

Red Grape Seed Extract (RGSE) declines Neuronal and Oxidative Damage in the Brain Regions of Alzheimer's Induced Wistar Rats.

M.Thulasi¹, K. Janardhana², C. Aruna³, M. Muniya Naik⁴, A. Govardhan Naik⁵, V. Uday Kiran

¹Department of Zoology, SVA Government College for men, Srikalahasti, Tirupati, Andhra Pradesh, INDIA.

²Department of Zoology, Government Degree College, Rajampeta, Andhra Pradesh, INDIA.

³Department of Botany, Dr.YSR Government Degree College, Vedurukuppam, Chittoor, Andhra Pradesh, INDIA.

⁴Department of Zoology, Govt. Degree College, Rayachoty, Andhra Pradesh, INDIA.

⁵Department of Zoology, SVU College of Sciences, Sri Venkateswara University, Tirupati, Andhra Pradesh, INDIA.

Submission Date: 17-09-2023; Revision Date: 13-10-2023; Accepted Date: 23-11-2023.

ABSTRACT

Objectives: This research explores Red Grape Seed Extract (RGSE) declines Neuronal and Oxidative Damage in the brain regions of Alzheimer's Induced Wistar Rats. **Materials and Methods:** A cohort of three-month-old Wistar rats were divided into two groups, receiving either a standard diet (control diet) or a diet supplemented with 2% RGSE over a 60-day period. RGSE, containing 592.5 mg/g dry weight of Total Phenolic Content (TPC), consisted of Gallic acid (49 mg/g), Catechin (41 mg/g), Epicatechin (66 mg/g), and Proanthocyanidins (436.6 mg catechin equivalents/g). **Results:** Long-term RGSE diet feeding proved well-tolerated, exhibiting no fatalities or behavioral abnormalities. Furthermore, no irregularities in food consumption or body weight were observed. The levels of Amyloid-beta (A β) in the brains of Wistar rats subjected to RGSE were notably lower compared to Alzheimer 's-induced Wistar rats on the control diet. Additionally, RGSE administration led to a reduction in amyloid plaques and microgliosis in the brains of Alzheimer's-induced Wistar rats. **Conclusion:** The polyphenol component of RGSE demonstrated a substantial decrease in brain A β load and microglia activation. Ultimately, polyphenol-rich RGSE exhibited the potential to inhibit A β deposition and alleviate neuronal and oxidative damage in the Wistar rat model, suggesting its promise in delaying the progression of Alzheimer's disease.

Keywords: Alzheimer's Disease, Red Grape Seed Extract, Neuronal and oxidative damage.

Correspondence:

Dr. V. Uday Kiran

Department of Zoology,
Loyola Degree College
(YSRR) Pulivendula,
Andhra Pradesh, INDIA.

Email:
vempati.uday6@gmail.
com

INTRODUCTION

Alzheimer's disease (AD) is the most prevalent type of senile dementia that develops later in life, and it is a leading cause of impairment and death among the elderly (WHO, 2003). With the world's population ageing, the number of people affected by Alzheimer's disease is expected to quadruple every 20 years, from 26.6 million presently to 106.8 million by 2050.^[1]

Alzheimer's disease (AD) is marked neuropathologically by deposits of Amyloid-beta peptides (A β), Neurofibrillary Tangles (NFTs), reactive microgliosis and astrogliosis, cerebral amyloid angiopathy, and neuronal loss, which culminates in the gradual decline of cognition and memory. According to the Amyloid hypothesis, the buildup of A β in the brain is the primary factor driving AD pathogenesis.^[2]

Disease begins at the molecular level and progresses to the cellular and tissue levels. When a disease starts to alter tissues, it is crucial to monitor it at the appropriate time.^[3] Using a microscope, histopathology is the scientific diagnosis and study of diseases at the cellular and tissue levels. Under a microscope, histopathology allows us to scientifically examine the alterations in the impacted tissues. Even though this is an old method, it

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DOI: 10.5530/ajbls.2023.12.72

is still used in medical sciences.^[6] For in-depth research on the majority of human and animal diseases. This field of scientific inquiry held a significant place in contemporary methods for accurately diagnosing disease.

The microarchitecture of tissue is highlighted in histopathology. The tissues are being stained with a variety of stain types for this reason. Even though this procedure takes a lot of time, certain advancements in protocol have been accomplished in the recent period. The previous traditional disease diagnostic processes were improved by the new breakthroughs in the fresh technology of today, allowing such practice to proceed quickly.^[5] Automated machines take the role of the manual protocol. The histological picture of life sciences is now processed in medical sciences in the same way as engineer images.^[6] The most widely accepted, effective, and trustworthy way to identify cancer and other diseases is through the use of medical image processing technologies, which have greatly improved as a result of information technology advancements.

Significance

- Histopathology is very useful in making a proper and accurate diagnosis and determining the severity and progression of a Disease.
- Understanding the normal structure and function of different tissues, essential for interpreting the changes that occur during Disease.
- Histopathology enables professionals to look for changes in cells that explain the actual cause of the patient's illness.
- Pathologists are able to reach a diagnosis by examining a small piece of tissue from various organs.
- This discipline is absolutely vital to understanding and detecting Diseases, which ultimately broadens and progresses treatment options in the majority of instances.

In view of the significance of the Histopathological observations in the assessment of the severity of Alzheimer's Disease, in my present study emphasis has been laid on the histological aspects of two selected brain regions related to learning and other cognitive functions in Rat model subjected to AD and I selected Red Grape Seed Extract as the test substance.

The most important characteristics of Alzheimer's disease are visible under a microscope: a cerebral cortex dotted with Senile Plaques (SPs) and Neurofibrillary Tangles (NFTs). Consequently, AD can be defined as the

dementia linked to these Histopathological anomalies. The limbic brain's amygdala and hippocampal regions, as well as certain cortical and subcortical regions, are typically home to the extracellular lesions known as neurotic plaques. The AD brain is characterised by various neuropathological changes in addition to these proteinaceous aggregates, such as atrophy, loss of synapses, selective depletion of neurotransmitter systems (e.g., acetylcholine), and in a small percentage of cases, Lewy bodies.^[9]

MATERIALS AND METHODS

Isolation of tissues

Six weeks after induction of Alzheimer's Disease the rats were treated with Red Grape Seed Extract for a period of 60 days. The rats were kept fasting for 12 hr before sacrificing them on selected days of experimentation i.e. 30th and 60th days. For all biochemical estimations, the above-mentioned four groups of rats were sacrificed by 'Cervical dislocation, the brain was instantly isolated and mounted on a plate of chilled glass. Different selected regions of the Brain viz. Cerebral cortex (CC), Hippocampus (HC), Cerebellum (CB) and Pons Medulla (PM) were isolated by ensuing standard anatomical marks,^[7] frozen in liquid nitrogen and preserved at -80°C for further usage. The tissues were thawed at the time of biochemical analysis and used. The results obtained were analysed statistically.

Quantitative Analysis of Phytochemicals

Total Phenolics and Flavonoids Content

In the present study, the results have demonstrated that the Total Phenolic compounds in Red Grape Seed extract was 364 mg Gallic Acid Equivalents (GAE)/g of dry Seed extract. Similarly, the results also revealed that the Total flavonoid content was 12.86 mg Quercetin Extract (QE)/g of dry seed extract.

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Table showing Total Phenolic and Total Flavonoid compounds in Grape Seed Extracts

Sample	Total phenolic compounds (mg/g) ^a	Total flavonoids (mg/g) ^b
Red Grape Seed (Ethanollic extract)	592.5±17.04	12.86±0.874

a: mg Gallic Acid Equivalents (GAE)/g of dry seed extract;

b: mg Quercetin Extract (QE)/g of dry seed extract. Each value is expressed as the mean±SD (n=3).

Seed extract which was relatively lower than as reported by,^[12] whereas in his studies the Total Polyphenols content was 506.25 mg Gallic Acid Equivalents (GAE)/100 g. However, the Total Flavonoids content in the present study of Red Grape Seed Extract was 12.86 mg Quercetin Extract (QE)/g of dry seed extract which was relatively lower in comparison to the research reports of^[10] where the Total Flavonoids in ethanolic extract was: 13.75 mg Quercetin Extract (QE)/g grape seed.

Histopathology

Histological examinations of various regions of the brain were followed according to Humason (1972).^[11]

Light Microscopy

On selected days of experimentation viz. 30th and 60th days, the rats were sacrificed by cervical dislocation and the selected brain regions such as Cerebral Cortex, Hippocampus, Cerebellum and Pons Medulla were isolated. Isolated tissue of control and experimental rats were gently rinsed with physiological saline (0.9% NaCl) to remove blood and debris adhering to the tissues. They were fixed in 5% formalin for 24 hr. The fixative was removed by washing through running tap water overnight. After dehydrating through a graded series of alcohols, the tissues were cleared in methyl benzoate, embedded in paraffin wax. Sections were cut at 6 μ thickness and stained with Haematoxylin^[9] and counter-stained with eosin (dissolved in 95% alcohol). After dehydration and clearing, sections were mounted with DPX. The stained sections were observed under a microscope and the histological changes were recorded with the help of a pathologist.

Haematoxylin and Eosin staining

The most commonly used staining system is called "H and E" (Haematoxylin and Eosin) which contains two dyes- 'Haematoxylin' and 'Eosin'. Haematoxylin, considered as a basic dye (general formula is: dye+Cl-) is actually a dye called 'Hematein' (obtained from the log-wood tree) which is used in combination with Aluminium ions (Al³⁺). It is used to stain acidic (or basophilic) structures a purplish-blue. Haematoxylin is not strictly a basic dye, but it is used with a 'mordant' that makes this stain act as a basic dye. The mordant (aluminium salts) binds to the tissue and then haematoxylin binds to the mordant, forming a tissue-mordant-haematoxylin linkage. Eosin is an acidic dye, negatively charged (the general formula for acidic dyes is: Na⁺ dye). It stains basic (or acidophilic) structures red or pink. This is also sometimes termed as 'Eosinophilic'.

RESULTS

Even though all biochemical estimations have been carried out in four selected regions of the brain, Histopathological studies were done only on two regions viz., Cerebral Cortex and Hippocampus because they are associated with higher cognitive functions.

Ethical Committee Resolution No: 09/(i)/a/CPCSEA/IAEC/ SVU/ KY Dt.08-07-2012.

Histopathological changes in Cerebral cortex

In the current study, Histopathological examinations of control and all the 3 experimental groups of rat brain on 30th and 60th day brain regions were performed. On 30th day, both the control (Group I) and RGSE alone treated group (Group II) showed the presence of highly active nerve cells with big prominent nuclei in abundance. On the contrary, observations on the histopathological components of the cerebral cortex in AD-model group rats revealed the formation of β -Amyloid plaques, mostly from the 30th day onwards, followed by the formation of neurofibrillary tangles, which were visible on the 60th day (Plate 1 and Plate 2). Furthermore, beta-amyloid proteins aggregated around the amyloid core and neurofibrillary tangles developed in the cytoplasm as long pink threads. However, neurons with hyperchromatin were seen in the protective group (AD-I+RGSE). Apart from these, from the 30th day onwards, several partially degraded neurons were also seen and by the 60th day, there had been significant recovery, in the architecture of neurons as evidenced by the presence of distinct and conspicuous nuclei.

Histopathological changes in Hippocampus

Similar to the Cerebral Cortex, Healthy and active neuronal cells with large circular vesicular nuclei, prominent nucleoli and amphophilic cytoplasm are present in the hippocampal regions of control and RGSE-treated rats. The Hippocampus region was also similarly affected and damaged in the AD-model group, exhibiting typical pathological characteristics such as Plaques and Tangles (Plate 3 and Plate 4). Chronic administration of D-Galactose for 30 days resulted in the formation of amyloid plaques surrounded by amyloid core, followed by hyperphosphorylation of tau proteins, which led to tau protein aggregation within the neurons and subsequently, the formation of Neurofibrillary Tangles on 60th day. Simultaneous treatment of AD-induced rats with RGSE reversed all of these pathological alterations and restored them to almost control conditions, thus signifying that Red

Plate 1: Sections of Cerebral Cortex region, stained with Hematoxylin and Eosin-30th day of experimentation (40 X).

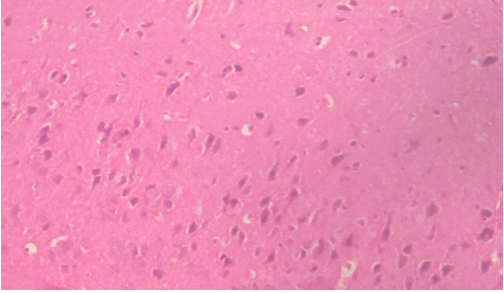
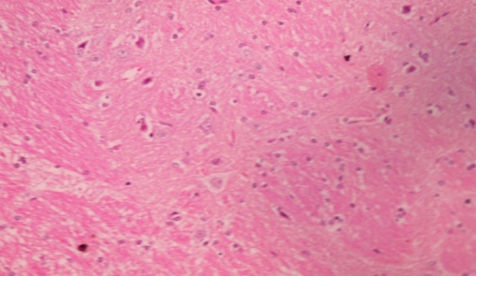
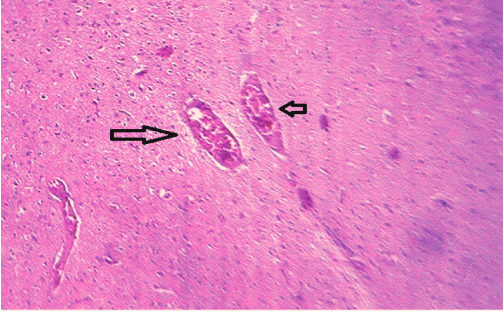
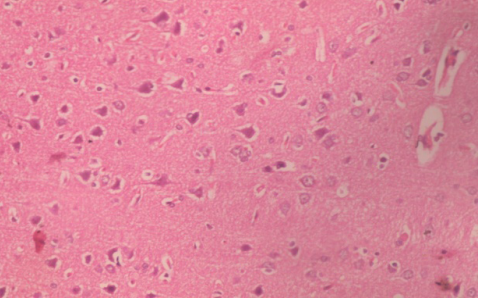
Group-I	Group-II
	
Control-30th day	RGSE Treated-30th day
Group-III	Group-IV
	
AD-I-30th day	AD-I and RGSE Treated-30th day

Plate 2: Sections of Cerebral Cortex region stained with Hematoxylin and Eosin-60th day of experimentation (40 X).

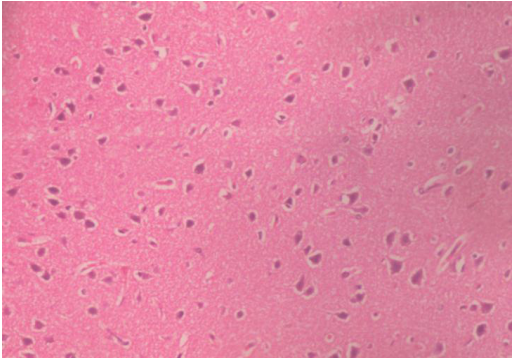
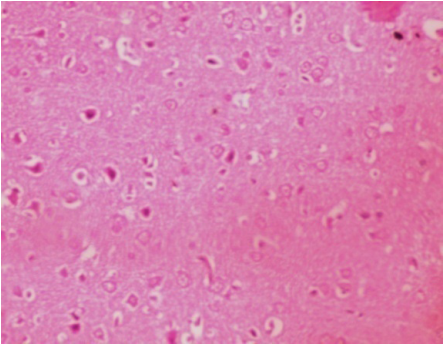
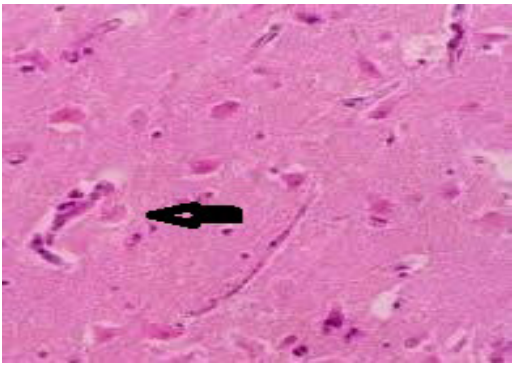
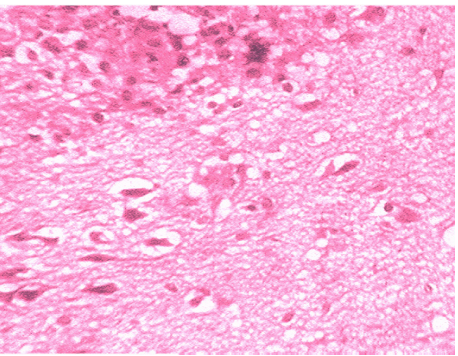
Group - I	Group - II
	
Control-60th day	RGSE Treated-60th day
Group-III	Group-IV
	
AD-I-60th day	AD-I and RGSE Treated-60th day

Plate 3: Sections of Hippocampus region stained with Hematoxylin and Eosin-30th day of experimentation (40 X).

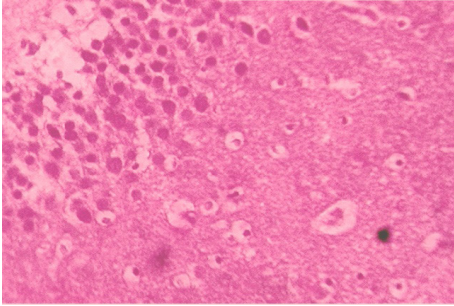
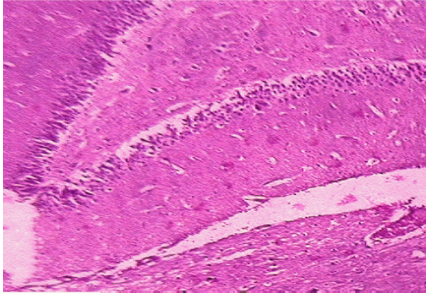
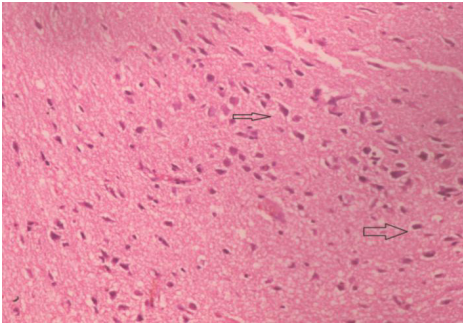
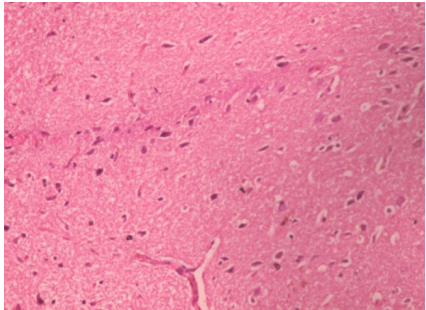
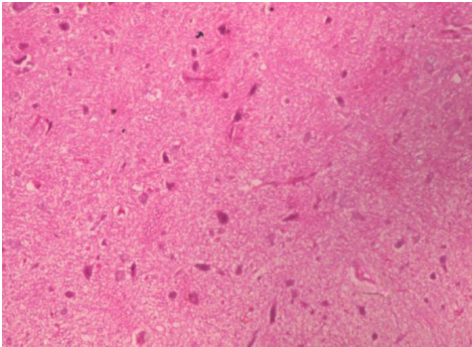
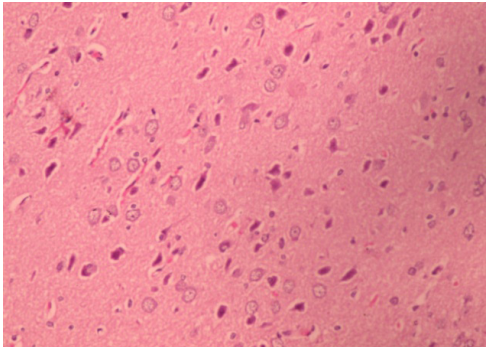
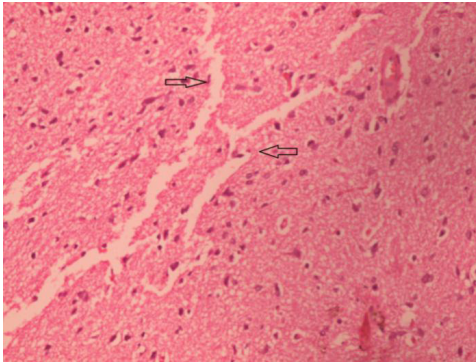
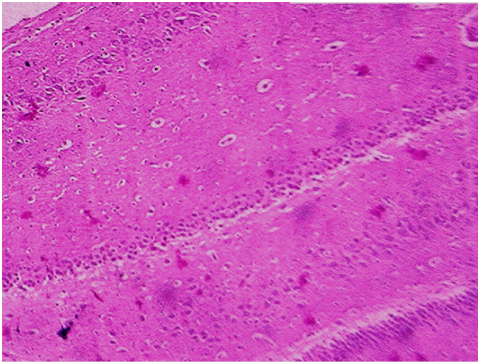
Group-I	Group-II
	
Control-30th day	RGSE Treated-30th day
Group-III	Group-IV
	
AD-I-30th day	AD-I and RGSE Treated-30th day

Plate 4: Sections of Hippocampus region stained with Hematoxylin and Eosin-60th day of experimentation (40 X).

Group-I	Group-II
	
Control-60th day	RGSE Treated-60th day
Group-III	Group-IV
	
AD-I-60th day	AD-I and RGSE Treated-60th day

Grape Seed Extract is involved in protecting nerve cells from damage incurred due to the induction of Alzheimer's Disease.

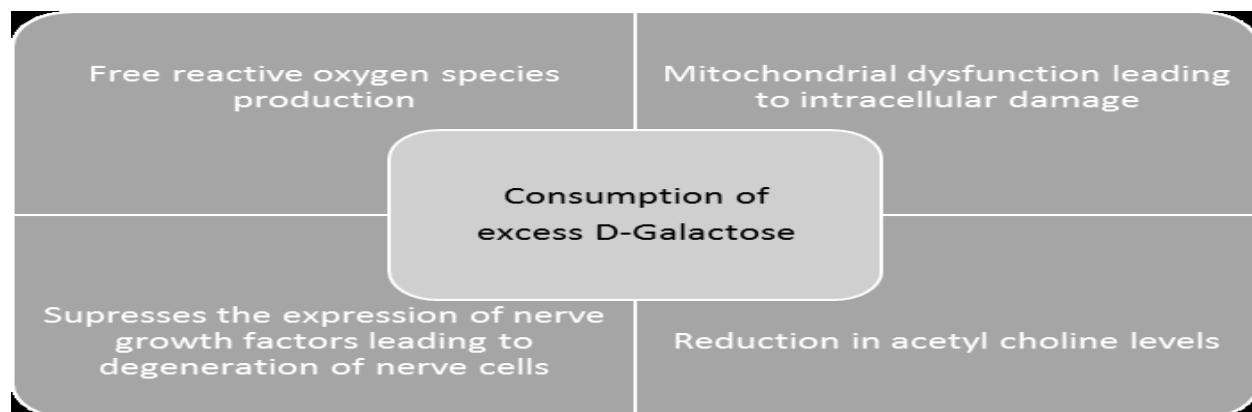
DISCUSSION

The study investigates cytoarchitectural alterations in the cerebral cortex and hippocampus of AD-induced rats given Red Grape Seed Extract (RGSE). Results reveal that RGSE treatment corrected these alterations, demonstrating its positive impact on Alzheimer's disease, Neuronal damage and Oxidative damage.

According to the study, prolonged treatment of D-galactose for 30 days caused the development of amyloid plaques, tau protein hyperphosphorylation, and neurofibrillary tangles. These results are consistent with the theory that amyloid plaques build up and lodge in the brain, resulting in Alzheimer's disease^[7,8] These lesions are extracellular deposits of an Amyloid Protein, each plaque measuring approximately 20 to 200 μm . Plaques are found in the Hippocampus, Amygdala and Neocortex and although there is usually relative sparing of primary motor and sensory cortices (this also applies to Neurofibrillary Tangles). The amyloid core is made up of less frequent elements such complement cascade components, pro-inflammatory cytokines, and apolipoproteins. It also contains aberrant proteins like A, which are produced when APP is processed. The results of the study showed that Red Grape Seed Extract (RGSE) treatment of AD-induced rat brain areas lowered the production of β -Amyloid plaques, decreased the number of vacuoles, and deactivated astrocytes. In a time-dependent way, oral treatment repaired cytoarchitectural damage. This backs up other studies that found D-Gal-treated rats had significant neuroanatomical changes, a common visible marker

of AD.^[19,20] finally responsible of AD. Further, a more recent study has demonstrated that continuous subcutaneous injection of D-gal in rats induced an increase in cell karyopyknosis, apoptosis and caspase-3 protein levels in hippocampal neurons involved in learning and memory.^[4] D-galactosioned rats have less neurons, reduced migration of neural progenitor cells, and more mortality of freshly generated neurons. This D-galactose-induced ageing model promotes ageing by reducing learning, memory, and neuronal damage.^[3,14] D-Galactose induces aging-inducible oxidative stress *in vivo*, which resembles the natural aging process in rats.^[13] D-Galactose is metabolized to galactose-1-phosphate at a normal concentration by D-galactokinase or galactose-1-phosphate uridyl-transferase, but at increased concentration, D-galactose is converted to galactitol, which accumulates in cells and then induces osmotic stress and generates 'ReactiveOxygenSpecies' (ROS) which induces mitochondrial dysfunction and oxidative stress, the major cause of intracellular damage.^[14] Thus, D-galactose could cause brain aging.^[2]

Apart from all these, D-Galactose also reacts with the free amines of amino acids in peptides and proteins forming 'Advanced Glycation End-products (AGE)'.^[17] D-Galactose increases replicative senescence markers p16 expression and telomere shortening but reduces double cortin (DCX) expression.^[18,20] Therefore, D-galactose continuously stimulates low-grade inflammation, which is associated with the acceleration of aging. High dosage of D-Galactose also suppresses the expression of nerve growth factors and its associated protein resulting in accelerated degeneration of astrocytes which caused cognitive deficits by reduction of Acetylcholine with simultaneous activation of AChE^[13,16] levels in various brain regions as shown in the figure below:



SUMMARY AND CONCLUSION

From the data obtained on the phytochemical constituents of RGSE presented in my research study, it was evident that RGSE contained several chemical substances such as: Flavonoids, Anthocyanins, Alkaloids, Phenols, Tannins, Terpenoids, Glycosides and Steroids, which have been shown to have Antioxidative properties, scavenge Reactive Oxygen and Nitrogen Species in vitro as well as Anti-ageing properties etc. For example, Polyphenols, the major constituent of RGSE plays major role as a chelating component in APP formation.

RGSE preventing Neuronal damage and LDL (bad) cholesterol from being oxidised and minimising damage to heart tissue under stress, RGSE may help lower the chance of developing heart disease. Thus, the memory boosting as well as Anti-Alzheimer's properties of various bioactive phytochemicals present in RGSE were substantiated by the above research findings of histopathology of rat brain.

FUNDING

There is currently no funding for this effort; all research expenses are entirely covered by me.

FUTURE SCOPE

Deeper comprehension of pathogenic mechanisms and novel treatment targets are provided by the unclear role of certain channels in AD pathogenesis. The therapeutic benefits of red grape seed extract require more investigation and testing.

ACKNOWLEDGEMENT

I am grateful that the research supervisor supported me with my research work. I would like to thank all of my co-researchers for their constant support. I would also like to thank the Head, Department of Zoology, Sri Venkateswara University, Tirupati, for providing computer lab space for this research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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Cite this article: Thulasi M, Janardhana C, Aruna M, Naik MA, Naik G, Kiran VU. Red Grape Seed Extract (RGSE) declines Neuronal and Oxidative Damage in the Brain Regions of Alzheimer's Induced Wistar Rats.. *Asian J Biol Life Sci.* 2023;12(3):551-7.