White Paper-Karnozin Extra

Carnomed is a company that produces and distributes all natural, high-quality food supplements. Our mission is to educate first ourselves, then patients, medical doctors, and other professionals from different disciplines about the importance of L-carnosine, its role in the organism, and its benefits in different medical conditions and problems. For those interested in delving deeper into the benefits of L-carnosine and its therapeutic potential for various diseases and conditions, I highly recommend visiting our company page on Karnopedia. This comprehensive resource offers detailed insights into how L-carnosine can aid in managing some conditions and diseases.

Abstract

The focus of the Carnomed research program is that by improving the cellular respiration in the mitochondria we can affect human health and prevent disease development. Our supplement Karnozin Extra beneficially affects several target functions in the body, reducing the risk of disease and improving overall well-being. Karnozin Extra is a high-quality food supplement with a wide range of physiological activities for many diseases and conditions. Its formulation is based on the principles of mitochondrial medicine. Karnozin Extra is improving the mitochondrial function of every single cell in the body, allowing it to produce enough energy necessary for the body to function properly.

Our R&D department is continuously designing and conducting many studies involving our main product, Karnozin Extra.

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White paper

Karnozin Extra – Carnomed Research Program

To enhance our expertise and boost our market competitiveness, we have decided to conduct several critical scientific experiments in collaboration with the Government of the Republic of Serbia and our partner universities across Serbia.

Preliminary in vitro study of Karnozin Extra stability

It is well-known that pure L-carnosine is quickly degraded in the blood by the enzymes called carnosinases, which split L-carnosine into 2 constituent amino acids beta-alanine and l-histidine, and therefore its bioavailability inside the cells is lower. During years of our work experience, we have been witnessing differences in patient's outcomes when using pure L-carnosine supplements and Karnozin Extra formulation. These observations suggested that L-carnosine in Karnozin Extra formulation is somehow protected from the carnosinase activity and in this *in vitro* study, we wanted to prove that point.

First, we have determined UV/VIS spectra for pure L-histidine (Figure 1.) and pure L-carnosine. (Figure 2.). The spectral analysis shows different spectra. Then we incubated pure L-carnosine and Karnozin Extra in the same concentration with carnosinase enzyme for 10 minutes. After that, we analyzed spectra.

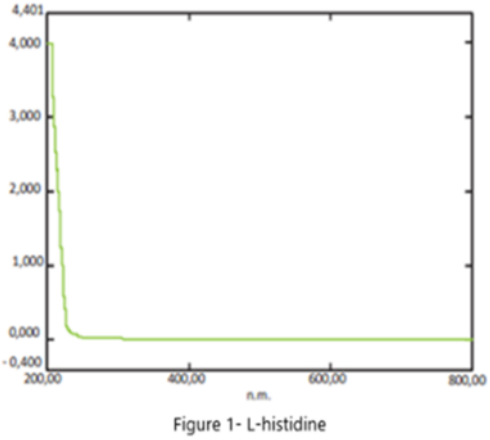

Figure 1- L-histidine

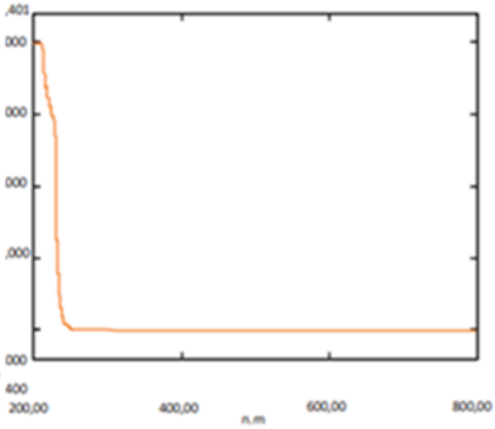

Figure 2 - pure L-carnosine

L-carnosine incubated with carnosinase enzyme showed different spectra compared to only L-carnosine (Figure 3.) (almost similar to the histidine spectra Figure 1.). These findings suggested that the carnosinase enzyme split L-carnosine into beta-alanine and L-histidine.

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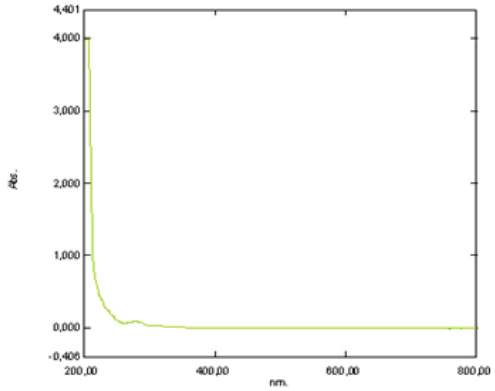

Figure 3. L-carnosine+ carnosinase

However, when Karnozin Extra in the presence of carnosinase enzyme was analyzed, results showed that there was no difference between spectra of Karnozin Extra with and without enzyme (Figures 4 and 5).

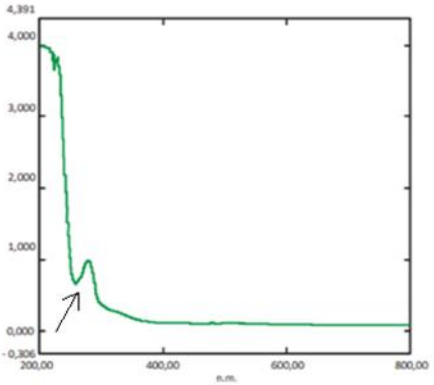

Figure 4. Karnozin Extra

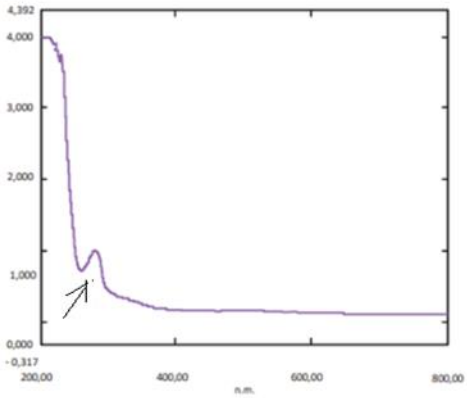

Figure 5. Karnozin extra+carnosinase

According to those results, there is indicia that L-carnosine in Karnozin Extra is stabilized. Although the exact mechanism hasn't been found so far, by performing these analyses we can proudly say that the reason why Karnozin Extra is showing far superior effects in patients compared to using supplements that contain pure L-carnosine substance is its protection from the carnosinase enzyme degradation.

These analyses have been performed at the Institute for Biological Research 'Siniša Stanković' in Belgrade.

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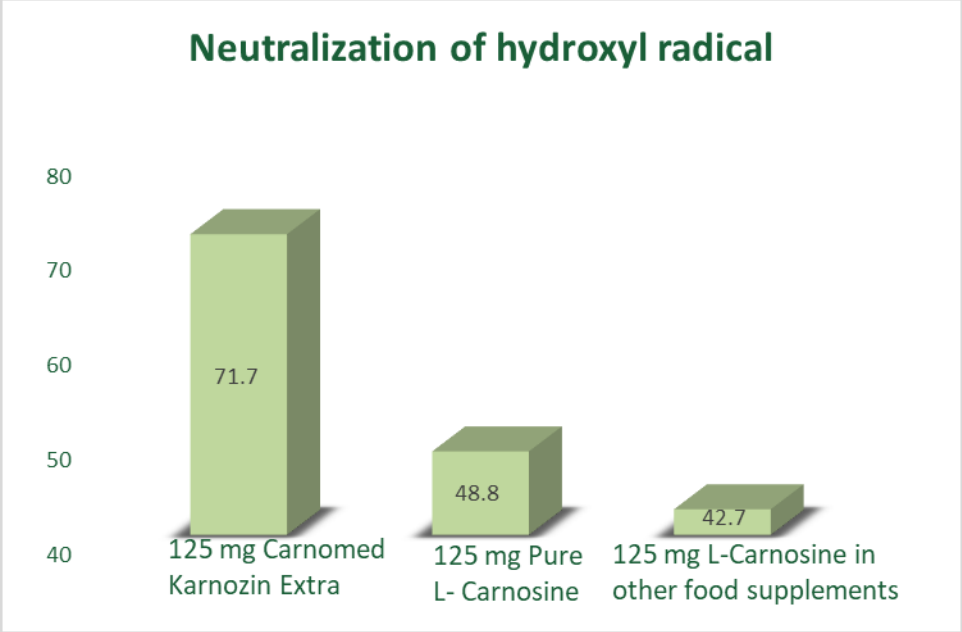
Karnozin Extra – Antioxidative capacity

The first research Carnomed has performed is *in vitro* research of the antioxidant capacity of our product Karnozin Extra. We quantitatively measured its ability to scavenge different types of free radicals. Also, we compared its antioxidant capacity with the capacities of using the same amount of pure L-carnosine ingredient and L-carnosine from competitive food supplement producers.

There is a variety of free radicals that are produced inside our cells. Some of them are useful in low concentrations, but others are extremely dangerous. Some free radicals are so harmful and reactive that they will steal an electron from anything, including DNA, proteins, lipids, and cell structures. **The hydroxyl radical** is highly unstable and it can damage virtually all types of macromolecules: carbohydrates, nucleic acids (mutations), lipids (lipid peroxidation), and amino acids. That is the most reactive radical known to chemistry.



The small, free radical molecule **nitric oxide** has been identified as a major signal transduction molecule in humans and animals. Nitric oxide free radical is involved in the process of apoptosis. When produced excessively, it can disregulate this important way of cell defense system. Also, nitric oxide is involved in oncogene expression, meaning that his role in forming cancer is well described. Moreover, it can damage our DNA through DNA oxidation.

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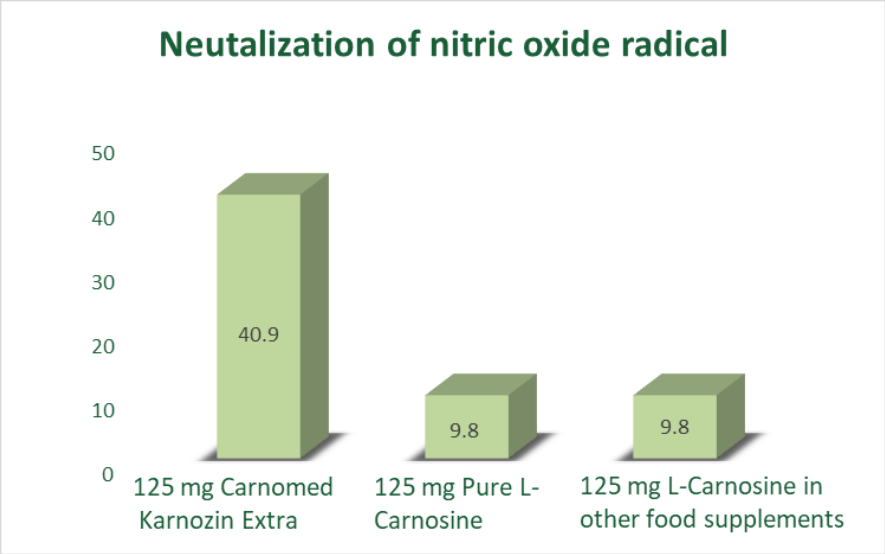
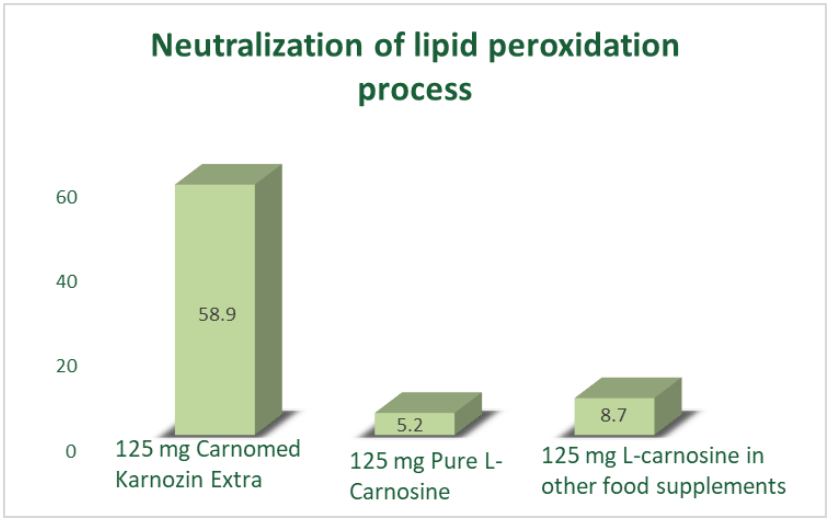


Lipid peroxidation is the oxidative degradation of lipids. It is the process where free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. Lipid peroxidation is implicated in the underlying mechanisms of several disorders and diseases, such as cardiovascular disease, cancer, neurodegenerative diseases, and aging.



These findings suggest that Karnozin Extra possesses far superior antioxidative potential comparing to pure L-carnosine compound and may be translated to any disease and disorder where oxidative stress play a large role.

Above mentioned experiments have been performed at the Medical Faculty, University of Novi Sad.
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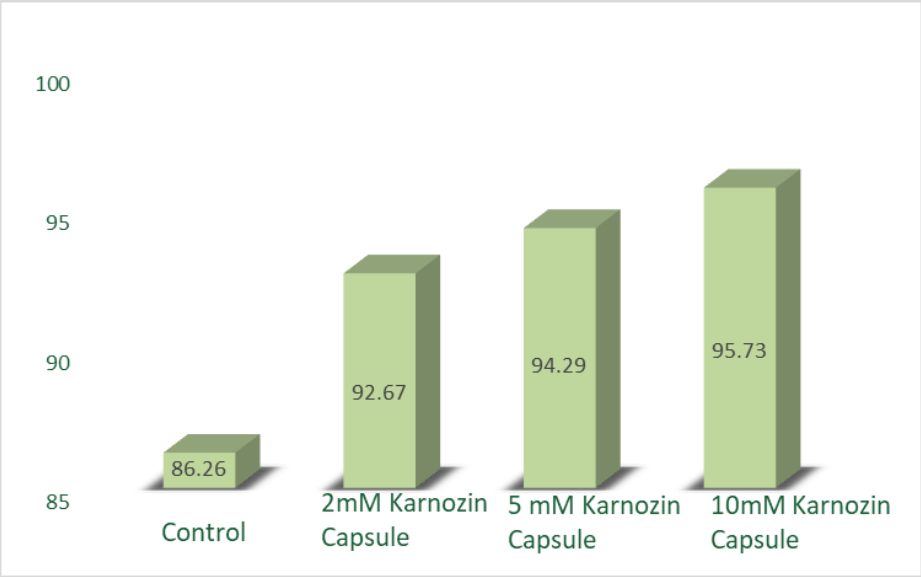
Karnozin Extra and Neuroblast cell line

The second research Carnomed performed was *in vitro* determining the neuromodulatory potential of Karnozin Extra on neuroblasts cell culture. Neuroblasts are precursors of neurons and despite those, neuroblasts still can divide. We used neuroblast cell culture in our research to explore what effect our product has on the brain and nerve tissue. When Karnozin Extra has been administered into the culture in increasing concentrations (2mM, 5mM, 10mM), increased proliferative power of neuroblast has been observed. This finding highlights the positive trophic potential of the Karnozin Extra (Carnomed) preparation on neuroblast cells under in vitro conditions, suggesting that Karnozin Extra functions similarly to a growth factor in nerve tissue. This could explain why Karnozin Extra demonstrates outstanding results in conditions characterized by neuron loss or reduced synaptic activity.Those disorders include autism, epilepsy, ischemic strokes, Alzheimer's, and Parkinson's disease.



The research has been conducted at the Medical faculty, University Of Novi Sad, under the supervision of:
https://www.researchgate.net/profile/Ivan_Capo
https://www.researchgate.net/profile/Dejan_Miljkovic3

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Karnozin Extra and *in vivo* Model of Multiple Sclerosis

The third research is an animal model of autoimmune encephalomyelitis, which is the model that in experimental conditions represents multiple sclerosis.

Multiple sclerosis (MS) is a condition of the central nervous system, that interferes with nerve impulses within the brain, spinal cord, and optic nerves. It is characterized by multiple lesions or scars. These scars occur within the central nervous system and depending on where they develop, manifest into various symptoms. MS affects more than two million people worldwide. Most people are diagnosed between the ages of 20-40, but it can affect younger and older people too. There is currently no known cure for MS and MS represents a significant clinical issue nowadays.

The study design was as following: There were 3 groups of mice, one control group, one that received the dose of 140mg/kg that corresponds to 2 capsules of Karnozin Extra in humans, and the third group received the dose of 420mg/kg that corresponds to 6 capsules of Karnozin Extra per day. We fed all 3 groups for 25 days, which corresponds to 2-2, 5 years in human lifespan.

Brief conclusions:

- Administering the aqueous suspension of Karnozin EXTRA (Carnomed) in a daily dose of 140 mg/kg and 420 mg/kg for 25 days did not lead to statistically significant changes in body weight of mice.

- Administering the aqueous suspension of Karnozin EXTRA (Carnomed) in a daily dose of 140 mg/kg for 25 days (equivalent to a daily human dose of 2 capsules) did **not cause histologically visible changes and damages** to the kidney, liver, spleen, heart, pancreas, testis, digestive tubes and brain.

- Administering the aqueous suspension of Karnozin EXTRA Carnomed in a daily dose of 420 mg/kg for a 25 day period (equivalent to a daily human dose of 6 capsules) **did not cause histologically visible changes and damage** to the kidney, liver, spleen, heart , pancreas, testis, digestive tubes and brain.

- Administering the aqueous suspension of Karnozin EXTRA (Carnomed) in a daily dose of 140mg/kg for period of 20 days (equivalent to a daily human dose of 2 capsules), to animals with previously induced autoimmune encephalitis, **did not make the significant neuronal and glial-protective potential** relative to the control group of individuals without treatment.

- Administering the aqueous suspension of Karnozin EXTRA (Carnomed) at a daily dose of 420 mg/kg for period of 20 days to animals with previously induced autoimmune encephalitis **produced a potentially significant neuronal and glial-protective potential** relative to the control group of the animals without treatment.

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Qualitative analysis of the tissue characteristics among the investigated groups with AIE showed that there were no significant differences between the control group AIE (group 1) and the experimental group with the use of Karnozin EXTRA in a dose of 140 mg/kg (group 2) concerning the intensity of brain damage. However, in the group of mice with 6 capsules per day, it is observed less demyelinating areas and less inflammation. Those mice were in a better shape and condition.

There were no observed morphological, histological and functional changes in lung, liver, kidney, heart, pancreas, brain, spleen and testis tissues, even in high doses (6 capsules per day). That qualifies our product Karnozin Extra as highly safe for usage.

Research has been conducted at the Medical faculty, University Of Novi Sad, under supervision of:

https://www.researchgate.net/profile/Ivan_Capo

Karnozin Extra and Mitochondrial Function of the Cancer Cells

We decided to investigate the effects of Karnozin Extra on oxidative phosphorylation, electron transport chain, and production of ATP in cancer cells. We have measured oxygen consumption in examined cell lines. The research has been completed at The Medical Faculty, University of Novi Sad.

The study was conducted on healthy cells treated with Karnozin Extra, where oxidative respiration was closely monitored. The results of this research, which provide valuable insights into the effects of Karnozin Extra on cellular respiration, are displayed in the accompanying image.

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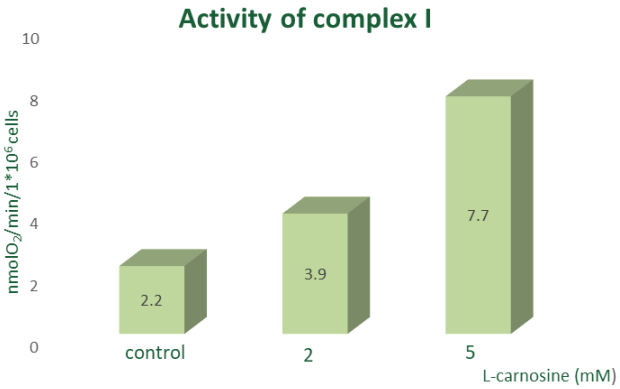

Figure 1. The activity of Complex I

Explains that 2 mM of L-Carnosine doubled the oxygen flow through the cells and 5 mM of L-Carnosine increased it almoust 4 times in complex I in ETS of mitohondria.

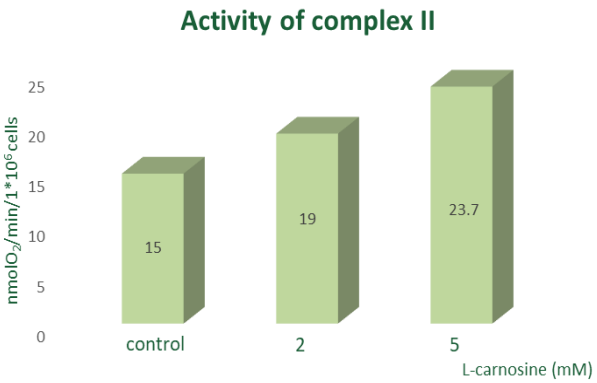

Figure 2. The activity of Complex II

Shows increased oxygen flow through cells in Complex II of ETC, versus the control group, with treatment with 2 mM and 5 mM L-carnosine even more.

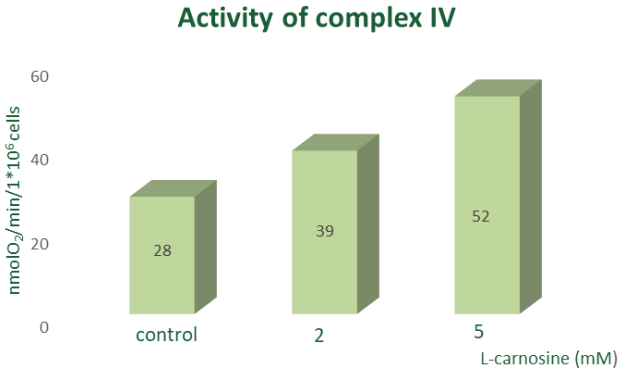

Figure 3. The activity of Complex IV

The most significant increase of oxygen flow through cells is noticed in the activity of complex IV ETC, where the concentration of 5mM of L-carnosine reaches its highest point.

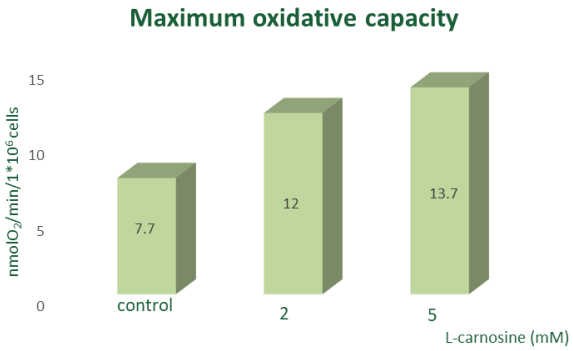

Figure 4. Maximum oxidative capacity of the electron transport system

After the application of FCCP shows us the positive effects of L-carnosine in concentrations of 2mM and 5mM in total.

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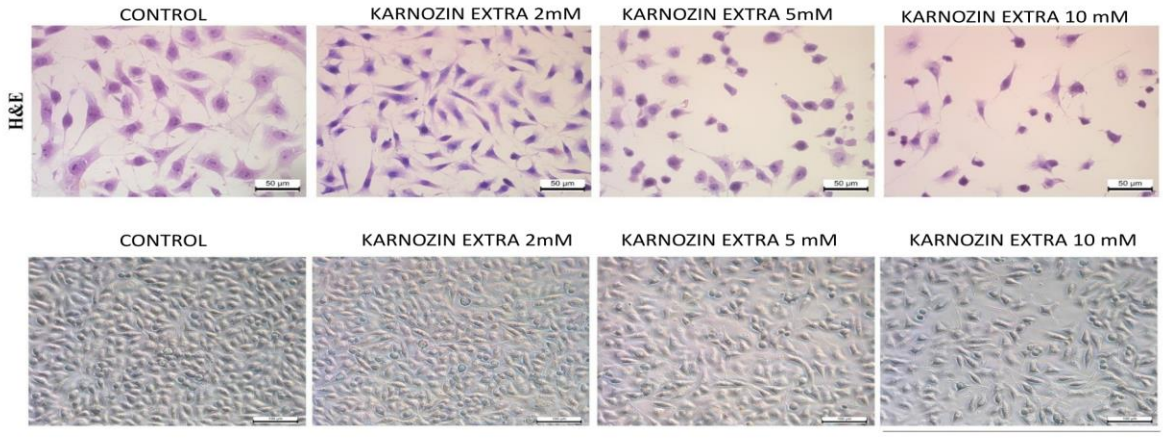
Having examined the effects of Karnozin Extra on healthy cell lines, we then shifted our focus to cancerous cell lines to further understand its potential therapeutic benefits. We selected the human breast cancer cell line (MCF-7) for this study and divided the culture into four groups. The first group served as the control. In the second group, we added 2 mM of Karnozin Extra, in the third group, we added 5 mM of Karnozin Extra, and the fourth group received 10 mM of Karnozin Extra.



As shown in the picture, Karnozin Extra leads to drastic changes in morphological characteristics that are dose-dependent with a noticeable cytotoxic effect. In the group, 2 mM is recorded in the presence of individual round cells, smaller in size than untreated cells. Condensation cytoplasm, impaired cell membrane integrity, reduced cell adhesiveness to flask walls as well as an increase in the number of floating cells in the medium are observed in groups 5 and 10 mM. In the 10 mM group, the presence of round cells with significantly smaller dimensions dominates visible condensation and fragmentation of the nucleus.

We have left the cancer cells to grow, and here's what we have observed after some time:

1. We have measured the basal respiration of the cancer cells. In other words, we measured how the cells breathe as a whole. We can see at this graph how 10mM of Karnozin Extra has significantly decreased the basal respiration of these cancer cells.

2. Furthermore, in the second measurement we added ADP to the cells to speed up the electron flow along the ETC. We measured the mitochondrial breathing or cellular respiration in this case. Again, the cellular respiration has significantly decreased after administration of 2, 5 and especially 10mM of Karnozin Extra

3. In the third measurement, we investigated how Karnozin Extra influences the activity of the first protein complex (NADH dehydrogenase) in the ETC. Again, a significant reduction of the first

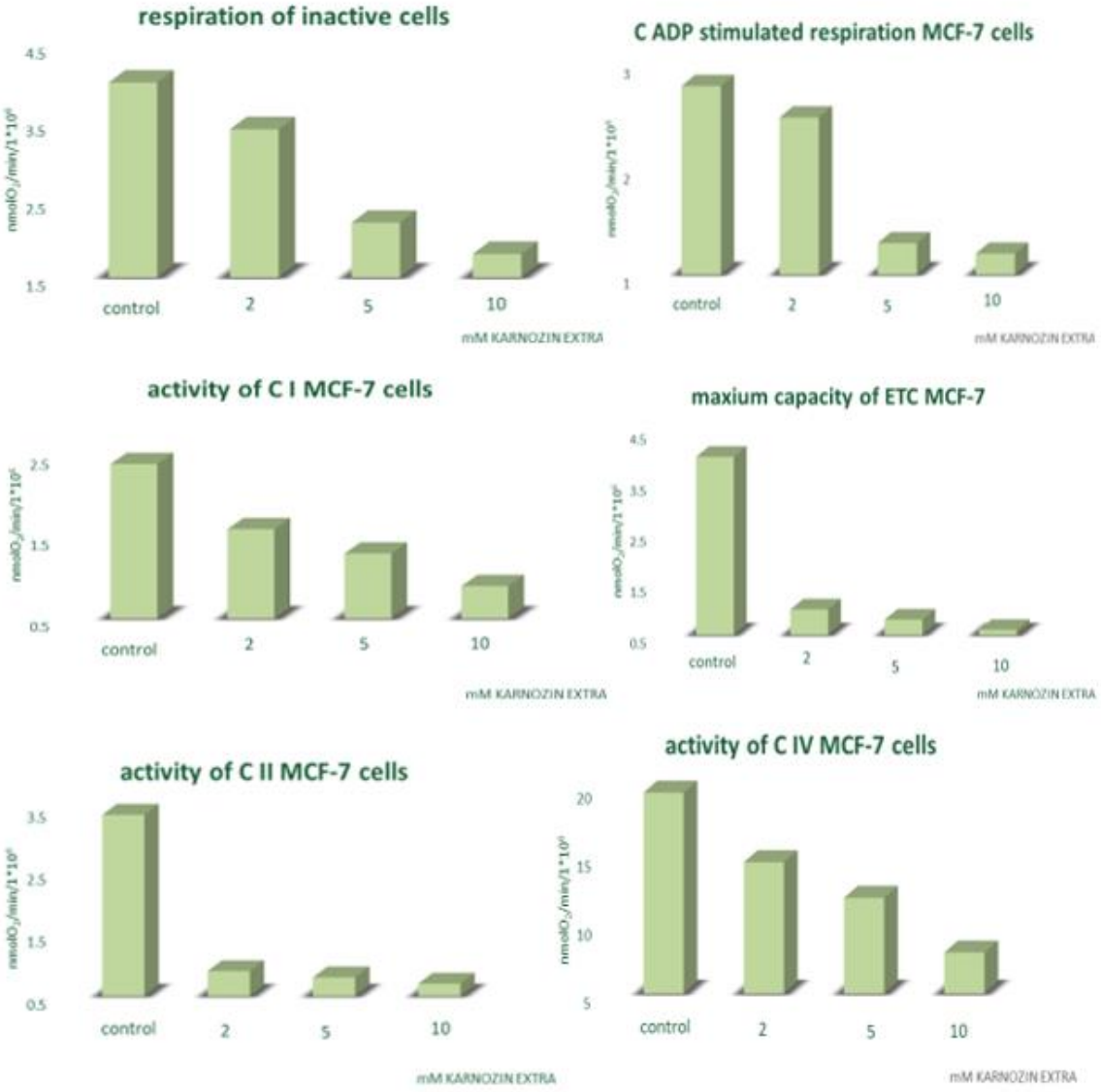
complex activity has been observed after the administration of Karnozin Extra, especially in higher concentration.

4. In the next measurement, we added the substrate to the cancer cells to speed up respiration to the maximum capacity. 2, 5 and 10 mM of Karnozin Extra succeeded in decreasing cellular respiration in this case.

5. In the 5th measurement, we have observed decreased activity of the second protein complex in the ETC after administration Karnozin of Extra. Actually, Karnozin Extra showed its greatest inhibitory effect on the complex II, succinate dehydrogenase.

6. And finally, complex IV, cytochrome C oxidase. Similarly, to other complexes, Karnozin Extra was able to reduce its activity in the dose dependent manner.

To summarize, Karnozin Extra blocks and inhibits the cellular respiration in the breast cancer cell line, acting on different components of the ETC in the mitochondria. The strongest inhibition is clearly seen on the complex II (succinate dehydrogenase). Moreover, Karnozin Extra shows its anticancer potential in the dose dependent manner. When higher concentrations of Karnozin Extra (10mM) were added to the culture, cancer cells have used less oxygen, and their respiration was compromised. Although this was in vitro examination on the cell cultures and not in the living organism, these findings suggest that it makes sense to further investigate Karnozin Extra anticancer potential in the animals and eventually show that Karnozin Extra can do the same in the human organism affected by cancer.



The results shown in the graph present the inhibitory effect of Karnozin Extra on energy metabolism in the MCF-7 cell line (breast cancer cell line) and ATP production. The application of Karnozin Extra preparation in all the examined concentrations after treatment of the MCF-7 cell line caused the inhibition of energy production and the rate of oxygen consumption both at the level of inactive cells and after cellular permeabilization membranes and by applying Krebs cycle substrates. An inhibitory effect on activity is also evident in all tested complexes I, II, and IV of the electron transport system, as well as the reduction of the maximum capacity of the respiratory chain in the production of ATP. In conclusion, there was a decrease in the number of viable cancer cells in this cell line.

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Karnozin Extra and Molecular Biology

Faculty of Natural Science in Novi Sad, Department for Molecular Biology has completed the research on rats using our supplements. We have fed middle aged rats with Karnozin Extra for 2 months (equivalent 6 years in human lifespan). The dosage we used in this experiment was only 2 capsules per day. **In this experiment we looked for their ability and will to run more than the control group.** We recorded their physical activity every 6 minutes, every day and night during 60 days, by using highly sophisticated software.

After statistical evaluation of data, it has been shown that group of rats that received Karnozin Extra run 40% more comparing to the control group.

The gathered data suggest that Karnozin Extra provided more energy to the rats, they were capable to run more and they had more will and initiative to move.

https://www.researchgate.net/profile/Silvana_Andric
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Preclinical and clinical study on metabolic syndrome

Metabolic syndrome is a cluster of conditions — increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or triglyceride levels — that occur together, increasing the risk of getting heart disease, stroke, and diabetes. Metabolic syndrome is closely linked to obesity and inactivity. It's also linked to a condition called insulin resistance. Today, we now that all metabolic diseases are also inflammatory diseases.

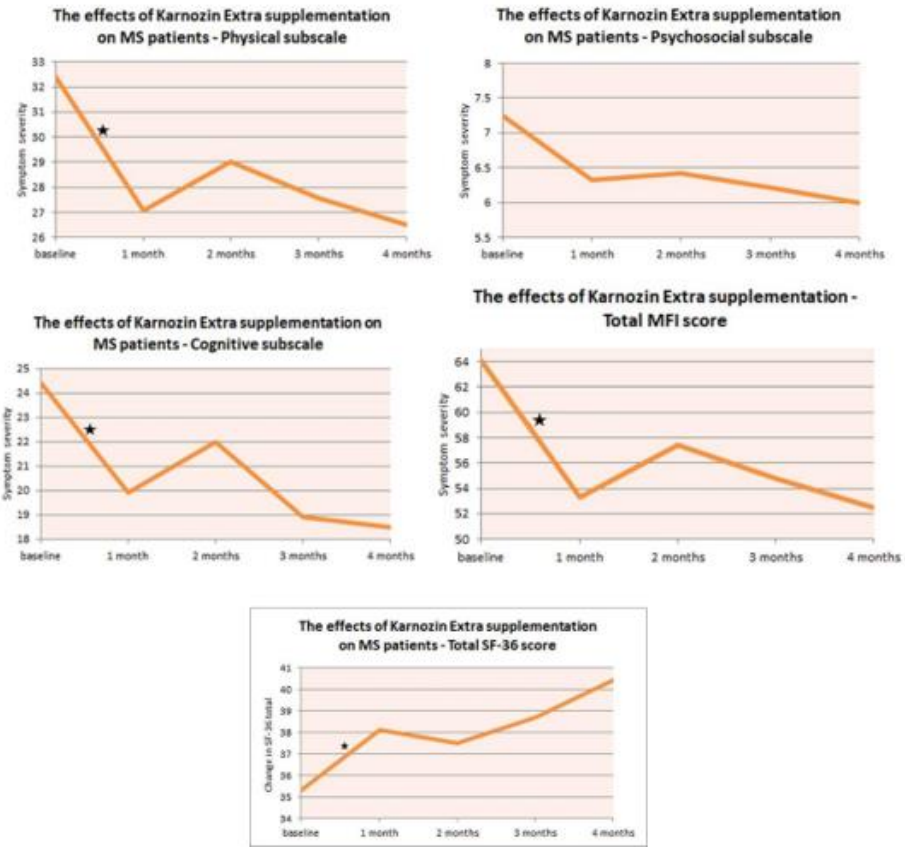
Since it has been already proven in scientific literature that pure L-carnosine inhibits glycation, oxidation of LDL cholesterol, and protects from atherosclerosis, we thought it would be a good idea to perform similar experiments with our product Karnozin Extra. We obtained all Regulatory and Ethics Committee approvals and the research is currently ongoing at the Department of Pharmacology at the Medical Faculty, University of Novi Sad. It includes *in vivo* model, more than 100 mice and rats are included, and 50 patients with diabetes type 2. We will be measuring their glucose, insulin and cholesterol levels, their antioxidant capacity and inflammatory markers, before and 3 months after taking our supplement. Also, the biological activity of Karnozin Extra will be quantitatively compared with commonly used drugs metformin and

simvastatin. Actually, this will be the PhD thesis of one of the students from the Medical Faculty, University of Novi Sad, Serbia.

Project leader: https://www.researchgate.net/profile/Aleksandar_Raskovic

Karnozin Extra and Multiple sclerosis – patient-reported outcomes

We have been invited to present our scientific abstract at the World Congress of Neurology which was held in Dubai on October 27-October 31. The abstract is related to 51 MS patients and their outcomes while taking the Karnozin Extra supplement. They have reported reduced fatigue and increased quality of life after just 4 months of supplementation. The abstract is available online. The poster presents the study which was presented at the World Congress of Neurology in Dubai.



Karnozin Extra and Multiple Sclerosis-pilot clinical study

Among other factors, it appears that low levels of tissue carnosine and mitochondrial dysfunction accompany MS (Keytsman et al., 2018), with oral carnosine might be applicable to tackle impaired bioenergetics and oxidative stress in MS, and perhaps win back neuromuscular function (Eijnde. Carnosine loading in MS. clinicaltrials.gov ID: NCT03418376). However, several formulations of carnosine have shown limited applicability due to restraints in brain delivery or tissue performance (Sariev et al, 2015) thus pushing both industry and researchers to find bioavailable and effective formulations of carnosine. No human studies so far evaluated the impact of innovative L-carnosine formulation (Karnozin Extra) in MS. Here, we will evaluate the impact of supplemental carnosine on neuromuscular performance, brain biomarkers of carnosine metabolism, and health-related quality of life in a case series of patients with MS.

We are the first in the world to investigate the association between L-carnosine and multiple sclerosis. The first clinical pilot study related to Karnozin Extra and Multiple Sclerosis (CARMUS) was initiated in June 2019 at Applied Bioenergetic Lab, University of Novi Sad. It included 3 MS patients, where we measured their work capacity, autonomic nervous system function, blood analysis, and levels of L-carnosine and other metabolites in the brain using MR spectroscopy before and after 8 weeks of supplementation with Karnozin Extra.

The study was completed in December 2019 and we obtained promising results. All patients experienced reduced fatigue accompanied by improved walking capabilities. Brain levels of significant metabolites increased, specifically there was an average increase of 18.9% in choline-containing substances, 21.2% in creatine, and 12.3% in myoinositol. Additionally, lactate levels decreased by 23.5%. Analysis of cardiovascular reflexes indicated normalized parasympathetic and sympathetic function in all patients. Total antioxidant capacity increased up to 49% after 8 weeks of Karnozin Extra administration These findings suggest that Karnozin Extra presents an important adjuvant treatment in multiple sclerosis. The results are published in the peer-reviewed journal Nutrition Research. The abstract was accepted for presentation at Nutrition 2020 Congress, held in Seattle, WA, the US from May 30 - June 2.

The Principal Investigator: **https://www.researchgate.net/profile/Sergej_Ostojic**

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Conclusion

Karnozin Extra is perfectly safe supplement that can be used in a wide range of diseases and disorders. It is well tolerated and has no identified side effects or adverse drug interactions. The dosage should be from 1-2 capsules for infants, and up to 10 capsules per day in neurodegenerative disorders that are present in the elderly population. As a prevention of the aging process, 2-4 capsules per day are recommended. With its multitargeted physiological actions, Karnozin Extra serves to prevent age-related diseases such as cognitive decline and dementia, to promote exercise comfort and performance, to slow the progression of metabolic conditions such as diabetes, and to defend against atherosclerosis and heart disease.

Education is very important to us, and so is spreading our knowledge, research and findings. We really hope that all our committed work, supported with scientific background, clinical and other studies, all evidence-based, will help us to reach and to introduce to as many people as possible the product that might change their lives.

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