ERAF
EIROPAS REĢIONĀLĀS ATTĪSTĪBAS FONDS
IEGULDĪJUMS TAVĀ NĀKOTNĒ
EIROPAS SAVIENĪBA
RĪGAS STRADIŅA UNIVERSITĀTE

PERSPECTIVES OF OBESITY AND OBESITY-ASSOCIATED DISORDER CORRECTION WITH OREGONIN

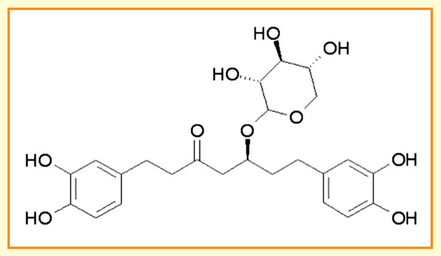
J. Krasilnikova[1], G. Telysheva[2], T. Dizhbite[2], O. Bikovens[2], I. Fizdel[3]

[1] **Rīga Stradiņš University**, Dzirciema 16, Riga, LV-1007, Latvia, e-mail: Jelena.Krasilnikova@rsu.lv;
[2] **Latvian State Institute of Wood Chemistry**, Dzerbenes 27, Riga, LV-1006, Latvia, e-mail: ligno@edi.lv;
[3] **Latvian Scientific-practical Centre of Treatment Dietology**, Dzintaru prospect 43, Jūrmala, LV-2015, Latvia, e-mail: info@dietologija.lv

This research work is related to the European COST programme, action CM0804. COST is an intergovernmental framework for European Cooperation in Science and Technology, allowing the coordination of nationally-funded research on a European level.

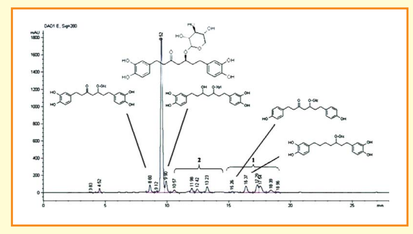
Chemical Biology with Natural Products:
"The main objective of the action is to *advance the use of natural products as tools for chemical biology. Applying modern techniques and advancing them, natural products will prove to be instrumental in discovering target proteins and biological pathways that are of relevance to diseases. This in turn, should facilitate and speed up subsequent drug discovery efforts in the pharmaceutical industry."*

For some *Alnus* species, oregonin, open chain diarylheptanoid, is found to be the major factor of the bark extract bioactivity.



Oregonin: 1,7-bis-(3,4-dihydroxyphenyl)-heptan-3-one-5-O-β-D-xylopyronoside

Composition of EtAc fraction of grey alder bark extract



The main part, more than 80% of EtAc fraction (or ~ 7% from o.d. bark), is identified as linear diarylheptanoids (DAH) (the data of FTIS, Pyr-GS/MS, HPLC UV/MS and NMR). More than 90% of these DAH are presented by 1,7-bis-(3,4-dihydroxyphenyl)-heptan-3-one-5-O-D-xylopyronoside or **oregonin**. The radical scavenging activity of **DAH fraction** 1 was a little higher, whereas of **DAH fraction** 2 lower than that for oregonin: IC_{50} values in the DPPH* test were, respectively, 3,0 ± 0,1; 8,8 ± 0,3 and 4,5 ± 0,3 mg/L.

The diet-related conditions as obesity and dysmetabolism in children are a global problem. Efficient and easy-in-use natural ethnopharmaceutical forms, with no side-effects, are intensively studied. The aim of our research was detection of the healing properties of the rich-in-diarylheptanoid (oregonin) extract from alder bark.

Effects of the extract in daily doses of 2 mg/kg were studied *in vivo* in rats (n = 21) and *in vitro* using blood plasma from volunteer patients – 10–14 years old children in a condition of obesity (n = 12).

The children with the diagnosis of alimentary dynamic obesity, diet-induced glucosaemia and triglyceridaemia were observed at the Dietology Centre.

The rats (Vistar line) in a condition of modelled metabolic syndrome with obesity prevalence were observed at the Rīga Stradiņš University Vivarium.
In the *in vivo* experiment, besides weight, cholesterol, its fractions, triacylglycerols, glucose, glutathione, malondialdehyde and lipase in blood were measured.
In vitro, the changes in glucose and triacylglycerols were detected after blood 5 min incubation with the extract. Analyses were performed in the European Standardized Medical Laboratory of Rīga Stradiņš University.
After 28-day addition of the extract to rat diets, their weight returned to normal, glucose decreased from 6.16±0.08 to 2.3±0.04 mM/L; HDL-cholesterol increased from 1.04 to 1.48 mM/L, lipase elevated by 11.8%, glutathione increased by 12.3%.

The *in vitro* experiment showed a decrease in basic glucosaemia and triacylglycerolaemia to normal ranges.

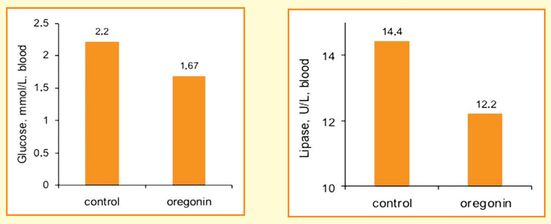
High potentials of the oregonin-rich extract for preparing metabolic food or nutritional supplements intended for treatment and prophylaxis of obesity and dysmetabolism-associated conditions were shown.

BIOMEDICAL PROPERTIES OF GREY ALDER PHENOLICS IN EXPERIMENTS *IN VIVO*

The results of the 2-month experiments *in vivo* on rats with induced hypercholesterolaemia have confirmed the conclusions drawn from the *in vitro* study.

Design of the experiment. The experiment on 21 white rats (Vistar line, weight 200 g) was conducted in the vivarium of Rīga Stradiņš University.
I step: Blood samples were taken from all healthy rats for biochemical analysis.
II step (duration 30 days): All rats had increased cholesterol levels (1.3 g per day that was twice above the normal level). The cholesterol solution was introduced *per os.* Blood samples were taken on the 30th day.
III step (duration 30 days): The control group was fed with a standard physiological ration. The experimental group was given oregonin (0.2 mg per every 100 g of animal weight) using 0.9% NaCl solution. Blood samples were taken on the 30th day.
The microscopic morphological investigation of animal tissues was carried out after the end of the III step of the experiment. The tissue samples for microscopy were prepared from the rat kidneys, liver and heart.

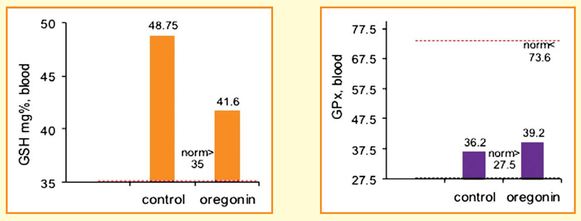
Biomedical properties of grey alder phenolics in experiments *in vivo*



The results of this experiment have confirmed the ability of **oregonin** to **normalize protein**, **lipid** and **carbohydrate metabolism**.

Biomedical properties of grey alder polyphenolics *in vitro*

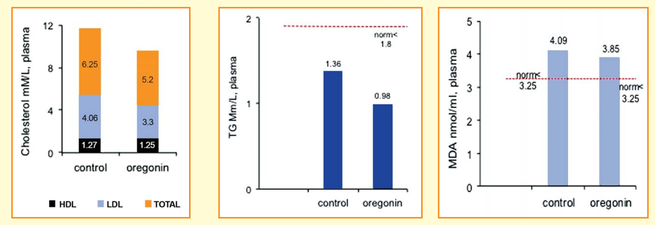
Glutathione, Superoxide Dismutase (SOD), Glutathionperoxidase (GPx)



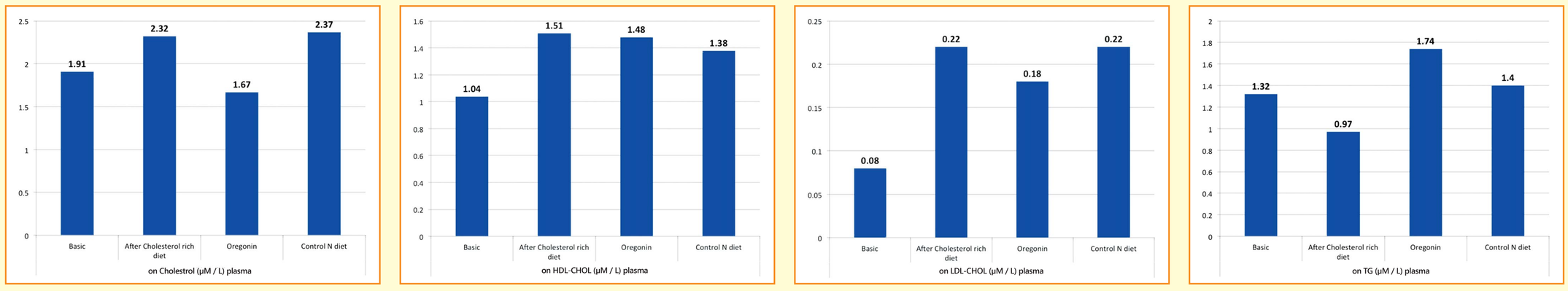
Glutathione content is often used as an actual parameter for the determination of various degenerative, age-related pathologies. In our experiment it was shown that **oregonin decreased glutathione content in the blood by ~15%.**
The level of SOD, GPx and catalase was normal both before and after incubation of blood samples with oregonin.
The data show that diarylheptanoids obtained from *Alnus incana* bark could be considered as a part of integral means for the prevention of free radicals and aging-related pathologies.

Biomedical properties of grey alder polyphenolics *in vitro*

Cholesterol, Triglyceride (TG), Malondialdehyde (MDA)

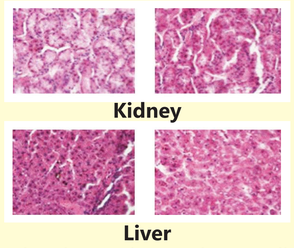


For the initial blood samples, an imbalance in the cholesterol pool was observed. As a result of the 30 min incubation with **oregonin**, the levels of **total** and **LDL-Chol decreased by ~20%** and **TG** level – **by ~40%**.
Oregonin reduced to some extent **peroxidation of lipids** that followed from the changes in **MDA** content in blood plasma.

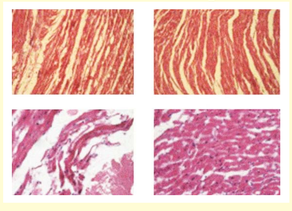


Biomedical properties of grey alder phenolics in experiments *in vivo*

Morphology of kidney and liver tissues



Morphology of heart tissues (myocardium and endocardium)



It has been shown that application of oregonin provided a high level of regeneration of kidney, liver, myocardium and vessel wall tissues after a diet with cholesterol.

The action of diarylheptanoid oregonin on the enzymes of the gastrointestinal tract was shown. The concentration of oregonin was calculated by reversal phases HPLC-UV. The amylase of saliva, pepsin and pancreatic enzymes don't digest oregonin. The concetration of oregonin decreased proportionally to the solution (Fig. 1).
Oregonin was stable in the acidic medium of gastric juice (pH~2).
Bile reduced the concentration of oregonin two times less, that may be the result of oregonin joining bile compounds (Fig. 2).

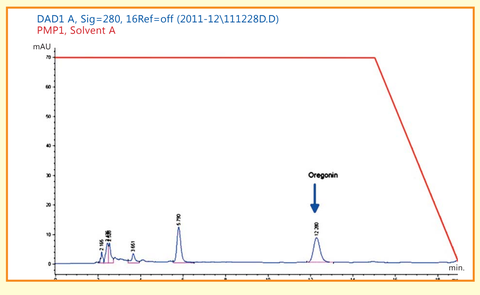


Figure 1.
HPLC-UV (λ=280 nm) chromatogram: after incubation of oregonin with pepsin

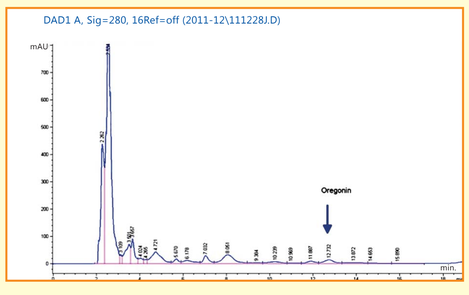


Figure 2.
HPLC-UV (λ=280 nm) chromatogram: after incubation of oregonin with bile