Antidiabetic Activity of Date Seed Methanolic Extracts in Alloxan-Induced Diabetic Rats

Seyed Abdulmajid Ayatollahi1,2, Mehdi Sharifi-Rad1, Amir Rooointan3, Navid Baghalpour1*, Bahare Salehi3*, Zabta Khan Shinwari6,7, Ali Talha Khalil8 and Javad Sharifi-Rad1

1Phytochemistry Research Center, Shahid Beheshhti University of Medical Sciences, Tehran, Iran; 2Department of Pharmacognosy, School of Pharmacy, Shahid Beheshhti University of Medical Sciences, Tehran, Iran; 3Zabol Medicinal Plants Research Center, Zabol University of Medical Sciences, Zabol, Iran; 4Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran; 5Student Research Committee, School of Medicine, Bam University of Medical Sciences, Bam, Iran; 6Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan; 7Qarshi University, Lahore, Pakistan; 8Department of Eastern Medicine and Surgery Qarshi University Lahore Pakistan

*Corresponding author: navid.bp1994@gmail.com; bahar.salehi007@gmail.com; shinwari2008@gmail.com; javad.sharifirad@gmail.com

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ABSTRACT
Phoenix dactylifera. L. (date palm) is one of the main fruits in North of Africa and Middle East. The date seeds are considered as wastes despite of their medicinal properties. Different seed preparations are used as expectorant, anti-diarrheic, hypoglycemic, tonic, and aphrodisiac agents. We established the anti-diabetic and anti-lipidemic activities of methanolic extracts of date seed in alloxan-induced diabetic rats. Animals were divided in to 6 groups (each group contained six animals), comprising of control group and diabetic rats. Animals were treated with different amounts of extract. To assess the hypolipidemic, and anti-hyperglycemic activity, cholesterol serum levels, low density lipoprotein (LDL), high density lipoprotein (HDL), creatinine, urea and alkaline phosphatase were measured in serum of the treated animals. The anti-diabetic potential was investigated through the levels of glucose and body weight. Moreover, to assess the extract’s safety, acute toxicity test was performed. In comparison with control group, significant reductions were observed in LDL, cholesterol, as well as blood glucose levels in diabetic rats that received date seed extract. Extract supplemented diabetic rats also showed a better tolerance to glucose, as well as reduced amounts of creatinine, urea, and alkaline phosphatase in their serum samples. In addition, no toxicity (acute toxicity), was detected even after high dosage of extract administration. Our results confirmed the anti-lipidemic and anti-diabetic potentials of date seed methanolic extract in diabetic rats.

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INTRODUCTION
Diabetes mellitus (DM) is a disorder having a huge economic burden worldwide (Zheng et al., 2018). The main feature of this ailment is high amount of sugar in blood. Elevated concentration of sugar in blood for a long time could cause different acute and chronic symptoms from frequent urination, increased hunger and thirst to serious problems like diabetic ketoacidosis, cardiovascular illnesses, kidney disorders, foot ulcers, eye damages and finally death. Modern life styles (sedimentary life style), obesity, and having unhealthy diets (consuming high amounts of red meat and sugar sweetened drinks), are the main factors that increased the global rate of DM (Semenovich et al., 2015; Zheng et al., 2018). Based on reports, the quantity of diabetic patients in 1980 was 108 million and this number has increased to 422 million cases in 2016 (Collaboration 2016). The global prevalence of DM is about 1 in 11 adults and Asia is the center of diabetes epidemic (Zheng et al., 2018). So far, different conventional therapies along with lifestyle management has been used to control diabetes (Garde et al., 2016). However, due to ineffectiveness of these agents and strategies, there have
been no cases with a full recovery from this disorder (Hasan and Mohrieldin, 2016). It seems that, there is an urgent need for a potent and effective therapeutic agent with ability to cure this tough disease.

Medicinal plants are known as great source of natural curative agents with huge therapeutic potential (Sharifi-Rad et al., 2018; Sharifi-Rad et al., 2018a). Use of medicinal herbs to cure a variety of human and animal diseases, have suggested their effectiveness in different medical areas (Sharifi-Rad et al., 2018b). During the last decades, different medicinal plants have been described as potent anti-diabetic agents. In general, plants are known as rich sources of different agents like amino acids, gallotannins, flavonoids, as well as other polyphenols with anti-oxidant, anti-hyperlipidaemic and anti-diabetic activities (Muruganandan et al., 2005). For treatment of diabetes, medicinal plants with high contents of anti-hyperglycemic agents are of utmost desire.

The date palm with scientific name of Phoenix dactylifera L. is a species belongs to the Arecaceae family (Besbes et al., 2009). This big family includes 200 genera and about 3000 species and mostly grown in dry and semidry areas, such as South Asia (Middle East) and Horn of Africa (Besbes et al., 2009). From many years ago, the date palm’s high nutrition values have been confirmed by different old and modern medicinal experts. With a little search in literature, one can find a huge number of scientific papers that checked and confirmed the beneficial effects of either fruit or seed extract of date on different human disorders and conditions, such as cardiovascular diseases for diverse medicinal purposes like cancer, diabetes, antiviral, hypertension, hepatoprotective, antimicrobial etc (Baliga et al., 2011). Moreover, both date’s fruits and date’s seeds are applied as antidiarrheics, aphrodisiac, expectorant, as well as hypoglycemic agents (Baliga et al., 2011). Unfortunately, after consumption of date’s flesh, the seed parts (pits), are treated as wastes. The seed forms 5-14% weight of the palm and is known as a good source of edible oils. In fact, besides having no toxic effects, the seed parts contain different nutrients with high energy values and are good sources of unsaturated fatty acids, which are valuable for humans (Azmat et al., 2010). In searching for more valuable features of date seed, the goal of this study was to evaluate the anti-diabetic activity of date seed methanolic extract (DSE), in diabetic rats.

**MATERIALS AND METHODS**

**Plant material and extraction:** The date seed (cultivar of Astamaran) were collected from Khuzestan Province in Iran (Coordinates: 31.3273°N 48.6940°E) (Fig. 1). Pulp materials were removed completely by accurate washing the seeds by water. The date seed dried for one week at room temperature (25±5 °C). In next step, the seed crushed into small pieces and then powdered with grinder. At finally, 1 kg of seeds powder was added to 1000 mL methanol. The material was reminded on shaker for overnight. After centrifugation (2000 rpm, 10 min), the pellet was discarded and the supernatant was collected and placed into a flask. The solvent was removed from the collected supernatant by evaporation.

**Animals:** In this study, all wistar albino rats (8–10 weeks) manipulations were carried out according to the Helsinki Convention (World Medical Association, 2001). All animals were housed in 6 groups of 6 per cages in a fine ventilated room and allowed for a period of 7 days to adapt to their environmental controlled conditions (25±5 °C and 12:12 hours light-dark cycle). They were having free access to food and water. The wistar albino rats were kept on fasting for 16 hours.

**Oral glucose tolerance assay:** The animals were divided in 6 groups (each group includes six animals). Before treatment, all animals did not receive food or water (fasted). Different groups treated as follows: group 1 that received 5% Tween 80 p.o, was retained as vehicle control, group 2 only received glucose, group 3 received 150 mg/kg date seed methanolic extract, group 4, received 300 mg/kg date seed methanolic extract, group 5 received 600 mg/kg date seed methanolic extract, and group 6 only received extracts (150 mg/kg, 300 mg/kg, and 600 mg/kg) in a vehicle, respectively. Thirty min after drug administration, animals of group 3, 4, and 5 received glucose (3 g/kg, p.o.). Just before drug administration, as well as at different time points after glucose loading (30, 90, and 150 min), blood samples were collected from retro orbital sinus. The level of glucose in animal’s serum was determined instantly via glucose assay kit (Sigma-Aldrich, USA; CBA086).

**Acute oral toxicity assays:** The experiment was performed at different orally administered doses (100 mg–3000 mg/kg) to the animal groups including all rats in each group. Mortality of animal was checked after 72 h. The acute toxicity was detected based on the Litchfield and Wilcoxon method (Litchfield and Wilcoxon 1949).

**Design of experimental in this study:** The study design was as described as follow: Six groups of animals, 6 rats in each groups received the following treatment plan. Group 1: as Normal control, only received Saline (for 14 days), Group 2: Alloxan treated control (150 mg/kg,ip), Group 3: Date seed extract (150 mg/kg, p.o) + Alloxan (150 mg/kg,ip), Group 4: Date seed extract (300mg/kg, p.o) + Alloxan (150 mg/kg,ip), Group 5: Date seed extract (600mg/kg, p.o) + Alloxan (150 mg/kg,ip), and Group 6: Alloxan (150 mg/kg,ip) + Glibenclamide (5 mg/kg, p.o). Cannula was used to administer the standard drug (glibenclamide, 5 mg/kg) and plant extracts. The animals in group 1 were the normal controls and animals in group 2-6 were diabetic control animals. The group 3 to group 6 that formerly received alloxan, were received a fixed dose of date seed extract 150 mg/kg, p.o, 300 mg/kg, p.o, and 600 mg/kg, p.o, as well as glibenclamide (5mg/kg), for 14 sequential days.

**Diabetes induction in animals:** To prepare diabetic models, animals received a single injection of Alloxan monohydrate (intraperitoneal 150 mg/kg) (Ahmed et al., 2010). The Alloxan was administered based on rat’s body weights and after solubilization with 0.2 ml saline (150 mM NaCl). Two days after Alloxan injection, the animals with plasma glucose levels of more than 140 mg/dl were
selected in the experiment. The treatments with the plant extracts were started 2 days after Alloxan injection.

**Blood sample collection of and blood glucose assays:** Blood was obtained from the retro-orbital plexus from overnight fasted animals and fasting blood sugar was measured (Giordano et al., 1989). Afterwards, serum was analyzed for the levels of cholesterol, HDL (Allain et al., 1974), LDL (Friedewald et al., 1972), creatinine (Bowers, 1980), urea (Wilson, 1966), and alkaline phosphatase (Sasaki, 1966).

**Statistical analysis:** In this study, all values of different assays were presented as mean±standard error. Post hoc Dunnet’s t-test, as well as one-way analysis of variance (ANOVA) were used for analyzing the obtained data. Differences among groups were considered significant at P<0.05.

**RESULTS**

The effects of DSE on serum levels of HDL, LDL, creatinine, cholesterol, urea and alkaline phosphatase are showed in Table 1. Compared to the diabetic control, we observed an increase in H.D.L levels in animals that received DSE. On the other hand, DSE caused a decrease in the levels of L.D.L in animal models. In addition, comparing with diabetic control, animals that received DSE showed decreased levels of urea, cholesterol, alkaline phosphatase, creatinine, and in their serum. All responses to DSE were seen in a dose responsive manner.

The glucose tolerance was enhanced after supplementation of DSE in the fasted normal rats (Fig. 2). At 90 minutes, serum glucose levels in animals decreased significantly and reduced differently at 150 minutes (P<0.05). DSE also revealed remarkable hypoglycemic effect after 90 minutes of treatment.

Fig. 3 shows the DSE’s anti-hyperglycemic effects on the fasting blood sugar concentration in diabetic rats. After two weeks of daily treatment with DSE, the observations revealed a dose responsive decrease in the blood sugar levels of animals (P<0.05).

After checking the body weight of animals in all groups during 14 days, it was observed that the diabetic rats lose weights more than other groups. On the other hand, there was a slight increase in body weights of animals in other groups (Fig. 4). No acute toxicity (mortality) was observed in animals that supplemented with high doses of DSE (up to 3000 mg/kg).
Table 1: Date seed extracts effect in various groups on serum profile in alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>H.D.L (mg/dl)</th>
<th>L.D.L (mg/dl)</th>
<th>Cholesterol (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Alkaline Phosphatase (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>61.99±0.1a</td>
<td>114.91±0.3f</td>
<td>95.94±1.3e</td>
<td>0.73±0.1e</td>
<td>28.51±0.3e</td>
<td>145.5±1.2f</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>45.89±2.1e</td>
<td>245.64±0.1a</td>
<td>199.22±0.5a</td>
<td>1.69±0.4a</td>
<td>78.45±1.2a</td>
<td>299.32±1.1a</td>
</tr>
<tr>
<td>Date seed extract (150 mg/kg,p.o + Alloxan)</td>
<td>52.92±1.3d</td>
<td>174.91±0.5b</td>
<td>182.55±1.1b</td>
<td>0.95±0.1b</td>
<td>52.33±0.1b</td>
<td>212.39±2.2b</td>
</tr>
<tr>
<td>Date seed extract (300 mg/kg,p.o + Alloxan)</td>
<td>57.77±1.5c</td>
<td>142.42±0.2c</td>
<td>154.71±0.2c</td>
<td>0.92±0.5c</td>
<td>45.9±1.4c</td>
<td>191.1±1.9c</td>
</tr>
<tr>
<td>Date seed extract (600 mg/kg,p.o + Alloxan)</td>
<td>59.63±0.2b</td>
<td>131.86±0.6d</td>
<td>134.67±0.3d</td>
<td>0.86±0.4d</td>
<td>36.54±1.3d</td>
<td>182.91±2.3d</td>
</tr>
<tr>
<td>Glibenclamide (5 mg/kg) + Alloxan</td>
<td>62.12±0.0a</td>
<td>120.39±0.5e</td>
<td>96.12±3.2e</td>
<td>0.73±0.0e</td>
<td>28.98±0.1e</td>
<td>173.44±2.9e</td>
</tr>
</tbody>
</table>

Values are shown as mean±SEM for groups. The values with different lower-case letters within a column are significantly different among different groups (P<0.05).

DISCUSSION

Currently, medicinal plants are considered as new, safe and inexpensive sources of different therapeutics. Therapeutic potential of different plant extracts and their beneficial effects in treatment of different disorders and health hazards have been shown by various research teams around the world. Application of herbal medicines for treatment of different diseases, such as diabetes dates back to many years ago. Here, the philosophy of using medicinal plants for treatment of diabetes is that they can escalate the regeneration of pancreatic beta cells, increase the insulin release and fight against insulin resistance (Hasan and Mohieldein, 2016). In folk medicine, date palm is known as a natural cure for hypertension, atherosclerosis, cancer, diabetes and different infectious diseases (Baliga et al., 2011). In parallel, scientific reports also showed the beneficial effects of date fruit on different disease conditions. Although, there are many reports related to the beneficial effects of date pulp, there is a limited number of experiments trying to find the probable effects of date seeds extract on disorders and health hazards. Date seeds, also known as date pits, can be safe and cheap sources of different vital nutrients and offer various health benefits for humans (Shi et al., 2014). Lately, there has been an increasing curiosity in exploration and extraction of polyphenols, essential oils, and dietary fiber from these precious seeds (Ardekani et al., 2010).

In the present experiment, we evaluated the probable anti-diabetic activity of date seed methanolic extract on alloxan-induced diabetic rats. Alloxan as a toxic analogue of glucose, with ability to destroy pancreatic beta cells (insulin producing cells), was used for induction of diabetes in animals (Macdonald Ighodaro et al., 2017). After preparing the date seeds and performing methanolic extraction, possible effects of the extract on serum profile of alloxan-induced diabetic rats were evaluated. After supplementation, the results showed the ability of the DSE in elevating the levels of HDL, while reducing the levels of LDL, cholesterol, urea, creatinine, and alkaline phosphatase in the serum of diabetic rats. Hypercholosteraemia and increased levels of L.D.L are among the common abnormalities in diabetic patients (Daniel, 2011). Handling the risk of cardiovascular problems in diabetic cases is considered as a big challenge for medical practitioners. Decreasing the load of cardiovascular ailment in diabetic patients must start with valuation and management of the raised LDL and cholesterol. In our experiment, the alloxan-induced diabetic rats (control group) showed higher amounts of LDL and cholesterol in their blood. On the other hand, animal groups that received DSE showed reduced amounts of these compounds in their blood. Such results indicated the potential of DSE in reducing the risk of cardiovascular problems in diabetic patients. According to some experiments, saponines and steroids have anti-hyperlipidaemic activities by hindering the absorption of lipids in intestine, as well as lowering the activity of lipase enzyme (Juarez-Rojop et al., 2012). Interestingly, DSE is rich in saponins, and the ability of reducing L.D.L and cholesterol could be related to these elements.

The escalation of some liver biomarkers like alkaline phosphatase has been reported in serum of diabetic cases. This can be a sign for damaged liver function resulted from hyperglycaemia. In our experiment, the alloxan-induced diabetic rats showed a higher level of ALP in their blood samples. On the other hand, animals that received DSE, showed reduced amounts of this enzyme in their blood samples in a dose responsive manner. Moreover, the significant decrease in the serum levels of ALP, as well as urea and creatinine in DSE supplemented animals revealed the protective effects of DSE on kidney and liver of diabetic rats. Such findings were in agreement with other experiments, where the date seed extract was able to reduce the serum levels and activities of ALP, in animal models (Hasan and Mohieldein 2016). The results of glucose tolerance tests showed the ability of DSE in elevating the glucose tolerance in the animal models. During time, the glucose levels in the DSE supplemented animals were reduced. These consequences can reflect an improved glycemic control, as well as better insulin level status in DSE supplemented animals. DSE also showed to be able to diminish the amounts of glucose in blood samples of diabetic animals during time. As it can be seen in Fig. 3, daily DSE supplementation caused a decrease in blood sugar levels of animals after 2 weeks. Such findings that revealed the anti-hyperglycaemic effects of the DSE are in agreement with other similar experiments (El Fouhil et al., 2013; Hasan and Mohieldein 2016).

Flavonoids, glycosides, and tannins are the main anti-diabetic components in medicinal plants. Moreover, previous experiments have shown that DSE contains all of the abovementioned phytoconstituents (Kalantaripour et al., 2012; Said et al., 2014). Hence, we can conclude that the anti-diabetic effects of the DSE might be due to these phytochemicals. The other point is that the DSE had no hazards. Date seeds, also known as date pits, can be safe and inexpensive sources of different therapeutics.
animals. In this experiment, diabetic animals that received DSE, showed no reduction, but a slight increase in their body weight. Maintaining the body weight in DSE supplemented diabetic animals might be due to the secretion of insulin, as well as glycemic control provided by active components in DSE. The obtained results were in line with other experiment that evaluated the anti-diabetic activity of the aqueous date seed extract on streptozotocin induced diabetic rats (Hasan and Mohieldein, 2016). In the following, to determine the probable acute toxicity of methanolic DSE, acute oral toxicity assay was conducted. The results showed no mortality caused by DSE in animal models, even after administration of a high dose of DSE (up to 3000 mg/kg). This finding supports the potential of the DSE as a safe applicant for treatment of diabetes.

Conclusions: The obtained data by the present experiment approves the anti-hyperlipidemic and anti-hyperglycemic features of methanolic date seed extract in alloxan-induced diabetic rats and introduced the date seed extract as a safe and efficient applicant in fighting against diabetes.

Authors contribution: All authors contributed to the manuscript. Conceptualization JSR and BS; validation investigation, resources, data curation, writing – all authors; review and editing, ZKS and ATK. All the authors read and approved the final manuscript.

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