

Chronic pantoprazole exposure induces behavioral deficits and region-specific molecular changes in the rat motor cortex and cerebellum

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Proton pump inhibitors are widely used, but their long-term effects on the central nervous system are not well understood. In this study, we investigated whether chronic pantoprazole use alters sensorimotor function, oxidative stress, inflammatory response, and apoptosis in the motor cortex and cerebellum. Twenty-one female Wistar rats were randomized to control (C), gavage control (GC), or pantoprazole (P; 20 mg/kg/day for 12 weeks) groups. Sensorimotor coordination (rotarod), water-maze swim velocity, and open-field locomotion were assessed as behavioral parameters. The cortical and cerebellar tissues were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA) for apoptosis, inflammation, and oxidative stress. Pantoprazole impaired sensorimotor coordination compared to both control groups. However, its effects on swim velocity and locomotor activity were primarily significant when compared to the naive control group, suggesting that gavage-related stress may have contributed to these behavioral outcomes. Bcl-2-associated X protein (BAX) and Bcl-2 associated agonist of cell death (BAD) protein levels and the BAX/Bcl-2 ratio increased with pantoprazole, particularly in the motor cortex, indicating enhanced pro-apoptotic activity. While tumor necrosis factor levels did not change, interleukin (IL)-6 and IL-1 β levels were significantly higher in the cerebellum, suggesting neuroinflammatory activation associated with both pantoprazole and gavage-induced stress. Furthermore, oxidative stress analyses revealed elevated malondialdehyde and oxidative stress index levels, as well as increased total antioxidant status, in specific regions, suggesting an imbalance between oxidative and antioxidant responses. Chronic pantoprazole administration resulted in modest motor deficits and region-specific molecular alterations, including a pro-apoptotic shift in the motor cortex. In addition, an inflammatory/compensatory antioxidant response in the cerebellum was observed due to both gavage-induced stress and pantoprazole administration. These findings highlight the need for further studies on dose-response, reversibility, and synaptic consequences, and suggest the importance of considering the risks of prolonged proton pump inhibitors exposure.

Key words: pantoprazole, cerebellum, motor cortex, sensorimotor coordination

INTRODUCTION

Proton pump inhibitors (PPIs) are among the most widely used medications worldwide for acid-related disorders, and pantoprazole is a commonly prescribed agent in this class (Shanika et al., 2023). Earlier phar-

macological studies have characterized the potent and sustained inhibition of gastric acid secretion by PPIs through irreversible binding to the H⁺/K⁺-ATPase (Kromer et al., 1990; Simon et al., 1991). PPIs are generally effective and considered to be safe in the short term. However, their long-term use has been associated with

several adverse outcomes, such as an increased risk of pneumonia and other gastrointestinal infections, bone fractures, and cancers of the digestive tract. This prolonged use has also been linked to a reduced absorption of essential vitamins and minerals. There's growing concern that its long-term use could have effects beyond the stomach, possibly affecting the nervous system (Gray et al., 2018; Zhang et al., 2022).

The evidence on the cognitive effects of PPIs is controversial. While some population studies have linked long-term PPI use with an increased risk of dementia (Li et al., 2019; Ahn et al., 2023), other large-scale studies and meta-analyses have found no clear causal relationship (Khan et al., 2020; Khan et al., 2024). Pre-clinical and clinical studies have suggested potential associations between PPI exposure and neurobiological alterations, including changes in cognitive function (Akter et al., 2015). For instance, a previous study showed that pantoprazole could be used as a supportive therapeutic agent in epilepsy (Taskiran et al., 2021). On the contrary, Alaeddin and his colleagues observed a correlation between PPI use and an increase in mean diffusivity in certain brain regions associated with cognitive function. This indicates the possibility of adverse effects on brain integrity (Alaeddin et al., 2024). These conflicting results highlight the limitations of observational data and the need for studies that examine the underlying mechanisms in controlled settings.

It is well-known that oxidative stress, neuroinflammation, and apoptosis are linked biological factors that trigger many neurodegenerative processes (Perfeito et al., 2013; Bliski et al., 2025). Increased levels of reactive oxygen and nitrogen species have been shown to damage lipids, proteins, and DNA, weaken mitochondrial function, and impair synaptic signaling. The presence of such redox changes is an early sign of Alzheimer's, Parkinson's, amyotrophic lateral sclerosis, and other neurodegenerative diseases (Cobb & Cole, 2015). In addition, experimental toxicology studies have reported broader systemic effects of PPIs, including potential developmental and reproductive toxicity under certain conditions (Mansel et al., 2011). These redox changes activate microglia and astrocytes, activate nuclear factor kappa B (NF- κ B) and inflammatory pathways, and stimulate the release of cytokines, including interleukin (IL)-1 β , IL-6, and tumor necrosis factor (TNF- α). Subsequently, the release of these inflammatory mediators suppresses antioxidant defenses and dysregulates mitochondrial dynamics, intensifying oxidative damage (Sugama et al., 2009; Chen et al., 2020; Simpson & Oliver, 2020). Synaptic dysfunction has been observed through oxidative stress and neuroinflammation, resulting in the disruption of neurotransmission, weakening of long-term potentiation and depression, and

alteration of the number and shape of dendritic spines, ultimately diminishing the strength and coordination of neural circuits (Donzis et al., 2014; Mottahedin et al., 2017; Tönnies & Trushina 2017). Taken together, oxidative stress, neuroinflammation, and apoptosis impair synaptic efficacy and impair the coordination of neural networks. As neuronal activity and plasticity are critical for learning, memory, motor control, and emotion, disturbances in these processes can have significant behavioral impairments. Brain regions with high metabolic demand and complex microcircuitry, particularly the cerebral cortex and cerebellum, are especially susceptible to redox imbalance and inflammatory signaling (Liu et al., 2024; Dash et al., 2025). These vulnerabilities make these regions key targets for studying how molecular stress leads to cognitive and motor dysfunction.

Although several studies have primarily focused on potential associations between proton pump inhibitors and cognitive outcomes such as memory impairment and dementia, neurodegenerative disorders including Alzheimer's and Parkinson's disease are also characterized by prominent motor dysfunction resulting from cortical and cerebellar involvement. The cerebral cortex and cerebellum both play a role in cognition, emotion, and motor control. These regions have a high energy demand and relatively modest antioxidant capacity, which makes them vulnerable to redox imbalance and inflammatory signaling. There are increasing reports linking long-term PPI use to effects on the central nervous system, however, mechanistic data on pantoprazole remains limited. In this study, we examined the molecular and behavioral effects of chronic pantoprazole exposure in the cortex and cerebellum of rats by measuring oxidative stress, inflammatory cytokines, and apoptosis-related proteins. With this approach, we aim to determine whether long-term pantoprazole administration disrupts brain homeostasis and contributes to neurodegenerative risk. These consequences directly impact synaptic plasticity, which is essential for evaluating the safety of a widely used gastrointestinal therapy.

METHODS

Experimental Animals and Design

Given the higher prevalence of long-term PPI use among women, and to reduce biological variability in this initial mechanistic study, only female animals were included in the experimental design to more clearly characterize pantoprazole-induced alterations in apoptotic, inflammatory, and oxidative stress markers in motor-related brain regions (Vakil et al., 2015; Shanika et

al., 2023). Twenty-one female Wistar rats (3-4 months; 250-300 g) were obtained from the Bezmialem Vakif University Laboratory Animal Center. Animals were housed 4-5 per cage under controlled conditions (12:12 h light-dark cycle; $20 \pm 2^\circ\text{C}$) with ad libitum access to standard rodent chow and water. All procedures followed the Guide for the Care and Use of Laboratory Animals (NIH, Bethesda, MD, USA) and were reported in accordance with the ARRIVE guidelines. The protocol was approved by Bezmialem Vakif University Animal Care and Use Committee (Approval No. 2024/8-1).

Rats were randomly assigned to three groups ($n=7$ each): a control group (C) that received no treatment; a gavage control (GC) group that received physiological saline by oral gavage to account for handling and gavage stress; and a pantoprazole group (P) that received 20 mg/kg/day pantoprazole (Panto 40 mg, Sandoz Pharmaceuticals Inc.) in physiological saline by oral gavage for 12 weeks. The dosage of pantoprazole was selected based on previous preclinical studies and is within the range commonly used to achieve pharmacologically relevant exposure in rodent models, while the 12-week treatment period was chosen to model chronic administration (Badiola et al., 2013).

Behavioral Experiments

To minimize the influence of acute gavage-related stress on behavioral performance, behavioral testing was performed approximately 24 h after the final daily gavage procedure. All groups were tested under the same timing conditions relative to their respective treatment procedures.

Rotarod

The motor coordination of the animals was assessed using a Ugo Basile Rota-Rod (model 47600). The apparatus is equipped with a rotating rod that functions in both directions and has high walls. The speed range of this rotating rod is from 0 to 40 rpm. For each trial, the rod accelerated from a starting speed of 4 rpm to 40 rpm over a duration of 120 seconds. The latency to fall, defined as the time the animal remained on the accelerating rod, was recorded for up to 300 seconds.

Swimming Velocity Analysis in a Water Maze Task

The testing apparatus consisted of a circular tank measuring 210 cm in diameter and 51 cm in height. The water was made opaque using a non-toxic food coloring

and maintained at $23 \pm 1^\circ\text{C}$ with an automatic temperature control system. The water depth was 45 cm. The swimming speed of the rats was monitored for 60 seconds using an automated video tracking system (EthoVision XT, Noldus Information Technology, Netherlands).

Locomotor Activity in Open Field Arena

The open-field apparatus consisted of a square arena (56 cm \times 56 cm) surrounded by opaque walls 30 cm in height. Each rat was gently placed in the center of the arena and given a 10-minute period of free exploration under ambient illumination of approximately 350 lux. Locomotor activity was automatically recorded using a video-tracking system (EthoVision XT, Noldus Information Technology, Netherlands) and expressed as the total distance traveled during the session. The arena was cleaned between trials to eliminate residual odor cues.

Sample Collection and Homogenization

Following behavioral testing, the animals were deeply anesthetized with ketamine/xylazine (90/10 mg/kg, i.p.) and then euthanized for the collection of tissue samples. The brain was extracted, and the cerebellum separately isolated on dry ice. The samples were transferred to sterile tubes and stored at -80°C until analysis. Brain sections were cut on a cryostat to isolate cerebral cortical tissue encompassing the motor cortex, according to the coordinates of the Paxinos and Watson rat brain atlas (2007). Frozen tissues were homogenized in 0.1 M phosphate-buffered saline (PBS) at a 1:5 (w/v) ratio using a bead-mill homogenizer (BeadBug; 300 m/s, 20 s). Homogenates were kept on ice for 20 min and centrifuged at 14,000 rpm for 15 min at 4°C . The resulting supernatants were aliquoted into sterile tubes and stored at -20°C until analysis.

Enzyme Linked Immunosorbent Assay (ELISA)

Homogenates were thawed at room temperature and diluted 1:2 in 0.1 M PBS. The ELISA procedure was applied according to the manufacturers' protocols (BT-LAB, Shanghai, China). Briefly, samples and standards were loaded into 96-well plates pre-coated with target-specific capture antibodies and incubated at 37°C . The plates were then washed, incubated with streptavidin-HRP, developed with substrate, and read at 450 nm using a Multiskan™ FC microplate reader (Thermo Fisher Scientific, Massachusetts, USA). The

concentrations were calculated from standard curves generated with known calibrators and reported as concentration per milligram of protein. The assays were performed on the followings: apoptosis markers (BAX (Bcl-2-associated X protein, Cat. No: E0034Ra), Bcl-2 (Cat. No: E1880Ra), BAD (Bcl-2-associated death promoter, Cat. No: E2645Ra), and Caspase-3 (Cat. No: E1648Ra), inflammatory markers (TNF- α , Cat. No: E0764Ra, IL-1 β , Cat. No: E0119Ra, and IL-6, Cat. No: E0135Ra), oxidative stress markers (Malondialdehyde (MDA, Cat. No: E0156Ra), Superoxide dismutase (SOD, Cat. No: E0168Ra), total oxidant status (TOS, Cat. No: E1512Ra), and total antioxidant status (TAS, Cat. No: E1710Ra)). ELISA measurements were performed in duplicate for all samples and standards.

Statistical analysis

Statistical analyses were performed in GraphPad Prism 10 (GraphPad Software Inc., San Diego, CA). Data are reported as mean \pm standard error of the mean (SEM), with the number of biological replicates (n) provided in the figure legends. Normality was assessed with the Shapiro–Wilk test. For normally distributed data, group differences were evaluated by one-way ANOVA followed by Tukey’s multiple comparison test. Values of $p \leq 0.05$ were considered statistically significant.

RESULTS

Behavioral Outcomes

To provide a comprehensive assessment of behavioral performance, we employed three complementary tests. The rotarod test was used to evaluate sensorimotor coordination, as indicated by latency to fall. Swim velocity in the water maze was used to assess general motor activity and task-related motivation. Spontaneous locomotor activity was quantified in the open-field test as total distance traveled.

A one-way ANOVA revealed a significant effect of group on rotarod performance ($F_{(2,20)}=4,897$, $p=0.02$). *Post-hoc* Tukey’s test showed that the P group exhibited a shorter latency than the GC group ($p=0.015$) while there is no differences between the C and GC groups (Fig. 1A).

Swim speed during the water-maze task showed a marginal trend among the groups ($F_{(2,20)}=3,287$, $p=0.06$). Tukey’s multiple comparisons indicated that the P group swam more slowly than the C group ($p=0.04$), whereas the GC group showed intermediate velocities that were not statistically different from either the C or P groups (Fig. 1B).

The total distance moved in the open field showed significant group differences ($F_{(2,20)}=3.381$, $p=0.05$). *Post hoc* Tukey’s test revealed that the C group traveled significantly longer distances than the GC ($p=0.04$) and P groups ($p=0.05$). However, the GC and P groups did not differ from each other ($p>0.05$, Fig. 2).

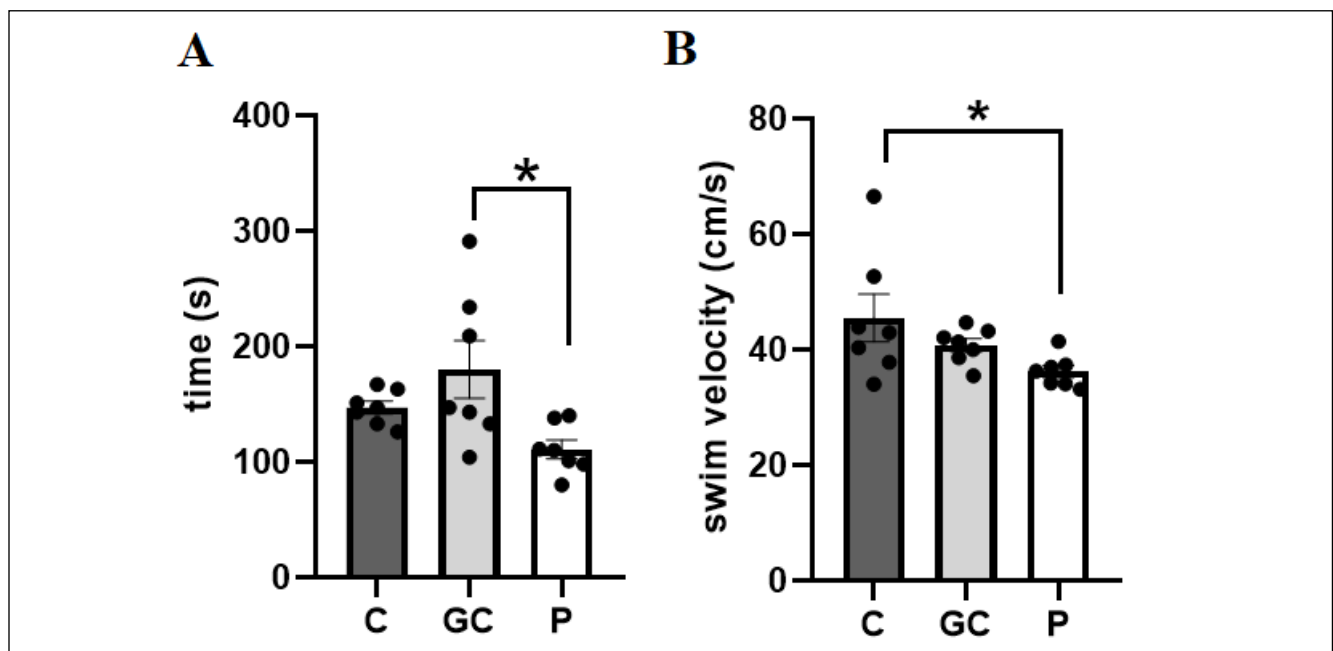


Fig. 1. Effects of chronic pantoprazole on motor performance. (A) Rotarod latency to fall (time, s) (B) Mean swim velocity (cm/s) in the water-maze task. Bars show mean \pm SEM; dots denote individual animals (n=7/group). * $p < 0.05$ represents *post-hoc* comparisons between groups. Abbreviations: C, control; GC, gavage control; P, pantoprazole.

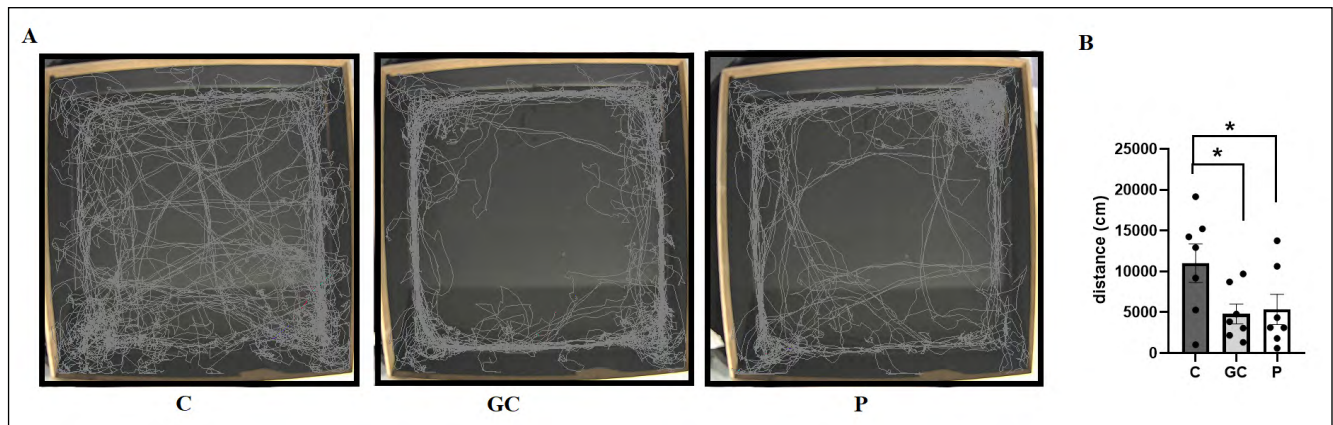


Fig. 2. Open-field locomotion after chronic pantoprazole. (A) Representative EthoVision tracking plots of 10-min exploration in the open-field arena. (B) Total distance traveled (cm) across groups. Bars show mean \pm SEM; dots denote individual animals ($n=7$ /group). * $p<0.05$ represents *post-hoc* comparisons between groups. Abbreviations: C, control; GC, gavage control; P, pantoprazole.

ELISA Results

The Effects of Pantoprazole on Apoptosis

To evaluate apoptotic signaling across different mechanistic levels, BAX and BAD were assessed as pro-apoptotic proteins, while Bcl-2 was included as an anti-apoptotic marker. While BAX acts as a key effector protein that directly mediates mitochondrial outer membrane permeabilization, BAD functions as an upstream regulator by neutralizing anti-apoptotic Bcl-2 family members and thereby facilitating BAX activation (Yang et al., 1995).

The results of the one-way ANOVA performed in the study yielded a statistically significant difference between the groups in BAX protein levels in the cerebellum ($F_{(2,18)}=4.41$, $p=0.03$). Pairwise comparisons revealed that the P group exhibited significantly higher BAX levels compared to the C group ($p=0.03$), while there was no change in its levels between the P and GC groups. The results obtained from the motor cortex tissue also suggest that the alteration in BAX levels is significant ($F_{(2,18)}=3.45$, $p=0.05$). According to *post-hoc* analyses, a considerable increase was observed in the P group in comparison to both the C and GC ($p=0.05$) groups (Fig. 3A). For Bcl-2, there is no significant difference in both its cerebellar levels [$F_{(2,18)}=3.22$, $p=0.06$] and its motor cortex levels among groups ($F_{(2,18)}=2.21$, $p=0.14$, Fig. 3B). Similarly, a significant effect was observed in the BAX/Bcl-2 ratio in the cerebellum [$F_{(2,18)}=5.37$, $p=0.015$] and motor cortex ($F_{(2,18)}=6.14$, $p=0.009$). *Post-hoc* analyses revealed that the P group showed a considerably higher BAX/Bcl-2 ratio only compared to the C group ($p=0.01$), but not the GC group in the cerebellum (Fig. 3C). In addition,

a considerable increase in the BAX/Bcl-2 ratio in the motor cortex was observed in the P group in comparison to both the C ($p=0.04$) and GC ($p=0.01$) groups (Fig. 3C).

For BAD, cerebellar levels did not differ among groups ($F_{(2,18)}=0.36$, $p=0.71$). Conversely, a significant effect was observed in the motor cortex, ($F_{(2,18)}=11.23$, $p=0.0007$). *Post-hoc* analyses revealed that the P group showed considerably higher BAD compared to both the C and GC ($p=0.002$) groups (Fig. 3D). However, there was no significant alteration in caspase-3 levels in both the cerebellum ($F_{(2,18)}=1.99$, $p=0.16$) and cortex ($F_{(2,18)}=0.61$, $p=0.55$, Fig. 3E).

The Effects of Pantoprazole on Inflammation

TNF- α , a proinflammatory cytokine, plays a critical role in the inflammatory process and alterations in its levels can be seen in cases of tissue damage. The one-way ANOVA analysis performed in our study demonstrated that there was no significant difference between the groups in terms of TNF- α levels. The data obtained from the cerebellum ($F_{(2,18)}=1.08$, $p=0.36$) and motor cortex ($F_{(2,18)}=0.114$, $p=0.89$) demonstrate that pantoprazole administration did not induce a statistically significant alteration in this cytokine levels (Fig. 4A).

IL-6 is a critical regulator of the inflammatory response and plays an active role in neuroinflammatory processes. The one-way ANOVA analysis revealed a statistically significant difference in IL-6 levels between the groups in the cerebellum ($F_{(2,18)}=6.09$, $p=0.009$). *Post-hoc* pairwise tests indicated higher IL-6 levels in both the P and GC groups relative to the C group ($p=0.02$ for both groups), with no observed difference

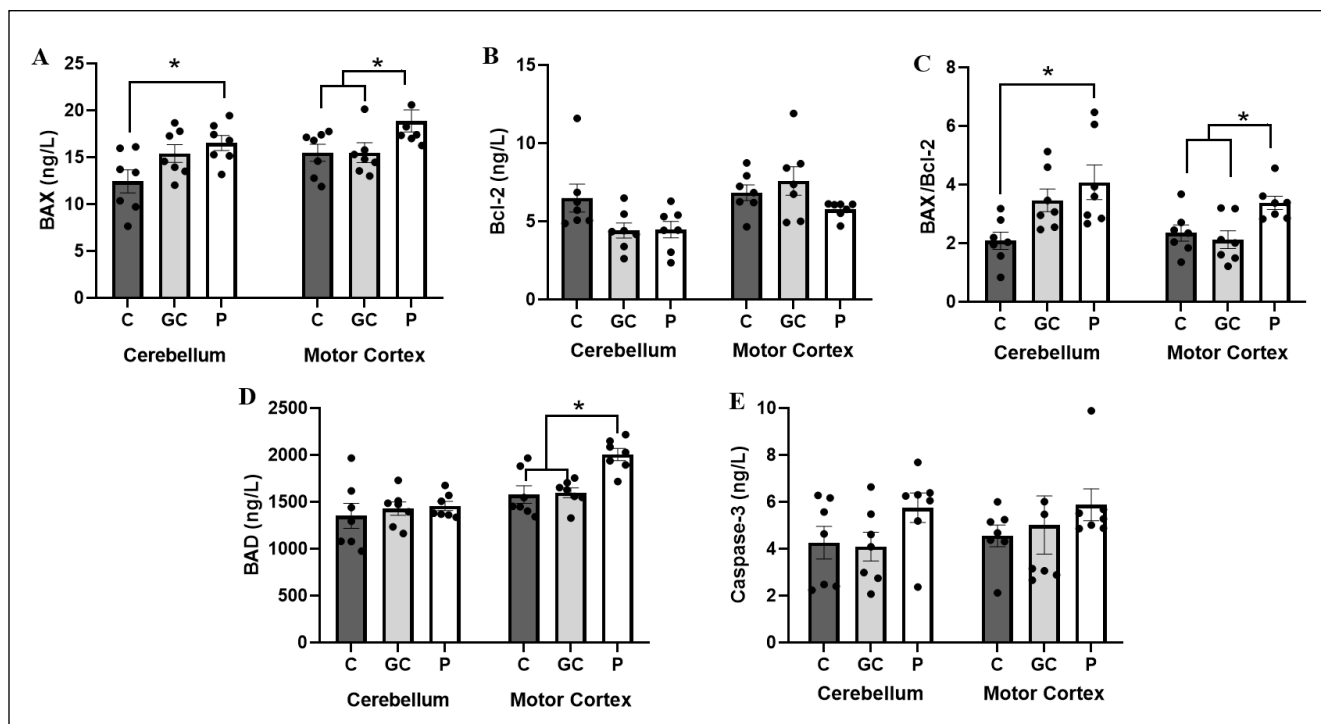


Fig. 3. Pro-apoptotic proteins after chronic pantoprazole. (A) BAX (Bcl-2-associated X protein) concentrations in cerebellum and motor cortex (B) BAD (Bcl-2-associated death promoter), concentrations in cerebellum and motor cortex. Values are ELISA measurements normalized to protein content and shown as mean \pm SEM with individual animals overlaid ($n=7$ /group; assays run in duplicate). * $p<0.05$ represents *post-hoc* comparisons between groups. Abbreviations: C, control; GC, gavage control; P, pantoprazole.

between the P and GC groups. Conversely, no substantial difference was detected between the groups regarding IL-6 levels in motor cortex ($F_{(2,18)}=0.28$, $p=0.76$, Fig. 4B).

IL-1 β is a proinflammatory cytokine that plays a crucial role in initiating and sustaining the inflammatory response in the central nervous system. In the cerebellum, the one-way ANOVA revealed a significant group effect on IL-1 β levels ($F_{(2,18)}=4.81$, $p=0.021$). Subsequent *post-hoc* comparisons indicated significantly higher levels of IL-1 β in the P group compared to only the C group ($p=0.02$). In the motor cortex, IL-1 β levels did not differ among groups ($F_{(2,18)}=0.46$, $p=0.64$, Fig. 4C).

The Effects of Pantoprazole on Oxidative Stress

SOD is a crucial antioxidant enzyme that catalyzes the dismutation of superoxide anions to hydrogen peroxide and oxygen, thereby limiting oxidative injury. The results of the one-way ANOVA analysis revealed that there was no statistically significant difference between the groups in terms of SOD levels in the cerebellum ($F_{(2,18)}=0.25$, $p=0.78$). Similarly, no significant difference was observed between groups in motor cortex ($F_{(2,18)}=0.26$, $p=0.78$, Fig. 5A).

MDA is defined as a final product of lipid peroxidation and is generally accepted as a marker of oxidative stress at the cellular level. The cerebellar MDA levels did not differ across groups, ($F_{(2,18)}=0.93$, $p=0.41$). In the motor cortex, a one-way ANOVA revealed a significant group effect on MDA concentrations ($F_{(2,18)}=3.80$, $p=0.04$). *Post-hoc* comparisons indicated higher cortical MDA levels in the P group relative to the C group ($p=0.04$) while there was no alteration in the MDA levels between the P and GC groups (Fig. 5B).

TAS is a significant parameter reflecting the total antioxidant capacity of the biological system in relation to its ability to counteract free radicals. In the cerebellum, a one-way ANOVA revealed a significant group effect on the TAS ($F_{(2,18)}=4.33$, $p=0.03$). According to the *post-hoc* comparisons, the P group showed higher TAS than only the C group ($p=0.03$). There is no difference between the P and GC groups in response to TAS levels in the cerebellum. In contrast, TAS did not differ across groups in the motor cortex ($F_{(2,18)}=1.15$, $p=0.34$, Fig. 5C).

TOS, a measure of the tissue's total oxidative status, showed no significant differences across groups in the cerebellum ($F_{(2,18)}=0.18$, $p=0.84$). A parallel examination of TOS levels in motor cortex tissue re-

vealed no statistically significant differences among the study groups ($F_{(2,18)}=1.72$, $p=0.21$, Fig. 5D).

OSI, which is calculated as the ratio of total oxidant status to total antioxidant status, offers an integrated

assessment of systemic redox balance. The OSI values in the cerebellum did not exhibit any significant differences among the groups ($F_{(2,18)}=0.58$, $p=0.57$). On the other hand, in the motor cortex, one-way ANOVA indicated a significant group effect on OSI values ($F_{(2,18)}=3.60$, $p=0.015$). Subsequent *post-hoc* comparisons indicated elevated OSI levels in the P group compared to only the C group ($p=0.04$) (Fig. 5E).

DISCUSSION

Pantoprazole, a PPI, suppresses gastric acid secretion by irreversibly binding to the gastric H^+/K^+ -ATPase and is widely used in acid-related disorders. Although earlier reports indicated good long-term tolerability (Moreira Dias, 2009), more recent evidence has raised safety concerns, including reduced bone mineral density, micronutrient deficiencies, increased infection risk, and renal, cardiac, and cognitive complications (Brisebois et al., 2018). These findings highlight the need to carefully consider the risks of prolonged use. Pantoprazole shows limited but measurable penetration across the blood-brain barrier, as evidenced by its detection in cerebrospinal fluid (2.79 ng/ml) to exert some effects on possible central nervous system targets (Sigaroudi et al., 2016). While some studies have suggested possible neurological associations, including dementia-related risks (Li et al., 2019; Ahn et al., 2023), its direct effects on brain regions involved in motor control remain largely unexplored. Therefore, the aim of our study was to investigate the effects of chronic pantoprazole administration on the motor cortex and cerebellum at both behavioral and molecular levels, focusing on apoptosis, inflammation, and oxidative stress.

Animals receiving pantoprazole exhibited impaired sensorimotor coordination on the rotarod and reduced spontaneous locomotion in the open field, with a trend toward decreased swim speeds in the water maze. However, reduced spontaneous locomotion may not solely be attributed to pantoprazole administration, but could also be influenced by gavage-induced stress, a well-documented factor known to elevate stress responses and promote freezing-like behavior in rodents (Brown et al., 2000; Li et al., 2026). The reduced distance traveled in the open-field test should be interpreted with caution, as it may reflect not only decreased locomotor activity but also anxiety-like behavior. Avoidance of the central area or increased freezing-like responses may shorten the total distance traveled without indicating a direct motor deficit. The findings, when taken together, suggest that pantoprazole could potentially diminish sensory

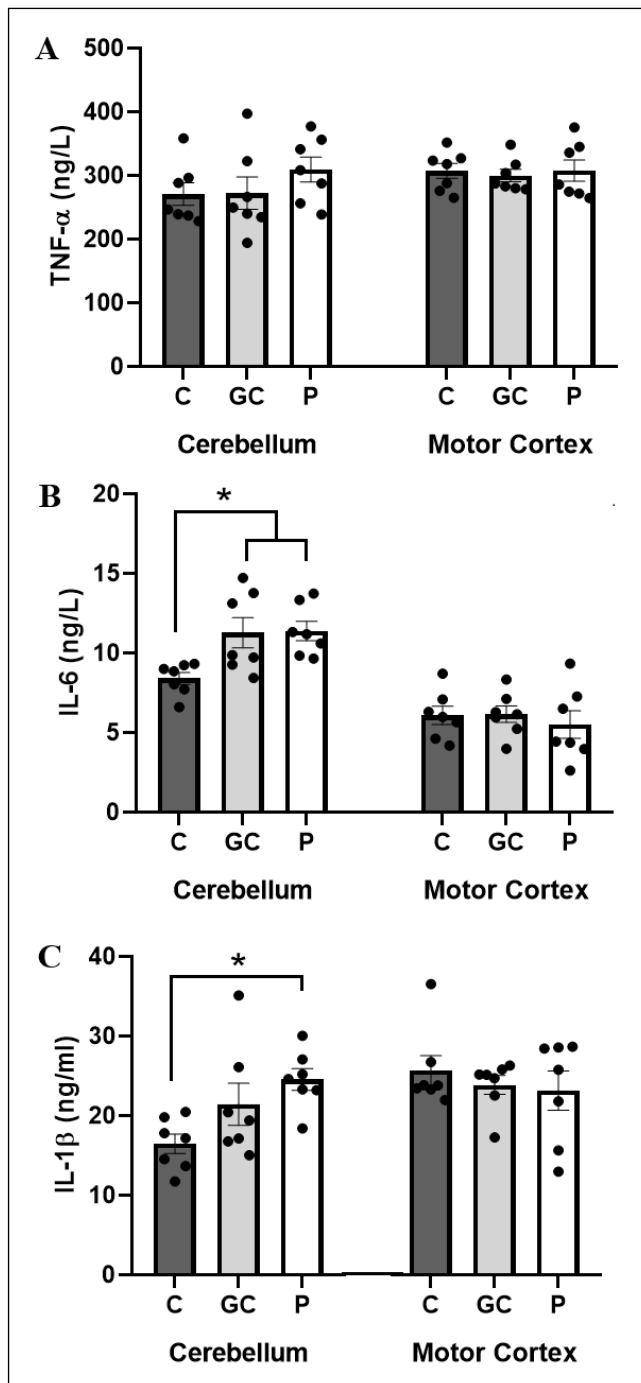


Fig. 4. Inflammatory cytokines after chronic pantoprazole. ELISA measurements of (A) TNF- α , (B) IL-6, and (C) IL-1 β in cerebellum and motor cortex. Bars show mean \pm SEM; dots denote individual animals ($n=7$ /group). * $p < 0.05$ represents *post-hoc* comparisons between groups. Abbreviations: C, control; GC, gavage control; P, pantoprazole.

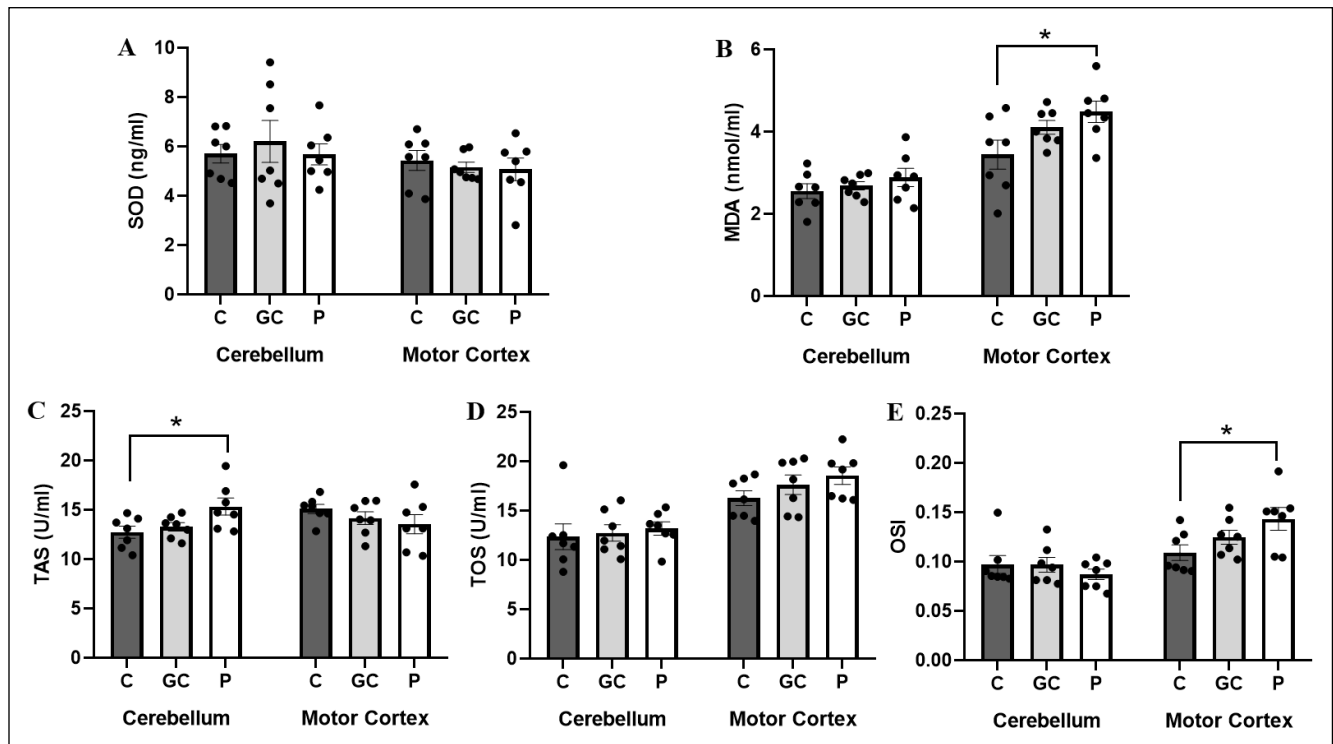


Fig. 5. Oxidative stress measures after chronic pantoprazole. ELISA measurements of (A) superoxide dismutase (SOD) activity, (B) malondialdehyde (MDA), (C) total antioxidant status (TAS), (D) total oxidant status (TOS), and (E) oxidative stress index (OSI) in cerebellum and motor cortex. Bars show mean \pm SEM; dots denote individual animals (n=7/group). * p<0.05 represents *post-hoc* comparisons between groups. Abbreviations: C, control; GC, gavage control; P, pantoprazole.

motor coordination rather than locomotor activity. Our behavioral findings are consistent with epidemiological signals that establish a link between the use of PPIs and an elevated risk of developing Parkinson's disease, a neurodegenerative condition that primarily affects motor function (Nielsen et al., 2012; Kim et al., 2022; Hong et al., 2023). The findings of these human studies suggest a potential correlation between vulnerabilities in motor circuits and micronutrient deficiencies, specifically magnesium (Mg), vitamin B12, and iron, in individuals who use PPIs at a daily prescribed dose for more than 3 months. A study conducted on rats have demonstrated that pantoprazole (oral dose of 1.3 mg/kg once daily for 4 weeks) use causes histopathological changes in the cerebellar cortex. These alterations have been associated with elevated levels of iNOS and GFAP (Alafify et al., 2022). The objective of our study is to understand the mechanism underlying motor function impairment by investigating the changes in proteins related to apoptosis, inflammation, and oxidative stress resulting from long-term pantoprazole use.

At the molecular level, pantoprazole activated pro-apoptotic pathways. BAX levels and the BAX/Bcl-2 ratio were elevated in both cerebellum and mo-

tor cortex, and BAD was selectively increased in the motor cortex. Importantly, the region-specific pattern of pro-apoptotic changes supports a direct effect of pantoprazole, as the P group showed significant differences from both GC and control groups in the motor cortex, while in the cerebellum, significance was observed only *versus* C group, arguing against a generalized stress response and favoring a selective cortical vulnerability. BAX and BAD function as regulatory proteins within the intrinsic mitochondrial pathway, and these elevations suggest an alteration in pro-apoptotic signaling, particularly within the motor cortex. In addition, there was no significant effect in the caspase-3 levels in the cerebellum and in the motor cortex. The findings of a recent cancer study demonstrated that pantoprazole treatment inhibited cell viability or growth of glioma cells and induced cell death through altering pro- and anti-apoptotic proteins. (Geeviman et al., 2018). Furthermore, a slight increase in the cerebellar BAX/Bcl-2 ratio in the GC group points the stress-related activation of the apoptotic pathways as previously observed (Zlatković & Filipović, 2012). The elevation of BAX and BAD in the motor cortex may be consistent with the upstream activation of mitochondria leading to outer

membrane permeabilization after pantoprazole exposure, in accordance with the findings of Geeviman et al. (2018). An additional study has indicated that PPIs inhibit choline-acetyltransferase (ChAT), suggesting that this inhibition may induce apoptosis in brain tissue (Kumar et al., 2020). These results support our findings of an increase in the levels of BAX and BAD, and BAX/Bcl-2 ratio that was observed following pantoprazole use.

It has been demonstrated that PPIs may promote apoptosis by modulating the secretion of inflammatory factors and enhancing oxidative stress (Li et al., 2023). Besides gastric acid suppression, pantoprazole could impact additional biological processes. Breedveld et al. (2005) noted that pantoprazole has the ability to interact with efflux transporter systems, including Bcrp1/ABCG2 and P-glycoprotein, which are critical to the distribution of drugs across the blood-brain barrier. Furthermore, Fawzy et al. (2022) demonstrated that pantoprazole, especially when combined with vincamine, altered oxidative stress and inflammation as well as the signaling pathways of MAPK/NF- κ B and apoptosis, in a study of renal ischemia/reperfusion. Thus, the effects of pantoprazole are likely to vary with the type of tissue, the dose, the duration of the treatment, as well as the relevant pathology. In the current study, an increase in both IL-6 and IL-1 β was observed in the cerebellum of animals as a combination effect of pantoprazole with gavage-related stress, while TNF- α levels remained unchanged. In the motor cortex, no statistically significant differences were observed in the levels of inflammatory cytokines among the different groups. IL-6, a context-dependent cytokine with both pro- and anti-inflammatory actions (Kerkis et al., 2024), increased significantly in the cerebellum of the P group relative to the C group. However, a similar increase also occurred in the GC group, indicating that the elevation probably represents gavage-related stress rather than a drug effect. Intragastric gavage has been shown to induce physiological stress, which has been linked to elevated plasma corticosterone levels, thereby modulating cytokine signaling, including IL-6 (Bethin et al., 2000; Brown et al., 2000). In parallel with our findings, a study conducted on mice has demonstrated that PPIs enhance the levels of certain pro-inflammatory cytokines, including IL-1 β (Hung et al., 2015). Contrary to this, studies in the literature have demonstrated that the administration of PPIs in pathological conditions is associated with a decrease in IL-1 β levels (Tanigawa et al., 2009; Li et al., 2023; Kiecka et al., 2023). However, neuroinflammation has been demonstrated to impair not only memory-related neural networks but also motor circuits, including

those located in the motor cortex, basal ganglia, and cerebellum (Felger & Treadway 2017; Fakorede et al., 2025). This neuronal damage can further impair motor balance and coordination as seen in our study.

The presence of inflammatory mediators within the central nervous system has been demonstrated to increase oxidative stress. Increased oxidative stress has been shown to result in neuronal damage in motor-related areas, such as the cerebellum and motor cortex (Sims-Robinson et al., 2013). The present study demonstrated that oxidative stress markers exhibited evidence of regionally distinct responses. While no change in SOD levels was observed in either brain region, levels of MDA, a marker of lipid peroxidation, increased only in the motor cortex following pantoprazole administration. Parallel to this, the OSI ratio elevated in the motor cortex reflecting a net redox imbalance in the motor cortex. The high level of MDA and OSI in the P group was not significantly different from that of the GC group suggesting the stress-related increase in the oxidative stress rather than pantoprazole effect in the motor cortex. A recent *in vitro* study investigated the antioxidant activity of PPIs using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay. Omeprazole and esomeprazole exhibited clear scavenging activity, while pantoprazole demonstrated weak free radical neutralization (Abed et al., 2020). These results indicate that the antioxidant properties of PPIs are variable. Furthermore, another study performed on rats demonstrated that sub-chronic PPI treatment (16 mg/kg once a day) causes moderate vascular endothelial dysfunction and renal dysfunction by reducing nitric oxide and increasing oxidative stress (Taneja et al., 2020).

Overall, these findings suggest a region-specific redox response to pantoprazole. While SOD levels remained unchanged, the cerebellum exhibited a compensatory increase in overall antioxidant capacity (higher TAS) without signs of lipid peroxidation or an oxidative shift (Bennet et al., 2007). In contrast, the motor cortex exhibits higher lipid peroxidation and a higher oxidative stress index even though the total oxidant level is relatively similar. This suggests insufficient antioxidant capacity rather than an increase in oxidants. This may reflect differences in baseline antioxidant defenses, lipid composition, mitochondrial activity, or glial support across brain regions (Lee et al., 2020; Vinokurov et al., 2021).

A notable limitation of the present study is the use of oral gavage as a route of administration, which is a well-recognized source of stress in experimental animals. Gavage-induced stress has been shown to elevate physiological stress responses, including glucocorticoid release, and may consequently influ-

ence both behavioral performance and inflammatory processes (Walker et al., 2012). Therefore, the contribution of stress-related factors should be considered when evaluating the behavioral and biochemical outcomes, and future studies employing less stressful administration methods may help to further clarify these effects. In addition, the lack of morphological assessments is another limitation for this study to further clarify the cellular and circuit-level effects of pantoprazole.

CONCLUSION

Our findings suggest that pantoprazole administration is associated with slight motor impairment and activation of pro-apoptotic signaling in the motor cortex, however, the absence of cleaved caspase-3 and the lack of functional mechanistic studies limit definitive conclusions regarding apoptosis and its causal contribution to the observed behavioral changes. In addition, pantoprazole administration further amplifies stress-related increases in neuroinflammation pointing to a potentiating effect that aggravates the inflammatory response in the cerebellum. These observations have clinical relevance and suggest that prolonged exposure to pantoprazole could affect motor circuitry differently which needs further investigations. Together, such studies could guide safer prescribing, especially for patients who require prolonged therapy.

ACKNOWLEDGEMENT

This work is part of a research project funded by the Istinye University Research Council (Grant number: 2024-8-1).

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