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ORIGINAL ARTICLE

Therapeutic Aspects of Red Grape Seed Extract (*Vitis vinifera*.L) on The Cholinergic Constituents in AD-induced Rat Brain

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ABSTRACT
Alzheimer's Disease (AD), one of the most seriously prevailing neurological diseases, is characterized by slow and progressive neurodegeneration in different brain regions. D-Galactoseis a potent and slow symptomatic neurotoxic chemical, implicated in the neuropathogenesis of AD. The present study was aimed to evaluate the possible neuroprotective potential of Red Grape Seed Extract (Vitis vinifera.L) in D-Galactose-induced AD in male albino rats with reference to the Cholinergic system. From the observations, it was obvious that AD induction caused a significant decrease Acetylcholine (ACh) content while elevated the AChE levels in all four regions of Rat brain thus revealing that RGSE has neuroprotective impact on the cholinergic system
Keywords: *RGSE, D-Galactose, Cholinergic agents, AD-rats, Moriss Water maze test.*

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INTRODUCTION

Alzheimer's Disease (AD), a neurodegenerative brain disorder that is incurable, irreversible and progressive is caused by nerve cell abnormalities that result in the death of brain cells. Dementia affects 46.8 million people worldwide, with the figure expected to be double for every 15-20 years (1). Alzheimer's Disease, Parkinson's Disease and Multiple Sclerosis are just a few of the degenerative diseases that can affect the human brain. Despite decades of research, no specific therapeutic medicine for the permanent cure of Alzheimer's Disease is now available. Current medicines on the market are designed to keep the Acetylcholine levels in the brain at a constant pace, balanced by inhibiting Acetylcholinesterase (AChE,) which is a membrane- bound the enzyme that hydrolysis the neurotransmitter, ACh. As per the Cholinergic Hypothesis (16) and the available literature, Acetylcholine is known to play an important role in memory and learning and also works as a neruromodulator in the Central Nervous System (CNS) as well as the Peripheral Nervous System (PNS) (2). Cholinesterases are a group of enzymes that catalyse the hydrolysis of ACh into choline and acetic acid, which is a crucial step in restoring cholinergic neuronal activity. Moreover, Cholinesterases are identified in both invertebrates and vertebrates (3) and can be found even where ACh is not a neurotransmitter. The effects of majority of medications are mediated by changes in cholinergic transmission (17). Alzheimer's Disease is a solely progressive Neurodegenerative disease of the brain associated with Dementia and is manifested in the form the severe forgetfulness and patient's enability to carry out routine daily activities and finally loss of behavioral and intellectual functionalities (4).Hence, the present study was focused on to identify and assess the neuroprotective role of RGSE in AD-induced Male Albino rat with reference to the Cholinergic system, not only because of its significant role in AD as already cited above, but also majority of the currently available medications were targeted on these 2 constituents.

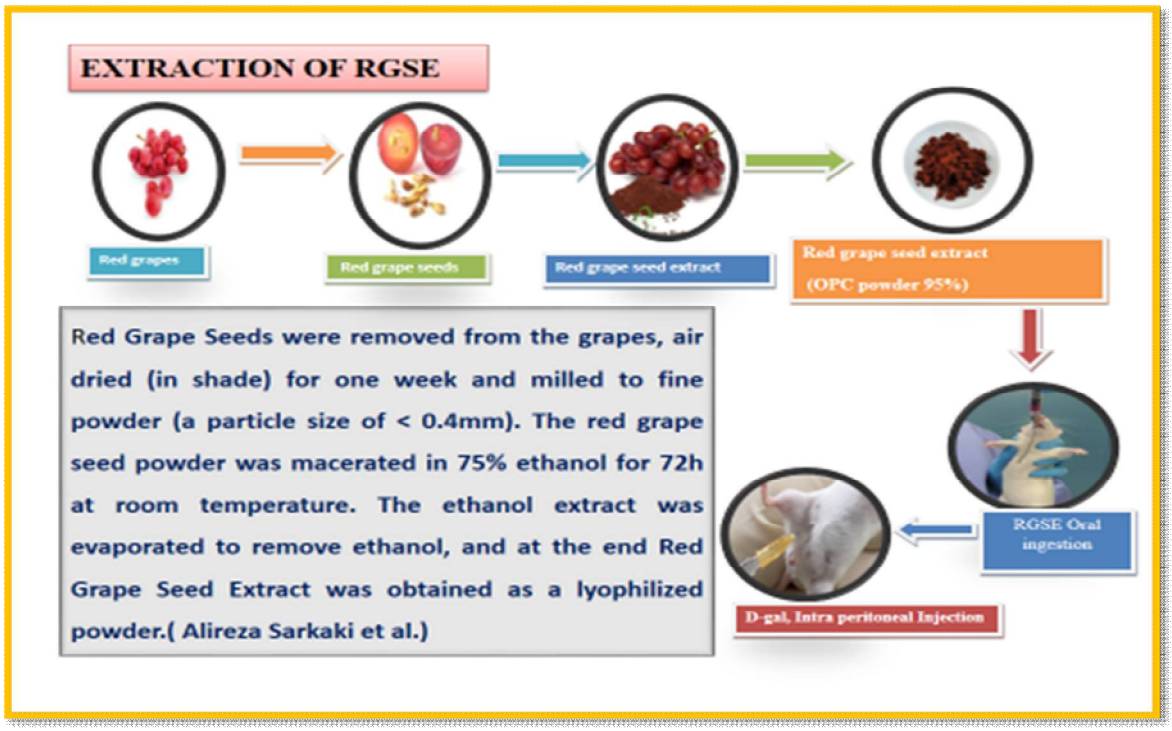
MATERIAL AND METHODS

The present study was focused on evaluation of the protective effects of Grape Seed extract on Cholinergic constituents in selected regions of experimentally induced AD rats brain. Male Albino rats were divided in to 3 groups as follows.

Grouping of Rats and Experiment Design

Group-I (Control Group):	Rats injected with saline (1ml/kg body weight) subcutaneously.
Group-II(AD-I Group)	D-galactose (160 mg/kg body weight) was intra-peritoneally injected into rats throughout the experiment.
Group-III(Protective Group :PG (AD-I+RGSE**)**	D-galactose (160 mg/kg b.w.) was injected into rats for six weeks, and then RGSE (10 ml/kg body weight of rat;12x 108 CFU/ml) was given orally at the same time for another 60 days.

Preparation Of Grape Seed Extract:



Administration of test substance

RGSE at the rate of 100mg/kg body weight (0.2 ml/rat), dissolved in distilled water was delivered by a gavage through oral route.

Isolation of tissues:

After treatment of different groups of rats either with D-galactose or the test substance for 60 days, were fasted for 12 hours before being scarified on the 30th and 60th days of the experiment. On these 2 days, the selected brain regions, such as the Cerebral Cortex, Hippocampus, Cerebellum and Pons Medulla, were quickly collected from each rat brain, washed thoroughly with ice cold saline, frozen in liquid nitrogen and used for estimation of Ach content and also AChE) by employing the following techniques.

NAME OF THE PARAMETER	METHODS EMPLOYED
Cholinergic System	
A. Acetylcholine (ACh)	**[11]**
B. Acetylcholinesterase (AChE)	**[6]**

RESULTS

Acetylcholine (ACh) Content: (Graph-1) :

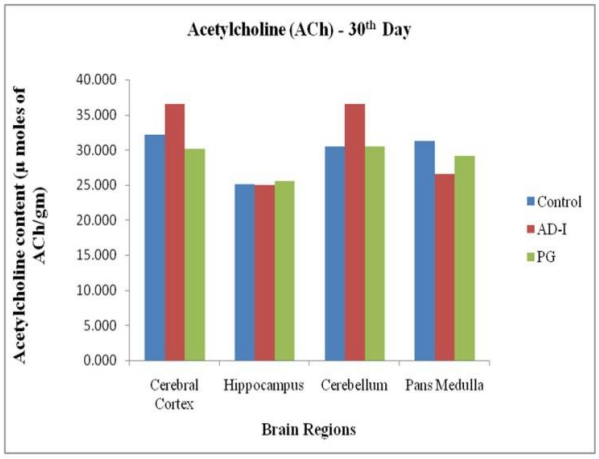
On 30thday:From the data analyzed after completion of the experimental parameters, it was obvious that in control groups of rats the ACh content was highest in Cerebral Cortex (32.127 µ moles of),lowest in Hippocampus (25.140µ moles)and in the other 2 regions as follows : Cerebellum (30.472 µ moles) and

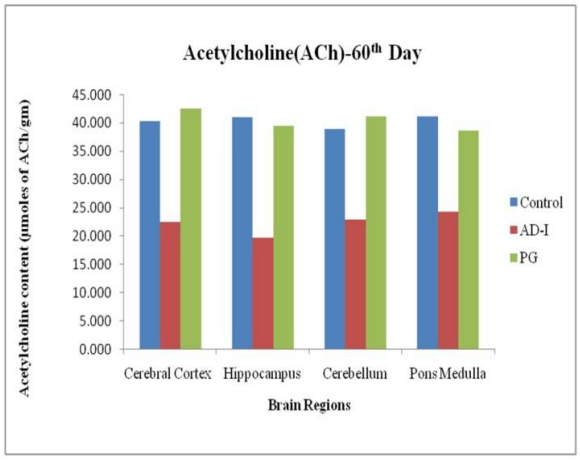
Pons Medulla (31.231 µ moles). Interestingly, AD induction caused a sudden decline in ACh levels in following brain regions in the order of Pons Medulla (26.53µ moles of ACh/gm) followed by Hippocampus(25.03µ moles). However, treatment of AD-Induced rats with, RGSE simultaneously for 30 days resulted in significant elevation in ACh content in all the 4 brain regions (Cerebellum (30.47µ moles);Cerebral Cortex (30.15µ moles); Pons Medulla (29.08µ moles) and Hippocampus (25.57µ moles).

On 60th Day: (**Graph-2) :**

As far as the results on the cholinergic system on 60th Day was concerned, RGSE excreted profound effect on ACh content while the trend remained the same as in the case of 30th Day. For example, the control group rats showed significant improvement in ACh content in all regions in the order of Cerebral Cortex (40.211 µ moles), Cerebellum (38.868 µ moles), Pons Medulla (41.109 µ moles) and Hippocampus (40.926 µ moles), while D-Gal administration caused a sudden and drastic decline in ACh levels. An interesting observation was that the reversal effect of RGSE was directly related to the duration of AD induction and RGSE treatment, i.e. longer the duration more recovery.

Graph: 1 & 2 Graphical representation of changes in **Acetylcholine content (µmoles of ACh/gm)** in selected regions of brain from Control and Experimental groups of rats on 30th and 60thDays of experimentation.





Acetylcholinesterase (AChE) activity:

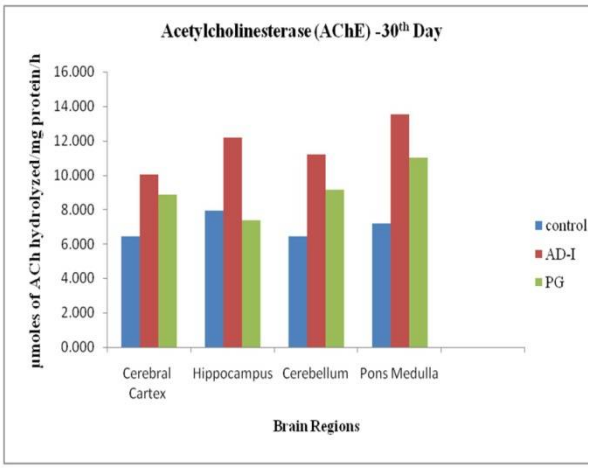
On 30th Day: (Graph-3):

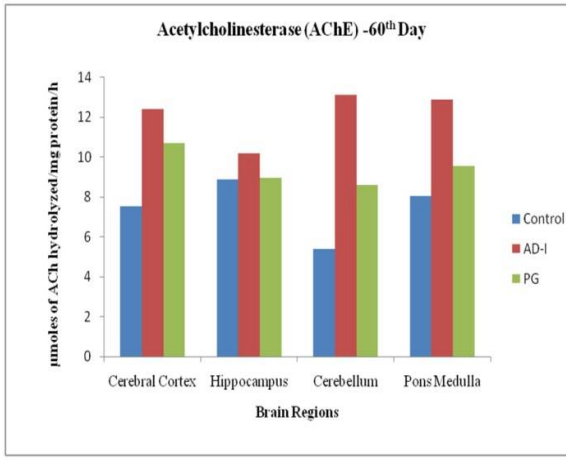
In control group of rats, among the four regions of brain, Hippocampus region registered highest level of AChE activity (7.913 µmoles) followed by Pons Medulla (7.166µmoles), Cerebellum (6.459 µmoles), and Cerebral Cortex (6.419 µmoles). In contrast to Ach content, the activity levels of AChE in AD-induced rat brain were significantly elevated in all regions, of course to different extent on 30th Day of experimentation. However, as in the case of ACh, RGSE Administration caused significant decrease in AChE levels in all regions of brain in the order of Pons Medulla >Cerebellum>Cerebral Cortex>Hippocampus, thus clearly revealing the reversal potential of RGSE on AChE activity.

On 60th day: (Graph-V1):

In control rats, the AChE content in different regions was in the order of Hippocampus which recorded the highest level(12.305µmoles)then in the order comes Pons Medulla(10.476µmoles) followed by Cerebral Cortex(7.530 µmoles) and Cerebellum(7.301 µmoles of ACh). In AD-induced rat, drastic increase in AchE activity levels was noticed in all regions where the Cerebral Cortex region showed the maximum level (Hippocampus 9.97µmoles) and Cerebellum region the lowest (Cerebellum 8.56 µmoles of ACh) of AChE respectively. As in the case of ACh, AChE also showed significant recovery in all regions on treatment with RGSE.

Graph:3 & 4: Graphical representation of changes in Acetylcholinesterase activity levels (µmoles of ACh hydrolyzed/mg protein/h) In selected regions of brain from Control and Experimental groups of rats treated on 30thand 60thDays of experimentation.





DISCUSSION

The results on the Cholinergic system clearly demonstrated that showed that in general, RGSE treatment to AD-induced rats restored whatever changes that were caused by AD-induction in both Acetylcholine (ACh) content and the Acetylcholinesterase (AChE) activity levels in all selected brain regions to almost control levels, thus demonstrating the reversal effect of RGSE. However, it was interesting to note that the percent of recovery was more pronounced on 60th Day when compared to 30th day, with a similar trend. It is a well-established fact that during the process of neurotransmission, in a cholinergic neuron, Acetyl Choline (ACh) is released at the synaptic junction from the presynaptic nerve terminal where it binds to the receptors on the post-synaptic membrane, relaying the signal from the nerve(5 & 18).These Cholinergic neurons, particularly innervating the Cortex and Hippocampus in the forebrain are closely associated with cognitive function and memory. Any kind of damage or degeneration of neuronal cells in theses brain regions due to trauma or infections will be reflected in the form of dementia (6 & 14).Further, in this connection, it is appropriate to state that Acetylcholine (ACh), the first neurotransmitter in the brain essential for short-term memory, when disturbed either anatomically or physiologically, eventually causes neurological diseases including Alzheimer's Disease (7).Moreover, the nucleus basalis and entorhinal cortex are the first areas of the brain where general neuronal loss occurs in Alzheimer's patients. Up to 90% of cholinergic neurons die as the disease advances which is reflected in proportional deterioration of learning and memory abilities(8).Observations in the present study might be justified with the previous reports where the Cholinergic markers in the Hippocampus and Cerebral Cortex were changed during the gradual progression AD (9,15) which were linked to its pathology and degree of cognitive impairment. This has piqued interest in the function of the basal forebrain cholinergic system. The present study was the first of its kind to examine the neuropathological markers of senile dementia, including memory loss, neuronal degeneration and plaque accumulation. Further, according to (10), ageing is a progressive process characterized by accumulation of oxidative damage, during which the brain experiences morphological and functional changes. It has been proposed that one of the causes of the degeneration of cholinergic neurons is lack of the neurotransmitter ACh (11 & 19). At this juncture, the present findings that RGSE exerted reversible effects on the AD-indeced negative by virtue of its anti-cholinergic properties finds appropriate place. Several reports from our research lab on the Cholinergic system proved that the natural compounds from different plants viz. *Lysomniainnermis* [11] ; Phyllanthus (12] ; Sea weed, Squaleen [19] exerted Anti-Alzheimer's properties. It was further supported by similar results wherein mice, scopolamine-induced dementia caused inhibitory effect on Acetylcholinesterase activity in a dose-dependent manner (12 & 13).The positive and beneficial effect of RGSE may be attributed to the presence of several bioactive compounds as given in the below table.

Table 1: Isolation of bioactive compounds

Phytochemical Constituent	Ethanolic extract of Grape seed
Flavonoid	+++
Anthocyanins	++
Alkaloids	++
Phenols	++
Tannins	+++
Terpenoids	+
Saponins	++
Glycosides	++
Steroids	++

It has already been reported that [3], all the aboe mentioned Phytochemicals (Flavonoids, Iisoflavonoids, Anthocyanidins, Phytoestrogens, Terpenoids, Carotenoids, Limonoids, Phytosterols, Glucosinolates Alkaloids, Glycosides), which are the organic chemical elements that exist naturally in Red grape (*Vitis vinifera*.L) Seed Extract, not only confers protection to animals including human beings but also play a key role in defending the plants against environmental dangers including stress, drought, pathogenic attack, etc. Further, it also constitutes an important source of alternative medical system to treat a number of diseases and disorders in general and AD in particular which was not possible with allopathic medicines.

CONCLUSION

Improvement in several cognitive deficits caused by AD-induction by administration of RGSE provides strong clue that RGSE has bioactive phytochemicals which act as potential target drugs.Based on the results obtained in the present investigation which were substantiated by several supporting research findings, it was finally inferred that RGSE has neuroprotective role against D-Galactose induced Alzheimer's Disease.

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