

Full Length Research Paper

## Hypoglycemic and hypolipidemic effects of *Cephalotaxus sinensis* in STZ-induced diabetic rats

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Accepted 9 July, 2012

Hypoglycemic and hypolipidemic activities of *Cephalotaxus sinensis* extracts were studied in streptozotocin-induced diabetic rats. The animals were divided into normal control, diabetic control, diabetic treated and control treated group ( $n = 6$ ). Effect of oral administration of 80% aqueous ethanolic extract (aq.EE), water extract (WtE), ethyl acetate fraction (EaF), and butanol fraction (BtF) of *C. sinensis* (200 mg/kg) based on the body weight for 28 days, level of blood glucose, total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) were determined. It was found that the ethyl acetate fraction of *C. sinensis* significantly lowered the elevated blood glucose level, cholesterol, triglycerides, LDL-C, and it showed a significant increase in body weight and HDL-C level ( $p < 0.01$ ). These results obviously indicated that *C. sinensis* possesses promising hypoglycemic and hypolipidemic effects, which could exert a beneficial action against high glucose level as well as reduced effect on HDL.

**Key words:** *Cephalotaxus sinensis*, hypoglycemic, hypolipidemic.

### INTRODUCTION

Diabetes mellitus (DM) is a chronic disease that affects an estimated 220 million people worldwide and fourth or fifth leading cause of death in the world. DM is an endocrine and metabolic disorder characterized by chronic hyperglycemia (Nickavar and Yousefian, 2011). Asia is the most affected by DM and expected to rise by two-to three folds by 2030 (Shaw et al., 2010). Certainly, diabetes will be one of the most challenging health problems in the 21st century. The management of diabetes is a huge burden on societies because the treatment on insulin is not economically beneficial. Beside that, insulin is mainly administered through injection; hence, it is not patient-friendly due to associated side effects. Alternative remedy is the only way out in the management of this disease. Considering the resistance level against the

orthodox medicine, ethnomedicine becomes the only way out. The ethnopharmacological use of herbal remedies for the treatment of diabetes mellitus is a vital important area, and has been widely researched. The advantages of herbal medicine are numerous, among which are the availability, absence of chemical in the preparation and inexpensive with quick therapeutics effects (Li et al., 2009).

*Cephalotaxus sinensis* (Rehd et Wile) Li, commonly known as Chinese plum yew, belongs to the family Cephalotaxaceae widely distributed in Southern China. It is an indigenous medicinal plant and used for the treatment of dyspepsia, helminthiasis, ascariasis, inflammation and cough. The branches, roots, leaves and seeds are source of many alkaloids, which are used to treat

leukaemia and lymphosarcoma (Duke and Ayensu, 1985). Ren and Xue (1981) has isolated the antitumor constituents from *C. sinensis*. Previously, we have studied free radical scavenging activity of *C. sinensis* leaves (Saeed et al., 2007a). However, to the best of our knowledge, no other biochemical investigation had been carried out on the effect of *C. sinensis* in STZ-induced diabetic rats on serum glucose, triglycerides (TG) and total cholesterol (TC). This investigation was therefore carried out to study the hypoglycemic and hypolipidemic effects of *C. sinensis* in streptozotocin-induced diabetic rats.

## MATERIALS AND METHODS

Leaves of *C. sinensis* were collected from Anhui province, People's Republic of China, and sample was authenticated by Professor Dr. Yulin Deng (Dean, School of Life Science and Technology, Beijing Institute of Technology, Beijing, People's Republic of China) and Associate Researcher Bing Wen (Xishuanbanna Tropical Botanical Garden, Chinese Academy of Sciences, People's Republic of China). Voucher specimen (no. S20041101) was deposited at the School Herbarium for future references. The leaves were shade dry at room temperature.

### Preliminary screening

The ethanolic extract was subjected to preliminary screening for various active phytochemical constituents, such as alkaloids, carbohydrates, steroids, protein, tannins, phenols, flavonoids, mucilage, glycosides, saponins and terpenes, according to establish procedures (Evans, 1989).

### Acute toxicity studies

Healthy albino rats of either sex (200 - 250 g), starved overnight (12 h) were divided into four groups ( $n = 6$ ) and the aqueous ethanolic extract of *C. sinensis* in increasing dose levels of 100, 500, 1000 and 3000 mg/kg body weight were orally fed by peritoneal tube (Ghosh, 1984). The experiments were done for ethanol extract to determine the minimal dose that kills all mice and the maximal dose that fails to kill any animal. The rats were observed continuously for 2 h for behavioral, neurological and autonomic profiles and after a period of 24 and 72 h for any lethality or death (Turner, 1965).

### Extraction and fractionation

In brief, 1 kg of completely air dried aerial parts of *C. sinensis* were ground and extracted by soxhlet extractor with 3 L of 80% ethanol at 80°C for 3 h. The aforementioned procedures were repeated three times. Subsequently, the combined ethanolic extract was concentrated in a rotary evaporator at reduced pressure to obtain about 345 g (34.5%, w/w) of extract. The ethanol extract was then suspended in distilled water and partitioned sequentially with petroleum ether, chloroform, ethyl acetate and n-butanol.

### Experimental animals

The effects of *C. sinensis* supplementation on glucose and lipid were studied using 42 Wistar rats (270 - 315 g), which were divided

into normal and *C. sinensis* supplemented groups given (100, 200 mg/kg body wt) for 4 weeks. The animals were individually housed in stainless-steel cages in a temperature ( $22 \pm 2^\circ\text{C}$ ) and light/dark (12/12 h) control room and fed standard laboratory diet and water *ad libitum* and all animals were observed daily for any clinical signs of disease. The food consumption and weight gain was measured daily and weekly. Animal studies were conducted according to the Institute Animal Ethics Committee regulations approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

### Streptozotocin-induced hyperglycemia

A freshly prepared solution of streptozotocin (65 mg/kg, body weight) in 0.1 M citrate buffer (pH 4.5) was injected intraperitoneally in a volume of 1 ml/kg. Streptozotocin (STZ)-injected animals exhibited massive glycosuria and hyperglycemia within 2 days (Siddique et al., 1989). Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration 96 h after the injection of STZ. The rats with blood glucose level  $>250$  mg/dl were considered to be diabetic and were used in the experiment.

### Experimental procedure

The rats were divided into seven groups (six in each group): Group I, normal rats + vehicle (solution of normal saline and 3.0% tween 80); Group II, untreated diabetic + vehicle; Group III, diabetic + Tolbutamide (25 mg/kg body wt); Group IV, diabetic + 80 % aqueous ethanolic extract (aq.EE); Group V, diabetic + water extract (WtE); Group VI, diabetic + ethyl acetate fraction (EaF) and Group VII, diabetic + Butanol fraction (BtF) (200 mg/kg body wt) daily by gastric intubations for a period of 28 days. All the extracts and fractions were dissolved in vehicle (solution of normal saline and 3.0% tween 80). All groups were sacrificed on the 28<sup>th</sup> day in fasting condition by cervical dislocation. Blood samples were collected weekly and serum was separated immediately by centrifuging at 3000 rpm for 15 min for various biochemical estimations and stored in an Eppendorf tube in freezer at  $-18^\circ\text{C}$ . Body weights of all the animals were recorded weekly, prior to the treatments and sacrifice.

### Measurement of hypoglycemic activity

Blood samples (0.5 ml) were obtained by orbital sinus puncture (Waynforth, 1980) using capillary tube and the glucose concentration in the serum samples was analyzed immediately by the glucose oxidase method (Braham and Trinder, 1972), using glucose oxidase (GOD) assay kit and a spectrophotometer (UV-VIS 2250 Shimadzu, Japan). Oral glucose tolerance test was performed according to method described by Whittington et al. (1991). Briefly, after over night fasting, zero-min blood sample (0.2 ml) was taken from the rats in normal, diabetic control, diabetic + aq.EE; diabetic + WtE; diabetic + EaF; diabetic + BtF (200 mg/kg body wt) and diabetic + Tolbutamide (25 mg/kg body wt) groups by orbital sinus puncture. Glucose solution (2 g/kg body wt) was administered orally immediately. Four more samples were taken at 30, 60, 90 and 120 min after glucose administration and used for the estimation of blood glucose.

### Determination of hypolipidemic activity

Total serum cholesterol (TC) was estimated by method as described by Allain et al. (1974). High-density lipoprotein cholesterol (HDL-c) was determined by the method of Burstein et al.

**Table 1.** Effect of 4-week treatment with various extracts and fractions of *C. sinensis* on body weight (g) in STZ induced diabetes rats.

Group	Duration of treatment and body weight			Change in body wt
	Day 1	Day 14	Day 28	
Group I(+) control	290.12 ± 3.5	316.90 ± 3.7	332.82 ± 4.3	+ 42.7
Diabetic (-) control	280.33 ± 3.2	265.70 ± 2.9	262.73 ± 3.1	-17.6
Diabetic + tolbutamide	304.65 ± 4.1**	317.20 ± 4.2**	324.95 ± 4.3**	+20.3**
Diabetic + aq.EE	274.35 ± 2.3*	280.62 ± 2.4*	283.55 ± 2.4*	+ 9.2*
Diabetic + WtE	266.20 ± 2.2#	269.50 ± 2.3#	272.00 ± 2.2#	+5.8#
Diabetic + EaF	310.85 ± 4.2**	319.75 ± 4.4**	326.95 ± 4.3**	+16.1**
Diabetic + BtF	283.41 ± 3.1*	293.62 ± 3.2*	296.01 ± 3.3*	+12.6*

All values are expressed as mean ±SD (n = 6); Diabetic control was compared with normal control and extracts/fractions were compared with the diabetic control. #P >0.05, \*P<0.05, \*\*P <0.001; TB: Tolbutamide 25 mg day<sup>-1</sup>; for 28 days; aq. EE: 80% ethanolic extract of *C. sinensis* 200 mg kg<sup>-1</sup> day<sup>-1</sup> for 28 days. EaF: ethyl acetate fraction of *C. sinensis* 200 mg kg<sup>-1</sup> day<sup>-1</sup> for 28 days; BtF: butanol fraction of *C. sinensis* 200 mg kg<sup>-1</sup> day<sup>-1</sup> for 28 days.

(1970). Low-density lipoprotein cholesterol (LDL-c) levels were calculated by using the formula of Noda et al. (2000). Serum triglycerides (sTG) were anticipated colorimetrically with enzymatic method (Fossati and Lorenzo, 1982) using spectrophotometer (UV-VIS 2250 Shimadzu, Japan). Also, atherogenic index was calculated using the following formula (Kayamori and Igarashi, 1994):

$$\text{Atherogenic index} = \frac{\text{Total cholesterol} - \text{HDL-C}}{\text{HDL-C}}$$

#### Histopathological studies

Rats were anesthetized with ether following a 16-h fast. Histopathological studies of pancreas were conducted in control and diabetic rats treated with control vehicle or extracts and fractions of *C. sinensis*. The whole pancreas from each animal was removed after sacrificing the animal and was collected in 10% formaline solution, and immediately processed by the paraffin technique. Sections of 5 µM thickness were cut and stained by haematoxylin and eosin (H & E) for histological examination.

#### Statistical analysis

Results were expressed as mean ± standard deviation (SD). The significance of the differences between the means of tests and control studies were established by Student's t-test for independent samples with one tail and *p* values less than 0.05 will be considered significant.

## RESULTS AND DISCUSSION

The use of herbal extracts and herbal formulations in the literature in recent times been reviewed and have gained importance for the control of type-II diabetes. For the preparation of many modern drugs, they are directly or indirectly used (Ghosh et al., 2012). The aim of the present work was to elucidate the *C. sinensis* effects on hyperglycemia and hyperlipidemic in STZ-diabetic rats, to provide an introductory approach for the evaluation of its traditional usage in order to scientifically validate the

therapeutic preparation of this plant in the control of diabetes. To the best of our knowledge, this is the first report that analyzes the hypoglycemic and hypolipidemic effects of *C. sinensis* extracts and fractions in experimental diabetes.

#### Preliminary chemical tests

The phytochemical studies indicated that the ethanolic extract of leaves of *C. sinensis* contains polyphenols, alkaloids, flavonoids, glycosides, saponins, terpenes and steroids, while mucilage and proteins showed negative results.

#### Acute toxicity studies

In performing preliminary tests for pharmacological activity in rats, aqueous ethanolic extract did not produce any significant changes in the autonomic, behavioral or neurological responses up to doses of 3000 mg/kg body wt. According to a toxicity classification (Loomis, 1968), *C. sinensis* extract is non-toxic. Toxicity studies showed that the hematological and biochemical parameters were within normal range.

#### Effect on body weight

Streptozotocin treatment caused significant weight reduction in rats as compared to the vehicle treated normal rats (Ahn et al., 2006). After treatment for 28 days, the gain in weight were EaF: +16.1 (*P* < 0.01); BtF: +12.1 (*p*<0.05) and aq. EE: +9.2 g (*p*<0.05) (200 mg/kg body wt.), respectively. However, the standard drug tolbutamide (25 mg/kg body wt) also exhibited significant improvement in body weight loss of the diabetic animals following 28 days of treatment (+20.3 g). The changes in

**Table 2.** Effect of 4-week treatment with various extracts and fractions of *C. sinensis* on glucose level in STZ induced diabetes rats.

Groups	Day 0 (before treatment)	Day 1 (96 h after STZ injection)	Day 14	Day 28
Normal (+) control	81.72 ± 2.3	83.40 ± 2.5	82.90 ± 2.7	86.50 ± 2.3
Diabetic (-) control	78.12 ± 1.7	311.52 ± 8.2	320.70 ± 8.9	327.63 ± 9.1
Diabetic + TB	80.65 ± 1.9**	309.61 ± 8.1**	147.60 ± 4.2**	92.25 ± 2.3**
Diabetic + aq.EE	77.50 ± 1.3*	302.35 ± 7.3*	218.72 ± 5.6*	148.32 ± 4.5*
Diabetic + WtE	79.30 ± 2.1 <sup>#</sup>	306.30 ± 7.5 <sup>#</sup>	258.70 ± 6.5 <sup>#</sup>	178.50 ± 2.2 <sup>#</sup>
Diabetic + EaF	80.41 ± 2.2**	314.62 ± 8.3**	162.83 ± 4.7**	115.35 ± 3.9**
Diabetic + BtF	82.15 ± 2.3*	305.70 ± 8.1*	193.62 ± 5.1*	139.80 ± 4.3*

All values are expressed as mean ±SD (n = 6); Diabetic control was compared with normal control and extracts/fractions were compared with the diabetic control. <sup>#</sup>P >0.05, \*P <0.05, \*\*P <0.001; TB: Tolbutamide 25 mg day<sup>-1</sup>; for 28 days; aq. EE: 80% ethanolic extract of *C. sinensis* 200 mg kg<sup>-1</sup> day<sup>-1</sup> for 28 days. EaF: ethyl acetate fraction of *C. sinensis* 200 mg kg<sup>-1</sup> day<sup>-1</sup> for 28 days; BtF: butanol fraction of *C. sinensis* 200 mg kg<sup>-1</sup> day<sup>-1</sup> for 28 days.

body weights in all groups of animals are given in Table 1. The ability of *C. sinensis* to protect massive body weight loss seems to be due to its ability to reduce hyperglycemia and enhance glucose utilization.

### Oral glucose tolerance test

Hyperglycemic was induced in rats with oral glucose (2 g/kg body wt) and significant hyperglycemia was observed during 120 min. The blood glucose varied from 78.2 ± 3.2 to 290.3 ± 0.22 mg/dl (n = 6). Figure 1 shows the changes in the levels of blood glucose in normal and experimental groups. In EaF (200 mg/kg body wt) and tolbutamide (25 mg/kg body wt) treated animals, blood glucose concentration was significantly decreased (p < 0.01) after 1 and 2 h. In BtF and aq. EE treated animals, blood glucose concentration was also significantly decrease (p < 0.05). STZ irreversibly damages the insulin secreting β-cells of the pancreas (Arambewela et al., 2005; MacSweeney et al., 1995). Therefore, the antidiabetic effect of *C. sinensis* may be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after sulphonylurea administration.

### Blood glucose

STZ treatment showed significant elevation of serum glucose level compared to the normal control rats as noted at different periods of study. The fasting blood glucose levels before and after treatment in all the groups of animals are given in Table 2. Results indicated that the fasting blood glucose levels of untreated diabetic rats were significantly higher than those in the normal rats. Moreover, a significant decrease in blood glucose levels was observed in diabetic treated with EaF (200 mg/kg body wt) group from an initial level of 314.62 ± 8.3 to the level of 115.35 ± 3.9 mg/dl (p < 0.01) after treatment, followed by BtF (200 mg/kg body wt) (p < 0.05) group

which was comparable to the standard drug tolbutamide (25 mg/kg body wt). In contrast, no significant reduction of blood glucose levels was observed in diabetic rats after the oral administration of the water extract (initial value 93.5 ± 2.5 and after treatment 104 ± 9.0 mg/dl) (p > 0.05), while no significant decrease in blood glucose levels was observed in normal group. The total β-cell mass reflects the balance between the renewal and loss of these cells. Similar effects in streptozotocin-treated diabetic animals were reported by pancreas tonic (Rao et al., 1998), ephedrine (Xiu et al., 2001) and *Gymnema sylvestre* leaf extracts (Shanmugasundaram et al., 1990).

### Effect on lipid profile

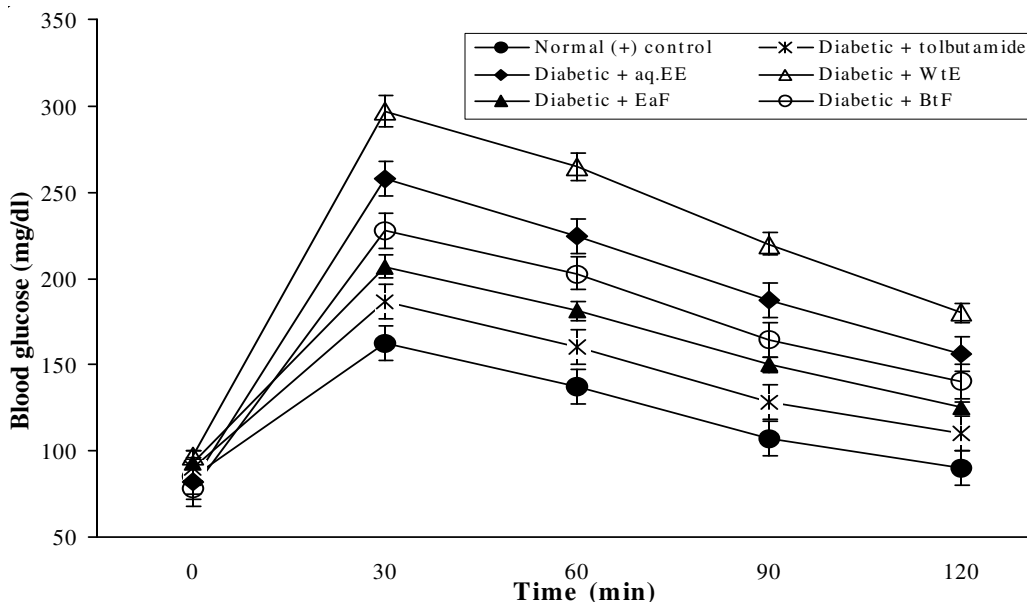
Hyperlipidemia is one of the major cardiovascular risk factors. It has been demonstrated that insulin deficiency in diabetes mellitus leads to a variety of derangements in metabolic and regulatory processes, which in turn leads to accumulation of lipids such TG and TC in diabetic patients (Kumar et al., 2012). Streptozotocin treatment showed significant elevation of triglycerides, total cholesterol, LDL-C and reduction in HDL-C levels compared to the normal control rats as noted at different periods of the study (Table 3). There was a significant reduction in serum triglycerides, total cholesterol and LDL-C levels of diabetic rats treated with EaF, BtF and aq. EE (100 and 200 mg/kg, o.p.) compared to tolbutamide (25 mg/kg body wt) at various time intervals. However, there was a significant (p < 0.01) elevation in the HDL-C level in EaF (100 and 200 mg/kg, o.p.) treated diabetic rats on the 28<sup>th</sup> day as compared to the diabetic control group, while the water extract did no show significant effect (p > 0.05).

Our finding was similar to Niu et al. (2006) who reported that the ethanol extract of *C. sinensis* leaves could decrease the serum level of TC and TG in rats with hyperlipidemia significantly. The glucose lowering action of the *C. sinensis* could be due to the consequence of an improved lipid metabolism apart from the direct interaction with glucose homeostasis. The triglycerides lowering

**Table 3.** Effect of 4-week treatment with various extracts and fractions of *C. sinensis* on lipid profile and atherogenic index in STZ induced diabetes rats.

Groups	TG (mg/dl)	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	Atherogenic index (units)
Normal (+) control	82.5 ± 2.4	120.3 ± 4.1	49.3 ± 1.3	54.5 ± 1.6	1.20 ± 0.2
Diabetic (-) control	148.3 ± 3.2	258.5 ± 6.3	196.5 ± 5.3	32.4 ± 1.2	6.97 ± 0.9
Diabetic + TB	85.4 ± 2.3**	118.4 ± 3.4**	48.7 ± 2.6	52.6 ± 1.5**	1.25 ± 0.2**
Diabetic + aq.EE	99.2 ± 2.8*	154.8 ± 4.8*	94.7 ± 3.2*	40.2 ± 1.3*	2.85 ± 0.3*
Diabetic + WtE	127.7 ± 4.6 <sup>#</sup>	190.2 ± 5.7 <sup>#</sup>	128.1 ± 3.3 <sup>#</sup>	36.5 ± 1.2 <sup>#</sup>	4.21 ± 0.8 <sup>#</sup>
Diabetic + EaF	90.5 ± 2.1**	132.7 ± 3.6**	65.4 ± 2.4**	49.2 ± 1.4**	1.69 ± 0.5**
Diabetic + BtF	94.8 ± 2.6*	141.6 ± 3.9*	77.2 ± 2.7*	45.4 ± 1.4*	2.11 ± 0.4*

All values are expressed as mean ±SD (n = 6); Diabetic control was compared with normal control and extracts/fractions were compared with the diabetic control. <sup>#</sup>P >0.05, \*P <0.05, \*\*P <0.001; TB: Tolbutamide 25 mg day<sup>-1</sup>; for 28 days; aq. EE: 80% ethanolic extract of *C. sinensis* 200 mg kg<sup>-1</sup> day<sup>-1</sup> for 28 days. EaF: ethyl acetate fraction of *C. sinensis* 200 mg kg<sup>-1</sup> day<sup>-1</sup> for 28 days; BtF: butanol fraction of *C. sinensis* 200 mg kg<sup>-1</sup> day<sup>-1</sup> for 28 days.

**Figure 1.** Oral glucose tolerance test in the normal and experimental animals.

property *C. sinensis* extract and fractions could indirectly contribute to the overall antihyperglycemic activity through a mechanism of so called glucose-fatty acid cycle (Randle et al., 1963).

### Histological examination of pancreas

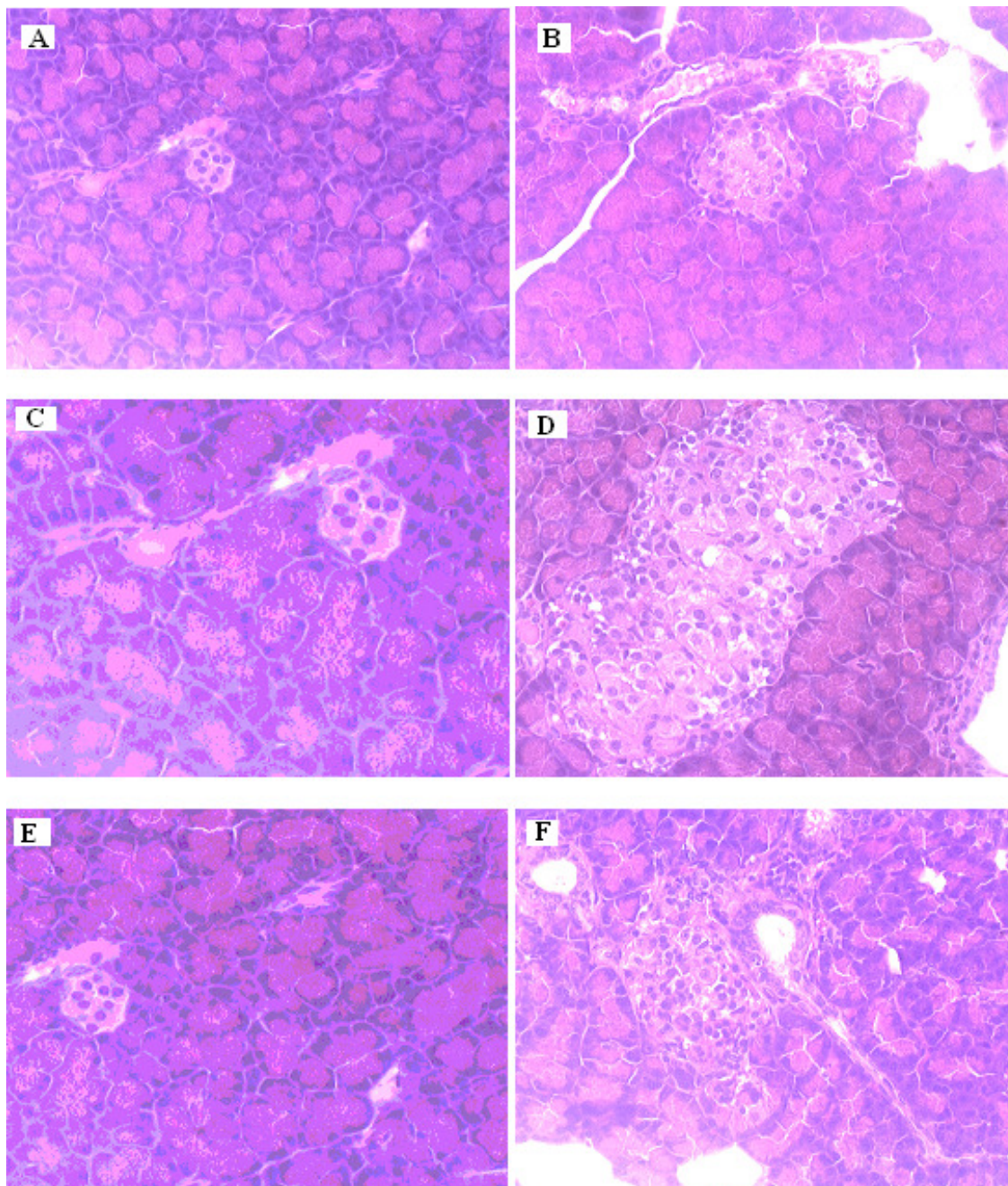
Photomicrographs (Figure 2A) showed normal acini and normal cellular population in the islets of Langerhans in pancreas of vehicle-treated rats. Extensive damage to the islets of Langerhans and reduced dimensions of islets in STZ-induced diabetic rats and the restoration of normal cellular population size of islets with hyperplasia by tolbutamide was also shown. In our studies, the damage of pancreas in STZ-treated diabetic control rats (Figure 2B) and regeneration of  $\beta$ -cells by tolbutamide (Figure

2C) was observed. A comparable regeneration was also shown by ethylacetate, butanol fractions and aqueous ethanolic extract of *C. sinensis* leaves (200 mg/kg, body wt, o.p.) (Figure 2D to F). The cause of diabetes may be modified by a variety of compounds, including alkaloids, flavonoids, glycosides, polysaccharides, peptidoglycans, hypoglycans and terpenoids (Maridass et al., 2008). Various flavonoids had been previously reported from this plant (Saeed et al., 2007b). The photomicrographical data in our studies reinforces healing of pancreas by *C. sinensis* extract and fractions as a possible mechanism of their antidiabetic activity.

### Conclusion

Ethyl acetate and butanol fractions of *C. sinensis* leaves





**Figure 2.** Photomicrographs of rats pancreas stained by haematoxylin and eosin in untreated control (A), STZ-induced diabetic (B), diabetic + tolbutamide (C), diabetic + aq. ethanol extract (D), diabetic + ethyl acetate fraction (E), diabetic + butanol fraction (F) of *C. sinensis*. Microscope magnification (400 $\times$ ).

exhibited significant antihyperglycemic activities in STZ-induced diabetic rats. These extract and fractions also showed improvement in parameters like body weight and lipid profile, as well as regeneration of  $\beta$ -cells of pancreas and so might be of value in diabetes treatment.

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