

## ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF *GALANTHUS GRACILIS* AND *G. XVALENTINEI* NOTHOSUBSP. *SUBPLICATUS*

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Alzheimer's disease (AD) is one of the most common cause of dementia in the elderly people. It is known to affect about 36 million people around the world. Acetylcholinesterase (AChE) inhibitors are major drugs for the symptomatic treatment of AD. Natural products constitute an important source for AChE inhibitors. For example galanthamine, an Amaryllidaceae alkaloid is used in the treatment of mild to moderate AD. Moreover, some Amaryllidaceae alkaloids have been found to exhibit similar or more potent AChE inhibitory activity when compared to galanthamine (1, 2). Therefore, Amaryllidaceae family is considered a major source to find better AChE inhibitors. Among the Amaryllidaceae genera found in Turkey, the genus *Galanthus* is represented by 14 taxa and one hybrid (3). In the present study, the AChE inhibitory potentials of *Galanthus gracilis* Čelak. and a naturally occurring *Galanthus* hybrid, *G. xvalentinei* (J. Allen) Beck nothosubsp. *subplicatus* (N. Zeybek) A. P. Davis were determined. A microplate assay modified from *in vitro* Ellman's method (4) is used to evaluate the AChE inhibitory activity of the alkaloidal extracts prepared from the bulbs and aerial parts of the above-mentioned plants. The final concentrations of the extracts in the assay ranged between 0.25–150 µg/ml.

The enzyme inhibitory activity was calculated as a percentage compared to blank. IC<sub>50</sub> values were evaluated by software package GraphPad Prism V3.0 (GraphPad Software, San Diego, CA). All of the extracts prepared from *G. gracilis* (bulbs: IC<sub>50</sub>=11.82 µg/ml, aerial parts: 25.5 µg/ml) and *G. xvalentinei* nothosubsp. *subplicatus* (bulbs: IC<sub>50</sub>=21.31 µg/ml, aerial parts: 16.32 µg/ml) showed remarkable AChE inhibitory activity. Galanthamine was used as a positive control (IC<sub>50</sub>=0.043 µg/ml).

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## SCAVENGING EFFECTS OF HYDROLISATES OBTAINED FROM THE SEA URCHINS COELOMIC FLUID

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Sea urchins possess an innate immune system and are regarded as a potential source of bioactive substances due to their coelomic fluid (CF). Coelomocytes constitute the defence system, which is capable of chemotaxis, phagocytosis, and production of cytotoxic metabolites. Bioactive substances from marine source may be produced by enzymatic hydrolysis of marine organisms and isolated tissues. Peptides and amino acids of the hydrolysates may exhibit significant antioxidant properties (1). Coelomic fluid from the fresh green sea urchins *Strongylocentrotus droebachiensis* were collected and hydrolyzed immediately during 1.5 and 3 hours at 50 °C. The enzymes used were: Alcalase 2.5 L (A), Flavourzyme 1000 L (F) (Novozymes, Denmark) and Protex 6L (P6L) (Genen-

cor International, Netherlands). Process of hydrolysis was stopped by heating in boiling water for 10 min to inactivate proteases. The coelomic fluid hydrolysates (CFH) were centrifuged (10 min at 7,000 rpm) and the supernatants were lyophilized and stored in a refrigerator until use. Amino acids (AA), peptides levels (P) in CFH and scavenging effect of CFH on  $\alpha$ ,  $\alpha$ -diphenyl- $\beta$ -picrylhydrazyl (DPPH) free radical were determined spectrophotometrically by OPHA (2), Warburg-Christian (3) and DPPH *in vitro* (4) methods, respectively.

The highest levels of amino acids and peptides were obtained after hydrolysis for 3 hours with Flavourzyme 1000 L: 12.0±0.5 and 22.4±0.5%, respectively. This hydrolysate possessed the most effective abilities as

Characteristics of the obtained hydrolisates

Sample	AA, %		P, %		DPPH: IC <sub>50</sub> , mg/ml	
	1.5 h	3 h	1.5 h	3 h	1.5 h	3 h
Native CF*	3.5±0.3	4.4±0.3	9.5±0.3	10.2±0.4	8.2±0.2	8.5±0.3
A	4.5±0.2	8.2±0.4	13.9±0.3	19.3±0.4	5.5±0.3	5.0±0.3
F	11.0±0.4	12.5±0.5	15.7±0.4	22.4±0.5	4.4±0.2	3.5±0.2
P6L	4.5±0.2	7.2±0.3	12.0±0.4	15.5±0.3	7.1±0.3	5.9±0.4

\*heating without enzymes

DPPH free radical scavengers: IC<sub>50</sub> = 3.5±0.2 mg/ml in comparison with native CF and other CFHs.

**References:** (1) H.-C. Wu et al. (2003) Food Res. Intern., 36: 949–957. (2) F.C. Church et al.

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## PRELIMINAR ANALYSIS OF THE COMPOSITION AND ACTION MECHANISM OF THE COMPLEX PREPARATION OF CODFISH LIVER

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The preparation includes diverse biological substances obtained from multistep processing of codfish liver. Previous studies demonstrated strong anti-inflammatory activity in contact dermatitis model (1). The purpose of this work was to characterize in outline composition and possible action mechanism of the preparation. Primary fractionation on molecular weight (MW) and estimation of effect on functional activity of professional phagocytes (on their capability to produce reactive oxygen species, ROS) were carried out. Lipophilic fraction was separated from aqueous preparation suspension by centrifugation; other fractions were obtained by sequential ultrafiltrational exclusion of components with

MW exceeding 10 kDa and 1 kDa. The ratio of fractions (fig.1) demonstrates the prevalent presence (54%) of low-MW components, which showed no reliable effect on ROS production (fig.2).

The strongest effect was detected in lipophilic fraction («lipids», fig. 2) and this effect four times exceeds one of total preparation (at the same concentration) and of 0,01% zymosan suspension. The exact relation between stimulatory effect on phagocytes and general anti-inflammatory action of the preparation require further investigations.

**Reference:** (1) K.L. Kryshen et al. (2012) Exp. Clinic. Dermatocos. 2: 38–42.

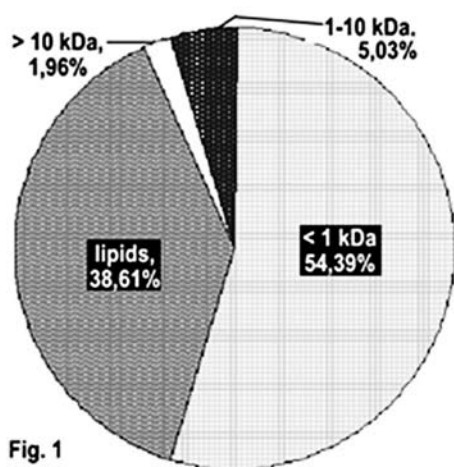


Fig. 1

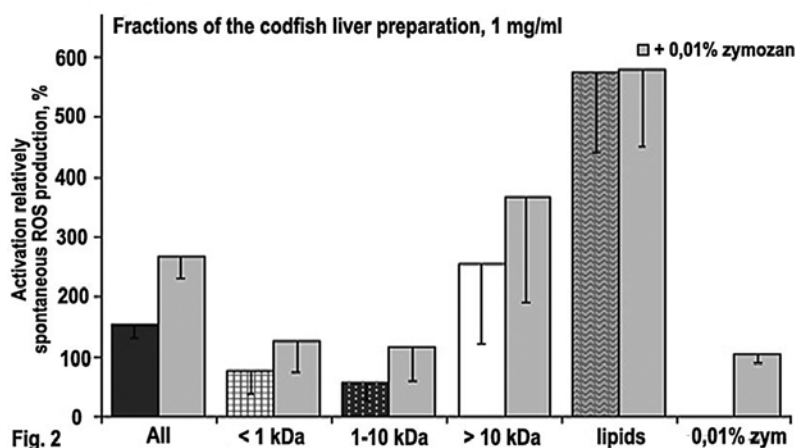


Fig. 2