Available online at www.ijpcr.com
International Journal of Pharmaceutical and Clinical Research 2013; 5(4): 145-149

ISSN- 0975 1556

Research Article

Study the Hypoglycemic Effect of *Crataegus Laevigata* in Diabetic Rats

*Alaghawani W, Naser I

Department of Pharmaceutical Science, Faculty of Pharmacy, the International University for Science and Technology (IUST), Jepap, Darra.

Available online: 17th September, 2013

ABSTRACT

The background and aim: to investigate the effect of *Crataegus laevigata* ethanolic extract in streptozotocine-induced diabetic rats.

Materials and methods: streptozotocine (50 mg/ kg) was used to induce the diabetes mellitus in rats. Afterwards, the diabetic rats were divided into groups in which they were injected by increased doses of ethanolic extract (200, 400, 600, 800, 1000, 1200 mg/kg). There was a groups given the standard oral hypoglycemic agent: *Glipizide* as reference to evaluate the effect of the extract. Indeed, oral glucose tolerance test was performed and the role of the previous extract was detected with comparison to Glipizide.

Results: The extract significantly reduced plasma glucose and this effect was dose- dependent. The effect of Glipizide was generally more potent than that of extract. In oral glucose tolerance test, the extract (1200 mg/ dl) obviously enhanced the glucose tolerance in rats.

Conclusion: The *Crataegus laevigata* has clear hypoglycemic effect but it should be further investigated and the active ingredients must be identified.

Key words: *Crataegus laevigata*, Diabetes Mellitus, Streptozotocine (STZ), Glipizide, Glucose tolerance test.

INTRODUCTION

Hawthorn (*Crataegus laevigata*) is a berry-like fruit of trees from the species of Crataegus that grows commonly in northern temperate regions around the world. It has been documented as a food in many regions around the world. Typically the leaf and flower, berry, or a combination of all three are consumed as a powder, tea, or liquid extract (1).

Crataegus laevigata, sometimes called Chinese hawthorn, is one of the most commonly used remedies in Asia and Europe and has long been used as a herbal medicine (2, 3). While traditional indications for use of hawthorn include asthma, diabetes, and neurasthenia, it is also used for the treatment of various cardiovascular diseases such as myocardial weakness, tachycardia, hypertension and arteriosclerosis (4, 5). Recent studies were reported that *Crataegus laevigata* ethanolic extracts have beneficial effects, including antioxidant, antimicrobial (6) and anti-inflammatory effects (7). Indeed, during the last decades Hawthorn has received much attention because of its potential to reduce plasma cholesterol and triacylglycerol (TAG) concentrations (8, 9). However, the scientific evidence of these beneficial effects of Hawthorn still needs to be further substantiated, including identification of its bioactive compounds and the underlying mechanisms of action. Similar to many countries in Asia, Hawthorn is seen widely in many parts of Syria. In general, Hawthorn plants grow in village gardens and as border plants in the arid areas of this region. In recent years, Hawthorn fruits have been sold in the local markets. Therefore, the determination of the economic and therapeutically values of this crop become important. The principle active components are flavonoids (10, 11): non-toxic phytochemicals that are widespread in fruit and vegetables and have health benefits. Among the most important ingredients of flavonoids are Quercetin and hyperoside (12). This important plant, that has rich and well-documented phytotherapy record, is really worth to investigate its hypoglycemic effect through preliminary studies. In fact, we could not find any scientific reports about this property. Nevertheless, the encourage to detect anti-diabetic effect can come from the traditional use as well as, and probably the most important, from some essential constitutes like Quercetin that has been reported to exhibit hypoglycemic properties (13). So that the aim of this research is to study the hypoglycemic effects of ethanolic extract of Crataegus laevigata in streptozotocin-induced diabetic rats in comparison with oral hypoglycemic agent: Glipizide.

MATERIAL AND METHODS

Plant material: the berries of Crataegus laevigata were collected at morning in western mountain area near Damascus. The identification was made with the help of an expert in botany (faculty of science, Damascus University)

*Author for correspondence: E-mail: waelsy74@yahoo.com

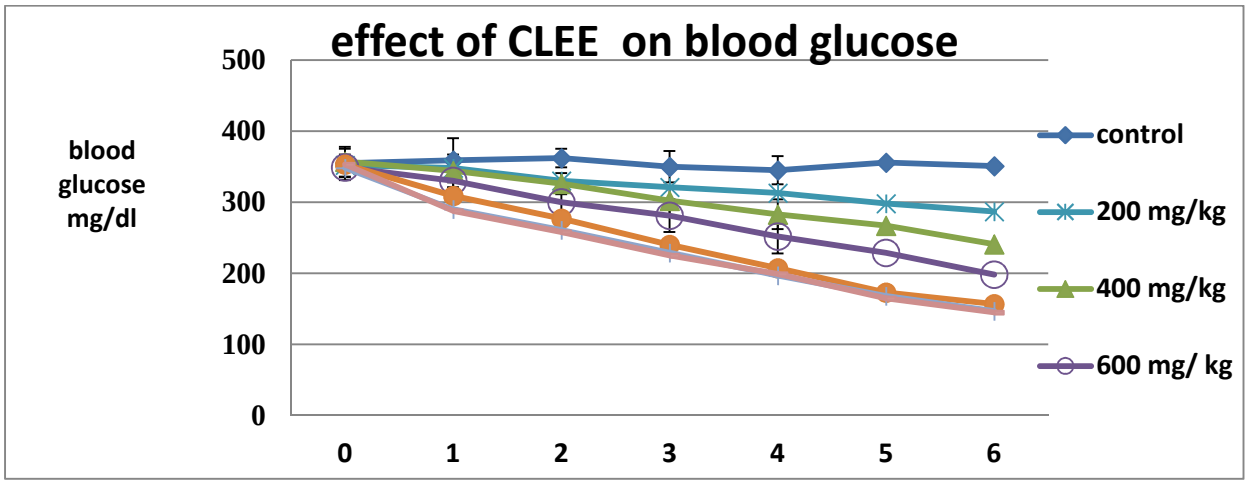

Figure 1: effect of Crataegus laevigata ethanolic extract (CLEE) in diabetic rats

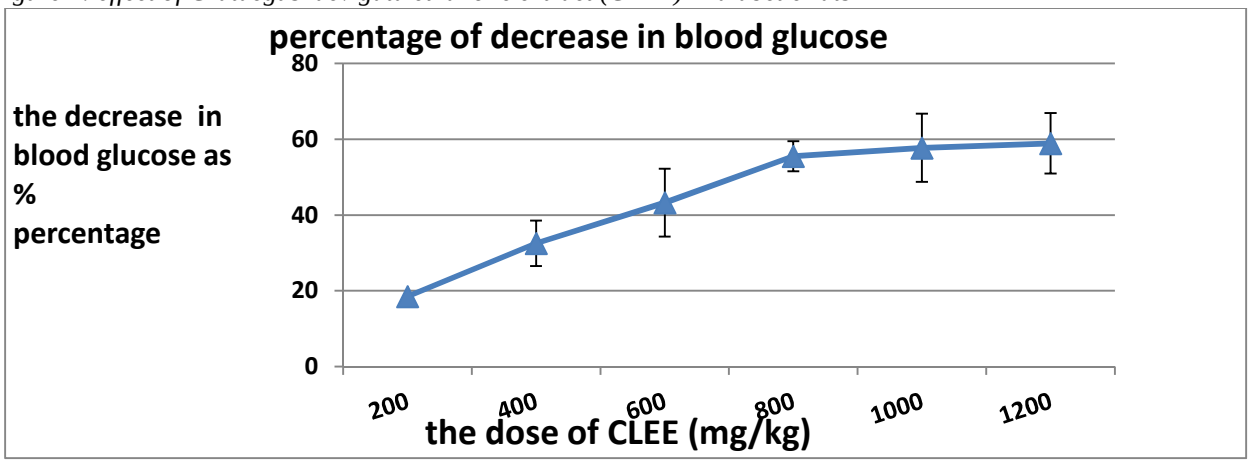

Figure 2: the effect of different doses of CLEE on blood glucose in diabetic animals

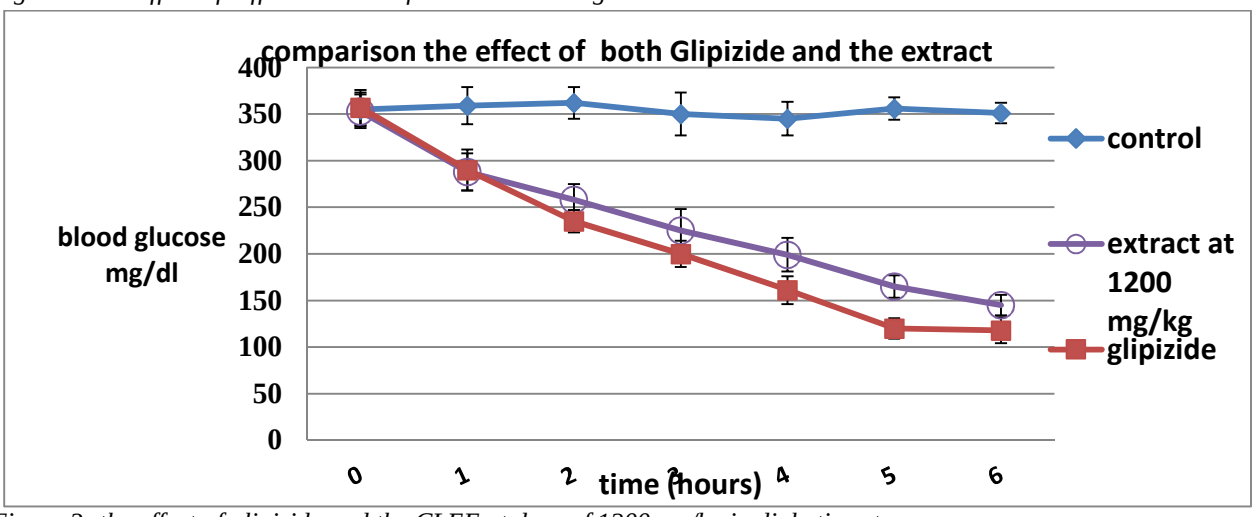

Figure 3: the effect of glipizide and the CLEE at dose of 1200 mg/kg in diabetic rats.

by means of comparisons among different herbarium Hawthorn samples.

Preparation of Crataegus laevigata ethanolic extract (CLEE): The plant extract was prepared by decoction followed by lyophylization. Ground, dried leaves (10 g) were added to 100 ml mixture of 30 ml de-ionized water and 70 ml ethanol and allowed to shake for 24 hours. Solid material was removed by filtration, then the ethanol was evaporated under vacuum and the remaining aqueous extract was lyophilized to dryness. Dry extract yield was approximately 5% (w/w) of crude material. Working solutions of extract were prepared daily at a concentration of 80 mg/ ml in distilled water.

Chemicals: streptozotocin (STZ) (Sigma). Glipizide was obtained from Unipharma company (Damascus, Syria).

Animals: Sprague-Dawley rats were used (150-180 g), all of them were purchased from Leen company, Damascus, Syria. They were housed in standard boxes, allowed free access to tap water and food in an air conditioned room (25^0 C) under 12-h light: 12-h dark cycle prior to the experiments.

Table 1: summary of hypoglycemic effects of CLEE in diabetic rats

Hours groups	0	1	2	3	4	5	6
control	355	359	362	350	345	356	351
CLEE **200** mg/kg	352	348	330	321	313	298	287
CLEE 400 mg/kg	357	344	326	302	283	267	241
CLEE 600 mg/kg	349	330	300	281	252	229	198
CLEE 800 mg/kg	353	309	277	240	207	173	157
CLEE 1000 mg/kg	348	290	261	229	197	168	147
CLEE 1200 mg/kg	353	288	258	225	199	165	145
Glipizide 10 mg/kg	357	290	235	200	161	120	118

Induction of experimental diabetes: Diabetes was induced by a single intraperitoneal injection of a freshly prepared streptozotocin (STZ) solution (50 mg/ kg in acetate buffer 0, 1 M, ph 4.5) to overnight-fasted rats. Control rats received only the buffer. Diabetes was identified by measuring non-fasting plasma glucose levels 48 h after injection of STZ. Animals did not develop more than 250 mg/dl glucose levels, were rejected.

Experimental groups and study the effect of *Crataegus laevigata* in diabetic rats: the diabetic animals were classified into eight groups (1-8) each of them with 12 rats. Group 1 served as a control and received 1.5 ml of physiological NaCl-solution (vehicle), group 2 was given a standard oral hypoglycaemic agent, Glipizide (10 mg/kg body weight), in the same vehicle. Groups 3- 8 received: 200, 400, 600, 800, 1000, 1200 mg/kg respectively of CLEE. The extracts were re-dissolved in 1.5 ml of physiological NaCl-solution and administered orally by a canula. Blood samples were collected just prior to extract's administration (time 0) and at 1, 2, 3, 4, 5, 6 h after administration.

Oral glucose tolerance test: fasted normal rats were divided into 4 groups of 12 rats in each. Group 1 served as a control and received distilled water with tween 80. Group 2 received standard drug Glipizide 10 mg/kg as an aqueous suspension. Groups 3 and 4 received extracts at dose of 800, 1200 mg/kg. After 45 min of extracts and drug administration 2,5 g/kg of glucose was orally given to all groups. Blood glucose samples were collected before and at 25, 60, 90 min following glucose loading. Glucose levels were measured immediately by Glucometer.

Collection of blood and determination of blood glucose: blood samples (0.025 ml) for all experiments were taken through puncture of the tail vein. Glycemic levels were determined in Accutrend Sensor Comfort apparatus using reactive strips (Roche).

Statistics: Student's –test and a probability of $P < 0.05$ were chosen as the criteria for statistical signifigance. Values reported as mean± standard error of the mean (SEM).

RESULTS

Activity in diabetic rats: STZ administration at a dosage of 50 mg/ kg to normal rats significantly (P< 0,001) elevated the blood glucose levels compared with rats injected citrate buffer alone.

In our diabetic rats the extract showed significant hypoglycemic effects, figure 1 and table 1. This effect increased with time comparing with control (P< 0.05). The maximum effect was at 6th hour following the administration of the extract. Indeed the hypoglycemic effect was a dose dependent with maximum effect (Emax) for the dose of 1200 mg/kg. Fig 2

Glipizide (10 mg/kg) caused a hypoglycemic action, and this effect was compared with the extract at 1200 mg/kg (Emax). Fig 3.

Oral glucose tolerance test: After 60 min following oral glucose loading (2.5 g/ kg), the blood glucose levels reached its maximum comparing with fasting level, then afterwards they gradually reduced. The extract (CLEE) was obviously effective in reducing the values of glucose levels especially at min 30 and 60 comparing with control group (P< 0.05), table 2 and figure 4.

DISCUSSION

The efforts for finding effective treatment for diabetes are always in progress. Despite the available of several groups of oral hypoglycemic agents, there are always approaches

Table 2: effect of CLEE on oral glucose test in normal rats

Groups	Min 0 (before glucose loading)	Min 25 (mg/dl)	Min 60 (mg/dl)	Min 90 (mg/dl)
control	95	137	166	131
CLEE (800 mg/kg)	93.25	118.25	129.5	112
CLEE (1200 mg/kg)	97.25	112.75	117	101.5
Glipizide 10 mg/kg	103	111	100	98

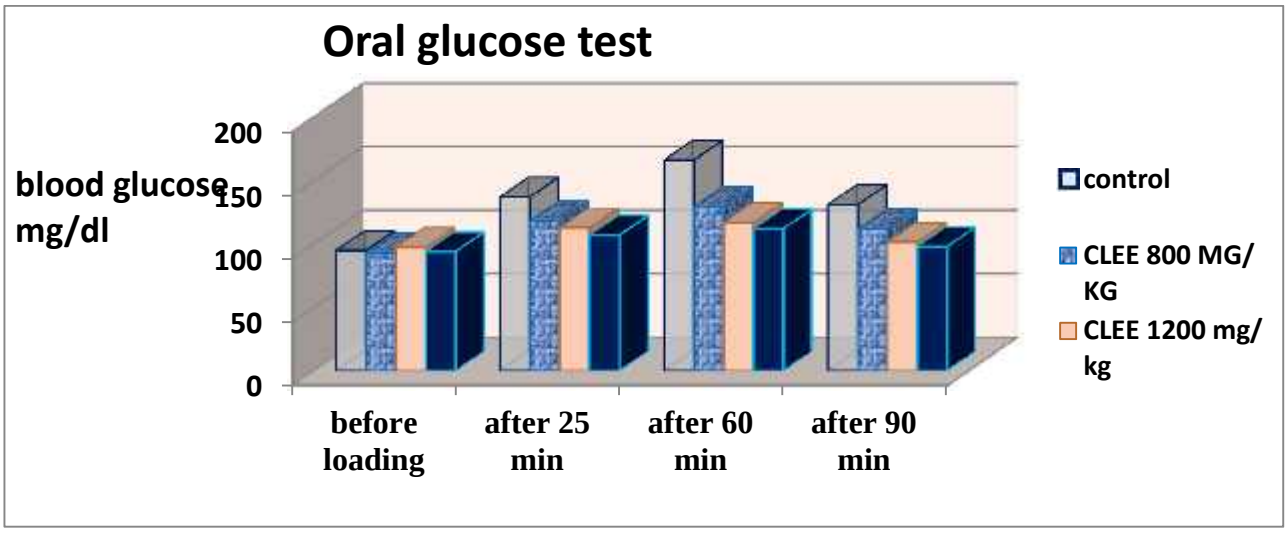

Figure 4: results of oral glucose test

for developing new drugs with more effective effect regarding controlling hyperglycemia. The matter, however, doesn't only restricted to chemical industrial side but it is also relevant to natural resources especially plants. So that it is scientifically common to investigate deeply some plants as they contain bioactive compounds and this may lead to the possibility of hypoglycemic effects. Some plants were certainly approved to have antidiabetic activities (14, 15). In this research, our results clearly indicate a hypoglycemic properties of *Crataegus laevigata*.

The direct procedures for detecting antidiabetic effects of CLEE exhibit a profound hypoglycemic trace of the extract. Indeed, it was a dose-dependent, as shown in figure 2, attempt was done to determine the dose that has the maximum effect (Emax) of CLEE and this dose was 1200 mg/kg. This dose induced a 60% reduction in blood glucose, from 353 to 145 mg/ dl at the 6^{th} hours following the administration of the dose. This effect was evaluated with the effect of glipizide, the standard hypoglycemic agent. As data showed, the glipizide's effect was slightly more potent than that of maximum dose of CLEE. Glipizide, which belongs to Sulphonyl urea, was used in relating research as standard reference (16, 17). In one of those researches, Punitha et al found that his plant, *Pongamia pinnata*, had decreased the hyperglycemia in rats as deeply as glipenclamide, another sulphonyl urea's drug, so the researcher described his extract as promising antidiabetic (18).

The oral glucose test is sometimes a strongly recommended procedure to emphasize the hypoglycemic characteristic of herbal extract (19). By referring to figure 4 and table 2, we can see that CLEE greatly enhances the tolerance of glucose, especially with dose 1200 mg/kg comparing to the control. Indeed this effect was close to that of glipizide. This may suggest that the extract may improve the sensitivity of insulin as well as increase the utilization of glucose throughout the body. Liu Z et al concluded that the improvement in insulin act can be explained by the reduction in insulin's peripheral resistance (20). This finding has also supported by other researcher like Deng YX and Brockman DA who related the improvement in glucose's tolerance after loading to overall enhancement in insulin performance in most body's tissues (21, 22).

CONCLUSION

In conclusion, the ethanolic extract of *Crataegus laevigata* (CLEE) showed a profound hypoglycemic effect. This effect is partly related to increasing the sensitivity of insulin. Anyway, it should be further investigated to clarify the mechanism and the principle ingredients that have this properties.

REFERENCES

1. P. E. McGovern, J. Zhang, J. Tang et al. Fermented beverages of pre- and proto-historic China. *Proceedings of the National Academy of Sciences of the United States of America*, 2004, (101): 51, pp. 17593–17598.
2. Gary N Asher et al. Effect of hawthorn standardized extract on flow mediated dilation in prehypertensive and mildly hypertensive adults: a randomized, controlled cross-over trial. *BMC Complementary and Alternative Medicine* 2012, 12:26.
3. Satrani B, Farah A, Talbi M. Fractional distillation effect on the chemical composition and antimicrobial activity of Moroccan Myrtle. *Acta Bot Gallica*. 2006; 153:235–242.
4. Mary C. Tassell et al. Hawthorn (*Crataegus* spp.) in the treatment of cardiovascular disease. *Pharmacogn Rev*. 2010; 4(7): 32–41.
5. Pittler MH, Guo R, Ernst E. Hawthorn extract for treating chronic heart failure. *Cochrane Database Syst Rev*. 2008, 23; (1).
6. Tadić VM et al. Anti-inflammatory, gastroprotective, free-radical-scavenging, and antimicrobial activities of hawthorn berries ethanol extract. *J Agric Food Chem*. 2008, 10; 56 (17):7700-9.
7. Li C, Wang MH. Anti-inflammatory effect of the water fraction from hawthorn fruit on LPS-stimulated RAW. *Nutr Res Pract*. 2011; 5(2):101-6.

8. Robert M Littleton et al. Whole plant based treatment of hypercholesterolemia with Crataegus laevigata in a zebrafish model. *BMC Complementary and Alternative Medicine* 2012, 12:105.
9. Xu H, Xu HE, Ryan D. A study of the comparative effects of hawthorn fruit compound and simvastatin on lowering blood lipid levels. Am J Chin Med. 2009; 37(5):903-8.
10. Çalişkan et al. Phytochemical characterization of several hawthorn (*Crataegus* spp.) species sampled from the Eastern Mediterranean region of Turkey. *Pharmacogn Mag*. 2012 Jan-Mar; 8(29): 16–21.
11. Edwards JE et al. A review of the chemistry of the genus Crataegus. *Phytochemistry*, 2012; 79:5-26.
12. Liu P, Kallio H, Yang B. Phenolic compounds in hawthorn (Crataegus grayana) fruits and leaves and changes during fruit ripening. *J Agric Food Chem.* 2011 26; 59 (20):11141-9.
13. Soo-Mi Jeong, Min-Jung Kang, Ha-Neul Choi, Ji-Hye Kim, and Jung-In Kim. Quercetin ameliorates hyperglycemia and dyslipidemia and improves antioxidant status in type 2 diabetic db/db mice. *Nutrition Research and Practice* (*Nutr Res Pract*) 2012; 6 (3):201-207.
14. Misra H, Soni M, Silawat N, Mehta D, Mehta BK, Jain DC. Antidiabetic activity of medium-polar extract from the leaves of Stevia rebaudiana Bert. (Bertoni) on alloxan-induced diabetic rats. *J Pharm Bioallied Sci*. 2011; 3 (2):242-8.
15. Adeneye AA, Agbaje EO. Hypoglycemic and hypolipidemic effects of fresh leaf aqueous extract of Cymbopogon citratus Stapf. in rats. *J Ethnopharmacol*. 2007 25; 112 (3):440-4.
16. Ortiz MI, Castañeda-Hernández G, Izquierdo-Vega JA, Sánchez-Gutiérrez M, Ponce-Monter HA, Granados-Soto V. Role of ATP-sensitive K^+ channels in the antinociception induced by non-steroidal anti-inflammatory drugs in streptozotocin-diabetic and non-diabetic rats. *Pharmacol Biochem Behav*. 2012 Jul; 102 (1):163-9.
17. Biswas A, Chatterjee S, Chowdhury R, Sen S, Sarkar D, Chatterjee M, Das J. Antidiabetic effect of seeds of *Strychnos potatorum Linn*. in a streptozotocin-induced model of diabetes. *Acta Pol Pharm*. 2012; 69 (5):939-43.
18. Punitha R, Manoharan S. Antihyperglycemic and antilipidperoxidative effects of Pongamia pinnata (Linn.) Pierre flowers in alloxan induced diabetic rats. *J Ethnopharmacol*. 2006, 21; 105 (1-2):39-46.
19. Shivanna N, Naika M, Khanum F, Kaul VK. Antioxidant, anti-diabetic and renal protective properties of Stevia rebaudiana. *J Diabetes Complications*. 2012, 6. pii: S1056-8727(12)00280-2.
20. Liu Z, Wang LJ, Li X, Hu JN, Chen Y, Ruan CC, Sun GZ. Hypoglycemic effects of malonyl-ginsenosides extracted from roots of Panax ginseng on streptozotocin-induced diabetic mice. *Phytother Res*. 2009; 23 (10):1426-30.
21. Brockman DA, Chen X, Gallaher DD. Consumption of a high β-glucan barley flour improves glucose control and fatty liver and increases muscle acylcarnitines in the Zucker diabetic fatty rat. *Eur J Nutr*. 2012 Dec 11. [Epub ahead of print]
22. Deng YX, Zhang XJ, Shi QZ, Chen YS, Qiu XM, Chen B. Anti-hyperglycemic effects and mechanism of traditional Chinese medicine Huanglian Wan in streptozocin-induced diabetic rats. *J Ethnopharmacol.* 2012; 144(2):425-3