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RESEARCH ARTICLE

SYNERGISTIC EFFECT OF MULTIPLE EXPOSURE OF LAMBDA-CYHALOTHRIN AND STRESSOR ON REDOX IMBALANCE IN RAT BRAIN

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ABSTRACT

Our previous research indicated that exposure to psychological stress as immobilization stress, (IMS) and physical stress as forced swim stress (FSS) worsened lambda-cyhalothrin (LCT) induced brain damage in rats. To understand the underlying mechanisms, we investigated the impact of IMS or FSS on LCT's effects on oxidative stress markers in the brain. Rats exposed to IMS, FSS, or LCT alone showed no significant changes in markers of oxidative damage (lipid peroxidation, protein carbonyl levels) or antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) compared to controls. However, pre-exposure to IMS or FSS before LCT treatment significantly increased oxidative damage and decreased antioxidant enzyme activity. These findings suggest that repeated stress exacerbates LCT's neurotoxicity by disrupting the brain's redox balance. Notably, the effects were more pronounced in rats pre-exposed to psychological stress (IMS) compared to physical stress (FSS) and LCT exposure. On the other hand, modification in these redox imbalances was more concentrated in LCT treated rats pre-exposed to psychological stressor as compare to those pre-exposed to physical stressor.

Keywords: *Redox imbalance, Psychological stress, Lambda-cyhalothrin, Physical stress,* Anti-oxidant, Free radicals

INTRODUCTION

Stress, a common element of daily life, can manifest in various forms [1, 2]. While the body has mechanisms to counteract these changes, both acute and chronic stress can disrupt physiological and hormonal balance [3, 4, 5]. The activation of the hypothalamic-pituitary-adrenal (HPA) axis is a key mechanism in the stress response, resulting in an increased release of glucocorticoids such as cortisol and corticosterone [6]. These hormones influence physiological and neuropharmacological responses. The intensity and duration of stressors significantly modulate the neuroendocrine response, allowing the body to adapt appropriately to different levels of stress[2, 7].

While stress can impact multiple bodily systems as cardiovascular, gastrointestinal etc, the nervous system, the brain is particularly vulnerable and susceptible to its detrimental and serious effects [8]. Interestingly, the elevated cortisol levels, associated with stress, have been implicated in neurodegenerative disorders like Alzheimer's and in the progression of the parkinson's diseases [9]. Although low level of glucocorticoids plays a role in neuronal development, neuronal survival and other brain functioning, however the excessive levels of glucocorticoid can disrupt metabolism and antioxidant defenses in the brain [10, 11]. Stressful situations can also trigger biochemical changes that increase free radical generation, leading to oxidative stress and heightened vulnerability of the brain, potentially affecting its pharmacological and physiological functions [12].

Stress-induced disruption of the blood-brain barrier (BBB) is another mechanism implicated in various studies previously [13, 14]. The extent of BBB permeability changes can vary depending on the type, intensity, and duration of stress. Consequently, certain chemicals or toxicants that normally cannot penetrate the BBB can easily enter the brain under stress conditions [15, 16].

The connection between stress and enhanced chemical toxicity was highlighted by the experiences of veterans returning from the Persian Gulf War [14]. Psychological and physiological disturbances, mood disorders, and brain-related neurodisorders led to the recognition of stress as a significant factor [17]. Studies suggested that under stress conditions the chemicals/toxicants can easily crosses the BBB and poses the significant disturbances in brain functioning [13, 14]. Epidemiological studies further support the notion that the interaction between stress and environmental toxins can contribute to disease development [18, 19]. Recognizing the vital role of neurotransmitters in regulating synaptic transmission, many experimental studies have explored the impact of various stressors—such as immobilization, forced swim, footshock, and exposure to cold or heat—on

pharmacological responses and physiological changes [5, 45]. Additionally, experimental studies have explored the impact of drugs and chemicals, such as metals and pesticides to assess the impact of stress [20].

Synthetic pyrethroids are widely preferred over other pesticides due to their potent insecticidal activity and bio-efficacy [21, 22, 23]. They account for approximately 40% of global insecticide consumption [5, 13]. A number of pesticides pyrethroid formulations been developed in the recent years with low toxicity and thus making them applicable for use in agriculture for pest control [24, 25]. Lambda-cyhalothrin (LCT), a newer-generation type II synthetic pyrethroid, shares similarities with cyhalothrin. Its high insecticidal and larvicidal properties make it valuable for controlling a wide range of insects and pests in agriculture, homes, and greenhouses. LCT's effectiveness against disease vectors has led to its frequent use in community health programs [26]. However, indiscriminate use of LCT poses a risk to non-target organisms, including mammals and aquatic invertebrates, due to its neurotoxic effects. Free radicals and oxidative stress have been implicated in the neurotoxicity of LCT [27, 28]. The studies carried out suggest the potential involvement of free radicals and oxidative stress governing the neurotoxicity of LCT.

Humans routinely encounter stress, everyday from varying sources, while also being exposed to environmental toxicants, even at low levels. The degree of exposure to these chemicals can vary significantly depending on occupational settings and specific circumstances. In natural conditions, various exposure scenarios are possible, including pre-exposure to stressors followed by neurotoxicants, simultaneous exposure to both, pre-exposure to neurotoxicants followed by stressors, or exposure to neurotoxicants during a stressful period.

Even though, the potential involvement of both physical and psychological are associated with the environmental chemicals toxicity. However the role and mechanism of the neurotoxicity of LCT in stress is still uncovered. With all this, the present study been designed to identify the involvement of redox imbalance on IMS (psychological stressor)and FSS (physical stressor) in governing the neurotoxicity of lambda-cyhalothrin (LCT), a new generation type II synthetic pyrethroid. The study will evaluate the relative impact of these two type of stressors on LCT-induced neurotoxicity in rats.

MATERIALS AND METHODS

Animals treatment and Procedure of Study:

Male Wistar rats (200 ± 10 grams) obtained from the CSIR-Indian Institute of Toxicology Research [CSIR-IITR] animal breeding colony, Lucknow, were used for the current study. The animals are accommodated in polypropylene cages under proscribed temperature (25 ± 2ºC)

and with the 12 hours light / dark cycle in the animals house. These rats were acclimatized for five days before start the experiment. A standard pellet diet and water were provided ad libitum. The experimental protocols were reviewed and approved by the CSIR-IITR Institutional Animal Ethics Committee, following the guidelines established by the CCSEA.

After a 15-day acclimatization period, the animals were randomly assigned to six treatment groups. Details of the subsequent exposure of rats to stressors and lambda-cyhalothrin are provided below.

Group I: Served as Control. Rats were served with vehicle i.e. corn oil orally for three days (26, 27 and 28 Day).

Group II: Rats were treated to lambda-cyhalothrin (3.0 mg/kg body weight, p.o. suspended in corn oil) for three days (26, 27 and 28 Day)

Group III: Rats exposed to immobilization stress daily for 28 days. Immobilization stress was given by placing them in to acrylic tubes (22 cm length and 7 cm diameter; 1 session of 15 min/day).

Group IV: Rats Exposed to immobilization stress similar like in groups III for 28 days. Followed by the rats were then treated with to lambda-cyhalothrin with the dose of (3.0 mg/kg body weight, p.o.) suspended in corn oil] for three days (Day 26, 27 and 28) identical as group II.

Group V: Rats exposed to forced swim stress for 28 days. The stress was given by placing them individually in a glass cylinder (45 cm high, 20 cm in diameter) filled with water (temperature around 25 + 2°C) up to a height of 30 cm (1 session of 3 min / day).

Group VI: Rats exposed to forced swim stress for 28 days same as group V and been treated lambda-cyhalothrin (3.0 mg/kg body weight, p.o. suspended in corn oil) for three days (Day 26, 27 and 28) same like group II.

The co-exposure of LCT with stressor, rats were first exposed to stressor i.e r immobilization and forced swim stress, followed by exposure to LCT. The treatment of lambda-cyhalothrin and stress procedure was carried out between 0900 – 1200 hrs.

After the last dose of LCT, the rats were sacrificed by the cervical dislocation. The five rats from each group were sacrificed and the brain was removed quickly and isolated and dissected in brain parts hippocampus, frontal cortex, corpus stratum and hypothalamus using the standard procedure.

Assessing Oxidative Stress: To understand how much oxidative stress occurred after exposure to stress, lambda-cyhalothrin, or both combined, we measured things like how much reactive oxygen species (ROS) were produced, the levels of harmful substances like lipid peroxidation and protein carbonyls, and antioxidant defenses like reduced glutathione. We also looked at the

activities of enzymes that protect against oxidative damage, such as superoxide dismutase, catalase, and glutathione peroxidase in different parts of the brain. The methods we used to measure these are explained briefly below.

Measuring Lipid Peroxidation: To determine how much lipid peroxidation (damage to fats) occurred, we measured the formation of malonaldehyde (MDA) in brain regions using a method described by Ohkawa et al. [29]. This method involves a chemical reaction between MDA and a chromogenic reagent. When one MDA molecule reacts with two molecules of the reagent, it produces a stable colored compound. The process began by breaking up brain tissue in a phosphate buffer, then incubating it with sodium dodecyl sulfate (SDS) and acetic acid. After that, the mixture was heated with thiobarbituric acid, causing it to form a pink color. We measured the color's intensity at a wavelength of 532 nm. Lipid peroxidation was quantified using a standard formula and expressed as micromoles of MDA produced per hour per milligram of protein.

Measuring Protein Carbonyl Levels: To assess oxidative damage to proteins, we measured protein carbonyl levels using a method by Levine et al. [30], with 2,4-dinitrophenylhydrazine (DNPH) as the key reagent. Brain tissue was homogenized, and the solution was spun in a centrifuge to separate the liquid [supernatant]. We divided the supernatant into two parts for each sample. One part was mixed with DNPH, while the other was mixed with hydrochloric acid (HCl) to act as a blank. Both samples were incubated for an hour, and proteins were then separated by adding trichloroacetic acid (TCA) and cooling on ice. After washing away any unreacted DNPH or lipids, the remaining protein was suspended in guanidine hydrochloride. The absorbance difference between DNPH-treated samples and blanks was measured at 375 nm using a spectrophotometer, and the protein carbonyl content was determined based on a standard reference value. Results were expressed as nanomoles of carbonyl per milligram of protein.

Measuring Reduced Glutathione (GSH) Levels: The levels of reduced glutathione (GSH) in specific brain regions were measured using the method described by Hasan and Haider [31]. In summary, a 10% brain homogenate was deproteinized by mixing it with an equal volume of 10% trichloroacetic acid (TCA) and incubated at 4°C for 1 hour. The mixture was then centrifuged at 3000×g for 15 minutes, and 0.5 ml of the supernatant was mixed with Tris-HCl buffer (0.4 M, pH 8.9) and EDTA (0.02 M). Subsequently, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, 0.01 M) was added, and the final volume was brought to 3 ml with distilled water. The absorbance of the resulting yellow color was measured at 412 nm using a spectrophotometer. A GSH standard was used concurrently, and GSH levels were expressed as micrograms per gram of tissue.

Measuring Superoxide Dismutase (SOD) Activity: Superoxide dismutase (SOD) activity in brain regions was assessed using a modified version of the method by Kakkar et al. [32]. The final reaction mixture (3 ml) consisted of 1 ml distilled water, 1.4 ml sodium pyrophosphate buffer [0.082 M, pH 8.3], 0.1 ml phenazine methosulfate (186 µM), 0.3 ml nitroblue tetrazolium (300 µM), 0.2 ml NADH (780 µM), and 0.1 ml post-mitochondrial brain fraction in phosphate buffer (0.1 M, pH 7.4). The reaction was initiated by adding NADH and incubated at 37°C for 90 seconds. It was terminated by adding 1 ml glacial acetic acid, followed by thorough mixing with 4 ml n-butanol. After allowing the mixture to stand for 10 minutes, it was centrifuged at 3000×g for 10 minutes. The intensity of the purple chromogen in the butanol layer was measured at 560 nm. SOD activity was expressed in units per minute per milligram of protein, where one unit is defined as the amount of enzyme required to inhibit chromogen formation by 50%.

Measuring Catalase Activity: Catalase activity in brain regions was assayed using the method of Aebi [33] with hydrogen peroxide (H2O2) as the substrate. The final reaction volume (1 ml) contained phosphate buffer (0.1 mM, pH 7.4), the post-mitochondrial brain fraction (100 µl), and H_2O_2 (30 mM). The reduction in optical density was recorded for 150 seconds at 240 nm using a spectrophotometer. The catalase activity was calculated using a molar extinction coefficient of 43.6 M cm-1, and the values were expressed as micromoles of H_2O_2 decomposed per minute per milligram of protein.

Measuring Glutathione Peroxidase [GPx] Activity

Glutathione peroxidase (GPx) activity in brain regions was assessed using the method described by Flohe and Gunzler [34]. A 10% brain homogenate was initially centrifuged at 1500×g for 10 minutes at 4°C, and the supernatant was further centrifuged at 10,000×g for 30 minutes at 4°C. The final supernatant was utilized for the GPx assay. The reaction mixture [1 ml] consisted of phosphate buffer (0.1 M, pH 7.4), reduced glutathione (2 mM), sodium azide (10 mM), hydrogen peroxide (H, O, , 1 mM), and the enzyme preparation. The mixture was incubated at 37°C for 15 minutes, and the reaction was terminated by adding 10% trichloroacetic acid (TCA). Following centrifugation at 1500×g for 5 minutes, the supernatant was transferred to a separate tube containing phosphate buffer and DTNB (0.4 mg/ml). Absorbance was recorded at 420 nm, and GPx activity was expressed as nanomoles of GSH oxidized per minute per milligram of protein.

Protein Content Estimation

Protein content was determined following the method of Lowry et al. [35], with bovine serum albumin used as the standard. A standard curve was generated

using varying concentrations to calculate the protein content in the samples.

Statistical Analysis: To examine the effects of lambda-cyhalothrin, stressors (immobilization or forced swim stress), and their interactions, we used a two-way analysis of variance (ANOVA), followed by the Bonferroni post hoc test to compare all pairs of columns and assess significance. The data are expressed as mean ± SEM, and differences with p-values below 0.05 were considered significant.

RESULTS:

Assessment of Pro- and Antioxidant Stress in Selected Brain Regions

Lipid Peroxidation (MDA Levels): Similar to ROS, no significant change in malondialdehyde (MDA) levels was found in any brain region following IMS, FSS, or LCT treatment alone compared to controls. Pre-exposure to IMS or FSS followed by LCT treatment significantly elevated MDA levels in the frontal cortex, corpus striatum, hippocampus, and hypothalamus compared to both control rats and LCT-alone rats (Figure 1). Furthermore, MDA levels were higher in rats pre-exposed to IMS or FSS followed by LCT compared to those exposed to IMS or FSS alone (Figure 1). The two-way ANOVA showed that stress exposure modulated LCT's effect on MDA levels, but no significant interaction was found between LCT and stress in the frontal cortex or hippocampus.

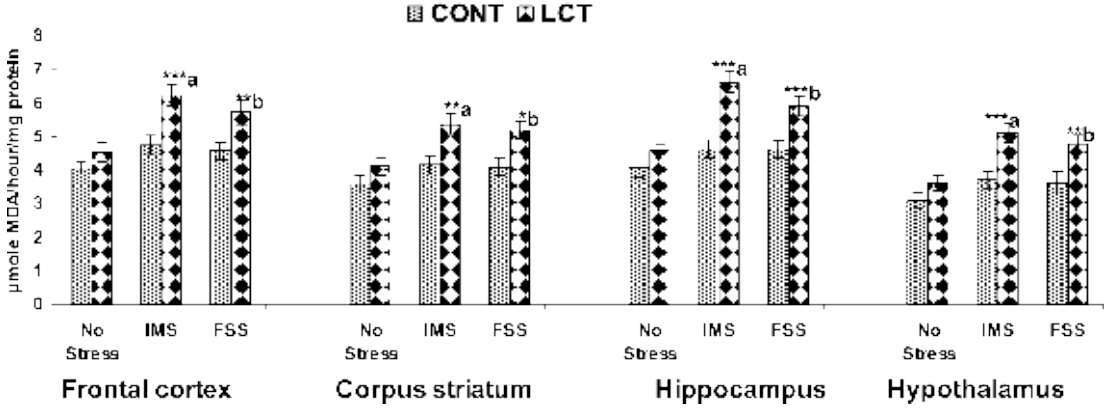

Figure 1: Effect on brain lipid peroxidation following exposure of rats following repeated exposure of rats to stressors for 28 days and lambda-cyhalothrin for 3 days

Rats were exposed to IMS (by placing in plastic restrainer for 15 min/day) or FSS (by placing in vertical glass cylinder filled with water for 3 min/day) alone for 28 days or with LCT (3.0 mg/kg body weight, p.o suspended in corn oil) for 3 days on day 26, 27 and 28 of stress.

Values are mean ± SEM of five animals in each group. The data was analyzed by two-way ANOVA followed by Bonferroni test. Significantly differs (*p < 0.05, **p < 0.01, ***p < 0.001). a-compared to immobilization stress, b-compared to forced swim stress group. CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress

Protein Carbonyl Levels: There were no significant changes in protein carbonyl levels in any brain region in rats exposed to IMS, FSS, or LCT treatment alone compared to controls. However, pre-exposure to IMS or FSS followed by LCT treatment significantly increased protein carbonyl levels in the frontal cortex, corpus striatum, hippocampus, and hypothalamus compared to both controls and LCT-alone rats (Figure 2). Additionally, protein carbonyl levels were higher in rats pre-exposed to IMS or FSS followed by LCT compared to those exposed to IMS or FSS alone. The two-way ANOVA revealed that stress exposure modified the effect of LCT on protein carbonyl levels, though no interaction was observed between LCT and stress exposure in the frontal cortex (Figure 2).

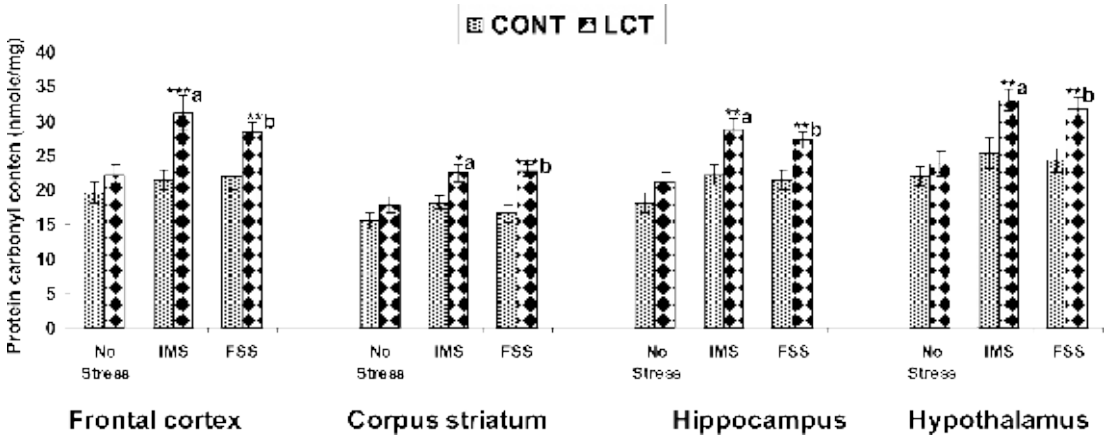

Figure 2: Effect on brain protein carbonyl levels following exposure of rats following repeated exposure of rats to stressors for 28 days and lambda-cyhalothrin for 3 days

Rats were exposed to IMS (by placing in plastic restrainer for 15 min/day) or FSS (by placing in vertical glass cylinder filled with water for 3 min/day) alone for 28 days or with LCT (3.0 mg/kg body weight, p.o suspended in corn oil) for 3 days on day 26, 27 and 28 of stress.

Values are mean ± SEM of five animals in each group. The data was analyzed by two-way ANOVA followed by Bonferroni test. Significantly differs (*p < 0.05, **p < 0.01, ***p < 0.001). a-compared to immobilization stress, b-compared to forced swim stress group.

CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress

Reduced Glutathione (GSH) Levels: No significant change in GSH levels was detected in any brain region of rats exposed to IMS, FSS, or LCT treatment alone compared to controls (Figure 3). However, pre-exposure to IMS or FSS followed by LCT treatment significantly reduced GSH levels in the frontal cortex, corpus striatum, hippocampus, and hypothalamus compared to both control and LCT-alone rats. Similarly, pre-exposure to IMS or FSS followed by LCT also caused lower GSH levels in these regions compared to IMS or FSS alone (Figures 3). The two-way ANOVA indicated that stress exposure modulated the effect of LCT on GSH levels, but no significant interaction between LCT and stress was found.

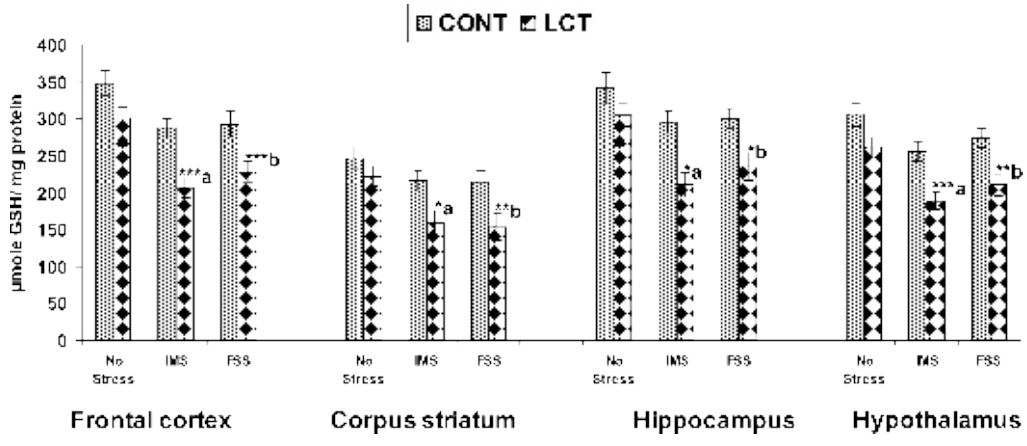

Figure 3: Effect on brain reduced glutathione levels following exposure of rats following repeated exposure of rats to stressors for 28 days and lambda-cyhalothrin for 3 days

Rats were exposed to IMS (by placing in plastic restrainer for 15 min/day) or FSS (by placing in vertical glass cylinder filled with water for 3 min/day) alone for 28 days or with LCT (3.0 mg/kg body weight, p.o suspended in corn oil) for 3 days on day 26, 27 and 28 of stress.

Values are mean ± SEM of five animals in each group. The data was analyzed by two-way ANOVA followed by Bonferroni test. Significantly differs (*p < 0.05, **p < 0.01, ***p < 0.001). a-compared to immobilization stress, b-compared to forced swim stress group. CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress

Superoxide Dismutase (SOD) Activity: No significant change in SOD activity was found in any brain region of rats exposed to IMS, FSS, or LCT treatment alone compared to controls. However, pre-exposure to IMS or FSS followed by LCT treatment significantly reduced SOD activity in the frontal cortex, corpus striatum, hippocampus, and hypothalamus compared to both control and LCT-alone rats (Figures 4). Additionally, pre-exposure to IMS or FSS followed by LCT treatment resulted in lower SOD activity in these regions compared to IMS or FSS alone. The two-way ANOVA revealed that stress exposure modified the effect of LCT on SOD activity, though no significant interaction between LCT and stress was observed in any brain region (Figures 4).

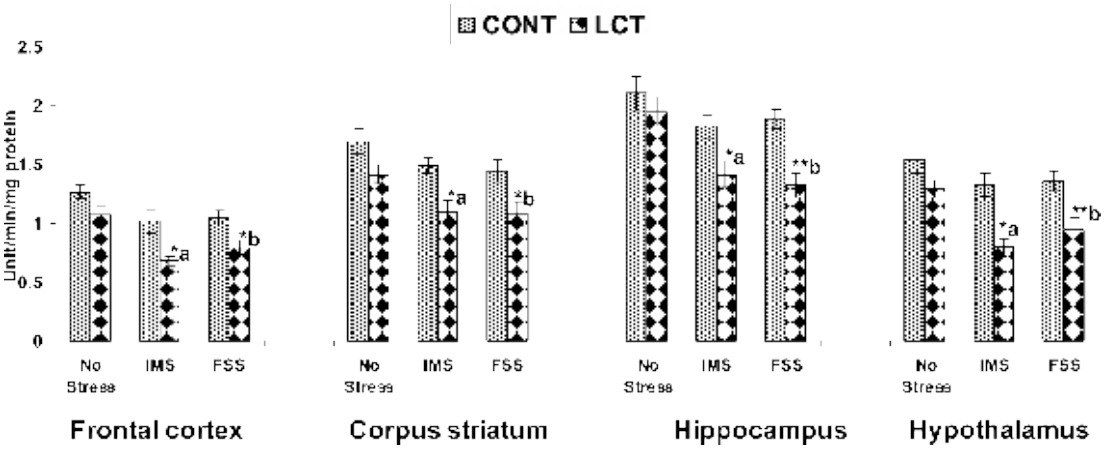

Figure 4: Effect on brain superoxide dismutase activity following exposure of rats following repeated exposure of rats to stressors for 28 days and lambda-cyhalothrin for 3 days

Rats were exposed to IMS (by placing in plastic restrainer for 15 min/day) or FSS (by placing in vertical glass cylinder filled with water for 3 min/day) alone for 28 days or with LCT (3.0 mg/kg body weight, p.o suspended in corn oil) for 3 days on day 26, 27 and 28 of stress.

Values are mean ± SEM of five animals in each group. The data was analyzed by two-way ANOVA followed by Bonferroni test. Significantly differs (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). a-compared to immobilization stress, b-compared to forced swim stress group. CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress.

Catalase Activity: Catalase activity showed no significant change in any brain region in rats exposed to IMS, FSS, or LCT treatment alone compared to controls. However, pre-exposure to IMS or FSS followed by LCT treatment significantly reduced catalase activity in the frontal cortex, corpus striatum, hippocampus, and hypothalamus compared to both control and LCT-alone rats (Figures 5). Pre-exposure to IMS or FSS followed by LCT also caused lower catalase activity in these regions compared to IMS or FSS alone (Figures 5). The two-way ANOVA indicated that stress exposure modulated the effect of LCT on catalase activity, though no significant interaction between LCT and stress was found in any brain region.

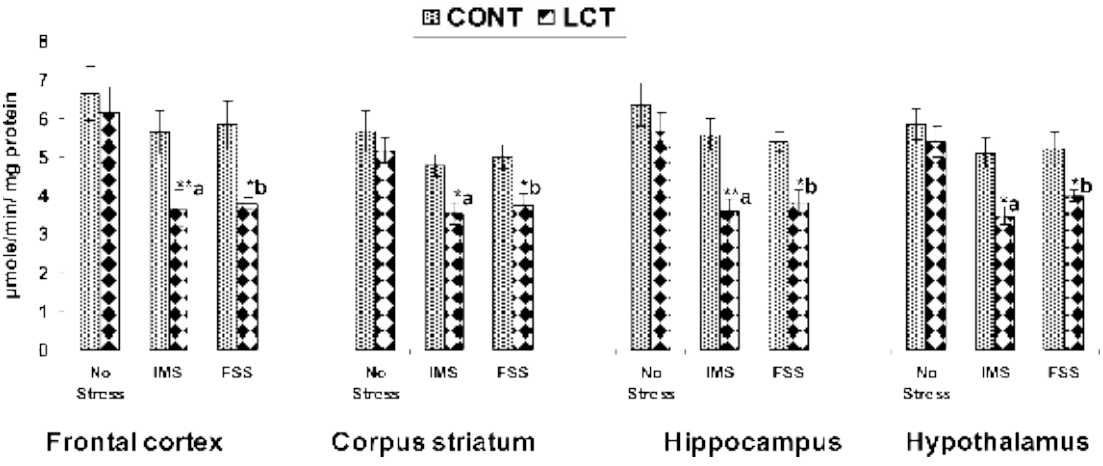

Figure 5: Effect on brain catalase activity of rats following exposure of rats following repeated exposure of rats to stressors for 28 days and lambda-cyhalothrin for 3 days

Rats were exposed to IMS (by placing in plastic restrainer for 15 min/day) or FSS (by placing in vertical glass cylinder filled with water for 3 min/day) alone for 28 days or with LCT (3.0 mg/kg body weight, p.o suspended in corn oil) for 3 days on day 26, 27 and 28 of stress.

Values are mean ± SEM of five animals in each group. The data was analyzed by two-way ANOVA followed by Bonferroni test. Significantly differs (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). a-compared to immobilization stress, b-compared to forced swim stress group. CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress

Glutathione Peroxidase (GPx) Activity:No significant changes were observed in GPx activity in any brain region of rats exposed to IMS, FSS, or LCT treatment alone compared to controls. However, pre-exposure to IMS or FSS followed by LCT treatment significantly

reduced GPx activity in the frontal cortex, corpus striatum, hippocampus, and hypothalamus compared to both control and LCT-alone rats (Figures 6). Pre-exposure to IMS or FSS followed by LCT treatment also caused lower GPx activity in these regions compared to IMS or FSS alone. The two-way ANOVA revealed that stress exposure altered LCT's effect on GPx activity, though no significant interaction between LCT and stress was found in any brain region (Figures 6).

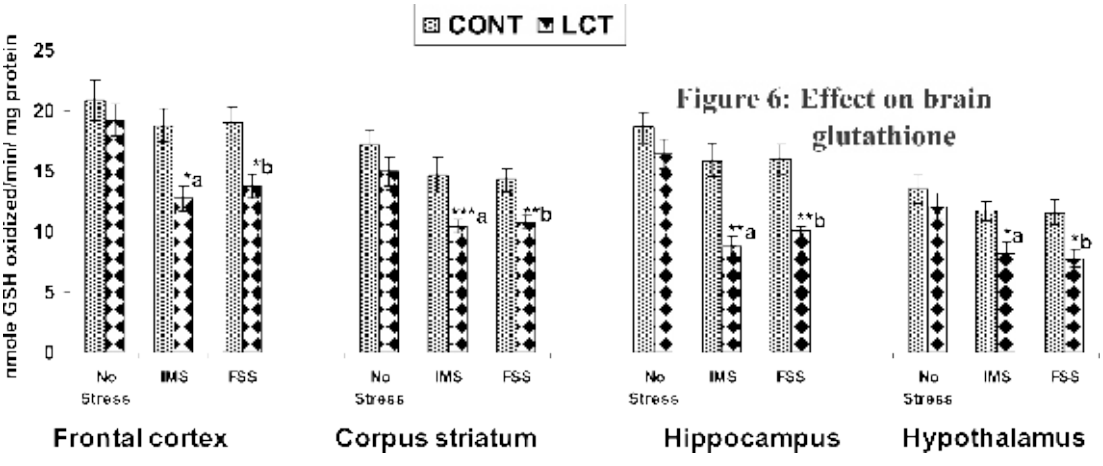

Figure 6: Effect on brain glutathione

Figure 6: Effect on brain glutathione peroxidase activity following repeated exposure of rats to stressors for 28 days and lambda-cyhalothrin for 3 days

Rats were exposed to IMS (by placing in plastic restrainer for 15 min/day) or FSS (by placing in vertical glass cylinder filled with water for 3 min/day) alone for 28 days or with LCT (3.0 mg/kg body weight, p.o suspended in corn oil) for 3 days on day 26, 27 and 28 of stress.

Values are mean ± SEM of five animals in each group. The data was analyzed by two-way ANOVA followed by Bonferroni test. Significantly differs (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). a-compared to immobilization stress, b-compared to forced swim stress group. CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress

DISCUSSION:

Elevated glucocorticoid levels in plasma/serum, often indicative of stress and its severity, have been widely documented [1, 36]. Research suggests that exposure to environmental neurotoxicants can also trigger this increase [19, 27, 28]. For instance, Soderlund et al. [46] observed heightened plasma corticosterone in rats exposed to low-dose deltamethrin, a synthetic pyrethroid, implying potential impacts on neuroendocrine and autonomic systems. Similarly, Righi and Palermo-

Neto [36] noted elevated serum corticosterone in rats exposed to higher doses of LCT (3.0 mg/kg body weight/day) for 7 days, but not at lower doses (1mg/kg body weight/day), while examining its behavioral effects. These findings underscore the complex relationship between environmental chemical exposure, stress response, and potential neuroendocrine disruption. Initial studies on rats, exploring the combined impact of restraint stress (lasting 5 to 60 minutes) and low-dose pyridostigmine bromide, revealed a significant cortisol spike at the 15–20-minute stress mark [13, 15]. Mirroring this, our current study showed no significant plasma corticosterone change in rats exposed to LCT (3.0 mg/kg/day) for 3 days. Similarly, isolated exposure to either IMS (10 minutes/day) or FSS (3 minutes/day) for 28 days didn't alter corticosterone levels. However, previous work by Shukla et al. [37, 38] demonstrated that pre-exposure to IMS or FSS for 28 days, followed by 3 days of LCT, heightened both plasma corticosterone and blood-brain barrier permeability. This suggests a potential synergistic effect between prior stress and LCT exposure, leading to a pronounced HPA axis response and redox imbalance.

A landmark study by Friedman et al. [14] revealed that stress could compromise the blood-brain barrier (BBB) in mice. This vulnerability was further exacerbated when combined with exposure to chemicals like pyridostigmine bromide, DEET, and permethrin [13, 15, 23]. Our current findings align with this, demonstrating that while neither stress nor LCT alone significantly impacted BBB permeability, the sequential exposure to stress (IMS or FSS) followed by LCT treatment did. This suggests a heightened susceptibility of the BBB to chemical insult under prior stress conditions. However, the precise mechanisms underlying this interaction remain to be elucidated [37, 38].

Experimental studies have shown varying degrees of oxidative stress in the brain following exposure to stress, likely due to differences in the types of stressors, experimental models, methodologies, and durations of stress procedures [36, 39]. Additionally, stress-induced changes in neurotransmitter and metabolite levels have been observed, often suggesting an adaptive response [19]. In our study, rats exposed to LCT (3 mg/kg body weight) for 3 days showed no significant changes in brain pro- and antioxidant levels, suggesting the dose was well-tolerated and non-neurotoxic. However, when rats were pre-exposed to IMS or FSS before LCT treatment, we observed decreased antioxidant levels and increased pro-oxidant levels in the hypothalamus, frontal cortex, corpus striatum, and hippocampus.

The brain is highly susceptible to various stressors, including physical, psychological, social, and environmental factors. Research has shown that stress can increase the production of reactive oxygen species (ROS) in the brain by altering biochemical pathways [22]. This

heightened ROS production, coupled with the brain's limited antioxidant capacity and high levels of polyunsaturated fatty acids (PUFAs), makes it particularly vulnerable to oxidative damage [40]. While ROS play essential roles in biological processes, excessive ROS generation can lead to lipid peroxidation, compromising cellular structure and function [41]. Studies have demonstrated that even short-term psychological stress can increase lipid peroxidation in the brain without affecting other organs like the liver. Chronic stress has also been linked to elevated lipid peroxidation and nitrite levels in the hippocampus, accompanied by cognitive impairments. Our study found that rats exposed to prolonged immobilization stress (IMS) or forced swimming stress (FSS) exhibited increased lipid peroxidation, protein carbonyl levels, and decreased antioxidant defenses in various brain regions [3]. However, these effects were not observed in rats subjected to shorter durations of stress, highlighting the importance of stress duration in causing these changes. Overall, exposure to acute or chronic stress can disrupt biochemical pathways, generate free radicals, and damage biomembranes, ultimately affecting cellular integrity and function in the brain [40].

Research suggests a strong link between stress and neuropsychiatric disorders. Stress-induced oxidative stress can trigger inflammation and disrupt the immune system, contributing to neurodegenerative diseases [42]. Observations from veterans after the Gulf War highlighted how stress can heighten the toxic effects of drugs on the central nervous system, even those considered safe under normal conditions [13]. Experimental studies have confirmed that stress can significantly increase the neurotoxicity of various drugs, environmental chemicals, metals, and pesticides. Furthermore, stress has been shown to worsen the neurotoxic effects of these substances [16]. The blood-brain barrier (BBB) plays a critical role in defensive the brain from injurious chemicals. However, stress can compromise the integrity of the BBB, allowing substances that would normally be blocked to enter the brain and cause damage [13, 15]. This study observed that pre-exposure to stress followed by LCT treatment, or simultaneous exposure to both, increased lipid peroxidation and decreased reduced glutathione (GSH) in rat brains [45]. The GSH depletion likely stems from its increased use in conjugation and/or antioxidant activities to neutralize reactive free radicals [44]. LCT toxicity may also be attributed to cyanohydrins, unstable free radical species under physiological conditions [46]. The simultaneous stress and LCT exposure impaired the antioxidant defense in rat brains, evidenced by decreased superoxide dismutase, catalase, and glutathione peroxidase activity, likely due to excessive free radical generation. Reduced catalase and Gpx activity, key enzymes in hydrogen peroxide degradation, may elevate brain-toxic hydrogen peroxide levels. Increased free radicals could disrupt the blood-brain

barrier, impacting brain homeostasis and metabolic functions.

This study's findings suggest that stress intensity and duration significantly impact neurobehavioral changes. Rats exposed to IMS for 6 hours/day or FSS for 15 minutes/day over 28 days exhibited these changes, whereas those exposed to IMS for 15 minutes/day or FSS for 3 minutes/day did not. The LCT doses used were well-tolerated and didn't independently affect neurobehavioral endpoints. However, concurrent exposure to IMS (exposure time 6 hours/day per session) or FSS (exposure time 15 minutes/day per session) and LCT worsened certain neurobehavioral parameters related to brain cholinergic and dopaminergic systems [37, 38]. Notably, pre-exposure to IMS (15 minutes/day) or FSS (3 minutes/day) combined with LCT led to brain cholinergic alterations, impacting learning and memory, and brain dopaminergic alterations, causing motor deficits. These changes appear linked to increased corticosterone levels, disrupted blood-brain barrier permeability, mitochondrial dysfunction, heightened oxidative stress, and apoptosis [22, 44].

While the study shows that psychological stress (IMS) may cause more intense oxidative stress changes in rats exposed to LCT compared to physical stress (FSS), it is evident that both types of stress significantly increase LCT's neurotoxic effects. This finding is particularly important considering a large portion of the population experiences stress regularly.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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