Medical Journal of Babylon
Vol. 14- No. 2 : 402 – 408 , 2017
http://www.medicaljb.com
ISSN 2312-6760©2017 University of Babylon

Original Research Article

Hypoglycaemic Activity of *Ceratonia siliqua* Leaves Extracts in Alloxan-Induced Diabetic Rats

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Accepted 9 July, 2017

Abstract

Assessment of the proposed antidiabetic properties of either of cold and hot hydrous extracts of *Ceratonia siliqua* plant, on fasting serum glucose levels and serum lipids profile levels in alloxan-induced diabetic rats were studied. Both, hot hydrous and cold aqueous extract of the herb leaves of *Ceratonia siliqua* elicited a significant anti diabetic activity with a dose of 250 mg/kg p.o. The corresponding histological pictures of the pancreases of rats that were formerly degenerated by "alloxan" revealed their restoration after treatment with hot and cold aqueous extracts.

Key Words: Antidiabetic effect, *Ceratonia siliqua* leaves, hydrous extract, Alloxan, cold extract.

دراسة فعالية مستخلصات اوراق نبات الخروب على تخفيض نسبة السكر في دم الجرذان المصابة بداء السكري باستخدام مادة الوكسان.

الخلاصة

في ضوء الفعالية المضادة لداء السكري المقترحة، تم دراسة تأثير المستخلصات المائية الحارة والباردة لأوراق نبات الخروب على تخفيض نسبة السكر والتحليل الكيميائي لدم الجرذان الصائمة المصابة بداء السكري المحدثة باستخدام مادة الوكسان. كانت النتائج المستخلصة من الدراسة عن فعالية عالية لأوراق نبات الخروب لعلاج داء السكري وبجرعة ٢٥٠ ملغم / كغم وزن فموي لكل من المستخلص المائي والبارد. كذلك اثبتت الدراسة النسيجية لبنكرياس الحيوانات المختبرية المستخدمة فعالية وقدرة فائقة لمستخلصات اوراق نبات الخروب في تجديد خلايا البنكرياس التي تلفت نتيجة استخدام مادة الوكسان.

Introduction

The diabetes mellitus (DM) is an enduring, hereditary and/or acquired disease due to lack or shortage in production of insulin fromβ-cell of the pancreas, or due to insensitivityof the cells tothe released insulin. So, inadequate insulin causesanelevation of blood glucose level, that eventually harms the body tissues,especially the blood vessels, eye, renal and nervous system. Due to increase inthe rates of individuals who suffer from diabetesglobally, the disease has consumed a growing portion from the national and international budgets that allocated for health care sector.

It is expected to bea main disable and murderers within the following 25 years. Areas with largestprospection are Africa and Asia, whereas, the rates of diabetic disease could increase2-3 timesas compared with the current rates. The herbal medications have been

recommended as one ofthe current available therapeutic choices for treatment of diabetic patients. Nowadays, the traditional medicinal plants are prescribed throughout the world for control of diabetes disease. Among the curative plants are trees of carob '*Ceratonia siliqua* leaves' (*C. silique* leaves)which belongs to' fabaceae' family, and they have 7-12m of height. This perennial green trees are frequently hermaphrodite and they have flower clusters and leaves with pinnate shape[1]. They have a type of droopy pod fruits, with plumpy sheath and their lengths are 10–30 cm. The number of hard seedsare 12-16 within the pod.

C. *siliqua* is present locally in Mediterranean region and in Iran, where it prevalent in province of Fars, nearby Shapoor cave in Kazerun. In some region, seeds of carob are utilized like coffee and tea [2]. Indeed, it is used as anappropriatealternative for cocoa, due to its absencesfromtheobromine and caffeine. Carob podis used fortreatment of cough, moreover, its styptic and bark are useful remedy for diarrhea [3]. Pulp of *Ceratonia* has been prepared to curethe patients with elevated serum cholesterol [4], as well as, for curingof mouth inflammation [5]. Additionally, the seeds of C. *siliqua*are beneficial in management and improvement the symptoms of diabetic disease and this attributed to presence of fibers, phytosterols and tocopherol in their contents [6,7].

The studies have been reported that Carob seeds are safe and there is no restrictionon their consumption [8]. So, because of increased numbers of diabetic patientsall over the world and the severeside effects associated with use of hypoglycemic agents, it become essential to introduceasafe medicine with minimum complications on long term therapy. In fact,the curative herbs are natural and have minor adverse effects and they are currentlyhaving a value in treatment and control diabetic disease. There is no adequate data regarding the effectiveness of C.*siliqua* seed extracts on diabetic patients.So,according to the mentioned facts, the present study was carry out to assess effects of both hot and cold aqueous extracts of C.*siliqua* leaves on levels of blood glucose and lipids profile of diabetes.

Materials and Methods

Herbal Material

C. *silique* leaves the fresh and soft leaves were collected from private gardens in Baghdad, and was authenticated by Iraqi National Herbarium in Baghdad.

Grinding of leaves

The green leaves of C. *silique* were shade and dried at ordinary room temperature. After drying, they were exposed to grinding process to reduce their sizesand render them into coarse powder by using dry electrical grinder. The coarse powder then passed through sieve with mesh no 40 to get a fine powder.

Hot Aqueous extraction.

The fine powder that obtained from C. *siliqua* l leaves was transfer to thimble of the soxhlet apparatus. Next, the distilled water was added to the soxhlet which then turn on for 8 hours. Finally, the gained extract (7.5%) was put in oven and underwentdrying at 45°C untilit turn into solid to semisolid form [9].

Cold extraction.

Cold aqueous extract was prepared by maceration method. 100 g of the fine powder of C.*siliqua*leaves was weigh, then transferred into 2 Lcapacity a conical flask. 500 ml of distilled water was added to the conical flask followed byaddition of 10 ml of chloroform as a preservative. Then the extract left up to 7 dayswith agitation from time to time in average of 2 hours/daily by using a mechanical stirrer. At end time of extraction, the extract was clarifiedby using muslin cloth. The marc was thrown away and the remaining liquid (8.1%) was dehydrated by using an oven adjusted at 45°C until solid-slightly solid form obtained.

All extracts were kept in tightly closed containerswhich then stored in refrigerator under 10°C. Normal saline was used as solvent in preparation of the solutions of hot and cold hydrous extracts that attended for administration to the experimental rats [9].

Testing Animals

Both females and males Wistar albino rats weighed 50-200 g and Wistar albino mice weighed 20-25 g were get from Indian Institute of Sciences, Bangalore, India. They were nourisheda typical diet from Gold Mohr, Lipton India Ltdbefore and during research time.The testing animals were divided randomly into a number of groups. Before starting of research, the rats were accustomed for 7 days' interval under standard surroundingsincluding room temperature, moisture, and dark-light succession. They were become fasted by deprivation them 16 hours from diet and liquids *ad libitum* [9].

Blood specimens:

Samples of blood were obtained by puncture from retro-orbital plexus. The blood glucose levels were assessed using glucostix (Bayer diagnostic India Ltd) and an electronic glucometer [Miles Inc., USA).

Investigational Design

The studied animals were divided randomly into five groups each of them was contain six animals. The animal groups (I), (II), and (III) were given saline solution, alloxan, and glibenclamide, 10 mg/kg, respectively [9]. While, group IV and group V were administeredhot hydrous extract and cold extract of *C. siliqua* leaves (250 mg/kg/dayp.o), respectively [10].

Evaluation of Extracts on Alloxan-treated rats

A single dose of alloxan monohydrate injection (Loba Chemie, Bombay: 150 mg/kg Rats) was injected into rats intraperitoneal to render them diabetic [11]. Alloxan dose was first calculated and weighed individually according to weight of each animal. It then dissolved in 0.2ml saline (154 m MNaCl) to become ready to injection. Two days next alloxan injection, plasma glucose levels were measured and the rats with plasma glucose levels of >140 mg/dl were involved in the study. Treatment with herbal extracts was begin 48 hours later alloxan injection. Blood samples were taken from the animals a weekly for three consecutive weeks.Measurement of fasting blood glucose levels and body weight were done on the end day of 1, 2 and 3 weeks of the study.

On day 21, blood sampleswere collected by cardiac puncture from overnight fasted rats under mild ether anesthesia. Then, the fasting serum glucose levels were measured [12]. Sera wereinvestigated for total cholesterol; T-ch [13], triglycerides (TG) and measured using an enzymatic colorimetric method [14], serum high-density lipoprotein; HDL[15], serum low-density lipoprotein; LDL [16], serum creatinine [17], serum urea [18] and serum alkaline phosphatase by hydrolyzed phenol amino antipyrine method [19].

After sacrificingof the animals, the entire pancreasesofthe study animals were removed and retained in 10% formalin solution, and processed directly by the paraffin technique. Histological examination of pancreatic was done. The pancreas was cut into sections of5µm in thickness, which were stained with hematoxylin and eosin (H&E). The micrographsof the rat pancreases are illustrated in fig (2); A-E.

Statistical treatments

Data of animal body weights, their fasting blood glucose, and biochemical investigations were calculated as mean ± standard error of mean (SEM). Their statistical comparison was done by Student t test.

Results

The hypoglycemic properties of the *Ceratonia siliqua* extracts on the levels of fasting serum glucose of alloxan-induced diabetic rats are presented in figure[1]. Administration of alloxan to the rats in a dose of 150 mg/kg, intraperitoneal caused about 1.5-fold rising in measurements of fasting serum glucose. The incrementwas sustainedinto 3 weeks of the study time. The daily treatment with the herbal extractsthat lasted three weeks led to 25-62% dose-dependent decrease in blood glucose levels of the studied rats. The maximum therapeutic effects of the extracts were reached after two weeks of usagewhich continued persistent during the 3rd week of treatment. The control rats

were maintaining their body weight, while diabetic rats were show a significant decrease in their body weight on 21st dayof the study (Table 1). Administration of alloxanto the experimental animals were cause a weight reduction, that was overturned after 7 days of treatment with hydrous and cold extracts of *C. siliqua*.

Afterward 21 consecutive days of therapy of the rats with glibenclamide (group III), hydrous extract (group IV) and hot extract (group V) of *C. siliqua*, the measurements of serum T-ch, serum Tg, serum LDL, serum urea, serum creatinine,and serum alkaline phosphatase levels were significantly reduced in group III; glibenclamide treated rats (P< 0.005), group IV; hydrous extract treated rats (P< 0.001), and group V; cold extract treated rats (P<0.01) in comparison to group II; alloxan-induced diabetic control, while HDL levels were significantly increased in in group III; glibenclamide treated rats (P<0.001), group IV; hydrous extract treated rats(p < 0.001) and group V; cold extract treated rats (p<0.01) in comparison to group II; alloxan-induced diabetic control as mentioned in table 2.

Histological examinations of pancreases of the rats were demonstrated in photomicrographs that presented in figure 2, which revealed ordinary acini, and ordinary cellular numbers in the islets of Langerhans in pancreas of vehicle-treated rats (photomicrograph A), while, a wide damage to the islets of Langerhans and reduced sizes was shown in alloxan-induced diabetes rats (photomicrograph B).However, regeneration of normal cellular population size of islets with hyperplasia was shown in glibenclamide treated rats (photomicrograph C). There was a partial repair of normal cellular population and distended size of the pancreatic β-cells with hyperplasia showed in each group of rats treated separately withhydrous and cold extracts of *C. siliqua* (photomicrographs D & E).

Table 1: Influence of 3 weeks daily treatment with glibenclamide, hydrous and cold extracts of *Ceratonia siliqua* on the weight (g) of alloxan-induced diabetic rats (150 mg/kg I.P. alloxan).

Group No. Treatment Dose (mg/kg PO)	**Mean of the body weight (g) ± SEM**			
	Day 1	**Day 7**	**Day 14**	**Day 21**
Gr. I Vehicle control 0.2 ml a	201.9±1.8	201.8±1.1	203.05 ±1.09	205.85± 1.62
Gr. II Alloxan-induced diabetic control 0.2 ml b	207.1±1.9	178 ± 5.2	160.33 ± 2.51	149.82±1.62
Gr. III Glibenclamide10	208.8±2.2	197.1±1.31**	194.21± 2.3***	190± 3.96***
Gr. IV Hydrous extract 250	206.2±2.06	195.15 ±1.7*	191.20 ±1.6**	180.21 ± 4.2**
Gr.V Cold extract 250	205.8 ±1.82	196.1±2.1*	188.71 ± 3.1**	179.4± 5.1**

Values of mean body weight (g) ±SEM were given for each studied ratgroup with six rats/group.

a. Isotonic saline solution dose.
b. Isotonic saline solution containing alloxan dose.
c. Significancesversuscontrol * P< 0.05, **P<0.01, ***P<0.001.

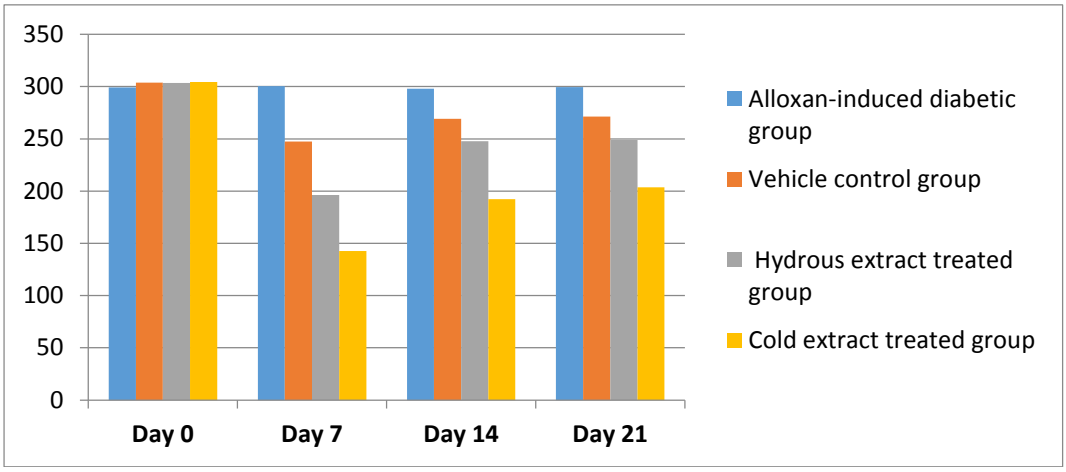

Figure 1: Comparison of the therapeutic effects of hydrous and cold extract of leaves of *Ceratonia siliqua* on serum glucose levels of alloxan-induced diabetic rats (150 mg/kg).

Table 2: Effects of glibenclamide, hydrous and cold extracts of *Ceratonia siliqua* on serum lipid profile levels, creatinine, urea, alkaline phosphatase and glucose of alloxan-induced diabetic ratsafter3 consecutive weeks of treatment.

Group No	Dose mg/kg	Serum Cholesterol	Serum Triglyceride	Serum HDL	Serum LDL	Serum Creatinine	Serum Urea	Serum alkaline phosphatase	Serum glucose
I	Vehicle	0.2 ml a	150+5.2	85.9+5.1	35.1+1.5	92.3+1.5	0.53+0.1	22.666+1.3	115.2+2.5100.3±6
II	alloxan	0.2 ml b	268.3±14.9	200.9±11.1	30±1.4	198.1±1.4	1.4±0.1	60±1.7	313.5±5.9300.3±4
III	Glibenclamide	10	145.8±5.1	108±5.1	50.5±1.7	72.7±6.7	0.56±0.1	30.1±2.1	129.1± 4.3 265±4
***	***	***	***	***	***	*****			
IV	Hydrous extract	250	153.8 ±3.8	114±5.2	40.1. ±1.2	03.5±2.7	0.6±0.1	30.8±1	132.6±5.6250±5
***	***	**	***	***	***	*****			
V	Coldextract	250	160.8± 4	120±5.8	44.2±1.6	101±3.5	0.7±0.1	34.2±1.7	139.2±4202±3
**	**	**	**	**	**	** **			

a. Isotonic saline solution dose.
b. Isotonic saline solution containing alloxan dose.
c. Significances versus control * P < 0.05, **P < 0.01, ***P < 0.001.

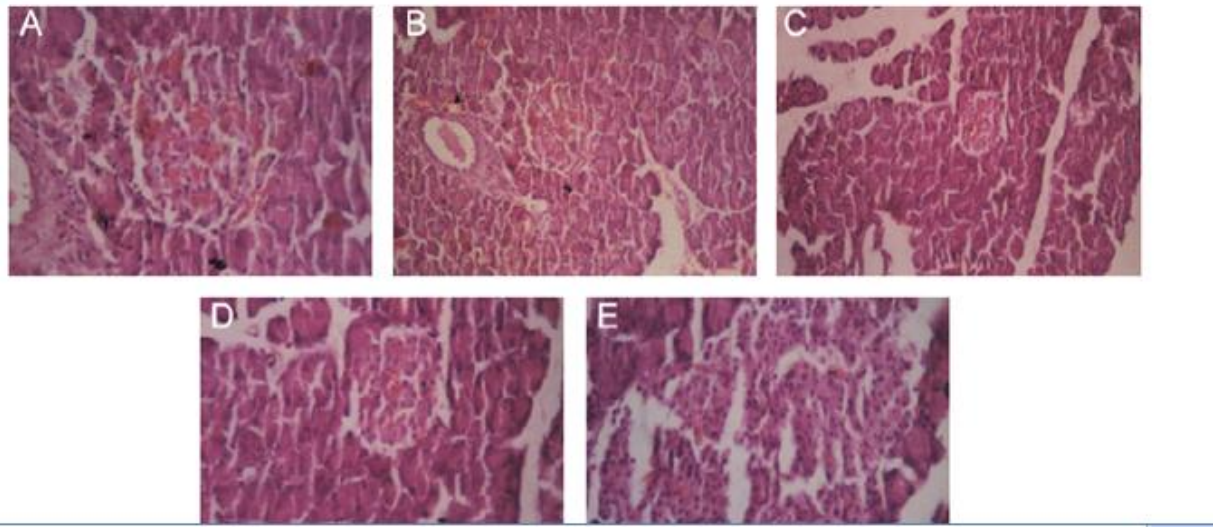

Figure 2: Photomicro graphs with 40x. magnification power ofthe rat pancreasesspotted withstains of eosin and haematoxylin of vehicle-treated rats (A), alloxan induced-diabetes rat (B), glibenclamide treated rats (C), hydrous extract treated rats (D), and cold extract treated rats with *Ceratonia siliqua* (E).

Discussion

The biochemical and histological resultsin this study were showedthatboth hot hydrous extract and cold extract of *Ceratonia siliqua* leaves have hypoglycemic effects on therats, whereas, both extracts exhibited significant hypoglycemic effects in alloxan-induced diabetic rats with insignificant alteration in their body weightiness.

According to the study parameters which include, bodymass, lipid profiles together with serum creatinine, serum urea, and serum alkaline phosphatase in alloxan-induced diabetes, these extracts were also improved the health conditions of diabetic rats. The numbers of physiological active β-cells in pancreas of rats is criticalfor the progression and consequences of diabetes. Regeneration of pancreatic β-cells of the diabetics have been studied in quite a lot of experimental animal samples. The entiremass of β-cell reveals the equilibriumamong the regeneration and damage of the cells. It was also proposed that renewal or restoration of islet β-cells of the pancreas after the damage induced by alloxan may be the principal reason of healing of alloxan–injected guinea pigs from the effects of the drug [20,21]. *Vincarosea* extract [22] has also revealed to act by means of β-cell restoration. Comparable outcome in streptozotac in treated diabetic animals had been reported by pancreas tonic [23], ephedrine [24], and *Gymnema Sylvestre* leaves extracts [25].

In the presentstudy, the injury to β-cells of pancreas of diabetic rats (figure 2-B), and restoration of the damaged cells by glibenclamide (figure 2-C) was recorded. Moreover, asimilarrestoration was also obtained after treatment of rats with aqueous and cold extracts of *Ceratonia siliqua* leaves as showed in figures 2-D and 2-E. This influencemightbe attributeto existence of β-carotenethathas been reported as one of *Ceratonia siliqua* constituents [26]. The valuable role of β-carotene in reducing the complications of diabetes such as glycosylation in diabetes rats [10] had been describedformerly. The available information from photomicro-graphics of rat pancreases in our studies showed a partial repair of normal cellular population and distended size of the pancreatic β-cells with hyperplasia in each group of rats treated separately with hot hydrous and cold extracts of C. *siliqua* (photomicrographs D & E). This approve the healing of pancreas by cold and hot C. *siliqua* leaves extracts and it considered as a reasonable mechanism of their hypoglycemic effect.

Conclusion

The extracts of C. *siliqua*leaves demonstrated a significant improvement of hyperglycemic control, body weight, serum lipid profile as well as regeneration of β-cells of pancreas of alloxan-induced diabetic rats and might be promising herb in in treatment of diabetes.

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