The influence of amaryl on genetic alterations and sperm abnormalities of rats with alloxan-induced hyperglycemia

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Abstract: Amaryl (Glimepiride) is the third generation antidiabetic sulphonylurea known to possess the antioxidant effect in streptozotocin (STZ) induced diabetes. In this study, the antimutagenic activity of amaryl (0.03mg/kg.po daily for 30 days) was evaluated against the cytotoxic effect of alloxan (150mg/kg) on somatic and germinal cells of male and female albino rats . Somatic cells included bone marrow (for chromosome abnormality and micronucleus tests) and liver (for DNA fragmentation test). In germinal cells , sperm shape and count were studied . The present results showed that the glucose levels significantly increased in alloxan diabetic rats compared to those found in the controls. The alloxans diabetes of rats (males or females) had higher frequencies of structural and numerical chromosome aberrations compared to normal control. The diabetic condition in both male and female rats also increased the populations of each of micronucleated erythrocytes and DNA fragmentation. Moreover, the diabetic condition of male rats significantly increased the sperm shape abnormalities besides significant reducing of caudal sperm count. In contrast , the administrations of amaryl (glimepiride) to the alloxan diabetic rats had reduced the blood glucose level, abnormalities of genetic materials (chromosomal aberrations, the population of micronucleated erythrocytes, DNA fragmentation) and sperm shape abnormalities besides enhancing the sperm count. In conclusion, the present findings add that the antioxidant property of amaryl could have contributed for its ability to decrease the alloxan mediated defects in somatic and germinal cells.

[Abeer H. Abd El-Rahim, Hasnaa A. Radwan, Omaima M. Abd El-Moneim, Ibrahim M. Farag and Somaia A. Nada. **The influence of amaryl on genetic alterations and sperm abnormalities of rats with alloxan-induced hyperglycemia**. Journal of American Science 2010; 6(12):1739-1748]. (ISSN: 1545-1003).
http://www.americanscience.org.

Key words: Hyperglycemia, amaryl, alloxan, genetic alterations, sperm abnormalities, rats.

1. Introduction

Several studies showed that hyperglycemia and oxidative stress play a central role in diabetic tissue damage (Mastrocola et al., 2005; Somefai. et al., 2006; Kuhad et al., 2008; Rabbani et al., 2009). High level of blood sugar determines overproduction of reactive oxygen species (ROS) by the mitochondria electron transport chain. Also, in diabetic condition, both nitric oxide levels and mitochondrial nitric oxide synthase expressions were found to be increased in brain mitochondria, whereas antioxidant defences, such as glutathione (GSH) peroxidase activity and manganese superoxide dismutase protein content were reduced (Mastrocola et al., 2005; Rabbani et al., 2009). ROS virtually damages all cellular components, leading to DNA and protein modification (Rehman et al., 1999; Rabbani et al., 2009). In consequence, the diabetic patients suffer from an increased risk of oxidative stress-related diseases not only in the present generation but can also transmit the nuclear defects to their progeny (Blasiak et al., 2004).

Induction of diabetes in laboratory animals is a convenient and useful strategy in the understanding and treatment of the disease. An appropriate dose of alloxan was used to induce experimental diabetes. Alloxan (AL) was the first cytotoxic compound reported to cause inhibition of glucose – induced insulin secretion and selective beta-cell damage (Lenzen and Panten, 1988). The cytotoxic activity of this compound seems to be achieved through its penetration into the pancreatic beta-cells, a phenomenon which by itself depends on the expression of the glucose transporter proteins-2 (Schnedl et al., 1994; Bloch et al., 2000). An addition contributor to beta cells sensitivity to alloxan or to various toxins related to their poor antioxidant enzyme defence system (Tiege et al; 1997).

Glimepiride is a sulfonylurea was known to possess antioxidant effect against the oxidative stress induced by streptozotocin –diabetes (Krauss et al., 2003; Rabbani et al., 2009). The mutagenic studies conducted by battery of in vitro and in vivo methods concluded that glimepiride do not have mutagenic potential (Donaubauer and Mayer, 1993; Rabbani et

al., 2009). Since quenching the free radicals generated in the oxidative stress is one of the possible mechanisms to prevent the mutagenic defects in diabetes and there is a need for antidiabetic regimen that also reduce the ROS induced health complications (Johansen et al., 2005; Rabbani et al., 2009), the present study has been planned to evaluate the anti-mutagenic effect of amaryl (glimepiride) in alloxan – induced diabetic rats.

2.Materials and Methods

2.1. Materials

2.1.1. Animals:

Males and females of adult albino rats weighing 150-160 g, 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*.

2.1.2.Drugs:

Alloxan and glucose oxidase peroxidase diagnostic enzyme kit was 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 / d 1 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 (Sato et al., 1993; Nieszner et al., 2002).

2.2. Methods:

2.2.1. 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 (El-Shabrawy and Nada. 1996). 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 (Kuhad et al., 2008 and Rabbani et al., 2009). The rats with serum glucose level above 200 mg / d1 were selected and used for the present study (Kuhad et al., 2008).

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.

2.2.2. Experimental design:

Male or 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 (D), and third group consisted of diabetic animals treated with amaryl (DT).

2.2.3. Chromosome preparations:

For chromosome analysis both treated (D or DT) and control animals were sacrificed by cervical dislocation at the end of experiment. One hour and half or two hours before sacrifice, rats were injected with 4 mg colchicine / kg. b.w. Femurs were removed and the bone marrow cells were aspirated using saline solution. Metaphase spreads were prepared using the method of Preston et al. (1987). Fifty metaphase spreads per animals were analyzed, for scoring the different types of chromosome aberrations.

2.2.4. Micronucleus test:

Bone marrow slides were prepared according to the method described by Krishna and Hayashi (2000). The bone marrow was washed with 1 ml of fetal calf serum and then smeared on clean slides. The slides were left to air dry and then fixed in methanol for 5 minutes, followed by staining in May-Grunwald-Giemsa for 5 minutes then washed in distilled water and mounted. For each animal, 2000 polychromatic erythrocytes (PCEs) were examined for the presence of micronuclei.

2.2.5. DNA fragmentation:

Liver samples were collected immediately after sacrificing the animals. The tissues were lysed in 0.5 ml lysis buffer containing 10mM tris-HCL (PH.8), 1 mM EDTA, 0.2 % triton X-100, centrifuged at 10000 r.p.m. (Eppendorf) for 20 minutes at 4ºC. The pellets were resuspended in 0.5 ml of lysis buffer. To the pellets (P) and supernatants (S), 1.5 ml of 10 % trichloroacetic acid (TCA) was added and incubated at 4ºC for 10 minutes. The samples were centrifuged for 20 minutes at 10000 r.p.m. (Eppendorf) at 4ºC and the pellets were suspended in 750 µl of 5 % TCA, followed by incubation at 100 ºC 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 (Gibb et al., 1997). The proportion of fragmented DNA was calculated from absorbance reading at 600 nm using the formula:

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

2.2.6. 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 (1978) and Farag et al. (2002). At least 3000 sperms per group were assessed for morphological abnormalities. The sperm abnormalities were evaluated according to standard method of Narayana (2008). Epididymal sperm count was also determined by hemocytometer as described by Pant and Srivastava (2003).

2.2.7. 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 treatment in the same sex. However, two-way analysis of variance (ANOVA) followed by Duncan's post hoc test were used in the DNA fragmentation test. Moreover Chi-Square test was used for comparison between male and female rats for inducing micronuclei and chromosome aberrations in diabetic condition and amaryl-treated diabetes. Results were reported as mean ± S.E. and differences were considered as significant when $P<0.05$.

3. Results

3.1. Blood glucose levels:

Blood glucose levels significantly increased in alloxan diabetic rats (Fig. 1, Table 1) compared to those in the normal controls (normal males or normal females). Whereas the blood glucose levels were significantly reduced in alloxan diabetic rats after 30 days of amaryl treated compared to those found in the D groups.

Fig.1:Alloxan - diabetic rats treated with amaryl (4mg/Kg)

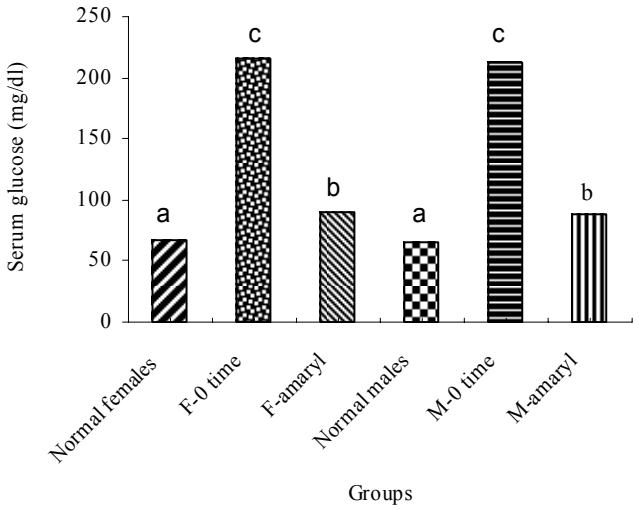

Table (1): Effect of amaryl (4 mg/Kg) on alloxan-induced diabetic female and male rats

Female			Male		
Normal females	F-0 time	F-amaryl	Normal males	M-0 time	M-amaryl
66.33± 1.35 [a]	215.58± 2.46 [c]	89.92 ± 1.36 [b]	66.00 ± 2.24 [a]	212.50 ± 1.86 [c]	88.75 ± 1.86 [b]

Data were expressed as mean ± S.E.
Means with different superscript letters (a, b, c) are significantly different ($P<0.05$)
F= Females M=Males

3.2. Chromosome examinations in diabetic male rats:

Chromosome examinations (Table, 2) showed that there were structural and numerical chromosome aberrations. Structural aberrations consisted of chromatic gaps and breaks, deletions, fragments, centromeric attenuation (C.A) and endomitosis. Numerical aberrations were aneuploidy and polyploidy. From the present results it was found that, the diabetic male rats (D group) had higher frequencies of structural and numerical aberrations than control group. Deletions, C.A., endomitosis and aneuploidy were more frequent than other aberrations. Statistical analysis showed that there were significant differences between diabetic and normal rats for the frequencies of total structural aberrations (except fragments) and total numerical aberrations.

On the other hand, the diabetic males treated with amaryl drug (DT group) had decreases in the frequencies of structural and numerical aberrations compared to D group. These decreases were significant ($P< 0.05$) for the frequencies of gaps, C.A, total structural aberrations, aneuploidy and total numerical aberrations.

3.3. Chromosome examinations in diabetic female rats:

Chromosome examinations (Table, 3) showed that the diabetic female rats had higher frequencies of structural and numerical chromosome aberrations compared to normal females. Statistical analysis showed that there were significant differences between diabetic and normal rats for the frequencies of chromatic gaps and breaks, deletions, fragments, C.A, endomitosis, total structural aberrations and polyploidy.

In contrast, the diabetic females treated with amaryl drug (DT) had decreases in the frequencies of structural and numerical chromosome aberrations compared to D group. These decreases were significant for the frequencies of deletions, fragments, C.A and total structural aberrations.

From the present study, it was observed that structural chromosomal aberrations were increased in diabetic females than those found in diabetic males

(Table, 4). These increases were significant ($P < 0.05$). On the other hand, numerical aberrations (especially aneuploidy) were more frequent in diabetic males than those found in diabetic females. However, the statistical analysis showed that the differences for the frequencies of numerical aberrations between the two sexes were not significant.

Moreover, the treatment with amaryl drug led to decrease of the structural and numerical chromosome aberrations in both diabetic sexes. However, the DT females were more response for decreasing of structural aberrations than DT males. Whereas, the frequencies of numerical aberrations were lowered in DT males than those of DT females.

Table (2): Effect of amaryl on the frequency of chromosome aberrations in alloxan-induced diabetic male rats

Treatment	Structural chromosomal aberrations							Numerical chromosomal aberrations		
	Gap	Break	Deletion	Fragment	C.A	End.	Total structural	Aneuploidy	Polyploidy	Total numerical
Control	1.0± 0.26 ^{b}	0.0± 0.0 ^{b}	0.67± 0.33 ^{b}	0.17± 0.17 ^{a}	2.33± 0.21 ^{b}	0.17± 0.17 ^{b}	4.33± 0.33 ^{c}	2.50± 0.50 ^{b}	0.0± 0.0 ^{b}	2.50± 0.50 ^{b}
D	2.67± 0.42 ^{a}	1.33± 0.42 ^{a}	3.0± 0.37 ^{a}	1.0± 0.52 ^{a}	5.0± 0.52 ^{a}	2.5± 0.34 ^{a}	15.50± 1.23 ^{a}	6.50± 1.20 ^{a}	2.0± 0.68 ^{a}	8.50± 1.18 ^{a}
DT	1.17± 0.31 ^{b}	0.83± 0.31 ^{ab}	2.67± 0.56 ^{a}	0.67± 0.33 ^{a}	3.17± 0.48 ^{b}	2.0± 0.58 ^{a}	10.50± 0.76 ^{b}	4.17± 0.31 ^{b}	0.83± 0.40 ^{ab}	5.0± 0.68 ^{b}

Data were expressed as mean ± S.E.
Means with different superscript letters (a, b, c) are significantly different ($P<0.05$)
D: diabetic condition
DT: diabetic treated with amaryl
C.A: Centromeric attenuations, End.: Endomitosis

Table (3): Effect of amaryl on the frequency of chromosome aberrations in alloxan- induced diabetic female rats

Treatment	Structural chromosomal aberrations							Numerical chromosomal aberrations		
	Gap	Break	Deletion	Fragment	C.A	End.	Total structural	Aneuploidy	Polyploidy	Total numerical
Control	0.50± 0.22 ^{b}	0.17± 0.17 ^{b}	1.50± 0.22 ^{b}	0.0± 0.0 ^{b}	3.0± 0.37 ^{b}	0.33± 0.21 ^{b}	5.50± 0.72 ^{c}	3.17± 0.70 ^{a}	0.33± 0.21 ^{b}	3.50± 0.76 ^{a}
D	3.50± 0.50 ^{a}	2.0± 0.68 ^{a}	3.83± 0.75 ^{a}	1.83± 0.31 ^{a}	6.50± 0.76 ^{a}	2.0± 0.26 ^{a}	19.67± 1.14 ^{a}	4.67± 1.17 ^{a}	2.17± 0.79 ^{a}	6.83± 1.92 ^{a}
DT	2.50± 0.43 ^{a}	1.33± 0.42 ^{ab}	1.17± 0.48 ^{b}	0.83± 0.40 ^{b}	4.17± 0.70 ^{b}	1.17± 0.40 ^{ab}	11.17± 0.91 ^{b}	4.50± 0.43 ^{a}	0.83± 0.40 ^{ab}	5.33± 0.72 ^{a}

Data were expressed as mean ± S.E.
Means with different superscript letters (a, b, c) are significantly different ($P<0.05$)
D: diabetic condition
DT: diabetic treated with amaryl
C.A: Centromeric attenuations, End.: Endomitosis

Table (4): Comparison between male and female rats for inducing chromosomal aberrations in diabetic condition and amaryl-treated diabetes

Treatment	Structural chromosomal aberrations							Numerical chromosomal aberrations		
	Gap	Break	Deletion	Fragment	C.A	End.	Total structural	Aneuploidy	Polyploidy	Total numerical
Control ♂	1.0±0.26	0.0±0.0	0.67±0.33	0.17±0.17	2.33±0.21	0.17±0.17	4.33±0.33	2.50±0.50	0.0±0.0	2.50±0.50
Control ♀	0.50±0.22	0.17±0.17	1.5±0.22	0.0±0.0	3.0±0.37	0.33±0.21	5.50±0.72	3.17±0.70	0.33±0.21	3.50±0.76
X^2 values	1.02	1.0	1.96	1.0	0.53	0.33	0.92	2.22	2.0	1.06
D ♂	2.67±0.42	1.33±0.42	3.0±0.37	1.0±0.52	5.0±0.52	2.5±0.34	15.50±1.23	6.50±1.20	2.0±0.68	8.50±1.18
D ♀	3.50±0.50	2.0±0.68	3.83±0.75	1.83±0.31	6.50±0.76	2.0±0.26	19.67±1.14	4.67±1.17	2.17±0.79	6.83±1.92
X^2 values	0.71	0.83	0.65	1.51	1.32	0.35	4.57*	2.04	0.04	1.29
DT ♂	1.17±0.31	0.83±0.31	2.67±0.56	0.67±0.33	3.17±0.48	2.0±0.58	10.50±0.76	4.17±0.31	0.83±0.40	5.0±0.68
DT ♀	2.50±0.43	1.33±0.42	1.17±0.48	0.83±0.40	4.17±0.70	1.17±0.40	11.17±0.91	4.50±0.43	0.83±0.40	5.33±0.72
X^2 values	3.02	0.71	3.66	0.11	0.89	1.36	0.15	0.09	0.0	0.07

*Significant at $P<0.05$.
C.A: Centromeric attenuations, End.: Endomitosis
D: diabetic condition
DT: diabetic treated with amaryl

3.4. Micronucleus assay:

In male groups: As shown in table (5), the frequencies of micronuclei were significantly higher ($P < 0.01$) in diabetic males (D group) than those found in the control group. On the other hand, the frequencies of micronuclei significantly decreased ($P < 0.05$) in diabetic males which treated with amaryl drug (DT group) compared with those found in D group.

Table (5): Effect of amaryl on the frequency of micronucleated polychromatic erythrocytes in alloxan-induced diabetic male rats

Treatment	No. of animals	No. of examined cells	Mean values of MNPE
Control	6	12,000	6.50±0.99 [c]
D	6	12,000	16.50±1.78 [a]
DT	6	12,000	11.67±1.05 [b]

Data were expressed as mean ± S.E.
Means with different superscript letters (a, b, c) are significantly different (P<0.05)
D: diabetic condition
DT: diabetic treated with amaryl

In female groups:

Micronuclei results in female groups (Table, 6) were similar with those recorded in male groups.

Moreover, the diabetic females had more frequencies of MNPE than diabetic males. However, statistical analysis showed no differences between the two sexes (Table, 7). On the other hand, the treatment of amaryl drug led to decrease of MNPE in both sexes. However, the DT females were more response for amaryl treatment and had the lowest frequencies of MNPE than DT males.

Table (6): Effect of amaryl on the frequency of micronucleated polychromatic erythrocytes in alloxan-induced diabetic female rats

Treatment	No. of animals	No. of examined cells	Mean values of MNPE
Control	6	12,000	6.33±0.88 [c]
D	6	12,000	20.50±1.48 [a]
DT	6	12,000	12.83±0.83 [b]

Data were expressed as mean ± S.E.
Means with different superscript letters (a, b, c) are significantly different (P<0.05)
D: diabetic condition
DT: diabetic treated with amaryl

Table (7): Comparison between male and female rats for inducing micronucleated polychromatic erythrocytes in diabetic condition and amaryl-treated diabetes.

Treatment	MNPE
Control ♂	6.50±0.99
Control ♀	6.33±0.88
X^2 values	0.01
D ♂	16.50±1.78
D ♀	20.50±1.48
X^2 values	2.61
DT ♂	11.67±1.05
DT ♀	12.83±0.83
X^2 values	0.33

D: diabetic condition
DT: diabetic treated with amaryl

3.5. DNA fragmentation:

In male groups:

The present results (Table, 8) showed that, the rates of DNA fragmentation were significantly increased ($P < 0.05$) in diabetic males than those in control group. On the other hand, the rates of DNA fragmentation significantly decreased ($P < 0.05$) in diabetic males treated with amaryl drug (DT) compared to D group of males.

In female groups:

The diabetic females had more frequent ($P < 0.01$) of rates of DNA fragmentation. In contrast, the rates of DNA fragmentation significantly decreased ($P<0.05$) in diabetic females treated with amaryl drug (DT) compared to D group of female.

Moreover, diabetic females had higher frequent ($P<0.05$) of DNA fragmentation than diabetic males. On the other hand, the treatment with amaryl drug led to decrease the rates of DNA fragmentation in both diabetic sexes. The DT females were more response for decreasing of DNA fragmentation than DT males. However, statistical analysis showed no differences for the frequencies of DNA fragmentation between two sexes (DT males and DT females).

Table (8): Effect of amaryl treated upon DNA fragmentation of rat livers. The livers were obtained from control, diabetic and amaryl-treated diabetic rats

Sex	Treatment	Rate of DNA fragmentation
Males	**Control**	19.78 ± 0.32 [de]
	D	34.17 ± 1.19 [b]
	DT	23.16 ± 1.38 [cd]
Females	**Control**	19.46 ± 0.22 [e]
	D	41.02 ± 2.08 [a]
	DT	24.68 ± 0.19 [c]

Data were expressed as mean ± S.E. Means with different superscript letters are significantly different (P<0.05)
D: diabetic condition DT: diabetic treated with amaryl

3.6. Sperm-shape analysis:

Sperm examination (Table, 9) showed that the sperm abnormalities (head and tail) were more frequent in diabetic male rats than those of the control. Statistical analysis showed the differences of the frequencies of head abnormalities (such as amorphous, total head abnormalities) and total sperm abnormalities (head and tail) were significant ($P < 0.05$ or $P < 0.01$) between diabetic and normal rats. On the other hand, the sperm abnormalities especially in head decreased in males diabetic treated with amaryl drug (DT) compared to those of the diabetic animals (D group). These decreases were significant ($P < 0.05$) in the frequencies of amorphous, without hock, total head and total sperm abnormalities. Exception to this,

the frequencies of tail abnormalities were few in each of D (0.17) and DT (0.33) groups; these abnormalities non-significant increased in DT group than those found in D group.

Sperm count:

Sperm counts significantly decreased ($P < 0.01$) in diabetic rats than those found in control group. In contrast, sperm counts significantly increased ($P < 0.05$) in diabetic rats treated with amaryl drug (DT) compared to those of the D group.

Table (9): Sperm abnormalities in diabetic condition of male rats and amaryl-treated diabetes

Treatment	Head abnormalities						Tail abnormalities	Total abnormalities	Sperm count
	Amorphous	Without hock	Small shape	Big shape	Banana	Total	Coiled		
Control	0.67± 0.33 [b]	2.33± 0.42 [c]	0.50± 0.34 [a]	0.0± 0.0 [a]	0.17± 0.17 [a]	3.67± 0.84 [c]	0.0± 0.0 [a]	3.67± 0.84 [c]	76.60± 4.06 [a]
D	4.50± 1.06 [a]	5.83± 0.60 [a]	1.17± 0.40 [a]	0.33± 0.21 [a]	0.33± 0.21 [a]	12.17± 1.35 [a]	0.17± 0.17 [a]	12.33± 1.26 [a]	46.96± 3.64 [c]
DT	2.17± 0.54 [b]	4.0± 0.52 [b]	0.33± 0.21 [a]	0.0± 0.0 [a]	0.33± 0.21 [a]	6.83± 0.60 [b]	0.33± 0.21 [a]	7.17± 0.54 [b]	62.33± 2.73 [b]

Data were expressed as mean ± S.E.
Means with different superscript letters (a, b, c) are significantly different ($P<0.05$)
D: diabetic condition
DT: diabetic treated with amaryl

4. Discussion

In the present study, the blood glucose levels significantly increased in alloxan diabetic rats compared to those in the normal controls. Our results are in agreement with that reported on observed hyperglycemia in alloxan diabetic mice (Diamond et al., 1989) and rats (El-Shabrawy and Nada, 1996). The observed hyperglycemia may be due to cytotoxic effect of alloxan compound on level of glucose transporter protein-2 (GLUT-2) expression in the pancreatic beta-cells causing inhibition of glucose-induced insulin secretion (Lenzen and Panten, 1988; Schnedl et al., 1994, Bloch et al., 2000). The administration of amaryl for 30 days at dose of 0.03mg/g b.wt. caused a significant reduction of the serum glucose level in alloxan diabetic rats. Similarly, Rabbani et al. (2009) found that the administration of glimepiride to nicotinamide (NA)-streptozotocin (STZ) diabetic rats had reduced the serum glucose level. As known amaryl (glimepiride) belongs to third generation sulphonylureas and chemically it is a carboxamido phenyl pyrroline sulphonylurea. 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 (Kramer et al., 1996; Rabbani et al., 2009). The extra pancreatic glucose reducing effects include inhibition of gluconeogenesis, ketogenesis, stimulation of peripheral glucose transport, glucogen synthase activity and glycerol-3-P-acyltransferase activity (Muller et al., 1995; Rabbani et al., 2009). Also, Kecskemeti et al. (2002) and Yassin and Mwafy (2007) indicated that the main effect of the sulfonylureas is enhancement of insulin secretion and improvement of metabolism both by pancreatic and extra-pancreatic mechanisms.

The present results showed that the alloxan diabetes of rats (males and females) had higher frequencies of structural and numerical chromosome aberrations compared to normal control. The diabetic condition in male and female rats also increased the populations of each of micronucleated erythrocytes and DNA fragmentation. Moreover, the diabetic

condition of male rats significantly increased the sperm shape abnormality besides significant reducing of caudal sperm count. These effects appeared to be mediated through the oxidative stress and inducing of ROS generated due to hyperglycemia (Vikram et al., 2007; Shrilatha and Muralidhara, 2007). The generation of ROS in diabetes was considered to be the major cause for mutagenecity including chromosome aberrations, DNA fragmentation, micronuclei and sperm abnormalities (Chauhan et al., 2000; Otton et al., 2004; Rabbani et al., 2009). Cytogenetic analysis by Tollinger et al.(1974) and Bloch et al. (2000) indicated that the majority of alloxan- induced diabetic rats was composed of hypodiploid cell with a chromosome number of 38 to 41 (2n=42). Also, chromosome abnormalities (aneuploidies and polyploidies) have been reported to be increased frequency in embryos of diabetic mice (Yamamoto et al., 1971). The influence of alloxan diabetes on first meiotic segregation behaviour in female and male mice was studied by Wauben-Penris and Prins (1983) who found in primary spermatocytes higher chiasma frequencies in the translocation multivalent in diabetic males than in controls. Also, the analysis of metaphase -II cells in the females revealed less 3:1 segregation and more adjacent-II segregation in the diabetics. So, they concluded that diabetes influences the meiotic segregation behaviour of chromosomes and that chromosomes showing higher incidence of unbalanced segregation behaviour. Also, alloxan diabetes caused fragmentation of nuclear beta-cell DNA (Okamoto, 1996). The occurrence of DNA fragmentation in lymphocytes obtained from alloxan – induced diabetic rats was found to be 81% compared to 45% of untreated cells from the control (Otton et al., 2004).So, an association between diabetes and both chromosomal abnormalities and DNA fragmentation has been found, and alloxan has been used as a means of studying this association. Despite, the fact that alloxan has been used in chromosomal and DNA fragmentation studies, no information is available concerning alloxan as a cause of micronuclei and sperm abnormalities. However, the increase micronuclei frequency and sperm abnormality has been reported in streptozotocin (STZ) diabetes of rats and mice(Vikram et al., 2007; Shrilatha and Muralidhara, 2007). Also, the results of Rabbani et al. (2009) indicated that STZ diabetes of rats increased the population of micronucleated erythrocytes and sperm shape abnormality besides reducing of caudal sperm count. Several studies reported that suggested mechanism for inducing damage of nuclear component and sperm abnormalities in diabetic condition include the activation of several damaging pathways by the ROS such as accelerated formation of advanced glyccation end production (AGE), polyol pathway, hexosamine pathway, protein kinase (PKC) or increase of lipid peroxidation (LPO) (Piconi et al., 2003; Valko et al., 2007 and Rabbani et al., 2009).

LPO occurs when ROS attacked the poly unsaturated fatty acid residues of phospholipids of cell membrane which is extremely sensitive to the oxidation. Host cell like spermatozoa are highly susceptible to the damage by excess concentrations of ROS due to high content of polyunsaturated fatty acid within their plasma membrane. Increased LPO and altered membrane can affect the sperm function through impaired metabolism, motility, acrosome reaction as well as oxidative damage to sperm DNA leading to increase of morphological changes in sperm and decrease of caudal sperm count (Tramer et al., 1998; Kumar et al., 2002; Sanchez et al., 2006; Rabbani et al., 2009). Also, Sikka (2001) reported that the decrease in sperm count can be attributed to the influence of hyperglycemia on late stages of spermatogenesis, possibly through an increase of reactive oxygen species (ROS). The consequences of such oxidative damage could include loss of motility due to lipid peroxidation. Moreover, Hemachand and Shaba (2003) indicated that the increased hydroperoxide level can affect the spermatogenesis process, since germ cells are more susceptible to peroxidative damage.

In contrast, the administration of amaryl (glimepiride) to the alloxan (AL) diabetic rats in the present study had reduced the abnormalities of genetic materials (chromosomal aberrations, DNA fragmentation, the population of micronucleated erythrocytes) and sperm shape abnormalities besides enhancing the sperm count. These findings indicated that amaryl (glimepiried) inhibited the AL mediated changes in the genetic materials and sperm abnormality and enhanced the antioxidant defence. These observations suggest that the antioxidant property of amaryl could have contributed for its ability to decrease the AL mediated defects in somatic and germinal cells. Similar results were observed by Rabbani et al. (2009) who reported that the administration of glimepiride to the STZ diabetic rats had reduced the population of micronucleated erythrocytes and sperm shape abnormalities besides enhancing the sperm count compared to diabetes control. They also found that this drug has enhanced the serum levels of antioxidant enzymes (CAT, SOD and GPx) and reduced the LPO and hyperglycemia. So they suggested that the increasing levels of CAT, SOD and GPx and reducing the LPO could minimize the cytogenetic damage in somatic and germinal

cells. The same authors indicated that the compound possessing glucose lowering property along with an antioxidant effect play a beneficial role in preventing the ROS mediated DNA damages. Krishnamoorthy et al. (2007) reported that antioxidants limit the nuclear damage by preventing the free radical action.

The antioxidant activity of glimepiride on rats with streptozotocin -induced hyperglycemia was also previously reported by Krauss et al. (2003) who found that the administration of glimepiride had increased the plasma levels of SOD, GPx besides reducing the levels of H2O2 and malondialdehyde. So, they suggested that glimepiride by increasing the level of antioxidant enzymes lead to decrease the ROS mediated damage in the host cells. Kono (1978) and Valko et al. (2007). Rabbani et al. (2009) reported that SOD is an enzyme that catalyses the dismutation of superoxide ion in to oxygen and hydrogen peroxide, thus protecting the cell from the superoxide toxicity. Moreover, Valko et al. (2007) and Rabbani et al. (2009) indicated that the function of GPx is to remove the H2O2 generated by metabolic action or oxidative stress. Another study by Yassin and Mwafy (2007) found in diabetic rats, that serum triglycerides, cholesterol and urea concentration were markedly elevated. However, glimepiride therapy returned such changes towards normal.

In conclusion, the present findings add that the antioxidant property of amaryl could have contributed for its ability to decrease the alloxan mediated defects in somatic and germinal cells.

5. Refrences:

1. Blasiak, J., Arabski, M., Krupa, R., Wozniak, K., Zadrozny, M., Kasznicki, J., Zurawska, M. and Drezewoski, J. (2004): DNA damage and repair in type 2 diabetes mellitus. Mutat. Res., 554 (1-2): 297-304.
2. Bloch, K. O., Zemel, R., Bloch, O. V., Grief, H. and Vardi, P. (2000): Streptozotocin and alloxan – based selection improves toxin resistance of insulin-producing RINm cells. Int. Experimental Diab. Res., 1: 211-219.
3. Chauhan, L. K. S., Pant, N., Gupta, S. K. and Srivastava, S. P. (2000): Induction of chromosome aberrations, micronucleus formation and sperm abnormalities in mouse following carbafuran exposure. Mutat. Res., 465 (1): 123-129.
4. Diamond, M.P., Moley, K.L., Pellicer, A., Vaughn, W. K. and De Cherney, A. H. (1989): Effect of streptozotocin – and alloxan- induced diabetes mellitus on mouse follicular and early embryo development. J. Reprod. Fert., 86 (1): 1-10.
5. Donaubauer, H. H. and Mayer, D. (1993): Acute, subchronic and chronic toxicity of the new sulfonylurea glimepiride in rats. Drug Res., 43 (5): 547-549.
6. El-Shabrawy, O. A. and Nada, S. A. (1996): biological evaluation of multicomponent of tea used as hypoglycemic in rats. Fitoterapia, Volume LXVII (2): 99-102.
7. Farag, I.M., Abdou, H.S.A., Ayesh, A.M. and Osfr, M.M.H. (2002): Chromosomal and sperm studies on the mutagenic effect of over heated meat and the protective role of green tea and gingeng on rats. Al-Azhar Bull. Sci., 13: 105 - 120.
8. Gibb, R., K., Taylar D.D., Wan, T., Oconnor, D. M., Doering, D.L., and Gercel- Taylor, C., (1997) Apoptosis as a measure of chemo sensitivity to cisplatin and taxol therapy in ovarian cancer cell lines. Gynecologic Oncology, 65: 13-22.
9. Hemachand, T. and Shaba, C. (2003): Functional role of sperm surface glutathione s-transferases and extracellular glutathione in the haploid spermatozoa under oxidative stress. FEBS lett., 538:14 -18.
10. Johansen, J. S., Harris, A. K., Rychly, D. J. and Ergul, A. (2005): Oxidative stress and the use of antioxidants in Diabetes: Linking basic science to clinical practice. Cardovasc. Diabetol. , 4 (1): 5-16.
11. Kecskemeti, V. Bagi, Z. and Pacher, P. (2002): New trends in development of oral antidiabetic drugs . Curr. Med. Chem., 9 (1): 53 – 71.
12. Kono, Y. (1978): Generation of superoxide radical during antoxidation of hydroxylamine and an assay for superoxide. Arch. Biochem. Biophys., 186: 189-195.
13. Kramer, W., Mullet, G. and Geisen, K. (1996): Characterization of the molecular mode of action of sulphonylurea, glimepiride at beta cells. Horm . Metab. Res., 28 (9): 464- 468.
14. Krauss, H., Kozlik, J., Grzymislawski, M., Sosnowski, P. Mikrut, K., Piatek, J. and Paluszak, J. (2003): The influence of glimepiride on the oxidative state of rats with streptozotocin-induced hyperglycaemic. Med. Sci. Monit., 9 (11): 389-393.
15. Krishna, G. and Hayashi, M. (2000): In vivo rodent micronucleus assay: protocol, conduct and data interpretation. Mutation Research, 455 (1-2): 155 – 166.

16. Krishnamoorthy, G. Venkataraman, P., Arunkumar, A., Vignesh, R., C., Aruldas, M. M.and Arunakaran, J. (2007): Ameliorative effect of vitamins (α-tocopherol and ascorbic acid) on PCB (Aroclor 1254) induced oxidative stress in rat epididymal sperm . Reprod. Toxicol., 23 (2): 239 -245.
17. Kuhad, A., Sethi, R .and Chopra, K. (2008): Lycopene attenuates diabetes –associated cognitive decline in rats. Life sciences, 83 (3-4) : 128 – 134 .
18. Kumar, T. R., Doreswamy, K. Shrilatha, B. and Muralidhara, (2002): oxidative stress associated DNA damage in testis of mice: Induction of abnormal sperms and effects on fertility. Mutat. Res., 513(1-2): 103-111.
19. Lenzen, S. and Panten, U. (1988): Alloxan: history and mechanism of action. Diabetologia, 31 (6): 337 – 342.
20. Mastrocola, R., Restivo, F., Vercellinatto, L., Danni, O., Brignardello, E., Aragno, M., and Boccuzzi, G., (2005): Oxidative and nitrosative stress in brain mitocondria of diabetic rats. Journal of Endocrinology, 187: 37 -44
21. Muller, G., Satoh, Y. and Geisen, K. (1995): Extrapancreatic effects of sulphonylurea- a comparison between glimpiride and conventional sulphonylureas. Diabetes Res, Clin. Pract., 28: 115-137.
22. Narayana, K. (2008): An aminoglycoside antibiotic gentamycin induces oxidative stress, reduces antioxidant reserve and impairs spermatogenesis in rats. J. Toxicol. Sci., 33: 85 – 96.
23. Nieszner, E, Posa, I., Pogatsa, G et al (2002): Influence of diabetic state and that of different sulfonylureas on the size of myocardial infraction with and without ischemic preconditioning in rabbits. Exp. Clin. Endocrinol Diabetes, 110 (5): 212- 218 .
24. Okamoto, H. (1996) : Okamoto model for B-cell damage : recent advances , In : Lessons from animal diabetes , edited by Shafrir ,E. pp, 97-112 , Boston , Birkhauser.
25. Otton, R., Soriano, F. G., Verlengia, R. and Curi, R. (2004): Diabetes induces apoptosis in lymphocytes. Journal of Endocrinology,182: 145-156.
26. Pant, N. and Srivastava, S. P. (2003): Testicular and spermatotoxic effect of quinaphos in rats . J. Appl. Toxicol., 23 (4):271-274.
27. Piconi, L., Quagliaro, L. and Ceriello, A. (2003): Oxidative stress in diabetes. Clin. Chem. Lab. Med. 41: 1144 - 1149.
28. Preston, R. J., Dean, B. J., Galloway, S., Holden, H., McFee, A. F., and Shelby, M. (1987): Mammalian in vivo cytogenetic assays: Analysis of chromosome aberrations in bone marrow cells. Mutat. Res., 189 (2):157-165.
29. Rabbani, S. I., Devi, K. and Khanam, S. (2009): Inhibitory effect of glimepiride on nicotinamide – streptozotocin induced nuclear damage and sperm abnormality in diabetic Wister rats. Indian Journal of experimental Biology, 47: 804-810.
30. Rehman, A., Nourooz-Zadeh, J., Moller, W., Tritschler, H., Pereira, P. and Halliwell, B., (1999): Increased oxidative damage to all DNA bases in patients with type - II diabetes mellitus. FEBS Letters, 448(1):120-122.
31. Sanchez E. E. T., Marquette M. L., Brown D. B. and Ansari N.H. (2006): The effect of oxidative stress on human sperm morphology . Fertil. Steril. , 86 (suppl 1). S444. In: Am. Soc. Reprod. Med. 62 nd Annual Meeting.
32. Sato, J., Ohsawa, I., Oshida, Y., et al. (1993): Effects of glimepiride on vivo insulin action in normal and diabetic rats. Diabetes Res. Clin. Pract., 22: 3-9.
33. Schmid, W. (1975): The micronucleus test. Mutat. Res., 31: 9 – 15.
34. Schnedl, W. J., Ferber, S., Johnson, J. H. and Newgard, C.B. (1994): STZ transport and cytotoxicity. Specific enhancement in GLUT-2 expressing cells. Diabetes, 43(11): 1326 – 1333.
35. Shrilatha, B. and Muralidhara, (2007): Early oxidative stress in testis and epididymal sperm in streptozotocin –induced diabetic mice: in progression and genotoxic consequences. Report. Toxicol, 23(4):578-587.
36. Sikka, S.C. (2001): Relative impact of oxidative stress on male reproductive function. Curr. Med. Chem., 8(7): 851 – 862.
37. Somfai, G. M., Knippel, B., Ruzicska, E., Stadler, K., Toth, M., Salacz, G., Magyar, K., Somogyi, A. (2006): soluble semicarbazide – sensitive amine oxidase (SSAO) activity is related to oxidative stress and subchronic inflammation in streptozotocin – induced diabetic rats. Neurochemical International, 48(8):746 -752.
38. Tiege M., Lortz S Drinkgern J. and Lenzen S. (1997): Relation between antioxidant enzyme gene expression and antioxidative defense status of insulin-producing cells. Diabetes, 46: 1733- 1742.
39. Tollinger, C. D., Chrisman, C. L. and Doolittle, D. P. (1974): Alloxan – induced aneuploidy in mice. The Journal of Heredity, 63 : 345-348

40. Tramer, F., Rocco, F., Micali, F., Sandri, G. and Panfili, E. (1998): Antioxidant systems in rat epididymal spermatozoa . Biol. Reprod, 59(4): 753-758.
41. Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur M. and Telser, J (2007): Free radicals and antioxidant in normal physiological function and human disease. Int J biochem. Cell Biol., 39: 44-84.
42. Vikram, A. Tripathi, D. N., Ramarao, P. and Jene, G. B. (2007): Evaluation of streptozotocin genotoxicity in rats from different ages using the micronucleus assay. Regulatory Toxicology and Pharmacology , 49, (3): 238-244
43. Wauben-Prins, P. J. J. and Prins, J.-B. (1983): Meiotic behavior of alloxan –treated diabetic and non diabetic T (1; 13) 70H/+mice . Human Genetics, 63 (3): 268-273.
44. Wyrobek, A. J. and Bruce, W. R. (1978): The induction of sperm shape abnormalities in mice and humans. Chem. Mutagens, 5: 257 – 285.
45. Yamamoto, M., Endo, A., Watanabe, G. and Ingalls T.H. (1971): Chromosomal aneuploidies and polyploidies in embryos of diabetic mice. Archs Enviro. Health, 22(4):468-475
46. Yassin, M.M. and Mwafy, S.N. (2007): Protective potential of glimepiride and Nerium oleander extract on lipid profile, body growth rate, and renal function in streptozotocin – induced diabetic rats. Turk J Biol., 31: 95 – 102.

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7/12/2010