Neuroprotective potential of spermidine against rotenone induced Parkinson's disease in rats

NEUROPROTECTIVE POTENTIAL OF SPERMIDINE AGAINST ROTENONE INDUCED PARKINSON'S DISEASE IN RATS

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Abstract

Parkinson's disease is second leading late life movement disorder after AD. It is a hypokinetic disorder characterized by selective and immense loss of dopaminergic neurons in substantia nigra pars compacta region of mid-brain. Neurodegeneration in PD occurs due to excessive formation of free radicals, neuroinflammation, disturbancrs of intracellular calcium homeostasis and glutamate excitotoxicity. Spermidine is a polyamine which counteracts age associated cell death by scavenging free radical formation, activates authophagic machinery by enhancing formation of autophagosome, and antagonize NMDA receptor. Therefore this study was designed to explore the neuroprotective effect of spermidine against rotenone induced PD in rats. Rats were treated subcutaneously with rotenone 1.5mg/kg daily for 28 days. Spermidine 5&10mg/kg was administered orally one hour prior to rotenone administration from 15 to 28. Rotenone administration caused significant reduction in motor functioning, endogenous antioxidants such as glutathione and significant increase in nitrite and lipid peroxidation evidenced by increased malondialdehyde. Apart from oxidative stress, it also activated proinflammatory cytokines and enhanced inflammatory mediators such as IL-1β, IL6 and TNF-α. The neurochemical analysis revealed a significant decrease in serotonin, norepinephrine, dopamine and their metabolites accompanied by a significant loss of dopamine (DA) neurons in the substantia nigra pars compacta area upon ROT injection. However, treatment with spermidine rescued DA neurons in substantia nigra pars compacta area and nerve terminals in the striatum from the ROT insult. Spermidine treatment also restored antioxidant enzymes, prevented depletion of glutathione, and inhibited lipid peroxidation. Following treatment with spermidine, proinflammatory cytokines were also reduced. Results of our study suggest that spermidine has promising neuroprotective effect against degenerative changes in PD, and the protective effects are mediated through its antioxidant and anti-inflammatory properties.

Keywords: Parkinson's Disease; Spermidine; Rotenone; Oxidative Stress; Catecholamines; Neurodegeneration.

1. Introduction

In the new century, Parkinson's disease (PD) ranks among the most common late life neurodegenerative diseases, affecting approximately 1.5% to 2.0% of the population over the age of 60. PD is a long-term degenerative disorder of the central nervous system which affects movement and speech. It is a progressive disease marked by tremor, muscular rigidity, and slow, imprecise movement associated with the degeneration of the dopaminergic neurons in substantia nigra pars compacta region of brain. These neurons project to the striatum and their loss leads to alterations in the activity of the neural circuits within the basal ganglia that regulate movement, in essence an inhibition of the direct pathway and excitation of the indirect pathway. The direct pathway facilitates movement and the indirect pathway inhibits movement, thus the loss of these cells leads to a hypokinetic movement disorder (1). Many factors are speculated to operate in the mechanism of nigrostriatal dopaminergic degeneration, including oxidative stress and cytotoxicity of reactive oxygen spices (ROS), disturbances of intracellular calcium homeostasis and excitotoxicity due to overactivation of NMDA receptor through NR2B subunit. (2).

The most effective Parkinson's drug is levodopa. Several other treatment therapies are also used such as ropinirole, pramipexole, and pergolide - reduce muscle rigidity, improve speed and coordination of movement, and relieve tremor (Ravina et al., 2003). Some surgical treatments are also available such as stem cell therapy, deep brain stimulation, thalamotomy, pallidotomy, transcranial magnetic stimulation, gamma knife surgery. Still, there are much more limiting points in PD therapy. As all these therapies only provide symptomatic relief. No therapy has yet been shown to slow or reverse the disease. Surgical treatment has become a mainstay of late-stage management, although not all patients can afford it. There is a growing interest in the use of antioxidants and anti-inflammatory agents found endogeneously or exogeneously, which might prevent cell death and damage associated with the use of various neurotoxins (3).

Polyamines such as spermine, spermidine, putrescine and thermospermine are found in green vegetables, milk products, and meat. It belongs to an ubiquitous family of organic polycations, the polyamines, which exert diverse roles in cell proliferation, differentiation, survival and death (Büttner

et al., 2014). A decrease in polyamine levels is known to play a significant role in the aging process and the pathophysiology of neurodegenerative disorders including PD.

Spermidine is a polyamine that counteracts age associated cell death via activation of the autophagic machinery. It belongs to an ubiquitous family of organic polycations, the polyamines, which exert diverse roles in cell proliferation, differentiation, survival and death (4). An age dependent decrease of polyamine levels has been described in many organisms, including the brain of drosophila, rodents and humans. Interestingly, dietary spermidine supplementation as well as genetically enforced biosynthesis of spermidine showed to protected cognitive aging (5). It acts by scavenging the free radical formation, antagonize NMDA receptor and enhance the formation of autophagosome.

Furthermore, results from our laboratory have shown that Spermidine exhibit beneficial effects in 3-nitropropionic acid induced neurodegeneration in rat by combating oxidative stress, inflammation, apoptosis and mitrochondrial dysfunctioning in invitro model (6).

Hence the administration of spermidine can improve motor function and cell survival, which can become beneficial in PD. The present study was designed to standardize the rotenone induced rat model of PD and to study the effect of spermidine on rotenone induced neurobehavioural deficits, biochemical neuroinflammatory and neurochemical alterations in rats.

2. Materials and methods

2.1 Experimental animals

In the present study, experiments were carried out in male Wistar rats (200-250gm) procured from Panecia biotech and kept in the central animal house of ISF College of Pharmacy, Moga, Punjab (India) with food and water ad libitium. The animals were kept in polyacrylic cages and maintained under standard husbandary conditions (room temperature 22±2ºC and relative humidity of 55-60%) with a 12-h light/dark cycle (lights turned on at 7 AM). All the behavioral assessments were carried between 19:00 and 23:00 h i.e., in the active phase of animals. The experimental protocol was approved (ISFCP/CPCSEA/18/313) by the Institutional Animal Ethics committee (IAEC) and experiments were carried out in accordance with Indian National Science Academy (INSA) guidelines for the use and care of experimental animals.

2.2 Drugs and chemicals

Rotenone and spermidine was purchased from (Sigma Aldrich, St. Louis, MO, USA). Rotenone was dissolved in DMSO (dimethyl sulphoxide) and administered s.c. daily for 28 days. Whereas spermidine was dissolved in double distilled water and administered p.o. from 15-28 days daily one hour prior to the rotenone administeration. All other chemicals used in the study were of analytical grade. Solutions of the drugs and chemicals were freshly prepared before use.

2.3 Experimental procedure:

Animals were randomly assigned into 5 groups (n = 6 for each group). Group I –Normal control, Group II – Rotenone (1.5mg/kg, s.c. for 28 days), Group III – Rotenone+ spermidine (5mg/kg, p.o.), Group IV – Rotenone+ spermidine (10mg/kg, p.o.), Group V – Rotenone+ropinirole(0.5mg/kg i.p.). Spermidine and ropinirolr were administered one hour prior to rotenone administration for 14 days i.e. from day 15 to 28. Behavioral assessments were carried out on 14, 21 and 28 day. On day 29 animals were sacrificed for neurochemical, neuroinflammatory, biochemical and histopathological assessment. The experimental procedure is summarized in (Fig. 1).

Experimental design

Rotenone was dissolved in 1 % DMSO solution and administered subcutaneously daily for 28 days at a dose of 1.5mg/kg. Spermidine was dissolved in double distilled water and administered at a dose of 5 and 10 mg/kg by the per oral route. It was given one hour prior to the rotenone administration from day 15 to 28. Behavioral parameters like grip strength, narrow beam, rotarod, and locomotor activity were assessed on day 14^{th}, 21^{st} and 28^{th} day. Terminally on 29^{th} day, animals were sacrificed to isolate brain. Then striatum was separated and used to estimate biochemical parameters (LPO, nitrite, and reduced GSH) neurotransmitter analysis (DA, NE, serotonin, DOPAC, HVA, 5-HIAA, glutamate and GABA). The levels of pro-inflammatory cytokines (IL-1b, IL-6, and TNF-a) were estimated using ELISA kits.

2.4 Parameters

2.4.1 Measurement of body weight

The body weight of animals was measured prior to Rotenone administration (1st day) and on the last day of the study (28^{th}day). The percentage change in body weight was calculated as follows: Change in bodyweight= bodyweight (1st day−28^{th}day) / 1st day body weight × 100.

2.4.2 Assessment of behavioral parameter

Open Field Test: Open field test is used to monitor spontaneous locomotor_activity using wooden, rectangular, light brown-colored open field apparatus measuring 100 9 100 9 40 cm. The floor of the apparatus was divided into 25 rectangular squares by pencil lines. The experimental room was illuminated by 40 watt white bulb located 150 cm above the test apparatus. The animal was placed for 12 in the center and numbers of squares crossed in last 10 min were recorded. Each crossing was considered only when all four paws were in another square. After, each trial apparatus was cleaned properly and readings were taken (7)

Rotarod Activity: The motor coordination and grip performance of the animals were evaluated using the rotarod apparatus. The rats were exposed to a prior training session to acclimatize them to rotarod performance. Rats were placed on a rotating rod having a diameter of 7 cm (speed 25 rpm). The cut off time was 180 s and the average time of the fall was recorded (8)

Grip Strength Measurement: Grip strength of the fore limbs was measured using digital grip force meter (DFIS series, Chatillon, Greensboro, NC, USA). The rat was positioned to grab the grid with the fore limbs and was gently pulled to record the grip strength (9). The grip strength was recorded in Kgf.

Beam-Crossing Task: This task requires an animal to walk on across a narrow wooden beam, measuring its motor coordination ability. The beam consisted of two platforms (8 cm in diameter) connected by a wooden beam (0.5 mm in thickness, 2.0 cm in width, and 120 cm in length). The beam was elevated 50 cm above ground. A box filled with sawdust was placed below the beam, serving as protection for a falling rat. In order to adapt to the elevated beam, a rat was allowed to explore it for 5 min before training. A training trial started by placing the rat on the platform at one end. When a rat walked across the beam from one end to the other end, slipping of its feet occurred. Number of slips and time taken to cross the beam in each trial was recorded (8)

2.4.3 Measurement of biochemical parameters

Dissection and homogenization

On 29th day, animals were randomly divided into three groups, one group for biochemical estimations, second for neuroinflammatory markers estimations and third for neurotransmitters estimation immediately after the behavioral assessments and samples were run in duplicate. The brains were removed; striatum was separated by putting on ice, weighed and homogenized using 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000g for 15 min and aliquots of the supernatant was separated and used for biochemical estimations.

Measurement of lipid peroxidation: The quantitative measurement of lipid peroxidation in the brain striatum was performed according to the method of (10) The amount of malondialdehyde (MDA), a measure of lipid peroxidation, was measured by reaction with thiobarbituric acid at 532 nm using a Shimazdu spectrophotometer (Kyoto, Japan). The values were calculated and expressed as a percentage of the vehicle-treated group.

Estimation of nitrite: The accumulation of nitrite in the striatum supernatant, an indicator of the production of nitric oxide (NO), was determined by a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) ethylene diamine dihydrochloride, 1%sulfanilamide and 2.5% phosphoric acid) as described by Greenet al (11). Equal volumes of supernatant and Greiss reagent were mixed and incubated for 10 min at room temperature in the dark. Absorbance was measured at 540 nm with a Shimazdu spectrophotometer (Kyoto, Japan). The nitrite concentration in the supernatant was determined from the standard curve and expressed as a percentage of the vehicle-treated group.

Estimation of glutathione levels: Reduced glutathione (GSH) in striatum was estimated according to the method described by Ellman (12). Results were calculated and expressed as percentage of vehicle group.

Protein estimation: The protein was measured by the Lowry method using Folin phenol reagent (13).

2.4.4 **Estimation of striatal neuroinflammation:** The quantifications of TNF-α, IL-1β and IL-6 were done by immunoassay kit (KRISHGEN BioSystem,Ashley Ct, Whittier, CA). The quantikine rat TNF-α, IL-1β and IL-6 immunoassay is a 4.5 h solid phase ELISA designed to measure rat TNF- α, IL-1β and IL-6 levels. It is a solid- phase sandwich enzyme linked immunosorbent assay (ELISA)

using a microtitre plate reader. Concentrations of TNF- , IL-1 and IL-6 were calcu-lated from the standard curves.

2.4.5 Neurochemical analysis

Brain catecholamines estimation by HPLC-ECD: Catecholamines (Dopamine, serotonin and nor-epinephrine)and their metabolites (HVA, DOPAC and 5-HIAA) levels were estimated by HPLC using electrochemical detector. Waters standard system consisting of a high pressure isocratic pump, a 20µl manual sample injector valve, C18 reverse phase column and electrochemical detector were used in the study. Mobile phase consisted of sodium citrate buffer (pH 4.5) acetonitrile (87:13, v/v). Sodium citrate buffer consisted of 10 mM citric acid, 25 mM NaH2HPO4,25 mM EDTA, and 2 mM of 1-heptane sulfonic acid. Electrochemical conditions for the experiment were +0.75 V, sensitivity ranges from5 to 50 nA. Separation was carried out at a flow rate of 0.8 ml/min. Samples (20 µl) were injected manually. On the day of experiment frozen brain samples were thawed and homogenized in 0.2 M perchloric acid. After that samples were centrifuged at 12,000g for5 min. The supernatant was filtered through 0.22 mm nylon filters before injecting in the HPLC sample injector (14). Data were recorded and analyzed with the help of breeze software. Concentrations of neurotransmitter and their metabolites were calculated from the standard curve generated by using standard in a concentration range of 10–100 ng/ml.

Brain GABA and glutamate estimation by HPLC-ECD: The estimation of GABA and glutamate was done by method described by Donzanti and Yamamoto (15) with slight modifications. GABA and glutamate were estimated by HPLC using electrochemical detector. Waters standard system consisting of a high pressure isocratic pump, a 20 µl manual sample injector valve, C18 reverse phase column and electrochemical detector were used in the study. The mobile phase was comprised of 100 mM disodium hydrogen phosphate anhydrous, 25 mM EDTA and 22%methanol (pH-6.5). Electrochemical conditions for the experiment twere +0.65 V, sensitivity ranges from 5 to 50 nA. Separation was carried out at a flow rate of 1.2 ml/min and column temperature was maintained at 40◦C. Samples (20µl) were injected manually through rheodyne valve injector. On the day of experiment frozen brain samples were thawed and homogenized in 0.2 M perchloric acid. After that samples were centrifuged at 12,000g for 15 min. The supernatant was derivatized using OPA/ -ME

and then filtered through 0.22 mm nylon filters before injecting into the HPLC sample injector. Data were recorded and analyzed with the help of breeze software. Concentrations of amino acids were calculated from the standard curve generated by using standard in a concentration range of 10–100 ng/ml. The values are expressed as percentage of normal control group.

Pre-column derivatisation procedure: Amino acids were measured as OPA/β-ME derivatives according to the method of Donzanti and Yamamoto (15). Stock solution of amino acids standards were prepared at the level of 1 mg/ml. Stock solution of OPA/β-ME derivatizing reagent were prepared by dissolving 27 mg OPA in 1 ml methanol. 5 µl of β-ME and 9 ml tetraborate buffer (0.1 M Sodium tetraborate, pH-10.3) were then added. This solution is stable upto 5 days when protected from light. The working OPA/β-ME derivatizing reagent was prepared by diluting 2.5 ml of stock OPA/β-ME solution with 7.5 ml of tetraborate buffer. This solution must be prepared fresh daily. Brain samples were derivatized by mixing sample and reagent in ratio of 1:1.5(Sample: reagent ratio).

2.5 Statistical Analysis: The data obtained are expressed as mean ± SEM. The behavioral data were analyzed using two-way analysis of variance (ANOVA) followed by Bonferroni post hoc test for multiple comparison. p\0.05 was considered statistically significant. For biochemical parameters, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test is used for comparison. p\0.05 was considered statistically significant.

3. Results

3.1 Behavioral Parameters

(A) Effect of spermidine on body weight in rotenone treated rats. Rotenone treated rats showed a gradual decrease in body weight during the course of administration and significant decrease was recorded at the end of 4th week of rotenone exposure ($p<0.001$). Treatment with spermidine significantly and dose dependently restored decrease in the body weight as compared to rotenone treated group ($p<0.05$). Among the selected doses of spermidine (5 & 10mg) , spermidine (10mg) was found to be more effective in restoration of rotenone induced decrease in body weight rats (Fig.2).

(B) Effect of spermidine treatment on narrow beam walk parameters (time taken to cross the beam and no. of foot slips) in rotenone treated rats. Rats treated with rotenone showed increase in transfer latency to cross the narrow beam runway and foot errors as compared with vehicle treated rats (p<

0.001). Treatment with spermidine significantly and dose dependently prevented the increase in time taken to cross the beam and foot errors as compared to rotenone treated groups ($p<0.005$). Among selected doses, spermidine (5 & 10mg), spermidine (10mg) was found to be more effective in preventing narrow beam walk activity (Fig.3a and 3b).

(C) Effect of spermidine treatment on grip strength activity in rotenone treated rats. Rotenone infusion significantly reduced gripping ability in rats as compared to vehicle treated groups. spermidine significantly and dose dependently ameliorated rotenone induced impairment in gripping ability on 14^{th}, 21^{th}, 28^{th} days. Among selected dose, spermidine (5 & 10mg), spermidine (10mg) was found to be more effective in improving gripping strength (Fig.4)

(D) Effect of spermidine treatment on open field (locomotor activity) test in rotenone treated rats. Daily administration of rotenone for 28 days significantly decreased locomotor activity in open field test as compared with vehicle treated group. Our treatment drug spermidine significantly and dose dependently ($P<0.05$) attenuated rotenone induced decrease in motor activity and restored toward normal. Among all selected dose spermidine (10 mg) was found to be more effective in improving motor activity in rats (Fig.5).

(E) Effect of spermidine treatment on rotarod activity in rotenone treated rats. During the course of treatment with rotenone, it showed a gradual decrease in motor co-ordination (rotarod activity). Our treatment drug spermidine significantly and dose dependently ($P<0.05$) attenuated rotenone induced decrease in motor activity and restored toward normal. Among all selected dose spermidine (10 mg) was found to be more effective in improving motor activity in rats (Fig. 6).

3.2 Biochemical parameters

Effect of spermidine on brain oxidative stress (LPO, nitrite, GSH) in rotenone treated rats. MDA (melan dialdehyde,) nitrite levels were found to be increased significantly ($p<0.001$) and GSH was decreased significantly ($p<0.001$) in rotenone treated rat brain striatum as compared to control. However treatment with spermidine (5 & 10 mg) produced significant and dose dependently decrease in MDA, nitrite and restored the levels of reduced glutathione in rotenone infused groups ($p<0.05$) as shown in table 1.

3.3 Neuroinflammatory markers

(A) Effect of spermidine treatment on striatal pro-inflammatory cytokines levels (TNF-α, IL-1β, IL-6) in rotenone treated rats. Rotenone treatment in rats produced significant elevation in striatal TNF-α, IL-1β and IL-6 levels as compared with vehicle treated group ($P<0.001$), indicating increased neuroinflammation. Spermidine (5 & 10 mg) significantly and dose dependently ($P<0.05$) attenuated Rotenone induced deficits in pro inflammatory cytokines levels ($p<0.05$) (Fig. 7).

3.4 Neurochemical estimation

(A) Effect of spermidine treatment on striatal catecholamines levels (DA, NE, 5-HT) in rotenone treated rats. The catecholamines (DA, NE, and 5-HT) were found to be significantly reduced in Rotenone treated rats as compared to the normal control group ($p<0.001$). Treatment with spermidine significantly prevented the decline in catecholamines levels in the striatum ($p<0.005$). among selected doses, spermidine (5 & 10mg) was found to more effective in restoring the level of catecholamines (Fig.8).

B) Effect of spermidine treatment on striatal catecholamines metabolites levels (DOPAC and HVA) in rotenone treated rats. Repeated rotenone treatment in rats showed increase in DOPAC and HVA level as compared with vehicle treated group(Fig) ($P<0.001$). Spermidine (5 & 10 mg) significantly and dose dependently decrease in DOPAC and HVA level in Rotenone treated rats as compared to 2.5 mg dose (Fig.9)

(C) Effect of spermidine treatment on striatal catecholamines metabolites levels (5-HIAA) in rotenone treated rats. Repeated subcutaneous rotenone treatment in rats showed decrease in 5-HIAA level as compared with vehicle treated group (Fig) ($P<0.001$). Spermidine (5 & 10 mg) significantly and dose dependently increase the 5-HIAA level in Rotenone treated rats (Fig.10).

(D) Effect of spermidine treatment on striatal GABA level in rotenone treated rats. The group treated with rotenone showed a significantly low GABA level when compared to the Rotenone group ($p<0.001$). Treatment with spermidine significantly and dose dependently prevented the alteration in GABA levels in the striatum ($p<0.005$). Among selected doses, spermidine (5 & 10mg/kg) showed more effective in prevention of alteration of GABA levels (Fig.11).

(E) Effect of spermidine treatment on striatal glutamate level in rotenone treated rats.

Rotenone treatment for28 days showed a significantly high level of glutamate when compared to the Rotenone group ($p<0.001$). Treatment with spermidine significantly and dose dependently prevented the alteration in glutamate levels in the striatum ($p<0.005$). Among selected doses, spermidine (5 & 10mg/kg) showed more effective in prevention of alteration of glutamate levels (Fig.12).

4. Discussion

In the current study, we had investigated the neuroprotective effect of spermidine in the rotenone model of Parkinson's disease (PD). We had administered rotenone subcutaneously at a dose of 1.5mg/kg body weight for 4 weeks to induce the progressive dopaminergic degeneration in SNPc region. Rotenone can easily cross the BBB because of its high lipophilic nature and inhibits the activity of complex-1 in the mitochondrial respiratory chain, thereby increase oxidative insult, inflammation and decreased neurotransmitters. It resembles many of key pathological features of human PD. In our study rotenone administration significantly decreased motor coordination which was confirmed by the reduced body weight, hypokinetic movement in open field test, decreased rotarod and grip strength activity. These findings were in corroboration with other published reports which also observed impairment in behavioral parameters, oxidative defense, and neurotransmitters, resulting in dopaminergic degeneration (16).

Whereas, the administration of spermidine significantly and dose-dependently increased the body weight and locomotor activity, no. of foot slips was also reduced whereas latency time was significantly increased. Recent reports from our lab have also shown improvement in motor coordination by spermidine in quanillinic acid induced HD (6).

Oxidative and nitrosative damage was also increased significantly by the rotenone administration as compared to the normal animals. It was observed by the increased lipid proxidation (elevated level of MDA) and nitrite and decreased levels of GSH in animals. These plays key role in the brain by removing free radicals formed during the metabolism. Recent reports from our laboratory show, that such kind of changes are compelling features of early PD (17). However, treatment with spermidine significantly and dose dependently reversed the effect of rotenone induced oxidative damage. As

described by previous findings, the beneficial effect of spermidine on oxidative stress parameters is due to inhibition of lipid peroxidation, free radical scavenging, and antioxidant property (18).

In progression of parkinson's disease neuroinflammation plays a critical role. The outcomes of present study shows that administration of rotenone results in activation of pro-inflammatory markers which shows significant elevation in the levels of IL-6, IL-1β, and TNF-α in striatum. They are detrimental for the proteins and DNA. These cytokines can directly activate inflammatory and apoptotic pathway, resulting in Neurodegeneration. The observations of our current study was in agreement with previous studies (19). Whereas treatment with spermidine (5 and 10 mg/kg/day orally) significantly prevented the alteration in levels of these cytokines in striatum. Inhibition of IL-1b (reduced excitotoxicity) and inhibition of microglia activity, result in decrease in the expression of TNF-a, iNOS (protein and mRNA), and NO release, leading to less neuronal degeneration and apoptosis (20).

GABA and glutamate are major inhibitory and excitatory neurotransmitters of the mammalian CNS respectively. Neuronal excitotoxicity is defined as neuronal death arising from prolonged stimulation of NMDA receptor and excessive influx of calcium ions into the cell. Striatum receives large glutamatergic input from corticostriatal afferents and is at high risk of glutamate-mediated excitotoxic injury. Rotenone produces excitotoxicity by the activation of NMDA receptors. In contrast to several studies which shows altered levels of catecholamines and their metabolites in PD animals, patients, and postmortem brains. Rotenone treatment causes decrease in release of glutamate and subsequent sensitization of NMDA receptor. A recent study from our lab suggested that rotenone treatment produces decline in NE, DA, and 5-HT levels as compared to normal (17). Findings from our study also suggests that 28 day administration of rotenone by subcutaneous injection can result in loss of dopaminergic, cholinergic and GABAergic neuronal degeneration. HPLC analysis of homogenized brain tissue also shows that rotenone increases the levels of HVA and DOPAC and decreases the level of 5-HIAA. However, treatment with spermidine at dose of 5 and 10 mg/kg orally significantly prevented the alteration in catecholamines and their metabolites level. The preventive effect of

spermidine against rotenone mediated excitotoxicity is attributed to antagonistic action of spermidine on NMDA receptors (6).

5. Conclusion

In conclusion the study demonstrated the protective effect of spermidine against rotenone induced dopaminergic degeneration. Our observations suggest that spermidine possess immense potential as a candidate drug and warrants further research to extrapolate preclinical findings into clinical studies for better understanding of its role in the treatment and management of PD. Based on the current observations it can be concluded that the observed beneficial effects of spermidine on motor functions may be due to its neuroprotective potential which can be correlated with its antioxidant, anti-inflammatory activities and its ability to restore striatal neurochemistry.

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Legands for tables and figures

Fig 1: Plan of work

Fig 2: Effect of spermidine on body weight in rotenone treated rats (n=6). Columns represent mean of values, and error bars represent SEM. Data analyzed by one way ANOVA followed by Tukey's post hoc test. [a]p<0.001 versus NC, [b]p<0.05 versus RT, [c]p<0.05 versus SPM (5mg), [d]p<0.05 versus SPM (10mg). NC normal control, RT-Rotenone (1.5mg), SPM- spermidine (5 mg), SPM- spermidine (10 mg).

Fig. 3: Effect of spermidine on narrow beam walking in rotenone treated rats (n=6). Columns represent mean of values, and error bars represent SEM. Data analyzed by repeated measures two-way ANOVA followed by Bonferroni's multiple comparison. [a]p< 0.01 versus NC, [b]p< 0.05 versus RT, [c]p< 0.05 versus SPM (5mg), [d]p< 0.05 versus SPM (10mg). NC normal control, RT-Rotenone (1.5mg), SPM- spermidine (5 mg), SPM- spermidine (10 mg).

Fig. 4 Effect of spermidine on grip strength in rotenone treated rats (n=6). Columns represent mean of values, and error bars represent SEM. Data analyzed by repeated measures two-way ANOVA followed by Bonferroni's multiple comparison. [a]p< 0.01 versus NC, [b]p< 0.05 versus RT, [c]p< 0.05 versus SPM (5mg/kg), [d]p< 0.05 versus SPM (10mg). NC normal control, RT-Rotenone (1.5mg), SPM- spermidine (5 mg), SPM- spermidine (10 mg).

Fig. 5 Effect of spermidine treatment on open field (locomotor activity) test in rotenone treated rats (n=6). Columns represent mean of values, and error bars represent SEM. Data analyzed by repeated measures two-way ANOVA followed by Bonferroni's multiple comparison. [a]p< 0.01 versus NC, [b]p< 0.05 versus RT, [c]p< 0.05 versus SPM (5mg/kg), [d]p< 0.05 versus SPM (10mg). NC normal control, RT-Rotenone (1.5mg), SPM- spermidine (5 mg), SPM- spermidine (10 mg).

Fig. 6 Effect of spermidine treatment on rotarod activity in rotenone treated rats (n=6). Columns represent mean of values, and error bars represent SEM. Data analyzed by repeated measures two-way ANOVA followed by Bonferroni's multiple comparison. [a]p< 0.001 versus NC, [b]p< 0.05 versus RT,

[c]p< 0.05 versus SPM (5mg), [d]p< 0.05 versus SPM (10mg). NC normal control, RT-Rotenone (1.5mg), SPM- spermidine (5 mg), SPM- spermidine (10 mg).

Fig 7: Effect of spermidine on TNF-α, IL-1β and IL-6 levels in rotenone treated rats. Values are expressed as mean ± SEM. Data analyzed by one way anova followed by turkey's post hoc test. [a]P<0.001vs vehicle, [b]p<0.001 vs Rotenone, [c]p<0.05 vs Rotenone + Spermidine (5mg), [d]p<0.05 vs Rotenone + Spermidine (10mg), ep<0.05 vs Rotenone + Ropinirole(0.5mg).

Fig 8: Effect of spermidine on striatal catecholamine level in rotenone treated rats. Values are expressed as mean ± SEM. Data analyzed by one way anova followed by turkey's post hoc test. [a]P<0.001vs vehicle, [b]p<0.001 vs Rotenone, [c]p<0.05 vs Rotenone + Spermidine (5mg/kg), [d]p<0.05 vs Rotenone + Spermidine(10mg), ep<0.05 vs Rotenone + Ropinirole (0.5mg).

Fig 9: Effect of spermidine on striatal catecholamine in rotenone treated rats. Values are expressed as mean ± SEM. Data analyzed by one way anova followed by turkey's post hoc test. [a]P<0.001vs vehicle, [b]p<0.001 vs Rotenone, [c]p<0.05 vs Rotenone + Spermidine (5mg), [d]p<0.05 vs Rotenone + Spermidine (10mg), [e]p<0.05 vs Rotenone + Ropinirole (0.5mg).

Fig 10: Effect of spermidine on striatal catecholamine in rotenone treated rats. Values are expressed as mean ± SEM. Data analyzed by one way anova followed by turkey's post hoc test. [a]P<0.001vs vehicle, [b]p<0.001 vs Rotenone, [c]p<0.05 vs Rotenone + Spermidine (5mg), [d]p<0.05 vs Rotenone + Spermidine 10mg), [e]p<0.05 vs Rotenone + Ropinirole (0.5mg).

Fig 11: Effect of spermidine on striatal GABA level in rotenone treated rats. Values are expressed as mean ± SEM. Data analyzed by one way anova followed by turkey's post hoc test. [a]P<0.001vs vehicle, [b]p<0.001 vs Rotenone, [c]p<0.05 vs Rotenone + Spermidine (5mg/kg), [d]p<0.05 vs Rotenone+ Spermidine (10mg/kg), [e]p<0.05 vs Rotenone + Ropinirole (0.5mg/kg).

Fig 12: Effect of spermidine on striatal glutamate level in rotenone treated rats. Values are expressed as mean ± SEM. Data analyzed by one way anova followed by turkey's post hoc test. [a]P<0.001vs

vehicle, [b]p<0.001 vs Rotenone, [c]p<0.05 vs Rotenone + Spermidine (5mg/kg), [d]p<0.05 vs Rotenone + spermidine 10mg/kg), [e]p<0.05 vs Rotenone + Ropinirole (0.5mg/kg).

Table 1. NC normal control, ROT- rotenone (1.5 mg), SPM- spermidine (5mg), SPM- spermidine (10mg). [a]p< 0.01 versus NC, [b]p< 0.05 versus ROT, [c]p< 0.05 versus SPM (5mg/kg), [d]p< 0.05 versus SPM (10mg), [e]p < 0.05 versus Ropinirole.

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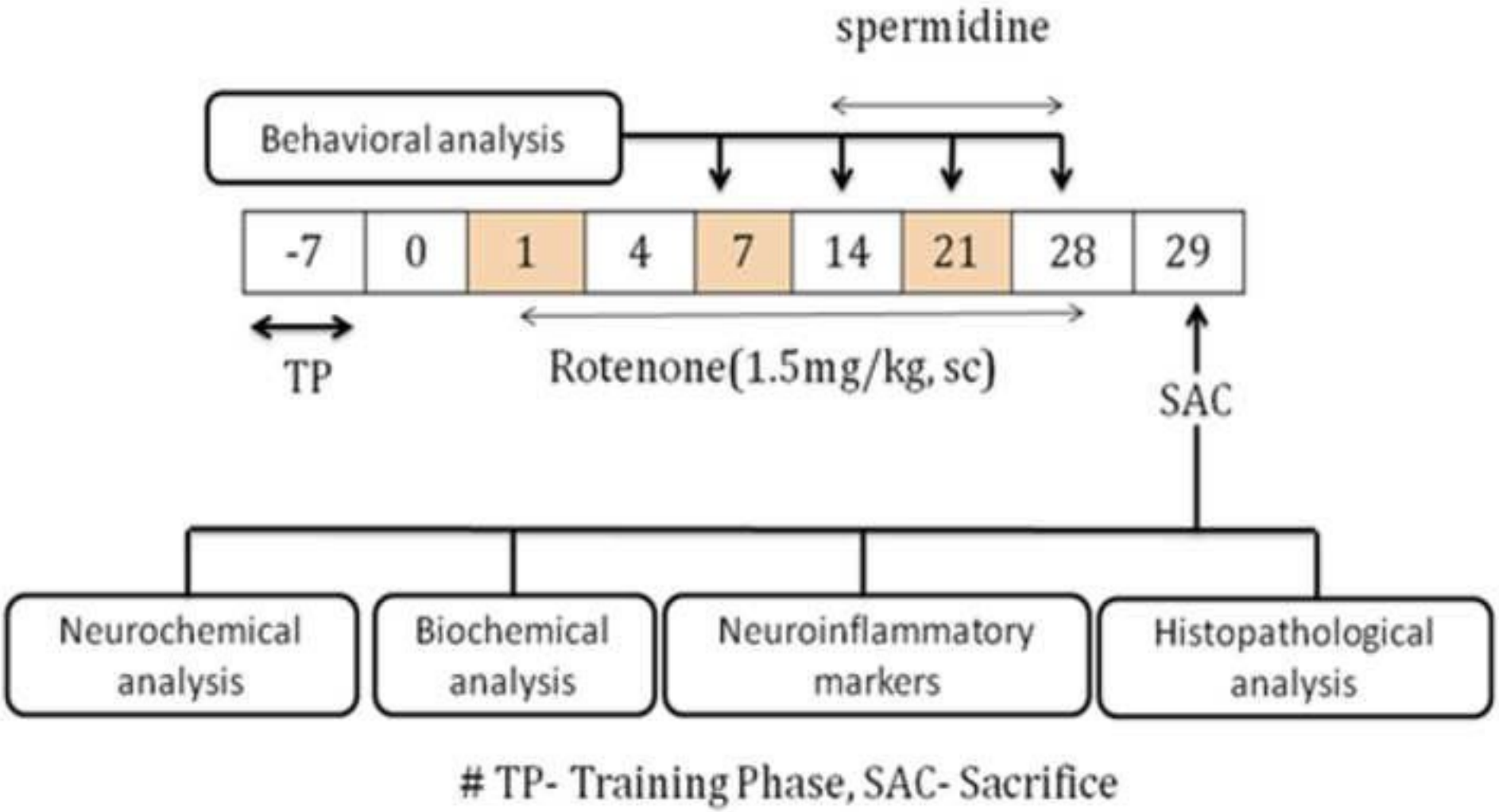

TP- Training Phase, SAC- Sacrifice

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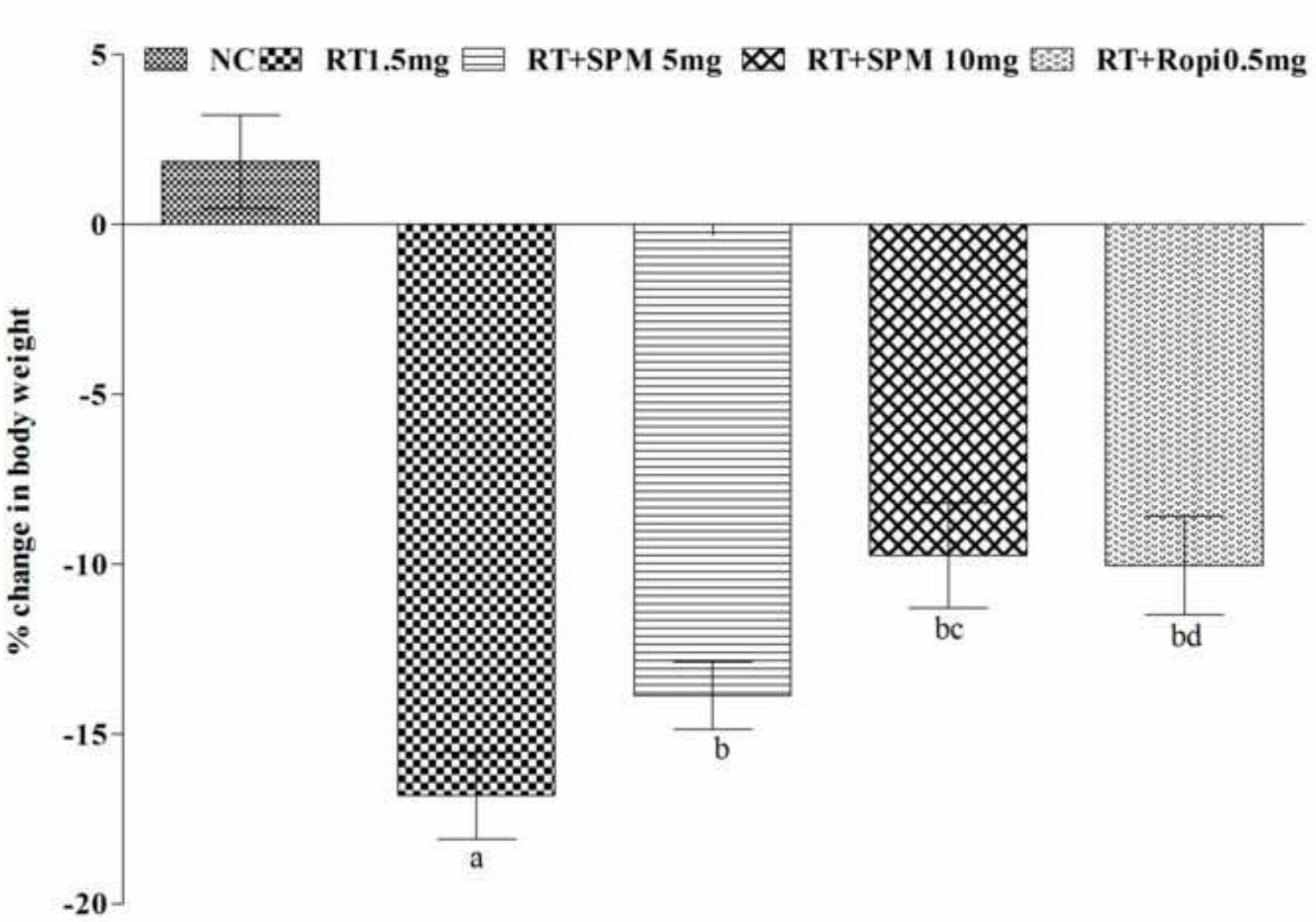

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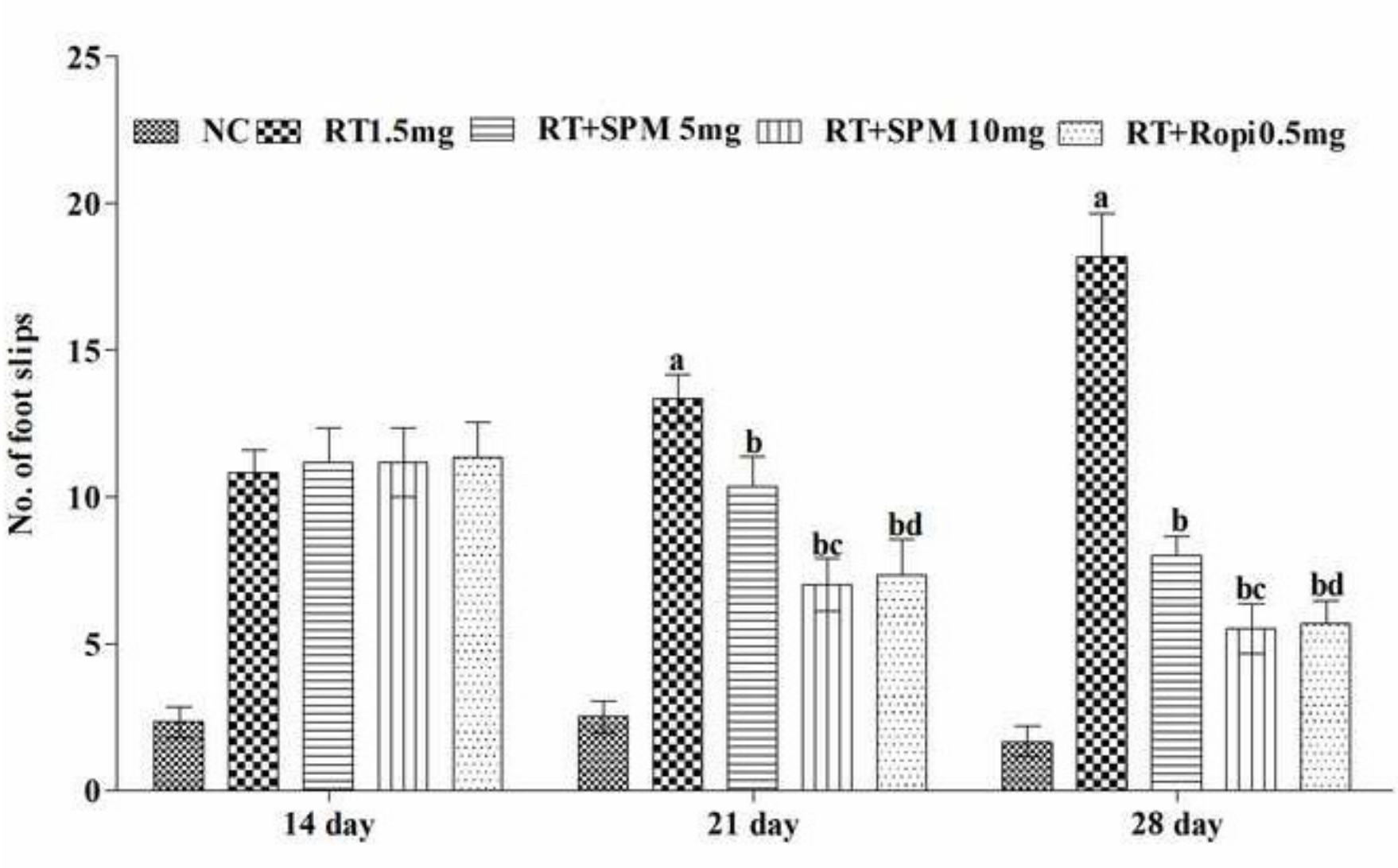

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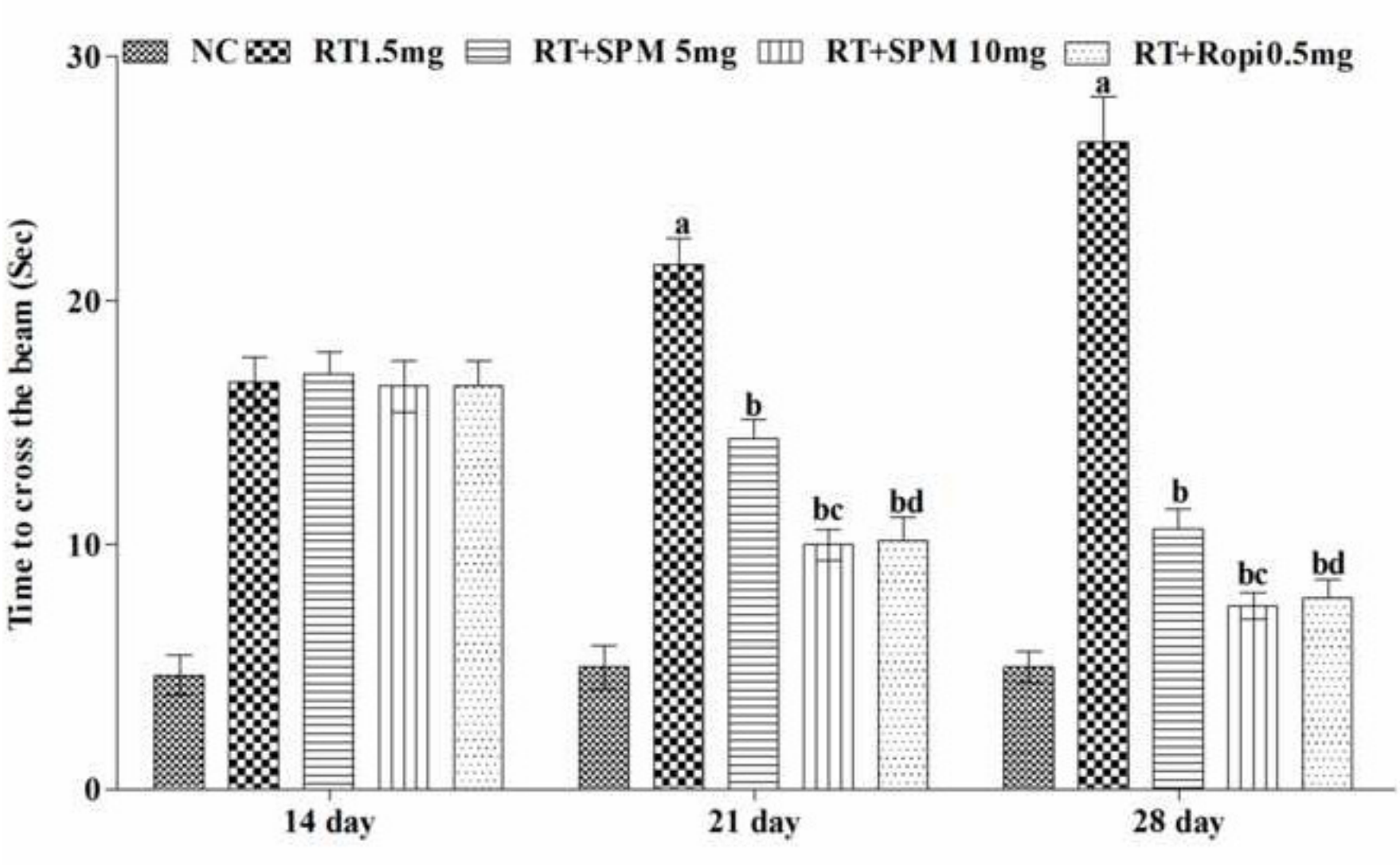

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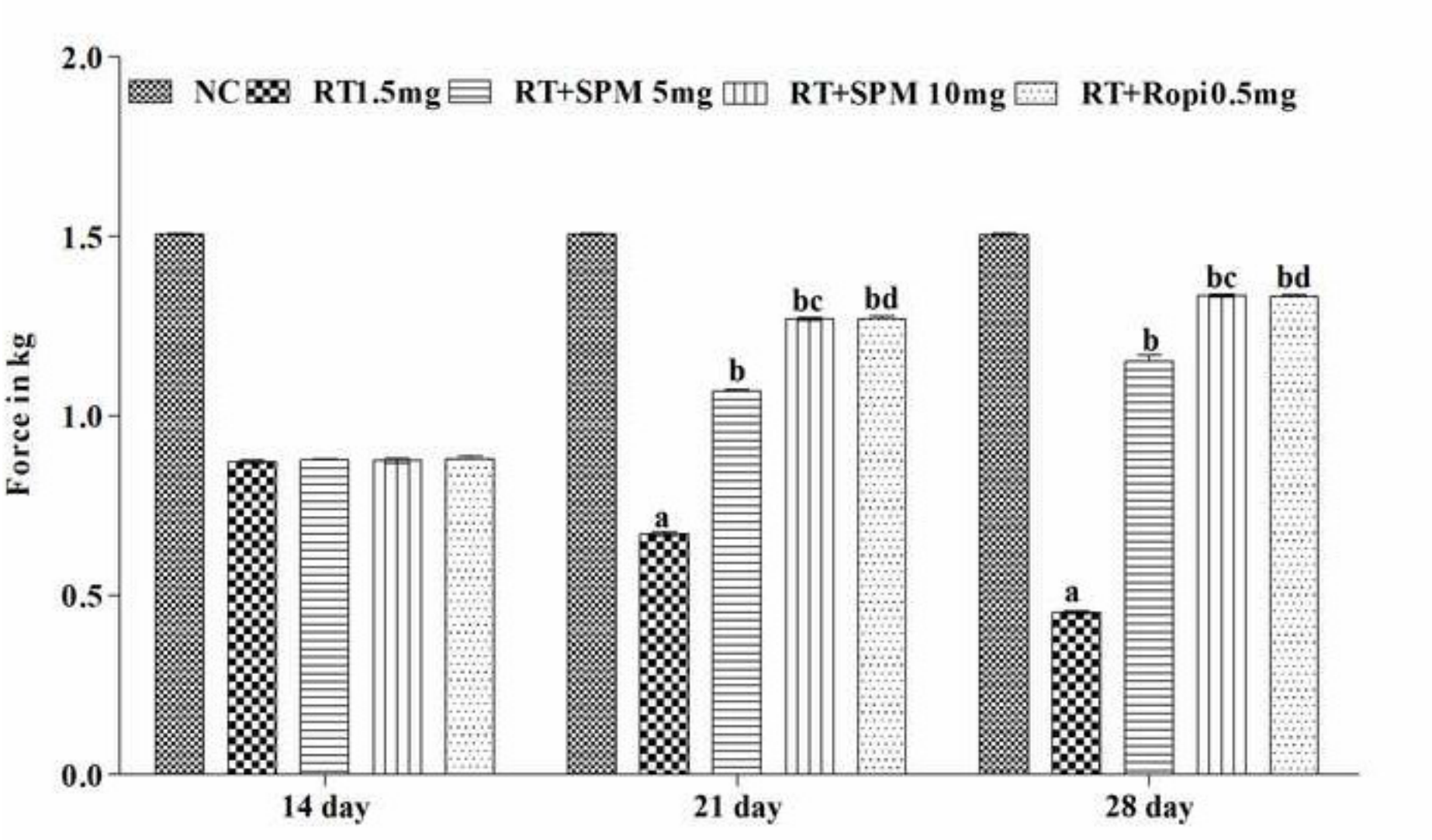

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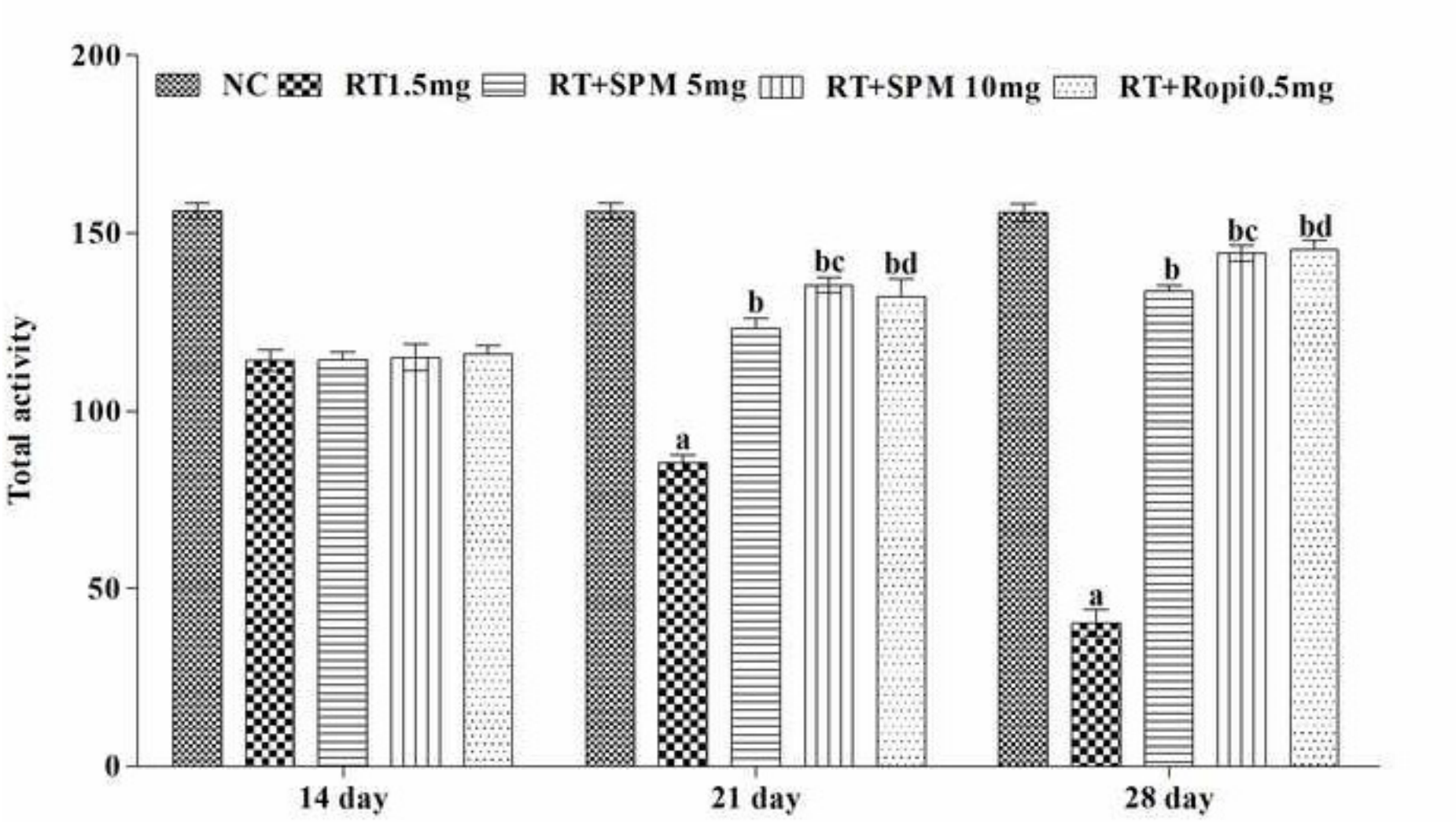

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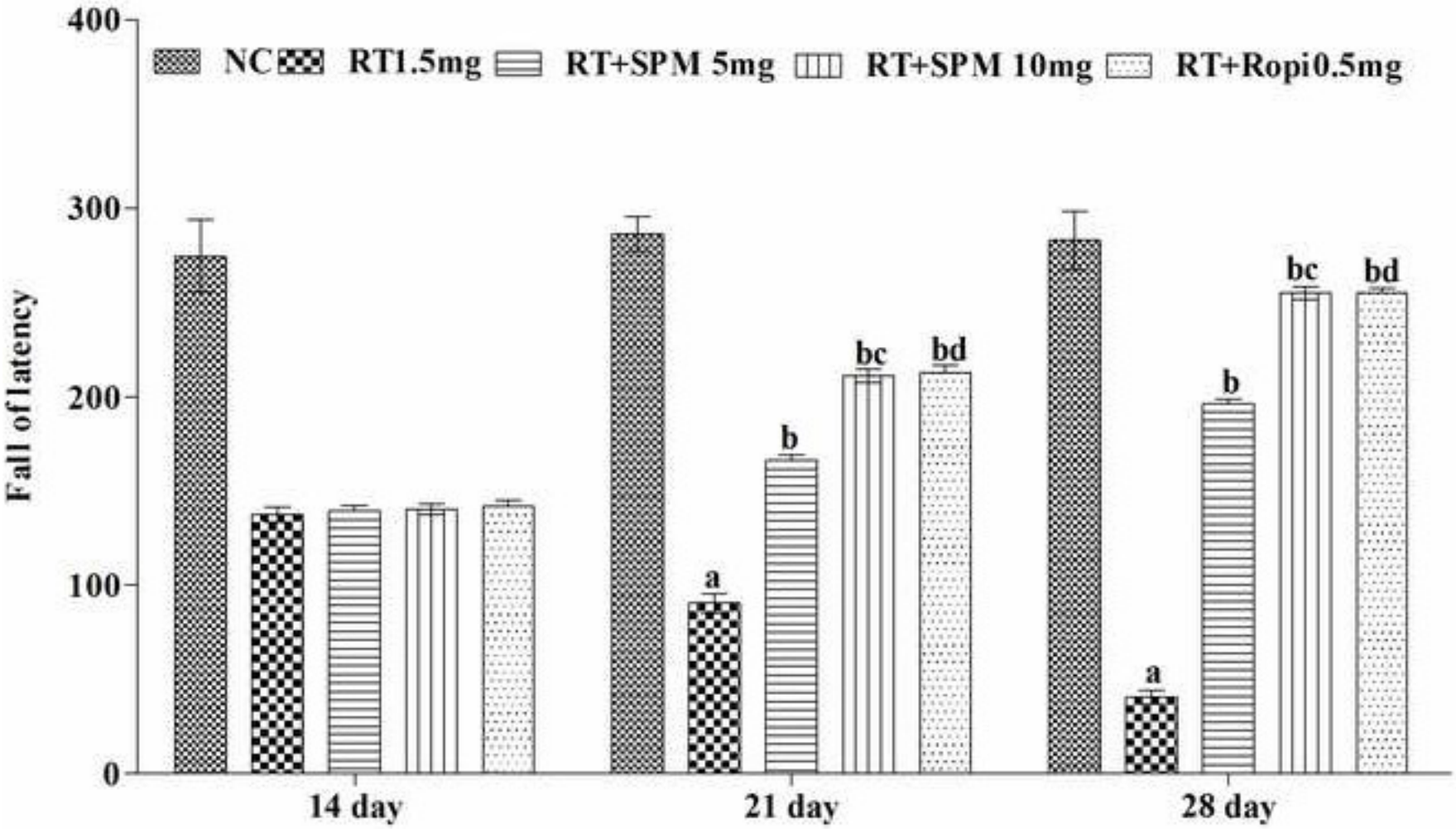

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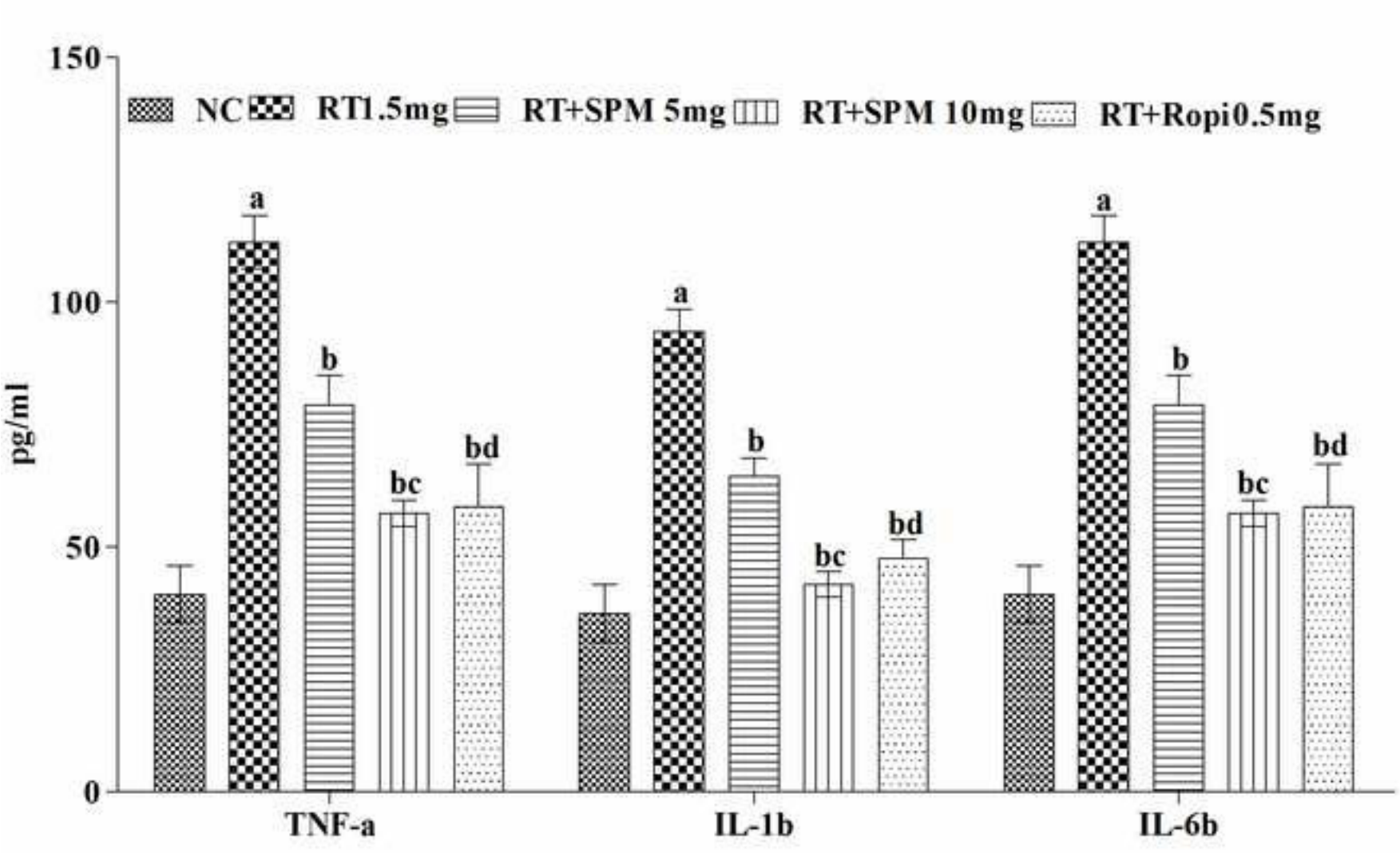

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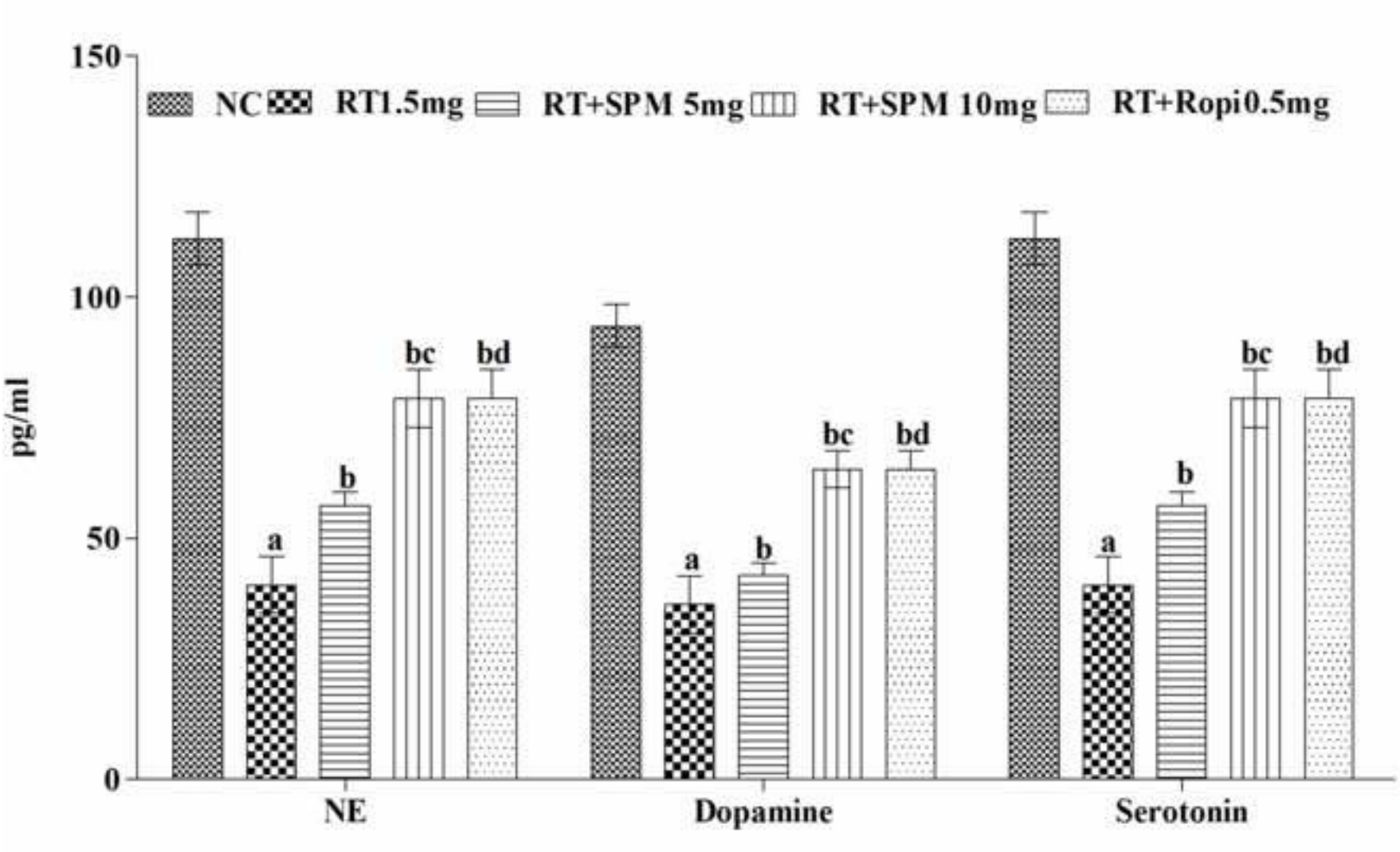

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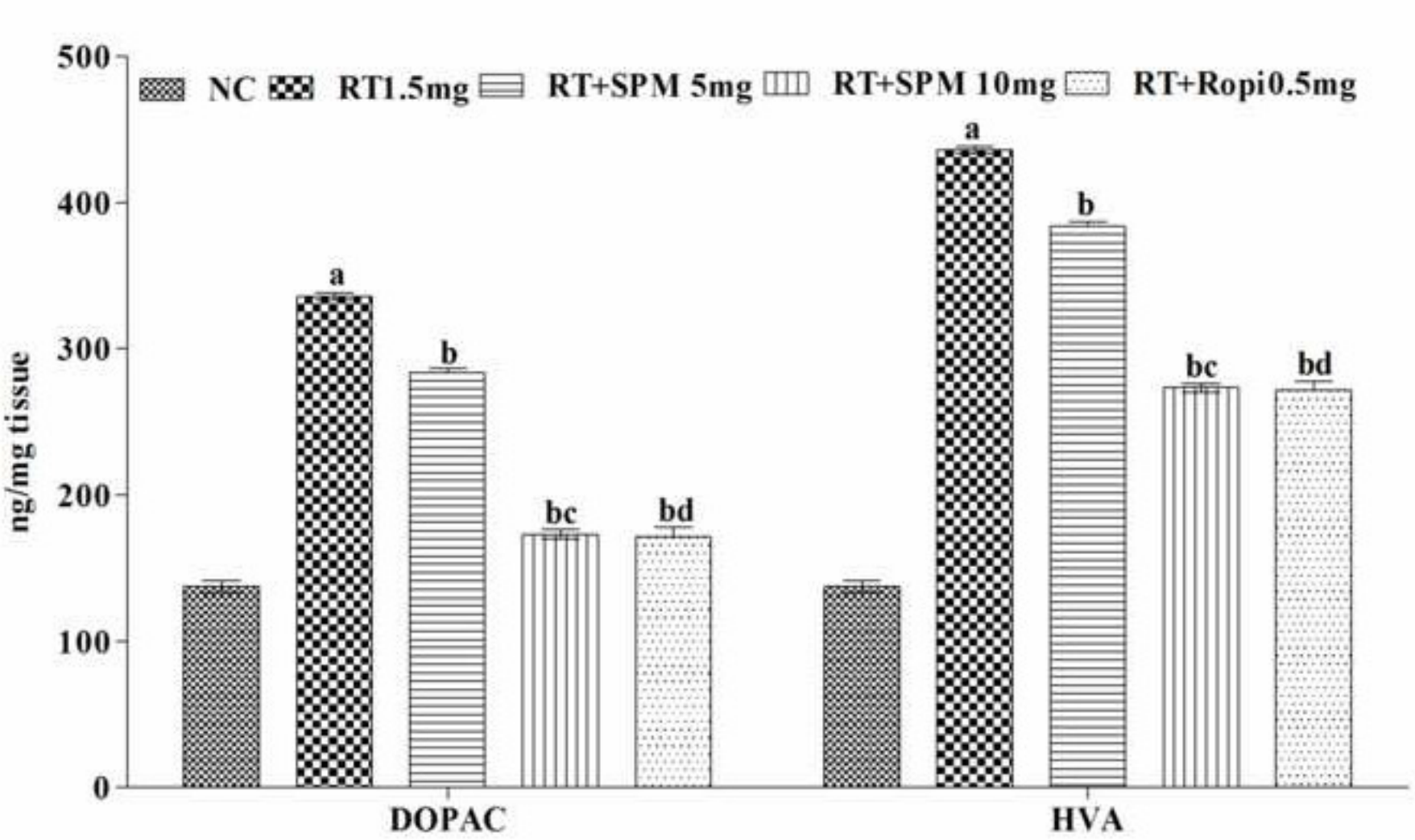

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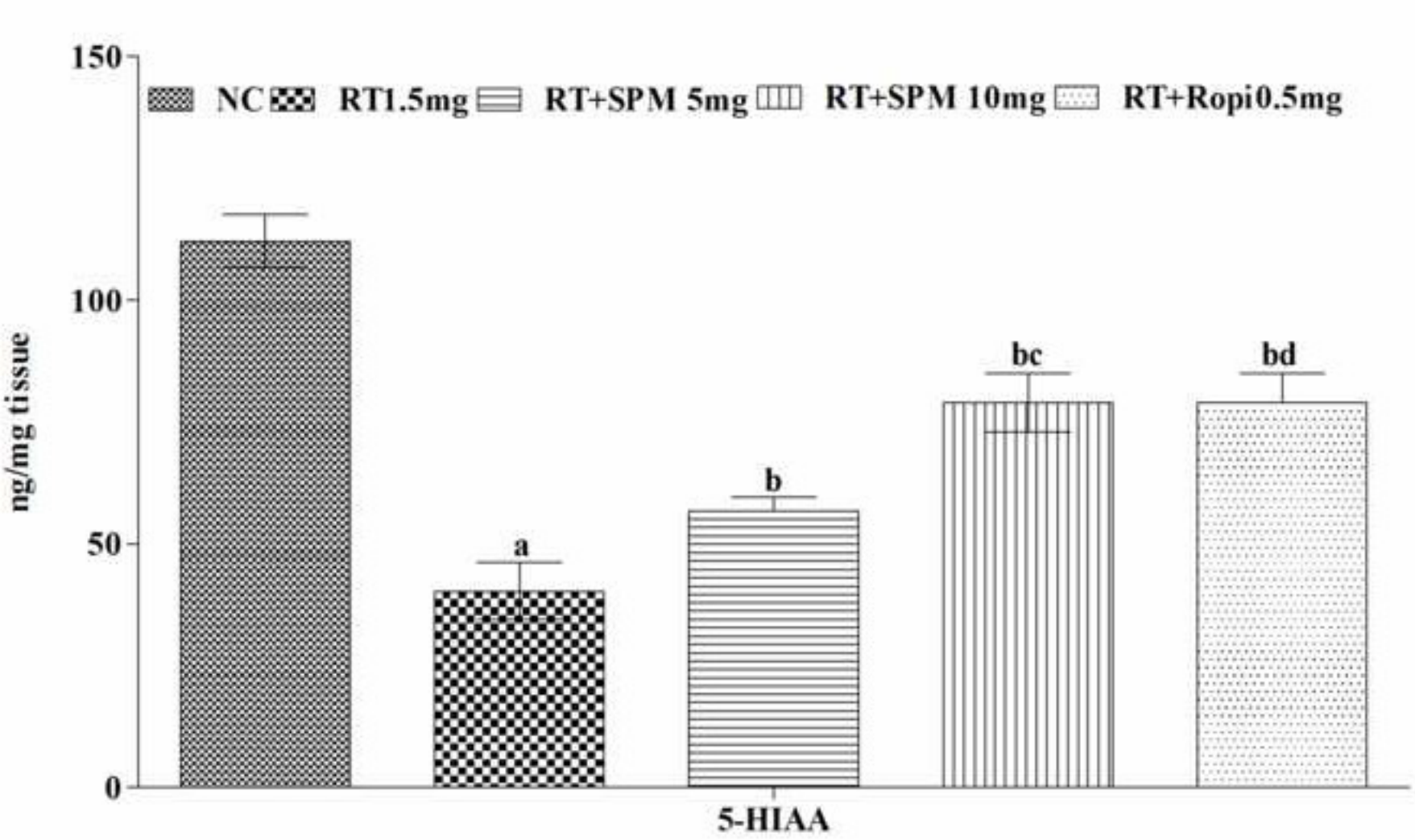

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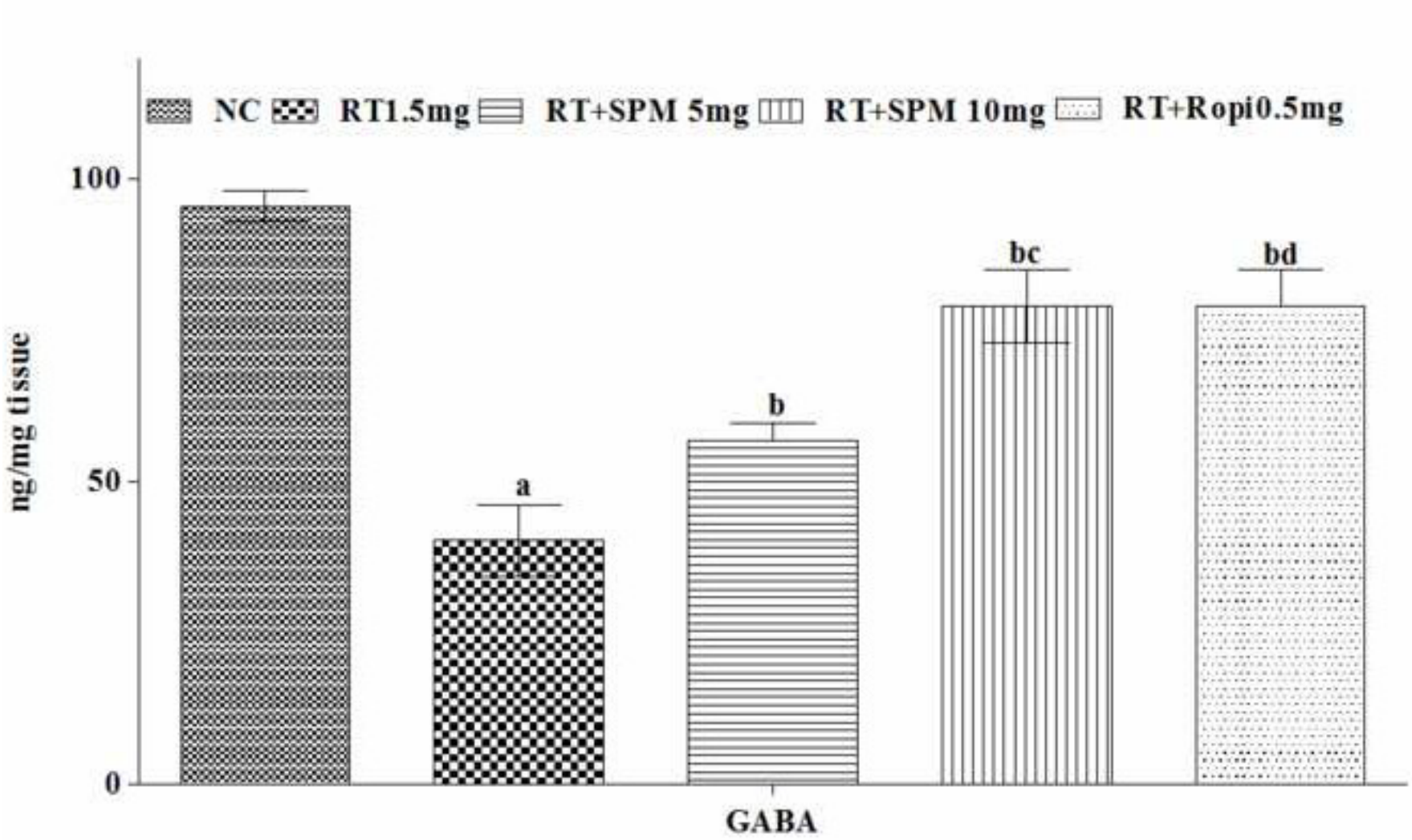

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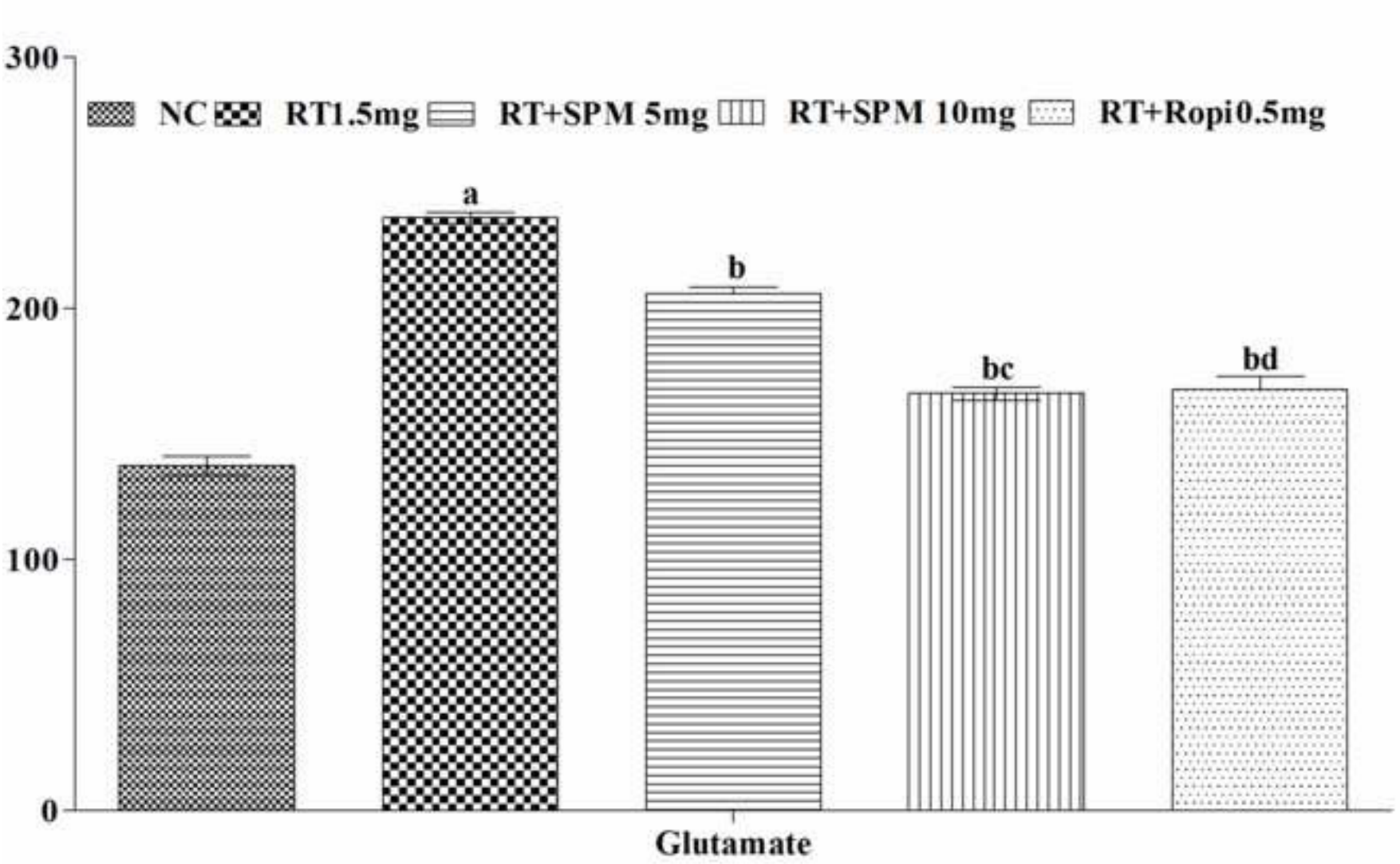

Table

Table 1

Experimental Groups	MDA (n mol/mg pr)	Nitrite (μg/ml)	GSH (μmol/mg pr)
Normal control	2.11 ± 0.62	122 ±79	0.113 ± 0.016
ROT+NC	7.76 ± 0.57[a]	256 ± 10.61[a]	0.027 ± 0.006[a]
ROT + SPM(5mg/kg)	6.03 ± 0.49[b]	212 ± 4.67[b]	0.059 ± 0.010[b]
ROT + SPM (10mg/kg p.o.)	4.33 ± 0.49[bc]	170 ± 5.76[bc]	0.081 ± 0.010[bc]
ROT + Ropinirole (0.5mg/kg i.p.)	4.12 ±0.43[bd]	153±10[bd]	0.111±0.017[bd]