PHYSICAL CHEMISTRY 2021



SPECIFIC METHODS FOR FOOD SAFETY AND QUALITY

September 22nd 2021, Vinča Institute of Nuclear Sciences - National Institute of the Republic of Serbia, University of Belgrade, Belgrade, Serbia

PROCEEDINGS

SPECIFIC METHODS FOR FOOD SAFETY AND QUALITY

7th WORKSHOP: SPECIFIC METHODS FOR FOOD SAFETY AND QUALITY

September 22nd, 2021, Belgrade, Serbia

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EFFECTS OF CHRONIC ORAL D-GALACTOSE TREATMENT ON GENERAL HEALTH STATUS IN MALE WISTAR RATS

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ABSTRACT

D-galactose (d-gal) is an important physiological nutrient. According to the widely accepted aging metabolic theory d-gal at high levels can be converted into aldose and hydroperoxide, resulting in the overproduction of reactive oxygen species (ROS). Increased ROS levels may subsequently cause oxidative stress, inflammation, mitochondrial dysfunction, and apoptosis which are hallmarks of natural senescence as well as various pathological conditions. We investigated the effects of chronic oral d-gal intake (200 mg/kg and 500 mg/kg for 6 weeks) on physiological, neurological and toxicity parameters in 3 months old male Wistar rats. The obtained results indicate that body weight, food intake, serum glucose, neurological and toxicity status remained unaffected while urine proteins were significantly increased in d-gal treated rats. Although there was no effect on the general health status of the animals, our findings suggest that chronic oral d-gal administration may lead to renal dysfunction.

INTRODUCTION

D-galactose (d-gal), a reducing sugar that occurs naturally in the body, is primarily found in foods as a structural part of lactose and to a lesser extent in legumes and some fruits and vegetables. It is crucial for human metabolism, with an established role in energy delivery (due to conversion into glucose) and galactosylation of complex molecules [1]. Its maximal recommended daily dose for healthy adults is 50 g, and most of it can be metabolized and excreted from the body within 8 h [2].

However, recent studies have demonstrated that chronic administration of d-gal leads to immune system dysregulation, sex hormone deficiencies, increased inflammatory cytokine levels, cellular apoptosis, and diminished total antioxidant capacity. Taken together, these effects mimic aging and initiate the development of age-related diseases [3]. In animal aging studies, chronic treatment with d-gal induced oxidative brain damage and cognitive dysfunction, accompanied by several prominent features of the aging brain, such as impairment of synaptic plasticity and neurogenesis. Other organs might also be affected by d-gal treatment [3, 4, 5].

Interestingly, the majority of published studies are based on intraperitoneal or subcutaneous administration of d-gal, while data regarding the oral intake of d-gal are scarce. Only a few studies investigated the alternation of biochemical parameters provoked by oral d-gal treatment, and all of them were focused on brain tissue [6, 7]. The present research is focused on the impact of chronic d-gal treatment on general rats' health, with additional interest on several biochemical markers (glucose, proteins, blood, ketones, and pH), neurological and toxicity status.

EXPERIMENTAL

All experimental procedures complied with the Ethical Committee for the Use of Laboratory Animals of VINČA Institute of Nuclear Science – National Institute of the Republic of Serbia, University of Belgrade (protocol number 02/11) and European Communities Council Directive (2010/63/EU) guidelines. For the 6 weeks-lasting treatment, 3 months old male Wistar rats were randomly assigned to three groups: animals that drank tap water (Control, n = 4), rats receiving either 200 mg/kg (n = 4) or 500 mg/kg d-gal (n = 4) dissolved in tap water.

Body weight was monitored weekly while food intake was monitored daily to evaluate the general health. At the end of treatment, a battery of neurological and clinical toxicity tests was performed, along with the analysis of blood glucose levels and biochemical parameters in urine, using commercially available test strips (Insight Urinalysis Reagent Strips, Acon Laboratories, Inc., USA). The neurological test included scoring of following sensory - motor parameters: consciousness (0-1), respiration (0-1), spontaneous activity (0-3), forepaw outstretching (0-2), climbing (0-1), visual placing (0-1), cage grasp (0-1), gait posture (0-2), geotaxis (0-1), hearing and pacing/circling (0-1). The total score of neurological tests was expressed as the sum of average scores of all investigated parameters and graded on a scale of 0 to 15. The toxicity test included: agitation, convulsion, piloerection, sleepiness and lethargy. All toxicity parameters were valued as: – (no effect), + (mild effect), ++ (moderate effect) and +++ (major effect). In pre-treatment control testing, none of the animals exhibited any physiological deficit.

Obtained data were analyzed by One-way analysis of variance followed by Tukey's post hoc test. Results are presented as mean \pm SEM, and the *p* value less than 0.05 is considered significant.

RESULTS AND DISCUSSION

As presented in Figure 1., all animals similarly and gradually increased body weight without statistical differences. Moreover, food intake in all groups was stable and remained within tight boundaries from the beginning until the end of the experiment (data not shown).



Figure 1. Body weight.

At the end of the six-week treatment, there was no significant change in glycemic levels between animals in Control and d-gal treated groups, demonstrating that d-gal does not interfere with glucose metabolism. This trend was also observed in other studies, regardless of the route of d-gal application [3, 6]. Concerning the urine parameters, oral d-gal treatments increased only urine protein levels (Table 1.), which is often a sign of renal dysfunction that occurs during aging. Indeed, recent studies indicate that renal aging could be induced by chronic d-gal treatment, initiating the disturbance of molecular and histopathological parameters [4, 5].

Table 1 Dischamical nonomatons

Table 1. Diochemical parameters.			
Experimental group/biochemical parameters	Control	200mg/kg	500mg/kg
Serum glucose (mmol/l)	6.9	6.6	6.3
Proteins	47.5	100***	166.7^{***}
Blood	/	/	/
Ketones	12.5	23.3	15
pН	7.25	7.5	8

Although we previously reported that oral application of d-gal compromises the memory [8], results of the current study, presented in Table 2., indicate unimpaired sensory-motor functions and non-toxic effect of d-gal. It suggests that d-gal could successfully be used for evoking alterations that correspond to mild cognitive impairment [6].

Table 2. Results of neurological and toxicity tests scoring.

	Control	200mg/kg	500mg/kg
Sensory - motor functions	15	15	14.75
Toxicity signs	-	-	-

CONCLUSION

Although the results of the current study indicate unaltered general health status (unchanged body weight, food intake, glucose level, neurological markers and most of the toxicity indicators), we also report the renal dysfunction in d-gal chronically treated animals, which highlights the importance of highly controlled d-gal intake.

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