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Anti-diabetic activities of traditional Chinese herbal medicine in streptozotocin-induced diabetic rats

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Summary: Zhen Qi Hypoglycemic Capsules (ZQHC) is a traditional Chinese herbal medicine containing medical activities by ougi (*Astragalus membranaceus*) and ousei (*Polygonatum rhizome*). Although ZQHC has been traditionally utilized as an anti-diabetic medicine in China, there is no evidence. Therefore, this study investigated the beneficial effects of ZQHC against diabetes using streptozotocin (STZ)-induced diabetic rats by biochemical and morphological methods. Eight-week old male Fisher strain rats were intraperitoneally injected with STZ (50 mg/kg of B.W.) to induce diabetes and were fed ad lib feeding with normal diet containing 4% ZQHC for 30 days. Blood and urine samples were collected for biochemical analysis, and liver and pancreas samples were prepared for morphological analysis. Values of blood glucose, AST and ALT of ZQHC oral administrated diabetic rats were lower than those of diabetic rats without administration. Morphological analysis revealed that ZQHC induced sustainment of insulin secreted β cells survival and suppression of hepatocellular fat droplet accumulation. These results suggested that oral administration of ZQHC has anti-diabetic activities those were mainly associated with improvement of liver metabolism.

Introduction

Currently, several oral diabetic medicines with different pharmacological actions, for examples, biguanides involving suppression of glucose generation and appetite, α-glucosidase inhibitors involving blocking intestinal glucose absorption, and sulfonylureas involving enhancement of insulin secretion, have been used. Thus, adverse effects of these medicines, including impairment of gastrointestinal function, hypoglycemia, and liver dysfunction[1–4], have been major concerns. Besides these chemical-based medicines, several herbs, for examples, myrobalan fruit (*Terminalia chebula*), chard (*Beta vulgaris* var. *cicla*), and parsley (*Petroselinum crispum*), have been widely used as oral diabetic medicines in Chinese herbal medicine[5–7]. Besides using single herb, hachimi-jiogan, which is a mixture of plant extracts, was used to promote the production and secretion of insulin and enhance the glucose uptake capacity of the liver[1]. Thus far, wide varieties of herbal medicines have been consumed not only for treatment of diabetes but also for the prevention and treatment of diabetic complications[7], especially in China.

It is apparently that several herbal medicines could put forth certain hypoglycemic action by inhibiting glucose generation and inducing the upregulation of glucose metabolism. Among these, Zhen Qi Hypoglycemic Capsule (ZQHC), an herbal anti-diabetic medicine being manufactured and sold in China, was brought into our attention. ZQHC composes of astragalus root (*Astragalus membranaceus*, milkvetch: English, ougi: Japanese) and polygonatum rhizome (*Polygonatum* spp., ousei: Japanese). Astragalus, a member of the legume family, could exert several effects including immunological enhancement, diuretic effect, anti-neuphritic effect, nourishing tonic effect, and antioxidant activity[8, 9]. Polygonatum,

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a member of the lily family, is used for exerting diuretic effect, improving dry mouth, and lowering blood glucose level[2, 6, 10].

It is a matter of fact that diabetic animal model is necessary for studying therapeutic effects of anti-diabetic drugs. Regards, streptozotocin (STZ) was shown to effectively induce insulin-deficiency symptoms and develop into type I diabetes in laboratory animals, especially rat. Thus, STZ-induced diabetic rats have been used to elucidate substances involving their hypoglycemic activity and mechanism of action[4, 7, 11]. In this study, we investigated the anti-diabetic activity of ZQHC on biochemical changes in blood, urine and morphological changes of liver and pancreas of the STZ-induced rats.

Materials and Methods

Animals

This study was approved by the Animal Experiment Ethics Committee of Rakuno Gakuen University (H18A52). Regarding, five 8-week old male Fischer rats (purchased from Charles River, Japan), weighing 150–170 g, were divided into the experimental and control group. The experimental group was subdivided into the STZ- and STZ+ZQHC-treated group. For the induction of diabetic condition, 200 μL of sterile physiological saline containing 50 mg/kg body weight of STZ (No. S0130, Sigma-Aldrich) was given intraperitoneally to the rats of the experimental group. Control group was comprised of one rat, and both STZ- and STZ+ZQHC-treated groups were comprised of two rats, respectively. In the control group, that without STZ was given via similar route. At 48 hours after the STZ administration, individual rat, that exhibited an elevation of blood glucose level to more than 250 mg/dL by using glucose pilot method (Aventir Biotech, LLC, Carosbad, CA) and positive urinary glucose result by using glucose oxidase colorimetric method (BM Test Glucose 5000, Roche Diagnostics), were considered to develop diabetes and used for further experiments. Normal diet (MF, Oriental Yeast Co., Tokyo, Japan) was given to the STZ-treated and control groups. However, this diet was mixed with 4% ZQHC prior to giving to the STZ+ZQHC-treated group. All groups were allowed freely to access diet and water for 30 days. The daily amounts of water and diet ingested by these rats were measured. At the end of the experiment, weight of all rats was measured and differences from their initial weights were calculated.

The blood and urinary glucose levels were measured as described above. But the fasting blood glucose level was measured after fasting at least 12 hours. Afterward, the rats were anesthetized by intraperitoneal injection of pentobarbital (1 mL/kg BW, Somnopentyl, Sankyo, Tokyo, Japan) prior to collecting blood samples from left ventricle for further serobiochemical analysis. Then, laparotomy was performed to remove liver and pancreas for light microscopy analysis, preceding the sample collection for transmission electron microscopy analysis, a cannula was inserted into its left ventricle of the rat and flushed with physiological saline to remove the residual blood. Afterward, the animals were perfused and fixed with 0.1 M phosphate-buffered 3% glutaraldehyde solution (pH7.4). After the tonic cramp was confirmed, liver and pancreas were excised for the continuing processes.

Serobiochemical analysis

Blood samples were centrifuged with 10,000x *g* at 4°C for 10 minutes to collect serum for immediately analyzing the levels of insulin, glycosylated albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and triglycerides (TG), with different methods. Henceforth, radioimmunoassay was applied for the measurement of insulin, the standard operating procedure established by the Japanese Committee for Clinical Laboratory Standards for AST and ALT, and enzyme assay for TG, respectively.

Morphological analysis

a) Light microscopy

The removed organs were rapidly trimmed and immediately fixed in Bouin solution. Paraffin embedding technique was applied and then continuous slices of 4-μm thickness were produced by the conventional method. Each tissue slice was stained with hematoxylin and eosin (H&E) and observed under an optical microscope. To investigate the pancreatic islets, slices of pancreas were treated with anti-insulin antibody (polyclonal guinea pig anti-Insulin, Dako, Japan) or proliferating cell nuclear antigen (PCNA; Cappel™, MP Biomedicals, LLC, Tokyo, Japan) antibody. Streptavidin-biotin method (Nichirei Biosciences, Tokyo, Japan) was applied to visualize the target cells. Furthermore, the area of immunopositive cells and the occupancy rate of β cells were measured in twenty randomly selected pancreatic islets by use of analysis software (Image J, Ver.1.30, NIH).

b) Transmission electron microscopy (TEM)

The glutaradehyde fixed organs were cut into pieces of 1 × 1 × 1 mm and re-immersed in the same fixative for 2 hours. After several rinses with 0.1 M phosphate buffer, the samples were post-fixed with 1% osmium (VIII) oxide solution, dehydrated in an ethanol series, substituted with QY-1, embedded in Quetol 812, and subjected to thermal polymerization at 60°C. Ultrathin slices were then obtained using an ultramicrotome (Reichert Supernova, Leica). The slices were collected on a copper grid and applied to double electron staining with uranyl acetate and lead citrate. The tissue samples were observed under the transmission electron microscope (JEM-1220, JEOL, Tokyo, Japan) at 80 kV.

Table 1. Consumption and weight change

	Control	STZ-treated	STZ + ZQHC-treated
Average drinking water (mL/day/rat)	23.1	92.7	54.2
Average diet (mg/day/rat)	19.1	24.0	21.5
Weight gain (g/30 days/rat)	116.0	–14.0 ± 38.2	55.0 ± 50.2

Table 2. Biochemical analysis

	Control	STZ-treated	STZ + ZQHC-treated
Blood glucose (mg/dL) (Start point)	112.0	434.0 ± 43.1	379.0 ± 20.5
(End point)	108.0	538.0 ± 39.6	215.0 ± 46.7
Urinary glycose (mg/dL)	ND	3500.0 ± 2121.3	1500.0 ± 2121.3
Insulin (mU/mL)	25.20	7.20 ± 0.92	8.30 ± 1.41
Glycsylated albumin (%)	7.60	16.10 ± 7.28	10.10 ± 0.21
AST (IU/L)	142.0	1933.0 ± 422.8	403.0 ± 268.7
ALT (IU/ L)	84.0	804.0 ± 207.2	234.0 ± 166.2
TG (mg/dL)	117.0	213.0 ± 57.3	150.0 ± 48.8

Statistical analysis

Kruskal-Wallis test was used to compare overall differences among three groups, and median of all groups were compared using Dunn's Multiple Comparison test. $P < 0.05$ was considered as significant.

Results

General observations

Table 1 showed that the average daily water consumption of each individual rat during the experiment was 23.1 mL in control group, 92.7 mL in the STZ-treated group, and 54.2 mL in the STZ+ZQHC-treated group, respectively. The average daily food intake of each rat was 19.1 g in control group, 24.0 g in STZ-treated group and 21.5 g in STZ+ZQHC-treated group, respectively. Average weight gain was found only in the control (116.0 g) and STZ + ZQHC-treated group (55.0 ± 50.2 g). However, rat of STZ-treated group lose weight at the average of 14.0 ± 38.2 g.

Serobiochemical analysis

Table 2 showed the results of biochemical analysis of the blood and urine samples at the end of experimental period. Immense changes in both the non-fasting and fasting blood glucose levels were observed among the three groups--112.0 and 108.0 mg/dL in the control group, 434.0 ± 43.1 and 538.0 ± 39.6 mg/dL in the STZ-treated group, and 379.0 ± 20.5 and 215.0 ± 46.7 mg/dL in the STZ+ZQHC-treated group, respectively. Urinary glucose level was not detected in the control group, whereas it was 3,500.0 ± 2,121.3 mg/dL in the STZ-treated group and 1,500.0 ± 2,121.3 mg/dL in the STZ + ZQHC-treated group.

The following data were presented in comparison between the control vs STZ-treated vs STZ + ZQHC-treated groups, respectively, which included blood insulin (25.20 vs 7.20 ± 0.92 vs 8.30 ± 1.41 μU/mL), glycosylated albumin (7.60 vs 16.10 ± 7.28 vs 10.10 ± 0.21%), AST (142.0 vs 1,993.0 ± 422.8 vs 403.0 ± 268.7 IU/L), ALT (84.0 vs 804.0 ± 207.2 vs 234.0 ± 166.2 IU/L), and TG (117.0 vs 213.0 ± 57.3 vs 150.0 ± 48.8 mg/dL). It was apparently that AST and ALT levels in the STZ + ZQHC-treated group were much less than those of the STZ-treated group, but still higher than those of control group. Moreover, TG level in the STZ + ZQHC-treated group was slightly lower than that of the STZ-treated group.

Morphological analysis

(a) Light microscopy

(i) Liver

In the control group, the central veins close to the centers of the hepatic lobules, the hepatic cord formed by hepatocytes radiating outwards from central vein, and the interlobular triad at marginal lobules, were observed (Fig. 1 A–1 and A–2). In the STZ-treated group, although the hepatic cords were also observed, their hepatocytic population surrounding the interlobular triad was found to contain numerous diffusive intracellular vacuoles. Such vacuoles became larger in the hepatocytes locating close to the portal triads (Fig. 1 B–1 and B–2). In contrast, the hepatocytic vacuoles were not observed both in the control and STZ+ZQHC-treated groups (Fig. 1 C–1 and C–2).

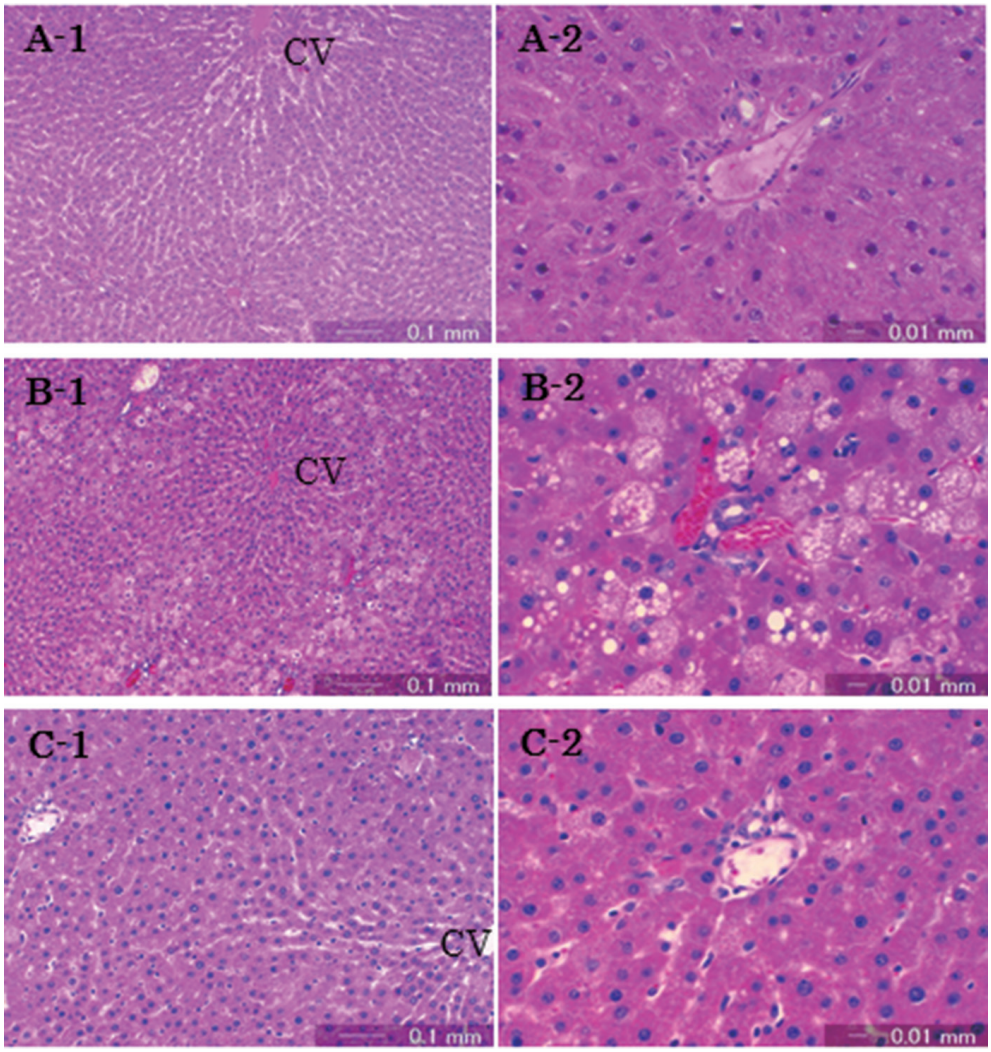

Fig. 1. Morphology of liver with H&E staining in the control group (A–1 and A–2), STZ-treated group (B–1 and B–2), and STZ + SQHC-treated group (C–1 and C–2). CV: central vein.

(ii) Pancreas

In pancreas of the control group, clear pancreatic islets were observed within the exocrine portion (Fig. 2 A–1). But only extremely small islets with certain amount of pyknoses were also observed in the STZ-treated group (Fig. 2 B–1). In the STZ+ZQHC-treated group, even the comparative clear islets were observed, only a few pyknosis were observed (Fig. 2 C–1). The immunostaining for insulin could reveal an extensive distribution of the insulin-positive cells within the islets both in the control and STZ + ZQHC-treated groups (Fig. 2 A–2, C–2). However, only a few positive cells were seen in the STZ-treated group (Fig. 2 B–2). Proportion of the insulin-positive cells in the pancreatic islets was 66.60 ± 9.58% in control group, 13.70 ± 7.52% in STZ-treated group, and 33.50 ± 16.42% in STZ + ZQHC-treated group, respectively. This result indicated that the substantial decrease of the insulin-positive cells in the islets in both STZ-induced diabetic conditions. However, their decreasing degree was diminished by the ZQHC administration (Fig. 2–2).

The immunostaining for PCNA was also able to exhibit the distribution of the positive cells throughout the islets in the control group (Fig. 2 A–3). In contrast, almost none of the positive cells were found in the STZ-treated group (Fig. 2 B–3). The positive cells in the STZ+ZQHC-treated group were much fewer than that in the control group, but still much more than that in the STZ-treated group (Fig. 2 C–3).

(b) Transmission electron microscopy

(i) Liver

The polygonal hepatocytes containing spherical nuclei, numerous mitochondria and glycogen gran-

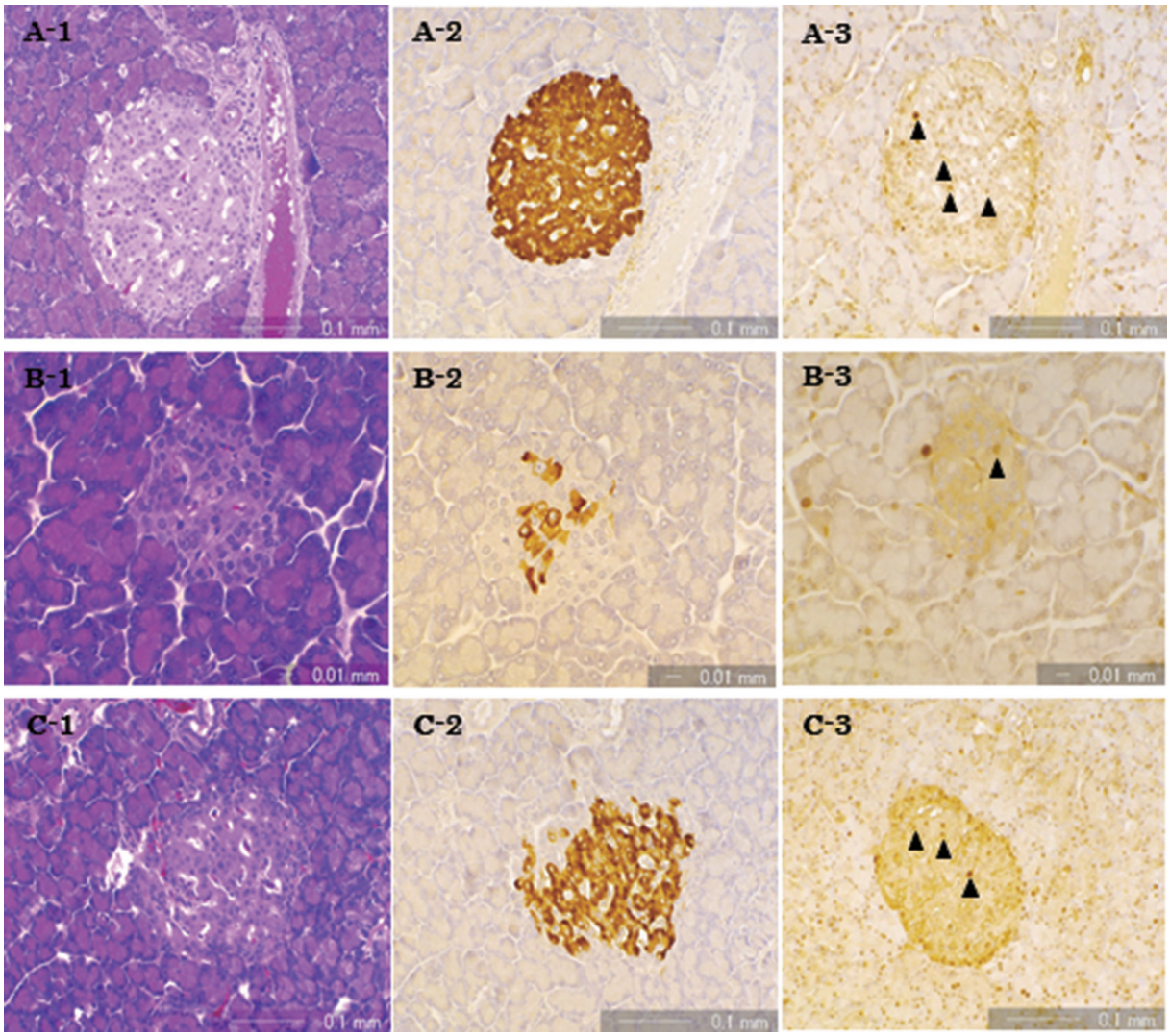

Fig. 2-1. Morphology of pancreatic islets with H & E staining (A–1, B–1 and C–1), immunostaining for insulin (A–2, B–2 and C–2) and immunostaining for PCNA (A–3, B–3 and C–3). Fig. 2–1 showing light micrographs of the Control group (A), STZ-treated group (B) and STZ + ZQHC-treated group (C). Arrow head indicates the PCNA positive cells. Fig. 2–2 showing proportion of insulin-positive cells in the pancreatic islets by the whiskers of box plots encompassing the minimum to maximum. Statistical significance were represented by asterisk ($P < 0.05$).

ules, were observed in the control group (Fig. 3A). In the STZ-treated group, glycogen granules were hardly observed in hepatocytes. In addition, numerous fat droplets of various sizes and swollen mitochondria without apparent cristae were observed in the hepatocytes of this group (Fig. 3B). In the STZ+ZQHC-treated group, hepatocytes containing small fat droplets, un-distorted mitochondria and numerous glycogen granules were observed (Fig. 3C).

(ii) Pancreas

In the control group, small spherical secretory granules with high electron density locating in numerous vacuoles in β cells were observed. These cells were found distributing near the center of the islets (Fig. 4A). In the STZ-treated group, β cells containing only vacuoles but without secretory granule were seen. In addition, numerous lacunae of various sizes and multiple membranous structures were observed in the islets of this group (Fig. 4B). In the STZ+ZQHC-treated group, β cells with similar characteristics to those of the control group were observed; however, the similar lacunae as of those in the STZ-treated group were hardly found (Fig. 4C).

Numerous α cells, that contained the high electron dense secretory granules being surrounded by a halo around the margins of the islets, were observed in the control group (Fig. 4A). Much more numerous α cells with similar distribution were also observed in both the

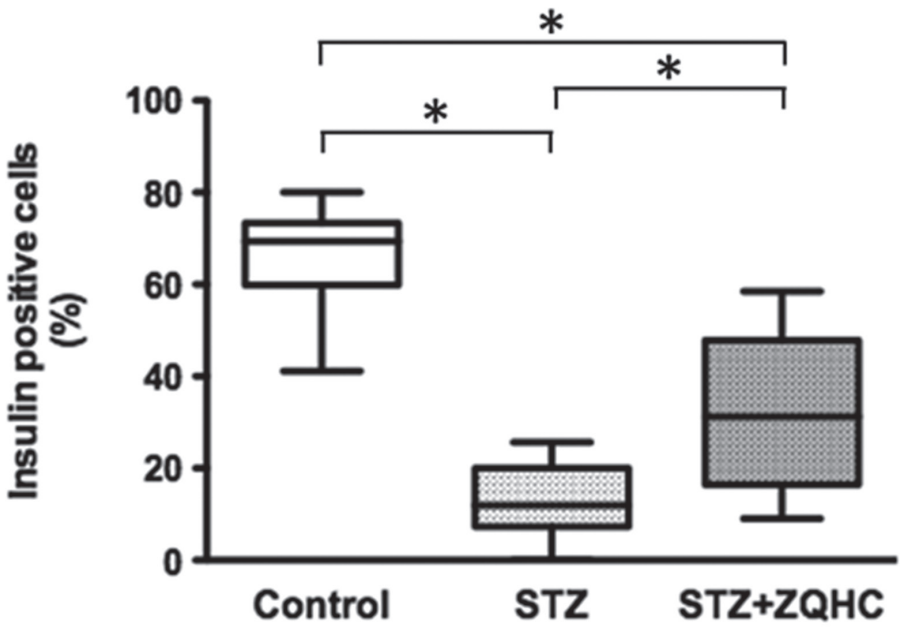

Fig. 2-2. Morphology of pancreatic islets with H & E staining (A–1, B–1 and C–1), immunostaining for insulin (A–2, B–2 and C–2) and immunostaining for PCNA (A–3, B–3 and C–3). Fig. 2–1 showing light micrographs of the Control group (A), STZ-treated group (B) and STZ + ZQHC-treated group (C). Arrow head indicates the PCNA positive cells. Fig. 2–2 showing proportion of insulin-positive cells in the pancreatic islets by the whiskers of box plots encompassing the minimum to maximum. Statistical significance were represented by asterisk ($P < 0.05$).

STZ-treated and STZ + ZQHC-treated groups (Fig. 4B, 4C).

Discussion

Since diabetes mellitus is one of the most critical diseases in all countries, accumulating information for the effective treatment and prevention of this disease is indeed urgently. In particular, oral anti-diabetic treatment has been considered as the most simple and important non-invasive regime for this life-long ailment. Currently, several oral medications, including biguanides, α-glucosidase inhibitors and sulfonylureas, has become major concerns regarding the risks of their side effects[1,5-7]. Since several plant-derived ingredients have been found to exert potent anti-diabetic activities, numerous plant-based oral medicines against diabetes have been developed[1, 6, 8, 9, 12].

Zhen Qi Hypoglycemic Capsules (ZQHC), containing astragalus and polygonatum, is an oral product that has been traditionally used as an anti-diabetic herbal medicine in Chinese society. It was a surprise that there is no definite evidence involving the efficacy against diabetes of this oral preparation. Thus far, astragalus has been reported to enhance immune system and exert diuretic effect, antinephritic effect, nourishing tonic effect, and antioxidant activity[8, 9]. Polygonatum has been documented to lower the blood glucose level, improve dry mouth, and exert diuretic effect[2, 7, 10, 13]. To confirm its efficacy upon the anti-diabetic effects, oral administration of ZQHC to the type I diabetic rats being induced by STZ were performed and several evidences were shown afterwards.

The decline in blood insulin level, rise in blood glucose level, and morphological changes in liver and pancreas in the STZ-induced diabetic rats receiving oral ZQHC were the strong evidences upon the improvement of diabetic condition[4, 6, 7, 14] (Table 2). Hitherto, these results suggested that the oral ZQHC possesses potential to control blood glucose level independently of insulin (Table 2)[5]. This suggestion was also supported by the changes in blood ALT and AST values and morphology of liver and pancreas.

Regarding the morphological analysis, the presence of normal pancreatic β cells observable only in the ZQHC-administrated diabetic rats confirmed that ZQHC did not reduce the secretory granules in the β cells (Fig. 2–2 and Fig. 4). Usually, the generation of free radicals in the β cells involves reduction in number of secretory granules. The following depletion of NAD shall finally bring these cells to death through nuclear deformation and chromatin clumping[3]. In consideration of its anti-diabetic activity, astragalus[8] in the oral ZQHC could suppress the free radical generation for the sustainment of insulin secreting β cells, regardless of blood insulin level

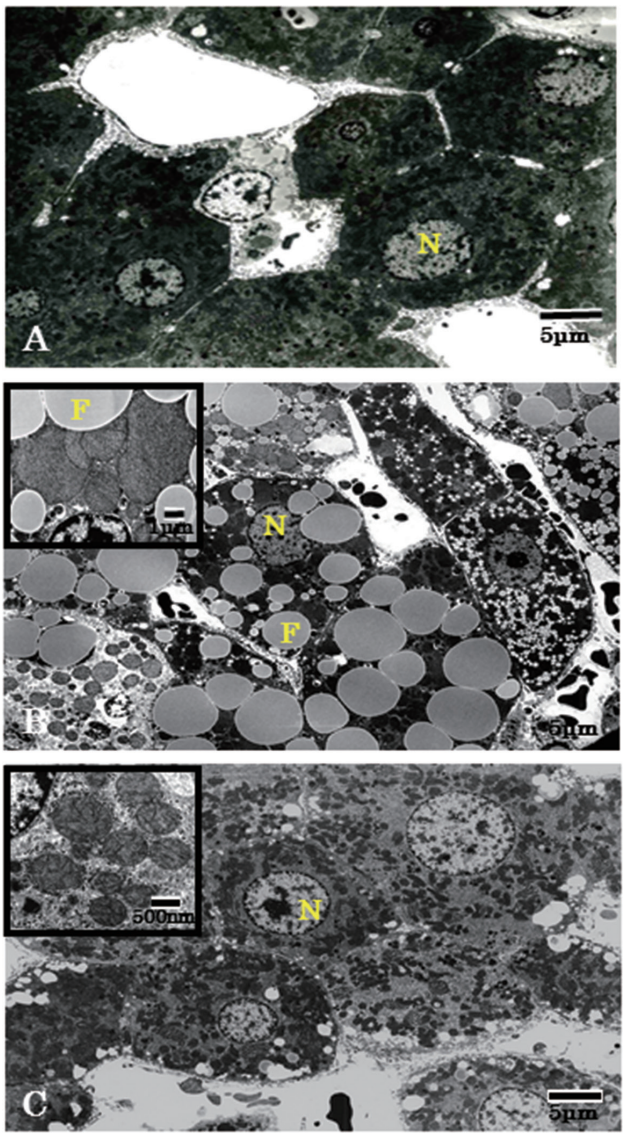

Fig. 3. TEM photographs of liver of the Control (A), STZ-treated (B) and STZ + ZQHC-treated group (C). The square inserts in B and C are magnification areas.

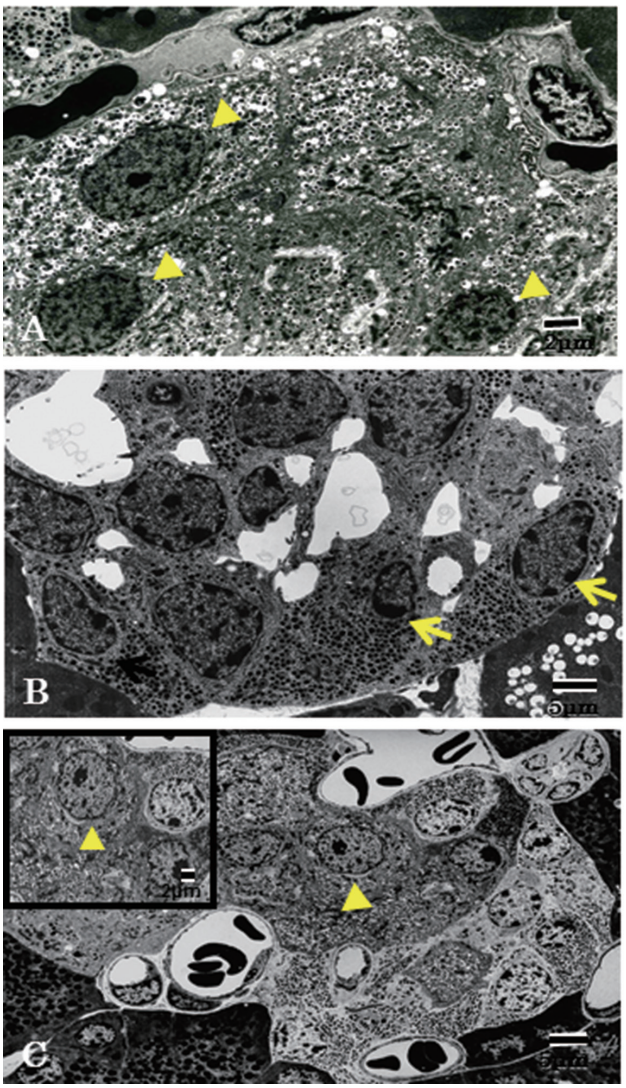

Fig. 4. TEM photographs of pancreas of the Control (A), STZ-treated (B) and STZ + ZQHC-treated group (C). The square insert in A is a magnification area. Arrow indicates α cell. Arrow head indicates β cell.

(Fig. 2–1, 2-2 and Table 2).

Generally, insulin deficiency promotes the metabolic activity of the fat stored in the adipose tissue and enhances the accumulation of fat in hepatocytes. Such activity could impair membrane permeability of the liver cells, and in consequence, hepatic dysfunction and increase of AST and ALT levels are induced[5, 6, 9]. Levels of AST and ALT in the ZQHC-administrated diabetic rats were lower than those in the diabetic rats (Table 2). The numerous fat droplets as seen in the hepatocytes of the non-ZQHC-administered diabetic rats were not observed in those of the ZQHC-administrated diabetic rats. Only less number of small droplets were seen per se (Fig. 1 and 3). The capability in reduction of blood glucose, improvement of insulin sensitivity and glucose uptake of hepatocytes in the STZ-induced diabetic rats[6, 8] of astragalus and polygonatum would suggest that the anti-diabetic activity of ZQHC were mainly induced by the improvement of liver function through the synergistic effect of ZQHC components.

In this study, the apparent enhancement effects on blood insulin level in severe diabetic condition was not observed. However, polygonatum having inhibition effect for α-glucosidase activity on small intestinal mucosal surface to induce support the insulin secretion from β cell via delaying glucose absorption[2, 10, 13]. Thus, there is possibility of suppression to decrease blood insulin level in moderate diabetic condition by oral ZQHC adminis-

tration through the sustainment of survival of vigorous β cells and control of glucose metabolism in the liver.

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