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Research article

Whey protein concentrate supplementation suppresses DNA damage and regenerates insulin release in streptozotocin-induced diabetic rats

Khaled G. Abdel-Wahhab[1]*, Laila K Hassan[2], Enas A El-Hussieny[3], Mahmoud Ashry[4], Mohamed A. Mustafa[5], Doaa M Khaled[6], Fathia Abd Elwahid Mannaa[1]

[1]*Medical Physiology Department, National Research Centre, Dokki, Cairo, Egypt.*
[2]*Dairy Department, National Research Centre, Cairo, Egypt.*
[3]*Zoology Department, Faculty of Science, Ain-Shams University, Cairo, Egypt.*
[4]*Zoology Department, Faculty of Science, Al-Azhar University, Assiut, Egypt.*
[5]*Basic Centre of Science, Misr University for Science and Technology, Cairo, Egypt.*
[6]*Histology Department, Faculty of medicine, Helwan University, Cairo, Egypt.*

Key words: Whey protein, DNA, diabetes, STZ, rats, lipids, antioxidant.

***Corresponding Author: Khaled G. Abdel-Wahhab,** Medical Physiology Department, National Research Centre, Dokki, Cairo, Egypt.

Abstract

Improving potential of milks' proteins on carbohydrate metabolism has been suggested. It is proposed that supplementation of diet with concentrated milk proteins could might ameliorate diabetes deteriorations and differentially protect against DNA damage; therefore, this study aimed to estimate the efficiency of whey protein concentrate (WPC 80%) in STZ-induced diabetic rats. The hyperglycemic groups were made with a single (ip) dose of STZ (55mg/kg). Five groups of rats were used; (I) normal control group, (II) WPC (200 mg/kg/day) treated group, (III) STZ-diabetic rats acted as positive control; (IV) group included diabetic rats treated orally with (0.1 mg/kg/day) Amaryl®1mg; and (V) included diabetic rats treated with WPC. After six weeks of the study, treatment of diabetic animals with WPC markedly exhibited anti-diabetic, dyslipidemia, anti-DNA damage, hepato-renal protection and anti-oxidative stress potentials. These effects were monitored from the significant reduction in glucose, cholesterol, triglycerides, LDL, DNA fragmentation, ALAT, ASAT, urea, creatinine, MDA and NO levels concomitant with a significant raise in insulin, HDL, GSH, SOD, GPx and CAT values close to the corresponding values of healthy ones. Also, WPC succeeded to modulate STZ-induced histological distortion. In conclusion, WPC exhibits multi-health benefits with promising potentials against STZ-induced diabetes; this behavior may be attributed to its antioxidant and free radical scavenging mechanisms that due to vie its thiol-rich amino acids and antioxidant, as evidenced by in vitro investigations.

Introduction

Diabetes mellitus (DM) is the most endocrine disorder that includes the degeneration and reduction of the function of β–cells; this defect is guiding research into finding effective alternate sources. Insulin is the only true specific marker identified for β-cells; its level reflects clearly the function of β-cell but not dependently the cell mass [1].

Streptozotocin (STZ) is the antibiotic that produces pancreatic β-cell destruction. In 1960, it was initially extracted from *Streptomyces achromogenes*, and described with its diabetogenic properties later by Junod *et al.* [2] who had concluded that the diabetogenic effect of STZ is due to selective destruction of pancreatic islet β-cells. Many animal species, including rats, mouse, and monkey, are of high response to the pancreatic β-cell cytotoxic potential of STZ, with the rabbit being less so [3]. Currently, STZ is most often used to induce diabetic animals' models. Moreover, STZ induces diabetes via generating of reactive oxygen species (ROS), which in its turn leads to β-cell β-cell destruction [4]. Besides to its diabetogenic property, STZ has serious biological actions, including carcinogenesis, the production of acute and chronic cellular injury, teratogenesis and mutagenesis [5].

An effective therapeutic strategy is suggestible and absorption and production, some of them adjust the action of insulin [6] to reduce or delay the development of pancreatic β-cell disturbance and dysfunction. In spite many studies, carried out on rodents, have suggested that β-cells are able to be regenerated after being seriously degenerated [7, 8]; others are currently trying to explore natural substances that are efficient in reducing the severity of diabetes itself and/or its complications. In this target, many natural compounds are able to suppress the effect of certain enzymes included in glucose metabolism. Latest studies have shown that certain compounds present in plants or other natural sources can suppress β-cell

apoptosis and improve the hypoglycemic potential of insulin [9].

Several reports showed that the use of natural products is helpful in prophylaxis or treatment of diabetes; given that they possess marked efficacy, low cost, relative safety, and importantly low incidence of side effects [10]; however synthetic anti-diabetic agents possibly induce serious side effects, as hypoglycemic coma as well as deterioration of the liver and kidneys [11].

The liberation of oxygen radicals is normally stabilized by the presence of enough endogenous antioxidant batteries [12]. Oxidative stress has been involved in the initiation mechanism of diabetes mellitus [12, 13], a disease marked by a prolonged inflammatory period that increases the time required for recovery.

Milk contains primary sources of protein; whey, with bioactive peptides, is one of them. The important physiological behaviors of whey protein on the control of food intake and glucose metabolism have been reported [14]. Also, the exact health importance of these proteins or bioactive peptides are categorized as binding, antithrombotic, immunomodulatory, mineral cytomodulatory, antioxidant, antimicrobial, blood-pressure lowering (angiotensin converting enzyme, ACE-inhibitory), opioid like and cholesterol-lowering [15]. The physiological property of bioactive peptides depends on their activity to maintain integral state during transport to the various functional systems of the body [16]. Studies suggested that the anti-diabetic properties of milk protein are primarily attributable to its content of bioactive peptides which, could arouse the secretion of gut-derived hormones and/or inhibit enzymes involved in glucose homeostasis.

Whey protein concentrate (WPC) includes both essential and nonessential amino acids and is a rich source of glutamine and the branched-chain amino acids that are require to cell growth [17]. Several researches have evidenced the potential of glutathione, which is elevated by whey protein (WP), as effective antioxidant [18, 19]. WP can reduce the intensity of oxygen radicals and lipid peroxidation through raising the antioxidant battery of glutathione, thus ameliorating epithelization and the proliferation of fibroblasts as well as modulating the secretion of both pro- and post-inflammatory cytokines. Also, it has been found to markedly reduce hydroperoxide and ROS levels in white cells, cutaneous tissues and liver by recharging the antioxidant glutathione [20]. In respect to the favorable and promising properties of WPC, the present study aimed to explore the efficiency of WPC to alleviate STZ-induced DNA-fragmentation besides to its anti-diabetogenic, protective and antioxidant effects in STZ-induced diabetic male rats.

Materials and methods

Chemicals and drugs

Streptozotocin (STZ, Sigma 85882), Sodium citrate (Sigma C0909) and Citric acid (Sigma C1909) were purchased by Egyptian International Center for Import, 22 AbuZer El-Ghafary St., Nasr City Cairo, Egypt. Amaryl® 1mg (glimepiride 1mg, obtained from Sanofi, 3 el assaneh St. Zietoun, Cairo, Egypt) dissolved in slight alkali (pH 6.8) distilled water after testing its optimum solubility pH; whey protein concentrate (80%, purchased from the Milky Whey, Inc., 910 Brooks St 203, Missoula, MT 59801, USA). It dissolved in distilled water. Other chemicals and solvents were of high gradient and analar.

Composition and solubility of WPC-80

The composition of WPC-80 was analyzed again; the analyses included the assessment of the proteins, amino acids, fatty acids, vitamins, as well as minerals content. The humidity and calorific values of WPC-80 were also evaluated. Whey proteins solubility was determined according to the method described by Morr *et al.* [21]; several aliquots of 5.85 g/L NaCl solution were prepared, then (0.5 g) of dry WPC was accurately weighed and added with stirring to form a smooth paste. Additional 5.85 g/L NaCl solution was then added to bring the total volume of the dispersion to about 40ml; the mixture was hold circulating in hot water bath and the temperature was maintained in agreement with the interest of each experiment (40-90°C). The pH values were adjusted between 5.0 - 6.8 using NaOH (4.0 g/L) and HCl (3.65 g/L) solutions. After agitation of the samples, the dispersion was completed with NaCl 5.85 g/L and the solution was cooled (4°C) and centrifuged at 13500 rpm for 30 minutes, then the supernatant was filtered (Whatman paper no 2). The soluble protein content of three aliquots was determined using the micro-Kjeldahl method. The soluble protein percentage was calculated through the following equation:

$$\text{Soluble protein content (g/100g)} = \left[\frac{A(g/L)}{W(g)x(\frac{S}{100})}\right]x100$$

Where; A is supernatant protein concentration [g/L]; W is sample weight [g]; S is sample protein concentration [g/100g]. Each experiment was accomplished in triplicate, being the soluble protein content the resulting average of the two values.

Experimental design

This study was conducted on adult male Wistar albino rats (140-160g) obtained from Animal Colony, National Research Centre, Cairo, Egypt. The animals were housed in suitable plastic cages for one week for acclimation. Excess tap water and standard rodent pellets [20.3% protein (20% casein and 0.3% DL-Methionine), 5% fat (corn oil), 5% fibers, 3.7% salt mixture and 1% vitamin mixture, obtained from Meladco Company, El-Obour City, Cairo, Egypt] were always available. All animals

received human care in compliance with the standard institutional criteria for the care and use of experimental animals according to the NRC ethical committee (FWA 00014747).

Induction of diabetes in rats

After fasting 16 hours, the animals were intraperitoneally injected with (55mg/kg) streptozotocin dissolved in ice cold sodium citrate-citric acid buffer [20ml of sodium citrate (0.1 M) with 30ml of citric acid (0.1M), pH=4.0) followed by oral administration of 2-3 ml sucrose solution 10% (w/v) for one day next; then animals were fasted overnight and one drop blood sample was obtained by nicking the rats' tail lateral-vein using a sterile surgical scissors, and immediately the blood glucose level was determined using Gluco Dr SUPER SENSOR AGM-2200 glucometer (Korea). Animals with blood glucose level above 240 mg/dl were considered to be diabetic [22].

Study animal groups

After induction of diabetes, both normal and diabetic rats were rearranged randomly in five groups (10 rats/group); first group included normal rats administrated orally with 2 ml distilled water (pH 6.8) and pointed as control; second group included normal rats daily ingested with whey protein concentrate (80%, dissolved in distilled water pH 6.8) at dose of 200 mg protein /kg [~250 mg of WPC 80%]**;** third group was STZ-diabetic rats; fourth group included diabetic rats treated orally with (0.1 mg/kg/day) glimepiride (Amaryl®1mg, dissolved in distilled water pH 6.8) for a similar period [23]; and fifth group comprised diabetic rats treated with WPC same dose and period**.**

Blood and tissue sampling

At the end treatment period (six weeks), rats were weighed then fasted overnight and blood glucose level of each animal was determined using Gluco Dr set through blood specimens from the rats' tail. Following anesthesia (inhalation with diethyl ether), blood specimens were withdrawn from the retro-orbital plexus using heparinized and sterile glass capillaries; whole blood specimens were cool-centrifuged at 3000 rpm for 10 minutes using and the sera were separated, divided into aliquots and stored at -80°C till biochemical measurements could be carried out as fast as possible. After blood collection, the animals were sacrificed soon; then both the liver and kidneys of each animal were dissected out. One kidney and part of the liver of each animal were washed in saline, dried, rolled in a piece of aluminum foil and stored at -80°C for either biochemical or DNA fragmentation determinations. Another portion of the liver was soaked in formalin-saline (10%) buffer for histological processing and microscopic examination.

Tissue Homogenization

A specimen from each organ (liver and kidney) was homogenized in ice-cold phosphate buffer (50 mM, pH 7.4) to give 10% homogenate (w/v); the homogenate was centrifuged at 5000 rpm for 20 minutes to remove the nuclear and mitochondrial fractions; the supernatant was divided into aliquots and stored at -80°C till the biochemical measurements.

Biochemical determinations

Blood glucose level was determined using Gluco Dr SUPER SENSOR AGM-2200, Korean glucometer through blood sample obtained from the lateral tail vein using a sterile surgical scissors. Insulin level was determined in serum by using ELISA kits purchased from Immunospec, Canoga Park, USA. Serum urea, creatinine, ASAT, ALAT, cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol levels; as well as hepatic and renal GSH, NO, SOD, GPx and CAT levels were estimated spectrophotometrically using reagent kits obtained from Biodiagnostic, Dokki, Giza, Egypt. Kidney and liver MDA level was determined chemically as described by Ruiz-Larnea *et al.* [24] through MDA reaction with thiobarbituric acid (TBA) forming a pink complex that can be measured photometrically; In this method, 0.5 ml liver homogenate supernatant was added to 4.5 ml working reagent [0.8 g TBA was dissolved in 100 ml percloric acid 10%, and mixed with TCA 20% in a volume ratio 1 : 3, respectively); then in a boiling and shaking water bath, the sample-reagent mixture was placed for 20 minutes, then carried to cool at room temperature and centrifuged for 5 minutes at 3000 rpm; finally, the absorbance of the clear pink supernatant was measured at 535 nm against reagent blank (0.5 ml distilled water + 4.5 ml working reagent); MDA level (nmol/g tissue) was calculated according to the following formula: MDA (nmol/g tissue) = [{$A_{535}\times 10^9$ / (1.56×10^5) × 10^3} x AD] × 10. Where**,** 1.56x105 $M^{-1}L^{-1}cm^{-1}$ is MDA extinction coefficient and AD is assay dilution that equals 10.

DNA fragmentation percentage

The degree of DNA fragmentation was determined by separating the cleaved DNA from the intact chromatin by centrifugation and measuring the amount of DNA present in the supernatant and pellet using the diphenylamine assay according to the quantitative method used for grading the DNA damage [25]. The degree of DNA fragmentation refers to the ratio of DNA in the supernatant to the total DNA in the supernatant and pellet. The liver tissues were lysed in 0.5 ml of hypotonic lysis buffer containing 10 mM Tris–HCl (pH 8), 1 mM EDTA and 0.2% Triton X-100, and centrifuged at 14,000 ×g for 20min at 4 °C. The pellets were resuspended in hypotonic lysis buffer. To the resuspended pellets and the supernatants, 0.5 ml of 10% trichloroacetic acid (TCA)

was added. The samples were centrifuged for 20 min at10, 000 ×g at 4 °C, and the pellets were suspended in 500 µl of 5% TCA. Subsequently, each sample was treated with a double volume of diphenylamine (DPA) solution [200mg DPA in 10 ml glacial acetic acid, 150µl of sulfuric acid and 60µl acetaldehyde] and incubated at 4°C for 48h. The proportion of fragmented DNA was calculated from the absorbance reading at 578 nm using the following equation:

$$\text{DNA fragmentation \%} = \frac{\text{A of supernatant}}{\text{A of supernatant} + \text{A of pellet}}$$

Histopathology

Paraffin sections of 5µm thick were stained with hematoxylin and eosin [26] and investigated by light microscope.

Statistical analysis

The obtained data were subjected to ANOVA followed by Duncan multiple post hoc test at level of $p \leq 0.05$ according to Steel and Torrie [27] using a statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

Results

In respect to WPC solubility, the results of *in vitro* investigation (Figure 1) declared that, regard any temperature, the degree of solubility was the lowest at pH 5.0. At 40°C, it was noticed that at pH values more than 5 the solubility raised up. Also, it could be detected that near to neutral pH (pH=6.8) the solubility lowered with the temperature. In contrast, at the pH 5 and 6, WPC solubility elevated with the temperature. As expected, the result of the composition test demonstrated that protein is the major constituent of WPC-80 and it accounted 77.4% of the WPC (80%) mass. Also, carbohydrates, lipids, dietary fiber and other constituents such as ash and vitamins were found; the caloric level was 400 kcal%**.** The resultant protein formula was found close to aspartic (7.78), therionine (5.37), serine (3.81), glutamic (13.63), proline (4.42), glycine (1.88), alanine (4.65), valine (4.93), methionine (1.78), isoleucine (4.67), leucine (8.56), tyrosine (2.62), henylalanine (2.86), histidine (1.59), lysine (6.87), argnine (2.70) and cystine (1.72).

In compare to control group, the diabetic animals recorded a significant reduction in body weight gain (BWG) at the end of experimental period, while those administrated with WPC performed slight elevation. Favorably, post- treatment of diabetic rats orally with either the pharmaceutical drug (Amaryl®) or WPC significantly improved BWG to levels close to that of healthy control group when both groups were compared with the diabetic animal group; WPC was the most effective (Figure 2).

The *in vivo* results performed a significant decrease in insulin level coupled with a significant increase in blood glucose in STZ-induced diabetic group when compared with the control group. Interestingly, administration of diabetic rats with WPC improved both insulin and glucose serum levels towards normal values as it significantly increased insulin and significantly decreased the glucose levels compared to diabetic rats. This reflects the analogue of anti-diabetogenic potential of WPC similar to that of the pharmaceutical drug Amaryl® data (Table 1).

Administration of healthy rats with WPC never damaged DNA, while diabetic group showed a significant elevation in DNA fragmentation percentage compared to control animals. In comparison with diabetic animals, post-treatment of diabetic rats with Amaryl® or WPC resulted in significant reductions in the percent of DNA fragmentation; WPC was the most potent (Figure 3).

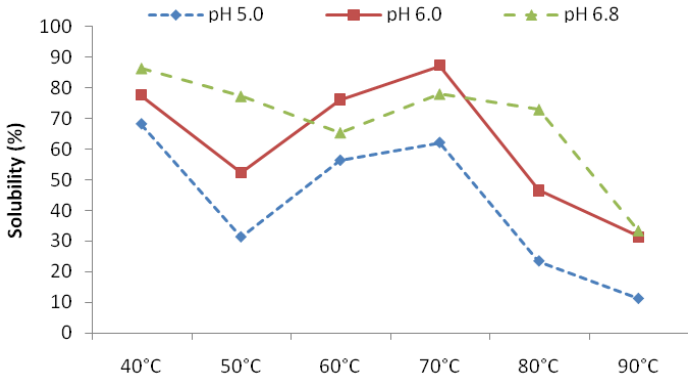

Figure 1. shows the solubility behavior of whey protein concentrate at different pH values and temperature degrees.

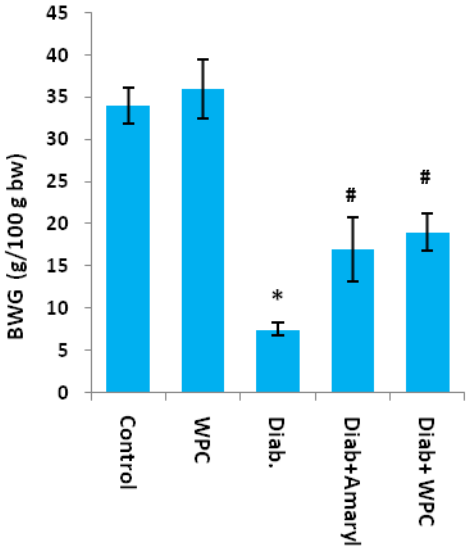

Figure 2. Shows the percentage of body weight gain (BWG) of control, diabetic and diabetic-treated (either with WPC or Amaryl®) rats' groups. * is significantly different from control group, while # is significantly different from diabetic group ($p \leq 0.05$).

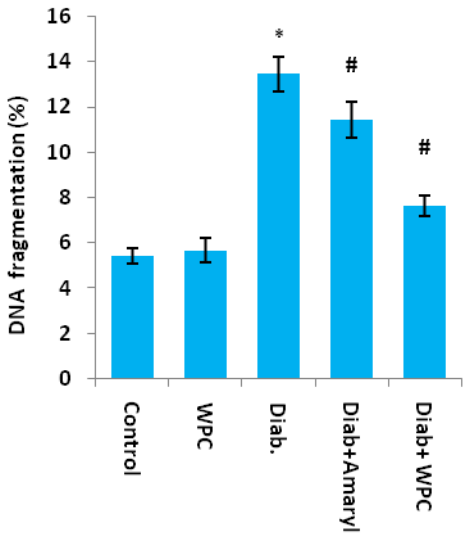

Figure 3. Shows the percentage of hepatic DNA fragmentation of control, diabetic and diabetic-treated (either with WPC or Amaryl®) rats' groups. * is significantly different from control group, while # is significantly different from diabetic group ($p \leq 0.05$).

Table 1. Serum glucose and insulin concentrations of normal, diabetic and diabetic-treated rats.

	Glucose (mg/dl)	Insulin (ng/ml)
Control	93±2.3 ^{d}	2.8±0.03 ^{a}
WPC	95±4.3 ^{d}	3.0±0.07 ^{a}
Diab.	541±15^{a}	0.5±0.03 ^{b}
Diab+Amaryl®	140±2.3 ^{c}	2.6±0.05 ^{a}
Diab+ WPC	156±3.9 ^{b}	3.5±0.04 ^{a}

Data are presented as mean ±standard error; within each column, means with superscript different letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Duncan post hoc test; WPC, whey protein concentrate.

Oral administration of healthy rats with WPC never disturb liver and kidney functions, while single intraperitoneal injection of STZ resulted in a significant elevation of liver and kidney function biomarkers; this is monitored from the marked elevated serum liver enzymes (ALAT and ASAT) and the raised serum kidney markers (urea and creatinine) compared to their normal counterparts. Administration of WPC or Amaryl® to diabetic rats ameliorated significantly the diabetes-induced changes in the measured serum liver function enzymes and kidney function markers (Table 2). As illustrated in Table 3 and compare to healthy control group, administration of healthy rats with WPC didn't deteriorate the lipid profile, while intoxication with STZ led to an atherosclerotic initiation; this was achieved from the significant raise of serum total cholesterol, triglycerides and LDL-cholesterol coupled with the marked reduction in HDL-cholesterol. In contrast, post-treatment of diabetic rats with either Amaryl® or WPC improved all lipid profile parameters; fortunately, WPC was more effective when both groups compare to untreated diabetic one.

Injection of rats with STZ resulted in sharp damages their livers and kidneys as evidenced by the marked depletion in hepatic and renal GSH level and GPx, SOD and CAT activities, matched with a significant increase in MDA and NO levels as compared to the normal control group. Fortunately, post-treatment of diabetic rats with WPC or the reference drug (Amaryl®) significantly recharged liver and kidney GSH battery and significantly increased the activities of GPx, SOD and CAT; moreover, WPC succeeded in reducing both hepatic and renal NO level, but Amaryl® failed to ameliorate NO level in the kidney in compare to the corresponding values of diabetic rats (Tables 4-5).

Histopathological Examination

The microscopic examinations of the liver sections of the normal, diabetic or treated rats are illustrated in the figures (4-8). Concerning histopathological examination of liver tissue; liver sections of both normal control and WPC-treated healthy rats' groups exhibited normal hepatic cells each with well-defined cytoplasm, prominent nucleus, central vein and blood sinusoids (Figures 4 & 5). STZ-intoxication resulted in a wide cloudy and hydropic degenerations in most of the hepatocytes, vacuolization, and congestion of sinusoid (Figure 6). Post-treatment of diabetic rats with WPC exerted moderate improvement; as WPC succeeded to improve the histological structure of liver sections of rats, it is presented in normalized appearance of liver as well as the formation of normal hepatic cords. Minimal cytoplasmic vacuolization of some of the hepatocytes were still seen (Figure 8).

Table 2. Serum liver and kidney functions of normal, diabetic and diabetic-treated rats.

	ALAT (U/L)	ASAT (U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Control	118±6.2^{d}	290±19^{c}	36±3.1^{c}	0.6±0.04^{b}
WPC	111±6.8 ^{d}	217±15 ^{c}	37±2.2^{c}	0.6±0.03^{b}
Diab.	261±5.5^{a}	429±14^{a}	67±2.5^{a}	1.2±0.04^{a}
Diab+Amaryl®	133±8.3^{c}	253±13^{b}	42±1.3^{b}	0.8±0.02^{c}
Diab+ WPC	167±8.5^{b}	265±17^{b}	44±5.2^{b}	0.7±0.04^{bc}

Data are presented as mean ±standard error; within each column, means with superscript different letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Duncan post hoc test; WPC, whey protein concentrate.

Table 3. Serum lipid profile of normal, diabetic and diabetic-treated rats.

	Cholesterol (mg/dl)	Triglycerides (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Control	83±4.1^{d}	71±3.3^{c}	55 ±4.2 ^{cd}	40±1.9^{a}
WPC	78±2.6^{d}	69±4.1^{c}	51±2.1^{d}	42±3.4^{a}
Diab.	254±7.9^{a}	208±9.4^{a}	152±8.2^{a}	28±3.3^{c}
Diab+Amaryl®	183±8.6^{b}	144±4.8^{b}	111±3.1^{b}	35±9.9^{b}
Diab+ WPC	135±7.6^{c}	94±5.9^{d}	58±6.8^{c}	38±4.4 ^{ab}

Data are presented as mean ±standard error; within each column, means with superscript different letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Duncan post hoc test; WPC, whey protein concentrate.

Table 4. shows hepatic and renal malondialdehyde (MDA), nitric oxide (NO) and reduced glutathione (GSH) concentrations of normal, diabetic and diabetic-treated rats.

	MDA (nmol/g tissue)		NO (µmol/g tissue)		GSH (mg/g Tissue)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Control	112±4^{d}	42±2^{d}	56.9±4.2^{c}	2.9±0.1^{bc}	199±3.1^{a}	65.1±0.6^{a}
WPC	110±5^{d}	39±2^{d}	58.2±4.7^{c}	3.1±0.1^{b}	194±0.8^{a}	63.6±2.7^{a}
Diab.	249±11^{a}	206±4 ^{a}	78.7±2.7 ^{a}	4.3±0.3^{a}	115±1.3^{b}	34±1.3^{c}
Diab+Amaryl®	163±4^{b}	175±3^{b}	61.8±6.9^{b}	4.1±0.3^{a}	128±2.1^{b}	50±1.4^{b}
Diab+ WPC	134±5^{c}	125±3^{c}	63.1±4.9^{b}	3.2±0.3^{b}	143±0.7^{c}	56±1.4^{b}

Data are presented as mean ±standard error; within each column, means with superscript different letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Duncan post hoc test; WPC, whey protein concentrate.

Table 5. shows hepatic and renal glutathione peroxidase (GPx) superoxide dismutase (SOD) and catalase (CAT) concentrations of normal, diabetic and diabetic-treated rats.

	GPx (U/g Tissue)		SOD (U/g Tissue)		CAT (nmol/g Tissue)	
	Liver	Kidney	Liver	Kidney	Liver	Kidney
Control	15.3±0.8 ^{a}	5.2±0.27 ^{a}	3318±89^{a}	2823±65^{b}	2.5±0.1^{a}	1.7±0.13^{c}
WPC	14.8±0.6 ^{a}	5.6±0.32 ^{a}	3336±73^{a}	2685±43^{b}	2.6±0.4^{a}	1.8±0.14^{c}
Diab.	6.4±0.3 ^{c}	2.3±0.21 ^{b}	1306±55^{d}	1798±85^{c}	1.5±0.1^{c}	1.2±0.1 1^{a}
Diab+Amaryl®	11.6±0.9 ^{b}	4.7±0.33 ^{a}	2470±76^{b}	2077±34^{a}	1.8±0.1^{b}	1.3±0.11^{a}
Diab+ WPC	12.2±0.9 ^{b}	4.9±0.41 ^{a}	2925±66^{b}	2217±28^{a}	1.8±0.1^{b}	1.5±0.11^{b}

Data are presented as mean ±standard error; within each column, means with superscript different letters are significantly different at $p \leq 0.05$ using one way ANOVA followed by Duncan post hoc test; WPC, whey protein concentrate.

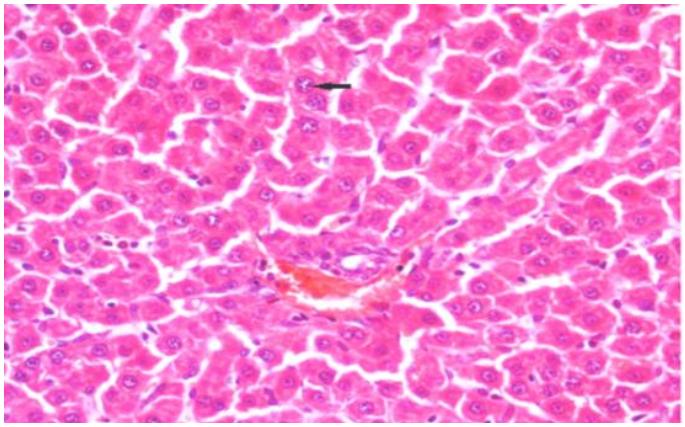

Figure 4. A photo micrograph for liver section of normal rats group showed normal appearance of the hepatocytes (x400, H&E).

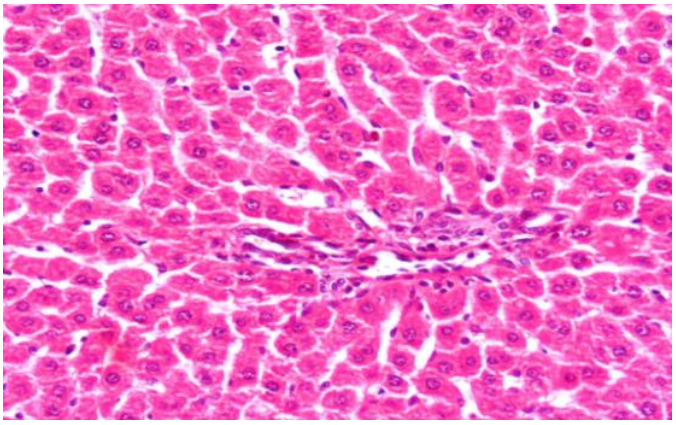

Figure 5. A photo micrograph of liver section of normal rats treated with WPC showing normal appearance of the hepatocytes (x400, H&E).

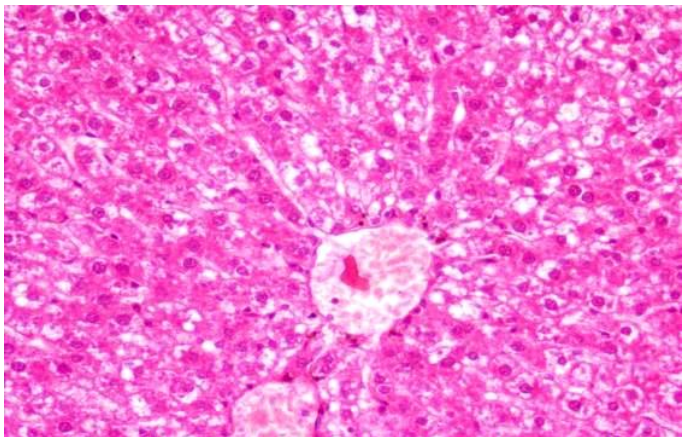

Figure 6. A photo micrograph of a section in the liver of diabetic rat shows wide cloudy and hydropic degenerations in most of the hepatocytes (H&E, x 400).

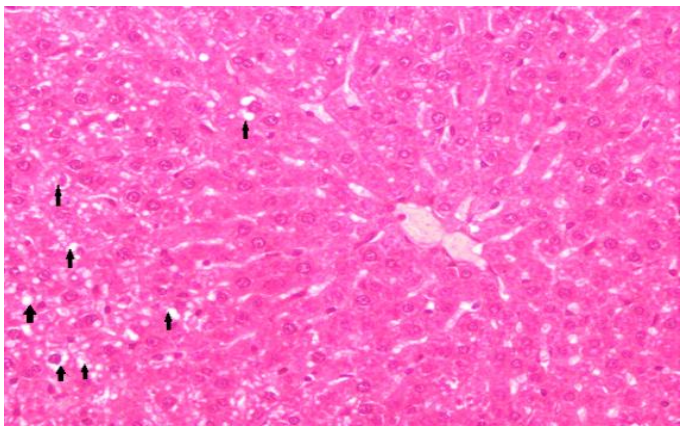

Figure 7. A photo micrograph of liver section of diabetic rat treated with Amaryl® shows some hepatocytes with cloudy hydropic degeneration and few steatosis vacuoles (the arrows) (x400, H&E).

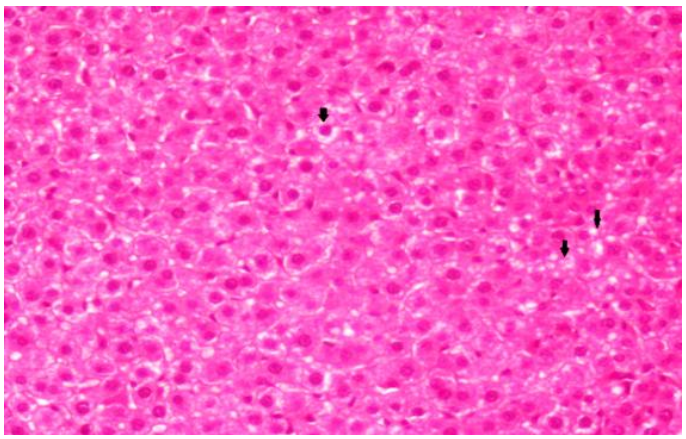

Figure 8. A photo micrograph of liver section of diabetic rat treated with WPC shows minimal cytoplasmic vacuolization of some of the hepatocytes (arrows), in otherwise normal liver parenchyma (x400, H&E).

Discussion

High solubility of the material used in scientific research, especially in biology, pharmacology and medicine, is of importance to reach and use the minute dose in treatment; in this respect, WPC was tested for determination of the optimum pH and temperature at which highest solubility occur without affecting the nature of its constituents. The result of this *in vitro* test declared that at any temperature, the solubility was the smallest at pH 5, i.e. near isoelectric point of whey proteins, as the protein-protein interactions increase due to the minimum electrostatic forces and low protein-water interaction. At 40°C, as the protein structure was under low stress as a consequence to heat action, it was pointed that at pH values over 5 (β-lactoglobulin isoelectric point), the solubility raised up, this could be attributed to the fact that protein has both positive and/or negative net charges that lead to high protein protein-water interaction. Also, it could be noticed that near to pH 6.8, the solubility lowered by the temperature; this might reasoned to the effect of the temperature on the bonds responsible both secondary and tertiary structures stabilization, where the protein unfolding triggering the interaction among the hydrophobic groups and consequently decreasing the protein-water interactions, reflecting the occurrence of thermal protein denaturation. In contrast, at the pH 5 and 6 WPC solubility raised up by the temperature, indicating neither coagulation nor aggregation between the protein molecules was present, this might be due to the fact that β-lactoglobulin is a dimmer dissociates into monomers at 50°C and only above 60°C (at pH 5.0) or 70°C (at pH 6.0) the proteins unfold and the hydrophobic groups react. That is why we use water of pH 6.8 for WPC high solubility

Studies on diabetes mellitus revealed raise in the occurrence of oxidative stress due to the increase on the level of free radicals and diminish cells antioxidant capabilities that in turn can cause oxidative stress and tissue degeneration in diabetic subjects [28]. These characteristics of exogenous and endogenous antioxidant, represents a key role in the protection against free radicals [29]. Streptozotocin (STZ) has been extensively used for induction of diabetes mellitus in rats where it has selective potential to pancreatic islet β-cell cytotoxicity, also interferes with cellular metabolic oxidative mechanisms [30]. Hyperglycemia is an essential reason for the liberation of free radicals that develop the diabetic complications, reactive oxygen species (ROS can be produced by means of both enzymes and/or non-enzymatic chemical reduction of molecular oxygen [31]. The significant reduction in both BWG and serum insulin level coupled with the significant elevation of glucose of the untreated diabetic rats herein goes in line with many previous reports of [32, 33]. It was stated that body weight loss is common diabetic sign. In spite of insulin deficiency (similarly recorded in our study), increased appetite, reduced anabolic processes and accelerated catabolic processes, contributing further to body weight loss, which is already occurring by polyuria polyuria and

glycosuria [34]; therefore, the reduction in BWG of diabetic rats in this study might be a result of distortion of structural proteins due to unavailability of carbohydrates for usage as a source of energy. The deficiency of insulin in diabetic subjects results in suppressed synthesis and elevated breakdown of proteins that elevates the level of blood amino acid levels and subsequently increased gluconeogenesis [35].

The estimation of oxidative stress, either quantitatively or qualitatively, is carried out using a number of biological redox markers including the determination of ROS capacity, determination of the values of separate antioxidants and assessment of lesions resulted from ROS and their derivatives; as the total antioxidant capacity is not the sum of concentrations and activities of these separate antioxidant agents [36]. ROS are highly reactive and attack in their vicinity various classes of biomolecules including proteins, DNA and lipids such as polyunsaturated fatty acids (PUFAs) via a process known as oxidative stress leading to the stable end product, malondialdehyde [37-39]. Similarly, elevated NO production is recognized as an important mediator of physiological and pathological processes [40]. This suggestion is confirmed herein via the significant raise in hepatic and renal MDA and NO levels in consequence to STZ-diabetogenic effect.

The elevated serum values of ASAT and ALAT, urea, creatinine in STZ-induced diabetic rats are in consistent with many studies [41-43]. These results declare the occurrence of liver and kidney damages after the administration of STZ to the rats. Diabetes exhibits high oxidative stress, it reduces of the antioxidant protective system and promotes the generation of free radicals [31, 44] causing hepatic and renal damage; consequently leakage of ASAT and ALAT leading to their elevation in serum ASAT and ALAT. Calisti and Tognetti [45] attributed this deterioration in the hepatic function to the loss of integrity of the hepatocyte membrane [46]. Also, hyperglycemia resulted in hemodynamic changes within the kidney tissue and free radical generation as well renal dysfunction [47]. This mechanism could explain the marked raise in serum levels of urea and creatinine in our study; this finding goes hand in hand with report of Aboulthana *et al.* [48].

WPC is contains sulpher-rich amino acids (cysteines and methionines) that are essential materials in the biosynthesis of GSH which is a major agent of cellular antioxidants, has high detoxifying characteristics, triggers white blood cells synthesis and activity, and improves immune functions [49].

Attention should be drowning here that treatment of diabetic rats with WPC significantly ameliorated and inhibited DNA-fragmentation; this property might be attributed to the antioxidant activity of the whey proteins as stated before [43]. This finding is strengthen with the remarkable reduction of renal and hepatic MDA and NO combined with elevation of SOD, GPx and CAT of activity and GSH storage. Similarly, WPC was found able to improve liver and kidney functions in diabetic rats, this effect could be due to improvement in the anti-oxidative battery that evidenced by the sharp reduction of renal and hepatic MDA and NO with its efficiency in restoring SOD, GPx and CAT of activity and GSH levels; that resulted from the synergistic effect of thiol-rich amino acids such as methionine and cystine.

On the same line, WPC markedly ameliorated the disturbed cholesterol, triglycerides, LDL and HDL levels after its administration to diabetic rats; this finding was in accordance with several intervention studies [50-52]. The mechanisms behind the favorable effect on lipid profile could be due to the effect of WPC on *de novo* cholesterol biogenesis in the liver [53] and/or the prevention of cholesterol absorption in the intestine controlled by β-lactoglobulin [54].

The improvements that recorded in the biochemical measurements were further confirmed by the repair in hepatocytes' distortion resulted from STZ injection. Liver sections of both normal and normal-WPC treated groups displayed normal architecture and appearance of portal tract and hepatocytes. Liver toxicity (elevation of serum ASAT and ALAT) producing acute necrosis since the mitochondrial ASAT is released only when the cells are severely disintegrated [48]. This result was confirmed by disorganization of the architecture of the hepatocytes. ALAT levels elevated in plasma due to large bile duct obstruction by STZ induced oxidative stress [55] and this was appeared in the histopathological examination which revealed hyperplasia of biliary epithelium with formation of newly formed bile ductules, biliary cyst formation and intense fibroblastic proliferation which extend to infiltrate the surrounding hepatic [32]. It was previously reported that WPC possesses hepatoprotective and antioxidant activity [56]. The antioxidant potential of WPC is basically comes from its ability to increase the cellular GSH [57-59] through its rich content of cysteine, β-lactoglobulin, α-lactalbumin, and bovine serum albumin [60, 61].

Conclusion

WPC exhibits multi-health benefits with promising potentials against STZ-induced diabetes; this behavior may be attributed to its antioxidant and free radical scavenging mechanisms that due to vie its thiol-rich amino acids and antioxidant, as evidenced by in vitro investigations.

Recommendation

The ability of WPC to repair and prevent oxidative DNA damage in the tissues of diabetic rats should contribute to its use in the management of diabetes.

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