Study of Therapeutical Role of Chromium-Enriched Yeast
(Saccharomyces cerevisiae) on Genetic Alterations and
Sperm Abnormalities in Streptozotocin-Induced Hyperglycemic Rats

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Abstract: The present study was designed to evaluate the therapeutical role of chromium-enriched yeast saccharomyces cerevisiae (Sc Cr) on genetic alterations and sperm changes in STZ-induced hyperglycemic rats. Six groups of male rats were used and included a group of non-diabetic control animals, the second group was of diabetic animals, third, fourth, fifth and sixth groups consisted of diabetic animals treated with amaryl (AM), yeast without chromium (Sc), yeast with low level (ScCr) of chromium (0.2 ppm) and yeast with high level of (ScCr) of chromium (0.4 ppm), respectively. The treated diabetic animals were orally given the AM, Sc, ScCr1 and ScCr2 for 30 days. AM treatment (as a standard treatment) was used for comparison with yeast treatments. Also, Sc treatment was used as control for chromium yeast treatments. The results showed that STZ diabetic rats had significant elevation of blood glucose level (BGL), genetic alterations (including increases of DNA fragmentation, absence or disappear of some base pairs of DNA according to ISSR-PCR analysis and increases of the frequencies of chromosome aberrations) and sperm-shape abnormalities compared to normal control. Whereas, the administration of amaryl and yeasts with or without chromium to diabetic rats led to significant decreases of BGL, genetic alterations and sperm abnormalities compared to untreated diabetic animals. When comparing the AM group with yeast groups, it can be seen that the BGL decreased in AM group than those of yeast groups (Sc, ScCr1 and ScCr2), this decrease was significant than those of Sc group and not significant than those of ScCr1 or ScCr2 groups. However, the yeast groups (Sc or ScCr1 or ScCr2) had significant decreases of DNA fragmentation compared to AM group. Also, ISS-PCR analysis especially by using B14 primer showed that many fragments of base pairs of DNA which disappeared in diabetic or amaryl treatments, they have been existed in yeasts with chromium treatments especially ScCr1 group. Moreover, the diabetic animals treated with yeasts with or without chromium had significant decreases of most frequencies of chromosome aberrations compared to AM group. Sperm abnormalities were also decreased in yeast groups with or without chromium than those of AM group and significant differences were observed between AM and Sc groups especially for total sperm head abnormalities (TSHA) and total sperm abnormalities (TSA) (head + tail) and between AM and chromium yeasts groups especially for TSA, tail abnormalities (TA) and TSA. When comparing the yeast groups with each other, it was observed that chromium yeasts groups had significant decreases of BGL, genetic alterations and sperm abnormalities (especially in ScCr1) than Sc group. On the other hand, the BGL was approximately similar in each of ScCr1 and ScCr2 treatments and there were no significant differences between the two groups. However, ScCr2 treatment was the more effective for decreasing the most of genetic alterations and sperm abnormalities than ScCr1 and there were significant difference between the two groups. In conclusion, the administration of amaryl and yeasts with or without chromium to diabetic rats led to significant decreases of BGL in hyperglycemic rats. Moreover, the treatments with chromium yeasts had succeeded in counteracting of genetic aberrations and sperm abnormalities in diabetic disease and they were more effective than amaryl treatment.

Keywords: Hyperglycemia • Rats • Saccharomyces cerevisiae • Molecular Genetics • Cytogenetics • Sperms

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INTRODUCTION

Diabetes mellitus had been reported to be often associated with the presence of hyperglycemia condition [1-4]. Hyperglycemia status has been known as production sources of free radicals and other reactive oxygen species (ROS) [5, 6]. Oxygen-derived radicals and ROS are known to attack cell membranes and cellular components resulting in DNA and protein modification and lipid peroxidation [4, 7, 8]. DNA fragmentation, micronuclei populations and chromosome aberrations have been observed to be significant higher in hyperglycemia condition of alloxan diabetic rats compared to normal animals [9]. On the other hand, the risk of oxidative stress not only reported in the present generation of diabetic patients but can also transmit the nuclear defects to their progeny [4, 10]. Some studies have been published suggesting that semen quality and fertility are commonly affected by hyperglycemia condition in man and rats [4, 10, 11]. The streptozotocin (STZ) or alloxan diabetic condition of male rats significantly increased the sperm shape abnormalities besides significant reducing of caudal sperm count [4, 10]. Moreover, the total antioxidant status in diabetic mellitus had been found to be lower than of age-matched controls, a finding which might be attributable to lower levels of antioxidant micronutrients including trace elements in blood [6, 12-14]. Chromium is an important trace element in the regulation of blood glucose and immune response in humans and laboratory species [15]. Chromium occurs in both trivalent and hexavalent forms. Trivalent chromium (Cr III) is considered the biologically active form and known to be a structural one of a glucose tolerance factor which potentiates the action of insulin and is an essential trace mineral for normal metabolism of carbohydrates and lipids [16, 17]. Pattar et al. [18] reported that trivalent chromium (Cr III) supplementation may lower blood glucose by altering the plasma membrane composition of cholesterol in fat and muscle cells. However, the plasma membrane cholesterol content of cells from non-diabetic subjects with normal insulin sensitivity and glucose tolerance would be lower and not be affected by Cr III. Moreover, some studies reported that the supplementation of well-controlled type diabetes with chromium-enriched yeast is safe and can result in improvements in blood glucose variables, carbohydrates and lipid metabolism and oxidative stress [19, 20]. On the other hand, Lai [6] reported that chromium could also improve cellular antioxidant capacity in rats. Furthermore, a number of in vivo studies have not find any toxicity [12] or mutagenic potential [21, 22] of Cr III in laboratory animals or humans. Therefore, the present study was designed to evaluate the therapeutical role of chromium-enriched yeast on the potential mutagenic effects and sperm changes in STZ-induced hyperglycemic rats.

MATERIALS AND METHODS

Experimental Animals: Male adult albino rats weighing 150-160 g, bred in the animal house lab. National Research Center, Cairo, Egypt, were used in the 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).

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 at dose 0.03 mg/kg b.wt/dl for 30 days. This dose equals the dose of acceptable daily intake of amaryl for human (4 mg/kg), after modification to suit the small weight of rats. The dose of amaryl was based on previous studies [23, 24]. Amaryl (Glimepiride) treatment (as a standard treatment) was used in this study for comparison with other yeast treatments.

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 center, Cairo, Egypt.

Sc was grown in 500 ml conical flaks containing 100 ml of sterilized growth medium, 100 ml of sugar cane molasses 50% fermentable sugars, 4.0 of Diamonium phosphate, 0.5 of magnesium sulphate, 0.02 of chromium trichloride. The conical flaks were sterilized by autoclaving at 121°C for 20 minutes. The cooled sterilized flaks 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 demand 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 et al. [25]. The powder of Saccharomyces cervisiae (Sc) or the powder of chromium rich yeast level, (ScCr) or level2 (ScCr), were dosed at 1x10⁶⁰ Cfu in 0.6 ml distilled water and given once-a-day daily treatment.

**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 [26]. 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 [4, 8]. 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 [27]. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

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

**ISSR-PCR Analysis**

**DNA Extraction:** Genomic DNA was isolated from the liver of the tested animals according to the method of Sharma et al. [28]. 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 procured 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 Taq Polymerase (Promega, USA). Reactions were performed in a thermocycler (Biometra, GmbH).

ISSR amplification was performed according to Zietkiewicz et al. [29] 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 2 min.

**Table 1:** Primer sequences used for ISSR amplification:

<table>
<thead>
<tr>
<th>ISSR names</th>
<th>Primer sequences</th>
</tr>
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<tbody>
<tr>
<td>HB-10</td>
<td>5′ GAG AGA GAG AGA CC 3′</td>
</tr>
<tr>
<td>HB-12</td>
<td>5′ CAC CAC CAC GC 3′</td>
</tr>
<tr>
<td>HB-14</td>
<td>5′ CTC CTC CTC GC 3′</td>
</tr>
</tbody>
</table>
and a final extension at 72°C for 7min. 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 electrophoretic 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:** 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 [30]. 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 [31]. 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 [32], Wyrobek et al. [33] and Farag et al. [34]. At least 3000 sperms per group were assessed for morphological abnormalities. The sperm abnormalities were evaluated according to standard method of Narayana [35].

**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 in the same sex. 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 present results (Table 2 and Fig. 1) showed that the blood glucose levels (BGL) significantly increased in diabetic animals (D group) than those of control group. Whereas, the BGL were significantly decreased in diabetic animals treated with amaryl (AM group) or Sc or ScCr, or ScCr1, than those of D group. The BGL decreased in AM group than those of yeast groups (Sc or ScCr1 or ScCr2) and this decrease was significant than those of Sc group and not significant than those of ScCr1 or ScCr2 groups. On the other hand, the ScCr1 or ScCr2 groups had significant decreases of BGL than Sc group. However, the difference between ScCr1 and ScCr2 for BGL was not significant.

**DNA Fragmentation:** The results in Table 3 showed that the rates of DNA fragmentation significantly increased in diabetic animals (D group) than those of control group. Whereas, the diabetic animals treated with amaryl (AM group), Sc or ScCr, or ScCr1 had significant decrease of DNA fragmentation than D group. On the other hand, the yeast groups (Sc or ScCr1 or ScCr2) had significant decreases of DNA fragmentation compared to AM group.

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**Table 2:** Effect of yeasts with or without chromium on blood glucose levels (BGL) in diabetic rats after 30 days of treatments

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Streptozotocin</th>
<th>AM</th>
<th>Sc</th>
<th>ScCr1</th>
<th>ScCr2</th>
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<tr>
<td>BGL</td>
<td>63.60±1.75</td>
<td>B 165.07±3.55</td>
<td>C 93.80±4.14</td>
<td>D 117.60±2.18</td>
<td>C 103.00±1.95</td>
<td>C 99.00±2.59</td>
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</tbody>
</table>

- 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
- Sc = Treatment with yeast without chromium
- ScCr = Treatment with chromium-enriched yeast at level1 (0.2 ppm)
- ScCr1 = Treatment with chromium-enriched yeast at level1 (0.2 ppm)

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Table 3: Effect of AM, Sc, ScCr₁, and ScCr₂ treatments on the rates of DNA fragmentation in STZ-induced hyperglycemic (diabetic) male rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% of DNA Fragmentation M ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.25±0.1a</td>
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<tr>
<td>D</td>
<td>20.57±0.74e</td>
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<tr>
<td>D + AM</td>
<td>17.29±0.40d</td>
</tr>
<tr>
<td>D + Sc</td>
<td>13.87±0.56c</td>
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<td>D + ScCr₁</td>
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<tr>
<td>D + ScCr₂</td>
<td>10.23±0.49b</td>
</tr>
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</table>

- Data were expressed as mean ± S.E.
- Means with different superscript letters (a, b, c, d, e) are significantly different (P<0.05)
- D = Diabetic (hyperglycemic) condition. - AM = Amaryl treatment
- Sc = Treatment with yeast without chromium
- ScCr₁ = Treatment with chromium-enriched yeast at level (0.2 ppm)
- ScCr₂ = Treatment with chromium-enriched yeast at level (0.4 ppm)

Moreover, the rates of DNA fragmentation significantly decreased in ScCr₁ or ScCr₂ groups than those of Sc group. In addition to ScCr₁ group, had the lowest rate of DNA fragmentation compared to ScCr₂ group and there were significant differences between the two groups.

**ISSR-PCR Analysis:** Three ISSR primers; HB10, HB12 and HB14 were used to detect the chromium effects in yeast addition for hyperglycemia comparing with Amaryl drug in rats (Fig. 2 and Table 4). Using primer HB10, diabetic rats and Amaryl drug samples revealed an equal four fragments which were differed from the control samples in a 150 bp fragments. On the other hand, the two yeast samples with low and high chromium as well as yeast without chromium displayed four amplified fragments, while a 610 bp fragments disappeared comparing with the control, diabetic and Amaryl drug samples.

Moreover, ISSR primer HB-12 revealed a total six fragments in the control and in ScCr₁ groups and seven fragments in ScCr₂ group. However, in diabetic, Am and Sc groups the primer Hb-12 revealed five fragments. Diabetic and Am groups were similar with each other and they differed than control of 230 bp fragment. On the other hand, 500bp and 390bp fragments were disappeared in the control, diabetic and Am groups, while, they existed in Sc, ScCr₁ and ScCr₂ groups. However, 700 and 590 bp fragments were disappeared in Sc and ScCr₁ groups and they were existed in other groups. Whereas, 230bp fragment was existed in the ScCr₁ and control group and disappeared in the other groups.

The obtained results by using the primer HB-14 revealed total No. of fragments (8) existed in yeast samples with low chromium concentration and control. The results were evidently observed that the 490 pb, was existed only in yeast samples with low and high chromium concentration and control while it disappeared in each of diabetic rats and Amaryl drug samples. Also, the fragment of 290 bp was also existed in yeast without chromium, yeast samples with low and high chromium concentration and control, while, it disappeared in diabetic rats and amaryl drug samples. On the other hand, the fragment of 250bp was disappeared in diabetic group, while it was existed in other group. Moreover, the fragment of 50 bp was found in each of yeast without chromium, yeast with low chromium concentration and control and disappeared in each of diabetic, amaryl and ScCr₂ groups.

**Chromosome Examination:** Cytogenetic or chromosome examination (Table 5) showed that the frequencies of structural and numerical chromosomes aberrations were significantly increased in diabetic animals than those of control group. In contrast the diabetic animals treated with amaryl (AM group)
Fig. 2: ISSR amplified products using three ISSR primers; HB-10, HB-12 and HB-14
M= Marker. Diab= Diabetes  
Yeast = Yeast without chromium  
ScCr₁ = Treatment with chromium-enriched yeast at level 1 (0.2ppm). 
ScCr₂ = Treatment with chromium-enriched yeast at level 2 (0.4ppm).
Table 4: ISSR analysis using three ISSR primers; HB-10, HB-12 and HB-14

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<th>ISSR primers</th>
<th>Band No.</th>
<th>M <a href="bp">1</a></th>
<th>Control</th>
<th>Diab.</th>
<th>AM</th>
<th>Sc</th>
<th>ScCr1</th>
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Sc = Treatment with yeast without chromium
ScCr1 = Treatment with chromium-enriched yeast at level (0.2 ppm)
ScCr2 = Treatment with chromium-enriched yeast at level (0.4 ppm)

Table 5: Effect of AM, Sc, ScCr1 and ScCr2 treatments on the frequency of chromosome aberrations in STZ-induced hyperglycemic (diabetes) male rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Gaps</th>
<th>Deletions</th>
<th>Breaks</th>
<th>C.A.</th>
<th>EEA</th>
<th>Rings</th>
<th>Structural Chromosomal Aberrations</th>
<th>Total structural chromosomal aberrations</th>
<th>Peri diploidy</th>
<th>Poly ploidy</th>
<th>Numerical aberrations</th>
<th>Total numerical aberrations</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.6±0.24</td>
<td>0.0±0.0</td>
<td>0.4±0.24</td>
<td>1.2±0.2</td>
<td>0.2±0.2</td>
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<td>1.4±0.24</td>
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</tr>
<tr>
<td>D</td>
<td>2.2±0.2</td>
<td>7.8±0.48</td>
<td>3.2±0.58</td>
<td>3.4±0.4</td>
<td>3.2±0.4</td>
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<tr>
<td>D + AM</td>
<td>1.4±0.24</td>
<td>4.2±0.49</td>
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<td>4.2±0.58</td>
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</tr>
<tr>
<td>D + Sc</td>
<td>1.4±0.24</td>
<td>4.2±0.49</td>
<td>1.0±0.32</td>
<td>4.2±0.58</td>
<td>1.8±0.37</td>
<td>0.4±0.24</td>
<td>14.2±0.58</td>
<td>3.2±0.37</td>
<td>1.2±0.37</td>
<td>4.4±0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D + ScCr1</td>
<td>1.16±0.31</td>
<td>1.16±0.17</td>
<td>0.5±0.22</td>
<td>0.5±0.22</td>
<td>0.16±0.4</td>
<td>0.33±0.21</td>
<td>3.8±0.4</td>
<td>2.66±0.21</td>
<td>0.0±0.0</td>
<td>2.66±0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D + ScCr2</td>
<td>0.8±0.37</td>
<td>2.8±0.3</td>
<td>0.8±0.2</td>
<td>0.4±0.24</td>
<td>0.4±0.24</td>
<td>0.2±0.2</td>
<td>5.4±0.4</td>
<td>2.6±0.4</td>
<td>0.6±0.24</td>
<td>3.2±0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- 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 = hyperglycemic (diabetic) condition
- AM = Amaryl treatment
- Sc = Treatment with yeast without chromium
- ScCr1 = Treatment with chromium-enriched yeast at level (0.2 ppm)
- ScCr2 = Treatment with chromium-enriched yeast at level (0.4 ppm)
- C.A. = Centromeric Attenuations
- EEA = End to End Associations
Table 6: Sperm abnormalities in hyperglycemic (diabetic) condition and hyperglycemia (diabetes) treated with AM, Sc, ScCr1 and ScCr2 of male rats

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total abnormal sperms [1] (head)</th>
<th>Total abnormal sperms [1] (head and tail)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.7±0.25*</td>
<td>15.75±1.2*</td>
</tr>
<tr>
<td>D</td>
<td>28.8±1.3*</td>
<td>39.0±0.1*</td>
</tr>
<tr>
<td>D + AM</td>
<td>24.5±1.5*</td>
<td>34.75±2.2*</td>
</tr>
<tr>
<td>D + Sc</td>
<td>18.5±1.2*</td>
<td>26.7±1.6*</td>
</tr>
<tr>
<td>D + ScCr</td>
<td>16.25±0.75*</td>
<td>22.25±1.3*</td>
</tr>
<tr>
<td>D + ScCr2</td>
<td>20.0±0.7*</td>
<td>27.5±1.3*</td>
</tr>
<tr>
<td></td>
<td>Data were expressed as mean ± S.E.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Means with different superscript letters (a, b, c, d, e) are significantly different (P&lt;0.05)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D = Diabetic (hyperglycemic) condition</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AM = Amaryl treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sc = Treatment with yeast without chromium</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ScCr1 = Treatment with chromium-enriched yeast at level1 (0.2 ppm)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ScCr2 = Treatment with chromium-enriched yeast at level2 (0.4 ppm)</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Induction of diabetes in laboratory animals is a convenient and useful strategy in the understanding and treatment of the hyperglycemia disease. The hyperglycemia condition was induced in the present work by injection of an appropriate dose of streptotozocin.

The present results showed that the blood glucose levels were significantly increased in streptozotocin (STZ) diabetic rats compared to those found in normal healthy animals (control). These findings of the presence of hyperglycemia condition are in agreement with that reported in other studies on STZ rats [4,36] and also similar with that found in alloxan diabetic mice [37] and rats [9, 38]. The induction of hyperglycemia may be due to cytotoxic effect of STZ on pancreatic beta-cells causing insulin deficiency [39, 40]. Also, Weir et al. [2] and Arulmozhi et al. [3] proved basal hyperglycemia and abnormal glucose tolerance after 6 weeks of age of STZ rats. Also, previous observations by Portha et al. [1] found in the insulin secretion studies of the 10-16 week-old STZ rats, that there was a complete loss of B-cell sensitivity to glucose.

or Yeast group (Sc or ScCr1 or ScCr2) had significant decreases of most frequencies of structural and numerical chromosome aberrations compared to D group. On the other hand, the diabetic animals treated with Sc or ScCr1 or ScCr2 had significant decreases of most frequencies of chromosome aberrations compared to AM group. Moreover, chromosome aberrations were lowered in ScCr1 or ScCr2 groups than those of Sc group. The differences between Sc and ScCr1 or ScCr2 groups were significant especially for total structural aberrations. However, the differences for the frequencies of chromosome aberrations between ScCr1 or ScCr2 were not significant and the only exception to this the ScCr1 had significant decrease of deletions than ScCr2.

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 (AM group) or Sc or ScCr1 or ScCr2 had significant decreases of sperm abnormalities than D group especially for total sperm head abnormalities (TSHA) in AM group and for most frequencies of sperm abnormalities (head or tail) in yeast (Sc or ScCr1 or ScCr2) groups. On the other hand, sperm abnormalities were decreased in yeast groups than those of AM group and significant differences were observed between AM and Sc groups especially for TSA and total sperm abnormalities (TSHA) (head + tail). Also significant differences were found between AM and ScCr1 or ScCr2 groups especially for TSA, tail abnormalities (TA) and TSA. Moreover, ScCr1 group had the lowest frequencies of sperm abnormalities compared to Sc or ScCr2 groups. Significant differences were observed between ScCr1 and Sc for TA and TSA and between ScCr1 and ScCr2 for TSHA. However, the differences between ScCr2 and Sc groups for sperm abnormalities were not significant.
The present study showed that the administration of yeasts (including Sc, ScCr, and ScCr2) and amaryl drug led to significant reduction of blood glucose levels (BGL) in diabetic rats compared to non-treated diabetic animals. The BGL decreased in AM group than those of yeast groups and this decrease was significant than those of Sc group and not significant than those of ScCr1 or ScCr2 groups. The ScCr1 or ScCr2 groups had significant decreases of BGL than Sc group. However, the difference between ScCr1 and ScCr2 for BGL was not significant.

The amaryl drug was used in this study as a standard treatment, because the amaryl (Glimepiride) belongs to the third generation antidiabetic sulphonylurea known to possess the ability for reduction of blood glucose levels [4, 41]. Our results on amaryl were similar with that reported of effect of glimepiride on reduction of blood glucose levels (BGL) in STZ or alloxan diabetic rats [4, 9]. The primary mechanism of action of glimepiride in lowering the blood glucose appears to be dependent on stimulating the release of insulin from functioning pancreatic beta cells [4, 41].

Our findings on Cr III were also supported by some studies that reported that the supplementation of well-controlled type diabetes with chromium-enriched yeast is safe and can result in improvements in blood glucose variables, carbohydrates and lipid metabolism and oxidative stress [19, 20]. Moreover, several studies showed that the trivalent chromium (Cr III) was known to possess the ability for reduction of blood glucose levels in diabetes [16, 17, 42]. The mechanism of action of Cr III for reducing the blood glucose showed in diabetic subjects by altering (or correcting) the plasma membrane composition of cholesterol in fat and muscle cells, or by inducing a loss of plasma membrane cholesterol [18, 42]. Also, the chromium treatment improve the first phase of secretion of insulin or facilitate post-receptor insulin sensibility as a way of potentiating the insulin action [43].

Concerning, the effect of hyperglycemia condition on genetic alterations and sperm abnormalities, the present results showed that STZ diabetic rats had significant increases of each of DNA mutation (including increases of DNA fragmentation and absence or disappear of some base pairs of DNA structure according to ISSR-PCR analysis), frequencies of chromosome aberrations and sperm-shape abnormalities.

The induction of DNA fragmentation in the present results were similar with that reported of other studies on alloxan diabetic condition. In these studies 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 control [44]. Also, the hyperglycemia condition due to alloxan treatment in both male and female rats significantly increased the rates of DNA fragmentation compared to controls [9].

Also, ISSR analysis showed loss base pair fragments of DNA structure of individuals with hyperglycemia condition compared to normal control. ISSR assay is a powerful analysis that has been applied to illustrate the genetic variation between and within a lot of species of plants [45, 46]. Also, this technique was used to investigate the effect of salinity condition on genetic stability of fish (redtilapia) [47]. 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 [47, 48].

The observed DNA mutation in the present study may be due to the presence of hyperglycemia condition, that is known to be a good marker for overproduction of ROS. Virtually, ROS attack the DNA structure causing scission, fragmentation and cross-linking and consequently leading to DNA mutation [4, 44, 49, 50].

The induction of chromosome aberrations in rats with hyperglycemia condition in the present study, were supported by other reports of several studies, Yamamoto et al., [51] found numerical chromosome aberrations (aneuploidy and polyploidy) in embryos of diabetic mice. Tollinger et al. [52], reported hypodiploid cells with a chromosome number of 38 to 41 (2n=42) in alloxan-induced diabetic rats. Abd El-Rahim [9], observed higher frequencies of structural and numerical chromosome aberrations in bone marrow cells in alloxan diabetes of rats (males or females) compared to normal controls. Also, in previous study, Wauben-Pennis and Prins [53] found in primary spermatocytes higher chiasma frequencies in the translocation multivalent in diabetic male mice than in controls.

The observed chromosome aberrations in this study may be due to the presence of DNA mutation including DNA fragmentation and the loss genetic composition of DNA according to ISSR-PCR analysis [4, 54].
Concerning the induction of sperm abnormalities in rats with hyperglycemia condition in the present study, the findings are in agreement with that reported by Rabbani et al. [4] and similar with that observed by Abd El-Rahim et al. [9] who found significant increases of sperm shape abnormalities besides significant reducing of caudal sperm count in STZ or alloxan diabetic rats.

Diabetes mellitus has also been previously known to be often associated with sexual dysfunction in men [11] and delayed sexual maturation and infertility or compromised semen quality [11, 55].

The sperm aberrations in diabetes may be due to the potential generation of ROS in hyperglycemia condition. The ROS attack the polyunsaturated fatty acid residues of phospholipids of cell membrane occurring lipid peroxidases (LPO). Science the sperms have a high content of polyunsaturated fatty acids in the plasma membrane, they are highly sensitive to oxidative stress. Increased LPO and altered membrane can affect the sperm DNA leading to sperm abnormalities [4, 56-58].

In the present study, the administration of amaryl and yeasts (Sc, ScCr and ScCr) led to significant decreases of genetic alterations (DNA fragmentation, loss or disappear of some base pair fragments of DNA according to ISSR analysis and chromosome aberrations) and sperm abnormalities compared to diabetic control. However, these findings showed that the yeast treatments especially the treatment with chromium-enriched yeast in controlling the diabetic disease (genetic alterations and sperm abnormalities) have been observed to be over expressed than amaryl drug. The chromium yeasts had significant decreases of DNA fragmentation as well as the ISSR analysis showed the repair of DNA mutation by the presence of a lot of the base pair fragments (which were previously lost by hyperglycemia condition) especially by using primer HB-14 compared to amaryl treatment. Moreover, there were approximate similar of results between ScCr and ScCr treatments in curing the diabetic disease.

The present findings concerning the effect of amaryl on genetic alterations and sperm abnormalities in diabetes were similar with that reported by Abd El-Rahim et al. [9] who found that the administration of amaryl to alloxan diabetic rats had decreased the abnormalities of genetic materials (chromosome aberrations, the populations of micronucleated erythrocytes and DNA fragmentation) and sperm abnormalities, besides enhancing the sperm count compared to diabetic control. Also, Rabbani et al. [4] found that the glimepiride treatment had reduced the sperm abnormalities in STZ diabetic rats compared to diabetic control.

To our knowledge, the effect of chromium yeast on repairing of genetic alterations (such DNA mutation, chromosome abnormalities) and sperm abnormalities in hyperglycemia condition has not discussed previously. However, the decreasing of genetic alterations and sperm abnormalities in amaryl and yeast treatments (especially chromium-enriched yeast) in the present study, may be due to the reduction of hyperglycemia condition (reduction of high blood glucose levels) or the possible antioxidant activity of amaryl and chromium yeast and consequently lead to reduction of potential generation of ROS.

Concerning, the reduction of hyperglycemia in this study, the amaryl [4, 9, 41] and trivalent chromium [16-18] treatments were found to possess the ability of reduction of blood glucose levels in diabetes. On the other hand, the possible antioxidant activity of amaryl and chromium yeast has been shown in diabetes patients of several studies. Considering the antioxidant activity of amaryl, Kramer et al. [41], Krauss et al. [59] and Rabbani et al. [4] 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, H2O2 and malondialdehyde. Also, the antioxidant activity of chromium yeast has been shown by scavenging ROS or other aqueous peroxyl radicals [6, 60].

Furthermore, in another studies, the chromium treatment has been shown to protect rats from oxidative damage related to carbon tetrachloride exposure [61] and decrease the lipid peroxidation in isolated rat hepatocytes [62]. Moreover, chromium was found in some studies to be essential for maintaining the structural stability of proteins and nucleic acids and the animals fed diet low in chromium had a significantly lower sperm count and decreased fertility compared to chromium-supplemented controls [63, 64].

In conclusion, there were no significant differences between AM and chromium yeasts treatments for reduction of BGL in hyperglycemic rats. However, the chromium yeast was the best than yeast without chromium for the same purpose Moreover, the treatments with chromium-enriched yeasts (ScCr or ScCr) for controlling the diabetic disease by reduction or repairing the genetic alterations as well as improving the reproductive characteristic (by decreasing the sperm changes) had succeeded in counteracting these abnormalities and they were more effective than amaryl treatment.
REFERENCES


