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Rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside as a means of antioxidative defense against tetrachloromethane induced hepatotoxicity in rats

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It is known that poisoning by many industrial and commercial toxins, as well as some drugs, is accompanied by development of liver toxicity, and by activation of the process of lipid peroxidation (LPO) [1]. One of the contemporary approaches to the treatment of toxic hepatitis is to employ antioxidative therapy. Among the antioxidants, flavonoids of plant origin are well regarded, some of which exhibit pronounced hepatoprotective properties [1]. Rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside is a recently isolated flavonoid glucoside [2].

Flavonoids have been shown to elicit antitumoral, antiplatelet, antiischemic, antiallergic and anti-inflammatory activities [3]. The biological activities of the flavonoids are thought to be related to their antioxidant properties [4]. Hepatotoxicity of CCl_4 is accompanied by the activation of LPO in animals [5]. The present study was designed to investigate the antioxidative properties of rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside in experimental hepatitis induced by poisoning with CCl_4.

Toxicity of CCl_4 was accompanied by the activation of LPO in animals of the control group. As can be seen from Table I, on day 7 of the experiment, the content of TBARS reflecting LPO in the serum of control animals was 51% higher than in intact animals. On day 15 of the experiment (Table 1) this difference was also observed and TBARS content was 41% higher. An increased LPO was also seen in the liver of control animals (Table 2). By the end of the experiment, the content of TBARS in the liver of control rats (Table 2) was 44% higher than their content in intact animals.

The use of Rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside led to decreased levels of TBARS in the serum and the liver of experimental animals compared with controls. So, for instance, in the middle and at the end of the experiment, the content of TBARS in the serum and the liver of experimental animals (Table 1 and 2) were more than 1.5 times lower than in the control animals. On day 15 of the experiment, the content of TBARS in the serum and the liver of animals in the experimental group was almost similar to the content of animals in the intact group.

Table 1: Content of TBARS in the serum of rats (n mol/mg lipid)

Day	Intact	Control	Experimental
7	1.9 ± 0.1*	3.88 ± 0.25	2.3 ± 0.12*
15	1.8 ± 0.1*	3.05 ± 0.19	1.9 ± 0.20*

* $P < 0.001$, n = 9, student's t-test (mean ± s.m.e.)

Table 2: Content of TBARS (n mol/g tissue) in the liver of rats on day 15

Intact	Control	Experimental
41.25 ± 3.7*	73.62 ± 6.4	47.5 ± 3.84**

* $P < 0.001$, ** $P < 0.01$, n = 9, student's t-test (mean ± s.m.e.)

Table 3: Serum antioxidant activity (AOA) (μM) of rats

Day	Intact	Control	Experimental
7	425 ± 25**	285.5 ± 27.5	627 ± 19.34*
15	424.2 ± 25.4*	200.02 ± 44.66	374 ± 22**

* $P < 0.001$, ** $P < 0.01$, n = 9, student's t-test (mean ± s.m.e.)

The development of toxic hepatitis not only led to an increase in TBARS levels in serum and liver, but also to the lowering of serum antioxidant activity (AOA). In particular, on days 7 and 15 of the experiment, the serum AOA of control animals were correspondingly 33% and 53% lower than those in intact animals (Table 3). Such a decrease of serum AOA of control animals was apparently the result of an increased usage of endogenous antioxidants under conditions of developing oxidative stress, induced by CCl_4.

Administration of the flavonoid drug to animals of the experimental group was accompanied by a different change in AOA of the serum. So, for instance, in the middle of the experiment, the serum AOA of experimental animals was 54% and 32% higher than that of the corresponding control and intact groups ($p < 0.001$). By the end of the experiment, the serum AOA of the animals of the experimental group was 46% higher than the serum AOA of control animals and was quite indistinguishable from that of intact animals.

These results suggest that the novel flavonoid has antioxidant activity against liver toxicity. Here, animals in the experimental group exhibited a lower level of TBARS in serum and liver and showed also an increase in serum AOA compared with that of the control group. This showed that the process of LPO in the liver and serum, during CCl_4 poisoning are tightly interconnected, and also demonstrated the effectiveness of the flavonoid drug as a means of antioxidative defense.

Experimental

Experiments were conducted on 27 male Wistar strain rats, weighing 155–220 g. They were maintained on a standard vivarium diet. Animals were divided into three groups: control, experimental, and the intact group. Experimental hepatitis was induced with CCl_4 by a well-established procedure [6]. Rats in the control and experimental group were administered 4 ml/kg of CCl_4 for 4 days. Animals in the experimental group received the drug (rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside) 4 days prior to the first administration of CCl_4 and for the duration of the experiment. The drug was administered per os in the form of a water-crystalline suspension at 100 mg/kg, using a catheter. On day 7 after the first injection of CCl_4 (day 7 of the experiment) a blood sample was taken from all of the animals via the tail vein. Fourteen days after the administration of CCl_4 (day 15 of the experiment) animals were killed by decapitation under light ether anesthesia. Serum was obtained by centrifugation at 1500 × g for 15 min. The extracted liver was washed in physiological saline, blotted with filter paper, and used to prepare a 10% homogenate in buffer containing 40 mM KH_2PO_4 and 100 mM KCl (pH 7.45). All the procedures were conducted at 4 °C.

The level of LPO in the serum and in the liver was estimated by contents of substances, which reacted with 2-thiobarbituric acid (TBARS) measured spectrofluorometrically after addition of 25 μl of 45 mM of an ethanol solution of butylated hydroxytoluene [7]. Accumulation of TBARS in serum was expressed in nmol/mg of lipid and in the liver in nmol/g of raw tissue. The content of total lipids was established with the help of commercial Bio-Lachema-Test (Brno, Czech Republic) kits. The method is based on the fact that lipids, after their hydrolysis with concentrated sulphuric acid, react with a phosphovaniline reagent yielding a colored product, which can than be determined spectrophotometrically at 530 nm.

Serum AOA was estimated by the measurement of the chemiluminescence (CL) in the haemoglobin-hydrogen peroxide-luminol system [8]. The reaction solution of 5 ml contained 0.21 μM luminol (Serva, Germany) in phosphate buffer (50 mM KH_2PO_4, 100 μM EDTA, pH 7.4). the initiation of free radical oxidation of luminol was started by the introduction of 27 μM of hydrogen peroxide. All experiments were conducted at 37 °C under constant stirring on a chemiluminometer (Turner, USA). The CL signal was obtained by the production of luminol-derived radicals gener-

ated from the hemoglobin-hydrogen peroxide interaction. The latent period of the CL, which was found as the time between the initiation of luminol oxidation and the beginning of CL was used for measurement. AOA of serum was determined by the lengthening of the latent period in the model system after addition of 10 μl serum and it was expressed as the concentration of an equivalent ascorbate solution (μM), which was chosen as a reference inhibitor. Results were treated by the Student's t-test and expressed as mean values ± standard mean error.

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DNA-binding properties and cytotoxicity activity of novel aromatic amidines in cultured human skin fibroblasts

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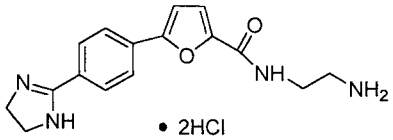
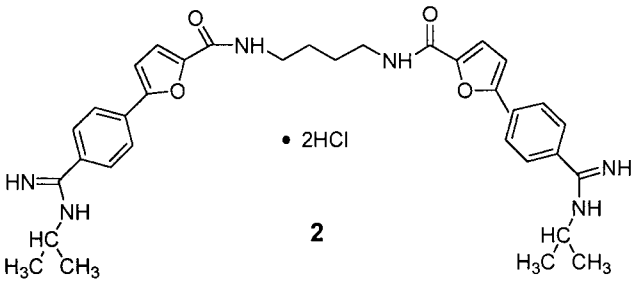
A number of natural and synthetic compounds are known to bind to DNA double helix in a nonintercalative manner [1]. Minor groove binding drugs typically have several aromatic rings, such as pyrrole, furan or benzene connected by bonds possessing torsional freedom [1]. Cationic charges provide affinity for the tunnel of negative potential in the groove and in addition many minor groove ligands possess H-bond donating or accepting atoms [2]. The minor groove binding drugs exhibits a wide spectrum of antimicrobial, antiviral, and antitumour properties [3, 4]. These drugs are thought to exert their biological effects by interfering with the template function of DNA and either blocking gene transcription or inhibiting DNA replication [4, 5]. In the course of our investigations of minor groove binding drugs, new aromatic amidines **1** and **2** were synthesized and tested for DNA-binding properties (Table).

The apparent DNA binding constants (K_{app}) of compounds **1** and **2** to calf thymus DNA, poly(dA-dT) · poly(dA-dT), T4 DNA and poly(dG-dC) · poly(dG-dC) were determined using the ethidium displacement assay [6, 7] and were compared to those of distamycin and netropsin (Table). These data demonstrate that compounds **1** and **2** can bind to the DNAs studied. The association binding constants for T4 coliphage DNA for **1** and **2** gave evidence of their minor-groove selectivity, because the major groove of T4 coliphage DNA is blocked by α-glycosylation of the 5-

Table: Association constants ($K_{app} \times 10^5\ M^{-1}$) of ligands with polynucleotides



1



2

Ligand	Calf thymus DNA	T4 DNA	poly(dA-dT) · poly(dA-dT)	poly(dG-dC) · poly(dG-dC)
Netropsin	8.7	8.3	875	2.5
Distamycin	7.5	6.4	340	2.0
1	2.2	2.6	3.9	1.8
2	1.7	1.6	3.4	1.4

The error for netropsin, distamycin and compounds **1** and **2** is $\pm 0.2 \times 10^5\ M^{-1}$.