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ORIGINAL ARTICLE

Mechanistic *In vitro* Evaluation of *Prosopis farcta* Roots Potential as an Antidiabetic Folk Medicinal Plant

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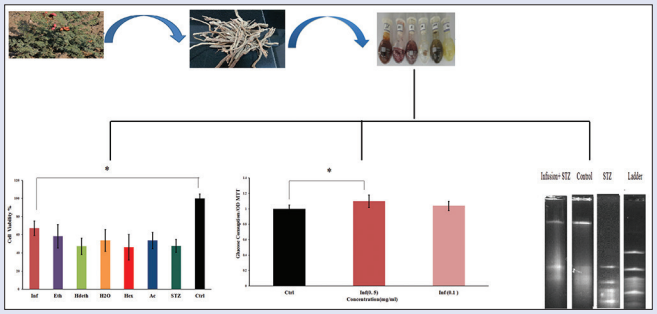
ABSTRACT

Objective: *Prosopis farcta* has been used as a traditional herbal medicine for treating *Diabetes mellitus*. The aim of this study is to investigate the antidiabetic mechanisms of infusion (INF) extract of *P. farcta* and discovering the active extract for the first time. **Materials and Methods:** Six different extracts of *P. farcta* were prepared using five different solvents (ethanol, *n*-hexane, acetone, ethanol:water (1:1 v/v), and water). Cytotoxicity and cell proliferation assays were performed on mouse pancreatic β-cells (β-TC_3) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium method. The effects of *P. farcta* on glucose metabolism (in a hepatocellular carcinoma cell line [HepG2]) and glucose diffusion across a dialysis membrane (as a model of cellular glucose absorption) were evaluated. The protective effect of various *P. farcta* extracts on cytotoxicity, mitochondrial membrane potential (MMP), and streptozotocin (STZ)-induced apoptosis in β-TC_3 cells was investigated. **Results:** Cytotoxicity study indicated that extracts were safe on β-TC_3 and HepG2 (≤0.5 mg/ml). INF protected β-TC_3 cells from apoptosis induced by STZ and improved cell viability for 20% and significantly decrease depolarization of MMP ($P < 0.005$). The results showed that INF inhabited breaking/streaking the DNA. Proliferation study showed no significant increase in the number of cells either at single or multiple doses. In moderate hyperglycemia (11.1 mmol/l), a significant glucose-lowering effect was observed but glucose diffusion was not the probable mechanism of extracts antidiabetic effect. In conclusion, only INF, the traditionally used extract, has an antidiabetic potential by attenuating the death and apoptosis induced by STZ in β-TC_3 cells and increase glucose consumption. **Conclusion:** The present study demonstrates that only INF extract have an antidiabetic potential by attenuating the death and apoptosis induced by STZ in β-TC_3 cells and increase glucose consumption.

Key words: β-TC_3 cells, diabetes mellitus, hepatocellular carcinoma cell line, *Prosopis farcta*

SUMMARY

- Six different extracts from *P. farcta* were prepared using five different solvents [ethanol, n-hexane, acetone, ethanol: water (1:1 v/v), and water]
- The protective effect of various *P. farcta* extracts on cytotoxicity, mitochondrial membrane potential (MMP), and Streptozotocin-induced apoptosis in β-TC_3 cells were investigated.
- Infusion has an antidiabetic potential by attenuating the death and apoptosis induced by STZ in β-TC_3 cells and increase glucose consumption
- The effect of infusion extract on glucose consumption in hepatocellular carcinoma cell line cells (a) and effect of infusion extract on glucose consumption in hepatocellular carcinoma cell line cells adjusted by optical density MTT (b). Significance was calculated by analysis of variance (* $P \leq 0.05$). MTT: 3 (4,5 dimethylthiazol 2 yl) 2,5 diphenyltetrazolium.



Abbreviations used: AC: Acetone extract; ANOVA: Analysis of variance; BSA: Bovine serum albumin; β-TC_3: Mouse pancreatic β-cells; DMEM: Dulbecco modified Eagle medium; DMSO: Dimethyl sulfoxide; ETH: Ethyl acetate extract; FBS: Fetal bovine serum; HDETH: Hydroethanolic extract; HepG2: Hepatocellular carcinoma cell line; HEX: Hexane extract; INF: Infusion; KUMS: Kermanshah University of Medical Sciences; MMP: Mitochondrial membrane potential; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; NaCl: Natrium chloride; OD: Optical density; spp: Species; STZ: Streptozotocin; Tag: T-antigen; USA: United States of America.

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INTRODUCTION

Diabetes mellitus is one of the most common metabolic disorders worldwide, affecting approximately 8.5% of the world's population and is anticipated to cross 5.4% by the year 2025.[1] Twenty percent of the world population is estimated to be affected by this disease.[2,3] Recent studies have shown that currently, the incidence of diabetes in Iran is about 12.3% in 2014.[4]

Thus far, approximately 800 plants worldwide have shown antidiabetic potential.[5] Plants have traditionally been used for the treatment of *diabetes mellitus*, and they provide a great source of drugs and biological compounds.[6]

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Antidiabetic plants are widely distributed in Iran and India.[7] The families containing the most antidiabetic plants include Fabaceae, Leguminosae, Lamiaceae, Liliaceae, Cucurbitaceae, Asteraceae, Moraceae, Rosaceae, and Araliaceae.[8] A previously published study indicated that the roots of the Fabaceae family have antioxidant and antidiabetic potential.[9] In particular, these plants have a β-cell protective, regenerative, and insulinogenic properties. *Prosopis* species (spp) such as *P. glandulosa* has been shown to stimulate insulin secretion and proliferation of β-cells[10] and a leaf extract from *P. cineraria* significantly reduced the lipid and glucose levels in diabetic rats.[11]

Prosopis spp (Fabaceae) are hardy evergreen and deciduous trees or shrubs found in temperate climates of arid and semiarid regions. *P. farcta* is an established introduced spp in parts of Iran, including Khuzestan, Gilan, Fars, Hormozgan, Baluchestan, Khorasan, and Tehran.[12] Three spp of *Prosopis* have been found in Iran, including *Prosopies cineraria*, *P. koelziana*, and *P. farcta*.[12] The *Prosopis* spp have been used as a folk remedy for diabetes,[13] healing wounds,[14] cardiac or chest pain,[14] as antidiuretics,[15] and anticancer,[16] in bacterial infection (INF),[17] and in weight deficiency.[18]

Moreover, a traditional study in Jordanian population reported that a decoction of *P. farcta* roots contains antidiabetic agents.[19] However, to the best of our knowledge, no studies have been performed on the antidiabetic mechanisms of *P. farcta*. In this study, we investigated the antidiabetic mechanisms of *P. farcta* and discovered the active extract for the first time. We studied the effects of the extract using a cell proliferation and cytotoxicity assay in mouse pancreatic β-cells, β-TC_3. Its protective effect on streptozotocin (STZ)-induced cell death in β-TC_3 cells was investigated. Because of the central role of the liver in the metabolism of glucose, the glucose-lowering potential of the *P. farcta* extract in hepatocellular carcinoma cell line (HepG2), a human hepatocellular carcinoma cell line, was also evaluated. Finally, the effect of the *P. farcta* extract on glucose diffusion across a dialysis membrane, which is a suitable model for assessing cellular glucose absorption, was evaluated *in vitro*.

MATERIALS AND METHODS

General instruments and chemicals

Glucose Assay Kit (Sigma) [Reagents: glucose oxidase/peroxidase g-3660, o-dianisidine reagent d-2679 glucose standard solution G-3285], 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT), dimethyl sulfoxide (DMSO), rhodamine 123, Triton X-100, and STZ was purchased from Sigma Aldrich (United States of America [USA]). Dulbecco modified Eagle medium (DMEM), fetal bovine serum (FBS), bovine serum albumin (BSA), penicillin-streptomycin, and trypsin were purchased from Ato-cell, Hungary. Agarose gel was obtained from Geneon Company (Germany). Novel juice was obtained from Genedirex, Taiwan. All the solvents used for extraction obtained from Merck Chemical Company (Germany).

Plant material

Roots of *P. farcta* (Banks and Sol.) were collected from Ilam surroundings, Iran. Plant materials were identified by Dr. Shahram Miraghayi, Medical Biology Research Center, Kermanshah University of Medical Sciences, Kermanshah and compared to herbarium voucher specimen of Herbarium of Agricultural Faculty of Razi University, Kermanshah, Iran.

Sample preparation

Preparation of infusion

Plant material was powdered after air drying and 400 g was added to 3200 ml boiling water and mixed for 30 min on a hot bath. After filtration and concentration *in vacuo*, the dried extract was kept in 20°C for further purification called as INF.

Preparation of organic extracts

Two 60 g aliquots of ground powder of *P. farcta* roots were extracted with *n*-hexane and acetone (250 ml of each) separately using Soxhlet apparatus for 3 h for each solvent. Another three aliquots were extracted through maceration method using ethanol, ethanol-water (50:50), and water at room temperature (with 600 ml of each solvent for 3 days). These five extracts were concentrated *in vacuo* and kept in −20°C for further purification as, hexane extract (HEX), AC, ethyl acetate extract (ETH), hydroethanolic extract (HDETH), and H_2O, respectively.

Effect of *P. farcta* extracts on *in vitro* glucose diffusion

A method described by Gallagher *et al.*[20] was used to evaluate the effects of plant extracts on glucose movement *in vitro* which consists of a dialysis tube (6 cm × 15 mm, cutoff 2000) into which 6 ml of extract (0.5 mg/ml) and 2 ml of natrium chloride (NaCl) (0.15 M) with D-glucose (1.65 mM) were added. The dialysis tube was sealed at each end and placed in a centrifuge tube containing 45 ml NaCl (0.15 M). The tubes were placed on an orbital shaker water bath and incubated at 37°C for 3 h. The movement of glucose into the external solution was provided. The concentration of glucose within the dialysis tubing was measured, and control test was conducted in the absence of extracts. Glucose concentration was analyzed by enzymatic method using glucose oxidase kit.[21,22]

Cell-culture conditions

β-TC_3 (a mouse beta pancreatic cell line) and HepG2 cells (human liver hepatocellular carcinoma cell line) were purchased from the Iran genetic resources center. They were cultured in 25 cm^2 culture flasks using DMEM supplemented by 10% v/v FBS and penicillin/streptomycin (100 U/ml, 100 mg/ml) at 37°C in a humidified atmosphere of 5% CO_2. The cells were subcultured regularly using trypsin (Atocell, Australia). The growth medium was changed every 2–3 days and subcultured until use.

Cell viability assay

The cytotoxicity effect of different extracts was determined against β-TC_3 and HepG2 cell lines by a colorimetric assay using MTT and compared with the untreated control. Cells were plated onto 96-well plates at a density of 5.0×10^4 cells/ml and in a volume of 180 µl. Stock solutions of HEX and AC were prepared in DMSO. The final concentration of the DMSO in the medium was always 0.5%. One day after seeding, 20 µl of the various extracts at several concentrations was added to each well. After 24 h, 20 µL of MTT (5 mg/ml) was added to each well and then the plates transferred to a 37°C incubator for 3 h, the medium was removed by aspiration and the reduced MTT dye was solubilized with DMSO (150 µl/well). Absorbance was determined on an ELISA plate reader (synergy H1, Biotek, USA) with a test wavelength of 540 nm and a reference wavelength of 630 nm to obtain sample signal (optical density [OD] 540–OD630). Percentage of toxicity was calculated using formula1:[23]

Percent of control proliferation = (OD test/OD control) ×100 (1)

Inhibition of streptozotocin-induced β-TC_3 cell death

To measure IC_{50} of STZ, β-TC_3 cells (5×10^4 cells/ml) were seeded in each well of a 96-well culture plate. After overnight incubation, 20 µL of STZ at different concentration (3-60 µg/µl) was added to the cells. The cells were further incubated for 24 h then the MTT assay was performed to measure the cell viability as mentioned in section 2.5.

To measure the inhibition effect of *P. farcta* extracts, nontoxic concentrations of extracts were used either before adding

STZ (pretreatment) or together with STZ (coadministration). Then, cell viability was measured by MTT assay.

Glucose consumption

HepG2 cells were grown in DMEM (5.5 mmol/L glucose) containing 10% FBS. Two days before the experiments, the cells were plated into 96-well tissue culture plates with some wells left blank. After the cells reached confluence, the medium was replaced by DMEM supplemented with 0.2% BSA and glucose. After 12 h, the medium was removed, and 0.2% BSA-DMEM containing extracts were added to wells as well as the blank wells. Finally, the medium was removed, and its glucose concentration was assessed by the glucose oxidase method. The volume of glucose consumption was evaluated by the glucose concentration of blank wells subtracting the remaining glucose in cell plated wells.[24]

$$\text{Amount of glucose}(\text{mg}) = \frac{(\Delta A540 \text{ of test})\ (\text{mg glucose in standard})}{\Delta A540 \text{ of standard}} - \frac{(\Delta A540 \text{ of test})(0.05)}{\Delta A540 \text{ of standard}} \quad (2)$$

β-cell proliferation assay

In vitro cell proliferation was assessed using MTT assay, and cells were seeded at a density of 1×10^4 cells/ml in a 96-well plate and incubated for 24, 48, 72, and 96 h at 37°C in 5% CO_2. Approximately, 24 h after seeding, the cells were treated with INF extract in concentration of 0.5 mg/ml and continuously monitored for up to 96 h. For multiple doses, the culture medium was replaced by culture medium containing INF (0.5 mg/ml) every day. The cells which were not treated with any chemical agents served as control cells. Cell proliferation was monitored every 24 h using the MTT assay as mentioned in section 2.4. OD was converted to cell count using standard curve.[22]

Measurement of mitochondrial membrane potential

Mitochondrial dysfunction has been shown to participate in the induction of apoptosis. In this study, mitochondrial membrane potential (MMP) was measured by using rhodamine 123 fluorescent dye. Depolarization of MMP during cell apoptosis results in the loss of rhodamine 123 from the mitochondria and a decrease in intracellular fluorescence intensity. β-TC_3 cells (1×10^5 cells/ml) were seeded in 24 well plates (NEST, China) 24 h before experiments. Cells were treated with INF in the culture medium at 37°C for 24 h. Then, cells were incubated with rhodamine 123 for 30 min at 37°C. Cells were washed three times with phosphate-buffered saline (PBS) and lysed on ice with lysis buffer (1% Triton X-100 in PBS) for 1 h. The fluorescence was measured at an excitation wavelength of 488 nm and an emission wavelength of 520 nm using a fluorescence microplate reader (BioTek, H1M, USA). The mean fluorescence intensity was normalized to the amount of protein present in the sample using Bradford assay.[25]

DNA fragmentation analysis

β-TC_3 cells (2×10^6 cells/well) were seeded in 6 well plates and allowed to adhere for 24 h and then treated with 200 µL of STZ alone or with INF (0.5 mg/ml). Plates were then incubated for 24 h at 37°C in humidified atmosphere of 95% air and 5% CO2. Total DNA from β-TC_3 cells was extracted using high pure isolation kit (Roche, Mannheim, Germany) according to the manufacturer instruction. 20 µl of DNA eluted sample was mixed with 4 µl of novel juice dye and run on 1.2% agarose gel at 75 V for 1 h and visualized using an ultraviolet transilluminator and then photographed.[26]

Statistical analyses

All tests were performed in triplicate, and the results were presented as means ± standard deviation. Significant differences between the means of the experimental groups were identified with analysis of variance (ANOVA), followed by the *Tukey–Kramer* multiple comparisons test (GraphPad version 5.0; GraphPad Software Inc., San Diego, CA, USA). Statistical significance was set at $P < 0.05$.

RESULTS

Effect of extracts on glucose diffusion

The present study was undertaken to investigate the effects of extracts on glucose movement across dialysis membrane into external solution, which is a convenient model for assessing factors affecting glucose absorption *in vitro*. Extracts did not demonstrate significant inhibitory effects on glucose movement across dialysis membrane into external solution compared to control [Figure 1].

Determination of nontoxic concentration of extracts on β-TC_3 and hepatocellular carcinoma cell line cell lines

To set concentrations which are nontoxic to cells but could prevent cytotoxicity of STZ, we examined the effects of different concentrations of INF, HEX, AC, ETH, HDETH, and H_2O on cell viability. For each concentration and time course study, there was a control sample that remained untreated and received the equal volume of solvent. The cells were treated for 24 h with various extracts, and cell viability was determined by MTT assay. The results were shown in Figures 2 and 3. The extracts showed no cytotoxic effect in β-TC_3 and HepG2 cell lines at 0.1 mg/ml while INF was not cytotoxic even at 0.5 mg/ml.

Cell viability of β-TC_3 cell line after exposure to streptozotocin

The viability of β-TC_3 cell was evaluated after 24 h of exposure to different concentrations of STZ. Cell viability was evaluated by the MTT method. As shown in Figure 4, STZ-induced cytotoxicity was dose dependent in the cell line, and the IC_{50} value was 48 µg/µl in β-TC_3 cell line.

Inhibition of streptozotocin-induced β-TC_3 cell death

Exposure to STZ (48 µg/µl) induced about 50% death of β-TC_3 cells. Among different extracts, only INF extract significantly protected β-TC_3 cells against STZ [Figure 5].

Protection effect of INF on cytotoxicity of STZ was also evaluation by study of cell' morphology. Morphological changes in treated cells either with STZ or with STZ and extracts for 24 h were observed in comparison

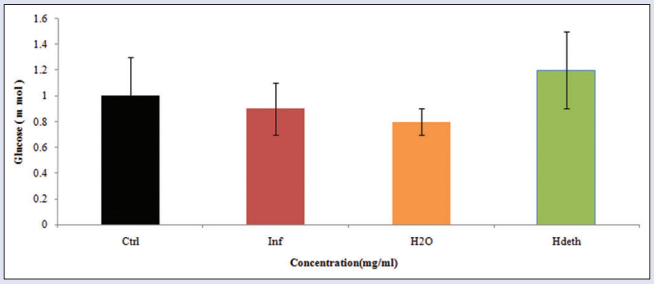

Figure 1: Effect of various *Prosopis farcta* extracts on *in vitro* glucose diffusion. Significance was calculated by analysis of variance (*$P \leq 0.05$)

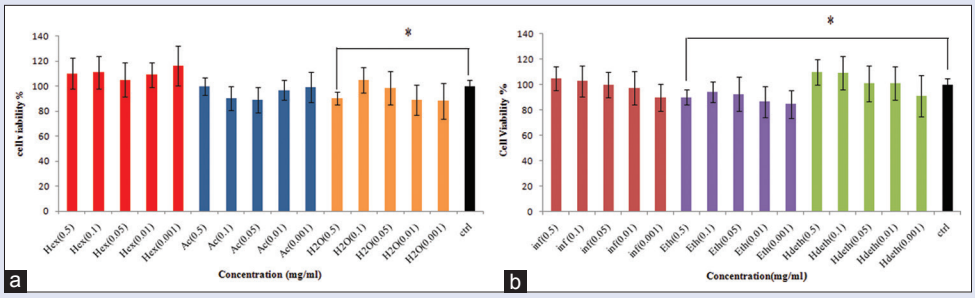

Figure 2: Cytotoxicity of infusion and ethyl acetate extract and hydroethanolic extract (a) hexane extract and Ac and H_2O (b) extracts on β-TC_3 cell line after 24 h. The cell viability was determined by MTT assay. Data are expressed as the mean ± standard error of the mean of three separate experiments. Significance was calculated by the analysis of variance (*$P \leq 0.05$). MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

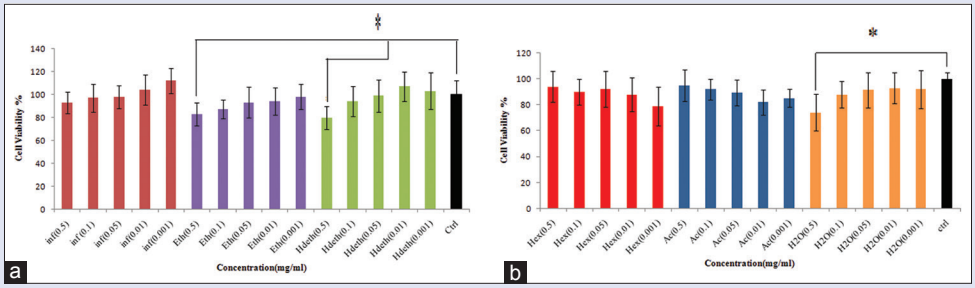

Figure 3: Cytotoxicity of infusion and ethyl acetate extract and hydroethanolic extract (a) hexane extract and Ac and H_2O (b) extractson hepatocellular carcinoma cell line cell lineafter 24 h. The cell viability was determined by MTT assay. Data are expressed as the mean ± standard error of the mean of three separate experiments. Significance was calculated by analysis of variance (*$P \leq 0.05$). MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

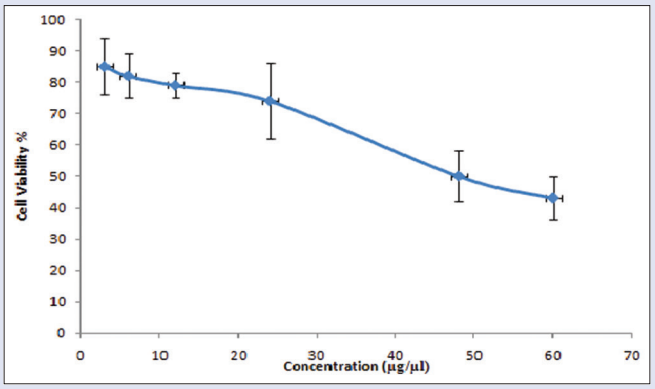

Figure 4: The effect of streptozotocin on β-TC_3 cell viability. The cell viability was determined by MTT assay. Data are expressed as the mean ± standard error of the mean of three separate experiments. Significance was calculated by analysis of variance (*$P \leq 0.05$). MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

to control cells [Figure 6]. Visualization of the control (untreated) cells indicated that the cells maintained their original morphology form containing several nucleoli while cell population was significantly decreased and cell morphology was changed into round and spherical in STZ-treated wells. After 24 h treatment with 0.1 mg/ml INF, nearly 90% of surface in the control wells was confluent, and 70% of the surfaces were covered by cells in INF treated wells which indicated significant increase in compare to STZ-treated wells.

To evaluate the effect of coadministration and pretreatment of INF on inhibition of STZ cytotoxicity, β-TC_3 cells were pretreated with INF (0.1 mg/ml) for 2 and 6 h; then the medium was changed, and cells were treated with IC_{50} concentration of STZ (48 µg/µl) for another 24 h. As shown in Figure 7, pretreatment could also inhibit cell death induced by STZ, and protection effect was not increased by increasing incubation time of pretreatment from 2 to 6 h. In addition, there was no significant difference between pretreatment and coadministration.

Effects of infusion extract on mitochondrial membrane potential

MMP was determined using a cell permeable cationic fluorescent dye. Depolarization of mitochondria membrane potential induced by the damage of the outer membrane resulted in the loss of the dye from the mitochondria and a decrease in intracellular fluorescence compared with the untreated control group. The results indicated that INF was able to significantly increased intracellular fluorescence ($P < 0.005$) of β-TC_3 cells [Figure 8].

DNA fragmentation

Apoptosis is a crucial cellular mechanism that is involved in inflammation, cell differentiation, and cell proliferation. Caspase activation has two main pathways: the extrinsic and the intrinsic. Both pathways trigger a cascade of downstream caspases that induce DNA fragmentation. Apoptosis assays can be classified into six major groups: (1) cytomorphological alterations, (2) DNA fragmentation, (3) detection of caspases, cleaved substrates, regulators, and inhibitors, (4) membrane alterations, (5) detection of apoptosis in whole mounts, and (6) mitochondrial assays.[27,28]

DNA fragmentation was very important because it occurs in the later phase of apoptosis, DNA fragmentation pattern of β-TC_3 treated with STZ alone, and STZ with INF extract monitored by agarose gel electrophoresis is shown in Figure 9. Breaking/streaking DNA induced

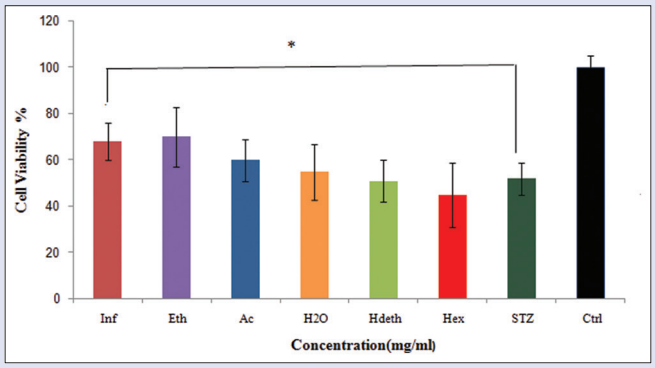

Figure 5: The effect of infusion extracts on streptozotocin-induced cytotoxicity after 24 h in β-TC_3 cells. The cell viability was determined by MTT assay. Data are expressed as the mean ± standard error of the mean of three separate experiments. Significance was calculated by analysis of variance (*$P \leq 0.05$). MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

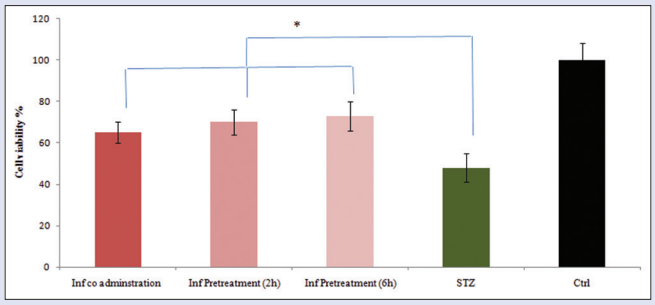

Figure 7: The effect of coadministration and pretreated (for 2 and 6 h) infusion extracts on streptozotocin-induced cytotoxicity in β-TC_3 cells after 24 h. The cell viability was determined by MTT assay. Data are expressed as the mean ± standard error of the mean of three separate experiments. Significance was calculated by the analysis of variance (*$P \leq 0.05$). MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

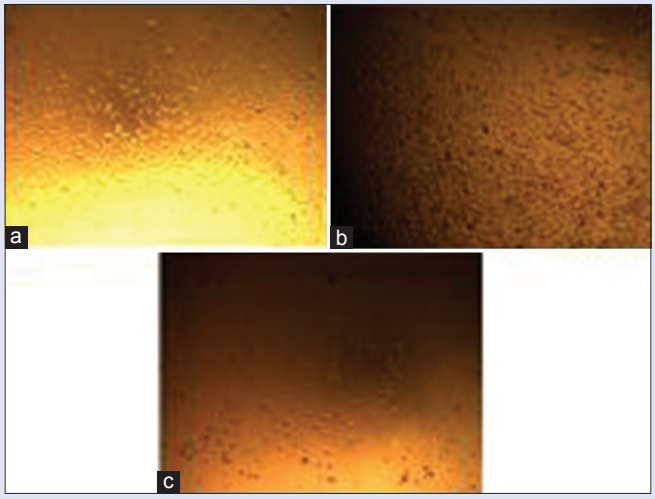

Figure 6: Representative photomicrograph shows morphological changes of the β-TC_3 cells. Cells were coadministration treated with infusion of *Prosopis farcta* extract and streptozotocin (48 μg/ml) (a) and cells were treated with infusion of *Prosopis farcta* extract (b) and control (c) for 24 h imaged by inverted phase-contrast microscope

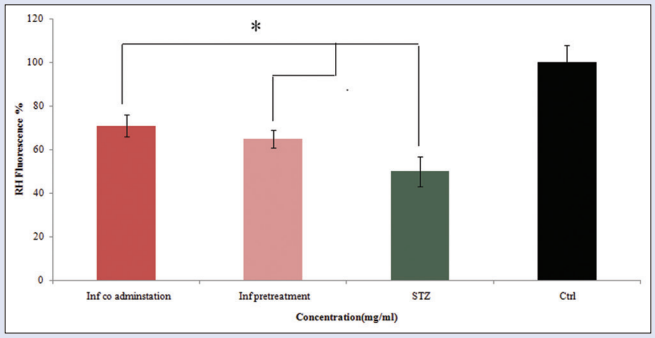

Figure 8: The effect infusion on streptozotocin (48 μg/μl)-induced mitochondrial membrane potential collapse after 24 has detected by rhodamine 123 using plate reader. Data are expressed as the mean ± standard error of the mean of three separate experiments. Significance was calculated by analysis of variance (*$P \leq 0.05$)

by STZ was not observed in cell treated by INF extract. As a result, INF can prevent breaking/streaking DNA induced by STZ.

Effect of infusion extract on β-cell proliferation

INF at a concentration of 0.5 mg/ml did not significantly ($P < 0.05$) increase the proliferation of cells in the multiple dose [Figure 10a] and single dose [Figure 10b] compared to control.

Effect of infusion extract on glucose consumption in hepatocellular carcinoma cell line cells

Subsequent to 24 h incubation of HepG2 cells with 0.5 mg/ml of INF extract at 11.1 mmol/l glucose concentration, glucose consumption of hepatic cells was assessed [Figure 11a]. The glucose consumption had been adjusted by MTT OD [Figure 11b]. In moderate hyperglycemia (11.1 mmol/l), a significant glucose-lowering effect of INF was observed. The glucose-lowering effect of INF was not due to an increment of cell number because the viability of treated cells was not significantly decreased with the increase of concentration of INF. In addition, the glucose-lowering was normalized by viable cells.

DISCUSSION

Traditional medicines suggest a wide range of remedies for the management of symptomatologies associated with chronic disorders including diabetes mellitus. Natural products such as plant extracts and phytochemicals are increasingly attracting attention to be considered as new drug leads to treat and prevent diabetes mellitus. Plants with antidiabetic properties might provide a viable approach, either as a drug or as a supplement in the treatment of diabetic complications.[29]

Natural agents from plant show their protective and therapeutic effect on diabetes mellitus through several cellular mechanisms, including regeneration of pancreatic b cell, limitation of glycogen degradation and gluconeogenesis, anti-inflammatory, immuneregulatory, anti-apoptosis, antioxidative stress, as well as modulation of intracellular signaling transduction pathways.[30]

The tumor cell line β-TC_3 has been established from insulinomas derived from transgenic mice by expression of the SV40 T antigen carrying a hybrid insulin promoter.[31,32]

The β-TC_3 cells express high steady-state levels of proinsulin messenger RNA and produce both proinsulin I and II.[33] STZ is a broad-spectrum antibiotic that causes direct irreversible damage to β-cells of pancreatic islets of Langerhans and induces type 1 diabetes in mice, which is similar to diabetes mellitus with nonketosis hyperglycemia.[34]

According to the literature, STZ enters the cell through a glucose transporter 2 (GLUT2) selectively expressed in pancreatic cells.[35]

Some hypoglycemic plants have the ability to inhibit glucose diffusion. In the glucose diffusion study, *P. farcta* extracts did not demonstrate significant inhibitory effects on glucose movement into the external solution across dialysis membrane compared with control conditions. These results suggest that antidiabetic actions of *P. farcta* do not involve intestinal absorption. Recently, growing evidence suggests the involvement of cation channels, transporters in the regulation of intestinal glucose absorption. The classical pathway of glucose absorption is across the intestinal brush-border membrane, which was predominantly mediated by SGLT1, a membrane protein that couples two molecules of Na + together with one molecule of glucose. The passive move out of the basolateral surface of enterocytes contains a facilitated-diffusion GLUT2, which allows glucose to move from the IEC into the extracellular medium near the blood capillaries.[36] Effect of *Prosopis farcta* extract on the ion channel and transporter is not determined yet. However, this is inconclusive considering the limitations of our experimental model where in the time for glucose to fully diffuse through the dialysis tube is not directly comparable to the timing of cellular mechanisms of glucose absorption within the gut.[20] This is supported partly by a previous study reporting an inhibitory effect of the plant on glucose diffusion when administered at higher concentrations. Viscous polysaccharides reduce postprandial hyperglycemia in normal and diabetic humans. Previous reports suggested a direct link between viscosity of the plant soluble polysaccharides, the polysaccharides concentration, molecular mass of soluble fibers, and the plant ability to inhibit glucose absorption.[20,37] Therefore, we suggest that *P. farcta* may have an inhibitory effect on glucose movement when administered at higher concentrations.

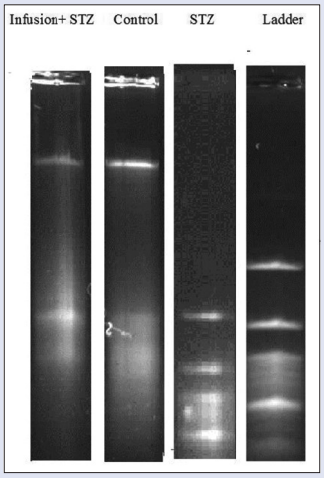

Figure 9: The effect of infusion on fragmentation of DNA induced by streptozotocin (48 μg/μl). DNA fragmentation was viewed on gel agarose (1.2%) and photographed by ultraviolet illumination

We also evaluated the protective effect of *P. farcta* against STZ-induced apoptosis, a major contributor to the development of diabetes mellitus. First, we carried out cell viability studies using the MTT assay to identify the cytotoxic doses of the plant extract. Gulalp *et al.* reported that *P. farcta* does not induce toxicity in humans.[38] Besides, Asadollahi *et al.* showed that *P. farcta* extract possesses antioxidant properties and alleviates hepatotoxicity. These protective features may possibly be the results of polyphenols.[39] In our cell line, we observed that *P. farcta*extract does not affect the viability of β-TC_3 and HepG2 cells up to 0.5 mg/ml.

When investigating STZ effect, our results showed that the STZ decreases β-TC_3 cell viability. Furthermore, DNA fragmentation and MMP

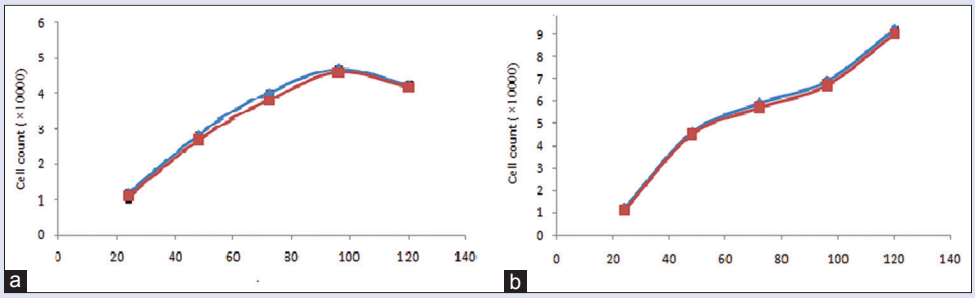

Figure 10: The effect of infusion extract (single dose (a) and multiple dose,(b)) on β-cell proliferation for 24, 48, 72, and 96 h

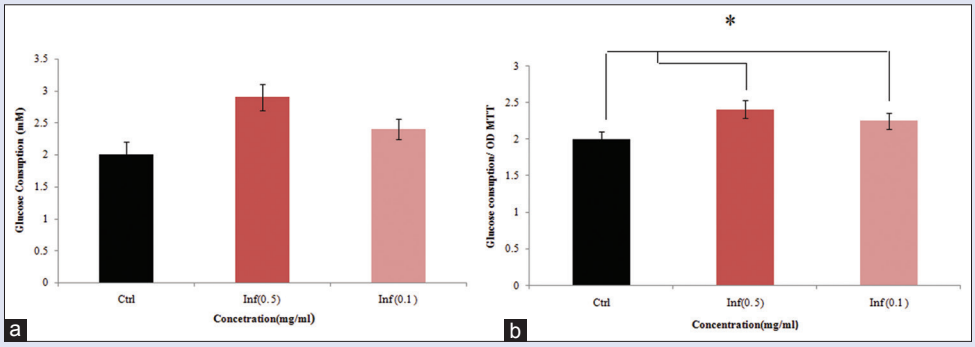

Figure 11: (a) The effect of infusion extract on glucose consumption in hepatocellular carcinoma cell line cells (b) and effect of infusion extract on glucose consumption in hepatocellular carcinoma cells adjusted by optical density MTT. Significance was calculated by analysis of variance (*$P \leq 0.05$). MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

results confirmed that STZ-mediated cytotoxicity is mainly induced by apoptosis. Mitochondria play an important role in the apoptotic cascade. Interestingly, INF extracts application reduced toxicity induced by STZ and increased MMP in β-TC_3 cells.

According to our results, INF was the only extract that could protect cells against STZ and was hence selected for further studies. This is the form which is used traditionally for this plant.[40] Reported compounds in the roots were caffeic acid, luteolin, and 5-deoxyluteolin tannins.[41] The antidiabetic activity of *Prosopis farcta* was most likely promoted by the antioxidant activity of its constituents.[42,43] Antioxidants have shown promise as a possible therapy for the prevention and treatment of diabetes through free radical scavenging activity and the inhibiting lipid peroxidation. Free radical scavenging activity mimics preconditions of pathological situations such as cancer, ischemia, inflammation, age-related macular degeneration, and diabetes.[44,45] Our pretreatment data with *P. farcta* suggest that this extract can be used as a potential protective compound to reduce the risk of diabetes. Antioxidant treatment may indeed delay or prevent diabetes.

Recent studies provided evidence that β-cells possess the potential to regenerate through proliferation of preexisting β-cells under both physiological and pathological conditions. As such, a strategy that induces β-cell proliferation, thus preserving functional β-cell mass, could potentially prevent the onset of diabetes-relatedβ-cell dysfunction and death attributed to the inability to produce sufficient amounts of insulin.[45] Lack of insulin production results from a shortage of ATP produced within the mitochondria.[46] In our experiment, exposing β-TC_3 cell line to P. farcta extract did not increase their proliferation potential. According to the previous studies, alkaloid and flavonoid that stimulate β-cell proliferation are not present in the P. farcta root. This may explain the lack of the extract effect on β-TC_3 cell proliferation.[47]

The liver plays a central role in glucose homeostasis and blood glucose control. Glucose homeostasis is the result of a balance between gluconeogenesis and glycolysis. Glycolysis is the pathway of breakdown of glucose into pyruvate/lactate, making it a catabolic pathway in muscles and other various tissues. Gluconeogenesis is essentially the reverse of glycolysis that results in carbohydrate synthesis from pyruvate/lactate in liver and kidney cells. Lowering the level of plasma glucose contributes to enhancement of glycolysis and decrease in glycogen.[48]

Another mechanism of hypoglycemic herbs is glucose uptake enhancement by muscle tissues and glucose absorption inhibition from the liver. INF showed antidiabetic action through increased glucose consumption. This may be attributed to caffeic acid which reduces hepatic glucose output and enhancement of adipocyte glucose uptake. In a similar study, a Fabaceaous plant-enhanced glucose transport and glucose metabolism.[49] In type 2 diabetes, the control of hepatic glucose metabolism and hepatic glucose output are disturbed. In addition, the inability of the liver to respond to insulin results in severe defects in glucose homeostasis such as increased hepatic glucose output and hyperglycemia. Clearly, an increase in triglycerides plays a causal role in the development of hepatic or systemic insulin resistance.[50] *P. farcta* has also an antihyperlipidemic effect that may promote its antidiabetic activity.[51] It is widely accepted that elevated plasma triglyceride concentrations may impair insulin action through an over activity of the Randle cycle. Thus, one would expect a lowering in plasma triglyceride concentration can be associated with an improvement of insulin-mediated glucose uptake.[52]

Hajinezhad *et al.* showed that hydroalcoholic extract of *P. farcta* pods could prevent diabetes-associated histopathological changes of the liver.[53] On the other hand, severe and fatal liver injury during clinical use of antidiabetic drugs, such as troglitazone, metformin, and glibenclamide, has been reported.[54,55] These results propose INF as a suitable alternative to treat or prevent diabetes.

CONCLUSION

This study demonstrated that different extracts of *P. farcta* did not show cytotoxicity in β-TC_3 and HepG2 cell lines. Only INF attenuates the death and apoptosis induced by STZ in β-TC_3 cells. It could exert a significant glucose-lowering effect.

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Conflicts of interest

There are no conflicts of interest.

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