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Red Grape Seed Extract (*RGSE*) has a Positive Effect on Systemic Organ Dysfunctions Caused by Long-Term Exposure to D-galactose (IP) in the Alzheimer's Disease Rat Model

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ABSTRACT: Objectives: The purpose of the study is to investigate the effects of D-Galactose exposure on cognitive, motor, physical, and organ dysfunction in Alzheimer's disease rats. Materials and Methods: A total of 48 healthy male Wistar rats (*Rattus norvegicus*) aged 3 months, weighing approximately 180±20g. Rats were divided into four groups and treated with intraperitoneal (i.p.) injections of D-Galactose (120 mg/kg body weight) dissolved in distilled water. Experiments were conducted for 30 and 60 days. Cognitive Functions Evaluated using Y maze, nest building, and Morris water maze tests. Motor Functions: Assessed using wire hang and rotarod tests. Physical Condition: Monitored via symptom score. Sacrifice and Analysis: On both the 30th and 60th day of the experiment, the rats were sacrificed by cervical dislocation. Their organs were weighed, and organ damages were examined. Results: D-Galactose administration in rats boosted Iba-1 and CD3 positive cells, but did not improve cognitive impairment or amyloid β protein deposition, resulting in sarcopenia and heart damage in AD model albino rats. Discussion: The study demonstrated that continuous brain exposure to D-Galactose leads to sarcopenia and cardiac injury in AD model rats. However, this exposure did not exacerbate cognitive impairment or lead to amyloid β protein deposition, suggesting that D-Galactose's impact on systemic organ dysfunction may occur independently of its effects on cognitive function in this AD model.

Keywords: Alzheimer's disease, D-Galactose, Brain exposure, Intraperitonial injection.

INTRODUCTION

Alzheimer's disease (AD) is characterised by a progressive deterioration in cognitive function. Alzheimer's disease has a greater impact on persons 65 and older, with a steady decline in memory, thinking, language, and learning capacity. Alzheimer's disease is a progressive neurological ailment characterised by memory loss, cognitive dysfunction, behavioural problems, and difficulties with daily activities. According to the Alzheimer's Association, Alzheimer's disease affects around 4 million people in India, and 44 million people worldwide. In general, Alzheimer's medicines such as donepazil modify the haemoprofile and impact the heart rhythm. Elderly people usually have diminished age-related cardiac function. As a result, chronic usage of these anti-alzheimer drugs raises cardiovascular problems, which may worsen cardiovascular functioning in the body Hanaa *et al.* (2015); Zec & Burkett 2008). In addition to being a great source of antioxidants to treat a variety of degenerative diseases, medicinal plants have long been used to treat a number of human diseases (Sochorova *et al.,* 2020). Cholines from these plants also help the brain synthesis ACh, which enhances cognitive and memory functions. This helps prevent the side effects of anti-alzheimer's drugs (Nelson *et al.,* 2009; Mishra and Palanivelu 2008).

MATERIALS AND METHODS

A. Animals

Sri Venkateswara Enterprises, Bangalore, provided 48 healthy male Wistar rats (Rattus norvegicus) that were 3 months old and weighed about 180±20g. In accordance with the "Institutional Animal Ethical Committee" requirements, the animals were subjected to a photoperiod of 12 hours of light and 12 hours of dark. Under typical laboratory circumstances, the animals were kept in separate cages and given access to unlimited water and a regular pellet meal. Before the experimentation day, they fasted for the entire night. Room temperature was kept at 27°C while the animals were kept in the departmental animal home.

B. Induction of Alzheimer's disease

An intraperitoneal (i.p.) injection of D-galactose (120 mg/kg body weight) that had been dissolved in distilled water was used to cause memory impairment.

Isolation of Tissues. All four of the aforementioned groups of rats were slaughtered by cervical dislocation on the thirty and sixty-first days of the experiment in order to obtain biochemical estimations. The separated tissue was quickly put on a glass plate that had been refrigerated, frozen in liquid nitrogen at 180°C, and then kept at 70°C until needed again. The tissues were thawed and used for the biochemical analysis. Statistics were used to analyse the outcomes.

Experimental Design. Age matched rats were divided into 4 groups of six in each group:

Group I–Normal Control: Rats injected with saline (1ml/kg body weight) subcutaneously.

Group II– AD-model group: Rat, intraperitoneally (IP) administered with D-Gal (120mg/kg body weight) up to end of the experiment.

Group III–Red Grape Seed Extract* [RGSE Group]*: Six weeks of saline injection were followed by seven weeks of oral administration of Red Grape Seed Extract (RGSE) ethanol extract (100 mg/kg body weight) for a duration of sixty days.

***Group IV*–Administered with D-Gal+*Red Grape Seed Extract* (AD+RGSE):** Rats were given intraperitoneal injections of D-galactose (120 mg/kg body weight) for six weeks, and for an additional sixty days, they were also given 100 mg/kg body weight of Red Grape Seed Ethanol Extract orally.

Biological effect of D-Gal induced Alzheimer's Disease. D-galactose, a reducing sugar, can be injected intraperitoneally (I.P.) or taken orally to promote ageing (Rahman *et al.,* 2012; Ben Youssef *et al.,* 2021). It quickly interacts with free amines in amino acid proteins and peptides in vitro and in vivo to create Advanced Glycation End-products (AGE) by non-enzymatic glycation (Zino *et al.,* 2016).

The Advanced Glycation End-product stimulates the formation of free radicals by activating its receptors, which are linked to metabolic pathways (Rahman *et al.,* 2012). Although D-galactose ($C_6H_{12}O_6$) is a necessary food, an excess of it can cause abnormalities in metabolism. The advanced glycation end products are produced by the oxidative metabolism of D-galactose (Gao *et al.,* 2015).

Reactive oxygen species (ROS) that outnumber the cells' ability to remove them, resulting in cellular membrane, structural, and gene expression impairments (Rahman *et al.,* 2012). Prolonged D-Galactose consumption accelerates the ageing process in experimental animals, impacting age-related cognitive impairment due to ROS production and mitochondrial malfunction.

Furthermore, chronic D-Galactose supplementation in experimental animals reduces nerve growth factor expression and related proteins, which is associated with nerve cell degeneration and, as a result, lowers acetylcholine levels in brain areas (Mishra & Palanivelu 2008). Thus, long-term oral or intraperitoneal injections of D-Galactose promote Alzheimer's disease in normal rats. The experimental duration in this study was 90 days. D-Gal was administered for the first 14 days in rats to examine AD signs and test cognitive skills. Furthermore, AD-induced mice were administered with D-Gal and APRE simultaneously over a 90-day period. On specific days, rats were submitted to behavioural examinations to assess their cognitive abilities (Loureiro *et al.,* 2017; Zhang *et al*., 2006).

C. Behavioural Aspects

(i) Elevated plus-maze. The elevated plus-maze functioned as a behavioural model for evaluating external stimulation-related memory in mice. The strategy, technique, and endpoint for evaluating memory were followed according to the standards provided by investigators working in the field of psychopharmacology (Saito and Saido 2018; Kilroy *et al.,* 2019). The raised plusmaze for mice included two open arms (16cm × 5cm) and two covered arms (16cm × 5cm × 12cm) that extended from a central platform (5cm × 5cm). The maze was raised to a height of 25 cm above the floor. On the research day, the experimental animals were placed in an open arm, distant from the central platform. Transfer latency (TL) was defined as the time (in seconds) required for the animal to go from an open arm to a covered arm utilising all four legs. Each animal's TL was recorded on the first day of training (Kilroy *et al.,* 2019). The animals were permitted to walk through the maze before returning to their home cage. The retention of this learnt task (memory) was tested 24 hours after the first day of trialling and on specific days to assess cognitive function improvements. A significant decrease in the TL value of retention showed an improvement in memory.

(ii) Morris water maze test. The water maze, initially developed to test rodents' learning and memory abilities, was used in behavioural research. The tank was filled with water (21-26°C) to a height of 30 cm, and the transparent escape platform, which measured 10 cm in diameter and 29 cm in height, was submerged 1.5 cm below the surface of the water in a fixed location. Water was rendered opaque by adding powdered nonfat milk (Fu *et al.,* 2021). The platform was not visible from just above the water's surface, and transfer trials revealed that visual or other proximate signals did not facilitate escape onto the platform. The time spent by the animal to reach the hidden platform was dubbed the Escape Latency and was utilised as a memory index (Morris, 1984).

(iii) Y-maze test. The Y-maze test recorded spontaneous alternation in a single session, which was used to evaluate the short-term memory of mice. The polywood Y-maze utilised in this investigation included three identical arms that measured 35 cm in length, 8 cm in height, and 15 cm in breadth. The arms were placed at a 120-degree angle in a single piece. The animals were given the appropriate medicines, then left to wander freely within the maze for eight minutes after being placed at the end of one arm. Every mouse's entrance movement in each arm was captured on video (Samimi and Edalatmanesh 2016). The animal's four paws must be inside the arm passage for the entry to be counted. The animal's motion into the arm points to a general locomotor activity. The arms of the maze were cleaned between sessions with 10% ethanol.

(iv) Organ weight. The animal's numerous organs under investigation were removed, and then they were

weighed. The weights of the kidneys, testes, uterus, ovaries, adrenal glands, liver, heart, lungs, thymus glands, spleen, and kidneys were noted and examined for any unusual weight growth or loss. This provides an initial confirmation of the side effects (if any) of the medication being tested (Fu *et al.*, 2021).

Statistical Analysis. The data obtained from animal experiments are expressed as mean ± SEM (standard error of mean).

RESULTS AND DISCUSSION

A. Morphological Features

All of the animals in Group I of the Alzheimer's inducer had lower observed body weights than the animals in the other treatment groups. There was a variation of 12% between groups I and II, 10% between groups I and IV for 60 days, 17.5% between groups I and II, and 15.4% between groups I and IV for 90 days. In contrast to the control group, AD-model rats gradually lost hair and developed stiff, thin, and sagging skin. When compared to the AD-model group, the concurrent treatment of the AD-induced group with RGSE in protective groups III & IV had resulted in a considerable rise in total body weight.

B. Visceral organ Index

Compared to the AD-model group, the organ index of protective groups III and IV showed a greater increase in organ weight. All treatment groups showed a small rise in visceral organ weight, with a slight change in weight between the 30th and 60th day of the trial.

Table 1: Organ index from different groups of experimental rats for 30 and 60 Days treatment.

Parameter	Brain		Kidney		Liver		Spleen	
Group	30 Days	60 Days	30 Days	60 Days	30 Days	60 Days	30 Days	60 Days
CONTROL	1.95±0.36	2.08±0.13	1.92±0.18	1.95±0.25	9.34±0.52	9.88±1.21	1.37±0.13	1.26±0.24
A.D.	1.76±0.18	1.81±0.25	1.78±0.21	1.99±0.16	7.59±0.18	8.82±0.82	0.93±0.15	1.22±0.26
G.S.E	1.98±0.07	2.13±0.11	1.98±0.18	2.05±0.10	9.92±1.04	10.19±0.93	1.82±0.31	1.76±0.18
A.D.+GSE	1.94±0.10	1.80±0.09	2.13±0.20	2.31±0.24	8.56±0.95	9.7±1.47	1.44±0.14	1.49±0.38

Note: The above values are expressed in grams.

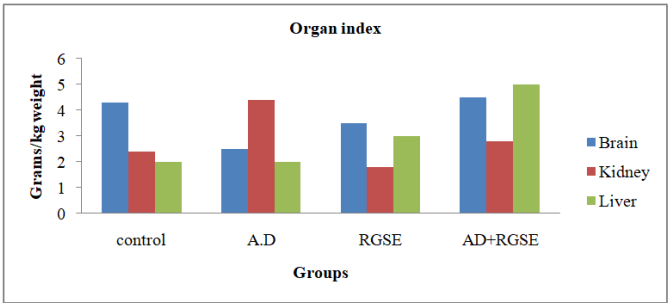

Fig. 1. Organ index from different groups of experimental rats (Grams /body weight) for 30 and 60 Days treatment.

SUMMARY

The hydro-alcohol (30:70) was used to extract the dry powder of red grape seeds at a temperature not to exceed 60> C. The anti-Alzheimer's activity of extracts was evaluated in rats. It was discovered that the components of RGSE included tannins, phenols, alkaloids, saponins, and flavonoids. The therapy groups showed an increase in their organ index and body weight morphologically. RGSE improves memory, as demonstrated by behavioural testing. A 150 and 300 mg/kg hydroalcoholic extract of RGSE had demonstrated an improvement in the cognitive system's cholinergic system. RGSE has enhanced antioxidant status, which benefits AD caused by stress.

CONCLUSIONS

There are numerous pharmaceutical applications for the berry fruit *Vitis vinifera.* Although the pharmacological activities of roots have been studied previously, leaves and seeds have not. The results of this study show that the hydroalcoholic root extract contains alkaloids, which may be able to treat Alzheimer's disease. Subsequent research ought to be designed to clarify the precise components at the core and determine the whole therapeutic range of RGSE.

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