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Dasatinib and quercetin increase testosterone and sperm concentration in mice

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ABSTRACT

Cellular senescence is a defense mechanism to arrest proliferation of damaged cells. The number of senescent cells increases with age in different tissues and contributes to the development of age-related diseases. Old mice treated with senolytics drugs, dasatinib and quercetin (D+Q), have reduced senescent

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cells burden. The aim of this study was to evaluate the effects of D+Q on testicular function and fertility of male mice. Mice (n = 9/group) received D (5 mg kg^{-1}) and Q (50 mg kg^{-1}) via gavage every moth for three consecutive days from 3 to 8 months of age. At 8 months mice were breed with young non-treated females and euthanized. The treatment of male mice with D+Q increased serum testosterone levels and sperm concentration and decreased abnormal sperm morphology. Sperm motility, seminiferous tubule morphometry, testicular gene expression and fertility were not affected by treatment. There was no effect of D+Q treatment in β-galactosidase activity and in lipofuscin staining in testes. D+Q treatment also did not affect body mass gain and testes mass. In conclusion, D+Q treatment increased serum testosterone levels and sperm concentration and decreased abnormal sperm morphology, however did not affect fertility. Further studies with older mice and different senolytics are necessary to elucidate the effects in the decline of sperm output (quality and quantity) associated with aging.

KEYWORDS

Senotherapeutics, flavonoids, cellular senescence, fertility, aging

INTRODUCTION

Cellular senescence is a defense mechanism to arrest proliferation of damaged cells [1]. Senescent cells can avoid apoptosis and have a senescence-associated secretory phenotype (SASP) to attract macrophages for their clearance [2]. The number of senescent cells increases with age in different tissues [3–5] and it is associated with increased inflammation and tissue dysfunction, accelerating the development of age-related diseases [3]. Recently, it was shown that old mice treated with senolytic drugs, dasatinib and quercetin (D+Q), have reduced number of senescent cells [6]. Consequently, several health parameters are improved and mice have a longer lifespan [6, 7]. Long-term treatment with D+Q has been described as safe and efficient at low doses both *in vitro* and *in vivo* [6]. The combination of D+Q also has senolytic effects in humans [8]. However, long-term studies to understand the effects of D+Q on fertility are scarce.

Aging decreases reproductive function in males and females by various mechanisms, and some remain unclear. Although the increased burden of senescent cells with aging in several tissues is known [2, 9], there is no data about it in male reproductive organs. Fertility in males does not undergo the same sharp decline with age that is commonly observed in females, however, there is a progressive decline in testosterone levels [10], sperm motility, concentration and quality with age [11]. Additionally, decreased testosterone levels are associated with deficient sperm production [12]. As cellular senescence is a naturally occurring phenomena in several tissues, its occurrence in testes need to be characterized, as well as its effect on male fertility.

Several life-extending therapies have been shown to delay reproductive aging. Among these, caloric restriction (CR) was shown to increase longevity of different species [13] and reduce ovarian aging in mice [14]. Interestingly, moderate CR increased testosterone levels in rats [15] and monkeys [16] even during CR, suggesting it may prevent the decrease in spermatogenesis with age. However, others found that CR decreased testosterone levels [17] and induced sperm defects in rats [16]. In addition, rapamycin - CR mimetic - can increase longevity of mice [18], but has negative effects on sperm production, testosterone levels and fertility [19]. Therefore, it

is important to understand the effects of senolytics drugs on the male reproductive system to consider its clinical use in humans. Based on this, the aim of this study was to evaluate the effects of the senolytics drugs D+Q on testicular function and fertility of male mice.

MATERIALS AND METHODS

Animals and treatment

For this study, 3-month-old male C57BL/6 mice (n = 18) were evaluated in controlled conditions (22 ± 2 °C, 12 h light dark cycle, 40–60% humidity). Mice were divided into the intervention group (n = 9), which received an oral gavage of dasatinib (5 mg kg^{-1}, LC Laboratories, Woburn, MA, USA) and quercetin (50 mg kg^{-1}, Sigma, St. Louis, MO, USA) dissolved in phosal (60%, Lipoid, Ludwigshafen, Germany), polyethylene glycol 400 (30%, Synth, São Bernardo do Campo, SP, Brazil) and ethanol (10%, Synth) at three consecutive days once a month, according prior studies [6, 9], and control group (n = 9), which received placebo via gavage. All mice received water and standard diet *ad libitum* throughout the experiment. Treatment started at three months and was maintained until eight months of age in a total of six treatments before euthanasia. Throughout the experiment the mice were observed daily for clinical signs of prostration and feeding through individual mass gain. Mice were weighed every two weeks during the experiment. At the end of the treatment, the males were mated with 3-month-old females (not receiving any treatment) in the proportion of one male to two females for 10 days. The pregnancy rate and litter size were checked after delivery up to 22 days after the last day of breeding. We also checked the number of males that impregnated at least one female of the pair. After mating, the males were anesthetized, euthanized, dissected and testes, epididymis and semen were collected for further analysis. The blood also was collected for analysis. All procedures were approved by the Animal Experimentation Ethics Committee from the Universidade Federal de Pelotas, protocol number 9015–2020.

Semen collection

For epidydimal semen collection, after euthanasia and an incision on the scrotum, the cauda of the epididymis was placed in a microtube containing 300 µL of Phosphate-buffered saline (PBS 1×), pre-heated to 36.5 °C. The epididymis was gently fractionated with scissors and agitated for 3 min in order to release the sperm [11].

Semen analysis

For sperm concentration analysis 25 µL of the semen solution was collected and diluted in 25 µL of formaldehyde-saline solution. Concentration was evaluated by counting sperm cells on a haemocytometer counting chamber [20]. The analysis of sperm motility was performed on a microscope (Axio Scope A1®, Zeiss, Jena, Germany) coupled to a computer-assisted semen analysis system (CASA, SpermVision®, Minitube, Tiefenbach, Germany). For this, 6 µL of semen sample was placed on a pre-heated glass slide with coverslip and at least six fields were evaluated for each sample/mouse. The variables assessed by the CASA system were: velocity average path (VAP), velocity curved line (VCL), velocity straight line (VSL), beat cross

frequency (BCF), amplitude of lateral head displacement [16], total motility (TMO), and progressive motility (PMO).

The membrane integrity of sperm cells was assessed by hypoosmotic swelling test (HOS) [21]. For this, 10 µL of the semen solution were diluted into 90 µL of HOS solution (sodium citrate, fructose and distilled water adjusted to 93 mOm) and incubated at 36.5 °C for 30 min. After incubation, 200 cells were counted for swollen and non-swollen tails in a microscope (Olympus®, América INC, São Paulo, SP) at 20× magnification. As a control, 6 µL of semen solution before incubation were smeared on a glass slide and 200 cells were counted for swollen and non-swollen tails. The percentage of membrane integrity were calculated by the proportion of sperm with swollen tails before and after incubation in the hypoosmotic solution [22].

For the acrosome integrity analysis, 3 µL of semen solution was stained with lectin from *Arachis hypogaea* FITC conjugate (Sigma, Saint Louis, MO, USA) and incubated at room temperature in the dark for 15 min. In this analysis, 100 cells were differentially evaluated by observing the emission of green fluorescence by intact acrosomes. This analysis was performed on an epifluorescence microscope at 40× magnification (Olympus B×51, América INC, São Paulo, SP, Brazil), using WU filter (450–490 nm excitation and 516–617 emission) [23].

Lastly, cells were evaluated for sperm morphological defects. For this, a drop of each semen sample was smeared on a glass slide and dried. Samples were then stained using the fast panoptic method. One hundred cells were evaluated on a microscope (Nikon Eclipse E200, Nikon, Tokyo, Japan) at 100× magnification using immersion oil and differentially counted as normal, or with defects on tail, head and midpiece [24].

Serum steroids assays

Blood samples were collected and centrifuged at 2000×*g* for 10 min to separate the serum. Testosterone levels were measured by eletrochemiluminescence (Modular Analytics E170 Cobas, Roche Diagnostics International Ltd, Rotkreuz, Switzerland).

Testes histology

After epididymal semen collection, the testes were excised. One of the testes was fixed in paraformaldehyde 4%, dehydrated in alcohol, cleared in xylol and then embedded in paraplast (Sigma, Saint Louis, MO, USA). Paraplast blocks were transversally cut into 5 µm sections at the central region of the testis. The sections were then dewaxed in xylene, rehydrated in descending series of alcohol and stained with hematoxylin and eosin (HE). Stained sections were photographed by a camera coupled to a microscope (Nikon Eclipse E200, Nikon, Tokyo, Japan) using the software TC Capture (Tucsen Photomics Co.) at 10× magnification. For each animal twenty round or near round seminiferous tubes were randomly chosen. The perimeter, area and diameter of the seminiferous tubes and its lumen were measured using the Motic 3.0 software (Motic®, Hong Kong, China). For statistical comparison, the average measurements from the 20 seminiferous tubes from each mouse was used.

Lipofuscin staining

Lipofuscin staining was performed using the Sudan black dye (Sigma, Saint Louis, MO, USA) on a subset of the testes histological slides from each mouse. Lipofuscin is a heterogeneous

pigmented by-product due to failure of intracellular catabolism, conventionally found in the lysosomes or cytosol of post-mitotic aged cells [25]. We used a modified protocol [26]. Briefly, the histological slides were dewaxed with xylol, washed in an alcohol gradient until reaching 70% alcohol and rehydrated in water. After diluting the Sudan black in 70% alcohol, avoiding its precipitation, a 10 mL syringe with a filter was used to drop the Sudan black solution on a clean slide, which was positioned downwards over the slide with the tissue for approximately 2 min. The slide was then washed with 50% alcohol and distilled water. A glycerol droplet and a coverslip were placed over the section and observed under the light microscope. Images obtained at 10× magnification (3 sections per mouse) were used to quantify the lipofuscin positive area (dark blue). The area of pixels in the images was calculated using the Image J software (National Institutes of Health - USA) and presented as percentage relative to the total area of the section.

β-Galactosidase activity

The β-galactosidase activity test was performed using a commercial kit (Mammalian β-Galactosidase Assay Kit, Pierce Biotechnology, RockFord, IL, USA). Frozen testes were used following the manufacturer's instructions. Briefly, protein was extracted using a RIPA buffer (50 mM Tris-HCl, 150 mM sodium chloride, 1% triton X-100, 2 mM EDTA, pH 7.5). The extracted solution was mixed with the β-galactosidase assay reagent, incubated for 30 min and measured on a plate reader at 562 nm. β-galactosidase activity was expressed relative to the weight of each sample.

Gene expression

Testes were homogenized in Trizol reagent (Invitrogen, Carlsbad, CA, USA) for RNA extraction, according to the manufacturer recommendations. RNA concentration and the A260/280 ratio were measured in a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). Reverse transcription was performed using the iScript Synthesis kit (Biorad, Hercules, CA, USA) in a volume of 15 µL using 1 µg of RNA in a MyCycle^{TM} Thermo Cycler (Bio-Rad). Real-time PCR was performed using GoTaq qPCR Master Mix (Promega, Madison, WI, USA) in a volume of 15 µL in a StepOnePlus thermal cycler (Applied Biosystems, Waltham, MA, USA). Each reaction was performed in duplicate, using 4 µL of cDNA (20 ng), 5 µL of GoTaq, 0.75 µL of each primer (5 µM) and 4.5 µL of ultrapure water. For each assay, 45 cycles (95 °C for 15 s and 60 °C for 1 min) were performed and a dissociation curve was included at the end of reaction to detect the amplification of a single PCR product. The genes β-2 microglobulin (*β2m*), Actin beta (*Actb*) and Peptidylprolyl isomerase A (*Ppia*) were evaluated as candidate reference genes using the geNorm software [27]. The relative expression was calculated by equation $2^{A-B}/2^{C-D}$, using the geometric mean of gene expression of *β2m*, *Actb* and *Ppia* internal controls. The target genes evaluated were genes related to cellular senescence: Cyclin Dependent Kinase Inhibitor 2A (P16/*Cdkn2*), cyclin-dependent kinase inhibitor 1A (P21/*Cdkn1*), Matrix Metallopeptidase 12 (*Mmp12*), C-C Motif Chemokine Ligand 2 (*Mcp1*/*Ccl2*), transformation related protein 53 (*Trpp53*), Interleukin 1 alpha (*Il1a*), Stanniocalcin 1 (*Stc1*) and genes related to spermatogenesis: Growth Differentiation Factor 15 (*Gdf15*) and mammalian target of rapamycin (*Mtor*). The primers used are represented in Supplemental Table 1.

Statistical analysis

Statistical analysis was performed using the software GraphPad Prism 6. When parametric, variables were compared between groups by a *T*-test and when non-parametric, by a Mann-Whitney test. Number of females pregnant was evaluated with Fisher's exact test. Values were presented as mean ± standard error of the mean and *P* values lower or equal to 0.05 were considered significant.

RESULTS

Body mass and testes mass

No differences were observed in body mass ($P = 0.66$, Fig. 1A), testes mass ($P = 0.85$, Fig. 1B) and relative testes mass ($P = 0.91$, Fig. 1C) after D+Q treatment.

Sperm and fertility parameters

Mice treated with D+Q had higher sperm concentration ($P = 0.02$, Table 1) and serum testosterone concentration (Fig. 2). Sperm kinetics, membrane and acrosome integrity were not different between groups (Table 1). However, the D+Q treatment reduced the percentage of abnormal sperm in comparison to placebo treated male mice ($P = 0.01$, Fig. 3).

The percentage of fertilized females ($P = 0.72$) and the number of pups/litter ($P = 0.91$) were also not affected by the treatment of male mice with D+Q (Table 1).

The total area, perimeter and diameter of the seminiferous tubules and lumen of seminiferous tubules was not different between groups (Supplemental Fig. 1).

Testicles senescence and SASP

Testicular β-galactosidase activity was not different between control and D+Q-treated mice ($P = 0.21$, Fig. 4C). Lipofuscin staining in testes also was not different between groups ($P = 0.55$, Fig. 4A, B and D). There was no difference in the relative expression of genes related to spermatogenesis (*Gdf15* and *Mtor*, Fig. 5A and B) and cellular senescence (*Cdkn2*, *Cdkn1*, *Trpp53*, *Ccl2*, *Il1a*, *Mmp12* and *Stc1*, 5 C-I) between groups.

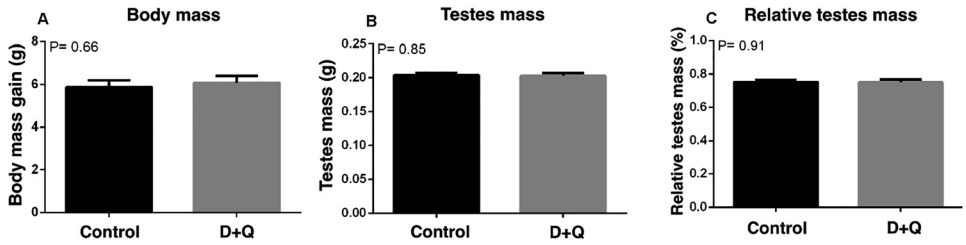

Fig. 1. Body mass (a), testes mass (b) and relative testes mass (c) in placebo (control) or dasatinib and quercetin (D+Q) treated male mice. All data are presented as mean ± SEM. Values of $P \leq 0.05$ were considered significant

Table 1. Sperm parameters and fertility in male mice treated with dasatinib and quercetin (D+Q) or placebo (Control)

	Control	D+Q	*P* value
Sperm Parameters			
Epididymal sperm concentration ($\times 10^6$ cells/mL)	4.77 ± 0.59	6.84 ± 0.49	0.02
Motility (%)	61.08 ± 3.06	58.27 ± 3.10	0.53
Progressive motility (%)	44.16 ± 2.98	44.76 ± 3.21	0.89
Average path velocity	74.78 ± 5.70	83.47 ± 5.02	0.27
Curvilinear velocity	145.60 ± 10.04	154.30 ± 7.90	0.50
Straight line velocity	50.26 ± 5.25	57.17 ± 4.04	0.31
Amplitude of lateral head	8.18 ± 0.36	7.36 ± 0.57	0.26
Beat cross frequency	17.56 ± 1.01	20.12 ± 1.67	0.22
Hypoosmotic swelling test (Δ%)	25.75 ± 1.73	26.89 ± 3.39	0.78
Acrosomal integrity (%)	42.75 ± 1.82	45.00 ± 2.92	0.54
Fertility Parameters			
Fertilized females (%)	33.3 (6/18)	25.0 (5/20)	0.72
Number of pups/litters	6.50 ± 0.62	6.40 ± 0.60	0.91
Males that fertilized at least one female (%)	44.44 (4/9)	50.00 (5/10)	1.00

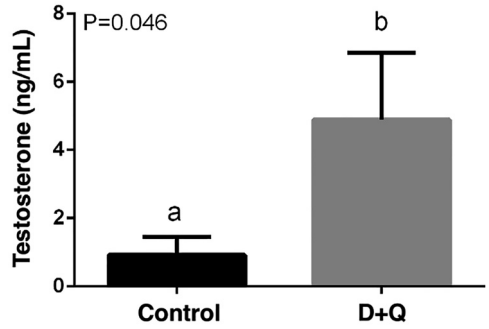

Fig. 2. Serum testosterone levels in male mice treated with placebo (control) or dasatinib and quercetin (D+Q). All data are presented as mean ± SEM. Values of $P < 0.05$ were considered significant. Different letters indicate $P \leq 0.05$

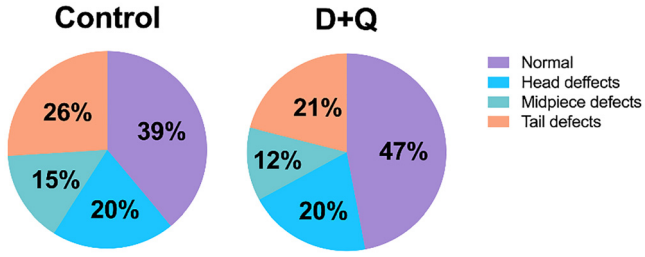

Fig. 3. Percentage of normal sperm, or sperm with head, midpiece or tail defects in male mice treated with placebo (control) or dasatinib and quercetin (D+Q) treated. Asterisks indicate $P \leq 0.05$

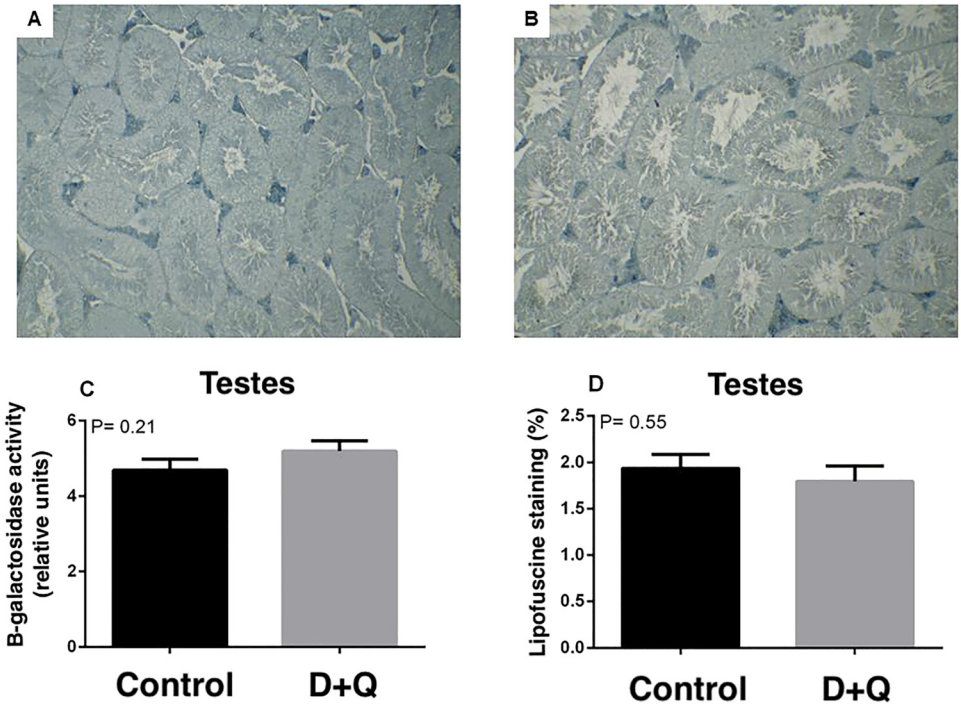

Fig. 4. Representative lipofuscin staining sections of seminiferous tubules at 10× magnification from placebo (Control - a) and D+Q (b) treated male mice. β-galactosidase activity (c) and lipofuscin staining (d) in testes of mice treated with dasatinib and quercetin (D+Q) or placebo (control group). All data are presented as mean ± SEM. Values of $P \leq 0.05$ were considered significant

DISCUSSION

The treatment of male mice with D+Q from three to eight months of age increased serum testosterone levels, sperm concentration and decreased the percentage of abnormalities observed in sperm. No difference in testicular β-galactosidase activity or lipofuscin staining were observed between control and D+Q-treated mice. D+Q treatment also did not affect body mass gain, sperm motility, seminiferous tubule morphometry and testicular gene expression or fertility.

β-galactosidase activity has been used as a reliable marker for senescent cell burden in various organisms both *in vivo* and *in vitro* [28]. There is an association between increasing age and number of β-galactosidase positive cells in the hippocampus, liver, kidneys and lungs of mice [29]. The failure to observe a difference may be due to not accumulating enough senescent cells for detection at eight months of age, requiring a longer intervention. It should be noted that, in this study, our goals were to use younger mice to test the influence of the treatment in males at peak reproductive life. However, previous studies with D+Q usually treat much older mice to observe significant senolytic effects in different tissues [29, 30]. The use of D+Q for four months improved muscle regeneration after injury in 24-month-old mice, whereas it impaired

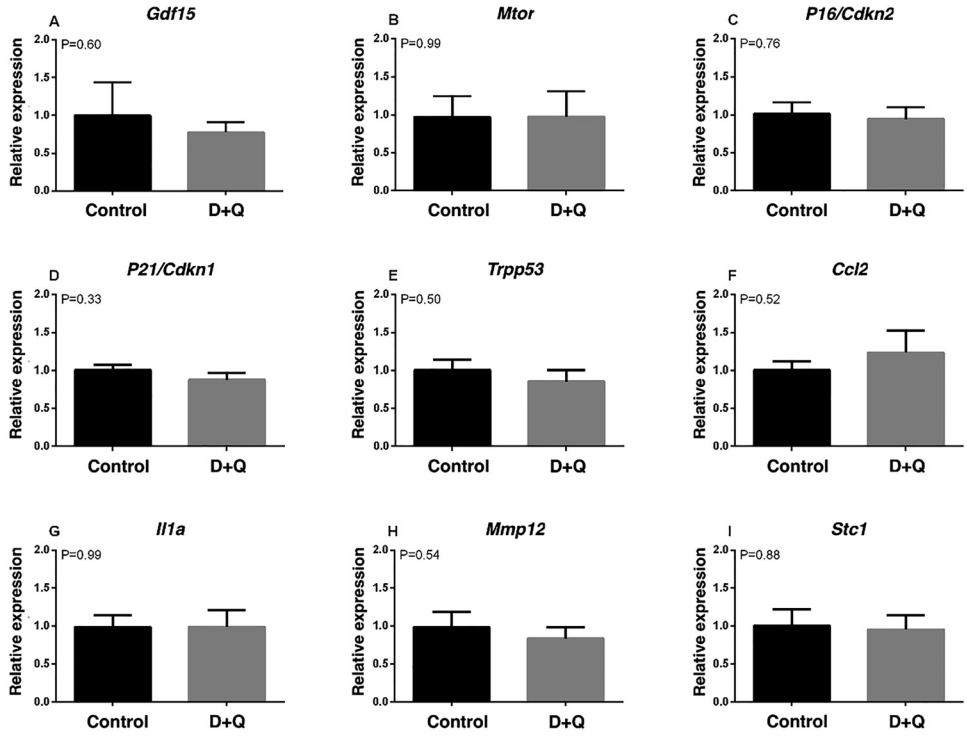

Fig. 5. Analysis of relative gene expression related to spermatogenesis and cellular senescence in testes of male mice treated with placebo (control) or dasatinib and quercetin (D+Q). Growth Differentiation Factor 15 (Gdf15 – a), mammalian target of rapamycin (Mtor – b), Cyclin Dependent Kinase Inhibitor 2A (P16/Cdkn2 – c), cyclin-dependent kinase inhibitor 1A (P21/Cdkn1 – d), transformation related protein 53 (Trpp53 – e), C-C Motif Chemokine Ligand 2 (Ccl2 – f), Interleukin 1 alpha (Il1a – g), Matrix Metallopeptidase 12 (Mmp12 – h), Stanniocalcin 1 (Stc1 – i). All data are presented as mean ± SEM. n = 7–9/group. Values of $P \leq 0.05$ were considered significant

regeneration in seven-month-old mice [30], indicating this age-dependent effect. The small proportion of parenchyma, and a seminiferous epithelium composed mostly of germ cells in differentiation, can also contribute to the presence of fewer senescent cells. Additionally, the blood-testis barrier is a physical barrier isolating the events of spermatogenesis within the seminiferous tubules in an immune-privileged environment [31]. Therefore, the absence of macrophages in the seminiferous epithelium in physiological conditions [32] may contribute to this reduced inflammation and senescent burden.

Lipofuscin is also used as a marker of cellular senescence and consists of an aggregate of oxidized proteins that accumulate with advancing age [33]. Among the morphological alterations of Leydig cells normally observed in aged testis, there is a tendency to form small clusters of lipofuscin [34]. We observed a small accumulation of lipofuscin in testes, which was not affected by D+Q treatment. Our previous study in aged female mice revealed a significant increase in lipofuscin in ovaries from three to 12 months-of-age [35], suggesting differences

between male and female gonadal aging. Similarly, there was no change in the level of gene expression of SASP components in the testes. SASP components can change depending on the tissue evaluated [36]. P16/Cdkn2, P21/Cdkn1, Mmp12, Mcp1/Ccl2, Trpp53, Il1a, Stc1 are considered SASP markers for different tissues [36]. However, data suggest that SASP is very heterogenous among tissues [36]. Therefore, more studies are needed to indicate which are the best markers for cellular senescence in testes. However, as we did not detect any difference in other senescence markers (β-galactosidase and lipofuscin) it would be expected that SASP markers would be similar between groups.

We observed that D+Q treated mice had increased testosterone levels, which could explain the increased concentration and reduced sperm defects in this group. Testosterone levels progressively decline with aging in men [10], along with sperm motility, concentration, and quality, leading to infertility [11]. Changes in sperm parameters (concentration, morphology and motility) are also age-dependent in mice [10–12]. Testosterone is essential for the maintenance of the blood-testis barrier, spermatogenesis, regulation of LH and FSH secretion, stimulation of Sertoli cells, germ cell division, and sperm production and release [12, 37]. In the absence of testosterone signaling, spermatogenesis is interrupted, and mature sperm are phagocytosed by Sertoli cells [38]. The concentration of testosterone in serum and seminal plasma is correlated with sperm concentration, percentage of motile sperm and other sperm characteristics [39]. In addition, in the absence of testosterone, the conversion of round spermatids into elongated spermatids is blocked [38], increasing the proportion of abnormal sperm. Thus, in addition to affecting sperm concentration, testosterone also regulates spermiogenesis [40], affecting sperm morphology. Therefore, our observation of reduced sperm defects and increased concentration can be testosterone dependent. Abnormal sperm morphology is related to changes in sperm quality [10]. In the present study, sperm concentration and percentage of normal sperm were higher in D+Q treated males, along with increased serum testosterone levels. However, treatment with D+Q did not affect fertility and parameters of sperm motility. This small increase in sperm parameters was not enough to result in increased pregnancy rate, as sperm quality and concentration was adequate also in the control group. Previous studies showed that sperm motility was reduced only after 20 months of age in mice something similar happened with the number of pups/litters [41]. Therefore, changes in fertility, due reduced sperm quality, are observed only when mice are much older than the mice used in the current study. Future studies should focus on long-term treatment of older mice in order to observe possible fertility improvements.

It is still unclear how D+Q increased testosterone levels in mice. The beneficial effects of D+Q in testosterone levels could be attributed to the antioxidant potential of quercetin [42]. One of the main causes for decreased semen quality is the accumulation of reactive oxygen species (ROS) that accompanies testicular aging [43]. Aging induces the accumulation of ROS in sperm mitochondria and Leydig cells [43]. Since, Leydig cells are responsible for the production of testosterone through LH stimulation [12], their senescence may contribute to the decreased testosterone levels observed with aging. However, we did not observe reduced senescence in D+Q treated mice. Additionally, flavonoids such as quercetin have been suggested to influence the entry of cholesterol into the mitochondria, leading to increased production of testosterone by Leydig cells [37]. It is worth noting that the effects of quercetin on sperm quality and concentration depend on the dose and duration of treatment [44]. Others observed decreased sperm concentration and no change in testosterone levels after two weeks of treatment with

quercetin around time of puberty in mice [44]. There are no studies on the effect of dasatinib on testicular parameters. The suggestion is the beneficial effects of D+Q on testosterone levels may only be observed when used in combination. Senescent cells can activate protective networks of the senescent cell anti-apoptotic pathway (SCAPs) [8]. Dasatinib promotes apoptosis caused by dependence receptors, such as ephrins and down-regulates tyrosine kinase, c-KIT and PRGFRA to inhibit SCAPs [45]. The c-KIT and PRGFRA proteins are expressed in the testes and regulate testosterone production [31]. Senolytic flavonoids, such as quercetin, inhibit members of the BCL-2 family, as well as HIF-1α in the SCAP network [45]. Quercetin also suppresses the Janus kinase/signal transducer and activator of transcriptional proteins (JAK/STAT) pathway, which regulate Leydig cell steroidogenesis [38].

One of the limitations of the current study was the use of reproductive age, young animals, to evaluate the effect of senolytics on aging and testicular senescence. However, we did observe that treatment with D+Q for a prolonged period did not harm fertility of these mice, and even improved some fertility parameters. We were not able to observe an effect of D+Q on testicular senescence, however it is also possible that D+Q can reduce senescence in other tissues and have a benefit in the sperm parameters observed. Therefore, this need to be determined in different tissues. The evaluation of oxidative stress in the testes would also be interesting to observe the impact of senolytics compounds in future studies. Also, in this sense, the inclusion of two more groups with D and Q treatments alone, would help better understand the role played by these components. Additionally, future studies should focus on the mechanisms by which D+Q can affect testosterone concentration. There is a scarcity of studies evaluating the effects of D+Q on the male reproductive function, therefore studies in older mice and using different types of senolytics compounds are needed to understand how we can delay or reverse the expected decline in sperm quantity and quality with advancing age. Since there is a growing trend towards late paternity, alternatives that can prolong fertility, prevent testosterone decline and preserve sperm quality are of paramount importance.

CONCLUSION

Treatment with D+Q from three to eight months of age in male mice increased plasma testosterone levels, sperm concentration and decreased the percentage of abnormal sperm. Despite that, D+Q did not affect the fertility rate and testicular cellular senescence of these mice. Further studies with older mice, and different senolytics are necessary to elucidate the effects of senescence in the decline in sperm quality and quantity associated with aging.

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Declaration of competing interest: The authors declare no competing financial interests.

SUPPLEMENTARY MATERIAL

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