

Research Project Proposal (Comprehensive Project)

1. Project Title

"Evaluation of neuro-immunomodulatory mechanisms, the microbiome-spinal cord axis, and neuromuscular junction plasticity during post-traumatic recovery from canine paraplegia under the influence of a standardized bioactive phytocomplex."

2. Project Objectives

- **Primary Objective:** To demonstrate biological efficacy and elucidate the cellular/molecular mechanisms by which the treatment induces nerve regeneration and restores motor control in dogs with hind-limb paralysis.
- **Secondary Objectives:**
 - o To identify changes in gut microbiome composition before and after treatment.
 - o To quantify markers of systemic and spinal inflammation (M1 vs. M2 cytokines).
 - o To evaluate the integrity and re-innervation of the neuromuscular junction in effector muscles.

3. Experimental Design & Work Stages (Work Packages)

To ensure high-impact research, the project is structured around three main pillars: the Retrospective Study, the Prospective Clinical Study, and Laboratory Analysis (mechanistic).

POLENOPLASMIN Research Project		
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WP1: RETROSPECT (Database)	WP2: PROSPECTIVE (New active cases)	WP3: MOLECULAR (Lab Analysis)

□ WP1: Retrospective Study (Leveraging historical data)

- **Action:** Centralizing the several hundred previously monitored cases into a scientific database.
- **Parameters** to be extracted: Breed, age, type of trauma (e.g., Grade 5 disc herniation, vehicular trauma), time elapsed from paralysis onset to treatment initiation, initial neurological score (OFS scale – Olby Canine Spinal Cord Injury Score), and time to recovery of ambulation.
- **Outcome:** Generation of the world's first robust statistical analysis based on such a large cohort of canine subjects treated with this protocol.

□ WP2: Prospective Clinical Study and Microbiome-Spinal Cord Axis Monitoring

For new cases enrolled in the study, non-invasive samples will be collected at key time points (Day 0, Day 30, Day 60, Day 90):

- Microbiome Metagenomics (16S NGS Sequencing): Fecal analysis to demonstrate the direct correlation between phytocomplex administration, the increase in butyrate-producing bacteria (*Lactobacillus*, *Bifidobacterium*, *Faecalibacterium*), and neurological improvement.
- Cytokine Profile (Blood/Cerebrospinal Fluid): ELISA-based measurement of pro-inflammatory markers (TNF- α , IL-1 β – specific to the toxic M1 phenotype) and anti-inflammatory markers (IL-10, TGF- β – specific to the regenerative M2 phenotype) to demonstrate neuro-immunomodulation.

⚡WP3: Analysis of the Neuromuscular Junction and Nerve Conduction

- Electromyography (EMG) and Motor Nerve Conduction Velocity (MNCV): Periodic assessment of electrical conductivity from the spinal cord to the hind limb muscles. This will scientifically demonstrate whether the nerve impulse successfully crosses the injured area and whether the neuromuscular junction has been functionally re-innervated.
- Plasticity Biomarkers (Optional/Blood): Monitoring levels of BDNF (Brain-Derived Neurotrophic Factor) and GAP-43 (growth-associated protein 43).

4. Required Strategic Partnerships

Such a project requires multidisciplinary expertise, ethical accreditations, and the establishment of partnerships with:

1. **A Faculty of Veterinary Medicine** (Neurology and Pathophysiology Departments) – for clinical infrastructure, imaging (veterinary MRI/CT), and Bioethics Committee approval.
2. **A Genetics/Molecular Biology Laboratory** – for microbiome sequencing and cytokine analysis.
3. **The manufacturer of the phytocomplex (Deniplant)** – to ensure standardized product batches and potential co-financing (private research grant).

5. Deliverables and Scientific Impact

- **Publication of ISI Articles:** Publishing results in prestigious international journals with high impact factors (e.g., *Journal of Neurotrauma*, *Frontiers in Veterinary Science*, *Nutrients*).
- **Method Patent/Protocol:** Standardization of an officially recognized complementary therapeutic protocol for managing spinal cord trauma in companion animals.