Effects of selenium supplementation on oxidative stress in the brain

Efeitos da suplementação de selênio estresse oxidativo no cérebro

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ABSTRACT
Selenium is known to produce great effect on the oxidative stress of cells, both systemic and cerebral. Brain degeneration occurs as a result of several factors, one of them is the oxidative stress. We aim To analyze selenium effect on rats' brains, investigating serum and immunohistochemical oxidative stress markers, and the neural structural pattern. Selenium supplementation with 48 μg chelated selenium and with 96 μg chelated selenium for 60 days, followed by an evaluation of oxidative stress markers, as well as immunohistochemical markers. Lower TBARS was observed in the rats subjected to 44mcg Se supplementation when compared to the other groups. Among the other oxidative stress markers, and immunohistochemical markers, little variation was observed. Improvement was observed in TBARS levels. However, the other measurements showed little statistical relevance. This raises some questioning regarding the dose used or the formulation and its bioavailability as influential factors in the oxidative response.

Keywords: brain alteration neurology oxidative stress, selenium, selenium supplementation.

RESUMO
O selênio é conhecido por produzir grande efeito sobre o estresse oxidativo das células, tanto sistêmicas quanto cerebrais. A degeneração cerebral ocorre como resultado de vários fatores, um deles é o estresse oxidativo. Nosso objetivo é analisar o efeito do selênio no cérebro de ratos, investigando marcadores de estresse oxidativo sérico e imuno-histoquímico e o padrão estrutural neural. Suplementação de selênio com 48 μg de selênio quelatado e 96 μg de selênio quelatado durante 60 dias, seguida de uma avaliação dos marcadores de stress oxidativo, bem como dos marcadores imuno-histoquímicos. Foi observada uma TBARS inferior nos ratos submetidos à suplementação com 44mcg Se em comparação com os outros grupos. Entre os demais marcadores de estresse oxidativo e os marcadores imuno-histoquímicos, pouca variação foi observada. Foram observadas melhorias nos níveis de TBARS. Mas as outras medidas mostraram pouca relevância estatística. Isto levanta alguns questionamentos em relação à dose utilizada para a formulação e sua biodisponibilidade como fatores influentes na resposta oxidativa.

Palavras-chave: neurologia da alteração cerebral estresse oxidativo, selênio, suplementação de selênio.

1 INTRODUCTION

Selenium was considered a toxic element to humans for many years. However, it started to draw interest after being found in a detoxifying enzyme in 1973, and from then onwards a series of favorable research has been developed investigating its use in the human body. Such studies have also determined its importance in the prevalence of cardiovascular problems in immunological functions (Chen and Berry, 2003)

Currently, selenium is known to act as antioxidant, antimutagenic, anticarcinogenic, and anti-inflammatory agent. It also presents immunological function, helps in reproduction, as well as the gastrointestinal, respiratory, endocrinological, and neuropsychiatry systems. (Valentine *et al.*, 2008) (Santos *et al.*, 2022) (Ferreira, Neto and Nalesso, 2021)

In the nervous system, it acts as a protective factor against oxidative stress modulating neurotoxicity. Reduction in selenium and in the 25 selenium-binding proteins causes neurological deficiency, which might be corrected with the supplementation of this element (Valentine *et al.*, 2008). The glutathione peroxidase enzymes help to protect cortical neurons against exogenous oxidative stress. (Conrad, 2009; Schomburg and Schweizer, 2009; Avery and Hoffmann, 2018)

Results have been reported in which the deficiency of this element, in Parkinson's disease, cognitive decline, epilepsy seizures, Alzheimer's disease (Pillai, Uyehara-Lock and Bellinger, 2014), mood disorders,(Whanger, 2016), and cerebral ischemia (Ansari *et al.*, 2004), tends to worsen the patterns of these disorders.

Selenium dosage, its formulation, and its bioavailability are not completely established, which generates great variability in the doses used in different studies.(Nève, 1995; Burk *et al.*, 2006)

This study aimed to analyze oxidative stress and histomorphological changes in the presence and absence of selenium supplementation, in the brain degeneration process.

2 MATERIAL AND METHODS

The sample calculation was carried out using the GPower 3.1.9.4(Faul *et al.*, 2007). The sample power used was 80% with a 10% error. Forty-eight Wistar rats (*Rattus novergicus)* obtained from the UEPG central vivarium were included in the study.

The rats were divided into 3 groups according to the selenium supplementation, so that each group contained 12 rats, namely:

- G0 (control group): received 0.5 mL physiological serum per gavage;
- G1 (group 1): received 0.2 mL chelated selenium (48 μg) dissolved in 0.3 mL physiological serum per gavage;
- G2 (group 2): received 0.4 mL chelated selenium (96 μg) dissolved in 0.1 mL physiological serum per gavage.

The experiment lasted six weeks. Between he second and sixth weeks, the gavage was carried out.

At the end of the 6th week, the rats were euthanized. The procedure consisted in emerging the animals in a recipient containing1mL/1mL isoflurane inhaling anesthetics and, after anesthesia, each rat was subjected to thoracic abdominal transection using a scalpel. Next, cardiac puncture was performed to for blood collection and biochemical analysis. In addition, the animals were beheaded for brain extraction.

The frontal lobe was sent to immunohistochemical analysis, while the remaining material was preserved for histological and oxidative stress analyses. The samples destined to the histological analysis were stored in formalin at room temperature, while those destined to the biochemical analysis were kept in 0.9% physiological serum and stored in a laboratory freezer at -80ºC.

To determine the concentration of non-protein thiol (NPSH) and quantification of non-protein sulfhydryl groups (SH-NP), the method described by Ellman in 1955 was carried out in the rats' tissues. A calibration curve was performed for the final result determination (Ellman, 1959). The determination of lipid peroxidation levels (TBARS) was carried out following the method described by Schumacher et. Al 2011(Schumacher *et al.*, 2011). Spectrophotometry was employed to determine catalase. activity (Aebi, 1984), the catalase activity calculation was carried out according to (Aebi, 1984; Neto *et al.*, 2008)

The peroxide determination concentration was carried out using four different methods, and the results were expressed in mM H_2O_2/g tissue/min (MINOZZO, no date) and in mM H_2O_2/ml S1/min (RAVSKI, Leandro Carlos and Vellosa, no date). The H2O2 degradation speed was measured using the method proposed by Neto, et al 2008 (Neto *et al.*, 2008)

The encephala were deposited on positively charged slides and then stained with hematoxylin and eosin, and immunohistochemical dye. In the immunomarking, they were incubated for 12 hours at 4ºC with the primary antibodies anti-NF Kappa Beta, Caspase-3, COX-2, CD68, and BCL-2. Next, they were incubated with specific secondary antibodies: rabbit biotinylated secondary antibody against goat IgG (Vector Laboratories, BA-500, diluted at 1:200), except for the binding protein that was incubated with streptavidin conjugated with peroxidase. The primary antibody binding was revealed using DAB.

Two hundred and forty-five slides prepared from the material of 25 brains were analyzed since some material was lost. The electronic microscopy technique was employed.

2.1 STATISTICAL ANALYSIS

Initially, a descriptive analysis of the variables was carried out estimating mean, median, standard deviation, and interquartile interval of the variables in general. Next, the Shapiro-Wilk

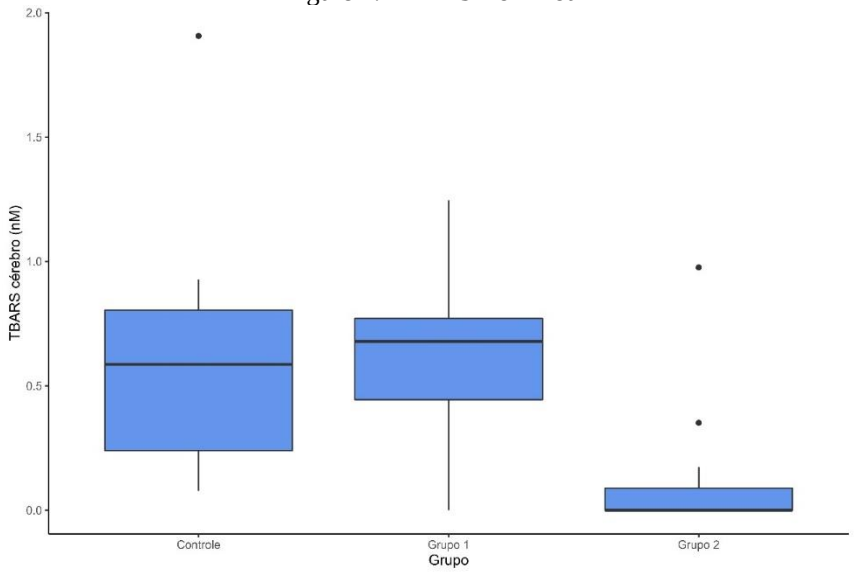
test was applied to verify normal distribution, and we opted for the non-parametric data approach when $p < 0.05$, and the parametric approach when $p > 0.05$. The differences between groups were verified using the ANOVA or Kruskall-Wallys tests, followed by the Tukey or Dunn tests as multiple comparison. To improve the result visualization, we produced boxplot graphs differentiating the groups. The tests were considered significant when $p < 0.05$ and the analyses were carried out in R environment (R Core Team, 2019).

3 RESULTS

This study comprised 48 rats, out of which 36 were female and 12 male animals that were three months old and whose average weight was 232.7 g (+- 17.4) (IC 95%; p=0.09).

In the THIOL average, groups 1 and 2 showed little variation one from another. However, when compared to the control group, the latter showed higher mean than the intervention groups. Regarding TBARS, group 1 showed a 0.64 mean, while group 2 presented a 0.14 mean, and the control group obtained a 0.61 mean (p between groups 0.003) (**figure** 1). As regards CATALASE, the variation between groups was small, and the lowest mean was that of the control group (**table 1)**. The TBARS multivariate analysis was statistically significant in the group 1 x group 2 comparison (p0.005) and in the control X group 2 comparison (p0.010).

Figure 1. TBARS Box Plot



Source: The author

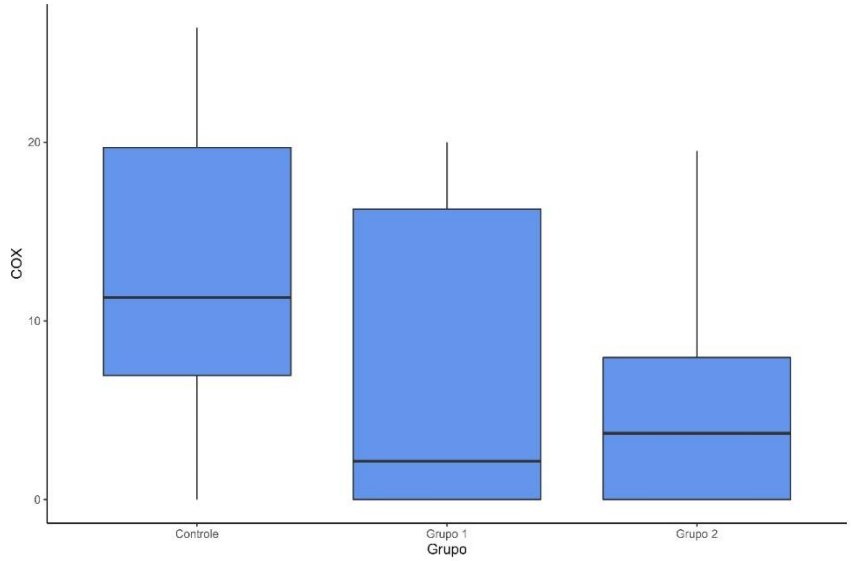
Table 1. Encephalon oxidative stress markers

Marker	Group	M	MD	1Q	3Q	SD	p-value between groups
THIOL	Control	47.83	46.22	27.99	61.43	24.68	0.919
	Group 1	44.36	45.71	28.34	59.86	21.16	
	Group 2	44.37	43.8	29.26	62.03	24.74	
TBARS	Control	0.61	0.59	0.20	0.84	0.51	0.003
	Group 1	0.64	0.68	0.44	0.77	0.32	
	Group 2	0.14	0.00	0.00	0.17	0.30	
CATALASE	Control	0.06	0.04	0.03	0.09	0.04	0.147
	Group 1	0.08	0.09	0.07	0.10	0.02	
	Group 2	0.07	0.06	0.04	0.09	0.04	

M=mean; MD=median; SP=standard deviation; IQI= interquartile interval; *ANOVA in the parametric approach; **Kruskall-Wallys test (equivalente to ANOVA in the non-parametric approach).
Source: The authors.

In the brain immunohistochemical analysis, we observed a reduction in the COX in groups 1 (mean = 7.2) and group 2 (mean = 4.8) when compared to the control group (mean = 11.9) (**figure 2).** In CD68, the control group, group 1 and group 2 means were 18.7; 21.8; and 23.3 respectively. Other results are presented below (**table 2**).

Figure 2. Boxplot graph of the COX variable according to the groups



Source: the authors.

Table 2. immunohistochemical analysis

Marker	Group	M	MD	SD	IQI	p- value between groups
BCL-2	Control	5.0	0.0	8.7	5.4	0.693**
	Treated 1	7.4	2.3	10.0	10.4	
	Treated 2	5.7	4.7	5.9	8.1	
HE	Control	19.9	22.4	8.2	9.0	0.634*
	Treated 1	17.1	15.8	7.7	8.3	
	Treated 2	19.3	19.4	6.6	5.0	
CASP	Control	0.9	0.0	3.0	0.0	0.287**
	Treated 1	0.0	0.0	0.0	0.0	

Marker	Group	M	MD	SD	IQI	p- value between groups
	Treated 2	2.6	0.0	5.7	0.0	
	Control	18.7	17.0	7.8	6.6	
CD68	Treated 1	21.8	21.7	12.2	19.0	0.609*
	Treated 2	23.3	23.4	12.8	18.3	
	Control	11.9	11.3	8.3	12.8	
COX	Treated 1	7.2	2.2	8.6	16.3	0.111**
	Treated 2	4.8	3.7	5.9	8.0	
	Control	4.3	0.0	7.2	5.7	
NFKB	Treated 1	9.8	9.8	9.1	13.8	0.240**
	Treated 2	7.9	9.8	6.9	11.6	
	Control	22.0	20.5	6.8	5.8	
GFAP	Treated 1	19.8	20.0	12.1	13.0	0.692*
	Treated 2	23.3	20.6	10.5	12.2	

M=mean; MD=median; SP=standard deviation; IQI= interquartile interval; *ANOVA in the parametric approach; **Kruskall-Wallys test (equivalente to ANOVA in the non-parametric approach).

Source: The authors.

4 DISCUSSION

This study showed that selenium supplementation reduced the levels of oxidative stress markers, THIOL, and TBARS. As for the catalase, no difference was observed between groups. When exposing the cells to hypoxia for 48 hours, selenium inhibited ROS formation and increased the concentration of the Glutathione peroxidase enzyme (Sarada *et al.*, 2008). After exposing them to patulin, selenium controlled the increase in TBARS, protein carbonyl, and the oxygen reactive species. However, this result was not observed for THIOL (Song *et al.*, 2014). In the exposure to high doses of prednisone, selenium prevented TBARS increase, and resulted in a recruitment of antioxidant enzymes such as catalase and glutathione for the brain (Beytut *et al.*, 2018). In his review, Schweizer et.al , concluded that selenium proteins acted as a neuron protector agent, promoting antioxidant action and protecting the brain (Schweizer *et al.*, 2004). Therefore, selenium was seen to have a protecting effect against oxidative stress and increases the production of antioxidant enzymes.

According to our results, selenium supplementation resulted in lower COX and CD68 concentration, after the stressing factor. Regarding the other markers evaluated, no difference was observed after supplementation. Selenium deficiency provokes increased levels of RNAm of inflammatory citokines in the brain (prostaglandin E synthase, cyclooxygenase-2 (COX-2), nuclear factor-kappa B (NF-κB), and inducible nitric oxide synthase)(Zhang *et al.*, 2020). Selenium supplementation, in hepatotoxicity, leads to the reduction in pro-inflammatory citokines and Cd68, in addition to controlling the increase in transaminases, indicating a control of the inflammatory process in the presence of Se (Hamid *et al.*, 2017) . In hypoxy, selenium supplementation caused reduction in cytochrome C and increased BCL2, indicating apoptosis

reduction. (Sarada *et al.*, 2008) Thus, selenium plays a role in the apoptosis and inflammation control in several events.

In his review, Nève, 1995 found out that the selenium dosage in the studies ranged from 10 to 100 ug Se/ day. He also reported variations in the chemical formulation, varying from inorganic and simple organic to more complex forms such as the Se-enriched yeast or Se-rich food. The literature also reports that variations in time, dosage, and formulation presented different results regarding the glutathione peroxidase activity. (Nève, 1995). In this study, different Se doses also caused variations in the results.

Selenium has been considered an important element in the oxidative stress control, and has been more and more investigated by countless studies and findings related to its role in the inflammatory process physiology and in the oxidative stress control. In the central nervous system, there is growing evidence of several stressing factors being controlled by its supplementation. The main issues to be still investigated are related to the dosage, formulation, and bioavailability of selenium supplementation as influence factors in the oxidative response, which still requires further investigations.

5 CONCLUSION

Selenium contributes to a reduction in the oxidative stress markers and those linked to the central nervous system inflammation. Further studies are still required to elucidate selenium bioavailability and dosage needed to obtain a therapeutical effect on the CNS.

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