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Cytoarchitectural improvement in Leydig cells of diabetic rats after treatment with aqueous and ethanol extracts of *Dracaena arborea* (Dracaenaceae)

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ABSTRACT

Background and aim: Recent studies have demonstrated the androgenic effects of *Dracaena arborea* in castrated and diabetic rats, but the cytoarchitectural mechanism at the level of Leydig cells (LCs) justifying this improvement in androgens production in diabetic rats has never been examined. We investigated the effects of aqueous and ethanol extracts of *D. arborea* on diabetes-induced cytoarchitectural impairments of LCs in rats.

Experimental procedure: Besides a normal group, 4 groups of diabetic rats were treated orally with Millipore water (10 ml/kg, diabetic), sildenafil citrate (1.44 mg/kg), aqueous (500 mg/kg) and ethanol (100 mg/kg) extracts of *D. arborea* for 21 days. On day 22, rats were sacrificed and the testes were removed and prepared for electron microscopic analyses of LCs ultrastructure.

Results and conclusion: The ultrastructure of LCs in control rats was normal, while that in diabetic rats exhibited large heterochromatinization in the nuclei, reduced amount of smooth endoplasmic reticulum with no lipid droplets in the cytoplasm, many autophagosomes and degenerated mitochondria containing lots of electron dense granules in the matrix. Interestingly, treatment with *D. arborea* especially its aqueous extract (500 mg/kg) alleviated these impairments, characterized by a rarification of heterochromatinization in the nuclei coupled to an increase and the presence in the cytoplasm of prominent smooth endoplasmic reticulum and a reduction of electron dense granules in the matrix of mitochondria. These alleviating properties of *D. arborea* on LCs ultrastructure of diabetic rats could explain its androgenic potential. These results are useful for the management of patients suffering from diabetes-induced hypogonadism.

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1. Introduction

In mammals, the maintenance of male fertility depends on germ cells and Leydig cells (LCs), which are responsible for spermatogenesis (production of spermatozoa) and steroidogenesis (testosterone secretion) respectively.^{1,2} LCs is the main cell type

containing all enzymes necessary for the synthesis of sex hormones.^{3,4} Sex hormones production starts in the mitochondrion by the cleavage of cholesterol giving pregnenolone which is progressively metabolized enzymatically to ultimately be converted into testosterone within the smooth endoplasmic reticulum (er).^{3,4} It has been shown that, in the testicular interstitium, testosterone is primarily produced in LCs with active autophagy and, autophagy deficiency in this organelle is associated with reduced levels of serum testosterone in rats, mice and humans.^{5,6} During autophagy, cytosolic constituents such as organelles, nucleic acids, or proteins as well as other biological macromolecules are engulfed by

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List of abbreviations

CNH	Cameroon National Herbarium
Cyp11A1	Cytochrome P450 side chain clivage
<i>D. arborea</i>	<i>Dracaena arborea</i>
DNA	Deoxyribonucleic acid
DM	Diabetes mellitus
Ers	Estradiol receptors
LC3	Protein 1 light chain 3
LH	Lutropin
StAR	Steroidogenic acute regulatory protein
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus

autophagosome and they then fuse with lysosomes for degradation and recycle of the engulfed components within the autolysosome to maintain cellular homeostasis or to help cells survive under stress.^{7,8} Normally, LCs ultrastructure contains: euchromatin nuclei predominantly, abundant smooth endoplasmic reticulum, numerous mitochondria with tubular cristae and a variable number of lipid droplets in the cytoplasm, which participate actively in hormonal biosynthesis.⁹ Autophagy can either protect cells or promote cell death depending on the cellular and environmental context and, there is ample evidence supporting an active role for autophagy in the pathophysiology of diabetes mellitus.¹⁰ The real impact of DM on testicular function remains a matter of great debate. Untreated DM-induced testicular oxidative stress and mitochondria-related apoptotic cell death are associated to hypogonadism-induced sexual dysfunctions and infertility in rats and mice.^{11,12} Moreover, since risks of stroke, heart attack, prostate tumorigenesis and reduced spermatogenesis have been reported in hypogonadic patients using modern drugs such as exogenous testosterone,^{13,14} there is an increasing demand for new natural medications with less side effects capable of targeting the reproductive ultrastructure impaired by diabetes.

Although many studies have demonstrated the efficacy of medicinal plants in the treatment of hypogonadism-induced sexual dysfunction and infertility in diabetic rats,^{15,16} studies analysing the implication of the ultrastructure of LCs in the occurrence of these pathologies as well as the effects of plant extracts on this ultrastructure are scarce. The study of cytoarchitectural appearances of LCs in plant-treated rats under diabetic conditions, will shed light on our knowledge about these preoccupations. Cameroonian traditional medicine indicates that the mixture of the roots of *D. arborea* (Wild) Link (Dracaenaceae) with palm wine possess aphrodisiac potentials. In a pilot study, we demonstrated that *D. arborea* extracts stimulate the copulatory activity in normal and androgen-deprived (castrated) rats.¹⁷ Moreover, we demonstrated recently that streptozotocin-induced diabetes in rats altered the ultrastructure of seminiferous tubules¹⁸ and the sexual behavior¹⁹ and, these detrimental effects were reversed after treatment with *D. arborea* extracts. In all these studies, the possibility of an androgenic potential of these plant extracts was incriminated to justify the observed activities.^{17–19} Interestingly, these androgenic properties of *D. arborea* extracts have been recently successfully proven through the increase in intratesticular and serum testosterone concentrations recorded in diabetic rats treated with these plant extracts.²⁰ Even though the androgenic effects of *D. arborea* extracts have been proven in diabetic rat, the underlying ultrastructural mechanism at the level of LCs explaining these effects has never been examined. Therefore, we hypothesized that these androgenic properties observed previously in diabetic rats could be

related to the improvement of the cytoarchitecture of LCs by *D. arborea* extracts. Therefore, in order to explain the enhancement in testosterone production induced by *D. arborea* extracts in diabetic rats, the present study was undertaken to investigate the effects of the aqueous and ethanol extracts of *D. arborea* on the cytoarchitectural impairments of LCs induced by diabetes in rats. Since many studies have demonstrated that sildenafil citrate is a reference drug with androgenic properties in normal and diabetic mice and rats,^{20–22} it has been selected as a positive control in the present study.

2. Materials and methods

2.1. Plant material: collection and preparation of plant extracts

Fresh root barks of *D. arborea* (Willd) Link (Dracaenaceae) were harvested in January (2 p.m., local time, dry season) in Bagnoun, West Region of Cameroon, and authenticated at the Cameroon National Herbarium (CNH) under the voucher number 25361/SFR/Cam. They were cut into small pieces, dried at room temperature and ground into fine powder. The aqueous and ethanol extracts used in this study were prepared at a final concentration of 100 mg/ml in Millipore water and the doses used: 100 mg/kg bw for ethanol extract and 500 mg/kg bw for aqueous extract, were chosen based on our previous studies.^{17–20} Phytochemical screening previously done by our research team on the aqueous and ethanol extracts of *D. arborea* indicated the presence of chemical compounds such as phenols, flavonoids, sterols and saponins.¹⁷

2.2. Reagents

Streptozotocin (Sigma-Aldrich N.V/S.A. K. Cardijnplein 8, B-2880 BORNEM) and sildenafil citrate (Pfizer) (Cluj-Napoca, Romania) were used in this study.

2.3. Animal models

Male Wistar rats (90 days old) were obtained from the animal house of "Iuliu Hațieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania, and acclimatized for one-week in the Department of Cell and Molecular Biology, in the Faculty of Medicine of the above-mentioned university. Animals were housed in groups of 3 rats per cage (40 × 25 × 20 cm plexiglass cages, using pine wood shavings as bedding material) at room temperature, under a 12:12 light: dark cycle. These animals were fed twice per day with standard food pellets for rodents (Cantacuzino Institute, Bucharest, Romania) and water *ad libitum*. This study was approved by the Ethics Committee of the University of Medicine and Pharmacy "Iuliu Hațieganu", Cluj-Napoca, Romania (Permit Number: 687A/30.10.2012), in accordance with the guidelines for laboratory animal use and care as described by the Council of the European Economic Community.²³

2.4. Induction of type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM) was induced in overnight fasted rats by a single intraperitoneal (i.p.) injection of streptozotocin (STZ; 50 mg/kg bw) dissolved in ice-cold 0.1 M sodium citrate buffer, pH 4.5 and administered i.p. as previously described.^{18–20} All streptozotocin-treated animals received an intraperitoneal injection of glucose 33%, to prevent severe hypoglycaemia which could lead to the death of rats due to the pancreatic β-cell destruction and massive release of insulin after STZ injection. 48 h after STZ treatment, the fasting blood glucose level was measured using reagent strips (Accu-Chek®, Roche) with a drop of blood obtained by tail-

vein puncture. Animals were considered diabetic and used in this study, if they manifested diabetic symptoms such as hyperglycaemia (blood glucose values higher than 200 mg/dl), polyuria and polydipsy as described previously.²⁰

2.5. Animals care

In order to ensure a good health status of animals, the bedding material in each cage were changed two times per day after STZ injection to provide dry bedding for polyuric animals. Selected type 1 diabetic rats were housed in groups of 3 rats per cage to reduce the amount of urine in bedding. These diabetic animals were kept untreated for 2 weeks before the beginning of the experiment to let appear diabetes-related reproductive complications. No adverse events were observed during the experiment.

2.6. Animal groups and treatment

At the start of the experiments, 25 Wistar rats (200–250 g) were used. Two weeks after the induction of diabetes, besides 5 normal rats treated with Millipore water (10 ml/kg bw, group 1: control group), 20 diabetic animals were randomly distributed into 4 groups of 5 animals each and treated as follows: group 2, diabetic rats receiving Millipore water (10 ml/kg bw, diabetic group); group 3, diabetic rats administered with sildenafil citrate (1.44 mg/kg bw); groups 4 and 5, diabetic rats treated with the aqueous (500 mg/kg bw) and ethanolic (100 mg/kg bw) extracts of *D. arborea* respectively. The vehicle (Millipore water) and test solutions were orally administered to rats once a day (7 a.m., local time, at home cage) for 3 weeks using an endogastric canule. The sample size was chosen according to our previous study.¹⁸

2.7. Experimental procedure for electron microscopic observations

In the present study, ultrastructural analysis of Leydig cells has been carried out using the same specimens of testes previously used to assess the effects of aqueous and ethanol extracts of *D. arborea* in streptozotocin-induced ultra-structural spermatogenic alterations in Wistar rats.¹⁸ At the end of the treatment period (3 weeks), rats were sacrificed through chloroform (because its effects took place rapidly) inhalation. Testes were removed, and 3 small pieces of about 2 mm² from each testis were collected and prefixed with 2.7% glutaraldehyde in 0.1 M phosphate saline buffer for 2 h. After being washed 4 times in the same buffer (the first 3 for 1 h and the last, overnight), samples were post-fixed with 1% osmium tetroxide in 0.15 M phosphate saline buffer for 2 h and washed again 2 times (30 min each) in the same buffer. The samples were next dehydrated in acetone solutions (prepared in Millipore water), once in 30%, 50%, 70%, 80%, 90% and three times in 100% for 15 min respectively, except that of 50% which was done for 30 min. All the steps from the beginning up to 70% acetone were performed at 4 °C while the others (from 80 to 100%) were done at room temperature. After complete dehydration, the samples were infiltrated with Epon 812 in 4 steps: acetone-epon 2:1 (45 min), acetone-epon 1:1 (1 h), acetone-epon 1:2 (1 h), pure epon (overnight). The samples were then placed in gelatin capsules with fresh epon, and polymerized at 60 °C for 72 h. Trapezoidal ultrathin sections of 70–80 nm thickness were obtained from 3 Leydig cells of 4 blocks from each group with glass knives using a Bromma 8800 ULTRATOME III (LKB, Sweden) and contrasted next with solutions of uranyl acetate (15 min) and lead citrate (8 min). The examination of these sections was performed on a Jeol JEM 1010 transmission electron microscope (Jeol, Tokyo, Japan). Images were captured using a Mega VIEW III camera (Olympus, Soft Imaging System, Munster, Germany), and introduced in a database using a Soft

Imaging System software (Soft Imaging System, Munster, Germany). Ultrastructural features of the prepared Leydig cells sections of each group were then evaluated. The experimenters were blinded to the pharmacological treatment while making ultrastructural micrograph examinations.

3. Results

3.1. Electron microscopic observations of the effects of different treatments on diabetic rats Leydig cells

3.1.1. Control group

Normal ultrastructure of Leydig cells was observed in the control group, where Leydig cells were seen either alone or in small clusters, displaying large round or oval shaped nuclei (Fig. 1A and B), predominantly euchromatic but with a thin rim of heterochromatin, and in which prominent nucleoli were present (Fig. 1B). In the cytoplasm, smooth endoplasmic reticulum was abundant (Fig. 1A–D), and some lipid droplets were observed (Fig. 1A). Mitochondria had round shapes (Fig. 1A–D) with visible cristae (Fig. 1C and D) and many of them contained one or several small electron dense inclusions (Fig. 1A–D).

3.1.2. Diabetic group

In the diabetic group, Leydig cells had nuclei containing a high amount of heterochromatin and a low amount of smooth endoplasmic reticulum without lipid droplets in the cytoplasm (Fig. 2A–D). In some cells, autophagosomes were also observed (Fig. 2C). Most of the mitochondria had normal round or oval shapes (Fig. 2A–D), but polymorphous/enlarged mitochondria were also identified (Fig. 2B, D). However, the most important ultrastructural aspect in this group involved the mitochondria which were loaded with high amount of small electron dense inclusions (Fig. 2A–D), and in some cases with large size and/or very high amounts of electron dense inclusions (Fig. 2A–C).

3.1.3. Treatment group 1 (sildenafil citrate 1.44 mg/kg bw)

In the Leydig cell sections of diabetic rats treated with sildenafil citrate, nuclei looked normal (Fig. 3A–C) and a relative high amount of smooth endoplasmic reticulum compared to the diabetic group (Fig. 3A–D) as well as some lipid droplets (not shown). Some mitochondria were normal with only 1–2 small dense inclusions (Fig. 3A–D), but many had rarefied matrices either with small or without dense inclusions (Fig. 3A–D). In some regions due to the presence of fibroblast (not shown), collagen fibres were observed (Fig. 3A). Lysosomes were also observed in some cells (Fig. 3C) while autophagosomes were absent.

3.1.4. Treatment group 2 (*D. arborea*, 500 mg/kg bw, aqueous extract)

In most of the Leydig cells of diabetic animals treated with aqueous extract of *D. arborea*, the general ultrastructure of nuclei, smooth endoplasmic reticulum and most of mitochondria, with small dense inclusions, was similar to those observed in control group (Fig. 4A–C). There were two exceptions concerning mitochondria: a few had rarefied matrices (Fig. 4B, C) and others were overloaded with dense inclusions (Fig. 4A–C). In some Leydig cells, the single ultrastructural change observed when compared to the control group was the prominent smooth endoplasmic reticulum (Fig. 4D) while nuclei and mitochondria with relatively small inclusions were normal. Many fibroblasts were also observed in this group (Fig. 4A) as well as the presence of collagen (Fig. 4A, D). Some lipid droplets were also seen in the cytoplasm of cells (Fig. 4C).

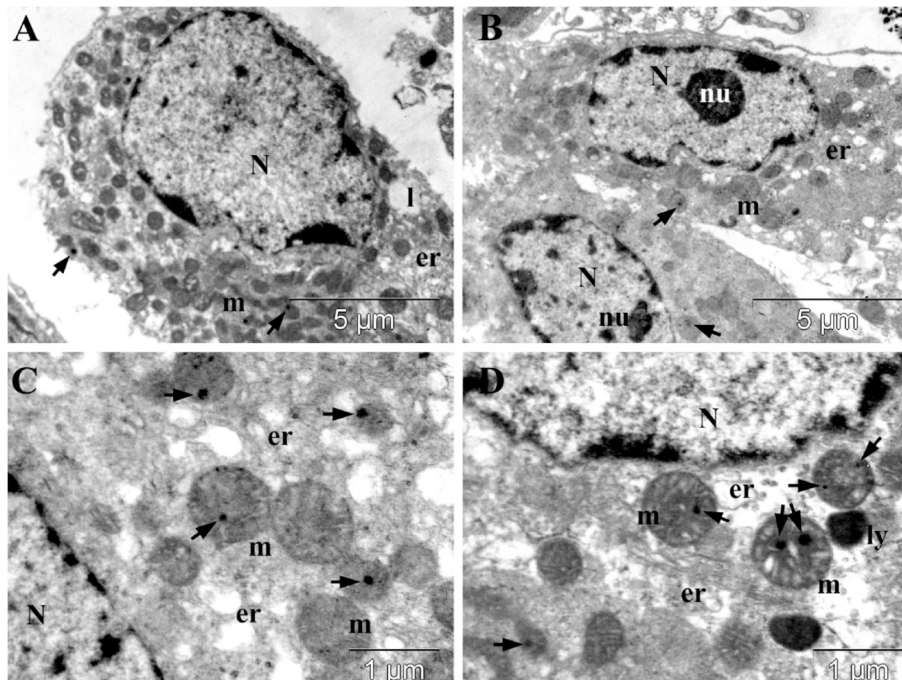


Fig. 1. Microphotograph showing ultrastructure of Leydig cells in control rats treated with Millipore water (10 ml/kg bw) presenting normal cytoarchitecture. N: nuclei, er: smooth endoplasmic reticulum, nu: nucleoli, m: mitochondrion, ly: lysosomes, l: lipid droplet, arrows: small electron dense inclusions.

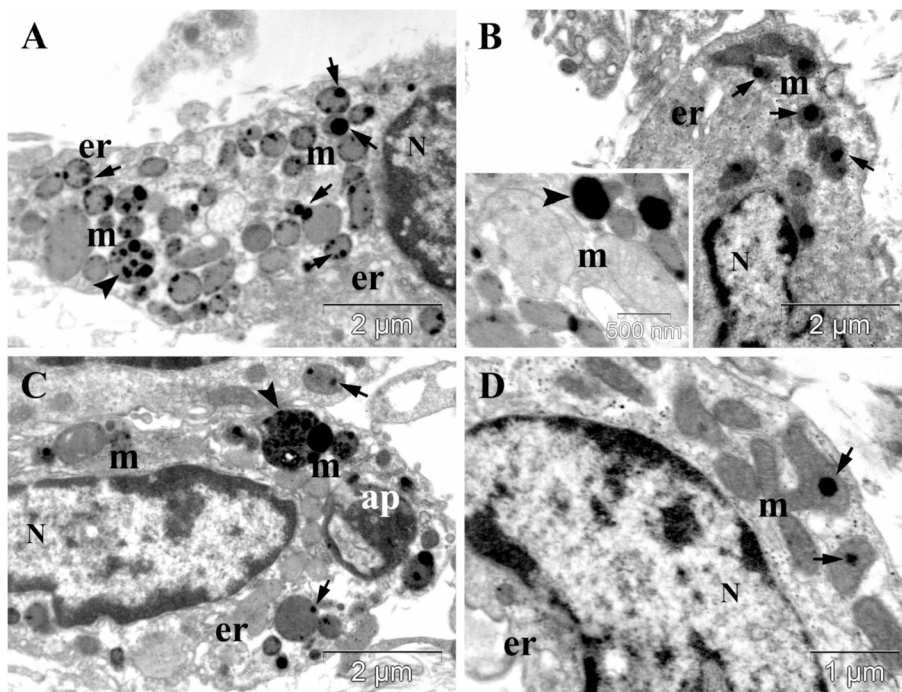


Fig. 2. Microphotograph showing ultrastructure of Leydig cells section in diabetic rats treated with Millipore water (10 ml/kg bw) with many alterations in Leydig cells cytoarchitecture. N: nuclei, er: smooth endoplasmic reticulum, m: mitochondrion, ap: autophagosomes, arrows: small electron dense inclusions, arrowheads: very high amounts of large electron dense inclusions.

3.1.5. Treatment group 3 (*D.arborea*, 100 mg/kg bw, ethanol extract)

In many Leydig cells of diabetic rats treated with ethanol extract of *D. arborea*, nuclei had normal aspects (Fig. 5A and B) while in other cells, chromatin fragmentation was observed (Fig. 5C). Mitochondria displayed a heterogeneous ultrastructure: most of

them looked similar to those observed in the control group (Fig. 5A–D), without dense inclusions or with a low number of small dense inclusions (Fig. 5A, C); in some cases, mitochondria with dilated cristae were observed (Fig. 5A, C); in the cells with severe lesions, mitochondria with rarefied matrix were present,

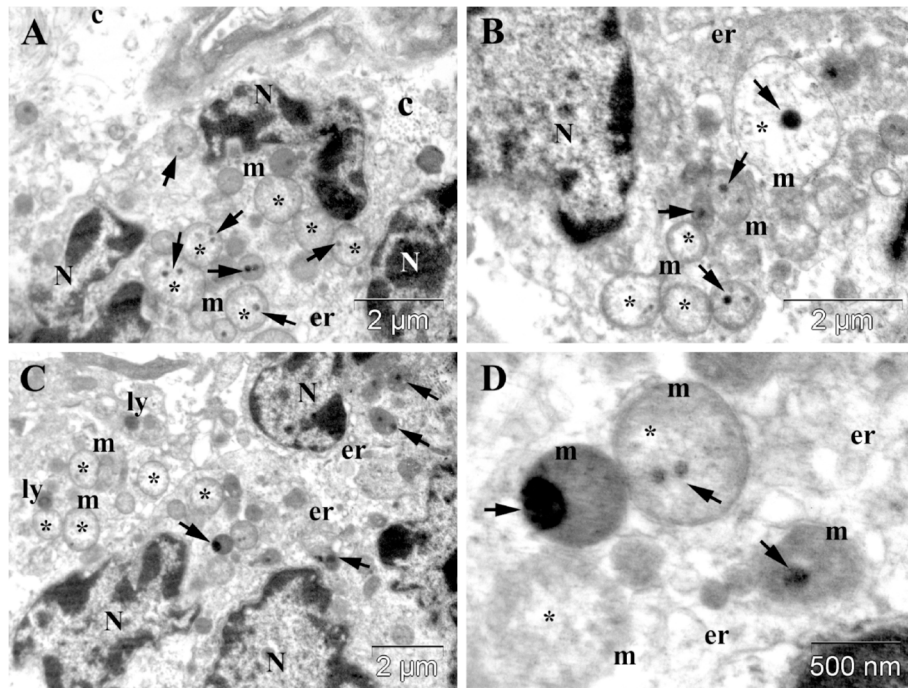


Fig. 3. Microphotograph showing ultrastructure of Leydig cells section of diabetic rats treated with sildenafil citrate (1.44 mg/kg bw). N: nuclei, er: smooth endoplasmic reticulum, m: mitochondrion, arrows: small electron dense inclusions, *: mitochondrion with rarefied matrix, c: collagen fibres, ly: lysosomes.

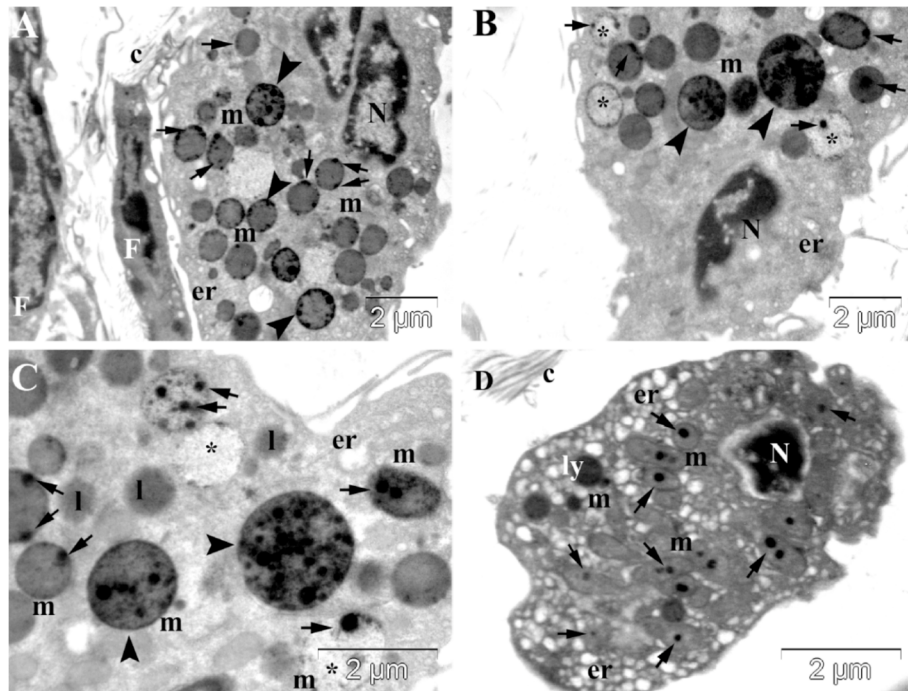


Fig. 4. Microphotograph showing ultrastructure of Leydig cell section of diabetic rats treated with aqueous extract of *D. arborea* (500 mg/kg bw). Most Leydig cells in this group looks similar to those observed in the control group. N: nuclei, er: smooth endoplasmic reticulum, nu: nucleoli, m: mitochondrion, arrows: small electron dense inclusions, arrowheads: very high amounts of dense inclusions matrix, *: mitochondrion with rarefied matrix, c: collagen fibres, ly: lysosomes, F: fibroblasts.

still containing small dense inclusions (Fig. 5 B, D). Rare mitochondria overloaded with dense granules were present in some cells (Fig. 5 B). Smooth endoplasmic reticulum looked similar in the unaffected regions of the cells (Fig. 5A–D) and autophagosomes were identified in some cells (Fig. 5C).

4. Discussion

Recent studies have demonstrated the androgenic effects of *Dracaena arborea* in castrated and diabetic rats but, the cytoarchitectural mechanism at the level of Leydig cells (LCs) justifying this

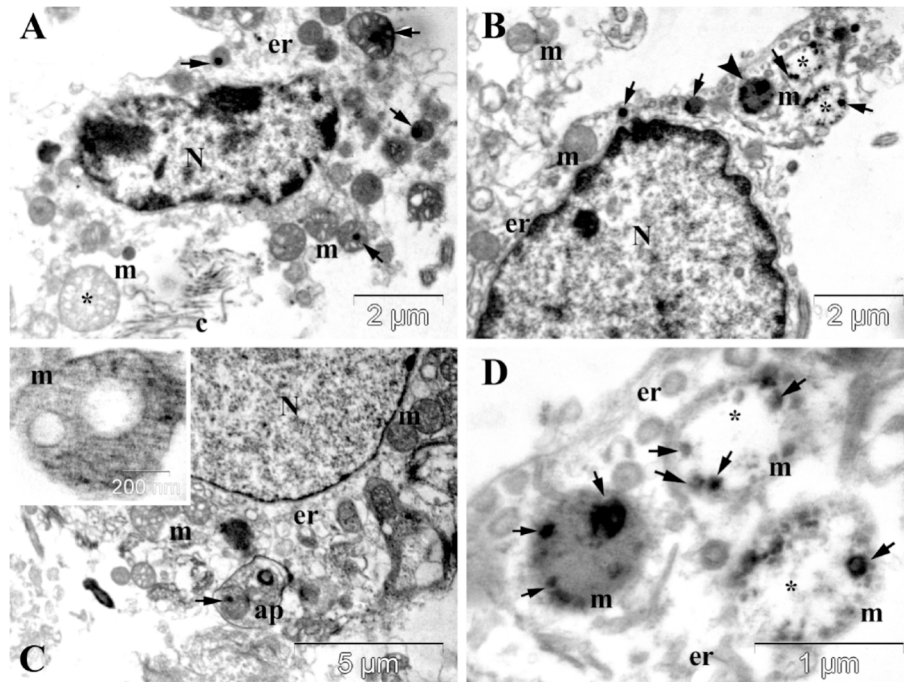


Fig. 5. Microphotograph showing ultrastructure of Leydig cells section of diabetic rats treated with ethanol extract of *D. arborea* (100 mg/kg bw).

N: nuclei, er: smooth endoplasmic reticulum, m: mitochondrion, ap: autophagosomes, arrows: small electron dense inclusions, arrowheads: very high amounts of dense inclusions matrix, *: mitochondrion with rarefied matrix, c: collagen fibres.

improvement of androgens production in diabetic rats has never been examined. The objective of the present study was to assess the effects of *D. arborea* on the cytoarchitecture of LCs in diabetic rats. Electron microscopic analyses of LCs sections indicated normal ultrastructure in the control group while those of untreated diabetic rats showed apoptosis and necrosis in this organelle, characterized by fragmentation and condensation of chromatin as well as reduction in the amount of smooth endoplasmic reticulum with no lipid droplets in the cytoplasm. Moreover, the presence of many autophagosomes in some cells as well as multiple degenerated and/or enlarged mitochondria with fewer ridges and a lot of electron dense granules in their matrix in this group are characteristics of cellular suffering. Since autophagy is a cellular metabolic process that uses lysosomal degradation of cellular components to provide raw materials to help cells survive under stress conditions,²⁴ the cytoplasmic autophagosomes seen in altered LCs in the present study could be a sign of a defective autophagy activity related to a low secretion of testosterone in these cells.^{10–12,25} This result agrees with the data of many other authors who explained that the reduction in testosterone production in autophagy-deficient LCs in rats, mice and humans, which is similar to the symptoms of LOH (Late-Onset-Hypogonadism) is due to the accumulation of Na⁺/H⁺ exchanger regulatory factor 2 (NHERF2) in LCs resulting in the down-regulation of scavenger receptor class B, type I (SR-BI) and leading to insufficient cholesterol supply.^{5,6} However, the evaluation in future studies of autophagy biomarkers such as BECLIN 1 (involved in the formation of the pre-autophagosomal structure) and microtubule-associated protein 1 light chain 3, LC3 (which is cleaved and associated with the autophagosome membrane indicating that autophagy is indeed occurring) will be important to confirm this hypothesis. The heterochromatinization observed in the nuclei of LCs is another indicator of apoptosis which could be associated to androgen secretion impairment in untreated diabetic rats. Indeed, in the nuclei of LCs in untreated diabetic rats, the transformation of chromatin into heterochromatin (inactive

chromatin), is responsible for the partial or total suppression of certain androgen-dependent gene expressions as well as the repression of DNA replication and active transcription, leading to the inhibition of DNA, proteins synthesis and enzymes involved in the steroidogenesis process.^{26,27}

The reduction in the amount of smooth endoplasmic reticulum mainly observed in untreated diabetic rats could also be attributed to diabetes complications as reported by other authors.¹⁵ Smooth endoplasmic reticulum is one of the most important organelle of LCs and its alteration/reduction coupled with the absence of cytoplasmic lipid droplets as observed in the present investigation, may result in the inhibition of LCs differentiation and proliferation, and ultimately to the inhibition of testosterone production.²⁸

In this study, enlarged mitochondria as well as mitochondria overloaded with large and high amount of electron dense inclusions observed in LCs sections in untreated diabetic rats, are manifestations of mitochondrial dysfunction probably due to defective-autophagy. Although mitochondria are the first site of androgen synthesis in the LCs, they are also the primary site of the production of reactive oxygen species (ROS) and, in certain conditions such as diabetes, an imbalance in ROS production relative to the cytoprotective action of autophagy may lead to the accumulation of ROS with overproduction and release of cytochrome c, activation of caspases 9 and 3, and ultimately cell death.²⁹ Similar results have been demonstrated by Petersen et al.³⁰ This mitochondrial alterations in untreated diabetic rats attributed to defective autophagy could definitely affect the function of the steroidogenic transport protein (StAR) and the steroidogenic enzyme P450scc located in their membranes.³¹ Moreover, since autophagy is required in the production of testosterone, an anticipated consequence of impaired autophagy is the accumulation of dysfunctional organelles, such as mitochondria, within the cell.¹⁰ However, since there were no accumulation of cholesterol in form of lipid droplets in the cytoplasm of LCs in untreated diabetic rats, the damage of StAR signalling pathway by diabetes could not

be directly involved in the impairment of androgen synthesis, as previously reported.²⁶ Therefore, we strongly believe that the damage of mitochondrial CYP11A1 enzyme is the main pathway explaining the hypo-secretion of androgens in the LCs of diabetic rats. Large and high amount of electron dense inclusions accumulated in the mitochondria of LCs observed in this study could be lipid material that migrated from the cytoplasm to the mitochondrial membrane, but which has not been converted into pregnenolone, probably due to the absence or alteration of cytochrome P450scc (CYP11A1) enzyme by diabetes.

Alterations in the cytoarchitecture of LCs observed in untreated diabetic rats could be attributed to the diabetes-induced apoptosis and decrease in testosterone levels, which is itself related to the lack of insulin, increased aromatization of testosterone with the inhibition of estrogen sulfotransferase and/or the inhibition of kisspeptin. Insulin acts indeed, as an anti-apoptotic factor capable of promoting proliferation of LCs and steroidogenesis through the maintenance of LH receptors on the membrane of these cells.^{32,33} Additionally, estrogen resulted from the normal aromatization of testosterone in LCs plays a major role in the overall growth, development and function of these cells.³⁴ When LCs are subjected to an alkylating toxic agent such as obesity and/or diabetes, increased aromatization to estradiol coupled to the inhibition of estrogen sulfotransferase, SULFT_{1E1} (enzyme in charge to eliminate estrogen resulted from aromatization of testosterone)^{35,36} and subsequent estradiol exposure will lead to the down regulation of the hypothalamic-pituitary axis, the blockage in the reappearance of mature LCs and ultimately the inhibition of steroidogenesis via the inhibition of luteinizing hormone (LH) in the mature LCs.^{37,38} Additionally, under normal physiology, kisspeptin excites the hypothalamic GnRH neurocytes to produce GnRH into circulation, subsequently stimulating the production of gonadotrophs LH and FSH, which stimulates sex organs in humans to produce testosterone and sperms³⁶; Low levels of kisspeptin have been incriminated in hypogonadotropic hypogonadism in humans and animal models due to the decreased release of gonadotrophins and testosterone in males with type 2 diabetes.³⁸ All these abnormalities observed in the LCs ultrastructure in untreated diabetic rats were in harmony with the results obtained by Orth et al.³⁹

Interestingly, the administration of *D. arborea* extracts and sildenafil citrate to diabetic rats for three weeks alleviated these detrimental effects of diabetes on the cytoarchitecture of LCs without having antihyperglycaemic properties.¹⁸ The highest effects were observed in rats administered with the aqueous extract (500 mg/kg bw) where aspects of cells, nuclei and mitochondria were similar to those of control rats and, the presence of some lipid droplets and prominent endoplasmic reticulum in the cytoplasm of LCs indicated the restarting of the cells activity in androgen synthesis. Indeed, the presence of many fibroblasts observed in microphotographs of the LCs sections in this group is substantial evidence of the reparation of injuries caused by diabetes.^{40,41} These results further justify the androgenic properties of *D. arborea* extracts demonstrated recently.²⁰ Phytochemical compounds revealed in the *D. arborea* extracts such as phenols, flavonoids and steroidal saponins¹⁷ might be responsible for the reverse properties of these plant extracts as demonstrated previously.^{17–20} Phenols and flavonoids possess powerful antioxidant effects at the level of LCs and are involved in the inhibition of cyclooxygenase-2 (COX2), the enzyme responsible for the inhibition of StAR gene, leading to the improvement of steroidogenesis.⁴² In addition, it is demonstrated that, some steroidal saponins have the potential to increase the level of steroidogenic enzymes as well as the production and the activity of testosterone.^{43,44} Since the features of LCs ultrastructure were almost the same in all sections of the 5 rats in each group, it would be important to conduct such observational studies

(where statistical analyses is not needed) with a reduced number of 3 animals in each group as recommended by the ARRIVE (Animal Research: Reporting In Vivo Experiments) guidelines checklist, in order to fulfill the 3R principle (Replacement, Refinement and Reduction of Animals in Research).

Based on the results obtained in the present study, the ultrastructural impairments of the nuclei, mitochondria and smooth endoplasmic reticulum of LCs are mainly responsible for the alteration of spermatogenesis, sexual activity and low secretion of testosterone in untreated diabetic rats observed previously.^{17–20} Alleviating properties of *D. arborea* extracts on the cytoarchitectural impairments of LCs through the reduction of defective-autophagy in diabetic rats in the present study could justify the androgenic properties of this plant extracts in diabetic rats demonstrated recently.²⁰ This androgenic potential of *D. arborea* extracts could then be responsible for the aphrodisiac properties of this plant.^{45,46} Moreover, similarities between LCs sections of diabetic rats treated with sildenafil citrate, whose androgenic properties have already been demonstrated,^{21,22} and those of diabetic rats administered with *D. arborea* extracts could be considered as additive arguments supporting the androgenic potential of these plant extracts.

5. Conclusion

Overall, these findings support the androgenic properties of *D. arborea* extracts in diabetic rats, and the mechanism could involve could the alleviation of the cytoarchitectural impairments of LCs, probably through the reduction of defective autophagy in the nuclei, mitochondria and smooth endoplasmic reticulum of LCs in streptozotocin-induced diabetes in rats. These outcomes represent important stools for the management of hypogonadism-induced infertility in patients with diabetes.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

Conflicts of interest

The authors declare that there is no conflict of interest concerning this work.

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