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Effect of Repeated Exposure to Lambda-Cyhalothrin and Immobilization or Forced Swim Stress on Oxidative Stress in Rat Brain

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Abstract

The present study has been undertaken to investigate role of immobilization stress (IMS), a psychological stressor and forced swim stress (FSS), a physical stressor on the neurobehavioral toxicity of lambda-cyhalothrin (LCT), a new generation, type-II synthetic pyrethroid with extensive uses to control insects and ectoparasites. Rats subjected to IMS (placed in plastic restrainer for 6 hour/day) or FSS (one session of 15 min/day) for 28 days exhibited a significant increase in lipid peroxidation and protein carbonyl levels in frontal cortex, corpus striatum, hippocampus and hypothalamus as compared to controls. Further, decrease in the levels of reduced glutathione and activity of superoxide dismutase, catalase and glutathione peroxidase was also observed in these brain regions of rats subjected either IMS or FSS as compared to rats in the control groups. No significant change in any of the parameters was observed in any of the brain regions of rats treated with LCT (0.5 mg/kg body weight, p.o., 28 days) as compared to controls. Simultaneous exposure of rats to LCT and IMS or FSS resulted to cause marked increase in protein carbonyl levels and lipid peroxidation as compared to controls. Further, decrease in the activity of superoxide dismutase, catalase and glutathione peroxidase, enzymes involved in antioxidant defense in these brain regions was also observed in rats simultaneously exposed to IMS or FSS and LCT as compared to controls. The data of the present study exhibit that both psychological and physical stress enhances the neurotoxicity of lambda-cyhalothrin by enhancing oxidative stress in rat brain.

Keywords: Immobilization stress, Forced swim stress, Lambda-cyhalothrin, Oxidative stress

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1. Introduction

Stress is inevitable in the present day life to which humans are exposed in one way or the other in different forms. It has been found that stress disrupts the normal homeostasis of the organisms and activates the HPA axis.[1] As a result, release of glucocorticoid (cortisol in humans and corticosterone in rodents) is the primary effect in response to stress.[2] Enhanced corticosterone by sustained / persistent stress has been found to be a risk factor for a number of diseases.[3] Increased incidences of cardiovascular and neuropsychiatric disorders have frequently been associated with stress and are a cause of concern.[1] Excessive cortisol could be neurotoxic and affect the brain structure and associated functions.[4]

Stress has been found to affect brain dopaminergic, cholinergic and serotonergic systems and associated functions.[5] Stress could affect the cognitive functions in the affected individuals although severity of changes depends on timing and intensity of exposure during the life.[3] A number of studies have found that chronic stress could disrupt the blood brain barrier (BBB) permeability

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and thus the drugs and chemicals that do not cross the BBB easily target the brain and cause neurobehavioral toxicity.[6, 7] Of various mechanisms associated in the etiology of neurotoxicological disorders, enhanced oxidative stress has been reported by a number of investigators both in experimental and clinical studies.[8, 9] Exposure to stress situations may affect the biochemical pathways and enhance free radical generation in brain.[9] Further, impairment in antioxidant defense has also been reported in brain of animals subjected to stress.[10] Increased corticosterone levels in brain have been associated with enhanced ROS generation in experimental models.[11] Decrease in the activity of superoxide dismutase and glutathione peroxidase, the enzymes involved in the antioxidants defense on corticosterone treatment further contribute to enhanced oxidative stress.[11] Increased oxidative stress therefore may enhance vulnerability of the brain and could affect the pharmacological and physiological processes.[9]

Impact of stress on the functioning of brain has been assessed using different physical, psychological and environmental stressors on experimental models.[12] Further, potential of anti-stress agents has also been screened.[13] Acute stress has been found to cause transient changes while chronic stress may cause long lasting and at times permanent deficits.[2] As stress could significantly enhance the toxicity of drugs and chemicals, a number of experimental studies have been carried out to understand the impact of different stressors on the neurotoxicity of environmental chemicals.[6, 14-16] Use of synthetic pyrethroids has increased significantly in recent years due to their high bioefficacy and low mammalian toxicity.[17] A number of formulations of pyrethroids are commercially available for their specific uses.[18] LCT, a new generation type II synthetic pyretrhroid has extensive uses both as indoor and outdoor pesticide.[19] It is used to control insects, pests and ectoparasites in agriculture and veterinary practices.[20] Besides, use of LCT is also preferred in homes, gardens and public health programmes.[19] While pyrethroids are considered safe, presence of high levels of their metabolites in urine and blood in children and exposed individuals have aroused a great concern due to associated health effects including neurobehavioral toxicity.[21, 22] Further, presence of residues of LCT in vegetables, cattle milk and meat enhances the risk of human exposure and is a cause of concern.[17, 23] Inspite of the fact that chronic exposure to LCT could cause neurobehavioral toxicity, effect of stressors and their impact on the severity of changes is not known. The present study has therefore been carried out to investigate the effect of immobilization stress, a psychological stressor and forced swim stress, a physical stressor on the integrity of key parameters associated with oxidative stress in rat brain.

2. Materials and Methods

Animals and housing conditions: Adult male rats of Wistar strain weighing 200 ± 10 gms were used for the present study. Rats were obtained from the animal breeding colony of CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Lucknow and housed in polypropylene cages in the experimental animal room. The temperature of the experimental room was set at 25 ± 2°C with a 12 hours light / dark cycle. Pellet diet, procured commercially and water were available *ad libitum* to the animals.

Treatment procedure: After acclimatization for at least 5 days, rats were randomly divided in to six groups. Group I rats were handled daily and given corn oil orally for 28 days and served as controls, Group II rats were exposed to LCT (0.5 mg/kg body weight, p.o. suspended in corn oil) for 28 days, Group III rats were subjected to IMS daily for 28 days by placing them in to acrylic tubes (22 cm length and 7 cm diameter; 1 session of 6 hr/day) following the procedure as described by Rao et al.[23], Group IV rats were simultaneously exposed to IMS and LCT identically as in groups II and III respectively for 28 days, Group V rats were subjected to FSS daily for 28 days 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 15 min / day) following the procedure as described by Badowska-Szalewska et al.[24], Group VI rats were simultaneously exposed to FSS and LCT identically as in groups V and II respectively for 28 days.

Exposure of rats to stress and LCT was given between 09:00 – 12:00 hrs. In case of simultaneous exposure to stressors and LCT, rats were first subjected to either IMS or FSS followed by LCT exposure after a gap of 15 minutes. The animals were sacrificed by cervical dislocation between 09:00 – 16:00 hrs, 24 hrs after the last exposure

to stressors and LCT. Brain was removed immediately and dissected into corpus striatum, frontal cortex, hippocampus and hypothalamus following the standard procedure by Glowinski and Iversen.[25] The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of CSIR-IITR, Lucknow and all the experimental procedures were carried out in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests (Government of India), New Delhi, India.

Assessment of oxidative stress: To understand the extent of oxidative stress following exposure to stressors, LCT and their co-exposure, effect on the levels of lipid peroxidation, protein carbonyls and reduced glutathione and activity of superoxide dismutase, catalase and glutathione peroxidase was assessed in selected brain regions.

Assay of lipid peroxidation: Formation of malonaldehyde (MDA) was estimated in brain regions to assess the extent of lipid peroxidation, following the procedure as described by Ohkawa et al.[26]

Assay of protein carbonyl levels: The isolated brain regions were homogenized separately in phosphate buffer (50mM, pH 7.4, 10% w/v) and centrifuged at 11,000xg for 15 min. The supernatant was separated and used to assess the protein carbonyl levels following the methods of Levine et al.[27] using 2,4 - dinitrophenylhydrazine (DNPH) as a substrate.

Assay of reduced glutathione levels: The method of Hasan and Haider,[28] was followed to measure the levels of reduced glutathione (GSH) in specific brain regions.

Assay of superoxide dismutase activity: Activity of superoxide dismutase in brain regions was measured

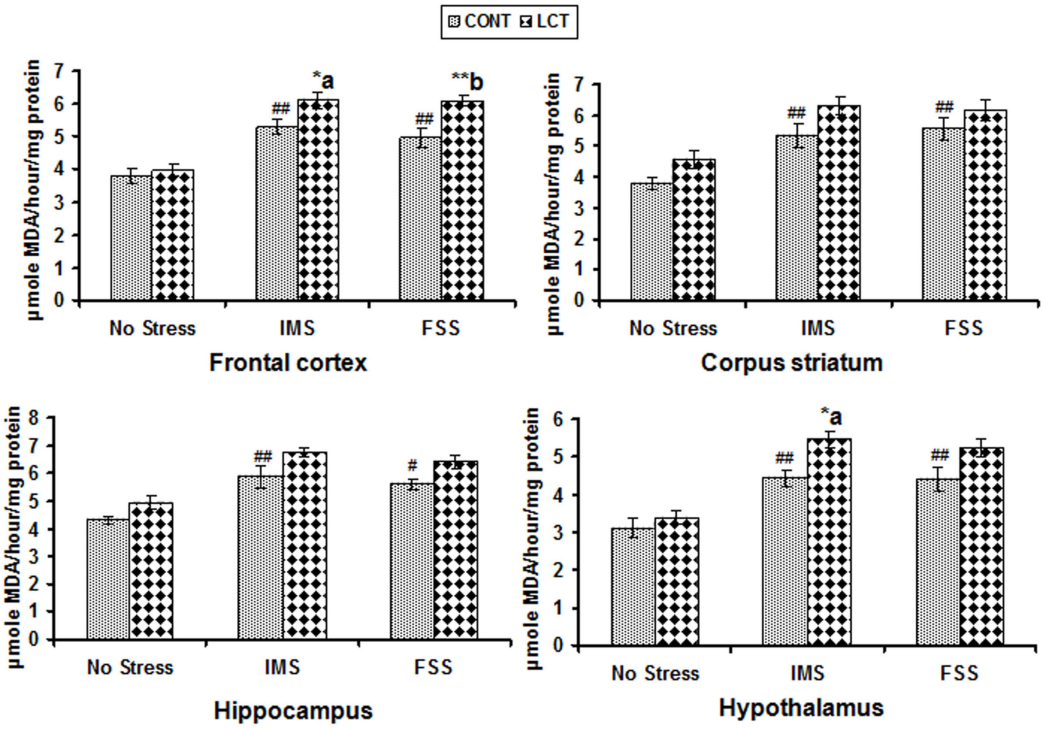


Figure 1. Effect on lipid peroxidation in brain regions following exposure of rats to immobilization stress, forced swim stress and lambda-cyhalothrin alone or in combination for 28 days

Values are mean ± SEM of five rats in each group. #-compared to control group. a-compared to IMS group, b-compared to FSS group. Significantly differs (#p < 0.05, ##p < 0.01; ###p < 0.001); (**p < 0.01, ***p < 0.001). CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress.

following the method of Kakkar et al.[29] with minor modifications.

Assay of catalase activity: The method of Aebi et al.[30] was followed to assess the activity of catalase in brain regions using H_2O_2 as substrate.

Assay of glutathione peroxidase activity: Assay of glutathione peroxidase activity was done following the method of Flohe and Gunzler.[31]

Protein estimation: Protein content was measured following the method of Lowry et al. [32] Bovine serum albumin was used as a reference standard and a curve using its varying concentrations was constructed to calculate the protein in samples.

3. Statistical analysis:

In the present study, two-way ANOVA was carried out using GraphPad prism (3.0) software to analyze the changes in parameters of oxidative stress endpoints on exposure to either of the stressors (immobilization or forced swim stress) or lambda-cyhalothrin and to assess the interaction in each brain regions. Further, the general linear regression model had been used to assess the effect level of immobilization stress, forced swim stress and lambda-cyhalothrin. One way ANOVA was used to compare among the groups while interaction was found significant. Bonferroni post hoc test was carried out to assess the level of significance. The data has been expressed as mean ± SEM and values up to $p<0.05$ are considered statistically significant.

4. Results

Effect on lipid peroxidation in brain regions: The two-way ANOVA revealed that exposure to stress and LCT resulted to increase the levels of MDA in frontal cortex, corpus striatum, hippocampus and hypothalamus. However, no interaction was observed between LCT and stress exposure in any of the brain regions (Figure – 1).

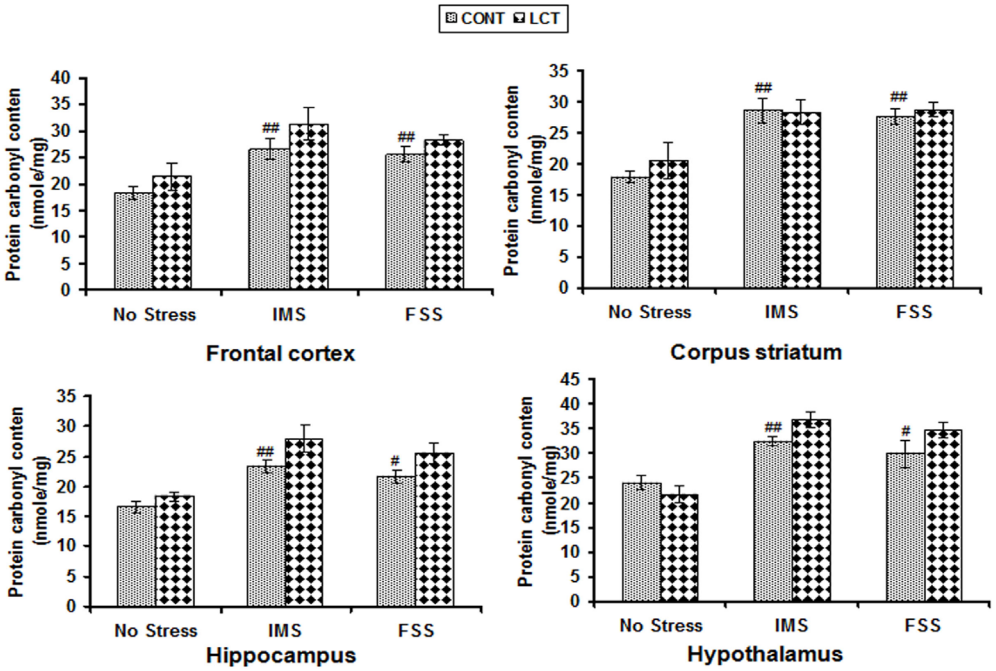


Figure 2. Effect on protein carbonyl levels in brain regions following exposure of rats to immobilization stress, forced swim stress and lambda-cyhalothrin alone or in combination for 28 days

Values are mean ± SEM of five rats in each group. #-compared to control group. a-compared to IMS group, b-compared to FSS group. Significantly differs (#$p < 0.05$, ##$p < 0.01$; ###$p < 0.001$); (**$p < 0.01$, ***$p < 0.001$). CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress.

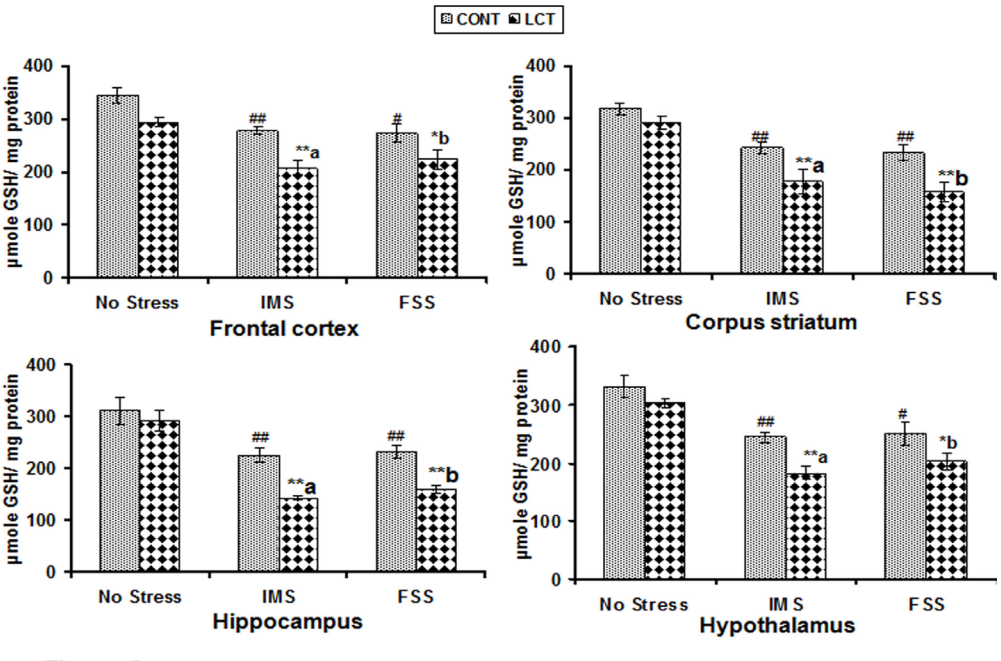


Figure 3. Effect on reduced glutathione levels in brain regions following exposure of rats to immobilization stress, forced swim stress and lambda-cyhalothrin alone or in combination for 28 days
Values are mean ± SEM of five rats in each group. #-compared to control group, a-compared to IMS group, b-compared to FSS group. Significantly differs (#p < 0.05, ##p < 0.01); (*p < 0.05, **p < 0.01). CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress.

Effect on protein carbonyl levels in brain regions: A significant increase in protein carbonyl levels was observed on exposure of rats to stress and LCT in frontal cortex, corpus striatum, hippocampus and hypothalamus as revealed by two-way ANOVA (Figure – 2). There was however, no interaction between LCT and stress exposure in any of the brain regions.

Effect on reduced glutathione levels in brain regions: Although stress and LCT affected the GSH levels in frontal cortex, corpus striatum, hippocampus and hypothalamus, there was no significant interaction between LCT and stress exposure on GSH levels (Figure – 3). The regression analysis further showed significant decrease in the GSH levels in frontal cortex, corpus striatum, hippocampus and hypothalamus as compared to controls.

Effect on superoxide dismutase activity in brain regions: The two-way ANOVA exhibited exposure to stress and LCT resulted to decrease the activity of superoxide dismutase, an enzyme involved in the dismutation of superoxide radicals in frontal cortex, corpus striatum, hippocampus and hypothalamus while there was no interaction between LCT and stress exposure in any of the brain regions (Figure – 4).

Effect on catalase activity in brain regions: A significant decrease in catalase activity was observed on exposure of rats to stress and LCT in frontal cortex, corpus striatum, hippocampus and hypothalamus as revealed by the two-way ANOVA (Figure – 5). There was however, no interaction between LCT and stress exposure in any of the brain regions.

Effect on glutathione peroxidase activity in brain regions: The two-way ANOVA showed that exposure to LCT and stress resulted to decrease the activity of glutathione peroxidase in frontal cortex, corpus striatum, hippocampus and hypothalamus No interaction was however observed between LCT and stress exposure in any of the brain regions (Figure – 6).

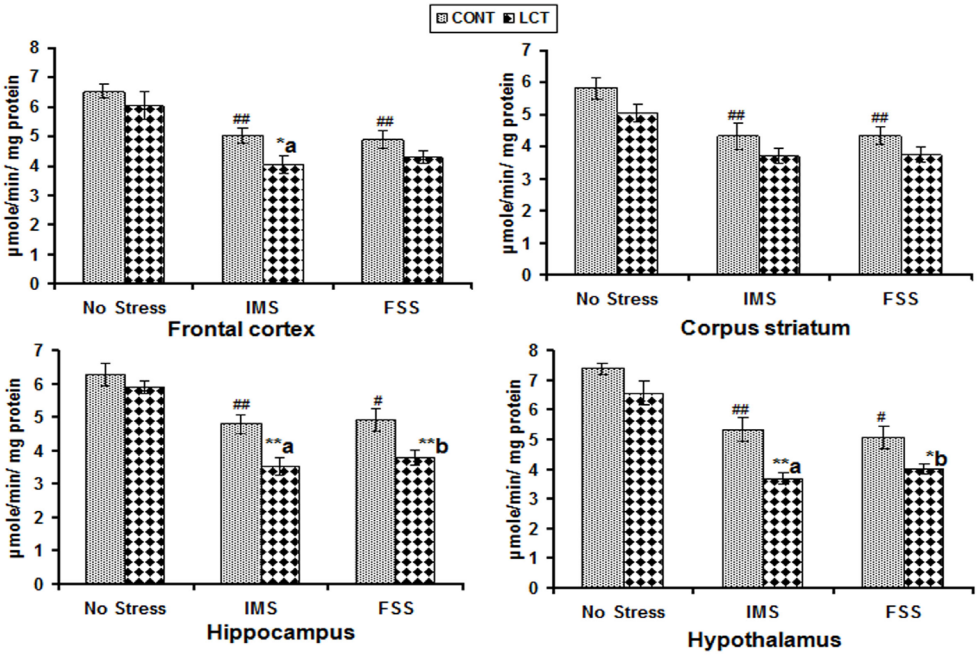


Figure 5. Effect on catalase activity in brain regions following exposure of rats to immobilization stress, forced swim stress and lambda-cyhalothrin alone or in combination for 28 days

Values are mean ± SEM of five rats in each group. # - compared to control group, a-compared to IMS group, b-compared to FSS group. Significantly differs (#p < 0.05, ##p < 0.01); (**p < 0.01, ***p < 0.001). CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress.

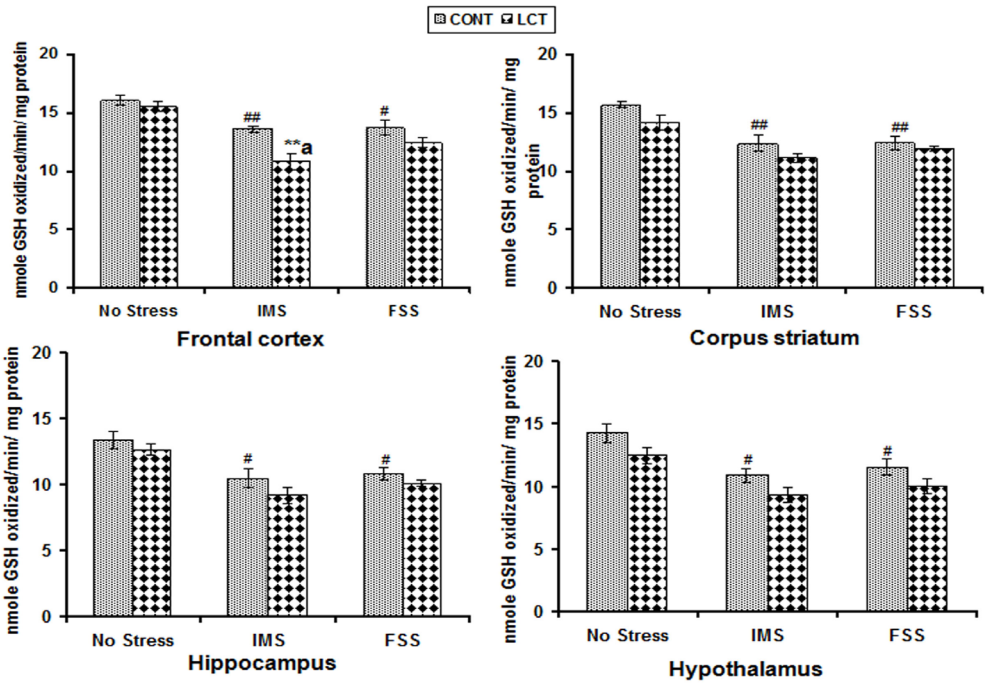


Figure 6. Effect on glutathione peroxidase activity in brain regions following exposure of rats to immobilization stress, forced swim stress and lambda-cyhalothrin alone or in combination for 28 days

Values are mean ± SEM of five rats in each group. # - compared to control group, a-compared to IMS group, b-compared to FSS group. Significantly differs (#p < 0.05, ##p < 0.01); CONT – Control, LCT - Lambda-cyhalothrin, IMS – Immobilization stress, FSS – Forced swim stress.

5. Discussion:

Although synthetic pyrethroids primarily target the voltage-dependent sodium channels leading to hyperpolarization,[33] enhanced oxidative stress in brain has been found to be an important contributor in the neurotoxicity of LCT.[17] Early life exposure to LCT resulted to increase lipid peroxidation and protein carbonyl levels associated with decrease in the levels of reduced glutathione and activity of superoxide dismutase, catalase and glutathione peroxidase in different brain regions of developing rats in one of our earlier study. [8] The intensity of changes was more in rats exposed to LCT at a high dose (3 mg/kg body weight) as compared to those exposed at a low dose (1 mg/kg body weight) for 28 days.[8] Interestingly, the changes in these parameters remained persistent in rats exposed to LCT at a high dose (3 mg/kg body weight) even on withdrawal of exposure for 15 days.[8] Enhanced oxidative stress in brain has also been observed on exposure to deltamethrin and fenvalerate, the other pyrethroids in experimental animals.[34, 35 33, 34] In view of involvement of free radicals in chemical induced neurotoxicity, a number of studies have been carried out to assess the impact of oxidative stress to understand the associated mechanism.[17] In the present study, no significant change was observed in lipid peroxidation, protein carbonyl and reduced glutathione levels and also in the activity of superoxide dismutase, catalase and glutathione peroxidase in any of the brain regions of rats treated with LCT (0.5 mg/kg) for 28 days and could possibly be due to low dose of LCT.

Brain as compared to other organs is more vulnerable to different stressors including physical, psychological, social and environmental. A number of experimental studies have found that stress could significantly enhance the ROS generation by modulating the biochemical pathways in brain.[9, 17, 36] Impairment in the antioxidant defense as a result of stress has also been observed by a number of investigators.[9, 18] Further, brain is an easy target of ROS due to inefficient anti-oxidant capacity and therefore, imbalance in the pro-oxidant and anti-oxidant ratio may cause oxidative damage due to presence of high levels of PUFA in the brain.[10] Generation of reactive oxygen species in the biological reactions is obvious and impart different role, however, increased ROS generation may enhance lipid peroxidation which could affect the integrity of cellular structure and function.[37] In an interesting study, psychological stress for 4 hours in rats resulted to increase lipid peroxidation in the brain although no such effect on lipid peroxidation was observed in liver and serum suggesting enhanced vulnerability of brain to stress [ ] (Matsumoto 1999). Increase in lipid peroxidation and nitrite levels in hippocampus was observed in rats subjected to chronic restraint stress.[38] Interestingly, these changes were associated with cognitive dysfunctions in these rats.[38] In the present study, rats subjected to IMS (6 hour/day) or FSS (15 minutes/day) for 28 days exhibited enhanced lipid peroxidation and protein carbonyl levels in the frontal cortex, corpus striatum, hippocampus and hypothalamus. Consistent with this, decrease in reduced glutathione levels and activity of superoxide dismutase, catalase and glutathione peroxidase was also observed in these brain regions of rats subjected either to IMS or FSS for 28 days. Exposure to acute or chronic stress may affect the biochemical pathways by generating free radicals,[10, 39] which may damage biomembranes and affect the integrity and functions of cells.

Epidemiological and clinical evidences suggest that stress is one of the important factor in the etiology of neuropsychiatric disorders.[1, 40] Stress induced oxidative stress has been found to enhance inflammation and disrupt immune functions with increased vulnerability of human to cause neurodegenerative disorders.[41] Interestingly, soon after the gulf war, it was also realized that stress may modify the toxic response of drugs and affect the functioning of CNS which are not toxic in normal situation in the veterans.[42] Experimental studies further revealed that stress could significantly modify the neurotoxicity of drugs and environmental chemicals including metals and pesticides.[15, 43, 44] Stress has also been found to exacerbate neurotoxicity of drugs and chemicals in a number of experimental studies.[6, 14] It has been convincingly demonstrated that blood brain barrier is an important determinant in the neurotoxicity of drugs and chemicals.[7, 42] As a result, the chemicals that do not cross the BBB easily penetrate the brain due to modification or disruption in the BBB permeability.[7, 42] In the present study, simultaneous exposure to stress and LCT resulted to increase lipid peroxidation and decrease reduced glutathione levels in brain regions of rats. The depletion of GSH levels in the brain regions of rats could be due to high consumption of GSH for conjugation and/or contribution of GSH as an antioxidant in neutralizing reactive free radicals.[17] In another report, LCT induced toxicity has been suggested due to the cyanohydrins, considered to be highly unstable free radical species in

physiological conditions.[45] Further, decrease in the activity of superoxide dismutase, catalase and glutathione peroxidase in different brain region of rats simultaneously exposed to stress and LCT in the present study indicates impairment in the antioxidant defense which could be due to excessive free radical generation. As catalase and glutathione peroxidase are primarily involved in the degradation of hydrogen peroxide to oxygen and water, decrease in the activity of these enzymes may be associated with enhanced levels of hydrogen peroxide, considered to be toxic to the brain.[46, 47] Further, disruption of blood brain barrier permeability due to increased generation of free radical species could disrupt the homeostasis and metabolic activities of the brain.[48]

Although lambda-cyhalothrin alone had no significant effect on the parameters associated with oxidative stress, exposure to immobilization and forced swim stress resulted to increase the oxidative stress in rat brain. The data further exhibit that both immobilization and forced swim stress could significantly enhance oxidative stress in brain of LCT exposed rats. The results of the present study further suggest that psychological and physical stressors could significantly contribute to enhance LCT neurotoxicity.

6. Acknowledgment

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7. Conflict of Interest

None Declared

8. References

1. Cohen S, Janicki-Deverts D and Miller GE. Psychological stress and disease. Jama. 2007; 298: 1685-7.
2. Herman JP and Seroogy K. Hypothalamic-pituitary-adrenal axis, glucocorticoids, and neurologic disease. Neurologic clinics. 2006; 24: 461-81.
3. Sapolsky RM. Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. Archives of general psychiatry. 2000; 57: 925-35.
4. Cory-Slechta DA, Stern S, Weston D, Allen JL and Liu S. Enhanced learning deficits in female rats following lifetime Pb exposure combined with prenatal stress. Toxicological Sciences. 2010; 117: 427-38.
5. Fink G. Stress: definition and history. Stress Science: Neuroendocrinology. 2010: 3-9.
6. Friedman A, Kaufer D, Shemer J, Hendler I, Soreq H and Tur-Kaspa I. Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response. Nature medicine. 1996; 2: 1382-5.
7. Hanin I. The Gulf War, stress and a leaky blood—brain barrier. Nature medicine. 1996; 2: 1307-8.
8. Ansari RW, Shukla RK, Yadav RS, et al. Cholinergic dysfunctions and enhanced oxidative stress in the neurobehavioral toxicity of lambda-cyhalothrin in developing rats. Neurotoxicity research. 2012; 22: 292-309.
9. Gandhi S and Abramov AY. Mechanism of oxidative stress in neurodegeneration. Oxidative medicine and cellular longevity. 2012; 2012.
10. Madrigal JL, Olivenza R, Moro MA, et al. Glutathione depletion, lipid peroxidation and mitochondrial dysfunction are induced by chronic stress in rat brain. Neuropsychopharmacology. 2001; 24: 420-9.
11. Zafir A and Banu N. Modulation of in vivo oxidative status by exogenous corticosterone and restraint stress in rats. Stress. 2009; 12: 167-77.
12. Bhatia N, Maiti PP, Choudhary A, et al. Animal models in the study of stress: A review. J Pharm and Healthc Manag. 2011; 2: 42-50.
13. Jaggi AS, Bhatia N, Kumar N, Singh N, Anand P and Dhawan R. A review on animal models for screening potential anti-stress agents. Neurological Sciences. 2011; 32: 993-1005.
14. Abdel-Rahman A, Abou-Donia SM, El-Masry EM, Shetty AK and Abou-Donia MB. Stress and combined exposure to low doses of pyridostigmine bromide, DEET, and permethrin produce neurochemical and neuropathological alterations in cerebral cortex, hippocampus, and cerebellum. Journal of Toxicology and Environmental Health, Part A. 2004; 67: 163-92.
15. Hancock S, Ehrich M, Hinckley J, Pung T and Jortner BS. The effect of stress on the acute neurotoxicity of the organophosphate insecticide chlorpyrifos. Toxicology and applied pharmacology. 2007; 219: 136-41.
16. Pung T, Klein B, Blodgett D, Jortner B and Ehrich M. Examination of concurrent exposure to repeated stress and chlorpyrifos on cholinergic, glutamatergic, and monoamine neurotransmitter systems in rat forebrain regions. International journal of toxicology. 2006; 25: 65-80.
17. Fetoui H, Garoui EM, Makni-ayadi F and Zeghal N. Oxidative stress induced by lambda-cyhalothrin (LTC) in rat erythrocytes and brain: attenuation by vitamin C. Environmental Toxicology and Pharmacology. 2008; 26: 225-31.

18. Fetoui H, Feki A, Salah GB, Kamoun H, Fakhfakh F and Gdoura R. Exposure to lambda-cyhalothrin, a synthetic pyrethroid, increases reactive oxygen species production and induces genotoxicity in rat peripheral blood. Toxicology and industrial health. 2015; 31: 433-41.
19. He L-M, Troiano J, Wang A and Goh K. Environmental chemistry, ecotoxicity, and fate of lambda-cyhalothrin. Reviews of environmental contamination and toxicology. Springer, 2008, p. 71-91.
20. Fetoui H, Garoui EM and Zeghal N. Lambda-cyhalothrin-induced biochemical and histopathological changes in the liver of rats: ameliorative effect of ascorbic acid. Experimental and Toxicologic Pathology. 2009; 61: 189-96.
21. Barr DB, Olsson AO, Wong L-Y, et al. Urinary Concentrations of Metabolites of Pyrethroid Insecticides in the General U. S. Population: National Health and Nutrition Examination Survey 1999-2002. Environmental health perspectives. 2010; 118: 742-8.
22. Muhammad F, Akhtar M, Rahman Z, Farooq H, Khaliq T and Anwar M. Multi-residue determination of pesticides in the meat of cattle in Faisalabad-Pakistan. Egypt Acad J Biol Sci. 2010; 2: 19-28.
23. Rao BS, Madhavi R, Sunanda and Raju T. Complete reversal of dendritic atrophy in CA3 neurons of the hippocampus by rehabilitation in restraint stressed rats. Current science. 2001; 80: 653-9.
24. Badowska-Szalewska E, Klejbor I, Cecot T, Spodnik JH and Moryś J. Changes in NGF/c-Fos double staining in the structures of the limbic system in juvenile and aged rats exposed to forced swim test. Acta neurobiologiae experimentalis. 2009; 69: 448-58.
25. GItiwinski J and Iversen L. Regional studies of catecholamines in the rat brain. The disposition of 3H-norepinephrine, 3H-dopamine and3H-DOPA in various regions of the brain. J Neurochem. 1966; 13: 655-69.
26. Ohkawa H, Ohishi N and Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. 1979; 95: 351-8.
27. Levine RL, Garland D, Oliver CN, et al. Determination of carbonyl content in oxidatively modified proteins. Methods in enzymology. 1990: 464-78.
28. Hasan M and Haider S. Acetyl-homocysteine thiolactone protects against some neurotoxic effects of thallium. Neurotoxicology. 1988; 10: 257-61.
29. Kakkar P, Das B and Viswanathan P. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984; 21: 130-2.
30. Aebi H. [13] Catalase in vitro. Methods in enzymology. 1984; 105: 121-6.
31. Flohé L and Günzler WA. Assays of glutathione peroxidase. Methods in enzymology. 1984; 105: 114.
32. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the Folin phenol reagent. J biol Chem. 1951; 193: 265-75.
33. Ray DE and Fry JR. A reassessment of the neurotoxicity of pyrethroid insecticides. Pharmacology & therapeutics. 2006; 111: 174-93.
34. Prasanthi K and Rajini P. Fenvalerate-induced oxidative damage in rat tissues and its attenuation by dietary sesame oil. Food and chemical toxicology. 2005; 43: 299-306.
35. Kilian E, Delport R, Bornman M and Jager Cd. Simultaneous exposure to low concentrations of dichlorodiphenyltrichloroethane, deltamethrin, nonylphenol and phytoestrogens has negative effects on the reproductive parameters in male Spraque-Dawley rats. Andrologia. 2007; 39: 128-35.
36. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S and Rezaiee A. Pesticides and oxidative stress: a review. Medical Science Review. 2004; 10: RA141-RA7.
37. Mancuso C, Scapagini G, Curro D, et al. Mitochondrial dysfunction, free radical generation and cellular stress response in neurodegenerative disorders. Front Biosci. 2007; 12: 1107-23.
38. Abidin I, Yargicoglu P, Agar A, et al. The effect of chronic restraint stress on spatial learning and memory: relation to oxidant stress. International journal of neuroscience. 2004; 114: 683-99.
39. Olivenza R, Moro MA, Lizasoain I, et al. Chronic stress induces the expression of inducible nitric oxide synthase in rat brain cortex. Journal of neurochemistry. 2000; 74: 785-91.
40. Esch T, Fricchione GL and Stefano GB. The therapeutic use of the relaxation response in stress-related diseases. Medical Science Monitor. 2003; 9: RA23-RA34.
41. de Pablos RM, Herrera AJ, Espinosa-Oliva AM, et al. Chronic stress enhances microglia activation and exacerbates death of nigral dopaminergic neurons under conditions of inflammation. J Neuroinflammation. 2014; 11: 34.
42. Abdel-Rahman A, Shetty AK and Abou-Donia MB. Disruption of the blood–brain barrier and neuronal cell death in cingulate cortex, dentate gyrus, thalamus, and hypothalamus in a rat model of Gulf-War syndrome. Neurobiology of Disease. 2002; 10: 306-26.
43. Habr SF, Macrini DJ, Spinosa HdS, Florio JC and Bernardi MM. Repeated forced swim stress has additive effects in anxiety behavior and in cathecolamine levels of adult rats exposed to deltamethrin. Neurotoxicology and teratology. 2014; 46: 57-61.
44. Terçariol SG, Almeida AA and Godinho AF. Cadmium and exposure to stress increase aggressive behavior. environmental toxicology and pharmacology. 2011; 32: 40-5.
45. Organization WH. Cyhalothrin. 1990.
46. Reiter RJ, Tan D-x, Osuna C and Gitto E. Actions of melatonin in the reduction of oxidative stress. Journal of biomedical science. 2000; 7: 444-58.
47. Mates J. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology. 2000; 153: 83-104.
48. Škultétyová I, Tokarev D and Ježová D. Stress-induced increase in blood–brain barrier permeability in control and monosodium glutamate-treated rats. Brain research bulletin. 1998; 45: 175-8.