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Protection with Electrolyzed Reduced Water on Gut Microbiota in Rats Exposed to Permethrin during Postnatal Development

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Objectives: Electrolyzed reduced water (ERW) is a functional drinking water with antioxidant activity due to its negative oxidation reduction potential (ORP = –300 mV) that release electrons by hydrogen (400 ppb).

Here, the effect of ERW was investigated in an animal model developing neurodegenerative disease after exposure to permethrin pesticide (PERM) in early life. In particular, the impact of ERW on gut microbiota and permeability was studied.

Methods: Rats were treated in early life with permethrin (PERM), vehicle (control), PERM + ERW. At adolescent age (60 days old), feces of rats were collected and submitted to microbiota identification by 16S rRNA gene-amplification (MiSeq Illumina). In addition, fecal short chain fatty acids (SCFA) were measured by GC.

In order to measure intestinal barrier permeability, rats were gavaged with a permeability tracer, FITC-dextran 4 Da (500 mg/kg) and 2 h later, blood was withdrawn.

Results: PERM group showed an increase of intestinal permeability, a lower abundance of bacterial species and changes of bacterial composition in the feces compared to CONTROL group.

The co-treatment with electrolyzed reduced water could not prevent at all the abnormal intestinal permeability induced by PERM but could attenuate the leakiness of the intestinal barrier.

Gut microbiota analysis shows that the co-treatment with ERW was effective to increase the bacterial diversity. Higher diversity in bacterial OTUs is advantageous because there are less dominant species who monopolize most of the resources at the expense of the others.

Analyzing the composition of bacteria phyla, we observed that ERW increased *Firmicutes* (55.33%) at the expense of *Bacteroides* (43.06%), whereas CONTROL and PERM groups did not differ from each other.

The co-treatment with ERW was effective to prevent the negative effect of PERM by increasing the abundance of many members of the *Lachnospiraceae* family.

ERW had a positive effect on fecal metabolites with respect to CONTROL group.

Conclusion: ERW is an alkaline (pH = 9) and hydrogen molecule-rich water with ROS-scavenging activity. A moderate consumption of alkaline water predisposes to bowel reducing environment promoting the growth of *Lachnospiraceae* family. These bacteria family have been associated with a low grade intestinal inflammation state which prevents gut barrier alteration and disease development.

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Effect of Electrolyzed Reduced Water in an Animal Model of Parkinson-Like Disease

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Objectives: Early life environmental factors, life style and diet have a profound impact on the organism's later development and subsequent onset of age-related diseases such as neurodegenerative diseases. In previous studies, we developed an animal model where the early life exposure (from postnatal day 6 to 21) to low dose of pesticide permethrin (PERM, 34 mg/kg) induced Parkinson-like neurodegeneration in rats characterized by decreased dopamine, Nurr1 and glutathione levels in the basal ganglia, altered immune responses and gut disbiosis. The animal model was exposed to a neurotoxin PERM that is an insecticide widely used for indoor and outdoor applications (i.e. carpets, kitchen worktops other treated wood furniture, lawn, mosquito control). The presence of its metabolites in the urine of 98% of population makes this insecticide a reliable environmental risk factor to health.

The present study aims to test the effect of electrolyzed reduced water (ERW) on this animal model developing neurodegenerative disease after exposure to PERM pesticide in neonatal age.

Methods: The effect of ERW is determined by cognitive tests, dopamine and Nurr1 levels measured in basal ganglia and Tyrosine Hydroxylase (TH) immunostaining in the Substantia Nigra pars compacta (SNc) in 60-day-old rats.

Results: When working memory is assessed in a T-maze, PERM group has a worst performance compared with healthy controls, whereas the performance of PERM+ERW is similar to control group. The same trend is observed for the average of perseverative errors.

With regard to dopamine levels measured by HPLC in basal ganglia, decreased levels are observed in PERM group compared to control group, whereas the co-treatment with PERM+ERW protects against the damage induced by the pesticide even increasing the levels of dopamine. Similar results are obtained with regard to Nurr1 levels measured by western blot in basal ganglia.

Immunohistochemical analysis with anti-TH antibody marking dopamine neurons in SNc shows a reduced number of TH-positive neurons in PERM rats compared to control rats, whereas a slight increase (not statistically significant) was observed in the PERM+ERW group with respect to PERM group.

Conclusion: The use of ERW as functional water could be helpful as a therapeutic tool in the prevention of neurodegenerative diseases.