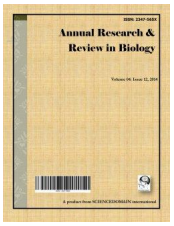

Annual Research & Review in Biology

26(6): 1-14, 2018; Article no.ARRB.41988
ISSN: 2347-565X, NLM ID: 101632869

Comparative Study between Powder and Nanoparticles of Dried Cactus (*Opuntia ficus-indica* L.) Fruit Peels in Streptozotocin-induced Diabetic Rats: Anti-microbial and Anti-genotoxic Capacity

Eman M. Hegazy[1], Naglaa A. Hafiz[2], Nehad N. Rozik[3] and Wagdy K. B. Khalil[2*]

[1]Department of Food Toxicology and Contaminants, National Research Centre, 33-Bohouth st., P.O.12622 Dokki, Giza, Egypt.
[2]Department of Cell Biology, National Research Centre, 33-Bohouth st., P.O.12622 Dokki, Giza, Egypt.
[3]Department of Polymers and Pigments, National Research Centre, 33-Bohouth st., P.O.12622 Dokki, Giza, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Authors EMH and WKBK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NAH and NNR managed the analyses of the study. Author WKBK managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2018/41988
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Mohamed M. Abdel-Daim, Suez Canal University, Egypt.
(2) Daniela Hanganu, Iuliu Haţieganu University of Medicine and Pharmacy, Romania.
(3) Martin Potgieter, University of Limpopo, South Africa.
Complete Peer review History: http://www.sciencedomain.org/review-history/24818

Original Research Article

Received 16th March 2018
Accepted 22nd May 2018
Published 26th May 2018

ABSTRACT

Aim: To overcome the toxic effects attributed to the use medicinal treatments against diabetes there is a desire toward using natural food and folk remedies. So, the aim this study was to use nanoparticles of dried cactus fruit peels (*Opuntia ficus-indica*) compared with powder materials to control blood glucose in streptozotocin (STZ)-induced diabetic rats.
Place and Duration of Study: Food Toxicology and Contaminants Department, Polymer and Pigments Department, and Cell Biology Department, National Research Centre, Egypt, between

**Corresponding author: E-mail: wkbassaly@gmail.com;*

April 2017 and March 2018.
Methodology: Powder and nanoparticles were used to determine the fatty acids content and assessment the dietary fiber contents, mycotoxin contamination as well as examine the antimicrobial activity. Moreover, male albino rats were treated with single i.p. dose of STZ to induce diabetes. STZ induced-rats were divided into several groups and treated daily with 50, 100 and 200 mg/kg b.wt of cactus fruit peels powder or nanoparticles orally for 2 months. At the end of the experimental period, blood samples were aspirated to determine glucose levels as well as liver and pancreas tissues were collected for the biological analyses.
Results: The results of the present study exhibited that both extracts of cactus fruit peels either powder or nanoparticles were able to reduce significantly the glucose levels and increase the expression of insulin and insulin receptor genes in induced-diabetic rats. Moreover, cactus fruit peels extracts exhibited antifungal and antibacterial activities and increase in the antioxidant enzymes (GPx and CAT) as well as anti-genotoxic effects in DM-induced rats. Furthermore, nanoparticles of dried cactus fruit peels were more effective in control glucose levels, gene expression, antimicrobial and anti-genotoxic activities compared with powder materials even in its low dose.
Conclusion: The results conclude that the nanoparticles form of cactus fruit peels extracts was much more effective in the therapeutic action than powder form. The anti-diabetic effect of cactus fruit peels nanoparticles could be attributed to its content from dietary fiber. Moreover, the antifungal and antibacterial activities as well as the anti-genotoxic ability of cactus fruit peels nanoparticles could be attributed to fatty acids and/or GABA contents which were more able to control oxidative stress.

Keywords: Streptozotocin; cactus fruit peels; nanoparticles; mycotoxins; antimicrobial and anti-genotoxic capacity; Gene expression.

1. INTRODUCTION

One of the most critically diseases threat human health beside cancer, heart diseases and Alzheimer is the Diabetes mellitus (DM). It has been recorded that approximately two hundred million people are suffering from DM worldwide [1]. Additionally, the WHO reports estimates that the DM disease will increase in number to reach three hundred and sixty million people by 2030 [2,3].

There are several ways to treat diabetes such as exercise and diet therapies, coincided with clinical and pharmacotherapy as well as supplementation with hyperglycemia inhibitors, insulin and other medical therapies. On the other hand, lethal side effects could be occurring with using such treatments such as lactic acidosis and hypoglycemia [4]. To overcome the toxic effects attributed to the use medicinal treatments to maintain glucose levels in blood at normal profile levels, there is a desire to use natural food and folk remedies [1]. Additionally, many scientists are conducting experiments to discover substances extracted from food ingredients and natural resources which could reduce glucose levels in the blood [5-7].

One of the imperative studies regarding treatment of diabetes with natural products is that on *Opuntia* species [1]. The stem of *Opuntia ficusindica* has been used in Mexican folk remedies for treatment of Diabetes Mellitus. Although the normal people can't digest the dietary fiber and other hard parts of *Opuntia* species the Mexican population are eating the stem of *Opuntia ficusindica* for diabetes treatment. In this regards, Trejo-González et al. [8] have been used extracts of *Opuntia* species to reduce blood glucose levels in diabetic-induced mice using streptozotocin (STZ). They are founding that diabetic-induced mice treated with *Opuntia* species extracts reduced the glucose levels in the blood. They are reporting that although the action mechanism of *Opuntia* species extract reducing blood glucose levels is not fully understood, they suggested that this action could be attributed to the dietary fiber in the extract of *Opuntia* species.

So, it important to understand the role of dietary fiber in reducing the blood sugar levels. It has been reported that dietary fibers are categorized in 2 classes as water soluble and non-water soluble dietary fiber [1]. The water soluble fiber is consists of hemicelluloses, gum, mucus and pectin components, however, non-water soluble

fiber is consists of hemicelluloses, lignin and cellulose. Several reports suggested that the glucose decreased in the blood by water soluble dietary fiber, because the intestine extends passage of food including the dietary fiber compared with other food without fiber contents [1,9,10].

Newly, nanotparticles have been used in therapeutic strategies in pharmacotherapy and medical application as a basic science technology [11,12]. Nanoparticles have several advantages in pharmaceutical and clinical applications such as having very low side effects, can be used in very low concentration, having wide surface area, having efficient carrier possibility and low toxicity.

Despite of fruit drying is considered as one of the best method for preservation of fruits, production of several types of mycotoxins in dried fruits due to sugar content and harvesting tools of fruits are still a significant problem [13]. Thus, assessment of mycotoxins in dried cactus fruit peels in the present study was carried out before using its materials in the biological study. Therefore, the aim of the current study was to use nanoparticles of cactus fruit peels (*Opuntia ficus-indica*) dried by means of solar energy compared with powder materials to control blood glucose in STZ-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Drugs and Chemicals

Streptozotocin (STZ) was purchased from Sigma–Aldrich (USA). Trizol was bought from Invitrogen (Carlsbad, CA, USA). The reverse transcription and PCR kits were obtained from Fermentas (Glen Burnie, MD, USA). SYBR Green Mix was purchased from Stratagene (La Jolla, CA, USA). All other chemicals and reagents were of analytical grade and obtained from standard commercial suppliers.

2.2 Animals

Male Wistar rats (120–150 g) were obtained from the Animal House Colony, National Research Center, Giza, Egypt. They were housed in stainless steel wire meshed cages under environmentally controlled conditions. The ambient temperature was 25 ± 2ºC and the light/dark cycle was 12/12 h. The animals were provided free access to water and a standard rodent chow diet. All animals received human care in compliance with guidelines of the Ethical Committee of National Research Centre, Egypt, which follows the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.3 Induction of Experimental Diabetes

Diabetes was induced in 12 h fasted rats with single Intraperitoneal (i.p.) injection of STZ (50 mg/kg, [14] dissolved in citrate buffer (0.01 M, pH 4.5). Control group was injected with only citrate buffer. Animals were considered diabetic when their blood glucose level exceeded 250 mg/dl [15] and were included in the study after 72 h of STZ injection.

2.4 Plant Material

The dried Cactus fruit peels (*Opuntia ficus-indica*) were collected from private farm in Giza Governorate and dried immediately by means of solar energy (50 and 60ºC). The Cactus fruit peels were authenticated by Department of Botany, Agriculture and Biology Research Division, National Research Center, Giza, Egypt.

2.5 Nanoparaticles Preparation of Cactus Fruit Peels

2.5.1 Materials preparation

Cactus fruit peels were dried by means of solar energy at a temperature varying between 50 and 60ºC and crushed by a mechanical crusher into fine powder particles.

2.5.2 Synthesis of nanoparticles

Cactus fruit peels solution was prepared by dissolving its powder in ethanol. The solution was added to boiling water in drop-wise manner under ultrasonication condition with an ultrasonic power and frequency of 50 kHz. The solution was sonicated for about 30 min. After sonication, the mixture was stirred at 800 rpm for about 20 min. Afterwards, the supernatant was discarded and the Cactus fruit peels powder obtained was used for biological study after examining its size and shape by transmission electron microscopy. JEOL-JEM-1011 version of transmission electron microscopy (TEM) was used for observing the shape, size and particle size distribution of the prepared nanoparticles (Fig. 1) [16].

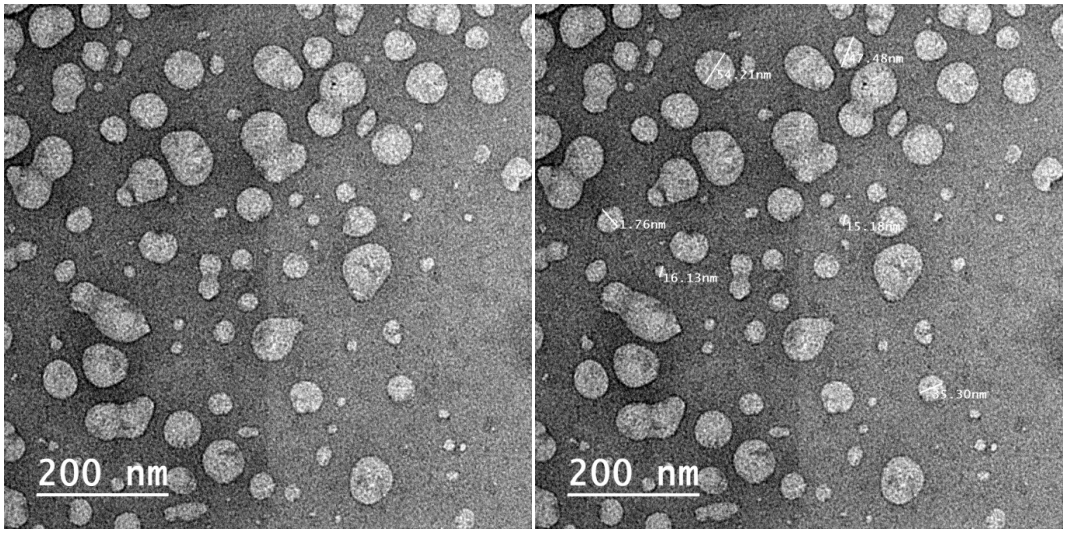

Fig. 1. Transmission electron microscope (JEOL JEM-1230) image of the prepared Cactus fruit peels nanoparticles

2.6 Fatty Acid Analysis of Cactus Fruit Peels Materials

Fatty acids of the cactus fruit peels extract in the powder and nanoparticles forms were transmethylated to form fatty acid methyl esters (FAME) using 0.5 N NaOH in methanol and 14% boron trifluoride in methanol according to De Wit et al. [17]. FAMEs from fat were quantified using a Varian 430 flame ionization GC, with a fused silica capillary column, Chrompack CPSIL 88 (100 m length, 0.25 mm ID, 0.2 mm film thicknesses). FAME in hexane (1 ml) were injected into the column using a Varian 8400 auto sampler at a split ratio of 100:1. Galaxy Chromatography Software recorded the chromatograms. Fatty acid methyl ester samples were identified by comparing the retention times of FAME peaks from samples with those of standards obtained from Supelco (Supelco 37 Component Fame Mix 47885-U, Sigma–Aldrich, USA).

2.7 Insoluble Dietary Fiber Content (IDF) and Soluble Dietary Fiber Content (SDF)

Insoluble dietary fiber was quantified according to the method of Prosky et al. [18,19]. Soluble dietary fiber was quantified according to the method of Hwang et al. [1].

After the completion of the above process, content of dietary fiber was calculated as follows:
Blank (B) = $R - P - A$,

Total dietary fiber (%) = $(R - P - A - B)/ M\times$ 100

Where R is weight of sediments after enzyme treatment, P is, protein amount, A is ash amount, and $M$is weight of sample.

2.8 Mycotoxicns Determination

All samples of powder and nanoparticles of dried cactus fruit peels were analyzed for 3 mycotoxins including ochratoxin A, aflatoxins (B1, B2, G1 and G2) and zearalenone based on the methods reported previously by Wang et al. [20]. With minor modification. Briefly, an aliquot of 5.0 g dried fruits was mixed with 5 mL of 50 mM citric acid in a 50 mL polypropylene centrifuge tube. Then, 20 mL acetonitrile was added and the mixture was homogenized with a high speed blender (Ultra-Turrax T25, IKA, USA) for 3 min. After the addition of 2 g NaCl, the mixture was shaken vigorously for 1 min and centrifuged at 10,000 rpm for 5 min at 10ºC. Afterwards, the supernatant was analyzed for the 3 mycotoxins by UPLC-MS/MS.

2.9 Antibacterial Bioassay

The antibacterial activity test of the cactus fruit peels extract in the powder and nanoparticles forms was carried out using the agar-disc diffusion method [21]. The labeled plates were seeded with the test organisms. Sterile filter paper discs of 6mm in diameter were impregnated with various concentrations of the extract and placed on the seeded plates. These

were incubated at 37ºC for 24 h. After 24 h of incubation, each plate was examined and the diameters of zones of inhibition were measured in millimeters [mm]. Using the Kirby Bauer classification method of inhibitory zone ≤12mm below was regarded as moderately sensitive/ resistant while from ≥15mm and above was considered highly sensitive.

2.10 Antifungal Bioautography Assay

The procedures of antifungal activity of the cactus fruit peels extract in the powder and nanoparticles forms were carried out according to Wedge et al. [22]. The cactus fruit peels extract of endophytic fungi and plants were applied at 160 µg/spot in chloroform onto a silica plate. Technical fungicide grade standards benomyl, cyprodinil, azoxystrobin, and captan (Chem Service Inc., West Chester, PA, USA) were used as positive controls at 2 mM in 2 µL of 95% ethanol and the sterile potato dextrose agar (PDA, Sigma–Aldrich, St. Louis, Missouri) extract was used as the negative control.

2.11 Biological Study

2.11.1 Experimental design

Animals (n=80) were divided into following 8 groups. Each group consists of 10 rats. Group 1: animals served as control and treated with oral saline (C). Group 2: rats were injected by single i.p. dose of STZ (50 mg/kg dissolved in citrate buffer to induce diabetes. After 1 week of STZ treatment, animals with fasting blood glucose of 250 mg/dL were selected for the further groups, by measuring blood glucose of blood from the tail vein with a blood glucose monitoring device (Accu-Chek, Germany) [1]. Groups 3-5: STZ induced-rats treated daily with 50, 100 and 200 mg/kg b.wt of cactus fruit peels powder dissolved distilled water orally for 2 months, respectively [1]. Groups 6-8: STZ induced-rats treated daily with 50, 100 and 200 mg/kg b.wt of cactus fruit peels nanoparticles (C. peels NPs) dissolved distilled water orally for 2 months, respectively. At the end of the experimental period, blood samples were aspirated to determine glucose levels, animals were anesthetized with ether, and were then rapidly sacrificed. Liver and pancreas tissues were collected for the biological analyses.

2.12 Expression of Diabetes Related Genes

I. Isolation of total RNA

Total RNA was extracted from the pancreas tissues from treated animals by the standard TRIzol® Reagent extraction method (Invitrogen, Germany). The RNA pellet was dissolved in diethylpyrocarbonate (DEPC)-treated water by passing solution a few times through a pipette tip. Total RNA was treated with 1 U of RQ1 RNAse-free DNAse (Invitrogen, Germany) to digest DNA residues, re-suspended in DEPC-treated water. Purity of total RNA was assessed by the 260/280 nm ratio (between 1.8 and 2.1). Additionally, integrity was assured with ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis. Aliquots were used immediately for reverse transcription [23].

II. Reverse transcription (RT) reaction

The complete Poly(A)$^+$ RNA isolated from male rats samples was reverse transcribed into cDNA in a total volume of 20 µl using RevertAidTM First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). The RT reaction was carried out at 25 °C for 10 min, followed by 1 h at 42 °C, and the reaction was stopped by heating for 5 min at 99 °C. Afterwards the reaction tubes containing RT preparations were flash-cooled in an ice chamber until being used for DNA amplification through quantitative real time-polymerase chain reaction [24].

III. Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

PCR reactions were set up in 25 µL reaction mixtures containing 12.5 µL 1× SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd.,

Table 1. Primer used for qRT-PCR amplification

Gene	Primer sequences (5'-----'3)	
Insulin 1	F-CCT GTT GGT GCA CTT CCT AC	R-TGC AGT AGT TCT CCA GCT GC
Insulin receptor	F-TTC ATT CAG GAA GAC CTT CGA	R-AGG CCA GAG ATG ACA AGT GAC
β-actin	F-GGT ATG GAA TCC TGT GGC ATC CAT GAA A	R-GTG TAA AAC GCA GCT CAG TAA CAG TCC G

Germany), 0.5 µL 0.2 µM sense primers, 0.5 µL 0.2 µM antisense primer, 6.5 µL distilled water, and 5 µL of cDNA template. At the end of each qRT-PCR a melting curve analysis was performed at 95.0°C to check the quality of the used primers**.** Each experiment included a distilled water control. The quantitative values of RT-PCR (qRT-PCR) of insulin1 and insulin receptor were normalized on the bases of ß-actin expression (Table 1). The relative quantification of the target to the reference was determined by using the $2^{-\Delta\Delta CT}$ method.

2.13 The Comet Assay

Comet assay was performed referring to the protocol developed by Blasiak et al. [25], with minor modifications. Rats liver cells of each treatment were mixed with low-melting-point agarose (ratio of1:10v/v), then pipetted to precoated slides with normal-melting-point agarose. The slides were kept flat at 4°C for 30 min in dark environment. The third layer of low melting point agarose was then pipetted on slides, left to solidify at for 30 min 4°C. The slides were transferred to pre-chilled lysis solution, kept for 60min at 4°C. After that, slides were immersed in freshly prepared alkaline unwinding solution at room temperature in the dark for 60 min. Slides were subjected to an electrophoresis run at 0.8 V/cm, 300 mAmps at 4°C for 30 min. The slides were rinsed in neutralizing solution followed by immersion in 70% ethanol and then air-dried. Ethidium bromide was used for slides stain then and visualized by using Zeiss epifluorescence microscope (510–560 nm, barrier filter 590 nm) with a magnification of ×400. 100 cells per animal were scored then analyzed with DNA damage analysis software (Comet Score, TriTek corp., Sumerduck, VA22742).

2.14 Analysis of Antioxidant Enzymes

Liver tissues were homogenized with corresponding buffer according to the protocols of commercially available kits and centrifuged at 1500 g for 20 min at 4ºC. The supernatant was used for the measurement of glutathione peroxidase (GPx) and catalase (CAT) following the commercial kit instructions [26].

2.15 Statistical Analysis

All data were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System [27] followed by Scheffé-test to assess significant differences between groups. The values are expressed as mean±SEM. All statements of significance were based on probability of $P<0.05$.

3. RESULTS

3.1 Characterization of Cactus Fruit Peels Nanoparticles

Fig. 1 shows the Transmission electron microscope (TEM) image of cactus fruit peels nanoparticles. The micrograph shows spherical ultrafine particles in the nanograde with diameter ranges from 15-71nm. They also indicate the presence of cavities distributed on a heterogeneous surface.

3.2 Glucose Level Measurements

Table 2 shows the levels of glucose in serum of diabetic rats at the day one (The day when blood sugar reach >250 mg/dl, 2-4 days after STZ treatment) and at the end of experiment (2 months later). The results revealed that both powder and nanoparticles extracts of cactus fruit peels were able to reduce significantly the glucose levels in induced-diabetic rats. However, the nanoparticles of cactus fruit peels decreased the glucose level to be near to that in control rats.

3.3 Contents of Dietary Fiber in Cactus Fruit Peels Extracts

Table 3 shows the content of contents of dietary fiber in cactus fruit peels extracts. The results showed that the powder materials of cactus fruit peels exhibited 42.7% soluble and 17.2% insoluble dietary fiber, however, nanoparticles of cactus fruit peels exhibited 57.4% soluble and 13.2% insoluble dietary fiber.

3.4 Fatty Acids Composition

The results of fatty acid composition analysis of cactus fruit peels extract (powder and nanoparticles) are summarized in Table 4. The results exhibited 9 fatty acid compounds with retention time ranging from 7.053 to 18.52 min. The maximum peak of fatty acid components was identified as Lauric acid methyl ester with percentage of 31.09% in the powder and 35.35% in nanoparticles extracts. The followed identified fatty acid components were Heptadecenoic acid with percentage of 19.25%

in the powder and 21.70% in nanoparticles extracts followed by Cis-5, 8, 11-eicosatrienoic acid with percentage of 19.25% in the powder and 19.93% in nanoparticles extracts. Additionally, Gamma-linolenic acid (GLA) was indentified with percentage of 9.86% in the powder and 11.29% in nanoparticles extracts. The minimum peak was showed in Octanoic acid with acid with percentage of 0.0% in the powder and 2.03% in nanoparticles extracts.

3.5 Mycotoxicns Determination

The results showed that no detection of the three mycotoxins including ochratoxin A, aflatoxins (B1, B2, G1 and G2) and zearalenone was found in either powder or nanoparticles of dried cactus fruit peels.

3.6 Antifungal and Antibacterial Activities

Table 5 shows the antifungal and antibacterial activities of cactus fruit peels extract in both forms as powder and nanoparticles. Several fungal (*A. flavus* and *A. ocherceu*s) and bacterial (*B. cereus, S. aureus* and *E. coli*) strains were used to determine the activity of the cactus fruit peels extracts against these strains. The results showed that the highest mean growth inhibition of cactus fruit peels extract was determined against *E. coli* with mean values 13.3±3.6 in the powder and 17.8±1.55 in nanoparticles extracts. The second mean growth inhibition of cactus fruit peels extract was determined against *S. aureus* with mean values 9.3±0.29 in the powder and 11.0±0.50 in nanoparticles extracts. The third mean growth inhibition of cactus fruit peels extract was determined against *A. flavus* with mean values 7.5±0.29 in the powder and 12.3±2.02 in nanoparticles extracts. The minimum growth inhibition of cactus fruit peels extract was determined against *B. cereus* with mean values 8.0±0.50 in the powder and 9.5±0.50 in nanoparticles extracts.

3.7 Alteration in the Expression of Diabetes Related Genes

Figs. 2 and 3 show the expression levels of diabetes related genes encoding insulin and insulin receptor in pancreas tissues of DM-induced rats treated with different doses of powder and nanoparticles (NPs) of cactus fruit peels extracts. The results of this current study exhibited that DM-induced rats decreased significantly ($P<0.01$) the mRNA expression levels of insulin and insulin receptor genes, respectively, compared with healthy control rats.

In contrary, the results showed that expression levels of insulin gene in pancreas tissues of DM-induced rats treated with all doses of powder extract of cactus fruit peels increased significantly ($P<0.05$) compared with those in pancreas tissues of DM-induced rats. Furthermore, expression levels of insulin receptor gene in pancreas tissues of DM-induced rats treated with all doses of powder extract of cactus fruit peels increased compared with those in pancreas tissues of DM-induced rats but it was significant ($P<0.05$) only with the medium and high doses of the powder extract of cactus fruit peels.

The expression levels of insulin and insulin receptor genes in pancreas tissues of DM-induced rats treated with low dose of nanoparticles extract of cactus fruit peels increased significantly ($P<0.05$) compared with those in pancreas tissues of DM-induced rats.

Moreover, expression levels of insulin and insulin receptor genes in pancreas tissues of DM-induced rats treated with medium and high doses of nanoparticles extract of cactus fruit peels increased significantly ($P<0.01$) compared with those in pancreas tissues of DM-induced rats and reached the expression levels near that in healthy control rats.

3.8 Effect of Cactus Fruit Peels Extracts on the DNA Damage Assessed by Comet Assay

Assessment of the DNA damage in liver tissues of DM-induced rats collected from different treated groups with powder and nanoparticles of cactus fruit peels extracts was summarized in Table 6.

The results exhibited that rates of DNA damage in the liver tissues of DM-induced rats increased significantly ($P<0.01$) compared with the healthy control rats. However, DNA damage in the liver tissues of DM-induced rats treated with low dose of powder extract of cactus fruit peels decreased significantly ($P<0.05$) compared with those in pancreas tissues of DM-induced rats. Moreover, medium and high doses of powder extract of cactus fruit peels as well as low and medium

Table 2. Effect of powder and nanoparticles forms of Cactus (*Opuntia ficus-indica*) peel on serum glucose level in diabetic rats

Treatment	Glucose level (mg/dl)	
	Day 1*	Mon2
Control	86.2 ± 8.2^{b}	85.7 ± 8.2^{e}
DM	324.3 ±16.2^{a}	331.2 ± 13.7^{a}
DM+ C. peels L	325.2 ± 14.8^{a}	262.3 ± 18.2^{b}
DM+ C. peels M	322.6 ± 17.4^{a}	189.5 ± 13.6^{c}
DM+ C. peels H	323.9 ± 22.1^{a}	157.9 ± 16.7^{cd}
DM- C. peels NPsL	324.7 ± 21.8^{a}	172.4 ± 11.4^{cd}
DM+ C. peels NPsM	318.6 ± 15.2^{a}	128.4 ± 8.5^{d}
DM+ C. peels NPsH	321.3 ± 22.6^{a}	101.6± 10.2^{de}

Day 1: The day when blood sugar reach >250* mg/dl *(2-4 days after STZ treatment); DM: Diabetus mellitus; NPs: Nanoparticles; [a, b] Mean values within column with unlike superscript letters were significantly different ([a:] P<0.001, [b:] P<0.01).*

Table 3. Content of soluble and insoluble dietary fiber in powder and nanoparticles of cactus fruit peels extract

Dietary fibers	Powder extract (%)	Nanoparticles extract (%)
Soluble dietary fiber	42.68 ± 3.22	57.43 ± 4.16
Insoluble dietary fiber	17.19 ± 3.62	13.15 ± 2.13

Table 4. Fatty acid composition (%) detected in cactus fruit peels extract (powder and nanoparticles)

Retention time	Compound	Fatty acid (%) in cactus fruit peels extracts	
		Powder	Nanoparticles
7.053	C8 Octanoic acid	--	2.03
11.08	C15 Pentadecanoic acid	5.4	5.25
13.31	C17:1 Heptadecenoic acid	19.25	21.7
14.64	C18:2 Linoleic acid	--	3.1
16.21	C18:3 n-6 Gamma-linolenic acid (GLA)	9.86	11.29
16.51	C20:1 n-9 all-cis-5,8,11-eicosatrienoic acid	19.25	19.93
17.51	C13 Lauric acid methyl ester	31.09	35.35
17.9	C20:3 n-6 Dihomo-γ-linolenic acid	4.95	1.91
18.52	C20:3 n-3 Eicosatrienoic acid	6.32	3.32

doses of nanoparticles of cactus fruit peels extracts decreased with high significant differences (P<0.01) the rates of DNA damage compared with those in DM-induced rats. Also, high dose of nanoparticles of cactus fruit peels extracts decreased the rates of DNA damage with high significant differences (P<0.001) to the level near to that in healthy control rats.

Table 5. Antifungal and antibacterial activities of powder and nanoparticles of Cactus fruit peels extracts

Type	Strain	Mean growth inhibition (mm) ± SE			
		Negative control	Positive control	Cactus fruit peels extracts	
				Powder	Nanoparticles
Fungi	*A. flavus*	0	32.3±1.04	7.5±0.29^{b}	12.3±2.02^{b}
	A. ocherceus	0	29.6±0.76	8.7±0.76^{ab}	10.2±1.04^{bc}
Bacteria	*B. cereus*	0	26.2±1.04	8.0±0.50^{b}	9.5±0.50^{c}
	S. aureus	0	25.5±0.76	9.3±0.29^{ab}	11.0±0.50^{bc}
	E. coli	0	25.2±2.02	13.3±3.6^{a}	17.8±1.55^{a}

Mean inhibitory clear zones and standard errors were used to determine the level of antifungal and antibacterial activities against each fungal and bacterial species.

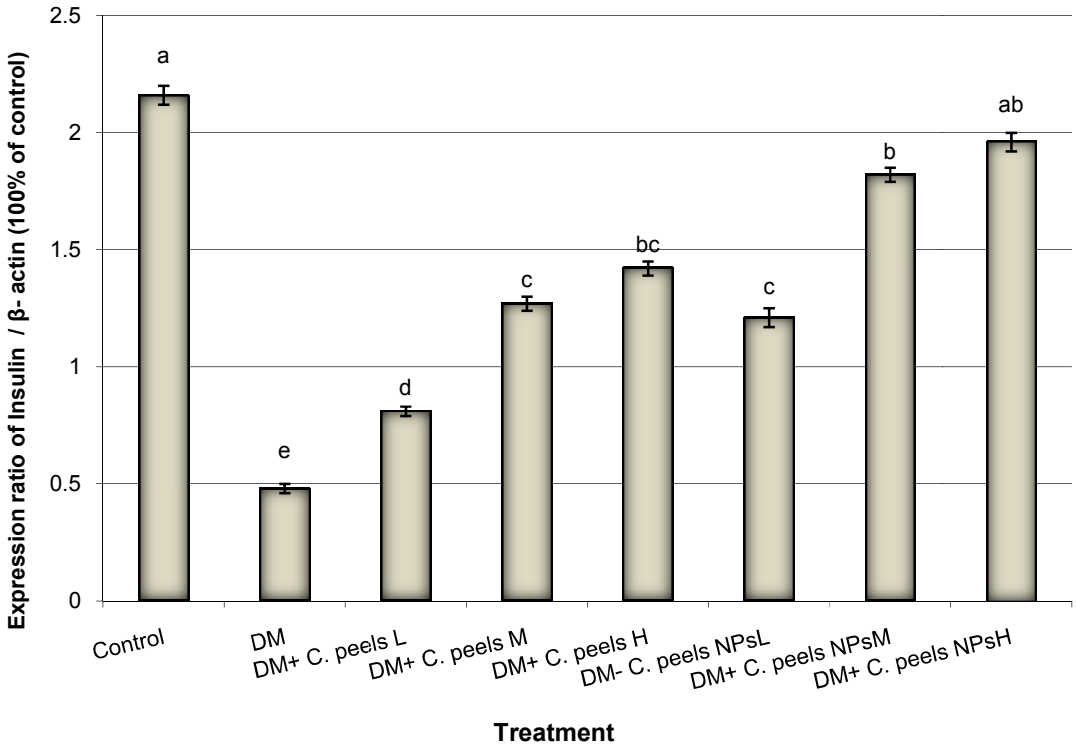

Fig. 2. The alterations of the expression of insulin gene in pancreas tissues collected from DM-induced rats treated with low (L), medium (M) and High (H) doses of powder and nanoparticles (NPs) extracts of cactus fruit peels.

Data are presented as mean ± SEM. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different ([a:] P<0.01, [b, c,d] P<0.05)

3.9 Effect of Cactus Fruit Peels Extracts on the Antioxidant Enzyme Activities

Fig. 4 shows the levels of antioxidant enzyme activities including GPx and CAT in liver tissues of DM-induced rats collected from different treated groups with powder and nanoparticles of cactus fruit peels extracts.

The results showed that levels of GPx activities in the liver tissues of DM-induced rats decreased significantly (P<0.01) compared with the healthy

Table 6. Rate of DNA damage in liver tissues of DM-induced rats treated with low (L), medium (M) and High (H) doses of powder and nanoparticles (NPs) extracts of cactus fruit peels using comet assay

Treatment	No. of cells		Class[¥] of comet				DNA damaged cells
	Analyzed	Total comets	0	1	2	3	(mean ± SEM)
Control	500	27	473	18	9	0	5.4±0.24[d]
DM	500	103	397	31	43	29	20.6±0.42[a]
DM+ *C. peels L*	500	76	424	23	28	22	15.2±0.62[b]
DM+ *C. peels M*	500	58	442	19	24	15	11.6±0.52[c]
DM+ *C. peels H*	500	52	448	21	17	14	10.4±0.36[c]
DM- *C. peels* NPs *L*	500	59	441	23	25	11	11.8±0.65[c]
DM+ *C. peels* NPs *M*	500	46	454	26	17	3	9.2±0.28[cd]
DM+ *C. peels* NPs *H*	500	33	467	24	7	2	6.6±0.42[d]

[¥]: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.(*): No of cells analyzed were 100 per an animal

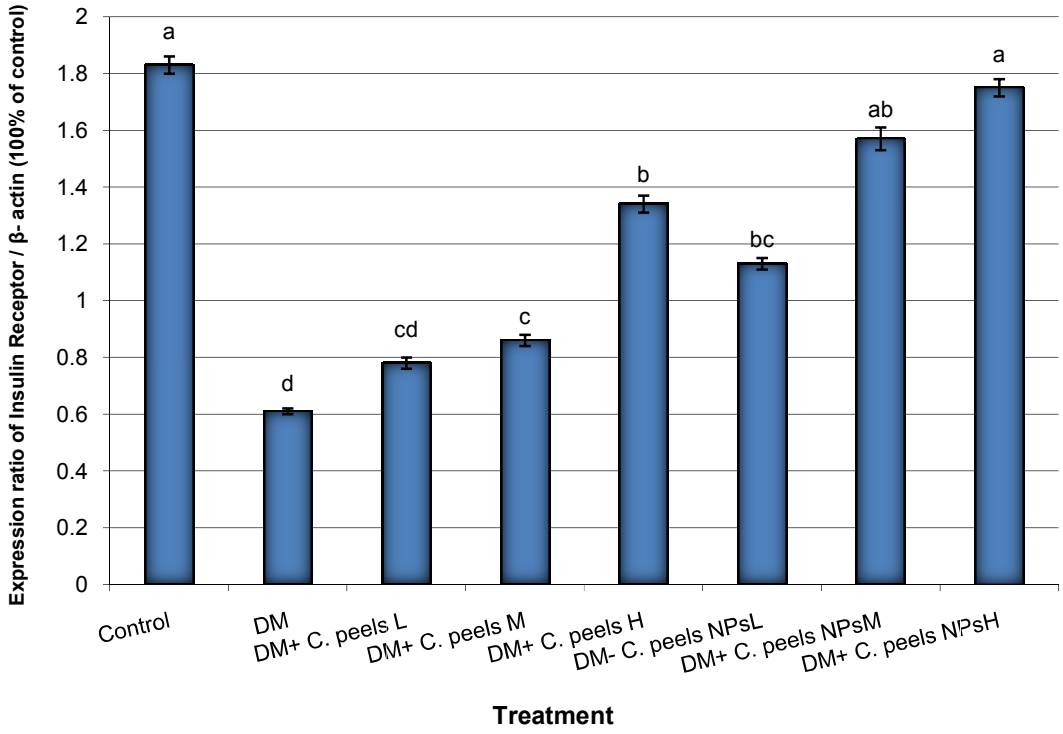

Fig. 3. The alterations of the expression of insulin receptor gene in pancreas tissues collected from DM-induced rats treated with low (L), medium (M) and High (H) doses of powder and nanoparticles (NPs) extracts of cactus fruit peels

Data are presented as mean ± SEM. [a,b,c]Mean values within tissue with unlike superscript letters were significantly different ([a:] P<0.01, [b, c,d] P<0.05)

control rats. However, GPx activities in the liver tissues of DM-induced rats treated with medium and high doses of powder and nanoparticles extract of cactus fruit peels increased significantly (P<0.05, P<0.01, respectively) compared with those in pancreas tissues of DM-induced rats.

In the same line, levels of CAT activities in the liver tissues of DM-induced rats decreased significantly (P<0.01) compared with the healthy control rats. However, CAT activities in the liver tissues of DM-induced rats treated with high dose of nanoparticles extract of cactus fruit peels

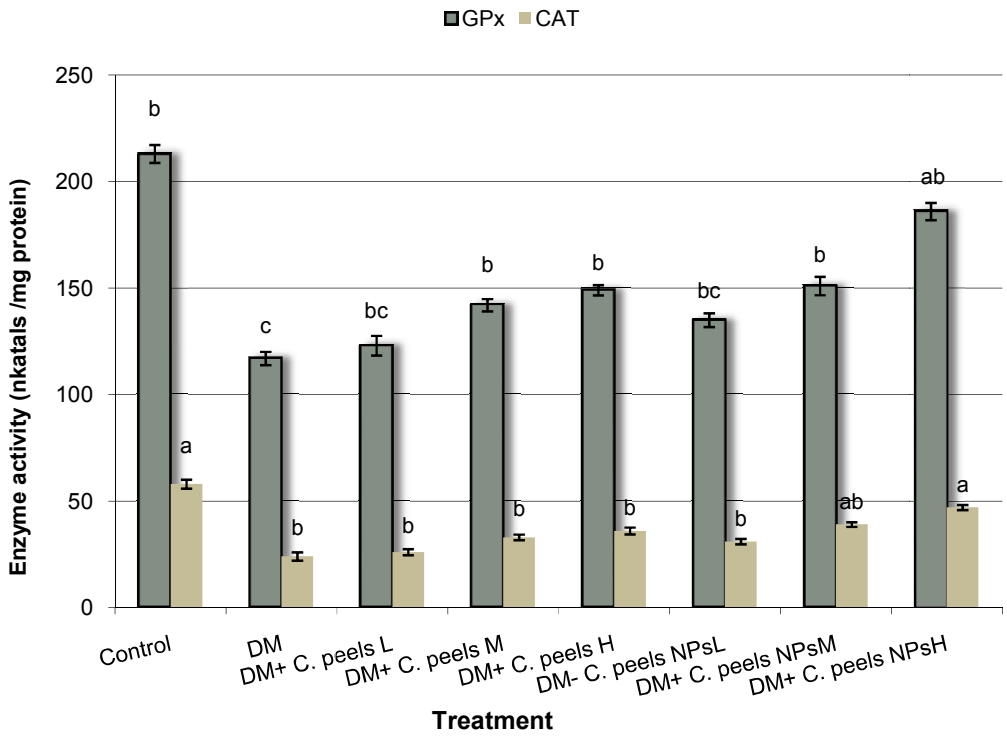

Fig. 4. Glutathione peroxidase (GPx) and catalase (CAT) activities (nkatals/mg protein) in the liver tissues of DM-induced rats treated with low (L), medium (M) and High (H) doses of powder and nanoparticles (NPs) extracts of cactus fruit peels. [a,b]: Within each column, means superscripts with different letters are significantly different (P < 0.05)

increased significantly (P<0.05) compared with those in pancreas tissues of DM-induced rats.

4. DISCUSSION

The aim of the current study was to investigate the role of dried cactus fruit peels (*Opuntia ficus-indica*) nanoparticles to control blood glucose in STZ –induced diabetic rats compared with powder materials. The results of the present study exhibited that both extracts of cactus fruit peels either powder and nanoparticles were able to reduce significantly the glucose levels in induced-diabetic rats. However, the nanoparticles of cactus fruit peels decreased the glucose level to be near to that in control rats. In agreement with our results, Trejo-González et al. [8] have been used extracts of *Opuntia* species to reduce blood glucose levels in diabetic-induced mice using streptozotocin (STZ). They are founding that diabetic-induced mice treated with *Opuntia* species extracts reduced the glucose levels in the blood. Additionally, Hwang et al. [1] reported that fresh Nopal (*Opuntia ficus-indica*) extract decreased blood glucose levels in diabetic rats. Although, they found that *Opuntia ficus-indica* extract decreased the glucose levels below 150 mg/dl during the time intervals of zero to 120 min after food deprivation, our study found that the glucose levels of diabetic rats decreased to 101.6 mg/dl after 2 month intake of *Opuntia ficus-indica* nanoparticles.

In the present study nanoparticles of cactus fruit peels was more effective in control glucose levels compared with powder materials even in low dose of its nanoparticles form. In the same line, Rani et al. [28] reported that glycyrrhizin (an active constituent of the roots and rhizomes of *Glycyrrhiza glabra*) in the nanoform was supplement to diabetic rats. Glycyrrhizin-NPs exhibited significant anti-diabetic impacts even though they contained approximately one quarter of the dosage compared to the pure form.

The anti-diabetic effects of cactus and dietary fibers have been investigated in several studies on the STZ-induced diabetic rats [29], DM patients [6,30]. Moreover, Ou et al. [31]

investigated the ability to absorb glucose *in vitro* by the dietary fiber as anti-diabetic. They reported that glucose levels were lowered by using several types of dietary fibers. In the current study we have found that the powder extract of cactus fruit peels exhibited high levels of soluble dietary fiber compared with the insoluble dietary fiber which it could be responsible for lowering the glucose levels in DM-induced rats.

The mechanism of action of dietary fiber in reducing glucose levels in the blood is that dietary fiber could be able to absorb glucose and suppress its transmission and, then prevent the α-glucosidase activity and finally delay the release of glucose from starch into blood stream [31]. Moreover, this study showed that expression levels of insulin and insulin receptor genes in pancreas tissues of DM-induced rats treated with cactus fruit peels (powder and nanoparticles) increased significantly compared with those in pancreas tissues of DM-induced rats.

In the same line with our results Indrowati et al. [32] reported that treatment of diabetic rats with *Artocarpus altilis* leaf extract associated with active compounds Gamma Amino Butyric Acid (GABA) decreased the blood glucose levels and increased the expression of insulin gene in pancreas cells. Filannino et al. [33] reported that Gamma Amino Butyric Acid (GABA) has been identified in Cactus pear (*Opuntia ficus-indica* L.). They suggested that Gamma Amino Butyric Acid could be used as antidiabetic products. Therefore, it is important to use natural materials including GABA as anti-diabetic agents.

It has been reported that GABA could be used as antioxidant and anti-inflammatory agents [33]. In addition, Coelho et al. [34] suggested that treatment of Wistar rats with vigabatrin as γ-aminobutyric acid transaminase inhibitor increase DNA damage in hippocampus and blood cells assessed by comet and micronucleus tests. Moreover, Berraaouan et al. [35] reported that fatty acids in the oil extracts of cactus pear seed (CPSO) exhibited high antioxidant activity alloxan-induced diabetes in mice preventing DNA structure from damage.

Our results are also in consistent with these findings, since the current results found that cactus fruit peels extracts decreased the rates of DNA damage assessed with comet assay compared with those in DM-induced rats. Moreover, the present results revealed that cactus fruit peels extracts exhibited 9 fatty acid compounds which could be responsible for its antioxidant capacity. So, the anti-genotoxicity effect of cactus fruit peels extracts in the current study which it increased with using nanoparticles form could be attributed with increase the GABA levels and fatty acids contents in the nanoparticles form compared with the powder form of cactus fruit peels.

The cactus fruit peels extract in the current study exhibited no mycotoxin contents and antifungal and antibacterial activities in both forms as powder and nanoparticles. The results showed that the highest mean growth inhibition of cactus fruit peels extract was determined against several bacteria species and then fungal species which consequently inhibit mycotoxin progression. Xie et al. [36] investigated the gastroprotective effect of GABA on ethanol-induced gastric mucosal and epithelial cells injury. They found that pretreatment with GABA reduced significantly the gastric ulcer index. Also, they found that GABA pretreatment was able to control oxidative stress due to increase the antioxidant activities of superoxide dismutase and catalase in gastric cells, but also decreased the pro-inflammatory factors (IL-6 and TNF-α) levels in gastric cells.

The present study found that the activities of antioxidant enzymes such as GPx and CAT in the liver tissues of DM-induced rats treated with cactus fruit peels extracts increased significantly compared with those in pancreas tissues of DM-induced rats. Therefore, the antimicrobial activity and anti-genotoxic ability of cactus fruit peels could be attributed to the activity of GABA content which increases the antioxidant activities of GPx and CAT enzymes.

5. CONCLUSION

The nanoparticles form of cactus fruit peels extracts was much more effective in the therapeutic action than powder form. The anti-diabetic effect of cactus fruit peels extracts could be attributed to its content from dietary fiber which is responsible for reducing glucose levels in the blood. Moreover, The antifungal and antibacterial activities as well as the anti-genotoxic ability of cactus fruit peels could be attributed to GABA content which is able to control oxidative stress due to increase the antioxidant activities of GPx and CAT.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Hwang SH, Il-Jun Kang IIJ, Lim SS. Antidiabetic effect of fresh nopal (*Opuntia ficus-indica*) in low-dose streptozotocin-induced diabetic rats fed a high-fat diet. Evidence-Based Complementary and Alternative Medicine. 2017;4380721:8.
2. WHO. Fact sheet no 310: The top 10 causes of death. Updated May 2014. World Health Organization, Geneva; 2015.
3. WHO. World Health Organization. Global report on diabetes. Geneva. World Health Organization; 2016.
4. Frati-Munari A, Altamirano E, Rodriguez-Barcenas N, Ariza CR, Lopez-Ledesma R. Hypoglycemic effect of Opuntia streptacantha Lemaire: Research with crude extracts. Archivos De Investigacion Medica. 1986;20:321–325.
5. Leem KH, Kim MG, Hahm YT, Kim HK. Hypoglycemic Effect of *Opuntia ficus-indica* var. Saboten is Due to Enhanced Peripheral Glucose Uptake through Activation of AMPK/p38 MAPK Pathway. Nutrients. 2016;8:800.
6. Halmi S, Benlakssira B, Bechtarzi K, Djerrou Z, Djeaalab H, Riachi F, Hamdi Pacha Y. Antihyperglycemic activity of prickly pear (*Opuntia ficus-indica*) aqueous extract. Int. J. Med. Arom. Plants. 2012; 2:540–543.
7. Frati-Munari AC, Licona-Quesada R, Araiza-Andraga CR, Lopex-Ledesma R, Chavez-Negrete A. The action of Opuntia streptacantha on healthy subjects with induced hyperglycemia. Archivos de Investigacion Medica. 1990;21(2):99–102.
8. Trejo-González A, Gabriel-Ortiz G, Puebla-Perez AM, et al. Apurified extract fromprickly pear cactus (*Opuntia fuliginosa*) controls experimentally induced diabetes in rats. Journal of Ethnopharmacology. 1996;55(1):27–33.
9. Zhu X, Sun X, Wang M, Zhang C, Cao Y, Mo G, Liang J, Zhu S. Quantitative assessment of the effects of beta-glucan consumption on serum lipid profile and glucose level in hypercholesterolemic subjects. Nutr Metab Cardiovasc Dis. 2015;25(8):714-23.
10. Kitano Y, Murazumi K, Duan J, Kurose K, Kobayashi S, Sugawara T, Hirata T. Effect of dietary porphyran from the red alga, Porphyra yezoensis, on glucose metabolism in diabetic KK-Ay mice. J Nutr Sci Vitaminol (Tokyo). 2012;58(1):14-9.
11. Karakoti AS, Hench LL, Seal S. The potential toxicity of nanomaterialsethe role of surfaces. JOM. 2006;58:77-82.
12. Bhattacharyya SS, Paul S, Khuda-Bukhsh AR. Encapsulated plant extract (*Gelsemium sempervirens*) poly (lactide-co-glycolide) nanoparticles enhance cellular uptake and increase bioactivity in vitro. Exp. Biol. Med. (Maywood). 2010; 235(6):678-688.
13. Wei D, Wang Y, Jiang D, Feng X, Li J, Wang M. Survey of Alternaria Toxins and Other Mycotoxins in Dried Fruits in China. Toxins (Basel) 2017; 9(7): 200.
14. Hounsom L, Horrobin DF, Tritschler H, Corder R, Tomlinson DR. A lipioc acid-gamma linolenic acid conjugate is effective against multiple indices of experimental diabetic neuropathy. Diabetologia. 1998; 41:839–43.
15. Cam M, Yavuz O, Guven A, Ercan F, Bukan N, Ustundag N. Protective effects of chronic melatonin treatment against renal injury in streptozotocin-induced diabetic rats. J Pineal Res. 2003;35:212–20.
16. Khalil WKB, El-Bassyouni GT, Booles HF. Nano-encapsulated Form of *Citrus medica* for Osteoporosis Treatment in Animal Model. International Journal of Pharmaceutical and Clinical Research. 2016;8(1):49-59.
17. De Wit M, Hugo A, Shongwe N. South African cactus pear seed oil: A comprehensive study on 42 spineless burbank *Opuntia ficus-indica* and *Opuntia robusta* cultivars. Eur. J. Lipid Sci. Technol. 2018;120:1700343.
18. Prosky L, Asp NG, Schweizer TF, DeVries JW, Furda I, Lee SC. Determination of soluble dietary fiber in foods and food products: collaborative study. Journal of AOAC International 1994;77(3):690–694.
19. Prosky L, Asp NG, Furda I. Determinaton of total dietary fiber in foods, food products, and total diets: Interlaboratory Study. Journal of the Association of Official Analytical Chemists. 1984;67(6):1044–1052.
20. Wang M, Jiang N, Xian H, Wei D, Shi L, Feng XY. A single-step solid phase extraction for the simultaneous

determination of 8 mycotoxins in fruits by ultra-high performance liquid chromatography tandem mass spectrometry. J. Chromatogr. A. 2016; 1429:22–29.
21. Harley JP, Prescott LM. Laboratory exercise in Microbiology [3rd edition]. WCB. McGraw-Hill, Boston. 1996;150-151.
22. Wedge DE, Klun JA, Tabanca N, Demirci B, Ozek T, Baser KHC, et al. Bioactivity-guided fractionation and GC/MS fingerprinting of *Angelica sinensis* and *Angelica archangelica* root components for antifungal and mosquito deterrent activity. J Agric Food Chem. 2009;57:464–70.
23. Salem NA, Wahba MA, Eisa WH, El-Shamarka M, Khalil W. Silver oxide nanoparticles alleviate indomethacin-induced gastric injury: A novel antiulcer agent. Inflammopharmacology; 2017. DOI: 10.1007/s10787-017-0424-2.
24. Khalil WKB, Booles HF, Hafiz NA, El-Bassyouni GE. Ameliorative effects of *Brachidontes variabilis* Calcium Carbonate against bone loss in Ovariectomized rats. International J. Pharmacology. 2018;14: 477-487.
25. Blasiak J, Arabski M, Krupa R, Wozniak K, Zadrozny M, Kasznikcki J, Zurawska M, Drzewoski J. DNA damage and repair in type 2 diabetes mellitus. Mutation. Mutation Res. 2004;554(1-2):297-304.
26. Elmegeed G, Khalil WKB, Abdel Raouf A, Abdelhalim MM. Synthesis and *in vivo* anti-mutagenic activity of novel melatonin derivatives. Eur J Med Chem. 2008;43: 763-70.
27. SAS Institute. SAS User's Guide: Statistics. 1982 Edition, SAS Institute Inc., Cary, NC; 1982.
28. Rani R, Dahiya S, Dhingra D, Dilbaghi N, Kim KH, Kumar S. Evaluation of anti-diabetic activity of glycyrrhizin-loaded nanoparticles in nicotinamide-streptozotocin-induced diabetic rats. Eur J Pharm Sci. 2017;106:220-230.
29. Bwititi P, Musabayane CT, Nhachi CFB. Effects of *Opuntia megacantha* on blood glucose and kidney function in streptozotocin diabetic rats. Journal of Ethnopharmacology. 2000;69(3):247–252.
30. Frati AC, Jimenez E, Ariza CR. Hypoglycemic effect of *Opuntia ficus indica* in non insulin-dependent diabetesmellitus patients. Phytotherapy Research. 1990;4 (5);195–197.
31. Ou S, Kwok KC, Li Y, Fu L. *In vitro* study of possible role of dietary fiber in lowering postprandial serum glucose. Journal of Agricultural and Food Chemistry. 2001;49 (2);1026–1029.
32. Indrowati M, Pratiwi R, Rumiyati, Astuti P. Levels of blood glucose and insulin expression of beta-cells in streptozotocin-induced diabetic rats treated with ethanolic extract of Artocarpus altilis Leaves and GABA. Pak J Biol Sci. 2017;20(1):28-35.
33. Filannino P, Cavoski I, Thlien N, Vincentini O, De Angelis M, Silano M, Gobbetti M, Di Cagno R. Lactic acid fermentation of Cactus Cladodes (*Opuntia ficus-indica* L.) generates flavonoid derivatives with antioxidant and anti-inflammatory properties. PLoS One. 2016; 11(3):e0152575.
34. Coelho VR, Sousa K, Pires TR, Papke D, Vieira CG, de Souza LP, Leal MB, Schunck R, Picada JN, Pereira P. Genotoxic and mutagenic effects of vigabatrin, a γ-aminobutyric acid transaminase inhibitor, in Wistar rats submitted to rotarod task. Hum Exp Toxicol. 2016;35(9):958-65.
35. Berraaouan A, Abderrahim Z, Hassane M, Abdelkhaleq L, Mohammed A, Mohamed B. Evaluation of protective effect of cactus pear seed oil (*Opuntia ficus-indica* L. MILL.) against alloxan-induced diabetes in mice. Asian Pacific Journal of Tropical Medicine 2015;8:532-537.
36. Xie M, Chen H, Nie S, Tong W, Yin J, Xie M. Gastroprotective effect of gamma-aminobutyric acid against ethanol-induced gastric mucosal injury. Chem Biol Interact. 2017;272:125-134.

© 2018 Hegazy et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24818