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Evaluation of Antihyperglycemic Action of Oyster Mushroom (*Pleurotus ostreatus*) and Its Effect on DNA Damage, Chromosome Aberrations and Sperm Abnormalities in Streptozotocin-Induced Diabetic Rats

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Abstract: The present study was carried out to evaluate the antihyperglycemic action of an extract of oyster mushroom, *Pleurotus ostreatus* and its effect on potential DNA damage, chromosome aberrations and sperm abnormalities in streptozotocin (STZ) - induced diabetic rats. Five groups of adult male albino rats (five animals each) were used in this study. The first group consisted of normal animals (control), the second group was of diabetic (hyperglycemic) animals, the third, fourth and fifth groups included hyperglycemic animals that orally treated with amaryl (AM) (0.03 mg/kg.b.wt/dl), low ($Mush_1$) (100 mg/kg.b.wt/dl) and high ($Mush_2$) (200 mg/kg.b.wt/dl) levels of mashroom extract, respectively, for 30 days. The treatment with AM (as a standard treatment) was used for comparison with mushroom treatments. The results revealed a significant increases of each of blood glucose level (BGL), genetic changes (DNA fragmentation, deletion or disappear of some base pairs of DNA according to ISSR-PCR analysis and chromosome aberrations) and sperm abnormalities in hyperglycemic animals compared to normal animals. On the other hand, the treated hyperglycemic animals with AM, $Mush_1$ and $Mush_2$ had significant ($P<0.05$) decreases of BGL, genetic alterations and sperm abnormalities than untreated hyperglycemic animals. The AM group had the lowest level of BGL when comparing with mushroom groups ($Mush_1$ or $Mush_2$) and there were significant ($P<0.05$) differences between the AM and mushroom treatments. However, the result showed that the rates of DNA fragmentation were significantly ($P<0.05$) decreased in mushroom groups than those observed in AM group. Also, ISSR-PCR analysis showed (especially by using primer HB-14) that some fragments of base pairs of DNA which disappeared in diabetic animals or in AM group have been existed in mushroom groups. Furthermore, the diabetic animals treated with mushroom extract (low or high level) had significant ($P<0.05$) decreases of most frequencies of each of chromosome aberrations and sperm abnormalities compared to AM group. Moreover, the results showed that the treatment with high level of ($Mush_2$) was better than the treatment with low level of ($Mush_1$) of mushroom extract for reduction of each of BGL, genetic alterations and sperm abnormalities in hyperglycemic animals. In conclusion, the present study showed that the treatment with mushroom *Pleurotus ostreatus* extract (especially high level) could reduce the high blood glucose level in hyperglycemic rats but less than amaryl treatment. However, the mushroom treatment were more effective for decreasing the genetic alterations and sperm abnormalities in diabetes conditions than amaryl treatment.

Key words: Hyperglycemia · Rats · Mushroom (*Pleurotus ostreatus*) · DNA damage · Chromosome aberrations · Sperm abnormalities

INTRODUCTION

Diabetes mellitus is a major endocrine disorder and growing health problem in most countries [1, 2]. At least 171 million people worldwide have diabetics. This figure is likely to be more than double by 2030 to reach 366 million [3, 4]. Increasing evidence in both experimental and clinical studies suggests that there is a close link between hyperglycemia and diabetic complications [5]. Numerous physiological processes derived of presence of hyperglycemia condition usually lead to overproduction of oxygen-center free radicals and other reactive oxygen

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species (ROS) [4- 7]. ROS can overwhelm antioxidant defense (protective enzymes) like superoxide dismutase, catalase and peroxidase and can cause destructive and lethal cellular effects (e.g. apoptosis) by oxidizing membrane lipids, cellular proteins, DNA and enzymes [7]. Diabetes mediated oxidative stress is known to damage the nuclear component of the host cells and is considered to be a vital cause for the mutation related somatic and germinal cell disorders. The somatic cell defects has the tendency to cause various types of neurological defects, heart ailments, carcinogenesis aging etc., while the germinal cell damage results in infertility [5, 8]. Moreover, DNA damage, chromosome aberrations and sperm abnormalities were the major observations in hyperglycemic rats [5, 9, 10]. Oxidation processes due to hyperglycemia may be inhibited by antioxidant agents. Artificial antioxidants such as butaleted hydroxyanisole (BHA) butelated hydroxytoluene (BHT) and n-propyl gallate, exhibit strong antioxidant activity against several oxidation systems. However, because artificial antioxidants pose potential risks in vivo, their use is restricted or prohibited in some countries [7, 11]. Safer antioxidants from natural sources, mainly medical plant extracts, have therefore been investigated. Edible mushrooms are an important source of nutrients and of physiologically beneficial, non - toxic medicines [12]. These medicinal plants are an important source of strong antioxidants and have potent free radical-scavenging activities [13-17]. Moreover, many medicinal properties have been attributed to mushrooms [18], including inhibition of platelet aggregation [19], reduction of blood cholesterol concentrations [20], prevention or alleviation of heart disease [21] and have also been reported to block induced liver peroxidation [22, 23]. Since research has tended to focus on the dietary value of edible mushrooms, there is relatively little information pertaining to their antihyperglycemic agent and their possible use to treat diabetes conditions. So, the present study was designed to evaluate the potential antihyperglycemic action of an extract of the oyster mushroom, *Pleurotus ostreatus* and its effect on potential DNA damage, chromosome aberrations and sperm abnormalities in streptozotocin (STZ) -induced diabetic rats.

MATERIALS AND METHODS

Experimental Animals: Male albino rats weighing 150-160 g, bred in the Animal House Lab., National Research Centre, Giza, Egypt, were used in the present 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.*

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.

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 [24, 25]. Amaryl treatment (as a standard treatment) was used in this study for comparison with mushroom treatments.

Induction of Diabetes: The experimental group of animals was fasted for 24 h and then intraperitoneally injected with a single dose of 65 mg / kg body weight of freshly prepared steptozotocin dissolved in citrate buffer PH 4.5 to induce diabetes [26]. Diabetes was confirmed after 48 (or72) h of steptozotocin 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 [2, 5]. The rats with serum glucose level above 160 mg / dl were selected [5] 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: Mal rats were randomly selected and divided into five groups (five animals each). The first group included non-diabetic control animals (C group). The second group included diabetic animals. The third, fourth and fifth groups consisted of diabetic animals treated with amaryl drug (AM group), low level of mushroom ($Mush_1$) and high level of mushroom ($Mush_2$), respectively. The third group was orally given, 0.03 mg/ kg.b. wt./dl of amaryl. While, the fourth and fifth groups were orally given low and high levels of mushroom, 100 mg and 200 mg, respectively/ kg.b.wt./dl. The period of treatments for diabetic animals was extended for 30 days. At the end of experiment, the animals were sacrificed by cervical dislocation for investigating 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 of lysis buffer containing, 10 mM tris-HCl (pH. 8), 1 mM EDTA, 0.2% triton X-100, centrifuged at 10000 r.p.m. (Eppendorf) for 20 min at 4°C. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and the 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 min. 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 conc. sulfuric acid and 60 µl of acetaldehyde solution) was added and incubated at room temperature for 24 hours [27]. The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

$$\text{DNA fragmentation} = \frac{\text{O.D. of fragmented DNA}}{\text{OD of fragmented DNA + OD of intact DNA}} \times 100$$

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 primer 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 25 µl containing 10 x PCR buffer (50 mM KCl, 10 mM Tris- HCl, 1.5mM $MgCl_2$, pH 9.0, 2mM dNTPs, 10mM primer, 50 ng of template DNA and 0.5 u of Taq Polymerase (Promega, USA). Reactions were performed in a thermocycles (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 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 electrophoretic patterns of the PCR products were recorded digitally using a Gel- Doc 2000 image analysis system (Bio-Rad) according to the instruction manufactory.

Chromosome Preparations: For chromosome analysis in both treated (AM, $Mush_1$ and $Mush_2$) 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* [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 (0.075M 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 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 3000sperms per group were assessed for morphological abnormalities. The sperm abnormalities were evaluated according to standard method of Narayana [35].

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

Statistical Analysis: Statistical analysis was carried out 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. On the other hand, ISSR bands were scored using Gel- Doc (Bio-Rad) Gel analysis program as present (+) or absent (0) and they were examine to estimate differences or relationships among the investigated treatments.

RESULTS

Blood Glucose Level: The present results (Table, 2 and Fig. 1) showed that blood glucose level (BGL) significantly (P< 0.05) increased in diabetic rats than those of the control group. Whereas, the BGL were significantly (P< 0.05) decreased in diabetic animal treated with amaryl (AM group) or with low ($Mush_1$, group) and high ($Mush_2$, group) levels of mushroom compared to those of D group. On the other hand, $Mush_1$ or $Mush_2$ groups had significant (P< 0.05) increases of BGL than AM group. However, $Mush_2$ group had significant (P< 0.05) decrease of BGL than $Mush_1$ group.

DNA Fragmentation: The present results (Table 3) showed that the rates of DNA fragmentation significantly (P< 0.05) increased in diabetic animal (D group) than those of the control group. In contrast, the diabetic animals treated with amaryl (AM group) or with low ($Mush_1$, group) and high ($Mush_2$, group) levels of mushroom had significant (P< 0.05) decreases of DNA fragmentation than D group. Moreover, the $Mush_1$ or $Mush_2$ groups had significant (P< 0.05) decreases of DNA fragmentation than AM group. On the other hand, the $Mush_2$ group had the lowest rat of DNA fragmentation compared to $Mush_1$ group, however, there were no significant d (P< 0.05) differences between the two groups.

ISSR-PCR Analysis: Three ISSR primers; HB10, HB12 and HB14 were used to detect the effect of the mushroom extracts on hyperglycemia condition comparing with amaryl treatment in diabetic rats (Fig. 2 and Table 4). Primer HB10, revealed in diabetic rats and amaryl samples an equal four fragments which were differed from the control samples in a 150 bp fragment. Also, the two groups of rats which treated with low ($Mush_1$) and ($Mush_2$) concentrations of mushroom displayed four amplified fragments, while, 610 bp fragment disappeared comparing with control, diabetic and amaryl drug samples. On the other hand, ISSR primer HB12 revealed a total of six fragments in the control and a group of rats treated with high concentration of mushroom. Whereas, diabetic rats, amaryl samples and a group of rats treated with low concentration of mushroom lacked a 230 bp fragment that was existed in each of control and samples treated with high concentration of mushroom. Moreover, using the primer HB14 revealed eight fragments in control samples, while this primer revealed four and five fragements in each of diabetic and amaryl groups respectively, which they were differed from control samples in 490, 290, 250 and 50 bp fragments (for diabetic samples) and in 490, 290 and

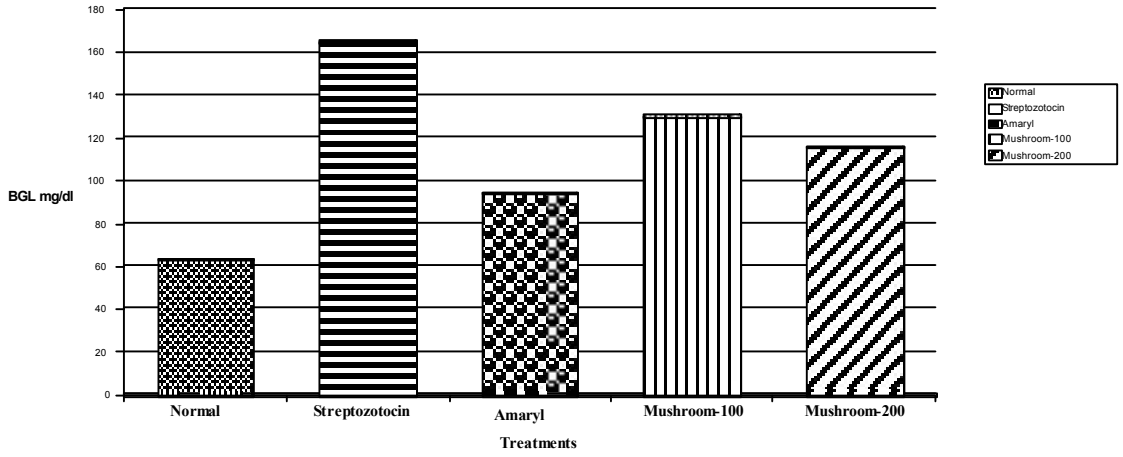

Fig. 1: Effect of Mushroom (100 and 200 mg/kg/b.wt.) on blood glucose levels (BGL) in diabetic rates after 30 days of treatments

Table 2: Effect of Mushroom (100 and 200 mg/kg/b.wt.) on blood glucose levels (BGL) in diabetic rats after 30 days of treatments

Normal	STZ	AM	Mush-100	Mush-200
A	E	B	D	C
63.60 ±1.75	165.07 ±3.55	93.80 ±4.14	130.12 ±2.24	116.00 ±2.17

- Data were expressed as mean ± SE
- Means with different superscript letters (A, B, C, D, E) are significantly different ($P < 0.05$)
- STZ = Streptozotocin treatment
- D = Diabetic(hyperglycemic) condition. - AM = Amaryl treatment
- Mush-100 = Mushroom treatment at level 100 mg/kg/b.wt
- Mush-200 = Mushroom treatment at level 200 mg/kg/b.wt

Table 3: Effect of AM, Mush1 and Mush 2 on the rates of DNA fragmentation in STZ- induced hyperglycemic male rats

	% of DNA Fragmentation
Treatment	M±S.E.
Control	7.25 ± 0.1^{a}
D	20.57 ± 0.74^{d}
D + AM	17.29 ± 0.40^{c}
D + $Mush_1$	14.68 ± 0.48^{b}
D +$Mush_2$	13.5 ± 0.43^{b}

- Data were expressed as mean ± SE
- Means with different superscript letters (a,b,c,d) are significantly different ($P < 0.05$)
- D = Diabetic (hyperglycemic) animals
- AM= Amaryl treatment
- $Mush_1$ = Mushroom treatment (with low concentration, 100 mg/ kg. b.wt.)

50bp fragments (for amaryl samples). Samples treated with low ($Mush_1$) and high ($Mush_2$) concentrations of mushroom revealed an equal seven fragments which were differed from the control samples in only one fragment of 290 bp for samples treated with low concentration of mushroom and in 490 bp for samples treated with high concentration of mushroom. On the other hand, the obtained results evidently observed that 490, 290 and 50 bp fragments which existed in $Mush_1$ group, they disappeared in diabetic rats. Also, the 490 and 50bp fragments were absent in amaryl samples, while they were existed in $Mush_1$ group. Furthermore the 290, 250 and 50bp fragments which were existed in $Mush_2$ group, they disappeared in the diabetic samples. Also, 290 and 50 bp fragments disappeared in amaryl samples, while, they were existed in $Mush_2$ group.

Chromosome Examination: Chromosome examination (Table, 5) showed that the frequencies of structural and numerical chromosome aberrations were significantly ($P< 0.05$) increased in diabetic animals (D group) than those of the control group. In contrast, the diabetic animal treated with amaryl (AM group) or with low ($Mush_1$) and

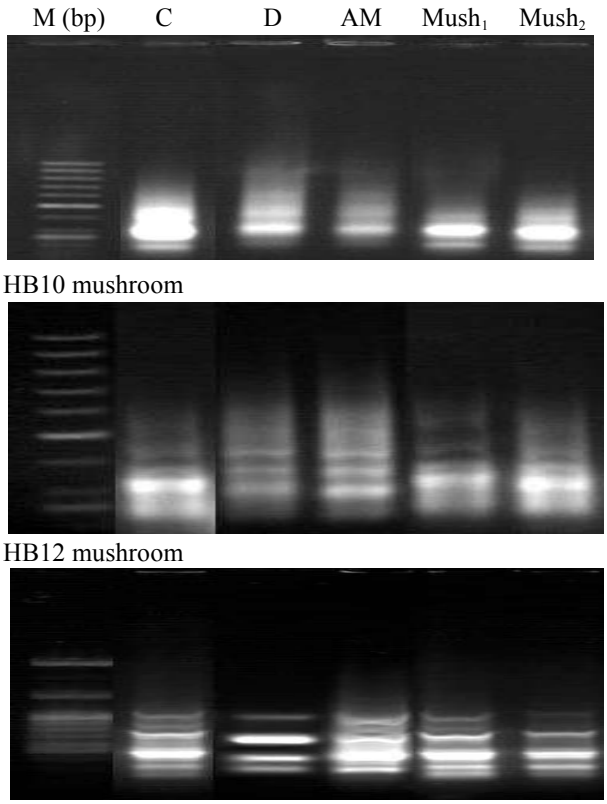

Fig. 2: SSR amplified products using three ISSR primers; HB-10, HB-12 and HB-14

M = Marker.
C = Normal Control.
D = Diabetes (hyperglycemia).
AM = Amaryl treatment.
$Mush_1$ = Mushroom treatment (with low concentration, 100 mg/ kg. b.wt.).
$Mush_2$ = Mushroom treatment (with high concentration, 200 mg/ kg. b.wt.).

high ($Mush_2$) levels of mushroom had significant ($P< 0.05$) decreases of most frequencies of structural and numerical chromosome aberrations than D group. On the other hand, the diabetic animals treated with low or high levels of mushroom had low frequencies of most structural and numerical chromosome aberrations compared to amaryl group. The differences between $Mush_1$ and AM groups were significant ($P< 0.05$) for the frequencies of deletions, centromeric attenuations (C.A) and total structural aberrations. Also, the differences between $Mush_2$ and AM group were significant ($P< 0.05$) for the frequencies of deletions, C.A, total structural aberrations, polyploidy and total numerical aberrations. Moreover, the $Mush_2$

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

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

M = Marker. Diab = Diabetes(hyperglycemia)

AM = Amaryl treatment. Conc. = Concentration

Table 5: Effect of AM, $Mush_1$ and $Mush_2$ treatments on the frequency of chromosome aberrations in STZ- induced hyperglycemic male rats

	Structural chromosomal aberrations						Total Structural chromosomal aberrations	Numerical aberrations		
Treatments	Gaps	Deletions	Breaks	Centromericatt enuations	End to End attenuations	Rings		Peridiploidy	Polyploidy	Total numerical aberrations
Control	0. 6±0.24^{a}	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±0.0^{a}
D	2.2±0.2^{b}	7.8±0.48^{d}	3.2±0.58^{c}	3.4±0.4^{b}	3.2±0.4^{c}	0.2±0.2^{a}	20.0±0.55^{d}	5.0±0.7^{c}	2.0±0.44^{c}	7.0±0.31^{c}
D+AM	1.4±0.24^{a}	4.2±0.49^{c}	2.2±0.2^{bc}	4.2±0.58^{b}	1.8±0.37^{b}	0.4±0.24^{a}	14.2±0.58^{c}	3.2±0.37^{b}	1.2±0.37^{bc}	4.4±0.5^{b}
D+ $Mush_1$	1.2±0.37^{a}	2.2±0.37^{b}	1.2±0.37^{ab}	1.8±0.37^{a}	1.4±0.5^{ab}	0.4±0.25^{a}	8.2±0.37^{b}	2.4±0.4^{ab}	1.2±0.2^{b}	3.6±0.5^{b}
D+$Mush_2$	0.6±0.24^{a}	2.2±0.2^{b}	1.2±0.37^{ab}	1.6±0.24^{a}	1.6±0.51^{b}	0.0±0.0^{a}	7.2±0.37^{b}	2.0±0.31^{ab}	0.2±0.2^{a}	2.2±0.2^{a}

- Data were expressed as mean ± SE

- Means with different superscript letters (a,b,c,d) are significantly different ($P < 0.05$)

- D = Diabetic (hyperglycemic) conditions. - AM= Amaryl treatment

- $Mush_1$ = Mushroom treatment (with low concentration, 100 mg/ kg. b.wt.)

- $Mush_2$ = Mushroom treatment (with high concentration, 200 mg/ kg. b.wt.)

Table 6: Sperm abnormalities in each of hyperglycemic condition and hyperglycemia treated with AM, $Mush_1$ and $Mush_2$ of male rats

	Types of sperm head abnormalities						Total head abnormalities	Total tail abnormalities	Total abnormal sperms (head +tail)
Groups	Amorphous	Without hook	Banana shape	Sperm with Two heads	Big shape	Small shape			
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^{b}	0.8±0.48^{a}	1.0±0.3^{b}	0.2±0.4^{a}	0.6±0.24^{a}	28.8±1.3^{d}	10.6±0.4^{b}	39.0±0.1^{c}
D+AM	11..0±0.7^{c}	11.25±0.25^{b}	0.75±0.4^{a}	0.75±0.25^{ab}	0.25±0.5^{a}	0.5±0.28^{a}	24.5±1.5^{c}	10.25±0.75^{b}	34.75±2.2^{c}
D+Mush 1	7.75±0.62^{b}	7.75±0.25^{a}	0.5±0.28^{a}	0.5±0.28^{ab}	0.25±0.5^{a}	025±0.25^{a}	17.25±1.2^{b}	8.5±1.1^{b}	25.75±1.7^{b}
D+Mush 2	6.75±0.25^{b}	6.0±0.4^{a}	0.5±0.2^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	13.5±0.86^{ab}	9.5±0.28^{b}	23.0±0.7^{b}

- Data were expressed as mean ± SE. - Means with different superscript letters (a,b,c,d) are significantly different ($P < 0.05$)

- D = Diabetic (hyperglycemic) conditions. - AM= Amaryl treatment

- $Mush_1$ = Mushroom treatment (with low concentration, 100 mg/ kg. b.wt.)

- $Mush_2$ = Mushroom treatment (with high concentration, 200 mg/ kg. b.wt.)

group had more low frequencies of most chromosome aberrations than $Mush_1$ group, however, the differences between the two group were only significant ($P< 0.05$) for the frequencies of total numerical chromosome aberrations, especially polyploidy.

Sperm - Shape Analysis: Sperm examinations in Table, 6 showed that sperm–shape abnormalities significantly increased in diabetic animals than those of normal group. In contrast, the diabetic animals treated with amaryl or $Mush_1$ or $Mush_2$ levels had decreases of sperm abnormalities than D group, these decreases were significant ($P< 0.05$) in AM group, especially for total sperm head abnormalities (TSHA) and significant ($P< 0.05$) in $Mush_1$ or $Mush_2$ groups especially for TSHA and total sperm abnormalities (head + tail) (TSA). On the other hand, sperm abnormalities were lowered in $Mush_1$ or $Mush_2$ groups than those of AM groups and there were significant ($P< 0.05$) differences between AM and $Mush_1$ or $Mush_2$ groups, especially for TSHA and TSA. Moreover, the treatment with $Mush_2$ level was better than the treatment with $Mush_1$ level for decreasing the sperm abnormalities in treated diabetic animals, however, the differences between the two groups were not significant.

DISCUSSION

The Pleurotus species of oyster mushroom have been proven to be a good source of almost all essential amino acids that have many medicinal properties and antioxidant activities [23, 36, 37]. Levostatin, a cholesterol- lowering drug derived from Pleurotus species and its analogues were reported to be the best therapeutic agents for correcting hypercholesterolemia [23, 36]. However, the effect of mushroom extracts on high blood glucose levels or hyperglycemia condition in diabetes was not clear in previous studies. Hence, the present investigation was carried out to test the putative antihyperglycemic action of oyster mushroom, *Pleurotus ostreatus* and its effect on potential DNA damage, chromosome aberrations and sperm abnormalities in STZ diabetic rats. On the other hand, the present study used the amaryl treatment as a standard treatment. 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 STZ- induced diabetes [5, 38].

Concerning the inducing of hyperglycemia condition and its effect on genetic materials and sperm shape in the present study, the results showed that the blood glucose level was significantly increased in STZ diabetic rats than those of undiabetic or normal animals. This hyperglycemia condition may be due to the cytotoxic effect of STZ on pancreatic beta-cells causing hypoinsulinemia and a complete loss of sensitivity of B-cells to glucose [39-41]. These abnormalities in pancreatic β cells were found to be extended after injection with STZ in rats for a period of 10-16 weeks [40] or for 6 weeks [39,41] of age. Our findings are in agreement with that reported in STZ rats [4, 5] and similar with that found in alloxan diabetic rats [10], these authors observed significant increases of blood glucose levels in such diabetic rats than those found in normal controls.

Also, the present results showed that STZ diabetic rats had higher rates of DNA fragmentation compared to undiabetic animals (normal control). These findings were supported by other studies [9, 10]. Otton *et al.* [9] observed that the occurrence of DNA fragmentation in lymphocytes collected from alloxan- induced diabetic rats has been found to be 81% compared to 45% of untreated cells from the control. Abd El-Rahim *et al*. [10] reported that the presence of hyperglycemia condition due to alloxan treatment in both male and female rats led to significant increases of rates of DNA fragmentation compared to normal controls. Moreover, the present results showed by using ISSR-PCR analysis that a lot of base pair fragments of DNA disappeared in STZ diabetic rats, however, all or most of disappeared fragments were existed in normal control. 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 [42, 43]. Our findings showed by using ISSR analysis that some base pair fragments of DNA in hyperglycemic rats were deleted or disappeared. However, the disappeared fragments of DNA in diabetes were existed in normal control. The loss of base pairs of DNA may be due to the presence of hyperglycemia condition. This condition is a good marker for overproduction of ROS, that attack the DNA structure causing DNA mutations [5, 9, 10]. The present results also showed that hyperglycemic rats had significant increases of chromosome aberrations compared to normal control. These results were similar with that reported by Abd El-Rahim *et al*. [10] who found that the alloxan diabetes of male and female rats has significant increases of structural and numerical chromosome aberrations than normal controls. On the other hand, Rabbani *et al.* [5] observed increase frequencies of bone marrow micronucleus (a good indicator for the presence of chromorome aberrations) in polychromatic and normochromatic

erythrocytes in streptozotocin (STZ) diabetic rats than those found of normal animals. Also, in previous studies, Tollinger *et al*. [44] and Block *et al*. [45] observed that hypodiploid cells with a chromosome number of 38 to 41 (2n = 42) were the majority cells in alloxan–induced diabetic rats. In embryos of diabetic mice, Yamamoto *et al*. [46] found higher rates of numerical chromosome aberrations (aneuploidy and polyploidy) compared to those of normal animals.

Furthermore, the present results showed that the sperm abnormalities were significantly increased in STZ diabetic rats than those observed of normal animals. These findings are in agreement with that reported by Rabbani *et al.* [5] who found significant increases of sperm shape abnormalities besides significant reducing of caudal sperm count in STZ diabetic rats. Also, the same findings were observed in alloxan diabetic rats by Abd El-Rahim *et al*. [10]. Moreover, the delyed sexual maturation and abnormal semen quality were the main findings in diabetes mellitus [47, 48].

The present abnormal findings in diabetic rats including genetic alterations (DNA fragmentation, loss of some base pairs of DNA according ISSR analysis and chromosome aberrations) and sperm abnormalities may be due to (as discussed above) the presence of hyperglycemia condition, that was found in several studies to be a main factor for inducing overproduction of reactive oxygen species (ROS) [9, 49-51]. ROS attack the cell membrane, nucleus and genetic materials causing DNA and protein modification [45, 52] and consequently lead to chromosome aberrations and sperm abnormalities [5, 9, 53]. In other mean, the consistently high incidence of DNA fragmentation and chromosome aberrations, as a result of potential formation of ROS, may be indicative of a general susceptibility of these animals in the present study for inducing DNA fragmentation and consequently chromosome derangements of gonadal cells causing abnormalities in sperm shape [54-56]. Moreover, 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) [5, 8, 57]. 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 concentration 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, [5, 58-60].

On the other hand, in this study, the supplementation of amaryl and mushroom to the hyperglycemic rats led to reduction of blood glucose level (BGL) compared to untreated diabetic animals. The BGL in amaryl group was significantly lowered than those observed in mushroom groups. Moreover, the BGL was significantly decreased in diabetic animals treated with high level of mushroom than those of diabetic rats treated with low level of mushroom. The present findings on the effect of amaryl on the BGL are in agreement or similar with that reported by Rabbani *et al.* [5] and Abd El-Rahim *et al.* [10], who found significant decreases of BGL in STZ or alloxan diabetic rats treated with amaryl than those of untreated diabetic animals. 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 [5, 38]. Concerning the effect of mushrooms on hyperglycemic condition, although, several researches were focused on the therapeutic effects of the medicinal mushrooms (for cardiovascular disease, dental conditions, bacterial infection and other potential applications include brain ischemia Alzheimer's disease and arthritis), little information is available about their antihyperglycemic properties. However, the medicinal mushrooms were found in some studies to have positive effect for reduction the lipid profile (LP), obesity (OB) and hypertriglyceridamia (HGD), these factors (LP, OB, HGD) are considered a main cause for inducing the hyperglycemia condition. In an animal model of diabetes, Huang *et al.* [61] demonstrated favorable effect of medicinal mushrooms on lipid profiles. Also, some studies found that mushroom extracts contain chitosan component (N-acetyl -D- glucosamine / D-glucosamine ratio), that was considered to be a prime therapeutic agent for prevention or treating the obesity diseases [62-64], this obesity causes not only hypertriglyceridaemia but also peripheral insulin resistance [65-67]. Several studies on animals and humans have reported that chitosan reduces body weight and cholesterol [62, 63, 68]. Furthermore, Neyrinck *et al.* [64] demonstrated that dietary supplementation with chitosan counteracted triglyceride accumulation in the liver (decreased of triglyceride content by 39%) and in the muscle (decreased of triglyceride content by 66%) as well as the reduction of each of blood triglycerides and hyperinsulinaemia in high-fat (HF) diet-induced obese mice.

In the present study the supplementation of amaryl and mushroom extract to the STZ diabetic rats led to reduction 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 untreated diabetic animals. The mushroom treatments (at low or high levels) were found to be more effective than amaryl treatment. Moreover, the diabetic animals treated with high level (200 mg/ kg.b.wt) of mushroom had the lowest frequencies of genetic alterations and sperm abnormalities. These decreases or repairing of genetic abnormalities 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 treatment in diabetes were found to possess the ability of reduction of blood glucose levels [5, 10, 38]. Also, the mushroom treatment has important role for reduction of lipid profile, cholesterol, obesity and hypertriglyceridameia that consequently lead to avoid of inducing of hyperglycemia condition [38,61,63]. On the other hand, the possible antioxidant and antimutagenic activities of amaryl and medicinal mushrooms (that had been reported in several studies) may be the main factor for reduction or repairing of genetic and sperm abnormalities in the present study. Concerning the antioxidant activity of amaryl, Rabbani *et al.* [5],Kramer *et al.* [38]and Krauss *et al.* [69] and observed that the supplementation of amaryl to diabetes increases of plasma levels of antioxidant enzymes (CAT, SOD and GPX) and reduces the levels of LPO, H_2O_2 and malondialdehyde. Also, the antimutagenic activity of amaryl in diabetes was reported by Rabbani *et al.,* [5] and Abd El-Rahim *et al.* [10]. Rabbani *et al.* [5] reported that the administration of amaryl (glimepiride) to the STZ hyperglycemic rats caused reduction of populations of micronucleated erythrocytes and sperm abnormalities besides enhancing the sperm count compared to untreated diabetes. Abd El-Rahim *et al.* [10] observed that treatment with amaryl in alloxan-induced hyperglycemic rats had reduced genetic alterations (populations of micronucleated erythrocytes, DNA fragmentation and chromosome aberrations) and sperm abnormalities besides enhancing the sperm count compared to untreated diabetes. Concerning, the antioxidant activity of mushrooms, these medicinal plants were found to have potent free radical-scavenging activities. [13-17]. Mau *et al.* [70] prepared methanolic extracts from medicinal mushrooms and studied their antioxidant properties. The results showed an excellent antioxidant activity by scavenging and chelating abilities on 1, 1-diphenyl-2-picrylhydrazyl radical. Total phenols were the major naturally occurring antioxidant components found in methanolic extracts from medicinal mushrooms. Lakshmi *et al.* [71] examined the protective effect of medicinal mushrooms extracts against lipid peroxidation induced by γ-radiation and certain chemicals such 2.2 Azobis (2-amidopropane) dihydrochloride (AAPH), which generates two potent ROS capable of inducing LP, namly hydroxyl radical (OH) and peroxyl radical (ROO) using rat livers and brain mitochondria as model systems. The results showed that mushroom extracts (either methanolic or ethyl acetate extracts) significantly inhibited the formation of lipid perioxidation. Also, Jayakumar *et al.* [23] found that an ethanolic extract from medicinal mushroom is able to confer protection against acute hepatotoxicity induced by administrations of carbon tetrachloride (CCL4) in Wistar rats. In rats receiving the mushroom extract and CCL4 were found to be near normal levels of hepatic constituents (malondial- ehyde (MDA), glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx)) and serum enzymes (glutathione oxaloacetic transaminase (SGOT), glutamic pyruvate transaminase (SGPT) and alkaline phosphatase (SALP)), in contrast to alterations in all these parameters in rats receiving CCL4 alone. Nitha *et al.* [72] examined the aqueous-ethanol extract of mushrooms for its ability to scavenge super oxide, hydroxyl, nitric oxide, 2,2-diphenyl-1-picrylhydrazl (DPPH) and 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radicals as well as for inhibition of lipid peroxidation. The extract efficiently scavenged all these radicals and also inhibited lipid peroxidation. Moreover, Aydin *et al.* [53] observed that mushrooms have antioxidant activity in direct hyperbiliribinaemic conditions and may protect DNA structure and liver tissue by reducing oxidative damage in obstructive jaundice as a palliative agent. Concerning the antimutagenic activities of mushrooms, chemical analyses have allowed the isolation and purification of some of relevant antimutagenic compounds of these medicinal plants expecially polysaccharides [73-75] and Lentinan [76,77] that possess strong imunomodulation and anticancer or antitumor activities. Polysaccharides have been shown to potentiate the host's innate (non-specific) and acquired (specific) immune responses and to 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 [74,78]. Also, several studies suggested that these polysaccarides have immunomodulating properties, including the enhancement of lymphocyte proliferation and antibody production [73] as well as producing both anti-genotoxic and antitumor promoting activities [73,80 -82]. The mechanism of action include the inhibition of proliferation, induction of apoptosis, induction of cell cycle arrest, inhibition of invasive behavior and suppression of tumor angiogenesis in many experimental system including prostate cancer [83]. In the present study, the results showed that the treatment with high level (200 mg/kg.b.wt.) was better than the treatment with low level (100 mg/kg.b.wt.) of extract of oyster mushroom, *Pleurotus ostreatus* as a therapeutic effect on each of hyperglycemia, genetic alterations and sperm abnormalities. These findings were supported by another studies that reported that the extract of leaves of mushroom Kalanchoe pinnata Pers. [84] or extract of mushroom *Pleurotus ostreatus* [23] when administrated in a dose of 200 mg/kg.b.wt., was effective and exhibited hepatoprotective activity in albino rats with CCL4-induced hepatotoxicity. In conclusion, the present study showed that the treatment with mushroom *Pleurotus ostreatus* extract (especially high level) could reduce the high blood glucose level in hyperglycemic rats but less than amaryl treatment. However, the mushroom treatments were more effective for decreasing the genetic alterations and sperm abnormalities in diabetes conditions than amaryl treatment.

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