

## Some anti-diabetic properties of *Prosopis farcta* extracts in alloxan induced diabetic in adult rats

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### Abstract

*Prosopis farcta* is belonged to Mimosaceae family, commonly known as mesquite. It was chosen to investigate their effect on  $\alpha$ -glucosidase inhibitory activity (*in-vitro*), serum glucose and liver functions. Roots, fruits, and leaves of *P. farcta*, were extracted by *n*-hexane, ethyl acetate, and methanol.  $\alpha$ -glucosidase inhibition was analyzed by using ELISA technique then half maximal inhibitory concentration IC<sub>50</sub> was found. The blood glucose levels were determined with a glucose analyzer model. The serum alkaline phosphatase (ALP), Alanine transaminase (ALT), aspartate aminotransferase (AST) and serum total bilirubin (TB) were estimated by using the Cobas diagnostic kit with a fully automated chemical analyzer. Diabetes was done by a single dose of 120 mg alloxan/kg b.w with subcutaneously injection. Ethyl acetate extracts of *P. farcta* showed the higher  $\alpha$ -glucosidase inhibitory activity, and the best one was root extract. Treatments of the alloxan-induced diabetes rats were done by daily oral administration of different concentrations with *P. farcta* extracts of ethyl acetate for 28 days and the dose 200 mg/kg BW was the effective one. The root extract was the best one for reduction of serum glucose followed by leaves then fruits. Administration of root extract of *P. farcta* showed a decrease in the levels of ALP and TB in alloxan-induced diabetes rats. The fruit extract of *P. farcta* showed decreasing in the level of ALP in alloxan-induced diabetes rats. In conclusion, the *P. farcta* extracts for ethyl acetate have properties of hypoglycemic effect as well as improving some parameters related with diabetic complications of liver functions.

**Keywords:** *Prosopis farcta*, Diabetes, Rats

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### بعض الصفات المضادة للسكري لمستخلصات *Prosopis farcta* في الجرذان البالغة المستحدث فيها السكري بالالوكسان

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### الخلاصة

نبات *Prosopis farcta* يعود الى عائلة Mimosaceae والاسم الشائع له هو Mesquite. اختير هذا النبات لمعرفة تأثيره على الفعالية التثبيطية لأنزيم الفالوكوسايديز (خارج الجسم الحي) ومقاييس كلوكوز الدم ووظائف الكبد. تم استخلاص جذور وثمار وأوراق نبات الينبوت بواسطة الهكسان والأثيل اسيتيت والميثانول. قيست الفعالية التثبيطية لأنزيم الفالوكوسايديز باستعمال تقنية الاليزا ومن ثم إيجاد التركيز التثبيطي الأقصى حتى النصف. قيست مستويات كلوكوز الدم بواسطة جهاز محلل الكلوكوز وقيست مستويات أنزيمات الفوسفاتاز القلوي ALP وناقلة أمين الألائين ALT وناقلة أمين الاسباراتات AST والبيليروبين الكلي في الدم TB باستخدام عدة التشخيص الخاصة بجهاز Cobas. تم استحداث السكري بواسطة الجرعة المفردة للالوكسان ١٢٠ ملغم/كغم من وزن الجسم بالحقن تحت الجلد. أظهرت

مستخلصات الأثيل اسيتيت لنبات *Prosopis farcta* اعلى تثبيط لأنزيم الفالوكوسايديز وكانت الأفضلية في التثبيط لمستخلص الجذور. تم علاج الجرذان المستحدث فيها السكري بالالوكسان بالإعطاء الفموي اليومي لتراكيز مختلفة من مستخلصات الأثيل اسيتيت لنبات *P. farcta* ولمدة ٢٨ يوما فكانت الجرعة ٢٠٠ ملغرام / كغم من وزن هي الجرعة المؤثرة. مستخلص الجذور كان الأفضل في تخفيض كلوكوز الدم وأعقبه مستخلص الأوراق وأخيرا مستخلص الثمار. أدى إعطاء مستخلص الجذور الى خفض في مستويات ALP والبيليروبين الكلي في الدم TB في الجرذان المستحدث فيها السكري بالالوكسان. أدى إعطاء مستخلص الثمار الى خفض مستوى ALP في الجرذان المستحدث فيها السكري بالالوكسان. ويمكن الاستنتاج بان مستخلصات الأثيل اسيتيت لنبات *P. farcta* يمتلك صفة تثبيط السكر بالإضافة الى تحسينه لبعض تعقيدات السكري المتعلقة بوظائف الكبد.

## Introduction

Diabetes is characterized by increasing the level of blood sugar due to insulin resistance, lack of insulin secretion or an abnormal increase of glucagon secretion (1,2). At the level of villi of the small intestine wall the  $\alpha$ -glucosidase breaks down disaccharides to monosaccharides which is considered the end step of digestion of the sugars. Substances that inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase will prevent degradation of carbohydrates in the small intestine and decrease the postprandial blood glucose in the circulation (3). There is a good history of using *P. farcta* in the treatment of diabetes in traditional medicine because it contains an active compound such as daucosterol, epicatechin,  $\beta$ -sitosterol and lupeol that have anti-diabetic effects (4,5). *P. farcta*, commonly known as Syrian mesquite in Arabic countries and as Kahurak and Jeghjehgeh in Iran, is a woody perennial dwarf legume, which has been distributed from India to Jordan. Different species of the *Prosopis* genus have been utilized for gum, paint and cordage (6) and as dietary supplements for feeding ruminants (7) as well as for medicinal purposes. Beans and leaves of *P. farcta* have traditionally been used for the treatment of some diseases and disorders such as diabetes (8,9) healing lesions (10), diarrhea, and colds (11).

The liver is the main organ for the regulation of carbohydrate metabolism, as it uses glucose as a fuel, it has the capability to store glucose as glycogen and synthesize glucose from non-carbohydrate source. This key function of the liver makes it vulnerable to diseases related to metabolic disorders, particularly diabetes (12). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. Liver Function Tests (LFTs) are used for indication of liver disease. The most common liver function tests include the serum aminotransferases (ALT, AST), alkaline phosphatase, bilirubin, and albumin. Increased activities of liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and  $\gamma$ -glutamyl transferase (GGT) are indicators of hepatocellular injury. Increased levels of these markers are associated with insulin resistance (13). We conducted this study to explain the effect of *P. farcta* on glucose levels, functions of the liver in type 1 diabetes.

## Materials and methods

The roots, leaves, and fruits of *P. farcta* were collected from Koya in Erbil province, Iraq. The plant was identified in the Herbarium of Medicinal Plants and Drugs Research Institute, Shahid Beheshti University in Tehran-Iran.

The plant materials were dried at room temperature and ground by an electrical grinder, 10 g was extracted with 50 ml of each solvent and the solvents were *n*-hexane, ethyl acetate and methanol then macerated on a magnetic stirrer for 1 hr. The extracts were dryness by rotatory evaporator under reduced pressure at 45 °C, finally, we got solid powder extracts. High concentration for extracts 1000 ppm was used as stock solution for  $\alpha$ -glucosidase assay (*in-vitro*) by adding 1.4 mg of each extract to 1.4 ml (1400  $\mu$ L) of dimethyl sulfoxide (DMSO). The request volume of the extract solution for the alpha glucosidase assay was one micro litter from each extract samples. Different concentrations of extract samples were used in this assay. Enzyme Linked Immuno Sorbent Assay (ELISA) (CLARIO star Microplate Reader from BMG Labtech) was applied to do  $\alpha$ -glucosidase assay on extracts using the reaction mixture consisting 25  $\mu$ L of 2 mM p-nitrophenyl  $\alpha$ -D-glucopyranoside (Sigma Chemical Co.), 49.5  $\mu$ L phosphate buffer (pH 7.0) adding to flask contains 0.5  $\mu$ L of sample dissolved in DMSO at various concentrations 200-1000 ppm. The reaction mixture was pre-incubated for 5 minutes at 37 °C, the reaction was started by adding 25  $\mu$ L  $\alpha$ -glucosidase (Sigma) incubation was continued from 30 minutes. The reaction stopped by adding 1 ml of 0.01 M  $\text{Na}_2\text{CO}_3$ . The activity of  $\alpha$ -glucosidase was determined by measuring the release of p-nitro phenol at 400 nm. Acarbose (Glucobay®) positive control of  $\alpha$ -glucosidase. Inhibition of sample with  $\alpha$ -glucosidase activities was measured by increasing concentration of p-nitrophenyl  $\alpha$ -D-glucopyranoside as a substrate in the absence or presence of samples at different concentrations. Inhibition type was determined by Lineweaver-Burk plot analysis of the data, which were calculated from the result according to Michaelis of the data, which were calculated from the result according to Michaelis- Menten kinetics.  $\alpha$ -glucosidase inhibition % inhibition has been obtained using the formula, % inhibition = (1- average of sample slope/ average of

enzyme slope)  $\times 100$  (14) then  $IC_{50}$  was found by formula of  $y=a x+b$  where  $y=50$  and  $a, b$  was found from the plot.

Albino rats male rats 1.5-2 months old that weighed 190-220 g with a mean weight of 200 g. were used. Diabetes was induced by a single subcutaneous injection of 120 mg alloxan/kg b.w and rats with blood glucose levels above 200 mg/dl were considered as diabetic rats (15).

Rats were randomly divided into six groups of seven each. Because extract of ethyl acetate for roots of *Prosopis farcta* showed the best inhibition for 50% of alpha glucosidase activity, therefore it was selected for the next work to compare with an ethyl acetate extract for fruits and leaves of *P. farcta* as well as with the insulin. The extraction of *in-vivo* study for glucose and liver function parameters included mixing of 2kg of each part of the *P. farcta* (roots, leaves, fruits) with 10 liters of the ethyl acetate and the procedure continued as mentioned above. Different concentrations 50, 100, 150, 200, 250, 300 mg/kg of extracts were prepared by suspending the dried powder of each kind of the extracts in physiological saline.

The ethyl acetate extracts of *P. farcta* (roots, leaves, fruits) was orally administrated daily for 28 days with a different doses (concentrations) for each kind of extract. Group I, non-treated rats administered 1ml physiological saline; Group II alloxan-induced diabetic rats, administered 1ml physiological saline; Group III alloxan-induced diabetic rats, administered roots extract; Group IV alloxan-induced diabetic rats administered leaves extract ; Group V alloxan-induced diabetic rats administered fruits extract; and Group VI alloxan-induced diabetic rats subcutaneously injected with insulin (Actrapid. Novo Nordisk, Denmark) (10 I.U/Kg of body weight) (16).

The blood glucose levels were determined *in-vivo* with a glucose analyzer model (Contour ® TS, Bayer Pty, Ltd; Healthcare Division, Japan). The serum glucose was estimated by using the Cobas diagnostic kit with fully automated chemical analyzer (Cobas C 311). The serum ALP, ALT, AST and serum total bilirubin (TB) were estimated by using the Cobas diagnostic kit with a fully automated chemical analyzer (Cobas C 311 according to the manufacturer's protocol).

## Statistical analysis

SPSS version 24 statistical software was used to analyze the data. The ANOVA analysis was used to investigate the relationship of the parameters. Probability level of P value ( $P<0.05$ ) level of significance was considered to be statistically significant.

## Results

The results of the *in vitro* study, revealed that ethyl acetate extract for roots of *P. farcta* extract showed the best inhibition for 50% of  $\alpha$ - glucosidase activity ( $IC_{50}$ ) and it was better than acarbose 16.5  $\mu$ g/ml (Table 1).

Table 1:  $IC_{50}$  ( $\mu$ g/ml) values for  $\alpha$ -glucosidase inhibition activity of *P. farcta* extracts

Extracts	n-hexane	Ethyl acetate	Methanol
Roots	>125	10.9 $\pm$ 1.03 a	26.4 $\pm$ 1.42 b
Fruits	>125	68.1 $\pm$ 2.11 c	59.8 $\pm$ 1.9 d
Leaves	>125	54.7 $\pm$ 2.02 e	40.1 $\pm$ 1.16 f
Acarbose	16.5 $\pm$ 1.12 g		

Data are presented as mean  $\pm$ SD values of triplicate determination. Different letters for a given value within a column are significantly different from each other ( $P<0.05$ ). SEM: Standard Error Mean;  $IC_{50}$ : Half-maximal inhibitory concentration.

Table 2 elucidates the effects of *Prosopis farcta* extracts on serum glucose level in normal and diabetic male rats. Incidence of diabetes caused hyperglycemia in the rats. Among the extracts effects, the root extract was more reduced to the glucose. The highest reduction was in the third week of administration of the root extract as compared with other two extracts in diabetic rats, but their hyperglycemic effect was less than effect of insulin. The second one was leave extract then the extract of fruits. Also, the reduction of glucose was better in third week in these two kinds of extract as compared to other weeks.

Table 2: Effect of *Prosopis farcta* extracts on serum glucose level in alloxan-induced diabetic rats

Animal groups	Serum Glu. Before treat.	1 <sup>st</sup> week of treat.	2 <sup>nd</sup> week of treat.	3 <sup>rd</sup> week of treat.	Before T vs 3 <sup>rd</sup> week P value
Normal control rats	92.00 $\pm$ 4.461	96.20 $\pm$ 6.437	93.00 $\pm$ 5.831	93.00 $\pm$ 6.819	NS
Diabetic rats	465.0 $\pm$ 24.97	495.0 $\pm$ 24.60	451.0 $\pm$ 20.76	446.0 $\pm$ 17.20	NS
Diabetic rats + root extract	448.0 $\pm$ 30.36	284.0 $\pm$ 15.03	286.0 $\pm$ 10.77	176.2 $\pm$ 8.375	0.001
Diabetic rats + leaves extract	448.0 $\pm$ 28.04	304.0 $\pm$ 10.30	290.0 $\pm$ 7.071	274.0 $\pm$ 9.274	0.05
Diabetic rats + fruits extract	465.0 $\pm$ 25.00	306.0 $\pm$ 12.08	291.0 $\pm$ 6.403	300.0 $\pm$ 10.49	0.001
Diabetic rats + insulin	467.0 $\pm$ 22.51	153.0 $\pm$ 13.38	132.0 $\pm$ 11.47	112.4 $\pm$ 5.501	0.0001

Results express as mean  $\pm$  SEM. Glucose was measured by mg/100 ml.

Table 3 elucidates the changes of effect of *Prosopis farcta* extracts 200 mg/kg of body weight on the liver functions test parameters in normal and alloxan induced diabetic rats. ALP decreased in diabetic rats compared with normal rats. Root and fruit extracts decreased the enzyme of ALP in diabetic rats compared with control diabetic rats. There was no significant difference between them. Decreasing of insulin was higher than both extracts. AST

and ALT showed increasing in their levels in diabetic rats. No extracts could do any changes in the level of AST and ALT. Unlike insulin, that showed a reduction in the levels of both enzymes in diabetic rats compared with control diabetic rats. Total serum bilirubin increased in diabetic rats comparing with the normal control rats. Root extract and insulin showed reduction in level of total serum bilirubin in diabetic rats compared with those in control diabetic rats.

Table 3: Changes in the liver markers by *P. farcta* roots, leaves and fruits extracts in alloxan induced-diabetic rats

liver markers	Control groups		Treatment group (Diabetic rats)			
	Normal rats	Diabetic rats	Roots extract	Leaves Extract	Fruits Extract	Insulin
ALP	143.1±11.35 <sup>a</sup>	242.9±11.51 <sup>c</sup>	195.9±5.241 <sup>b</sup>	218.3±5.052 <sup>bc</sup>	200.0±10.80 <sup>b</sup>	157.1±4.199 <sup>a</sup>
AST	44.06±8.622 <sup>a</sup>	109.1±7.435 <sup>c</sup>	98.82±12.20 <sup>bc</sup>	94.64±2.833 <sup>bc</sup>	80.69±4.557 <sup>abc</sup>	60.25±4.727 <sup>ab</sup>
ALT	55.02±2.100 <sup>ab</sup>	80.28±2.163 <sup>c</sup>	75.68±1.875 <sup>c</sup>	76.81±1.190 <sup>c</sup>	75.28±1.670 <sup>c</sup>	65.88±2.307 <sup>b</sup>
Bilirubin	0.320±0.019 <sup>a</sup>	1.632±0.145 <sup>c</sup>	0.904±0.064 <sup>b</sup>	1.202±0.036 <sup>bc</sup>	1.206±0.036 <sup>bc</sup>	1.132±0.198 <sup>b</sup>

Results expressed as Mean ±St. error. The same letters mean non-significant difference, while the different letters mean significant difference at P<0.05.

## Discussion

Ethyl acetate extract for roots, leaves and fruits of *P. farcta*, showed most effective inhibition of  $\alpha$ -glucosidase and the ethyl acetate extract the roots of *P. farcta* was the best one for reduction of serum glucose in vivo. This effect may use in ease the risk of the post-prandial phase. This phase occurs after breaking down of disaccharides by alpha-glucosidase enzymes which located in the brush border of the enterocytes of the jejunum in the small intestine; they are immediately absorbed by capillaries in the upper jejunum causing hyperglycemia (17). Alpha glucosidase inhibitors compete with binding of disaccharides with alpha glucosidase enzymes so will prevent binding of them that will lead prevent cleavage to monosaccharides then prevent or slow entrance in the bloodstream and finally reduce post-prandial blood glucose level (18). Herbs like *Cyperus rotundus*, *Plumbago zeylanica*, *Symplocos racemosa* and *Terminalia arjuna* contain a component such as alkaloid, terpene, saponin, tannin, glycoside, flavonoid, and quinone have a high ability to inhibit alpha-glucosidase with minimum quantities and side effects as well as at the lowest cost (19,20).

Alloxan has been found to be selectively toxic to pancreatic beta cells as it preferentially accumulates in the beta cells as glucose analogues preventing insulin production leading to hyperglycemia. In addition, the cytotoxic action of alloxan is mediated mainly by the generation of reactive oxygen species (ROS) (21). The obtained results in this study revealed that diabetes induced by alloxan induced significant increases in the serum levels of ALP, AST and ALT. These enzymes are evidence of liver cells injury and leakage of enzymes from cells (22).

Also, they are considered enzyme biomarkers to monitor the liver status and aid to demonstrate the liver toxicity conditions (23). Furthermore, diabetic complications such as increase of gluconeogenesis and ketogenesis refers to the increase in the levels of these enzymes (24). Treatment with insulin decreased level all those enzymes in diabetic rats. The diabetic case is represented with hyperglycemia that is associated with liver injury, as indicated by elevated levels of ALP, AST, and ALT, treatment with insulin will decrease the hyperglycemia, improve case of liver and decrease those enzymes level (25,26). Administration of *P. farcta* extracts (Root and fruit) caused a detectable decrease of ALP in diabetic rats thus improving hepatic function. Similar results were reported by many authors for role of olive leaves extracts in improving hepatic function (27-30). In addition to what the artificial induction of diabetes is doing in body changes including liver, hyperglycemia has been found to play a key role in ROS generated damage (31). Reduction of this enzyme level may be through the ability of *P. farcta* to reduce sugar in diabetic rats that leads to normalizing the liver function including this enzyme (25). Total serum bilirubin increased in diabetic rats compared with the normal control rats. Diabetes can affect many parts of the body and is associated with serious complications. Oxidative stress is a major contributor in the pathogenesis of diabetic complications and bilirubin has been shown to have antioxidant effects so will increase its level in diabetic case (32). Insulin showed a reduction in the level of total serum bilirubin in diabetic rats. The characteristic sign of diabetes is hyperglycemia that plays a key role in ROS generated damage (31). High ROS ratios will be associated with an increase in antioxidant levels including bilirubin level (32). So, diminishing of hyperglycemia by insulin will lower levels of ROS and thus

reduce the level of antioxidants including bilirubin. Maybe, in the same way, the root extract of *P. farcta* can reduce the bilirubin level.

## Conclusion

We concluded from this study that *Prosopis farcta* extracts especially root extract could improve serum glucose and some liver function parameters in diabetic rats that may suggest it to use as a medicine against diabetes.

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