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The Effect of Thymoquinone Treatment on the Levels of Serum Autoimmune Anti-Islet Cell Antibodies in Type 1 Diabetic Rats

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ABSTRACT

The research purpose was to investigate the effect of thymoquinone (TQ) administration on the levels of main type 1 diabetes mellitus (IDDM) autoantibodies which are anti islet cell antibodies (ICA) with an attempt to find a relation between this immunological effect and histological or biochemical findings. We have evaluated with the help of ELISA kits the levels of ICA and serum insulin in male Sprague-Dawley rats with Streptozocin-induced IDDM in addition to pancreatic histological findings. The four groups (6 rats each) under study received or not different intraperitoneal doses of TQ for a period of 30 days. Daily intraperitoneal administration of TQ (either low dose 5 mg/kg or high dose 10 mg/kg) for up to 30 days to type1 diabetic rats effectively reduces levels of anti islet cell antibodies in addition, reduced level of insulin due to damaged Langerghans islet cell was significantly increased in the serum due to repairing tissue process in pancreatic tissues. These experimental results suggest that TQ treatment has a therapeutic protective effect against autoimmune reactions occur in IDDM. The data may provide new strategies for using TQ to be recommended as excellent candidate in the clinical management of IDDM.

Keywords: Thymoquinone, *Nigella sativa*, Type1 diabetes mellitus; anti islet, cell antibodies, serum insulin.

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INTRODUCTION

Type I diabetes mellitus or insulin-dependent diabetes mellitus (IDDM), is an autoimmune disease process in which pancreatic islet β-cells are targeted for destruction by an aberrant host immune system [1]. Autoimmunity directed against pancreatic islet cells results in slowly progressing ß-cell destruction, culminating over years in clinically manifested insulin-dependent diabetes mellitus (IDDM) [2]. Circulating serum autoantibodies directed against the endocrine cells of the islets of Langerhans are an important hallmark of this disease. Assays for these islet cell antibodies (ICA) have facilitated the investigation and understanding of several facets in the pathogenesis of autoimmune diabetes. Their applications have begun to extend into clinical practice and have opened new avenues for early preclinical prediction and preventive prophylaxsis in IDDM [2,3].

In subjects newly diagnosed with IDDM, up to 90% have antibodies to ICA [4]. These autoantibodies appear during the preclinical period of β-cell destruction before the clinical manifestation of diabetes [4]. The cell destruction is thought to result mainly from the action of T-lymphocytes, the key players in autoimmune disease development. The ICA autoantibodies are thought to signal a T-cell mediated immune response that sets the stage for beta cell destruction [5]. However, multiple environmental and genetic factors make the immune cells, particularly T lymphocytes to invade islet β cells and cause pancreatic inflammation. [2-5]

Experimental studies on animals especially rats showed that these animals develop a form of autoimmune diabetes that resembles human IDDM [6]. Studies with animal model have established that islet-infiltrating cell– reactive T-cells are the major effectors of ß-cell damage. However, other immune system cells are also crucial in the disease development. Among these cells, B-cells are essential in the onset and progression of IDDM [7], and although it is not fully understood when and how these cells participate in IDDM, it is known that they produce ICA autoantibodies against many ß-cell autoantigens [8], and act as antigen-presenting cells [9]. On the other hand, the production of specific ICA autoantibodies directly correlates with the progression of IDDM in both humans and laboratory animals. [7-9]

Due to the increasing worldwide prevalence and financial burden of diabetes, it has become increasingly important to find pharmacological remedies to alleviate the symptoms and complications of these conditions. In particular the use of natural remedies such as Nigella sativa or black seeds has become popular as both preventative and treatment alternatives. [10]

Thymoquinone (TQ), the active component of Nigella sativa oil, has been shown to have various biological effects including disease treatment and prevention [11-13]. TQ is believed to share similar properties to the benzoquinones already in use as therapeutic drugs [12]. Therefore, many clinical and experimental studies have demonstrated the therapeutic benefits of TQ alone or within Nigella sativa, including immunomodulative [14], anti-oxidant and anti-inflammatory [15], antitumour [16,17], anti-cardiovascular effects [18], and antidiabetic effects [10,14].

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To date, there are no successful treatment interventions that have been found to delay the onset of type 1 diabetes although several studies have been carried out to explain the controversial effect of TQ on the non-specific immune responses in IDDM but the exact role on the disease process still unclear. Thus, present study was carried out to investigate the effect of TQ administration on the levels of main IDDM autoantibodies which are anti islet cell antibodies with an attempt to find a relation between this immunological effect and histological and/or biochemical findings.

MATERIALS & METHODS

Experimental Animals

Twenty four male Sprague-Dawley rats with an average weight of 150-250g and an average age of 12-16 weeks were used throughout the experiment, obtained from Nano Life Quest Company, Malaysia. The rats were acclimatized for a period of 21 days. A standard environmental condition such as temperature (20-22^{o}C), relative humidity (45-55%) and 12 hrs dark/light cycles was maintained. The animals were fed daily with rodent pellet diet and tap water ad-libitum under strict hygienic conditions.

Ethical clearance for performing the experiment on animals was approved by Animal Care and Use Committee (ACUC), Faculty of Medicine, University Technology MARA (UiTM) Malaysia that conforms to the Guide for the Care and Use of Laboratory Animals [19] and all efforts were made to minimize animal suffering and the number of animals used.

Chemicals

Streptozotocin (STZ) used in the present study was purchased from Nano Life Quest Company; Thymoquinone TQ (2-isopropyl-5-methyl-1,4-benzoquinone, C10H12O2) was purchased from Nano Life Quest Company. The TQ was administered once a day by intraperitoneal injection (i.p) at a dose of (5 mg/kg and 10mg/kg) for 30 days.

Induction of type 1 diabetes mellitus and treatment of rats

A single injection of STZ is widely used to generate a rat model of type I diabetes, which results from the selective toxicity of STZ towards the insulin-producing β-cells in pancreatic islets [20-21]. IDDM was induced in overnight fasted animal group by intraperitoneal injection with a single dose of STZ (65 mg/kg body weight) (Sigma). This dose of STZ lies within the range used in most of studies to produce IDDM, in which blood glucose levels are 3–4 times normal, by causing substantial depletion of pancreatic insulin [20]. STZ was dissolved in sodium citrate buffer solution (PH 4.5) immediately before use. The development of IDDM was confirmed by the presence of hyperglycemia with blood glucose above 13.9mmol/L (250 mg/dL), which last for at least three days. The rats were divided into four groups comprising 6 rats each. Group A

(GA; control group), rats were injected with an equal volume of vehicle (citrate buffer, 65 mg/ Kg body weight) ; Group B (GB; untreated STZ-diabetic rats) ; Group C (GC; STZ-diabetic rats treated with 5 mg⁄ kg, i.p., TQ) ; Group D (GD; STZ-diabetic rats treated with10 mg⁄ kg, i.p.,TQ).

The treatment by TQ was started for a period of 30 days. During this period, Control and STZ-treated animals had free access to standard diet and water until 6pm. None of the rats was treated with insulin at any time during the experiment. Animals were sacrificed at 30th day of experiment immediately after measuring blood glucose. [22] Blood glucose levels were tested every morning (at 8 am). Blood was collected from the tail of fasting (14 h) animals. A drop of blood was used for the blood glucose test with the help of a One Touch Glucometer GX.

Laboratory tests

On the last day (30th day) and after completion of the experimental protocols, blood samples were collected from overnight fasting rats by sacrificing each diabetic and control rats. The animals were anesthetized in a chamber containing diethyl ether. Cardiac puncture was done using a heparin syringe and blood was collected into a heparin containing container. Immediately after collection, 2.0 ml of blood was transferred into fresh tube and centrifuged at 3000 rpm for 10 minutes. The serum was collected and stored at – 80°C until serological analysis.

Serum was assayed for anti islet cell antibodies (ICA) and serum insulin using enzyme-linked immunosorbent assay (ELISA) using a commercially available kits (USCNK, CHINA). Also pancreatic tissues were collected for histological examination.

Summary of histopathological procedures

Pancreatic tissues were harvested from the animals and they were fixed in 10% neutral formaline solution, embedded in paraffin, and then stained with hematoxylin and eosin (H&E) [23]. The preparations were evaluated by means of a bright-field microscope, and photographed (Optiphot 2; Nikon, Tokyo, Japan).

Statistical analysis

The data are expressed as mean ± SE. with 'n' referring to the number of rats used. Two way analysis of variance (ANOVA) was carried out using SPSS 16 software to assess the overall effects and interaction of treatment and time on parameters and followed by repeat one way analysis of variance (ANOVA) with post hoc least significant difference (LSD) test to determine the effect of treatments on differences among means when the analysis of variance indicated a significant result. $P < 0.05$ was taken to indicate significance.

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RESULTS AND DISCUSSION

Immunological and Biochemical Findings

The diabetic animals exhibited consistent hyperglycemia (figure.1), meanwhile, TQ treatment caused a decrease in the elevated serum glucose (figure. 1), and however, figure 2 demonstrated the changes occurred to the body weight of all rat groups during the experiment. Rat body weight was elevated by treatment in both TQ doses.

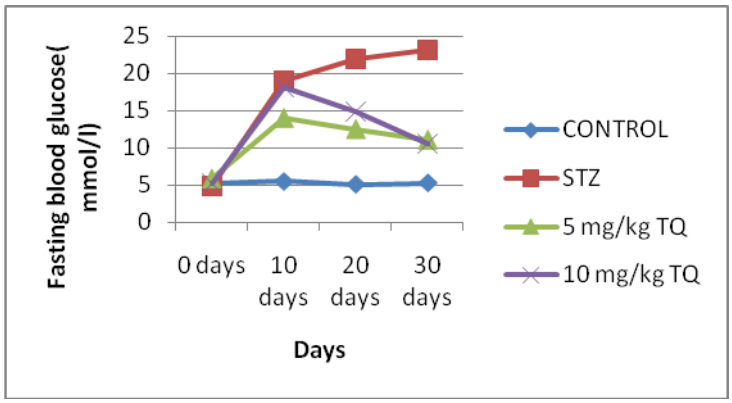

Figure1: Levels of FBG for, GA; control group, GB; untreated diabetic rats; GC, diabetic rats treated with 5 mg/kg , i.p., TQ .GD, diabetic rats treated with 10 mg ⁄ kg, i.p.,TQ. Data are expressed as mean ±SD.

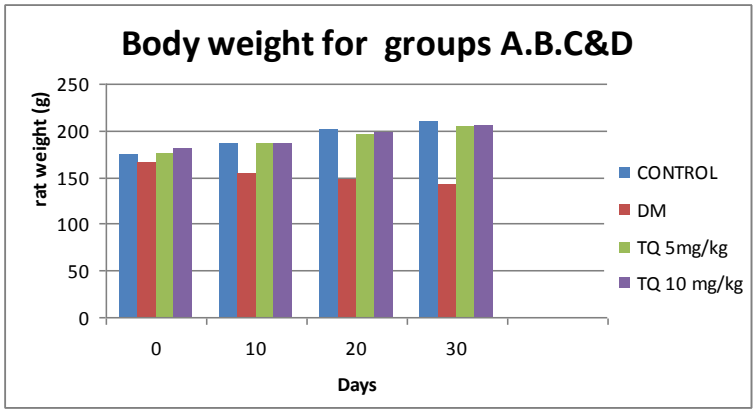

Figure2. Effects of TQ on the body weight on different groups at a different time of the experimental time. GA normal, GB non treated diabetic group, GC diabetic rats treated with (TQ 5mg/kg), GD diabetic rats treated with (TQ 10mg/kg).

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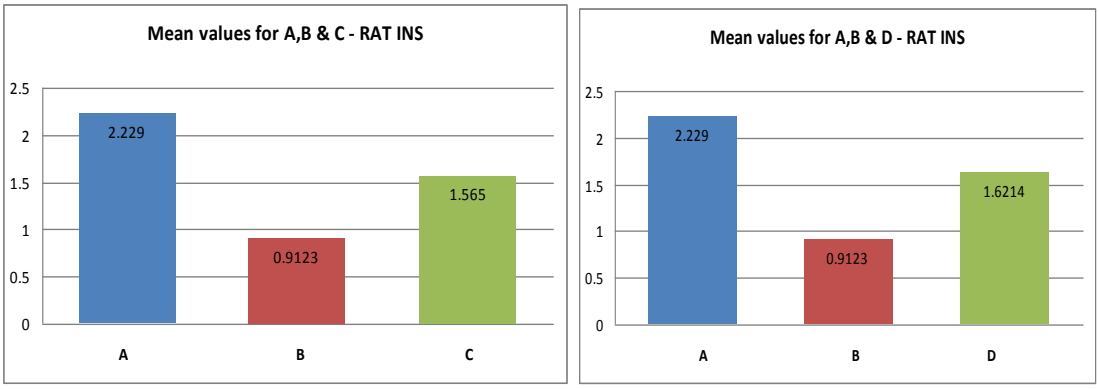
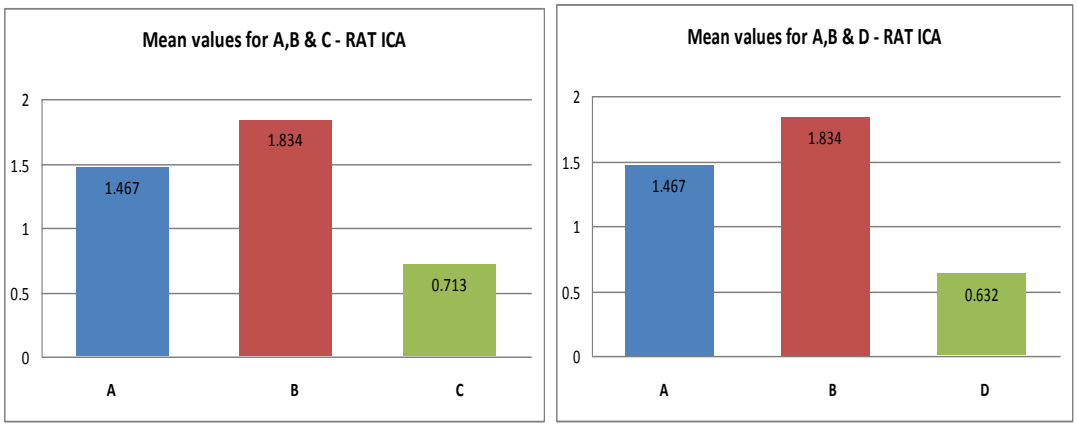


Low dose (5mg/kg) High dose (10mg/kg)

Fig 3: Effect of TQ administration on insulin level production.



Low dose (5mg/kg) High dose (10mg/kg)

Fig 4: Effect of TQ administration on the ICA levels

There is an increase (P=0.001) in the lowered serum insulin concentrations in STZ induced diabetic rats by the end of the experiment. (Figure 3)

Figure 4 shows the effect of TQ (5-10 mg/kg) on the different groups after 4 weeks of treatment. After induction of IDDM, the diabetic animals (GB) showed increase the levels of anti islet cell auto antibodies ICA, however, by the end of the experiment a significant decrease (P= 0.001) in the levels of ICA was observed in GC and GD diabetic rat groups treated with 5 mg/kg, 10 mg/kg repectively (Figure 4).

Histological findings

In the control rats group (GA) the histological sections showed normal structure (fig.5a). The islet of langerhans appeared regular in shape surrounded by thin capsule of connective tissue with lightly stained round clusters of cells embedded in the exocrine tissue. The islets consisted of polygonal cells with pleomorphic nuclei. However, in STZ diabetic rats with no

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treatment (GB), the findings in the histologic sections of pancreatic tissues stained with H&E were degenerative and necrotic changes, and shrinkage in the islets of Langerhans (fig.5b). The islets were relatively small, atrophied, and showed a reduction in the number of polygonal islet cells. The nucleus of necrotic cells indicated either pyknosis or marginal hyperchromasia. Meanwhile, figure 5c shows the islets of langerhans after TQ treatment (5mglkg) (GC), that revealed lightly stained, elongated islets of similar size to those seen in the control group (GA).the islet consisting of polygonal cells with variously shaped nuclei. This figure will be much better with high dose of TQ in last group (GD) (Fig. 5d) since there was protection to the majority of the Langerhan islets' cell which appeared regular in shape and so much similar to the normal shape in GA.

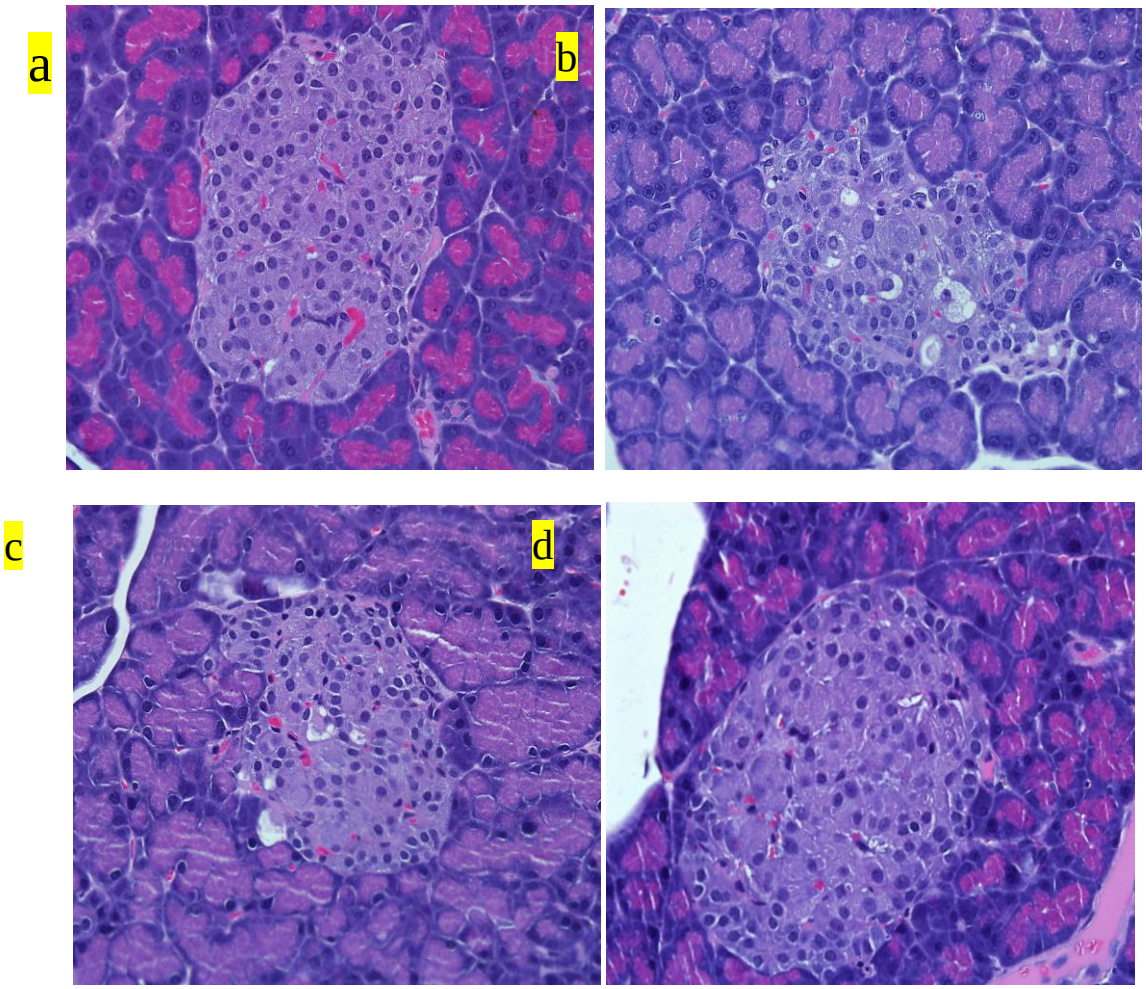

Fig 5: **Microphotographs of pancreatic tissue. (H&E 40). a.** Control group, showing normal cells in the islet of Langerhans. **b:** Diabetic group. Shrunken islets of Langerhans displaying degenerative and necrotic changes in diabetic rats with no treatment. **c:** TQ-treated group (5mg/kg): TQ protected the majority of cells in the islet of Langerhans; **d**. TQ-treated group 10mg/kg; looks like normal control group.

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DISCUSSION

This study demonstrates for the first time, to the best of our knowledge, the effect of thymoquinone, the major component of Nigella sativa on the islet cell autoantibodies production in IDDM with additional biochemical and histological evidences.

Our principal findings are: (1) daily intraperitoneal administration of TQ (either low dose 5 mg/kg or high dose 10 mg/kg) for up to 30 days to type 1 diabetic rats effectively reduces levels of anti islet cell antibodies which are the main antibodies produced in autoimmune process of the disease; and (2) the elevated hyperglycemia was reduced under the effect of TQ in low and high doses; (3) reduced level of insulin due to damaged Langerghans islet cell was significantly increased in the serum due to repairing tissue process due to TQ administration.

While the therapeutic anti diabetic effects of Thymoquinone on type2 diabetes mellitus are numerous and well-documented [13,14, 19, 24], evidence presented in this study actually shows a novel immunomodulatory effect of TQ in STZ induces type1 diabetic rats. This finding is made more interesting by the fact that we and others have shown that TQ is hypoglycemic in both types of diabetes through biochemical evidences while in this study we have proved that TQ has significant effect on the production of main autoimmune antibodies in IDDM means can affect the disease process of this disease and so on decrease the effects of the disease complications.

Researchers have determined that during the first stage of IDDM, ICA antibodies are synthesized that act against the insulin-producing cells of the pancreas [4-9]. The consequence of these autoantibodies is a destruction of the insulin-producing beta cells of the islets of Langerhans cells and an absence or deficiency of circulating insulin [9]. Indirect immunofluorescence stains of human pancreas sections demonstrate that nearly majority of recently diagnosed diabetics have ICA [4]. The autoimmune attack of these antibodies appears to destroy ß cells selectively. These ICAs can lead to lysis of the islet cells. [8,9]

Many trials regarding Nigella sativa and or its major component TQ have been done to show the immunopotentiating effect on different diseases including diabetes [25-30]. They have significant effect in STZ-diabetic hamsters with regard to macrophage phagocytic activity [29], they exerted a stimulatory effect on macrophages through interleukin (IL)-3, which was secreted by T-lymphocytes under the effect of N. sativa oil and TQ which also found to increase the percentage of CD4-positive subset of T-lymphocytes (T helper 2 subset) that secrete IL-3), which activates macrophages and regulates their activity [27-30].

The present study showed additional biochemical evidence to that of immunological effect of TQ. This result indicates that TQ affects blood glucose and insulin level. Persistent hyperglycemia in diabetes brings about a decrease of insulin secretion and insulin resistance of peripheral tissues, which further worsens the control of blood glucose levels in diabetes. TQ ameliorated blood glucose and insulin levels in the present study, in accordance with data

reported previously [11, 13,14]. The hypoglycemic effect of TQ in diabetic rats was may be mediated through decrease in hepatic gluconeogenesis and glucose production. The significant increase in insulin level after TQ treatment was may be attributed to the antioxidant activity of TQ, which may alleviate damage to ß-cells in the pancreas caused by STZ.

In the current study we examined the effects of TQ on cell damage in IDDM in STZ-induced rats in concordance with immunological and biochemical effects. STZ is cytotoxic to ß-cells [20, 31]. Although the ß-cell cytotoxic action of STZ is not fully understood, it is thought to be mediated by the inhibition of free radical scavenger-enzymes, which enhances the production of superoxide radicals. In the present study, almost all of the insulin-producing ß-cells were degranulated, degenerated, or necrosed in the STZ-treated rats (fig. 5b), which led to a decrease in insulin secretion and an increase in blood glucose levels. STZ induced a significant decrease in the area of insulin immunoreactive ß-cells. STZ causes IDDM. However, TQ treatment protected the majority of the Langerhans islet cells and prevented degeneration of ß-cells (fig.5d).

CONCLUSIONS

These preliminary findings suggest that TQ treatment has a therapeutic protective effect against autoimmune reactions occurs in IDDM and immune defense in IDDM can be significantly improved by the administration of TQ. Our data may provide new strategies for using TQ to be recommended in the clinical management of IDDM.

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Disclosure: There are no conflicts of interest to declare.

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