# Effect of Glibenclamide and Fruit extract of *Zizyphus spina-christi* on Alloxan- induced Diabetic Dogs

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## ABSTRACT

In traditional medicine, several plants have been used for diabetes treatment. However, the antidiabetic activity of the fruits of *Zizyphus spina-christi* had never been investigated in experimental diabetic dogs. Twelve adult dogs were divided into three equal groups each one consisting of four dogs. One was the diabetic control, the second was the glibenclamide treated, and the other was the Zizyphus extract treated group. The present study aimed to test the 10 days oral administration of *Z. spina-christi* fruit hydroalcoholic extract (500 mg/kg) for its effects in alloxan-induced diabetic dogs in comparison with glibenclamide (0.2 mg/kg) antidiabetic activity. The results showed that treatment with glibenclamide nonsignificantly reduced the blood glucose level ($p > 0.05$) and significantly increased the serum insulin level of diabetic dogs ($p < 0.05$). In contrast to glibenclamide, the hydroalcoholic extract of *Z. spina-christi* resulted in a significant decrease in the level of blood glucose, with a concomitant significant increase in the serum insulin level, in diabetic dogs ($p < 0.05$). The extract had a mild, but significant, blood glucose lowering effect and the long-term use of this agent may be advantageous over chemical drugs in alleviating some of the chronic diseases and complications caused by diabetes.

## INTRODUCTION

In the dog and cat, the most common disorder of the endocrine pancreas is diabetes mellitus, which results from an absolute or relative insulin deficiency due to deficient insulin secretion by the beta cells (Chakrabarti and Rajagopalan, 2002; Jelodar et al., 2007). The incidence of diabetes mellitus is similar for the dog and cat, with the reported frequency varying from 1 in 100 to 1 in 500. Different types of oral hypoglycemic agents are available, along with insulin for the treatment of diabetes. Despite the presence of known antidiabetic medicine

in the pharmaceutical market, diabetes, and the related complications continued to be a major medical problem (Jahodar, 1993; Hussein et al., 2006). Recently, several medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies (Marles and Farnsworth, 1995; Jung et al., 2006; Gad et al., 2006; Grover et al., 2002; Prabhakar and Doble, 2008). Spina-christi is a tree indigenous to the south of Iran. Plant leaves are used in Iranian folk medicine as an antiseptic, antifungal and anti-inflammatory agent, and for healing skin diseases such as atopic dermatitis. The fresh fruits of the plant are also used to promote the wound healing, dysentery control, and treatment of bronchitis, coughs, and tuberculosis (Nazif, 2002). This plant has several pharmacologic effects such as analgesic effect (Adzu et al., 2001; Adzu and Haruna, 2007). The extract of *Zizyphus spina-christi* leaves improved glucose utilization in diabetic rats (Glombitza et al., 1994; Abdel-Zaher et al., 2005). The Zizyphus fruit is contained fructose, xylose, glucose, and rhamnose, mucilage, and lipids (Nazif, 2002). Upon review of the current literature, nothing was reported concerning the hypoglycemic effect of this fruit in the dog. Therefore, the present study was conducted to investigate the effect of hydroalcoholic extract of *Z. spina-christi* on the blood glucose and serum insulin level of dogs which have been experimentally diabetic by alloxan.

## MATERIALS AND METHODS

### Animals

Twenty adult mongrel dogs of both sexes weighing 10.2 to 20.46 kg were used. All dogs appeared healthy, as determined by results of physical examination, normal hemogram, and clinical chemical profiles. The dogs were housed separately in a controlled environment and fed a home-made diet containing chicken and rice. Water was available ad libitum. The animal care was done under supervision of suitably qualified veterinarian. The study was performed under control of Iranian Society for the prevention of cruelty to animals.

### Experimental Protocol

The dogs were allocated to three groups of four dogs each:

- Group A: Alloxan (Sigma, England) was administrated intravenously at a single dose of 60 mg/kg in normal saline.
- Group B: After induction of diabetes similar to group A; Glibenclamide (Poursina Co., Iran) was administered orally at a dose of 0.2 mg/kg daily, by gavages for 10 days.
- Group C: After induction of diabetes similar to group A; Hydromethanolic extract of *Z. spina-christi* fruit (which prepared by maceration method) was administered orally at a dose of 500 mg/kg- daily, by gavages for 10 days.

### Blood Sampling

The blood samples were collected from cephalic veins. Blood glucose concentration was measured by a glucometer (Cleverchek, Taiwan) using strips before alloxan administration and every day later. The dogs, which had blood glucose more than 200mg/dl, were concidered diabetic and introduced to study drug evaluation (Jelodar et al., 2007). Insulin concentration was measured by an ELISA kit (Diametra, Italy) before, 3 and 10 day after induction of diabetes.

### Data Analysis

The arithmetic mean of blood glucose and insulin were compared between groups using paired samples t-test, repeated measures analysis of variance and Dunnett tests (SPSS, version 10, SPSS Inc., Chicago, IL, USA). The level of significance was set at 0.05.

## RESULTS

### Clinical Findings

After alloxan administration, the dogs became diabetic within 72 hours. Clinical findings included polyuria, polydipsia, emesis, diarrhea, anorexia, and depression. Some dogs were resistance to alloxan-induced diabetes, which excluded then from the study.

Therapeutic application of Zizyphus extract and glibenclamide improved clinical status of dogs and decreased clinical signs. The dogs in group B and C returned to normal status at the end of study. Treatment was done for dogs in group A at end of study.

**Blood Glucose Cncentration**

After alloxan administration, the dogs had increased in glucose concentration within 24 to 72 hours. The mean of blood glucose concentration was increased from 83.00±3.32 mg/dl before alloxan administration and reached to 355.08±15.02 mg/dl after 72 hours. Therapeutic application of Zizyphus extract and glibenclamide significantly decreased blood glucose concentration with the time (Figures. 1 to 3).

The repeated measures analysis of variance showed that there are significant differences between groups on blood glucose ($p=0.026$). In addition, time and time-drug interaction had significant effect on blood glucose ($p<0.001$). The analysis with Dunnett test showed that the blood glucose between groups A and C was significantly different ($p=0.015$) but not between groups A and B ($p=0.2$). The fluctuation of blood glucose was not significant during time of study in group A ($p>0.2$), but were significant in groups B and C ($p<0.001$).

**Serum Insulin Concentration**

After alloxan administration, the mean of serum insulin concentration was decreased from 28.67±1.68 IU/ml before alloxan administration and reached to 3.94±0.55 IU/ml after 72 hours. Therapeutic application of Zizyphus extract and glibenclamide significantly decreased blood glucose concentration with the time (Figure 4). The repeated measures analysis of variance showed that there are significant differences between groups on serum insulin level ($p<0.001$). In addition, time and time-drug interaction had significant effect on insulin concentration ($p<0.001$). The analysis with Dunnett test showed that the serum insulin between groups A and B and also between groups A and C was significantly different ($p<0.001$). The mean of insulin level in group A were 3.63 and 3.83 IU/ml before and 10 days after diabetes induction, respectively that were not significantly different. The mean of insulin in group B were 4.76 and 21.33 IU/ml before and 10 days after diabetes, respectively which were significantly different ($P=0.015$). The mean of insulin in group C were 3.43 and 26.46 IU/ml before and 10 days after diabetes, respectively. These means was different significantly ($P=0.001$).

## DISCUSSION

The data showed that alloxan increased blood glucose and decreased serum insulin levels. Alloxan selectively destroys the islets of langerhans and decreases insulin production which conducted to diabetes (Lenzen et al., 1996). Also, alloxan increases free radical production and cause pancreatic injury (Szkudelski, 2001; Ananthan et al., 2004). The dog is more susceptible to alloxan–induced diabetes mellitus (Kim et al., 2006).

Treatment with Zizyphus extract reduced blood glucose of dogs, while serum insulin level increased. These data are similar to some other studies (Hussein et al., 2006; Cisse et al., 2000). The presence of saponin in Zizyphus fruit may have glucagon decreasing effect and may enhance glucose utilization and lower blood glucose. It is reported that saponin stimulates insulin release from pancreas (Norberg et al., 2004). In otherwise, glibenclamide exerts hypoglycemic action by stimulation insulin secretion and inhibition of glucagon release. The remaining intact pancreatic cells are stimulated by Zizyphus extract or glibenclamide and the serum insulin level is increased and the blood glucose is decreased. The tannins in Zizyphus fruit have antioxidative effect. Oxidative stress is one of the important factors in tissue injury in diabetes mellitus (Baynes, 1991; Santini et al., 1997; Feillet-Coudray et al., 1999). These potent antioxidants may protect beta cells and increase insulin secretion in diabetic dogs. Also, tannins may inhibit insulin degradation and improve glucose utilization (Peungvicha et al., 1998; Mohamadin et al., 2003). The relative highly content of fructose (78% of

***Figure 1.*** *Mean±SE of serum glucose level in alloxan-induced diabetic dogs (group A).*

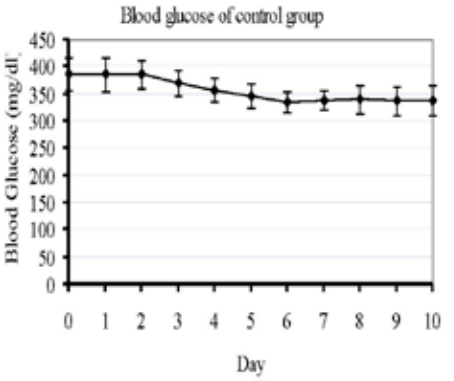



***Figure 2.*** *Mean±SE of serum glucose level in alloxan-induced diabetic dogs treated with glibenclamide (group B).*

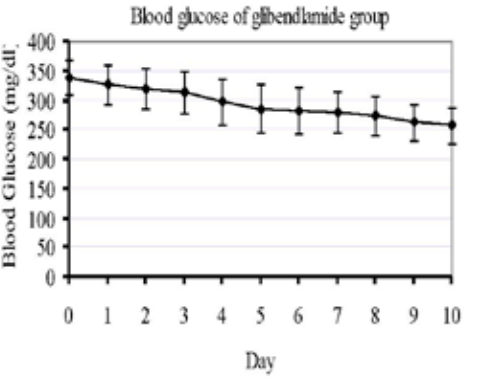



***Figure 3 .*** *Mean±SE of serum glucose level in alloxan-induced diabetic dogs treated with Ziziphus spina-christi extract (group C).*

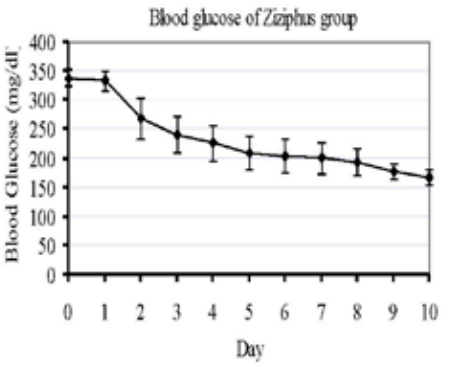
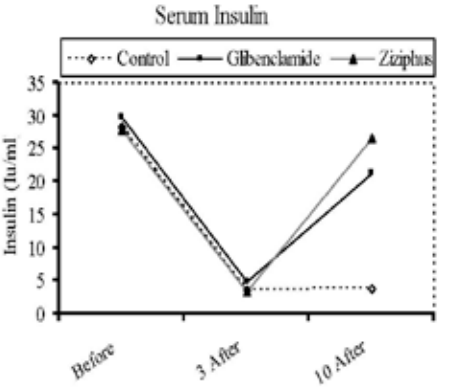



***Figure 4****. Mean±SE of serum insulin level in alloxan-induced diabetic dogs.*




the total free sugars) makes Zizyphus fruit useful for diabetic patients (Nazif, 2002).

## CONCLUSION

The hydroalcoholic extract of the fruits of *Z. spina-christi* had a mild, but significant, blood glucose lowering effect and the long-term use of this extract may be advantageous over chemical drugs in alleviating some of the chronic diseases and complications caused by diabetes. Additionally, the use of this natural agent in conjunction with conventional drug treatments, such as a chemical agent or insulin, permits the use of lower doses of the drug and/or decreased frequency of administration which decreases the side effects which most commonly observed. So, the antihyperglycemic activity of *Z. spina-christi* was experimentally born out but it has to be standardized for common use.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