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RESEARCH

Effects of Melatonin and Cortistatin on Nucleus Accumbens Damage Induced by Chronic Cellulosic Thinner Inhalation in Rats

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Abstract

Background Chronic inhalation of cellulosic thinner, which contains toluene and other volatile solvents, induces oxidative stress–mediated neurotoxicity, particularly within the nucleus accumbens (NAc). This study evaluated the neuroprotective effects of melatonin and cortistatin against thinner-induced NAc injury.

Methods Ninety male Wistar rats were assigned to nine groups (n = 10): control, thinner-only, thinner + cortistatin, thinner + melatonin, thinner + melatonin + cortistatin, and four vehicle controls. Rats were exposed to thinner vapour twice daily (1 h/session) for six weeks. Melatonin (10 mg/kg) and/or cortistatin were administered intraperitoneally once daily. NAc tissue was analysed by light microscopy, transmission electron microscopy, and biochemical assays of lipid peroxidation (MDA) and antioxidant enzymes (GPx, SOD, CAT).

Results Thinner inhalation induced behavioral changes, fur discoloration, and significant weight loss ($p < 0.05$). Histology and ultrastructure showed neuronal loss and cellular damage in the nucleus accumbens, least evident with melatonin + cortistatin. Catalase activity showed no differences among groups ($p > 0.05$). Glutathione peroxidase was reduced in the thinner group vs. control (0.36 vs. 0.56, $p = 0.046$). Superoxide dismutase activity was lower in thinner (1.93 vs. 3.67, $p < 0.001$) and thinner + cortistatin (2.82 vs. 3.67, $p = 0.028$) groups, with melatonin mitigating reductions. Lipid peroxidation was elevated in the thinner group (3.59 vs. 2.38, $p = 0.042$), though overall group differences were not significant ($p = 0.127$).

Conclusions Chronic thinner inhalation causes oxidative stress–related degeneration of the NAc. Melatonin and cortistatin exert neuroprotective effects, with their combination offering superior preservation of cellular and biochemical integrity. These findings highlight potential therapeutic avenues for solvent-induced neurotoxicity.

Keywords Cortistatin · Inhalation · Melatonin · Nuc. accumbens · Thinner

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1 Introduction

Organic solvents constitute a large and diverse group of industrial and household chemicals widely used in paints, coatings, adhesives, and cleaning agents. Among them, cellulosic thinner is a volatile solvent mixture containing toluene and other hydrocarbons that is frequently abused for its psychoactive properties [1, 2]. Experimental studies indicate that chronic inhalation of toluene-containing solvents activates mesoaccumbal dopaminergic pathways via stimulation of the ventral tegmental area, leading to enhanced dopamine release in the nucleus accumbens (NAc) and contributing to addictive behavior and neurotoxicity [3, 4]. The NAc, a key component of the brain's reward circuitry, plays a critical role in reinforcement, motivation, and goal-directed behavior, and is highly vulnerable to oxidative damage induced by repeated solvent exposure [5, 6].

Animal studies have shown that prolonged paint thinner inhalation causes significant behavioral and cognitive impairments, along with histopathological alterations in brain regions including the hippocampus and striatum [7]. These changes are associated with reduced levels of neuroplasticity markers such as brain-derived neurotrophic factor (BDNF) and cAMP response element-binding protein (CREB) and increased oxidative stress [8–10]. Therefore, given the increasing rates of solvent misuse, developing effective brain-protective approaches to prevent neurological damage caused by paint thinner exposure has emerged as a critical area of scientific investigation.

Melatonin, an indoleamine synthesized primarily by the pineal gland, exhibits potent antioxidant, anti-apoptotic, and mitochondrial-protective properties. It enhances the activities of endogenous antioxidant enzymes, including superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT), while reducing lipid peroxidation markers [11, 12]. Its neuroprotective effects are mediated through multiple mechanisms, including free radical scavenging, metal chelation, mitochondrial membrane stabilization, and modulation of inflammatory responses [13, 14]. Due to its high lipophilicity, melatonin readily crosses the blood–brain barrier and directly protects central nervous system structures [15]. Cortistatin, a neuropeptide structurally related to somatostatin, also possesses antioxidant and anti-inflammatory properties, and has been shown to modulate neuronal excitability and protect against oxidative stress-related injury [16]. However, its role in preventing damage to the NAc induced by thinner remains poorly understood.

This study aims to investigate the potential protective effects of melatonin and cortistatin against damage induced by chronic cellulosic thinner inhalation in the rat nucleus accumbens, with emphasis on oxidative stress parameters and histopathological outcomes.

2 Materials and Methods

2.1 Subjects

Ninety young male Wistar rats (*Rattus norvegicus*), each weighing 400–450 g, were procured from the Akdeniz University Experimental Animals Unit. All animal procedures were approved by the Akdeniz University Local Ethics Committee (Protocol No: 1034/ 2020.02.013) and conducted in accordance with institutional guidelines and ARRIVE standards.

2.2 Experimental Design

Rats were randomly assigned to nine groups (n = 10 per group; Fig. 1 a, Fig. 1 b). Rats were randomly assigned to nine groups (n = 10 per group). The schematic representation of the experimental protocol showing the random assignment of 90 male rats (Fig. 1 a) and the graphical illustration of the experimental protocols applied to the groups (Fig. 1 b) are shown in the figures. All protocols were reviewed and approved by the Akdeniz University Faculty of Medicine Animal Care and Use Committee.

- **Groups 1 (Control):** No treatment.
- **Groups 2–5:** Twice-daily, 1-h sessions of thinner inhalation for 6 weeks.
  - **Group 3:** received concurrent cortistatin (i.p.).
  - **Group 4:** received concurrent melatonin (i.p.).
  - **Group 5:** received both cortistatin and melatonin (i.p.).
- **Group 6**: Daily intraperitoneal ethanol + saline for 6 weeks (vehicle control for melatonin solvent).
- **Group 7:** Daily intraperitoneal cortistatin for 6 weeks.
- **Group 8:** Daily intraperitoneal melatonin for 6 weeks.
- **Group 9:** Daily intraperitoneal distilled water for 6 weeks (vehicle control for cortistatin).

All rats had unrestricted access to standard commercial chow and tap water. Both right and left nucleus accumbens (NAc) tissues were harvested for ultrastructural, biochemical, and histological analyses.

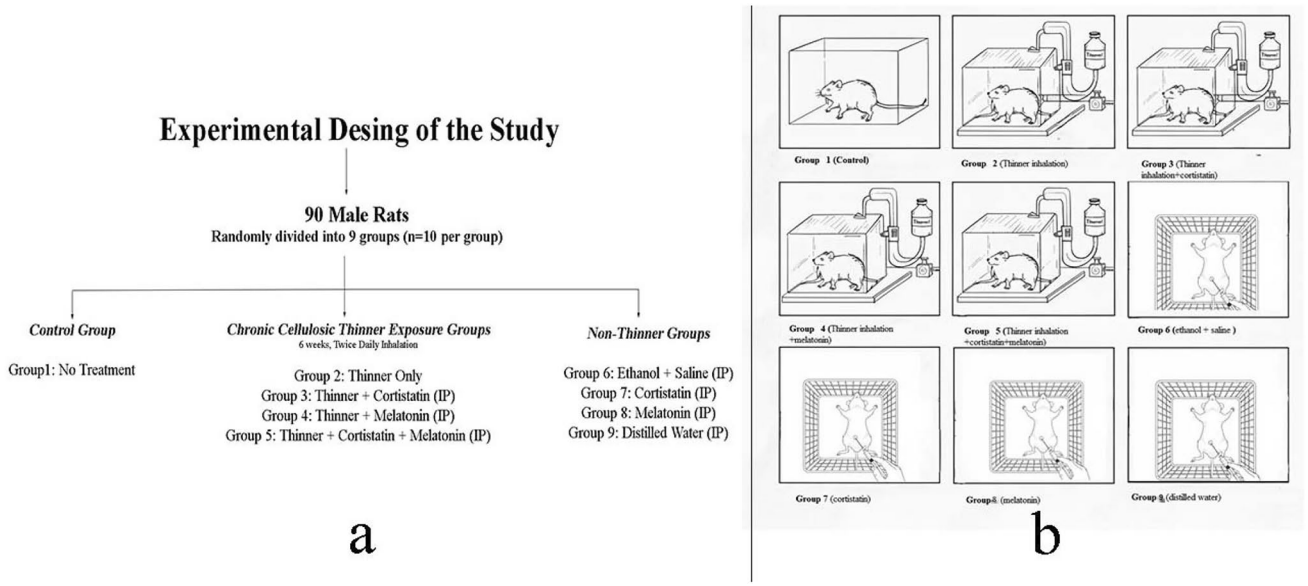


Fig. 1 Experimental groups

2.3 Thinner Inhalation Procedure

Rats in Groups 2–5 were placed in custom glass chambers (100 × 65 × 72 cm) maintained at 20–22 °C and 760 mmHg. Industrial cellulose thinner was aerosolized using a pressure-sprayer pump twice daily for 1 h for 6 weeks. Targeted solvent concentrations (toluene 1800 ppm; acetone 500 ppm; isobutyl acetate 7000 ppm; isobutanol 6000 ppm) were measured twice daily with a Dräger gas detector (Drägerwerk, Germany), following [17]. Post-exposure, cage air was vented outdoors. Flammable solvents required that no ignition sources or electric devices were present, and handlers wore protective clothing, goggles, gloves, and masks.

2.4 Melatonin Administration

Following each inhalation session, melatonin (10 mg/kg; Sigma M5250) was administered intraperitoneally daily at 18:00–19:00 for 6 weeks. Melatonin was dissolved in ethanol and saline. To control solvent effects, Group 6 received the same volume of ethanol + saline vehicle i.p.

2.5 Cortistatin Administration

Following inhalation, cortistatin (Cayman, Cortistatin-29 (1–13) (rat) 2474) was administered intraperitoneally daily at 18:00–19:00 for 6 weeks, dissolved in distilled water. Group 9 served as a vehicle control, receiving equivalent volumes of distilled water i.p.

2.6 Sacrifice and Tissue Collection

At the end of week 6, animals were anesthetized with xylazine HCl (15 mg/kg, Rompun®) and ketamine (100 mg/kg, Ketalar®) i.p. Thoracotomy was performed, and transcardiac perfusion was carried out using 250 mL heparinized saline followed by fixative solution.

Brains were extracted via the foramen magnum, and a coronal section containing the NAc was identified using a brain matrix. Bilateral NAc tissues were collected and placed in 10% formalin, 4% glutaraldehyde, or PBS depending on the downstream analysis.

2.7 Light Microscopy

NAc tissue was fixed in 10% formalin for 24 h, dehydrated through graded ethanol, cleared in xylene, and embedded in paraffin. Sections (5 µm) were stained with hematoxylin–eosin and examined under light microscopy Layer thicknesses and histopathological changes were assessed qualitatively. For each group, 5 randomly selected slides were evaluated and examined and photographed under a × 40 objective using a Zeiss Axioplan light microscope.

2.8 Transmission Electron Microscopy (TEM)

Samples were fixed in 4% glutaraldehyde (0.1 M Sorensen phosphate buffer, 4 °C) for 2 h, post-fixed in 1% osmium tetroxide, dehydrated in graded ethanol series, and embedded in Araldite CY212. Semi-thin Sects. (300 nm) were stained with toluidine blue; ultrathin Sects. (70 nm) were contrasted

using uranyl acetate and lead citrate, then visualized with a LEO 906 EM transmission electron microscope.

2.9 Biochemical Analyses

2.9.1 Lipid Peroxidation

Lipid peroxidation was quantified using the TBARS assay (OxiSelect™ TBARS Assay Kit, STA-330). Malondialdehyde (MDA) levels were measured spectrophotometrically at 532 nm and expressed as nmol per gram of protein.

2.9.2 Antioxidant Enzyme Activity

After 6 weeks, tissue homogenates were prepared from all experimental groups for the determination of antioxidant enzymes. The antioxidant enzymes were measured in these samples as follows: Glutathione peroxidase (GPx): Cayman Assay Kit 703,102. Activity measured by NADPH consumption at 340 nm. Superoxide dismutase (SOD): Cayman Assay Kit 706,002. Catalase (CAT): Cayman Assay Kit 707,002. Activity quantified via formaldehyde standard curve as µmol formaldehyde/min.

2.10 Statistical Analysis

For histological evaluation, five randomly selected sections per group were analyzed qualitatively. Descriptive statistics were presented as mean, standard deviation (SD), median, minimum (min), and maximum (max) values. In the biochemical analysis, the assumption of normality was checked with the Shapiro–Wilk test. As the data did not meet the assumption of normal distribution, the Mann–Whitney U test was used to analyze the difference between the measurement values of two groups, and the Kruskal–Wallis test was used for the comparison of more than two groups. The Bonferroni-Dunn test was applied as a post-hoc test for significant results. The analysis was performed using the SPSS 20.0 software package. P-values less than 0.05 were considered statistically significant ($p < 0.05$).

3 Results

3.1 General Health and Behavioral Observations

The initial clinical manifestations observed in rats exposed to thinner inhalation included subjects lying on top of one another attempting to hide their faces and a tendency to escape to the corners of the specialized glass cage ($100 \times 65 \times 72$ cm). As the duration of thinner exposure increased, rats exhibited excessive grooming behavior, frequent blinking, salivation, dyspnea, rapid escape movements from one end of the cage to the other, and when the toluene concentration within the chamber reached saturation levels (1800 ppm), tremors in the hind limbs and loss of balance were observed. On the eighth day of thinner inhalation, yellowing of body fur was observed in subjects receiving thinner inhalation, which differed from the control group (Fig. 2 a, Fig. 2 b). This discoloration persisted throughout the entire experimental period.

3.2 Histopathological and Ultrastructural Evaluations

3.2.1 Light Microscopy Observations

For morphological evaluation of the nucleus accumbens tissue layers in experimental groups exposed to thinner inhalation and the control group not exposed to thinner inhalation, specimens were stained with Hematoxylin–Eosin (H&E). Hematoxylin–Eosin staining was performed to visualize pyramidal cells in the rat nucleus accumbens.

In Hematoxylin–Eosin staining, we determined that the number of pyramidal cells in the nucleus accumbens was normal in the control group and non-thinner groups (evaluated qualitatively) (Fig. 3 a., f., g., h., ı.). In the thinner-inhaling group, numerical reduction occurred in cells along with morphological changes in their structure (Fig. 3 b). When Group 3 (Thinner + Cortistatin), Group 4 (Thinner + Melatonin), and Group 5 (Thinner + Melatonin + Cortistatin) were compared, numerical reduction in cells along with morphological structural changes showed the least

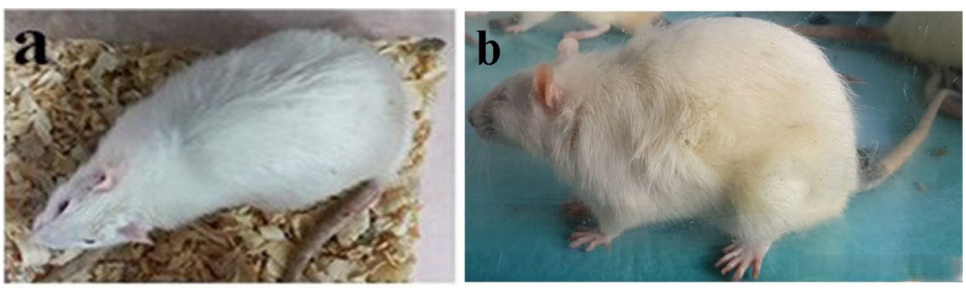


Fig. 2 **a** Control group, **b** Subjects inhaled thinner

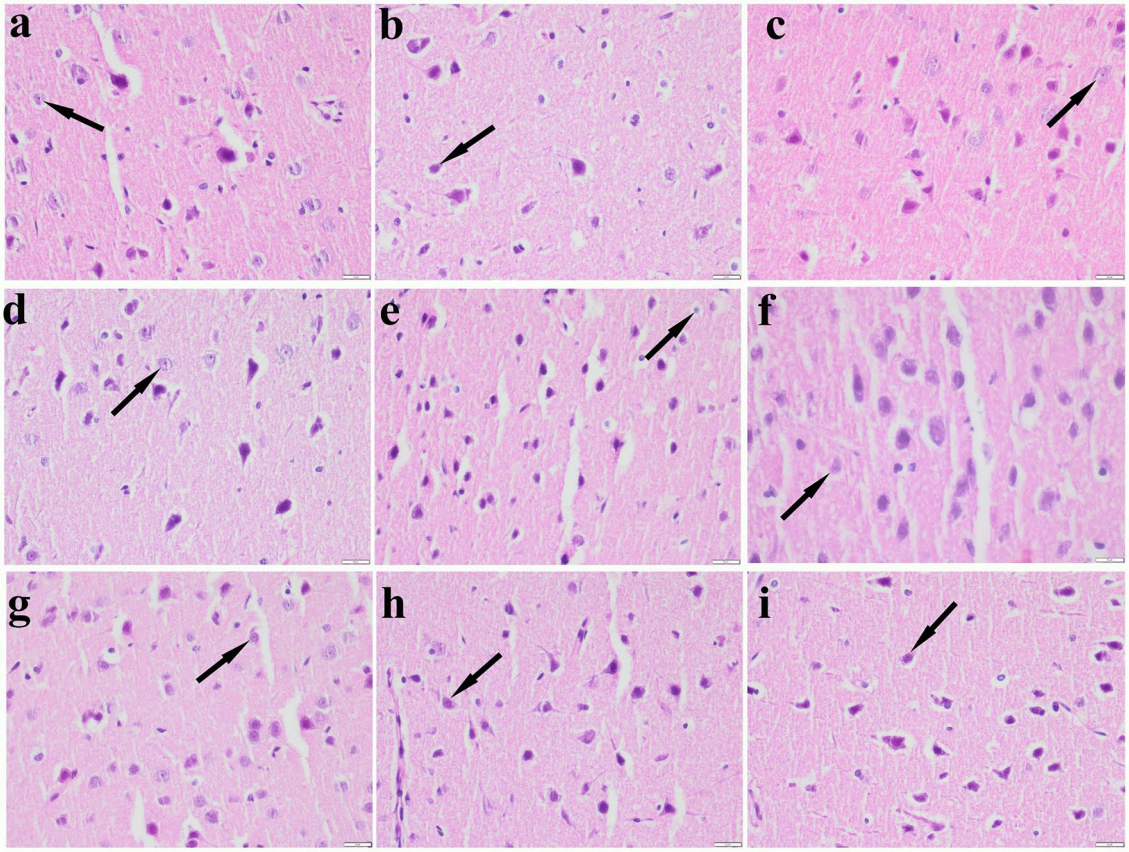


Fig. 3 Hematoxylin–Eosin staining of pyramidal cells in the nucleus accumbens. **a** Group 1 (Control Group), **b** Group 2 (Thinner inhaled group), **c** Group 3 (Thinner + Cortistatin), **d** Group 4 (Thinner + Melatonin), **e** Group 5 (Thinner + Melatonin + Cortistatin), **f** Group 6 ethanol + physiological serum, **g** Group 7 Cortistatin, **h** Group 8 melatonin, **i.** Group 9: distilled water (Scale bar, 20 µm)

alteration in Group 5, followed by Group 4, and then Group 3, respectively (Fig. 3 c., d., e.).

3.2.2 Ultrastructural Evaluation Findings

3.2.2.1 Control Group In semi-thin sections of the control group, an oval nucleus with granular chromatin was observed in the cell layer. While cytoplasmic density appeared normal, abundant mitochondria were observed. Endoplasmic reticulum and ribosomes had normal structure (Fig. 4 a.).

3.2.2.2 Thinner Inhalation Group When the cell layer in semi-thin sections of the thinner inhalation group was compared with the control group, condensation was observed in the oval nucleus while cytoplasmic density decreased. Cell membrane deterioration, reduction in ribosome and mitochondria numbers were observed, and endoplasmic reticulum could not be distinguished. Vacuolization and dense basilar cells were encountered (Fig. 4 b).

3.2.2.3 Cortistatin Administration and Thinner Inhalation Group (Group 3) When the cell layer in semi-thin sections of the group receiving thinner inhalation and cortistatin was compared with the control group, condensation was observed in the oval nucleus while cytoplasmic density decreased. Vacuolization was present but organelles were distinguishable. Small amounts of mitochondria, ribosomes, and endoplasmic reticulum were observed (Fig. 4 c).

3.2.2.4 Melatonin Administration and Thinner Inhalation Group (Group 4) In semi-thin sections of the group receiving thinner inhalation and melatonin, when the cell layer was compared with the control group, slight condensation was observed in the oval nucleus while cytoplasmic density showed minimal decrease. While slight deterioration was observed in the nuclear membrane, no significant reduction was observed in ribosome and mitochondria numbers; however, reduction in endoplasmic reticulum number and structural deterioration were observed. Vacuolization and dense basilar cells were encountered (Fig. 4 d).

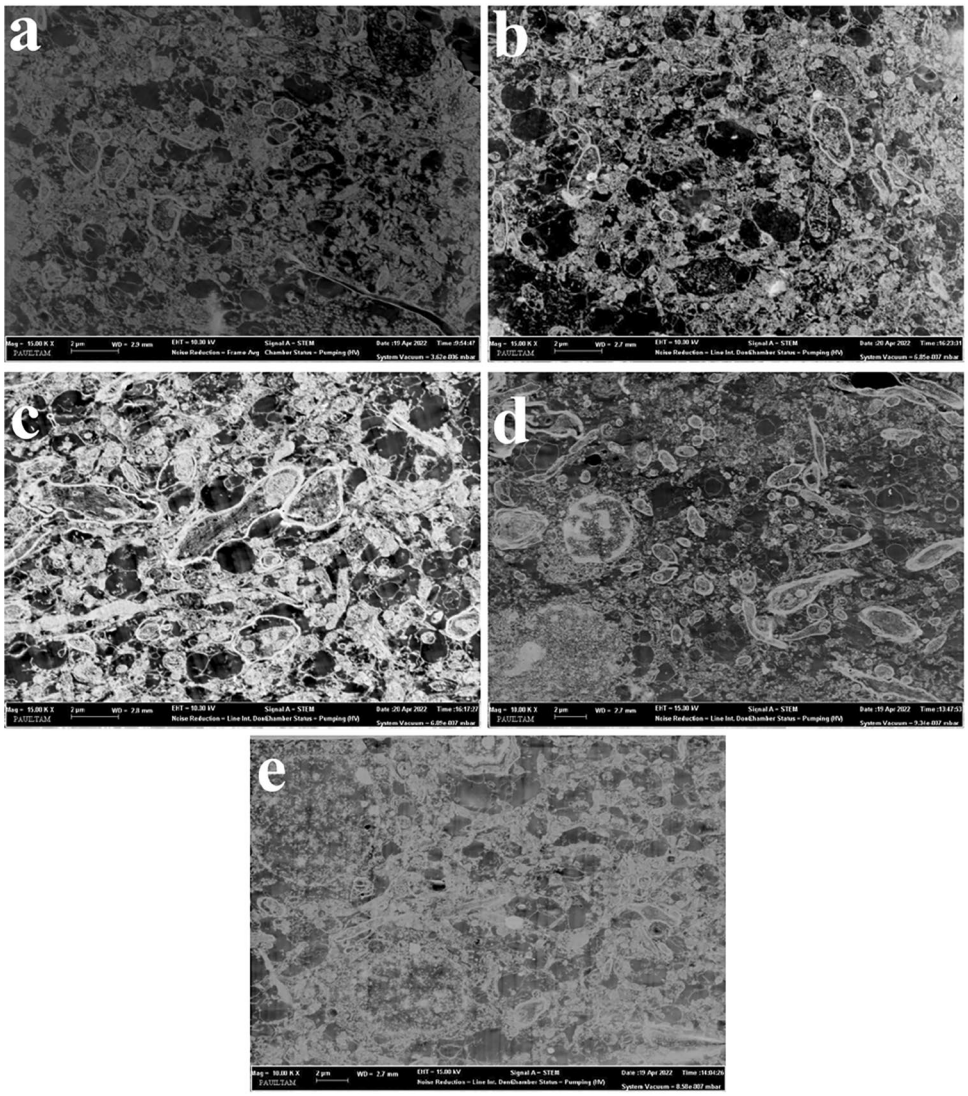


Fig. 4 Nuc. accumbens region-pyramidal cell Electron microscopy findings **a** Group 1 (Control Group), **b** Group 2 (Thinner inhaled group), **c** Group 3 (Thinner + Cortistatin), **d** Group 4 (Thinner + Melatonin), **e** Group 5 (Thinner + Melatonin + Cortistatin)

3.2.2.5 Melatonin, Cortistatin Administration and Thinner Inhalation Group (Group5) When the cell layer in semithin sections of the group receiving thinner inhalation and treated with melatonin and cortistatin was compared with the control group, condensation was observed in the oval nucleus while some reduction in cytoplasmic density was detected. Slight deterioration in the nuclear membrane and reduction in ribosome and mitochondria numbers was detected, and endoplasmic reticulum structure was similar to the control group (Fig. 4 e).

3.3 Biochemical Evaluations

3.3.1 Catalase Enzyme Activity

3.3.1.1 Nucleus Accumbens Tissue Catalase Enzyme Activity Levels Table 1. presents the catalase/protein values of subjects according to study groups. When the catalase/pro-

Table 1 MDA/protein values of subjects according to study groups

Group	MDA/protein (nmol/mg protein)			
	mean standard deviation	Median(min–max)	p^1	p^2
1	2,45 ± 0,63	2,38(1,84–3,7)	–	0,127
2	3,64 ± 1,18	3,59(2,14–5,4)	**0,042**	
3	2,34 ± 0,81	2,34(1,28–3,54)	0,755	
4	3,21 ± 1,07	3,34(1,59–4,56)	0,181	
5	2,96 ± 0,82	2,76(1,99–4,39)	0,138	
6	3,25 ± 1,42	2,94(1,45–5,96)	0,165	
7	2,13 ± 0,66	2,12(1,41–2,81)	0,731	
8	3,25 ± 0,87	3,21(2,05–4,24)	0,073	
9	3,01 ± 1,33	3,12(1,58–5,22)	0,694	

1 MDA/protein values of the groups compared to the control group (Group 1) Mann–Whitney U test

2 MDA/protein values of all groups Kruskal–Wallis test

Table 2 MDA/protein values of subjects in the first 5 groups

Group	MDA/protein (nmol/mg protein)		
	mean standard deviation	Median(min–max)	p
1	2,45 ± 0,63	2,38(1,84–3,7)	0,083
2	3,64 ± 1,18	3,59(2,14–5,4)	
3	2,34 ± 0,81	2,34(1,28–3,54)	
4	3,21 ± 1,07	3,34(1,59–4,56)	
5	2,96 ± 0,82	2,76(1,99–4,39)	

Kruskal–Wallis test

Table 3 Catalase values of subjects according to study groups

Group	Catalase /protein (k/mg protein)			
	mean standard deviation	Median(min–max)	p^1	p^2
1	0,78 ± 0,34	0,75(0,33–1,29)	–	0,499
2	0,47 ± 0,19	0,45(0,22–0,78)	0,121	
3	0,69 ± 0,28	0,72(0,27–1,16)	0,613	
4	0,65 ± 0,2	0,65(0,39–0,96)	0,445	
5	0,7 ± 0,22	0,64(0,48–1,1)	0,628	
6	0,6 ± 0,05	0,62(0,51–0,64)	0,268	
7	0,57 ± 0,15	0,55(0,41–0,76)	0,295	
8	0,55 ± 0,17	0,56(0,28–0,81)	0,209	
9	0,66 ± 0,26	0,67(0,26–0,99)	0,456	

1Catalase/protein values of the groups compared to the control group (Group 1) Mann–Whitney U test

2Catalase/protein values of all groups Kruskal–Wallis

Table 4 Catalase values of subjects in the first 5 groups

Group	Catalase /protein (k/mg protein)		
	mean standard deviation	Median(min–max)	p
1	0,78 ± 0,34	0,75(0,33–1,29)	0,297
2	0,47 ± 0,19	0,45(0,22–0,78)	
3	0,69 ± 0,28	0,72(0,27–1,16)	
4	0,65 ± 0,2	0,65(0,39–0,96)	
5	0,7 ± 0,22	0,64(0,48–1,1)	

Kruskal–Wallis test

Table 5 GPx/protein values of subjects according to study groups

Group	GPx/protein (U/mg protein)			
	mean standard deviation	Median(min–max)	p^1	p^2
1	0,59 ± 0,21	0,56(0,33–1,03)	–	0,390
2	0,37 ± 0,22	0,36(0,14–0,81)	**0,046**	
3	0,57 ± 0,26	0,5(0,29–1,06)	0,755	
4	0,51 ± 0,22	0,42(0,28–0,9)	0,536	
5	0,54 ± 0,13	0,62(0,36–0,65)	0,943	
6	0,46 ± 0,13	0,47(0,27–0,62)	0,228	
7	0,60 ± 0,14	0,64(0,36–0,76)	0,699	
8	0,47 ± 0,16	0,42(0,23–0,67)	0,336	
9	0,54 ± 0,22	0,55(0,28–1,01)	0,574	

1GPx/protein values of the groups compared to the control group (Group 1) Mann–Whitney U test

2GPx/protein values of all groups Kruskal–Wallis test

tein values of other study groups were compared to the control group (Group 1), no statistically significant difference was observed ($p > 0.05$). No statistically significant difference was determined among the catalase/protein values of all study groups ($p = 0.499$). Bold values indicate statistically significant differences ($p < 0.005$).

Table 2. compares the catalase/protein values of Groups 1, 2, 3, 4, and 5. Accordingly, the catalase/protein values of the groups were found to be statistically similar ($p = 0.297$).

3.3.2 Glutathione Peroxidase Enzyme Activity Determination

3.3.2.1 Nucleus Accumbens Tissue GPx Enzyme Levels Table 3. presents the GPx/protein values of subjects according to study groups. When theGPx/protein values of other study groups were compared to the control group (Group 1), it was found that Group 2's median GPx/protein value (0.36 [min–max: 0.14–0.81]) was significantly lower than the control group (0.56 [min–max: 0.33–1.03]) ($p = 0.046$). No statistically significant difference was detected among the GPx/protein values of all groups ($p = 0.390$).

Table 4. compares the GPx/protein values of Groups 1, 2, 3, 4, and 5. Accordingly, no statistically significant difference was observed among the groups' GPx/protein values ($p = 0.277$).

3.3.3 Superoxide Dismutase Activity Determination

3.3.3.1 Nucleus Accumbens Tissue SOD Enzyme Levels Table 5. presents the SOD/protein values of subjects according to study groups. When the SOD/protein values of other study groups were compared to the control group (Group 1), it was determined that the median SOD/protein values of Group 2 and Group 3 (1.93 [min–max: 1.59–3.45]and 2.82 [min–max: 1.23–5.6]) were significantly lower than the control group (3.67 [min–max: 2.86–4.68]) ($p < 0.001$ and $p = 0.028$). When all groups' SOD/protein values were compared, it was found that Group 2's SOD/protein values were significantly lower than Groups 1 and

Table 6 GPx/protein values of subjects in the first 5 groups

Group	GPx/protein (U/mg protein)		
	mean standard deviation	Median(min–max)	p
1	0,59 ± 0,21	0,56(0,33–1,03)	0,277
2	0,37 ± 0,22	0,36(0,14–0,81)	
3	0,57 ± 0,26	0,5(0,29–1,06)	
4	0,51 ± 0,22	0,42(0,28–0,9)	
5	0,54 ± 0,13	0,62(0,36–0,65)	

Kruskal–Wallis test

Table 8 SOD/protein values of subjects in the first 5 groups

Grup	SOD/protein (U/mg protein)		
	mean standard deviation	Median(min–max)	p
1	3,81 ± 0,6	3,67(2,86–4,68)	**0,003**
2	2,33 ± 0,81	1,93(1,59–3,45)	
3	2,96 ± 1,24	2,82(1,23–5,6)	
4	3,59 ± 0,43	3,75(2,81–4,07)	
5	3,67 ± 1	3,32(2,9–5,8)	

Kruskal–Wallis test

4 ($p = 0.014$). Bold values indicate statistically significant differences ($p < 0.05$).

Table 6. compares the SOD/protein values of Groups 1, 2, 3, 4, and 5. Accordingly, it was determined that Group 2's SOD/protein values were statistically lower than Groups 1 and 4($p = 0.003$).

3.3.4 Lipid Peroxidation Levels

3.3.4.1 Nucleus Accumbens Tissue TBARS Evaluation Table 7. presents the MDA/protein values of subjects according to study groups. When the MDA/protein values of other study groups were compared to the control group (Group 1), it was determined that Group 2's median MDA/protein value (3.59 [min–max: 2.14–5.4]) was significantly higher than the control group (2.38 [min–max: 1.84–3.7]) ($p = 0.042$). No statistically significant difference was observed among all groups' MDA/protein values ($p = 0.127$). Bold values indicate statistically significant differences ($p < 0.05$).

Table 8. compares the MDA/protein values of Groups 1, 2, 3, 4, and 5. The highest median MDA/protein value was determined in Group 2 followed by Group 4; however, this difference was not statistically significant ($p = 0.083$). Bold values indicate statistically significant differences ($p < 0.05$).

Table 7 SOD/protein values of subjects according to study groups

Grup	SOD/protein (U/mg protein)			
	mean standard deviation	Median(min–max)	p^1	p^2
1	3,81 ± 0,6	3,67(2,86–4,68)	–	**0,014**
2	2,33 ± 0,81	1,93(1,59–3,45)	**< 0,001**	
3	2,96 ± 1,24	2,82(1,23–5,6)	**0,028**	
4	3,59 ± 0,43	3,75(2,81–4,07)	0,694	
5	3,67 ± 1	3,32(2,9–5,8)	0,463	
6	3,02 ± 0,82	2,84(2,2–3,92)	0,171	
7	3,31 ± 1,62	2,76(2,2–6,41)	0,081	
8	3,24 ± 1,08	3,01(2,31–5,56)	0,054	
9	3,22 ± 0,49	3,38(2,42–3,67)	0,142	

1SOD/protein values of the groups compared to the control group (Group 1) were analyzed using the Mann–Whitney U test

2SOD/protein values of all groups were compared using the Kruskal–Wallis test, and post hoc comparisons were performed using Bonferroni

4 Discussion

This study examined the neurotoxic effects of cellulosic thinner inhalation on the *nucleus accumbens* region of the rat brain, focusing also on the potential protective roles of melatonin and cortistatin. Toluene, a volatile component of thinner, has been widely documented to exert neurotoxic effects [18–20]. In established rat models of thinner addiction, toluene is administered either alone or combined with thinner [21–23].

In this study, rats weighing 400–450 g were exposed to cellulosic thinner inhalation twice daily for six weeks. The thinner contained precisely quantified concentrations of toluene, acetone, isobutyl acetate, and isobutanol. This protocol aligns with previously described animal models [24–26], although the chemical composition and duration differed.

The clinical manifestations observed following thinner inhalation were consistent with prior reports [25, 27] and included yellowing of fur, excessive licking, increased secretions, huddling in a cage corner, dyspnea, and frequent blinking.

A key novelty of this work is the targeted analysis of thinner-induced neurotoxicity in the *nucleus accumbens*. Our literature review revealed no prior studies addressing this brain region in the context of thinner exposure; thus, our results were compared with studies on other brain regions.

Transmission electron microscopy (TEM) revealed that pyramidal cells in controls exhibited an oval-shaped nucleus, whereas in the thinner-only group, this nuclear morphology

was markedly disrupted. This aligns with [28] who described ultrastructural mitochondrial, neurofilament, and plasma membrane damage in the cerebellum after paint thinner exposure. Qualitative assessment showed that nuclear integrity was better preserved in the melatonin + thinner, cortistatin + thinner, and melatonin + cortistatin + thinner groups compared with the thinner-only group, suggesting a cell-protective effect.

Light microscopy revealed hypoxic-like changes and moderate-to-severe pyramidal cell degeneration in the thinner-only group. These observations are consistent with [29] who reported significant neuronal loss in the gyrus dentatus after toluene inhalation. In our study, pyramidal cell counts in the *nucleus accumbens* were reduced, with damage graded as mild-to-moderate or moderate-to-severe. All histopathological evaluations were conducted by a single pathologist to ensure consistency.

The elevation in lipid peroxidation supports the conclusion that thinner inhalation induces oxidative stress. Consistent with prior work [30, 31] our findings show significantly higher median MDA/protein values in the thinner-only group ($p = 0.042$) compared with controls. This supports the hypothesis that thinner's neurotoxicity is mediated via enhanced lipid peroxidation. Higher TBARS levels in the thinner-only group relative to all treatment groups further confirmed oxidative stress, demonstrating concordance between microscopic and biochemical results.

Antioxidant enzyme activity analysis revealed significant reductions in GPx ($p = 0.046$) and SOD ($p < 0.001$) in the thinner-only group compared with controls, indicating overwhelmed endogenous defenses. Importantly, SOD activity was significantly higher in the melatonin + thinner group than in the thinner-only group ($p = 0.014$), statistically validating melatonin's potent antioxidant capacity against thinner-induced oxidative damage.

No previous studies have evaluated CAT, SOD, MDA, or GSH-x levels in the *nucleus accumbens* following melatonin and cortistatin treatment after thinner inhalation, highlighting the novelty of our biochemical findings.

Both agents, alone or combined, attenuated thinner-induced neuronal damage. Light and electron microscopy revealed milder pyramidal cell injury in the combined treatment and melatonin + thinner groups compared with the thinner-only group, suggesting potential additive or synergistic neuroprotective mechanisms.

Although limited, available studies on cortistatin indicate anticonvulsant, neuroprotective, and neuroregenerative properties. Our data extend these observations by demonstrating cortistatin's ability to reduce thinner-induced injury, both independently and when co-administered with melatonin [32–34].

5 Conclusion

Chronic cellulosic thinner inhalation induces pronounced histological, ultrastructural, and biochemical alterations in the rat nucleus accumbens, including laminar thinning, nuclear degeneration, and elevated oxidative stress. Administration of melatonin or cortistatin individually attenuated these changes, preserving neuronal architecture and reducing oxidative imbalance. Notably, combined treatment conferred superior protection against cellular and ultrastructural injury, suggesting a synergistic neuroprotective effect. These findings position melatonin and cortistatin as potential therapeutic agents for mitigating solvent-induced neurotoxicity and warrant further investigation into their combined mechanisms of action.

5.1 Limitations

Although this study provides valuable insights into the potential neuroprotective effects of melatonin and cortistatin against thinner-induced damage in the nucleus accumbens, several limitations should be taken into account. Although histological, biochemical, and ultrastructural evaluations were included in the experimental design, behavioral and cognitive tests were not included; therefore, functional implications were limited.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical Approval The study conformed to the Declaration of Helsinki and was approved by the ethics committees at Akdeniz University, including the institutional review board (Protocol No: 1034/2020.02.013).

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