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Aminoguanidine mitigates apoptosis, testicular seminiferous tubules damage, and oxidative stress in streptozotocin-induced diabetic rats

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ABSTRACT

This study aimed to investigate the effect of aminoguanidine (AG) against testicular damage streptozotocin (STZ) induced diabetes. Thirty two rats were separated into four groups: control, AG, STZ and STZ + AG.

In the STZ group, 12.5 ± 1.3% of tubules were seen as containing sloughed spermatogenic cells into the lumen, 28.7 ± 1.8% of tubules were atrophic, 46.2 ± 2.1% of tubules were degenerative and 8.5 ± 0.9% of tubules contained giant cells. Statistically, the affected tubule number was significantly lower in the STZ + AG group than in the STZ group. Intensely stained caspase-3 cells showed a statistically significant increase in the STZ group, while it decreased in the STZ + AG group. The enzyme activities of catalase (CAT), superoxide dismutase (SOD) and glutathione (GSH) level decreased and the level of malondialdehyde (MDA) and nitric oxide (NO) increased in the STZ group, while AG treated diabetic rats showed an increase of CAT, SOD activity and GSH level and a decrease in MDA and NO levels.

This study shows that the oxidative stress, increased NO level and apoptotic cell death play an important role in diabetic rat testicular damage and that AG treatment of diabetic rats results in protection of spermatogenic cells against oxidative stress and apoptotic cell death.

1. Introduction

Diabetes mellitus develops as a result of hyperglycemia due to either a deficiency in insulin or an insufficiency of the effect of insulin. Different experimental studies and clinical observations indicate that hyperglycemia directly and indirectly increases the formation of free radicals, thus leading to oxidative stress (Liptakova et al., 2002; Agardh et al., 2002). Increase in oxidative stress and changes in antioxidant capacity play a significant role in the pathogenesis of chronic diabetes mellitus (Baynes and Thorpe, 1999; Wolff et al., 1991; Armagan et al., 2006).

The nitric oxide (NO) molecules, which increase in diabetes, may cause tissue damage and inflammation through the production of nitrated and oxidated intermediate products and the inhibition of many enzymes; they may also lead cells to necrosis or apoptosis through the creation of highly toxic peroxynitrite radicals (Nilsson, 1999; Eiserich et al., 1998).

Clinical and experimental studies have established that diabetic patients are subject to changes such as spermatogenesis dysfunction, lower sperm count and motility, reduced seminal fluid volume and a fall of testosterone level (Amaral et al., 2008). Additionally, diabetes-related histologic changes are observed in the testes, seminiferous tubules and interstitial connective tissue (Armagan et al., 2006).

Aminoguanidine (AG), a phenylhydrazine compound, reduces NO production by selectively inhibiting the synthesis of NO (through the intermediary of the inducible nitric oxide synthase (iNOS). It has also been reported to have antioxidant properties by scavenging radicals like peroxynitrite (Ihm et al., 1999; Philis-Tsimikas et al., 1995).

This study aimed to investigate the therapeutic effects of AG on diabetes induced testicular damage by using histopathological semiquantitative evaluation and oxidative stress markers in damaged testicular tissue in rats.

2. Materials and methods

2.1. Animals and experimental protocol

The study was performed on 32 adult male Sprague–Dawley rats provided by the Experimental Animal Research Center of Inonu University (Malatya, Turkey). After weighing, rats were randomly

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assigned to one of four groups of eight animals each: Control group (C); animals given AG only (AG); diabetic animals (STZ) and animals treated by AG after provoking diabetes (STZ + AG). Diabetes was induced with a single dose of streptozotocin (STZ) (Sigma, USA), 45 mg/kg body weight intraperitoneally (i.p.). In the previous studies, STZ was given as a single injection of 40–60 mg/kg to provoke diabetes in the rat (Maiti et al., 2004; Usta et al., 2004; Khaki et al., 2010). Blood glucose levels were measured with a glucometer (Accu-Check Glucose Meter, Roche, Switzerland) after 72 h of STZ injection. Animals with blood glucose levels of 270 mg/dl and above were taken into the study. AG, 1 g/L (Yavuz et al., 2001) was prepared by dissolving thoroughly in tap water and given to the animals every morning at 09:00 a.m. During the 10 weeks of study, all animals were fed with standard rat pellet food. The drinking water intake of each animal was measured and the daily AG dose calculated in mg. The animals were weighed and their blood glucose levels were measured at the end of the study.

2.2. Collecting the testes

The rats were sacrificed under ketamine and xylazine (1.2–1.4 g/kg) anesthesia. The testes were excised and weighed. The left testis of each animal was frozen for biochemical studies and the right one used for histological examination.

2.3. Histological and immunohistochemical methods

The testis tissues were fixed in 10% formalin solution, and then embedded in paraffin. The paraffin blocks were cut 5 μm and stained with hematoxylin and eosin (H&E). For immunohistochemical analyses, sections were mounted on polylysine-coated slides. After rehydrating, the samples were transferred to citrate buffer (pH 7.6) and heated in a microwave oven at 65 °C for 20 min. After cooling for 20 min at room temperature, the sections were washed with phosphate buffered saline (PBS). Then the sections were kept in 0.3% hydrogen peroxide (H_2O_2) for 7 min, followed by a wash with PBS. Subsequently, the sections were incubated with primary rabbit-polyclonal cysteine aspartate specific proteinase (caspase-3) (Neomarker, USA), rinsed in PBS and then incubated with biotinylated goat anti-polyvalent for 10 min and streptavidin peroxidase for 10 min at room temperature. The staining procedure was completed with chromogen + substrate for 15 min and the slides were counter-stained with Mayer's hematoxylin for 1 min. Caspase-3 was used according to the manufacturer's instructions with a minor revision.

2.4. Semi-quantitative evaluation

The diameter and germinative cell layer thickness of the seminiferous tubule (ST) from twenty different areas of each testis were measured using Leica Q Win Plus Image Analysis System (Leica Micros Imaging Solutions Ltd., Cambridge, United Kingdom) at 10×. Histopathological evaluations were performed according to the methods of Sayım (2007). Histologic changes were detected by counting 100 tubules in slides stained with H&E. One hundred tubules per animal were examined and classified as normal, contain sloughed spermatogenic cells into the lumen, atrophied, tubules contain degenerated germ-cell or multi-nucleated giant cell based on the degree of seminiferous tubule degeneration. The tubules with sloughing were those that showed disrupted cell association. Tubules showing few or no germ cells were classified as atrophic tubules. Tubules with abnormal cells or interrupted spermatogenic cells at various stage of mitotic phase were classified as tubules with germ-cell degeneration. Tubules with cells that have more than one nucleus were classified as tubules with multi-nucleated giant cell.

Caspase-3 staining was also evaluated semiquantitatively as follows: weak (+), moderate (++), and strong (+++), according to the intensity of staining. Cells positive for caspase-3 stained as a brown color. Stained cells with caspase-3 were counted using the Leica Q Win Image Analysis System and classified according to three intensity categories.

2.5. Biochemical analysis

2.5.1. Homogenization

Tissues were homogenized (PCV Kinematica Status Homogenizator) in ice-cold phosphate buffered saline (pH 7.4). The homogenate was sonified with an ultrasonifier (Bronson sonifier 450) by 3 cycles (20-s sonications and 40-s pause on ice). The homogenate was centrifuged (15,000 × *g*, 10 min, 4 °C) and cell-free supernatant was subjected to enzyme assay immediately.

2.5.2. Catalase (CAT) assay

CAT activity was measured at 37 °C by following the rate of disappearance of H_2O_2 at 240 nm ($\varepsilon_{240} = 40\ M^{-1}\ cm^{-1}$) (Luck, 1963). One unit of CAT activity is defined as the amount of enzyme catalyzing the degradation of 1 μmol of H_2O_2 per min at 37 °C and specific activity corresponding to transformation of substrate (μmol H_2O_2) per min per mg protein.

2.5.3. Superoxide dismutase (SOD) assay

SOD activity was assayed using the nitroblue tetrazolium (NBT) method (Sun et al., 1988). The samples were subjected to ethanol–chloroform (62.5/37.5%) extraction prior to the assay. NBT was reduced to blue formazan by the superoxide ion (O_2^-), which has a strong absorbance at 560 nm. One unit (U) of SOD is defined as the amount of protein that inhibits the rate of NBT reduction by 50%. The calculated SOD activity was expressed as U/mg tissue protein.

2.5.4. Total glutathione (GSH) assay

The formation of 5-thio-2-nitrobenzoate is followed spectrophotometrically at 412 nm (Theodorus et al., 1981). The amount of GSH in the extract was determined as nmol/mg protein utilizing a commercial GSH as the standard. The results are expressed as nmol/mg protein.

2.5.5. Lipid peroxidation assay

The analysis of lipid peroxidation was carried out as described previously (Buege and Aust, 1978) with a minor modification. The absorbance of the supernatant was recorded at 532 nm. Malondialdehyde (MDA) results were expressed as nmol/mg protein in the homogenate. The protein content of the samples was determined by the colorimetric method of Lowry et al. (1951). The absorbance measurement was taken at 595 nm using a UV-VIS spectrophotometer (Shimadzu UV-1601). Bovine serum albumin was used as protein standard.

2.5.6. Measurement of nitric oxide

The end products of *in vivo* reactions of NO are nitrites (NO^{2-}) and nitrates (NO^{3-}). The respective proportions of these products being variable, the best index of total NO (tNO) production is the measurement of NO^{2-} and NO^{3-} together. The tNO levels in testicular tissue were measured using the Griess reaction, by fluorimetry of NO^{-2} and NO^{-3} (Cayman Inc., Ann Arbor, MI, USA).

Table 1
Body and testis weights (g) and blood glucose levels (mg/dl) are shown for the start and the end of the study.

Parameters	Groups			
	C	AG	STZ	STZ + AG
Beginning body weight	282.1 ± 4.3	290.6 ± 49	319.4 ± 3.7	319.7 ± 3.6
Final body weight	323.9 ± 6.4	326.4 ± 8.18	207.1 ± 6.3[a]	294.6 ± 18.2[b]
Beginning blood glucose	128.6 ± 2.5	132.25 ± 6	383.3 ± 14.1	283.58 ± 6.1
Final blood glucose	154.4 ± 3.4	167.0 ± 13.2	587.0 ± 5.0[a]	334.6 ± 7.8[b]
Testicular weights of right testis	1.4 ± 0.003	1.55 ± 0.003	0.7 ± 0.1[a]	1.55 ± 0.07
Testicular weights of left testis	1.6 ± 0.1	1.51 ± 0.008	0.8 ± 0.1	1.53 ± 0.008[b]

[a] $p < 0.05$ vs control group.
[b] $p < 0.05$ vs STZ group.

2.6. Statistical analysis

A computer program (SPSS 11.0) was used for statistical analysis. The results were compared with Kruskal–Wallis variance analysis. Where differences among the groups were detected, group means were compared using the Mann–Whitney *U* test. Values of $p < 0.05$ were considered significant. All results were expressed as arithmetic mean ± standard error (SE). In-group change was evaluated by Wilcoxon's paired matched sample test. Results were considered statistically significant at $p < 0.05$.

3. Results

3.1. Body weight and blood glucose values

The body weights of the diabetic rats were significantly lower than those of the control group. On the other hand, loss of body weight was reduced by AG treatment. Hyperglycemia was present in the STZ group from 72 h following the STZ injection to the end of the study. The STZ + AG animals had significantly lower blood glucose levels than STZ group ($p = 0.001$). Body weights and blood glucose levels from beginning to the end of the study were shown in Table 1.

3.2. Testicular weight

Testis weight for both the left and the right organ at the end of the study was measured. Testicular weight was lower in diabetic animals (right testis 0.7 ± 0.1 g; left testis 0.8 ± 0.1) compared to the control animals (right 1.4 ± 0.003, left 1.6 ± 0.1 g); the difference was statistically significant ($p = 0.001$). AG administration prevented the lost of testicular weight (right 1.55 ± 0.07 and left 1.53 ± 0.008 g). No statistical difference could be seen between these weights and those in the control group ($p > 0.05$). Testicular weight by groups was shown in Table 1.

3.3. Histological findings

3.3.1. Control and AG groups

In control and AG groups, the seminiferous tubules were intact and germ cells organized in concentric layers. The lumen of seminiferous tubules were containing abundant amounts of spermatids and sperms. Spermatogenic cells at various stage of division (to spermatid from spermatogonia) were observed in the seminiferous tubules (Fig. 1A and B).

3.3.2. STZ and STZ + AG groups

Seminiferous tubule structures appeared shrunken and separated from each other (Fig. 2A). Histopathological observation showed that STZ caused obvious seminiferous tubule degeneration such as sloughing, atrophy, germ-cell degeneration and multinucleated giant cell formation. Numerous round germ cells which detected from spermatogenic layers were observed in the some seminiferous lumen of this group (Fig. 2B). Some of other the tubules were atrophic. There was a significant loss in the number of germ cells in this seminiferous tubules (Fig. 2C). Arrested spermatogenic cells at various stages of division and degenerated germ-cell in different forms were observed in some of the seminiferous tubules (Fig. 2D and E). Additionally, the multinucleated giant cells, formed as a result of the fusion of spermatids, were detected in a few of the seminiferous tubule epithelium (Fig. 2F). Moreover the mean of seminiferous tubule diameter and germinal cell layer thickness were decreased in the STZ group.

On the other hand, the number of intact seminiferous tubules in the STZ + AG group was increased significantly compared to STZ group ($p = 0.0001$). AG treatment did not completely alleviate the lesions, and milder degenerative changes such as deformed tubules (Fig. 3A) and arrested spermatogenic cells (Fig. 3B) were still present in tubule epithelium. Moreover AG administration, improved the decreased seminiferous tubule diameter and germinal cell layer.

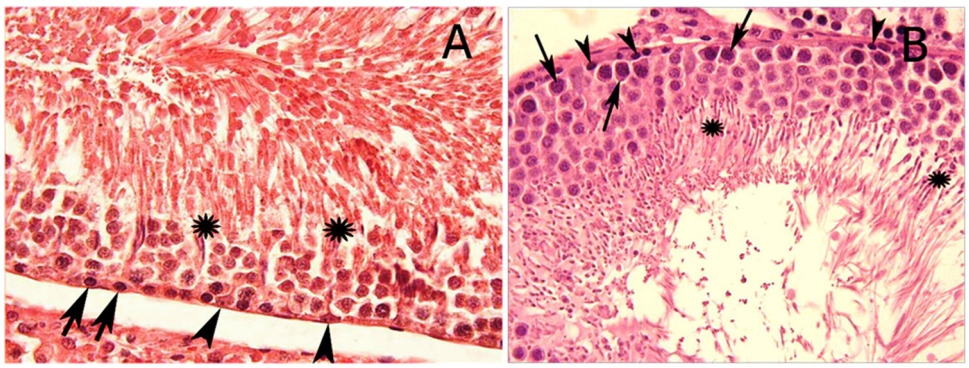

Fig. 1. (A) Control group; normal histological view of seminiferous tubules: spermatogonium (arrows), Sertoli cells (arrow heads), and spermatids (stars). (B) Aminoguanidine group; histological structure of seminiferous tubules is similar to control group; primer spermatocyte (arrows), spermatogonium (arrow heads) and spermatid (stars), H–E.

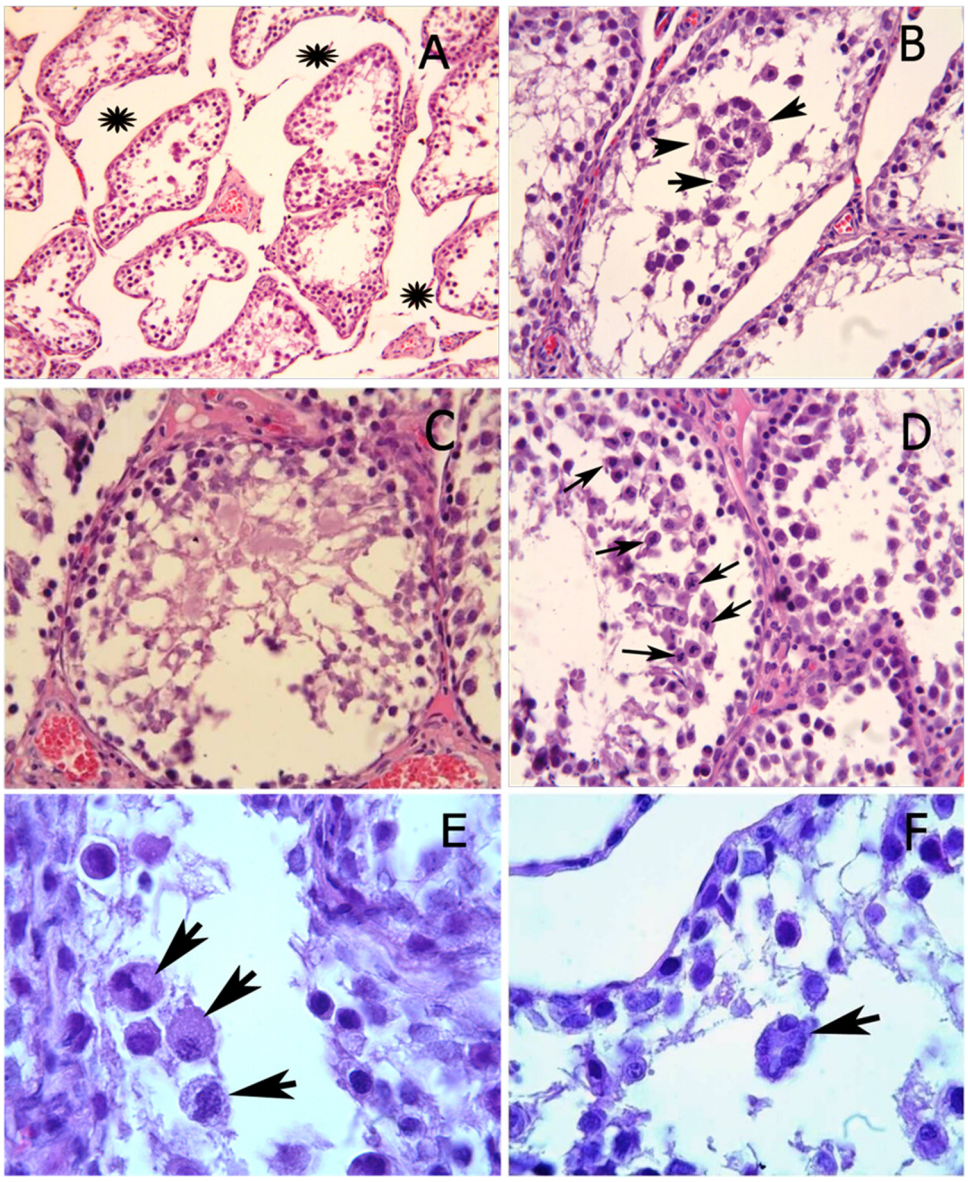

Fig. 2. (A) Diabetic group; tubular shrinkage and extensive intertubular area (stars). (B) Notice the accumulation of immature germ cells in the lumen (arrows). (C) Tubule shows severe tubular atrophy and loss or decrease of germ cells. (D) Arrested spermatocytes in different stage of division (arrows). (E) Degenerated spermatogenic cells (arrows) are visible in seminiferous tubule. (F) A giant multinucleated cell derivative from round spermatids (arrow) are evident, H–E.

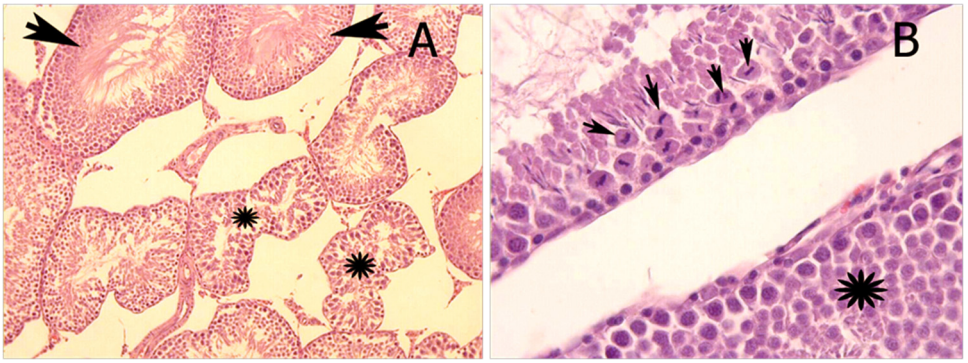

Fig. 3. (A) Aminoguanidin + Diabetic group; disordered testicular epithelium and deformed tubules (stars) are observed but the other tubules (arrows) show nearly normal histologic appearance. (B) Spermatogenic arrest (head arrows) are still evident in some tubules (arrows), star indicates intact tubule. H–E.

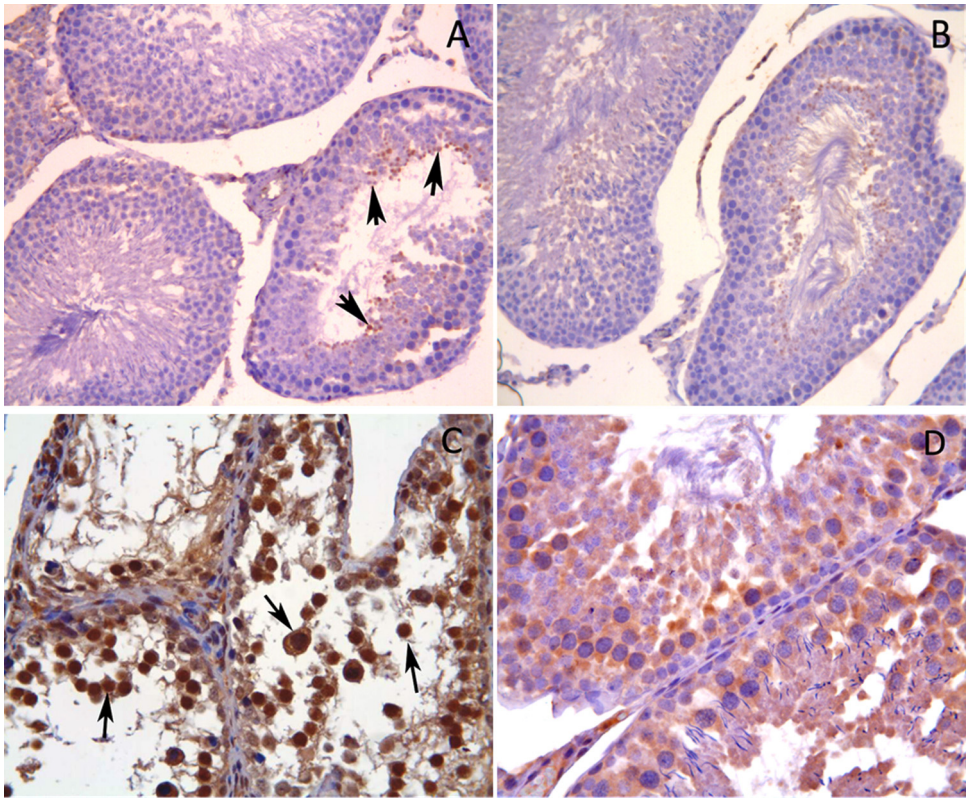

Fig. 4. (A) Control and (B) Aminoguanidine groups; caspase-3 positive staining of cytoplasmic residues of spermatids (arrows); (C) Diabetic group; germ cells are seen marked and diffuse caspase 3 staining. Arrows indicate caspase-3 immunoreactivity in cytoplasm or perinuclear region of germ cells. (D) Aminoguanidine + Diabetic group; germ cells are observed much less positive caspase-3 staining than diabetic group. There is a marked reduction number of caspase-3 positive cells and coloring intensity in all regions.

Table 2
Diameters seminiferous tubules (DST) and germinal cell layer thickness (GCLT).

Parameters	C	AG	STZ	STZ + AG
DST (μm)	254.6 ± 5.17	233.4 ± 6.39	142.0 ± 19.27[a]	226.3 ± 8.96[b,c]
GCLT (μm)	64.5 ± 0.83	60.1 ± 2.08	38.6 ± 5.42[a]	61.4 ± 1.93[b,c]

[a] Significantly decreased when compared with control group, $p < 0.05$.
[b] Significantly increased when compared with D group, $p < 0.05$.
[c] Not significant when compared with control group, $p > 0.05$.

Table 3
Percentage of histopathologic classification of seminiferous tubules in the testis.

Groups	Intact	Degenerated	Sloughing	Giant cell	Atrophic
C	97.8 ± 0.7%	0.0 ± 0.0%	0.9 ± 0.1%	0.0 ± 0.0	1.3 ± 0.0%
AG	93.8 ± 0.9%	1.4 ± 2.3%	2.4 ± 0.3%	0.0 ± 0.0	2.4 ± 0.0%
STZ	4.1 ± 0.8%[a]	46.2 ± 2.1%[c]	12.5 ± 1.3%[a]	8.5 ± 0.9%[c]	28.7 ± 1.8%[c]
STZ + AG	78.5 ± 0.8%[b]	8.2 ± 0.6%[d]	6.1 ± 0.5%	0.8 ± 0.1%[d]	6.4 ± 1.0%[d]

[a] Significantly decreased when compared with control group, $p < 0.001$.
[b] Significantly increased when compared with D group, $p < 0.001$.
[c] Significantly increased when compared with control group, $p < 0.001$.
[d] Significantly decreased when compared with D group, $p < 0.001$.

Table 4
The number of tubules staining with caspase-3 (CASP).

Groups	CASP 0	CASP +	CASP ++	CASP +++
C	11.3 ± 3.2	8.6 ± 3.2	0.0 ± 0.0	0.0 ± 0.0
AG	8.6 ± 1.9	11.3 ± 1.9	0.0 ± 0.0	0.0 ± 0.0
STZ	0.0 ± 0.0[a]	0.5 ± 0.5	6.1 ± 1.8[b]	13.1 ± 2.0[b]
STZ + AG	2.3 ± 3.6	7.5 ± 3.1[e]	9.0 ± 3.5[b,d]	1.1 ± 1.3[c,e]

[a] Significantly decreased when compared with control group, $p < 0.05$.
[b] Significantly increased when compared with control group, $p < 0.05$.
[c] Significantly decreased when compared with D group, $p < 0.05$.
[d] Not significant when compared with D group, $p > 0.05$.
[e] Not significant when compared with control group, $p > 0.05$.
(+, weak; ++, mild and +++, strong).

Table 5
The effect of aminoguanidine on STZ-induced changes in oxidative stress parameters and NO level.

	CAT (U/mg protein)	SOD (U/mg protein)	MDA (nmol/mg protein)	GSH (nmol/mg protein)	NO (nmol/mg protein)
C	7.70 ± 0.37	34.47 ± 0.92	1.57 ± 0.09	26.47 ± 0.97	307.15 ± 37.68
AG	6.27 ± 0.92	32.27 ± 0.43	1.16 ± 0.08	25.14 ± 2.50	201.28 ± 22.48
STZ	3.70 ± 0.23[a]	25.43 ± 0.81[a]	2.60 ± 0.24[c]	15.37 ± 1.12[a]	442.27 ± 38.03[c]
STZ + AG	6.20 ± 0.49[b]	32.66 ± 1.10[b,d]	1.24 ± 0.18	25.49 ± 0.77[b,d]	221.86 ± 21.7[d]

[a] Significantly decreased when compared with control group, $p < 0.05$.
[b] Significantly increased when compared with D group, $p < 0.05$.
[c] Significantly increased when compared with control group, $p = 0.05$.
[d] Not significant when compared with control group, $p > 0.05$.

Small intraluminal cytoplasmic fragments, considered as positive for caspase-3 staining, were observed in the tubules (Fig. 4A and B). No moderately (++) or strongly (+++) staining spermatogenic cells were encountered in these groups. Brown-staining, positive caspase-3 cells were visible in the seminiferous tubules of STZ-treated animals (Fig. 4C). There was a statistically significant increase in the number of germ cells immunostained with caspase-3 (+++) in the seminiferous tubule epithelium of the STZ group when compared with the control group ($p < 0.05$). The coloring density and number of germ cells staining for caspase-3 decreased in the STZ + AG group compared with the STZ group ($p < 0.005$) (Fig. 4D). Caspase-3 immunostaining was not observed in the Sertoli and Leydig cells of any of the groups. The results of the diameters seminiferous tubules (DST), germinal cell layer thickness (GCLT), histopathological classification of the seminiferous tubules and staining with caspase-3 were given in Tables 2–4.

3.4. Biochemical findings

Briefly, in the STZ group, the level of MDA, the end product of lipid peroxidation, statistically significantly increased ($p < 0.005$) while the SOD, CAT activities and GSH level statistically significantly decreased ($p < 0.005$) compared with the control group. On the other hand, AG administration caused a statistically significant decrease in MDA levels ($p < 0.005$) and an increase in SOD, CAT enzyme activities and GSH level ($p < 0.005$) when compared with STZ alone. NO levels were highest in the diabetic animal group (442.256 ± 38.03) followed by the controls (307.148 ± 37.68) and STZ + AG treatment group (221.86 ± 21.7); the lowest values were found in the AG treatment group (201.282 ± 22.48). AG administration resulted in a marked reduction of NO levels in diabetic rats, who had a significantly higher NO level than the controls ($p = 0.004$). The biochemical results were shown in Table 5.

4. Discussion

STZ penetrates β cells by glucose transporter GLUT-2, resulting in DNA alkylation (by binding methyl-ethyl groups to phosphates in the DNA skeleton) and the consequent activation of ADP ribosylation. This is followed by the appearance of hydrogen peroxide and hydroxyl radicals (Szkudelski, 2001). Following the realization that STZ is an NO provider, the role of NO in the cytotoxic action on β cells was confirmed by several reports (Turk et al., 1993). NO is not, however, the only molecule responsible for the cytotoxic action of STZ (Szkudelski, 2001). The diabetogenic action of STZ in β cells is known to occur through both NO production and that of reactive oxygen radicals. In fact, it has been observed that the administration of inhibitors of nitric oxide synthase (NOS) and antioxidants may arrest STZ hyperglycemia (Unlucerci et al., 2000; Aksoy et al., 2003; Cam et al., 2003).

A noticeable loss of testicular weight was observed in the diabetic rats in our study. Ricci et al. (2009) report a dramatic decrease in testicular weight in rats with a blood glucose higher than 12 mmol/L. Similarly, a 20% decrease in testicular weight of diabetic rats was noted compared to controls by Navarro-Casado et al. (2010). Male fertility depends on the continual renewal of spermatogonia and their differentiation into spermatogenic cells. Several authors have reported a narrowing of tubular diameter, disorganization of the germinative epithelium and shedding of germ cells at different stages of meiosis into the tubular lumen (Ricci et al., 2009; Navarro-Casado et al., 2010). We similarly observed atrophy of the seminiferous tubules and a dissociation with tubular shedding of immature spermatogenic cells in our diabetic animals. Sayım (2007) indicates that the dissociation of cells in the germinal epithelium and their tubular shedding is an index of the disruption in intercellular bonds. Ricci et al. (2009) report an irregular occludin distribution in some tubules, reaching complete absence in some others. Increase in oxidative stress has also been reported to substantially decrease e-cadherin and α-catenin expression (Ha et al., 2011).

Caspase-3, one of the 14 known members of the caspase family, is a key protease activated in the early stages of apoptosis (Brüne et al., 1998). Apoptotic cells in the testicular tissue were marked using caspase-3 activity in our study. An increase in oxidative stress levels in testicular tissue has been shown in earlier reports (Giugliano et al., 1996; Baynes and Thorpe, 1999; Kanter et al., 2012). Increased oxidative stress leads to cellular injury by different mechanisms, including oxidative DNA and protein damage, and membrane lipid peroxidation. Oxidative stress is known as a potent apoptosis mediator (Leon et al., 2005). One other way of apoptosis induction is the NO pathway. It has been established that the enzyme poly (ADP-ribose) polymerase (PARP), a caspase-3 substrate, is broken down in NO-mediated apoptosis. This means that NO production intensifies caspase activation. The caspase-3-mediated apoptotic process may therefore be inhibited by NO inhibition (Brüne et al., 1998). This study showed an important increase in caspase-positive tubule numbers and density in the diabetic animals, and also a reduction in apoptotic tubule numbers and activity in the group treated with AG. Hammes et al. (1995) report having preventing Mullerian cell apoptosis *in vivo* in diabetes by AG administration. We think that inhibition of both oxidative stress and NO production play a role in the reduction of apoptotic cell numbers following AG treatment.

In this study, diabetic animals showed an increase in MDA and NO, while SOD and CAT activity and GSH levels decreased. Hyperglycemia is known to increase glycolysis, activate the intracellular sorbitol (polyol) pathway, and increase free radical formation. Glucose is oxidated into reactive ketoaldehyde and superoxide radicals. Superoxide anions are transformed into H_2O_2 by superoxide dismutase (SOD). In the absence of breakdown by CAT or glutathione peroxidase (GPx), they lead to the production of reactive hydroxyl radicals. Also, superoxide anions may react with NO to form reactive peroxynitrite radicals (Maritim et al., 2003). An increase in nitrates and nitrites, end products of NO activity, has been reported in diabetic rats. Both of these radicals have a more potent oxidative and cytotoxic effect than NO (Anggered, 1994; Welsh et al., 1994; Anwar and Meki, 2003).

The cellular control of ROS is regulated by the antioxidative defense system, represented by enzymatic (SOD, CAT) and nonenzymatic (GSH) antioxidants. Excessive production of free radicals creates damage by binding to intracellular proteins and nucleic acids (Maritim et al., 2003). Additionally, low density lipoproteins in the membrane intensifies lipid peroxidation. MDA is the most widely used indicator of lipid peroxidation in tissues. An increase in MDA levels suggests membrane injury due to free radicals. Kanter et al. (2012) have indicated that MDA levels increase in diabetic testicular tissue. On the other hand, the spermatic plasma membrane is more sensitive than other cells to free radical-induced lipid peroxidation, as a result of its specific lipid composition including a high content of polysaturated fatty acids and sphingomyelin (Makker et al., 2009). A decrease in SOD and CAT enzymatic activity and in GSH levels increased oxidative stress. By decreasing cellular NADPH, hyperglycemia decreases GSH levels, thus making the cell vulnerable to ROSs and increasing oxidative stress. Glucose accumulated in the cells is metabolized by the polyol pathway. Activation of the polyol pathway decreases intracellular NADPH. The absence of this cofactor, which is necessary for making GSH, will reduce the level of the latter, an important element in the protection of cells against free radical damage (Bonnefont-Rousselot et al., 2000). Reactive metabolites are thus unable to bind to GSH, oxidating mainly tissue proteins, lipids and macromolecules (Kaplowitz, 2000). We observed that AG treatment was accompanied by an important fall in lipid peroxidation due to diabetes. Additionally, SOD and CAT activity as well as GSH levels were substantially higher in untreated diabetic animals. Philis-Tsimikas et al. (1995) has already reported the inhibition by AG of reactive oxygen radical production and lipid peroxidation. Giardino et al. (1998) studied the effect of AG on hydroxyl radicals, observing a decrease in hydroxylated benzoate concentration inversely correlated to that of AG. Agardh et al. (2002) indicated that AG may inhibit the glycooxidation process both by blocking reactive oxo groups and by scavenging dicarboxyls.

In conclusion, our study is the first report to demonstrate the beneficial effects of AG on diabetic testicular damage and apoptosis. This study emphasizes the potential of AG as an antioxidant and iNOS inhibitor to treatment for STZ-induced diabetes mellitus in rats. However, further studies are needed to elucidate the mechanism underlying the beneficial effects of AG therapy on diabetes.

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