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Antioxidant activity of rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside in the brain of aged rats

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Rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside is a flavonoid drug [1]. We have recently shown its antioxidative activity against tetrachloromethane-induced hepatotoxicity in rats [2]. Ageing of the central nervous system might, partly, be a result of the damage caused by oxygen free radicals and their intermediates [3, 4]. During ageing free-radical generation increases and production of small molecular weight antioxidants becomes insufficient leading to defective disposal of reactive oxygen species (ROS) [5, 6]. This leads to increased oxidative stress on cells and causes a large amount of oxidative damage to vital biomolecules [6, 7]. Also, Alzheimer's disease (AD), which is linked to ageing, may be, in part, due to oxidative stress in the brain [8]. Memory deficits induced by the neurotoxins – colchicines and ibotenic acid, proposed as animal models in AD are, at least, partly due to neurodegeneration induced by oxidative stress injury [9, 10].

Antioxidants are useful for preventing the formation free radicals and the deleterious actions of ROS that damage lipids, DNA and proteins [11].

The present study investigated the effect of rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside on the oxidative free radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and on lipid peroxidation (LPO), in order to assess the antioxidant effect of the drug in the brain of aged rats. The study was undertaken to develop the novel drug as an antioxidative therapy against ageing and related disorders like AD.

The Table shows LPO and antioxidant status in the brains of young and aged rats. A significant increase in LPO and significant reductions in the activities of SOD, CAT and GPx were observed in aged rats. These values returned to almost normal on 15 days administration of the drug in aged rats. No significant changes were observed in the brain of young rats.

There are certain merits of brain tissue in investigations of free-radical metabolism in relation to ageing: brain tissue has a relatively high lipid content, consumption of oxygen by the tissue is high and the brain has a relatively weak antioxidant defense system [17].

The high lipid content of neural tissue comprises large amounts of polyunsaturated fatty acids, which are highly susceptible to oxidative attack and this leads to changes in membrane fluidity, permeability and cellular metabolic function [18].

Though, cells can destroy free radicals by coordinated expression and functioning of an array of free-radical-scavenging enzymes including SOD, CAT and GPx, these antioxidant defense systems are not perfect and are subject to alteration during ageing [19]. As a result there is an age-dependant increase in the function of ROS and free radicals escaping these cellular defense systems, which eventually leads to the damage of cellular constituents [5]. So an effective antioxidant agent should be capable of augmenting intracellular concentrations of SOD, CAT and GPx, in finally reducing the lipid peroxidation [20].

The results of the present study show that rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside increased SOD, CAT and

Table: Effect of rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside on lipid peroxidation (LPO), activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)

Parameter	Young group			Aged group		
	Group 1.1 (control)	Group 1.2 (7-days treated)	Group 1.3 (15-days treated)	Group 2.1 (control)*	Group 2.2 (7-days treated)**	Group 2.3 (15-days treated)***
LPO[a]	1.62 ± 0.14	1.58 ± 0.13	1.45 ± 0.08	2.43 ± 0.20	1.89 ± 0.12	1.64 ± 0.13
SOD[b]	20.8 ± 2.21	20.3 ± 1.66	20.2 ± 2.61	14.3 ± 1.44	17.2 ± 0.92	19.43 ± 1.0
CAT[c]	23.6 ± 0.90	23.4 ± 1.32	24.0 ± 1.38	18.8 ± 1.67	20.2 ± 1.20	23.3 ± 1.26
GPx[d]	5.24 ± 0.42	5.35 ± 0.24	5.72 ± 0.56	3.81 ± 0.26	4.49 ± 0.37	5.12 ± 0.4

Values represent mean ± S.E.M, n = 9, student's t-test.
* All values in the column represent $P < 0.001$, as compared to group 1.1
** All values in the column represent $P < 0.05$, as compared to group 2.1
*** All values in the column represent $P < 0.001$, as compared to group 2.1
[a] nmol MDA liberated/ mg protein
[b] U/ mg protein
[c] U/ mg protein
[d] µmol reduced glutathione oxidized/min/mg protein

GPx activities in the aged brains and also lowered down the activity of lipid peroxidation, significantly. It may therefore be acting as an antioxidant in the ageing brain. It is pertinent to note here the bioavailability aspect of the drug. The drug is a flavonol having a short sugar moiety alongwith methoxyl and hydroxyl substituents [1]. Despite having this short hydrophilic sugar moiety, the drug has crossed the blood brain barrier and exerted present effects in the brain. Pharmacokinetic studies on the drug have revealed an octanol-water partition coefficient, log P = 1.68 [21], which is almost optimal for a compound to readily cross the blood brain barrier [22]. This study should open vistas for the further development of rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside as antioxidative drug.

Experimental

Male albino Wistar strain rats were used in the study. They were maintained in plastic cages with free access to pellet chow and tap water, under conventional conditions. Rats were divided into two separate groups: Group 1 (young rats, aged 3–4 months, weighing 125–150 g) and Group 2 (aged rats, aged > 24 months, weighing 350–400 g). These two groups were further subdivided into three each (for each subgroup, n = 9): one control group (Groups 1.1 and 2.1), 7-days treated group (Groups 1.2 and 2.2) and 15-days treated group (Groups 1.3 and 2.3). Rhamnazin-4′-*O*-β-[apiosyl(1→2)] glucoside was obtained in pure form (a generous gift from Dr. S. Kumar) and was administered per os in the form of a water-crystalline suspension at 100 mg/kg per day, using a catheter.

Groups 1.2 and 2.2 received the drug for 7 days and groups 1.3 and 2.3 received the same for 15 days. On completion of 7 and 14 days administration of the drug the rats were killed by decapitation. The brain was excised immediately and transferred to physiological saline. The tissue was homogenized in 1 ml of ice-cold triple distilled water and sonicated for 10 s. The homogenates were than centrifuged (10000 × g, 2 min) and the supernatants were used for the estimations of LPO [12], SOD [13], CAT [14] and GPx [15] and Protein content [16].

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ERRATUM

We apologize for a mistake in author names of the research paper "Synthesis of 1,2-unsaturated pyranosylphosphonate nucleosides from 3,4,6-*O*-acetyl-D-glycal", published in PHARMAZIE **56**, 534–535 (2001). The correct name of the single author is A. H. Ismail.

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Synthesis of 1,2-unsaturated pyranosylphosphonate nucleosides from 3,4,6-*O*-acetyl-D-glycal

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