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THE NEUROPROTECTIVE EFFECTS OF AN ETHANOLIC TURMERIC (Curcuma longa L.) EXTRACT AGAINST TRIMETHYLTIN-INDUCED OXIDATIVE STRESS IN RATS

--Manuscript Draft--

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The Editor

Nutritional Neuroscience

Dear Editor,

Re: THE NEUROPROTECTIVE EFFECTS OF AN ETHANOLIC TURMERIC (*Curcuma longa* L.) EXTRACT AGAINST TRIMETHYLTIN-INDUCED OXIDATIVE STRESS IN RATS

By Sapto Yuliani, Mustofa, and Ginus Partadiredja

We would like to express our gratitude to the editor and the reviewers for their comments on our manuscript in your email dated 31 January 2018. We also would like to thank the editor for giving us chances to improve our manuscript and thus to make it suitable for publication in the Nutritional Neuroscience journal. Please find our responses to the comments of the reviewer below and how we have improved our manuscript accordingly (additional sentences written in red):

Reviewer #1:
1. In the method section of the abstract please state the experimental design in more detail. There were six groups....., etc. Please list the doses of turmeric extract that were dministered.

Our response: We comply with the reviewer request. We have added more detailed information of the experimental design in the abstract.

2. The experimental design includes administration of turmeric extract before and after administration of trimethyltin. Since, in most cases, prophylactic treatment cannot be administered for neurodegenerative disorders because treatment starts after the disorder is

diagnosed, the strongest experimental design would be to administer the insult that produces neurodegeneration and then administer the treatment to see if neurodegeneration could be halted or reversed. However, the study design used does shown an effect on the oxidative stress markers in the brain. It is not clear if it is a protective effect or a treatment effect. In several places the discussion refers to the effect as protective. Please clarify the language to be consistent with the study design.

Our response: We have added an explanation on this preventive issue in paragraph 2 of the discussion section. It is true that the design of the experiment was for preventive.

3. The results show that turmeric has activity similar to the positive control at a dose of 200 mg/kg but not at 100 or 300 mg/kg. Is there a reason those 3 doses were chosen? If so, please include that information.

Our response: We have added the information about the reason and the reference for using the doses in the experimental design sub-section of the methods.

Inverted U curves are not uncommon in pharmacology studies and they usually represent some change in pharmacological activity as the concentration of test material at the receptor changes. Do you know what receptors or other targets turmeric might act on to alter oxidative stress? If so, it would be helpful to discuss a possible mechanism in the discussion, which could lead to further, more mechanistic studies.

Our response: We have added an explanation about this matter in the paragraph 3 of the discussion section.

4. The discussion suggests that the new formulation of turmeric may be less bioavailable. If possible, addition of PK data from plasma and brain tissue would strengthen the paper. If not possible for this study, please consider it for future studies.

Our response: We thank the reviewer for the important suggestion of incorporating PK data from plasma and brain tissue. However, it is not possible to be conducted at present since this study has been accomplished. Nevertheless, we have included the suggestion in the last sentence of paragraph 5 of the discussion section.

5. Please check for misspellings and typos. Page 14, third line from top, "oxygen" is spelled "oxygen".

Our response: We have made the correction of "oxigen" into "oxygen"

Once again, we thank the editor and reviewer for giving us positive comments, and therefore enabling us to improve our manuscript to be suitable for publication in the Nutritional Neuroscience journal. Please find the attached file where we have made amendments accordingly.

Yours sincerely,

Ginus Partadiredja

Oxidative stress

imbalance between ROS production and elimination by the antioxidant defense system (SOD,GSH,GPx, CAT)

Trimethyltin (TMT)

ROS/RNS

Neurodegeneration model in rats

Turmeric extract containing 28.08% of curcumin

THE NEUROPROTECTIVE EFFECTS OF AN ETHANOLIC TURMERIC (*Curcuma longa* L.) EXTRACT AGAINST TRIMETHYLTIN-INDUCED OXIDATIVE STRESS IN RATS

ABSTRACT

Introduction: Oxidative stress is known to contribute to the pathogenesis of neurodegenerative disorders. An ethanolic turmeric (*Curcuma longa* L.) extract containing curcumin has been reported to produce antioxidant effects.

Objective: The present study aims to investigate the possible neuroprotective effects of the ethanolic turmeric extract against trimethyltin (TMT)-induced oxidative stress in Sprague Dawley rats.

Methods: Rats were divided randomly into six groups, i.e. N group, which served as a normal control group; T group, which was given intra-peritoneal injection of TMT chloride; T-Cit group, which was treated with oral citicoline and TMT chloride injection; and three turmeric-treated groups (T-TE100, T-TE200, T-TE300) which were given 100, 200 and 300 mg/kg bw of turmeric rhizome extract orally, as well as TMT chloride. The treatments were administered to the TMT-exposed rats from day 1 to day 28 of the experiment. The TMT chloride injection was administered as a single dose of 8 mg/kg bw on day 8 of the experiment. The plasma and brain malondialdehyde (MDA) levels and reduced glutathione (GSH) levels and the activities of the superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) enzymes in the brain were examined at the end of the experiment.

Results: The TMT injection resulted in a significant increase in the plasma and brain MDA levels and a decrease in the SOD, CAT, and GPx enzyme activities as well as GSH levels in the

brain. The administration of 200 mg/kg bw of the ethanolic turmeric extract prevented oxidative stress by decreasing the plasma and brain MDA levels and increasing the SOD, CAT, and GPx enzyme activities and GSH levels in the brain. These effects seem to be comparable to those of citicoline.

Discussion: The ethanolic turmeric extract at a dose of 200 mg/kg bw may exert neuroprotective effects on TMT-exposed Sprague Dawley rats by preventing them from oxidative stress.

Keywords: neurodegeneration**;** *Curcuma longa* L.; trimethyltin; oxidative stress; antioxidant

1. Introduction

Oxidative stress, which is an imbalance between reactive oxygen species production and elimination by the antioxidant defense system, is considered to underpin the pathogenesis of neurodegenerative disorders [1]. Brain cells are very sensitive to oxidative damage, due to the high oxygen demand of the brain. Oxidative damage to lipids consisting of unsaturated fatty acids (lipid peroxidation) yields end products such as malondialdehyde (MDA), 4-hydroxy-2,3-nonenal (HNE), acrolein, and other compounds [2] that exert negative effects on human health.

Endogenous antioxidant enzymes, such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), serve to protect the cells from the oxidative damage [3]. However, while the unsaturated fatty acid content of the brain is high, the antioxidant defense system of the brain is weaker compared to that of other organs. Therefore, oxidative stress may easily cause the degeneration of the hippocampus, a major brain region for memory processing. In turn, such degeneration may play a role in the pathogenesis of neurodegenerative disorders, including dementia [4].

Trimethyltin (TMT) is an organometal that has neurotoxic effects. TMT enhances the formation of reactive oxygen species in the hippocampus. TMT also increases the expression of important factors related to the pathophysiology of Alzheimer's disease (AD) such as amyloid precursor protein (APP), presenilin and c-fos in the limbic system [5]. Therefore, TMT-intoxicated rats may serve as valuable models for studies of neurodegenerative disorders, such as Alzheimer's disesase [6].

Curcuma longa, L. or turmeric is widely known as a dietary food ingredient and traditionally used as a medicinal herb. The potential use of turmeric as an alternative medicine is attributed to its antioxidant properties. *In vitro* and *in vivo* studies showed that the active

compound of turmeric extract, curcumin, prevented oxidative stress-induced neurodegeneration in the nervous system [7,8]. Our previous studies have shown that an ethanolic turmeric extract prevented the deficits of the spatial memory and the estimated total number of hippocampal pyramidal cells [9], as well as inhibited the apoptosis of the pyramidal neurons in the CA2-CA3 region, but not in the CA1 region of the hippocampus of TMT-exposed rats [10]. The present study extends these studies and aims to determine the effects of the ethanolic extract of turmeric on the levels of oxidative stress markers in the brains and plasma of Sprague Dawley rats exposed to TMT. The effects of the turmeric extract were compared to those of citicoline (citidine-5-diphosphocholine or CDP-choline) (an endogenous compound that stabilizes membrane function and reduces free radical generation), which is a drug of choice for dementia.

2. Methods

2.1. Animals

Thirty-six adult male Sprague Dawley rats weighing approximately 195-215 g were obtained from the animal house of Universitas Gadjah Mada. The animals were housed individually in cages under standard conditions (room temperature: 24–26 °C; humidity: 60–65%; 12/12-h natural light/dark cycle). The animals had free access to food and water. The rats were acclimatized for at least 6 days prior to the study. The experimental protocols and animal handling procedures were approved by the Ethics Committee of the Integrated Research and Testing Laboratory, Universitas Gadjah Mada (approval number 130/KEC-LPPT/XII/2013).

2.2. Extraction of the Turmeric Rhizome

The turmeric rhizome was obtained from CV. Merapi Farma, Yogyakarta, Indonesia, and verified by a botanist from the Department of Biology, Universitas Ahmad Dahlan, Yogyakarta, Indonesia. The extract was prepared as follows: five hundred grams of the turmeric rhizome powder were macerated in 1.25 L of 96% ethanol (Merck, Darmstat, Germany). The maceration was repeated twice, followed by filtration. The resulting filtrate was concentrated using a vacuum rotary evaporator (Heidolph, Germany) under reduced pressure at 40°C. The final yield of the turmeric extract was 30.38 % *w*/*w*. The concentration of curcumin that represented the major active content of the extract was determined using a TLC scanner densitometer (CAMAG, Switzerland) operated at a wavelength of 426 nm. The curcumin level obtained was 28.08%. The turmeric rhizome extract was finally dissolved in a sodium-carboxymethyl cellulose (CMC-Na) solution prior to oral administration to the rats.

2.3. Experimental Design

The rats were randomly divided into six groups as follows: the N group served as a normal or vehicle group and was treated with the CMC-Na solution (the vehicle of turmeric extract) and an intraperitoneal injection of 0.9% saline; the T group served as control group and was orally administered the CMC-Na solution and intraperitoneally injected with TMT chloride (Sigma-Aldrich, Inc., St. Louis, USA) dissolved in 0.9% saline [11]; the T-Cit group served as a positive control group and was orally administered 200 mg/kg bw of a citicoline solution (Bernofarm Pharmaceutical Company, Indonesia) and injected with TMT chloride; and the T-TE100, T-TE200, and T-TE300 groups, which were orally administered 100 mg/kg bw, 200 mg/kg bw, and 300 mg/kg bw, respectively, of the ethanolic extract of turmeric and intraperitoneally injected with TMT chloride. The doses of the extract adopted in the present study referred to the curcumin doses applied in Awasthi et al.'s study [12]. The curcumin doses were converted into turmeric extract doses which were considered acceptable for humans. Each group consisted of six rats. The ethanolic extracts of turmeric and the citicoline solutions were administered daily from day 1 to day 28 of the experiment. The TMT chloride injection was administered as a single dose of 8 mg/kg bw on day 8 of the experiment. The turmeric extracts and the citicoline solutions were deliberately administered daily for a week prior to the TMT injection with the purpose to give a prophylactic protection to the rats. At day 36, the rats' blood was taken from the sinus orbitalis. These blood samples were used to determine the plasma MDA levels. Then, all rats were sacrificed and the cerebral hemispheres of the rats were dissected from their skulls. The left cerebral hemispheres were homogenized at 4°C using a homogenizer (Potter-Elvehjem Tissue Grinders, Wheaton, USA). The homogenates were used for the biochemical assays. In the present study the cerebral hemispheres were used for the experiment since in the brain of AD

patients, oxidative stress products are found not only in the hippocampus but also in other regions of the brain such as in the temporal lobe [13].

2.4. Experimental Procedures

2.4.1. Measurement of the Plasma Malondialdehyde (MDA) Levels

The plasma MDA levels were measured using a previously described method [14]. Briefly, 0.75 mL of phosphoric acid was poured into a tube filled with 0.25 mL of thiobarbituric acid (TBA) (Sigma Aldrich, Inc., St. Louis, USA). Subsequently, 0.05 mL of the blood plasma and 0.45 mL of water were added to the tube, and this mixture was blended with a vortex for 2 minutes. The mixture was then heated in a water bath (Memmert, Germany) for 60 minutes at 60°C. After being cooled for 1-2 hours, the mixture was poured into a Sep-Pak C18 (Waters) column to bind with MDA, and washed with 5 mL of methanol and water. The absorbance of the mixture was examined using a spectrophotometer (Shimadzu - 1601, Japan) at a wavelength of 532 nm. The absorbance of the mixture was compared with that of a 1,1,3,3 - tetraethoxypropane (TEP) solution (Sigma Aldrich, Inc., St. Louis, USA), which served as a standard. The MDA values were expressed as mmol/L plasma.

2.4.2. Measurement of the Brain MDA Levels

The brain MDA levels were determined according to the method described in the study by Colado et al. [15]. Five hundred microliters of the tissue homogenate dissolved in phosphate buffer solution (pH 7.4) were mixed with 300 μL of 30% trichloroacetic acid (TCA), 150 μL of 5 N HCl, and 300 μL of 2% w/v TBA. The mixture was heated at 90°C for 15 min and centrifuged at 12,000 g for 10 min. The absorbance of the supernatant was read in a spectrophotometer at a

wavelength of 532 nm. 1,1,3,3-tetraethoxypropane was used as a standard. The MDA level was expressed as nmol/mg tissue.

2.4.3 Measurement of the Brain Superoxide Dismutase (SOD) Activity

The SOD activity in the brain tissues was determined using an Assay Kit, Cat. K335 -100 (BioVision, USA). The tissues were homogenized in 0.2 mL of 0.1 M Tris/0.1 M HCl (pH 7.4) and centrifuged at 14.000 rpm for 5 min at 4°C. The supernatants of these mixtures were used to measure the activity of the SOD enzyme. Three blank solution wells were used for each sample. Twenty microliters of supernatant were added into the sample well and blank well 2. Meanwhile, 20 µL of distilled water were added into blank wells 1 and 3. At the same time, 200 µL of working solution were added to each well and 20 µL of buffer solution were added to blank wells 2 and 3. Twenty microliters of the enzyme solution was added into each of the wells and the mixture was homogenized by wrapping the microplate with aluminum foil and shaking it. These mixtures were then incubated at 37°C for 20 min. The absorbance of these mixtures was measured at a wavelength of 450 nm using a microplate reader (Bio Rad model 680 XR, USA). The SOD activity was normalized to the milligrams of protein used in the assay and expressed as ng/mg protein.

2.4.4. Measurement of the Brain Catalase (CAT) Activity

The CAT enzyme activity in the tissues was determined using an Assay Kit, Cat. K773-100 (BioVision, USA). In this assay, catalase first reacts with H_2O_2 to produce water and oxygen. The unconverted H_2O_2 reacts with an OxiRed™ probe to produce water and oxygen. This reaction is assessed by a colorimetric method. Briefly, the brain tissue homogenates were

centrifuged at 10.000 rpm for 15 min at 4°C in cold assay buffer, and the supernatants were collected for the assay. The assay was performed in triplicate using 96-well microplates. The rate of H_2O_2 decomposition was measured at a wavelength of 570 nm in a microplate reader (model 680 XR, Bio Rad laboratories, USA). One unit of catalase was defined as the amount of enzyme needed to decompose 1 μM of H_2O_2 in 1 min. The CAT activity was normalized to the milligrams of protein used in the assay and expressed as mU/mg protein.

2.4.5. Measurement of the Brain Reduced Glutathione (GSH) Levels

The GSH content of the brain tissues was analyzed using a colorimetric GSH detection kit according to the manufacturer's instructions (Cat. K261-100, BioVision, USA). One hundred milligrams of the brain tissue homogenates dissolved in 0.4 mL of glutathione buffer were mixed with 100 μL of 5% SSA, and centrifuged at 8,000 rpm for 10 min. The supernatant of the mixture was transferred into a fresh tube and used for the glutathione assay. One hundred sixty microliters of a reaction mixture containing the NADPH generating mix, glutathione reductase, and glutathione reaction buffer was added to 96-well plates and incubated at room temperature for 10 min to generate NADPH. Twenty microliters of either the GSH standard solutions or the supernatant was pipetted into the wells, and the plates were incubated at the room temperature for 10 min. The GSH concentrations in the sample solutions were calculated using the standard glutathione calibration curve and expressed as μg/mg protein.

2.4.6. Measurement of the Brain Glutathione Peroxidase (GPx) Activity

The GPx activity was determined using an assay kit (Cat. K762-100, BioVision, USA) according to the manufacturer's instructions. Approximately 0.1 g of the brain tissue homogenates dissolved in 0.2 mL cold assay buffer was centrifuged at 10,000 rpm for 15 min at

4°C. The supernatant of this mixture was collected and used for the assay. Ten microliters of the supernatants were transferred into 96-well plates. Forty microliters of reaction mix (containing 33 µL of assay buffer, 3 µL of a 40 mM NADPH solution, 2 µL of GR solution and 2 µL of GSH solution) were added to the plates to deplete the GSSG in the samples and this mixture was incubated for 15 min. Ten microliters of a cumene hydroperoxide solution was added to the mixture to start the GPx reaction. The GPx activity was measured at a wavelength of 340 nm at 5 min and 140 min. The NADPH levels were calculated based on a standard curve. The GPx activity was expressed as mU/mg protein.

2.4.7. Estimation of the Brain Protein Concentrations

The brain protein concentrations were determined using a biuret method. One hundred milligrams of the brain homogenates were mixed with 0.4 mL of distilled water and centrifuged at 8.000 rpm for 15 min. Forty microliters of the supernatant of this mixture was pipetted into tubes and distilled water was added until the volume of the mixture reached 150 µL. The mixture was then mixed with 100 µL of biuret reagent and incubated at the room temperature for 30 min. The absorbance of this mixture was measured at a wavelength of 540 nm using a microplate reader (model 680 XR, Bio-Rad Laboratories, USA). The protein concentrations were calculated based on a standard curve and expressed as mg/mL.

2.5. Statistical Analyses

All data were tested using the Shapiro-Wilk and Levene tests for the normality and homogeneity of the data, respectively. When the data were normally distributed and homogenous, the data were analyzed using one-way ANOVA, followed by the post hoc Tukey's

HSD test. When the data were not normally distributed or homogenous, they were analyzed using the Kruskal-Wallis test, followed by the Mann-Whitney U test. All data were analyzed using SPSS software version 21. The significance level was set at $p < 0.05$.

3. Results

Figures 1 – 6 present the data for the plasma MDA and brain MDA and GSH levels, as well as the SOD, CAT, and GPx enzyme activities. The injection of a single dose of 8 mg/kg bw of TMT in the T group significantly increased the plasma ($p = 0.002$) and brain MDA ($p = 0.002$) levels and decreased the activities of the SOD ($p = 0.004$), CAT ($p = 0.0001$), and GPx ($p = 0.002$) enzymes and the level of GSH ($p = 0.002$) in the brain compared to the N group.

The administration of 100 mg/kg bw of the ethanolic turmeric extract to the TMT-injected rats (T-TE100) significantly decreased the plasma MDA levels, but not the brain MDA levels. However, this dose of the ethanolic turmeric extract failed to reverse the TMT-induced decrease in the activities of the SOD ($p = 0.132$), CAT ($p = 0.466$), and GPx ($p = 0.310$) enzymes and GSH levels ($p = 0.602$) in the rats' brains.

The administration of 200 mg/kg bw of the ethanolic turmeric extract to the TMT-injected rats (T-TE200) significantly decreased the plasma ($p = 0.002$) and brain ($p = 0.002$) MDA levels. The activities of the SOD, CAT, and GPx enzymes and GSH levels in the brain were significantly increased after the administration of 200 mg/kg bw of the ethanolic turmeric extract ($p = 0.015$; $p = 0.045$; $p = 0.015$; and $p = 0.015$, respectively) compared to the T group.

The administration of 300 mg/kg bw of the turmeric extract decreased the plasma MDA levels ($p = 0.002$), but not the brain MDA levels, in the TMT-injected rats (T-TE300). This dose of the ethanolic turmeric extract was not able to prevent the TMT-induced decrease in the SOD, CAT, and GPx enzyme activities and GSH levels compared to the T group.

The administration of 200 mg/kg bw of citicolin to the TMT-injected rats (T-Cit) decreased the plasma ($p = 0.002$) and brain ($p = 0.002$) MDA levels. The current dose of citicoline also significantly increased the activities of the SOD ($p = 0.002$), CAT ($p = 0.006$) and

GPx ($p = 0.002$) enzymes in the rats' brains compared to the T group. There was no significant difference in the GSH levels between the T-Cit and N groups ($p = 0.070$) or between the T-Cit and T groups ($p = 0.117$) [16].

4. Discussion

In the present study, we found that rats injected with a single dose of TMT exhibited an increase in the plasma and brain MDA levels and a decrease in the activities of antioxidant enzymes (SOD, CAT, and GPx) and the levels of a non-enzymatic antioxidant co-factor (GSH). TMT is a potent neurotoxicant that can induce severe damage and neuronal death in both the human and animal limbic system, particularly in the hippocampus. TMT is currently regarded as a useful tool to obtain an animal model of neurodegeneration associated with cognitive impairment, such as dementia. However, the precise mechanisms of its effects are not entirely clear [17]. In general, it is thought that the toxicity of organotin compounds is related to the number and length of the alkyl groups attached to the tin atom. Its toxicity also depends on the compound's affinity for neurons [18]. Previous studies reported that TMT-induced neuronal damage was mediated by derivatives of reactive oxygen species (ROS) and reactive nitrogen species (RNS). At high concentrations, ROS, such as hydrogen peroxide (H_2O_2), superoxide anion (O2•−) and hydroxyl radical (HO•), can be important mediators of damage to cell structures, including peroxidation of lipids, oxidation of proteins, damage to nucleic acids, and enzyme inhibition [19]. In hippocampal cells, TMT intoxication increased membrane lipid peroxidation, ultimately leading to cell death. This impact of oxidative stress has been found to be implicated in a wide range of neurological disorders including stroke, trauma and dementia [20].

The 200 mg/kg bw dose of the ethanolic turmeric extract has been shown to exert the highest efficacy compared to both the lower and higher doses examined in the current study. The administration of turmeric extract prior and subsequent to TMT exposure has been shown to prevent the brain of rats from oxidative stress. TMT produces delayed toxicity where the most

dramatic reduction of pyramidal cell numbers of TMT-treated rats occurs between 14 and 28 days after TMT injection [21]. In this study the preventive effect of turmeric extract was evidenced by the decrease of the plasma and brain MDA levels and by the increase of the CAT, GPx, and SOD enzyme activities and GSH levels in the TMT-injected rats. All parameters were measured at the end of the experiment.

It is well known that curcumin, the major compound of the ethanolic turmeric extract, is capable of attenuating the production of reactive oxygen species (ROS) and neutralizing harmful free radicals such as superoxide anions, hydroxyl radicals and nitric oxide [22]. The phenolic and the methoxy groups on the phenyl ring appear to be important structural features for these actions [23]. Curcumin induces endogenous antioxidant defense mechanisms by modulating nuclear factors such as E2-related factor 2 (Nrf2) [24], activator protein-1 (AP-1), and nuclear factor kappa B (NFκB). This in turn implicates the expression of a variety of genes in response to oxidative stress [25]. Phosphorylation of Nrf2 allows the Nrf2 to translocate to the nucleus and bind to the antioxidant response element (ARE) in the regulatory region of the target genes and induce the transcription of antioxidant enzymes [26].

Pre-clinical studies have shown that orally delivered curcumin has neuroprotective effects [27] and has been found to reduce lipid peroxidation by maintaining the activities of antioxidant enzymes such as CAT, SOD, GPx and GSH levels in rats exposed with neurotoxic compounds [28,29]. The ability of curcumin to increase the GSH levels is due to its affinity to induce the transcription of the mRNAs for the GSH biosynthetic genes [30]. Clinical studies also demonstrated that oral curcumin remarkably improved the behavioral symptoms of patients with Alzheimer's disease [31] and ameliorated systemic oxidative stress on human with chronic pulmonary complication [32].

The particular method of preparation of curcumin may enhance the above-mentioned beneficial effects of curcumin. Curcumin emulsified in carboxymethyl cellulose has a potent anti-inflammatory effect [33]. Curcumin in its dissolved or emulsified form has been shown to be easily absorbed in the gastrointestinal system of rats [34]. Furthermore, curcumin administrated orally is considered to cross the blood-brain barrier as shown in a multi-photon microscopy study on in vivo Alzheimer's model of mice [35]. However, in the present study, it remains uncertain whether the reconstituted turmeric actually crossed the blood brain barrier, since we used turmeric extract instead of curcumin, and applied a different method of dissolution from that of Cui et al and Garcia-Alloza et al's studies [34,35]. Further investigation on the pharmacokinetics of turmeric extract is required to determine the bioavailability of curcumin in both the brain and plasma.

The present study did not observe a linear dose-response relationship between the effects of the ethanolic turmeric extract on TMT-induced oxidative stress. The higher doses of the ethanolic turmeric extract (300 mg/kg bw) did not correspond to increased activity of the extract. This may be due to paradoxical effects of curcumin, which can act as an antioxidant or pro-oxidant, depending on the concentration and the cellular environment [36]. The pro-oxidant activities appear to be mediated by the generation of phenoxyl radical of curcumin by the peroxidase–H_2O_2 system, which co-oxidizes cellular glutathione or NADH to form ROS [37]. Curcumin-mediated apoptosis is closely related to the increase in the intracellular ROS levels. The exact dose of curcumin that can cause the pro-oxidative effects remains to be determined.

The antioxidant activities of the 200 mg/kg bw dose of the turmeric extract seem to resemble those of citicoline in several parameters (plasma and brain MDA levels; SOD, GPx, and CAT enzyme activities). Citicoline is commonly used as a neuroprotective and memory

enhancing agent [38]. In this study, this drug was shown to reduce the plasma and brain MDA levels, as well as to increase the levels of GSH and the GPx, SOD and CAT enzyme activities in the brains of rats exposed to TMT. Another study reported that citicoline stimulated the biosynthesis of glutathione, activated the glutathione reductase enzyme, and prevented lipid peroxidation [39].

In summary, the ethanolic turmeric extract prevented TMT-induced oxidative stress at a dose of 200 mg/kg bw by decreasing the plasma and brain MDA levels and increasing the SOD, CAT, and GPx enzyme activities as well as GSH levels in the brain. These effects seem to be similar to those of citicoline. Further studies are required to investigate the potential use of this extract as a substitute treatment for neurodegenative disorders.

Declaration of Interest

The authors report no declarations of interest.

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Figure captions

Figure 1. Comparison of the plasma MDA levels in the normal (N), control TMT (T), Citicolin (T-Cit), 100 mg/kg bw of the ethanolic turmeric extract (T-TE100), 200 mg/kg bw of the ethanolic turmeric extract (T-TE200) and 300 mg/kg bw the ethanolic turmeric extract (T-TE300) groups of Sprague Dawley rats exposed to TMT. The values are expressed as the means ± SEM of each group (n = 6). *$p < 0.05$, compared to the T group; $^{\#}p < 0.05$, compared to the N group; Kruskal-Wallis test, followed by the Mann-Whitney U test.

Figure 2. Comparison of the brain MDA levels in the normal (N), control TMT (T), Citicolin (T-Cit), 100 mg/kg bw of the ethanolic turmeric extract (T-TE100), 200 mg/kg bw of the ethanolic turmeric extract (T-TE200) and 300 mg/kg bw of the ethanolic turmeric extract (T-TE300) groups of Sprague Dawley rats exposed to TMT. The values are expressed as the means ± SEM of each group (n = 6). *$p < 0.05$, compared to the T group; $^{\#}p < 0.05$, compared to the N group; Kruskal-Wallis test, followed by the Mann-Whitney U test.

Figure 3. Comparison of the brain SOD activity in the normal (N), control TMT (T), Citicolin (T-Cit), 100 mg/kg bw of the ethanolic turmeric extract (T-TE100), 200 mg/kg bw of the ethanolic turmeric extract (T-TE200) and 300 mg/kg bw of the ethanolic turmeric extract (T-TE300) groups of Sprague Dawley rats exposed to TMT. The values are expressed as the means ± SEM of each group (n = 6). *$p < 0.05$, compared to the T group; $^{\#}p < 0.05$, compared to the N group; Kruskal-Wallis test, followed by the Mann-Whitney U test.

Figure 4. Comparison of the brain catalase activity in the normal (N), control TMT (T), Citicolin (T-Cit), 100 mg/kg bw of the ethanolic turmeric extract (T-TE100), 200 mg/kg bw of the ethanolic turmeric extract (T-TE200) and 300 mg/kg bw of ethanolic turmeric extract (T-TE300) groups of Sprague Dawley rats exposed to TMT. The values are expressed as the means ± SEM of each group (n = 6). *p < 0.05, compared to the T group; $^{\#}$p < 0.05, compared to the N group; one-way ANOVA test, followed by Tukey's HSD test.

$F_{5, 30} = 7.533$; p <0.05

Figure 5. Comparison of the brain GSH levels in the normal (N), control TMT (T), Citicolin (T-Cit), 100 mg/kg bw of the ethanolic turmeric extract (T-TE100), 200 mg/kg bw of the ethanolic turmeric extract (T-TE200) and 300 mg/kg bw of the ethanolic turmeric extract (T-TE300) groups of Sprague Dawley rats exposed to TMT. The values are expressed as the means ± SEM of each group (n = 6). *p < 0.05, compared to the T group; $^{\#}$p < 0.05, compared to the N group; one-way ANOVA, followed by Tukey's HSD test.

$F_{5, 30} = 4.214$; $p < 0.05$

Figure 6. Comparison of the GPx activity in the normal (N), control TMT (T), Citicolin (T-Cit), 100 mg/kg bw of the ethanolic turmeric extract (T-TE100), 200 mg/kg bw of the ethanolic turmeric extract (T-TE200) and 300 mg/kg bw of the ethanolic turmeric extract (T-TE300) groups of Sprague Dawley rats exposed to TMT. The values are expressed as the means ± SEM of each group (n = 6). *p < 0.05, compared to the T group; #p < 0.05, compared to the N group; Kruskal-Wallis test, followed by the Mann-Whitney U test.

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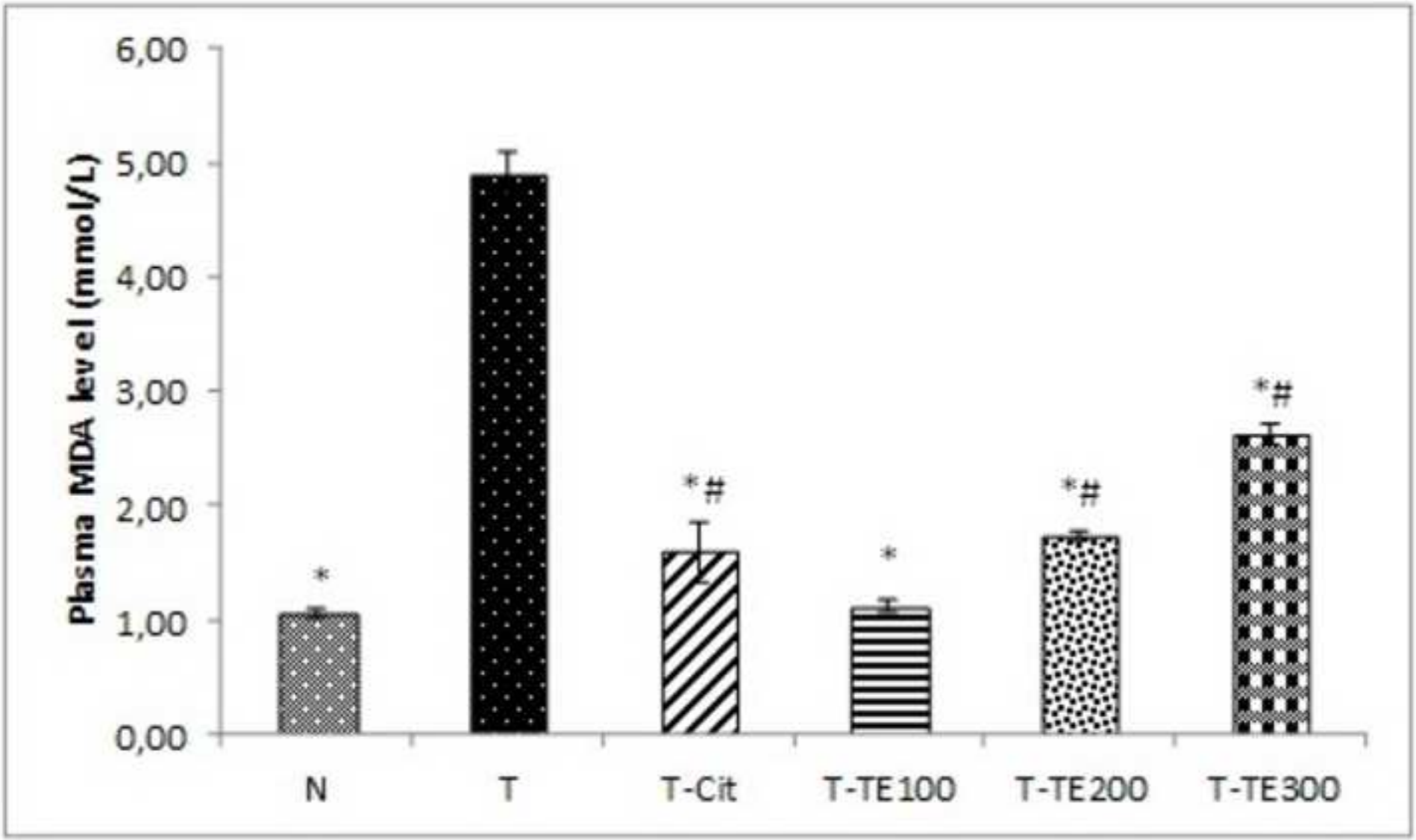

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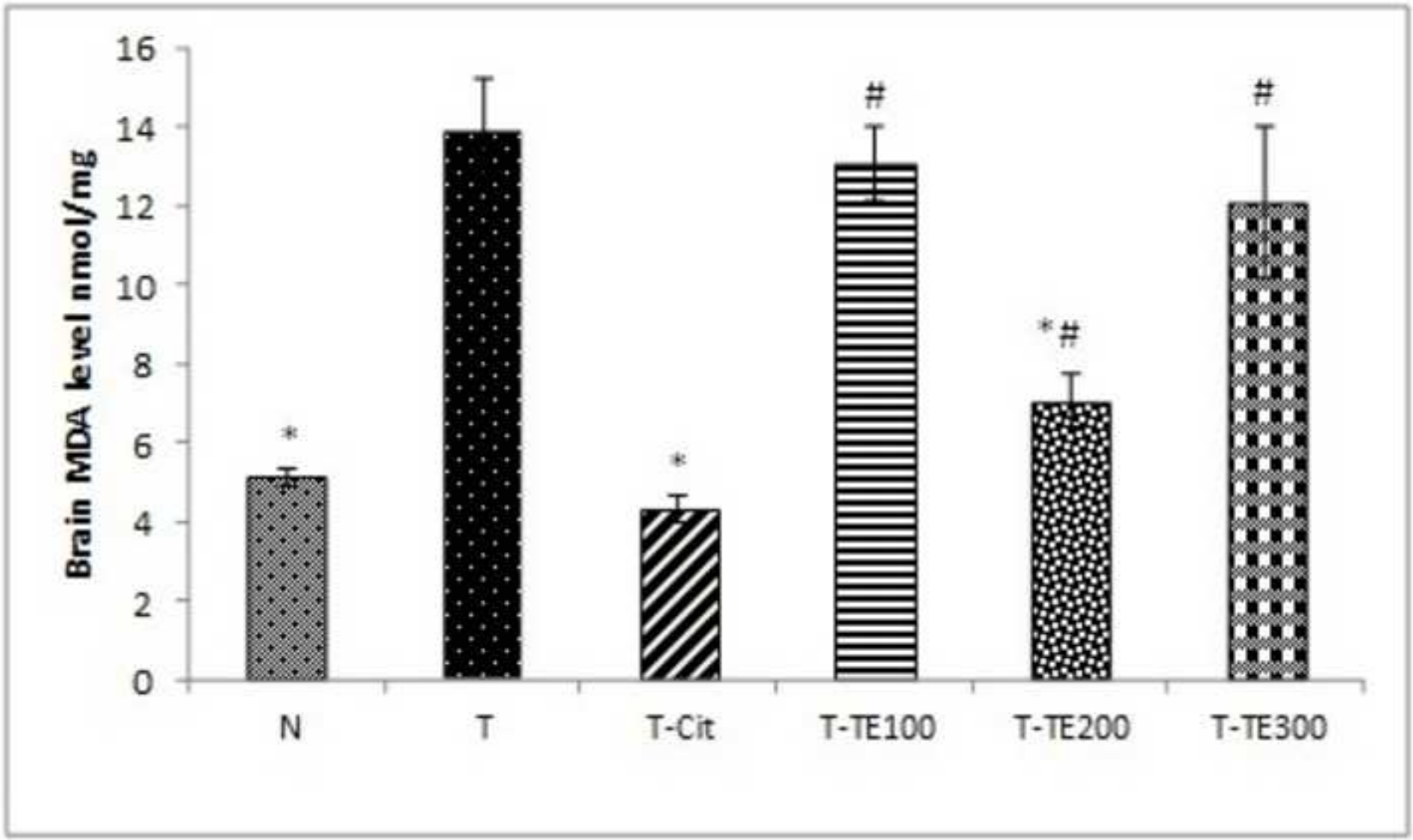

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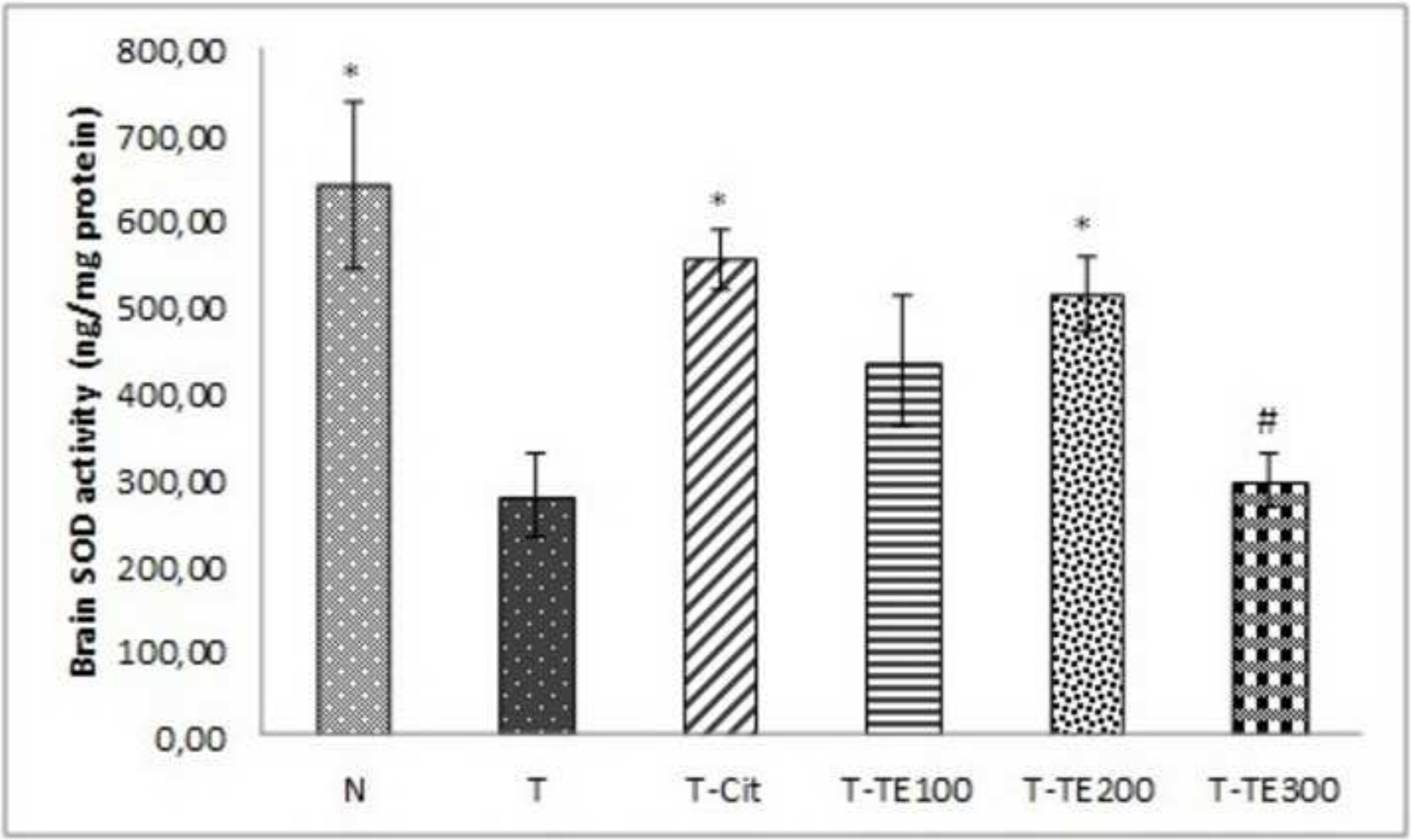

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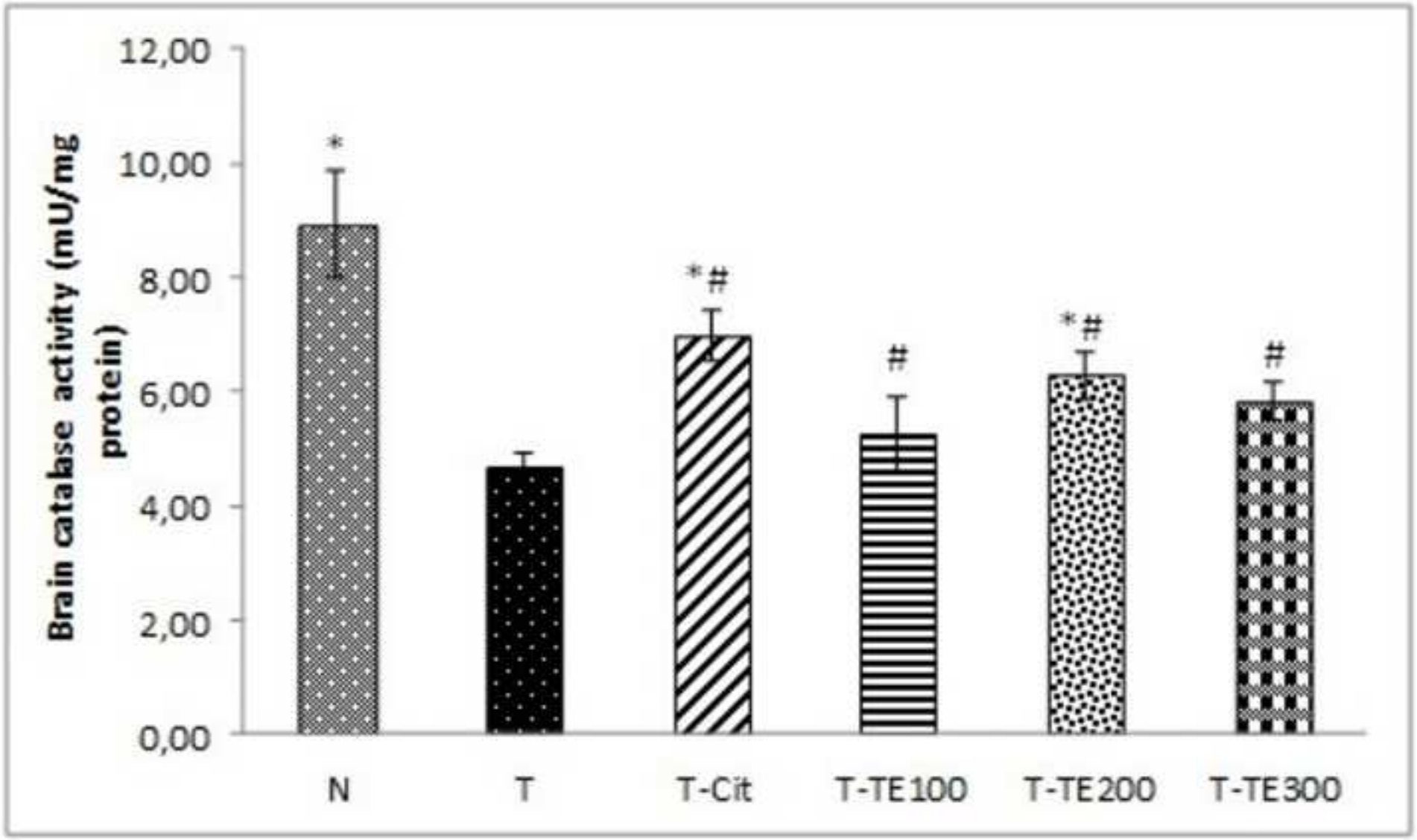

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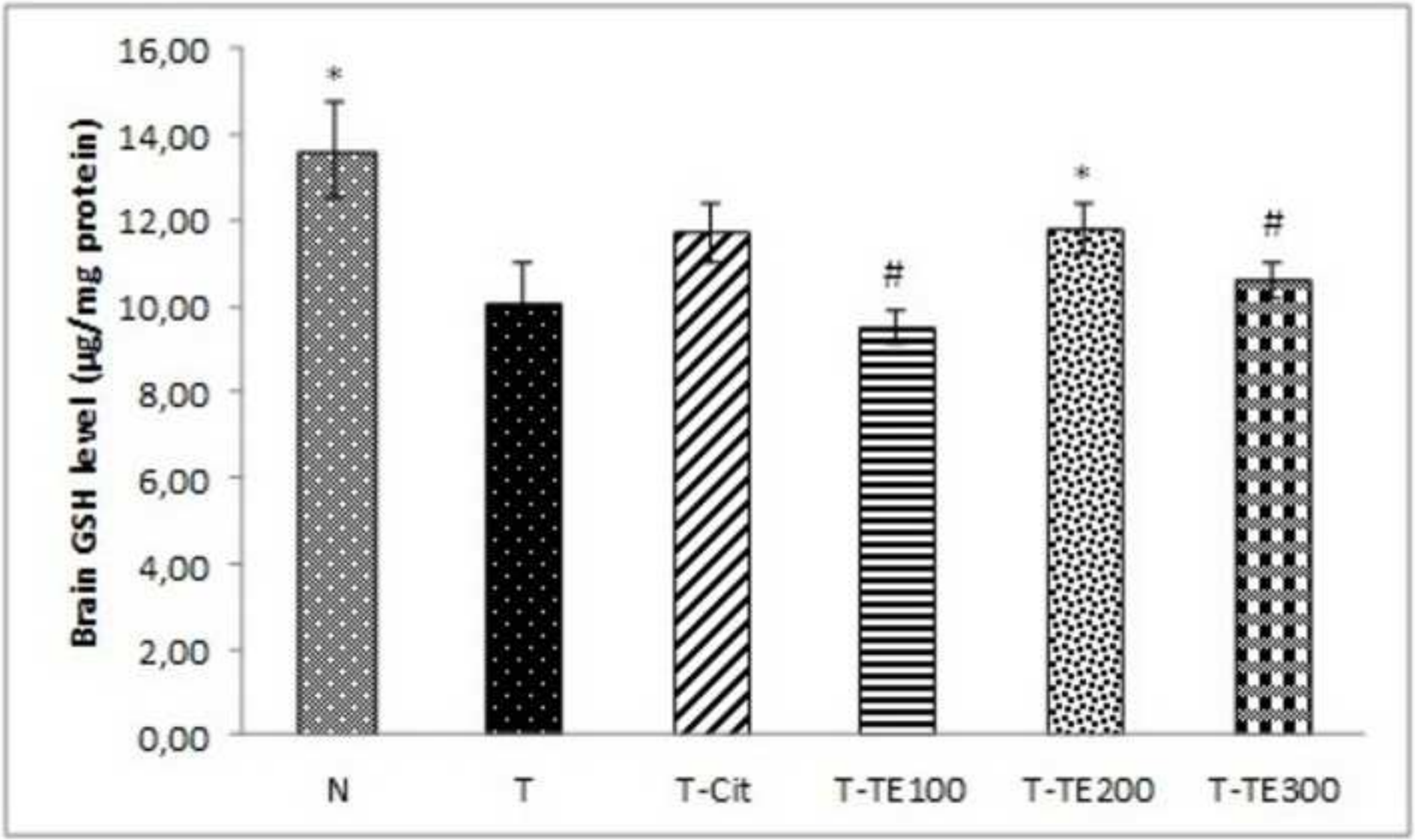

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