

Research Article



Effect of *Vitis vinifera* Fruit Seed Extract against Streptozocin Induced Diabetes Related Alzheimer's Disease in Wistar Rats

Chitra V^{*}, Udayasri N¹, J Narayanan¹

¹Department of Pharmacology, SRM College of Pharmacy, SRM University, Kattankulathur, Kancheepuram Dist, Tamil Nadu, India.

^{*}M.Pharm., Ph.D, Dept of Pharmacology, SRM College of Pharmacy, SRM University, Kattankulathur, Tamil Nadu, India.

^{*}Corresponding author's E-mail: akashnara07@gmail.com

Accepted on: 10-12-2014; Finalized on: 30-06-2015.

ABSTRACT

Diabetes mellitus is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism. Recently, herbal products have started gaining importance as complementary and alternative medicine to treat diabetic mellitus. The investigation was carried out on diabetes and cognitive impairment with relevance of the hypothesis, on Streptozotocin induced increased level of blood glucose, AchE, MAO and impaired behavioural performance. *Vitis vinifera* extract administration significantly ($P < 0.05$) reduced the blood glucose level and Acetylcholine esterase Enzyme and MAO, when compared with negative control group. The extract decreases diabetic and neurodegenerative and helps in memory retention activity. Based on the results in our study, we speculate that the ethanolic extract of *Vitis vinifera* seeds might be promising for the development of a standardized phytomedicine for the treatment of diabetes mellitus.

Keywords: Diabetes, Alzheimer's, Ethanol grape seed extract (EGSE), Streptozotocin (STZ).

INTRODUCTION

Diabetes mellitus is a global burden as its incidence is considered to be high (4–5%) all over the world.¹ The prevalence of diabetes mellitus is increasing with ageing of the population and lifestyle changes associated with rapid urbanization and westernization.

Diabetes and Alzheimer's disease are inter related and may eventually reveal new strategies to avoid Alzheimer's as a complication of diabetes. Especially type 2 diabetes is at higher risk of eventually developing Alzheimer's disease due to damaged blood vessels in brain caused by Diabetes.

The objective of the present study was to assess the effect of *Vitis vinifera* fruit seed extract against Streptozotocin induced Diabetes and Alzheimer's disease.

Vitis vinifera is one of the herbal drugs which belong to the family vitaceae. In traditional a system of medicine, whole plant is used for antifungal, skin diseases, antioxidant cytoprotective, chronic degenerative diseases, antiulcer, and antihypertension.

Information based on ethno medicinal survey reveals that the herbal preparations of seeds of *Vitis vinifera* had been considered as effective economical and safe treatments for curing various diseases in Indian traditional system of medicine.

MATERIALS AND METHODS

Plant Material

The seed of *Vitis vinifera* used in the present study were collected from the super market, Chennai, and the plant material was authenticated by Dr. P. Jayaraman Ph.D.,

Plant Anatomy Research Centre (PARC), Tambaram and a specimen copy was submitted to PARC/2011/1028.

Preparation of Seed Extract

Seed were dried in hot air oven at 50°C for 72 h. Then, dried seeds were ground to coarse powder by a grinder. About 500g of powder were mixed with 1000ml of 70% ethanol in distilled water and kept for 3 days at room temperature.

The extract were then filtered through a Buchner funnel and excess of solvent (ethanol/water) was removed using rotary evaporator under vacuum at 50°C.²

Induction of Diabetes and Alzheimer's

Diabetes were induced by 40mg/kg of Streptozotocin administered i.p. in citrate buffer (PH 4.0). After 48 hours blood glucose levels were measured to conformed the induction of diabetic rats with a glucose level above 200mg/dl were selected as diabetic rats included in the experiment.³

Grouping of Animals

Animals were divided into 5 groups (n=6), Male Wistar rats were acclimatized for 10 days prior to experimentation.

Animals were fasted overnight and 40mg/kg/day of inducing agent were i.p. to all animals for 7 days.

Group II and III were treated with grape seed extract of plant (150 and 300mg/kg).

Group IV and V were treated with grape seed extract of plant (150and 300mg/kg). Blood (1ml) were collected from all animals by tip of tail vein for estimation of parameters.



Animals were sacrificed at the end of the experimentation period. Brain are collected and homogenized to estimate Acetylcholine esterase enzyme, Mono amino oxidase enzyme, and Protein levels.

Parameters

Body Weight

Animal body weight is observed by weighing animals.

Blood Glucose Estimation

Blood were collected by retro orbital puncture and glucose were measured by using glucose kit.

Estimation of Total Protein

Total protein is estimated by tissue homogenate were measured by using protein kit.

Passive Avoidance Test

The apparatus was 50cm×25cm×25cm acrylic box, whose floor consisted of parallel 1.0 cm apart. A 7.0 cm wide, 2.5 cm high, 25.0 cm long platform occupied the centre floor. In the training session, immediately after stepping down placing their four paws on the grid animals received a 0.4 ma. 2.0 s scrambled foot shock. In test session no foot shock will be given and step-down latency was used as a measure of retention (to a ceiling of 300 S) one-trial step-down inhibitory avoidance in rats and mice involves the activation of separate memory types, short-term memory (STEP) system, and a long-term memory (LTM) system. Therefore retention tests will be carried out 90 min after training to evaluate STM and 7 days after training to evaluate long term memory⁴⁴. The same were used for both tests, as testing for STM has been found not to affect LTM retention scores in previous studies⁴.

Y-maze Task

Y-maze task is used to measure the spatial working through the spontaneous alternations of behaviour⁴⁶. The maze is made of black painted wood. Each arm is 40cm long, 13 cm, 3 cm wide at the bottom, 10 cm wide at the top and converges at an equal angle. Each rat is placed at the end of one arm and allowed to move freely through the maze during an 8-min session; rats tend to explore the maze systematically, entering each arm in turn. The ability to alternate required that rat know which arm they have already visited. The series of arm entries, including possible returns into the same arm recorded visually. Alternation is defined as the number of successive entries into the three arms, on overlapping triple sets. The percentage of alternation is calculated as the ratio of actual alternation, defined as the total number of arm entries minus two, and multiplied by 100.⁵

Elevated plus maze

Elevated plus-maze served as the exteroceptive behavioural model to evaluate memory in rats. The procedure technique and endpoint for testing memory were followed as per the parameters described by the

investigators working in the area of psychopharmacology. The Elevated plus-maze for rats consisted of two open arms (16cm×5cm) and two covered arms (16cm×5cm×12cm) extend from a central platform (5cm×5cm), and the maze was elevated to a height of 25cm from the floor. On the first day each rat was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was defined as the time (in sec) taken by the animal to move from the open arm into one of the covered arm with all its four legs. TL was record on the first day (training session) for each animal. The rat was allowed to explore the maze for another 2min and then returned to its home cage. Significant reduction in the TL value of retention indicated improvement in memory.⁶

Acetylcholinesterase (AChE) Enzyme

20mg of brain tissue per ml of phosphate buffer (pH 8, 0.1m) were homogenized in a homogenizer. A 0.4ml aliquot of brain homogenate was added to cuvette containing 2.6ml of 0.1m phosphate buffer (pH 8). 100µl of the DTNB reagent were added to photocell. The absorbance was measured at 412nm. 20µl of the acetylthiocholine were added changes in absorbent were recorded and a change in absorbance per minute was calculated.

The enzyme activity is expressed as µ moles per minute per mg tissue.⁷

MAO Assay

Rat brain mitochondrial fraction are prepared by cutting the brain sample in to small pieces and rinsed in 0.25m sucrose 0.1m Tris 0.02m EDTA (pH 7.4) to remove blood. The pieces were homogenized for 45 sec in a potter-elvehjem homogenizer with 400 ml of same medium. The homogenate were centrifuged at 800 rpm for 10 min and pellets were discarded. The supernatant was centrifuged at 1200 rpm for 20 min in the same medium. The precipitate were washed twice more with 100ml of sucrose Tris EDTA and resuspended in the 50ml of medium the protein concentration were adjusted to 1mg/ml.

MAO Activity was assessed spectrophotometrically. The assay mixture contains 4mm of serotonin as specific substrate for MAO-A, 250µl solution of mitochondrial reaction were allowed to proceed at 35°C for 20 min and stopped by adding 1m HCL(200µl), the reaction product were extracted with 5ml of butyl acetate, the organic phase were measured at wavelength of 280nm in a spectrometer. Blank sample was prepared by adding 1m (200µl) prior to the reaction and worked subsequently in the same manner.⁸

Estimation of Antioxidant Enzyme

Assay of Superoxide Dismutase

To 0.5ml of tissue homogenate, 0.25ml of absolute ethanol and 0.15ml of chloroform were added. After 15



min of shaking in a mechanical stirrer, the suspension was centrifuged and supernatant obtained constituted the enzyme content. The assay mixture contain 0.2m of Tris HCl buffer, 0.5ml of Pyrogallol 0.5m of aliquots of the enzyme extract and h₂o to give final autoxidant after the addition of the enzyme was noted at absorbance at 470nm at interval of 1min for 3min the enzyme is defined as the amount of enzyme, which inhibits the rate of pyrogallol auto oxidation by 50%. The SOD is expressed as units/mg protein.⁹

Assay of Glutathione Peroxidase

The reaction mixture consisting of 0.2ml each of EDTA, sodium azide and H₂O₂ 0.4 ml of phosphate buffer, 0.1 ml of suitable diluted tissue will be incubated at 37°C at different intervals.

The reaction will be arrested by addition of 0.5ml of TCA and the tubes will be centrifuged at 2000 rpm to 0.5ml of supernatant, 4ml of disodium hydrogen phosphate and 0.5 ml of DTNB will be added and the colour developed will be read at 420nm spectrophotometrically.¹⁰

The activity of GPx is expressed as μ moles of glutathione oxidized/minutes/mg protein.

Assay of Glutathione Reductase

The reaction mixture containing 1ml of phosphate buffer, 0.5ml of EDTA. 0.5ml of oxidized glutathione, 0.2ml of NADPH was made up to 3ml with water after the addition of 0.1 ml of suitable diluted tissue, the change in optical density at 340nm will be monitored at 2min 30 sec interval.¹¹

The activity of GR is expressed as n moles of NADPH oxidized/minute/mg protein.

Assay of Catalase

0.1 Ml of tissue homogenate was taken to which 1 ml of phosphate buffer and 0.5 ml of hydrogen peroxide was added and the reaction were acted. The reaction was arrested by the addition of 2 ml of dichromate acetic acid reagent. Standard hydrogen peroxide in the range if 2 to 4 μM were taken and treated similarly. The tubes were heated in a boiling water bath for 10 minutes. The green colour developed was read at 570nm.¹²

Catalase activity was expressed as μ moles of hydrogen peroxide utilized/min/mg protein under incubation condition.

Histopathological Studies

Histological evaluation was performed on brain, pancreas tissues. Fresh brain tissues were excised and then fixed in formalin for 24hr. The fixative were removed by washing through running tap water for overnight. After dehydration through a graded series of alcohols, the tissue were cleaned in methyl benzoate, embedded in paraffin wax section were cut into 5 um thickness and stained with hematoxylin and eosin after repeated dehydration and cleaning, the sections will be mounted and observed under light microscope with magnification of 100x for histological change.

Statistical Analysis

Statistical Analysis: Statistical analysis was carried out using InStat3 software. All results were expressed as Mean ± S.E.

Data analysis were done using ANOVA followed by Dunnet's test. A p≤0.05 level of probability were used as criterion for significance.

Table 1: Effect of EGSE on body weight of rats

Group	1 st Day	14 th Day	28 th Day
Group I	123.3 ± 1.054	106.7 ± 1.054	102.5 ± 1.708
Group IV	136.7 ± 2.108*	131.7 ± 2.108*	144.2 ± 1.537*
Group V	141.7 ± 1.667*	137.5 ± 1.708*	148.3 ± 1.054*

There was a significant difference between the negative control group (1) and treated groups in body weight of STZ induced diabetes rats. The EGSE treated groups show significantly increases the body weight, when compared with negative control groups. The low dose and high dose shown significant (P< 0.05) when compared with the negative control was shown.

Table 2: Effect of EGSE on Blood Glucose level

Group	1 st Day	14 th Day	28 th Day
Group I	213.5 ± 3.041	406.7 ± 4.723	419.2 ± 6.112
Group IV	208.3 ± 2.319 *	378.3 ± 7.813 *	82.17 ± 2.023*
Group V	207.8 ± 1.138*	423.8 ± 8.923*	73.83 ± 0.477 *

There was a significant difference between the negative control group and treated groups in blood glucose levels of STZ induced diabetes rats. The EGSE treated group show significant to decrease the blood glucose level. The low dose and high dose shown significance (P<0.05) when compared with negative control.

Table 3: Effect of EGSE on Y-Maze

Group	Group I	Group IV	Group V
Percentage Alteration	36.33 ± 1.453	46 ± 0.577*	47.67 ± 0.881*



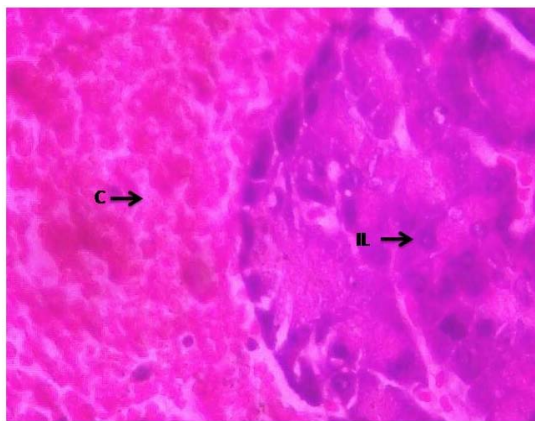
The STZ induced Alzheimer’s group (negative control) indicated increase in the percentage alteration of by (P<0.05) in comparison with treated groups. The results presented by the treatment groups shows significance by (P<0.05) increase in percentage alteration in respect of 150mg/kg of EGSE and 300mg/kg of EGSE when compared with of the negative control group.

Table 4: Effect of EGSE on Acetylcholine Esterase

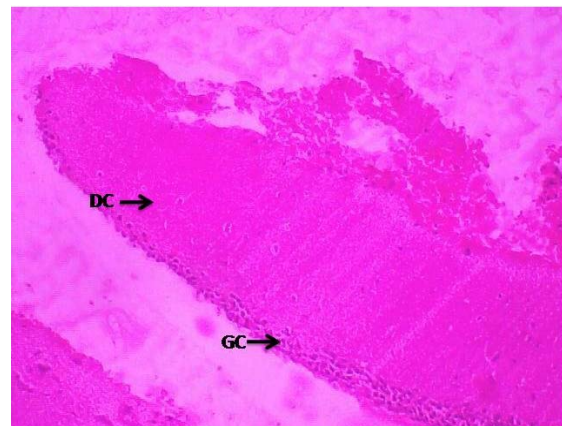
Group	Group I	Group IV	Group V
Micro Mole/min/mg Protein	6.234 ± 0.1554*	5.451 ± 0.1051*	4.554 ± 0.09*

Diabetes induced Alzheimer’s group significantly (P<0.05) increases the AChE activity when compared to the treated group. In treated group there was significant (P<0.05) reduction in enzyme level on both 150 and 300 mg/kg of EGSE treated rats as shown in the Figure 1.

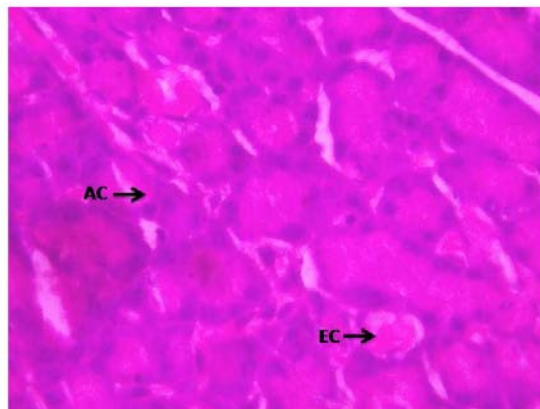
Histopathological Studies



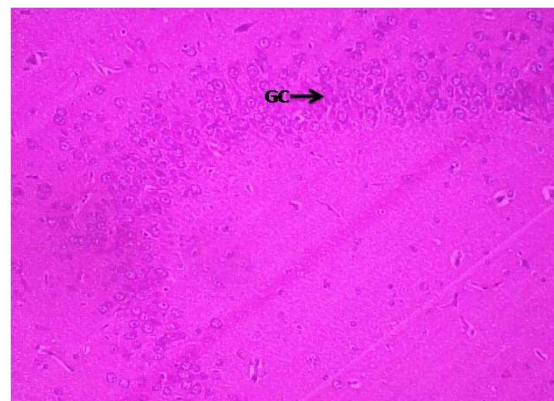
Group-I Pancreas 40X



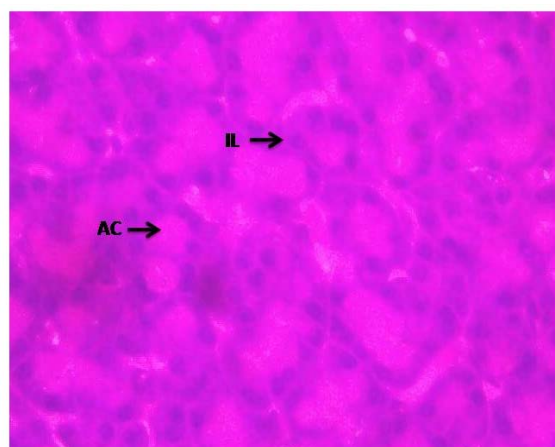
Group-I Brain 40X



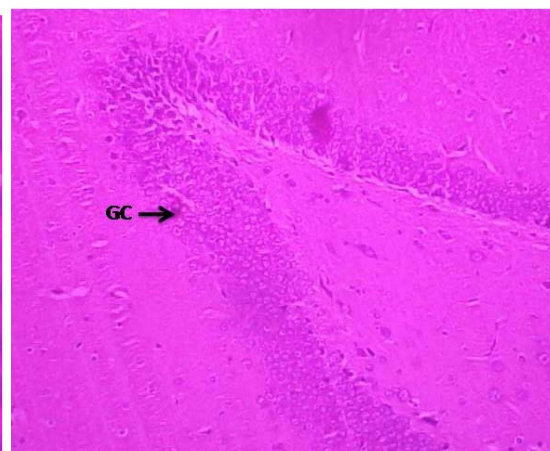
Group-IV Pancreas 40X



Group-IV Brain 40X



Group-V Pancreas 40X



Group-V Brain 40X

Group I: negative control
 Group IV: EGSE treated 150mg/kg
 Group V: EGSE treated 300mg/kg

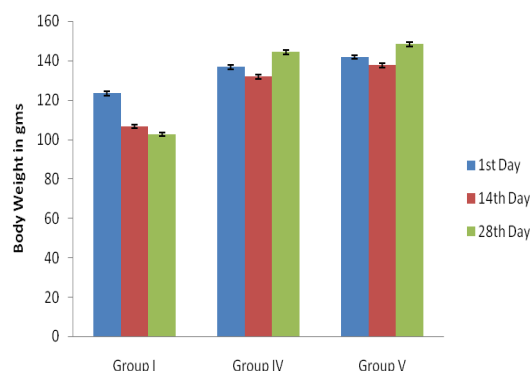


Figure 1: Effect of EGSE on body weight of rats

There was a significant difference between the negative control group (1) and treated groups in body weight of STZ induced diabetes rats. The EGSE treated groups show significantly increases the body weight, when compared with negative control groups. The low dose and high dose shown significant ($P < 0.05$) when compared with the negative control was shown.

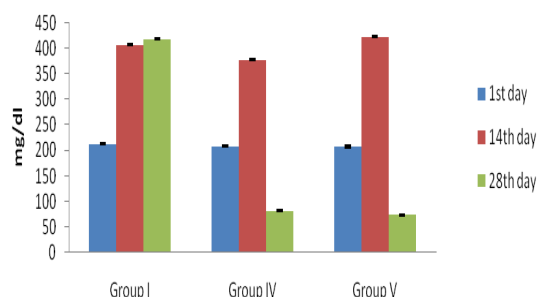


Figure 2: Effect of EGSE on Blood Glucose level

There was a significant difference between the negative control group and treated groups in blood glucose levels of STZ induced diabetes rats. The EGSE treated group show significant to decrease the blood glucose level. The low dose and high dose shown significance ($P < 0.05$) when compared with negative control.

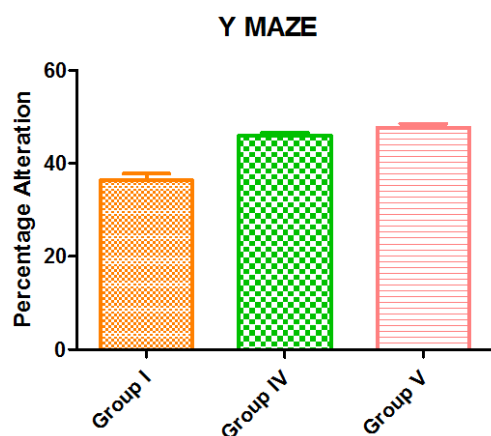


Figure 3: Effect of EGSE on Y-Maze

The STZ induced Alzheimer's group (negative control) indicated increase in the percentage alteration of by ($P < 0.05$) in comparison with treated groups. The results presented by the treatment groups shows significance by ($P < 0.05$) increase in percentage alteration in respect of 150mg/kg of EGSE and 300mg/kg of EGSE when compared with of the negative control group.

DISCUSSION

The present study demonstrates the beneficial effects of the standardized seed extract of *Vitis vinifera* in STZ induced Diabetes and Alzheimer's in the rats and proved that the extract significantly ameliorated the blood glucose level and cognitive defects in rats.

In the STZ induced diabetes rats, increased food consumption and decreased body weight were observed. This indicated polyphagic condition and loss of weight due to excessive breakdown of tissue proteins (Chatterjee & Shinde). Hakim have stated that decreased body weight in diabetic rats could be due to dehydration and catabolism of fats and proteins. Increased catabolic reaction leading to muscle wasting might also be the cause for the reduced weight gain by diabetic rats (Raj Kumar). It has been shown that grape seed extract improved the glucose tolerance in STZ induced diabetes

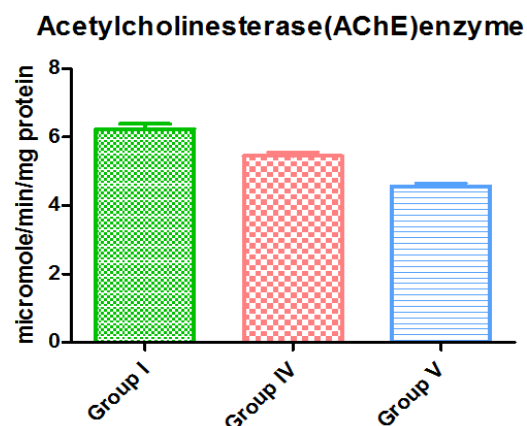


Figure 4: Effect of EGSE on Acetylcholine Esterase

Diabetes induced Alzheimer's group significantly ($P < 0.05$) increases the AChE activity when compared to the treated group. In treated group there was significant ($P < 0.05$) reduction in enzyme level on both 150 and 300 mg/kg of EGSE treated rats.

in rats as compared with negative control group dose dependent effect 300 mg/kg showed more reduction than 150 mg/kg.

Presently out study with regards of non enzymatic antioxidant, we found that treatment with EGSE ameliorated cognitive deficits in STZ induced rats, especially shows increase memory in passive avoidance task. EGSE exerted significantly decreased in number of escape failure. However, since the time used in our study was relatively short, it was possible that higher rates of memory improvement might be found with longer periods of treatment. We also examined the effect of EGSE in Y-maze. Y-maze task is used to measure the spatial working through the spontaneous alternation of behaviour. These results of our experiments have been consistent with its favourable effect on increasing the percentage of alternation in respect to the dose

dependent manner. Elevated plus maze test, which is used to evaluate the anxiety level in rat brain, consumption of EGSE showed dose dependent reduction in transfer latency indicated improvement in memory. It is possible that neuroprotection plays a role in favourable effects of EGSE on STZ induced Alzheimer's.

The AChE activity has been shown to be increased around A β plaques in Alzheimer's brain. The calcium influx followed by oxidative stress is involved in increase activity of AChE induced by STZ, decreasing cell membrane order ultimately leading to exposure of more active enzyme.

The observation that diabetes prolonged leads to Alzheimer's in rat brain showed increase in AChE activity indicated that it can be possible to ameliorate cholinergic function, by inhibiting STZ induced increase in AChE activity. The AChE activity in the brain was increased in rats treated with STZ when compared with EGSE treated.

MAO is an important enzyme involved in the metabolism of a wide range of mono amine neurotransmitters, including noradrenalin, dopamine and 5-hydroxytryptamine¹³. MAO exists in two forms, A and B, MAO-A in the metabolism of the major neurotransmitter monoamines. MAO-A inhibitors have been accepted to treat depression. MAO-A and MAO-B in the brain have been implicated in the etiology of Alzheimer's disease. Elevations in MAO-A in Alzheimer's neurons have been linked to increase in neurotoxin metabolites and neuronal loss. Free radical generation by STZ or other noxious stimuli could contribute to an imbalance between the production of nitric oxide and oxygen radicals and precipitate in oxidative stress.

The high metabolic rate of the brain the low concentration of antioxidant enzymes and the high proportion of polyunsaturated fatty acid, makes the brain a tissue particularly susceptible to oxidative damage super oxide dismutase (SOD), glutathione peroxidase (GPX) and catalase are three main enzymes involved in cellular protection against damage due to oxygen derived free radicals.

The position the GSH takes part in redox-sensitive signalling cascade has been receiving growing support. One of the most important GSH-dependent detoxifying processes involved in glutathione peroxidase (GPX) which place central role in removal on hydrogen and organic peroxidase and leads to the formation of oxidized glutathione(GSSG).

GSSG is reduced back to its thiols form (GSH) by the ancillary enzyme glutathione reductase (GRD) leading to the consumption of NADPH which is mainly produced in the pentose phosphate pathway. GSH also takes part in xenobiotic conjugation with the assistance of several Glutathione S-transferase iso enzymes GSH conjugate or GSSG can be eliminated from the cell by family ATP dependent transporter pump.

In present study anti oxidant (SOD, CAT, GPx, GRD) significantly increased in treated groups when compared with negative control group.

Histopathological studies of brain tissue showed structural damage indicate degenerative changes in negative control group. By observing drug treated of low and high dose, it inference that showed regenerative changes shows similar to normal architecture of hippocampus region brain.

Histopathological studies of pancreas tissue showed structural damages like vacuolization and congestion in negative control group. By observing drug treated of low and high dose, it inference that showed regenerative changes shows similar to normal cytoarchitecture of pancreas.

In conclusion, we suggest that markedly EGSE improves cognitive deficits induced by STZ and the effect is mediated by antioxidant properties of EGSE.

REFERENCES

1. William G. Epidemiology of diabetes mellitus. *Textbook of Diabetes*, Blackwell, Oxford. 1(2), 1997, 3.1–3.28.
2. Hemmati AA, Nazari Z, Mohammadian B, Hasanvand N., Grape seed extract can reduce the fibrogenetic effect of bleomycin in rat lung. *Iaran J Pharma Sci.*, 2(3), 2006, 142-150.
3. Maritim ADB, Sanders RA, and Watkins III JB. Effects of pycnogenol treatment on oxidative stress in streptozotocin in induced diabetic rats. *J Biochem moltoxicol*, 17, 2003, 193-199.
4. Barros DM, Izquierdo LA, Mello e Souza T, Ardenghi P, Pereirap, Medina JH., Molecular signalling path ways in cerebral cortex are required for retrieval of one-trail inhibitory avoidance learning in rats, *Brain. Res;* 9, 2009, 114-183.
5. Brain P, Tangui Maurice, Amnesia induced in mice by centrally administered β -Amyloid peptide involves cholinergic dysfunction, *Brain Research*, 706, 1996, 181-193.
6. Pa Patil, Veena Nayak., Anti depressant Activity of Fisinopril, Ramipril and Losartan but not of Lisinipril in depressive Paradigms of Albino rats and mice. *Indian Journal of Experimental Biology*, 46, 2008, 180-184.
7. Ellam GL, Courtney KD., Feather stone RM, A new and rapid colorimetric determination of acetyl cholinesterase activity. *Biochem Pharmacol*, 7, 1961, 88-95.
8. Charles M, MC Ewen J, Tabor H, Tabor CW (Eds.). *Mao activity in rabbit serum*, *Methods in Enzymologists*, XVIIIB, New York and London, Academic Press, 1977, 692-698.
9. Marklund S & Marklund G., Involvement of the superoxide anion radical in the autoxidant of Pyrogallol and a convenient assay of for superoxide dismutase. *European J. of biochem.* 47, 1974, 469-474.
10. Burk RF, Lawrence RA., Glutathione peroxidase activity in selenium-deficient rat liver. *Biochemical and Biophysical Research Communications*. 1976, 952-958.



11. Staal, GE. J, Visser. J and Veeger. C., Purification properties of glutathione reductase of human erythrocytes. *Biochim. Biophys. Acta [BBA]-Enzymology*. 185, 1969, 39-48.
12. Sinha AK., Colormetric assay of Catalase. *Anal Biochem.*, 1972, 389-394.
13. Casanova MF, Walker LC, Whitehoue PJ, Price DL., Abnormalities of the nucleus basalis in Down's syndrome. *Ann.Neurol.*, 18, 1985, 310-313.

Source of Support: Nil, **Conflict of Interest:** None.

