Benefits of the consumption of Brazil nut (*Bertholletia excelsa*) extract in male reproductive parameters of streptozotocin-induced diabetic rats

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Abstract

Objective The objective of this study was to evaluate the effects of intake of Brazil nut extract (BN) or sodium selenite solution on reproductive parameters of male diabetic animals.

Methods A total of 48 Wistar rats were distributed into six groups: diabetes ($n = 8$); diabetes and Brazil nut extract ($n = 8$); diabetes and sodium selenite (Na_2SeO_3) ($n = 8$); Brazil nut extract ($n = 8$); sodium selenite ($n = 8$) and control ($n = 8$). A single dose of streptozotocin (65 mg/kg) was injected intravenously to the rats to induce diabetes. BN or Na_2SeO_3 were administered by gavage for 56 days.

Results The diabetes caused critical alterations on body mass gain, reproductive parameters and antioxidant capacity. Treatments with both BN or Na_2SeO_3 were able to increase significantly the glutathione peroxidase and the daily sperm production, both in diabetic ($p < 0.01$ and $p < 0.05$) and in healthy animals ($p < 0.01$ and $p < 0.05$).

Conclusion The Brazil nut extract and sodium selenite were able to improve some reproductive parameters of diabetic rats. Moreover, we could infer that this effect is probably due to the natural selenium content of the BN.

Keywords Diabetes · Oxidative stress · Selenium · Infertility

Introduction

Diabetes mellitus (DM) is one of the most important metabolic disorders, and it has been affecting a large number of people around the world for thousands of years [1]. The incidence of type 1 Diabetes (DM1) has increased particularly in preschool children and the number may double in young people by 2020 [2, 3].

There is evidence that changes found in the hyperosmolar hyperglycemic state may be associated with oxidative stress [4]. This process results from an imbalance between oxidant

and antioxidant compounds [4, 5]. Diabetes, due to hyperglycemia and subsequent oxidative stress, causes various organs to be affected, including the reproductive system [1, 5, 6]. It is estimated that about 90% of diabetic patients have disturbances in sexual function, including decreased libido, impotence and infertility [7].

Clinical and experimental studies have revealed changes in spermatogenesis and sperm morphology. In addition, low sperm count and motility as well as lower levels of testosterone and seminal fluid were found in diabetic subjects [6, 8].

Treatment with antioxidants has been shown to be extremely effective in reversing or mitigating the damage caused by excessive production of reactive oxygen species [9, 10]. Among the antioxidants described in literature, the most commonly used are vitamins A, C, E, lipoic acid, bioflavonoids and antioxidant minerals (copper, zinc, manganese and selenium) [4, 11]. Selenium is known for its antioxidant activity, therapeutic aspects, chemopreventive function as well as anti-inflammatory and antiviral properties [12]. Moreover, it regulates the major component of the antioxidant defense mechanism in all living tissues, being a fundamental component of

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several enzymes such as the glutathione peroxidase (GPx) [12, 13].

The Brazil nut is known for being a source of selenium [14]. Furthermore, nuts in general have been identified as rich sources of antioxidants and phytochemicals, including phenolic compounds (tannins, ellagic acid and curcumin), flavonoids (luteolin, quercetin, myricetin, kaempferol and resveratrol), isoflavones (genistein and daidzein) and terpenes [15]. Brazil nuts have been shown to be a food rich in polyunsaturated fatty acids, vitamins such as thiamine, pyridoxine and tocopherol and magnesium, in addition to the selenium mentioned above. All of these compounds play an important role in reproduction and antioxidant activity, showing the importance of food and not just an isolated compound [14–17].

Brazil nuts have phenolic antioxidants which can effectively control oxidative stress in the body [15]. A recent study has demonstrated that patients on hemodialysis supplemented with Brazil nuts had their levels of selenium and GPx increased, suggesting that the nut can improve the condition of oxidative stress [16]. The same situation was verified by Cominetti et al. [17], who showed that the consumption of Brazil nuts increased the GPx activity and decreased the atherogenic risk in obese women.

The growing interest in the possible health benefits of eating foods rich in antioxidant compounds provides a compelling case for research and understanding of the effects of Brazil nut consumption, especially in conditions where oxidative stress is triggered. Therefore, the aim of this study was to evaluate the effects of Brazil nut extract consumption on reproductive parameters and testicular antioxidant capacity of diabetic rats.

Materials and methods

Animals

Forty-eight male adult Wistar rats (90-days-old) from the Centro de Desenvolvimento de Modelos Experimentais (CEDEME), at the Universidade Federal de São Paulo (UNIFESP) were used. The animals were kept under controlled conditions (24 ± 2 °C), light-dark cycle of 12 h and had free access to food (Chow diet containing low amounts of selenium, <1% of the daily recommendation) and water. All experimental procedures were conducted according to the International Guiding Principles for Biomedical Research Involving Animals, after approval by the Research Ethics Committee of UNIFESP (protocol no. 0603/11).

Experimental design

The experimental groups ($n = 48$) were divided into 6 groups: control group (CTRL), diabetes group (DM), Brazil nuts

extract group (BN), sodium selenite group (Na_2SeO_3), diabetes and Brazil nut extract group (DMBN), and diabetes and sodium selenite group ($\text{DMNa}_2\text{SeO}_3$).

A group receiving sodium selenite was included in the experimental design to test the impact of selenium alone, as sodium selenite is a common form of selenium supplementation in reproductive studies. The animals in DM, DMBN and $\text{DMNa}_2\text{SeO}_3$ groups received a single intravenous injection containing streptozotocin (STZ) (Sigma Aldrich, St. Louis, MO, USA) at 65 mg/kg bw (body weight), diluted in sodium citrate buffer (pH 4.5), after a fasting period of 12 h [18]. The non-diabetic groups received only the vehicle. After 1 week, the animals injected with STZ were evaluated for blood glucose levels to test the effectiveness of the application, with the aid of a glucometer (Accu-Check® Performa Nano). Animals considered diabetic were those with fasting blood glucose levels higher than 300 mg/dL. Glycemic evaluation was repeated on the day of euthanasia to measure the change in blood glucose during the experiment.

Preparation of Brazil nut extract and sodium selenite

The extract of the Brazil nut was obtained by grinding the nut for 3 min with water at 75 °C, according to Ferberg et al. [19] and Cardarelli & Oliveira [20]. The extract was strained in stainless steel and pasteurized at 72 °C for 20 min in a water bath. The extract was further concentrated by evaporation and the selenium content was then determined as 0.121 µg/g. Animals received a daily dosage of 1.1 g of the extract, which corresponds to 50% of the Recommended Dietary Allowance (RDA) for humans adjusted for rats considering average weight of a male human adult (70 kg). Sodium selenite (Sigma® St. Louis, MO, USA) was prepared to obtain the same amount of selenium in the Brazil nut extract. The substance was diluted with water, and the animals were treated daily with 1 g of sodium selenite by gavage.

It is important to note that, based on the RDA proposed by the National Institutes of Health (NIH), the US Department of Health and Human Services, and the US Department of Agriculture (USDA), nut consumption by patients should be one seed per day. This amount would guarantee the required amount of selenium and would not exceed the consumption limit for humans [21].

Biometric parameters

Body mass gain and absolute weight of reproductive organs

The value of body mass gain was determined according to the difference between the initial and final weight. Animals were weighed weekly during the treatment period. The absolute weights of the testes, epididymis, prostate and seminal vesicles (with coagulating glands) were determined. The organs

were removed immediately after sacrificing the animals by decapitation and weighed on an analytical balance.

Sperm parameters

Daily sperm production (DSP)

Sperm counting was performed according to Pires et al. [10]. The right testis was homogenized in 10 ml of sodium chloride 0.9% (saline) containing 0.5% Triton X-100 in tissue homogenizer for 1 min. The homogenate was diluted in a 1:10 proportion; subsequently the spermatids were counted in 4 Neubauer chambers (hemocytometers) to obtain the average count. Then, the number of spermatids per animal obtained by the counting was divided by 6.1 days for the conversion to average daily sperm production [22].

Sperm morphology

To evaluate the percentage of morphologically abnormal sperms, a sperm suspension was obtained by washing the tail of the epididymis with a phosphate buffer saline (PBS) solution. An aliquot of the suspension was dropped on slides and dried at room temperature [10]. Two hundred sperms per animal were examined microscopically at a 400x magnification, and the total percentage of sperm with tail and/or head defects was recorded [23].

Sperm count in the epididymis

The right epididymis was collected and weighed. The organ was separated into two portions: head/body and cauda. The epididymides were frozen in separate plastic tubes for future counting procedures. After homogenization, an aliquot of the solution was placed in a Neubauer chamber and counted [22].

Histomorphometric analysis

After removal, the left testis and epididymis were immediately fixed in an ALFAC solution (85% ethyl alcohol 80GL, 10% formaldehyde and 5% glacial acetic acid). Incisions were made at the testicular poles to facilitate the fixation. After 4 h, the testis was cut into two halves and retained in fixation for an additional 20 h. After this period, the testis was dehydrated by increasing the concentration of ethanol to 80%, diaphanized in xylene and embedded in Paraplast Plus® for histological analysis in cuts of 5 µm, followed by staining with hematoxylin/eosin.

The percentage of interstitial and tubular areas of the testis was determined by measuring the area occupied by the seminiferous tubules and interstitium in fifteen fields per animal [10, 24]. For each animal, thirty round tubules were selected randomly to measure the tubular diameter. This analysis was

performed using the AxioVision 4.8 software (Carl Zeiss) linked to a microscope (Carl Zeiss) at 200x magnification.

Histopathological analysis of spermatogenesis

To evaluate the histopathology of spermatogenesis, the Johnsen score was used as adapted by various authors [25, 26]. The analysis was performed on 50 randomly chosen seminiferous tubules per animal, as described by Lee et al. [27].

Plasma testosterone levels

Immediately after decapitation, blood samples were collected in EDTA tubes and centrifuged at 5000 rpm for 15 min at 4 °C. Plasma samples were kept at −80 °C before analysis. Plasma testosterone levels were determined by ELISA method, using the Salimetrics kit (SciencePro, São Paulo, Brazil) with adaptations.

Activity of testicular antioxidant enzymes

Activity of testicular antioxidant enzymes was assessed from a testicular portion (approximately ¼ of the right testis) frozen at −80 °C. This temperature is required for maintaining the integrity of the enzymes. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase enzyme activity assays were performed using specific kits from Cayman Chemical Company (Ann Arbor, Michigan, USA), according to the manufacturer's instructions.

Statistical analysis

The two-way analysis of variance (ANOVA) was used for the variables of body mass gain, DSP, organ weight, percentage of abnormal sperm morphology, sperm count in the epididymis, antioxidant enzyme activity and plasma testosterone levels. To study the behavior of the blood glucose, the repeated measures ANOVA was used, as two assessments were performed for each individual [28]. The software R Core Team (2016) (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) was used.

Results

Glycemic levels

After the single dose of streptozotocin, the glucose levels of the diabetic group increased significantly when compared to the control group ($p < 0.001$) (Table 1). Interestingly, the glycemic levels of diabetic animals treated with Brazil nuts (DMBN) were significantly lower than the DM group ($p <$

Table 1 Mean and standard deviation of variance of blood glucose levels after 7 days and the last day of the experiments (mg/dL) and body mass gain (g)

Diabetes	Treatment	7 Days	Last Day	Body mass gain
No	Control	105.5 ± 3.46	97.25 ± 8.7	92.2 ± 11.4
	Brazil nut	87.28 ± 9.96*	88.28 ± 8.85*	81 ± 18.9
	NaSe	87.87 ± 9.59*	86.25 ± 5.5*	84.8 ± 19.2
Yes	Control	540.4 ± 61.46*	473.4 ± 79.05*	75 ± 40.1*
	Brazil nut	406 ± 119.48	387 ± 97.93 ^A	99.8 ± 48.5 ^A
	NaSe	475 ± 75.16	480 ± 50.75	121.7 ± 32.1 ^A

*($p < 0.001$) compared to control without diabetes; A ($p < 0.001$) compared to control with diabetes

0.001). The same, however, was not observed in the Na_2SeO_3 group. In non-diabetic groups (BN and Na_2SeO_3), statistically significant lower glycemic values were observed when compared to control group ($p < 0.05$) (Table 1). Compounds present in Brazil nut capable of modulating antioxidant molecules that inactivate reactive oxygen species from diabetic hyperglycemia and the selenium present in Brazil nut that could also activate glucose transporters such as Glut 1 and Glut 2 are shown in Fig. 2.

Body mass gain

The body mass gain of the diabetic group was lower when compared to the CTRL group; on the other hand, the diabetic animals that received Brazil nut and sodium selenite presented a higher mass gain when compared to the control group with diabetes ($p < 0.001$) (Table 1). No changes in mass gain of BN and Na_2SeO_3 groups were observed when compared to the control group.

Weight of reproductive organs

The weight of the testis, epididymis, seminal vesicle, ventral and dorsolateral prostate were lower in diabetic animals when compared to control group ($p < 0.001$). Treatment with Brazil nut extract or sodium selenite did not improve these

parameters in diabetic groups. Non-diabetic animals did not differ from the control group (Table 2).

Morphometric analysis

Animals of the DM group showed reduced tubular diameter when compared to the CTRL group ($p < 0.001$). The diabetic group treated with Brazil nut (DMBN) had an increase in the percentage of tubular area and lower interstitial area when compared to the DM group ($p < 0.05$ and $p < 0.01$, respectively). In contrast, sodium selenite had no effect on morphometric parameters (Table 3 and Fig. 1).

Histopathologic analysis of spermatogenesis

In the histopathologic analysis (Johnsen score), no significant differences were observed in any of the evaluated groups, as shown in Table 3.

Testicular antioxidants

There was no difference in the activity of superoxide dismutase and catalase amongst groups (Table 4). However, the diabetic group had higher GPx activity when compared to the control group ($p < 0.01$). Moreover, the diabetic animals treated with Brazil nut and sodium selenite showed higher enzymatic activity when compared to the DM group ($p < 0.05$ and $p < 0.001$, respectively).

Healthy animals consuming Brazil nut or sodium selenite also showed higher GPx activity when compared to the control group ($p < 0.05$ and $p < 0.001$, respectively).

Daily sperm production (DSP)

While the diabetic group showed a significant decrease in daily sperm production when compared to the control group ($p = 0.001$), diabetic animals treated with Brazil nut extract or sodium selenite showed significantly higher sperm production when compared to DM group ($p = 0.003$ and $p = 0.001$, respectively). Likewise, BN and Na_2SeO_3 groups showed

Table 2 Reproductive organ weights expressed as means and standard deviation (g)

Diabetes	Treatments	Dorsolateral prostate	Ventral prostate	Seminal vesicle	Epididymis	Testis
No	Control	0.26 ± 0.04	0.37 ± 0.06	0.8 ± 0.22	1.26 ± 0.04	3.58 ± 0.18
	Brazil nut	0.29 ± 0.04	0.39 ± 0.09	0.89 ± 0.25	1.14 ± 0.11	3.46 ± 0.23
	NaSe	0.25 ± 0.08	0.37 ± 0.09	0.84 ± 0.23	1.21 ± 0.14	3.66 ± 0.28
Yes	Control	0.11 ± 0.06*	0.08 ± 0.07*	0.17 ± 0.12*	0.73 ± 0.29*	2.7 ± 0.77*
	Brazil nut	0.11 ± 0.06	0.16 ± 0.12	0.25 ± 0.17	0.78 ± 0.26	3.05 ± 0.63
	NaSe	0.32 ± 0.4	0.09 ± 0.05	0.14 ± 0.04	0.66 ± 0.24	2.71 ± 0.75

*($p < 0.01$) compared to control without diabetes

Table 3 Histomorphometrical analysis and Johnsen's score (mean values and standard deviations)

Diabetes	Treatments	Tubular area %	Interstitial area %	Tubular diameter μ^2	Johnsen's score
No	Control	79.88 $\pm$ 3.04	20.12 $\pm$ 3.04	285 $\pm$ 16.06	9.96 $\pm$ 0.08
	Brazil nut	69.13 $\pm$ 19.75	23.81 $\pm$ 2.65	295.42 $\pm$ 15.86	9.94 $\pm$ 0.09
	NaSe	63.95 $\pm$ 20.69	24.53 $\pm$ 3.37	302.41 $\pm$ 23.86	9.93 $\pm$ 0.09
Yes	Control	73.55 $\pm$ 5.77	26.45 $\pm$ 5.77	251.45 $\pm$ 34.69*	9.83 $\pm$ 0.24
	Brazil nut	80.87 $\pm$ 7.64 ^A	19.13 $\pm$ 7.64 ^A	266.1 $\pm$ 23.27	9.67 $\pm$ 0.39
	NaSe	77.39 $\pm$ 5.61	22.61 $\pm$ 5.61	256.24 $\pm$ 34.55	9.31 $\pm$ 1.54

*($p < 0.01$) compared to control without diabetes. A ($p < 0.01$) compared to control with diabetes

higher sperm production when compared to CTRL ($p = 0.003$ and $p = 0.001$, respectively) (Table 5).

Consumption of Brazil nut and sodium selenite showed no effects in any condition (Table 5).

Sperm morphology

The diabetic group showed a lower percentage of morphologically normal sperm when compared to CTRL ($p = 0.004$). Neither sodium selenite nor consumption of Brazil nut extract were able to reverse this change triggered by hyperglycemic condition. No effect was observed when the extract or the sodium selenite were administered to healthy animals (Table 5).

Epididymal sperm count

Diabetic animals showed lower values of epididymal sperm count in both the head/body and the cauda portions when compared to control ($p < 0.001$). Brazil nut extract and sodium selenite did not alter this parameter in any condition (Table 5).

Testosterone levels

Testosterone levels of animals in the diabetes group were lower when compared to the control group ($p < 0.001$).

Discussion

This study has demonstrated the harmful effects of diabetes mellitus and the positive effects of Brazil nut extract in some of the evaluated parameters. The increase in glucose levels induced by streptozotocin agrees with the results of Chougala et al. [1] and Shrilatha et al. [29]. According to Etuk [30], this effect is due to the drug action in inducing cell death after changes in the DNA of pancreatic β cells.

The consumption of Brazil nut extract at a dietary dose (50% of selenium in RDA) attenuated some of the induced changes. The treatment significantly decreased the levels of circulating glucose in diabetic and healthy animals. This may be due to the complex composition of Brazil nuts. It is well established that the Brazil nut is rich in vitamin E and fatty acids, which may have positively contributed to the reduction of blood glucose levels observed in this study. Stapleton [31] reported that selenium could also activate important proteins in the insulin signaling cascade. Selenium can also increase the activity of glucose transporters such as GLUT-1 and

Fig. 1 **a** Seminiferous tubules of control animals without diabetes. **b** Altered seminiferous tubules of animals from control group with diabetes. Presence of atrophied tubules with increased of the luminal portion (Bar = 100 μ m to a and b)

Table 4 Enzymatic activity of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) (mean values and standard deviations)

Diabetes	Treatments	SOD (U/mL)	CAT (nmol/min/mL)	GPx (nmol/min/mL)
No	Control	6.55 ± 0.9	438.32 ± 109.93	147.63 ± 27.25
	Brazil nut	5.76 ± 1	377.38 ± 119.43	164.33* ± 46.69
	NaSe	5.55 ± 1.09	341.67 ± 93.72	185.56* ± 32.35
Yes	Control	6.37 ± 0.81	360.86 ± 95.2	164.66 ± 10.12*
	Brazil nut	4.77 ± 0.61	327.94 ± 86.01	191.15 ± 37.76 ^A
	NaSe	6.67 ± 1.67	246.92 ± 120.43	217.29 ± 30.44 ^A

*($p < 0.01$) compared to control without diabetes; A ($p < 0.01$) compared to control with diabetes

GLUT-2, thereby decreasing the hyperglycemic condition [32]. Sodium selenite as positive control showed effects only in healthy animals. This effect was obtained in different investigations by Pillai et al. [33] and Guney [34]. This is probably due to the effect of selenium to mimic the action of insulin [33]. The persistence of high glucose levels in diabetic rats treated with sodium selenite was assessed by Erbayraktar et al. [35]. The authors found that animals treated with selenomethionine, the organic form of selenium supplementation, similar to the one found in Brazil nuts, showed reduction in blood glucose levels, unlike those exposed to sodium selenite. Conversely, Ayaz et al. [36] found no reduction in glucose levels, although the treatment with sodium selenite could prevent diabetes-induced changes in the heart. It was suggested that selenium, in its organic form, has a higher gastrointestinal absorption rate and consequently superior bioavailability when compared with the inorganic compound (selenite or selenate) [35, 36].

Oxidative stress resulting from diabetic hyperglycemia activates the antioxidant defense mechanism that involves enzymatic and non-enzymatic systems [4]. In this study, however, no changes in the activity of superoxide dismutase and catalase after induction of diabetes were observed. Differently, other studies reported reduction in enzyme activity, possibly attributable to the excessive increase of reactive oxygen species [29, 37, 38]. According to Masaki et al. [39], catalase is active when hydrogen peroxide is present at low concentrations,

whereas GPx acts as the main catalyst enzyme when these molecules are abundant.

In our study, SOD activity was not significantly different amongst the groups. Indeed, there was not a consensus on possible changes of SOD in tissues under diabetic conditions. Published results on this matter show no trends or associations with gender of animals, species, duration of diabetes or tissue under investigation [4, 38].

Although no differences in SOD activity were found, a significant increase in glutathione peroxidase of diabetic animals was observed, agreeing with previous studies [40, 41], showing increased activity in rat tissues, such as liver, kidney, heart, blood and testis. Furthermore, it is reported that in diabetes there is induction of enzymes that act on hydrogen peroxides and reduction of SOD activity by oxidative stress and mainly hydrogen peroxide [42]. According to Sozmen et al. [42], patients with diabetes showed increased catalase activity and reduced SOD activity.

According to Unlucerci et al. [40], increased enzymatic activity demonstrates adaptive change in mechanism of antioxidant defense. Furthermore, diabetic animals treated with Brazil nut extract or Na_2SeO_3 also showed significant increase in GPx activity, suggesting that selenium (an essential part of the glutathione peroxidase molecule) may play a role in this change [16]. The presence of selenium as catalytic group guarantees a fast reaction with the hydroperoxide and a faster reduction of glutathione [43]. Such additional increase observed in the DMBN group may represent an increment in the testicular

Table 5 Daily sperm production (DSP) ($\times 10^6$), sperm morphology (SM) (%), sperm count in the epididymis ($\times 10^6$) and testosterone levels (pg/mL) presented in mean and standard deviation

Diabetes	Treatments	DSP	SM	Epididymis count cauda	Epididymis count head/body	Testosterone
No	Control	28.44 ± 6.51	69.12 ± 9.25	154.05 ± 40.65	83.49 ± 25.55	273.1 ± 142.79
	Brazil nut	34.77 ± 7.45*	57.61 ± 18.37	160.4 ± 47.02	78.94 ± 34.13	340.5 ± 92.6
	NaSe	46.78 ± 20.8*	61.25 ± 10.71	160.3 ± 39.71	85.23 ± 25.67	296.1 ± 107.3
Yes	Control	18.15 ± 6.2*	49.95 ± 9.32*	46.5 ± 46.12*	33.35 ± 19.70*	207.9 ± 89.36*
	Brazil nut	28.57 ± 9.43 ^A	51.78 ± 22.03	53.74 ± 28.03	44.35 ± 29.95	207.1 ± 97.3
	NaSe	24.81 ± 6.05 ^A	46.71 ± 8.51	32.6 ± 15.36	39.76 ± 22.42	159.6 ± 35.98

*($p < 0.01$) compared to control without diabetes. A ($p < 0.01$) compared to control with diabetes

antioxidant defense. We believe there was an increase in SOD activity at an early (unchecked) time due to hyperglycemia. After an increase in GPx activity, some adaptation mechanism caused a reduction in SOD activity and only glutathione peroxidase acted. After all, SOD is the first line of defense and with Brazil nut treatment hyperglycemia has decreased, further increasing glutathione peroxidase activity.

Although it was not possible to evaluate phytochemicals, Brazil nuts are a food rich in many micronutrients, vitamins and fatty acids, which seems to play important roles in male reproduction. In addition to selenium, already mentioned, magnesium is also present in nuts and may have played an important role in this improvement. Magnesium has been shown to play an important role in diseases related to oxidative stress, such as preeclampsia, systemic scleroderma, diabetes and β -thalassemia [44, 45]. The relationship between magnesium and selenium has already been discussed as being fundamental for antioxidant defense and maintenance of reproductive cells, demonstrating the importance of consumption of Brazil nuts, a food that has both compounds [46]. Besides that, Brazil nuts have several vitamins in their composition, among them thiamine and pyridoxine. Such vitamins can also play important roles in reproduction, elucidating such results. Thiamine may undergo transformation in vivo resulting in conformation of the thiazole open ring with sulfhydryl (SH) group capable of reducing oxidative stress (Fig. 2) [47]. Sulfhydryl groups (HS) are considered the largest and most frequent antioxidants in plasma [48–50]. Thiamine (vitamin B1) causes activation of intermediate metabolism pathways, improving cellular respiration and decreasing ROS generation [51].

The hyperglycemic state may also be related to hormonal and metabolic changes, such as reduction in body mass and serum testosterone levels, the latter influencing the reproductive parameters [8]. As observed in this study, loss of body mass in hyperglycemic animals has also been verified by other authors

[36, 43, 52]. It was suggested that the abnormal metabolism of carbohydrates, lipids and proteins, as well as decreased insulin levels, might lead to weight loss under conditions of diabetes mellitus [42]. Plasma testosterone levels were also lower in diabetic animals of our study, confirming previous observations [8, 28]. The reactive oxygen species, resulting from hyperglycemia, can inhibit steroidogenesis by interfering with cholesterol transport in mitochondria or in its conversion to pregnenolone by the action of the mitochondrial enzyme P450_{scc} (P450 cholesterol side-chain cleavage enzyme) [52]. The lowered levels of testosterone observed in the non-treated diabetic animals may also be related to decrease in weight of reproductive organs such as testis, epididymis and prostate gland, and also to the reduced sperm production.

Although it is known that selenium can increase testosterone synthesis through modulation of StAR protein (steroidogenic acute regulatory protein) [53–56], the intake of Brazil nut extract or sodium selenite did not change plasma testosterone levels. However, both nut extract and sodium selenite significantly improved the sperm production of diabetic and healthy animals.

In treated diabetic animals, the selenium intake might have increased the intratesticular testosterone levels by modulating the oxidative stress, improving steroidogenesis and, consequently, the sperm production.

As argued by Walker (2011) [57], testosterone is the key androgen for spermatogenesis maintenance through complex signaling pathways mainly involving the Sertoli cells. Moreover, levels of intratesticular testosterone are higher than those observed in the serum [58, 59]. Additionally, it has been established that the intratesticular testosterone levels are much higher than those necessary to maintain a normal spermatogenesis [57, 58]. It was found that testosterone levels ranging from 20 to 46% of the normal intratesticular values are enough to support a normal spermatogenesis [56, 59, 60].

Fig. 2 Compounds present in Brazil nut capable of modulating antioxidant molecules that inactivate reactive oxygen species from diabetic hyperglycemia. Selenium present in Brazil nut can also activate glucose transporters such as Glut 1 and Glut 2

Considering all previous data, we can suggest that the values of intratesticular testosterone (not measured) in the animals exposed to Brazil nut extract or sodium selenite, may have reached high enough levels to maintain a normal sperm count. Such minimum recovery of testicular testosterone may not be sufficient to reflect an increase or even normal values in plasma, as observed in our results.

Healthy animals receiving sodium selenite also showed improvement in sperm production. Similar results were found in healthy animals treated with trans-resveratrol. Authors attributed the effect to its antioxidant capacity, counteracting the constitutive oxidative stress within the seminiferous tubules [61]. In addition to the antioxidant effect of selenium, this micronutrient plays important roles in the testicular structure, development of germline cells and normal development of sperm during spermatogenesis [13, 62].

Sperm count in the epididymis was reduced in diabetic animals, and it was not affected by the intake of Brazil nut extract. These results can be related to the fact that selenium is preferentially absorbed by the testis, impairing the distribution to other organs like epididymis [62]. Studies on human semen show no correlation between selenium levels, epididymis sperm count and sperm motility [63–65]. In addition, according to Hawkes et al. [66], selenium supplementation had no effect on the sperm count and morphology of the epididymis, agreeing with the results of our research.

It has been explained that epididymis is more susceptible to diabetic-induced changes than testis, becoming the stored sperm more exposed to oxidative stress, without protection of the microenvironment provided by the Sertoli cells [8, 67]. In this context, the observed reduction of morphologically normal sperm of DM group corroborates with previous studies [28]. Furthermore, treated animals showed no improvement.

Singh et al. [67] reported that the decrease in testosterone levels is also related with decrease in tubular diameter, which was observed in DM group. This event can be intensified by damaged epithelium, disruption of spermatogenesis and fragmentation of chromatin, previously described in diabetic animals [37]. Another consequence of hyperglycemic condition is the vascular disruption, impairing the nutritional and oxygen support to germline cells [18] and leading to edema in the interstitial area [23]. This state was not observed in DM group. However, consumption of Brazil nut extract reduced the interstitial area in diabetic animals, possibly due to the role of selenium on testicular structure and to the hypoglycemic effects observed after Brazil nut treatment.

Although we have evidenced diabetes-induced moderate and variable histomorphometric changes in testis, the same was not observed in the histopathological analysis according to the Johnsen score. This result may be related to the fact that moderate reduction of testosterone is not severe enough to affect spermatogenesis, as previously reported [68]. Nevertheless,

histopathological changes, such as vacuolization, disruption of spermatogenesis and exfoliation, were present in group DM, although not uniformly distributed among individuals.

In conclusion, Brazil nut extract improved some of the reproductive parameters and the antioxidant capacity of diabetic rats. The positive effects were mostly, but not only, due to the relevant selenium content, as demonstrated by the positive control group (Na_2SeO_3).

Thus, Brazil nut extract showed, probably because it contains important antioxidant compounds, protective action on fundamental parameters. We can state that there is a fundamental role of Brazil nut consumption in male reproduction, even in healthy individuals, demonstrating the synergistic effect of these nutrients. It is important to highlight that Brazil nuts are a complex food, not only with organic selenium, but also with other nutrients and bioactive compounds, which were not evaluated in this study.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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