Global Veterinaria 8 (1): 22-34, 2012
ISSN 1992-6197

Molecular Genetic, Cytogenetic and Sperm Studies on the Remedial Effects of Whey Protein and α-Lactalbumin in Streptozotocin-Induced Hyperglycemic Rats

[1]Hoda F. Booles, [1]Hasnaa A. Radwan, [1]Inas S. Ghaly, [1]Ibrahim M. Farag and [2]Somaia A. Nada

[1]Department of Cell Biology, National Research Centre, Giza, Egypt
[2]Department of Pharmacology, National Research Centre, Giza, Egypt

Abstract: The aim of the present study was to investigate the effect of treatments with whey protein (WP) and α-lactalbumin (α-lac) on genetic alterations and sperm abnormalities in streptozotocin (STZ) - induced hyperglycemic rats. Twenty five of male adult albino rats were used and divided into five groups (five animals each). The first group was normal animals. The second group consisted of diabetic animals.Whereas, the third, fourth and fifth groups included diabetic animals treated with amaryl (AM) (0.03 mg/kg.b.wt./dl), WP (200 mg/kg.b.wt./dl) and α-lac (200 mg/kg.b.wt./dl), respectively for 30 days. The results showed that blood glucose level (BGL), genetic alterations (including DNA fragmentation, disappear of some base pairs of DNA according to ISSR-PCR analysis and chromosome aberrations) and sperm abnormalities were significantly increased in STZ diabetic rats than those of normal control. In contrast, the treatments with AM, WP and α-lac in diabetic rats significantly decreased the BGL, genetic alterations and sperm abnormalities than untreated diabetes. When comparing the AM treatment with the protein (WP and α-lac) treatments, it was observed that AM group had significant decrease of BGL than WP group, while α-lac group had un-significant decrease of BGL than AM group. However, the WP and α-lac groups had significant decreases of rates of DNA fragmentation than AM group. Also, ISSR-PCR analysis showed that a lot of fragments of base pairs of DNA which disappeared in diabetic or amaryl treatments, they have been existed in protein treatments especially in α-lac group. Moreover, the WP and α-lac groups had significant decreases of most frequencies of each of chromosome aberrations and sperm abnormalities compared to AM group. On the other hand, the comparison between WP and α-lac groups showed that α-lac group had significant decrease of BGL than WP group. Also the treatment with α-lac in diabetic animals was more effective for decreasing the genetic alterations and sperm abnormalities than treatment with WP and there were significant differences between the two treatments. In conclusion, WP and α-lac have a role in controlling the diabetic disease by reviving the hyperglycemia condition to the normal health state and repairing the genetic alterations as well as improving the reproductive condition by decreasing the sperm abnormalities. These effects of protein treatments especially α-lac have been found to be over expressed than amaryl treatment.

Key words: Hyperglycemia · Rats · Whey Protein · α-Lactalbumin · Molecular Genetics · Cytogenetics · Sperms

INTRODUCTION

Diabetes mellitus is a group of syndromes characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and protein and an increased risk of complication of vascular diseases [1]. The minimum defining characteristic feature to identify diabetes mellitus is chronic and substantiated elevation of circulating glucose concentration [1,2]. Induction of diabetes in laboratory animals is a convenient and useful strategy in the understanding and treatment of the disease. An appropriate dose of streptozotocin (STZ) was used to induce experimental diabetes. Streptozotocin selectively destroyed pancreatic β-cells, resulting in hypoinsulinemia, basal hyperglycemia and abnormal glucose tolerance [1-4]. High level of blood sugar (or the presence of hyperglycemia condition) has been known to be a good marker for inducing overproduction of free radicals and

Corresponding Author: Hoda F. Booles, Department of Cell Biology, National Research Centre, Postal Code: 12622, Dokki, Giza, Egypt.

other reactive oxygen species (ROS) [5-8]. ROS virtually damage all cellular components, leading to DNA and protein modification and lipid peroxidation [9-11]. More frequencies of DNA fragmentation, populations of micronucleated erythrocytes and chromosome aberrations have been observed in rats with alloxan-induced hyperglycemia than those of normal animals [12]. In addition, diabetic patients are reported to have reduction of antioxidant defenses, such as superoxide dismutase and decreased levels of antioxidants [10, 11]. In consequence, the diabetic patients suffer from an increased risk of oxidative stress-related diseases not only in the present generation but can also transmit the nuclear defects to their progeny [11, 13]. Some studies reported that the hyperglycemic condition has been found to be major reason for inducing sperm shape abnormalities besides reducing the caudal sperm count in each of STZ diabetic rats [11] and alloxan diabetic rats [12].

The curing or the treatment for the hyperglycemia condition by using artificial drug such amaryl has been shown in several studies [11,12,14], this is because the amaryl is a third generation antidiabetic sulphonylurea known to possess the ability for reduction of blood glucose levels and the antioxidant effect in streptozotocin (STZ)- induced diabetes [11,14]. On the other hand, the identifying food components that decrease the blood glucose levels may be beneficial for developing low glycemic food and supplements than artificial drugs. Some proteins from certain foods have been reported to have the ability to reduce blood glucose excursion and scavenge active oxygen species [15, 16].

Milk protein in particular, appears to stimulate the increase in post-prandial insulin response with a corresponding reduction in postprandial blood glucose levels [17] and it was recently shown that this property is related to the whey protein fraction of milk [18]. Whey protein is particular high in branch-chain amino acids, in particular leucine [19]. These amino acids are insulinogenic, meaning that they have a higher capacity to increase an insulin response and consequently decrease the blood glucose levels [20, 21]. Moreover, Mortensen *et al.* [22] compared the effect of whey protein with effects of other proteins (such casein, cod and gluten) on postprandial lipid and incretion response to a high-fat meal in diabetes. Their results showed that free fatty acids were most pronouncedly suppressed after whey-meal and the glucose response was lower after the whey-meal than after the other meals.

In addition, possible antioxidant activity of whey protein has also been suggested. It is an essential source of amino acids such as histidine and tyrosine that were reported to have antioxidant activities by scavenging radicals or ROS and other peroxides [23- 27]. Also, whey protein is considered to be the main factor in delay lipid oxidation, because it was found to be related to the prevalence of hydrophobic amino acids that have this property [27]. Moreover, the antioxidant activity of whey protein may be due to its ability to elevate cellular glutathione (GSH) concentration, which in turn would increase the scavenging of free radicals produced by damage condition or mutagenic agents [28- 30]. On the other hand, the majority of whey protein are cysteine-rich, including α-lactalbumin, β-lactoglobulin and bovine serum albumin [31]. α-lactalbumin has been found to regulate of lactose synthesis [32] and to act as an antioxidant by having radical scavenging activity [27, 32]. Furthermore, cystine is known as an amino acid that regulates the in vivo concentrations of GSH biosynthesis. The latter was reported beside an antioxidant, it is an anti-apoptotic and anti-carcinogenic tripeptide, thereby improving protection against oxidant-induced cell damage [30].

So, the present work was designed to study the effect of treatments with whey protein and α-lactalbumin on potential genetic and reproductive abnormalities in STZ-induced hyperglycemic rats. In this respect, molecular genetic, cytogenetic and sperm studies have been investigated.

MATERIALS AND METHODS

Experimental Animals: Adult male albino rats weighting 150-160 g, bred in the animal house lab. National Research Center, Cairo, Egypt, were used for this study. The animals were housed under standard laboratory conditions, maintained on a 12 h light and dark cycle and provided water and pellet food ad libitum.

Chemicals and Drugs:

- Streptozotocin (STZ) and glucose oxidase peroxidase diagnostic enzyme kit were purchased from Sigma (st. Louis, Mo. USA).
- Standard drug: Amaryl (Glimepiride tablet) was obtained from local pharmacies, Cairo, Egypt and ground using a mortar. The powder was dissolved in distilled water and orally administrated to dose 0.03

mg/kg b.wt/d1 for 30 days. This dose equals the dose of acceptable daily intake of amaryl for human (4mg/kg), after modification to suit the small weight of rats. The dose of amaryl was 0.03 mg/kg.b.wt/dl according to previous studies [33, 34]. Amaryl treatment (as standard treatment) was used in this study for comparison with other treatments.

Protein Materials: Whey protein (WP) and α-lactalbumin (α-Lac) were purchased from Davisco Foods International, Inc (Eden Prairie, MN, USA). WP or α-Lac solutions {15% (w/w), pH 6.9} were separately prepared by dispersing WP or α-Lac powder in distilled water. The solution of WP or of α-Lac was individually administered at a dose of 200 mg/Kg.b.wt.

Induction of Diabetes: The experimental group of animals was fasted for 24 hours and then intraperitoneally injected with a single dose of 65 mg/kg body weight of freshly prepared streptozotocin dissolved in citrate buffer PH 4.5 to induce diabetes [35]. Diabetes was confirmed after 48 or (72) h of streptozotocin injection, the blood samples were collected via retro-orbital venous plexus and serum glucose levels were estimated by enzymatic GOD-PAP (Glucose oxidase peroxidase) diagnosis kit method [10, 11]. The rats with serum glucose level above 160mg/d1 were selected [11] and used for the present study.

Also, blood glucose values were determined just prior to killing the animals at the end of experiment. The animals were fasted for three hours then blood was collected from orbital sinus.

Experimental Design: Male rats were randomly selected and divided into five groups (five animals each). The first group included normal animals (control). The second group was diabetic animals (D group). Whereas, the third, fourth and fifth groups consisted of diabetic animals treated with amaryl drug (AM group), whey protein (WP group) and α-lactalbumin (α-lac group), respectively. The third group was orally given 0.03 mg/kg.b.wt/d1 of amaryl. While, the fourth and fifth groups were orally given WP (at level of 200 mg/kg.b.wt/d1) and α-lac (at level of 200 mg/kg.b.wt/d1), respectively. The period of treatments for diabetic animals extended for 30 days. At the end of experiment, the animals were sacrificed by cervical dislocation for performing of molecular genetic, cytogenetic and sperm studies.

Molecular Genetic Studies

DNA Fragmentation: Liver samples were collected immediately after sacrificing the animals. The tissues were lysed in 0.5 ml lysis buffer containing 10 mM tris-HCL (PH.8), 1 mM EDTA, 0.2% triton X-100, centrifuged at 10000 r.p.m. (Eppendrof) for 20 minutes at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and supernatants (S), 1.5 ml of 10% trichloroacetic acid (TCA) was added and incubated at 4°C for 10 minutes. The samples were centrifuged for 20 minutes at 10000 r.p.m. (Eppendorf) at 4°C and the pellets were suspended in 750 µl of 5% TCA, followed by incubation at 100°C for 20 minutes. Subsequently, to each sample 2 ml of DPA solution {200 mg DPA in 10 ml glacial acetic acid, 150 µl of sulfuric acid and 60 µl acetaldehyde} was added and incubated at room temperature for 24 hour [36]. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

$$\text{DNA fragmentation} = \frac{\text{OD of fragmented DNA (S)}}{\text{OD of fragmented DNA (S)} + \text{OD of intact DNA (P)}} \times 100$$

ISSR-PCR Analysis

DNA Extraction: Genomic DNA from the liver of the tested animals was isolated according to the method of Sharma *et al.* [37]. The concentration of DNA and its relative purity were determined using a spectrophotometer based on absorbance at 260 and 280 nm respectively. The integrity of extracted genomic DNA was checked by electrophoresis in 0.8% agarose gel using DNA molecular weight marker (Eurblio, Paris, France).

ISSR-PCR and Electrophoresis: Inter Simple Sequence Repeat (ISSR) analysis was performed using three different ISSR primers that were procedure from Integrated DNA technologies Inc. (San Diego, CA, USA), based on core repeats anchored at the 5' or 3' end as shown in table (1). Amplification reactions for ISSR analysis were used in a final volume of 25 µl containing 10 X PCR buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM MgCl2, pH 9.0), 2 mM dNTPs, 10 mM primer, 50 ng of template DNA and 0.5 U of Tag Polymerase (Promega, USA). Reactions were performed in a thermocycler (Biometra, GmbH).

Table 1: Primer sequences used for ISSR amplification:

ISSR names	Primer sequences
HB-10	5' GAG AGA GAG AGA CC '3
HB-12	5' CAC CAC CAC GC '3
HB-14	5' CTC CTC CTC GC '3

ISSR amplification was performed according to Zietkiewiez *et al.* [38] with an initial denaturation of 2 min. at 94°C followed by 40 cycles of 94°C for 30 sec. annealing at 52°C for 45 sec. extension at 72°C for 7 min. PCR products were analyzed using 1.2 % agarose gel electrophoresis and visualized with 10 ug/ul ethidium bromide staining. The sizes of the fragments were estimated based on a DNA ladder of 100 to 2000 bp (MBI, Fermentas). The electrohporetic patterns of the PCR products were recorded digitally using a Gel-Doc 2000 image analysis system (Bio-Rad) according to the instruction of manufactory.

Chromosome Preparations: For chromosome analysis, in both treated (AM, WP and α-lac) and control (C and D) animals, femurs were removed and the bone marrow cells were aspirated from both femurs of each animal in 5-6 ml of RPMI 1640 medium into sterile tubes; 0-2 ml of 0.05 colchicine was added to each tube in vitro [39]. Cultures were incubated at 37-38°C for 1 h. The cells were centrifuged at 1000 rpm for 10 min. and resuspended in prewarmed (37°C) hypotonic solution (0.075 M potassium chloride) for 20 min. at 37°C. The samples were centrifuged and fixed in cold 3:1 methanol : glacial acetic acid. Each sample was washed five times in fixative and slides were produced by the conventional method and stained with Giemsa stain [40]. In each animal, chromosome analysis was carried out in 50 metaphase spreads.

Sperm Analysis: For sperm-shape analysis, the epididimus excised and minced in about 8 ml of physiological saline, dispersed and filtered to exclude large tissue fragments. Smears were prepared after staining the sperms with Eosin Y (aqueous), according to the methods of Wyrobek and Bruce [41], Wyrobek *et al.* [42] and Farag *et al.* [43]. At least 3000 sperms per group were assessed for morphological abnormalities. The sperm abnormalities were evaluated according to standard method of Narayana [44].

Statistical Analysis: Statistical analysis was performed with SPSS software. Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for comparison between different treatments. The values were expressed as mean ± S.E. and differences were considered as significant when $P< 0.05$. Furthermore, ISSR bands were scored using Gel-Doc (Bio-Rad) Gel analysis program as present (+) or absent (0) and they were examined to estimate differences or relationships among the investigated treatments.

RESULTS

Blood Glucose Levels: The results in Table, 2 and Fig. 1 showed that the blood glucose levels (BGL) significant increased in streptozotocin diabetic rats (D group) than those of control group. Whereas, the BGL were significantly reduced in diabetic animals treated with

Table 2: Effect of AM, WP and α-lac on blood glucose levels (BGL) in diabetic rats after 30 days of treatments.

Normal (control)	STZ	AM	WP	α-Lact.
A	B	C	D	AC
63.60±1.75	165.07±3.55	93.80±4.14	117.60±2.18	81.80±2.60

- Data were expressed as mean ±SE
- Means with different superscript letters (A, B, C, D) are significantly-different (P<0.05).
- STZ = Streptozotocin treatment.
- AM = Amaryl treatment.
- WP = Whey Protein treatment.
- α -Lac = α -Lactalbumin treatment.

Table 3: Effect of AM, WP and α-lac treatments on the rates of DNA fragmentation in STZ-induced hyperglycemic (diabetic) male rats.

Treatments	% of DNA Fragmentation M ± S.E.
Control	7.25±0.1[a]
D	20.57±0.74[e]
D + AM	17.29±0.40[d]
D + WP	14.35±0.54[c]
D + α -Lac	10.52±0.71[b]

- Data were expressed as mean ± S.E.
- Means with different superscript letters (a, b, c, d, e) are significantly different (P<0.05).
- D = Diabetes (hyperglycemia).
- AM = Amaryl treatment.
- WP = Whey Protein treatment.
- α -Lac = α -Lactalbumin treatment.

Table 4: ISSR analysis using three ISSR primers; HB-10, HB-12 and HB-14.

ISSR primers	Band No.	M (bp)	Control	Diab	AM	WP	α-Lac
HB-10	1	610	+	+	+	+	+
	2	500	+	+	+	+	+
	3	420	+	+	+	+	+
	4	275	+	+	+	+	+
	5	150	+			+	+
	5		5	4	4	5	5
HB-12	1	700	+	+	+	+	+
	2	590	+	+	+	+	+
	3	500					+
	4	410	+	+	+	+	
	5	390					+
	6	360	+	+	+	+	+
	7	290	+	+	+	+	+
	8	230	+			+	+
	8		6	5	5	6	7
HB-14	1	650	+	+	+	+	+
	2	490	+				+
	3	400	+	+	+	+	+
	4	290	+				+
	5	250	+		+	+	+
	6	210	+	+	+	+	+
	7	120	+	+	+	+	+
	8	50	+				+
	8		8	4	5	5	8

M= Marker. Diab= Diabetes (hyperglycemia). AM = Amryl treatment.
WP= Whey Protein treatment. α-lac = α- lactabumin treatment.

Table 5: Effect of AM, WP and α -Lac treatments on the frequency of chromosome aberrations in STZ-induced hyperglycemic male rats.

	Structural Chromosomal Aberrations						Total structural chromosomal	Numerical aberrations		Total numerical
Treatments	Gaps	Deletions	Breaks	C.A.	EEA	Rings	aberrations	Peridiploidy	Polyploidy	aberrations
Control	0.6±0.24^{ab}	0.0±0.0^{a}	0.4±0.24^{a}	1.2±0.2^{a}	0.2±0.2^{a}	0.0±0.0^{a}	2.4±0.24^{a}	1.4±0.24^{a}	0.0±0.0^{a}	1.4±0.24^{a}
D	2.2±0.2^{c}	7.8±0.48^{d}	3.2±0.58^{c}	3.4±0.4^{b}	3.2±0.4^{d}	0.2±0.2^{a}	20.0±0.55^{e}	5.0±0.7^{c}	2.0±0.44^{c}	7.0±0.31^{c}
D + AM	1.4±0.24^{b}	4.2±0.49^{c}	2.2±0.2^{bc}	4.2±0.58^{b}	1.8±0.37^{c}	0..4±0.24^{a}	14.2±0.58^{d}	3.2±0.37^{b}	1.2±0.37^{bc}	4.4±0.5^{b}
D + WP	1.2±0.37^{b}	3.0±0.32^{b}	1.4±0.4^{ab}	1.2±0.37^{a}	0.6±0.4^{ab}	0.6±0.4^{a}	8.0±0.54^{c}	3.0±0.44^{b}	0.4±0.2^{ab}	3.2±0.37^{b}
D + α -Lac	0.33±0.21^{a}	2.16±0.166^{b}	0.8±0.3^{a}	0.5±0.34^{a}	1.3±0.21^{bc}	0.33±0.21^{a}	5.5±0. 4^{b}	2.6±0.21^{b}	0.5±0.34^{ab}	3.16±0.47^{b}

- Data were expressed as mean ± S.E.
- Means with different superscript letters (a, b, c, d, e, f) are significantly different (P<0.05).
- D = Diabetes (hyperglycemia).
- AM = Amaryl treatment.
- WP= Whey Protein treatment.
- α -Lac = α -Lactalbumin treatment.
- C.A. = Centromeric Attenuations.
- EEA = End to End Associations.

Table 6: Sperm abnormalities in each of hyperglycemic condition and hyperglycemia treated with AM, WP and α-Lac. of male rats.

	Types of sperm head abnormalities						Total head	Total tail	Total abnormal
Treatments	Amorphous	Without hook	Banana shape	Sperm with two heads	Big shape	Small shape	abnormalities	abnormalities	sperms[1] (head + tail)
Control	4.25±0.47^{a}	6.5±1.0^{a}	0.25±0.25^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	11.5±1.0^{a}	4.7±0.25^{a}	15.75±1.2^{a}
D	14.2±1.1^{d}	12.0±0.7^{d}	0.8±0.48^{a}	1.0±0.3^{b}	0.2±0.4^{a}	0.6±0.24^{b}	28.8±1.3^{e}	10.6±0.4^{b}	39.0±0.1^{g}
D + AM	11.0±0.7^{c}	11.25±0.25^{cd}	0.75±0.4^{a}	0.75±0.25^{ab}	0.25±0.5^{a}	0.5±-0.28^{ab}	24.5±1.5^{d}	10.25±0.75^{b}	34.75±2.2^{f}
D + WP	9.75±0.75^{bc}	9.25±0.47^{bc}	0.75±0.4^{a}	0.25±0.25^{a}	0.5±0.5^{a}	0.0±0.0^{a}	20.5±1.0^{c}	6.25±0.75^{a}	26.75±1.6^{e}
D + α-Lac	7.5±0.64^{b}	7.25±0.85^{ab}	0.5±0.5^{a}	0.25±0.25^{a}	0.0±0.0^{a}	0.0±0.0^{a}	15.5±0.64^{b}	5.25±0.47^{a}	20.75±0.75^{b}

Data were expressed as mean ± S.E.
Means with different superscript letters (a, b, c, d) are significantly different (P<0.05).
D = Diabetes (hyperglycemia).
AM = Amaryl treatment.
WP = Whey Protein treatment.
α-Lac = α-lactalbumin treatment.

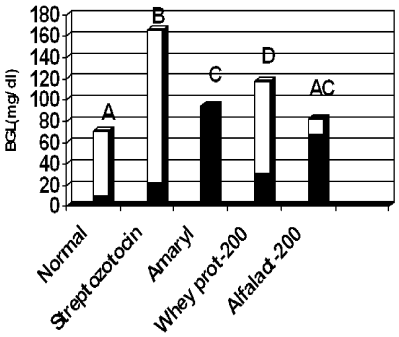

Fig. 1: Effect of AM, WP and α-Lac on blood glucose levels (BGL) in diabetic rats after 30 days of treatments.

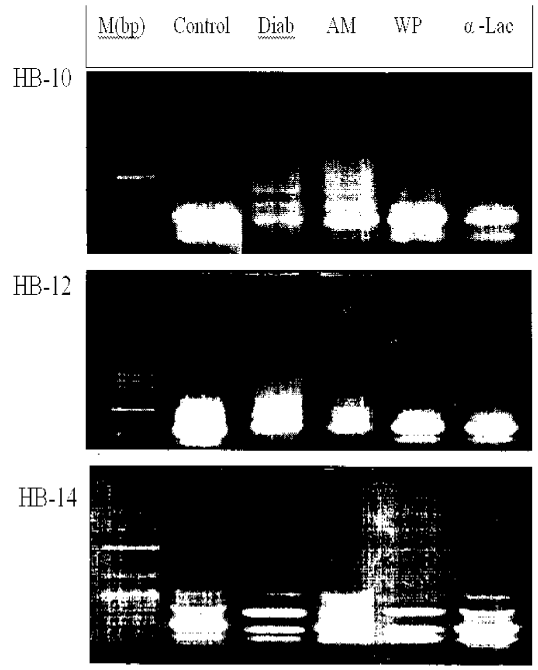

Fig. 2: ISSR amplified products using three ISSR primers; HB-10,HB-12 and HB-14
M = Marker.
Diab = Diabetes (hyperglycemia).
AM = Amaryl treatment.
WP = Whey Protein treatment.
α-Lac = α-lactalbumin treatment.

amaryl (AM group) or whey protein (WP group) or α-lactalbumin (α Lac group) compared to those found in D group. On the other hand, AM group had significant decrease of BGL than WP group, while the α-lac group had decrease of BGL than AM group, however this decrease was not significant. Moreover, the α-.lac group had significant decrease of BGL than WP group.

Molecular Genetic Results

DNA Fragmentation: The results in Table, 3 showed that the rates of DNA fragmentation were significantly increased in diabetic animals (D group) than those of control group. In contrast, the diabetic animals treated with amaryl (AM group) or whey protein (WP group) or α-lactalbumin (α- Lac group) had significant decreases of DNA fragmentation than D group. Moreover, the WP and α -Lac groups had significant decreases of DNA fragmentation than AM group. On the other hand, the treatment with α-lactalbumin in diabetic animals was more effective for decreasing the rates of DNA fragmentation than the treatment with whey protein and there were significant differences between the two protein treatments.

ISSR-PCR Analysis: Three ISSR primers; HB10, HB12 and HB14 were used to detected the whole and lacto protein effects for hyperglycemia comparing with Amaryl drug in rat (Fig. 2 and Table, 4). Using primer HB10, the diabetic rat and Amaryl drug samples revealed an equal four fragments which were different from the control samples in a 150 bp fragment. On the other hand, the two protein samples with whole WP and α-lac proteins displayed five amplified fragments similar to the control.

Moreover, ISSR primer HB-12 revealed a total of six fragments in the control and in the WP samples and seven fragments in α-Lac samples, however, diabetic rat and Amaryl drug samples lacked especially a 230 bp fragment that existed in the foremost samples. On the other hand, the α-lac protein samples revealed two unique fragments with 500 and 390 bp that disappeared in the other samples. However, α-Lac group lacked 410 bp fragment that existed in other samples. The obtained results by using the primer HB-14 revealed a total eight fragments in the control and α-Lac samples. However, the diabetic group lacked four fragments of 490, 290, 250 and 50bp that have also been lacked (except the 250 bp) in AM and WP samples. These disappeared fragments in diabetic, AM and WP samples were existed in each of control and α-Lac groups.

Cytogenetic or Chromosome Examination: Chromosome examination in Table, 5 showed that the frequencies of structural and numerical chromosome aberrations were significantly increased in diabetic animals (D group) compared to those found in the control group. In contrast, the diabetic animals treated with amaryl or WP or α-Lac had significant decreases for most frequencies of chromosome aberrations than D group. On the other hand, structural and numerical chromosome

aberrations were decreased in WP and α-Lac groups than those of amaryl (AM) group. These decreases were significant in WP or α-Lac for the frequencies of deletions, C.A, EEA and total structural aberrations or for the frequencies of chromatid gaps and breaks, deletions, C.A and total structural aberrations, respectively. Moreover, the most frequencies of structural and numerical chromosome aberrations were more lowered in α-lac group than those of WP group and there were significant differences between the two groups for the frequencies of chromatid gaps and total structural aberrations.

Sperm-Shape Analysis: Sperm examination (Table, 6) showed that sperm-shape abnormalities significantly increased in diabetic animals (D group) than those of control group. In contrast, diabetic animals treated with amaryl or whey protein or α-lactalbumin had significant decreases of sperm abnormalities compared to D group. On the other hand, the whey protein or α-lactalbumin groups had significant decreases of most frequencies of sperm abnormalities than AM group. Moreover, the α-lactalbumin group had the lowest frequencies of sperm abnormalities when comparing with whey protein group and there were significant differences between the two groups for total sperm head abnormalities and total sperm abnormalities (head + tail).

DISCUSSION

Several studies showed that the incidence of diabetes has been associated with the presence of hyperglycemia condition [1,11,14,45]. The treatment for this condition by using amaryl (glimepiride) drug has been reported, because the amaryl was known to possess the ability for reduction of blood glucose levels in diabetes [11,12,14]. On the other hand, the identifying food components such proteins that decrease the blood glucose levels has also been reported [18]. Proteins vary in their ability to decrease post-prandial glycemia (or hyperglycemic incidence). Comparing the insulinotropic characteristics of milk, gluten, cod, cheese and whey demonstrated that both milk and whey have the greatest impact on glucose metabolism by increasing both insulin secretion and glucose-dependent insulin tropic polypeptide [18].

Concerning the hyperglycemia condition in the present study, the blood glucose level was significantly increased in streptozotocin (STZ) diabetic rats than those of normal control. These findings are in agreement with that reported in other studies on STZ rats [11,46]. Also, the present results were similar with that observed in alloxan diabetic mice [47] and rats [11,48]. The observed hyperglycemia in this study may be due to cytotoxic effect of STZ on pancreatic β-cells causing insulin deficiency [45,49,50]. In the insulin secretion studies of the 10-16 week-old STZ rats, there was a complete loss of β cell sensitivity to glucose [2]. Also, by 6 weeks of age of STZ rats, these animals showed basal hyperglycemia and abnormal glucose tolerance [1,4].

The present results also showed that STZ diabetic rats had higher rates of DNA mutations (DNA fragmentation and deletion or disappear of some base pairs fragments of DNA according to ISSR-PCR analysis) comparing with normal control. ISSR analysis using primers HB10, HB12 and HB14 revealed that the total number of fragments of base pairs of DNA in control samples were existed to be 5, 6 and 8 respectively, however, in diabetic rat samples, some of these fragments had disappeared and the total No. of fragments were lacked to be 4 (lacked one fragment of 150 bp), 5 (lacked one fragment of 230 bp) and 4 (lacked four fragments of 490, 290, 250 and 50 bp) fragments, respectively. Moreover, the present diabetic condition in male rats significantly increased the frequencies of chromosome aberrations and sperm-shape abnormalities. These abnormal effects can be attributed to the dangerous role of the presence of hyperglycemia condition, that was found in several studies to be a good marker for inducing overproduction of reactive oxygen species (ROS) [5 - 8]. ROS attack the building structures of the cell membrane, nucleus and genetic material by causing scission, carbonylation, fragments, cross-linking and oxidation [51,52] leading to DNA modification [9,45] chromosome aberrations and sperm abnormalities [6,11,52].

Concerning the incidence of DNA fragmentation in the present study, the results were similar with that reported in other studies on individuals associated with alloxan diabetic condition. The occurrence of DNA fragmentation in lymphocytes obtained from alloxan-induced diabetic rats was found to be 81% compared to 45% of untreated cells from the control [6]. Also, the presence of hyperglycemia condition due to alloxan treatment in both male and female rats led to significant increases of rats of DNA fragmentation compared to normal controls [12].

On the other hand, it is reported that ISSR assay is considered to be a powerful method which used to illustrate the genetic variation between and within species of plants such as in citrus (Poncirus trifoliate L. [53]) and

peanut (Arachis hypogaea L. [54]). This technique has also been applied to study interspecific polymorphisms such as in rice (genus Oryza, [55]), tomato (genus lycopersicon, [56]), chickpea (genus Cicer, [57]), grevillea (proteaceae, [58]) and strawberry (Fragaria anonassa, [59]).

Moreover, ISSR analysis was used to investigate the effect of salinity condition on genetic stability of red tilapia fish [60]. ISSR fragments were found to be variety-specific markers. These markers were scored whether they were present or absent as an unique band for a given variety. Each band was assumed to represent an unique genetic locus [59,60].

ISSR analysis in the present study showed deletion or disappear of some base pair fragments of DNA in diabetes individuals. These disappeared fragments in diabetes were existed in normal control. The loss of genetic composition in diabetes may be due to the presence of hyperglycemia condition that is considered as discussed above the main factor for inducing overproduction of ROS. ROS attack the DNA structure causing DNA mutations [6,11,12]. Concerning the occurrence of chromosome aberrations in hyperglycemic rats of the present study, the findings were supported by several studies. For example, cytogenetic analysis by Tollinger *et al.*[61] and Block *et al.*[45], indicated that the majority of alloxan-induced diabetic rats was composed of hypodiploid cells with a chromosome number of 38 to 41 (2n=42). Chromosome abnormalities (aneuploies and polyploidies) have been reported to be increased in embryos of diabetic mice [62]. The influence of alloxan diabetes on first meiotic segregation behavior in female and male mice was studied by Wauben-Penris and Prins [63]. They found in primary spermatocytes higher chiasma freuquencies in the translocation multivalent in diabetic males than in controls. Also, the analysis of metaphase-II cells in the females revealed less 3 : 1 segregation and more adjacent-II segregation in the diabetics. So, they concluded that diabetes influences the meiotic segregation behavior of chromosomes and that chromosomes showing higher incidence of unbalanced segregation behavior. Moreover, Abd El-Rahim *et al.* [12] found that the alloxan diabetes of rats (males or females) had higher frequencies of structural and numerical chromosome aberrations of bone marrow cells compared to normal controls. Considering the incidence of sperm abnormalities in the present study, the results were concidence with other studies by Rabbani *et al.* [11] and Abd El-Rahim *et al.* [12] who found the STZ or alloxan diabetic condition of male rats significantly increased the sperm shape abnormalities besides significant reducing of caudal sperm count. In previous studies diabetes mellitus has been known to be often associated with sexual dysfunction [64] delayed sexual maturation and infertility or compromised semen quality [64, 65] in men. Several studies reported that the mechanism for inducing abnormalities in nuclear component and sperm-shape in hyperglycemia condition include the activation of several damaging pathways by the ROS such as accelerated formation of advanced glycation end production (AGE), polyol pathway, hexosamine pathway, protein kinase (PKC) or increase of lipid peroxidation (LPO) [11,66,67]. LPO occurs when ROS attack the poly unsaturated fatty acid residues of phospholipids of cell membrane which is extremely sensitive to the oxidation. Host cell like spermatozoa are highly susceptible to the damage by excess concentrations of ROS due to high content of polyunsaturated fatty acid within their plasma membrane. Increased LPO and altered membrane can affect the sperm function through impaired metabolism, motility, acrosome reaction as well as oxidation damage to sperm DNA leading to increase of morphological changes in sperm [11, 68-70]. In the present study, the administration of amaryl, whey protein (WP) and α-lactalbumin (α-lac.) to the STZ diabetic rats led to reduction of blood glucose level (BGL) and resulting in decreases of each of genetic alterations (DNA fragmentation, deletion or disappear of some base pair fragments of DNA according to ISSR-PCR analysis and chromosome aberrations) and sperm abnormalities compared to diabetic control (D group). The BGL in amaryl group (AM group) was lower than WP group, however, the BGL has been decreased in α-lac group than AM and WP groups. On the other hand, the WP and α-lac treatments in STZ diabetic rats led to decrease of the frequencies of DNA fragmentation compared to amaryl treatment. Also, ISSR analysis revealed that many fragments of base pairs of DNA had disappeared in diabetic rats or amaryl drug samples, however, the most or all of these fragments were existed in protein samples especially in the α-lactalbumin samples. Moreover, the frequencies of chromosome aberrations and sperm abnormalities were more lowered in protein samples than those of amaryl drug. The α-lac. group had the lowest frequencies of genetic alterations and sperm abnormalities. This could be explained the role of proteins (especially α-lac) in controlling the diabetic disease which over expressed than the amaryl drug.

Concerning the effect of amaryl drug on reduction of hyperglycemia condition in the present study, our findings were similar with that reported by Rabbani *et al.* [11] and Abd El-Rahim *et al.* [12], who found that the treatment with amaryl (glimepiride) led to reduction of blood glucose levels (BGL) in STZ or alloxan diabetic rats.

The primary mechanism of action of amaryl (glimepiride) in lowering the blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells [11,14]. Moreover, in the antidiabetic therapy, glimepiride has the advantage that it does not cause severe hypoglycemic complications due to sudden release of insulin as like the other sulphonylureas [11,14].

On the other hand, the effect of whey and α-lactalbumin proteins in lowering the blood glucose level have been reported by Frid *et al.* [16] who found that the addition of whey to meals stimulated insulin release and reduced postprandial blood glucose excursion in diabetic subjects, the insulin responses were higher after both breakfast (31%) and lunch (57%), when they were included in the meal than when whey was not included. After lunch, the blood glucose response was significantly reduced after whey ingestion. So, the authors, concluded that insulin tropic effect of whey protein may potentially attenuate the post-prandial blood glucose excursions over the day. Moreover, Mortensen *et al.* [22] compared the effects of the proteins casein, whey, cod and gluten on postprandial lipid and incretion response to a high-fat meal in diabetic persons. Their results showed that free fatty acids were most pronouncedly suppressed after whey-meal. The glucose response was lower after the whey-meal than after the other meals. In addition, Bushmarina *et al.*[32] reported that the main function of α-lactalbumin was found to be the regulate of lactose synthesis.

Concerning, the decreases of genetic alterations and sperm abnormalities in diabetic animals treated with amaryl and proteins (WP and α-lac) in the present study, these decreases or repairing of genetic and sperm changes may be due to the reduction of hyperglycemia condition by such treatments and consequently the reduction of potential generation of ROS.

As discussed above, the amaryl [11,12,14] and whey protein [16,22] treatments were found to possess the ability of reduction of blood glucose levels in diabetes. Also, the possible antioxidant activity of amaryl and proteins (WP and α-lac.) that has been reported in several studies, may be the main factor for reduction or repairing of genetic and sperm abnormalities in the present study. Kramer *et al.* [14], Krauss *et al.* [71] and Rabbani *et al.* [11] reported that the administration of amaryl (glimepiride) to diabetes had increased the plasma levels of antioxidant enzymes (CAT, SOD and GPx) besides reducing the levels of LPO, H_2O_2 and malondialdehyde.

In other studies, the effect of amaryl treatment on genetic alterations and sperm abnormalities in diabetes were observed. Rabbani *et al.* [11] reported that the administration of glimepiride to the STZ diabetic rats had reduced the populations of micronucleated erythrocytes and sperm abnormalities besides enhancing the sperm count compared to diabetic control. Also, Abd El-Rahim *et al.* [12] found that amaryl treatment in alloxan diabetic rats had reduced genetic alterations (populations of micronucleated erythrocytes, DNA fragmentation and chromosome aberrations) and sperm abnormalities besides enhancing the sperm count compared to diabetic control. On the other hand, possible antioxidant activity of whey protein has also been reported. It would include chelation of transition metals by serum albumin and lactoferrin, an iron-binding glycoprotein, as well as free radical scavenging activity by amino acids, such as tyrosine and cysteine [24,25]. Also, Pihlanto[27] reported that whey protein has ability to delay lipid oxidation, because WP was found to be related to the prevalence of histidine and hydrophobic amino acids, these amino acids have been shown to act as antioxidants. Moreover, Bushmarina *et al.*[32] and Pihlanto[27] showed that the α-lactalbumin has ability to act as an antioxidant by having radical scavenging activity.

In previous study, Zommara *et al.* [72] reported that a fermented milk product had an antiperoxidative action on rats fed a vitamin E deficient diet; the lactic acid bacteria and whey proteins in the product contributed partly to the observed antiperxidative action. Kullisaar *et al.* [73] noted that the consumption of fermented goat milk improved anti-atherogenicity in healthy subjects by prolonging the resistance of the lipoprotein fraction to oxidation, lowering the levels of peroxidized lipo-proteins and the glutathione redox ration and enhancing total anti-oxidative activity.

The reported mechanism for antioxidant effect of whey protein has been attributed to the increase in blood and tissue glutathione (GSH) concentration, which consequently increase the scavenging of free radicals produced by damage condition or mutagenic agents [28]. Also, in this respect, Czuczejko *et al.* [74] reported that the increase in the activity of GSH-dependent enzymes in

plasma may arise from increased formation of reactive oxygen species or from release of these enzymes from injured cells to plasma. So, the ability of treatments of amaryl, WP and α-lac for reduction of hyperglycemia besides they have antioxidant properties, could prevent or minimize the genetic alterations and sperm abnormalities by preventing ROS (or other free radical action), [11,75]. To our knowledge, the repairing effects (or antimutagenic property) of WP and α-lac on genetic and sperm abnormalities in diabetes have not been discussed previously. Moreover, it was observed in the present study that the repairing effects of proteins especially α-lac. for such damages were more effective than amaryl. In conclusion, whey protein and α-lactalbumin have effect in controlling the diabetic disease by reviving the hyperglycemia condition to the normal health state and reduction the genetic alterations as well as improving the reproductive condition by decreasing the sperm abnormalities. These repairing effects of the protein treatments especially of α-lactalbumin treatment have been found to be over-expressed than amaryl treatment.

REFERENCES

1. Arulmozhi, D.K., A. Veeranjaneyulu and S.L. Bodhankar, 2004. Neonatal Streptozotocin-induced rat model of type 2 diabetes mellitus: A glance. Indian J. Pharmacol., 36: 217-221.
2. Portha, B., L. Picolon and G. Rosselin, 1979. Chemical diabetes in the adult rat as the spontaneous evolution of neonatal diabetes. Diabetologia, 17: 371-377.
3. Bonnier-Weir, S., D.E. Trent, R.N. Honey and G.C. Weir, 1981. Response to neonatal islets to streptozotocin: Limited cell regeneration and hyperglycemia. Diabetes, 30: 64-9.
4. Weir, G.C., E.E. Clore, C.J. Zma-Chinsky and S. Bonnier-Weir, 1981. Islet secretion in new experimental model for non-insulin dependant diabetes. Diabetes Metabolism Rev., 30: 590-595.
5. Chauhan, L.K.S., N. Pant, S.K. Gupta and S.P. Srivastava, 2000. Induction of chromosome aberrations, micronucleus formation and sperm abnormalities in mouse following carbafuran exposure. Mutat. Res., 465: 123-129.
6. Otton, R., F.G. Soriano, R. Verlengia and R. Curi, 2004. Diabetes induces apoptosis in lymphocytes. J. Endocrinol., 182: 145-156.
7. Vikram, A., D.N. Tripathi, P. Ramarao and G.B. Jena, 2007. Evaluation of streptozotocin genotoxicity in rats from different ages using the micronucleus assay. Regulatory Toxicology and Pharmacol., 49: 238-244.
8. Shrilatha, B. and Muralidhara, 2007. Early oxidative stress in testis and epididymal sperm in streptozotocin-induced diabetic mice: Its progression and genotoxic consequences. Report. Toxicol., 23: 578-587.
9. Rehman, A., J. Nourooz-Zadeh, W. Moller, H. Tritschler, P. Pereira and B. Halliwell, 1999. Increased oxidative damage to all DNA bases in patients with type - II diabetes mellitus. FEBS Letters, 448: 120-122.
10. Kuhad, A., R. Sethi and K. Chopra, 2008. Lycopene attenuates diabetes -associated cognitive decline in rats. Life Sci., 83: 128-134.
11. Rabbani, S.I., K. Devi and S. Khanam, 2009. Inhibitory effect of glimepiride on nicotinamide-streptozotocin induced nuclear damage and sperm abnormality in diabetic Wister rats. Indian J. experimental Biol., 47: 804-810.
12. Abd El-Rahim, A.H., H. A. Radwan, O.M. Abd El-Moneim, I.M. Farag and S.A. Nada, 2010. The influence of amaryl on genetic alterations and sperm abnormalities of rats with alloxan-induced hyperglycemia. J. American Sci., 6: 1739-1748.
13. Blasiak, J., M. Arabski, R. Krupa, K. Wozniak, M. Zadrozny, J. Kasznicki, M. Zurawska and J. Drezewoski, 2004. DNA damage and repair in type 2 diabetes mellitus. Mutat. Res., 554: 297-304.
14. Kramer, W., G. Mullet and K. Geisen, 1996. Characterization of the molecular mode of action of sulphonylurea, glimepiride at beta cells.Horm. Metab. Res., 28: 464- 468.
15. Okada, Y. and M. Okada, 1998. Scavenging effect of water soluble proteins in broad beans on free radicals and active oxygen species. J. Agricultural and Food Chemistry, 46: 401-406.
16. Frid, A.H., M. Nilsson, J. J. Holst and I.M.E. Björck, 2005. Effect of whey on blood glucose and insulin responses to composite breakfast and lunch meals in type 2 diabetic subjects. American J. Clinical Nutrition, 82: 69-75.
17. Ostman, E.M., H.G.M.L. Elmstahl and I.M.E. Bjorck, 2001. Inconsistency between glycemic and insulinemic responses to regular and fermented milk products. Am. J. Clin. Nutr., 74: 96 -100.

18. Nilsson, M., M. Stenberg, A.H. Frid, J..J. Holst and I.M. Bjorck, 2004. Glycemia and insulinemia in healthy subjects after lactose-equivalent meals of milk and other food proteins: the role of plasma amino acids and incretins. Am. J. Clin. Nutr., 80: 1246-1253.
19. Layman, D.K., 2003. The role of leucine in weight loss diets and glucose homeostasis. J. Nutr., 133: 261S-267S.
20. Rocha, D.M., G.R. Faloona and R.H. Unger, 2004. Glucagon-Stimulating Activity of 20 Amino Acids in Dogs. J. Clin. Invest., 51: 2346-2351.
21. Petersen, B.L., L.S. Ward, E.D. Bastian, A.L. Jenkins, J. Campbell and V. Vuksan, 2009. A whey protein supplement decreases post-prandial glycemia. Nutrition J., 8: 47.
22. Mortensen, L.S., M.L. Hartvigsen, L.J. Brader, A. Astrup, J. Schrezenmeir J.J. Holst, C. Thomsen and K. Hermansen, 2009. Differential effects of protein quality on postprandial lipemia in response to a fat-rich meal in type 2 diabetes: comparison of whey, casein, gluten and cod protein. The American J. Clinical Nutrition, 90: 41- 48.
23. Taylor, M.J. and T. Richardson, 1980. Antioxidant activity of skim milk. Effect of heat and resultant sulfhydryl groups. J. Dairy Sci., 63: 1783-1795.
24. Colbert, L.B. and E.A. Decker, 1991. Antioxidant activity of an ultrafiltration permeate from acid whey. J. Food Sci., 56: 1249-1250.
25. Meucci, E., A. Mordente and G.E. Martorana, 1991. Metal-catalyzed oxidation of human serum albumin: Conformational and functional changes. J. Biological Chemistry, 266: 4692-4699.
26. Shinmoto, H., S. Dosako and I. Nakajima, 1992. Antioxidant activity of bovine lactoferrin on iron/ascorbate induced lipid peroxidation. Bioscience, Biotechnology and Biochemistry, 56: 2079-2080.
27. Pihlanto, A., 2006. Antioxidative peptides derived from milk proteins. International dairy J., 16: 1306-1314.
28. Bounous, G., 2000. Whey protein concentrate (WPC) and glutathione modulation in cancer treatment. Anticancer Res., 20: 4785-4792.
29. Abd EL-Aziem, S.H., Z.A. Saleh and A.A. Farrag, 2007. Impact of whey protein on the genotoxic effects of aflatoxins in rats. Int. J. Dairy Sci., 2: 126-137.
30. Peng, X., Y.L. Xiong and B. Kong, 2009. Antioxidant activity of peptide fractions from whey protein hydrolysates as measured by electron spin resonance. Food Chem., 113: 196-201.
32. Bushmarina, N.A., C.E. Blanchet, G. Vernier and V. Forge, 2006. Cofactor effects on the protein folding reaction: acceleration of alpha-lactalbumin refolding by metal ions. Protein Sci., 15: 659-671.
31. Morr, C.V. and E.Y.W. Ha, 1993. Whey protein concentrates and isolate processing and functional properties. Crit. Rev. Food Sci. Nutr., 33: 431-476.
33. Sato, J., I. Ohsawa, Y. Oshida, Y. Sato and N. Sakamoto, 1993. Effects of glimepiride on vivo insulin action in normal and diabetic rats. Diabetes Res. Clin. Pract., 22: 3-9.
34. Nieszner, E., I. Posa, E. Kocsis, G. Pogatsa, I. Preda and M.Z. Koltai, 2002. Influence of diabetic state and that of different sulfonylureas on the size of myocardial infraction with and without ischemic preconditioning in rabbits. Exp. Clin. Endocrinol. Diabetes, 110: 212-218.35.
35. Tuzcu, M. and G. Baydas, 2006. Effect of melatonin and vitamin E on diabetes-induced learning and memory impairment in rats. Eur. J. Pharmacol., 537: 106-110.
36. Gibb, R.K., D.D. Taylar, T. Wan, D.M. Oconnor, D.L. Doering and C. Gercel- Taylor, 1997. Apoptosis as a measure of chemosensitivity to cisplatin and taxol therapy in ovarian cancer cell lines. Gynecologic Oncol., 65: 13-22.
37. Sharma, K.K., M. Lavanya and V. Anjaiah, 2000. A method for isolation and purification of peanut genomic DNA suitable for analytical applications. Plant Mol. Biol. Rep., 18: 393-393.
38. Zietkiewicz, E., A. Rafalski and D. Labuda, 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20: 176-183.
39. Williams, D.L., A. Harris, K.J.Williams, M.J. Brosius and W. Lemods, 1984. A direct bone marrow chromosome technique for acute lymphoblastic leukemia. Cancer Genetics Cytogenetics, 13: 239-257.
40. Preston, R.J., B.D. Dean, S. Galloway, H. Holden, A. F. McFee and M. Shelly, 1987. Mammalian in vivo cytogenetic assays: Analysis of chromosome aberrations in bone marrow cells. Mutat. Res., 189: 157-165.

41. Wyrobek, A.J. and W.R. Bruce, 1978. The induction of sperm shape-abnormalities in mice and humans. Chemical Mutagens, 5: 257-285.
42. Wyrobek, A.J., G. Watchmaker and L.A. Gordon, 1984. Sperm Morphology testing in mice. In: Killbey, B.J. Legator, M. Nichols, M. Ramel, C. (Eds.), Handbook of Mutagenicity Test Procedures. Elsevier, Amsterdam, pp: 739-750.
43. Farag, I.M., H.S.A. Abdou, A.M. Ayesh and M.M.H. Osfr, 2002. Chromosomal and sperm studies on the mutagenic effect of over heated meat and the protective role of green tea and ginseng on rats. Al-Azhar Bull. Sci., 13: 105-120.
44. Narayana, K., 2008. An aminoglycoside antibiotic gentamycin induces oxidative stress, reduces antioxidant reserve and impairs spermatogenesis in rats. J. Toxicol. Sci., 33: 85-96.
45. Block, K.O., R. Zemel, O.V. Bloch, H. Grief and P. Vardi, 2000. Streptozotocin and alloxan-based selection improves toxin resistance of insulin-producing RINm cells. Int. Experimental Diab. Res., 1: 211-219.
46. Yassin, M.M. and S.N. Mwafy, 2007. Protective potential of glimepiride and Nerium oleander extract on lipid profile, body growth rate and renal function in streptozotocin - induced diabetic rats. Turk. J. Biol., 31: 95-102.
47. Diamond, M.P., K.L. Moley, A. Pellicer, W.K. Vaughn and A.H. De Cherney, 1989. Effect of streptozotocin - and alloxan-induced diabetes mellitus on mouse follicular and early embryo development. J. Reprod. Fert., 86: 1-10.
48. El-Shabrawy, O.A. and S.A. Nada, 1996. Biological evaluation of multicomponent of tea used as hypoglycemic in rats. Fitoterapia, LXVII: 99-102.
49. Weiss, R.B., 1982. Streptozotocin: a review of its pharmacology, efficacy and toxicity. Cancer Treat. Rep., 66: 427-38.
50. Schnedl, W.J., S. Ferber, J.H. Johnson and C.B. Newgard, 1994. STZ transport and cytotoxicity. Specific enhancement in GLUT-2 expressing cells. Diabetes, 43: 1326-1333.
51. Cakatay, U., R. Kayali and H. Uzun, 2008. Relation of plasma protein oxidation parameters and paraoxonase activity in the ageing population. Clinical and Experimental Medicine, 8: 51-57.
52. Aydin, S., E. Aytac, H. Uzun, T. Altug, B. Mansur, S. Saygili, N. Buyukpinarbasili and M. Sariyar, 2010. Effects of Ganoderma lucidum on obstructive jaundice-induced oxidative stress. Asian J. Surgery, 33: 173-180.
53. Fang, D.Q., M.L. Roose, R.R. Krueger and C.T. Federici, 1997. Fingerprinting trifoliate orange germ plasm accessions with isozymes, RFLPs and inter-simple sequences repeat markers. Theoretical and Applied Genetics, 95: 211-219.
54. Raina, S.N., V. Rani, T. Kojima, Y. Ogihara, K.P. Singh and R.M. Devarumath, 2001. RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification and phylogenetic relationships in peanut (Arachis hypogaea) cultivars and wild species. Genome, 44: 763-772.
55. Joshi, S.P., V.S. Gupta, R. K. Aggarwal, P.K. Ranjekar and D.S. Brar, 2000. Genetic diversity and phylogenetic relationships as revealed by inter simple sequence repeat (ISSR) polymorphism in the genus Oryza. Theoretical and Applied Genetics, 100: 1311-1320.
56. Kochieva, E.Z., N.N. Ryzhova, I.A. Khrapalova and V.A. Pukhalskyi, 2002. Genetic diversity and phylogenetic relationships in the genus Lycopersicon (Tourn) Mill. As revealed by inter-simple sequence repeat (ISSR) analysis. Russian J. Genetics, 38: 958-966.
57. Iruela, M., J. Rubio, J. Cubero, J. Gil and T. Millan, 2002. Phylogenetic analysis in the genus Cicer and cultivated chickpea using RAPD and ISSR markers. Theoretical and Applied Genetics, 104: 643-651.
58. Pharmawati, M., G. Yan and I. McFarlane, 2004. Application of RAPD and ISSR markers to analyse molecular relationships in Grevillea (Proteaceae). Australian Systematic Botany, 17: 49-61.
59. Hussein, T.S., A.A. Tawfik and M.A. Khalifa, 2008. Molecular identification and genetic relationships of six strawberry varieties using ISSR markers. International J. Agriculture and Biol., 10: 677-680.
60. Eshak, M.G., N.M. Ebeed, H.F. Booles and K.F. Fahmy, 2010. Genetic assessment of salinity tolerance in red tilapia. New York Science J., 3: 132-141.
61. Tollinger, C.D., C.L. Chrisman and D.P. Doolittle, 1974. Alloxan-induced aneuploidy in mice. The J. Heredity, 63: 345-348.
62. Yamamoto, M., A. Endo, G. Watanabe and T.H. Ingalls, 1971. Chromosomal aneuploidies and polyploidies in embryos of diabetic mice. Archs Enviro. Health, 22: 468-475.
63. Wauben-Penris, P.J.J. and J.B. Prins, 1983. Meiotic behavior of alloxan -treated diabetic and non diabetic T(1, 13) 70H/+mice. Human Genetics, 63: 268-273.

64. Rodriguez-Rigau, L.J., 1980. Diabetes and male reproductive function. J. Androl., 1: 105-110.
65. Bartak, V., M. Josifko and M. Horackova, 1975. Juvenile diabetes and human sperm quality. Int. J. Fertil., 20: 30-32.
66. Piconi, L., L. Quagliaro and A. Ceriello, 2003. Oxidative stress in diabetes. Clin. Chem. Lab. Med., 41: 1144-1149.
67. Valko, M., D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidant in normal physiological function and human disease. Int. J. biochem. Cell Biol., 39: 44-84.
68. Tramer, F., F. Rocco, F. Micali, G. Sandri and E. Panfili, 1998. Antioxidant systems in rat epididymal spermatozoa. Biol. Reprod. 59: 753-758.
69. Kumar, T.R., K. Doreswamy, B. Shrilatha and Muralidhara, 2002. Oxidative stress associated DNA damage in testis of mice: Induction of abnormal sperms and effects on fertility. Mutat. Res., 513: 103-111.
70. Sanchez, E.E.T., M.L. Marquette, D.B. Brown and N.H. Ansari, 2006. The effect of oxidative stress on human sperm morphology. Fertil. Steril. 86 (supp 1). S444. In: Am. Soc. Reproed. Med 62nd Annual Meeting.
71. Krauss, H., J. KoŹlik, M. Grzymisławski, P. Sosnowski, K. Mikrut, J. Piatek and J. Paluszak, 2003. The influence of glimepiride on the oxidative state of rats with streptozotocin-induced hyperglycemia. Med. Sci. Monit, 9: 389-93.
72. Zommara, M., H. Tougo, M. Sakana and K. Imaizumi, 1998. Prevention of peroxidative stress in rats fed on a low vitamin E-containing diet by supplementing with a fermented bovine milk whey preparation: effect of lactic acid and b-lactoglobulin on the antiperoxidative action. Biosci. Biotechnol. Biochem., 62: 710-717.
73. Kullisaar, T., E. Songisepp, M. Mikelsaar, K. Zilmer, T. Vihalemm and M. Zilmer, 2003. Antioxidative probaiotic fermented goats milk decreases oxidative stress-mediated atherogenicity in human subjects: Brit. J. Nutr., 90: 449-459.
74. Czuczejko, J., B.A. Zachara, E. Staubach-Topczewska, W. Halota and J. Kedziora, 2003. Selenium, glutathione and glutathione peroxidase in blood of patients with liver diseases. Acta Biochim. Pol., 50: 1147-1154.
75. Krishnamoorthy, G., P. Venkataraman, A. Arunkumar, R.C. Vignesh, M.M. Aruldas and J. Arunakaran, 2007. Ameliorative effect of vitamins (α-tocopherol and ascorbic acid) on PCB (Aroclor 1254) induced oxidative stress in rat epididymal sperm. Reprod. Toxicol., 23: 239-245.