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Research Article

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Potential Therapeutic Role of N-acetylcysteine Against Risperidone-Induced Oxidative Stress in *Caenorhabditis elegans*

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Abstract

Risperidone, an antipsychotic drug, is widely used for treating mental health disorders such as schizophrenia, psychosis, etc. Despite its therapeutic benefits, Risperidone has been linked to oxidative stress through several pathways, including increased lipid peroxidation, mitochondrial and lysosomal dysfunction, excessive production of reactive oxygen species (ROS), and depletion of intracellular antioxidants such as glutathione (GSH). These changes impair membrane integrity, disrupt cellular redox balance, and increase the cytotoxic consequences. To address this, the current study examined how N-acetylcysteine (NAC), a potent thiol antioxidant and glutathione precursor, protects against oxidative damage induced by Risperidone. We assessed oxidative stress indicators such as ROS, lipid peroxidation (MDA), antioxidant enzyme activities (GSH, SOD, CAT), and oxidative stress tolerance (tBHP assay) using *Caenorhabditis elegans* as a model organism. Results showed decreased worm survival, increased oxidative stress markers, and compromised antioxidant defences with 50 µM risperidone exposure. However, pretreatment with 10 mM NAC effectively reduced ROS levels, enhanced GSH, and reduced lipid peroxidation, thereby improving survival under oxidative stress conditions (tBHP assay), which supports its role in maintaining redox balance. These findings suggest that risperidone-induced toxicity is mediated through oxidative stress, and NAC may offer a protective effect, presenting a potential therapeutic approach to mitigate its damaging effects.

Keywords: Antipsychotic drug; Risperidone toxicity; N-acetylcysteine; Pretreatment; *Caenorhabditis elegans.*

1. Introduction

Antipsychotic drugs are a prominent class of drugs widely used for the treatment of mental health problems [1]. These drugs alter the brain chemistry to reduce symptoms like hallucination, delusion, aggression, and disordered thinking, though their mechanism of action is not entirely clear [2]. Risperidone is a second-generation antipsychotic drug (APD), approved by the FDA (Food and Drug Administration) for use in children and adolescents as well as adults [3]. It acts as an antagonist for dopamine D2, histamine H1, and α1 and α2 adrenergic receptors with a high 5-HT2A/D2 affinity ratio, and is metabolized in the liver before being excreted by the kidneys [4, 5]. Despite the drug's substantial therapeutic benefits, several studies have documented the adverse consequences of Risperidone, such as hyperprolactinemia, metabolic dysregulation, hepatotoxicity, reproductive toxicity, and neurotoxicity

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[6–9]. These side effects have been linked, at least in part, to its ability to induce oxidative stress [10–12].

There are several ways in which risperidone causes oxidative stress. It weakens the cellular defence system by increasing the production of reactive oxygen species (ROS), damaging the integrity of the mitochondrial and lysosomal membranes, and depleting important antioxidants like glutathione (GSH) [10, 11] as being involved in the release of hydrolytic enzymes, lysosomal damage tends to be more significant in further accelerating the generation of ROS and it further encourages lipid peroxidation, which damages cells and causes membrane instability [11, 13, 14]. These changes collectively disrupt the redox equilibrium and increase the cytotoxic potential of Risperidone, as shown in mammalian cells and invertebrate models [10–12].

To counter these effects, antioxidant supplementation has been explored. It is commonly known that N-acetylcysteine (NAC), an antioxidant that contains thiols and is a precursor to cysteine, can directly scavenge ROS and restore intracellular GSH pools [15–17]. NAC is a viable option against drug-induced oxidative damage because, in addition to its antioxidant action, it also has cytoprotective and anti-inflammatory properties [18, 19]. Previous studies have shown that NAC can mitigate oxidative damage caused by antipsychotics such as haloperidol and clozapine in animal models [18]. However, its protective potential against risperidone-induced toxicity remains underexplored.

Additionally, lab studies by May et al. (2019) demonstrated that low-dose Risperidone affects the hematopoietic system of the bone marrow and induces immunosuppression in mice [20]. Risperidone is also known to directly affect bone, contributing to bone loss beyond the effects of hypogonadism alone [9]. Other studies on risperidone concentrations above 100 µM were associated with deaths, teratogenic effects, and cardiotoxic and neurotoxic effects in zebrafish embryos [21]. Earlier, we reported risperidone-induced alterations in the feeding and locomotion behavior of C. elegans, suggesting adverse effects of the drug at low doses [22]. Risperidone and aripiprazole, second-generation APDs, also impact the touch response and pharyngeal pumping in *C. elegans*, affecting dopamine and serotonin receptors in the nematode. Notably, the behavioral changes induced by these APDs persist across generations, hinting at potential epigenetic modifications [23].

Another study has reported that treatment with APDs, such as Risperidone and haloperidol, increases free radical production, either through lipid peroxidation or by altering antioxidant enzyme activities, in rat neural stem cells [10]. In human blood lymphocytes, Risperidone at concentrations of 12, 24, and 48 nM is able to cause toxicity by inducing oxidative stress and damaging mitochondrial and lysosomal membranes [11]. Risperidone is also reported to cause reproductive toxicity in male rats by inducing oxidative stress and disrupting hormonal regulation [3, 12].

Caenorhabditis elegans (*C. elegans)* has been employed for the current study as a convenient and informative model organism for toxicological studies. Due to its completely sequenced genome, short life cycle, transparent body, and high predictive capacity, *C. elegans* is becoming an increasingly common test organism for studies of aging, genetics, stress, and toxicity screening [24, 25]. Despite its simplicity, *C. elegans* and mammals share a great deal of genetic, cellular, tissue, and organ conservation. Potential APD targets conserved in humans can be found in the genome of *C. elegans*. By leveraging these advantages, *C. elegans* serves not only as a powerful tool for elucidating the molecular mechanisms of APDs but also for discovering novel therapeutic targets and assessing potential toxicities. Its genetic tractability, physiological relevance, and experimental versatility make it a powerful tool for linking basic research to clinical applications in toxicology and pharmacology.

Considering the lack of substitutes for antipsychotic drugs, particularly Risperidone, and known adverse effects with Risperidone, there has been limited comprehensive therapeutic research using antioxidants in its toxicity. Hence, the present study evaluates the use of NAC as a potent antioxidant to alleviate risperidone-induced toxicity in *C. elegans*, an animal model. NAC, being a potent thiol antioxidant and anti-inflammatory agent, has been known to inhibit enhanced ROS production [15, 16]. NAC also serves as a precursor to L-cysteine, which helps replenish glutathione levels in the body, further strengthening resistance to oxidative damage caused by ROS [16, 17]. NAC has also been proven to be useful in preventing oxidative stress due to

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treatment with a few antipsychotic drugs, including haloperidol and clozapine, in a mouse model [26]. Based on this premise, our investigation aims to explore the oxidative stress caused by Risperidone and investigate the potential of NAC pretreatment in mitigating risperidone-induced oxidative stress in *C. elegans*.

2. Materials and Methods

2.1. Chemicals and Reagents

Risperidone powder (purity ≥ 98%) was obtained from Tokyo Chemical Industry Co. (Tokyo, Japan). Sodium hypochlorite solution, dimethyl sulfoxide, 2,7-dichlorofluoroscein diacetate (H_2DCF-DA), N-acetylcysteine, and 70% tBHP were obtained from Sigma-Aldrich (USA). Agar powder, Peptone (Bacteriological), Calcium Chloride, Sodium chloride, Dihydrogen Potassium Phosphate, and Magnesium Sulphate were obtained from Himedia, India. OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit (Catalog Number STA-312), TBARS Assay Kit (Catalog Number STA-330), Catalase Activity Assay Kit (Catalog Number STA-340), and Superoxide Dismutase Activity Assay (Catalog Number STA-339) were purchased from Cell Biolabs, USA. All glassware, pipettes, and plasticware used were of high research grade and well-calibrated.

2.2. C. elegans Strains and Culture Conditions

N2 Bristol (wild-type) *C. elegans* was obtained from the Caenorhabditis Genetics Center, University of Minnesota. The nematode strain was maintained and cultured at 20°C on a nematode growth medium (NGM) agar plate seeded with a bacterial lawn of *Escherichia coli* (*E. coli*) OP50 strain, which is a uracil-quiring mutant of *E. coli* [27]. The NGM was prepared as described by Brenner (1973), containing NaCl, peptone, agar, cholesterol, $CaCl_2$, $MgSO_4$, and phosphate buffer [28]. A synchronized worm population was obtained by bleaching with a sodium hypochlorite solution, following a previsouly described protocol [29].

2.3. Risperidone Exposure

Synchronized *C. elegans* at the L4 larval stage were used for risperidone exposure. The L4 stage is the final larval phase before adulthood, marked by distinctive morphological characteristics, including the presence of a developing vulva in hermaphrodites [26]. The L4 stage was selected to ensure physiological maturity and minimize reproductive variability. Worm synchronization was achieved by allowing L2 larvae (second larval stage, occurring after hatching and before the L3 stage, characterized by active feeding and growth) to grow under controlled conditions until they developed to the L4 stage, which were then treated with 50 µM of Risperidone from a stock concentration of 20 mM prepared in DMSO. The final concentration of DMSO in the risperidone solution of 50 µM equals 0.25%. Vehicle control used the same DMSO concentrations in the absence of Risperidone. The worms were incubated for four hours at 20˚C in a BOD incubator, followed by washing thrice in M9 buffer (3g KH_2PO_4, 6g Na_2HPO_4, 5g NaCl, 1ml 1M $MgSO_4$, H_2O to 1 litre) to remove excess *E. coli*. The incubation time period was selected based on our previous study [22].

2.4. NAC Pretreatment

N-acetylcysteine (NAC) solution was freshly prepared in Milli-Q water with minor modifications to previously described protocols [16, 30]. For pretreatment, synchronized worms at the L2 larval stage (as described in Section 2.3) were transferred to NGM plates seeded with *E. coli* OP50 and overlaid with NAC solution to achieve a final concentration of 10 mM. The worms were maintained at 20 °C until they reached the L4 stage (approximately 20-24 h), ensuring continuous NAC exposure throughout development. Control worms were treated with an equivalent volume of Milli-Q water.

2.5. Measurement of Intracellular Reactive Oxygen Species Formation

For measuring intracellular ROS levels, the previously used protocol was followed [31]. H_2DCFDA was used as the fluorescent probe in *C. elegans*, based on the formation of highly fluorescent 2,7-dichlorofluorescein from non-fluorescent H2DCF-DA through reaction with ROS. In *C. elegans*, the fluorescence intensity corresponds to intracellular ROS levels. For measuring ROS levels, synchronized young adult worms were collected and incubated for 4 hours in 50 µM risperidone,

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and then washed in 1X M9 buffer three times. Worm extracts were prepared by flash-freezing worm pellets in liquid nitrogen and subjecting them to three freeze-thaw cycles, followed by sonication (setting of 30 amplitudes, 12 cycles of 1 s pulse on/off, 5–8 times using a Diagenode sonicator in 1X PBS). The worm extract was centrifuged at 20,000 × g for 15 min at 4°C, and the protein concentration of the supernatant was determined using the Bradford protein estimation kit (Bio-Rad, USA). Supernatant containing 5 µg of protein was pre-incubated with 50 µM of H_2DCFDA in 100 µL of 1X PBS at 37 °C for 1 hour. Fluorescence intensity was measured using a SpectraMax M2e Multimode Microplate Reader at an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The assay was repeated in three independent experiments.

2.6. Lipid Peroxidation Assay

For the direct quantitative measurement of malondialdehyde (MDA), the OxiselectTM TBARS Assay Kit was used according to the manufacturer's protocol. 50 µL of the sample was homogenized after 4 hours of risperidone exposure in a liquid assay with 300 µL of phosphate-buffered saline containing three µL of 100× butylated hydroxytoluene. Homogenized samples were centrifuged at 12000 rpm and 4℃ for 5 minutes. The tissue lysate supernatant was collected and reacted with thiobarbituric acid at 95℃ for 60 minutes. After all sample tubes were cooled on ice, they were centrifuged at 3000 rpm for 15 minutes. The absorbance of the solution was measured at 532 nm using a SpectraMax M2e Multimode Microplate Spectrophotometer. For NAC pretreatment, worms were overlaid with 10 mM NAC at the L2 Stage and then allowed to reach the L4 stage, followed by risperidone exposure.

2.7. Superoxide Dismutase Assay

Activity of SOD was measured using the Oxiselect™ Superoxide Dismutase Activity Assay kit by following the manufacturer's protocol. The collected worms were homogenized in 400 µL of cold 1× lysis buffer. The crude homogenate was centrifuged for 10 minutes at 12000 rpm (4 °C), and the tissue lysate supernatant was collected and stored at –80 °C until analysis. Superoxide (O_2^-) was generated by the xanthine/xanthine oxidase system and then detected with chromogen solution by measuring the absorbance reading at 490 nm using a SpectraMax® M2e Multimode Microplate Spectrophotometer. For NAC pretreatment, worms were overlaid with 10 mM NAC at the L2 Stage and then allowed to reach the L4 stage, followed by risperidone exposure.

2.8. Catalase Activity

The activity of CAT was detected following the instructions of the kit Oxiselect™ Catalase Assay Kit. Briefly, treated worms were collected, homogenized and centrifuged at 12000 g for 10 min at 4 °C following the manufacturer's instructions with minor adaptations for *C. elegans* lysates. Catalase standards were freshly prepared from the supplied stock solution. For the assay, 20 µL of each sample or standard was incubated with 50 µL H_2O_2 working solution for exactly 1 min; the reaction was quenched with 50 µL catalase quencher, and 5 µL of each reaction well was transferred to a fresh well and developed with 250 µL chromogenic working solution for 40–60 min. Absorbance was measured at 520 nm using a SpectraMax M2e Multi-Mode Microplate Reader, and catalase activity (Units/mL) was calculated. For NAC pretreatment, worms were overlaid with 10 mM NAC at L2 Stage and then the worms were allowed to reach L4 stage followed by risperidone exposure. All assays were performed in duplicate and repeated in three independent biological experiments.

2.9. Total Glutathione Assay

Total GSH levels were determined using an Oxiselect™ Total Glutathione Assay kit. A worm sample with a volume of 50 µL was homogenized in 200 µL of ice-cold 5% metaphosphoric acid and centrifuged at 12000 rpm and 4℃ for 15 minutes. The collected supernatant was stored at -80℃. Absorbance was measured at 405 nm with a SpectraMax® M2e Multimode Microplate Reader. The total GSH content in samples was determined by comparison with a predetermined GSH standard curve. For NAC pretreatment, worms were overlaid with 10 mM NAC at the L2 Stage and then allowed to reach the L4 stage, followed by risperidone exposure.

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2.10. tBHP Oxidative Stress Tolerance Assay

For this assay, a previous methodology was followed with slight modifications [32]. tBHP plates were prepared freshly by adding 150 µL of tBHP to 100 mL of NGM media and stored in the dark. For risperidone exposure, synchronized L4 worms were incubated for 4 hours at 20°C with continuous rotation in liquid medium, then transferred to tBHP plates (10.8 mM), followed by scoring of worms for survival. For NAC pretreatment, worms at the L2 stage were exposed to 10 mM NAC until they reached the L4 stage, followed by exposure to 50 µM risperidone. Worm survival was scored at 2-hour intervals until all worms died.

2.11. Statistical Analysis

Results are presented as mean standard deviation (SD). Significance was determined with one-way ANOVA followed by Tukey's Test using GraphPad Prism 8 software (GraphPad Software, San Diego, CA, USA). A p-value <0.05 was considered statistically significant. All experiments were performed in triplicate, and all the represented graphs are based on biologically independent replicates.

3. Results and Discussion

The current study aimed to assess the therapeutic efficacy of NAC as a potent antioxidant in mitigating oxidative stress induced by Risperidone in *C. elegans*.

3.1. Measurement of intracellular Reactive Oxygen Species Formation

Figure 1 illustrates the measurement of intracellular Reactive Oxygen Species (ROS) formation across different treatment groups, quantified by fluorescence intensity. Treatment with 50 µM risperidone results in a substantial increase in ROS production compared to the vehicle control (VC), as indicated by the elevation in fluorescence intensity. Pretreatment with 10 mM NAC notably reduced ROS production, both when administered alone (NAC + VC) and in combination with Risperidone (NAC + RISP), relative to the RISP-only group. Statistical analysis shows that the differences between RISP and all other groups are highly significant (***p < 0.001 and **p < 0.01). In contrast, pretreatment with NAC + VC compared to VC alone shows no significant difference (p = ns). Results are presented as mean ± SD from three independent experiments, demonstrating that NAC effectively attenuates risperidone-induced ROS generation.

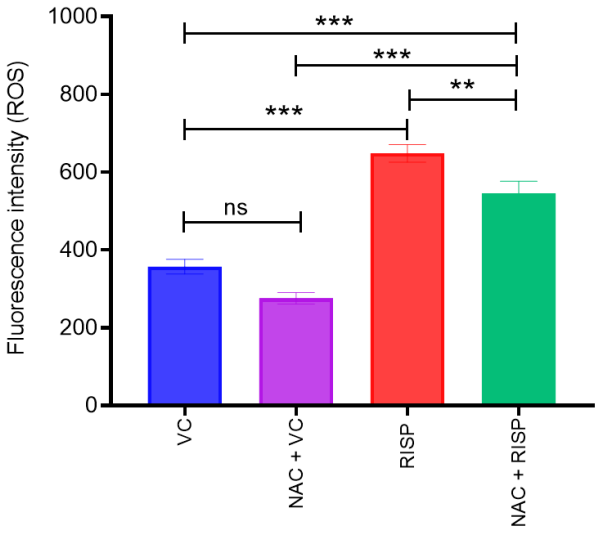

Figure 1. Measurement of ROS: ROS quantification by measuring fluorescence intensity in 50 µM risperidone treatment and 10 mM NAC pretreatment. Results are presented as mean ± SD of three independent experiments. All the asterisks indicate a significant difference between the groups. *(*p < 0.05), (**p < 0.01), (***p < 0.001),* ns- non significant.

3.2. Lipid Peroxidation

The MDA content in the VC group is approximately 20 µg/µL. Treatment with NAC alone does not significantly alter MDA levels compared to the VC group (ns). Risperidone treatment significantly increases MDA content to approximately 40 µg/µL, indicating elevated lipid peroxidation (p < 0.001 compared to VC). Pretreatment with NAC significantly reduces MDA levels induced by Risperidone to approximately 25 µg/µL (***p < 0.001 compared to Risperidone alone), although MDA levels remain slightly higher than in the VC group (ns).

Lipid peroxidation is a complex process, which can be considered as a sequence of events initiated by a hydrogen atom abstraction, followed by a reaction of oxygen with the subsequently formed radical, and by further free radical chain reactions[33]. MDA levels were higher in Risperidone-treated worms compared to the VC. This is in line with the drug's well-known pro-oxidative actions, which may be a factor in cellular damage in biological systems. The MDA level was enhanced by nearly 2-fold

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upon exposure to 50 µM risperidone compared with VC. However, when pretreated with 10 mM NAC, this increase was significantly reduced (**Figure 2**), indicating that NAC can prevent lipid peroxidation through antioxidant mechanisms. NAC most likely prevents oxidative damage to lipids by scavenging ROS and lowering oxidative stress.

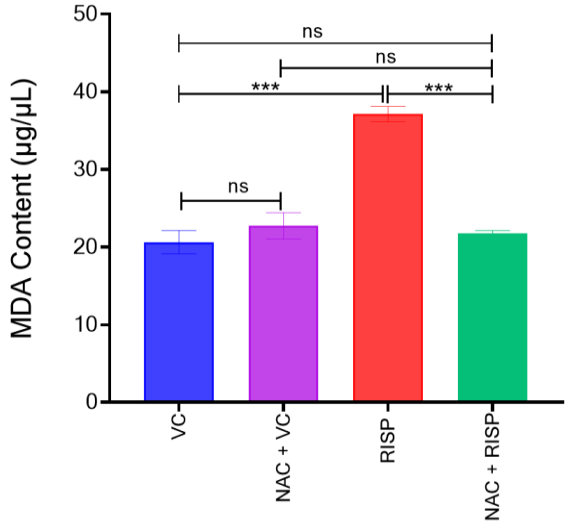

Figure 2. The bar graph illustrates the malondialdehyde (MDA) content, a marker of lipid peroxidation, in C. elegans under various treatment conditions. MDA levels (measured in µg/µL) are used to assess oxidative damage to lipids. Results are presented as mean ± SD of three independent experiments. All the asterisks indicate a significant difference between the groups. *(**p < 0.01), (***p < 0.001),* ns- non significant.

Remarkably, NAC alone has no significant effect on MDA levels in comparison to the VC group, suggesting that its effects are most pronounced under oxidative stress conditions, such as those induced by Risperidone. Although noteworthy, the MDA levels in the NAC + Risperidone group are significant but do not fully return to control levels. This implies that while NAC offers some protection, it may not completely reverse the lipid peroxidation induced by Risperidone. These results underscore the potential role of antioxidants, such as NAC, in mitigating oxidative damage induced by antipsychotic drugs and emphasize the importance of addressing oxidative stress in therapeutic contexts.

3.3. Enzymatic Assays

GSH is the most abundant non-protein thiol in mammalian cells, acting as a major reducing agent by maintaining tight control of redox status and thus serving as a cellular antioxidant. The total GSH activity was significantly decreased in the 50 µM risperidone exposure group compared to the control group. However, after pretreatment with 10 mM NAC, a significant increase in GSH Activity was observed (**Figure 3**).

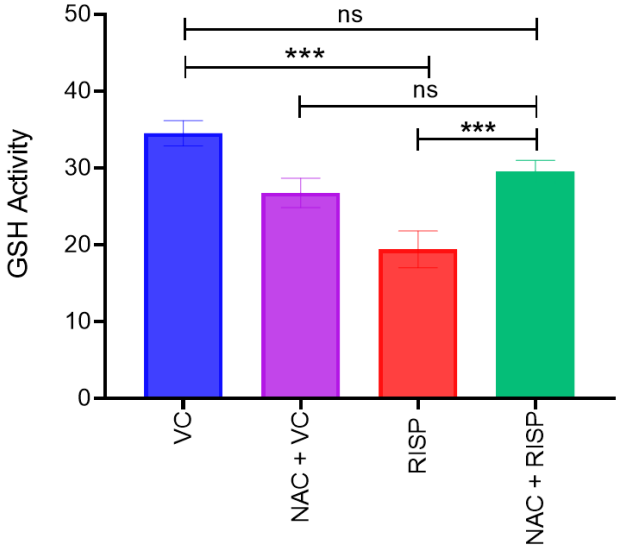

Figure 3. The graph illustrates the GSH Activity in various treatment groups: VC (vehicle control), NAC+VC (10 mM N-acetylcysteine with vehicle control), RISP (50 µM risperidone), and NAC+RISP (10 mM N-acetylcysteine with 50 µM risperidone). All the asterisks indicate a significant difference between the groups. *(**p < 0.01, ***p < 0.001),* ns-non-significant.

SOD converts superoxide radicals into hydrogen peroxide and molecular oxygen (O2). SOD activity was significantly increased ($p < 0.001$) in the C. elegans group exposed to 50 µM risperidone compared to the control group. At the same time, pretreatment with 10 mM NAC resulted in a slight increase (**Figure 4**).

The catalase enzyme is responsible for the dismutation reaction, where it aids in the destruction of H_2O_2. Fluorescence values were proportional to the catalase levels within the sample, and the catalase content in unknown samples was determined by comparison with the predetermined catalase standard curve. CAT activity was significantly reduced in C. elegans exposed to 50 µM risperidone compared to the control group. However, when pretreated with 10 mM NAC, a significant change was observed, as the level of CAT increased (**Figure 5**).

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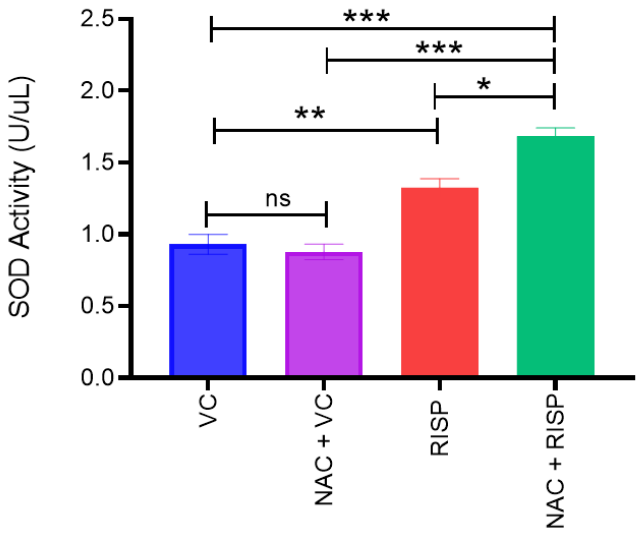

Figure 4. This graph illustrates SOD Activity across the different treatment groups, showing changes in SOD with 50 µM risperidone treatment and 10 mM NAC pretreatment. Results are presented as mean ± SD of three independent experiments. All the asterisks indicate a significant difference between the groups. *(*p < 0.05, **p < 0.01, ***p < 0.001),* ns- non-significant.

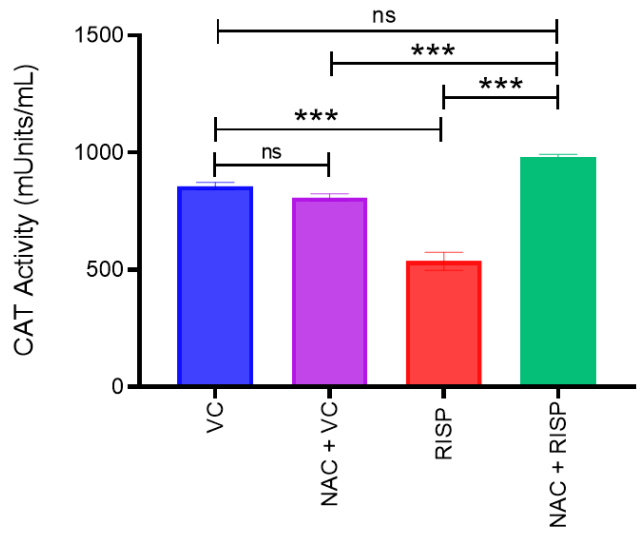

Figure 5. CAT Activity: Changes in catalase levels of *C. elegans* exposed to 50 µM risperidone treatment and 10 mM NAC pretreatment**.** Results are presented as mean ± SD of three independent experiments. All the asterisks indicate a significant difference between the groups. *(**p < 0.01, ***p < 0.001),* ns- non-significant.

The different enzymatic activities (GSH, SOD, and CAT) are represented in the above three graphs (**Figures 3-5**). The presented data demonstrate the effect of risperidone exposure on oxidative stress indicators in C. elegans, as well as the protective effect of pretreatment with NAC.

Antioxidant levels are continuously compromised by risperidone exposure in *C. elegans*. When exposed to Risperidone, GSH, an essential antioxidant that counteracts ROS, is markedly decreased, suggesting oxidative stress induction. CAT activity also decreases significantly with risperidone exposure, as it hampers the organism's capacity to break down hydrogen peroxide into water and oxygen. However, SOD activity is upregulated in response to risperidone exposure, which may be a compensatory mechanism to counteract the increased production of superoxide radicals under oxidative stress conditions.

Pretreatment with 10 mM NAC exhibited a protective effect against oxidative stress caused by Risperidone. Being the precursor of glutathione, NAC refills intracellular GSH pools, as observed by the partial restoration of GSH levels in the NAC+RISP group. In addition to being restored, CAT activity surpasses the baseline levels of the VC group, indicating improved hydrogen peroxide enzymatic detoxification. The NAC+RISP group exhibits additional increased SOD activity, demonstrating NAC's capacity to boost the organism's response to oxidative stress.

3.4. tBHP Oxidative Stress Tolerance Assay

This sentences goes like: The TBHP-induced oxidative stress tolerance assay results demonstrate the survival trends of worms under different treatment conditions (**Figure 6**). The survival rate of the worms in the vehicle control (VC) group drops steadily, with 50% survival by approximately 4 hours and total mortality by 8 hours. Worms treated with NAC alongside the vehicle show slightly improved survival compared to the VC group, especially after 6 hours. The survival rate of worms treated with Risperidone exhibits a pronounced sensitivity to oxidative stress, with survival decreasing more rapidly than in the VC group, highlighting its role in exacerbating oxidative stress. Complete death is observed before 8 hours. While pretreatment with NAC significantly increases worms' survival exposed to Risperidone. This demonstrates how NAC protects against oxidative damage, most likely by scavenging reactive oxygen species (ROS) and restoring

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intracellular glutathione levels. NAC alone may not significantly improve oxidative stress tolerance in these circumstances, as indicated by the lack of discernible survival improvement in the 10 mM NAC + VC group compared to the VC group. However, when paired with Risperidone, its protective impact becomes apparent. Overall, the data highlight how Risperidone can cause oxidative stress and how antioxidants, such as NAC, can be used therapeutically to mitigate these effects. Understanding the oxidative side effects of antipsychotics and investigating co-therapeutic approaches to lessen their toxicity are both impacted by these discoveries.

Risperidone, an often-prescribed atypical antipsychotic, has been linked to oxidative stress and cellular damage through various mechanisms, including the overproduction of reactive oxygen species (ROS), mitochondrial dysfunction, lipid peroxidation, and the reduction of natural antioxidants such as glutathione (GSH) [10, 11, 34]. This imbalance in redox status contributes to the cytotoxic effects and tissue injury observed in both mammalian and invertebrate models. In this research, *C. elegans* was utilized as an appropriate in vivo model to investigate the oxidative effects of risperidone exposure and to assess the potential protective role of N-acetylcysteine (NAC).

Exposure of *C. elegans* to 50 µM risperidone resulted in a significant rise in ROS levels, as shown by fluorescence intensity, confirming that Risperidone induces oxidative stress. The increased malondialdehyde (MDA) levels further indicated heightened lipid peroxidation, implying membrane damage caused by excessive ROS. At the same time, the marked decrease in total glutathione (GSH) and catalase (CAT) activity illustrated a loss of the worm's natural antioxidant mechanisms. Notably, the activity of superoxide dismutase (SOD) was heightened, potentially indicating a compensatory mechanism in response to the elevated superoxide radicals. Collectively, these changes confirm that Risperidone causes oxidative stress in *C. elegans*, paralleling findings in mammalian systems [11, 14].

Pretreatment with 10 mM NAC considerably alleviated these effects. NAC decreased the levels of ROS and MDA induced by Risperidone, indicating a reduction in oxidative and lipid damage. It also replenished GSH levels, thereby restoring the primary cellular antioxidant reservoir, and enhanced CAT activity, facilitating more effective detoxification of hydrogen peroxide. While SOD activity remained increased, the overall oxidative stress was notably diminished. Additionally, the tBHP oxidative stress tolerance assay demonstrated improved survival rates in worms following NAC pretreatment, validating its protective role in maintaining redox balance under stress conditions.

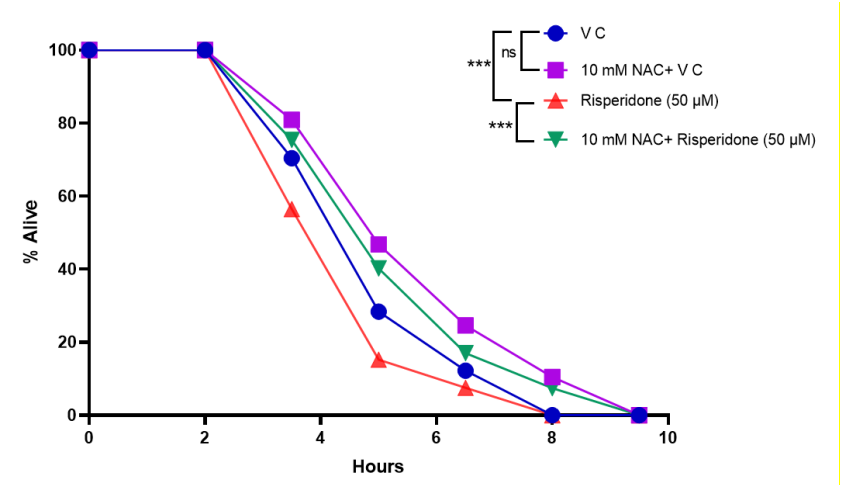

Figure 6. Tert-butyl hydroperoxide (tBHP)-induced oxidative stress tolerance assay: Survival analysis of *C. elegans* with 50 µM risperidone pretreated with 10 mM NAC. The percentage of worms alive was measured over 10 hours. A significant improvement in survival was observed in the NAC + Risperidone group compared to the Risperidone alone group, beginning at 4 hours ($p < 0.01$) and persisting until 8 hours ($p < 0.001$). NAC alone did not differ significantly from vehicle control at any time point. Data represent mean ± SD of three independent experiments. All the asterisks indicate a statistically significant difference between the groups. (**$p < 0.01$, ***$p < 0.001$), ns- non-significant.

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These observations align with previous reports, which show that NAC replenishes intracellular thiols, restores antioxidant enzyme activity, and protects against antipsychotic-induced oxidative injury [15, 26]. The results suggest that NAC acts both as a direct radical scavenger and as a precursor for GSH synthesis, thereby providing dual protection against oxidative stress. Notably, NAC alone did not alter oxidative markers compared to control worms, indicating that its antioxidant effects are pronounced primarily under oxidative stress conditions rather than at baseline.

In conclusion, increased ROS and lipid peroxidation, as well as weakened antioxidant defenses, are signs that Risperidone causes oxidative stress in *C. elegans*. By successfully reducing these alterations, NAC therapy improves survival under oxidative stress and restores redox homeostasis. These results underline the therapeutic potential of NAC in reducing oxidative toxicity caused by Risperidone and lay the groundwork for future research into the advantages of co-administration of NAC with antipsychotic drugs.

4. Conclusion

In conclusion, this study highlights the important prophylactic effect of NAC against oxidative stress in C. elegans induced by Risperidone. Our results suggest that pretreatment with 10 mM NAC can modulate an organism's response to oxidative stress by decreasing ROS production, lowering lipid peroxidation (as measured by MDA), maintaining GSH levels, and enhancing survival in oxidative environments induced by tBHP. These findings support the potential of NAC as a therapeutic agent in reducing risperidone-induced oxidative damage. By elucidating these protective mechanisms, the study paves the way for further exploration of NAC in managing oxidative stress-related toxicities. Future studies could explore dose optimization, long-term safety, and effectiveness in other model organisms and human systems to better translate these findings into clinical practice. Furthermore, examining the synergistic effects of NAC with other antioxidants or pharmacological agents may further enhance its therapeutic use in managing disorders related to oxidative stress.

Legends

ROS: Reactive Oxygen Species
VC: Vehicle Control
RISP: Risperidone
NAC: N-acetylcysteine
MDA: Malondialdehyde
GSH: Glutathione
SOD: Superoxide Dismutase
CAT: Catalase
tBHP: Tert-butyl hydroperoxide
C. elegans: *Caenorhabditis elegans*
H_2DCF-DA: 2,7-dichlorofluoroscein diacetate
SD: Standard Deviation
ns: Non-significant

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Conflict of interest

The authors did not report any conflict of interest.

Data availability

All data are available within the article.

Authors Contributions

Babita - Investigation, Methodology, writing original draft preparation, writing-review and editing, Formal analysis.
Rakhi Agarwal - Conceptualization, Review and Editing, Data curation, Supervision, Validation

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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