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Research report

# Oral administration of D-galactose induces cognitive impairments and oxidative damage in rats

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## HIGHLIGHTS

- D-Galactose by oral route induces novelty habituation deficit.
- D-Galactose by oral route induces spatial memory impairment.
- D-Galactose by oral route induces high thiobarbituric acid reactive species levels.
- D-Galactose by oral route induces increase of carbonyl group content.

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## ABSTRACT

D-Galactose (D-gal) is a reducing sugar that can be used to mimic the characteristics of aging in rodents; however, the effects of D-gal administration by oral route are not clear. Therefore, the aim of this study was to elucidate if the oral administration of D-gal induces cognitive impairments, neuronal loss, and oxidative damage, mimicking an animal model of aging. Male adult *Wistar* rats (4 months old) received D-gal (100 mg/kg) *via* the oral route for a period of 1, 2, 4, 6 or 8 weeks. The results showed cognitive impairments in the open-field test in the 4th and 6th weeks after D-gal administration, as well as an impairment in spatial memory in the radial maze test after the 6th week of D-gal administration. The results indicated increase of levels of thiobarbituric acid reactive species—TBARS—and carbonyl group content in the prefrontal cortex from the 4th week, and in all weeks of D-gal administration, respectively. An increase in the levels of TBARS and carbonyl group content was observed in the hippocampus over the entire period of D-gal treatment. In the 8th week of D-gal administration, we also observed reductions in synaptophysin and TAU protein levels in the prefrontal cortex. Thus, D-gal given by oral route caused cognitive impairments which were accompanied by oxidative damage. Therefore, these results indicate that orally administered D-gal can induce the behavioral and neurochemical alterations that are observed in the natural aging process. However, oral D-gal effect in rats deserve further studies to be better described.



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## 1. Introduction

D-Galactose (D-gal) is a reducing sugar or monosaccharide which is abundantly present in milk products, fruits and vegetables [1], and is usually converted into glucose by galactose-1-phosphate uridyltransferase and galactokinase [2]. However, D-gal administration over long periods of time can lead to an enzymatic overload, which impairs the body's natural ability to catalyze galactose into glucose, so causing an increase of galactitol and an activation of aldose reductase. This in turn causes a depletion in NADPH, which leads to an accumulation of hydrogen peroxide and other free radicals (Lai, 2009), causing oxidative damage to the cells [3,4]. In addition, at high levels, D-gal may react with the amino groups of proteins and peptides to form advanced glycation end products (AGE) *in vivo* [5]. AGE are increased during aging and have been associated with the pathogenesis of many diseases, such as diabetes [6], amyotrophic lateral sclerosis [7], and Alzheimer's disease [8].

Therefore, it has been postulated that D-gal may induce behavioral alterations that reproduce the natural aging processes in rats and mice [9,10]. Several studies have suggested that chronic systemic administration of D-gal could be used as a model of cognitive disorders and aging [11–14]. Aging is a natural process of changes that culminates in a progressive decline in both physiological and behavioral ability. The progression of aging tends to compromise the entire organism, showing particular severity within the central nervous system [15,16]. It is characterized by a gradual loss of cognitive performance, memory, and spatial ability [17]. These symptoms are accompanied by structural and functional changes within the brain, such as a decline in mitochondrial function [18] characterized by a decrease in ATP synthesis and oxidative damage [19]. These changes play a crucial role in the neurodegenerative disorders associated with the pathogenesis of age-related diseases.

According to data from studies, D-gal leads the field in creating biochemical abnormalities in experimental animals, such as; accumulations of reactive oxygen species, reductions of antioxidant enzymes, mitochondrial deficits and neuroinflammation/apoptosis. These changes in rodents are similar to those that occur in the aging human brain [11,13,20–22].

Moreover, chronic systemic (intraperitoneal or subcutaneous) administrations of D-gal can induce alterations like the ones observed in Alzheimer's disease (AD) [23,24]. Lin et al. [24] found that D-gal given *via* intraperitoneal administration significantly increased the content of amyloid beta (Aβ) in the hippocampus of mice. A previous study showed that intraperitoneal administrations of D-gal also increased the expression of the brains Aβ precursor protein [25]. It has been well described in literature that the aggregation and deposition of Aβ in the brain is a key step in the pathogenesis of AD, and that this process elicits a cascade of cellular events that ultimately leads to neuronal loss and dementia [26]. In addition, intraperitoneal or subcutaneous injections of D-gal lead to spatial learning impairments, oxidative stress and neuroinflammation, as well as activation of the NFκB signaling pathway in the brain of rodents [11,27–30]. β-Amyloid peptide, as AGEís, can activate the receptor for advanced glycation end products (RAGE), leading to oxidative stress and to the activation of the transcription factor NFκ-B signaling pathways, causing the transcription of inducible nitric oxide synthase and a variety of cytokines [8].

On the other hand, there is compelling evidence showing that the oral administration of D-gal induces protective effects in an animal model of AD induced by streptozotocin. A recent study compared both systemic and oral chronic administrations of D-gal, and the results demonstrated that the oral administration route, unlike the systemic method, can reverse cognitive deficits in a streptozotocin-induced model of AD, thus the protective effects of this sugar may well be concentration or administration route dependent [31]. Therefore, there is some controversy surrounding the use of D-gal *via* the oral route.

Considering that many studies related to aging focus on the animal model of D-gal administered by the intraperitoneal and subcutaneous routes, the administration of this carbohydrate by the oral route has not received sufficient attention. Therefore, in this study we are investigating if the oral administration of D-gal induces cognitive and biochemical abnormalities, since the oral route can be used as an alternative way of administrating D-gal over longer periods of time.

## 2. Material and methods

### 2.1. Animals

4 month old adult male *Wistar* rats, (weighing 350–500 g) were used in this research (total of 150 rats). The animals were acclimatized to the laboratory conditions at room temperature prior to any experimentation. The animals were kept under standard lab conditions of a 12 h light/dark cycle, with food and water available *ad libitum,* and were housed in plastic cages with soft bedding. All manipulations were performed between 8:00 a.m. and 5:00 p.m. The project was approved by the ethical committee of the Universidade do Extremo Sul Catarinense and all experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals, as well as under the Brazilian Society for Neuroscience and Behavior recommendations for animal care. This study was approved by the local ethics committee (Ethics Committee on Animal Use—CEUA of the Universidade do Extremo Sul Catarinense).

### 2.2. Drugs and treatment

D-Gal (D-galactose, Sigma–Aldrich, St. Louis, MO, USA) solution was used. It was dissolved in water for administration at the dose of 100 mg/kg [9,14,32,33] of body weight, and given by oral gavage, once a day, over a period of 1, 2, 4, 6 or 8 weeks. Animals were randomized into two groups: control animals (receiving water by oral gavage) or D-gal animals (receiving D-gal by oral gavage). The behavioral tests and biochemical analysis were undertaken on the 1st, 2nd, 4th, 6th and 8th weeks after the last administration of D-gal. Twenty-four hours after the last administration of D-gal in each period of treatment, the animals were weighed and subjected to the behavioral tests. After the completion of the open field task, or 72 h after the last administration of D-gal, the rodents were killed by decapitation without the use of anesthesia (the procedure was approved by the Ethics Committee) and their brain tissues were collected for use in the molecular studies.

### 2.3. Open-field test

Long-term retention of habituation in a novel environment can be considered a non-associative, non-aversive type of learning, which can be measured by a decrease in the amount of exploratory activity undertaken by the test subject. In rodents, it is assessed by the number of rearings performed in a test session carried out 24 h after the first exploration session [34]. This apparatus consists of a 45 cm × 60 cm brown plywood arena which is surrounded by 50 cm high wooden walls and fitted with a frontal glass wall. The floor of the open field was divided into nine rectangles (15 cm × 20 cm each) by black lines. The animals were gently placed on the left rear quadrant and then left to explore the arena. To investigate the effects of any drug treatment on spontaneous locomotor activity, the numbers of horizontal (crossings) and vertical (rearings) activities performed by each rat during a 5 min observation period were