Red Grape Seed Extract (Vitis vinifera.L) prevents memory deficits and the increase in Acetylcholinesterase activity in D-Galactose - induced Albino Rats (Wistar strain)

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Abstract:

The objective of this study was to see how administration of Red Grape Seed Extract (RGSE) affected memory and Acetylcholinesterase (AChE) activity in D-Galactose-induced Albino rats' Cerebellum, Cerebral Cortex, Hippocampus, and Pons Medulla. Four groups of six animals were formed. D-Galactose, RGSE, and D-Gal+ RGSE groups were studied. After 30 days of RGSE therapy, the animals were subjected to behavioural tests before being euthanized and their brain structures and blood were collected. The D-Galactose group demonstrated a decrease in step-down latency. D-Galactose-induced memory impairment was prevented by the RGSE group. In the open field test, there were no discernible differences between the groups. When compared to the control group, the D-galactose group displayed significantly higher AChE activity in all regions of the brain. However, AChE activity decreased significantly in the RGSE groups in the Cerebellum, Cerebral cortex, Hippocampus, and Pons medulla, whereas no significant alterations were seen in the combined therapy groups in any brain tissue when compared to the control group. Finally, the current data reveal that RGSE therapy inhibits the increase in AChE activity and, as a result, memory impairment in Albino rats, suggesting that this molecule can alter cholinergic neurotransmission and, as a result, improve cognition.

****Keywords: Red Grape Seed Extract Memory, Acetylcholinesterase, D-Galactose, Albino Rats.**

Introduction:

Cholinergic neuron degeneration in the cerebral cortex and subcortical regions is regarded to be the root cause of neurological diseases and cognitive impairments (**Francis *et al.,* 2020**). A connection between the cholinergic system and cognition has emerged from a number of exploratory studies. Experimental damage to the basal forebrain cholinergic system and muscarinic antagonist treatment of animals result in memory problems (**Tarciana Rafaela Barbosa Figueiroa Silva *et al.,* 2022**). Acetylcholinesterase overexpression in transgenic rats has been found to cause a gradual cognitive loss (**Vanessa Linares *et al.,* 2022**) cholinergic stimulation, on the other hand, can improve cognitive performance in both animals and humans (**Jennifer M *et al.,* 2020**). These results confirm acetylcholine's critical function in cognition and imply that cholinomimetic chemical replacement therapy may help patients with cognitive and memory problems brought on by neurological disease. These deficits have primarily been characterised by modest deficits in learning and memory, psychomotor slowness, and decreased mental flexibility (**Sela, Y *et al.,* 2022**). The only class of medications that has been consistently demonstrated to be beneficial in treating the cognitive and functional symptoms of neurological disorders are cholinesterase inhibitors (**Jill R. Storry *et al.,* 2019**). Treatment for neurological diseases is centred around cholinesterase inhibitors, of which four have been licenced for the symptomatic management of mild to moderate neurological diseases. These include galantamine, donepezil, rivastigmine, and tacrine, which are all aminoacridines (a tertiary alkaloid). Clinical differentiation between these medications may be based on differences in their tolerability profiles and ease of administration because they seem to have comparable efficacy (**Jill R. Storry *et al.,* 2019**). Because acetylcholinesterase is more selective than butyrylcholinesterase, cholinesterase inhibitors' tolerability profiles may vary (**Gallagher, M.W *et al.,* 2020**). There are some signs that butyrylcholinesterase activity could be connected to the aetiology of neurological illness (**Paramita Chaudhuri *et al.,* 2019**). As a result, it has been hypothesised that nonselective cholinesterase inhibitors, which inhibit both butyrylcholinesterase and acetylcholinesterase, may be more helpful to persons with neurodegenerative diseases than selective cholinesterase inhibitors, which only inhibit acetylcholinesterase (**Jill R. Storry *et al.,* 2019**). The relative importance of acetylcholinesterase versus butyrylcholinesterase inhibition in peripherally (salivation) and centrally (brain acetylcholine levels and tremor) mediated cholinergic responses to cholinesterase inhibitors was examined.

Red Grape Seed Extract (RGSE) is a rich source of polyphenols, anthocyanins, and pro-anthocyanins, which are primarily found in red wine and grapes and have a variety of biological activities that have been established, including roles as an antioxidant, anti-inflammatory, cardioprotective agent, and anticarcinogen (**ZhihaoWu *et al.,* 2018**). Recent research on the neuroprotective properties of resveratrol and grape phytochemicals has shown that these compounds reduce the toxicity caused by amyloid peptides (**Z.W. Han *et al.,* 2020; Fatemeh Khorshidi *et al.,* 2021**), guard against cerebral ischemic injury (**AytacTasci *et al.,* 2022**), and protect against kainic acid-induced excitotoxicity (**AytacTasci *et al.,* 2022**). The powerful antioxidant activity of RGSE, which in numerous studies has been demonstrated to protect the neural tissue against a range of neurodegenerative disorders brought on by oxidative stress, has been attributed to several neuroprotective qualities of RGSE (**Ates *et al.,* 2020; M.Garrab *et al.,* 2019**)

MATERIALS AND METHODS:

The objective of the current investigation was to assess the protective effects of grape seed extract on the cholinergic system in the brain tissue of rats with memory defects.

Purchase and Care of Experimental Animals:

Healthy Wistar strain Albino rats (Rattus norvegicus), obtained from Sri Venkateswara enterprises in Bangalore, was used as the experimental model in this study. It was the same age group as the subjects—3 months—weighed 160 ± 20 grammes. According to Behringer's instructions, the rats were acclimated before the trial (**Behringer's 1973**). They were kept in polypropylene cages in the department of zoology's animal house under carefully controlled circumstances, including a 28°C temperature, a 12-hour photoperiod, and a 75% relative humidity level. The rats were kept on a regular pellet diet provided by Sri Venkateswara Enterprises, Bangalore, and were given unlimited access to water.

Preparation of Grape Seed Extract:

Grape, as large clusters with red berries, was bought from a local fruit market in Tirupati, Pulivendula and Bangalore (Devanahalli) as *vitisvinifera(Linn)*. Grape seeds were removed from the grapes, air dried (in shade) for one week and milled to fine powder (a particle size of < 0.4mm). The grape seed powder was macerated in 75% ethanol for 72h at room temperature. The ethanol extract evaporated to remove ethanol, and grape seed extract was obtained as a lyophilized powder (**Alireza**

Sarkaki *et al.*, 2007). The resulting ethanolic crude extract was air dried and used in the present study.

Grouping of Animals:

The rats were randomly separated into four groups after becoming adjusted to the laboratory environment for 10 days before to the experiment. Once more divided into two subgroups of six each, each major group was kept in a separate cage. Red grape seed ethanol extract and D-Gal were administered in the following dosages to the several groups of rats, except the control group. All doses were administered once between the hours of 8 and 9 in the morning, taking into account the fact that rats behave differently at night compared to during the day.

Group-I (Control)	Control Rat received with normal saline
Group-II (AD)	D-Gal was intraperitoneally (IP) delivered to rats from the first day to the 60th day of the experiment (**Zhang *et al.*, 2006; Hua *et al.*, 2007**).
Group-III (RGSE)	Rat, orally administered with Red grape seed ethanol extract (100mg/kg bodyweight) for 30 days.
Group-IV (AD+RGSE)	Rat, Intraperitonealy injected with D-Gal (120 mg/kg body weight) once daily for first 30 days. From 31stday onwards rats were administered with Red grape seed ethanol extract (100mg/kg body weight) for 30 days.

The experimental period in the current study was 60 days. D-Gal was administered to rats for the first 30 days so that AD symptoms could be seen and their cognitive abilities could be evaluated (AD group). Additionally, D-Gal and Red grape seed ethanol extract were administered to AD-induced Rat at the same time.

Experimental induction of Memory Disorder :

10 mg/kg of D-Galactose, diluted in 1.5 ml of normal saline, was administered intraperitoneally to generate Memory dysfunction. Normal saline was administered in the same quantity to the age-matched control rats. To lower hypoglycemia shock-related deaths, rats treated with D-Gal were given 5% glucose for 24 hours instead of water after inducing memory loss.

Treatment with RGSE

Six rats each group were randomly assigned to the Control group, D-galactose group, RGSE group, and D-gal+RGSE group. The animals from the control group and the D-Gal group got 10 mg/kg of RGSE orally through oral gavage one week after the

induction of memory impairment. The animals from the control group and the D-Gal group received saline solution orally instead of the 10 mg/kg of RGSE. A dose of RGSE that did not exceed 0.1 ml/100 g of rat weight was given once daily for 30 days, between 10 and 11 a.m., freshly prepared in 75% ethanol.

Results:

Acetylcholinesterase (AChE):

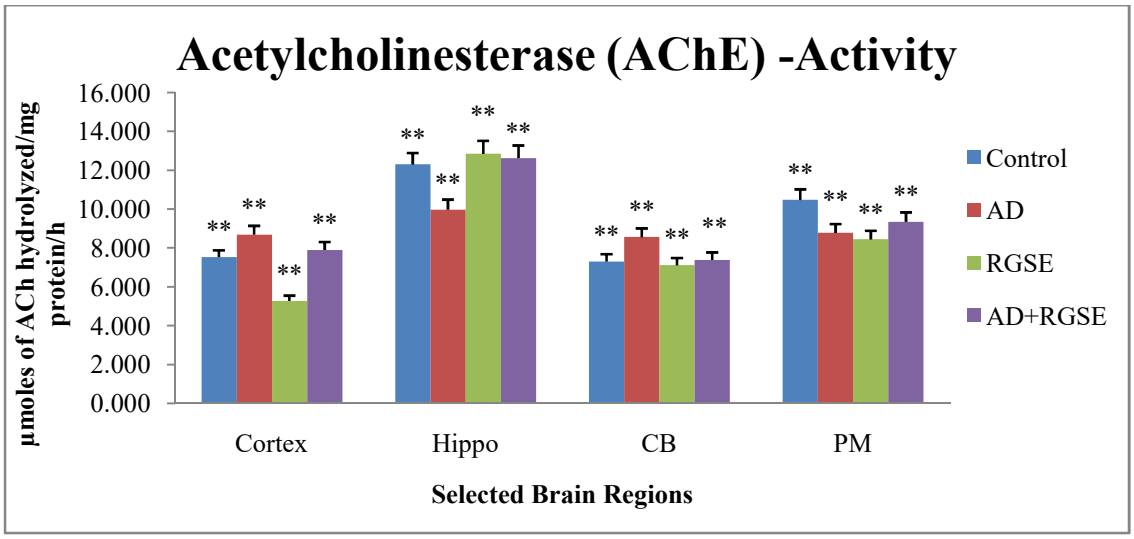
The activity levels of the enzyme, AChE showed a quite opposite trend to that of ACh content. Wherein AChE levels were drastically increased in AD-I group when compared to Controls. However, administration of RGSE to AD-I group could reverse AD induced effect and thus restored the AChE levels which were more than the controls. Hence, RGSE might be acting as a pro-cholinesterase compound by boosting its levels.

From the Experimental values revealed that, the Hippocampus (7.91 **µ moles of ACh hydrolyzed/mg protein/h**) had the highest level of AChE activity in control groups of rats, followed by the Pons Medulla(PM), Cerebellum(CB), and Cerebral Cortex(CC) (7.16, 6.45, and 6.41 **µ moles of ACh hydrolyzed/mg protein/h**). The Pons Medulla had a higher level of AChE content (88.68%) in the AD-induced group, while the Hippocampus had a lower level of AChE content (53.85 %). When rats were treated with RGSE and D-Galactose, the Pons Medulla (53.91 %) showed a larger percent change than the Hippocampus (-6.82 %).

Table – 1: AChE values in different brain regions of four groups animals

	Acetylcholinesterase (AChE) –Activity				
		Cerebral Cortex	Hippo campus	Cerebellum	Pons medulla
Mean ± SD	Control	7.530 ±0.36	12.305±0.59	7.301±0.38	10.476±0.55
	AD	8.691 ±0.45	9.973±0.52	8.565±0.45	8.776±0.46
	RGSE	5.272±0.28	12.852±0.67	7.116±0.37	8.446±0.44
	AD+RGSE	7.896±0.41	12.622±0.66	7.388±0.39	9.348±0.49

Fig.1:Changes in Acetylcholinesterase activity levels (moles of ACh hydrolyzed/mg protein/h) in the selected brain regions from the Control and Experimental groups of rats treated with RGSE, D-Galactose, D-Gal+ RGSE on the selected days of day of the experiment.



Discussion:

Cognitive dysfunctions brought on by D-Gal induction are accompanied by CNS structural and neurophysiological alterations (**Biessels *et al.*, 2008**). Additionally, polyphenolic substances have recently drawn a lot of attention because it has been demonstrated that they can shield neurons from a number of experimental neurodegenerative diseases, such as diabetes-related cognitive deficits (**Biessels *et al.*, 2008**). Although some research have looked into the neuroprotective benefits of RGSE in animal models of memory disorders (**Ates *et al.*, 2020**), there is no information in the literature about this compound's effects on cholinergic neurotransmission. Thus, in the current investigation, rats with D-Gal-induced memory impairment were used to examine how this polyphenol affected memory and AChE activity. In our investigation, we found that memory dysfunction rats showed a significant reduction in step-down latency during the inhibitory avoidance test, suggesting that these animals may have learning and memory impairment. These results suggest that RGSE therapy can prevent learning and memory loss brought on by memory dysfunction in rats. It has been established that AChE plays a fundamental role in learning and memory (**Faruk Karateke *et al.*, 2013**) and changes in its activity as well as in the level of the neurotransmitter acetylcholine are neurochemically linked with cognitive deficits seen in patients and in animal models of memory dysfunction. However, the precise mechanism by which D-Gal affects cognitive functions is still not fully understood. In the current

study, we found that memory-impaired rats' AChE activity increased throughout all analysed brain areas (cerebral cortex, hippocampus, pons medulla and cerebellum). Cerebellum and the hippocampus showed a less dramatic rise. The functional variability in the central cholinergic system may be reflected in the lack of uniformity in the AChE profile. Choline acetyltransferase-containing neurons can be found almost everywhere in the central nervous system. The membrane's integrity and permeability variations during synaptic transmission and conduction are caused by AChE, a key biological component of the membrane. Depending on the anatomical area, the G4 form makes about 60–90% of the total AChE in the mammalian brain whereas the remaining portion is made up of G1 and G2 forms. Based on these findings, we can hypothesise that oxidative stress and subsequent free radical generation in various brain regions may operate as a mediating factor in the AChE activation seen in memory impairment in Rat.

The findings of the present study showed that diabetic rats had impaired memory and learning, which was accompanied by a substantial increase in AChE activity throughout the entire brain.

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