Effect of Glucose Tolerance Factor (GTF) on cerebral cells alterations in Diabetic rats

Mona Shehadeh¹, Shifra Sela², Eilam Palzur³ & Nitsa Mirsky⁴

Abstract

Diabetes mellitus can lead to neuropathy and to cerebral cell death. Previously we showed that oral intake of Glucose Tolerance Factor (GTF) extracted from yeast reduced blood glucose and inhibited nephropathy and retinopathy in diabetic rats. The aim of our study was to evaluate the effects of diabetes on neurodegenerative processes in rat's brain and to examine the influence of GTF treatment on these parameters. Diabetes was induced by streptozotocin (STZ). Diabetic rats were divided into 3 groups: Healthy, Diabetic untreated, and Diabetic treated with GTF. The hippocampus, known as the most sensitive area in the brain to the deleterious effects of diabetes, was studied. Cerebral apoptotic cell death was evaluated by immunohistochemistry using the TUNEL assay, anti-glial fibrillary acidic protein (GFAP), and anti-neuron nucleus (NeuN) immunostaining. Blood glucose levels were remarkably increased in the STZ diabetic rats (healthy: 111.1±15.51, diabetic: 471.6±26.32 mg/ dl), and significantly decreased to 352±20.49 mg/ dl in GTF treated group. The number of TUNEL stained apoptotic cells was significantly higher in diabetic rats compared to healthy and diabetic treated with GTF. Our data shows that programmed cell death is increased in the hippocampus of diabetic rats, but can be decreased by an oral treatment with GTF.

Keywords: Diabetes, neurodegeneration, hippocampus, Glucose Tolerance Factor (GTF)
1. Introduction

Diabetes mellitus (DM) is one of the most prevalent chronic diseases with an emerging public health burden across the world [Roriz-Filho et al., 2009; Coleman et al., 2004]. Central nervous system (CNS) disorders are thought to be of the most debilitating complications of diabetes mellitus [Coleman et al., 2004]. Cognitive dysfunction in diabetic patients was first noted in 1922, when patients with diabetes, who were “free from acidosis but usually not sugar free”, were found to have impaired memory and attention on cognitive testing compared with controls [Kodl and Seaquist, 2008].

Although different domains may contribute to the cognitive deficits associated with diabetes, several reports have shown alterations and vulnerability of the hippocampus area in both diabetic patients and experimental models of diabetes [Beauquis et al., 2009; Fujioka et al., 1997]. Pharmaceutical research conducted over the past decades has shown that natural sources like herbs, medicinal plants and yeast extract, are potential sources for new drug candidates for many diseases in general, and diabetes in particular [Marles and Farnsworth, 1995].

The Glucose Tolerance Factor (GTF) is a dietary agent first extracted from Brewer's yeast [Schwartz and Mertz, 1959]. This natural substance reversed the impaired glucose tolerance of diabetic rats [Tuman et al., 1978; Mirsky, 1993], and diabetic patients [Grant and McMullen, 1982]. GTF can be extracted from several sources, like liver, black pepper, and kidneys. An especially rich source for GTF is brewer's yeast [Mertz, 1975; Anderson and Mertz, 1977; Mirsky et al. 1980]. The anti-diabetic activity of GTF extracted from yeast first demonstrated by Mertz and his group [Schwartz and Mertz, 1959; Mertz, 1975; Anderson and Mertz, 1977] has been successfully reproduced over the years by many research groups who showed the activity of the yeast derived GTF both in vivo and in vitro. GTF improved glucose tolerance and lipid profile both in diabetic animals [Weksler-Zangen et al., 2012; Nakhoul et al., 2006] and diabetic patients [Grant and McMullen, 1982].

In vitro studies with partially purified preparations of GTF showed stimulation of glucose metabolism in several tissues: GTF potentiated glucose oxidation to CO₂ in adipocytes [Anderson et al., 1978; Davies et al., 1985] and adipose tissue [Evans et al., 1973; Toepfer et al., 1977].
Several groups found an increased glucose transport to cardiomyocytes [Fischer et al., 1992], to yeast cells [Mirsky and Berdicevsky, 1994], and to adipocytes and myocytes [Weksler-Zangen et al, 2012], by the addition of GTF. Our group examined the oral administration of GTF in animal models for both types of diabetes, and found high and rapid anti-diabetic, hypolipidemic and antioxidant activity in diabetic untreated retinas [Mirsky, 2012], with a remarkable reduction in the complications of diabetes: nephropathy [Nakhoul et al., 2006], and retinopathy [Mirsky, 2012]. In addition, in vitro studies showed insulin mimetic and insulin potentiating activity for GTF [Weksler-Zangen et al, 2012; Mirsky, 2012].

We have studied healthy, diabetic, and diabetic rats treated for two weeks with GTF, and demonstrated large amount of Glial Fibrillary Acidic Protein (GFAP) staining in Muller cell layer in diabetic untreated retinas [Mirsky, 2012]. GFAP has been widely used as a cellular marker for retinal pathology. Normally it is expressed in retinal astrocytes, but under pathologic conditions like hyperglycemia or ischemia, GFAP can be detected in other retina's areas like Muller cells layer. A remarkable reduction in GFAP expression was demonstrated in retinas derived from diabetic animals treated with GTF, where GFAP could be seen only in the glial astrocytes layer, very similar to what was found for healthy retinas [Mirsky, 2012]. Additional studies from our laboratory detected the activity of sodium potassium ATPase, which is a marker for neuronal integrity, in retinas of healthy, diabetic, and diabetic rats treated with GTF, with a remarkably reduced activity in the diabetic retinas. This enzyme inactivation was prevented by GTF treatment: retinas removed from GTF treated rats showed restored sodium potassium ATPase activity [Mirsky, 2012].

Since it is known that diabetes can enhance neurodegenerative processes in the brain, and the retina is a part of the CNS, we propose that treatment with GTF will show beneficial effects on additional brain areas involved in the pathogenesis of neurodegenerative disorders, such as the hippocampus.

2. Materials and Methods

2.1. Experimental animals and study design

An experimental model of diabetic rats was used. All experiments were performed according to the guidelines of the Israeli council for animal care and experimentation at the Haifa University (IL0780610).
During the study the animals were housed in groups of 2-3 rats in a sterilized solid bottom cages with contact bedding, under controlled temperature and 12:12 h light/ dark cycle, and maintained on standard pellet diet and water supplied ad libitum. Five week old Sprague Dawley rats were injected with Streptozotocin (STZ) (60 mg/ kg body weight, intraperitoneal) to induce diabetes. Three groups (n=5 in each group) of rats were studied: 1. healthy control rats; 2. STZ diabetic untreated rats; 3. STZ diabetic rats treated with oral dose of 2 gr/rat of GTF every other day for 4 weeks, and 3 gr/rat for the following 4 weeks. All rats were killed after 8 weeks; the brains were excised and fixed in 4% formaldehyde in saline. The fixed brain tissues were analyzed for morphological changes and for cell death by Immunohistochemical staining.

2.2. Preparation of Glucose Tolerance Factor (GTF):

The isolation method is based on the procedure of Mirsky et al., [Mirsky et al., 1980]. Yeast extract (Merk, New Jersey, USA) was dissolved in methanol and refluxed for 3 hours. After cooling, the sediment was removed and the supernatant was evaporated to dryness, dissolved in water and loaded on DEAE-52 cellulose column (anion exchanger). The water wash eluted from the column was collected and loaded on a Dowex 50Wx8 column (cation exchanger) and eluted with 0.5N NH₄OH. The eluent was evaporated to dryness then dissolved in sterile water, filtered, and lyophilized, to yield partially purified material that was used in the study.

2.3. Blood samples collection

Blood glucose was measured once a week through the duration of the study, and also on the day prior to sacrifice. Glucose levels were measured with a Glucometer (Optium Exceed; Abbott Diabetic Care Ltd. UK) and test strips (Optium Plus; Abbott Diabetic Care Ltd. UK) by puncturing the rat tail with a single use capillary blood sampling device (Unistik 2 Normal; Owen Mumford Ltd. UK)

At the end of the study, rats were deeply anesthetized by intraperitoneal injection of Equithesin (4ml/Kg). The chest was rapidly opened, the blood was collected, a catheter was introduced into the ascending aorta, and the right atrium was incised. Heparin in saline (1000 I.U. heparin/ 1ml,) was perfused through the catheter
(20ml), at a rate of 25 ml/min followed by perfusion at the same rate of 40–50 ml of fixative (4% formaldehyde in saline).

The brains were carefully removed and stored in this fixative for at least 24 h, then placed in 10% formaldehyde for 12 h before gross examination. Brain coronal sections were embedded in paraffin. Sections (5-µm thick) were cut with a rotary microtome, part of the sections was stained with haematoxylin-eosin (H&E) and examined under light microscopy, while the other parts were stained for immunohistochemistry (see below).

HbA1c levels were determined in whole blood collected immediately following animals' killing, according to the MULTIGENT hemoglobin A1C assay on the ARCHITECT system (Abbott laboratories, USA, Illinois).

2.4. Immunohistochemistry

2.4.1. Tissue section preparation

Staining was performed on formalin fixed paraffin embedded sections. The sections were de-paraffinized with xylene and rehydrated with a graded series of ethanol before staining.

2.4.2. TUNEL assay

In order to perform a quantitative evaluation of brain cell death, we performed TUNEL assay using the ApopTag® Peroxidase In SituOligo Ligation (ISOL) Apoptosis Detection Kit (Chemiconinternational, Inc., Temecula, California).

2.4.3. Neuronal Nuclei (NeuN) staining

NeuN staining was performed with anti-NeuN, clone 60, Monoclonal Antibody (Millipore corporations, Temecula, California).

2.4.4. Glial Fibrillary Acidic Protein (GFAP) staining

GFAP staining was performed with Anti-Glial Fibrillary Acidic Protein; clone GA5, Monoclonal Antibody (Millipore corporations, Temecula, California).
2.5. Statistical Analysis

Results are presented as means ± SEM and were evaluated by using ANOVA followed by Bonferroni's Multiple Comparison Test. P < 0.05 was considered statistically significant.

3. Results

3.1. Effect of GTF administration on body weight and basal glucose levels

All three experimental groups of rats had similar body weights at the beginning of the study. Eight weeks from the injection of STZ, both diabetic groups showed a significant lower body weight compared with healthy rats. Still, diabetic treated with GTF had significantly higher body weight than untreated diabetic rats (Figure 1).

**Figure 1**: Rat's body weight at the beginning, 4 and 8 weeks of the study, of the different experimental groups. ANOVA followed by Bonferroni's Multiple Comparison Test were used, **p<0.01, ***p<0.001.**
The effect of GTF administration on basal blood glucose levels, following a night starvation, was determined in the three animal groups in the 6th and 7th week of the experiment.

As can be seen from Figure 2, there is a difference in the 6th week of the experiment between basal blood glucose of untreated diabetic rats and diabetic treated with GTF (397±46, and 284±89 mg/dl respectively). Moreover, the difference between these groups increased during the 7th week (430±69 and 217±87 mg/dl respectively). While glucose level constantly climbed in the untreated diabetic group, it was attenuated in the group of diabetic treated with GTF.

Figure 2: Basal glucose levels of the three experimental groups, measured after an overnight fast in the 6th and 7th week of the study. Values represent means ±SEM.

Figure 3 presents glucose profiles of the different groups through 6 hours experiment in the second week of the study (one week of treatment with GTF). Blood glucose levels were monitored every 30 minutes following GTF administration. All 3 groups were fed, and time zero values were taken before the administration of GTF (2gr/rat), or water (as placebo), to the animals. While glucose levels of untreated diabetic rats were constantly high through all the experiment, a single dose of GTF administered orally to STZ diabetic rats resulted in an immediate decrease in blood
glucose. The maximal glucose reduction was achieved within 180 min and then glucose values gradually increased.

**Figure 3**: Blood glucose levels in response to administration of 2 gram GTF/rat at zero time to fed animals. Values represent means±SEM, analyzed by Two-way ANOVA, followed by Bonferroni post-tests *p<0.05, ***p<0.001 between diabetic and diabetic treated with GTF (n=5).

3.2. Effect of GTF supplementation on glycosylated hemoglobin (HbA1c) level:

HbA1c levels were determined in whole blood collected after 8 weeks, immediately after the animals were killed. HbA1c levels of the GTF treated and non-treated diabetic rats were similar and significantly higher compared to the healthy group.
3.3. Effect of GTF supplementation on cerebral cell apoptosis in the CA1 area of the hippocampus:

Apoptotic cells were assessed semi-quantitatively by an examiner blinded to the individual animals group, using the TUNEL method. We counted only the overstained cells which had apoptotic morphology such as: cell shrinkage, rounding, and chromatin condensation.

The number of TUNEL stained apoptotic cells was significantly higher in the non-treated diabetic rats compared to the healthy group and to the diabetic treated with GTF. GTF had a beneficial effect on decreasing apoptosis in the hippocampus CA1 area of the brain of the diabetic rats (Figure 5).
Figure 5: Apoptotic cell count+SEM (detected by TUNEL assay) in hippocampus of healthy control rats, untreated diabetic rats and diabetic rats treated with GTF (A). Analyzed by one way ANOVA, followed by Bonferroni’s Multiple Comparison Test *p<0.05, ***p<0.001 (n=5). TUNEL staining in the hippocampus CA1 region (magnification x100) of healthy rats (B), diabetic (C) and diabetic treated with GTF (D). Apoptotic cells are indicated by arrows.

3.3.1. Effect of GTF supplementation on neuronal survival in the CA1 area of the hippocampus:

Only neurons containing visible nuclei were assessed semi quantitatively by a blinded examiner to the animal group. No significant effect on the neuronal counts was observed in the CA1 region of the hippocampus after 2 months of hyperglycemia in all three experimental groups (Figure 6A & 6B).
Figure 6: NeuN positive cells count+SEM of healthy controls, untreated diabetic rats and diabetic rats treated with GTF (A); Analyzed by ANOVA, followed by Bonferroni's Multiple Comparison Test (n=5). Neun positive stained neurons in the CA1 area of the hippocampus (magnification x100) of healthy rats (B), diabetic (C) and diabetic treated with GTF (D). NeuN cells are indicated by arrows.

3.3.2. Effect of GTF supplementation on glial cells in the CA1 area of the hippocampus:

GFAP positive cells were assessed semi quantitatively by a blinded examiner to the animal group. The cells counted were only these detected with nucleus and dendrites.

The estimated number of GFAP positive cells was significantly lower in both diabetic group and the GTF group, compared to the healthy group. No significant difference was observed between the GTF and the diabetic group (Figures 7A and 7B).
Figure 7: GFAP positive cells count±SEM of healthy controls, untreated diabetic rats and diabetic rats treated with GTF(A); Analyzed by ANOVA, followed by Bonferroni’s Multiple Comparison Test (n=5). *p<0.05, **p<0.01.

GFAP positive stained neurons in the CA1 area of the hippocampus (magnification x100) of healthy rats (B), diabetic (C) and diabetic treated with GTF (D). GFAP cells are indicated by arrows.

4. Discussion

This study describes the effects of a single daily dose of GTF administered orally for two months to STZ induced diabetic rats. Oral administration of GTF caused a significant decrease in blood glucose of the treated rats for the whole duration of the experiment, similarly to our previous results [Mirsky, 1993; Weksler-Zangen et al, 2012; Nakhoul et al., 2006]. On a daily basis a significant glucose reduction was achieved within 120-180 minutes following GTF intake, and lasted for several hours. However, HbA1c levels at the end of the study were similar in both diabetic groups, and significantly higher than those measured in the healthy group.

This can be explained by the turnover of the erythrocytes in Sprague Dawley rats, which is 61±1.3 days [Derelanko, 1987], meaning that the hemoglobin remains glycated until the erythrocyte turns over.
Animals receiving GTF had significantly lower basal glucose levels after an overnight fast, compared to untreated diabetic animals. These findings are in concert with our previous data on decreased blood glucose in diabetic rats following oral treatment with GTF [Mirsky, 1993; Weksler-Zangen et al., 2012; Mirsky, 2012]. We found in the present study that GTF treatment resulted also in increased body weight, suggesting that GTF modestly stimulated glucose incorporation into lipids and inhibited lipolysis in fat cells [Edens et al., 2002], or prevented utilization of body's reservoirs of glycogen and proteins for energy [Mirsky, 2012].

In the present study, although we found a significant decrease in blood glucose of the treated animals, and a significant change in the number of apoptotic cells in TUNEL reaction, still, in other neuronal and glial staining methods we could not show a difference between non treated diabetic animals and diabetic treated with GTF.

Based on the beneficial effect of GTF on retinopathy presented in our previous studies [Mirsky, 2012], it was of our interest to examine GTF effect on cerebral cell death in the brain of diabetic animals.

Apoptosis determined in diabetic brains by the TUNEL staining assay [Lechuga-Sancho et al., 2006; Li et al., 2002], showed that neuronal apoptosis occurs in hippocampus of diabetic rats. We demonstrated in the current study that the number of TUNEL positive apoptotic cells presented in the CA1 region in the hippocampus of rats, are significantly higher in the diabetic group. Our findings are in agreement with the literature, showing that apoptosis is increased in hippocampal region of diabetic rats [JafariAnarkooli et al., 2008]. In addition, to the best of our knowledge, our present study shows for the first time that GTF treatment decreased the number of TUNEL positive apoptotic cells in diabetic rat's brain.

Apoptosis has been implicated in several neurodegenerative disorders like Alzheimer's disease. The incidence of Alzheimer's disease among diabetic patients is almost twice higher than in the non-diabetic population [Ott et al., 1999]. Moreover, cognitive impairments are more common in diabetic population than in the non-diabetic population [Ferguson et al. 2003; Kodl and Seaquist, 2008]. Since neuronal death underlies the symptoms of many neurological disorders, and neuronal loss in type 1 diabetes is associated with cognitive impairment, we
examined whether neuronal loss and astroglial activation occurs in the hippocampus area of the brain, given that astrocytes are modulated in the brain of diabetic animals [Coleman et al., 2004; Baydas et al., 2003; Saravia et al., 2002], and play an important role in neuronal metabolism, function and survival. Apoptotic changes in this cell type may be implicated in the pathophysiology of the disease. Additional finding in our study shows a significant decrease in the estimated number of GFAP$^+$ cells in diabetic animals, compared to their counterparts. This decrease in GFAP$^+$ probably results from both decreased proliferation and increased cell death. GTF treatment did not affect the number of GFAP$^+$ cells observed in diabetic animals.

The changes reported in the literature for GFAP$^+$ level in diabetic rat hippocampus are conflicting, and several possibilities may account for the differences: a) Apparently the time of evaluation of diabetes had an effect on this parameter; several investigators reported changes in GFAP positive cells number only after 16 weeks [Saravia et al., 2002]; b) Astroglial responses may vary according to the severity of diabetes [Lechuga-Sancho et al., 2006], c) Not all astrocytes are identical [Hewett et al., 2009], and astrocytes in different brain regions respond differently to diabetes; d) The variations in the results between the different studies may arise from the different ways of STZ administration (IP/IV), dosages of STZ, species variation in response to diabetes, fixation techniques, and/or differences in the antibodies used.

In our study, two months of diabetes had no significant effect on the neuronal counts in the CA1 region of the hippocampus. The present results are supported by the literature [Li et al., 2002], as abnormalities were not present in 2-month diabetes, but became evident later, after 8 months of diabetes, indicating that neuronal loss has a time dependent effect and can occur only after a prolonged duration of diabetes. Several properties of astrocytes suggest that they may react early to diabetes-related changes in glucose metabolism [Lebed et al., 2008]: a) Astroglial cells represent a critically important constituent of a brain tissue being closely involved in blood-brain exchange; Astrocytes are ideally suited to be the cellular locus of glutamate-induced glucose uptake and utilization.

Thus, astrocytic end feet surround intra parenchymal capillaries, which represent the source of glucose; this cyto-architectural arrangement implies that astrocytes form the first cellular barrier that glucose entering the brain parenchyma encounters, and it makes them a likely site of prevalent glucose uptake [Pellerin and Magistretti, 1994]. This may actually explain their higher sensitivity to the high levels
of glucose compared to neurons; b) Astrocytes may increase glucose uptake to protect neurons (the traditional view of glucose metabolism); c) Recent studies suggest that astrocytes may metabolize glucose to lactate or other intermediates then shuttled to neurons (Astrocyte Neuron Lactate Shuttle Hypothesis—ANLSH) [Pellerin and Magistretti, 1994], so glucose cytotoxicity mainly affects astrocytes, while neurons may be affected later when astrocytes are largely damaged; d) Brain glycogen, which is localized predominantly in astrocytes, may serve as a local glucose deposit for later mobilization as a neuronal energy substrate.

If explained according to the traditional view of glucose metabolism, the formation of lactic acid should have the same effect on astrocytes and neurons, because glucose enters both cells.

Lactate is transported in and out of cells by proton-coupled Monocarboxylate transporters (MCTs). It seems that changes in expression of MCTs could alter the metabolic responses of a brain region upon activation [Pierre and Pellerin, 2005]. MCTs have been altered in the brain within hours by ischemia [Tseng et al., 2003], and after 6 weeks on diet induced ketosis [Leino et al., 2001]. The study of monocarboxylate transporters in the CNS may be of a great importance to fully evaluate how energy metabolism represents a critical factor for brain function.

Regarding GTF effect on astrocytes after two months of treatment, the rats in this study were exposed to severe hyperglycemia (>450mg/dl). Diabetic damage to astrocytes can be irreversible by causing intracellular acidosis to glial cells by the formation of lactic acid due to increased glucose concentrations, and its utilization to form lactate [Staub et al., 1996]. Preliminary studies done in our laboratory on neuronal cell cultures (PC12 and neuroblastoma SH cells), showed an insulin-like effect of GTF along insulin signaling pathway in these cells, very similar to our previous findings in adipocytes and myocytes [Mirsy, 2012; Weksler-Zangen et al., 2012].

We also found that while the viability of neuronal cells (detected by MTT and FACS reactions) decreased in media with x5 concentration of glucose, the addition of GTF to the medium increased the viability of the cells in a dose dependent manner [Mirsy et al, unpublished results]. In addition to the effects shown for GTF treatment on decreasing basal glucose level in diabetic rats [Mirsy, 1993; Weksler-
[Zangen, 2012], we also found in the present study decreased apoptosis induced in the hippocampus of diabetic rats treated with GTF when compared to diabetic untreated rats.

NeuN staining of the hippocampus neurons showed no difference in the cell counts between diabetic and GTF treated rats. We suggest that GTF does not affect certain cell type but all types of cells in the hippocampus.

We imply that GTF mediates its anti-apoptotic effect not only by affecting insulin signaling pathway but also by its antioxidant activity, as previously shown [Nakhoul et al., 2006]. The level of lipid peroxidation in kidneys and hearts of diabetic rats were reduced after two weeks of treatment with GTF. Many studies evaluated the effects of oxidative stress and antioxidant systems in the CNS in diabetes mellitus [Baydas et al., 2002; Celik et al., 2002]. Excessive production of free radicals is believed to be involved in many diabetic complications, including diabetic neuropathy [Sima and Sugimoto, 1999]. In addition, mitochondria isolated from the brain of hyperglycemic rats showed increased ROS levels [Mastrocola et al., 2005].

The direct glucose toxicity in the neurons is especially due to increased intracellular glucose oxidation [Nishikawa et al., 2000], which leads to an increase in ROS production [Bonnefont-Rousselot, 2002]. In both man and experimental diabetic rats, oxidative stress seems to play a central role in developing brain damage [Arvanitakis et al., 2004]. Emerging evidence shows that the increased oxidative stress and consequent oxidative damage observed in hyperglycemic conditions begins in the mitochondria, which is the major site of ROS production [Duchen, 2004]. ROS are by products of the mitochondrial respiratory chain that are physiologically counteracted by the intracellular antioxidant systems [Green et al., 2004]. The overproduction of ROS induced by enhanced glucose oxidation might overwhelm the antioxidant defenses, leading to cell damage. It has been reported that normalizing superoxide mitochondrial production blocks the pathways of hyperglycemic damage [Nishikawa et al., 2000].

A pitfall of this study lies in the absence of microglia staining, since mitochondrial dysfunction was found in microglial cells in several animal models of neurodegeneration and aging. Mitochondria are crucial regulators of cell death, a key feature of neurodegeneration [Lin and Beal, 2006]. Microglial cells in the brain are self-maintaining cells that are normally not renewed by bone marrow-derived progenitor
cells, and are therefore prone to the accumulation of mitochondrial damage [Ajami et al., 2007].

Hyperglycemia is a condition found to be associated with GLUT expression in the brain, specific increase in GLUT3 mRNA and protein were observed in the hippocampus of hyperglycemic animals [Reagen et al., 1999]. The levels of GLUT1 mRNA in micro vessels in hyperglycemic rats were increased [Simpson et al., 1999]. It is likely that regulation of GLUTs mRNA and protein expression is an important therapeutic target for the prevention of hyperglycemic brain damage. We suggest performing also GLUT staining to find out whether it might be affected by GTF treatment.

In summary, although GTF caused a significant decrease in blood glucose, only slight differences were found between diabetic and diabetic treated with GTF in neuronal and glial staining. Our data suggests that hyperglycemia causes a programmed cell death in the hippocampus, supporting the notion that damage to the brain can be one of the severe complications of diabetes. These studies have to be repeated with higher doses of more purified and better identified GTF, and for a longer period of treatment.

There has been gathered a substantial data on GTF composition and characterization, including recent data from our laboratory: It has a molecular weight below 1000 Dalton. It is cationic and soluble in water. GTF is stable to high and low pH and it retains its activity up to 12 months in 4°C. GTF is also stable to proteolytic enzymes. These findings enable an oral treatment with GTF, in contrast to insulin, which is a protein and has to be injected [Mirsky, 1993; Weksler-Zangen et al., 2012; Nakhoul et al., 2006; Mirsky, 2012]. Although the exact composition of GTF has not been fully elucidated, the activity of GTF as an anti-diabetic substance in general, and a potential treatment for diabetic brain complications in particular, position the research on GTF as one of the promising investigations in the future.
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