

Clinical Research Project

Mechanistic Evaluation of Polenoplasmin Efficacy in Canine Spinal Cord Injury

□ Proposed Entities and Partnerships

- **Project Promoter:** Deniplant Biomedicine Center.
- **Clinical Partners:** Department of Neurology and Neurosurgery, Faculty of Veterinary Medicine.
- **Technology Partners:** Molecular Genetics Laboratory (for Metagenomics) and Veterinary Pathophysiology/Neurology Laboratory (for Electromyography).

□ Pillar I: Retrospective Study (Validation of Historical Data)

The objective is to organize the more than 200 resolved cases into a statistical database accepted by ISI-indexed journals (e.g., *Frontiers in Veterinary Science*).

- **Standardization of Neurological Scores:** Aligning historical clinical records with the **Olby Scale (Canine Spinal Cord Injury Score)**, which evaluates motor parameters ranging from 0 (flaccid paralysis, no deep pain perception) to 14 (normal gait).
- **Correlation Analysis:** Statistical calculation of the recovery index in relation to:
 - o Time elapsed between injury and administration (groups: <7 days, 8–30 days, 31–90 days).
 - o Administered dosage (correlating the number of capsules with the animal's body weight).
- **Survival and Functional Outcome Curve:** Utilizing Kaplan-Meier analysis to scientifically demonstrate the mean time required to regain standing stability and coordinated gait.

❑ **Pillar II: Prospective Study – Molecular Decoding of the Microbiome-Spinal Cord Axis**

Spinal Cord Injury (SCI) triggers secondary bowel paralysis (neurogenic ileus), destroying beneficial bacteria and releasing endotoxins that block spinal cord regeneration. This is where your phytocomplex comes into play.

1. Fecal Metagenomics (16S rRNA Sequencing)

Samples will be collected at T0 (pre-treatment), T15, T30, and T60. The molecular hypothesis the project will test is:

- **Stimulation of Firmicutes and Bifidobacteria:** Oligosaccharides from carob and pollen act as prebiotics. We expect to see a massive increase in **Lactobacillus** strains and **Faecalibacterium prausnitzii**.
- **Butyrate Production (HDAC Inhibitor):** These bacteria synthesize short-chain fatty acids (SCFAs). Absorbed butyrate enters the circulation and acts within the spinal cord as a histone deacetylase (HDAC) inhibitor. This molecular phenomenon directly unlocks genes responsible for neuroplasticity and axonal regeneration.

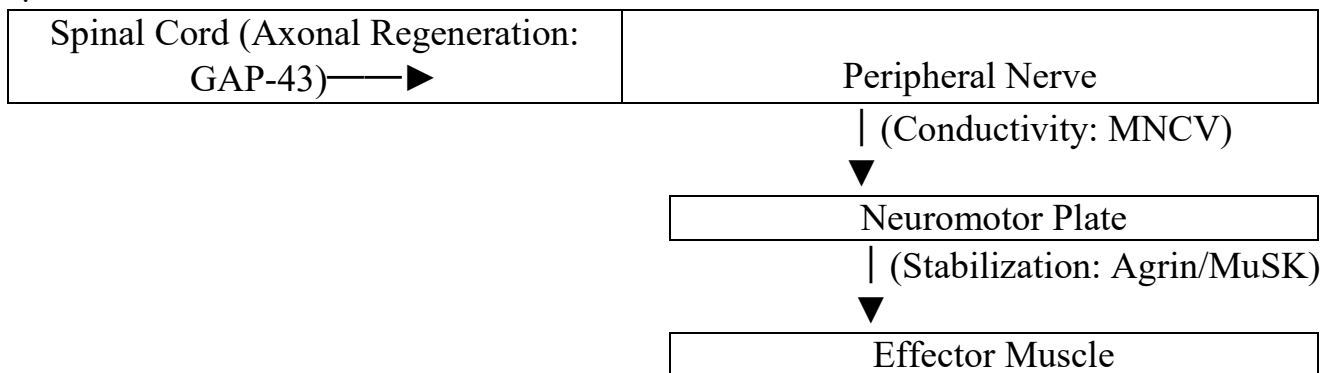
2. Cytokine-Mediated Neuroimmunomodulation (ELISA)

Monitoring serum inflammatory markers to demonstrate the shift from a neurotoxic state to a regenerative one:

- **Decrease in M1 markers (Neurotoxic):** Measuring the reduction of TNF- α and IL-1 β .
- **Increase in M2 markers (Neuroprotective):** Quantifying the upregulation of IL-10 and TGF- β , demonstrating that pollen polyphenols induce microglia to clear myelin debris without killing remaining neurons.

⚡Pillar III: Assessment of the Neuromuscular Junction and Peripheral Re-innervation

In cases of paralysis, even if the spinal cord begins to recover, the neuromuscular junction (NMJ) can undergo irreversible degradation due to denervation. Brewer's yeast (rich in nucleotides, B-complex vitamins, and minerals) and the amino acids found in pollen provide direct energy support for the restoration of the motor synapse.



1. Neurophysiological Protocol (EMG and MNCV)

- **Electromyography (EMG):** Assessment of fibrillation potentials in the gastrocnemius and tibial muscles. A reduction in fibrillations during treatment will serve as direct evidence of muscle fiber reinnervation.
- **Motor Nerve Conduction Velocity (MNCV):** Measurement of electrical conduction along the sciatic nerve to demonstrate axonal remyelination under the influence of Polenoplasmin components.

2. Determination of Neurotrophic and Synaptic Factors

- **GAP-43 (Growth-Associated Protein 43):** Plasma marker for axonal growth cone elongation.
- **Agrin-MuSK pathway:** Biochemical analysis (where possible, via fine-needle muscle biopsies or indirect markers) of how the formula's micronutrients stimulate the clustering of acetylcholine receptors (AChR) at the neuromuscular junction, restoring chemical signaling between nerve and muscle.

• • Regulatory and Commercial Alignment

The formulation offers a significant advantage: the ingredients are already classified in accordance with Regulation (EC) No 1831/2003 and Regulation (EU) No 68/2013.

The research project will not be hindered by the stringent testing requirements associated with new allopathic drugs; instead, it can be classified as **a clinical study on a nutritional product for special purposes (nutraceutical)**, thereby facilitating rapid approval by university bioethics committees.