Research Article

Effect of Amaryl and Variety of Dietary Supplementations on Gene Expression Alteration in Pancreas, Liver and Ovarian Tissues of Hyperglycemic Rats

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ABSTRACT

The present study was designed to evaluate the therapeutic role of amaryl (AM), whey protein (WP), α-lactalbumin (α-lac), *Saccharomyces cerevisiae* (Sc), Sc enriched with low (ScCr$_1$) or with high (ScCr$_2$) level of chromium and two levels [low (mush$_1$) and high (mush$_2$)] of Oyster mushroom extract on alterations of gene expression levels in hyperglycemic rats. The expressions of insulin (I and II) and coagulation factor genes were evaluated in streptozotocin-induced hyperglycemic male rats. Also, the effects of amaryl on the changes of expression levels of FSH, LH and CYP2E1 genes in alloxan-induced hyperglycemic female rats were investigated. The results showed that the expression levels of insulin I and insulin II genes were down-regulated in the pancreas tissue of hyperglycemic animals as compared to the control. The expression of coagulation factor gene was up-regulated in the liver tissue of hyperglycemic rats compared to the control. The ovarian tissue of the hyperglycemic rats, showed lower expression values of FSH and LH genes. However, over expression of the CYP2E1 gene was observed, in comparison to those of the control. The undesirable alterations of insulin and coagulation factor genes expression in the present study, due to streptozotocin-induced hyperglycemic condition were improved using amaryl or variety of dietary supplementations. The treatment with AM was more effective for such improvements. Also, the treatment with WP, ScCr$_2$ and Mush$_2$ significantly ameliorated the expression of ins. I and ins. II and coagulation factor genes than α-lac, Sc, ScCr$_1$ and Mush$_1$. These improvements were similar to those found with AM treatment. Moreover, the treatment of alloxan-induced diabetic rats with AM led to significant improvement in the expression of FSH, LH and CYP2E1 genes compared to the untreated diabetes. The levels of expressions of such reproductive genes in the amaryl treated group were similar to those of the normal control. In conclusion, the present study revealed that the treatment with AM, WP, and high levels of chromium yeast or mushroom extract could improve the expressions of insulin and coagulation factor genes in streptozotocin diabetic rats. However, AM treatment was more effective. Also, alloxan diabetic female rats treated with AM succeeded in overcoming the changes in their reproductive genes expression (FSH, LH and CYP2E1).

Keywords: Hyperglycemia, rats, amaryl, dietary supplementations, gene expression, RT-PCR.

INTRODUCTION

Hyperglycemia condition is a serious health problem among diverse populations worldwide. This condition is considered to be an indicator for inducing the type 2 diabetes mellitus (DM2). DM2 include impairment of peripheral insulin sensitivity and beta-cell dysfunction[1,2]. The hyperglycemia often exerts a glucotoxicity which damage beta – cell function due to its capacity to enhance islet oxidative stress[2]. Oxidative stress is a common pathogenic factor for the dysfunction of β-cells and endothelial cells. β-cells dysfunction results from prolonged exposure to high glucose, elevated free fatty acids level, or a combination of both[3] and they are particularly sensitive to reactive oxygen species (ROS) due to inadequate expression of free- radical quenching (antioxidant) enzymes such as catalase, glutathione peroxidase and superoxide dismutase[4]. The oxidative stress leads to mitochondrial damage and ultimately decreases insulin secretion[5,4]. Exposure of β-cells to ROS or other free radicals decreases flux of insulin mRNA, cytosolic ATP and calcium intocytosol[4]. Also, other studies suggested that high glucose concentrations induce mitochondrial ROS which suppresses the first phase of glucose – induced insulin secretion, at least in part, through the suppression of activity of glyceraldehyde 3-

phosphate dehydrogenase (GAPDH)[4]. ROS generation is a key component of NADPH oxidase, enzyme that generates the superoxide radicals[6]. Superoxide overproduction by the mitochondrial electron transport chain induced by hyperglycemia is considered to be a main cause for activation of all other pathways involved in the pathogenesis of endothelial dysfunction[4].

On the other hand, patients with type 2 diabetes have an increased prevalence of atherosclerotic vascular disease[7]. Hyperglycemia condition was found to be a major contributor to vascular disease[8]. High serum glucose has been shown to increase glycation of fibrinogen[9] and clots formed from glycated fibrinogen have a more compact structure and increased resistance to lysis[9]. In previous study, Boeri[10] found that prolonged exposure of cultured human endothelial cells to hyperglycemia increases tissue-factor gene expression. Rao[8] revealed that prolonged hyperglycemia induces activation of the tissue factor pathway of blood coagulation in young healthy individuals apparently free of preexisting atherosclerotic disease.

Moreover, hyperglycemia-related oxidative stress might be the trigger for many alterations on sexual function. The infertile patients with diabetes have been widely

reported in males. Whereas, very limited data are available in the literature regarding the reproductive function independent of diabetic female status, and restricted on polycystic ovary syndrome. Moran[11] concluded that women with polycystic ovary syndrome (PCOS) had elevated prevalence of impaired glucose tolerance (IGT) and type 2 diabetes mellitus (DM2). It has been reported that there are biomarkers of impaired glucose tolerance (IGT) and DM2 risk in women with PCOS. Concerning the diabetic males, it is well established that diabetes can produce erectile dysfunction and retrograde ejaculation in men[12]. Microscopic abnormalities in testicular morphology in diabetic young men with erectile dysfunction were observed by Andersson[13]. Several studies showed that diabetic males have reduction of fertility, prolificacy and libido[14,15].

As known FSH and LH hormones play important role in reproduction. Testicular and ovary functions are controlled by such hormones[16]. Decreases in the serum levels of FSH, LH, prolactin and growth hormone have been reported in hyperglycemic rats[17].

Moreover, the hypophysis of diabetic rats has a blunted response with a diminished stimuli-induced secretion of FSH and LH[18]. These results indicate that there is a relationship between glucose level and levels of FSH and LH in serum, however, this relationship has not clearly formulated in diabetic females.

Furthermore, the cytochromes of P_{450} (CYP) are a superfamily of haemoproteins that mediate the biotransformation of endogenous and exogenous compounds[19]. CYP2E1 is the classical ethanol-inducible CYP that has been shown to catalyse the bioactivity of several procarcinogens and protoxins including N-nitrosodimethylamine, benzene and Nalkylformamides[20]. The increased expression of CYP2EI has also been reported in association with obesity, type 2 diabetes and reproductive toxicity[21], however this association has not been reported in diabetic females.

Therapeutic interventions to diminish or prevent oxidative stress due to hyperglycemia could be therefore helpful in improving health of patients under diabetic condition.

Several studies by our laboratory showed that amaryl and variety of dietary supplementation (such whey protein, α-lactalbumin, Saccharomyces cerevisiae with chromium and mushroom) had appeared to be effective for both the control of blood glucose and the modification of the course of diabetic complications without side-effects[22-25] in rats with alloxan or streptozotocin-induced hyperglycemia.

These studies proved that the using of such variety of dietary supplementation was more effective than amaryl for diminishing of genetic alterations (DNA damage and cytogenetic aberrations) and sperm abnormalities in rats with streptozotocin-induced hyperglycemia. So, the present study was designed to investigate the effect of supplementation with amaryl, whey protein, α-lactalbumin, Saccharomyces cerevisiae with chromium and mushroom on expression disorders of insulin and coagulation factor genes in streptozotocin-induced hyperglycemic male rats. In addition to evaluate the effect of amaryl on expression changes of FSH, LH and CYP2E1 genes in alloxan-induced hyperglycemic female rats.

MATERIALS AND METHODS

1-Streptozotocin – induced diabetic rats

Experimental Animals

Adult male albino rats weighting 150-160g, bred in the animal house lab. National Research Centre, 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/dl 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[26]. Amaryl treatment (as standard treatment) was used in this study for comparison with other treatments.

- **Induction or 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[27]. 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[28]. The rats with serum glucose level above 160 mg/dl were selected[28] and used for the present study.

Protein Materials

Whey protein (WP) and α-lactalbumin (α-lac) were purchased from Daviscon 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.

Production of Chromium Trichloride Rich Yeast (Organic Chromium Yeast)

Yeast strain of *Saccharomyces cerevisiae* F. 707 (Sc) was obtained as lyophilized powder from Microbial Chemistry Dept., National Research centre, Cairo, Egypt.

Sc was grown in 500 ml conical flasks containing 100 ml of sterilized growth medium, 100 ml of sugar cane molasses 50% fermentable sugars, 4.0 of Diamomium phosphate, 0.5 of magnesium sulphate, 0.02 of chromium trichloride. The conical flasks were sterilized by autoclaving at 12 °C for 20 minutes. The cooled sterilized flasks were inoculated with yeast strain, then incubated on a rotary shaker for 72 h at 32°C. The biomass was obtained by centrifugation at 3000 rpm for 10 minutes and washed several times with distilled water, then over dried at 70°C till constant weight. The obtained yeast biomass was adjusted using dried yeast (control free from biochrome) to give the demond two levels (0.2 and 0.4 ppm) of chromium rich yeast which used in our experiment. The organic chromium was determined according to Hasten.[29] The powder of *Saccharomyces cerevisiae* (Sc) or the powder of chromium rich yeast level 1 (ScCr$_1$) or level 2 (ScCr$_2$) were dosed at I x10^{10} Cfu in 0.6 ml distilled water and given once-a-day daily treatment.

Preparation of the Mushroom Extract

Stem bodies of mushroom (*Pleurotus ostreatus*) were obtained in a dried form, at a local indigenous medicinal shop, Cairo, Egypt. The dried plant parts were powdered and five grams of the powder were extracted with 100 ml of 95 % ethanol. The residue was filtered and concentrated to a dry mass by vacuum distillation, the filtrate thus obtained was used as mushroom extract. This extract of mushroom was dosed at two levels, low level (Mush$_1$) of 100 mg/kg.b. wt. and high level (Mush$_2$) of 200 mg/kg.b. wt.

Experimental Design

Male rats were randomly selected and divided into ten groups (five animals each). The first group included normal animals (control). The second group was diabetic animals. Whereas, the third, fourth, fifth, sixth, seventh, eighth, ninth and tenth groups consisted of diabetic animals treated with amaryl drug (AM group), whey protein (WP group), α-lactalbumin (α-lac group), yeast (Sc), ScCr$_1$, ScCr$_2$, Mush$_1$ and Mush$_2$, respectively. The third group was orally given 0.03 mg/kg. b.wt/dl of amaryl. The fourth and fifth groups were orally given WP (200 mg/kg. b.wt/dl) and α-lac (200 mg/kg.b.wt/dl) respectively. The sixth, seventh and eighth groups were orally given 10^{10} Cfu of each type.

The period of above treatments for diabetic animals extended for 30 days.

At the end of experiment, the animals were sacrificed by cervical dislocation for performing of gene expression studies of insulin 1, insulin II and coagulation factor genes.

2-Alloxan – induced diabetic rats

Materials

Animals

Females of adult albino rats weighing 150-160g, bred in the Animal House Lab, National Research Centre, Cairo, Egypt, were used. These animals were maintained under standard laboratory conditions and provided a standard diet and water *ad libitum*.

Drugs

Alloxan was purchased from Sigma (St. Louis, MO., USA).

Methods

Induction and assessment of diabetes

A single dose of alloxan monohydrate (150 mg/kg) was prepared in 10 % saline solution and injected intraperitoneally to induce diabetes[30]. Diabetes was confirmed after 72 h of alloxan 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[28]. The rats with serum glucose level above 200 mg/d1 were selected and used for the present study[31].

Experimental Design

Female rats were randomly selected and divided in three groups of six animals each. First group consisted of non-diabetic control animals, second group was the diabetic control, and third group consisted of diabetic animals treated with amaryl. The amaryl was orally administrated to dose 0.03 mg/kg b.wt/dl for 30 days. At the end of the experiment, the animals were sacrificed by cervical dislocation for gene expression studies of FSH, LH and CYP2 El genes.

Gene expression

Semi-quantitative RT-PCR

First-strand cDNA synthesis from extracted rat RNA

Total RNA (Poly(A)$^+$RNA) was extracted from 50mg of pancreas, liver and ovary tissues using the standard TRIzol extraction method (Invitrogen, Paisley, UK) and recovered in 100 μL diethylpyrocarbonate (DEPC)-treated water by passing the solution a few times through a pipette tip.

Total RNA was treated with one unit of RQ1 RNAse-free DNAse (Invitrogen, Karlsruhe, Germany) to digest DNA residues, re-suspended in DEPC-treated water, and quantified photospectrometrically at 260 nm.

Total RNA was assessed for purity from the ratio between quantifications at 260 nm and 280 nm, and was between 1.8 and 2.1. Integrity was verified with the ethidium bromide-stain analysis of 28S and 18S bands using formaldehyde-containing agarose gel electrophoresis.

Aliquots were either used immediately for reverse transcription (RT) or stored at -80°C.

To synthesize first-strand cDNA, 5 µg of complete Poly(A)$^+$ RNA was reverse transcribed into cDNA in a total volume of 20 µL using 1 µL oligo (poly(deoxythymidine)$_{18}$) primer[32]. The composition of the reaction mixture was 50 mmol L^{-1} MgCl$_2$, 10x RT buffer, 200 U µL^{-1} reverse transcriptase (RNase H free, Ferments, Leon-Rot, Germany), 10 mmol L^{-1} of each dNTP, and 50 µmol L^{-1} of oligo(dT) primer.
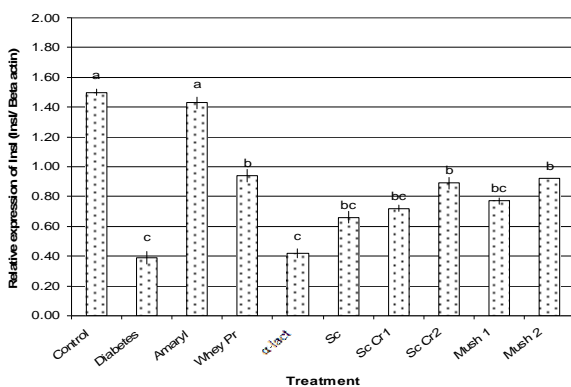
RT reaction was carried out at 25°C for 10 min, followed by 1 h at 42°C, and completed with denaturation at 99°C for 5 min. Reaction tubes containing RT preparations were then flash-cooled in an ice chamber until used for DNA amplification through polymerase chain reaction (PCR)[33].
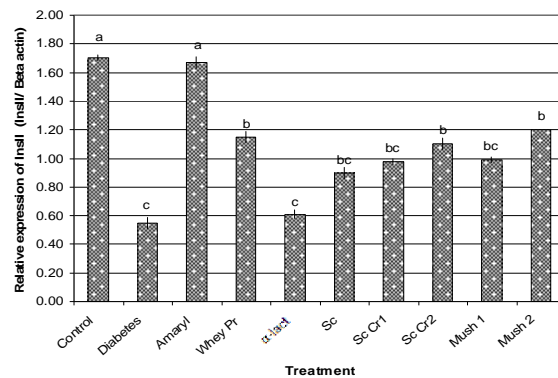
Statistical Analysis

All data were analyzed with one-way analysis of variance using SAS general linear models procedure[34] followed by Schaffer's test to assess differences between groups. The values are expressed as mean ± SEM. The level of significance was set at P<0.05.
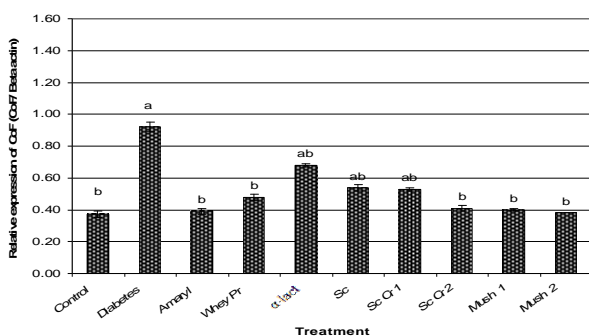
RT-PCR assay

First-strand cDNA was used as a template for semi-quantitative RT-PCR with a pair of specific primers in a 25-µL reaction volume. The sequences of specific primer and product sizes are listed in Tables 1 and 2. β-Actin was used to normalize mRNA levels of the target genes. The reaction mixture for RT-PCR consisted of 10 mmol L^{-1} dNTP's, 50 mmol L^{-1} MgCl$_2$, 10x PCR buffer, 1 U µL^{-1} taq polymerase, and autoclaved water. Tables 1 and 2 list the specific gene primer sequences and PCR cycling conditions. PCR products derived from β-actin were then loaded onto 2.0 % agarose gel. Each RT-PCR was repeated for each rat, generating at least ten new cDNA products per group.
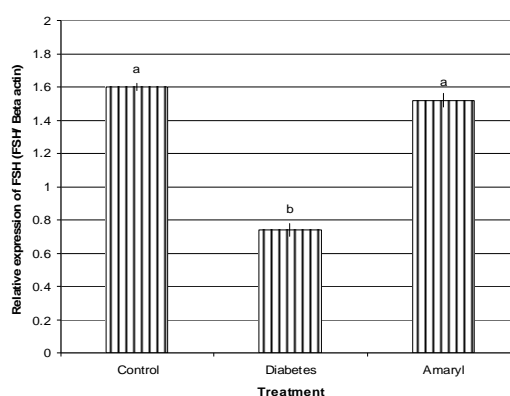

Figure 1: Semi-quantitative RT-PCR confirmation of Insulin I gene in pancreas tissues of streptozotocin-induced diabetic rats, diabetic rats treated with amaryl and diabetic rats treated with variety of dietary supplementations (whey protein, α-lac., Sc, ScCr$_1$, ScCr$_2$, Mush$_1$ and mush$_2$). Columns with different letters, differ significantly (P ≤ 0.05) for each other.

Figure 2: Semi-quantitative RT-PCR confirmation of Insulin II gene in pancreas tissues of streptozotocin-induced diabetic rats, diabetic rats treated with amaryl and diabetic rats treated with variety of dietary supplementations (whey protein, α-lac., Sc, ScCr$_1$, ScCr$_2$, Mush$_1$ and mush$_2$). Columns with different letters, differ significantly (P ≤ 0.05) for each other.
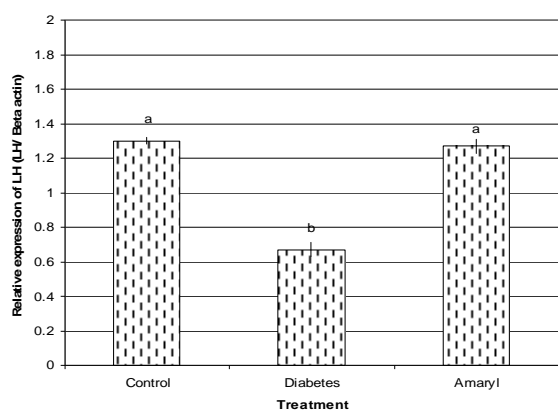

Figure 3: Semi-quantitative RT-PCR confirmation of coagulation factor gene in liver tissues of streptozotocin-induced diabetic rats, diabetic rats treated with amaryl and diabetic rats treated with variety of dietary supplementations (whey protein, α-lac., Sc, ScCr$_1$, ScCr$_2$, Mush$_1$ and mush$_2$). Columns with different letters, differ significantly (P ≤ 0.05) for each other.

Figure 4: Semi-quantitative RT-PCR confirmation of FSH gene in ovary tissues of alloxan-induced diabetic rats and diabetic rats treated with amaryl. "a" is significantly different from "b" (P≤ 0.05).
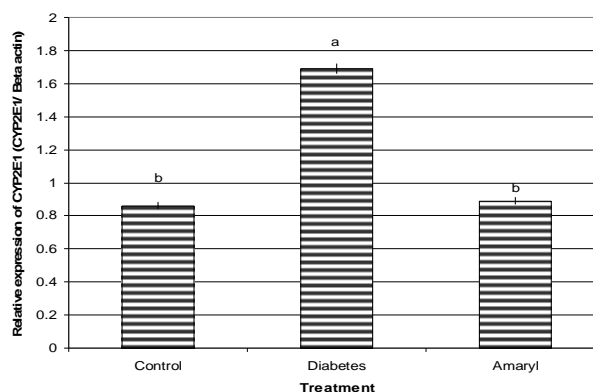
Figure 5: Semi-quantitative RT-PCR confirmation of LH gene in ovary tissues of alloxan-induced diabetic rats and diabetic rats treated with amaryl. "a" is significantly different from "b" (P≤ 0.05).

Figure 6: Semi-quantitative RT-PCR confirmation of CYP2E1 gene in ovary tissues of alloxan-induced diabetic rats and diabetic rats treated with amaryl. "a" is significantly different from "b" (P≤ 0.05).

Table 1: Primers and PCR thermo-cycling parameters for experiment 1

Gene	Primer séquence (5'-3')	Conditions of the PCR assay
Insulin I	CCT GTT GGT GCA CTT CCT AC	26 cycles: 95 °C for 30 sec, 58 °C for 1 min, and 72 °C for 30 sec. Final extension: 72°C, 5 min
	TGC AGT AGT TCT CCA GCT GC	
Insulin II	CAA CA TGG CCC TGT GGA TGC	26 cycles: 94°C, 30 s; 60°C, 30 s; 70°C, 1 min. Final extension: 72°C, 5 min
	AGT TGC AGT AGT TCT CCA GC	
Coagulation factor V	TCG AGA ACA AGA TAA ACC TAA AAC CAT	35 cycles: 94°C, 30 s; 55°C, 30 s; 69°C, 1 min. Final extension: 70°C, 5 min
	AGC TGG CGC TTT CAT CCA	
β-Actin	GTG GGC CGC TCT AGG CAC CAA	25 cycles: 94°C, 30 s; 65°C, 30 s; 68°C, 1 min. Final extension: 68°C, 2 min
	CTC TTT GAT GTC ACG CAC GAT TTC	

Table 2: Primers and PCR thermo-cycling parameters for experiment 2

Gene	Primer séquence (5'-3')	Conditions of the PCR assay
LH	GCA GGA TGG AGA GAT ACC AGG AG	40 cycles: 95 °C for 30 sec, 60 °C for 1 min, and 70 °C for 30 sec. Final extension: 72°C, 5 min
	ACA CTG GGC AGG CAT CGC	
FSH	AAT TCT GCA TCA GCA TCA ACA CC	40 cycles: 95 °C for 15 sec, 60 °C for 1 min, and 70 °C for 30 sec. Final extension: 72°C, 5 min
	GGT CTG ATC GGG TCC TTA TAT ACC	
CYP2E1	AGC ACA ACT CTG AGA TAT GG	32 cycles: 94°C, 30 s; 55°C, 30 s; 55°C, 1 min. Final extension: 70°C, 5 min
	ATA GCT ACT GTA CTT GAA CT	
β-Actin	GTG GGC CGC TCT AGG CAC CAA	25 cycles: 94°C, 30 s; 65°C, 30 s; 68°C, 1 min. Final extension: 68°C, 2 min
	CTC TTT GAT GTC ACG CAC GAT TTC	

RESULTS

Experiment 1: Assessment of the expression changes of *Insulin I*, *Insulin II* and *coagulation factor* genes in streptozotocin-induced hyperglycemic male rats.

The present results showed that the expression of Insulin1 gene was down-regulated in the pancreas tissues of streptozotocin-induced diabetic rats as compared to the control (Fig. 1).

The same trend observed in Insulin II gene, however, the expression value in Insulin II gene in the pancreas tissues

of diabetic rats was high compared with *Insulin1* gene (Fig. 1 and 2).

Supplementation of diabetic rats with amaryl increased highly significant the expression values of Insulin I and Insulin II genes in the pancreas tissues compared to diabetic rats alone. Furthermore, treatment of diabetic rats with whey protein, high dose of $ScCr_2$ (Sc plus 0.4 ppm of chromium) and high dose of mushroom (Mush$_2$)(200 mg/kg b.wt) increased significantly the expression values of Insulin I and Insulin II genes in the pancreas tissues compared to diabetic rats alone (Fig. 1 and 2). In addition, the treatment with Sc, low dose of $ScCr_1$ (Sc plus 0.2 ppm of chromium) and low dose of

mushroom (Mush$_1$) (100 mg/kg b.wt) increased the expression values of Insulin I and Insulin II genes in the pancreas tissues but without significant differences compared with diabetic rats alone. However, the expression values of Insulin I and Insulin II genes in the pancreas tissues of diabetic rats treated with α-lactalbumin didn't increase compared with diabetic rats alone (Fig. 1 and 2).

The expression of coagulation factor gene was up-regulated in the liver tissues of diabetic rats in respect to the control (Fig. 3). Treatment of diabetic rats with amaryl decreased significantly the expression value of the *coagulation factor* gene in the pancreas tissues compared to diabetic rats alone (Fig. 3). Furthermore, the expression level of *coagulation factor* gene was also significantly decreased due to treatment with whey protein and high dose of ScCr$_2$ (Sc plus 0.4 ppm of chromium) as well as low (Mush$_1$) (100 mg/kg b.wt) and high (Mush$_2$) (200 mg/kg b.wt) dose of mushroom in diabetic rats. The lowest expression level of the coagulation factor gene was observed when diabetic rats were treated with high dose of mushroom (200 mg/kg b.wt). Treatment of diabetic rats with Sc, low dose of ScCr$_1$ (Sc plus 0.2 ppm of chromium) and α-lactalbumin decreased the expression level of coagulation factor gene, however the decrease in the expression level was not significantly different compared with diabetic rats alone (Fig. 3).

Experiment 2: assessment of the expression changes of FSH and LH CYP2E1 genes in alloxan-induced hyperglycemic rats.

The present results showed that the expression values of FSH and LH genes in alloxan-induced hyperglycemic rats were down-regulated than those of the control (Fig. 4 and 5). FSH and LH genes were up-regulated in hyperglycemic rats supplemented with amaryl. The expression values were significantly higher than those assessed in diabetic rats alone (Fig. 4 and 5).

Assessment of the expression of CYP2E1 gene in alloxan-induced diabetic rats showed high level of expression as compared to the control (Fig.6). However, the lowest levels of expression of CYP2E1 gene were observed in diabetic rats treated with amaryl compared with those assessed in diabetic rats without any other treatment (Fig 6).

DISCUSSION

The present results showed down-regulation of the expression of insulin I and insulin II genes as well as increase in the expression of coagulation factor gene in streptozotocin-induced hyperglycemic rats as compared to the control. Moreover in alloxan-induced hyperglycemic rats, the levels of expression of FSH and LH genes were significantly decreased, however, the expression level of CYP2E1 gene significantly increased as

compared to the control. These effects appeared to be mediated through the oxidative stress and inducing of reactive oxygen species (ROS) due to the presence of hyperglycemia condition[35,36]. 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 other reactive oxygen species[35,36]. ROS attack the building structures of the cell membrane, nucleus and genetic material by causing scission, carbonylation, fragments, cross-linking and oxidation[37,38] inducing DNA modification[39] and consequently leads to changes in gene expression. Otton.[40] reported that the occurrence of DNA mutation in lymphocytes of diabetic rats was found to be 81% compared to 45% of untreated cells of the control. Also, Ghaly[23], Booles[24], and Ahmed[25] showed that STZ diabetic rats had higher rates of DNA mutation (DNA fragmentation and deletion or disappear of some base pairs of DNA according to ISSR-PCR analysis) comparing with normal control, these authors attributed such abnormal effects to the dangerous role of the presence of hyperglycemia condition, and they reported that this condition is a good marker for inducing overproduction of reactive oxygen species. Furthermore, the presence of hyperglycemia condition due to alloxan treatment in both male and female rats led to significant increases in rates of DNA fragmentation compared to normal control[22]. So, the down-regulation of expression of insulin genes in the present study may be due to the presence of hyperglycemia condition that induced the generation of ROS. ROS in hyperglycemia cases were found to decrease the flux of insulin mRNA[4]. Moreover, ROS due to high glucose concentration suppressed the first phase of glucose-induced insulin secretion[4].

The up-regulation of expression of coagulation factor gene in the present study was similar with that reported by Ceriello[41,42] who found that acute elevation of plasma glucose concentrations during oral glucose tolerance testing or intravenous glucose infusion had increased plasma factor VII coagulation (FV II C) activity and plasma prothrombin fragment 1.2 (F1.2) (as indicator of thrombin generation). Also, elevated plasma FVIIa, FVIIC, FVIIIC and other coagulation proteins have been reported in patients with type 2 diabetes and associated with pre-existing hyperglycemia[43].

Michiel[44] suggested that patients with hyperglycemia are susceptible to thrombotic events by a concurrent-insulin-driven impairment of fibrinolysis and a glucose-driven activation of coagulation.

On the other hand, the down-regulation of expression of FSH and LH genes in the present study is in agreement with the previous studies which reported that the presence of hyperglycemia condition was the main factor for reduction the levels of FSH and LH in hyperglycemic male rats[17]. Also, Seethalakshmi[18] reported that hypophysis of diabetic rats has a blunted response with diminished stimuli-induced secretion of FSH and LH. Moreover, the hyperglycemia-related oxidative stress

might be the trigger for many disorders of such reproductive hormones[14,15].

Concerning the over-expression of CYP2E1 in the present results were similar with that reported by Enriquez[21], who observed that the increased expression of CYP2E1 has been associated with obesity, type 2 diabetes and reproductive toxicity in diabetic males.

In addition, over-expression of CYP2E1 has been reported in association with diabetes[45]. The over-expression of such gene in this study might be due to the toxic effect of alloxan or alloxan-induced oxidative stress. This because the CYP2E1 is the classical ethanol-inducible CYP, and its expression was found to be highly significant in the presence of variety of toxins[20]. CYP has been shown to catalyze the bioactive of several procarcinogens and protoxins including N-nitrosodimethylamine, benzene and Nalkylformamides[20].

The undesirable alterations in gene expression levels in the present study due to streptozotocin or alloxan-induced hyperglycemic condition were diminished or improved using treatment with amaryl (Am) or with variety of dietary supplements (WP, α-lac., Sc, ScCr$_1$, ScCr$_2$, Mush$_1$ and Mush$_2$). The results showed that such materials were able to increase the expressions of Ins.I and Ins.II genes as well as to decrease the expression of coagulation factor gene than that was observed with untreated diabetic group. The treatment with AM was more effective for improvement of such gene expression than treatment with variety of dietary supplementations. Also, the treatment with WP, ScCr$_2$ and Mush$_2$ were pronounced for amelioration of expressions of insulin (I, II) and coagulation factor genes than α-lac, Sc, ScCr$_1$ and Mush$_1$, respectively. The improvement of expression of coagulation factor gene due to the treatment with ScCr$_2$, Mush$_1$ and Mush$_2$ were relatively similar to that found by treatment with amaryl. Moreover, the treatment with amaryl for alloxan-induced diabetic rats led to significant improvement in each of expression of FSH, LH and CYP2E1 genes compared to untreated diabetic group. The levels of expressions of such reproductive genes in amaryl group were relatively identical with that revealed in the normal control.

For our knowledge there are no data regarding the role of AM and variety of dietary supplementations (WP, α-lac, Sc, ScCr$_1$, ScCr$_2$, Mush$_1$ and Mush$_2$) on the expression of insulin and coagulation factor genes. Also, there is a lack of information concerning the effect of AM on expression of FSH, LH and CYP2E1 genes. However, in several studies the curing or the treatment of hyperglycemia condition and amelioration of genetic disorder has been shown in diabetic animals by using artificial drug (AM) or by natural products (WP, α-lac, Mush$_1$, Mush$_2$, Sc, ScCr$_1$, ScCr$_2$,)[22-25].

These studies revealed that such materials are important source of strong antioxidants and have potent free radical-scavenging activities and this can be explained as follows:

The antioxidant effect of amaryl

This drug was found to be a third generation antidiabetic sulphonylurea that known to possess the ability for reduction of blood glucose levels and the antioxidant effect in each of streptozotocin (STZ)-induced diabetes[28] and alloxan-induced diabetes[28,22].

Kramer[46] and Rabbani[28] reported that the primary mechanism of action of amaryl in lowering the blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells. These authors also observed that glimepiride has antidiabetic therapy advantage that it does not cause severe hypoglycemic complications due to sudden release of insulin as like the other sulphonylurease. Also, Krauss[47] and Rabbani[28] 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 respect to antimutagenic effect of amaryl, Rabbani[28] revealed that the administration of glimepiride to the STZ diabetic rats had reduced the populations of micro-nucleated erythrocytes and sperm abnormalities besides enhancing the sperm count compared to diabetic control. Also, Abd El-Rahim[22] found that amaryl treatment in alloxan diabetic rats had reduced genetic alterations (populations of micro-nucleated erythrocytes, DNA fragmentation and chromosome aberrations) and sperm abnormalities besides enhancing the sperm count compared to diabetic control. Ghaly[23]; Booles[24] and Ahmed[25] found that the treatment with amaryl in STZ diabetic rats significantly decreased the genetic alterations (DNA fragmentation, disappear or deletion of some base pairs of DNA according to ISSR-PCR analysis and chromosome aberrations) and sperm abnormalities as compared to untreated diabetic rats.

The antioxidant Effects of WP and α-lac

These protein materials have the ability to reduce blood glucose excursion and scavenge active oxygen species[48,24]. Whey protein is particular high in branch-chain amino acids in particular leucine. These amino acids are insulinogenic, meaning that they have a higher capacity to increase an insulin response and consequently decrease the blood glucose levels[49,50]. Also the amino acids such as histidine and tyrosine that are major structure in WP had been reported to have antioxidant activities by scavenging radicals or ROS and other peroxides[51]. Moreover, α-lactalbumin was found to be important agent for regulation the lactose synthesis[52] and to act as an antioxidant factor by having activity of radical scavenging[51,52].

Concerning the antimutagenic role of WP and α-lac: Booles[24] showed that the treatment with WP or with α-lac. in diabetic rats significantly decreased genetic alterations (DNA fragmentation, disappear or deletion of some base pairs of DNA according to ISSR-PCR analysis and chromosome aberrations) and sperm abnormalities

than untreated diabetes, the treatment with α-lac in diabetic animals was more effective for decreasing or repairing the genetic alterations and sperm abnormalities than treatment with WP and there were significant differences between the two treatments.

The antioxidant effect of chromium-enriched Saccharomyces cerevisiae

It was reported that the supplementation of well-controlled type diabetes with chromium-enriched yeast can result in amelioration in blood glucose variables, carbohydrates and lipid metabolism and oxidative stress[53,54]. Moreover, Duman[55] revealed that the trivalent chromium (CrIII) was known to possess the ability for reduction of blood glucose levels in diabetes. Duman[55] and Pattar[56] explained the mechanism of action of Cr III for reducing the blood glucose in diabetic subjects, this mechanism was showed by altering (or correcting) the plasma membrane composition of cholesterol in fat and muscle cells, or by inducing a loss of plasma membrane cholesterol. Also, Vladevea[57] reported that the treatment with chromium has been found to improve the first phase of insulin secretion or facilitate post-receptor insulin sensibility as a way of potentiating the insulin action. In addition, Duman[55] proved that chromium has able to enhance the action of insulin at adipocytes by increasing intracellular triglyceride synthesis and decreasing extracellular lipid. Moreover, the treatment with chromium has been observed to avoid rat cells from oxidative damage related to carbon tetrachloride exposure[58].

In respect to the antimutagenic role of Sc or ScCr: Ahmed[25] demonstrated that the administration of yeasts (Sc) with (ScCr$_1$ or ScCr$_2$)or without chromium (Sc) to diabetic rats led to significant decreases of genetic alterations (DNA fragmentation, absence or disappear of some base pairs of DNA according to ISSR-PCR analysis and chromosome aberrations) and sperm-shape abnormalities as compared to untreated diabetic animals, the authors found that chromium yeast groups had significant decreases of genetic alterations and sperm abnormalities (especially in ScCr$_1$ group) than Sc group. ScCr$_1$ treatment was the more effective for decreasing the most of genetic alterations and sperm abnormalities than ScCr$_2$ and there were significant differences between the two groups.

The antioxidant effect of mushrooms

These medicinal plants were found to have potent free radical-scavenging activities[59]. Methanolic extract from medicinal mushrooms was studied by Mau[60] and showed an excellent antioxidant activity (especially total phenol) by scavenging and chelating abilities on 1,1 – diphenyl-2-picrylhydrazl radical. Also, the ethanolic extract from medicinal mushroom was found to be able for protection against acute hepatotoxicity induced by administrations of carbon tetrachloride (CCL$_4$) in rats[61]. These authors reported that the levels of hepatic constituents

(malandaldohide (MDA), glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and serum enzymes (glutathione oxaloacetic transaminase (SGOT), glutamic pyruvate transaminase (SGPT) and alkaline phosphatase (SALP) were relatively similar with that found in the normal control and these levels of parameters are in contrast with that revealed in rats receiving CCL$_4$ alone.

Considering the antimutagenic activities of mushrooms: Several studies isolated and purificated some of relevant antimutagenic compounds of these medicinal plants especially poly-saccharides[62] and Lentiman[63]. These compounds were found to possess strong immunomodulation and anticancer or antitumor activities. Ooi and Liu[64] reported that polysaccharides have potentiate the host's innate (non-specific) and acquired (specific) immune responses and activate many kinds of immune cells that are important for the maintenance of homeostasis, e.g. host cells (such as cytotoxic macrophages, monocytes, neutrophils, natural killer cells, dendritic cells) and chemical messengers (cytokines such as interleukins, interferon, colony stimulating factors) that trigger complement and acute phase responses. Also, several studies by Wasser[65] and Mahajna[66] suggested that these poly-saccarides have immunomodulating properties, including the enhancement of lymphocyte proliferation and antibody production as well as producing both anti-genotoxic and antitumor promoting activities. Moreover, Ghaly[23] (2011) reported that the treated hyperglycemic rats with low level (100 mg/kg. b.wt/dl) or with high level (200 mg/ Kg. b.wt/dl) had significant decreases of genetic alterations (DNA fragmentation, deletion or disappear of some base pairs of DNA according to ISSR-PCR analysis and chromosome aberrations) and sperm abnormalities than untreated hyperglycemic animals. The results showed that the treatment with high level was better than the low level for reduction of each of genetic alterations and sperm abnormalities.

In conclusion, the present study revealed that the treatment with AM, WP, α-lac, chromium yeast and mushroom extract could improve the insulin genes (I and II) expressions in streptozotocin diabetic male rats. However, AM treatment was more effective.

The amelioration of coagulation factor gene expression in diabetic male rats was pronounced in the treatment with AM, WP, ScCr$_2$ and mushroom extract, and expression levels especially in ScCr$_1$ and extract treatments were similar with that found with AM treatment. Also, the treatment with AM for alloxan diabetic females has succeeded in counteracting the changes in reproductive genes expression (FSH, LH and CYP2E1).

of Microbial Chemistry Department, NRC for providing variety of *Saccharomyces cerevisiae* yeast.

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