Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2019.099

RESEARCH ARTICLE

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

Seyed Abdulmajid Ayatollahi[1,2], Mehdi Sharifi-Rad[3], Amir Roointan[4], Navid Baghalpour[1*], Bahare Salehi[5*], Zabta Khan Shinwari[6,7 *], Ali Talha Khalil[8] and Javad Sharifi-Rad[1*]

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

ARTICLE HISTORY (19-048)

Received: January 30, 2019
Revised: June 23, 2019
Accepted: August 22, 2019
Published online: September 11, 2019

Key words:
Date seed extract
Diabetes
Methanolic extract
Phoenix dactylifera L.

A B S T R A C T

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.

To Cite This Article: Ayatollahi SA, Sharifi-Rad M, Roointan A, Baghalpour N, Salehi B, Shinwari ZK, Khalil AT and Sharifi-Rad J, 2019. Antidiabetic activity of date seed methanolic extracts in alloxan-induced diabetic rats. Pak Vet J, 39(4): 583-587. http://dx.doi.org/10.29261/pakvetj/2019.099

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 (Semenkovich *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 (Gæde *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 Mohieldein, 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-hyperlipedaemic 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).

Fig. 1: The date seed used in this study

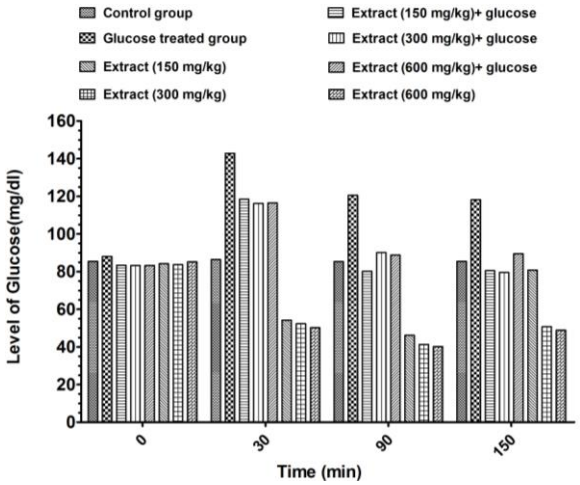

Fig. 2: Effects of methanolic seed extracts on glucose tolerance test.

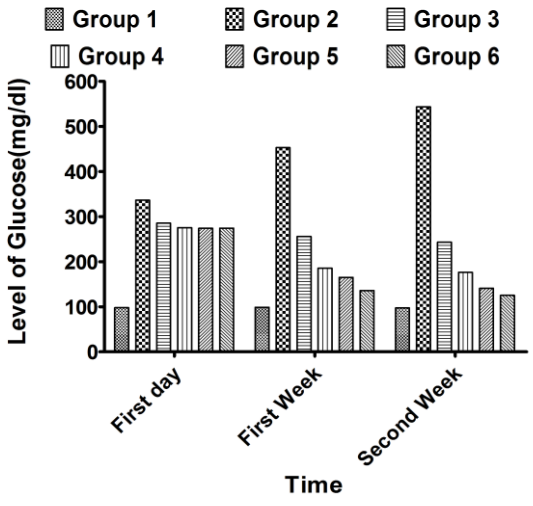

Fig. 3: Blood glucose level in alloxan-induced diabetic rats.

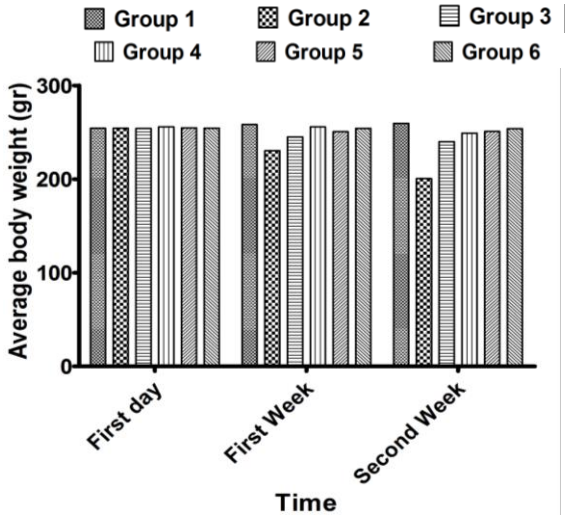

Fig. 4: The influences of date seed methanolic extract on body weight of diabetic rats.

Table 1: Date seed extracts effect in various groups on serum profile in alloxan-induced diabetic rats

Groups	H.D.L (mg/dl)	L.D.L (mg/dl)	Cholesterol (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Alkaline Phosphatase (mg/dl)
Normal control	61.99±0.1 a	114.91±0.3 f	95.94±1.3 e	0.73±0.1 e	28.51±0.3 e	145.5±1.2 f
Diabetic control	45.89±2.1 e	245.64±0.1 a	199.22±0.5 a	1.69±0.4 a	78.45±1.2 a	299.32±1.1 a
Date seed extract (150 mg/kg,p.o) + Alloxan	52.92±1.3 d	174.91±0.5 b	182.55±1.1 b	0.95±0.1 b	52.33±0.1 b	212.39±3.2 b
Date seed extract (300 mg/kg,p.o) + Alloxan	57.77±1.5 c	142.42±0.2 c	154.71±0.2 c	0.92±0.5 c	45.9±1.4 c	191.11±1.9 c
Date seed extract (600 mg/kg,p.o) + Alloxan	59.63±0.2 b	131.86±0.6 d	134.67±0.3 d	0.86±0.4 d	36.54±1.3 d	182.91±2.3 d
Glibenclamide (5 mg/kg) +Alloxan	62.12±0.0 a	120.39±0.5 e	96.12±3.2 e	0.73±0.0 e	28.98±0.1 e	173.44±2.9 e

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. Hyperchlosteraemia 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 (Juárez-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-hyperglycemic 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 hypoglycemic effects on the normal animals. Therefore, the methanolic DSE could be considered as a suitable anti-hyperglycaemic agent with no hypoglycaemic effect. Previous experiments have reported a remarkable decline in body weight of diabetic rats during time (Zafar *et al*., 2009). Such body weight reduction could be due to loss of structural proteins, which finally affect the body weight of

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.

REFERENCES

Ahmed MF, Kazim SM, Ghori SS, *et al*., 2010. Antidiabetic activity of Vinca rosea extracts in alloxan-induced diabetic rats. Int J Endocrinol 2010:6 pages.

Allain CC, Poon LS, Chan CS, *et al*., 1974. Enzymatic determination of total serum cholesterol. Clin Chem 20:470-5.

Ardekani MRS, Khanavi M, Hajimahmoodi M, *et al*., 2010. Comparison of antioxidant activity and total phenol contents of some date seed varieties from Iran. Iran J Pharm Res 9:141.

Azmat S, Ifzal R, Rasheed M, *et al*., 2010. GC-MS Analysis of n-hexane Extract from Seeds and leaves of phoenix dactylifera L. J Chem Soc Pak 32:672-6.

Baliga MS, Baliga BRV, Kandathil SM, *et al*., 2011. A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). Food Res Int 44:1812-22.

Besbes S, Drira L, Blecker C, *et al*., 2009. Adding value to hard date (*Phoenix dactylifera* L): compositional, functional and sensory characteristics of date jam. Food Chem 112:406-11.

Bowers LD, 1980. Kinetic serum creatinine assays I. The role of various factors in determining specificity. Clin Chem 26:551-4.

Collaboration NRF, 2016. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4.4 million participants. Lancet 387:1513-30.

Daniel MJ, 2011. Lipid management in patients with type 2 diabetes. Am Health Drug Benefits 4:312.

El Fouhil AF, Ahmed AM, Atteya M, *et al*., 2013. An extract from date seeds stimulates endogenous insulin secretion in streptozotocin-induced type I diabetic rats. Func Foods Health Dis 3:441-6.

Friedewald WT, Levy RI and Fredrickson DS, 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499-502.

Gæde P, Oellgaard J, Carstensen B, *et al.,* 2016. Years of life gained by multifactorial intervention in patients with type 2 diabetes mellitus and microalbuminuria: 21 years follow-up on the Steno-2 randomised trial. Diabetologia 59:2298-307.

Giordano B, Thrash W, Hollenbaugh L, *et al.,* 1989. Performance of seven blood glucose testing systems at high altitude. Diabetes Edu 15:444-8.

Hasan M and Mohieldein A, 2016. In vivo evaluation of anti diabetic, hypolipidemic, antioxidative activities of Saudi date seed extract on streptozotocin induced diabetic rats. J Clin Diagn Res 10:FF06.

Juárez-Rojop IE, Díaz-Zagoya JC, Ble-Castillo JL, *et al*., 2012. Hypoglycemic effect of Carica papaya leaves in streptozotocin-induced diabetic rats. BMC Comp Alt Med 12:236.

Kalantaripour T, Asadi-Shekaari M, Basiri M, *et al*., 2012. Cerebroprotective effect of date seed extract (*Phoenix dactylifera*) on focal cerebral ischemia in male rats. J Biol Sci 12:180.

Litchfield JJ and Wilcoxon F, 1949. A simplified method of evaluating dose-effect experiments. J Pharmacol Expe Ther 96:99-113.

Macdonald Ighodaro O, Mohammed Adeosun A and Adeboye Akinloye O, 2017. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. Medicina 53:365-74.

Muruganandan S, Srinivasan K, Gupta S, *et al*., 2005. Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. J Ethnopharmacol 97:497-501.

Said A, Kaoutrher D, Ahmed B, *et al*., 2014. Dates quality assessment of the main date palm cultivars grown in Algeria. Annu Res Rev Biol pp:487-99.

Sasaki M, 1966. A new ultramicro method for the determination of serum alkaline phosphatase. Use of Berthelot's reaction for the estimation of phenol released by enzymatic activity. Igaku to seibutsugaku. Med Biol 70:208.

Semenkovich K, Brown ME, Svrakic DM, *et al*., 2015. Depression in type 2 diabetes mellitus: prevalence, impact and treatment. Drugs 75:577-87.

Sharifi-Rad J, Tayeboon GS, Niknam F, *et al.,* 2018. *Veronica persica* Poir. extract-antibacterial, antifungal and scolicidal activities, and inhibitory potential on acetylcholinesterase, tyrosinase, lipoxygenase and xanthine oxidase. Cell Mol Biol 64:50-6.

Sharifi-Rad M, Fokou P, Sharopov F, *et al.,* 2018a. Antiulcer agents: from plant extracts to phytochemicals in healing promotion. Molecules 23:1751.

Sharifi-Rad M, Nazaruk J, Polito L, *et al.,* 2018b. *Matricaria* genus as a source of antimicrobial agents: From farm to pharmacy and food applications. Microbiol Res 215:76-88.

Shi LE, Zheng W, Aleid SM, *et al*., 2014. Date pits: Chemical composition, nutritional and medicinal Values, utilization. Crop Sci 54:1322-30.

Wilson B, 1966. Automatic estimation of urea using urease and alkaline phenol. Clin Chem 12:360-8.

World Medical Association, 2001. World medical association declaration of helsinki. Ethical principles for medical research involving human subjects. Bull World Health Org 79:373.

Zafar M, Naqvi SNH, Ahmed M, *et al*., 2009. Altered liver morphology and enzymes in streptozotocin induced diabetic rats. Int J Morphol Rev Endocrinol 14:88.