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Halonal, an original benzoylated phenobarbital derivative anticonvulsant: *in vivo* evaluation, chemometric and molecular docking studies of enantiomers

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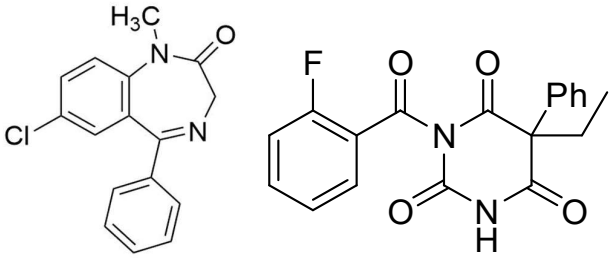
Experimental chemical part.

Halonal, derivative of barbituric acid, is a cyclic ureide. IUPAC name: 5-ethyl-1-(2-fluorobenzoyl)-5-phenylpyrimidine-2,4,6(1*H*,3H,5H)-trione, Chemical Formula: $C_{19}H_{15}FN_2O_4$, Molecular Weight: 354.33 g mol^{-1}. Odorless white crystalline powder with bitter taste; very slightly soluble in water, soluble in 96% alcohol, dimethylformamide, has a pronounced anticonvulsant effect due to interaction with the barbiturate center of the GABA-C1-ionophore complex [Figure S1].

Diazepam, benzodiazepine derivative, 7-chloro-1-methyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (general formula $C_{16}H_{13}ClN_2O$, molecular weight 284.7 g mol^{-1}, white odorless crystalline powder, practically insoluble in water, hardly soluble in ethyl alcohol, soluble in chloroform) with anticonvulsant and anxiolytic activity [Figure S1].



a **b**

Figure S1. Structural formulas: **a** - Diazepam; **b** – Halonal

Chiral HPLC resolution of enantiomers was conducted on high performance liquid chromatograph Agilent 1200 Compact LC equipped with UV detector. Chiral chromatographic column Agilent Ultron ES-OVM-C (150x4.6 mm, I.D., 5 μ) equipped with guard column with the same adsorbent was used as a stationary phase. The following eluting conditions were applied: mobile phase MeCN-phosphate buffer (0.02M, pH 4.4) in ratio 1:9 (isocratic elution); flow rate 1 mL/min, temperature of the column 30 °C; sample volume 10 μL, sample concentration 40% (halonal solution in MeCN-phosphate buffer 1:9); UV-detection at 200 nm.

Experimental biological part.

It was established that with a 10-day administration of *ortho*-fluorobenzonal, a barbiturate derivative with anticonvulsant activity due to increased GABA mediation (Halonal) at a dose of 65 mg kg^{-1} per day to male CBAxC57Bl/6) F1 mice, alcoholized for 6 months, decreased alcohol motivation in animals. The present study included 10-month-old healthy (control group) and long-term alcoholized (experimental group, which received 10% ethanol solution for 6 months) male mice of the (CBA x C57Bl/6) F1 line. The animals then received a course (10 days) of intragastric administration of Halonal at a concentration of 65 mg kg^{-1} per day in the form of a suspension of 1% starch mucus.

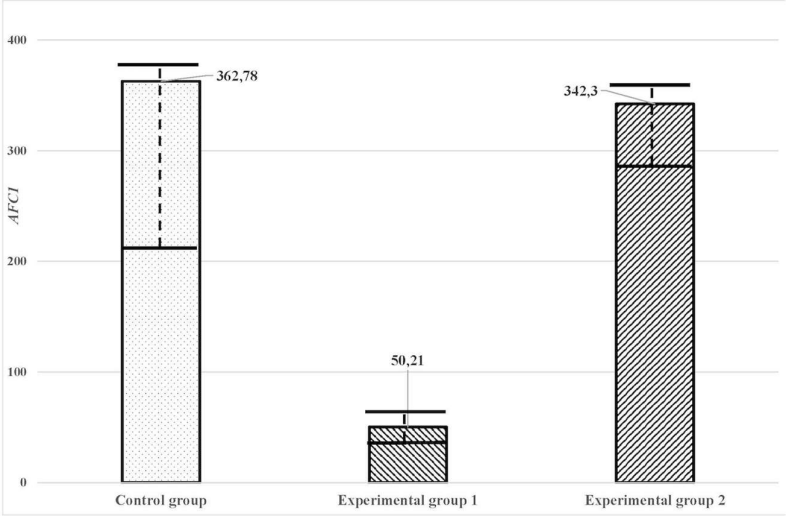


Figure S2. Relative number of antibody-forming spleen cells/10^6 nucleated cells (AFC1) in control and experimental groups

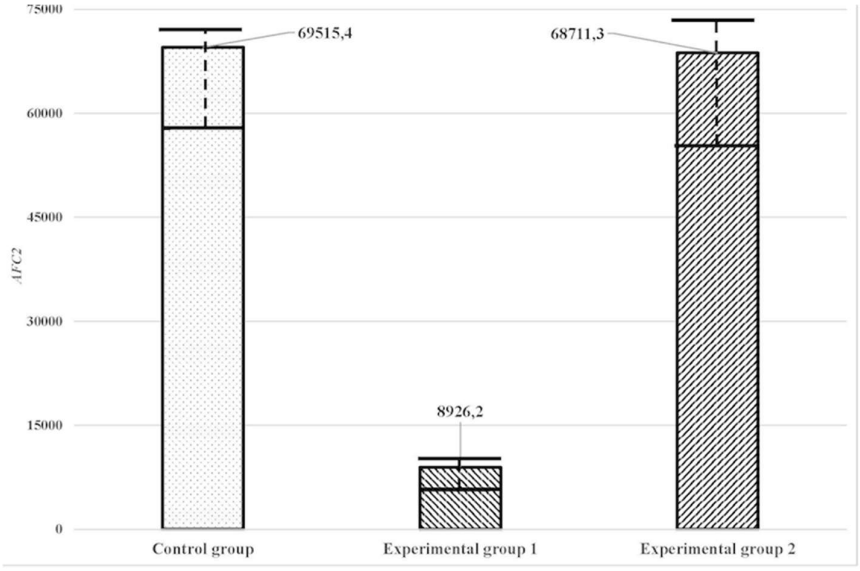


Figure S3. Absolute number of antibody-forming spleen cells (AFC2) in control and experimental groups

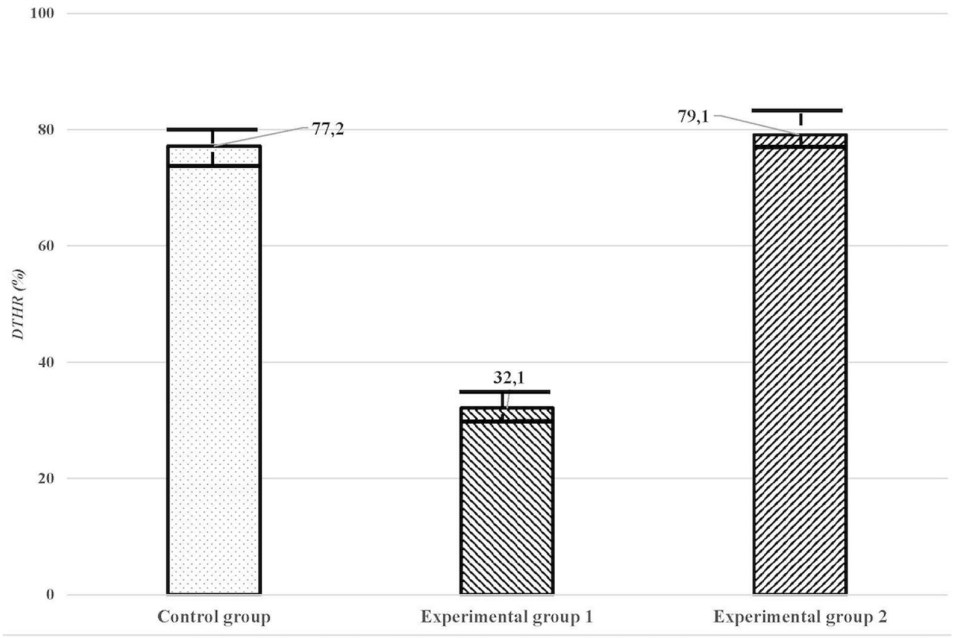


Figure S4. Delayed-type Hypersensitivity reaction (DTHR) (%) in control and experimental groups