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Chronic administration of thiamine pyrophosphate decreases age-related histological atrophic testicular changes and improves sexual behavior in male Wistar rats

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Summary. Aging is a multifactorial universal process and constitutes the most important risk factor for chronic-degenerative diseases. Although it is a natural process, pathological aging arises when these changes occur quickly and the body is not able to adapt. This is often associated with the generation of reactive oxygen species (ROS), inflammation, and a decrease in the endogenous antioxidant systems, constituting a physiopathological state commonly found in chronic-degenerative diseases. At the testicular level, aging is associated with tissue atrophy, decreased steroidogenesis and spermatogenesis, and sexual behavior disorders. This situation, in addition to the elevated generation of ROS in the testicular steroidogenesis, provides a critical cellular environment causing oxidative damage at diverse cellular levels. To assess the effects of a reduction in the levels of ROS, thiamine pyrophosphate (TPP) was chronically administered in senile Wistar rats. TPP causes an activation of intermediate metabolism routes, enhancing cellular respiration and decreasing the generation of ROS. Our results show an overall decrease of atrophic histological changes linked to aging, with higher levels of serum testosterone, sexual activity, and an increase in the levels of endogenous antioxidant enzymes in TPP-treated animals. These results suggest that TPP chronic administration decreases the progression of age-related atrophic changes by improving the intermediate metabolism, and by increasing the levels of antioxidant enzymes.

Key words: Aging, Free radicals, Antioxidant, Thiamine pyrophosphate

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Introduction

Natural aging is accompanied by a gradual decline in reproduction, associated with a chronic state of oxidative stress. This situation causes a functional deficit in Leydig, Sertoli and germ cells (Veldhuis, 2008; Pop et al., 2011). Previous studies have reported that while testicular function in aging is affected little as compared to females, there are constant changes in the functioning of many of the components of the male reproductive system (Fauser, 2000; Veldhuis et al., 2009). A progressive decrease in testosterone serum levels has been described, preceding alterations in the normal parameters of sperm (Neaves et al., 1984; Haidl et al., 1996). Other studies have reported an increase in the rate of arrest of spermatogenesis (Miething, 2005), among other things, due to the increase in apoptosis of germ cells (Walter et al., 1998; Kimura et al., 2003), with multiple histologic changes in the seminiferous tubules associated with aging (Honore, 1978; Johnson et al., 1986; Paniagua et al., 1987; Pal and Santoro, 2003), and a decrease in the number of sperm stem cells, decreasing exponentially with the increase in age (Suzuki and Withers, 1978). The decrease in steroidogenesis associated with the loss of balance between pro-oxidant and antioxidant endogenous systems, causes an

oxidative damage in diverse cellular processes. This oxidative damage, together with the accompanying lipid peroxidation, is particularly intense in tissues that synthesize steroids, since in addition to oxidative phosphorylation these tissues also use molecular oxygen for steroidogenesis (Hornsby, 1989). Furthermore, the excessive production of ROS alters mitochondrial cholesterol transport and catalytic function of P450 enzymes (Stocco et al., 1993). A further increase in lipid peroxidation (Rikans and Hornbrook, 1997) promotes oxidative toxicity and a decline in endogenous antioxidant systems (Mansour et al., 2002). To protect against the adverse effects of ROS, mammalian cells employ a variety of enzymatic and non-enzymatic antioxidant reactions. The main antioxidant enzymes are superoxide dismutase (SOD), glutathione system (GSH) and catalase (CAT). Regarding non-enzymatic antioxidants, the most relevant are estrogens (Hamden et al., 2008), growth factors (Xie et al., 2008), caloric restriction (Hamden et al., 2008) as well as vitamins, among others (Chen et al., 2005; Sener et al., 2005). There is evidence that the generation of ROS, inflammation and alterations of endogenous antioxidant systems, frequently found in aging, are also present in chronic illnesses such as diabetes mellitus type 2 (DM2) (Cholerton et al., 2011) and various neurodegenerative disorders (Orth and Schapira, 2001; Calabrese et al., 2008; Aviles-Olmos et al., 2012) such as Alzheimer's (Schuh et al., 2011; de la Monte et al., 2012) and Parkinson's Disease (Owen et al., 1996; Morris et al., 2011).

Thiamine pyrophosphate (TPP), the active form of vitamin B1, possesses two high-energy phosphates and is involved in intermediary metabolism reactions and cellular respiration, and contributes to the prevention and/or reduction in the formation of ROS. TPP is a coenzyme in multienzyme complexes such as pyruvate dehydrogenase (PDHC), α-ketoglutarate dehydrogenase (KDHC), transketolase, cytochromes and acetolactate synthetase (Martin et al., 2005; Ojano-Dirain et al., 2010). Experimental evidence has shown that TPP may decrease the generation and damage caused by ROS, with an improvement in the performance of aerobic respiration (Rozanov et al., 1990; Torres et al., 2009). Showing antioxidant capacity, TPP normalizes the levels of lipid peroxidation, and is a potent inhibitor of non-enzymatic glycosylation of proteins and improves antioxidant systems such as CAT and the glutathione system (Tolstykh and Khmelevskii, 1991; Booth et al., 1996; Senapati et al., 2000; Gibson and Zhang, 2002). Aging causes a decrease in the enzymatic activity of TPP-related compounds, with a progressive decrease in the activity of the protein kinase c-Jun, an enzyme that regulates the activation of the PDHC, causing a progressive decrease in metabolism (Zhou et al., 2009). Oxidative stress generates an inactivation of enzymes related to TPP, triggering a decrease in thiamine-dependent enzymes (Martin et al., 2005). Studies in thiamine-deficient animals have described a rise in markers of inflammation and oxidative stress like ICAM-1, heme oxygenase, endothelial nitric oxide synthase, redox active iron, microglial activation and an increase in ROS levels (Langlais et al., 1997; Todd and Butterworth, 1999; Gibson et al., 2000; Gibson and Zhang, 2002).

Inflammation and increased ROS generation play an important role in the onset and progression of changes related to aging. Therefore, a chronic treatment reducing ROS production could reduce the development of degenerative processes in different cellular components related to aging. The aims of this study were to determine the effect of TPP chronic administration on the testicular atrophic changes associated with aging, and in functional parameters such as sexual behavior and testosterone levels.

Materials and methods

Animals and treatments

Twenty male Wistar rats, aged 14 months old were employed for the experiments as follows: 10 for control group and 10 for study group. All animals were maintained in the animal house facility at a constant temperature of 25±3°C, under 12h controlled light:dark cycle, with access to water and fed *ad libitum* (LabDiet, Nutrition International, Brentwood, MO, USA). Both groups were treated for 14 months: The control group were given an interescapular subcutaneous saline solution administration, the study group received 1 mg/kg body weight TPP, twice a week (X-2, Instituto de Investigaciones Filosóficas y Científicas S.A. de C.V.). The handling of the animals and all experiments were performed in accordance with the specific regulations of the Mexican Health Ministry (NOM-062-ZOO-1999). After treatment, the rats were anesthetized with intraperitoneal ketamine (90 mg/kg) and xylazin (10 mg/kg), and sacrificed by cervical dislocation. Blood, liver, kidney and testicular tissue samples were obtained for posterior analysis. A second control group (n=8) was composed of young male Wistar rats 6 months old with full testicular maturity. This second control was employed to evaluate testicular weight and testosterone levels.

Histological evaluation

After sacrifice, testicular samples were weighed and immediately fixed in 3.5% paraformaldehyde (Sigma-Aldrich, Saint Louis, MO, USA) and processed according to the paraffin embedding technique. Samples were cut into 5-micron thick slices and these sections were mounted on slides and stained with hematoxylin-eosin (Wittekind, 2003). Once the samples were stained, double blind histopathological descriptions were performed with determination of the following parameters:

Diameter of the seminiferous tubules

Using a micrometer scale, seminiferous tubules were randomly chosen and their diameters measured in at least 25 different tubules for each specimen, comparing average tubule diameter for each group. Scale bars in each image correspond to 500 microns.

Cell count

This measurement was performed by determining the numbers of cells per 500 μm^2, 10 digital micrographs were chosen at random of a section of testicular tissue using a 10x magnification. Each of the micrographs represents a total area of 490 μm^2. The number of Sertoli and Leydig cells, spermatogonia, and the number of germ cells (spermatogonia) layers by tubule were determined. Sertoli cells were identified by their irregular nucleus containing multiple nucleoli, often positioned at the base of the membrane. Spermatogonia and spermatozoa were identified according to the characteristics described by Russell et al. (1990).

Testosterone determination

Measurements of plasma testosterone were determined in duplicate by radioimmunoassay as previously reported (Sinha and Swerdloff, 1993). The minimal detection limit was 0.01 ng/mL. The intra and interassay coefficients of variations were ±4% and ±8%, respectively.

Sexual behaviour test

Control and study animals were housed in groups of four or five and maintained under a reversed light/dark cycle (12/12 h) at constant room temperature (approximately 22°C) with free access to water and commercial rodent pellets. In addition, ovariectomized female Wistar rats were used as stimuli. These females had previously been treated with subcutaneous injections of 25 μg of estradiol benzoate (EB) (Sigma, St. Louis, MO, USA) and 1 mg of progesterone (Aldrich, St. Louis, MO, USA) 48-52 h and 4 h respectively, before the sexual test to induce receptivity. All the experiments were conducted during the dark phase of the reversed light-dark cycle, each test lasted 50 min. A stimulated female was placed in the mating cage 2 min before the male was introduced and allowed to sexually interact. During each session the following behaviours were recorded: number of mounts, intromissions and ejaculation; mounts, intromissions and ejaculation latencies and the post-ejaculatory inter-intromission and inter-copulatory intervals, as previously reported (Portillo et al., 2012).

Enzymatic activity assay

Liver and kidney samples were homogenized in 50 mM phosphate buffer, pH 7, containing 0.5 mM EDTA and 0.5% Triton, using a dismembrator (Kinematica, Switzerland) and centrifuged at 8,000 g for 15 min at 4°C. Cytosolic fractions were stored at -70°C until analysis. The following enzyme activities were determined in these samples: glutathione S-transferase (GST), CAT, and glutathione peroxidase (GPx). GST activity was measured in cytosolic fractions in the presence of 0.1% (w/v) BSA using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate, according to the method of Habig et al. (1974). GST activity was calculated using the extinction coefficient of 9.6 mM-1cm-1, and expressed as nM of CDNB–GSH conjugate formed per min per mg of protein. Protein concentration in the cytosolic fractions was determined by the bicinchoninic acid (BCA) protein assay (Pierce Inc., Rockford, IL), using bovine serum albumin (BSA) as standard. CAT activity was assayed by the method of Aebi (1974), which is based on the disappearance of H_2O_2 at 240 nm, 25°C for 30 seconds. CAT activity was expressed as μmol H_2O_2 consumed per min per mg of protein. GPx activity was measured by the method of Paglia and Valentine (1967). Briefly, this method is based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPx, which is then coupled to the recycling of GSSG back to GSH utilizing glutathione reductase and reduced ,-nicotinamide adenine dinucleotide phosphate (NADPH). The rate of NADPH consumption was monitored at 340 nm, 25°C for 2 min. GPx activity was calculated using the extinction coefficient of 6.22 mM^{-1} cm^{-1}, and expressed as nM NADPH consumed per min per mg protein. Reduced GSH was determined by the Ellman's method based on the ability of SH group to reduce 5, 5'dithiobis-(2-nitrobenzoic acid) (Ellman, 1959). GSH concentrations of the samples were derived from the standard curve prepared using known amounts of GSH. GSH levels were expressed relative to protein content as μM GSH per mg protein.

Antioxidant capacity

The Trolox Equivalent Antioxidant Capacity (TEAC) assay is based on the ability of a compound to scavenge the stable 2,2'-azinobis-(3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS) radical (ABTS•) converting it into a colorless product (Arts et al., 2004). The degree of decolorization induced by the compound is related to that induced by Trolox. The reduction in absorbance 6 min after the addition of the thiamine pyrophosphate was determined at 734 nm. The extinction coefficient of ABTS• at 734 nm is $1.5x10^4$ M^{-1} cm^{-1}. The TEAC of the thiamine pyrophosphate was calculated by relating this decrease in absorbance to that of a Trolox solution on a molar basis.

Image acquisition and statistical analysis

Samples were analyzed by using an Olympus BX51

fluorescence microscope (Olympus America Inc., Two Corporate Center Drive, Melville, NY, USA) with an attached digital camera (Evolution MP Color, Media Cybernetics, Melville, NY, USA). All images were digitally acquired using Image-Pro 6.2 Software (Optronics, Goleta, CA, USA). Statistical analyses were performed by using SigmaStat 3.1 (Systat Software, Inc., Point Richmond, CA, USA). The results are expressed by descriptive statistics mean and standard deviation. Comparisons of the results of the groups were performed using t test. Sexual behavior parameters were evaluated by using a nonparametric statistics Mann-Whitney U test for two independent groups. Differences were considered statistically significant at a $p<0.05$.

Results

Body and testicular weight

As shown in Fig. 1A, no significant differences were found regarding body weight between the control group (380±36 g; n=10), and the TPP-treated group (398±43 g; n=10). Testicular weight was significantly reduced ($p<0.05$) in the control (0.5±0.16 g) compared with the TPP-treated group (1.55±0.19 g). Testicular weight in TPP-treated animals was similar to 6 months-old control group (1.59±0.10 g), without significant differences between these two groups (Fig. 1B).

Histology: testicular morphology

Control animals showed a homogeneous decrease in the germ layers number, as well as in the number of spermatogonia in maturation (Fig. 2A,B). Similarly, in these samples there was also a reduced number in the observed spermatozoa in the seminiferous tubules and often arrest of spermatogenesis. There were areas of inflammation at the periphery of the seminiferous tubules and interstitial orchitis. Samples belonging to the study group showed seminiferous tubules and interstitium which appeared normal (Fig. 2C,D). Furthermore, we found processes in these samples related to normal spermatogenesis, with countless cells showing abundant spermatozoa within the tubule lumen. Leydig cells showed normal cytological features and there were no reported cases of fibrosis, inflammation, or testicular atrophy in the TPP-treated group.

Quantitative aspects

In agreement with the previous results, we observed a decline in the number of germ layers, spermatogonial cells and spermatozoa inside the tubules in the control group (Fig. 3A,B). In addition, we found a significant increase (23.5%) in the diameter of the seminiferous tubules in the TPP-treated group compared with the control group (Fig. 3C,D).

Subsequently, the number of Sertoli and Leydig cells for each group was determined. The results showed a significant difference in both cell populations for the control group and the TPP-treated group, which showed an increase in the number of these cell populations. In the study group, Sertoli cells showed a 30% increase (Fig. 3E) and Leydig cells presented a 39% increase (Fig. 3F) when compared to the control group. In addition, there was a significant difference between the germ layer number in control group (1.5 layers) versus the study group (4 layers, data not shown).

In addition, we determined the number of spermatogonial cells present in the seminiferous tubules for the two groups (Fig. 3G), and we found an average of 489 spermatogonial cells for the entire control group, compared to 1,078 spermatogonial cells for the entire TPP-treated group; a 54.6% significant increase.

Serum testosterone

Testosterone serum levels also showed significant differences ($p<0.05$). The control group showed testosterone levels of 0.5 ng/mL, significantly lower than those found in the TPP-treated group with 1.2 ng/mL, in addition these levels were similar to those found in the

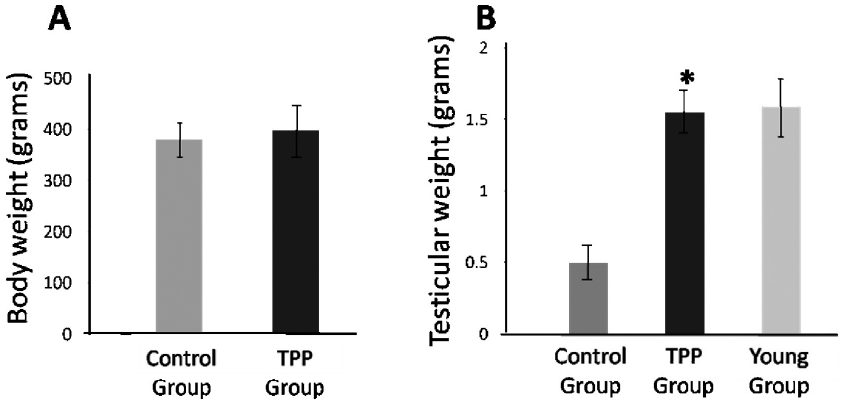


Fig. 1. Effect of chronic TPP administration on body and testicular weight. **A.** Body weight. **B.** Testicular weight. The asterisk shows a significant p value (<0.05).

young control group 1.3 ng/mL (Fig. 3H).

Sexual behavior

Since testis is the main source of testosterone production in males, subsequent trials were designed to determine testicular function by testing sexual behavior. When the two study groups were stimulated with receptive females, the results showed marked functional differences. The study group significantly increased the number of mounts, intromissions and especially the number of ejaculations during the tests. The study group showed better sexual conduct in all aspects evaluated. In addition, the mount, intromission and ejaculations latencies were significantly lower in this group. The inter-intromission and inter-copulatory intervals were also lower in the study group (Table 1). The increased performance in sexual behavior found in the study group is related to the histological findings in testicular tissue and serum testosterone levels.

Antioxidant enzymes

The determination of GSH levels showed a significant decrease in liver tissue for the study group (529.1 nmol/mg prot.), in relation to the liver controls (643.6 nmol/mg prot., Fig. 4A) ($p<0.001$). Moreover, there were significantly higher GSH levels in renal tissue for the study group (1137.6 nmol/mg prot., $p<0.001$) compared to controls (539.0 nmol/mg prot., Fig. 4A). Next, we determined the activity levels of GPx, and found a significantly higher activity in liver tissue for the study group (1235.3 nmol/min/mg prot., $p<0.001$) when compared to control group (1138.4 nmol/min/mg prot.). GPx activity in renal tissue showed no significant difference between the groups (604.0 and 630.7 nmol/min/mg prot., Fig. 4B). Subsequently, the GST activity was determined, and we found similar results

Table 1. Effect of TPP treatment on sexual behavior. Data are expressed as means±SEM.

Behavior	Control group Means±SEM	TPP group Means±SEM
Number		
Mounts	7.4±3 (6)	11,5±2 (8)
Intromissions	5.5±2 (6)	13±2 (8)**
Ejaculations	0.38±0.18 (3)	1.75±0.-37 (7)**
Percentages		
Moutns	75 %	100 %
Intromissions	75%	100%
Ejaculations	37.5%	87.5%
Latencies (seconds)		
Mounts	1084±261 (6)	199±53(8)**
Intromissions	1318±281 (6)	532±181 (8)**
Ejaculations	1224.3±1654 (3)	727.3±144 (6)*
Post ejaculatory interval (seconds)	ND (0)	270.25
Inter-intromission interval (second)	167±8,45	63.35±14.64
Inter-copulatory interval (seconds)	61,92±9,62	34-6±2

Asterisks indicate statistically significant differences compared to controls. *: $p<0.05$; **: $p<0.001$. Where: ND, not detected.

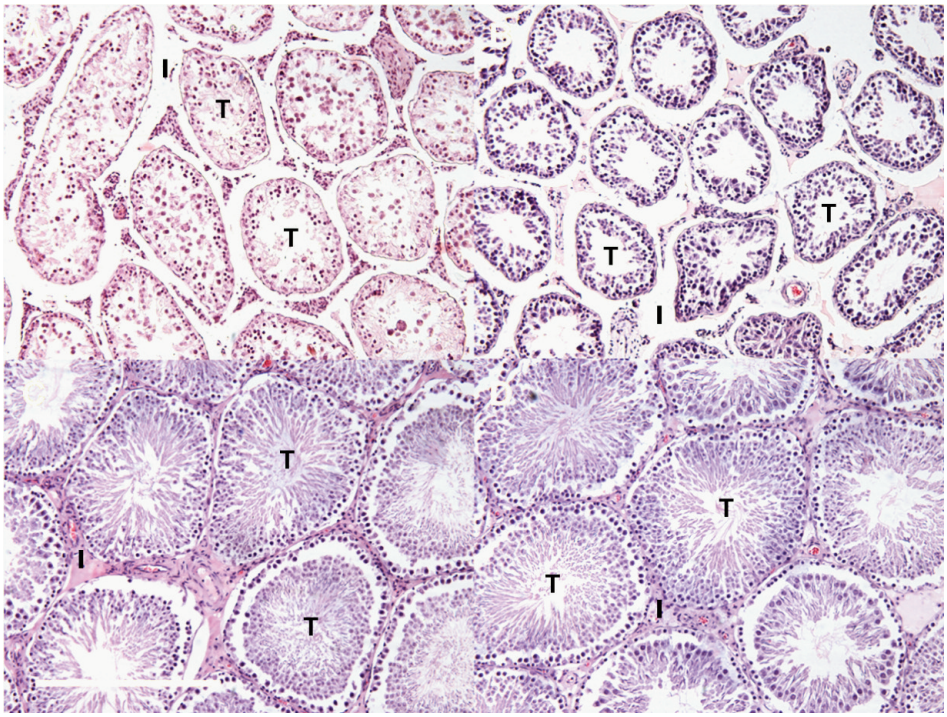
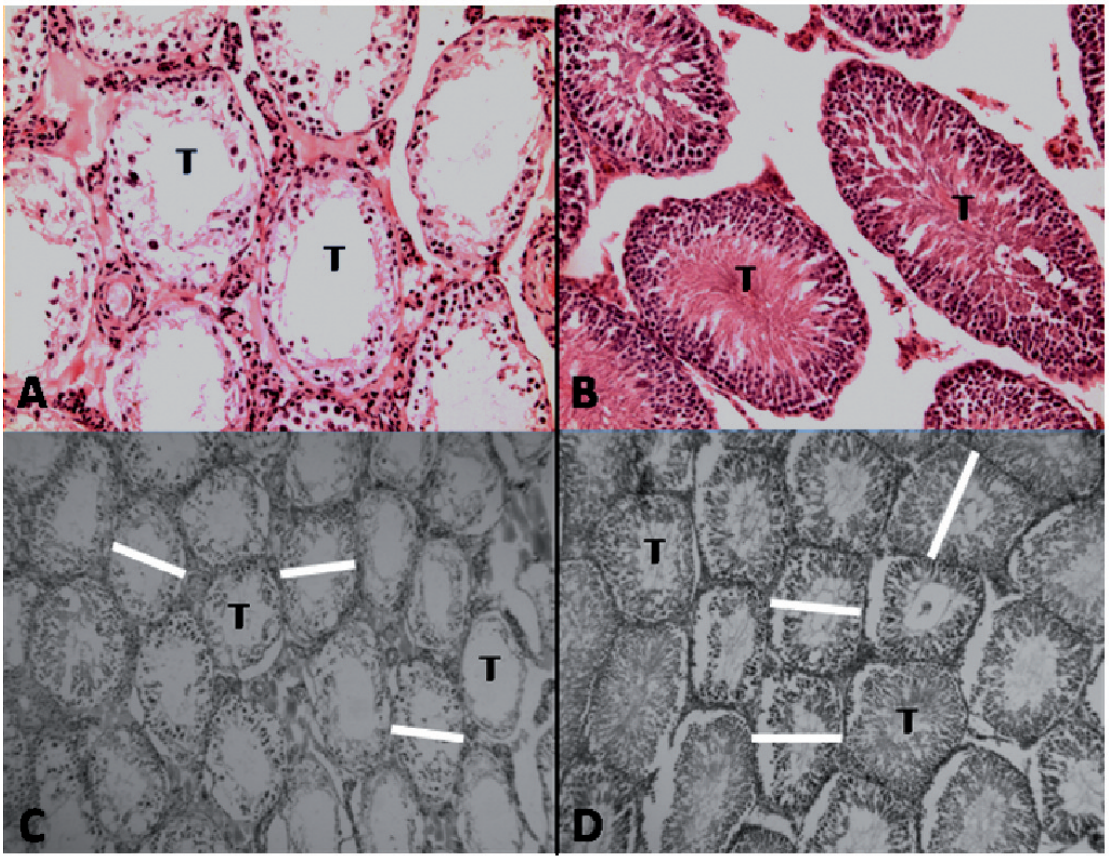
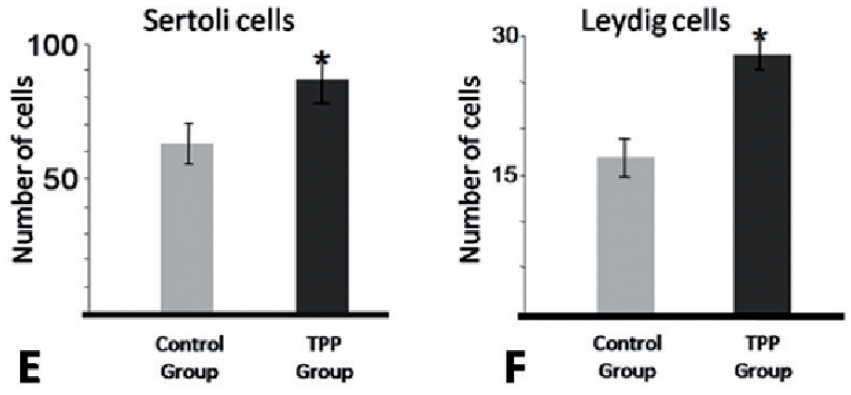


Fig. 2. Testicular histopathological changes observed in control and study groups. **A and B.** Control animals. **C and D.** Study group. T, tubule; I, insterstitium.





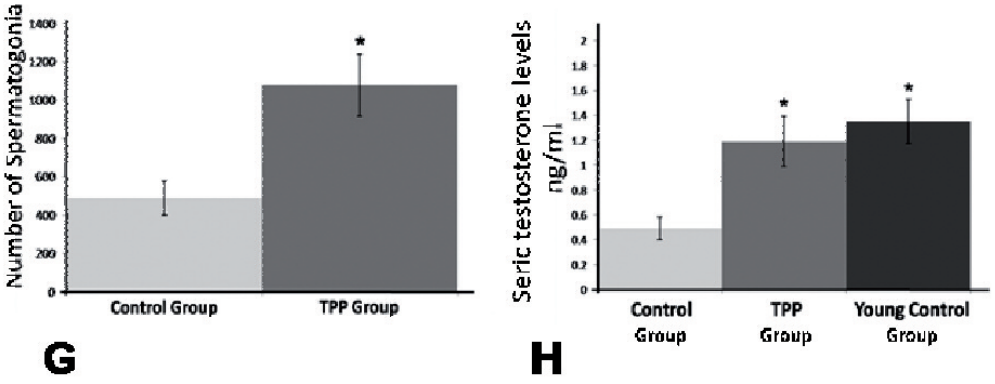


Fig. 3. Testicular histological changes and testosterone levels after TPP administration. **A.** Control testicular tissue. **B.** Study group. **C and D.** Tubule diameter for control and study group, respectively. **E.** Sertoli cell number. **F.** Leydig cell number for control and study groups. **G.** Spermatogonia cell number. **H.** Testosterone circulating levels for control, study and young control groups. T, tubule. White bars in panel C and D indicate the diameter of the tubules. The asterisk shows a significant p value (<0.05) compared to the control group.

compared to GPx, with a significant elevation in liver tissue for the study group (2506.0 nmol/min/mg prot., $p<0.003$) compared to the control group (2302.4 nmol/min/mg prot.). In kidney, GST activity was significantly lower for the study group (381.2 nmol/min/mg prot.) compared to the control group (436.5 nmol/min/mg prot., Fig. 4C, $p<0.001$). Finally, we determined the CAT activity and we found a significant increase for both tissues in the study group (1044.6 nmol/min/mg prot. and 756.0 nmol/min/mg prot. for liver and kidney, respectively, $p<0.001$) compared to the control group (730.1 nmol/min/mg prot. and 525.0 nmol/min/mg prot. for liver and kidney, respectively, Fig. 4D).

Antioxidant capacity test

This assay allows the determination of the TPP antioxidant capacity by using the ABTS compound. The results show that TPP has an antioxidant capacity of 23% compared on an equimolar basis to Trolox, a derivative of vitamin E, which has proven a powerful antioxidant activity.

Discussion

Aging is a physiological process exhibited by all organisms; however, there is a great variability in the intensity of the changes in individuals of the same species. There are several theories about aging and a diversity of pathological mechanisms involved; however, the generation of ROS is one of the most widely accepted theories (Gilca et al., 2007; Muller et al., 2007).

The pathology of aging progression is often based on mitochondrial alterations associated with chronic degenerative diseases, called mitochondrial diseases, sharing an initial mitochondrial damage which results in ATP depletion and pathologically increased ROS production (Harman, 1981).

There is also evidence that aging causes a decrease in PDHC and KGDHC and an increase in JNK activity, which inactivates TPP dependent enzyme complexes, leading to increased oxidative stress, decreased antioxidant capacity and energy production with glutathione depletion and mitochondrial dysfunction (O'Keeffe, 2000; Zhou et al., 2009).

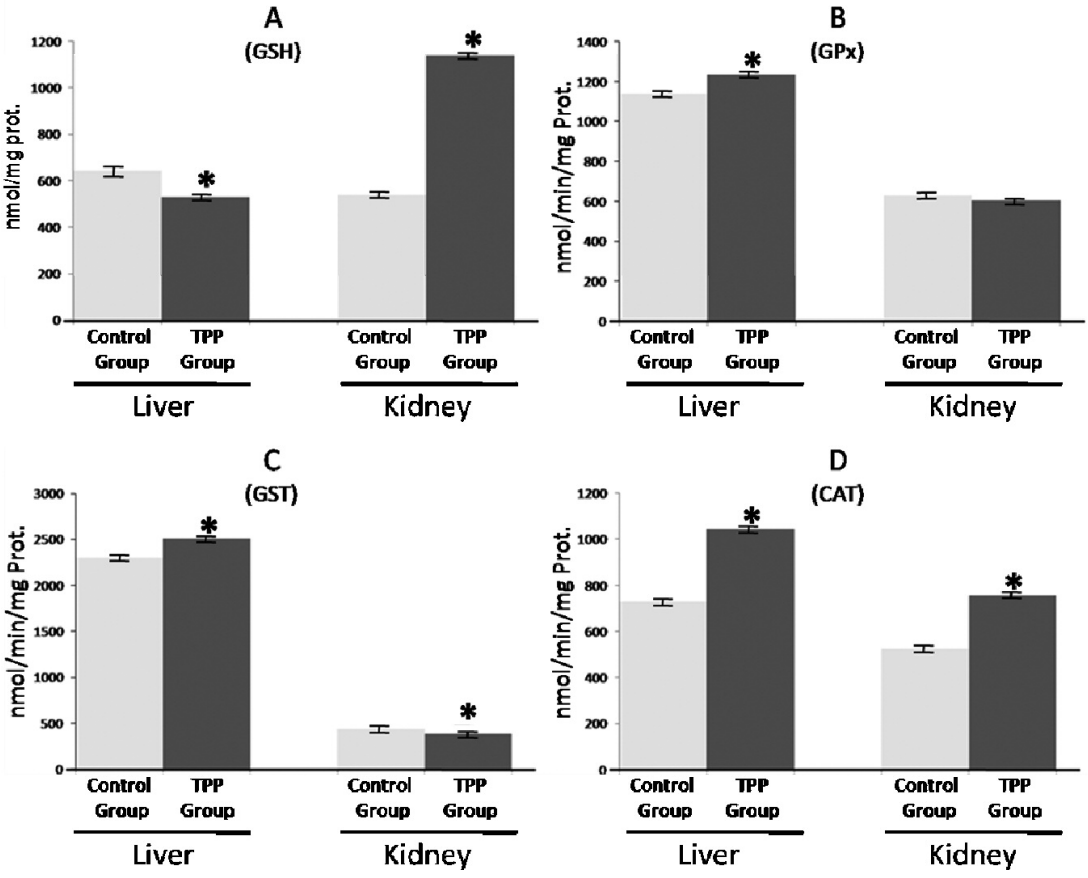


Fig. 4. Antioxidant enzyme levels in liver and kidney of TPP-treated group. GSH levels and GPx, GST and CAT activities are shown in hepatic and renal samples from control and study groups. The asterisk shows a significant p value (<0.05) compared to the control group.

In the testis, the results of TPP administration showed tissue preservation, with characteristics similar to those found in young individuals with a larger diameter in the seminiferous tubules due to preservation of the number of spermatogonia and spermatozoa. We highlight here the absence of neoplastic formations and inflammation in testicular tissue from the study group, as well as the presence of these lesions in the control group, which are frequent findings closely related to ROS production in this species, linking the activity of ROS and inflammation with the generation of various types of cancer (Federico et al., 2007; Perwez and Harris, 2007). The results could also be due to their participation in the system of SOD, CAT and glutathione system, to maintain a constant source of NADPH for the reduction of oxidized glutathione, allowing the proper disposal of toxic peroxide radicals (Hazell and Butterworth, 2009).

Sexual behavior

Histological changes associated with TPP chronic treatment allowed us to expect a positive impact on the sexual behavior for the treated males. Indeed, the study group showed a significant increase in the number of mounts, intromissions and ejaculations; additionally inter-intromission and inter-copulatory time intervals were also significantly decreased. There was not a post-ejaculatory interval in the control group, while several individuals exhibited it in the study group. Although atrophic changes associated with aging were described elsewhere (Suzuki and Withers, 1978; Ryu et al., 2006), preservation of cellular components and a preserved sexual functionality are associated with TPP treatment in this study.

Intermediary metabolism and antioxidant systems

The glutathione system represents one of the most important endogenous antioxidant systems, and we observed an increase in this system when TPP was administered. Intracellular GSH levels are used as a sensitive indicator of cell health and their ability to resist toxic assaults, elevated cell levels of the oxidized form of GSH (GSSG) could indicate pathological changes (Nair, 1991). The formation of ROS is a physiological process required to maintain general homeostasis, although when a certain threshold is exceeded cell damage begins by mitochondrial injury. The mitochondrial ratio GSSG/GSH increases with age, and antioxidant treatments protect against oxidation of the glutathione reserve (Garcia de la Asuncion et al., 1996). In this regard, GPx provides an important detoxification mechanism for intracellular peroxides, mainly lipid peroxides, which are also conjugated with GSH, thus having a crucial role in cellular protection against damage caused by ROS formed by peroxide decomposition. Furthermore, CAT provides an efficient detoxification mechanism for intracellular H_2O_2. Therefore, both enzymes prevent H_2O_2-mediated damage, a prerequisite for inflammation and a known risk factor for carcinogenesis (Mates et al., 1999). NF-E2-related factor-2 (Nrf2), a member of the cap 'n' collar-basic leucine zipper proteins (CNC-bZIP), is a short-lived transcription factor that can trans-activate the antioxidant response element (ARE) (Jaiswal, 2004). Therefore, the coordinated induction of the enzymatic activity of GST, GPx, SOD and CAT, as well as the GSH levels in liver and kidney of TPP-treated rats could be mediated through the Nrf2-ARE pathway.

A potential drawback in this study is that antioxidant enzyme levels were not measured directly on testis. However, although most of the tissues of the human body and animals are well provided with various antioxidant systems, tissue distribution of enzymatic and non-enzymatic antioxidants is usually lower in extrahepatic tissues, such as testis (Afolabi et al., 2012; Nishimura and Naito, 2006). Nevertheless, an induction of CAT and GPx, as well as an increase in GSH levels in rat testes after treatment with several antioxidant compounds has been observed (El-Demerdash et al., 2009; Afolabi et al., 2012).

Several studies have reported alterations that promote ROS on the antioxidant enzymes activity and intermediary metabolism. The overproduction of superoxide radicals has an inhibitory effect on the enzymes responsible for removing ROS, such as CAT and GPx (Pramod et al., 2006) and to inhibit KDHC and PDHC (Gibson and Zhang, 2002). The inhibition of CAT by superoxide radicals causes an increase in H_2O_2, inhibiting the activity of SOD and therefore causing an increase of superoxide radicals (Hassan and Fridovich, 1978). In addition, several changes of the glutathione system have been described in chronic degenerative diseases such as DM2 and several neurodegenerative diseases (Sian et al., 1994; Zeevalk et al., 2008) so that the use of TPP therapy would allow the recovery of CAT and the glutathione system. Indeed, we observed an increase in CAT, GSH and GST in response to TPP treatment.

TPP and neurodegeneration

TPP has been shown to decrease the rate of oxygen consumption and lactate production, as well as being a potent inhibitor of lipid peroxidation, lipotoxicity and non-enzymatic glycosylation of proteins (Bautista-Hernandez et al., 2008; Tolstykh and Khmelevskii, 1991). In addition, various chronic diseases course with normal serum levels of thiamin but lower levels of TPP dependent enzymes (Heroux et al., 1996). This suggests that phosphorylation mechanisms of thiamine in damaged organisms are deficient. Thus, neuro-degenerative diseases frequently include abnormalities in glucose metabolism (Ibanez et al., 1998), with low levels of PDHC, KGDHC, CAT and glutathione system (Mizuno et al., 1994; Heroux et al., 1996) and supplementation therapy decreases and restores the damage (Shen et al., 2000). Moreover, the processes of

insulin resistance and/or the central hypoinsulinemia with inflammation, overproduction of ROS and lipotoxicity, among others (Owen et al. 1996; Kidd, 2000; Orth and Schapira, 2001; Doherty, 2011; Schuh et al., 2011) have been implicated in various neurodegenerative disorders, and remain the centerpiece in the physiopathological development of most pathognomonic lesions found in Alzheimer's and Parkinson's diseases, even in asymptomatic patients (Cholerton and Craft, 2011; de la Monte, 2012; Umegaki, 2012). The use of TPP could be of relevance in these conditions, because the evidence found in this study in addition to previously published reports, positions the TPP as a potential candidate in the development of new therapies.

The key findings of this study can be circumscribed to an improvement of metabolic-oxidative state induced by chronic TPP-treatment, which reduces the generation of ROS through induction of endogenous antioxidant enzymes, and possibly a better aerobic metabolic performance. To our knowledge, this is the first study simultaneously characterizing TPP effect at a histological and functional level. Nevertheless, it is important to further clarify the role and mechanisms involved in TPP function.

Conclusions

Testicular histological changes associated with aging were less intense in the TPP-treated study group, due to the improvement of the intermediate metabolism and levels of the endogenous antioxidant enzymes. However, more studies are necessary regarding the effects and metabolic pathways used by TPP and its potential role as an adjuvant therapy in chronic conditions with inflammation, oxidative stress and depletion of endogenous antioxidant systems, such as neurodegenerative diseases.

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