Modulatory Effects of Grape Seed Extract on Brain Neurotransmitters and Oxidative Stress in Alloxan Diabetic Rats

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Abstract: Background**:** Hyperglycemia-mediated oxidative stress plays a crucial role in the progression of diabetic neuropathy (DN). Oxidative damage is the most common concluding pathway for various pathogenetic mechanisms of neuronal injury in diabetic neuropathy. Hence, the present study was hypothesized to explore the neuroprotective nature of grape seed extract (GSE) on diabetic rats by assessing markers of brain neurotransmitters secretion, oxidative stress, antioxidant competence and inflammatory marker in alloxan-induced diabetic rats. **Methods:** Four groups of rats were treated daily for ten weeks : (-ve) control, diabetic-control injected intraperitoneally with 150 mg kg^{-1} BW of alloxan monohydrate, diabetic-treated rats injected by alloxan and then treated with GSE 250 mg kg^{-1} BW and (+ve) control rats treated with the same previous dose of GSE**. Results**: In diabetic rats a significant increase in serum glucose and butyrylcholinesterase (BChE), while hypoinsulinemia were recorded. In addition a significant increase in brain neurotransmitters [epinephrine, noradrenaline (NA), serotonin (5-HT) and dopamine], MDA, superoxide dismutase (SOD) were recorded. Whereas there were a significant decrease in brain glutathione (GSH), Vitamin C, nitric oxide levels and glutathione peroxidase (GPx) activities were reported. There was non significant change in catalase (CAT) activity. GSE administration was found to be able to ameliorate most of the biochemical altered parameters in diabetic rats. **Conclusion:** The present results indicated that experimental diabetes produced metabolic disturbances in glucose, insulin that trigger brain enzymatic and non enzymatic oxidative stress that initiate disturbances in brain neurotransmitter, providing the incidence of nervous manifestation in diabetes. Administration GSE is valuable for enhancing the antioxidant defense against oxidative stress, neuroprotective, resulting in the modulation of brain neurotransmitters.
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1. Introduction

Diabetes mellitus (DM), long considered a disease of minor significance to world health, is now taking its place as one of the main threats to human health in the 21st century **(Zimmet, 2001).** It is the most common non communicable disease worldwide and the fourth to fifth leading cause of death in developed countries, ,the global figure of people with diabetes is set to rise from the current estimate of 150 million to 220 million in 2010 and 300 million in 2025 **(Tripathi *et al.*, 2006).**

Diabetes is a syndrome characterized by chronic hyperglycemia and disturbance of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action **(Prakash *et al.*, 2011).** Persistent hyperglycemia in diabetic patients despite appropriate therapeutic measures leads to several complications including retinopathy, nephropathy and neuropathy **(Saini *et al.*, 2007).**

Diabetic neuropathy (DN) refers to a group of debilitating, diabetes-related nerve disorders **(Boulton *et al.*, 2005).** It is one of the most important diabetes-related complications, affecting from 50% to 66% of all patients with diabetes and affects individuals with both types 1 and type 2 diabetes **(Zhao *et al.*, 2010).**

DN has serious detrimental effects on patients physical, emotional, and social functioning **(Jensen, *et al.*, 2007).** Many DN patients experience pain or discomfort, activity limitations, anxiety, and depression and have lost workdays or decreased work productivity as a result of DN **(Gore *et al.*, 2006).**

Oxidative stress has been suggested as a major contributor to the diabetic neuropathy **(Saini *et al.*, 2007).** Lipid, DNA, and protein are the cellular targets for oxidative stress, leading to changes in cellular structure and function. Increased oxidative stress within the cell lead to activation of the poly (ADP-ribose) polymerase (PARP) pathway, which regulates the expression of genes involved in promoting inflammatory reactions and neuronal dysfunctions **(Kuhad *et al.*, 2009).**

Insulin therapy and oral hypoglycemic agents offer effective glycemic control, but, insulin therapy

has shortcomings such as ineffectiveness on oral administration, short shelf life and requirement of constant refrigeration and in the event of excess dosage fatal hypoglycemia limit its usage. The use of oral drugs is limited due to adverse side effects including hematological, cutaneous and gastrointestinal reactions, hypoglycemic coma and disturbances of liver and kidney functions **(Prakash *et al.* 2011).**

In addition, there are in suitability for using during pregency. Nowadays there is considerable interest in the potential health benefits of natural remedies such as medicinal plants and their extracts. One of these natural extracts is GSE **(Gargari *et al.*, 2011).** GSE is a natural extract from the seed of *vitis vinifera*. It is a rich source of one of the most beneficial groups of plant flavonoids, proanthocyanidines oligomers **(Benzer *et al.*, 2012).** It has recently found that GSE has shown various pharmacological effects such as chemoprotective **(Nandakumar *et al.*, 2008)** and oxidative stress as well as being anti-inflammatory **(Terra *et al.*, 2009),** anti-bacterial **(Mayer *et al.*, 2008),** anticancer **(Kaur *et al.*, 2006).**

While there were shortage in the data and research in the effect of GSE on the neurotransmitters and relation with oxidative markers in induced diabetic rats. Therefore the aim of this study was to investigate possible protective effects of GSE supplementation on brain neurotransmitters and serum butyrylcholinesterase (BChE) activity. Beside its related effects on the level of enzymatic and non-enzymatic antioxidants, LPO and nitric oxide in brain of alloxan diabetic rats.

2. Materials and Methods

2.1. Experimental animals.

Fourty male albino rats (*Rattus norvegicus*) weighing about (140-150 g) were selected for the present study. They were obtained from the animal house of Research Institute of Ophthalmology, El-Giza, Egypt. The chosen animals were housed in metal (stainless steel) separate bottom cages at normal atmospheric temperature (25±5°C) and under good ventilation and received water and standard balanced diet.

2.2- Induction of experimental diabetes.

Diabetes mellitus was experimentally-induced in animals fasted for 12 hours by intraperitoneal (I.P) injection of (150 mg kg^{-1} BW) alloxan monohydrate, freshly prepared in normal saline, was immediately injected. The level of blood glucose of 200-250 mg/dl was taken as diabetic in this study.

2.3- Chemicals.

GSE is marketed as (Gervital®) by (Arab Co. for Pharmaceuticals & Medicinal Plants MEPACO-Egypt). It is available as capsules of 150 mg concentration. All other chemicals were analytical grade and were obtained from standard commercial suppliers.

2.4- Experimental design.

The experimental animals were divided in to four groups, each group comprises of ten rats detailed as follows: Group 1, served as normal control rats; group 2, was considered as diabetic (alloxan induced) keep without treatment for 10 weeks; group 3, alloxan diabetic rats was received GSE (250mg/kg b.wt/day) in aqueous suspension orally for 10 successive weeks by gastric intubation and group 4, normal animals were treated with GSE at dose level of (250mg/kg b.wt/day) for 10 successive weeks by oral administration.

During the experimental period, body weight, blood glucose, food and water consumption and physical examinations were determined at regular intervals. The dosage was adjusted every week according to any change in body weight to maintain similar dose per kg body weight of rat over the entire period of study for each group.

Blood and tissue sampling:

At the end of the treatment period, the rats were fasted overnight (10h) and then sacrificed under diethyl ether anesthesia. Blood samples were collected from each rat, allowed to coagulate at room temperature, and then centrifuged at 3000 rpm for 20 min and the clear serum separated. The brain was dissected out, rinsed with ice-cold saline and homogenized. Tissue homogenate and sera were kept at −20◦C until further analysis.

2.5 Biochemical analysis.

Fasting and post-prandial serum glucose level was estimated spectrophotometrically using reagent kits from Reactivos Spinreact Company (Spain) according to method of trinder (1969). Serum insulin was assayed by radioimmunoassay kits of DPC (Diagnostic Products Corporation, Los Angeles, USA) via the method of Marschner *et al.* (1974).

The lipid peroxidation products were estimated by measurement of malondialdehyde (MDA) reactive product at 532 nm according to the chemical method of Preuss *et al.* (1998). Superoxide dismutase (SOD EC 1.15.1.1), Catalase (CAT, EC 1.11. 1.6), glutathione peroxidase (EC 1.11.1.9) activity, reduced glutathione (GSH), Vitamine C (Ascorbic acid) and nitric oxide concentration in homogenates were determined according to the chemical method of Marklund and Marklin (1974), Cohen *et al*. (1970), Paglia and Valentine (1967), Beutler *et al*. (1963), Harris and Ray (1935) and Montgomery and Dymock, (1961) respectively.

The brain neurotransmitters were determined by Pagel *et al.,* (2000). Butyrylcholinesterase (BChE) activity in serum was determined according to Knedel and Bottger (1967) using reagent kits obtained from bio-diagnostic, Egypt.

Statistical analysis

The data were analysed using one-ways analysis of variance (ANOVA) (PC-STAT, 1995) followed by least significant difference (LSD) analysis to compare various groups with each other. Results were expressed as mean ± standard error and values of $P<0.05$ were considered statistically significant.

3. Results

3.1 Effect of GSE on serum glucose and insulin concentration.

The treatment of diabetic rats with GSE induced a highly significant decrease on the elevated fasting and post-prandial serum glucose concentration in comparison with diabetic rats (Table 1).

Alloxan treatment produced a significant decrease in the serum insulin level with respect to the control group. The administration of GSE significantly elevated the level of serum insulin compared to diabetic group (Table 1).

3.2. Effect of GSE on brain lipid peroxidation products (Malondialdehyde)

Alloxan produced a significant increase in brain MDA level of diabetic rats. The administration of GSE ameliorated the alloxan induced elevation in lipid peroxidation moreover, treatment of diabetic rats with GSE normalize value of MDA production as compared to control rats. The results are shown in table (2).

3.2-Effect of GSE on enzymatic and non-enzymatic antioxidants in the brain.

The results of antioxidant enzymes (SOD, GPx, CAT) activities in brain homogenate are illustrated in table (2) respectively. A significant increase in SOD activity was detected in diabetic animals while significant decrease ($P<0.001$) in the activity of GPx and non significant increase in CAT activity, on the other hand the data represented in table (2) illustrate that diabetic animals showed a significant ($p<0.001$) decrease in brain glutathione, ascorbic acid and nitric oxide concentration when compared with normal animals.

The increment in brain SOD activity was modulated by the oral administration GSE to diabetic rats, while CAT and GPx activity in brain were significantly increased by oral administration of GSE as compared to diabetic control group. The use of GSE showed a highly significant increase ($p<0.001$) in glutathione and nitric oxide level while a significant increase ($p<0.01$) in ascorbic acid concentration was reported in diabetic treated rats in comparison with diabetic group.

Effect of GSE on brain neurotransmitters

The effect of GSE on the levels of epinephrine, norepinephrine, dopamine and serotonin (5-HT) in the brain tissues of control and experimental groups of rats is illustrated in fig 1, 2, 3 and 4 respectively. Alloxan diabetic rats demonstrated a significant increase in epinephrine, norepinephrine, dopamine and serotonin (5-HT) compared to control group. Administration of GSE for 10 weeks resulted in significant decrease of the above mentioned biomarkers ($p<0.001$) as compared to diabetic group.

3.5. Effect of GSE on serum butyrylcholinesterase (BChE) activity.

Regarding the activity of BChE levels indicated in table (1) respectively, both serum BChE activity in the diabetic group was significantly increased when compared with their corresponding control group. The treatment of diabetic rats with GSE induced a highly significant decrease ($p<0.001$) in serum BChE activity.

Table (1):Changes in FBS, PPBS, Insulin levels and BChE activity in serum of different groups.

	Normal	Diabetic control	Diabetic+GSE	Normal with GSE	LSD at 5%
FBS (mg/dl)	**85±5.54^{a}**	**220±7.30^{b}**	**143±1.81^{c}**	**81±3.51^{a}**	**14.737**
PPBS (mg/dl)	**147±2.9^{a}**	**243.5±7.28^{b}**	**199.3±4.04^{c}**	**108.9±6.02^{d}**	**15.77**
Insulin (mIU/ml)	**0.45±0.04^{a}**	**0.12±0.04^{b}**	**0.78±0.08^{a}**	**0.34±0.07^{a}**	**0.19**
Butyryl cholinesterase (U\L)	**150±11.09^{a}**	**445.26±43.6^{b}**	**218.21±8.07^{a}**	**153.85±7.66^{a}**	**68.42**

Table (2): Changes in LPO and various antioxidants in brain of different groups

	Normal	Diabetic control	Diabetic+GSE	Normal with GSE	LSD at 5%
MDA (nmolMDA/g/hr) x100	3.43±0.13^{c}	12.1±0.30^{a}	10.1±0.40^{b}	1.48±0.12^{d}	0.008
Glutathione (nmol\100mg)x100	119.5±15.06^{a}	42±5^{c}	222±10.8^{b}	221±27.23^{b}	0.492
SOD (U\g)x100	142±0.93^{a}	178±2.3^{b}	138±2.3^{a}	152±1.4^{c}	0.055
GPx (mU\l00mg)	168.8±3.29^{a}	134±1.31^{c}	183.3±0.66^{b}	171.6±2.15^{a}	6.998
Catalase (k.10^{2})x100	6.9 ±0.46^{c}	8±0.42^{bc}	10± 0.90^{a}	9.9±0.71^{ab}	0.019
(VitC) (mg\L)	44.54±2.71^{a}	35.77±2.33^{b}	49.81±2.15^{a}	48.27±2.17^{a}	6.924
Nitric oxide (µmol\L)	44.52 ±2.02^{a}	19.81±2.08^{c}	29.95± 2.07^{b}	45.43±2.19a	6.188

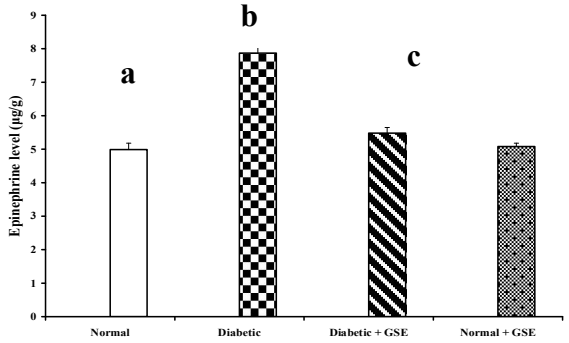

Fig 1·

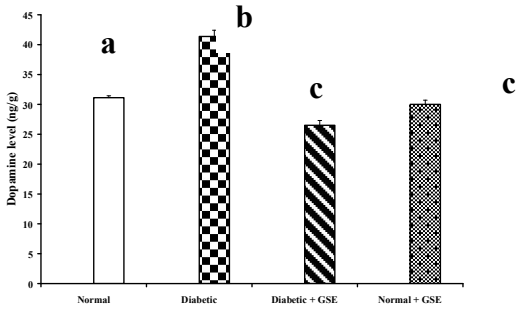

Fig. (3)

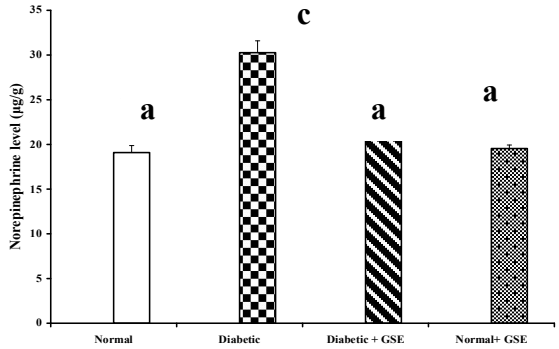

Fig. (2)

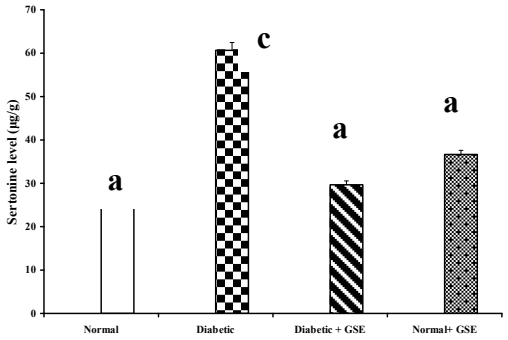

Fig (4)

4. Discussion

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia and insufficiency of secretion or action of endogenous insulin. Although the etiology of this disease is not well defined, viral infection, autoimmune disease, and environmental factors have been implicated **(Maritim *et al.*, 2003).** Alloxan-induced diabetes is a well-documented model of experimental diabetes.

Alloxan is known to destroy the β-cells of the islets of Langerhans resulting in a massive reduction in insulin release and hyperglycaemia **(Hashemi *et al.*, 2009).** In the present study the results obtained for fasting and post prandial serum glucose concentration of alloxan diabetic rats showed hyperglycemia as compared with the normal non diabetic rats. These results are in accordance with the results of **El-Alfy *et al.* (2005) and Bhattacharya *et al.* (2011)** who used alloxan diabetic animals. These effect were previously explained on the basis of alloxan's ability to produce hydrogen peroxide and other free radicals, including O_2• and OH• that damage β-cells hence leading to their death **(El-Alfy *et al.*, 2005).**

The alloxan-induced sustained hyperglycemia aggravates the oxidative stress status by auto oxidation of glucose and its primary and secondary adducts **(Sakurai and Tsuchiya, 1988).** The current study revealed that alloxan significantly induced hyperglycemia accompanied by hypoinsulinemia. Such effect might be explained by the possible pancreatic damage caused by rise in lipid peroxidation as well as inhibition in enzymatic and non-enzymatic antioxidants levels. The above result agree with **El-Alfy *et al.* (2005)** who reported that pancreatic β-cells are particularly susceptible to deleterious effects of ROS because of their low expression of the antioxidant enzymes genes as compared to other tissues.

Interestingly, the treatment of diabetic rats with GSE induced a profound increase in serum insulin level and in turn a decrease in fasting and post-prandial serum glucose level at the end of experiment. Such effect of GSE might be explained by the protective effect of GSE to restore the antioxidant status of pancreatic tissue and prevented the hyperglycemia and hypoinsulinemia induced by alloxan.

These results are in agreement with the finding obtained by **Pinent *et al*. (2004) and El-Alfy *et al.* (2005)** who observed the antihyperglycemic effects of GSE on diabetic rats. There have been increasing evidences suggesting that ROS generated under hyperglycemic conditions plays an important role in the development of diabetic complications, particularly vascular diseases involving both the macro-vasculature and micro-vasculature **(Yue *et al.*, 2005).** Diabetic neuropathy is one of the most common and assorted complications of long standing diabetes **(Negi *et al.*, 2011).**

Hyperglycemia increases oxidative stress through the overproduction of reactive oxygen species which results in an imbalance between free radicals and the antioxidant defense systems of the cell, such as antioxidants and antioxidant enzymes. Endogenous antioxidant enzymes (SOD, CAT, and GPx) are responsible for the detoxification of the deleterious oxygen species (Ugochukwu *et al.,* 2006).

The present data revealed that persistent hyperglycemia through alloxan generated ROS produced marked oxidant impact as evidenced by significant increase ($p<0.001$) in MDA levels in the brain tissue of diabetic rats higher than normal non diabetic animals. These findings are comparable to previous studies that have shown an increase in ROS concentrations in diabetic subjects **(Ugochukwu and Cobourne, 2003; Singh *et al*., 2005; Yue *et al.,* 2005).** In the current study, the GSE, being an antioxidant, is thought to suppress the over generation of ROS and therefore, eliminate the intracellular ROS level of the brain tissue in the experimental rats. GSE treatment post to the alloxan exposure has found to be effective in reducing the alloxan-induced brain oxidative stress under hyperglycemic condition.

Our results are in agree with the findings of **Chis *et al.* (2009)** who found that long-term administration of GSE offers enhanced antioxidant potential and protection of tissue lipid peroxidation and protein oxidation.

The enzyme SOD catalyzes the dismutation of the highly reactive superoxide anion (the first product of oxygen radical formation) to O_2 and to the less reactive species H_2O_2 **(Bhattacharya *et al*., 2011).** In this investigation, there is a significant increase in brain SOD activity of diabetic rats as compared with normal ones. This may be attributed to ROS that generated through glucose autoxidation and non-enzymatic protein glycation under hyperglycemia conditions in diabetes **(Wolff and Dean, 1987; Hunt *et al*., 1990).**

Moreover, ROS could be generated under diabetic conditions at early stages **(Ugochukwu and Cobourne, 2003).** Therefore, increase of SOD activity in brain is probably due to protective mechanism of the body in response to the increased generation of superoxide anion radicals. Similar results were found by Yue *et al.* (2005) in eyes and aorta of diabetic rats, SOD activity was significantly higher than that of normal group at 4 weeks. Different results were found by **Bhattacharya *et al.* (2011), Palsamy and Subramanian, (2011) and Choi *et al.* (2012)** who reported that, under hyperglycemia there is a reduction in the activity of SOD, which could be due to the glycation of this enzyme because of persistent hyperglycemia **(Yan and Harding, 1997).** Furthermore, H_2O_2 has been shown to inactivate SOD **(Bray *et al.,* 1974).** The hydrogen peroxide formed by SOD and other processes is scavenged by catalase (CAT), a ubiquitous heme protein that catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen **(Ugochukwu *et al.,* 2006).**

In the present study the activity of catalase in brain of diabetic and normal non-diabetic animals was not significant difference, this may be explained by **Kishi *et al.,* (2000**) who reported that the changes in antioxidant enzyme activity may related to duration of diabetes or due to post-translational modifications. This result in agreement with the result of **Yue *et al.* (2005)** who reported that CAT activities in the eyes and kidneys had no significant difference between the control and diabetic groups at both week 4 and week 8.

In contrast to our result, other studies reported an increase in CAT activity of the kidney of diabetic rats **(Ugochukwu and Cobourne, 2003)** or decreases according to other studies **(Bhattacharya *et al*., 2011).** However, the reason for the different response in CAT activities in different tissues to diabetes is not clear but this may be related to the concentration of H_2O_2 and/or the location of the enzyme besides the difference in the strain of the animals used, the duration of the experiment and/or the severity of diabetes.

GPx is an enzyme that detoxifies peroxides with GSH acting as an electron donor in the reduction reaction, producing GSSG as an end product **(Nogueira *et al.,* 2005)**.The enzyme activity was reduced in the diabetic animals as compared with normal. This attenuation may be due to ROS could cause inactivation of GPx activity and decreased availability of its substrate, GSH, which has been shown to be depleted during diabetes. This data agree with other results of some authors **(Ugochukwu *et al*., 2006; Umamaheswari *et al.,* 2009; Bhattacharya *et al.,* 2011 and Choi *et al.,* 2012)**. Glutathione (GSH), a tripeptide containing a sulfhydryl group is a highly distinctive amino acid derivative with several important roles including, protection from oxidative damage by serving as a sulfhydryl buffer and it is required to combat oxidative stress and maintain the normal reduced state in the cell. It is a peroxide scavenger that compensates the reduced effect of catalase thereby, trying to maintain the normal antioxidant defense status (Ugochukwu *et al.,* 2006). In addition to GSH, vitamin C and vitamin E are interrelated by recycling processes, because recycling of tocopheroxyl radicals to tocopherol is accomplished by reaction with ascorbic acid and dehydroascorbic acid is also produced in reaction with GSH. In the present study decrease glutathione and ascorbic acid concentration in brain of diabetic rats have been considered to be an

indicator of increased oxidative stress (Umamaheswari *et al.,* 2009). Lowered levels of GSH may also due to utilization of GSH by the GPx and Glutathione -S- transferase (GST) as their substrate (Umamaheswari *et al.,* 2009). The results are in agreement with the results of Palsamy and Subramanian, (2011) who reported that the levels of GSH, vitamin C and vitamin E are declined in the diabetic kidney portending the recycling of tocopheroxyl radicals to tocopherol may have been impeded that results in elevated lipid peroxidation (MDA).

The diabetic animals treated with GSE showed a significant amelioration in the elevated brain SOD activity while, improve in brain CAT, GPx activities, GSH and ascorbic acid (Vit. C) concentration. The data presented revealed that GSE offers enhanced antioxidant potential and protection against tissue lipid peroxidation.

A possible explanation for this effect is the antioxidant activity of grape seed polyphenols and their redox properties that allow them to act as reducing agents by donating hydrogen, quenching singlet oxygen or acting as metal chelators (Chis *et al*., 2009). The results obtained are in accordance with the findings of Anh *et al*. (2002) who reported that increased SOD and CAT activities are observed in liver tissue after feeding GSE and Sehirli *et al*. (2008) who showed that glutathione content increases significantly after consumption of GSE in humans.

GSE is a rich source of one of the most beneficial groups of plant flavonoids, procynadinies oligmers. These flavonoids exert many health promotings effects including the ability to increase the intracellular Vit. C levels decrease capillary permeability and fragility and scavenge oxidants and free radicals (Ozer *et al.,* 2011 and Benzer *et al.,* 2012). In contrast, Alía *et al.* (2003) reported that antioxidant enzymes such as SOD, catalase, and glutathione content did not change, but that glutathione peroxidase activity increased after consumption of grape seeds and grape skins.

Excessive ROS and oxidative stress in DM had been reported to be associated with subsequent impaired nitric oxide bioavailability (Ohtake *et al.,* 2007).

Nitric oxide (NO) plays an important role in cell signaling in the brain, and has been described as an unconventional neurotransmitter, because it is not stored in synaptic vesicles and not released upon membrane depolarization but released as soon it is synthesized and NO does not mediate its action by binding to membrane associated receptors but diffuses from one neuron to another and acts directly on intracellular components (Paul & Ekambaram, 2011). NO is synthesized in the brain upon demand as in cognitive condition for which NO activity is required. Neurons synthesize NO as a response to the activation of N-methyl-D-aspartate(NMDA) receptors by the excitatory amino acid glutamate (Paul & Ekambaram, 2011).

NO function as a neurotransmitter by stimulating soluble guanylyl cyclase to form the second messenger molecule, cyclic guanosine monophosphate (cGMP) in the target cells (Garthwaite & Boulton, 1995).

In the present study nitric oxide level in brain tissue of diabetic rats is significantly decrease as compared with normal rats as illustrated in table (2). This may be due to in diabetes mellitus, oxidants arise that interfere with the synthesis, diffusion, and action of NO on target proteins. In diabetic endothelial cells, oxidants may arise from endothelial nitric oxide synthase (eNOS), NADPH oxidase, or mitochondria

These oxidants inhibit endothelial function, by impairing the synthesis of NO, and in the case of superoxide anion (O_2^-), rapidly reacting with NO. The reaction between NO and O_2^- is key, because it is very rapid, and not only impairs the diffusion of NO to target cells, but also forms the very reactive product, peroxynitrite $ONOO^-$) which reacts with proteins, lipids, and DNA. Furthermore, oxidants arise in target cells exposed to elevated glucose and impair physiological function, via actions on ion channels and transporters important in NO function (Cohen, 2004).

The current study, indicated that diabetes has been decreased the synthesis of NO, induction of long-term potentiation and synaptic plasticity in the hippocampus of rats, suggesting that insulin deficiency and occurrence of blood sugar greater than normal level can result in an inhibition of NO synthesis and an impairment of cognitive behavior (Regan and McEwen 2002). GSE administration result in elevation of nitric oxide level in brain of diabetic rats and thus ameliorate cognitive condition.

Oxidative stress also plays a central role in diabetic tissue damage (Mastrocola *et al.* 2005). Besides the most common complications of the peripheral nervous system in diabetic patients (Bloomgarden, 2007; Dobretsov *et al*. 2007; Hoybergs *et al.* 2007), recent evidence has demonstrated that diabetes may also have negative impacts on the central nervous system (Gispen and Biessels, 2000; Trudeau *et al.* 2004; Li *et al.* 2005; Biessels and Gispen, 2005; Biessels *et al.* 2006;; Mijnhout *et al.* 2006 and Tuzcu and Baydas, 2006).

Oxidative damage to various brain regions constitutes the long-term complications, morphological abnormalities and memory impairments (Fukui *et al.* 2001). Diabetes mellitus has been reported to cause degenerative changes in

neurons of the central nervous system (Lackovic *et al*. 1990). The neurotransmitters, noradrenaline, serotonin and dopamine are important in the central nervous system regulation of many physiological processes including energy and glucose homeostasis (Halford *et al.,* 2005 and Gerozissis, 2008).

Neurotransmitters show significant alterations during hyperglycaemia resulting in altered functions causing neuronal degeneration. Neuropathic pain and neurons develop hyperexcitability in diabetic rats, attributed to disturbances in neurotransmitters pattern (Lackovic *et al*. 1990 and El-Seweidy *et al.,* 2009).

The data in the present study demonstrated that alloxan- induced diabetes result in a significant increase in the brain epinephrine (E), nor-epinephrine (NE), dopamine (D) and serotonin (5-HT) levels as compared to normal control. These results are in agreement with the result of Macro *et al.* (2008) who founded that tissues cells isolated from hyperglycemic animals demonstrated increased in norepinephrine and dopamine production/secretion. Brain neurotransmitters mainly norepinephrine, serotonin and dopamine showed a significant increase in STZ diabetic rats (El-Seweidy *et al.,* 2009).

Within the hypothalamus, increases in noradrenaline were observed following STZ treatment in the mouse (Chen and Yang, 1991) and rat (Barber *et al.,* 2003), while other workers have described no change (Stewart *et al.,* 1994) or a decrease (Ohtani *et al.,* 1997) in hypothalamic noradrenaline.

For instance, diabetes decreases the basal release levels of serotonin and dopamine in hippocampus (Yamato *et al.,* 2004), Ramakrishnan *et al.* (2005) reported an increase in the levels of dopamine. The apparent contradiction may be explained by the different time-points of the disease investigated. Altogether, a growing body of evidence suggests that diabetes impairs hippocampal neurotransmission (Baptista *et al.,* 2011).

Administration of GSE results in regulation of brain E, NE, DA and 5-HT levels. The protective effect of GSE against oxidative stress is due to grape seeds are a rich source of polyphenols, such as catechin, epicatechin, epicatechin gallate, epigallocatechin gallate, epigallocatechin, procyanidin B_1, and procyanidin B_2 (Choi *et al*., 2012) which inturn decrease deleterious effect of ROS on brain . In agreement with the current study Elseweidy *et al.* (2009) reported that Neuroprotective effects of green tea catechin were mostly attributed to its flavonoid content additionally its antioxidant effects.

There is evidence linking between BChE and diabetes mellitus (DM), where increased serum BChE activity is found in both type 1 and 2 forms of the disease in humans (Tvarijonaviciute *et al.* 2011).

In our study, we found a significant increase in serum BChE concentrations in rats with DM compared to control group. Increases of a similar magnitude have been reported in diabetic humans (Abbott *et al*., 1993) and also in dogs (Tvarijonaviciute *et al.,* 2011).

The cause of increased BChE concentration in DM remains unclear. Elevated glucose concentrations can stimulate BChE activity in vitro (Lunkes *et al.*, 2006), and a link between cholinesterase activity and lipid metabolism has been described in human diabetics (Alcântara *et al*., 2002), where serum BChE activity positively correlates with cholesterol and triglyceride concentrations (Abbott *et al*., 1993; Randell *et al.,* 2005).

In another study, no such correlation was found (Sridhar *et al.,* 2005) However, it has been postulated that BChE has a role in altered lipoprotein metabolism and in the hypertriglyceridaemia that occurs in DM (Iwasaki *et al*., 2007).

Insulin may have a direct effect on BChE activity that could account for the increases in BChE in type II DM, as it may stimulate the production of BChE by a CaCo-2 intestinal cell line (Randell *et al*., 2001), and increase the synthesis of BChE by the liver in cases of insulin resistance (Randell *et al*., 2005). In human patients, serum BChE activity has a significant positive correlation with serum levels of insulin and with insulin resistance in patients with type II DM and metabolic syndrome (Abbott *et al*., 1993; Cucuianu *et al*., 2002; Randell *et al*., 2005).

In the initial stages of Alzheimer's disease and diabetes mellitus, the activity of butyrylcholinesterase is increased whereas in the terminal stages of the disease and when these diseases are advanced butyrylcholinesterase activity is low (**Rao *et al.,* 2007**). Hence, when the concentrations of butyrylcholinesterase is increased it will lead to reduced levels of acetylcholine (whereas ACh is the principal vagus neurotransmitter) ACh is a neurotransmitter and has regulatory role on serotonin, dopamine and other neuropeptides (Rao *et al*., 2007). This may account for the decline in cognitive function (Sridhar *et al.,* 2010).

The diabetic animals treated with GSE showed a significant decrease in the level of serum BChE as compared to diabetic control. This decrease may be related to the regulation effect of GSE on hyperlipidemia and hyperglycemia. In agreement with our study, polyphenols of GSE was found to be of preventive effect on cardiovascular disease through their ability to reduce cholesterol absorption and plasma levels of triglycerides **(Zern and Fernandez, 2005)** beside antihyperglycemic effects of GSE were

also observed in diabetic rats (**Pinent *et al.*, 2004; Kiyic *et al.*, 2010)** and thus the high level of BChE is correlated. These effects of GSE polyphenols are due to their strong antioxidant activities of scavenging reactive oxygen (Shi *et al*., 2003).

The current results demonstrated a novel mechanism in which brain oxidative markers elevated and brain nitric oxide levels decreased leading to microvascular permeability changes during elevated brain neurotransmitter and vascular dementias in diabetic subjects. This is the first study to illustrate an association between oxidative stress, neurotransmitters (Epinephrine, Nor Epinephrine, dopamine, serotonin) and GSE supplementation and demonstrated that GSE improve the brain antioxidant activities and normalize the brain neurotransmitters.

Conclusion:

From the above mentioned results we can conclude that experimental diabetes produced metabolic disturbances in glucose, insulin that trigger brain enzymatic and non enzymatic oxidative stress that initiate disturbances in brain neurotransmitter, providing the incidence of nervous manifestation in diabetes. Administration of GSE has neuroprotective, hypoglycemic and antioxidants effects on diabetic rats and improving the biochemical alteration in diabetic rats. Therefore, GSE are expected to be effective for developing functional foods to avoid diabetic complications in neurotransmitters such as neurodegenerative disturbances.

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