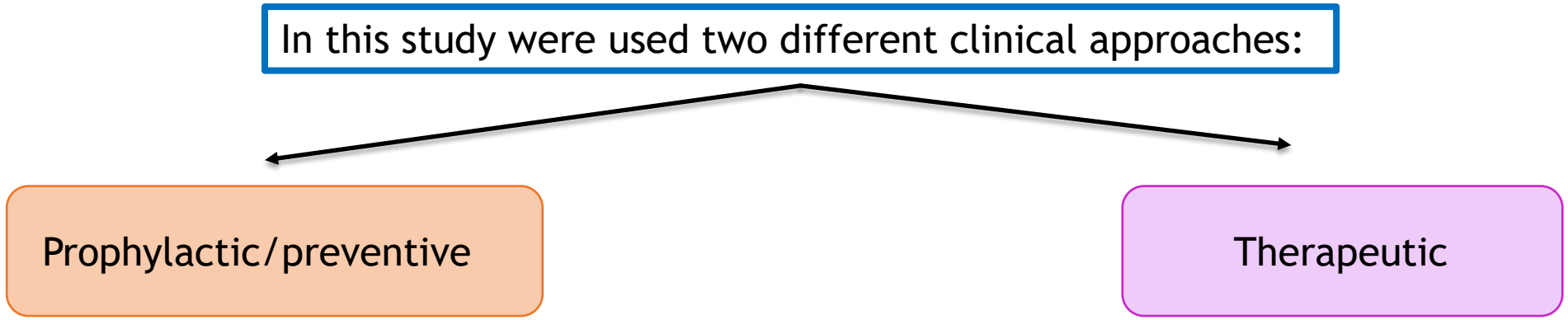
We have generated a *Lupinus angustifolius* protein hydrolysate (**LPH**) with the use of Alcalase

It possesses antioxidant and immunomodulatory effects *in vitro*, in murine models, and in a clinical food trial

LPH enhances the antioxidant status of the CNS, showing anxiolytic properties.

NO previous studies have examined the impact of food-derived peptides on the clinical symptoms of EAE

Considering these factors and recognizing that oxidative stress and inflammation play significant roles in neurodegenerative diseases, the primary objective of this study was to assess the impact of LPH on the clinical symptoms of EAE.



2. Objective

Lupinus angustifolius protein isolate was hydrolysed with Alcalase for 15 minutes at 50°C and pH 8 (= LPH).

1

The EAE inducer solution (MOG_{35-55} peptide emulsified in complete Freud adjuvant -CFA- containing heat-inactivated Mycobacterium tuberculosis) was subcutaneously inoculated.

2

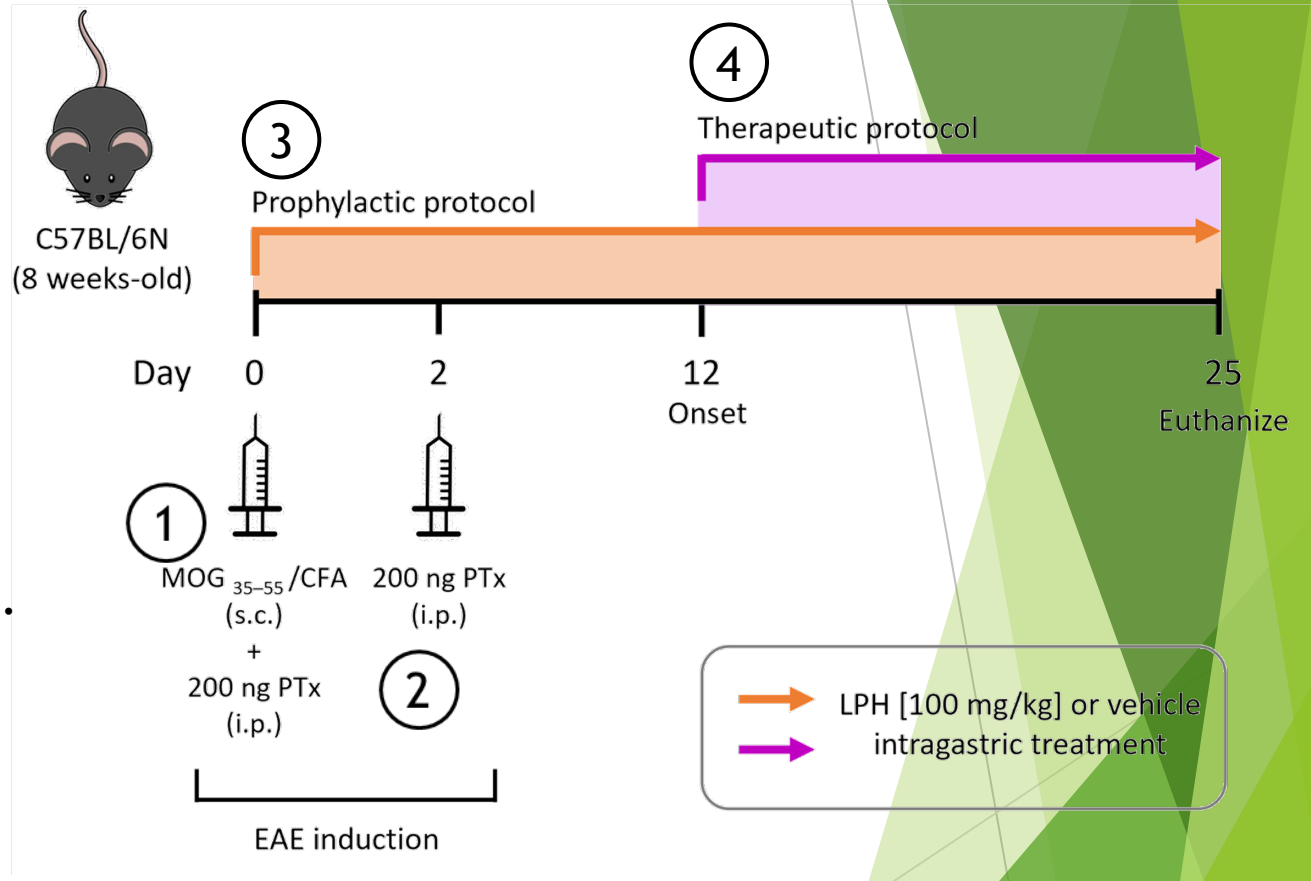
Two doses of pertussis toxin were administered intraperitoneally on days 0 and 2 post-induction.

3

Prophylactic protocol: daily treatment from day 0.

4

Therapeutic protocol: daily treatment from the beginning of clinical signs of EAE (day 12).

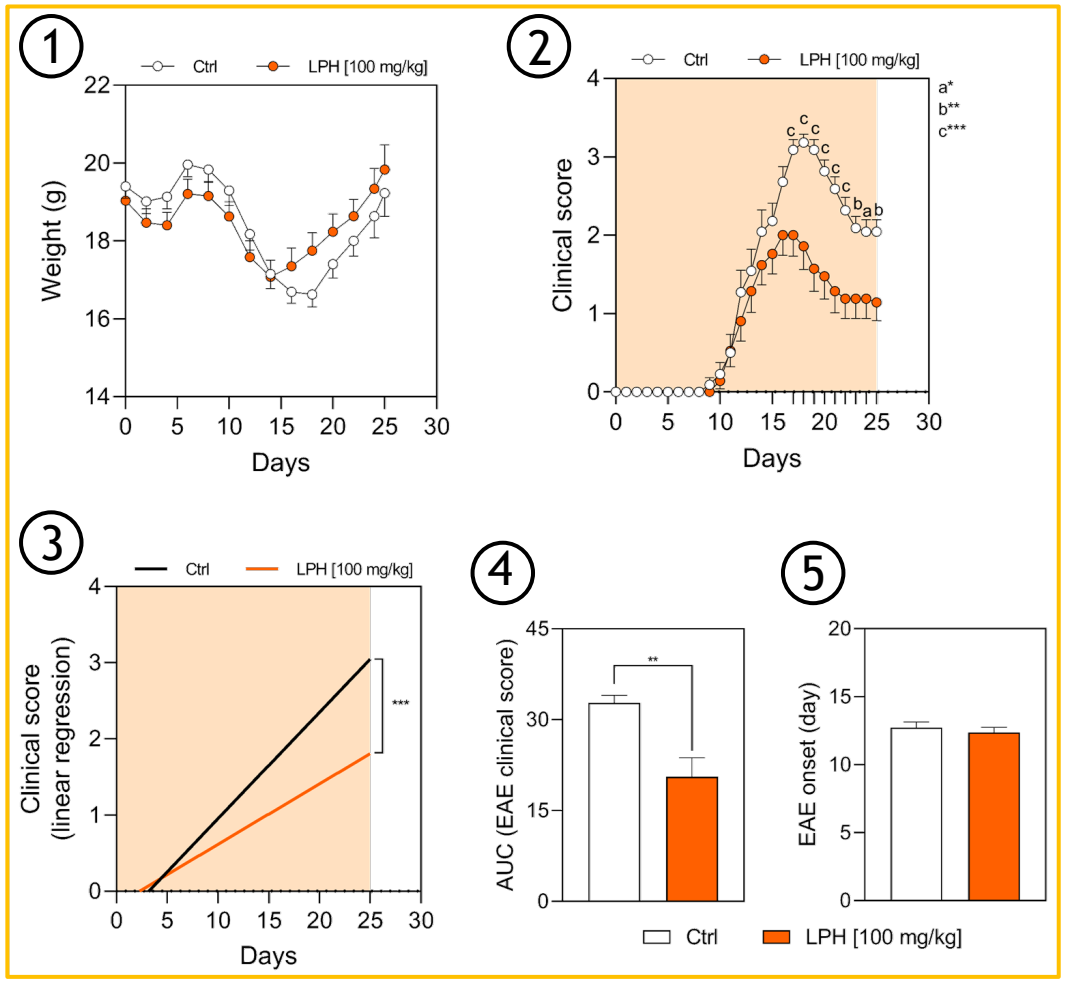


Data were represented as mean ± SEM and they were analysed either with the Mann-Whitney U test or with the Two-way ANOVA and post-hoc correction (GraphPad Prism 8, San Diego, CA, USA). *p*-value ≤ 0.05 was considered statistically significant.

3. Materials and Methods

Clinical score	Signs and symptoms
0	no clinical sign (normal mouse)
1	no tail tone
2	impaired righting reflex
3	paralysis of a hind limb
4	paralysis of both hind limbs
5	paralysis of both hindlimbs and one front limb
6	moribund or dead mouse

Prophylactic LPH treatment reduces the clinical severity of EAE

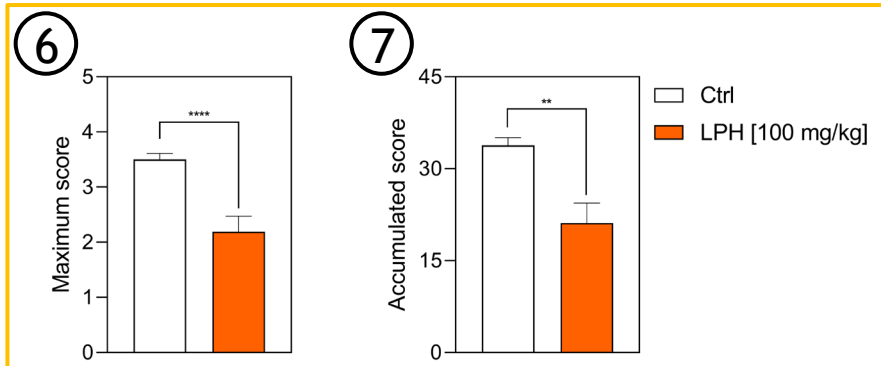


① The two experimental groups showed a normal weight fluctuation throughout the course of the EAE.

② LPH treatment significantly reduced the clinical signs of EAE. (day 18 p.i.: Ctrl: 3.2 ± 0.11 *vs.* LPH: 1.9 ± 0.29; $p = 0.0003$).

A linear regression of this EAE score curve ③ as well as the calculation of the area under the curve (AUC) ④ clearly showed the beneficial effect of LPH treatment, observing significant statistical differences.

⑤ LPH treatment did not modify the day of onset of the clinical signs.

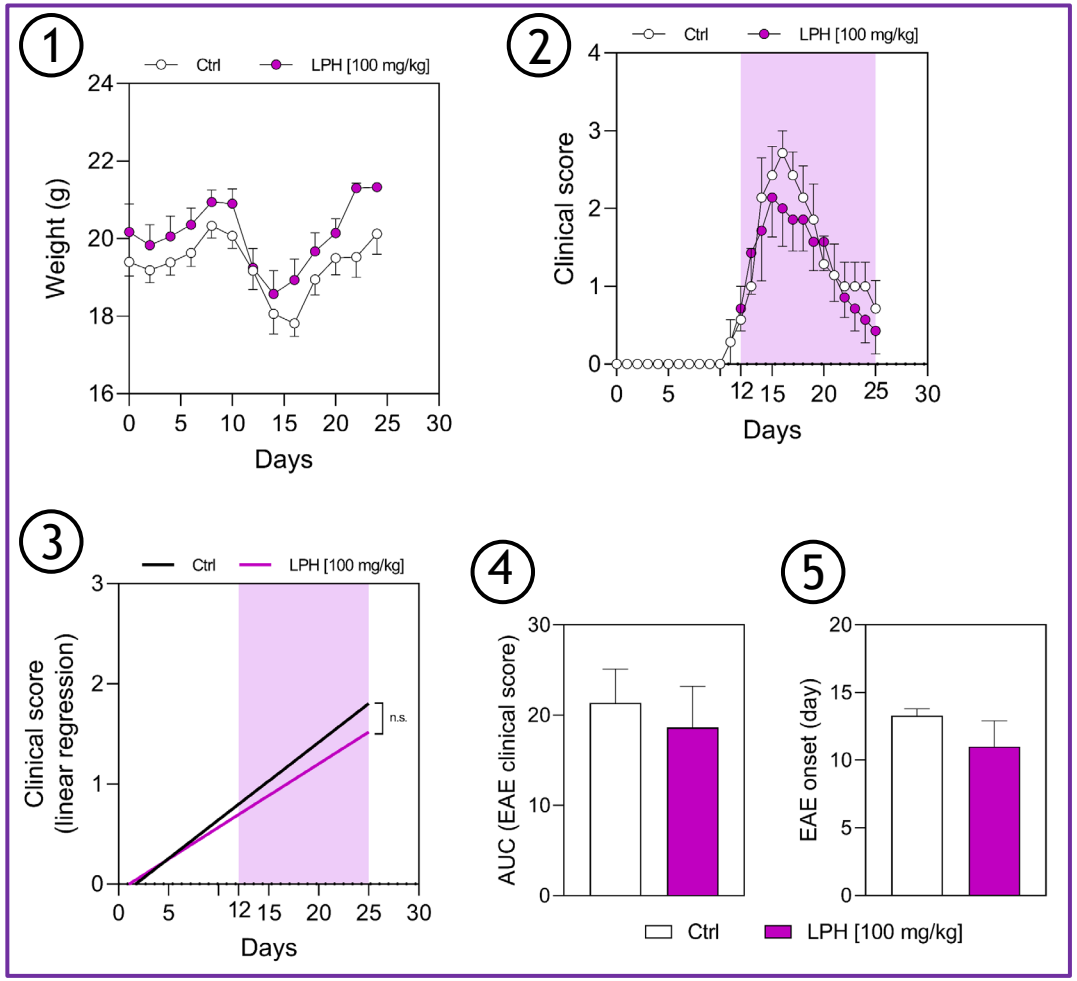


⑥ LPH-treated mice showed a mean maximum score of 2.2 ± 0.28, lower than the Ctrl group (3.5 ± 0.11); $p < 0{,}0001$.

⑦ The treatment with LPH decreased the total accumulated score (sum of all the daily scores) compared to vehicle-treated mice. (Ctrl: 33.82 ± 1.26 *vs.* LPH: 21.14 ± 3.23; *p*-value = 0.006)

Prophylactic protocol

Therapeutic LPH treatment does not ameliorate ongoing EAE



The body weight of the animals, (1)
as well as
the EAE score curve of the Ctrl group (2)
were similar to the prophylactic experiments

There were no significant differences in the:

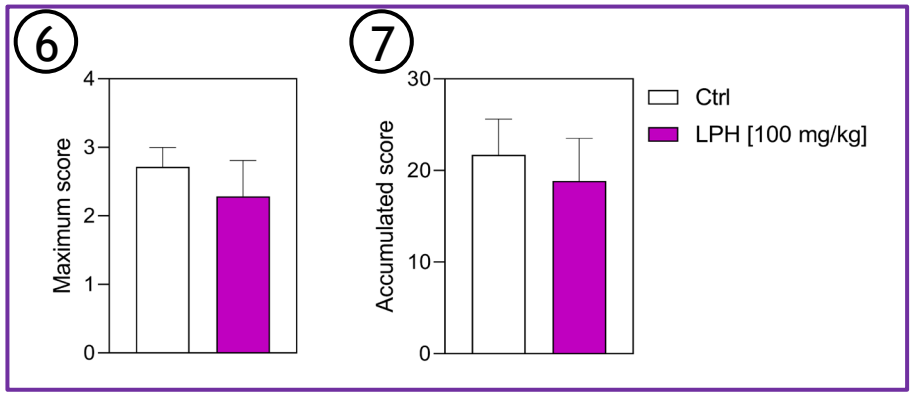
Clinical EAE score (2)

linear regression of the EAE (3)

AUC data (4)

between the two experimental groups

(5) The mean of the day of EAE onset was maintained around day 12 p.i. for both experimental groups, without significant differences.



(6) LPH treatment did not decrease the maximum score reached by mice (2.3 ± 0.5), compared to the Ctrl group .

(7) Accumulated score was not modified by LPH treatment with respect to the Ctrl group .

Therapeutic protocol

5. Conclusion

LPH daily treatment ameliorates the clinical symptoms of EAE, controlling the evolution of this debilitating disease.

LPH could be pointed out as a nutraceutical with very interesting clinical implications medium term.

Thanks

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