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Glucose Tolerance Factor – Insulin Mimetic and Potentiating Agent – A Source for a Novel Anti Diabetic Medication

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/54350>

1. Introduction

Diabetes is the world's most common metabolic disease and one of the leading causes of morbidity and mortality. The medications currently in use are limited in their potency, have many side effects, and cannot be tolerated by many patients. As a result of the global epidemic of diabetes, the need for new diabetes therapies is expected to grow dramatically during the next decade. An intense research has been conducted to identify new therapeutic targets and pharmacologic compounds that might correct the impaired glucose tolerance. Materials that mimic insulin action or augment the effect of residual endogenous insulin are likely to be beneficial for both type 1 and 2 diabetic patients. During the recent years many investigators have shown that natural products are a potential source for new drug candidates for many diseases in general, and diabetes in particular. A research aimed at revealing new natural sources to treat diabetes is of high importance.

A variety of traditional anti diabetic plants are known in the folk medicine. Although some of them have been studied for their anti diabetic effects, the knowledge on their efficacy and mechanism of action is very limited.

The Glucose Tolerance Factor (GTF) is a dietary agent first extracted from Brewer's yeast [1]. GTF reversed the impaired glucose tolerance of both diabetic rats and diabetic patients. In vitro studies with GTF showed remarkable increase in glucose transport into adipocytes, and cardiomyocytes. An increase in glucose incorporation into glycogen in rat hepatocytes was also found for GTF preparations [2].

Despite the high anti diabetic activity of this natural compound, GTF has not been fully characterized or identified, mainly due to the instability of the purified fractions. Our laboratory succeeded in extraction and partial purification of an active and stable GTF

preparation from brewer's yeast. We examined GTF effects in animal models for both types of diabetes, and found high and rapid anti diabetic activity. We also examined GTF effects on the cellular level and found high insulin mimetic and insulin potentiating activity for GTF. The mechanism of action of GTF along insulin signaling pathway was also studied.

2. Prevalence of diabetes mellitus

Diabetes is the world's most common metabolic disease and one of the leading causes of morbidity and mortality. According to WHO (World Health Organization) report [3], **346** million people worldwide are diabetic. The number is expected to grow to **438** million until 2030 [8% of the world population!] [4]. Diabetes is the greatest healthcare threat in both developed countries and the third world: diabetes is the third leading cause of death in most developed countries; moreover, it is epidemic in many third world nations. Diabetes imposes an increasing economic burden on national health care systems worldwide [5]. The global health expenditure on diabetes in 2010 was 376\$ billion and is expected to grow to 490\$ billion by 2030 [6].

3. Diabetes mellitus and its complications

Diabetes mellitus is a complex syndrome involving severe insulin dysfunction along with gross abnormalities in glucose homeostasis and lipid and protein metabolism. The disease is generally divided into two major types: Insulin Dependent Diabetes Mellitus (IDDM, or type 1), and Non Insulin Dependent Diabetes Mellitus (NIDDM, or type 2 DM). Both forms are devastating with respect to their latter complications. People with diabetes have a 25-fold increase in the risk of blindness, a 20-fold increase in the risk of renal failure, a 20-fold increase in the risk of amputation as a result of gangrene and a 2 to 6-fold increase in the risk of coronary heart disease and ischemic brain damage. In general, life expectancy for a person with diabetes is decreased by one-third [5].

4. Diabetes mellitus and oxidative stress

Oxidative stress and non enzymatic glycation play a major role in the pathogenesis of diabetes mellitus [7, 8]. During diabetes, persistent high concentrations of blood glucose increase the production of oxygen free radicals – OFRs. through auto oxidation of glucose Hunt et al., 1990, and also by non enzymatic lipid and protein glycation [9]. OFRs react with membrane phospholipids forming malondialdehyde (MDA) [10, 11]. Lipid peroxide levels, and especially oxidized LDL, are significantly higher in diabetic patients than in healthy individuals. [12-14]. These Major changes in lipid metabolism cause lipid peroxidation in plasma and cellular membranes which lead to micro and macro vascular pathologies [15].

The natural protective system of antioxidant enzymes like superoxide dismutase, glutathione peroxidase and catalase that provides the detoxification steps for the oxidative products, cannot overcome massive production of free radicals to prevent oxidative damage. [16]. It was shown that the activity of the antioxidant systems is decreased in

diabetic patients. [17, 18]. This leads to oxidative stress and to the development of diabetes complications.

5. Diabetes mellitus and aldose reductase

The reduction of glucose by the aldose reductase (AR) catalyzed polyol pathway has been linked to the development of secondary diabetic complications like cataract, nephropathy, retinopathy and neuropathy. Accumulation of sorbitol in the organs, due to AR-catalyzed reduction of glucose, causes osmotic swelling resulting in ionic imbalance and protein insolubilization leading to diabetes complications. [19]. Although treatment with AR inhibitors has been shown to prevent tissue injury in animal models of diabetes, the clinical efficacy of these drugs remains to be established. [20].

6. Treatment of diabetes mellitus

Daily injections of insulin are the only treatment for type 1 diabetes. The treatment for type 2 ranges from diet, to classical oral drugs (Sulfonyl urea and biguanides), and to Thiazolidinediones and the new GLP1 analogues. About 40% of type 2 diabetics use insulin in addition to oral drugs.

Although the pathogenesis of diabetes and its long-term complications are well known, optimal treatment remains elusive. The medications currently in use are limited in their potency, have many side effects, and cannot be tolerated by many patients. Only half of the patients achieve the recommended hemoglobin A1c target using conventional treatment [21]. As a result of the global epidemic of diabetes, the need for new diabetes therapies is expected to grow dramatically during the next years. [22]. 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 [23], and diabetes in particular [24].

7. Anti diabetic medicinal plants

Several reviews published in recent years screen many plant sources with anti-diabetic properties [24, 25, 26, 27, 28, 29]. Among these plants: *Trigonella foenum graecum*, *Allium cepa*. & *Allium sativum*, *Silybum marianum*, *Mordica charantia*, *Camellia sinensis*, *Morus nigra*, *Gymnema sylvestre* L., *Ginkgo biloba* L., and many others. Anti-diabetic health effects include increasing serum insulin, decreasing blood glucose, increasing glucose metabolism, and/or stimulating pancreatic function. Adverse effects, contraindications, and interactions between herbal medicines and synthetic drugs exist and may cause clinical consequences.

We shall briefly screen here some of the most potent anti diabetic sources.

Fenugreek (*Trigonella Foenum Graecum*), is one of the safest and most effective plants in treating diabetes. Clinical studies showed that fenugreek seeds have anti diabetic effects

[30]. "Bitter melon" (*Momordica Charantia*) fruit extract reduced blood glucose and was found effective in treating diabetes [31]. Garlic has been reported to possess a variety of medicinal properties including hypoglycemic, hypocholesterolemic and hypolipidemic activities [32]. Raw garlic extract reversed proteinuria in diabetic rats in addition to reducing blood glucose, cholesterol and triglyceride in diabetic rats [33]. Silybum Extract (*Silybum Marianum*) increases the cellular sensitivity to insulin and thus reduces blood glucose total cholesterol and LDL levels in diabetic patients [34]. Bitter cucumber plant fruit (*Momordica Charantia*) reduced blood glucose in patients with type 1 diabetes [35]. Green tea (*Camellia Sinensis*) can reduce blood sugar in diabetic patients. Studies show that the consumption of one and a half gram dry powder of green tea, improved the metabolism of blood sugar in diabetic patients [36]. Ginkgo biloba plant is capable of lowering glucose, fat, and lipid peroxide in diabetic patients [37]. The ethanolic extract of *Allium porrum* leaves had hypoglycemic effects on diabetic animals probably through the increase of insulin release [38].

Some nutritional factors, such as polyphenols, counteract insulin resistance and therefore may be beneficial for patients with type 2 diabetes mellitus through their insulin-potentiating, antioxidant, and anti-inflammatory properties. The common cinnamon (CN) has a long history of use as a spice, preservative, and pharmacological agent; CN is also a source of polyphenols. Several studies demonstrated that in animals and humans, CN and aqueous extracts of cinnamon improved the level of blood glucose, lipids and insulin, and may be beneficial to counteract the features of insulin resistance, metabolic syndrome, and the onset of type 2 diabetes mellitus [39, 40, 41, 42].

Although many medicinal plants have been traditionally used for treating diabetes [43, 44, 45], the influence of most of them has only rarely been scientifically tested and validated, and the knowledge on their efficacy and mechanisms of action is very limited.

7.1. Yeast as a natural source for anti diabetic material

Brewers' yeast is also included among the anti diabetic natural sources [46, 47]. Schwartz and Mertz were the first to discover the natural anti diabetic agent present in yeast and called it "Glucose Tolerance Factor" (GTF) [1].

8. Glucose Tolerance Factor (GTF) a natural anti diabetic agent

The Glucose Tolerance Factor (GTF) is a dietary agent first extracted from Brewer's yeast [1]. This natural compound reversed the impaired glucose tolerance of diabetic rats [48, 49], and of diabetic patients [50]. GTF can be extracted from several sources, among them: liver [51], black pepper, and kidneys. Especially rich source for GTF are brewers' yeast [52, 53, 54, 55].

Addition of partially purified GTF to the diet of glucose intolerant rats rapidly returned them to normal [56]. Doisy and his group found an improvement in glucose tolerance in elderly people who were treated for two months with GTF. In 50% of the patients, glucose tolerance was restored to normal values. [57].

Offenbacher and Pi Sunyer [46], examined 24 elderly subjects, who were fed daily for 8 weeks with brewers' yeast as a source for GTF. They found a considerable improvement in glucose tolerance and insulin sensitivity, and a reduction of total lipids in these patients.

Grant and McMullen [50] treated 37 type 2 diabetics for 7 weeks, in a double blind study, with either brewers' yeast as a source of GTF, or placebo. Supplementation of brewers' yeast significantly decreased HbA1c and increased HDL cholesterol in the treated group. Elwood [58] supplemented 11 normolipidemic and 16 hyperlipidemic subjects with brewers' yeast. They found that total circulating cholesterol was significantly reduced and the HDL levels were significantly increased in both the normo and hyperlipidemic subjects supplemented with brewers' yeast. Riales [59] reported that human subjects receiving 7g of brewers' yeast for 6 weeks had a significant decrease in serum LDL and an increase in HDL cholesterol.

In vitro studies with partially purified preparations of GTF, showed stimulation of glucose metabolism in several tissues. GTF potentiated glucose oxidation to CO₂ in adipose tissue [54, 60], or adipocytes [53, 61]. In those studies the enhancement was shown only in the presence of insulin, and the stimulation of CO₂ production by GTF in the absence of insulin was negligible [53, 54, 60, 61].

In contrast to the findings above, showing GTF activity only in the presence of insulin, other groups found an increase of glucose metabolism by adding GTF in the absence of insulin. Tokuda et al, [62] examined GTF obtained from yeast extract powder on glucose uptake in adipocytes. They found a stimulation of glucose uptake (5.6 times greater than the basal level) in the absence of insulin. Our group also showed an increase in glucose transport both to yeast cells [63, 64], and to animal cells [65].

Since GTF is supposed to be essential for normal glucose tolerance in mammals, and as muscle tissue consumes a major part of blood glucose in the post prandial state, it is most important to assess the effect of GTF on muscle tissue. Fischer and his group [66] examined the effect of GTF obtained by partial purification of yeast extract, on glucose transport in isolated cardiomyocytes. They found that GTF samples increased the rate of glucose transport in the isolated cells, 2 to 2.5 fold, in the absence of insulin. Hwang et al [67] showed enhancement of ¹⁴C -glucose oxidation into CO₂ in rat adipocytes by the addition of several fractions extracted from yeast. The authors found only insulin like activity and not insulin potentiating activity for the fractions examined.

The exact composition and structure of GTF are still obscure. Mertz and his group suggested that GTF is probably a small organic molecule comprising one trivalent chromium ion, two molecules of nicotinic acid, and three amino acids: glycine, cysteine and glutamic acid [54, 68, 69]. Its molecular weight is estimated to be around 500 daltons [54, 69]. It is cationic, soluble in water, and stable in physiological solutions [54, 68].

Several groups who tried to identify the active components present in brewers' yeast, claimed that they are quinoline derivatives [70], or phosphatidylinositol glycans [71]. Other investigators tried to further purify and identify the exact structure and composition of GTF. There is no standard accepted method to isolate GTF, and this fact can probably explain the

diversity of the results reported in the literature. In addition, a major problem related to GTF purification, is the instability of the partially purified fractions. This lability, can partially explain the complexity of the subject, and the fact that in spite of the long time since the material was discovered, its exact composition and structure have not been determined.

Tuman [48] who presented the activity of GTF and several synthetic complexes on lowering blood glucose found that in 10 days both the natural compound and the synthetic complexes lost their activity. Mertz reported that highly purified preparations of GTF from yeast or pork kidney tend to be unstable, and lost their activity very quickly [52]. Yamamoto [51] found that GTF like activity of the purified LMCr (low molecular weight chromium binding substance) reduced gradually, and finally there could not be detected any activity. Even at -20 °C, no recovery of the active material could be achieved. We can explain the instability of the purified fractions of GTF by a loss of a co-factor(s) which is probably responsible for the stability of the complex.

Most of the groups who tried to purify GTF from brewers' yeast agree that the GTF is a cationic compound. Only several researchers claimed that the GTF is an anionic compound: Votava and his group [72] reported that GTF is an anionic chromium complex of molecular weight 400-600, containing at least six amino acids. Since the authors measured only the absorption of the complex by rats, and no biological activity assay was done on it, it is hard to compare Votava's compound to other extracts exhibiting GTF activity.

A low molecular weight chromium binding substance (LMCr), was isolated from mouse or rabbit liver and bovine colostrum by Yamamoto and his group [51, 73]. LMCr appears as an anionic organic Cr compound, with a relative molecular mass of 1500 daltons. It is composed of glutamic acid, glycine, cysteine and aspartic acid in a ratio of Cr: Amino acid 1:4. The purified LMCr enhanced glucose conversion to CO₂ in rat epididymal adipocytes in the presence of insulin. The rate of glucose incorporation into lipid was stimulated by 30-40% with insulin, or by 15-23% without insulin [51]. Yamamoto and his colleagues were not able to detect nicotinic acid in the extract of LMCr, but some UV absorption was present [73]. This substance appeared to possess properties similar to GTF extracted from yeast.

Another question is related to the nature of the amino acids present in the GTF complex. Urumow & Wieland [74], suggested that GTF activity in stimulating ¹⁴C-glucose oxidation is attributable to the combined action of certain amino acids (aspartate, cysteine) and nucleosides (adenosine). Fischer [66] came to a conclusion that GTF activity is attributed to the presence of alanine. Hwang and his group [70] suggested that the GTF obtained was a quinoline derivative, which easily binds chromium.

While many research groups in the past agreed with the concept suggested by Mertz that GTF contains chromium [51, 55, 73, 75], accumulating data during the years indicates that there is no chromium present in the GTF preparation. Haylock and his group, who tried to purify and identify GTF for many years, did not find a correlation between chromium content and the biologic activity. They came to the conclusion that: "GTF from brewers' yeast can no longer be regarded as a chromium complex" [76]. Shepherd [77] also came to a similar conclusion.

Stearns [78] summarized the purification research that had been done on GTF and discussed the relation of the active component to chromium. She did not find a correlation between chromium and GTF activity. Stearns also investigated the issue of the essentiality of chromium to human health, and found that "no chromium-containing glucose tolerance factor has been characterized, the purpose of the low-molecular-weight chromium-binding protein is questionable, and no direct interaction between chromium and insulin has been found" [79]. Moreover, she criticized the dietary supplementation of chromium: "Chromium+3 may act clinically by decreasing the iron stores that are linked to diabetes and heart disease. This would make chromium+3 a pharmacological agent, not an essential metal" [79].

Eddens and his colleagues [47], isolated three separate fractions by eluting yeast extract from C18 column and found diverse activities in increasing glucose metabolism and inhibiting lipolysis for the different fractions, not connected to their chromium content.

Recently, our group also measured the chromium concentrations in the active fractions isolated from yeast extract and did not find any correlation between the chromium content and the biologic activity (Mirsky et al, unpublished data).

To summarize the chromium issue: Although the active material isolated from yeast (GTF) has been known for years to be a chromium complex, accumulating evidence during recent years show that the active anti diabetic fractions in GTF do not contain chromium.

Our laboratory succeeded in extraction and partial purification of an active and stable GTF preparation from brewer's yeast. We used several separation techniques including membranes with different molecular cut off, ion exchange columns and reversed phase HPLC. Our GTF preparation has a molecular weight below 1000 dalton. It was found to be very stable: it is stable to high and low pH and it keeps its activity up to 12 months in 4°C. Moreover, GTF is also stable to proteolytic enzymes. This finding enables an oral treatment with GTF, in contrast to insulin, which is a protein and has to be injected [49, 80, 81].

In the following paragraphs we shall present several of our findings on GTF both in vivo and in vitro. We examined GTF effects in animal models for both types of diabetes, and found high and rapid anti diabetic, hypolipidemic and antioxidant activity. We also found a remarkable reduction in the complications of diabetes: nephropathy and retinopathy, by treating the diabetic animals with oral doses of GTF [81].

In vitro studies done in our laboratory showed insulin mimetic and insulin potentiating activity for GTF [65].

9. In vivo effects of GTF

9.1. GTF decreases blood glucose in diabetic rat models

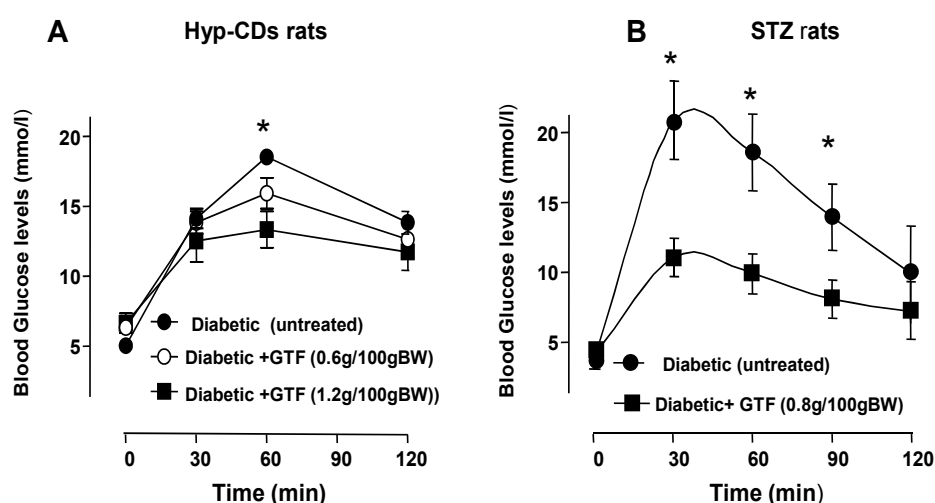
A single oral dose of GTF, orally administered to both types of diabetic animals, decreased immediately and remarkably glucose and lipid levels in their blood [49, 80]. Glucose reduction appeared immediately after the administration of GTF, reached a maximum within 2 hours, and lasted for several hours. When GTF was administered in concert with

marginal insulin doses, the reduction in blood glucose was much higher than for each agent alone, demonstrating a synergy between GTF and insulin [80].

9.1.1. GTF improves oral glucose tolerance test (OGTT) in diabetic rats

We examined GTF effects in two rat models exhibiting insulin deficiency: the streptozotocin (STZ) diabetic rat, which is characterized by the damage induced to beta cells by the drug, and the hyperglycemic Cohen diabetic-sensitive ((hyp-CDs) rat, which is characterized by beta cell dysfunction and decreased glucose stimulated insulin secretion (from Wexler-Zangen et al, [65].

Control-vehicle treated hyp-CDs and STZ rats exhibited an abnormal glucose-tolerance curve, characterized by elevated blood glucose levels (Figs 1A and B) Administration of an oral dose of GTF (at zero time) lowered the blood glucose area under the curve (BG-AUC) of both hyp-CDs and STZ rats compared to vehicle treated rats. The decrease in BG-AUC depended on the dose of GTF administered. Insulin secretion in response to glucose stimulation did not change significantly in GTF treated hyp-CDs rats (From Wexler-Zangen et al, [65], indicating that the glucose lowering effect of GTF is not related to stimulation of insulin secretion.



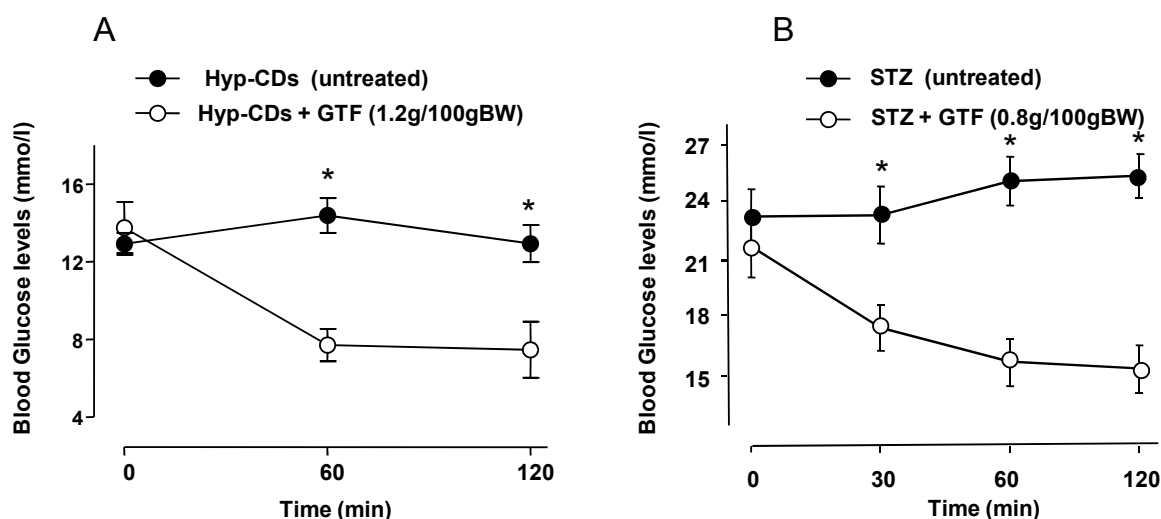
A: OGTT profiles of diabetic untreated Hyp-CDs rats (black circles), GTF (0.6g/100g BW) treated Hyp-CDs rats (white circles) and GTF (1.2g/100g BW) treated Hyp-CDs rats (black squares). B: OGTT profiles of diabetic untreated STZ rats (black circles) and GTF (0.8g/100g BW) treated STZ rats (black squares). GTF was administered at time 0. BG levels were measured after an overnight fast [0], and at 30, 60 and 120 min period after glucose (3.5g/kg for Hyp-CDs and 2g/kg for STZ rats) administration. Data are means $\pm$ SE for 5 or 6 rats per group. * $P < 0.001$, Diabetic vs. respective untreated control. (From Wexler-Zangen et al, [65])

Figure 1. Oral Glucose Tolerance Test (OGTT) of GTF treated diabetic rats

9.2. GTF reduces postprandial blood glucose concentration in diabetic rats

Post prandial (PP) blood glucose level is very high in both hyp-CDs and STZ diabetic rats. In the vehicle treated hyp-CDs and STZ rats, the markedly elevated BG concentrations remained high for more than 120 min (Figs 2A and B). A single oral dose of GTF

administered at zero time significantly reduced ($P<0.001$) the high BG concentrations in both hyp-CDs [33%) and STZ rats [38%).



A: PP glucose levels of control untreated Hyp-CDs rats (black circles), GTF treated (1.2g/100g BW) Hyp-CDs rats (white circles). At time 0 GTF was administered to the indicated groups. **B:** Postprandial glucose levels of untreated STZ rats (black circles) and GTF treated (0.8g/100g BW) STZ rats (white circles). At time 0 GTF was administered to the indicated groups. Data are means $\pm$ SE for 5 or 6 rats per group. * $P<0.001$, Diabetic vs. respective untreated control. (From Wexler-Zangen et al, [65])

Figure 2. GTF decreases Postprandial (PP) glucose levels in diabetic rats

9.3. GTF decreases tri glyceride and LDL cholesterol and increases HDL cholesterol

A remarkable decrease in triglyceride level was observed in diabetic animals administered with 5 daily oral doses of GTF (Figure 3). The treatment with GTF also remarkably decreased the level of LDL cholesterol (Figure 4A), and increased the level of HDL cholesterol (Figure 4B), (Ampel et al., unpublished data). The reduction in LDL cholesterol was also demonstrated in healthy animals treated with GTF, indicating a potential preventive effect of GTF in healthy subjects.

9.4. GTF reduces lipid peroxidation in the plasma, both in vivo and in vitro

GTF can inhibit the deleterious elevation in lipid peroxides induced by diabetes. Type 1 diabetic rats were treated with 5 daily oral doses of GTF. The animals were killed and the levels of lipid peroxidation products - malondialdehyde (measured as TBARS – thiobarbituric acid reactive substances), in healthy, diabetic and diabetic rats treated with GTF were determined. Figure 5 demonstrates the level of lipid peroxidation products detected in the plasma of healthy, diabetic, and diabetic rats treated with GTF, showing a major decrease in the level of plasma peroxides in diabetic animals treated with GTF. These results indicate high antioxidant activity of the GTF preparation (From Ampel et al., unpublished data).

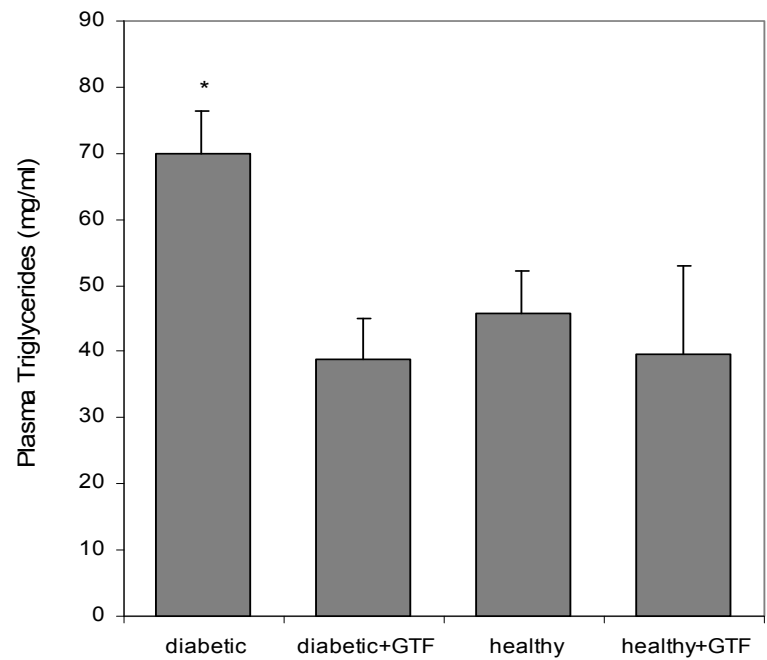


Figure 3. Effect of GTF on plasma triglycerides. Plasma triglyceride concentration in healthy rats, diabetic rats and diabetic rats treated with 5 daily oral doses of GTF [1,64g /rat). All animals were 15 weeks old, diabetic animals were 10 weeks after onset of diabetes. (from Ampel et al., unpublished data).

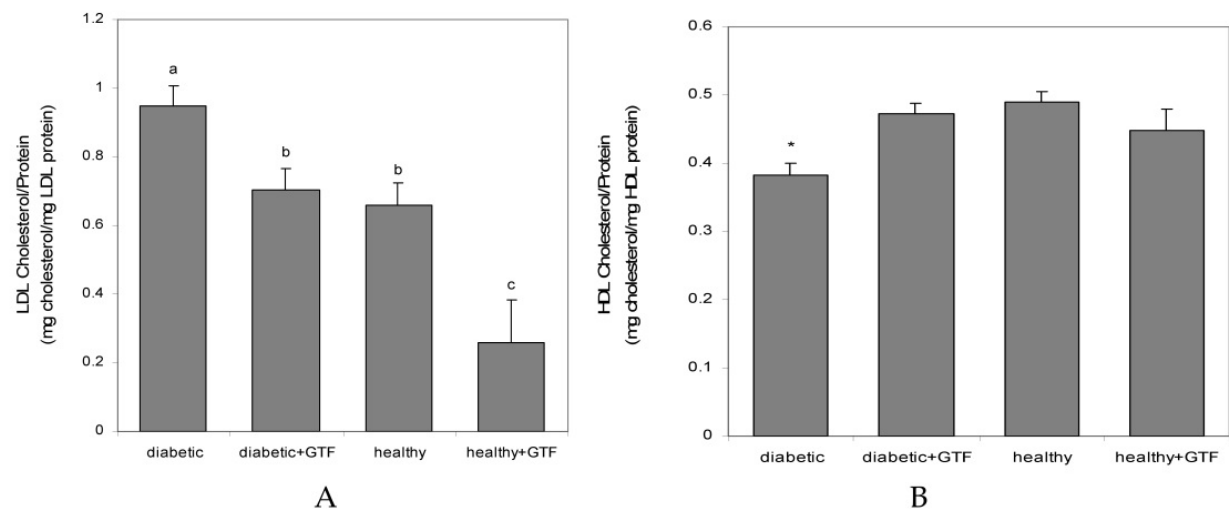


Figure 4. Effect of GTF on Plasma LDL and HDL concentrations. A. LDL composition: cholesterol/protein ratio in healthy rats, diabetic rats and diabetic rats treated with 5 daily oral doses of GTF [1,64g /rat). All animals were 15 weeks old, diabetic animals were 10 weeks after onset of diabetes. B. HDL composition: cholesterol/protein ratio in healthy rats, diabetic rats and diabetic rats treated with 5 daily oral doses of GTF [1,64g /rat). All animals were 15 weeks old, diabetic animals were 10 weeks after onset of diabetes. (from Ampel et al., unpublished data).

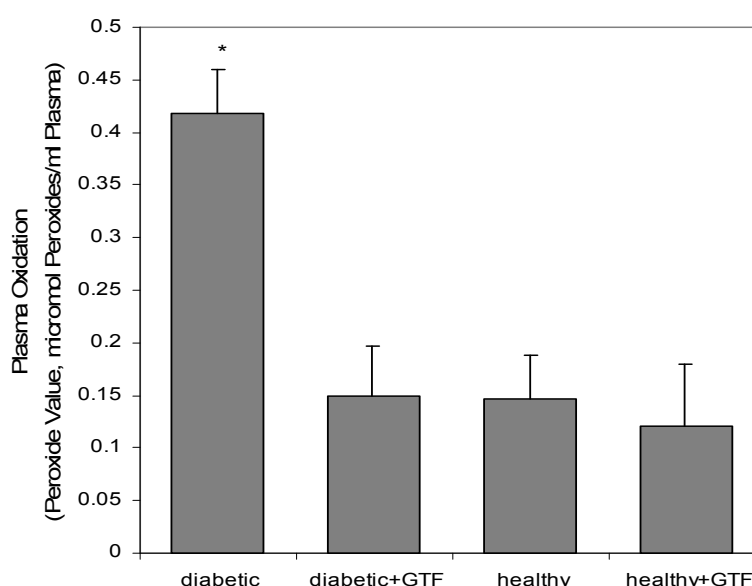


Figure 5. Effect of GTF on plasma peroxides. Plasma oxidation level, measured as peroxide concentration, in healthy rats, diabetic rats and diabetic rats treated with 5 daily oral doses of GTF (1,64g /rat). All Animals were 15 weeks old, diabetic animals were 10 weeks after onset of diabetes (From Ampel et al., 2000).

In vitro studies with GTF added to tubes containing human LDL, indicated a protective effect of GTF against LDL oxidation (Ampel et al., unpublished data).

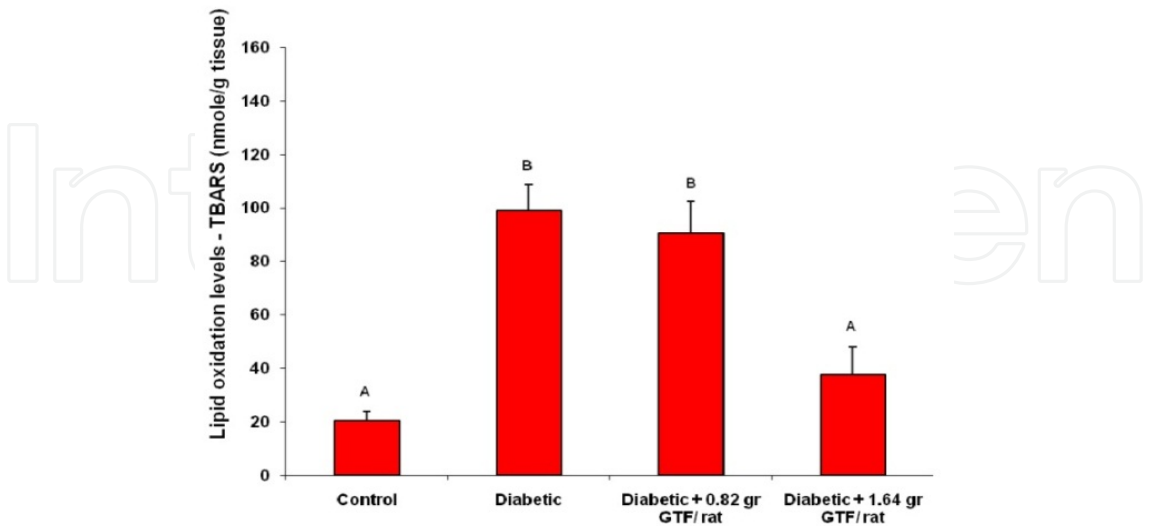
9.5. GTF reduces lipid peroxidation in the heart and kidneys of diabetic rats

The levels of lipid peroxidation products, TBARS, in the kidneys and hearts removed from healthy, diabetic, and diabetic rats treated with GTF are shown in figures 6 & 7 (From Nakhoul et al., [81] Figure 6 presents the level of lipid peroxidation products determined in the hearts of the various experimental groups. The value of lipid peroxides in untreated diabetic rats was significantly higher than the value detected for healthy animals. The level of lipid peroxides in the hearts of diabetic rats treated with 5 oral doses of GTF was very low - a higher effect seen for the higher dose of GTF, similar to the level found in healthy animals. A remarkable decrease in lipid peroxidation level is shown also in kidneys removed from diabetic rats treated with GTF (Figure 7). Peroxide values found in the animals treated with GTF were very similar to those found in healthy controls. The above assays show the remarkable effect of GTF treatment on lipid peroxidation level in diabetic animals, both in plasma and in cardinal organs.

9.6. GTF decreases aldose reductase activity

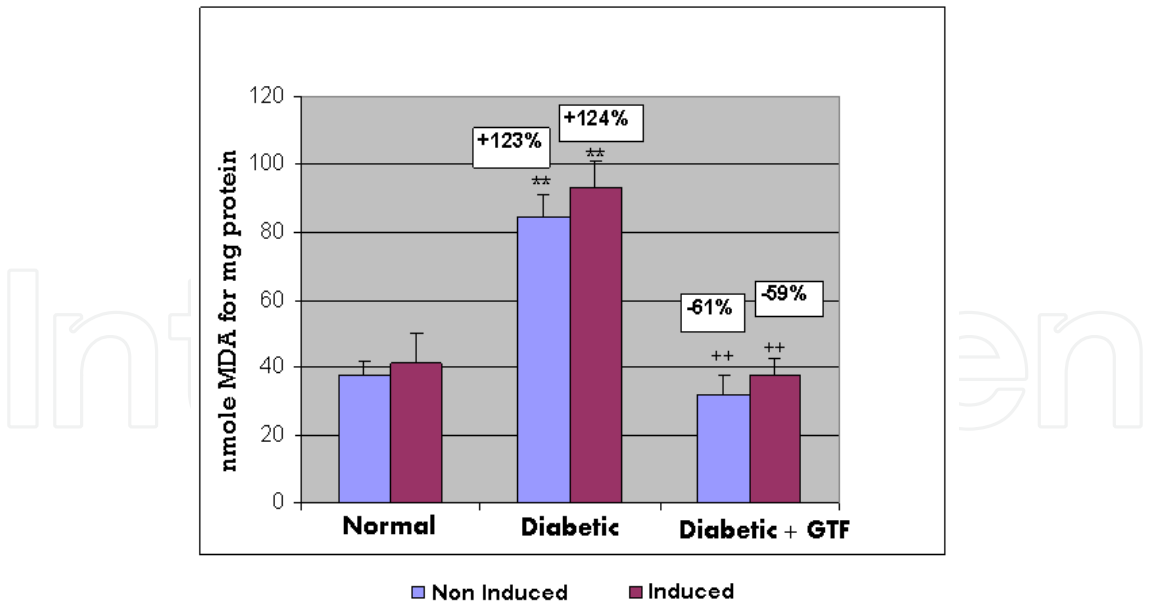
Elevated activity of aldose reductase (AR) in the organs is one of the events occurring in hyperglycemic conditions. We measured aldose reductase activity in the hearts of healthy, diabetic and diabetic treated with GTF (Figure 8). AR activity in the hearts of diabetic rats was much higher than that found in healthy rats. 5 oral doses of GTF remarkably reduced

the activity of the enzyme in the hearts of the treated diabetic animals in a dose dependent mode, indicating a potent aldose reductase inhibition activity in GTF preparation.



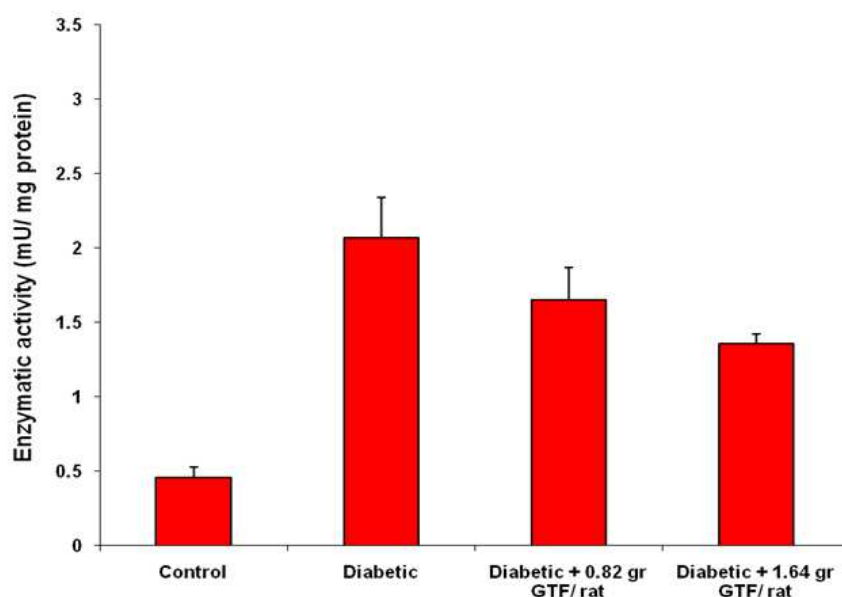
Effect of treatment with GTF on lipid peroxidation products measured as TBARS in the heart of control, untreated diabetic rats and diabetic rats treated with two different doses of GTF: 0,82 and 1,64 g/rat. The rats were treated with repeated doses for 5 days. The age of the rats was 9 weeks. Diabetes was induced with STZ injection (60mg/Kg BW) at the age of 5 weeks. Every group represents 8-11 animals. One way ANOVA test showed significant differences between groups ($P < 0,001$). Different letters above bars indicate significant differences between groups. (From Nakhoul et al., [81].

Figure 6. Effect of GTF on lipid peroxidation in the heart



GTF reduces the level of lipid peroxidation products in renal cortex of diabetic rats. Streptozotocin diabetic rats were administered with 5 repeated oral doses of GTF (1.64 g/rat). The level of lipid peroxidation products (TBARS nmole/g tissue) was determined in control rats ($n=5$), untreated diabetic rats ($n=8$), and diabetic rats treated with GTF ($n=8$). Products of lipid peroxidation were measured in the presence (induced) and absence (non-induced) of FeSO_4 . $P < 0.01$ compared with untreated diabetic animals. ** $P < 0.01$ compared with healthy controls. From Nakhoul et al., [81]

Figure 7. Effect of GTF on lipid peroxidation in renal cortex



Effect of treatment with GTF on aldose reductase activity in the hearts of control, untreated diabetic rats and diabetic rats treated with two different doses of GTF: 0,82 and 1,64 g/rat. The rats were treated with repeated doses for 5 days. The age of the rats was 9 weeks. Diabetes was induced with STZ injection (60mg/Kg BW) at the age of 5 weeks. Every group represents 8-11 animals. (From Mirsky et al unpublished data).

Figure 8. Effect of GTF on aldose reductase activity in the heart

9.7. GTF decreases nephropathy in diabetic rats

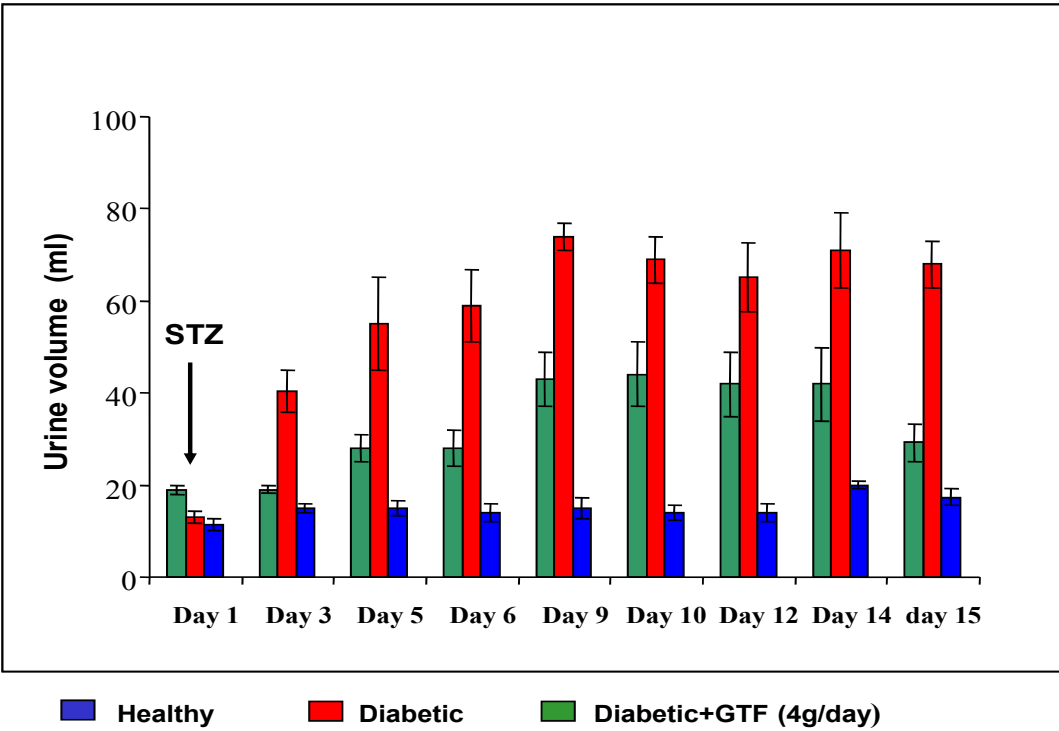
9.7.1. Decreased urine volume and urine protein in diabetic rats treated with GTF

We measured the urine volume and urine protein of healthy, diabetic, and diabetic rats treated for two weeks with oral doses of GTF. Diabetes was induced at zero time. The group of diabetic animals treated with GTF received daily doses of GTF mixed in their food immediately with the induction of diabetes. The animals were kept in metabolic cages, and urine was collected daily. Fig. 9 presents the urine volume and Fig 10 presents urine protein of the different groups, indicating a significant reduction both in urine volume and in urine protein of diabetic rats treated with GTF (from Nakhoul et al., [81]).

9.8. GTF decreases retinopathy in diabetic rats

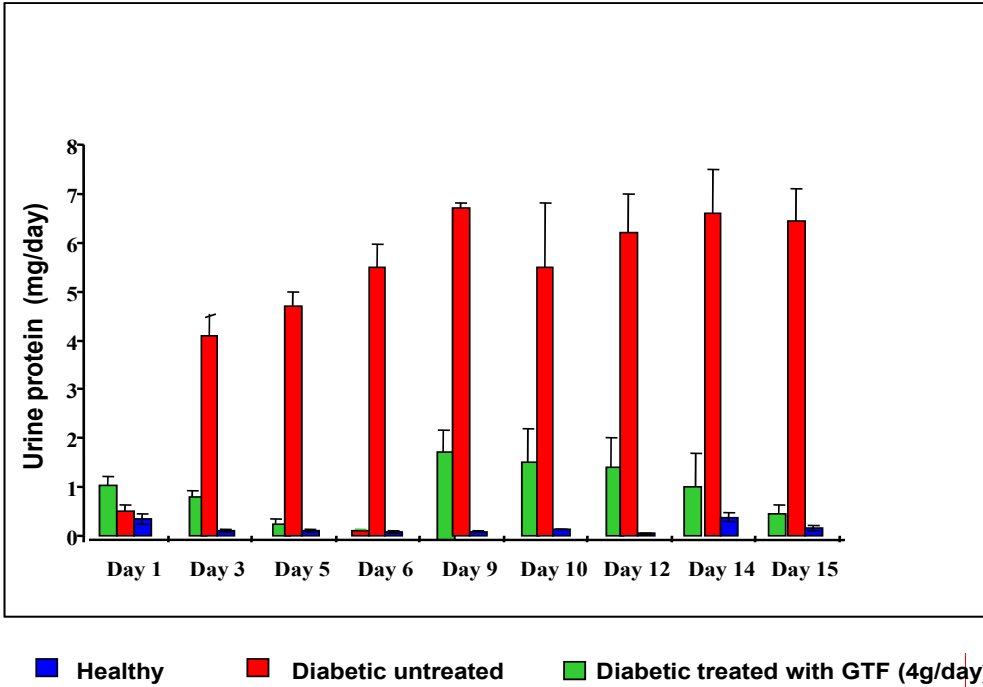
9.8.1. GFAP expression in healthy, diabetic and Diabetic rats treated with GTF

Glial Fibrillary Acidic Protein (GFAP) is normally expressed in retinal astrocytes. Under pathologic conditions like hyperglycemia or ischemia, GFAP can be detected in other retina's areas like Muller cells layer. GFAP has been widely used as a cellular marker for retinal pathology.



Daily urinary volume of healthy rats (n=5), diabetic rats (n=8) and diabetic rats treated with daily doses of GTF [4g/day) for 2 weeks. (From Nakhoul et al., [81].

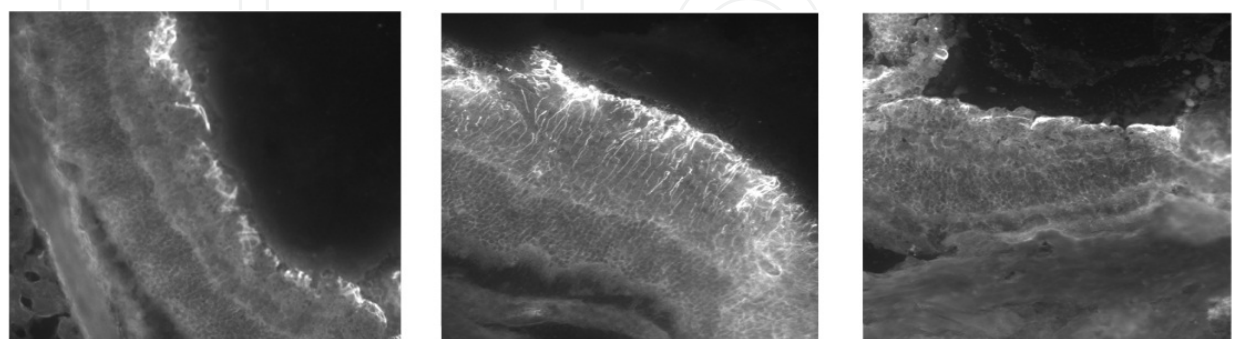
Figure 9. Effect of GTF on urine volume



Urine protein (mg protein / day) excreted by healthy (N=5), diabetic (N=8) and diabetic rats treated with GTF (N=8) (4g/day) for 2 weeks. (From Nakhoul et al., [81].

Figure 10. Effect of GTF on urine protein

In a study done in our laboratory on healthy, diabetic, and diabetic rats treated for two weeks with GTF, a large amount of GFAP staining in Muller cell layer was demonstrated in diabetic untreated retinas (Fig. 11). (Mirsky et al., unpublished data). 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 in the healthy retinas.



Healthy

Diabetic

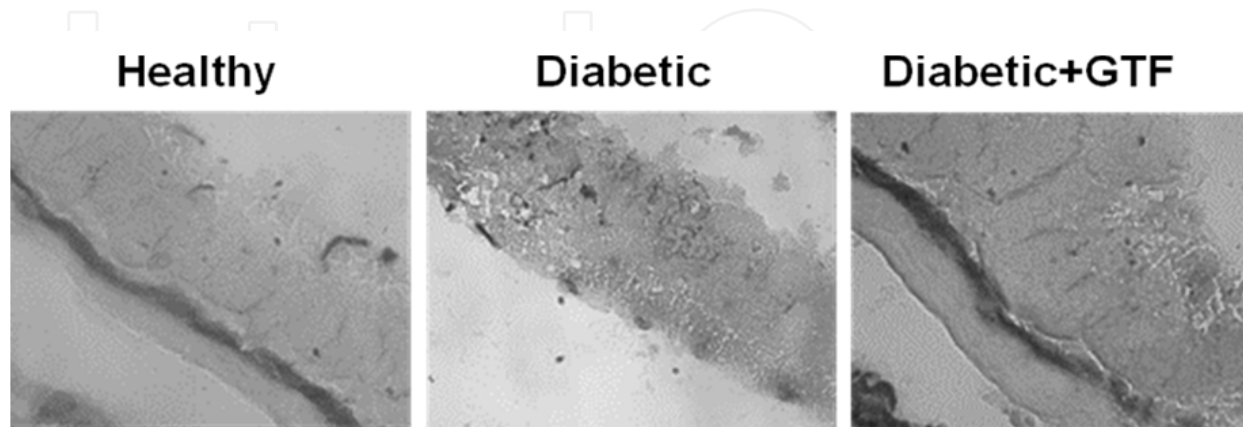
Diabetic+GTF

GFAP Immuno histochemistry in neural retina of healthy, diabetic and diabetic rats treated with GTF (4g/day) for 2 weeks (healthy, diabetic, and diabetic treated with GTF). Magnification X400 (Mirsky et al., unpublished data)

Figure 11. Effect of GTF on GFAP in the retina

9.8.2. GTF prevents the diabetic damage to Na/K ATPase in retinas

Sodium / Potassium ATPase is located in the pigment epithelial layer of the retina. In healthy retinas stained histochemically for Sodium / Potassium ATPase, the activity of the enzyme can easily be detected, whereas it was remarkably reduced in diabetic retinas. A prevention of the damage could be detected in retinas isolated from diabetic animals treated for two weeks with GTF, where the activity of the pump is similar to the activity shown for healthy controls. (Figure 12) (Mirsky et al., unpublished data).



Healthy

Diabetic

Diabetic+GTF

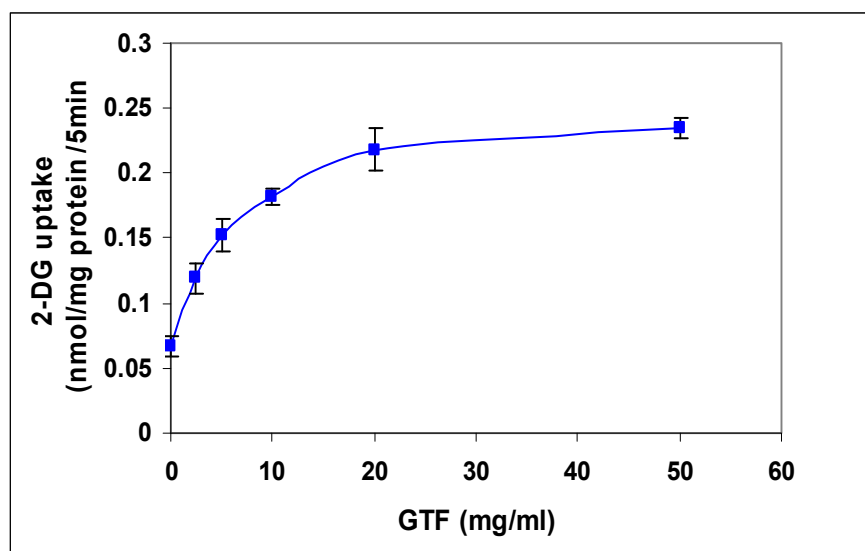
Histochemical localization of Sodium / Potassium ATPase in retinas of healthy, diabetic and diabetic rats treated for two weeks with GTF(4g/day) for 2 weeks. Magnification X400 (From Mirsky et al., unpublished data).

Figure 12. Effect of GTF on Sodium/Potassium ATPase in the retina.

10. Mechanism of action of GTF: Insulin mimetic activity

Binding of insulin to its receptor initiates a cascade of phosphorylations of several substrates, including insulin receptor substrate (IRS) proteins. IRS-1 is widely expressed in insulin-sensitive tissues, and it transmits the signal from insulin receptor to biological endpoints, such as glucose transport, protein, lipid, and glycogen synthesis. Phosphorylation of IRS-1 subsequently triggers the activation of downstream signal molecules such as phosphatidylinositol 3-kinase (PI3K), protein kinase B (PKB/AKT), several isoforms of protein kinase C (PKC), and mitogen-activated protein kinase (MAPK).

Studies done in our laboratory on L6 myoblasts and 3T3-L1 adipocytes presented a marked increase in 2-deoxy-glucose [2-DG) uptake induced by GTF, in a rate similar to insulin, indicating a high positive effect on glucose uptake (Figure 13) (From Weksler-Zangen et al., [65]).

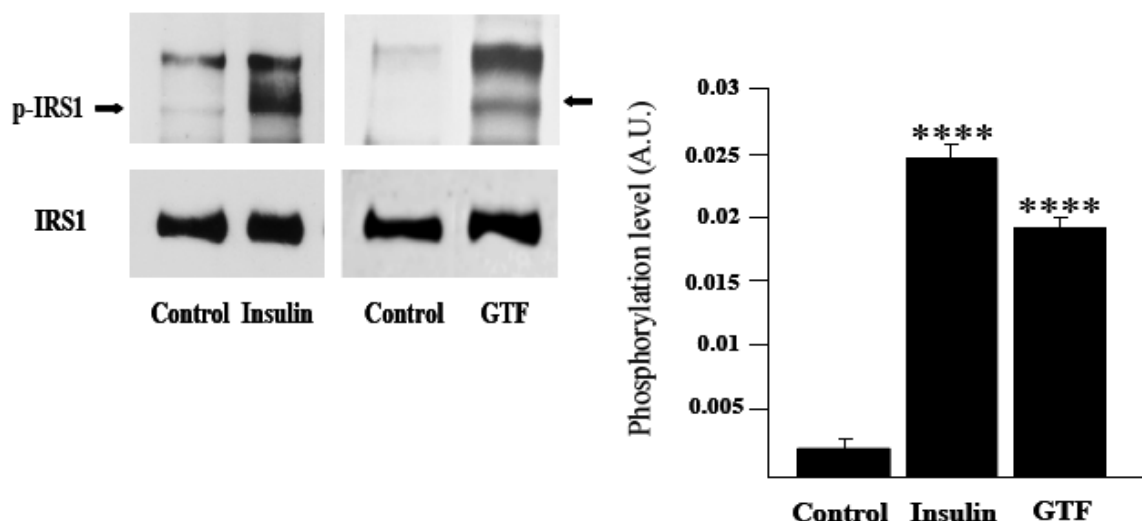


Glucose transport was measured in 3T3-L1 adipocytes in PBS (pH 7.4), at 37°C, with the addition of 0.05mmol/L (3H) 2-deoxyglucose. Different concentrations of GTF (0-20mg/ml) were assayed. Incubation time was 1 hour. Cells were dissolved in 1N NaOH, and aliquots were taken for scintillation counting and protein determination. Data are means $\pm$ SE n=3-4 plates in all experiments ($P<0.05$). (From Weksler-Zangen et al., [65]).

Figure 13. GTF increases 2-Deoxy glucose uptake to 3T3-L1 adipocytes.

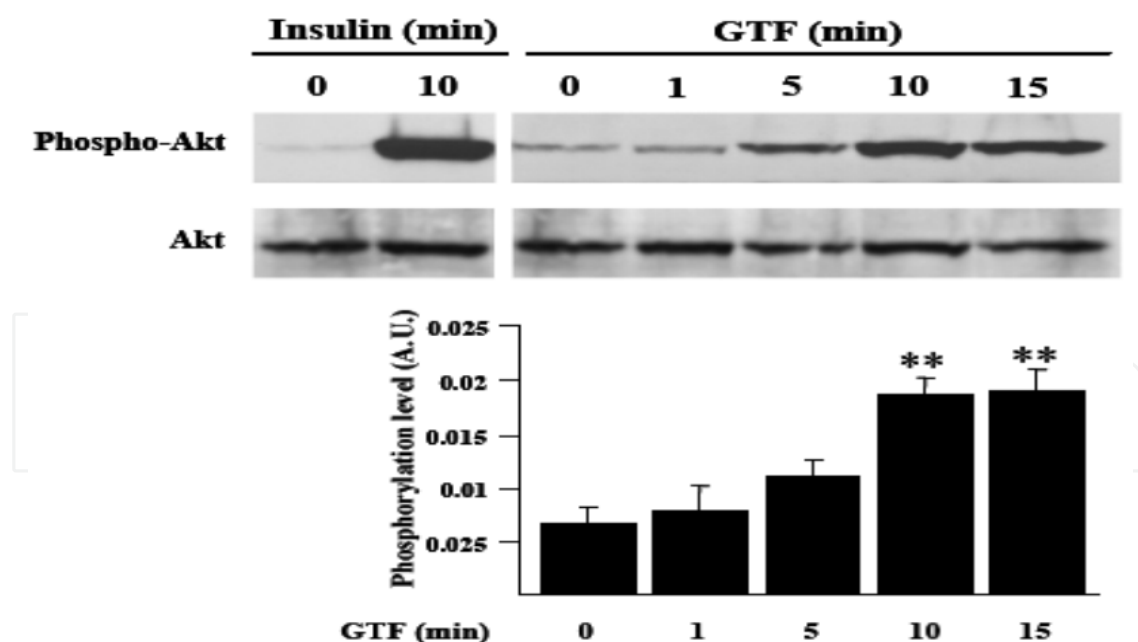
We also found that the increased glucose transport induced by GTF is dose dependent (Weksler-Zangen et al., [65]). A similar synergy between GTF and insulin that was demonstrated in diabetic animals in vivo was also found in vitro: The increase in 2-DG transport detected for the combination of GTF and insulin was much higher than for each agent alone. The rate of 2-DG transport found for the combined treatment exceeded the sum of the two separate treatments, indicating a synergy between GTF and insulin (Weksler-Zangen et al., [65]). We also found increased phosphorylation of key proteins along insulin signaling pathway, like IRS1, AKT, ccbl and MAPK, by the addition of GTF to the medium (Figures 14-16). GTF induced phosphorylation of key proteins was dose and time dependent. The phosphorylation obtained by GTF was similar to that induced by insulin. However, we

did not find any augmented phosphorylation of the insulin receptor following GTF addition, indicating a possible "by pass" of the insulin receptor by GTF.



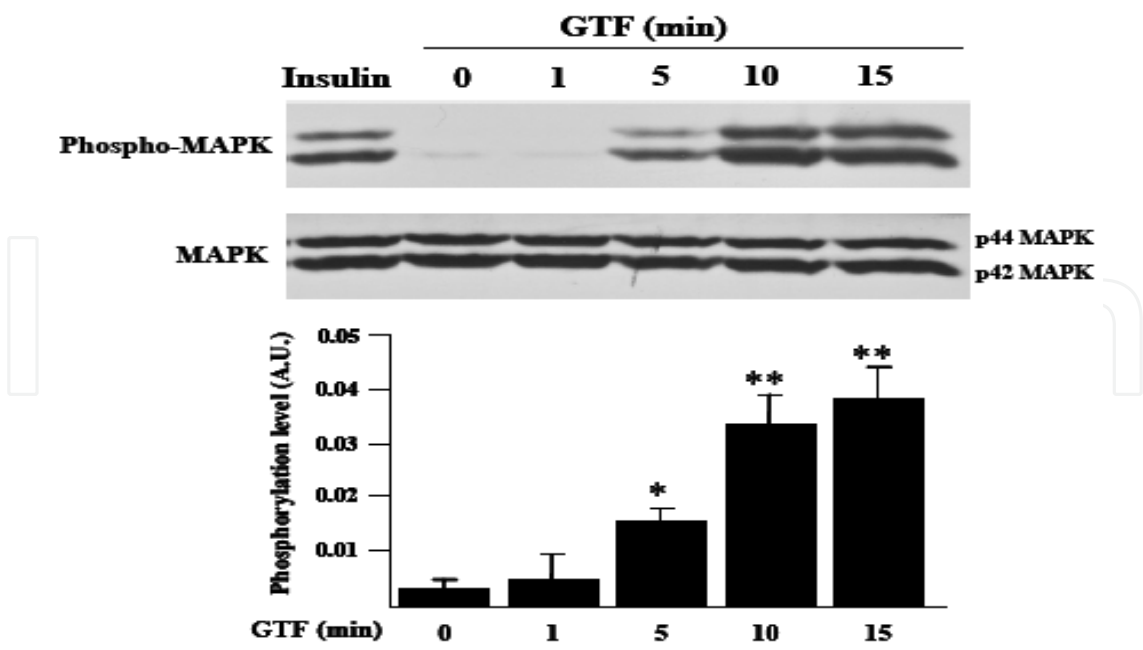
Differentiated 3T3-L1 adipocytes were serum starved for 18 hr. Cells were treated with 100nM insulin for 1 min or with 20 mg/ml GTF for 15 min. Cells were lysed and western blot analysis was performed with antibodies for phosphotyrosine followed by stripping and reblotting with antibodies for total IRS1 as a loading control. Quantification of the bands of P-IRS1 (mean $\pm$ SE) based on scanning densitometry of three independent immunoblots is represented by the bar graph (From Weksler-Zangen et al., [65]).

Figure 14. GTF stimulates tyrosine phosphorylation on IRS1.



Differentiated 3T3-L1 adipocytes were serum starved for 18 hr. Cells were treated with 100nM insulin for 10 min or with 20 mg/ml GTF for 1, 5, 10 or 15 min. Cells were lysed and western blot analysis was performed with antibodies for phospho-Akt followed by stripping and reblotting with antibodies for total Akt as a loading control. Quantification of the bands of P-AKT (mean $\pm$ SE) based on scanning densitometry of three independent immunoblots is represented by the bar graph (From Weksler-Zangen et al., [65]).

Figure 15. GTF stimulates AKT phosphorylation.



Differentiated 3T3-L1 adipocytes were serum starved for 18 hr. Cells were treated with 100nM insulin for 10 min or with 20 mg/ml GTF for 1, 5, 10 or 15 min. Cells were lysed and western blot analysis was performed with antibodies for phospho-p42/44 MAPK followed by stripping and reblotting with antibodies for total p42/44 MAPK as a loading control. Quantification of the bands of p42/44 MAPK (mean $\pm$ SE) based on scanning densitometry of three independent immunoblots is represented by the bar graph (From Weksler-Zangen et al., [65]).

Figure 16. GTF stimulates MAPK phosphorylation.

11. Conclusions

In a search for new and effective medications for diabetes mellitus, there is a growing interest in natural derived hypoglycemic agents such as medicinal plants, herbs, and yeast. The Glucose Tolerance Factor (GTF), which is an active anti diabetic material extracted from yeast, is presented in the current manuscript.

GTF effectively decreased the elevated blood glucose in diabetic animals and humans. It also decreased triglycerides and LDL cholesterol and increased HDL cholesterol in diabetic subjects. GTF treatment also prevented diabetes complications like nephropathy and retinopathy.

Not being a protein, GTF can be taken orally. GTF is both insulin mimicker and insulin potentiator: It can decrease glucose and lipids in the blood when given without any additional medication, but can also activate insulin effect: a small dose of insulin becomes more effective when administered with a dose of GTF.

GTF exerts insulin-mimetic and insulin-potentiating activity also in-vitro: glucose transport is increased by the addition of GTF. When GTF is added with insulin - an augmented glucose transport is detected.

In vitro studies shed light on the mechanism of action of GTF: GTF produces insulin-like effect by acting on cellular signals downstream of insulin receptor, regulating glucose

transport, glycogen, and protein synthesis and modulating nuclear activity in the same manner as insulin. These results demonstrate GTF as a potential natural source for a novel oral anti diabetic drug in the future.

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