World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

ALZHEIMER'S DISEASE (AD) INDUCED TOXICITY ON BRAIN TISSUE TOTAL ATPASE, Mg^{2+}AND Ca^{2+}ATPASES IN WISTAR STRAIN MALE ALBINO RAT WITH CONCERNING OF RED GRAPE SEED EXTRACT (RGSE)

S. Allabakash, V. Uday Kiran, Dr. K. Peera and *Prof. K. Yellamma

Department of Zoology, Sri Venkateswara University. Tirupati, Andhra Pradesh, India.

Corresponding Author: Prof. K. Yellamma
Department of Zoology, Sri Venkateswara University. Tirupati, Andhra Pradesh, India.

Article Received on 19/08/2020 **Article Revised on 09/09/2020** **Article Accepted on 29/09/2020**

ABSTRACT

D-Galactose toxicity can upset brain chemistry leading to depression, nervousness, and destabilized immunity. In people with Alzheimer's disease (AD), the increasing devastation of learning and memory eventually leads to a definitive diagnosis. The chief principle of the present study was to estimate the ameliorative effect of Red Grape Seed Extract (RGSE) on the brain of 24 male albino rats after introduction to D-Galactose toxicity. In the present study Age matched rats were be divided into 4 groups of six in each group and treated as follows: Group-I. Control (CN) rats received 0.9% saline(1ml/kg body weight).Group-II. Rats treated with intraperitoneally (IP) administered with D-Gal (120 mg/kg body weight) up to end of the experiment. Group-III. Rats injected with saline for first six weeks and from 7^{th} week onwards treated with Red Grape Seed Extract (RGSE) ethanol extract (100mg/kg body weight) orally for 60 days. Group-IV. Rats injected with D-Galactose intraperitoneally (120 mg/kg body weight) for six weeks, and then followed by simultaneous oral administration of Red Grape Seed ethanol extract (100mg/kg body weight) for another 60 days. The animals were sacrificed by cervical dislocation. Mean while isolated the Brain tissue and calculated the activity levels of Total ATPase, Mg^{2}+ and Ca^{2+}, while the treatment with RGSE affected the alterations in these parameters. AD induced experimental rats treated with Red Grape Seed Extract (RGSE) recorded a phenomenal elevation in Total ATPases, Mg^{2}+ and Ca^{2+}, ATP ases. Meanwhile the combination treatment (RGSE+D-Gal) reveals that significant elevation of Total ATPases, Mg^{2}+ and Ca^{2+}, ATPases was seen in experimental groups. Evidently, this review reveals that RGSE play a key role to enhance all energy metabolisms in AD induced rats and increase the neurotransmitters in brain system.

KEYWORDS: Red Grape Seed Extract (RGSE), D-Gal, Total ATPase, MG^{2+} and CA^{2+} ATPases, Brain Tissue and Male albino rat.

INTRODUCTION

Alois Alzheimer, a German neuropathologist, described about 51-year-old patient who developed dementia with predominant language and behavioral changes (Alzheimer A *et al*., 1907). According to Alois Alzheimer, About 70% of the risk is believed to be inherited from a person's parents, with many genes usually involved.(Ballard C *et al*., 2011). Other risk factors include: a history of head injuries, depression, and hypertension (other factors to be mentioned). In the brain, Alzheimer's disease is associated with progressive synaptic and neuronal loss, in particular of basal forebrain cholinergic neurons. In addition, the Alzheimer brain shows accumulation and spreading of two pathological features, i.e. intraneuronal neurofibrillary tangles consisting of phosphorylated Tau protein, and extra cellular senile plaques consisting of amyloid-β (Van der Beek, E.M. and Kamphuis, P.J., 2008). The oldest hypothesis, on which most currently available drug therapies are based, in the cholinergic hypothesis(Francis PT *et al*., 1999) which proposes that AD is caused by reduced synthesis of the neurotransmitter acetylcholine. The cholinergic hypothesis has not maintained widespread support, largely because medications intended to treat acetylcholine deficiency have not been very effective.(Martorana A *et al.*, 2010).

Brain aging is a risk factor of neurodegenerative diseases such as Alzheimer's disease (AD), the most common cause of dementia which accounts 70% of dementia causes in the most industrialized countries and is characterized by cell atrophy and extensive neuronal loss. It is a complex and heterogeneous disorder particularly prevalent in those over the 60 years of age. The incidence of AD rises from 2.8 per 1000 person in the 65-69year age group to 56.1 per 1000 person in the

older age group beyond 90 years. (Kukull, W.A., *et al*., 2002). A Japanese pedigree of familial.

Alzheimer's disease was found to be associated with a deletion mutation of codon 693 of APP. (Tomiyama T, 2010).

This mutation and its association with Alzheimer's disease were first reported in 2008. (Tomiyama T, *et al.*, 2008).

Grapes are considered the significant crop and well recognized worldwide. Grape Seed is one of the by-products of wine production, accounting for 38-52% of pomace on dry weight basis. Its importance is due to its high polyphenol content. These phenolic compounds are mostly known for their antioxidant properties (El Gengaihi S *et al*., 2014). Consumption of grape flavonoids has been shown to giveantioxidant protection, reduce thrombus formation and lead to the concentration of inflammatory biomarkers (Castilla *et al*., 2006).Flavonoids include colorless flavan-3-ols (such as catechin, epicatechin, their polymers, and their ester forms with galactic acid or glucose), colored flavanones (the most common flavanone in food is quercetin), and red and blue anthocyanins (Shi, J *et al*., 2003). The reported evidences of beneficial health effectsof phenolic compounds include inhibiting some degenerative diseases, (Shanmuganayagam*et al.,* 2007). Hence Flavonoids and other polyphenols found in grapes have the capacity to scavenge 'Reactive oxygen species' (ROS) (Rice-Evans *et al.,* 1996).

Thus in the present study revealed that An increasing evidences indicates that long-term systemic exposure of D-Galactose to rodents causes progressive decline in cognitive function and mimics aging progress, such as hippocampus-dependent cognitive dysfunction(Cui, X *et al*., 2006) neurodegeneration and impairments in antioxidant capacity. (Zhang, Q *et al*., 2005).

MATERIALS AND METHODS

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$\pm$ 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).

Biological effect of D-Gal

Old Age can be induced by intraperitonial (IP) injection of D-Galactose, a reducing sugar, which reacts readily with the free amines of amino acids in proteins and peptides both *in vivo* and *in vitro* to form Advanced Glycation End-products (AGE) through non-enzymatic glycation **(**Song F, Schmidt AM 2012**).** The Advanced Glycation End-product activates its receptors, which are coupled to biochemical pathways that stimulate free radical production **(**Yan H, Harding JJ, 1997**).** D-Galactose is a physiological nutrient, but over supply of D-Galactose will result in abnormality of metabolism. The oxidative metabolism of D-Galactose produces Reactive Oxygen Species (ROS), which surpass the ability of the cells to eliminate them, consequently causing impairment of cellular membrane, structure and gene expression (Zhang *et al.*, 2006). In addition, the non-enzymatic glycation is another pathway that can enhance oxidative lesions in ageing and age-associated disease such as Alzheimer's disease. Thus, long-term intraperitoneal injection of D-Galactose induces AD in normal rat (Zhang *et al.,* 2006; Fang and Liu, 2007).

Red Grape Extract Preparation

Grapes, as large clusters with red berries, were bought from a local supermarket in Tirupati, Bangalore and identified as *Vitis Vinifera (Linn).* Grape seeds were removed from the grapes, air dried in shade for 1 week and milled to a particle size of <0.4mm. The grape seed powder was macerated in 75% ethanol for 72h at room temperature. The ethanol extract was evaporated to remove ethanol, and grape seed extract was obtained as a lyophilized powder (Alireza Sarkaki*et al*., 2007).

Administration of Red Grape Seed Extract (RGSE)

Grape Seed extract (RGSE) 100 mg/kg body weight was dissolved in distilled water and given to the rat. A gavage tube was used to deliver the substance by oral route, which is clinically expected route for administration of RGSE. The volume of administration was kept at 0.2 ml to the animal.

Induction of Alzheimer's disease

Memory impairment was induced by an intraperitoneal (i.p.) injection of D-Galactose (120mg/kg body weight) and Sodium nitrite (90mg/kg body weight) by dissolving in distilled water. (Zhang *et al.*, 2006; Fang and Liu. 2007).

Isolation of Tissues

For biochemical estimations, all the above mentioned four groups of rats were sacrificed on both 30th day and 60th day of experimentation by cervical dislocation. The isolated tissue were immediately placed on a chilled glass plate and frozen in liquid nitrogen -180^0 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.

Grouping of Animals

After the rats were acclimated to the laboratory conditions for 10 days before the experimentation, they were randomly divided into four groups. Each main group was again divided in to 2 sub-groups of six each and was housed in separate cages. These different groups of rats except control were treated with selected doses of Red grape seed ethanol extract and D-Gal as given below. Keeping in view the altered activity of rats during the nights compared to day time, all doses were given once in the morning hours in between 8 A.M. to 9 A.M.

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, intraperitonealy (IP) administered with D-Gal (120 mg/kg body weight) up to end of the experiment (Zhang *et al.*, 2006; XiangdongHua *et al.*, 2007).

Group III –Red Grape Seed Extract **[RGSE Group]*:*** Rats injected with saline for first six weeks and from 7th week onwards treated withRed Grape Seed Extract (RGSE) ethanol extract (100mg/kg body weight) orally for 60 days.

Group IV **– Administered with D-Gal+*Red Grape Seed Extract* (AD+RGSE):** Rats injected with D-Galactose intraperitoneally (120 mg/kg body weight) for six weeks, then followed by simultaneous oral administration of Red Grape Seed ethanol extract (100mg/kg body weight) for another 60 days.

In the present study the experimental duration selected was 60days. D-Gal was given for first 6 weeks 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 for another 60 days.

RESULTS

ATPase System

In Energy metabolism, different ATPases viz., **Total ATPases, Ca^{2+}-ATPases and Mg^{2+} -ATPases** levels were observed in control rats and experimental rats treated with Red Grape Seed Extract recorded (RGSE). A significant elevation was observed in the energy metabolism when experimental rats were treated with RGSE.

In the present study we observe that changes occur in the enzyme metabolism and membrane transport functions, viz., Total ATPases, Mg^{2+} and Ca^{2+}-ATPases in different brain regions of control and experimental groups of mice at selected time intervals. The results clearly indicate that oral administration of RGSE significantly elevated the levels of Total ATPases, Mg^{2+} and Ca^{2+}-ATPases activity whereas the i.p. administration of D-Galactose inhibited the ATPase activity in brain region of rat, which could be reverted the changes to the normal level by the successive treatment with RGSE up to 60 days continuously.

1. TOTAL ATPases

In the control rats, the total ATPases activity levels were found to be highest and least were observed in AD rats treated with D-Gal. In general the AD induced experimental rats treated with Red Grape seed extract recorded (RGSE) a phenomenal elevation in Total ATPases and RGSE restores the total ATPases with experimental groups.

Table 1: Alterations in Total ATPases in (µmoles of Pi formed/mg protein/h) in brain tissue of control and experimental groups of rats.

Groups	Total ATPases
Control	23.0407 ± 1.09870
D-Gal	19.3330 ± 1.4570
RGSE	24.7588 ± 1.17922
D-Gal + RGSE	21.7588 ± 1.17922

Values are Mean ± SEM of six observations each from tissues pooled from 6 rats.

Values are significantly different from control at $p < 0.01$

TOTAL ATPASES (ANOVA)						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Control	Between Groups	10.537	5	2.107	0.000	0.000
	Within Groups	0.000	0			
	Total	10.537	5			
AD induced	Between Groups	40.506	5	8.101	0.000	0.000
	Within Groups	0.000	0			

	Total	40.506	5			
RGSE	Between Groups	6.953	5	1.391	0.000	0.000
	Within Groups	0.000	0			
	Total	6.953	5			
AD+ RGSE	Between Groups	6.036	5	1.207	0.000	0.000
	Within Groups	0.000	0			
	Total	6.036	5			

Ca^{2+}-ATPases

From the results it was observed that, Ca2+ ATPases levels were found to be highest in RGSE treated rats when compare to the other groups and least Ca^{2}+ ATPases levels were observed in AD rats treated with D-Gal. In general the AD induced experimental rats treated with Red Grape seed extract recorded (RGSE) a phenomenal elevation in total ATPases and RGSE restores the ca^{2}+ATPases with experimental groups.

Table 2: Alterations in Ca^{2+} –ATPases in (µmoles of Pi formed/mg protein/h) in brain tissue of control and experimental groups of rats.

Groups	Ca^{2+}-ATPases
Control	38.4407 ± .83614
D-Gal	39.1817 ± 1.79502
RGSE	39.2910 ± .40625
D-Gal + RGSE	39.0696 ±.6837

Values are Mean ± SEM of six observations each from tissues pooled from 6 rats.

Values are significantly different from control at $p < 0.01$.

CA^{2}+ATPases ANOVA						
		Sum of Squares	**df**	**Mean Square**	**F**	**Sig.**
Control	Between Groups	16.111	5	3.222	0.000	0.000
	Within Groups	0.000	0			
	Total	16.111	5			
AD induced	Between Groups	.825	5	.165	0.000	0.000
	Within Groups	0.000	0			
	Total	.825	5			
RGSE	Between Groups	2.328	5	.466	0.000	0.000
	Within Groups	0.000	0			
	Total	2.328	5			
AD+RGSE	Between Groups	3.496	5	.699	0.000	0.000
	Within Groups	0.000	0			
	Total	3.496	5			

Mg^{2}+ATPases

In the control rats the activity levels of Mg^{2+} ATPases were elevated in control and experimental rats when compare with AD rats. AD rats treated with RGSE showed progressive elevation than AD group and RGSE restores the mg^{2}+ ATPases with experimental groups.

Table 3: Alterations in ATPases Mg^{2+} in (µmoles of Pi formed/mg protein/h) in brain tissue of control and experimental groups of rats.

Groups	Mg^{2+} -ATPases
Control	38.6699 ± 1.05285
D-Gal	39.134 ± 046250
RGSE	39.1871 ± 1.79502
D-Gal + RGSE	39.0696 ± 068237

Values are Mean ± SEM of six observations each from tissues pooled from 6 rats.

Values are significantly different from control at $p < 0.01$

Mg^{2+}ATPASES ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Control	Between Groups	1.070	5	.214	0.00	0.00
	Within Groups	0.000	0			
	Total	1.070	5			
AD induced	Between Groups	16.111	5	3.222	0.000	0.000
	Within Groups	0.000	0			
	Total	16.111	5			
RGSE	Between Groups	2.328	5	.466	0.000	0.000
	Within Groups	0.000	0			
	Total	2.328	5			
AD+RGSE	Between Groups	5.542	5	1.108	0.000	0.000
	Within Groups	0.000	0			
	Total	5.542	5			

DISCUSSION

The present investigation demonstrates the changes that occur in the enzymes connected with enzyme metabolism and membrane transport functions, viz, Total ATP ase, Mg^{2+} and Ca^{2+} -ATPases in different brain regions of control and experimental groups of rats at selected time intervals. The results clearly indicate that oral administration of RGSE significantly elevated the levels of total ATP ases, Mg^{2+} and Ca^{2+}-ATPases activity whereas the i.p. administration of D-Galactose inhibited the ATPase activity in brain region of rat, which could be reverted the changes to the normal level by the consecutive treatment with RGSE up to 60 days continuously.

The membrane-bound ATPases are integral proteins responsible for the maintenance of ion homeostasis through active transport and control of delicate chemical gradient that is necessary for the optimal function of the central nervous system. Any alteration in the membrane lipid components of brain results in the inactivation of these membrane-bound enzymes (Barriviera and Hasson-Voloch, 1996). Loss of activity of ATPases is known to be involved in the development of a number of disorders such as neurological diseases, hypertension, diabetes, coronary artery diseases, stroke, tumor etc. The cation transport across the neuronal membrane mediated by the ATPases plays a significant role in many biological functions such as electron transport chain, biological oxidation in the mitochondria, synaptic transmission, and antioxidant enzyme functions. Disturbances in the ionic equilibrium of the cells as a result of inactivation of ATPases are believed to be the major factors in the pathogenesis of various neurological disorders (Vaillend *et al.,* 2002). Mitochondrial dysfunction contributes to tissue degeneration and results in the etiology of the degenerative disease of Alzheimers.

The energy needed for the active transport of these ions is provided by the hydrolysis of ATP. In brain, ~ 40% of the energy released by mitochondrial respiration is utilized by ATPases to maintain the ionic gradients across the cell membranes compared with 5% in other tissues This indicates that normal maintenance of membrane-bound enzymes in brain requires a high energy supply. Mitochondria represent potential hot spots for free radical-induced damage and the resultant mitochondrial dysfunction leads to a decline in the efficiency of ATP synthesis (Nicholls, 2002). Studies of mitochondria isolated from brains of rodents of different ages have provided evidence that the ability of mitochondria to generate ATP is compromised with advancing age and those mitochondria from old brain cells exhibit increased free radical-mediated damage (V. Toescu *et al.,* 2004). Impaired cellular energy metabolism may render neurons vulnerable to excitotoxic damage (M. Flint Beal & Robert J. Ferrante . 2004), particularly when neurons are subjected to additional stresses of Aβ and tau accumulations (Mark P. Mattson 2004). Oxidative stress may promote Aβ production by increasing APP cleavage by both β- and γ-secretases (Dong-Gyu Jo, *et al.,* 2010). Elevated intracellular Ca^{2+} levels resulting from age related increases in oxidative stress and Aβ toxicity may contribute to the increased amyloidogenic processing of APP in Alzheimer's disease (Bin Liang *et al.,* 2010). Hence disturbances in the ionic equilibrium of the cells as a result of inactivation of ATPases are believed to be the major factors in the pathogenesis of various neurological disorders.(Vaillend, C *et al.,* 2002). Neuronal membrane damage was evident from the decreased activities of membrane bound enzymes such as Na+ /K+, Mg^{2+} and Ca^{2+} -ATPases.

RGSE is used in the indigenous systems of medicine for the treatment of various nervous system ailments such as Alzheimer's disease, insomnia, anxiety, epilepsy, hysteria etc (Nadkarni, 1976). Preclinical and clinical studies have shown that *vitis vinifera* improves memory and mental function (Roodenrys *et al.,* 2002). Hence it is proved that consumption of grape flavonoids has been shown to confer antioxidant protection, reduce thrombus formation and lead to the concentration of inflammatory biomarkers (Castilla *et al.,* 2006). Naissides, M. *et al.,* 2006 said that Oxidative stress is a hallmark of various health problems. Resveratrol is a natural phytoalexin abundantly found in grapes and red wine, which has potent Antioxidant property.

D-Galactose, a normal sugar in the mammalian body, is converted into glucose in the presence of galactokinase (galE) and d-galactose-1-phosphate uridyltransferase (galD) At a higher level, however, it may lead to the formation of reactive oxygen species by galactose oxidase (Ho *et al.,* 2003). An increasing evidences indicates that long-term systemic exposure of D-Galactose to rodents causes progressive decline in cognitive function and mimics aging progress, such as hippocampal-dependent cognitive dysfunction (Cui *et al.,* 2006), neurodegeneration (Zhang *et al.,* 2005) and impairments in antioxidant capacity. The energy depletion of mitochondrial phosphorylation (ATP) due to the increased NO could lead to either necrosis or apoptosis (Zhen Pang and James W. Geddes, 1997) and disturbances of neuronal Ca2+ homeostasis. Brain oxidative metabolism is very active, mostly required to maintain cellular Na+/K+ gradients for keeping nerve impulse propagation, neurotransmitter release and cation homeostasis. Na^+, K^+ -ATPase activity decreased age-dependently in rat brain and was detected as a consequence of oxidative damage (Hemontika Chakraborty *et al.,* 2003). Inhibition of Na^+/K^+-ATPase can also result in increased concentrations of Ca^{2+} by stimulating Na^+/Ca^{2+} exchanger and produce cellular Mg^{2+} depletion since excessive calcium displaces magnesium from its binding sites thereby decreasing its functional availability (James K.Hoga *et al.,* 1992). The present observations of decreased levels of Mg^{2+}, Na^+/K^+ and Ca^{2+} -ATPase in D-Galactose and $NaNO_2$ exposed mice brain agree with the *above* hypothesis. Decrease in Mg^{2+} in turn inhibits Na^+/K^+-ATPase further, as ATP-Mg complex is the actual substrate for the enzyme.

Ca^{2+}-ATPase regulates Ca^{2+} pump activity and intracellular calcium functions as a second messenger in the control of cellular processes that plays a central role in mediating neurosecretion and release. Inhibition of Ca^{2+} ATPase activity can inturn increase intracellular concentration of Ca^{2+} and alter the signal transduction pathways and cellular fluidity and eventually results in cell death (GanesanMurali *et al.,* 2008). Calcium influx followed by D-Galactose and $NaNO_2$ exposure inhibited Ca^{2+}-ATPase in the rat brain. In the present study, administration of RGSE might have attenuated the influx of Ca^{2+} and favored its sequestration in the stores, thus maintaining the D-Galactose and $NaNO_2$ induced alteration in calcium ion homeostasis through its calcium antagonistic property.

CONCLUSION

Treatment of Alzheimer's disease (AD) rats with Red Grape Seed Extract (RGSE) enhanced brain histology and electrophysiology damages as dose dependent. Hence our findings proved that promote a possibility of therapeutic applications of RGSE for preventing neurodegenerative diseases, evidently, this review reveals that RGSE play a key role to enhance all energy metabolisms in AD Induced rats and increase the neurotransmitters in brain system.

ACKNOWLEDGEMENTS

The authors thankful to the Department of Zoology Sri Venkateswara University. Tirupathi (A.P) for providing necessary facilities to carry out this work. The authors also humble thanks to their Research supervisor, Prof.K.Yellamma (Retd) Department of Zoology S.V.University, Tirupati.

REFERENCE

1. Alzheimer A, Stelzmann RA, Schnitzlein HN, *et al*. An English translation of Alzheimer's paper, Uber eineeigenartige Erkankung der Hirnrinde. *Clin Anat*., 1907; 8: 429–431.
2. Ballard C, Gauthier S, Corbett A, Brayne C, Aarsland D, Jones E (March). "Alzheimer's disease". *Lancet*, 2011; 377(9770): 1019–31.
3. Van der Beek, E.M. and Kamphuis, P.J. The potential role of nutritional components in the management of Alzheimer's disease. European journal of pharmacology, 2008; 585(1): 197-207.
4. Francis PT, Palmer AM, Snape M, Wilcock GK (February). "The cholinergic hypothesis of Alzheimer's disease: a review of progress". *Journal of Neurology, Neurosurgery, and Psychiatry*, 1999; 66(2): 137–47.
5. Martorana A, Esposito Z, Koch G (August). "Beyond the cholinergic hypothesis: do current drugs work in Alzheimer's disease?". *CNS Neuroscience & Therapeutics*, 2010; 16(4): 235–45.
6. Kukull, W.A., Higdon, R., Bowen, J.D., Mc Cormick, W.C., Teri, L., Schellenberg, G.D., Van Belle, G., Jolley, L. and Larson, E.B. Dementia and Alzheimer's disease incidence: a prospective cohort study. *Arch Neurol*, 2002; 59: 1737-46.
7. Tomiyama T. "Involvement of beta-amyloid in the etiology of Alzheimer's disease". Brain Nerve, 2010; 62(7): 691–699.
8. Tomiyama T, Nagata T, Shimada H, Teraoka R, Fukushima A, Kanemitsu H, Takuma H, Kuwano R, Imagawa M, Ataka S, Wada Y, Yoshioka E, Nishizaki T, Watanabe Y, Mori H "A new amyloid beta variant favoring oligomerization in Alzheimer's-type dementia". *Ann Neurol*, 2008; 63(3): 377–387.
9. El Gengaihi S, Hassan E M., Aboul-ella F, Shalaby E and Abou Baker DH. Antioxidant Activity of Phenolic Compounds from Different Grape Wastes Baker J Food Process Technol, 2014; 5: 1-5.
10. Castilla, P., Echarri, R., Davalos, A., Cerrato, F., Ortega, H., Teruel, J.L., Lucas, M.F., Gomez-Coronado, D., Ortuno, J., and Lasuncion, M.A. Concentrated red grape juice exerts antioxidant, hypolipidemic, and anti-inflammatory effects in both hemodialysis patients and healthy subjects. *Am. J. Clin. Nutr*., 2006; 84: 252-262.
11. Shi, J., Yu, J., Pohorly, J. E., and Kakuda, Y. Polyphenolics in Grape Seeds—Biochemistry and Functionality. *J Med Food*, 2003; 6: 291–299.

12. Shanmuganayagam, D., Warner, T.F., Krueger, C.G., Reed, J.D., and Folts, J.D. Concord grape juice attenuates platelet aggregation, serumcholesterol and development of atheroma in hypercholesterolemic rabbits. *Atherosclerosis*., 2007; 190: 135–142.
13. Rice-Evans, C.A, Miller, N. J., and Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free RadicBiol Med.*, 1996; 20: 933–56.
14. Cui, X., Zuo, P., Zhang, Q., Li, X., Hu, Y., Long, J. *et al*., Chronic systemic D-Galactose exposure indices memory loss, neurodegeneration and oxidative damage in mice: protective effects of R-alpha-Lipoic acid. J NeurosciRes, 2006; 83(8): 1584-90.
15. Zhang, Q., Li, X.K., Cui, X. and Zuo, P.P., D-Galactose injured neurogenesis in the hippocampus of adult mice. Neurol. Res, 2005; 27: 552-556.
16. Zhang D, Liu GT, Shi JG and Zhang JJ. Coeloglossumviride var bracteatum extract attenuates D-Galactose and NaNO2 induced memory impairment in mice. J Ethnopharmacol, 2006; 104: 250-256.
17. Fang F and Liu G A novel cyclic squamosamide analogue compound FLZ improves memory improvement in artificial senescence mice induced by chronic injection of D-Gal and NaNO2. *Basic Clin Pharmacol Toxicol*, 2007; 101: 447-454.
18. Song F, Schmidt AM Glycation and insulin resistance: novel mechanisms and unique targets? Arterioscler ThrombVascBiol, 2012; 32: 1760-5.
19. Yan H, Harding JJ Glycation-induced inactivation and loss of antigenicity of catalase and superoxide dismutase. Biochem J, 1997; 328: 599-605.
20. Alireza sarkaki, The effect of grape seed extract (gse) on spatial memory in aged male rats, Pak j Med Sci., 2007; 23(4): 561-565.
21. XiangdongHua[a],MingLei[a],YongjieZhang,[a]JiongDing ,[a]QunyingHan,[a]GangHu,[b]MingXiao[ac] Long-term D-galactose injection combined with ovariectomy serves as a new rodent model for Alzheimer's disease. Life Sciences**,** 2007; 80(20**):** 1897-1905.
22. Barriviera ML, Hasson-Voloch A. Lipids associated with the (Na+-K+) ATPase of normal and denervated electric organs of Electrophorus electricus (L.). Z Naturforsch [c], 1996; 51: 883-892.
23. Vaillend C, Billard JM. Facilitated CA1 hippocampal synaptic plasticity in dystrophin-deficient mice: role for $GABA_A$ receptors? Hippocampus, 2002; 12: 713–717.
24. Nicholls DG, Ferguson SJ. Bioenergetics 3. Academic Press, 2002.
25. V. Toescu; s. L. Nuttall; u. Martin; p. Nightingale; m. J. Kendall; p. Brydon; f. Dunne changes in plasma lipids and markers of oxidative stress in normal pregnancy and pregnancies complicated by diabetes*clin sci (lond)*, 2004; 106(1): 93–98.
26. M. Flint Beal & Robert J. Ferrante. Experimental therapeutics in transgenic mouse models of Huntington's disease *Nature Reviews Neuroscience*, 2004; 5: 373–384.
27. Mark P. Mattson Pathways towards and away from Alzheimer's disease Nature, 2004; 430: 631–639.
28. Dong-GyuJo, *et al*., Evidence that γ-secretase mediates oxidative stress-induced β-secretase expression in Alzheimer's disease. Neurobiology of Aging, 2010; 31(6): 917-925.
29. Bin Liang, Bao-Yu Duan, Xiu-Ping Zhou, Jia-Xin Gong and Zhen-Ge Luo Calpain Activation Promotes BACE1 Expression, Amyloid Precursor Protein Processing, and Amyloid Plaque Formation in a Transgenic Mouse Model of Alzheimer Disease[*] The Journal of Biological Chemistry, 2010; 285: 27737-27744.
30. Vaillend, C., Mason, S.E., Cuttle, M.F. and Alger, B.E. Mechanisms of neuronal hyperexcitabikity caused by partial inhibition of Na+-K+-ATPases in the rat CA1 hippocampal region. J Neurophysiol, 2002; 88: 2963-2978.
31. Nadkarni A.K., Nadkarni K.M. Indian Materia Medica, Popular prakashan Bombay, 1976; 3(1): 263,269,1292.
32. Roodenrys, S., Booth, D., Bulzomi, S. *et al.* Chronic Effects of Brahmi (Bacopa monnieri) on Human Memory. *Neuropsychopharmacol*, 2002; 27: 279–281.
33. Castilla, P., Echarri, R.,Davalos, A., Cerrato, F., Ortega, H., Teruel, J.L., Lucas, M.F., Gomez-Coronado, D., Ortuno, J., and Lasuncion, M.A. Concentrated red grape juice exerts antioxidant, hypolipidemic, and anti-inflammatory effects in both hemodialysis patients and healthy subjects. *Am. J. Clin. Nutr.*, 2006; 84: 252-262.
34. Naissides, M., Mamo, J.C.L., James, A.P., Pal S. The effect of chronic consumption of red wine on cardiovascular disease risk factors in postmenopausal women. *Atherosclerosis*., 2006; 185: 438–45.
35. Ho, S., Liu, J. & Wu, R. Establishment of the mimetic aging effect in mice caused by D-galactose. *Biogerontology*, 2003; 4: 15–18.
36. Xu Cui et al., Chronic systemic D-galactose exposure induces memory loss, neurodegeneration, and oxidative damage in mice: Protective effects of R-α-lipoic acid Journal of neuroscience research, 2006; 83(8): 1584-1590.
37. Zhen Pang and James W. Geddes Mechanisms of Cell Death Induced by the Mitochondrial Toxin 3-Nitropropionic Acid: Acute Excitotoxic Necrosis and Delayed Apoptosis Journal of Neuroscience, 1997; 17(9): 3064-3073.
38. Hemontika Chakraborty[a]PapparaniSen[a]ArghyaSur[a] Uttara Chatterjee[b] Sasanka Chakrabarti Age-related oxidative inactivation of Na^+, K^+-ATPase in rat brain crude synaptosomes Experimental Gerontology, 2003; 38(6): 705-710.

39. James K.Hoga*WendyParris*MarvinGold*J. Bryan Jones† The substrate specificity of the muscle L-lactate dehydrogenase of spiny dogfish (*Squalus acanthis*)Bioorganic Chemistry, 1992; 20(3): 204-212.
40. GanesanMurali[a]KavinS.Panneerselvam[b]Chinnakkan nuPanneerselvam[a] Age-associated alterations of lipofuscin, membrane-bound ATPases and intracellular calcium in cortex, striatum and hippocampus of rat brain: protective role of glutathione monoester. International Journal of Developmental Neuroscience, 2008; 26(2): 211-215.