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REVITALIZATION OF ANTIOXIDANT ENZYMES BY RED GRAPE SEED EXTRACT (RGSE) DUE TO D-GALACTOSE INDUCED CHANGES ON ALZHEIMER'S DISEASE (AD) IN MALE ALBINO RATS

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ABSRACT

Neurodegenerative and terminal disease was first discovered by German psychiatrist and neuropathologist Alois Alzheimer in 1906 and was named after him. Generally, it is diagnosed in people over 65 years of age. In this study, the protective role of grape seed extract against D-Galactose dose-induced neural toxicity has been evaluated. The present study was carried out on the male albino rat, Age matched rats were be divided into 4 groups of six in each group and treated as follows: Group-I.Control (C) rats received 0.9% saline. Group-II. Rats treated with intraperitonealy (IP) administered with D-Gal (120 mg/kg body weight) up to end of the experiment (1^{st} day to 90^{th} day).Group-III. Rats treated orally administered with Red grape seed (GSE) ethanol extract (100mg/kg body weight) for 60 days. Group-IV. Rats were treated with intraperitonealy injected with D-Gal (120 mg/kg body weight) once daily for first 30 days. From 31^{st}day onwards rats were administered with Red grape seed ethanol extract (100mg/kg body weight) for 30 days. The animals were sacrificed on 60^{th}day of experimentation by cervical dislocation. Isolated the Brain tissue and measured the activity levels of Superoxidedismutase (SOD), Catalase (CAT), and Lipid Peroxidation (LPO). In the present study Data showed that AD induced rats treated with D-Gal showed maximum SOD levels in the brain tissue. Mean while SOD activity were significantly elevated in AD induced rats with RGSE (AD+RGSE). CAT activity were found in AD induced rat brain and considerable inhibitions was noticed, in AD induce rats treated with D-Gal. Catalase levels were significantly decreased in GSE. LPO levels were decreased in the AD induced rats treated with D-Gal, LPO levels were significantly increased in GSE alone treated and AD induced rats treated with RGSE. This study provides a new approach for the dealing of AD or, may at least improve the quality of life of patients with AD.

KEYWORDS: Red Grape Seed Extract (RGSE), D-Gal, SOD, CAT, and Lipid peroxidation.

INTRODUCTION

Alzheimer's disease (AD) is a chronic neurodegenerative disease with well-defined pathophysiological mechanisms, mostly affecting medial temporal lobe and associative neocortical structures. Leading to increase severe disability such as memory loss (amnesia), minimal to no communication (aphasia), the inability to perform activities of daily living (ADL) (apraxia), the impairment of the sensory input (development of agnosias**)**. In briefly, AD is a multifactorial neurodegenerative disorder that affects cognition (memory, thinking, and language abilities), quality of life and self-sufficiency. (Vanessa J De Paula *et al*., 2012). In briefly, AD is a multifactorial neurodegenerative disorder that affects cognition (memory, thinking, and language abilities), quality of life and self-sufficiency. It is characterised by loss of neurons and synapses in the cerebral cortex and certain subcortical regions. This loss results in gross atrophy of the affected regions, including degeneration in the temporal lobe and parietal lobe, and parts of the frontal cortex and cingulate gyrus. Many Researchers now believe that a combination of this lifestyle, environmental and genetic risk factors trigger an abnormal biological process in the brain that results in Alzheimer-type dementia.

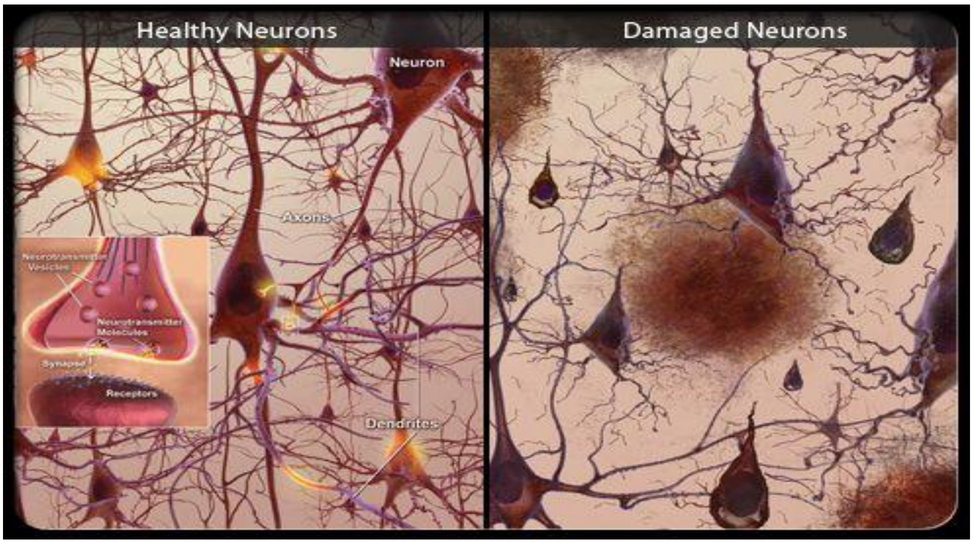

Fig.1: Before AD After AD

Morphologically, the disease is characterized by brain atrophy and by en- larged cerebral ventricles. From a biochemical point of view, the most clear-cut and consistent finding is a deficit of the cholinergic system, decreased levels of choline acetyltransferase, and other cholinergic markers. Histologically, AD is characterized by extra- cellular deposits, called cerebral plaques, composed of a dense proteinaceous core containing the Abpep- tide surrounded by dead and damaged neurons. Morphologically, the disease is characterized by brain atrophy and by en- larged cerebral ventricles. From a biochemical point of view, the most clear-cut and consistent finding is a deficit of the cholinergic system, decreased levels of choline acetyltransferase, and other cholinergic markers. Histologically, AD is characterized by extra- cellular deposits, called cerebral plaques, composed of a dense proteinaceous core containing the Abpep- tide surrounded by dead and damaged neurons. Morphologically, the disease is characterized by brain atrophy and by en- larged cerebral ventricles. From a biochemical point of view, the most clear-cut and consistent finding is a deficit of the cholinergic system, decreased levels of choline acetyltransferase, and other cholinergic markers. Histologically, AD is characterized by extra- cellular deposits, called cerebral plaques, composed of a dense proteinaceous core containing the Abpep- tide surrounded by dead and damaged neurons. Morphologically, the disease is characterized by brain atrophy and by en- larged cerebral ventricles. From a biochemical point of view, the most clear-cut and consistent finding is a deficit of the cholinergic system, decreased levels of choline acetyltransferase, and other cholinergic markers. Histologically, AD is characterized by extra- cellular deposits, called cerebral plaques, composed of a dense proteinaceous core containing the Abpep- tide surrounded by dead and damaged neurons.

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Epidemiological studies have shown that consumption of diets rich in anti-inflammatory agents, such as those found in fruits and vegetables, or anti-inflammation drugs, may lower the risk of developing age-related neurodegenerative diseases such as Parkinson's disease and AD (Lau et al. 2005; Barberger-Gateau et al. 2007; McGeer and McGeer 2.

Grape seed extract is a derivative of grape seeds (usually wine grapes) and is mostly made up of proanthocyanidins/procyanidins vitamin-E, flavonoids, and polyphenols .Epidemiological studies have shown that consumption of diets rich in anti-inflammatory agents, such as those found in fruits and vegetables, or anti-inflammation drugs, may lower the risk of developing age-related neurodegenerative diseases such as Parkinson's disease and AD (Lau et al. 2005; Barberger-Gateau *et al*,. 2007). Polyphenols from Red grape seeds extract (RGSE) have been suggested to be able to inhibit Aβ aggregation, reduce Aβ production, protect against Aβ neurotoxicity and attenuate oxidative stress in vitro (Bastianetto et al. 2000; Jang and Surh 2003; Ono et al. 2003, Mancuso et al. 2007; Riviere *et al*., 2007). GSE has been widely used as food additives in order to benefit health and chronic illness due to its anti-oxidation properties. The most interesting constituents of grape seeds (Fig.2) are the polyphenols (catechins). These tannin compounds, also called Procyanidins, leucoanthocyanins, pycnogenols, or oligomeric proanthocyanidins (OPC), are powerful antioxidants. Commercial extracts are generally standardized for OPC content.

Fig. 2: The Diagram exemplifies Red Grape seeds (Vitis vinifera L.).

Grape seed Proanthocyanidins refer to procyanidin mixtures extracted from grape (*Vitisvinifera*) seeds. Procyanidins are derivatives (Fig.3) of the flavan-3-o1 class of flavonoids. This class includes (+)-catechin, commonly referred to as catechin, and (-)-epicatechin, commonly referred epicatechin. Procyanidins are dimers and oligomers of catechin and epicatechin and their gallic acid esters. Procyanidins are widely distributed in the plant kingdom and, in addition to being found in grape seeds, are found in cocoa and chocolate, apples, peanuts, almonds, cranberries, blueberries and in the bark of pines, among other plant sources.

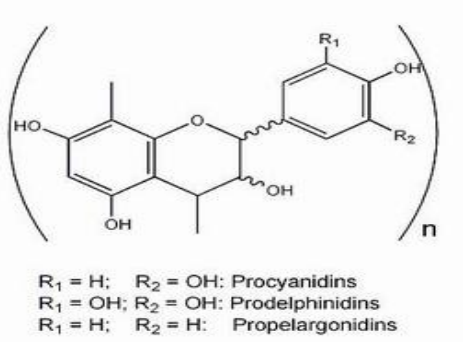


Fig. 3: Grape Seed Proanthocyanidins derivatives.

Grapeseed Proanthocyanidins are mainly comprised of dimers, trimers and tetramers of catechinand epicatechin and their Gallates. They also contain smaller amounts of pentamers, hexamers and heptamers of these flavan-3-o1s and their Gallates. The procyanidin dimersand oligomers are also known as oligomericprocyanidins (OPCs) and procyanidolic oligomers or PCOs. Grape seed proanthocyanidins comprise approximately 60 to 70% of the polyphenol content of grapes.

Alzheimer's disease is progressed by the formation of intra cellular deposition of Amyloid plaques and Hyperphosporylation of Tau protein forms Neuro Fibrillary Tangles outside the Neuron. This may result in malfunctions in biochemical communication between neurons and later death of Neurons in AD patients (Marvin M. Chun and Marcia K. Johnson 2011). Whether these plaques and tangles are the primary cause for the onset of Alzheimer's is still uncertain. Some people develop plaques in brain tissue as they age without developing Alzheimer's, and it's still unknown whether plaques cause the disease or whether they're a by-product of the Alzheimer's disease process.

The present study revealed that Grape Seed Extract (GSE) has many possible mechanisms for neuroprotection. It is an effective free radical scavenger that reduces lipid per oxidation. Grape seed extract provides superior antioxidant efficacy as compared to vitamin C and E at equal doses by weight. It also has anti-inflammatory action in association with its oxygen free radical scavenging, anti-lipid peroxoidation activity and reduces production of pro-inflammatory cytokines (McDonald et al., 1991).

MATERIALS AND METHODS

The present study was focused on the evaluation of the Antioxidant enzymes in Grape Seed extract which protect against on AD induced rats brain tissue treated with D-Gal.

Animals

Healthy young adult male albino rats of Wistar strain (*Rattus norvegicus*) (3months old 180±20g) were used in this study. The animals were maintained in a clean rodent room in standard conditions (28± 2^0C) and the animal room was well ventilated with a 12h light/ dark cycle, throughout the experimental period. The animals were housed in large spacious cages and were given standard pellet diet and water ad *libitum* throughout the experiment. Experimental animals were handled according to the regulations of the University and Institutional animal ethic committee.(Resolution No. 34/20122013/(i)/a/CPCSEA/IAEC/ SVU/KY dt.01.07.2012).

Chemicals

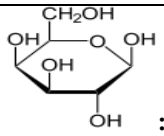
All chemicals used in the present study were of Analar Grade (AR) and were obtained from Sigma (St. Louis, MO, USA), Fisher (Pittsburg, PA, USA), Merck (Mumbai, India), Ranbaxy (New Delhi, India), Qualigens (Mumbai, India), Loba Chemicals (Bombay, India). In the present investigation, for biochemical assays, the following equipments have been used.

1. Barnstead Thermoline water purification plant for Nano pure water.
2. Hahnvapor Rotary Evaporator HS-2005V for extract preparation.
3. Kubota KR 2000T centrifuge for centrifugation of tissue homogenates.
4. Hitachi UV-2800 spectrophotometer for measuring Optical Density.

Physical Properties of D-Galactose

D-Galactose (D-Gal) was used to induce AD in rat as per (Zhang *et al.*, 2006; Fang and Liu., 2007).

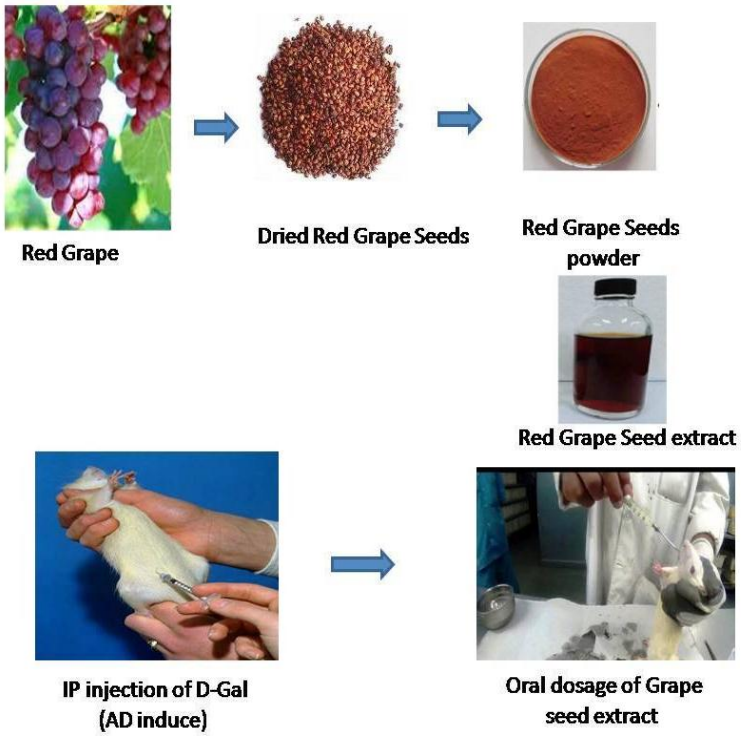
Table 1: D- Gal Structure and their Properties.

Molecular formula and Structure	 : $C_6H_{12}O_6$
IUPAC name	: (3R,4S,5R,6R)-6-(Hydroxymethyl)oxane- 2,3,4,5-tetrol
Synonyms	: D(+)-Galactose, Galactose, Brain sugar, d-galactose, D-Galactopyranose.
Molecular weight	: 180.156 g mol^{-1}
Melting point	: 165-168°C
Boiling point	: 527.1 °C at 760 mmHg
Solubility in water	: 683.0 g/L
Density	: 1.581 g/cm^3
PubChem ID	: 439357
Appearance	: White powder
Storage	: Store in cool and dry area and kept sealed away from direct light.

Preparation of the Grape Seed Extract (GSE)

Grape, as large clusters with red berries, was bought from a local fruit market in Tirupati, Pulivendula and Bangalore (Devanahalli) as *vitisvinifera (Linn)*. Red 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,2007). The resulting ethanolic crude extract was air dried and used in the present study.

Schematic Representation of Experimental Procedure



Isolation of Tissues

For biochemical estimations, all the above mentioned four groups of rats were sacrificed on 60day of experimentation by cervical dislocation. The isolated tissue were immediately placed on a chilled glass plate and frozen in liquid nitrogen (180^{o}C) and then stored at -70^{o}C until further use. At the time of biochemical analysis, the tissues were thawed and used. The results obtained were analyzed statistically.

Experimental Design

Group-I (Control)	Control Rat
Group-II (AD)	Rat, intraperitonealy (IP) administered with D-Gal (120 mg/kg body weight) up to end of the experiment (1st day to 90th day). (Zhang *et al.*, 2006; Hua*et al.*, 2007)
Group-III (RGSE)	Rat, orally administered with Red grape seed ethanol extract (100mg/kg body weight) for 60 days.
Group-IV (AD+RGSE)	Rat, intraperitonealy injected with D-Gal (120 mg/kg body weight) once daily for first 30 days. From 31st day onwards rats were administered with Red grape seed ethanol extract (100mg/kg body weight) for 30 days.

In the present study the experimental duration selected was 60days. D-Gal was given for first 30 days period to observe AD symptoms with the assessment of cognitive skills in rats (AD group). Further AD induced rats were again treated with D-Gal as well as Red grape seed ethanol extract simultaneously.

Biochemical Investigations

Superoxide Dismutase Activity

Superoxide dismutase activity was determined according to the method of Misra and Fridovich (1972) at room temperature. The brain tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at 0^0C in cold centrifuge. The supernatant was separated and used for enzyme assay. 100 µl of tissue extract was added to 880 µl (0.05 M, pH 10.2, containing 0.1 mM EDTA) carbonate buffer; and 20 µl of 30 mM epinephrine (in 0.05% acetic acid) was added to the mixture and measured the optical density values at 480 nm for 4 min on a Hitachi U-2000 Spectrophotometer. Activity expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit

Catalase (CAT – EC: 1.11.1.6)

Catalase activity was measured by a slightly modified version of Aebi (1984) at room temperature. The brain tissue was homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA to give 5% homogenate (w/v). The homogenates were centrifuged at 10,000 rpm for 10 min at 0^0C in cold centrifuge. The resulting supernatant was used as enzyme source. 10 µl of 100% EtOH was added to 100 µl of tissue extract and then placed in an ice bath for 30 min. After 30 min the tubes were kept at room temperature followed by the addition of 10 µl of Triton X-100 RS. In a cuvette containing 200 µl of phosphate buffer and 50 µl of tissue extract was added 250 µl of 0.066 M H_2O_2 (in phosphate buffer) and decreases in optical density measured at 240 nm for 60 s in a UV spectrophotometer. The molar extinction coefficient of 43.6 M cm^{-1} was used to determine CAT activity. One unit of activity is equal to the moles of H_2O_2 degraded / mg protein / min.

iii) Lipid Peroxidation (LPO): MDA (Malondialdehyde) levels were measured by the method described by Ohkawa *et al.*, (1979). The brain tissues (Cerebral Cortex and Hippocampus) were homogenized (5% - w/v) in 50 mM phosphate buffer (pH 7.0) containing 0.1 mM EDTA. The homogenates were centrifuged at 10,000 rpm for 10 minutes at 4^0C in cold centrifuge. The separated supernatant part was used for the estimation. 200µl of the tissue extract was added to 50µl of 8.1% sodium dodecyle sulphate (SDS), vortexed and incubated for 10 minutes at room temperature. 375 µl of 20% acetic acid (pH 3.5) and 375 pl of thiobarbituric acid (0.6%) were added and placed in a boiling water bath for 60 minutes. The samples were allowed to cool at room temperature. 1.25ml of Butanol –Pyridine (15:1) mixtureadded, vortexed and centrifuged at 1000 rpm for 5 minutes. The colored layer (500 µl) was measured at 532 nm using 1, 1, 3, 3-tetraethoxypropane as a standard. The values were expressed in µ moles of Malondialdehyde formed/gram wet weight of the tissue.

Statistical Analysis

Values of the measured parameters were expressed as Mean ± SEM. Repeated Measures of ANOVA was used to test the significance of difference among four different groups followed by Dunnet's Multiple Range Test (DMRT). Statistical analysis was performed by using Statistical Program of Social Sciences (SPSS) for windows (Version 19; SPSS Inc., Chicago, 1L, USA).

RESULTS AND DISCUSSION

In the present study, it was noticed that the Red Grape Seed Extract has significantly affected the antioxidant enzymes viz., Superoxide Dismutase, Catalase and Lipid peroxidation in brain tissue of control and experimental rats.

Superoxide Dismutase (SOD)

In the present Investigation, it was clear that, when compare with control and experimental rats AD induced rats treated with D-Gal showed maximum SOD levels in the brain tissue. When compared to control ones, SOD activity levels were elevated in AD induced rats treated

with GSE and reached to normal control level during the experiment.

With respect to the AD-induced group, SOD activity levels were up regulated in the RGSE and AD + RGSE group rats. (Table-1)

Superoxide dismutase is an enzyme that helps break down potentially harmful oxygen molecules in cells, which might prevent damage to tissues. It is being researched to see if it can help conditions where oxygen molecules are believed to play a role in disease. Superoxide dismutase (SOD) is found in virtually every oxygen-based organism, and its major function is to catalyze the dismutation of $O_2^{\bullet-}$ to H_2O_2. In the present study brain SOD activity was altered with Red Grape extract treatment. According to Khalindhar Basha *et al*.,(2013) reported in the kidney tissue SOD activity was more pronounced in young age rats than old age rats after treated with Red grape extract. *In vitro* studies showed that grape juice has significant antioxidant activity and can inhibit oxidation of low density lipoprotein (LDL) (Castilla *et al.,* 2006; O'Byrne *et al*., 2002). In addition to their antioxidant activity, polyphenols also possess many different biological properties. Normally phenolic compounds act by scavenging free radicals and quenching the lipid peroxidative side chain. It has been proposed that hydroxyl and hydroperoxy radicals initiate hydrogen abstraction from a free phenolic substrate to form phenoxy radicals that can rearrange to quinine methide radical intermediates which is excreted via bile (Rukkumani *et al.*, 2004). Dani *et al*., (2008) reported the SOD activity was elevated in rats when treated with organic grape juice.

Table-1: Changes in Antioxidative enzymes Superoxide Dismutase (superoxide anion reduced/mg protein/min), in brain from control and experimental groups of rat brain.

GROUPS	SUPEROXIDE DISMUTASE
Control	1.9657 ±0.20090
AD	2.6825 ±0.55644
RGSE	1.5615 ±0.21093
AD + RGSE	2.0316 ±0.01532

Values are Mean ± SEM of six observations each from tissues pooled from 6 rats. Values are significantly different from control at $p < 0.01$

ANOVA FOR SUPEROXIDE DISMUTASE						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
CONTROL	Between Groups	0.202	5	0.040	0.00	0.00
	Within Groups	0.000	0			
	Total	0.202	5			
AD	Between Groups	1.548	5	0.310	0.00	0.00
	Within Groups	0.000	0			
	Total	1.548	5			
RGSE	Between Groups	0.222	5	0.044	0.00	0.00
	Within Groups	0.000	0			
	Total	.222	5			
AD+RGSE	Between Groups	0.001	5	0.000	0.00	0.00
	Within Groups	0.000	0			
	Total	0.001	5			

Catalase

In control rats, the maximum CAT activity levels were found in AD induced rat brain and considerable inhibitions was noticed in AD induce rats treated with D-Gal. When compare with AD induced rats, Catalase levels were up regulated in RGSE alone treated as well as in AD induced rats treated with RGSE. When compare with Control rats the CAT activity was moderately inhibition in AD induced rats treated with RGSE. (Table-2).

Catalase (CAT) is an iron–containing enzyme found primarily in the small membrane–enclosed cell components called peroxisomes; it serves to detoxify H_2O_2 and various other molecules. Catalase (CAT) is an iron–containing enzyme found primarily in the small membrane–enclosed cell components called peroxisomes; it serves to detoxify H_2O_2 and various other molecules.CAT eliminates H_2O_2 is by catalyzing a reaction between two H_2O_2 molecules, resulting in the formation of H_2O and O_2. In addition, CAT can promote the interaction of H2O2 with compounds that can serve as hydrogen donors so that the H_2O_2 can be converted to one molecule of H_2O, and the reduced donor becomes oxidized (a process sometimes called the peroxidatic activity of catalase).

The combination of SOD and CAT provide an efficient mechanism for removal of free radicals from the cell (Husain *et al.*, 1996; Bhaskar Reddy, 2002). In *vitro* studies showed that grape juice has significant antioxidant activity and can inhibit oxidation of low density lipoprotein (LDL) (Castilla *et al.*, 2006; O'Byrne *et al*., 2002). In addition to their anyioxidant activity, polyphenols also possess many different biological properties. Rao *et al.,* (1990) reported that the CAT activity was decreased in the tissues of liver, brain and kidney with aging. They also reported mRNA levels in

the tissues of aged rats, which may result in the decreased activity of the enzyme in aged rats. Matlsuo *et al.,* (1992) also reported decreased CAT activity in the liver tissue between, 8, 14 and 32 months aged rats. The rates of mitochondrial superoxide and H_2O_2 generation were found to increase with age in mammals (Sohal *et al.*, 1990; Jhansi Lakshmi, 1998). Age related increase in the hydrogen peroxide concentration in the tissues leads to decrease in CAT activity and cause oxidative stress in the tissues. High reactive oxygen metabolites production especially O_2^- and H_2O_2 during aging process. Evidences suggest that O_2^- itself affect directly the CAT activity (Kono and Fridovich, 1982). It is also been reported that CAT is inactivated by hydroxyl radical (Piegeolet and Corbisier, 1990). The increased rate of reactive oxygen metabolites production frequently elicits, as a response, an increase in the level of antioxidants. Under high rate of free radicals input, the enzyme inactivation prevails and the enzymatic activities are reduced leading to autocatalysis of oxidative damage process (Escobar *et al*., 1996; Ray and Husain, 2002). Furthermore, iron is an essential co-factor in the catalase enzyme. An iron deficiency would not only impair oxygen transport in the body, but also compromise the body's antioxidant capacity by lowering catalase activity in cell (Halliwell and Gutteridge, 1999). If the animals take regularly the Red Grape Seed extract the activity of CAT would be altered.

Table-2: Changes in Antioxidative enzymes Catalase activity (µmoles of H_2O_2 degraded/mg protein/min) in brain from control and experimental groups of rat brain.

GROUPS	CATALASE ACTIVITY
CONTROL	0.8938 ±0.01340
AD	1.1628 ±0.00598
RGSE	0.7278 ±0.02962
AD + RGSE	1.0353 ±0.04996

Values are Mean ± SEM of six observations each from tissues pooled from 6 rats. Values are significantly different from control at $p < 0.01$

ANOVA FOR CATALASE ACTIVITY						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
CONTROL	Between Groups	0.001	5	0.000	0.00	0.00
	Within Groups	0.000	0			
	Total	0.001	5			
AD	Between Groups	0 .000	5	0.000	0.00	0.00
	Within Groups	0.000	0			
	Total	0.000	5			
RGSE	Between Groups	0.004	5	0.001	0.00	0.00
	Within Groups	0.000	0			
	Total	0.004	5			
AD+RGSE	Between Groups	0.012	5	0.002	0.00	0.00
	Within Groups	0.000	0			
	Total	0.012	5			

Lipid Peroxidation

In the present study, the LPO levels were relatively decreased in the AD induced rats treated with D-Gal when compare with control rat brain tissue. When compared with AD induced rats, LPO levels were significantly elevated in RGSE alone treated and AD induced rats treated with RGSE.(Table.3).

Malondialdehyde (MDA) is the organic compound with the nominal formula $CH_2(CHO)_2$. A colorless liquid, malondialdehyde is a highly reactive compound that occurs as the enol. It occurs naturally and is a marker for oxidative stress.

The results of the present study revealed that D-Gal induces significant alterations in the levels of LPO and certain enzymatic antioxidants status in both the selected brain regions of AD-induced rats. These changes were progressively reversed under prolonged treatment of AD-induced rats with RGSE. There is a strong regional correlation between lipid peroxides, antioxidant enzymes, presence of senile plaques, and NFTs in brain tissue from animal models as well as from AD patients .Previous studies have demonstrated that the long-term injection of D-Galactose (D-Gal) can lead to metabolic abnormality, excessive ROS formation, neuronal damage and a decline in learning and memory capacity in the treated rats or mice (Xu and Zhao, 2002; Holden *et al.*, 2003; Chen *et al.*, 2006). Brain function decreases following aging and central nervous system diseases such as AD, which is one of the most devastating neurodegenerative diseases. Deterioration of cognitive functions including learning and memory loss is a characteristic feature in aging and AD (R.D Terry, P Davies , 1980)

Several hypotheses have been presented to explain the process of aging in which oxidative stress was demonstrated to play a key role of senescence (Floyd and Hensley, 2002) which, subsequently causes mitochondrial dysfunction thus producing high-levels of ROS finally accelerating neurodegeneration (Zeevalk *et al.*, 2005). The brain is susceptible to free-radical damage due to its comparatively high levels of oxygen

metabolism and also relatively deficient in both free-radical scavenging enzymes and antioxidant molecules as compared with other organs (Halliwell and Gutteridge, 1995).

Table-3: Changes in Antioxidative enzymes Malondialdehyde levels (µ moles of Malondialdehyde formed/gram wet weight of the tissue) in brain from control and experimental groups of rat brain.

GROUPS	MDA
CONTROL	2.7958 ±0.55091
AD	1.3307 ±0.07029
RGSE	2.5744 ±0.19271
AD + RGSE	1.9341 ±0.21204

Values are Mean ± SEM of six observations each from tissues pooled from 6 rats. Values are significantly different from control at $p < 0.01$

ANOVA for MDA						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
CONTROL	Between Groups	1.517	5	0.303	0.00	0.00
	Within Groups	0.000	0			
	Total	1.517	5			
AD	Between Groups	0.025	5	0.005	0.00	0.00
	Within Groups	0.000	0			
	Total	0.025	5			
RGSE	Between Groups	0.186	5	0.037	0.00	0.00
	Within Groups	0.000	0			
	Total	0.186	5			
AD+RGSE	Between Groups	0.225	5	0.045	0.00	0.00
	Within Groups	0.000	0			
	Total	0.225	5			

CONCLUSION

In the present study, RGSE showed up regulations in the activity levels of SOD, CAT and with reduced MDA content in the region of rat brain. Elevated levels of SOD and CAT are observed in AD induced rats while decrease was observed in RGSE treated rats. Moderately elevated levels of SOD and CAT are observed in the AD+RGSE treated experimental group of rats. The constituent of grape seeds are the polyphenols (catechins) are powerful antioxidants that play a major role in treating AD.

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