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Protective Effect of Hesperidin, a Citrus Bioflavonoid, on Diabetes-Induced Brain Damage in Rats

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Abstract: 1- Among the numerous co-adjuvant therapies, which could influence the incidence and progression of diabetic complications, flavonoids, naturally occurring antioxidants, are currently being tested in several clinical and experimental trials. However, the protective efficiency of these compounds against complications in diabetic rat brain have rarely been investigated. 2- The aim of the present study is to evaluate the protective effect of hesperidin against diabetes-induced neuropathy in rat. 3- STZ-induced diabetes showed a significant ($p < 0.05$) increase in fasting plasma glucose, glycated Hb and fructosamine levels. The content of DNA; RNA and GSH significantly ($p < 0.05$) decreased whereas the content of lipid peroxide, as malondialdehyde (MDA), and nitric oxide (NO) significantly increased in rat brain. The activities of aldose reductase (AR); sorbitol dehydrogenase (SD) and cytochrome oxidase significantly increased whereas the activities of the antioxidant enzymes: glutathione reductase (GR); glutathione peroxidase (GPX); glutathione S-transferase (GST) and superoxide dismutase (SOD) significantly decreased in diabetic rat brain. 4- Oral administration of hesperidin (200 mg/kg b wt) to diabetic rats for 35 days resulted in significant ($p < 0.05$) improvement in the parameters studied. A decrease of plasma glucose, glycated Hb, brain NO levels and the activities of brain AR and cytochrome oxidase were observed along with the increase in brain DNA level and GST activity. Additionally, hesperidin administration produced a restoration of brain MDA; GSH and RNA levels and the activities of brain SD; GR; GPX and SOD. 5- The current results suggest that hesperidin exerts, efficiently, an attenuating effect on the progression of hyperglycemia and also on some diabetes-induced complications in rat brain.

Key words: Hesperidin, diabetic rat brain, polyol pathway, oxidative status, antioxidant systems, DNA and RNA levels.

INTRODUCTION

Neuropathy is quite common and, undoubtly, the major health problem among diabetic patients[1]. The development of diabetes-associated complications in the nervous system was found to be directly attributed to the increased glucose concentration as well as increased polyol pathway activity in brain of diabetic subjects. Many studies showed that hyperglycemia is among the contributing factors involved in most diabetic complications through excessive production of reactive oxygen spices (ROS)[2]. On the other hand, accumulation of sorbitol in nervous tissue of diabetic animals increased the cellular osmolarity resulting in water retention, cell oedema and an increase in cytosolic Na^+ concentration. Moreover, a novel monosaccharide phosphate, fructose-3-phosphate, was identified in the nervous system of diabetic animals. Such a compound is potent protein glycosylating agent and is an enzyme inactivator[3]. In addition to the changes in the intermediary metabolites of the polyol pathway, disturbances in NADPH and NADH balances, beside a reduction in glutathione level were also encountered in diabetes. All may contribute to the etiology of diabetic neuropathy[4].

Recently, there has been a growing interest in hypoglycemic agents from natural products especially those derived from plants[5,6], because plant sources are usually considered to be less toxic, with fewer side effects than synthetic sources. Over 200 pure phytochemicals are currently known to have hypoglycemic properties[7]. Several bioflavonoid, ubiquitously present in plants and common components of human diets, have been reported to improve hyperglycemia in diabetes mellitus by affecting glucose transport[8,9], insulin-like properties[10], and insulin-receptor function[11].

Hesperidin, a citrus bioflavonoid, exhibits biological and pharmacological properties, such as anti-inflammatory, anticarcinogenic, lipid-lowering and antioxidant activities[12,13]. A number of researchers have examined the antioxidant activity and radical scavenging properties of hesperidin using a variety of assay systems[14,15]. Recent studies examined the biochemical mechanism of the hypoglycemic effect of

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hesperidin[16], however, very little is known about the possible attenuating effect of such bioflavonoid on diabetes-induced neuropathy in rat.

Accordingly, our major goal, is to evaluate the neuroprotective efficiency of hesperidin administration against diabetes induced complications in rat brain.

Diabetes was induced by single i.p injection of streptozotocin (STZ) (50 mg/kg b wt). Hesperidin (200 mg/kg b wt) was supplemented to the diabetic rats for 5 successive weeks. The protective effect of hesperidin, on diabetic rat brain complications, was assessed by determinations of: polyol pathway enzymatic activities (aldose reductase (AR) and sorbitol dehydrogenase (SD)); oxidative stress biomarkers (lipid peroxide, as malondialdehyde (MDA), and nitric oxide (NO)); antioxidant systems (glutathione (GSH) level; glutathione reductase (GR); glutathione peroxidase (GPX); glutathione-S-transferase (GST) and superoxide dismutase (SOD) activities); apoptotic indicies (DNA; RNA levels and cytochromec oxidase activity). Also, plasma glucose; glycated Hb and fructosamine levels were investigated.

MATERIALS AND METHODS

Chemicals: Streptozotocin; hesperidin; enzymes; coenzymes were obtained from Sigma Co. (St. Louis, MD, USA). Other chemicals were from Analar grade or from purest grade available.

Animals and Treatment: Male albino wister rats, weighing 170-200 g, were injected intraperitoneally with STZ, (50 mg / kg b.wt.), freshly prepared in 0.1 M sodium citrate buffer, pH 4.5(17). During the first 24 hours of diabetes induction, STZ-treated animals were allowed to drink 5% glucose solution to overcome drug-induced hypoglycemia[18]. Treated and control animals were allowed free access to water and standard chow diet. Fourty eight hours after STZ administration, diabetes was confirmed by the presence of hyperglycemia and glucosuria. This was managed respectively by means of BM-hemoglucotest and glucotar strips. STZ-treated animals showed blood glucose less than 400 mg / dL and glucosuria lower than (+3) were discarded. This effectively minimized experimental variations due to different degress of the disease[19]. Ten days after the overt of diabetes, STZ-treated rats were randomly divided into two groups. The first group was the control diabetic one and the second was the treated group with hesperidin. Hesperidin was suspended in 0.5% sodium carboxymethyl cellulose and suspended in 0.5% sodium carboxymethyl cellulose and administered at daily oral dose of 200 mg/kg b wt for a period of 5 weeks. In addition to these diabetic groups, tow groups of normal control rats that received citrate buffer and sodium carboxymethyl cellulose were kept without treatment till the end of the experimental period.

Biochemical Analysis: At the end of the experimental period, all animals were sacrificed. Blood was collected in heparinized tubes, centrifuged at 600 xg for 15 minutes. The separated plasma was used for glucose estimation[20]. Portion of blood was collected in citrated tubes, centrifuged at 600 xg for 15 minutes. The separated serum was used for fructosamine estimation[21]. The remainder of blood was collected in citrated tubes and used for glycated Hb estimation(22). Meanwhile, the skulls were split on ice and salt mixture and the whole brains were frozen rapidly using a mixture of $CaCl_2$; NaCl and ice (-55°C). The frozen tissue was powdered, mixed and an accurately weighed amounts were treated differently for the separation and estimation of MDA[23]; NO[24]; GSH[25]; RNA[26] and DNA[27,28] levels. The isolated weighed brain tissues of another group of animals were used directly and treated differently for the separation and estimation of AR[29,30]; SD[31,32]; GR[33,17]; GPX[34,35]; GST[36]; SOD[37] and cytochromec oxidase[38] activities. The protein contents of the above supernatants were estimated by the method of Lowry *et al*[39].

Statistical Analysis: Results are given as the mean $\pm$ SEM. Comparison between groups were carried out by one way analysis of variance (ANOVA) followed by Kruskal-Wallis test for multiple group comparisons(40). P-values < 0.05 were considered significant.

RESULTS AND DISCUSSION

Our results showed that there is no significant difference between the obtained data of citrate-treated and sodium carboxymethyl cellulose-treated normal groups.

Effect on Plasma Glucose, Glycated HB and Fructosamine Levels: STZ-diabetes caused a marked elevation in blood glycated Hb, and serum fructosamine levels reaching to about 983%; 341% and 173%, respectively, compared to normal group values. Hesperidin treatment to diabetic rats, for 5 successive weeks, produced significant ($P < 0.05$) decrease in blood glucose and glycated Hb levels reaching to 47% and 50%, respectively, compared to diabetic group values (table 1).

Effect on Brain Polyol Pathway Enzymatic Activities: STZ-induced diabetes produced significant ($P < 0.05$) elevation in brain AR and SD activities reaching to about 241% and 147% respectively, of the normal values. Treatment with hesperidin to diabetic rats could normalize SD activity along with decrease significantly ($P < 0.05$) AR activity reaching to 62% of the diabetic brain values (table 2, Fig. 1).

Table 1: Effect of hesperidin treatment on plasma glucose; blood glycated Hb and serum fructosamine levels in diabetic rats.

Group / Parameters	Normal	Diabetic	Hesperidin treated
Plasma glucose mg/dL	122.6 ± 12.69	a 1210 ± 6.34	ab 571.2 ± 45.88
Blood Glycated Hb%	14.6 ± 1.34	a 49.8 ± 3.4	ab 25.3 ± 1.72
Serum Fructosamine m Mole / L	60.4 ± 5.14	a 104.5 ± 8.07	a 104.1 ± 5.85

Results expressed as means ± SEM.
a- Significantly different from the corresponding normal control at $p < 0.05$.
b- Significantly different from the corresponding diabetic control at $p < 0.05$

Table 2: Effect of hesperidin treatment on AR and SD activities in diabetic rat brains.

Group / Parameters	Normal	Diabetic	Hesperidin treated
AR n moles NADPH mg $prot^{-1}$. hr^{-1}	27.5 ± 2.13	a 66.2 ± 3.55	ab 41.5 ± 4.60
SD n moles NADH mg $prot^{-1}$. min^{-1}	35.6 ± 1.99	a 52.4 ± 2.48	b 37.8 ± 1.99

Results expressed as means ± SEM.
a- Significantly different from the corresponding normal control at $p < 0.05$.
b- Significantly different from the corresponding diabetic control at $p < 0.05$.

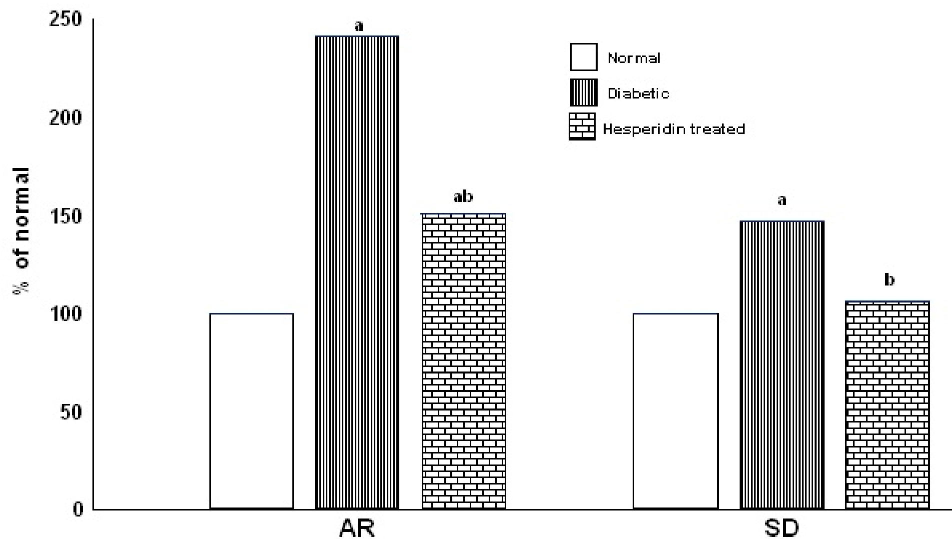

Fig. 1: Effect of hesperidin treatment on AR and SD activities in diabetic rat brains.
Results expressed as mean ± SEM.
a: Significant difference from the corresponding normal control at $p < 0.05$
b: Significant difference from the corresponding diabetic control at $p < 0.05$

Effect on Brain Oxidative Biomarkers: As shown in table (3) and Fig. (2), STZ-induced diabetes caused significant elevation in brain MDA and NO levels reaching to 209% and 264% respectively, compared to normal group values. Hesperidin administration to diabetic rats could normalize brain MDA level and decrease significantly ($P < 0.05$) NO level reaching to about 65% of the diabetic brain value.

Effect on Brain Antioxidant Defence Systems: Table (4) and Fig. (3) illustrated that STZ-diabetes significantly ($P < 0.05$) suppress all the studies antioxidant systems including GSH level; GR; GPX, GST and SOD activities reaching to about 46%, 73%, 74%, 78% and 61% of the normal group values, respectively. Treatment of the diabetic rats with hesperidin resulted in restoring all the studied

Table 3: Effect of hesperidin treatment on MDA and NO levels in diabetic rat brains.

Group / Parameters	Normal	Diabetic	Hesperidin treated
MDA m moles/gm t wt	40.2 ± 3.40	83.9 ± 4.98 [a]	40.4 ± 2.51 [b]
NO umoles/gm t wt	16.29 ± 1.483	43.1 ± 2.64 [a]	28.1 ± 2.2 [ab]

Results expressed as means ± SEM.
a- Significantly different from the corresponding normal control at $p < 0.05$.
b- Significantly different from the corresponding diabetic control at $p < 0.05$.

Table 4: Effect of hesperidin treatment on GSH level; GR; GPX; GST and SOD activities in diabetic rat brains.

Group / Parameters	Normal	Diabetic	Hesperidin treated
GSH µg/gm t. wt.	463.3 ± 11.72	214.7 ± 16.1 [a]	449.6 ± 18.1 [b]
GR u moles NADPH. mg $prot^{-1}$. hr^{-1}	121.0 ± 4.57	87.6 ± 3.48 [a]	131.4 ± 4.55 [b]
GPX m moles NADPH. mg $prot^{-1}$ hr^{-1}	79.8 ± 4.32	59.4 ± 4.71 [a]	90.2 ± 5.88 [b]
GST µ mole. mg $prot^{-1}.min^{-1}$	69.3 ± 1.5	53.8 ± 3.14 [a]	99.5 ± 6.15 [ab]
SOD µ/mg prot.	145.4 ± 4.63	88.2 ± 5.98 [a]	155.9 ± 6.01 [b]

Results expressed as means ± SEM.
a- Significantly different from the corresponding normal control at $p < 0.05$.
b- Significantly different from the corresponding diabetic control at $p < 0.05$.

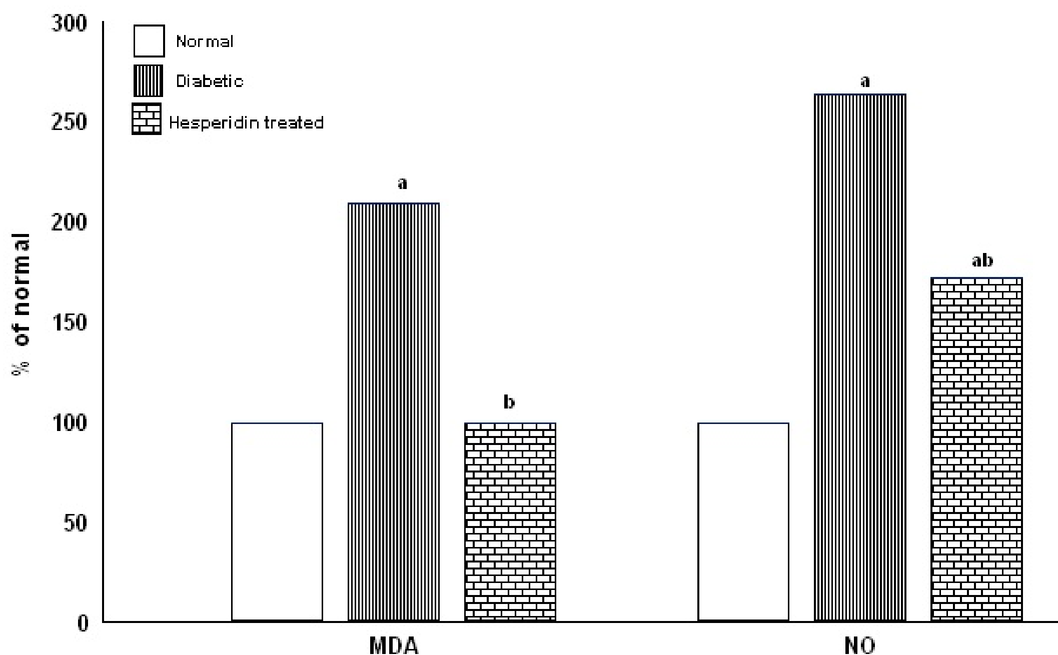

Fig. 2: Effect of hesperidin treatment on MDA and NO levels in diabetic rat brains.
Results expressed as mean ± SEM.
a: Significant difference from the corresponding normal control at $p < 0.05$
b: Significant difference from the corresponding diabetic control at $p < 0.05$

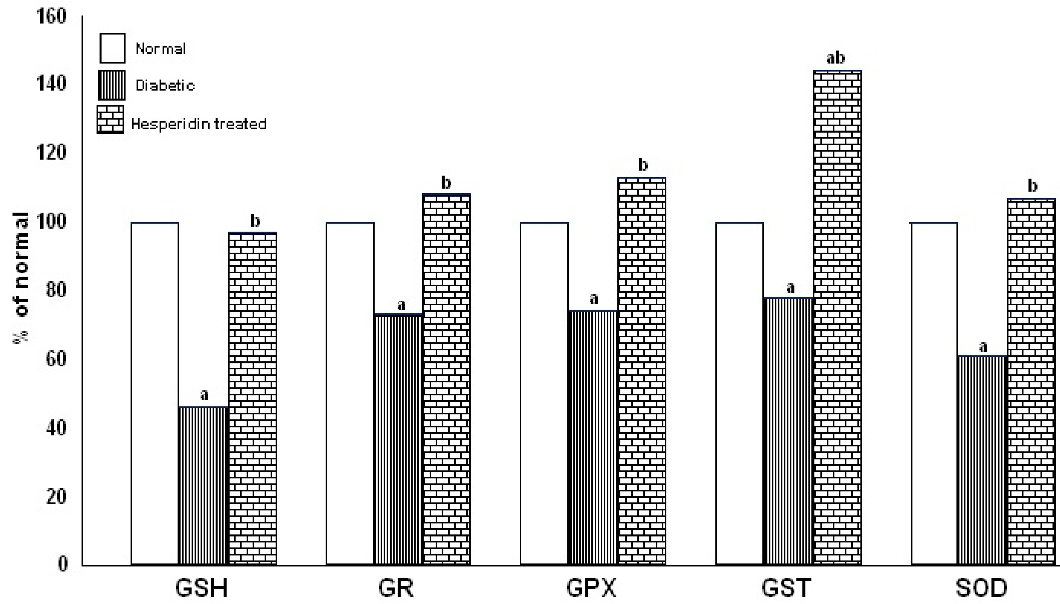

Fig. 3: Effect of hesperidin treatment on GSH level; GR; GPX; GST; and SOD activities in diabetic rat brains. Results expressed as mean ± SEM.
a: Significant difference from the corresponding normal control at $p < 0.05$
b: Significant difference from the corresponding diabetic control at $p < 0.05$

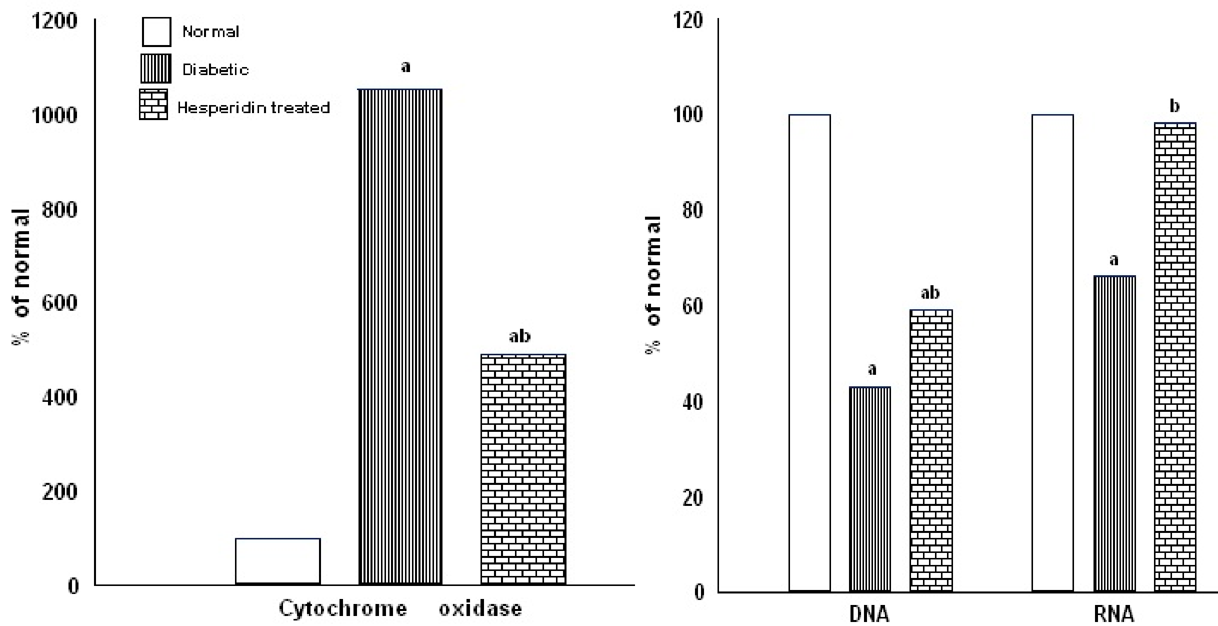

Fig. 4: Effect of hesperidin treatment on DNA;RNA levels and Cytochrome oxidase activity in diabetic rat brains.
Results expressed as mean ± SEM.
a: Significant difference from the corresponding normal control at $p < 0.05$
b: Significant difference from the corresponding diabetic control at $p < 0.05$

Table 5: Effect of hesperidin treatment on Cytochrome oxidase activity; DNA and RNA levels in diabetic rat brains

Group / Parameters	Normal	Diabetic	Hesperidin treated
Cytochrome c oxidase n moles reduced Cytochrome c mg $prot^{-1}$. hr^{-1}	10.4 ± 0.367	109.7 ± 8.338 a	50.9 ± 4.172 ab
DNA mg/gm t. wt.	10.39 ± 0.69	4.45 ± 0.185 a	6.09 + 0.22 ab
RNA mg/gm t. wt.	1.092 ± 0.067	0.72 + 0.035 a	1.072 ± 0.079 b

Results expressed as means ± SEM.
a- Significantly different from the corresponding normal control at p < 0.05.
b- Significantly different from the corresponding diabetic control at p < 0.05.

antioxidant parameters (GSH; GR; GPX and SOD) along with significant ($P < 0.05$) elevation in GST activity even compared to normal value.

Effect on Apoptotic Indices in Rat Brain: As shown in table (5) and Fig. (4), STZ-diabetes produced marked elevation in brain cytochrome oxidase activity reaching to about 1054% of the normal brain value, along with significant ($P < 0.05$) decrease in brain DNA and RNA levels reaching to about 43% and 66% of the normal group values respectively. Hesperidin supplementation could attenuate cytochrome oxidase activity, reaching to about 46% of the diabetic group value, together with significant increase in DNA level, reaching to about 137% as compared to diabetic group value, and normalization in RNA content.

Discussion: Dietary antioxidant compounds, such as bioflavonoids, may offer some protection against the development of diabetic complications. Previous studies[41,42] have shown that high blood glucose causes the deterioration of pancreatic B cells due to oxidative stress. Therefore, bioflavonoids, can have beneficial effects on pancreatic cells by neutralizing such oxidative stress. There is an inverse association between flavonoid intake and subsequent occurrence of several chronic diseases including diabetes mellities[43]. In the present study, hesperidin, supplementation for 5 successive weeks, was shown to lower, significantly, diabetes-induced elevation in the blood glucose level so that about 15% of the diabetic animals restored to the normal level, and the remainder animals exhibited lowered blood glucose values by about 53%, compared with the control diabetic group. Hesperidin plays an important role in preventing the progression of hyperglycemia, partly by increasing hepatic glycolysis and glycogen synthesis and/or by lowering hepatic gluconeogenesis[16]. Previously, hesperidin was found to reduce hyperglycemia induced by injection of alloxan in rat. This effect was stated to be linked to its ability to scavenge active oxygen radicals[44,45]. Recent study suggested that hesperidin is beneficial for improving hyperglycemia in type 2-diabetic animals by affecting the gene expression of glucose-regulating enzymes[46]. The metabolic control in diabetes mellitus can be assessed in several ways. The best studied parameter is the glycated Hb %, which is considered the method of choice for assessment of long term diabetic control[47]. As shown in our results, treatment with hesperidin was followed by significant decrease in glycated Hb %, reaching to 50% of the control diabetic group. Manuel *et al*[48] investigated that flavonoid could induce decrease in glycation as well as increase in the level and activities of plasma protein thiols as GPX. On the other hand, albumin and other serum proteins have been shown to undergo non-enzymatic glycosylation, both in vitro and in vivo, and in both animal and human[49,50]. Glucose bound to protein by aldimine linkage undergoes an amadori rearrangement to the ketomine, generically termed fructosamine[51]. All the diabetic rats, in the present study, were hyperglycemic with an increased Serum level of fructosamine which not modified by hesperidin, this result was agreed by Guillot *et al*[52].

In the present study, rats subjected to STZ-diabetes showed significant increase in brain AR and SD activities as compared to normal values. Previous researches revealed an accumulation of sorbitol and fructose in neural tissues of diabetic animals[53]. Increased activity of brain AR might attributed mainly to the marked elevation in glucose content demonstrated in diabetic rat brain by many investigators[54,29]. On the other hand, the increase in brain SD activity of diabetic animals might be considered as an enzymatic adaptation which facilitates the degradation of the accumulated sorbitol[55]. Enhanced metabolism of glucose via the polyol pathway play an important role in the pathogenesis of diabetic complications[56]. Sorbitol formation via the AR enzyme is coupled to the hexose-monophosphate shunt in oxidation-reduction reactions involving $NADP^+$ and NADPH. Sorbitol is converted to fructose by SD and a low activity of this enzyme may enhance the accumulation of sorbitol[2]. Although both AR and

SD mRNA were expressed in the rat Schwann cells, the levels of SD cDNA were much lower than those of AR cDNA[57]. SD over expression was found to stimulate reactive oxygen species generation in high glucose-exposed retinal pericytes and, subsequently, potentiate the cytopathic effects of glucose[53]. The ability of hesperidin to reduce significantly the increased brain AR activity might be attributed to its decreasing effect on the high brain glucose content via increasing its utilization especially through glycolysis. The stimulatory effect of hesperidin on the glycolytic pathway seems to be related to its powerful antioxidant property which may preserve the easily oxidizable sulfhydryl groups of rate limiting enzymes of the glycolytic pathway[16]. However, the ability of hesperidin in restoring the increased activity of brain SD, observed in diabetic rats, might be related to the obtained increase in the level of brain GSH by this treatment. Increased cellular GSH level was considered as one of the inhibitory factors for SD activity[58].

The present data revealed that rats subjected to chronic STZ-induced diabetes showed significant increase in brain MDA and NO levels, as a biomarkers of oxidative stress, as well as significant decrease in the antioxidant systems: GSH level; GR; GPX; GST and SOD activities. These results provide support for the previously reported diabetes-induced brain oxidative stress. Oxidative stress has emerged as a critical factor in the development of chronic diabetic complications[59]. In recently diagnosed type I diabetic patients, plasma levels of TBARS and lipid hydroperoxide were increased[60]. Increased oxygen free radical activity in diabetes has been ascribed to glucose-protein interactions[61], to auto-oxidation of glucose[62] and to glucose induced activation of the AR pathway[63]. High flux of glucose through the polyol pathway, as observed in our study, may consume NADPH, required for the reduction of GSH, which resulting in decreased level of the biologically active reduced form of this antioxidant metabolite. Additionally, GSH is involved in GSH peroxidase-catalyzed removal of peroxide formed by the scavenging action of SOD on oxygen free radicals[59]. Thus, oxidative stress causes depletion of intracellular GSH leading to serious consequences. Recent, in vitro, studies revealed that flavonoids may have considerable antioxidant activity in a wide range of chemical oxidation systems[64,67]. Flavonoids, as natural antioxidants, can scavenge various types of radicals in aqueous and organic environments[68]. In the present study, hesperidin administration could successefully ameliorate the oxidative stress damage, shown in the brain of diabetic rats, as represented by its powerful decreasing effect on the elevated brain MDA, reaching to about the normal value, as well as NO levels. In addition, hesperidin –treated diabetic rats showed well improvement for the endogenous antioxidant systems GSH; GR; GPX and SOD, reaching to near the normal level, as well as GST, reaching to higher activity even compared to the normal values. These findings suggest that hesperidin could suppress the oxidative stress shown in diabetic rat brain. A number of researchers have examined the antioxidant activity and radical scavenging properties of hesperidin using a variety of assay systems[15]. Hesperidin treatment has been previously demonstrated to improve GSH levels in liver and kidney of diabetic rats[69]. Hesperidin, in combination with Diosmin, has also been shown to inhibit the reactive oxygen radicals production in Zymosan-stimulated human polymorphonuclear neutrophils[45]. Recently, it was demonstrated that hesperidin administration could ameliorate the increased level of lipid peroxidation and also could improve level of endogenous antioxidant enzymes and GSH in liver after CCl_4 exposure which demonstrates the antioxidant effect of hesperidin[70]. Moreover, Kaur *et al*[71] investigated a beneficial effect of hesperidin in ameliorating of endotoxin-induced hepatic dysfunction possibly by preventing cytotoxic effects of NO and oxygen free radicals. Recently, Lee *et al.*[72] suggested that hesperidin may be a prodrug which is metabolized to hesperetin by intestinal flora. The data gained by Kim *et al*[73] showed that hesperetin can efficiently scavenge authentic peroxynitrite radical ($ONOO^-$) which is a reactive oxidant formed from superoxide O_2^- and nitric oxide (NO) that can oxidize several cellular components. Because of the lack of endogenous enzymes to thwart $ONOO^-$ activation, developing of specific $ONOO^-$ scavenger is remarkably important. Regarding the effect of hesperidin administration on the diabetes-induced decrease in brain GST activity, the present study revealed significant increase in the activity of such enzyme compared to both diabetic as well as normal group values. GST are a family of enzymes involved in the binding, transport and detoxification of a wide variety of endogenous and exogenous compounds[74]. GST play a role in cell defence by eliminating noxious reactive electrophilic xenobiotics and their metabolites as glutathione conjugates[75]. These electrophiles are potentially toxic species and can bind to nucleophiles, such as proteins and nucleic acids, causing cellular damage[76], as revealed in our study by marked decrease in DNA and RNA levels in diabetic rat brain. The activating effect of hesperidin on such enzyme might be related to that, cellular exposure to xenobiotics and antioxidants leads to coordinated induction of a battery of genes encoding detoxifing enzymes including GST[77]. Moreover, increase in brain GSH level, observed in the hesperidin-treated

diabetic group, may have a role in the increase of brain GST activity, since such enzyme make use of GSH in conjugating the xenobiotics and their metabolites.

Exposure to oxidants may lead to enhanced expression of the enzyme nitric oxide synthase (NOS), resulting in increased production of NO[78], as shown in the brain of STZ-diabetic rats. NOS has been identified as a source of ROS with special relevance to pathological condition. NO has limited radical reactivity and combine readily with O_2^-, and possibly H_2O_2, to produce the highly oxidizing, non radical compound, ONOO-[79]. $ONOO^-$ react with protein tyrosine residues to produce nitrotyrosine. Potential targets of tyrosine nitrosylation include many important cellular proteins. Results of the present study showed that hesperidin supplementation could significantly decrease the elevated brain level of NO radical shown in the diabetic group. This effect might be ascribed on the basis of the antioxidant property as well as the radicals scavenging efficiencies of hesperidin. The observed increased level of brain GSH, shown in hesperidin-treated diabetic group, might also participate in the lowering efficiency of hesperidin on the elevated brain NO level, since GSH could propably shift NO into a more stable antioxidant compound S-nitrosoglutathione. Previous data have shown that treatment with hesperidin suppressed production of PGE_2, nitrogen dioxide, and expression of iNOS protein using mouse macrophage cells[80,81]. Hesperetin, a hesperidin metabolite[72], showed time and dose-dependent inhibition of lipopolysaccharide -induced NO production and iNOS expression in macrophages[82]. Additionally, Timoshin *et al*[83] demonstrated that under the condition of CCL_4-induced oxidative stress, increase in the concentration of NO radical in rat heart and liver was observed, and after i.p. introduction of hesperidin, this effect was not observed. Moreover, previous study observed an inhibitory effect of flavonoids on lipid peroxidation and nitric oxide generation in endotoxin-induced shock in rat brain[84]. Also, it has been shown that some flavonoids were observed to inhibit the NADPH-diaphorase enzyme, prepared from the mouse brain, suggesting the ability of these compounds to inhibit the production of NO in the brain tissue[85,86]. The neuroprotective effect of flavonoids was reported by the observation that flavonoids attenuated up regulation of eNOS and iNOS in the brain and also reduced the BBB permeability disturbances, brain edema and cell injury[87].

Oxidative stress contributes to cellular damage and appears to be the common apoptotic mediator, most likely via lipid peroxidation[88]. The literature has supported the role of ROS in apoptotic cell death. Enhancement of ROS has been reported to elicit translocation of cytosolic bax to mitochondria and to activate bax to induce the release of cytochrom c, produced via the highly activated cytochrome oxidase reaction, shown in the diabetic rat brain, from mitochondria. Then, cytochrome c could stimulate caspases[89] and leading to apoptotic cell death[90], as revealed in our study by markedly decreased DNA and RNA levels shown in diabetic rat brain. Such results resemble that obtained by Kowluru and Koppolu[91] that increased oxidative stress in diabetes is involved in the activation of retinal caspase-3 and apoptosis of endothelial cells and pericytes. Accordingly, agents or antioxidants that can inhibit production of ROS or enhance cellular antioxidant defenses can possibly prevent apoptosis and protect cells from the damaging effects of oxygen radicals[92,93]. Hesperidin has shown in this study to exert some protective effects against the diabetes-induced brain apoptotic damage. This was represented by its improving effect on the decreased levels of DNA and RNA, reaching the later to about the normal level, as well as its attenuating effect on the highly activated cytochrome oxidase enzyme shown in the brain of diabetic rats. Such results were supported by previous observation that hesperidin has powerful protective effect on the radiation-induced DNA damage[94]. The attenuating efficiency of hesperidin on diabetes-induced brain apoptotic damage might be attributed to its decreasing effect on brain cytochrome oxidase activity as well as to its powerful antioxidant and free radicals scavenging efficiencies, as previously mentioned. Moreover, such effect might be related to the observed restoring effect of hesperidin on the active SD, shown in diabetic rat brain, which resulting in lowering of brain fructose level. Previous studies suggested that among sugars possessing high glycosylation potential, fructose and its metabolites[95]. Additionally, the markedly activating effect of hesperidin on brain GST may have an important role in such protection, since GST play a critical role in cell defence by eliminating noxious electrophilic xenobiotics and their metabolites, as previously mentioned. On the other hand, the increasing effect of hesperidin on brain RNA level, as compared to diabetic group value, might be attributed to its stimulating influence on the gene-expression of many of the glucose-regulating enzymes as reported by Jung *et al*[46].

In conclusion, our results demonstrated that STZ-diabetes induces a deleterious brain damage which is, in part, amenable to attenuation by hesperidin administration. The neuroprotective efficiency of hesperidin can be correlated directly to its improving effect on hyperglycemia as well as its antioxidant and radicals scavenging properties. Moreover, the attenuating effect of hesperidin on the active polyol pathway and the apoptotic damage, shown in diabetic rat brain, may also play a role in its neuroprotective efficiency.

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